The Study of Au(III) Compounds and their Interaction with Zinc Finger Proteins

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THE STUDY OF AU(III) COMPOUNDS AND THEIR INTERACTION WITH ZINC FINGER PROTEINS

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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May 2014
Acknowledgement

First and foremost, I would like to give thanks to God. If it weren’t for Him I would not be here today. It was my faith in Christ that helped me through all the rough times on this long and winding road called graduate school. I would also like to thank my parents for always encouraging and believing in me. I never would have made it this far if it weren’t for them and I will never be able to repay them for all they’ve done for me over the years. I would like to thank my boyfriend, Chris, for being there for me every step of this journey and always being patient and supportive. I would like to thank my advisor, Dr. Nicholas Farrell, for allowing me to join his group. He has taught me so much and always had a way of keeping me motivated and thinking positive. I would like to thank all of my group members, especially Samantha Tsotsoros and Daniel Lee, for their help and encouragement over the past five years. I would like to thank my committee members and all faculty members that had a role in my project for their help, advice and feedback. I would also like to thank the National Science Foundation for funding my research, as well as Altria for granting me a fellowship during my last year of research.
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**Figure 6.27.** High aggregate species is due to drug:SL2 complex. Lane 1: SL2, Lane 2: NCp7/SL2, Lane 3: NC/1mM \([\text{Au(dien)(9-EtG)}]^3^+\), Lane 4: 1mM \([\text{Au(dien)(9-EtG)}]^3^+\) + SL2.
**Figure 6.28.** HIV-testing results from the Southern Research Institute for [Au(dien)(5’GMP)]^{2+} with three HIV isolates

**Figure 7.1** Graphical representation of ZF/DNA antagonism

**Figure 7.2** Proposed Au(III)-N,C,C chelates.
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(A) Proposed TrxR Mechanism. First, the selenenylsulfide is reduced to the selenolate anion
(Se\(^-\)) with electrons received from NADPH via FAD. A second electron transfer from a
second molecule of NADPH reduces the active site thiol bonds with one Cys residue
stabilized by an interaction with FAD (Step 1). The selenolate anion then attacks the
disulfide bonds of Trx and the resulting enzyme-Trx mixed selenenylsulfide (Step 2), which
is then subsequently attacked by the neighboring Cys residue to regenerate the
selenenylsulfide (Step 3). This selenenylsulfide is then reduced by the active-site thiolate
from the other subunit (Step 4).

(B) Mechanism for H\(_2\)O\(_2\) reduction by TrxR. The nucleophilic selenolate is oxidized by H\(_2\)O\(_2\),
producing selenenic acid (-SeOH). One cysteine thiol reacts with the selenenic acid to
produce water and to reform the selenenylsulfide. A second thiol then attacks the bridge to
regenerate the selenol.

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Schematic representation of the interaction of Au(I)-N-heterocycle compounds with NCp7(F2).

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Various substitution reactions of [AuCl(dien)]\(^{2+}\) with water and strong nucleophiles.

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Reaction pathway of [Zn(bme-dach)]$_2$ with *trans*-\([\text{PtCl}(9\text{-EtGua})(\text{pyr})_2]\)^+ with formation of monothiolate bridged and metal exchanged species.
List of Abbreviations

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PR  progesterone receptor
PRE  progesterone DNA response element
py/pyr  pyridine
Q  glutamine
quin  quinoline
R  arginine
RNA  ribonucleic acid
RT  reverse transcriptase
salen  N,N'-ethylene bis(salicylideneimine)
SAMT  S-acyl-2-mercaptobenzamide thioesters
Sec  selenocysteine
Ser  serine
SIV  simian immunodeficiency virus
SL2  stem loop 2
Sp  specificity protein family
Sp1(F3)  specificity protein transcription factor 1 – zinc finger three
T  thymine
T-47D  breast cancer cell line
terpy  2,2’,2”-terpyridine
TDDP  transplatin, trans-diamminedichloroplatinum(II)
TFIIIA  transcription factor for polymerase IIIA
Thr  threonine
TMPyP  meso-tetrakis(N-methylypyridinium-4-yl)porphyrin
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List of Chemical Formulas

\[[\text{Au(dppe)}_2]\text{Cl}\] \quad \text{bis}[1,2\text{-bis(diphenylphosphino)ethane}]\text{gold(I) chloride}

\[[\text{Au(d}_{2}\text{pypp})_2]\text{Cl}\] \quad \text{bis}[1,3\text{-bis(di-2-pyridylphosphino)propane}]\text{gold(I) chloride}

\[[\text{(Et}_3\text{P})\text{AuCl}]\] \quad \text{chlorotriethylphosphinegold(I)}

\[[\text{(Ph}_3\text{P})\text{Au(I)}(\text{DMAP})]\text{]}^+ \quad 4\text{-dimethylaminopyridinetriphenylphosphinegold(I)}

\[[\text{Au(CN)}_2]^+ \quad \text{gold(I) cyanide}

\[[\text{AuCl(dien)}]\text{Cl}_2\] \quad \text{chlorodiethylenetriaminegold(III) dichloride}

\[[\text{Au(en}_2)]\text{Cl}_3\] \quad \text{bis(ethylenediamine)gold(III) trichloride}

\[[\text{Au(cyclam)}](\text{ClO}_4)_2\text{Cl}\] \quad 1,4,8,11\text{-tetraazacyclotetradecane}gold(III)

\quad \text{chlorobis(perchlorate)}

\[[\text{AuCl(terpy)}]\text{Cl}_2\] \quad \text{chloro(2,2',2"\text{-terpyridine})gold(III) dichloride}

\[[\text{Au(TMPyP)}]\text{Cl}_5\] \quad \text{meso-tetrakis(N-methlypyridinium-4-yl)porphyringold(III)}

\quad \text{pentachloride}

\[[\text{Au(salen)}]^+ \quad \text{N,N'}\text{-ethylene bis(salicylideneimine)gold(III)}

\[[\text{Au(dcbpb)}]^+ \quad 4,5\text{-dichloro-1,2-bis(2-(4-tert-}

\quad \text{butylpyridine)carboxamidogold(III)}

\[[\text{Au(phen)}]\text{Cl}_2]^+ \quad \text{dichlorophenanthrolinegold(III)}

\[[\text{Au(bipy)}]\text{Cl}_2]^+ \quad \text{dichloro(2,2-bipyridine)gold(III)}

\[[\text{AuCl(dien)}]\text{Cl(\text{ClO}_4)}\] \quad \text{chlorodiethylenetriaminegold(III) chloroperchlorate}

\[[\text{AuCl(E}_4\text{dien)}]\text{PF}_6\] \quad \text{chloro(N,N,N","N"-tetraethyl} \text{diethylenetriamine)gold(III)}

\[[\text{AuCl(E}_2\text{dien)}]\text{PF}_6\] \quad \text{chloro(N,N-diethyldiethylenetriamine)gold(III)}

\quad \text{hexafluorophosphate}
\[\text{[AuCl(Me}_4\text{dien})]\text{PF}_6\] chloro(1,1,7,7-tetramethyldiethylenetriamine)gold(III) hexafluorophosphate

\[\text{[AuCl(Me}_2\text{dien})]\text{PF}_6\] chloro(\text{N,N-dimethyl}diethylenetriamine)gold(III) hexafluorophosphate

\[\text{[HAuCl}_4\text{]}\] hydrogen tetrachloroaurate

\[\text{[AuCl}_3\text{(9-EtG)}]\] trichloro(9-ethylguanine)gold(III)

\[\text{[Au(dien)(9-EtG)](NO}_3\text{)}_3\] diethylenetriamine(9-ethylguanine)gold(III) trinitrate

\[\text{[AuCl(N-Medien)]Cl}_2\] chloro(2,2’-diamino-N-methyl-diethylenetriamine)gold(III) dichloride

\[\text{[Au(N-Medien)(9-EtG)](NO}_3\text{)}_3\] 2,2’-diamino-N-methyl-diethylenetriamine(9-ethylguanine)gold(III) trinitrate

\[\text{[Au(dien)(DMAP)]Cl}_3\] diethylenetriamine(4-dimethylaminopyridine)gold(III) trichloride

\[\text{[Au(N-Medien)(DMAP)]Cl}_3\] 2,2’-diamino-N-methyl-diethylenetriamine(4-dimethylaminopyridine)gold(III) trichloride

\[\text{[Au(dien)(4-picoline)](NO}_3\text{)}_3\] diethylenetriamine(4-picoline)gold(III) trinitrate

\[\text{[Au(dien)(2-amino-4-picoline)]Cl}_3\] (2-amino-4-picoline)diethylenetriaminegold(III) trichloride

\[\text{[Au(dien)(4-aminoquinaldine)]Cl}_3\] (4-aminoquinaldine)diethylenetriaminegold(III) trichloride

\[\text{[Au(dien)(guanosine)](NO}_3\text{)}_3\] diethylenetriamineguanosinegold(III) trinitrate

\[\text{[Au(dien)(5’GMP)](NO}_3\text{)}_2\] diethylenetriamine(5’guanosinemonophosphate)gold(III) dinitrate

\[\text{[Au(dien)(1-MetCyt)](NO}_3\text{)}_3\] diethylenetriamine(1-methylcytosine)gold(III) trinitrate

\[\text{[Au(dien)(1-MetCyt)]Cl}_3\] diethylenetriamine(1-methylcytosine)gold(III) trichloride
[Au(dien)(quinolone)]Cl$_3$  
\textit{diethylenetriaminequinolinegold(III) trichloride}

[Au(dien)(isoquinoline)]Cl$_3$  
\textit{diethylenetriamineisoquinolinegold(III) trichloride}

[Au(dien)(pyridine)](NO$_3$)$_3$  
\textit{diethylenetriaminepyridinegold(III) trinitrate}

[AuCl$_2$(dampa)]  
\textit{dichloro(2'[(dimethylamino)methyl]phenyl)gold(III)}

[Pt(dien)(9-EtG)]$^{2+}$  
\textit{diethylenetriamine(9-ethylguanine)platinum(II)}

[Pt(dien)(1-MetCyt)]$^{2+}$  
\textit{diethylenetriamine(1-methylcytosine)platinum(II)}

[Pt(NH$_3$)$_3$(1-MetCyt)]  
\textit{triammine(1-methylcytosine)platinum(II)}

[Pt(dien)(guanosine)]  
\textit{diethylenetriamineguanosineplatinum(II)}

[Pt(dien)(GMP)]  
\textit{diethylenetriamine(5’guanosinemonophosphate)platinum(II)}

[Pt(dien)(bztz)](NO$_3$)$_2$  
\textit{benzothiazolediethylenetriamineplatinum(II) dinitrate}

[Pt(dien)(quin)](NO$_3$)$_2$  
\textit{diethylenetriaminequinolineplatinum(II) dinitrate}

[Pt(dien)(pyr)](NO$_3$)$_2$  
\textit{diethylenetriaminepyridineplatinum(II) dinitrate}

[Pt(dien)(MeOpyr)](NO$_3$)$_2$  
\textit{diethylenetriaminemethoxypyridineplatinum(II) dinitrate}

[Pt(dien)(4-pic)](NO$_3$)$_2$  
\textit{diethylenetriamine(4-picoline)platinum(II) dinitrate}

[Pt(dien)(tz)](NO$_3$)$_2$  
\textit{diethylenetriaminethiazoleplatinum(II) dinitrate}

[Pt(dien)(CNpyr)](NO$_3$)$_2$  
\textit{cyanopyridinediethylenetriamineplatinum(II) dinitrate}

[Pt(dien)(DMAP)]$^{2+}$  
\textit{diethylenetriamine(4-dimethylaminopyridine)platinum(II)}

\textit{trans-}[PtCl$_2$(NH$_3$)$_2$]  
\textit{trans-diamminedichloroplatinum(II), transplatin}

[Pt(terpy)Cl]Cl  
\textit{chloro(2,2',2''-terpyridine)platinum(II) chloride}

(trans-[PtCl$_2$(NH$_3$)(Tz)])  
\textit{trans-amminedichlorothiazoleplatinum(II)}

\textit{cis-}[Pt(NH$_3$)$_2$(Cl)$_2$]  
\textit{cis-diamminedichloroplatinum(II)}

\textit{trans-}[PtCl(9-EtG)(py)$_2$]$^{+}$  
\textit{trans-chloro(9-ethylguanine)dipyridineplatinum(II)}
[PtCl(dien)]^+ \quad \text{chlorodiethylenetriamineplatinum(II)}

[Pd(dien)(9-EtG)]^{2+} \quad \text{diethylenetriamine(9-ethylguanine)palladium(II)}

[Pd(dien)(DMAP)]^{2+} \quad \text{diethylenetriamine(4-dimethylaminopyridine)palladium(II)}

[Zn(bme-dach)]_2 \quad \text{bis[N,N’-bis(2-mercaptoethyl)-1,4-diazacycloheptane]zinc(II)}
Gold compounds have been used in medicine dating back as early as 2500 BC. Over the years gold(I) and gold(III) compounds have been used and designed to target rheumatoid arthritis, cancer, and viral diseases. New drug targets have been found for gold compounds that give insight into their mechanisms of action. Here we focus on the synthesis of Au(III) compounds designed to selectively target zinc finger (ZF) proteins.

ZF proteins exhibit a variety of functions, including transcription, DNA repair, and apoptosis. Displacement of the central zinc ion, along with mutation of coordinated amino acids can result in a loss of biological function. Synthesis of complexes that selectively target zinc finger proteins, in turn inhibiting DNA/ZF interactions and therefore resulting in loss of protein function, is of great interest. Of particular interest here is the Cys₃His (Cys = cysteine, His = histidine) HIV nucleocapsid zinc finger protein, NCp7. NCp7 is involved in multiple steps of the HIV life cycle, thus making it a desirable drug target. Previous studies from our group show platinated nucleobases such as [Pt(dien)(9-EtG)]²⁺ (dien = diethylenetriamine; 9-EtG = 9-
ethylguanine) to stack effectively in a non-covalent manner with tryptophan of the C-terminal finger of HIV Nucleocapsid, NCp7(F2), a key residue involved in nucleic acid recognition. Due to the isoelectronic and isostructural relationship of Au(III) to Pt(II), we have expanded this system to Au(III)-(nucleobase/N-heterocycle) compounds.

Novel Au(III)(dien)(N-heterocycle) compounds, including the first Au(III)N₃(N-purine) examples, were synthesized. As previously reported for [AuCl(dien)]Cl₂, these compounds exhibit pH dependency of the ¹H NMR chemical shifts of the dien ligand. The acidity of the dien ligand is affected by the nature of the fourth ligand as a leaving group. The presence of an inert nitrogen donor, compared to that of the more labile Cl⁻, as the leaving group stabilizes the Au(III) metal center towards reduction, resulting in significant enhancement of π−π stacking interactions with tryptophan relative to platinum(II) and palladium(II) compounds.

The presence of a more inert N-donor as the leaving group slows down the reaction with the sulfur-containing amino acid N-Acetylmethionine (N-AcMet); essentially no reaction was observed for the Au(III)-N-heterocycle compounds. All compounds react readily with N-Acetylcysteine (N-AcCys), however lack of N-heterocycle ligand dissociation indicates, to our knowledge, the first long-lived N-heterocycle-Au-S species in solution.

Electrospray ionization mass spectrometry (ESI-MS) studies with NCp7(F2) indicate [Au(dien)(DMAP)]³⁺ (DMAP = 4-dimethylaminopyridine) to be the least reactive of the Au(III) compounds studied, showing the presence of intact NCp7(F2) zinc finger at initial reaction times. Reactivity of the Au-compounds was compared with that of Sp1(F3), a Cys₂His₂ ZF; in contrast, no intact ZF was observed for any of the compounds studied, suggesting the mode of action of these compounds is dependent on the nature of the zinc binding core.
ESI-MS studies were expanded to that of the full HIV NCp7 zinc finger. \([\text{Au}(\text{dien})(9-\text{EtG})]^3+\) reacts quickly with NCp7, resulting in immediate zinc ejection and replacement with up to three gold ions. Unlike with \([\text{Au}(\text{dien})(\text{DMAP})]^3+\), no intact NCp7 was observed. Addition of \([\text{Au}(\text{dien})(9-\text{EtG})]^3+\) to preformed NC-SL2 complex results in release of free RNA; based on EMSA (electrophoretic mobility shift assay) studies, \([\text{Au}(\text{dien})(9-\text{EtG})]^3+\) disrupts the NCp7-RNA complex with an IC$_{50}$ of ~450 µM. It is possible that this HIV nucleocapsid-nucleic acid antagonism may result in a loss of viral activity.
Chapter 1. Introduction

1.1 Gold compounds in medicine

The earliest use of gold in medicine dates back to 2500 BC when it was used as a therapeutic in Chinese medicine [1,2]. It was later discovered in 1890 that gold(I) cyanide, [Au(CN)₂]⁻, inhibited the growth of *Tubercle bacillus*, which led to the use of Au(I)-thiolate drugs, Figure 1.1, for the treatment of tuberculosis and rheumatoid arthritis with auranofin being approved for clinical use in 1985 [1,2]. Later studies suggested that gold decreases expression of important proinflammatory genes by inhibiting transcription mediated by the AP-1 transcription factor protein. AP-1 DNA binding was inhibited by gold(I)-thiolates by 50% at 5 \( \mu \text{M} \) concentrations of drug; the mechanism of action was hypothesized to be binding to cysteine residues in the DNA binding domain of AP-1 [3].

The discovery of the anticancer properties of cisplatin and its platinum(II) analogues and their clinical success in cancer treatments led to a great deal of interest in the area of metal-based
antitumor agents [4,5]. The fact that gold(I) compounds were already in the clinic for the treatment of rheumatoid arthritis led to their consideration as possible antiproliferative agents [4]. Early studies showed gold(I)-phosphine compounds to be fairly active in vitro but practically ineffective in vivo, likely due to the inherently ‘soft’ nature and, therefore, high thiol affinity of Au(I) [4,6,7]. This causes inactivation of the drug through extensive binding to serum proteins. In the case of auranofin, the tetraacetylthioglucose ligand rapidly dissociates in vivo followed by slower displacement and simultaneous oxidation of the phosphine to Et₃PO [8].

With the aim of reducing the high thiol reactivity, gold(I) complexes with chelated diphosgene ligands such as [Au(dppe)₂]Cl were synthesized and studied, Figure 1.2 [9]. It was found that these compounds accumulate in the mitochondria of tumor cells [1]. The high lipophilicity of these compounds, however, resulted in non-selective reactivity with both tumorigenic and non-tumorigenic cells, resulting in severe toxicity in the heart, liver, and lung as

![Figure 1.2. Structures of (A) [Au(dppe)₂]Cl (dppe = 1,2-Bis(diphenylphosphino)ethane) and (B) [Au(d2pypp)₂]Cl (d2pypp = 1,3-bis(di-2-pyridylphosphino)propane) [10].](image)
a result of mitochondrial dysfunction [1]. A similar compound, [Au(d2pypp)2]Cl, Figure 1.2, was designed with the idea to combine the two distinct features of Au(I)-phosphine drugs; the lipophilicity properties of the bis-chelated ligand allowing accumulation in the mitochondria, as well as enhancement of the reactivity towards protein thiols. This resulted in a compound that reacts selectively towards breast cancer cells but not normal breast cells [10].

Au(III) centers are isoelectronic and isostructural to those of platinum(II), both adopting a square planar geometry. Gold(III) compounds, therefore, looked appealing for cancer treatment as they might exhibit similar biological activity as previously studied platinum(II) drugs [4,5]. Gold (I), however, is thermodynamically more stable than gold(III); many gold(III) complexes are strong oxidizing agents and are easily reduced to Au(I), resulting in generally high toxicity [2]. Initial gold(III) compounds studied, therefore, although highly cytotoxic \textit{in vitro}, demonstrated a poor chemical stability and, also, a rather pronounced systemic toxicity [4].

![Figure 1.2](image1.png)

**Figure 1.2.** structures of Au(I) compounds shown to be more stable and exhibit toxic effects towards tumor cells. (1) [Au(en)2]Cl3, (en = ethylenediamine) (2) [Au(dien)Cl]Cl2, (dien = diethylentriamine) (3) [Au(cyclam)](ClO4)2Cl2(cyclam = 1,4,8,11-tetraazacyclotetradecane) (4) [Au(terpy)Cl]Cl2, (terpy = 2,2′:6′,2″-terpyridine) and (5) [Au(phen)Cl2]Cl (phen = 1,10-phenanthroline) [5,11].
Therefore, new classes of Au(III) compounds containing multidentate ligands such as polyamines and cyleams were synthesized to improve stability, Figure 1.3 [5,11]. All of these novel gold compounds turned out to be highly cytotoxic in vitro against representative human tumor cell lines [5]. The new cytotoxic gold compounds were often found to overcome resistance to cisplatin confirming the occurrence of a substantially different mode of action, which led researchers to speculate the existence of preferential protein targets for gold compounds [4,12].

Hard-soft acid base theory (HSAB) aids in understanding the predominant factors that drive chemical reactions. HSAB theory states that soft acids tend to react faster and form stronger bonds with soft bases. Soft acids and bases are characterized by the following characteristics: large atomic radius, high polarizability, low oxidation state, and low electronegativity [13]. Therefore, the ‘soft’ nature of gold(I) causes it to have a high preference for S-donors; gold drugs undergo ligand exchange with cysteine-rich peptides and proteins such as glutathione and albumin [14]. This reactivity with biological ligands such as cysteine may contribute to their pharmacological activity. The most studied targets of gold compounds are thioredoxin reductase (TrxR) enzymes [1]. TrxR and the thiol protein thioredoxin (Trx) play an integral role in intracellular redox regulation and have been implicated in chronic diseases such as certain cancers and rheumatoid arthritis [1,4,15]. TrxR catalyzes the NADPH-dependent reduction of thioredoxin, supplying electrons to a number of enzymes which rapidly regulates the level of hydrogen peroxide in the cell; refer to Scheme 1.1 for description of the involved mechanism [4,15]. Mammalian TrxRs have a conserved cysteine-rich catalytic site located in the flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide phosphate (NADPH) binding domain as well as a redox active selenocysteine (Sec) residue in the C-terminal active
site which is crucial for catalysis [1,4,15]. This high sulfur content explains the high reactivity of TrxR with gold compounds.

Auranofin inhibits TrxR activity with an IC$_{50}$ value of 4 nM [4]. Electrospray Ionization Mass Spectrometry (ESI-MS) studies of auranofin with a tetrapeptide used as a model for the TrxR C-terminus showed preferential binding of Au(I) to the Sec residue the C-terminal active site with subsequent loss of the thiosugar [16]. A variety of Au(III) drugs, including Au(III)-dithiocarbamates, are potent inhibitors of TrxR, resulting in apoptosis [1]. Some studies suggest
the inhibition of TrxR by Au(III) compounds is due to oxidative damage to the enzyme through oxidation of thiols or selenols rather than gold coordination as seen for Au(I)-drugs [4]. However, the Au(III) metal center may reduce to Au(I) in vivo, and therefore react similarly to Au(I) compounds [1].

Gold compounds have also been implicated as anti human immunodeficiency virus (HIV) agents [1,17]. The inhibition of the HIV reverse transcriptase (RT) by Au(I) aliphatic compounds was first shown in 1989 and the antiarthritic Au(I) drugs aurothioglucose, aurothiomalate and auranofin have all been investigated for their potential as drugs for treating acquired immunodeficiency syndrome (AIDS) [1]. AIDS patients treated with auranofin experienced an increase in CD4⁺ lymphocyte count, when normally the progression of HIV infection is marked by the decline of these cells [8,17]. Aurothioglucose was found to protect cells from HIV infection through ligand exchange with a cysteine (Cys) residue on the viral coat protein [1,8]. There are a few examples of Au(III) drugs that have been reported to show anti-HIV activity; these compounds are shown in Figure 1.4 [18]. The porphyrin complex, [Au(TMPyP)]Cl₅ (Fig 1.4) was found to successfully inhibit HIV-1 RT with an IC₅₀ value of 0.31 µM [17,18].

\[
\text{[Au}^{\text{III}}(\text{salen})]^{+}, \quad \text{[Au}^{\text{III}}(\text{dcbpb})]^{+}, \quad \text{[Au}^{\text{III}}(\text{TMPyP})]^{5+}
\]

**Figure 1.4.** Structures of Au(III)-chelate compounds that have shown anti-HIV properties. [Au(salen)]⁺ (H₂salen = N,N’-ethylene bis(salicylideneimine), [Au(dcbpb)]⁺ (H₂dcbpb = 4,5-dichloro-1,2-bis(2-(4-tert-butylpyridine)carboxamido) benzene), [Au(TMPyP)]⁵⁺ (H₂TMPyP)⁴⁺ = meso-tetrakis(N-methylpyridinium-4-yl)porphyrin) [18].
Inhibition by these Au(III)-porphyrin compounds has been shown to be linked to possible intercalation between nucleic acid base pairs of DNA, thus inhibiting the enzyme from converting viral ribonucleic acid (RNA) to complimentary-DNA (cDNA) [17,19].

As described later in this chapter, the HIV-1 nucleocapsid zinc finger protein, NCp7, is involved in many steps throughout the HIV life-cycle and is thus a desirable drug target. The thiol-rich nature of zinc fingers (ZF) merits the investigation of zinc finger proteins as specific targets of Au-drugs. A variety of Au(I)/(III) drugs react with zinc finger proteins, resulting in zinc ejection and loss of protein function; refer to Section 1.4. It is the goal of this work to synthesize Au(III) compounds that selectively target zinc finger proteins, one specifically being the NCp7 zinc finger protein.

1.2 Zinc Finger Proteins

Zinc fingers are involved in a variety of biological functions including DNA recognition, transcriptional activation, regulation of apoptosis, and protein folding and assembly. Zinc finger proteins comprise 2-3% of the entire human genome and are considered to be the most common DNA binding motifs found in human transcriptional factors [6,20]. In zinc fingers, zinc plays a

![Figure 1.5](image)

*Figure 1.5.* Representative coordination environments for zinc fingers - transcription factor Sp1 (Cys2His2, Finger 2); HIV nucleocapsid protein NCp7 (Cys3His, C-terminal Finger 2); nucleotide excision repair protein component XPA (xeroderma pigmentosum group A) (Cys4) [6].
structural role, typically coordinated to four protein derived Cys and histidine (His) residues. Therefore, zinc fingers are generally classified according to the number and type of amino acids involved in zinc(II) coordination, such as Cys$_2$His$_2$, Cys$_3$His, and Cys$_4$ zinc fingers. Figure 1.5 shows the structures of these three classes of zinc fingers [6].

It is understood that the Zn-cysteine bonds present in zinc fingers are susceptible to oxidation, which may lead to Zn(II) ejection [21,22]. Modification of the zinc finger core by a variety of electrophilic agents, including inorganic and transition metal ions, results in eventual zinc ejection and therefore inhibition of the nucleic acid binding capacity of the modified peptide [23]. It is with this knowledge that new drugs consisting of small inorganic molecules are being synthesized to target such zinc fingers to cause release of zinc and loss of function.

1.2.1 Cys$_2$His$_2$ (C$_2$H$_2$) Zinc Finger Proteins

Many Cys$_2$His$_2$ zinc fingers are transcription factors, which are proteins that bind to specific DNA sequences thereby controlling the flow of genetic information from DNA to messenger-RNA (mRNA) and regulating gene expression. Of the three zinc finger coordination environments shown in Figure 1.5, the Cys$_2$His$_2$ zinc finger motif has been shown to be the least nucleophilic and the least tightly packed [22].

The human transcription factor Sp1 is an example of a Cys$_2$His$_2$ zinc finger protein, where the zinc ion is tetrahedrally bound to two cysteine and two histidine residues. Specificity protein family (Sp) transcription factors regulate a variety of cancer-associated genes involved in cell cycle, cell proliferation and apoptosis [24]. The Sp family consists of several members that contain a highly conserved DNA-binding domain composed of three zinc fingers with typical $\beta\beta\alpha$ zinc finger folds at the C-terminus, Figure 1.6, and serine (Ser), threonine (Thr) and glutamine (Gln) rich transactivation domains at the N-terminus. Sp1 binds specifically to GC-
rich recognition elements (GC-box) mediated by its C-terminal zinc finger domain [25].

The overexpression of Sp1 is associated with tumor development and growth. Recent studies have shown that Sp1 can regulate the expression of human telomerase reverse transcriptase (hTERT), a key component responsible for telomerase activity. Inhibition of the Sp1 interaction with hTERT promoter DNA down regulates the expression of the telomerase gene, which is overexpressed in most malignant tumors [26-28]. These observations highlight the importance of Sp1 in tumor development and suggest that Sp1 is a potential therapeutic target.

There is increasing evidence that indicates the proteins regulated by Sp1 are also involved in the mechanisms of platinum drugs. Ctr1, a copper transporter protein, facilitates the cell uptake of cisplatin, and low levels of Ctr1 have been detected in cisplatin resistant cells. The DNA-dependent protein-kinase catalytic subunit (DNA-PKcs), a key enzyme involved in repairing DNA double strand breaks, protects cells from toxic effects of cisplatin. In addition, the vacuolar ATPase (ATP = adenosine triphosphate) is often involved in the acquired cisplatin resistance. All these proteins are regulated by the transcription factor Sp1. Studies by Chen et al.
show that the *trans*-platinum compound *trans*-\([\text{PtCl}_2(\text{NH}_3)(\text{Tz})]\) (\(\text{Tz} = \text{thiazole}\)) exhibited high activity with Sp1, disrupting its structure and function both *in vivo* and *in vitro*. [26]

1.2.2. Cys\textsubscript{3}His (C\textsubscript{3}H) Zinc Finger Proteins

One example of a Cys\textsubscript{3}His zinc finger is PARP-1, a protein consisting of four domains including a DNA binding domain composed of two zinc fingers motifs, positioned upstream from the catalytic domain, Figure 1.7A [29]. Poly(adenosine diphosphate (ADP)-ribose) polymerases (PARPs) are essential proteins with roles in maintaining genome integrity, and regulation of transcription, and cell death signaling pathways [29-31]. The catalytic activity of PARP-1 is stimulated by interaction with “nicked” or damaged DNA. Once PARP detects a single strand break, it binds to the DNA, causing a structural change and initiating the synthesis of poly (ADP-ribose) (PAR) chains as a signal for other DNA-repair enzymes. Nicotinamide adenine dinucleotide (NAD\textsuperscript{+}) acts as a substrate for generating ADP-ribose monomers. Severe

![Figure 1.7](image-url)

**Figure 1.7.** (A) Schematic representation of PARP-1 domain structure [29]. (B) Crystal structure of ZF2 bound to DNA (PDB: 30DC)
DNA damage may induce hyperactivation of PARP-1, which may deplete the level of cellular NAD\textsuperscript{+} and induce a depletion of ATP levels, which will simultaneously lead to necrotic cell death [30,31].

Mutational and deletion analysis have indicated that the Zn1 binding domain plays a key role in PARP-1 DNA-dependent activity \textit{in vitro}, whereas the Zn2 domain is not essential. Interestingly, studies show that the Zn1 domain has a weaker DNA binding affinity than that of Zn2, with K\textsubscript{d} values of 3.43±0.86 µM and 0.034±0.010 µM respectively [30]. Both PARP-1 zinc fingers bind similarly to the end of duplex DNA, each forming a continuous surface interaction with DNA through what has been termed “phosphate backbone grip” and “base stacking loop”. The Zn2 domain spans the DNA minor groove, Figure 1.7B, making additional contacts with the phosphate backbone of the complimentary DNA strand, which could contribute to a higher DNA affinity [30].

PARP-1 is considered an interesting drug target for the development of innovative therapeutic agents in diseases including cancer, inflammation, and diabetes. PARP-1 inhibitors have been considered as drugs for use in combinatorial therapies for cancer treatment. It is thought that use of PARP-1 inhibitors would allow for synergistic enhancement of DNA damaging therapeutic agents such as cisplatin and carboplatin. An increasing interest and understanding of the polypharmacology of PARP-1 inhibitors has caused questions to arise of the specificity and “off-target” activities of these drugs. Therefore, it is of great interest to synthesize highly specific drugs to target PARP-1 [31,32].

The HIV nucleocapsid protein NCp7 is another example of a Cys\textsubscript{3}His zinc finger protein and on which this dissertation primarily focuses. It contains two successive CysCysHisCys domains separated by a short linker sequence, RAPRKKG [33]. Although each of the two NCp7
zinc fingers shares the same retroviral zinc finger motif, the C-terminal finger (F2) is substantially more reactive than that of the N-terminal finger, with Cys\textsubscript{49} being the most labile site of the NCp7 protein [21]. NCp7 is characterized by the presence of two aromatic residues crucial for its activities, the distal domain containing a phenylalanine (Phe\textsuperscript{16}) and the proximal one containing a tryptophan residue (Trp\textsuperscript{37}) [33]. NMR structures show that the recognition of HIV nucleocapsid protein NCp7 with DNA/RNA occurs by stacking between guanine in the DNA/RNA and the tryptophan in the NCp7 protein, Figure 1.8 [34-36]. Mutation of the Trp and/or Phe residues significantly reduces the nucleic acid chaperone activity of NCp7, which correlates to inhibition of viral replication [37].

![Figure 1.8](image)

**Figure 1.8.** NMR structure (A) and sequence (B) of the full-length HIV-NCp7 protein and its interaction with RNA (C) [6, 36].

The HIV-NCp7 nucleocapsid protein has a variety of functions as shown in Figure 1.9. As a component of the Gag precursor, the NCp7 protein promotes the packaging of viral genomic RNA into virions, which involves recognition of a ~120 nucleotide RNA segment located near the 5’ end of the genome known as the Ψ-site [33,38]. In the precursor Gag, the NCp7 domain interacts with the genomic RNA to stimulate retrovirus particle assembly and
budding [33]. NCp7 also plays a role in the early stages of infection, as shown by its in vitro influence to promote initiation of reverse transcription through annealing of the tRNA\(^{\text{Lys,3}}\) to the primer binding site [33]. Most of the known functions of NCp7 involve interactions with nucleic acids. The NCp7 zinc finger protein is folded in such a way that allows specific binding to RNA. It has been shown that mutations that prevent zinc binding, such as Cys to alanine (Ala) or Ser, cause the peptide to unfold and produce disulfide bonds between Cys residues, which results in the production of noninfectious viruses lacking their genomic RNA [33,38]. It is clear, therefore, that there exists a strong relationship between the zinc finger folding and the functions of NCp7, thus NCp7 has become a major target in AIDS therapy.

1.2.2.1 Small Molecule Inhibitors of HIV-1

Numerous novel targets exist for anti-retroviral therapy, Figure 1.10 [39]. Attachment or entry inhibitors block the interaction between the cellular receptors and the antireceptor on the virus by binding to or altering the receptor sites. Chemokine receptor antagonists (examples are

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**Figure 1.9.** The various functions of NCp7 during the early and late phases of the HIV-1 replication cycle [33].
maraviroc, vicriviroc, aplaviroc, AMD070) are a novel class of HIV-1 entry inhibitors that disrupt binding of HIV-1 to either CCR5 or CXCR4 co-receptors. Since these agents target a cellular receptor instead of a viral enzyme or protein, emergence of viral resistance may occur more slowly than with other anti-retrovirals [39].

In HIV, RT is used to generate complementary DNA (cDNA) from the viral RNA genome; this process is called reverse transcription. Reverse transcriptase inhibitors, therefore, inhibit reverse transcriptase from being able to function and in turn inhibiting formation of viral DNA. Nucleoside and nucleotide analog reverse transcriptase inhibitors (NRTIs) contain faulty imitations of nucleotides found in a T-cell’s cytoplasm. Instead of incorporating a nucleotide into the growing chain of DNA, the imitation building blocks in NRTIs are inserted, preventing the double strand of DNA from becoming fully formed. Non nucleoside reverse transcriptase inhibitors block reverse transcription by attaching to the enzyme in a way that prevents it from functioning [39].
Other types of inhibitors include integrase inhibitors, drugs that inhibit the viral DNA from traveling to the nucleus, and protease inhibitors, which bind to the protease enzyme and prevent it from separating, or cleaving, the subunits. Researchers are also looking at drugs called maturation inhibitors, such as zinc finger inhibitors, which interfere with the packaging of the viral RNA into the nucleocapsid [39].

Owing to the importance of the two zinc fingers for the function of NCp7 in HIV-1 replication, the first inhibitors designed to target the protein were zinc ejectors. These consisted of electrophilic compounds that target the nucleophilic zinc-coordinating cysteine residues of NCp7 [19]. Early generation zinc ejectors showed potent antiviral activity, but suffered from limited selectivity and significant toxicity [40]. Figure 1.11 shows a variety of different zinc ejecting compounds. 3-nitrosobenzamide (NOBA) compounds are an example of a compound that showed good activity against NCp7 in vitro [33,41]. However, it did not show sufficient

![Figure 1.11. Structures of various zinc-ejecting compounds [33,43].](image-url)
selectivity, as they reacted with cellular zinc-binding proteins such as PARP [40,42]. Further studies led to the development of other electrophilic compounds including azodicarbonamide (ADA) and disulfide bond-containing compounds such as 2,2-dithiobis(benzamide) disulfides (DIBA). Thioester-based chemotypes such as S-acyl-2-mercaptopbenzamide thioesters (SAMT) were developed from the DIBA compounds in hopes of improving selectivity and reducing toxicity [33,40].

Zinc finger inhibitors have a common mechanism of reacting with a nucleophilic cysteine, forming a covalent adduct on the protein and leading to zinc ejection. Such adducts result in formation of intra- and inter-molecular disulfide bonds before zinc can bind again [33]. The SAMT compounds, however, acylate the cysteine residue and release a free thiol rather than use an oxidative mechanism, Figure 1.12 [33,40,41,43].

![Figure 1.12](image)

**Figure 1.12.** (A) Mechanism of zinc ejection from NCp7 by disulfide compounds, (B) Proposed thioester reaction mechanism with NCp7 [33,43].
Many problems are associated with the use of conventional antiretroviral (ARV) agents described at the beginning of this section including extensive drug resistance and short and long-term side effects [39]. This has caused a great need for newer, specific agents. Both components of the NC-nucleic acid chaperone activity have been targeted [44-48]. A study of approximately 2,000 small molecules from the NCI Diversity Set suggested a possible fluorescein-based pharmacore with a good correlation between tryptophan quenching and inhibition of NC-nucleic acid binding [44]. A second high-throughput screening of small molecules for inhibition of NC-mediated destabilization of the stem-loop structure of cTAR DNA (a sequence complementary to the transactivation response element) produced only five compounds with IC$_{50}$ values in the micromolar range from a total of 4800 compounds [45]. The inhibitory activity of 4 of the 5 correlated with their ability to compete with the nucleic acid for binding to NC [45]. Similarly, as discussed throughout this dissertation, we are currently working on synthesizing Pt(II)/Au(III) metal complexes designed to specifically target the NCp7 zinc finger protein and therefore inhibit binding to RNA.

### 1.3 Pt-nucleobase compounds act as electrophiles towards zinc finger proteins

Two strategies, have emerged in developing zinc finger inhibitors: the use of small molecule inhibitors that contain an electrophilic reactive group, and the use of metal-containing compounds that compete with zinc binding [33,40]. Previous studies in our group have used both of these strategies to develop a more selective type of zinc finger inhibitor. Coordination compounds such as cis-[Pt(NH$_3$)$_2$(Cl)$_2$] (Cisplatin), trans-[PtCl(9-EtG)(py)$_2$]$^+$ (9-EtG = 9-ethylguanine; py = pyridine), and [PtCl(dien)]$^+$ act as electrophiles towards zinc fingers, disrupting protein conformation and causing zinc ejection, Figure 1.13 [6]. To improve the
selectivity of these compounds, a small molecule approach using platinum-nucleobase compounds has been developed to target the retroviral zinc finger – DNA interaction [49-52]. As stated earlier, it is known that a key interaction between RNA and NCp7 is the π-stacking between the guanine bases of RNA and the planar aromatic amino acid Trp$^{37}$ of the zinc finger protein, Figure 1.8. It is thought that the nucleobase in these Pt compounds can π-stack with Trp$^{37}$ of NCp7, therefore blocking the interaction of RNA and inhibiting the function of the NCp7 zinc finger protein.

Fluorescence quenching studies have shown that metallation of nucleobase compounds significantly enhances the π-π stacking interactions with L-tryptophan. Metallation modifies HOMO-LUMO interactions, where the π-acceptor LUMO (lowest unoccupied molecular orbital) of the metallated nucleobase is lowered, thus improving the overlap and acceptor properties towards the π-donor HOMO (highest occupied molecular orbital) of the tryptophan [49,52]. These findings were extended to a biologically relevant system, that of the C-terminal peptide of the HIV-NCp7 zinc finger, F2. Upon incubation of F2 with [Pt(dien)(9-EtG)]$^{2+}$ and [Pt(dien)(5′GMP)] (5′GMP = 5’-guanosine monophosphate), fluorescence quenching was observed; Eadie-Hofstee analysis gave association constants of $7.5 \times 10^3$ and $12.4 \times 10^3$ M$^{-1}$,
respectively [51]. Though there is little difference between their binding with free tryptophan (Kₐ values are 6.8 and 6.9 x 10⁻³ M⁻¹ for [Pt(dien)(9-EtG)]²⁺ and [Pt(dien)(5’GMP)], respectively [49]), the extra phosphate group on the 5’GMP causes a significant increase in binding with F2. This may be due to enhanced binding interactions with the multiple lysine residues in the C-terminal fingers.

ESI-MS of [Pt(dien)(9-EtG)]²⁺ with F2 in a 1:1 ratio showed formation of a 1:1 adduct between the peptide and Pt-complex. MS-MS of this adduct peak shows dissociation to reactants, indicating it is most likely that this adduct formation is facilitated by the π-π stacking interactions between the tryptophan and the platinated nucleobase, Figure 1.14. Incubation of cis-[Pt(NH₃)₂(Guo)₂]²⁺ (Guo = guanosine) with F2 showed formation of the 1:1 adduct [Pt(NH₃)(Guo) / F2]³⁺. The binding is weaker, however, than that of [Pt(dien)(9-EtG)]²⁺, most likely due to the steric hinderance of the cis oriented nucleosides [49].

![Figure 1.14. ESI-MS of [Pt(dien)(9-EtG)]²⁺ mixed with F2 in a 1:1 ratio. Inset shows proposed molecular recognition [51].](image)
In these studies, \( \text{trans} \cdot [\text{PtCl}(9\text{-EtG})(\text{py})_2]^+ \) was the only compound used capable of covalent interactions due to the presence of the substitution-labile chloride. Due to the ‘soft’ nature of Pt(II), this compound has been shown to have a higher reactivity towards sulfur atoms (soft base) over nitrogen atoms (hard base), and it has also been shown to cause Zn ejection in model chelates. In the presence of a 1:1 stoichiometric ratio of \( \text{trans} \cdot [\text{PtCl}(9\text{-EtG})(\text{py})_2]^+ \), the ESI-MS of F2 showed numerous peaks pertaining to complex formation between F2 and [Pt(py)\text{2}]\text{Zn}. Another peak corresponding to an adduct ([F2(Pt[py]2)-Zn] shows two [Pt(py)]\text{2} units bound to the protein with resultant loss of Zn. Circular dichroism (CD) experiments revealed changes in the three-dimensional structure of the protein, which is consistent with the loss of tertiary structure due to Zn ejection [51]. Therefore, the proposed mechanism for attack of Pt-nucleobase compounds on zinc fingers consists of two steps: 1) recognition through \( \pi-\pi \) stacking of tryptophan with platinated nucleobase and 2) covalent interaction followed by zinc ejection, Figure 1.15.

![Figure 1.15. Proposed mechanism of zinc ejection from C-terminal peptide of the HIV-NCp7 [51].](image)

Platinum–nucleobase compounds are also of interest as models for understanding the formation of DNA-zinc-finger protein crosslinks mediated by platinum compounds [6]. Relevant biological examples are (i) the use of transplatin to cross-link HIV-NCp7 to RNA [53] and (ii)
ternary DNA-protein formation formed by DNA monoadducts of 2-amino-N-2-methylamino-2,2,1-bicycloheptanedicloroplatinum(II) with the *E. coli* zinc finger-containing UVrAB repair protein [54] and (iii) ternary DNA-protein crosslinks mediated by tri- and tetrafunctional dinuclear platinum compounds with the UVrAB protein and Sp1 [6,55,56]. To further examine the covalent attachment of metal compounds to zinc finger proteins, [PtCl(dien)]⁺ was reacted with NCp7(F2) [23]. ESI-MS studies showed formation of \{(ZF-Cl)-Pt(dien)\} by substitution of the Pt–Cl bond by a zinc-thiolate with concomitant transfer of Cl to Zn, Figure 1.16 [23]. This is the first suggestion that “structural” zinc, specifically that of zinc fingers, may expand its coordination sphere [23].

![Figure 1.16](image.png)

**Figure 1.16.** Proposed structure of \{(ZF-Cl)-ML₃\} adduct. ML₃ = Pt(dien) [23].

### 1.4 Gold complexes target zinc finger proteins

It is of interest to expand the work of Pt(II)-nucleobase compounds to that of Au(III), as they are isostructural and isoelectronic. The thiol-rich nature of zinc fingers merits their investigation as specific targets of Au-drugs. Many studies have been reported on the reaction of Au(I)/Au(III) compounds with various zinc finger proteins. Franzman *et al.* studied the reaction of Et₃P-Au-Cl with Cys₂His₂, Cys₃H, and Cys₄ zinc finger proteins [57]. UV/VIS spectroscopy
showed the presence of a thiolate to gold charge-transfer band and it was determined that Au(I) reacts with zinc finger peptides with a stoichiometry of one gold for every two cysteine residues. CD studies shown in Figure 1.17 show the difference in secondary structure of zinc finger, gold-finger, and reduced/oxidized peptide. In every case, the gold-fingers have a more ordered structure than the corresponding apo-peptide, yet portray a less alpha-helical structure than the zinc finger protein, indicated by a decrease in the maximum at 190 nm and a blue shift in the minimum present at 205 nm [57]. It is well known that the secondary structure of these zinc finger proteins is integral to their ability to bind to DNA. Therefore, it is reasonable to propose that the loss in structure of the zinc finger protein upon zinc ejection and gold binding could lead to a loss of DNA binding and a loss of protein function.

To test the hypothesis that the Au(I) drug aurothiomalate played a role in the inhibition of binding of certain transcription factors to regulatory elements in DNA, Handel et al. studied the interaction of a steroid hormone receptor, the progesterone receptor (PR), with its DNA response element (PRE) in the presence of aurothiomalate [58]. PR was used as a model zinc finger transcription factor containing two Cys4 zinc fingers. Studies showed that aurothiomalate inhibited binding of PR to PRE, with an IC$_{50}$ of approximately 3 µM [58]. This is significant due
to the fact that 3 μM is within the range of serum concentrations achieved during treatment of rheumatoid arthritis. Also, 3 μM is significantly lower than $K_i$ values reported for various enzymes inhibited by aurothiomalate [58]. To test the effect of aurothiomalate on PR-mediated transcription, the progestin-inducible expression vector pMSG-CAT was transfected into T-47D breast cancer cells. In the presence of 100 μM aurothiomalate there was 42±8% inhibition of chloramphenicol acetyl transferase activity. This shows that aurothiomalate can regulate gene expression and that inhibition of binding of a transcription factor to its response element is a likely mechanism of action [58].

Aurothiomalate was shown to inhibit both TFIIIA (Transcription Factor for Polymerase IIIA) and Sp1 (both Cys$_2$His$_2$ zinc finger transcription factors) in the micromolar range [59]. ESI-MS studies with a peptide modeled after the third finger of Sp-1, Sp1(F3), showed release of Zn$^{2+}$ and replacement with Au$^{+1}$, Figure 1.18A, with the gold having a 4-fold higher binding affinity than zinc. Isotopic cluster analysis showed that the Au(I) bound to one thiol and one thiolate residue [58]. CD spectroscopy showed a change in secondary structure of the peptide

![Figure 1.18.](image)

**Figure 1.18.** (A) ESI-MS spectrum showing 60% gold-finger formation after addition of aurothiomalate to pre-formed zinc finger (2 eq. zinc) and (B) CD spectrum showing change in conformational structure upon formation of gold-finger [59].
upon zinc ejection and concomitant gold binding. The CD spectrum of apo-Sp1(F3) showed features indicative of a random coil structure. Upon binding of Zn$^{2+}$, the CD spectrum showed less negative ellipticity, a red-shift in the minimum, and a positive ellipticity between 190 and 195 nm. The addition of aurothiomalate resulted in a slight red-shift of the minimum, however not to the degree caused by Zn$^{2+}$; there was also a decrease in the maximum at 195 nm when compared to the zinc finger spectrum (Figure 1.18B). This indicates that the gold-finger has an ordered structure with different conformational features than zinc finger, specifically less α–helical structure [59-61]. This is in agreement with what Franzman et al. observed with the Au(I) compound Et$_3$P-Au-Cl discussed earlier.

Many Au(I) and Au(III) compounds have been studied with the zinc finger protein PARP-1 described earlier in this section. Mendes et al. studied the effects of metal-based drugs including platinum, ruthenium, and gold complexes [31]. The gold complexes included the Au(I) drug auranofin and two Au(III) compounds, [Au(phen)Cl$_2$]$^+$ and [Au(bipy)Cl$_2$]$^+$ (phen = 1,10-phenanthroline and bipy = 2,2-bipyridine). To calculate IC$_{50}$ values, PARP-1 was incubated with each compound at various concentrations for 24 hours before assessing its activity spectrophotometrically by measuring the incorporation of biotinylated poly(ADP-ribose) onto histone proteins. When compared to the reference PARP-1 inhibitor, 3-aminobenzamide, all Au compounds reported lower, more potent, IC$_{50}$ values in the nanomolar range [31]. It is interesting to note, however, the Au(I) compound auranofin had an IC$_{50}$ value one order of magnitude lower than both Au(III) compounds. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICRMS) studies were performed using the N-terminal domain of PARP-1 to investigate the nature of these compounds inhibitory effects. When reacted with apo-peptide, it was seen that the Au(III) compounds had a higher reactivity that any other compounds studied, binding up to three
Au ions. Isotope cluster analysis indicates that the first Au ion remains in the $3^+$ oxidation state, whereas the additional gold ions are reduced and bind as Au(I) [31]. Experiments were also performed in which the Au-compounds were added to pre-formed zinc finger protein as well as added simultaneously with zinc acetate to mimic competitive binding conditions. All results were similar; within minutes, an equilibrium between the Zn(II) and Au-bound species was

![Figure 1.19](image)

**Figure 1.19.** ESI FT-ICR MS of (A) apo-ZF-PARP with zinc acetate and [Au(III)(bipy)Cl$_2$]$^+$ in a 1:2:2 ratio under competitive conditions (10 minute incubation), and (B) of pre-formed PARP zinc finger with subsequent addition of auranofin (24 h) in a 1:2:2 ratio. Notice the 3:1 and 1:1 ratios of Au:Zn for Au(III) and Au(I), respectively [31].
established. A ratio of Au- to Zn-bound protein was approximately 3:1 for Au(III) compounds and 1:1 for the reaction with auranofin, Au(I), indicating a higher binding affinity for Au ions than Zn(II) ions and a more efficient adduct formation for Au(III) ions than Au(I), Figure 1.19 [31]. The lower binding affinity of Au(I) is in agreement with its lower IC₅₀ value. This competition of Au for the same binding site of Zn could be reason for loss of zinc finger function and high inhibition of PARP-1 activity.

Casini et al. have investigated the interaction of multiple Au(III) compounds, including Au(III) heterometallic compounds, as well as mono- and bi-nuclear Au(I) complexes with the

![Figure 1.20](image_url)  
\[ \text{Figure 1.20. Au(III) and Au(I) compounds studied by Casini, et al. for their interaction with zinc finger PARP-1 [62,63].} \]
zinc finger protein PARP-1; the structures of these compounds are shown in Figure 1.20 [61,62]. All compounds studied showed interesting cytotoxicity effects in human ovarian carcinoma cell lines, both sensitive (A2780) and cisplatin resistant (A2780/R), human mammary carcinoma cells (MCF7), and non-tumorigenic human embryonic kidney cells (HEK293). Compounds containing the 2-(2’-pyridyl)imidazolate ligand, compounds I and V in Figure 1.20, showed selectivity towards the cancer cells with respect to the non-tumorigenic cells, and the dinuclear and trimetallic compounds were more cytotoxic than their mononuclear counterparts [62,63]. Interestingly, the heterometallic compounds VII and VIII were found to not react with plasmid DNA [62]. This, along with the fact that these Au-complexes had significantly higher cytotoxicity than cisplatin in the resistant A2780/R and MCF7 cell lines, supports the idea of different mechanisms of action than cisplatin [62].

Further studies, therefore, were performed to investigate their interaction with the zinc finger protein PARP-1, due to its involvement in DNA repair and relevance to the mechanism of cancer cell resistance to cisplatin. The trinuclear Au-Fe compound VII (Figure 1.20) was found to be a potent inhibitor of PARP-1 with an IC$_{50}$ value of 1±0.5 µM [62]. The active dinuclear compound V (Figure 1.20) was able to inhibit PARP-1 activity by 24% in A2780 cell extracts. It did not, however, have any effect in A2780/R cell extracts, indicating other proteins are competing with PARP-1 for gold binding [63]. An ESI-MS study of a 3:1 reaction of V with ubiquitin (Ub) after 1 hour incubation showed two main species; ubiquitin and a gold-containing species, Ub-AuPPh$_3$, in which the 2[(2’-pyridyl)imidazolato ligand was completely dissociated [63].

Gold(I) complexes of the formula [(PPh$_3$)Au(L)]$^+$ with L = substituted N-heterocycle pyridine have been studied by Abbehausen et al. to target the HIV nucleocapsid zinc finger
protein, NCp7 [64]. As stated earlier for Pt(II)-nucleobase compounds, the N-heterocycle ligand is used to specifically target the tryptophan residue in the C-terminal finger of NCp7. Fluorescence quenching studies showed the Ph$_3$P-Au-DMAP (DMAP = 4-dimethylaminopyridine) compound to π-stack effectively with free N-acetyltryptophan with a $K_a$ of 25.0 x 10$^3$ M$^{-1}$ [64]. ESI-MS studies of these Au(I) compounds with the C-terminal finger (F2) of NCp7 show the presence of a long-lived {($\text{PPh}_3$)Au}-S-peptide species resulting from dissociation of the pyridine ligand by zinc-bound cysteine followed by displacement of Zn$^{+2}$, Scheme 1.2 [64]. Interestingly, this species is similar to that reported by Serratrice, et al. in the study of dinuclear Au(I) compounds with the model peptide ubiquitin described earlier [64].

![Scheme 1.2](image)

**Scheme 1.2.** Schematic representation of the interaction of Au(I)-N-heterocycle compounds with NCp7(F2) [64].

The reactivity of NCp7(F2) was then compared to that of the Cys$_2$His$_2$ transcription factor, Sp1(F3) (F3 = zinc finger 3). As seen previously for the Au(I) drug aurothiomalate, immediate zinc ejection was observed followed by replacement of Au(I). No apoSp1F3-{Au(PPh$_3$ )} was observed for any of the compounds studied; however, when L = DMAP was used, ESI-MS peaks were present corresponding to a mass of [apoSp1F3-{(PPh$_3$)Au$_2$}] implying the presence of one coordinated Au(PPh$_3$) unit and one Au without any starting ligands [64]. The reaction with the Sp1 finger is thus more ligand-specific since no evidence for any equivalent Au(PPh$_3$) species is seen for any of the other Au(I) compounds studied. CD spectra show clear
differences in the conformation changes of NCp7(F2) and Sp1(F3) after reaction with the Au(I)-N-heterocycle compounds. The results suggest the possibility of electronic and steric variations of “carrier” group PR₃ and “leaving” group L as well as the nature of the zinc finger in modulation of biological activity [64].

The interaction of [AuCl(dien)]Cl₂ with NCp7(F2) has also been studied by ESI-MS and CD [23]. After 5 minutes of incubation of a 1:1 reaction, MS showed total loss of ligands and binding of 2 and 4 gold ions (i.e. formation of “gold-finger”) with Zn²⁺ ejection [23]. Whereas for Pt(II) direct electrophilic attack on the cysteine sites may occur, it is possible that in the presence of [AuCl(dien)]Cl₂ a redox reaction can occur, producing Au(I) species as well as oxidized peptide [23]. The nature of the gold ion upon binding to the protein is unknown; however, based on previous studies with other Au(III/I) compounds discussed above it is possible that Au(III) reduces to Au(I) followed by release of bound ligand and incorporation of the gold.

Figure 1.21. CD spectra for the reaction of NCp7(F2) and [MCl(dien)]⁺ (M=Pt(II), Pd(II), and Au(III)) [23].
ion as Au(I) [23]. The CD spectrum of NCp7(F2) is characterized by a broad band with a slight positive maximum at 210 nm and a stronger negative band at 200-205 nm. In the absence of zinc there is an increase in the negative band as well as a slight blue shift indicative of a random coil. In the presence of [AuCl(dien)]Cl₂, after 15 minutes of incubation the CD spectrum resembles that of free peptide, Figure 1.21 [23]. This suggests complete zinc ejection, which is in agreement with the ESI-MS results.

1.5 Chemistry of \([\text{AuCl(dien)}]\text{Cl}_2\)

\([\text{AuCl(dien)}]\text{Cl}_2\) was first prepared in 1963 and the crystal structure was solved in 1986, Figure 1.22 [65]. Three of the four equatorial sites around the gold(III) metal center are occupied by the nitrogens from the dien ligand (Au-N bond length is 2.04 Å), and the fourth site is occupied by a chlorine atom (Au-Cl bond length is 2.28 Å). The two chloride counterions are situated in the axial positions, with an elongated Au-Cl distance of 3.15 Å [65]. The X-ray absorption spectrum confirmed the oxidation state of Au to be 3⁺ indicated by an intense peak.
attributed to the 2p to 5d electronic transition that occurs for the Au(III) d⁸ species, but not for the d¹⁰ Au(0) or Au(I) species [65].

When in aqueous solution, [Au(dien)]²⁺ has been shown to lose a proton from one of the nitrogens on the dien with a pKa value of 4.0 in 0.5M ClO₄⁻ according to the following equation [66,67]:

\[
[AuCl(dien)]^{2+} \leftrightarrow [Au(dien-H)Cl]^+ + H^+
\]

Nardin et al. confirmed by single crystal x-ray diffraction of [AuCl(dien)]Cl(ClO₄) and [AuCl(dien-H)]Cl(ClO₄) that the deprotonation occurs from the central, secondary nitrogen, with a Au-Cl bond length of 2.33 Å, significantly longer than that of 2.273 Å for the parent amine complex [68]. In the protonated complex, the four donor atoms are essentially in a plane; a pronounced tetrahedral distortion of the coordination geometry was observed for the dien-H species [63].

The substitution reaction of -Cl by other nucleophiles has been studied for both the protonated and deprotonated Au(III)-dien compounds, [AuCl(dien)]²⁺ and [AuCl(dien-H)]⁺, respectively. Second-order rate constants for chloride exchange as well as a ring-opening mechanism were predicted for [Au(dien)Cl]²⁺ using water and strong nucleophiles, N₃⁻ and SCN⁻, Scheme 1.3 [69]. For the dien-H complex, the Au-Cl bond is weaker than the Au-N2 bond due to its trans position to the deprotonated secondary amine, and the Au-N1,N3 bonds are also weaker. However, Au-Cl bond order decreases 24% compared to only 8% for the Au-N1,3 bonds. Therefore, it is expected that the ring-opening should be less likely at pH values above the pKₐ of 4.0 due to rapid chloride exchange [69]. For the aquation reaction, which is important for the activation of metallodrugs, chloride exchange was found to be much faster in neutral and basic media, \( k_2 \sim 0.4 \text{ M}^{-1}\text{s}^{-1} \) versus \( k_2 \sim 10^{-7} \text{ M}^{-1}\text{s}^{-1} \) for pH < 4 [69]. The substitution reactions,
including ring-opening, were predicted to be faster in the presence of stronger nucleophiles, N$_3^-$ and SCN$^-$, with rate constants for chloride exchange being $10.1 \times 10^1$ and $5.5 \times 10^3$ M$^{-1}$s$^{-1}$, respectively [69]. Interestingly, the ring opening reactions were also predicted to proceed at rapid reaction rates for N$_3^-$ and SCN$^-$ nucleophiles, however these processes are highly unfavorable when compared to chloride exchange [69].

This pH dependency of [AuCl(dien)]Cl$_2$ can be observed by $^{13}$C and $^1$H NMR Spectroscopy. Figure 1.23 shows NMR spectra of 10 mM [AuCl(dien)]Cl$_2$ in D$_2$O at various pH values [67]. In the $^{13}$C NMR, Figure 1.23A, both resonances were pH dependent with peak 1 (corresponding to atoms C$_c$ and C$_b$) showing a downfield shift of 4.9ppm and peak 2 (corresponding to atoms C$_a$ and C$_d$) an upfield shift of 1.5ppm upon pH increase from pH 3 to 6.5. These shifts were fitted to a pK$_a$ value of 4.74±0.04 and attributed to the deprotonation of the central secondary amine group. Similar pH dependent results were seen for $^1$H NMR as shown in Figure 1.23B. Peak 1 (assigned to the methylene protons of C$_b$ and C$_c$) was broad over...
the whole pH range and shifted 1.09 ppm upfield from pH=3 to 7. Peak 2 (assigned to protons C\textsubscript{a} and C\textsubscript{d}) had a smaller shift difference. This peak was broad at low pH values, but turned into a sharp triplet at pH 4 and above. The pH dependent shifts of these peaks were again attributed to the deprotonation of the central secondary amine group [67].

![Figure 1.23](image)

**Figure 1.23.** 13\textsuperscript{C} (A) and 1\textsuperscript{H} (B) NMR of [AuCl(dien)]\textsubscript{2} in D\textsubscript{2}O at various pH values. The numbering system for the carbon atoms is shown in the center figure [67].

The addition of methyl groups to the tridentate diethylenetriamine ligand further adds to the steric hindrance of the compounds as well as decreases substitution reaction rates [70]. Methylated dien complexes including [AuX(Me\textsubscript{2}dien-H)]\textsuperscript{+} (Me\textsubscript{2}dien = N,N-dimethyldiethylenetriamine), [AuX(Me\textsubscript{4}dien)-H]\textsuperscript{+} (Me\textsubscript{4}dien = 1,1,7,7-tetramethyldiethylenetriamine), and [AuX(Et\textsubscript{2}dien)-H]\textsuperscript{+} (Et\textsubscript{2}dien = N,N-diethyldiethylenetriamine) were synthesized by Fant \textit{et al.} and the dissociation constants of their conjugate acids were measured, as shown in Table 1.1 [71]. Interestingly, as the number of alkyl
groups increase, the pKa of the center nitrogen decreases (the acidity of the complex increases). The much higher acidity of the [AuCl(Et₄dien)]PF₆ (Et₄dien = N,N,N",N"-tetraethyldiethylenetriamine) compared to [AuCl(dien)]Cl₂ is attributed to solvation effects [71,72]. The more positive acid is more solvated than its conjugate base for both alkyl-substituted and unsubstituted complexes. However, this difference, known as the energy of solvation, is smaller for the substituted complex because of its larger size. This results in a higher acidity for the alkyl-substituted ion [71,72].

<table>
<thead>
<tr>
<th>Complex</th>
<th>pKa</th>
</tr>
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<tbody>
<tr>
<td>[AuCl(dien)]Cl₂</td>
<td>4.7  [66]</td>
</tr>
<tr>
<td>[AuCl(Me₂dien)]PF₆</td>
<td>4.2</td>
</tr>
<tr>
<td>[AuCl(Me₄dien)]PF₆</td>
<td>3.8</td>
</tr>
<tr>
<td>[AuCl(Et₂dien)]PF₆</td>
<td>3.6</td>
</tr>
<tr>
<td>[AuCl(Et₄dien)]PF₆</td>
<td>2.2</td>
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Table 1.1. Acid dissociation constants for methylated Au(III)-dien complexes [71].

Kinetic studies showed the methylated dien complexes [Au(Me₂dien)Cl]PF₆ and [AuCl(Me₄dien)]PF₆ to undergo bromide substitution by the usual two-path mechanism common to low-spin d⁸ metal complexes, a simple displacement mechanism proceeding via a trigonal-bipyramidal-based transition state in which the entering ligand occupies an equatorial site, Figure 1.24A [71,73]. The ligand exchange rate was found to be higher for [AuCl(Et₂dien-H)]⁺ when bromide was the entering group; substitution was hypothesized to proceed via a ring-opening mechanism, Figure 1.24B, as described earlier [71]. It was postulated that this increase in substitution rate was due to either (1) the role of the higher effective positive charge on the gold
metal center in the complex with the 1,1-Et$_2$-dien-H ligand and/or (2) a distinct substitution mechanism passing through an open-ring intermediate, where the Au-N(Et)$_2$ bond is broken [70].

Gold(III) tetrarnitrogen donor complexes have previously been synthesized using porphyrin and cyclam-based ligands, see Figures 1.3 and 1.4 [5,6,74]. Nitrogen-donor planar amines are good σ-donor and π–acceptor groups, and the kinetics of L(N-heterocycle) substitution by Cl$^-$ in AuCl$_3$L + Cl$^- \rightarrow$ [AuCl$_4$]$^-$ + L showed a linear relationship between the basicity of the leaving group and the reactivity with respect to chloride substitution, with the rate of displacement decreasing with an increase in ligand basicity [75]. To investigate the interaction of [AuCl$_4$]$^-$ with DNA, the complex [AuCl$_3$(9-EtG)] was synthesized and isolated. X-ray crystallography confirmed binding of 9-EtG to the Au(III) metal center via N7 of the purine ring [76]. Solution studies show that guanosine(inosine) monophosphate (5’GMP/IMP) and L-His bind with high affinity to gold(III) centers [77]. The reactivity of the studied nucleophiles was L-His > 5’-GMP > 5’-IMP > inosine [77].

Figure 1.24. (A) Energy diagram for synchronous bimolecular substitution of X by Y and (B) Scheme showing ring-opening mechanism for [Au(Et$_2$-dien-H)Cl]$^+$ [71,75].
1.6 Dissertation outline

It is the goal of this dissertation to combine the chemistry of \([\text{AuCl(dien)}]^{2+}\) and the previously mentioned \([\text{AuCl}_3\text{L}]\) compounds and to synthesize novel \(\text{AuN}_4\) species, where three of the nitrogen donors are from the tridentate diethylenetriamine (dien) ligand and the fourth is a planar amine/heterocycle ligand (Figure 1.25). The dien ligand acts as a “carrier ligand”, allowing for the effects of only one leaving group, either a substitution-labile \(\text{Cl}^-\) or a substitution-inert nitrogen donor of a planar ligand, to be studied. The \([\text{ML(dien)}]^{n+}\) coordination sphere also allows the study of the effect of metal ion on the kinetics of the interaction. \(\text{MN}_4\) compounds serve two purposes: 1) templates for non-covalent molecular recognition through tryptophan stacking, 2) serve to examine the factors involved in “activation” of the inert coordination sphere and covalent binding upon initial recognition.

![Figure 1.25. \([\text{ML(dien)}]^{n+}\) structure allows for systematic reactivity studies.](image-url)
This dissertation discusses the synthesis, characterization, and reactivity of novel Au\textsuperscript{III}(dien)(N-heterocycle) compounds designed with the goal of slowing down the reaction with the NCp7 zinc finger protein, aiding in improving their selectivity through targeting the $\pi$-stacking interaction with tryptophan. The term “selectivity” is used often throughout this dissertation. As previously discussed, due to the high thiol affinity of gold, Au(I) and Au(III) compounds react rapidly with sulfur containing peptides. If our compounds are not selective, they can react extensively with multiple proteins containing Cys residues, for example serum proteins such as HSA (human serum albumin), and essentially become inactive before reaching the desired target. Therefore, it is important that we synthesize compounds that are selective to one particular protein, in our case the HIV NCp7 zinc finger protein.

Because these compounds are the first isolated Au\textsuperscript{III}N\textsubscript{3}(N-purine)/heterocycle compounds, it is necessary to study their chemistry in solution. The synthesis and pH dependency of these compounds is discussed in depth in Chapter 2. The enhanced stability of the Au(III) metal center by the addition of a more inert N-donor is also discussed.

Chapter 3 discusses the reactivity of the Au(III)-dien-nucleobase compounds with model amino acids including the planar aromatic amino acid N-Acetyltryptophan and the sulfur-containing amino acids N-Acetylmethionine and N-Acetylcyesteine. As previously discussed, Au(I)/(III) compounds have a high thiol affinity as a result of their ‘soft’ nature. The addition of a more inert N-donor as a leaving group, resulting in a AuN\textsubscript{4} coordination sphere, slows down this reaction. Comparison of the $\pi$-stacking ability of [Au(dien)(DMAP)]\textsuperscript{+3} with its Pt(II) and Pd(II) analogues is highlighted in Chapter 4.

Study with the model amino acids gives greater insight into how the Au(III) compounds will interact with the zinc fingers themselves, also discussed in Chapter 3. Zinc finger cysteinates
vary in chemical reactivity, and nucleophilicity is very dependent on the nature of the Zn binding core [55]. Therefore, the Cys$_3$His zinc finger NCp7(F2) and the Cys$_2$His$_2$ zinc finger Sp1(F3) were both studied to determine any difference in reactivity and possible binding selectivity. Lastly, studies were expanded to that with the full NCp7 zinc finger protein. Chapter 5 compares the reactivity of [M(dien)(9-EtG)]$^{n+}$ where M = Au(III), n=3 and Pt(II), n=2. This chapter ends with the suggestion that the Au(III)-9-EtG compound may induce protein crosslinking. Appendix A is a review on cobalt- and platinum- DNA-protein crosslinks as probes for zinc finger conformation and reactivity.

The majority of the published work consists of studies performed with [Au(dien)(9-EtG)]$^{3+}$ and [Au(dien)(DMAP)]$^{3+}$, however many additional Au(III)-N-heterocycle compounds were synthesized, as is discussed in Chapter 6. This chapter focuses on synthetic procedures as well as characterization techniques, both successful and unsuccessful. Experimental results are discussed, giving additional information about the solution behavior and reactivity of these compounds.

Published work is included in a format as close as possible to that in which it was published.

1.7 References


Chapter 2. Synthesis and Properties of the first \([\text{Au}(\text{dien})(\text{N-heterocycle})]^{3+}\) Compounds

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2.1 Abstract:

Novel \(\text{Au}^{III}\)(dien)(N-heterocycle) compounds, including the first \(\text{Au}^{III}\text{N}_3(\text{N-purine})\) examples are reported. The acidity of the dien ligand is affected by the nature of the fourth ligand as a leaving group. The metal center of \([\text{Au}(\text{dien})(\text{N-heterocycle})]^{3+}\) compounds was shown to be more stable to reduction than when \(\text{Cl}^-\) is present, with consequences for reactivity with biomolecules: specifically significant enhancement of \(\pi-\pi\)-stacking interactions with tryptophan relative to isostructural and isoelectronic platinum(II) and palladium(II) compounds.

2.2 Introduction:

We are currently exploring the use of small-molecule coordination compounds as electrophiles to alter zinc finger peptide structure and function [1-2]. Electrophilic attack by \([\text{MCl}(\text{dien})]^{n+}\) \((\text{M} = \text{Pt}^{II}, \text{Pd}^{II}, \text{Au}^{III}; \text{dien} = \text{diethylenetriamine})\) on the C-terminal finger of HIV Nucleocapsid NCp7(F2) results in conformational changes and ejection of \(\text{Zn}^{2+}\) [3]. Platinated nucleobases such as \([\text{Pt}(\text{dien})(\text{9-EtG})]^{2+}\) \((\text{9-EtG} = \text{9-ethylguanine})\) with a more substitution-inert nitrogen donor as the “leaving group” stack effectively in a noncovalent manner with tryptophan of NCp7(F2) [4]. We therefore wanted to study the more reactive gold(III) system for comparison as well as to examine models for the interaction of \([\text{AuCl}(\text{dien})]^{2+}\) with DNA [5]. In
this paper we report the synthesis and characterization of [Au\textsuperscript{III}(dien)X] chelates with purines and nitrogen-donor heterocycles, the first isolated Au\textsuperscript{III}N\textsubscript{3}(N-purine)/heterocycle compounds (Figure 2.1). The acidity of the primary and secondary amines is a long-recognized feature of [AuCl(dien)]\textsuperscript{2+} chemistry [6-11]. The presence of the pyridine or purine affects this acidity and the redox properties of the gold(III) complex as a whole. These differences further translate to variations in reactivity with biomolecules, where for 9-EtG, tryptophan stacking is significantly enhanced over that of the platinum(II) and palladium(II) analogs.

![Figure 2.1. Structures of [Au\textsuperscript{III}(dien)L]\textsuperscript{2+/3+} chelates studied (A) and the C-terminal finger of HIV Nucleocapsid NCp7(F2) (B).](image)

Tetranitrogen donor complexes of gold(III) are known for porphyrin- [12] and cyclam-based macrocycles [13] but the [Au\textsuperscript{III}(dien)X] species discussed herein are the first to be synthesized where X is a purine or planar N-heterocyclic ligand. Nitrogen-donor planar amines are good \(\sigma\)-donor and \(\pi\)-acceptor groups, and the kinetics of \(L(N\text{-heterocycle})\) substitution by \(\text{Cl}^-\) in \(\text{AuCl}_3L + \text{Cl}^- \rightarrow \text{[AuCl}_4]^- + L\) showed a linear relationship between the basicity of the leaving group and the reactivity with respect to chloride substitution [14]. The complex [AuCl\textsubscript{3}(9-EtG)]\textsuperscript{−} has been isolated, confirming Au–N7 binding [15], and solution studies show
that guanosine(inosine) monophosphate and histidine bind with high affinity to gold(III) centers [16].

2.3 Results and Discussion

The reaction of [AuCl(dien)]Cl₂ with 9-EtG in the presence of 3 equiv. of AgNO₃ afforded orange [Au(dien)(9-EtG)](NO₃)₃. NMR and potentiometry show three major distinct species for this complex over a wide pH range (Figures 2.2-2.4). The initial pH* of a 10 mM

![Image](image_url)

**Figure 2.2.** ¹H NMR spectra showing (A) H₈ proton of 9-EtG and (B) dien region in [Au(dien)(9-EtG)](NO₃)₃ over pH range.
aqueous solution is 2.63, where pH* is the measurement of the pH meter. The $^1$H NMR spectrum shows that as the pH is increased the purine H8 signal first broadens and shifts upfield (Figure 2.2A). This is followed by the appearance of sharp peaks in the intermediate pH 5-7 range and eventually purine dissociation at basic pH. Potentiometric titration also showed three midpoints with approximate pK$_a$ values of 3.3, 5.3, and 7.5 (Figure 2.3).

![Figure 2.3. pH titration curve of [Au(dien)(9-EtG)](NO$_3$)$_3$ shows three distinct equivalence points over broad pH range.](image)

The pK$_a$ for deprotonation of the secondary central amine in [AuCl(dien)]$^{2+}$ is in the range of 4.0-4.7 depending on conditions [7,11]. The dien region of the $^1$H and $^{13}$C NMR spectra for [Au(dien)(9-EtG)](NO$_3$)$_3$ was similar to that reported for [AuCl(dien)]Cl$_2$ (Scheme 2.1 and Figures 2.2B and 2.4) [11]. The $^{13}$C chemical shifts are a sensitive diagnostic of chelate deprotonation [11] and were fitted in this case to a pK$_a$ of 3.3 for the central nitrogen on the dien. At pH* > 6.3, new signals appear, attributed, as for [AuCl(dien)]Cl$_2$, to hydrolysis of the gold(III) species [11], also supported by dissociation of 9-EtG at basic pH. A reasonable explanation for the three species observed is therefore a “fully” protonated species, a species with dien-H but N1 protonated, and a fully “deprotonated” species. The pK$_a$ of the N1H of 9-EtG

47
(9.57) is reduced to 8.17 in [Pt(dien)(9-EtG)]\(^{2+}\) [17]. Binding to gold(III) further decreases this value to 5.3.

![Chemical structure](image-url)

**Figure 2.4.** \(^{13}\)C NMR spectrum showing dien region of [Au(dien)(9-EtG)](NO\(_3\))\(_3\) over a broad pH range. Peak 1 corresponds to C\(_b\) and C\(_c\) and peak 2 corresponds to C\(_a\) and C\(_d\).

![Graph](image-url)

**Scheme 2.1.** Proposed structures for [Au(dien)(9-EtG)](NO\(_3\))\(_3\) over broad pH range

To confirm these interpretations, analogous [Au\(^{III}\)(N-Medien)\(X\)] (N-Medien = 2,2’-diamino-N-methylidethyamine) compounds were prepared. In comparison to the free ligand, the \(^1\)H NMR N-Me peak shifts significantly downfield by approximately 1.35 ppm for both Cl\(^-\) and 9-EtG. The pH dependency of the \(^1\)H NMR spectrum shows only two major peaks, as expected (Figure 2.5). At pH\(^*\) 2.96, one peak is present, likely representing the fully protonated species.
At pH* 4.05, an additional upfield peak appears, indicating the NH deprotonation. At pH* > 5.6, the 9-EtG ligand is completely dissociated. A pKₐ of 5.7-6.8 has been assigned for deprotonation of the primary amine in [AuCl(N-Medien)]²⁺ [18]. Such deprotonation may destabilize the Au-9EtG bond.

**Figure 2.5.** ¹H NMR spectra of [Au(N-Medien)(9-EtG)][NO₃]₃ at various pH values

**Figure 2.6.** Absorption spectra of [Au(dien)(DMAP)]Cl₃ over pH range showing one isosbestic point for deprotonation of the chelate ligand dien→dien-H.
We further prepared \([\text{Au(dien)(DMAP)}]^3^+\) \([\text{DMAP} = 4\text-(\text{dimethylamino})\text{pyridine})]\) because the more basic DMAP \(4\text{-Me}_2\text{Npyr}\) has a \(pK_a\) of 9.1, compared to that of 2.7 for 9-EtG [17]. The UV/Vis spectra in the 3-5 pH range shows one isosbestic point (Figure 2.6). In the \(^{13}\text{C}\) NMR spectrum at pH* up to 6.4, two \(^{13}\text{C}\) signals were observed, assigned to \(C_b\) and \(C_c\) (Peak 1) and \(C_a\) and \(C_d\) (Peak 2) (Figure 2.7). The pH dependence of the peak shifts were fitted to a \(pK_a\) of 4.7 for the secondary central amine of the dien, higher than that for 9-EtG but similar to \([\text{AuCl(dien)}]^2^+\) [7,11]. Additional peaks appear at pH* > 6, peaks 1a and 2a in Figure 2.7, but the DMAP ligand remains bound, thus protecting the compound from hydrolysis (Figure 2.8). The extra peaks may be due to deprotonation of the primary amines.

![Figure 2.7](image_url)

**Figure 2.7.** \(^{13}\text{C}\) NMR chemical shift values of dien C’s versus pH for 50 mM \([\text{Au(dien)(DMAP)}]\text{Cl}_3\) in D\(_2\)O and proposed structures over pH range shown.

The contrast between \(X = \text{Cl}^-\) and the N-heterocycle as the fourth ligand is clearly seen in the \([\text{Au(N-Medien)X}]^{n^+}\) case. The \(^1\text{H}\) NMR N-Me peak in the DMAP complex is shifted downfield similar to \(\text{Cl}^-\) and 9-EtG. In excess \(\text{Cl}^-\), chelate displacement occurs for \([\text{AuCl(N-}
Medien)]^{2+} by a ring-opening mechanism with a large absorption band at 317 nm indicative of production of [AuCl₄]⁻ [18]. A similar trend is seen at low pH even in absence of Cl⁻, with a large absorption band at 310 nm (Figure 2.9A). The development of this band is not seen for the DMAP case, suggesting that no ring opening occurs and that the N-Medien ligand is stabilized.

Figure 2.8. ¹H NMR spectra of [Au(dien)(DMAP)]Cl₃ over broad pH range. The chelate dien region is shown in (A) and DMAP ligand region is shown in (B) giving evidence that DMAP does not dissociate at high pH.
by the DMAP ligand (Figure 2.9). At low pH*, one dien N-Me peak is present in the $^1$H NMR spectrum of both compounds, with a new peak appearing 0.15 ppm upfield at higher pH* (Figure 2.10). At pH* 6.1, these peaks are present at 50:50 integration, suggesting that the $pK_a$ of both compounds is $\sim$6.1 under these conditions and indicating deprotonation of the primary amines on

Figure 2.9. Absorption spectra of 1 mM [AuCl(N-Medien)]Cl₂ (A) and 1 mM [Au(N-Medien)(DMAP)]Cl₃ (B) at various pH values.
Figure 2.10. $^1$H NMR Spectra of [AuCl(N-Medien)]Cl$_2$ (A) and [Au(N-Medien)(DMAP)]Cl$_3$ (B) over broad pH range. Upon further expansion of aromatic region of DMAP ligand (C), additional peaks can be seen at pH$^*$>6.5 (inset). Red circles indicate free DMAP.
the N-Medien ligand, as reported [18]. At high pH*, the DMAP ligand remains bound to the gold(III) center (Figure 2.10C).

The basic and more substitution-inert N-donor stabilizes the gold(III) metal center. Cyclic Voltammetry (CV) of [Au<sup>III</sup>(dien)X] (X = Cl<sup>-</sup>, 9-EtG and DMAP) in phosphate buffer (to maintain a constant pH for all compounds) showed only one peak indicating a one-step reduction of Au<sup>III</sup>/Au<sup>0</sup> but with more negative reduction potentials for the nitrogen-donors when compared to the AuN<sub>3</sub>Cl species, indicating a more stable metal center (Table 2.1). CV in water gave two reduction peaks for separated Au<sup>III</sup>/Au<sup>1</sup> and Au<sup>1</sup>/Au<sup>0</sup> steps. Stabilization was not observed for [Au(N-Medien)(9-EtG)]<sup>3+</sup> attributed to the observed dissociation of the purine ligand, while the low peak potential for [Au(N-Medien)(DMAP)]<sup>3+</sup> at pH 7.4 may suggest that deprotonation of the primary amines affects the stability of the metal center.

<table>
<thead>
<tr>
<th>Complex&lt;sup&gt;a&lt;/sup&gt;</th>
<th>E&lt;sub&gt;p&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (V)</th>
<th>E&lt;sub&gt;p1&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt; (V)</th>
<th>E&lt;sub&gt;p2&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt; (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[AuCl(dien)]&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>-0.280</td>
<td>0.1</td>
<td>-0.418</td>
</tr>
<tr>
<td>[Au(dien)(9-EtG)]&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>-0.349</td>
<td>0.064</td>
<td>-0.446</td>
</tr>
<tr>
<td>[Au(dien)(DMAP)]&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>-0.328</td>
<td>N/A</td>
<td>-0.563</td>
</tr>
<tr>
<td>[AuCl(N-Medien)]&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>-0.232</td>
<td>0.043</td>
<td>-0.407</td>
</tr>
<tr>
<td>[Au(N-Medien)(9-EtG)]&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>-0.263</td>
<td>0.047</td>
<td>-0.358</td>
</tr>
<tr>
<td>[Au(N-Medien)(DMAP)]&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>-0.238</td>
<td>0.0482</td>
<td>-0.5504</td>
</tr>
</tbody>
</table>

<sup>a</sup> Anions omitted for clarity. <sup>b</sup> 50 mM Phosphate Buffer, 4mM NaCl pH=7.4. <sup>c</sup> H<sub>2</sub>O, 0.1M NaCl

A primary reason for the synthesis of these compounds has been to compare their potential biological properties with those of their isoelectronic and isostructural platinum analogs. Fluorescence studies with tryptophan, a planar amino acid present in the C-terminal
finger of the NCp7-HIV Zn finger (Table 2.2) shows that the π-stacking affinity of the Au(III)(9EtG) compounds is increased over their platinum(II) and palladium(II) analogs [19-20]. All DMAP compounds give $K_a$ values significantly higher than those for their direct 9-EtG analogs, reflecting the greater basicity of the DMAP ligand [21]. The low $K_a$ for [Au(N-Medien)(9EtG)]$^{3+}$ in Tris buffer, pH 7.4 (but not in water) may be explained again by the dissociation of the 9EtG at biological pH.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_a \times 10^3$ M$^{-1}$</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>free 9-EtG</td>
<td>3.3$^{19}$</td>
<td>0.1$^{19}$</td>
</tr>
<tr>
<td>[Pt(dien)(9-EtG)]$^{2+}$</td>
<td>7$^{19,20}$</td>
<td>0.1$^{19}$</td>
</tr>
<tr>
<td>[Pd(dien)(9-EtG)]$^{2+}$</td>
<td>5.1$^{19}$</td>
<td>0.3$^{19}$</td>
</tr>
<tr>
<td>[Au(dien)(9-EtG)]$^{3+}$</td>
<td>16.7 (16.6)$^b$</td>
<td>1.5 (1.9)</td>
</tr>
<tr>
<td>[Au(N-Medien)(9-EtG)]$^{3+}$</td>
<td>10.0 (16.7)</td>
<td>0.4 (0.5)</td>
</tr>
<tr>
<td>free DMAP</td>
<td>29.0</td>
<td>1.1</td>
</tr>
<tr>
<td>[Pt(dien)(DMAP)]$^{2+}$</td>
<td>25.0$^{20}$</td>
<td>0.9$^{20}$</td>
</tr>
<tr>
<td>[Au(dien)(DMAP)]$^{3+}$</td>
<td>25.5 (26.5)</td>
<td>0.3 (0.9)</td>
</tr>
<tr>
<td>[Au(N-Medien)(DMAP)]$^{3+}$</td>
<td>31.2 (26.1)</td>
<td>0.8 (0.3)</td>
</tr>
</tbody>
</table>

*a In Tris HCl buffer, pH=7.4. Anions omitted for clarity.

*b Values in parenthesis were obtained in water.

## 2.4 Experimental

**Materials and Reagents:** N-Medien was obtained from TCI America; all other materials were obtained from Sigma Aldrich, USA. Purity of all compounds was confirmed by $^1$H NMR Spectroscopy and Elemental Analysis (performed by QTI Laboratory, USA).

**NMR Spectroscopy:**

$^1$H NMR spectra were obtained on a Varian Mercury-300MHz Spectrometer. $^{13}$C NMR spectra
were obtained on a Bruker AVANCEIII 600MHz instrument. Chemical shifts were referenced to the residual signal in D$_2$O (4.80ppm). For pH dependency experiments, 10-12 mM and 50 mM solutions were prepared in D$_2$O for $^1$H and $^{13}$C NMR respectively. pH was adjusted using 1 M DNO$_3$ and 1 M NaOD; 0.1 M solutions were used for tighter pH ranges. Readings of the pH meter for D$_2$O solutions were not corrected for deuterium isotope effects and are designated as pH* values.

**UV/VIS Spectroscopy:** Experiments were carried out on a JASCO V-550 UV/VIS Spectrophotometer. For pH dependency experiments, 1 mM solutions were used and pH was adjusted using 0.1 M NaOH and 0.1 M HNO$_3$/HCl.

**Fluorescence Spectroscopy:** Methods were adapted from those previously published [19]. A 3 mL solution of N-AcTrp (5 µM) in 20 mM Tris-HCl buffer was titrated with aliquots of the corresponding quenching compound (7.5 mM) in the range [quencher]/[N-AcTrp]) 1-10, rather than 10-100 as previously reported.

**Potentiometric Titration:** Potentiometric titrations were performed using a Metrohm 798 MPT Titrino Autotitrator with Tiamo v 2.2 software. A 1mM solution of each compound in water was prepared. 25mL of water was added to allow the electrode to be fully submerged in solution. Aliquots of 0.01M NaOH was used as titrant.

**Cyclic Voltammetry:** Cyclic voltammetry experiments were conducted with a CH Instruments Potentiostat using a three-electrode cell having a platinum disk working electrode (1.7 mm in diameter), a platinum mesh counter electrode, and a Ag/AgCl (0.1 M KCl) reference electrode. All measurements were performed on solutions degassed with nitrogen for 10-15 minutes. Measurements were run in both 50 mM phosphate buffer (4 mM NaCl, pH=7.4) and water (0.1 M NaCl).
[AuCl(dien)]Cl₂ was prepared as previously reported [23]. Ep (50mM Phosphate buffer, 4mM NaCl pH=7.4) = -0.280 V; Ep (water, 0.1M NaCl) = 0.1, -0.418 V.

[Au(dien)(9-EtG)](NO₃)₃ • 2H₂O was prepared by reacting [AuCl(dien)]Cl₂ with 0.98 equivalents of 9-EtG and 2.8 equivalents of AgNO₃ in water in the dark at room temperature for 3 days. The reaction mixture was filtered to remove precipitated AgCl, and the orange filtrate was evaporated to dryness. Acetone was added to precipitate the orange product (78% yield) which may be crystallized from H₂O/acetone. Anal. Calcd. for C₁₁H₂₆O₁₂N₁₁Au: C, 18.84; H, 3.74; N, 21.97%. Found: C, 19.01; H, 3.32; N, 21.77%. At 1mM (pH=3.54): λmax₁=204 nm, log ε₁=4.79; λmax₂=248 nm, log ε₂=4.25; λmax₃=279 nm, log ε₃=4.04; λmax₄=362 nm, log ε₄=3.06. Ep (50mM Phosphate buffer, 4mM NaCl pH=7.4) = -0.349 V; Ep (water, 0.1M NaCl) = 0.064 V, -0.446 V.

[Au(dien)(DMAP)]Cl₃ • H₂O was prepared by reacting [AuCl(dien)]Cl₂ with 0.98 equivalents of DMAP in water at room temperature for 1 hour. Reaction mixture was evaporated to dryness. Acetone was added to precipitate orange product (85% yield) which may be crystallized from H₂O/acetone. Anal. Calcd. for C₁₁H₂₅AuCl₃N₅O: C, 23.78; H, 4.72; N, 12.60%. Found: C, 23.79; H, 4.65; N, 12.74%. At 1mM (pH=5.19): λmax₁=195 nm, log ε₁=4.31; λmax₂=211 nm, log ε₂=4.3; λmax₃=278 nm, log ε₃=4.33; λmax₄=380 nm, log ε₄=3.19. Ep (50mM Phosphate buffer, 4mM NaCl pH=7.4) = -0.328 V; Ep (water, 0.1M NaCl) = -0.563 V.

[AuCl(N-Medien)]Cl₂ was prepared as previously reported [18]. Ep (50mM Phosphate buffer, 4mM NaCl pH=7.4) = -0.232 V; Ep (water, 0.1M NaCl) = 0.043 V, -0.407 V.

[Au(N-Medien)(9-EtG)](NO₃)₃ • 2H₂O was prepared analogously to [Au(dien)(9-EtG)](NO₃)₃ • 2H₂O, using [Au(N-Medien)Cl]Cl₂ as the starting material (61% yield). Anal. Calcd. for C₁₂H₂₈AuN₁₁O₁₂: C, 20.15; H, 3.95; N, 21.54%. Found: C, 19.88; H, 3.62; N,
21.82%. At 1mM (pH=4.03): $\lambda_{\text{max}1}=204$ nm, log $\varepsilon_1=4.69$; $\lambda_{\text{max}2}=249$ nm, log $\varepsilon_2=4.14$; $\lambda_{\text{max}3}=260$ nm, log $\varepsilon_3=4.04$. $E_p$ (50mM Phosphate buffer, 4mM NaCl pH=7.4) = -0.263 V; $E_p$ (water, 0.1M NaCl) = 0.047 V, -0.358 V.

$[\text{Au(N-Medien)(DMAP)}]Cl_3\cdot2H_2O$ was prepared analogously to $[\text{Au(dien)(DMAP)}](Cl_3)\cdotH_2O$, using $[\text{Au(N-Medien)Cl}]Cl_2$ as the starting material (80% yield). Anal. Calcd. for C$_{12}$H$_{29}$AuCl$_3$N$_5$O$_2$: C, 24.9; H, 5.05; N, 12.1%. Found: C, 24.9; H, 4.72; N, 12.12%. At 1mM (pH=5.87): $\lambda_{\text{max}1}=195$ nm, log $\varepsilon_1=4.21$; $\lambda_{\text{max}2}=212$ nm, log $\varepsilon_2=4.22$; $\lambda_{\text{max}3}=281$ nm, log $\varepsilon_3=4.16$. $E_p$ (50mM Phosphate buffer, 4mM NaCl pH=7.4) = -0.248 V; $E_p$ (water, 0.1M NaCl) = 0.0482 V, -0.5504 V.

2.5 Conclusion

In summary, use of N-heterocycle ligands allows for manipulation of metal-ligand electronic communication leading to new insights into well-known chemistry including (i) the first isolated Au$^{\text{III}}$N$_3$(N-purine)/heterocycle compounds, (ii) recognition of the effect of the N-heterocycle on the acidity of the Au(dien) ligands, (iii) stabilization of the gold(III) center, and (iv) enhancement of $\pi$-$\pi$ stacking interactions relative to isostructural and isoelectronic platinum(II) and palladium(II) compounds. While many diverse gold(III) complexes have been reported with interesting cytotoxicity, information on speciation and stability at physiological pH is lacking. The use of more substitution-inert ligands within the MN$_4$ chemotype allows for greater control and more specific interactions with biomolecules, especially those with strongly nucleophilic thiolates [22].
2.6 References


Chapter 3. Au(III)-dien-N-heterocycles: reactivity with biomolecules and 
Cys$_3$His HIV-NCp7(F2) and Cys$_2$His$_2$ Sp1(F3) zinc finger peptides

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In preparation to be submitted to *Inorganic Chemistry*

3.1 Abstract:

The reaction of novel Au(III)-dien-N-heterocycle compounds with model amino acids and zinc finger proteins is reported. NMR studies show the presence of a more inert N-donor as the leaving group slows down the reaction with the sulfur-containing amino acids N-acetylmethionine (N-AcMet) and N-acetylcysteine (N-AcCys). Lack of ligand dissociation upon reaction with N-AcCys indicates, to our knowledge, the first long-lived N-heterocycle-Au-S species in solution. Reactions with zinc finger proteins show a higher reactivity with the Cys$_3$His zinc finger than the Cys$_2$His$_2$ zinc finger, likely due to the presence of fewer reactive cysteines. Of the Au(III) compounds studied, [Au(dien)(DMAP)]$^{3+}$ appears to be the least reactive, with ESI-MS studies showing the presence of intact zinc finger at initial reaction times. Though these compounds are still too reactive to result in any binding specificity, these results in combination with previous reported characterization and pH dependency will further aid in optimizing the structure of these AuN$_4$ species to obtain a reactive yet selective compound for targeting zinc finger proteins.
3.2 Introduction:

Zinc finger (ZF) domains are made up of amino acids with a combination of cysteine and histidine residues coordinated to a tetrahedral zinc ion core [1-4]. ZF proteins are critical for a variety of biological functions, including transcription, DNA repair, and apoptosis. Displacement of the central zinc ion, along with mutation of coordinated amino acids can result in a loss of biological function [5]. There has been an increasing interest to synthesize complexes that selectively target zinc finger proteins, in turn inhibiting DNA/ZF interactions and therefore resulting in loss of protein function. A zinc finger of special interest is the HIV nucleocapsid NCp7 protein, a significant target for HIV intervention [6,7]. A key interaction between RNA and NCp7 is the π-stacking between the guanine bases of RNA and the planar aromatic amino acid Tryptophan37 (Trp37) of the zinc finger protein [8,9]. Mutation and/or deletion of this residue from the zinc finger significantly decreases the chaperone activity of HIV NCp7 and inhibits viral replication [10].

Coordination compounds such as cis-[Pt(NH$_3$)$_2$(Cl)$_2$] (Cisplatin), trans-[PtCl(9-EtG)(py)$_2$]$^+$ (9-EtG = 9-ethylguanine; py = pyridine), and [PtCl(dien)]$^+$ (dien = diethylenetriamine) act as electrophiles towards zinc fingers, disrupting protein conformation with zinc ejection [5,11-13]. An approach to target specifically the C-terminal finger of HIV-NCp7, F2, is to use platinated nucleobases as in [Pt(dien)(Nucleobase)]$^{2+}$ as electrophiles capable of tryptophan molecular recognition and eventual attack on the highly nucleophilic zinc-cysteinate residues [12,14-16]. Pt(II)-N-heterocycle compounds with, for example 4-NMe$_2$pyr (DMAP) may also interact with the HIV-NCp7 C-terminal zinc finger through non-covalent interactions between tryptophan in the zinc finger and the nucleobase ligand [17].
Given the isoelectronic and isostructural relationship of Pt(II) and Au(III) we have been exploring analogous Au(III) chemistry. The properties of \([\text{Au(III)}(\text{dien})(\text{Nucleobase/N-heterocycle})]^3^+\) show that the N-donor ligand stabilizes the Au(III) oxidation state, the N-donor ligand affects the acidity of the central secondary amine proton of the dien ligand and that π-π stacking with the simple amino acid tryptophan is significantly enhanced over their Pt(II) analogs [18].

The “parent” \([\text{AuCl(dien)}]\text{Cl}_2\) reacts rapidly with the C-terminal finger of the HIV nucleocapsid protein NCp7 with Zn\(^{2+}\) ejection and formation of \text{AuF/Au}_2\text{F/Au}_4\text{F “gold fingers”} [13]. The Au(III) compound also reacts much faster than its Pt(II) analog, attributed to the ‘soft’ nature and high thiol affinity of Au(III) [5,13]. The presence of a more substitution-inert ligand

![Diagram](image)

**Figure 3.1.** Structures of (A) Au(III)-complexes studied, (B) N-Acetylmethionine, N-AcMet, (C) N-Acetylcysteine, N-AcCys, (D) NCp7(F2) ZF, and (E) Sp1(F3) ZF, \(X_1 = \text{RFMSDHL}\).
such as a nucleobase or planar amine may allow tuning of the Au(III) reactivity aiding in the overall selectivity of the reaction with peptides. Following our description of the basic chemistry of \([\text{Au(III)}(\text{dien})(\text{Nucleobase/N-heterocycle})]^{3+}\) [18], we now report on their reactivity with relevant biomolecules, specifically toward the sulfur containing amino acids N-AcMet (N-Acetylmethionine) and N-AcCys (N-Acetylcysteine) and zinc finger peptides differing in zinc coordination sphere: the Cys$_2$His$_2$ finger-3 of the Sp1 transcription factor (Sp1(F3)) and the Cys$_3$His C-terminal finger of HIVNCp7 nucleocapsid protein (NCp7(F2)), Figure 3.1. The Au(III)-N-heterocycle compounds reacted slower than \([\text{AuCl(dien)}]_2\) with both N-AcMet and N-AcCys, the latter producing a N-heterocycle-Au-S species, unlike previous results seen for Au(I)-N-heterocycle compounds [19]. Differences in reactivity of Cys$_3$His and Cys$_2$His$_2$ ZF are also discussed.

3.3 Results and Discussion

3.3.1 Reactivity with Biomolecules

Competition assays of the DNA-Ethidium Bromide (EtBr) interaction showed little fluorescence quenching of the intercalator in the presence of Au(III)-compounds I-V, Figure 3.2, confirming that DNA is unlikely to be the principal target for these compounds. \([\text{Au(dien)}(4\text{-picoline})]^{3+}\), compound IV, appears to bind to DNA slightly, resulting in a loss of fluorescence intensity. Based on these results, it is likely that there will be little competitive binding between the drug and DNA, a necessary prerequisite if we are to target zinc finger proteins.

The soft nature of Au(III) predicts reactivity with sulfur-containing amino acids. The reactions of \([\text{AuCl}_4]\) with L-Methionine (L-Met) and L-Cys and the influence of tridentate N-
donors varying in π-donor ability on stability of the Au(III) oxidation state have been reported [20-21]. The dien chelate affords the least reactive compounds of those studied. Given that the MN₄ compounds stabilize further the Au(III) oxidation state relative to [AuN₃Cl] [18], we compared the reactivity of the AuN₄ species with both N-AcMet and N-AcCys to verify whether this stabilization will further slow down the reaction.

The reaction of [AuCl₄]⁻ with L-Met results in oxidation of the thioether and concomitant Au(III) -> Au(I) reduction [20-21]. Little or no reaction has been reported for [AuCl(dien)]²⁺ and L-Met based on UV-VIS spectra. In this study, we used a 2.5 fold excess of N-AcMet and ¹H NMR spectral changes were noticed over time (Figure 3.3). The initial pH of the solutions was approximately 3.0. Clear downfield shifts of the S-CH₃ H₈ and S-CH₂ H₆, indicate binding of N-AcMet to gold (Figure 3.3). Multiple peaks appear for the S-CH₃ singlet shifted downfield from the free ligand at δ = 2.13 ppm. In the case of [AuCl(dien)]²⁺, a second singlet appears at 2.7
ppm and increases slightly in intensity over time; these shifts may be due to different Au-Met(SCH$_3$)$_3$ species or, as has been suggested [21], reflect the presence of oxidized methionine sulfone or sulfoxide. The dien ligand dissociates as indicated by both a loss of the broad singlet at 3.65ppm indicative of [AuCl(dien)]$^{2+}$ and by the appearance of a multiplet centered at $\sim$ 3.46 ppm (Figure 3.3A). This multiplet is similar in shape and chemical shift when compared to the free dien salt. For [AuCl(N-Medien)]$^{2+}$, the situation is somewhat different with up to three S-
CH$_3$ species clearly distinguishable (Figure 3.3B). In this case, the singlet of the N-Medien ligand suffers an upfield shift to shielding, consistent with the formation of Au-Met trans to the Au-NMe bond. No evidence for production of free N-Medien was seen throughout the reaction but it is possible that deprotonation of the primary amines on the N-Medien occurs allowing further ligand substitution, as noted previously in the solution behavior of [AuCl(N-Medien)]$^{2+}$ [18]. These complex highly pH-dependent reactions were not investigated further since our primary goal was to compare the reactivity of the Cl versus N-heterocycle compounds. A summary of chemical shifts is reported in Table 3.1.

**Table 3.1 Chemical shifts of N-AcMet and N-Me peaks upon reaction with AuN$_3$Cl species.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>S-CH$_3$ 1 (ppm)</th>
<th>S-CH$_3$ 2 (ppm)</th>
<th>S-CH$_3$ 3 (ppm)</th>
<th>S-CH$_2$ (ppm)</th>
<th>N-Me (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I + NAcMet</td>
<td>2.45 (+0.32)</td>
<td>2.72 (+0.59)</td>
<td>N/A</td>
<td>2.93 (+0.31)</td>
<td>N/A</td>
</tr>
<tr>
<td>II + NAcMet</td>
<td>2.64 (+0.51)</td>
<td>2.69 (+0.24)</td>
<td>2.8 (+0.67)</td>
<td>2.93 (+0.31)</td>
<td>3.37 (-0.25)</td>
</tr>
</tbody>
</table>

** Values in parenthesis refer to change in chemical shift from free, unreacted ligand (S-CH$_3$ = 2.13 ppm, S-CH$_2$ = 2.62 ppm, N-Me = 3.62 ppm)

NMR studies showed that the presence of the more inert N-donor ligand does results in slower reactivity and the absence of downfield shifts for the S-CH$_3$ protons indicated no significant reaction with N-AcMet. Further, no evidence for significant loss of dien ligand or release of the N-heterocycle was observed. For [Au(N-Medien)(DMAP)$^{3+}$ small peaks corresponding to N-AcMet substitution can be observed after 15 h. (Figure 3.4).

The thiol-containing amino acid L-cysteine quickly reduces Au(III) in aqueous solution, the reaction being complete in the first minute [20-21]. The initial rapid substitution reaction results in an unstable Au(III)-S complex that is quickly reduced to Au(I/0) in a slower electron-transfer process [21]. The addition of a tridentate ligand, dien, stabilizes the Au(III) metal center.
somewhat [21-22], and the addition of the more inert N-donor leaving group is expected to stabilize it even further [18]. Both Au(III) N₃Cl compounds are very reactive with N-AcCys, due to its greater nucleophilicity. Both AuN₃Cl species show similar results to that of the N-AcMet reaction, resulting in loss of dien/NMedien ligands and precipitation of white solids as has been
observed previously [21] (Figure 3.5). The final pH of all reaction mixtures upon addition of N-AcCys is approximately 3. In the case of the reaction with AuN₄ compounds, the dien ligand also dissociates, however there is no evidence for concomitant release of the DMAP N-heterocycle ligand, as indicated by no ligand peak shifts in the NMR spectra, Figure 3.6. The pH of [Au(dien)(DMAP)]³⁺ decreases from 5.2 to 3.3. The broad peak at ~ 3.6 ppm (Figure 3.6)
represents the dien protons when bound to Au. This broad peak decreases over time, while the multiplet at 3.4ppm (free dien) increases, again showing the loss of dien over time. As shown in Figure 3.6 the DMAP ligand does not dissociate over time; the presence of free DMAP would be indicated by peaks at 8.15 and 6.7ppm. This situation is in contrast to that of Au(I) where the reaction of \([\text{Ph}_3\text{P})\text{Au}(I)(\text{DMAP})]^1^+\) with 1 equivalent of N-AcCys, results in DMAP displacement and formation of a P-Au-S coordination unit [19].

Similar spectra are seen in the case of 9-EtG. The pH of \([\text{Au(dien})(9\text{-EtG})]^3^+\) increases from 2.7 to 3.0. The H8 peaks of the 9-EtG ligand shift upfield upon addition of NAcCys a result of the change in pH of the reaction mixture (Figure 3.7). This is not due to ligand dissociation as the spectrum is identical to that of III alone at pH ~3.0 [18]. The fact that the dien ligand dissociates but the N-heterocycle remains bound is an interesting result. The spectra could reflect the slower substitution kinetics of the \([\text{Au(dien)(N-heterocycle})]\) species or the presence of
mixed Au/N-AcCys/DMAP(9-EtG) products. Despite the complexity of the reaction it does appear that the stabilization of the Au(III) oxidation state in [Au(dien)(N-heterocycle)]^{3+} does extend to reactions with biomolecules and overall the results suggest that [AuN₄] systems might be capable of fine-tuning to distinguish substitution from redox reactions.

3.3.2. Reactivity with Zinc Finger Proteins:

Reaction of [AuCl(dien)]Cl₂ with NCp7(F2) results in immediate Zn ejection and formation of gold fingers [13]. It was our goal to slow down this interaction by replacing the labile -Cl with a more inert N-donor. As seen for Pt(II) species, it is expected that the planar amine will π-stack with the tryptophan residue in the C-terminal finger of HIV-NCp7.

Fluorescence studies with free N-AcTrp show that Au(III) compounds exhibit a greater stacking interaction when compared to Pt(II) analogs, Table 3.2 [18]. Compounds containing DMAP as the leaving group have K_a values significantly higher than those of their 9-EtG

![Figure 3.7. NMR spectra of [Au(dien)(9-EtG)]^{3+} (III) at initial pH*, pH*=3.05, and upon initial reaction with N-AcCys.](image-url)
analogues, reflecting the greater basicity of the DMAP ligand [14,17-18]. It was hypothesized that \([\text{Au}(\text{dien})(4\text{-picoline})]^{3+}\) would stack more efficiently than \([\text{Au}(\text{dien})(9\text{-EtG})]^{3+}\) due to the higher \(pK_a\) of the ligand; \(pK_a\) of 4-picoline is 6.05 whereas the \(pK_a\) of N7 of 9-EtG is 2.7 [23-24]. The \(K_a\) values for all 4-picoline compounds, however, are much lower than all other compounds studied, with a \(K_a\) of the free ligand being too low to determine. Though the \(K_a\) values are lower than expected, it is still important to note the more than 2-fold increase of Au(III) vs. Pt(II) as similarly seen for the \([\text{M}(\text{dien})(9\text{-EtG})]^{n+}\) compounds. It is also interesting to note the large effect of the N-dimethyl group of the DMAP ligand on the \(\pi\)-stacking ability of the complex.

**Table 3.2. Association Constants of N-AcTrp with Au(III)-N-heterocycle compounds and their Pt(II) analogues.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>(K_a) ((x10^3 \text{ M}^{-1}))</th>
<th>SD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Pt}(\text{dien})(9\text{-EtG})]^{2+})</td>
<td>6.8</td>
<td>0.1</td>
<td>14</td>
</tr>
<tr>
<td>([\text{Au}(\text{dien})(9\text{-EtG})]^{3+})</td>
<td>16.7</td>
<td>1.5</td>
<td>18</td>
</tr>
<tr>
<td>([\text{Pt}(\text{dien})(4\text{-pic})]^{2+})</td>
<td>1.34</td>
<td>0.48</td>
<td>--</td>
</tr>
<tr>
<td>([\text{Au}(\text{dien})(4\text{-pic})]^{3+})</td>
<td>3.61</td>
<td>0.58</td>
<td>--</td>
</tr>
<tr>
<td>([\text{Pt}(\text{dien})(\text{DMAP})]^{2+})</td>
<td>25</td>
<td>0.9</td>
<td>17</td>
</tr>
<tr>
<td>([\text{Au}(\text{dien})(\text{DMAP})]^{3+})</td>
<td>25.5</td>
<td>0.3</td>
<td>18</td>
</tr>
</tbody>
</table>

A fluorescence quenching assay is typically performed to calculate a \(K_a\) value for the compounds with the C-terminal finger of NCp7, due to the presence of a tryptophan residue. In the case for the Au(III) compounds studied, the fluorescence decreased significantly over time indicating loss of zinc and change in peptide structure (Figure 3.8A). Therefore, a representative \(K_a\) value could not be calculated. Both planar amine compounds exhibited a slightly larger decrease in fluorescence upon addition of drug, which could be due to their \(\pi\)-stacking ability.

CD experiments show that all Au(III) compounds studied result in some degree of zinc ejection when reacted with the C-terminal finger of the NCp7 zinc finger protein. The CD
spectrum of NCp7(F2) is characterized by a positive band at ~220 nm. The Au compounds cause a decrease in intensity of this band and a significant increase in the negative ellipticity with a slight blue shift of the 195-200 nm band, which are indicative of conformational changes from ordered structure to random coil [25-27]. After 15 minutes incubation, [AuCl(dien)]^{2+} resembles the free peptide spectrum. [Au(dien)(9-EtG)]^{3+}, III, and [Au(dien)(DMAP)]^{3+}, V, both result in zinc ejection and loss of ordered structure, however to a lesser degree than [AuCl(dien)]^{2+}, Figure 3.8B. After 1 hour incubation, the spectrum of III + NCp7(F2) resembles that of free

Figure 3.8 (A) Fluorescence quenching over time upon addition of I, III, and V. (B) CD spectrum of I, III, and V with NCp7(F2) after 15 minutes incubation.
peptide, however the spectrum of V + NCP7(F2) does not change over time, indicating [Au(dien)(DMAP)]^{3+} is the least reactive of the three gold compounds studied.

The ESI-MS of [AuCl(dien)]^{2+} with NCP7(F2) shows loss of ligand and complete zinc ejection, with incorporation of two or four Au ions [13]. ESI-MS spectra of both [Au(dien)(9-EtG)]^{3+} and [Au(dien)(DMAP)]^{3+}, Figure 3.9, show that both N-heterocycle compounds stay

![Figure 3.9](image_url)

**Figure 3.9.** Initial ESI-MS spectra of (A) [Au(dien)(9-EtG)]^{3+} and (B) [Au(dien)(DMAP)]^{3+} with NCP7(F2).
intact upon initial reaction with NCp7(F2) indicating the AuN₄ species are more stable to reduction. Interestingly, both compounds exhibit fragmentation resulting in partial loss of the dien ligand. This was also seen in ESI-MS recorded of the compounds alone. The most prominent peaks for the reaction between [Au(dien)(9-EtG)]^{3+} (III) and NCp7(F2) are 740.9927 (3⁺) and 1110.9861 m/z (2⁺) corresponding to apo-peptide. Smaller peaks at 872.3034 (3⁺) and 1307.9520 m/z (2⁺) represent Au₂/F. Therefore, like the -Cl complex, compound III results in formation of gold fingers, however incorporation of four Au ions was not observed. The ESI-MS spectrum of [Au(dien)(DMAP)]^{3+} (V) and NCp7(F2), however, shows peaks corresponding primarily to intact zinc finger, 571.7282 (4⁺) and 762.6356 m/z (3⁺). There is some zinc ejection, as indicated by a peak at 1111.4963 m/z (2⁺), which represents oxidized apo-peptide. This is in agreement with the CD data, which shows compound III to react the slowest. The CD spectrum results from a sum of different conformations in solution; therefore the negative ellipticity may be due to the presence of some apo-peptide in solution. At 4 hours reaction time, these zinc finger m/z peaks are no longer present. It is difficult to interpret the spectra at later time points; however, it does not appear that Au₂/F is present as is the case for the 9-EtG analogue.

Zinc finger cysteinate varies in chemical reactivity; the Zn-Cys49 thiolate of NCp7 is most susceptible to electrophilic attack. The Cys₂His₂ zinc finger motif has also been shown to be less nucleophilic and less tightly packed [28] and therefore it is expected that it would be less reactive with the Au(III) compounds. To examine this point further, we compared the reactivity of ZF NCp7 with that of Sp1(F3), the third zinc finger of the Sp1 transcription factor. As indicated by CD spectroscopy (Figure 3.10) there is a difference in the rate of reactivity between the Cys₃His and Cys₂His₂ ZFs, the latter taking ~ 5 hours for complete loss of ordered structure,
whereas the Cys3His ZF shows similar results at 15 minutes (Figure 3.8A). Compounds I and III appear to react similarly, resulting in complete Zn ejection and loss of structure at 5 hours. Compound V, [Au(dien)(DMAP)]^{3+}, appears to react slower than compounds I and III; though a slight blue shift occurs at ~200nm compared to that of zinc finger, an increase in negative ellipticity does not, indicating less zinc ejection. However, upon further examination, the CD spectrum in Figure 3.10C is similar to that of oxidized Cys2His2 peptide (See Figure 1.17 in Chapter 1), which indicates zinc is in fact being ejected and disulfide bonds are being formed.

**Figure 3.10.** CD spectra of (A) [AuCl(dien)]^{2+}, I (B) [Au(dien)(9-EtG)]^{3+}, III and (C) [Au(dien)(DMAP)]^{3+}, V, with Sp1(F3) at 15 min, 1 hr, and 5 hr incubation times.
ESI-MS of [AuCl(dien)]^{2+} and Sp1(F3) show prominent peaks for apo-peptide (674.3635 m/z, 5^+) and Au/F (713.7566 m/z, 5^+), Figure 3.11A. Unlike the reaction with NCp7(F2), incorporation of more than one gold ion was not observed initially. However, small peaks representing Au_2/F appeared after 1 hour incubation. Unlike the Cys_3His zinc finger, the [Au(dien)(9-EtG)]^{3+} does not stay intact upon reaction with the Sp1(F3) Cys_2His_2 zinc finger indicated by a large peak at 180.0883 m/z representing free 9-EtG ligand. Immediately after adding the drug, the prominent peaks in the mass spectrum are apo-peptide (842.4415 m/z, 4^+)

![Figure 3.11](image.png)

**Figure 3.11.** ESI-MS of (A) [AuCl(dien)]^{2+}, (B) [Au(dien)(9-EtG)]^{3+}, and (C) [Au(dien)(DMAP)]^{3+} with Sp1(F3) at initial time points.
and Au/F (891.9345 m/z, 4⁺), Figure 3.11B. Over time the Au/F peaks increase in intensity; however, Au₂/F is not present as seen for the Cl species. Unlike both the –Cl and –9-EtG species, the Au-DMAP species does not result in immediate formation of Au/F. The initial ESI-MS shows a small peak at 391.1222 m/z indicating a fragment of [Au(dien)(DMAP)]³⁺; loss of CH₂NH₂ from the dien ligand. Other peaks present represent apo-peptide (561.9996 m/z, 6⁺), Figure 3.11C. This peak refers to the mass of apo-peptide minus two protons, indicating the formation of a disulphide bond, which agrees with the CD data. After 1 hour, Au/F begins to appear at 713.3511 m/z, 5⁺, increasing in intensity over 24 hours, however apo-peptide remains the prominent species. An equimolar reaction of the Au(I) drug aurothiomalate and pre-formed Sp1 zinc finger (excess Zn present) resulted in 60% Au/F and 40% zinc finger [27]. Similar to our results, binding of more than one metal ion was not observed. [Au(dien)(DMAP)]³⁺, V, appears to be the least reactive of the Au(III) compounds studied for both the Cys₂His₂ and Cys₃His zinc finger proteins. Though zinc ejection and formation of gold-finger still occurs, the rate of the reaction has decreased with the presence of DMAP as the leaving group.

Upon binding to the peptide, the gold ions may remain Au(III) and bind in a square planar fashion or reduce to Au(I) and bind linearly. Table 3.3 shows the expected and observed m/z values for the reaction of the Au(III) compounds with Sp1. To calculate the expected values, it was assumed the Au would bind in a 4-coordinate geometry, similar to that of Zn²⁺. When the expected and observed m/z values are compared, we observe m/z values within 0.1 of what is expected, suggesting that when bound to the Cys₂His₂ zinc finger, Sp1, the Au remains in the 3⁺ charge state. CD spectra of Au/F formed from the reaction of aurothiomalate (Au⁺¹) and Sp1 showed a slight red shift in the minimum, however not to the same degree of ZF, indicating a difference in conformational features such as less α-helix [27]. Based on the difference in CD
spectra between the Au(III) compounds (large increase in negative ellipticity) studied here and various Au(I) compounds (decrease in negative ellipticity) in the literature [19,27], it is likely that Au(III) binding results in a different conformational changes than either Au(I) or Zn(II). This change in conformation can then, presumably, result in inhibition of DNA binding and therefore loss of function.

<table>
<thead>
<tr>
<th>Charge state</th>
<th>Observed m/z</th>
<th>Calculated m/z *</th>
<th>Delta m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>2+</td>
<td>1307.952</td>
<td>1307.964</td>
<td>-0.012</td>
</tr>
<tr>
<td>3+</td>
<td>872.303</td>
<td>872.309</td>
<td>-0.006</td>
</tr>
</tbody>
</table>

* Calculated values assume Au binds in same 4-coordinate geometry as Zn

It is slightly more complicated to determine how the Au atoms are binding in the Cys₃His zinc finger, NCp7(F2), due to the fact that multiple gold ions bind. Expected values match closest to those observed when it is assumed the first gold binds as 3⁺ and the second gold atom binds minus a proton, perhaps binding in a linear geometry as Au(I) (Table 3.4). This is similar to what was observed for Au(III) compounds when reacted with the Cys₃His zinc finger PARP-1 [29].

<table>
<thead>
<tr>
<th>Charge state</th>
<th>Observed m/z</th>
<th>Calculated m/z *</th>
<th>Delta m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>3+</td>
<td>1188.255</td>
<td>1188.240</td>
<td>0.015</td>
</tr>
<tr>
<td>4+</td>
<td>891.432</td>
<td>891.430</td>
<td>0.002</td>
</tr>
<tr>
<td>5+</td>
<td>713.347</td>
<td>713.344</td>
<td>0.003</td>
</tr>
<tr>
<td>6+</td>
<td>594.624</td>
<td>594.620</td>
<td>0.004</td>
</tr>
<tr>
<td>7+</td>
<td>509.679</td>
<td>509.817</td>
<td>-0.138</td>
</tr>
</tbody>
</table>
3.4 Experimental

**Materials:** HAuCl$_4$ was purchased from Strem Chemicals; N-Medien was purchased from TCI America; all other reagents were purchased form Sigma Aldrich, USA. Both NC and Sp1 peptides were purchased from GenScript Corporation.

**Synthesis:** Synthesis of [AuCl(dien)]Cl$_2$ (I), [AuCl(N-Medien)]Cl$_2$ (II), [Au(dien)(9-EtG)](NO$_3$)$_3$•2H$_2$O (III), [Au(dien)(DMAP)]Cl$_3$•1.5H$_2$O (V), have been previously published [18,30].

**Synthesis of [Au(dien)(4-pic)](NO$_3$)$_3$•H$_2$O (IV).** 0.98 equivalents of 4-picoline (4-pic) and 2.98 equivalents of AgNO$_3$ were added to a solution of [AuCl(dien)]Cl$_2$ in water. The reaction mixture was stirred at room temperature in the dark for 1 hour. The solution was filtered to remove AgCl and the yellow filtrate was evaporated to dryness. Acetone was added to precipitate orange product. Anal. Calcd. for C$_{10}$H$_{21}$AuCl$_3$N$_5$:  C, 20.11; H, 3.71; N, 16.42%. Found:  C, 19.67; H, 3.25; N, 16.01%. At 1 mM: $\lambda_{\text{max}1}$=206 nm, log $\varepsilon$1=4.63; $\lambda_{\text{max}2}$=247 nm, log $\varepsilon$2=3.92; $\lambda_{\text{max}3}$=357 nm, log $\varepsilon$1=2.87.

**Ethidium Bromide (EtBr) Assay:** CT-DNA (calf-thymus DNA) was prepared in 1 mM phosphate buffer (50 mM NaCl, pH=7.4). The concentration of both DNA and EtBr were determined by absorption measurements at 260 nm and 338 nm, respectively. Stock solutions of the CT-DNA incubated with the gold(III) compounds at a ratio of 0.1 were prepared and incubated at 37°C for 24 hours for I-II (covalent binding) and 1 hour for III - V (non-covalent binding). Varying amounts of ethidium bromide in the range of $r_i = 0 – 0.25$ was added to CT-DNA (100 µM) and CT-DNA (100 µM) treated with I-V. Fluorescence emission spectra were recorded on a Cary Eclipse (Varian) fluorometer at 25°C. Fluorescence was excited at $\lambda_{\text{ex}}$= 525 nm and registered at $\lambda_{\text{em}}$= 600 nm. Spectra were recorded in triplicate and averaged.
NMR Spectroscopy with N-AcMet and N-AcCys: 0.006-0.009 mmol solutions of compounds I-V were added to a solution containing 2.5 equivalents N-AcMet or 1 equivalent of N-AcCys. The reaction was followed over time by NMR. NMR spectra were referenced to the residual signal of D2O (1H, 4.80ppm).

Zinc finger preparation: Free peptide was dissolved in water (concentration was dependent on experiment/technique being performed). The pH was adjusted to 7.2-7.4 with a concentrated solution of NH4OH. 1.3 equivalents of Zn was added using a concentrated solution of zinc acetate. In both cases, the ZF formed immediately and no incubation was necessary. ZF formation was confirmed by both CD and MS. The data obtained were in agreement with values previously reported in the literature [25-27].

Tryptophan Quenching by Fluorescence Spectroscopy: For fluorescence quenching studies, methods were adapted from those previously published [15]. A 3 mL solution of N-AcTrp (N-acetyltryptophan) or NCp7(F2) (5 µM) was titrated with aliquots of the corresponding quenching compound (7.5 mM) in the range [quencher]/[N-AcTrp]) 1-10. For time studies performed with zinc finger proteins, 1 equivalent of Au-compound was added to 5µM NCp7 and fluorescence was monitored over time.

Circular Dichroism Spectroscopy: Methods were adapted from those previously published [13]. A 0.2 mg/mL solution of peptide in water was prepared. CD was run before and after adding zinc (1.3 eq.) to ensure correct formation of zinc finger. The reaction of ZF:drug was performed in a 1:1.3 eq ratio and followed over time.

Mass Spectrometry: ESI-MS experiments were performed on an Orbitrap Velos from Thermo Electron Corporation. Instrument parameters were as follows: electrospray voltage = 2.3 kV; capillary temperature = 230 °C; flow rate = 0.7 µL/min. A concentrated ZF solution was
prepared in water (450 µM) and 1 equivalent of Au(III) compound was added. Samples were
diluted as follows for analysis by mass spectrometry: 20 µL sample, 20 µL methanol, 200 µL of
20 µM acetic acid.

3.5 Conclusions

The Au(III) compounds studied are the first isolated Au(III)-dien-N-heterocycle
compounds. We have recently shown the effect of the nature of the N-heterocycle on the acidity
of the Au-dien ligand, stabilization of the Au(III) metal center, and the π-stacking ability with
free N-Acetyltryptophan when compared to their Pt(II) analogs [18]. Expanding on that work,
we report here the reactivity of these compounds with model amino acids and Cys3His and
Cys2His2 zinc finger protein cores. NMR studies show a decrease in reaction rate for AuN4
species with both N-AcMet and N-AcCys when compared to AuClN3 species. Upon reaction
with N-AcCys, the dien ligand completely dissociates. In the case of the AuN4 species, the N-
heterocycle ligand remains bound, suggesting formation of a N-heterocycle – Au – S species.
Interestingly, this was not the case for Au(I)-N-heterocycle compounds with N-AcCys, which
resulted in loss of the N-heterocycle ligand and formation of a P—Au—S species [19].

Many studies have been reported on the reaction of Au(I)/Au(III) compounds with
various zinc finger proteins. Such reactions result in rapid displacement of Zn2+ with
concomitant replacement of Au(I)/(III) in which all gold-coordinated ligands are lost
[13,27,29,31]. Though this zinc ejection may result in loss of ZF function, it is necessary to
develop drugs with selectivity. The Au(III) drugs discussed herein were designed to selectively
target the NCp7 ZF through π-stacking interactions with the planar amino acid tryptophan.
Though fluorescence experiments show a significant enhancement of π-stacking with free
NAcTrp for Au(III) compounds over their Pt(II) analogues, studies with NCp7(F2) resulted in quenching over time suggesting rapid zinc ejection. The initial mode of recognition may result from the non-covalent interaction of the N-heterocycle ligand with the tryptophan residue, however the reaction is still too fast to observe any intermediate species.

In agreement with small-scale NMR reactions with model amino acids, ESI-MS and CD experiments with zinc finger proteins also showed a decrease in the rate of reactivity for AuN₄ species. ESI-MS studies showed both Au(III)-N-heterocycle compounds remained intact at initial timepoints when reacted with NCp7(F2), unlike that observed for [AuCl(dien)]²⁺ [13]. CD and ESI-MS studies showed [Au(dien)(DMAP)]³⁺ to react the slowest, with evidence of intact zinc finger present at early time points. CD showed the Cys₂His₂ zinc finger, Sp1(F3), to react slower than the Cys₃His zinc finger, NCp7(F2), taking 5 hours to result in complete zinc ejection for [AuCl(dien)]²⁺ and [Au(dien)(9-EtG)]³⁺. [Au(dien)(DMAP)]³⁺ was the only compound studied that did not result in formation of Au/F. When calculated m/z values were compared to those observed, it was determined that the gold remains in the 3⁺ oxidation state upon binding to Sp1. In the case of NCp7(F2), the primary gold-finger species observed in NCp7(F2) reactions was Au₂/F. Based on calculations, it was determined that the first gold remains Au(III) upon binding and the second gold binds Au(I).

Previous pH dependency studies show [Au(dien)(DMAP)]³⁺ to be the most stable of the Au(III)-N-heterocycle compounds studied [18]. The DMAP ligand stabilizes the dien ligand and does not dissociate at low or high pH values [18]. The studies reported herein support the fact that the Au-DMAP species is the most stable, resulting in the least amount of zinc ejection and gold-finger formation for both zinc finger peptides studied. Though these compounds are sill
relatively reactive and unlikely to bind with selectivity, the loss in ordered structure indicated by CD suggests DNA would not bind and therefore would result in a loss of function.

3.6 References


Chapter 4. Modulation of the stacking interaction of MN₄ (M=Pt, Pd, Au) complexes with tryptophan through N-heterocyclic ligands

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**As supporting author for the following work, I have contributed all work pertaining to [Au(dien)(DMAP)[3+] including synthesis and fluorescence quenching data with N-Acetyltryptophan (N-AcTrp). I also played a significant role in understanding the inner filter effect in regards to the fluorescence quenching assay. At concentrations above 50 µM, [Au(dien)(DMAP)]³⁺ absorbs at the wavelength at which the fluorophore, NAcTrp, emits, thus resulting in more quenching than actually present and a higher Kᵳ value. Therefore, it was necessary to titrate 1-10 molar equivalents of drug : NAcTrp, rather than 10-100 as previously reported for platinum(II) compounds in the literature. The absorbance of [Au(dien)(DMAP)]³⁺ at or below10 molar equivalents (50 µM) was <0.05 absorbance units.

4.1 Abstract

A survey of selected N-heterocycle ligands showed that platination of 4-N-dimethylaminopyridine (DMAP) in [Pt(dien)L]²⁺ (dien = diethylenetriamine) gave especially
strong $\pi-\pi$ stacking interactions with tryptophan and the tryptophan-containing C-terminal zinc finger (ZF) of the HIV (human immunodeficiency virus) nucleocapsid protein NCp7. The association constants (all at $10^3 \text{ M}^{-1}$) were significantly stronger (25.0 and 28.1 for tryptophan and ZF respectively) than those previously measured for the purine nucleobase 9-ethylguanine (9EtG) in $[\text{Pt(dien)}(9\text{EtG})]^{2+}$ (6.88 and 7.55 for tryptophan and ZF, respectively). Extension to Pd and Au complexes also confirmed the utility of DMAP in assisting stacking interactions. The results confirm the utility of a “bioinorganic” approach to targeting and inactivation of medicinal chemistry targets using the dual approach of target recognition (non-covalent) followed by target fixation (covalent).

4.2. Introduction

Zinc finger (ZF) proteins constitute 2–3% of the human genome and play a diverse role in many cell processes, such as transcription, DNA repair, cellular signaling and apoptosis. Their structures are characterized by zinc coordination to four amino acids, typically cysteine and histidine. Elimination of zinc from zinc finger proteins causes a loss of 3-dimensional structure leading to loss of protein function [1–3]. The 2-zinc finger nucleocapsid protein human immunodeficiency virus (HIV1) NCp7 remains an interesting drug target, complementary to the current therapies aimed at inhibiting HIV reverse transcriptase and protease. While this approach has been successful in reducing the viral loads of many patients, there are problems of resistance, drug toxicity and latent viral reservoirs that are contained in long-lived cell populations. NCp7 is an attractive target because it is highly conserved in all viral strains and mutations in the sequence have been shown to render the virus non-infectious [4,5].

The C-terminal finger of NCp7 (ZF) contains a tryptophan residue while the N-terminal
finger contains a phenylalanine. A critical feature in NCp7-DNA/RNA recognition is the stacking of these aromatic residues with purine and pyrimidine bases (guanine, cytosine) on DNA/RNA [6,7]. N-quaternization of nucleobases enhances the stacking interaction, in part by lowering the energy of the lowest unoccupied molecular orbital (LUMO) of the nucleobase, making it closer in energy to the highest occupied molecular orbital (HOMO) of the Trp [8,9]. N-quaternization may be achieved by protonation, alkylation or metallation and it is possible that the lowering of the energy of the LUMO may be due in part to the removal of the lone pair of electrons on the nitrogen of the nucleobase [8,9]. We have been systematically examining the coordination chemistry of NCp7 with platinum–metal compounds based on the [M(dien)(nucleobase)]^{n+} (MN₄) motif (dien=diethylenetriamine). On average, free 9-ethylguanine (9EtG), GMP, and CMP have Kₐ values for association with tryptophan of 3x10³ M⁻¹ whereas platination increases the Kₐ for the three species to 6.8, 6.9, and 7.0x10³ M⁻¹, respectively [9–12]. Extension to the Trp-containing C-terminal finger of NCp7 with [Pt(dien)(9EtG)]^{2+} and [Pt(dien)(GMP)] gave Kₐ values of 7.5 and 12.4x10³ M⁻¹, respectively [13].

The design of [M(dien)(nucleobase)]^{n+} as a peptide-specific drug may be envisaged as a classic “two-step” approach — target recognition through tryptophan stacking followed by fixation” through zinc ejection and/or covalent binding to the peptide. Recently, we have shown that the incorporation of a Trp residue increases the rate of reaction, and perhaps specificity, between [Pt(dien)(9EtG)]^{2+} and a short 4-amino acid peptide [14]. While it is attractive to modulate drug–target interactions based on nature's motif, it is important to examine whether we are restricted to purine and pyrimidine motifs for targeting and how to improve these association constants in order to more effectively recognize NCp7. In this contribution, we explore the
manipulation of the \( \pi \)-stacking interaction between tryptophan, both free in solution and as part of a peptide sequence, and metal complexes containing non-natural (N-heterocycle pyridine and thiazole) aromatic ligands to mimic the Trp/Gua interaction. The strong donor 4-Me\(_2\)N-pyridine gives significantly enhanced stacking over the 9EtGua analog; these results were extended to the isostructural Pd and Au systems.

### 4.3 Results and Discussion

![Chemical structures](image)

**Figure 4.1.** Structures of the metal (M= Pt, Pd, Au) N-heterocycle complexes studied.

To examine the \( \pi \)-stacking potential of platinated N-heterocycle ligands an initial systematic survey was performed; Fig. 4.1 shows the structures of the synthesized complexes. Pyridine was used as the comparison point when referring to the effects of electron donating or withdrawing groups.

Fig. 4.2 shows the % quenching of tryptophan fluorescence by the various platinum compounds. The previously reported \([\text{Pt(dien)}(9\text{EtG})]^{2+}\) displayed a fluorescence quenching of
94.34%. The pyr, MeOpyr, and 4-pic and thiazole complexes gave less than 10% fluorescence quenching.

**Figure 4.2.** Percent fluorescence quenching of N-acetyltryptophan by platinum-N-heterocycle, [Pt(dien)L]^{2+}, complexes. **The percent fluorescence quenching for [Pt(dien)(DMAP)]^{2+} refers to the quenching at 10 molar equivalents. The value for all other compounds refers to the percent fluorescence quenching at 100 molar equivalents.**

Three complexes displaying high quenching – containing bztz, CNpyr and DMAP ligands – were evaluated to determine an association constant (Table 4.1). While the bztz and CNpyr compounds showed significant decrease in fluorescence intensity, the calculated K_{a}s, 3.32 and 6.73x10^3 M^{-1} respectively, are similar to that of the 9EtG complex (6.88x10^3 M^{-1} [9]). In contrast, the presence of the DMAP ligand results in a compound with a significant increase in K_{a} (25.0x10^3 M^{-1}) over that of 9EtG.

There is no clear trend in this series with respect to the simple quenching except that all ligands represent potentially delocalized systems. This trend is reinforced by the observation that the addition of a delocalized \pi system in quinoline increased the fluorescence quenching over pyridine, albeit not to as significant an extent as that of DMAP and benzothiazole, and no K_{a} was
Table 4.1. Association constants for metal-N-heterocycle complexes with N-acetyltryptophan and the C-terminal zinc finger of HIVNCp7. Published values for the 9-ethylguanine complex were taken from a — Ref. [10] and b — Ref. [13] and for \([\text{Au(dien)}(\text{DMAP})]^{3+}\) was taken from c — Ref. [19].

<table>
<thead>
<tr>
<th>Compound</th>
<th>(K_a \times 10^3 \text{ M}^{-1})</th>
<th>N-AcTrp</th>
<th>ZF</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Pt(dien)}(9\text{EtG})]^{2+})</td>
<td>6.88 ± 0.3 \text{ a}</td>
<td>7.5 \text{ b}</td>
<td></td>
</tr>
<tr>
<td>([\text{Pt(dien)(bztz)}]^{2+})</td>
<td>3.32 ± 0.36</td>
<td>3.20 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>([\text{Pt(dien)}(\text{CNpyr})]^{2+})</td>
<td>6.73 ± 0.26</td>
<td>7.72 ± 0.79</td>
<td></td>
</tr>
<tr>
<td>([\text{Pt(dien)}(\text{DMAP})]^{2+})</td>
<td>25.0 ± 0.90</td>
<td>28.1 ± 1.76</td>
<td></td>
</tr>
<tr>
<td>([\text{Pd(dien)}(\text{DMAP})]^{2+})</td>
<td>23.2 ± 0.31</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>([\text{Au(dien)}(\text{DMAP})]^{3+})</td>
<td>25.5 ± 0.30 \text{ c}</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

The \(pK_a\) for the platinated nitrogen may also give an indication of the strength of the stacking interaction between platinum complexes and Trp. The \(pK_a\)'s under similar conditions for 9EtG, CNpyr, bztz and DMAP are 1.2 [15], 1.9 [16], 3.1 [16], and 9.6 [17], respectively. Clearly the strong donor DMAP has the most profound effect, even though the platinated compound is actually slightly lower than free ligand, in contrast to the situation for free 9EtG. Basicity considerations and use of electron-donating substituents would seem important for identification of a wider group of improved N-heterocycle ligands.

The \([\text{M(dien)}] \) structure allows for systematic study of electronic and steric effects (substitution on the dien ligand) and substitution effects where the isoelectronic \(\text{M=Pt(II), Pd(II) and Au(III)}\) compounds can be studied for effects of substitution lability of the central metal ion. We have contrasted the reaction products of C-terminal HIV NCp7 with \([\text{MCl(dien)}]^{n+}\) where \(\text{M=Pt, Pd and Au [18]. It was therefore of interest to extend the DMAP findings to Pd(II) and Au(III) because of the strong stacking interaction the Pt-DMAP complex displays. In both cases, the calculated association constants confirmed the utility of the DMAP ligand, being
25.5x10^3 M^{-1} for [Au(dien)(DMAP)]^{3+} [19], and 23.2x10^3 M^{-1} for [Pd(dien)(DMAP)]^{2+} (Fig. 4.3). Both values are significantly higher than the corresponding 9-EtG complexes [10,19]. No reaction was seen between [Pd(dien)(DMAP)]^{2+} with N-AcTrp at pH* 7.52 when followed by 1H NMR over 2.5 h (data not shown). This implies improved complex stability over [Pd(dien)(9EtG)]^{2+}, which has been shown to react with N-AcTrp through displacement of the 9EtG ligand at increasing pH [11]. Interestingly, the changes in the chemical shifts for the DMAP protons are largest for Au(III) over that of the Pd(II) and Pt(II) complexes, Table 4.2. This may reflect the short central M-N bond observed in [AuCl(dien)]^{2+} and the demonstrated acidity of the dien ligand when bound to Au(III) [19].

Table 4.2. Major NMR ligand chemical shifts for [M(dien)(DMAP)]^{n+} complexes. Values in parentheses are the difference in chemical shift (ppm) from free ligand followed by the 3JH–coupling constants (Hz).

<table>
<thead>
<tr>
<th>Compound</th>
<th>δ^1H(Δδ^1H) ppm, J_{H,H}Hz</th>
<th>H2/H6</th>
<th>H3/H4</th>
<th>N-Me$_2$</th>
<th>δ^{195}Pt(ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Pt(dien)(DMAP)]^{2+}</td>
<td>6.64 (0.15), 5</td>
<td>8.02 (-0.20), 7.5</td>
<td>3.06 (0.06)</td>
<td>-2816</td>
<td></td>
</tr>
<tr>
<td>[Pd(dien)(DMAP)]^{2+}</td>
<td>6.66 (0.17), 7.5</td>
<td>7.92 (-0.31), 5</td>
<td>3.04 (0.04)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>[Au(dien)(DMAP)]^{3+}</td>
<td>6.90 (0.41), 5</td>
<td>8.02 (-0.21), 5</td>
<td>3.22 (0.22)</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>
These results were then extended to the C-terminal ZF of NCp7, which contains a single tryptophan in its sequence (Fig. 4.4). Generally, the association constants for the nucleobase complexes increase for the ZF of NCp7 when compared to N-AcTrp [9]. This trend is not followed for all N-heterocycles. The $K_a$s for $[\text{Pt(dien)}(bztz)]^{2+}$ and $[\text{Pt(dien)}(\text{CNpyr})]^{2+}$ are approximately the same for the ZF when compared to N-AcTrp and the $K_a$ for $[\text{Pt(dien)}(\text{DMAP})]^{2+}$ increases slightly. The interaction of the Au(III) and Pd(II) compounds with HIV NCp7 resulted in rapid displacement of the central Zn$^{2+}$ ion; no association constant could be measured for this interaction [Spell and Farrell, unpublished results].

![Figure 4.4](image)

**Figure 4.4.** A — structure of the HIVNCp7 zinc finger peptide and B — a model of the $\pi$-stacking interaction between $[\text{Pt(dien)(9EtG)}]^{2+}$ and N-acetyltryptophan

The results show that a limited number of N-heterocycle ligands, when metallated, also effectively engage in $\pi$-stacking interactions with tryptophan and tryptophan-containing peptides. These findings further expand the structural variation of the MN$_4$ chemotype where we have suggested that use of a substitution-inert ligand such as an N-heterocycle group instead of the substitution-labile Cl$^-$, in for example $[\text{PtN}_3X]$, where $X =$ leaving group, is an approach to selectivity for specific peptide-substitution reactions and templates for design of specific protein inhibitors [14]. The DMAP complexes all bind very strongly with Trp and the C-terminal ZF of NCp7, suggesting that they are suitable for further study with zinc finger proteins.
complementary to our studies with metallated natural nucleobases.

4.4 Experimental

Materials and reagents

The complexes [MCl(dien)]Cl (M = Pt, Pd, Au; dien = diethylenetriamine) were prepared by literature methods [20,12,21]; purity was confirmed by 1H and 195Pt NMR Spectroscopy, and Elemental Analysis (performed by QTI Laboratory, USA). All reagents were purchased from Sigma Aldrich, USA and used without further purification. The NCp7 C-terminal peptide sequence (KGCWKCGKQEHQMKDCTER) was purchased from GenScript Corporation.

Synthesis of N-heterocycle platinum complexes

Complexes of general formula [Pt(dien)L](NO\textsubscript{3})\textsubscript{2} for L = quin, MeOpyr, pyr, 4-pic, CNpyr, tz and bztz were prepared by standard procedures [10] and characterized by 1H and 195Pt NMR Spectroscopy and Elemental Analysis (performed by QTI Laboratory, USA).

\[ \text{[Pt}(\text{dien})(\text{quin})]\text{(NO}_3\text{)}\text{2} \]: [PtCl(dien)]Cl was stirred overnight in DMF with 1.98 equivalents of AgNO\textsubscript{3} in the dark. The solution was filtered through celite to remove the precipitated AgCl. Quinoline (3 eq.) was added to the filtrate and the solution was stirred overnight. The volume was reduced by rotary evaporation to near dryness, methanol and ether were added. The final product was obtained upon centrifugation and dried in vacuo (50% yield). Anal. Calcd for C\textsubscript{13}H\textsubscript{20}N\textsubscript{6}O\textsubscript{6}Pt: C, 28.32; H, 3.66; N, 15.24. Found: C, 27.76; H, 3.45; N, 14.88. \textsuperscript{1}H NMR (D\textsubscript{2}O): 3.00 (8H, m), 7.60 (1H, m), 7.90 (1H, m), 8.10 (2H, m), 8.50 (1H, m), 9.50 (2H, m). \textsuperscript{195}Pt NMR: -2837 ppm.

\[ \text{[Pt}(\text{dien})(\text{bztz})]\text{(NO}_3\text{)}\text{2} \cdot 0.75\text{H}_2\text{O} \]: [Pt(dien)(bztz)](NO\textsubscript{3})\text{2} was synthesized in a similar manner as [Pt(dien)(quin)](NO\textsubscript{3})\text{2} using benzothiazole as the planar amine ligand and
water as the solvent (29% yield). Anal. Cald for C_{11}H_{19.5}N_{6}O_{6.75}Pt: C, 23.14; H, 3.44; N, 14.72. Found: C, 23.14; H, 3.29; N, 14.26. ^1H NMR (D$_2$O): 3.00 (8H, m), 7.70 (1H, m), 7.80 (1H, m), 8.10 (1H, m), 8.80 (1H, m), 9.70 (1H, s). $^{195}$Pt NMR: -2861 ppm.

[Pt(dien)(pyr)](NO$_3$)$_2$: [Pt(dien)(pyr)](NO$_3$)$_2$ was synthesized in a similar manner as [Pt(dien)(bztz)](NO$_3$)$_2$ using pyridine as the planar amine ligand (62% yield). Anal. Cald for C$_{9.75}$H$_{19.5}$N$_6$O$_{6.75}$Pt: C, 20.99; H, 3.82; N, 16.32. Found: C, 21.28; H, 3.34; N, 15.86. ^1H NMR (D$_2$O): 3.00 (8H, m), 7.50 (2H, m), 8.00 (1H, m), 8.70 (2H, m). $^{195}$Pt NMR: -2833 ppm.

[Pt(dien)(MeOpyr)](NO$_3$)$_2$ • H$_2$O: [Pt(dien)(4-mopyr)](NO$_3$)$_2$ was synthesized in a similar manner as [Pt(dien)(bztz)](NO$_3$)$_2$ using 4-methoxypyridine as the planar amine ligand (24% yield). Anal. Cald for C$_{11}$H$_{22}$N$_6$O$_8$Pt: C, 21.86; H, 4.04; N, 15.30. Found: C, 21.31; H, 3.91; N, 15.41. ^1H NMR (D$_2$O): 3.00 (8H, m), 3.90 (3H, s), 7.10 (2H, d), 8.40 (2H, d). $^{195}$Pt NMR: -2824 ppm.

[Pt(dien)(4-pic)](NO$_3$)$_2$ • 0.75H$_2$O: [Pt(dien)(4-pic)](NO$_3$)$_2$ was synthesized in a similar manner as [Pt(dien)(bztz)](NO$_3$)$_2$ using picoline as the planar amine ligand (48% yield). Anal. Cald for C$_{10.75}$H$_{21.5}$N$_6$O$_7.5$Pt: C, 22.71; H, 4.10; N, 15.89. Found: C, 22.82; H, 3.69; N, 15.39. ^1H NMR (D$_2$O): 2.4 ppm (3H, s), 3.00 (8H, m), 7.40 (2H, d), 8.50 (2H, m). $^{195}$Pt NMR: -2835 ppm.

[Pt(dien)(tz)](NO$_3$)$_2$: [Pt(dien)(tz)](NO$_3$)$_2$ was synthesized in a similar manner as [Pt(dien)(bztz)](NO$_3$)$_2$ using thiazole as the planar amine ligand (39% yield). Anal. Cald for C$_7$H$_{16}$N$_6$O$_8$SPt: C, 16.57; H, 3.17; N, 16.56. Found: C, 16.74; H, 2.67; N, 16.07. ^1H NMR (D$_2$O): 3.00 (8H, m), 7.75 (1H, d), 7.94 (1H, d), 9.20 (1H, s). $^{195}$Pt NMR: -2820 ppm.
[Pt(dien)(CNpyr)](NO$_3$)$_2$: [Pt(dien)(CNpyr)](NO$_3$)$_2$ was synthesized in a similar manner as [Pt(dien)(bztz)](NO$_3$)$_2$ using 4-cyanopyridine as the planar amine ligand (23% yield). Anal. Calcd for C$_7$H$_{16}$N$_6$O$_8$SPt: C, 22.25; H, 3.45; N, 18.16. Found: C, 21.93; H, 3.19; N, 18.11. $^1$H NMR (D$_2$O): 3.00 (8H, m), 7.94 (1H, d), 9.00 (1H, d). $^{195}$Pt NMR: -2839 ppm.

Synthesis of [Pt(dien)(DMAP)](NO$_3$)$_2$ (DMAP=4-dimethyaminopyridine)

[PtCl(dien)]Cl was stirred overnight in water with 1.98 equivalents of AgNO$_3$ in the dark. The solution was filtered through celite to remove the precipitated AgCl. DMAP (1 eq.) was added to the reaction and the solution was stirred at room temperature overnight. The volume was reduced by rotary evaporation to near dryness. The final product was precipitated using acetone, filtered and washed with acetone and ether, and dried in vacuo (26% yield). $^1$H NMR (D$_2$O): 3.0 (8H, br, m), 3.06 (6H, s), 6.64 (2H, d), 8.02 (2H, d). $^{195}$Pt NMR: -2816 ppm.

Synthesis of [Au(dien)(DMAP)]Cl$_3$ • H$_2$O

[AuCl(dien)]Cl$_2$ was stirred with 0.98 equivalents of DMAP in water at room temperature for 1 h. The solvent was removed by rotary evaporation and acetone was added to precipitate the orange product (85% yield). Anal. Calcd. for C$_{11}$H$_{25}$AuCl$_3$N$_5$O: C, 24.17; H, 4.61; N, 12.81. Found: C, 23.96; H, 4.21; N, 12.68.

Synthesis of [Pd(dien)(DMAP)]Cl$_2$ • 1.1H$_2$O

[PdCl(dien)]Cl was stirred in water with 1 eq. of DMAP at room temperature overnight. The solvent was removed by rotary evaporation and acetone was added to the resulting solid. The solution was stirred overnight, the acetone was decanted and the solid dried in vacuo (60% yield). Anal. Calcd. for C$_{11}$H$_{25}$N$_5$O$_1$.1Cl$_2$Pt: C, 31.27; H, 6.01; N, 16.58. Found: C, 31.53; H, 6.11; N, 16.10. $^1$H NMR (D$_2$O): 3.0 (8H, br, m), 3.04 (6H, s), 6.6 (2H, d), 8.0 (2H, d).

Preparation of the zinc finger
The peptide was dissolved in deionized water at a concentration of 1 mg/mL. The pH was adjusted to 7.2 using NH4OH and zinc acetate (1.3 molar eq.) was added to the solution. For fluorescence experiments, a 5μM solution of ZF was prepared by diluting the concentrated stock solution [13].

**Nuclear magnetic resonance spectroscopy**

All samples were prepared in D2O at a concentration of ~4 mg/mL for 1H NMR and ~15 mg/mL for 195Pt NMR. For 1H NMR, the solvent peak (D2O) was referenced to 4.80 ppm. 195Pt NMR samples were measured using Na2PtCl6 as an external reference.

**Fluorescence spectroscopy**

Fluorescence studies were recorded on a Varian Cary Eclipse fluorometer with a single-cell Peltier accessory. Samples were irradiated with 280 nm light and spectra were recorded from 300 to 450 nm with a scan rate of 600 nm/min at 25 °C. The experiments were carried out in 20 mMTris buffer with 50 mM NaCl at pH 7.4. 5 μM N-acetyl tryptophan or zinc finger was titrated with [M(dien)L]n+ at molar ratios of drug from 1 to 10 for DMAP complexes and 10 to 100 for all other complexes. The absorbance for the DMAP compounds at 362 nm at 10 molar equivalents and all other compounds at 100 molar equivalents was <0.05; therefore, the inner filter effect was disregarded [22]. The emission maximum (362 nm) was measured after each titration. The K_a was determined from Eadie–Hofstee plots from an average of 3 trials using the equation: ΔF = (K_a)^-1 * ΔF / [quencher] + ΔF_c.

4.5 Conclusions

With respect to zinc proteins in general, target selectivity is a major issue for specific inhibition using small molecules. In the case of zinc fingers, the results here extend the concept
of “weak electrophiles” from organic chemistry to substitution-inert MN₄ platinum compounds in attempts to gain selectivity and distinguish between the cysteine nucleophilicity of the various coordination spheres Cys₂His₂, Cys₃His, and Cys₄ [23,24]. The notable increase in association constant of [M(dien)(DMAP)]ⁿ⁺ on the zinc finger through the “non-covalent” π–π stacking interaction allows further avenues for design of specifically targeted inhibitors and further confirms the viability of the medicinal chemistry dual approach of target recognition (non-covalent) followed by target fixation (covalent) [14].

4.6 References


Chapter 5. A new class of HIV nucleocapsid protein (NCp7)-nucleic acid antagonists.

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In preparation to be submitted to Journal of Inorganic Biochemistry

** As primary author of this paper, I have contributed all work pertaining to [Au(dien)(9\text{-EtG})\textsuperscript{3+}. All work pertaining to [Pt(dien)(9-EtG)]\textsuperscript{2+} was performed by Samantha Tsotsoros. FTICRMS studies were performed by Dr. John Mangrum at the University at Albany - SUNY in Dr. Daniele Fabris’ laboratory. EMSA and FP studies were performed by Erica Peterson at Virginia Commonwealth University.

5.1 Abstract

The zinc finger HIV nucleocapsid protein HIV NCp7 (NC) is a nucleic acid chaperone involved in both the early and late steps of the HIV-1 cycle. Development of NC-nucleic acid antagonists is an attractive strategy for AIDS treatment. We report a new class of potential NC-nucleic acid antagonists, based on the intrinsic ability of metallated nucleobases to mimic the reaction of nature. The formally substitution-inert [MN\textsubscript{3}(Nucleobase)]\textsuperscript{n+} systems are capable of
(i) NC molecular recognition through tryptophan-nucleobase stacking and (ii) subsequent peptide covalent bond formation through manipulation of the reactivity of the coordination sphere. They represent the first class of inorganic molecules capable of systematic study on the HIV zinc finger and may be the only set of compounds with intrinsic selectivity.

5.2 Introduction

Identification of novel targets and development of new agents for the management of HIV-AIDS is a global priority. The nucleocapsid protein HIV NCp7 (NC) is a small basic protein containing two zinc finger (or zinc knuckle) CysCysHisCys motifs [1,2]. This motif is highly conserved in all known retroviruses, and mutation of the zinc-chelating residues results in noninfectious viruses. NC is critically involved in both the early and late steps of the HIV-1 cycle, mainly through its ability to chaperone nucleic acids toward their most stable conformation [1,2]. The chaperone activity includes control of nucleic acid aggregation, destabilization and modulation of the kinetics of protein-nucleic acid interactions. The rearrangement of nucleic acids is essential for many viral replication processes including reverse transcription and recombination. For these reasons, development of NC-nucleic acid antagonists is an attractive strategy for AIDS treatment complementary to HAART (Highly Active Antiretroviral Therapy) [3,4]. Further, the high conservation in all HIV-1 strains raises the possibility that drugs targeted against NC may be effective in those strains resistant to anti-reverse transcription and anti-protease drugs [5].

Zinc proteins in general are important targets for inhibition and drug development [6, 7]. Zinc protein sites are generally divided according to function into catalytic, where there is usually one substitution-labile aqua ligand, and structural with four strongly-bound donors,
usually N (histidine) and S(cysteinate). Zinc finger thiolates constitute soft nucleophilic sites and a corollary of zinc thiolate alkylation in nature is that such a process – alkylation or oxidation of the liganding moieties - will inactivate the zinc finger and inhibit target (DNA/RNA) recognition. On the basis of the physical properties of structural and catalytic Zn-binding sites in proteins, it has been suggested that the target for drug design should be the metal ion (chelation) for coordination sites with \( \leq 1 \) Cys and the Zn(II) ligands (electrophilic attack) for sites with \( \geq 2 \) Cys [8]. The underlying principle is that the increase in Cys ligands renders the Zn(II) a weaker Lewis acid with concomitant weakening toward chelation. In general, cysteine modification is accompanied by conformational changes and zinc ejection. The report of the use of aromatic C-nitroso compounds to inhibit HIV-1 infectivity ushered in an intensive search for suitable drug candidates based on modification of the zinc-binding ligands [9,10]. The sparse clinical trials on zinc finger inhibitors as putative NC-interacting drugs for HIV treatment have been summarized [4]. S-acyl 2-mercaptobenzamide thioesters (SAMTs) reduce infectious virus in a transgenic mouse model [11]. In a proof-of-concept study using simian immunodeficiency virus SIV/DeltaB670 in cyanomolgus macaques SAMT-19 decreased infectious virions in peripheral blood mononuclear cells (PBMCs) [12].

Assessment of protein packing, steric accessibility and electrostatic screening of zinc finger cores has recognized the enhanced reactivity of Zn-Cys\(_3\)His coordination spheres over their Cys\(_2\)His\(_2\) counterparts [13,14]. Selectivity for the former, and the ability to distinguish between different zinc finger-coordination spheres, can be found in nucleophilic discrimination through reaction of weak “organic” electrophiles with the highly nucleophilic zinc-cysteine residues [13,14]. In coordination chemistry the equivalent of weak “inorganic” electrophiles has been suggested by us to be formally substitution-inert MN\(_4\) compounds with four strong M-N
bonds typical of the quintessential soft electrophiles Pt(II) and Au(III) [15]. Compounds containing a Pt(Au)-Cl bond are likely to be far too reactive for protein selectivity – exemplified by the often quoted statement that < 5% of the administered dose of the anticancer drug cisplatin, cis-[PtCl₂(NH₃)₂], is considered to get to its cellular target DNA.

A significant source of selectivity for HIV-1 inhibition lies in identification of nucleocapsid NC-nucleic acid antagonists [9,16,17]. Agents which interrupt the interaction with ‘natural’ substrate combined with either electrophilic attack or covalent modification may be classed as a discrete approach to zinc finger inhibition. The aromatic amino acids tryptophan (Trp) and phenylalanine (Phe) are critical for the NC-nucleic acid molecular recognition. The mutation of even one of these residues significantly decreases NC’s nucleic acid chaperone activity, and correlates with inhibition of viral replication [18]. Trp interacts with nucleobases through both H-bonding and π-stacking with the indole ring of the W37 residue inserted between adjacent C and G bases and stacked on the latter [19-22]. Metallation of nucleobases, as with protonation and alkylation, enhances π-π stacking interactions with tryptophan, in part due to lowering of the HOMO-LUMO gap [23]. Using the C-terminal finger of the HIVNCp7 (F2, residues 34-52) we have demonstrated the targeting of the critical tryptophan residue with metallated nucleobases [24,25].

We have now extended these studies to the ‘full” two-zinc finger NC and its complex with SL2 RNA (SL2). In the presence of the metallated nucleobases [M(dien)(9-EtG)]ⁿ⁺ (M=Au, n=3, I; M=Pt, n=2, II; 9-EtG = 9-ethylguanine) the NC•SL2 interaction is inhibited in an antagonist fashion and expands the chemistry of this important HIV target in hitherto unrecognized directions. Incorporation of a functionalized nucleobase within the MN₄ structure as in [M(dien)(Nucleobase)]ⁿ⁺ gives agents capable in principle of (i) molecular recognition
through the non-covalent tryptophan-nucleobase interaction and (ii) subsequent peptide covalent bond formation through manipulation of the reactivity of the coordination sphere. The contrast in substitution kinetics between the isoelectronic and isostructural Au(III) and Pt(II) compounds provides valuable insights into the antagonist mechanism, allowing for further systematic enhancement of this new biological role for platinum metal complexes. The proposed chemistry represents the first class of inorganic molecules capable of systematic study on the HIV zinc finger with the potential for intrinsic selectivity. The structures of the complexes and biomolecules are shown in Figure 5.1.

Figure 5.1. Structures of A) [M(dien)(9-EtG)]^{n+} (M= Au, I, n=3; Pt, II, n=2); B) SL2RNA (SL2) and C) NCp7 (NC).

5.3 Results and Discussion

Incubation of [Au(dien)(9-EtG)]^{3+} (I) with intact NC resulted in the detection of species produced by ejection of both Zn^{2+} ions and incorporation of up to 3 Au ions – AuF, Au_{2}F and Au_{3}F (Figure 5.2). There was no evidence for intermediate Au-ligand-peptide species, in contrast to the reaction of the C-terminal finger of NC with [Au(I)(PPh_{3})(L)]^{+} [26].
The CD spectrum of NC is characterized by a positive maximum observed at ~215 nm. The Au compound caused a decrease in intensity of this band and a significant increase in the negative ellipticity with a slight blue shift of the 195-200 nm band, which are indicative of conformational changes from ordered structure to random coil (Figure 5.3A) [27,28]. The pronounced changes are consistent with a loss of structural integrity in NC upon treatment with I.

The effects of these conformational changes on the NC-SL2 interaction were investigated by FT-ICRMS by adding SL2 to an NC sample that was pre-incubated with I for 30 min, or by adding I to preformed NC-SL2 complex. In the former case, only free unbound SL2 was observed, thus indicating that the major structural changes induced by Zn\(^{2+}\)-Au\(^{3+}\) replacement abrogated the binding capabilities of NC (Figure 5.4). In the latter case, a direct comparison of data obtained from NC-SL2 in the presence/absence of I showed a significant increase of free SL2 in solution (Figures 5.5 and 5.6A), thus suggesting that the gold compound is capable of inducing dissociation of the peptide from its cognate RNA. In addition, minor signals
corresponding to [(Au,ZnNC)•SL2] and [(Au2NC)•SL2] were detected, which are consistent with direct displacement of Zn$^{2+}$ from the intact NC•SL2 complex (Figure 6B).

An advantage of the [M(dien)(9-EtG)]$^{n+}$ system is that both isostructural and isoelectronic systems can be studied. The CD spectrum in the presence of [Pt(dien)(9-EtG)]$^{2+}$ (II) case shows that there is little disruption of the 3D structure in the presence of the Pt(II) complex, as evidenced by the maintenance of the positive ellipticity, (Figure 5.3A). Incubation of the intact NC with II results in a concentration-dependent decrease in fluorescence from which an

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**Figure 5.3.** A. CD spectra of the reaction of NC and [M(dien)(9-EtG)]$^{n+}$ at 15 minutes incubation. B. NC Fluorescence quenching upon addition of [Pt(dien)(9-EtG)]$^{2+}$ from 10-100 molar ratios.
Figure 5.4. FT-ICRMS spectrum (negative ion mode) of 1:1 [Au(dien)(9-EtG)]^{3+} / NC mixture (incubated for 30 minutes) followed by addition of SL2 RNA.

Figure 5.5. FT-ICRMS spectrum (negative ion mode) of NC:SL2 RNA complex in a 2:1 ratio. Note that under these MS conditions, no free RNA is present and the complex remains intact. These conditions were used before addition of any metallated nucleobase.
Figure 5.6. (A) FT-ICRMS spectra (negative ion mode) of 2:1 NC•SL2 complex upon addition of 1 eq. [Au(dien)(9-EtG)]^{3+} (top t=0, run immediately; bottom after 15 mins reaction). (B) Expanded region of 2:1 NC/RNA complex reacted with 1 eq. [Au(dien)(9-EtG)]^{3+} showing presence of possible RNA-Au-NC crosslinking.
association constant ($K_a$) of $2.0 \times 10^4 \text{ M}^{-1}$ was calculated, compared to $7.5 \times 10^3 \text{ M}^{-1}$ for the C-terminal finger alone, [24], Figure 5.3B.

In the case of the Pt complex, analysis under identical experimental conditions as the Au(III) case revealed only weak association between II and NC. Nevertheless, pre-incubation significantly retarded the kinetics of formation of the NC•SL2 complex. Furthermore, addition of II to preformed NC•SL2 complex induced the release of free SL2, similar to the observations afforded by the gold compound (Figure 5.7). In this case, the release of nucleic acid was also accompanied by the detection of a relatively abundant species corresponding to a $[\text{Pt(dien})(9\text{-EtG})]^2^+•\text{SL2}$ adduct. Biophysical studies on the interaction of II with Calf Thymus DNA indicated little or no reaction [15], but the tertiary structure of RNA may enhance association.

**Figure 5.7.** ESI-MS Spectrum (negative ion mode) of NC-SL2 (2:1) in presence of 2.5 eq. of $[\text{Pt(dien})(9\text{EtG})]^2^+$ showing liberation of SL2 with decrease in intensity of NC-SL2 species and observation of SL2-[Pt(dien)(9EtG)] species.
Electrophoretic mobility shift assays (EMSA) confirm the general trend of these results (Figure 5.8). Increasing concentrations of [Pt(dien)(9-EtG)]^{2+} results in a decrease in intensity of the NC•SL2 band. Concomitantly, the free SL2 band changes to an upward smear, which again may indicate interaction of the compound itself with the RNA. In contrast, there is no diminution of the NC•SL2 band in the presence of the Au compound, which could be explained by the absence of any interaction, in contradiction to the MS and CD results. An alternative explanation is the possible cross-linking of the two biomolecules by the gold compound, or metabolite or substitution product thereof. In this case, EMSA analysis may be precluded from properly resolving the cross-linked adduct by the large size of the NC•SL2 complex.

**Figure 5.8.** Effect of Metal-Nucleobase Compounds on SL2 RNA -NCp7 protein interaction. See SI for full experimental conditions.
A: Control Experiment. ^{32}P end-labeled SL2 RNA (2nM) incubated with varying concentrations of NCp7 in binding. Lane 1 contains SL2; Lanes 2-8 1µM, 500, 250, 125, 62.5, 31.3, and 15.6 nM NCp7 respectively.
B: 250nM NCp7 is Incubation of NC (250 nM) with [Pt(dien)(9-EtG)]^{2+} for 1hr followed by addition of SL2 (2 nM) in binding buffer and further incubation for 1 h. Lane 1 contains SL2 only, Lane 2 SL2 and NCp7 only; Lanes 3-9 contain NC, SL2 and 500, 250, 125, 62.5, 31.3, 15.6, and 7.8 µM of I respectively.
C: Identical to B in all respects but Lanes 3-9 contain 7.8, 15.6, 31.3, 62.5, 125, 250, and 500 µM [Au(dien)(9-EtG)]^{3+} respectively.

The cross-linking hypothesis was further investigated by fluorescence polarization experiments using labelled SL2-[Fle] (Fle = fluorescein). A control sample consisting of NC and
SL2-[Flc] afforded results consistent with formation of the NC•SL2 complex (Table 5.1). Upon incubation of NC for 1 hour with increasing concentrations of [Au(dien)(9-EtG)]^{3+} addition of SL2-RNA-[Flc] gave polarization values that were higher than those obtained from the control sample. Although it would be tempting to explain the strengthening of the RNA-protein interaction with the formation of a stable [NC-Au-SL2] species, the fluorescent anisotropy experiments cannot per se provide direct proof of its formation. Nevertheless, the minor products observed in the MS spectra (Figure 5.6B) are consistent with such a hypothesis. The gold compound does not interact with RNA itself, as indicated by the values of polarization afforded by this control (Table 5.1). In agreement, no [Au(dien)(9-EtG)]-SL2 adducts were observed from the MS analysis of Figure 5.6. The “parent” [AuCl(dien)]^{2+} also has only weak affinity for DNA. [29] These results can be rationalized by considering that upon NC interaction, the Au ligands are lost (confirmed by MS and CD studies, Figures 5.2 and 5.3A) and it is possible that coordinatively unsaturated Au-S or Au-OH(H\textsubscript{2}O) species could then bind to nucleotides. Of relevance, trans-[PtCl\textsubscript{2}(NH\textsubscript{3})\textsubscript{2}] (trans-DDP) produces cross-links between NC and HIV-1 RNA [30], presumably by taking advantage of the long-lived nature of monofunctional trans-DDP-biomolecule adducts [31].

<table>
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<th>SL2 (-) I</th>
<th>SL2 (+) I</th>
<th>SL2 + NC (-) I</th>
<th>SL2 + NC (+) 0.01 µM I</th>
<th>SL2 + NC (+) 0.08 µM I</th>
<th>SL2 + NC (+) 1.25 µM I</th>
<th>SL2 + NC (+) 10 µM I</th>
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<td>106</td>
<td>101</td>
<td>173.5</td>
<td>169</td>
<td>175</td>
<td>191.5</td>
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Table 5.1. Fluorescence Polarization (mP) of NC (100 nM) incubated with indicated concentrations of [Au(dien)(9-EtG)]^{3+} (I) followed by addition of SL2-[Flc] (10 nM). See Experimental.
5.4 Experimental

Synthesis and Biomolecule Preparation: The Pt and Au compounds were prepared as described previously [14,22]. For the MS studies the NC was prepared in the Fabris lab. Briefly, Nucleocapsid (NC) protein of HIV-1 was obtained by in vitro expression in E. coli, subsequently purified under non-denaturing conditions to preserve the coordination of Zn$^{2+}$ by its characteristic zinc-finger domains. For the gel shift and biophysical studies, the NC (identical sequence) was a generous gift of Dr. R.J. Gorelick, NIH. RNA oligonucleotides corresponding to the SL2 stemloop domain of the HIV-1 genome packaging signal (Ψ-RNA) were purchased from IDT (Coralville, IA) and desalted using ultrafiltration against 150 mM ammonium acetate.

Mass Spectrometry:

Experiments were conducted on a modified Bruker Daltonics (Billerica, MA) SolariX FTICR-MS equipped with a 12T superconducting magnet. Analyses were carried out by direct infusion using tapered quartz nanospray emitters loaded with 5-10 µL of sample with a spray voltage between 800-1100 V relative to the capillary inlet supplied by an inserted stainless steel wire. Solutions were prepared as follows:

A. 1:1 reaction of NC with [Au(dien)(9-EtG)]$^{3+}$

A solution of [Au(dien)(9-EtG)]$^{3+}$ (7.5 µM in 1 µL H$_2$O) was added to a solution of NC (7.5 µM in 1 µL H$_2$O) and volume was brought up to 10 µL with 7µL 150 µM ammonium acetate and 1 µL isopropyl alcohol. The solution was analyzed immediately in positive ion mode.

B. 1:1:1 reaction of NCp7/[Au(dien)(9-EtG)]$^{3+}$ with SL2-RNA, Figure S1.

A solution of NC (7.5 µM in 1 µL H$_2$O) and [Au(dien)(9-EtG)]$^{3+}$ (7.5 µM in 1 µL) was incubated for 30 minutes at room temperature. SL2-RNA (7.5 µM in 1 µL), 6 µL 150 mM
ammonium acetate, and 1 µL isopropyl alcohol were added and the mixture was analyzed immediately in negative ion mode.

C. Control experiment showing formation of NC/SL2 (2:1) complex, Figure S2

A solution of NC (7.5 µM in 4 µL H₂O) was added to a solution of SL2 (7.5 µM in 2 µL H₂O) and volume was brought up to 10 µL with 3 µL 150 mM ammonium acetate, and 1 µL isopropyl alcohol. The solution was analyzed immediately. No significant incubation was necessary.

D. 2:1:1 reaction of NC/RNA complex with [Au(dien)(9-EtG)]³⁺

A solution of NC (7.5 µM in 4 µL H₂O) was added to a solution of SL2 (7.5 µM in 2 µL H₂O) to form the 2:1 NC/RNA complex. The complex formed immediately; no significant incubation was necessary. A solution of [Au(dien)(9-EtG)]³⁺ (7.5 µM in 2 µL) was added and volume was brought up to 10 µL with 1 µL 150 mM ammonium acetate and 1 µL isopropyl alcohol. The mixture was analyzed in negative ion mode immediately (time = 0) and after 15 minutes incubation.

E. 2:1:2.5 reaction of NCp7/RNA complex with [Pt(dien)(9-EtG)]²⁺, Figure S4.

A solution of NC (7.5 µM in 4 µL H₂O) was added to a solution of SL2 (7.5 µM in 2 µL H₂O) to form the 2:1 NC/RNA complex. Complex formed immediately; no significant incubation was necessary. A solution of [Pt(dien)(9-EtG)]²⁺ (37.5 µM in 1 µL) was added and volume was brought up to 10 µL with 2 µL 150 mM ammonium acetate and 1 µL isopropyl alcohol. The mixture was analyzed immediately in negative ion mode.

**Circular Dichroism:**

Methods were adapted from those previously published [14,29].
**Fluorescence Spectroscopy:**

Methods were adapted from those previously published [14]. A 3 mL solution of NC (5 µM) in water was titrated with aliquots of the corresponding quenching compound (7.5 mM) in the range \([\text{quencher}/[\text{N-AcTrp}]) 10-100.

**Gel Shift Assays:**

As a control experiment, $^{32}$P end-labeled SL2 RNA (2 nM) was incubated with varying concentrations of NC in binding buffer (50 mM Tris-HCl, 40 mM MgCl$_2$, 200 mM NaCl, 0.1 mM ZnCl$_2$, 5% glycerol, and 1% BME) for 1 hr at 30°C. In all subsequent reactions, 250 nM NC was incubated with increasing concentrations of [Au(dien)(9-EtG)]$^{3+}$ or [Pt(dien)(9-EtG)]$^{2+}$ for 1 hr in binding buffer. Subsequently, 2 nM SL2 was added to the buffer and the mixture incubated for an additional hour.

**Fluorescence Polarization:**

In 250 µl total volume, increasing concentrations of NC were incubated with 10 nM fluorescein-labeled SL2 RNA for 4 hrs in minimal buffer (25 µM phosphate buffer, pH 7.2 and 225 mM NaCl) at room temperature to afford control values. In all subsequent experiments, in 250 µl total volume, 100 nM of NC was incubated in minimal buffer (25 µM phosphate buffer, pH 7.2 and 225 mM NaCl) with varying concentration of drug before addition of 10 nM SL2-RNA[Flc]. The reactions were incubated 4hrs at room temperature and analyzed on a Synergy™ NEO HTS multi-mode microplate reader system.

**5.5 Conclusions**

In summary, the results are consistent with the ability of the chemotype \([\text{M(dien)}(9-EtG)^{n+}\) to act as antagonist of the NC\(•\)SL2 interaction, with significant potential for optimization
as a structurally discrete new class of agents capable of disrupting the chaperone activity of NC, Figure 5.9. The differences in profile between the two metallated nucleobases reflect the different substitution kinetics of the central metal ions. Thus, we see immediate displacement of Zn$^{2+}$ by Au$^{3+}$ (MS, CD) whereas with the kinetically inert Pt$^{2+}$ an association constant can be measured and there is no evidence of Zn$^{2+}$ dislocation from the NC under these conditions – the stacking reaction is observed by fluorescence and CD. In this case, purely non-covalent approaches appear to allow physical blocking of the interaction. It is reasonable to expect the Au compound to react in the same way – the subsequent loss of ligands upon covalent reaction with peptide is just too fast. Model studies with N-AcTrp confirm the greater stacking of the Au(III) species relative to Pt(II) [32]. Thus, a classical “two-step’ approach of molecular recognition followed by target fixation (electrophilic attack on the zinc finger core) is suggested [24]. The greater reactivity of the Au(III) species is also reflected in the possible formation of the higher-order aggregates.

Both components of the NC-nucleic acid chaperone activity have been targeted [33-37]. A study of approximately 2,000 small molecules from the NCI Diversity Set suggested a possible fluorescein-based pharmacore with a good correlation between tryptophan quenching
and inhibition of NC-nucleic acid binding [33]. A second high-throughput screening of small molecules for inhibition of NC-mediated destabilization of the stem-loop structure of cTAR DNA (a sequence complementary to the transactivation response element) produced five selected hits from a total of 4800 compounds [34]. The inhibitory activity of 4 of the 5 correlated with their ability to compete with the nucleic acid for binding to NC [24]. Along with platinum compounds, [9,24], gold drug binding to single zinc fingers has been reported by a number of authors, [26,38-40] but this report is the first of coordination compounds reacting on a “multiple (>1)” zinc finger peptide. The chemotype has the potential for intrinsic selectivity for this important target over other zinc fingers – the formally substitution-inert species are the equivalent of ‘weak’ electrophiles proposed as one approach to selectivity [14,15].

5.6 References


Chapter 6: Synthesis and characterization of additional Au(III) compounds designed to target zinc finger proteins (unpublished data)

6.1 Introduction

The following is an account of additional, unpublished work completed on the synthesis and characterization of Au(III) compounds (Figure 6.1). This chapter will focus on synthetic procedures as well as characterization techniques, both successful and unsuccessful. Ongoing and suggested future work is also discussed.

Figure 6.1. Structures of Au(III) compounds discussed in this chapter.
6.2 Synthesis and Characterization:

The following compounds are additional [Au(dien)(N-heterocycle)]^{3+} compounds that were successfully synthesized. These compounds have not yet been published in the literature. Synthesis of [Au(dien)(9-EtG)]^{3+} and [Au(dien)(DMAP)]^{3+}, compounds 10 and 11 in Figure 6.1 respectively, is reported in Chapter 2.

[Au(dien)(2-amino-4-picoline)]Cl\textsubscript{3} (1): A solution containing 0.98 equiv. of 2-amino-4-picoline (7.8mg, 0.072mmol) in 1 mL H\textsubscript{2}O was added to a solution of [AuCl(dien)]Cl\textsubscript{2} (30.0mg, 0.074 mmol) in 3mL H\textsubscript{2}O. Reaction mixture was stirred at room temperature for approximately 1 hour. Upon addition of 2-amino-4-picoline, solution changed from yellow to orange in color. After stirring for 1 hour, solution was evaporated to dryness, resulting in an orange solid. Solid was dried in the dessicator and analyzed by NMR. \textsuperscript{1}H-NMR in D\textsubscript{2}O: δ 7.65 (d, 1H), 6.81 (s, 0.92H), 6.77 (d, 1.04H), 3.40 (t, 4.74H), 2.44 (broad s, 2.92H), 2.37 (s, 3.70H). Anal. Calcd. for C\textsubscript{10}H\textsubscript{21}AuCl\textsubscript{3}N\textsubscript{5}: C, 23.34; H, 4.11; N, 13.61%. Found: C, 23.02; H, 4.04; N, 13.37%. ε\textsubscript{200nm} = 37344 M\textsuperscript{-1} cm\textsuperscript{-1}, ε\textsubscript{230nm} = 12690 M\textsuperscript{-1} cm\textsuperscript{-1}, ε\textsubscript{294nm} = 7354 M\textsuperscript{-1} cm\textsuperscript{-1}.

[Au(dien)(4-aminoquinaldine)]Cl\textsubscript{3} \cdot H\textsubscript{2}O (2): A solution containing 0.98 equiv. of 4-aminoquinaldine (35.0mg, 0.221 mmol) in 1 mL H\textsubscript{2}O (not completely soluble) was added to a solution of [AuCl(dien)]Cl\textsubscript{2} (91.9mg, 0.226 mmol) in 2mL H\textsubscript{2}O. The reaction mixture was stirred at room temperature until all solid was dissolved, approximately 4 hours. Upon addition of 4-aminoquinaldine, solution changed from yellow to orange in color. After all solid was dissolved, solution was evaporated to dryness, resulting in an orange solid. Solid was dried in the dessicator and analyzed by NMR. \textsuperscript{1}H-NMR in D\textsubscript{2}O: δ 7.94 (d, 1.07H), 7.81 (t, 1.11H), 7.55 (m, 2.16H), 6.48 (s, 1.00H), 3.38 (t, 2.87H), 2.51 (s, 3.27H), 2.41 (broad s, 2.03H). Anal. Calcd. for C\textsubscript{14}H\textsubscript{25}AuCl\textsubscript{3}N\textsubscript{5}O: C, 23.86; H, 4.32; N, 12.02%. Found: C, 28.93; H, 3.91; N, 11.91%.
\[ \varepsilon_{191\text{nm}} = 9661 \text{ M}^{-1} \text{cm}^{-1}, \varepsilon_{198\text{nm}} = 11034 \text{ M}^{-1} \text{cm}^{-1}, \varepsilon_{214\text{nm}} = 23905 \text{ M}^{-1} \text{cm}^{-1}, \varepsilon_{235\text{nm}} = 27132 \text{ M}^{-1} \text{cm}^{-1}, \varepsilon_{319\text{nm}} = 10244 \text{ M}^{-1} \text{cm}^{-1}, \varepsilon_{329\text{nm}} = 9101 \text{ M}^{-1} \text{cm}^{-1}. \]

\[ \text{[Au(dien)(guanosine)](NO}_3\text{)}_3 \cdot \text{HCl (3): A mixture of [AuCl(dien)]Cl}_2 \text{ with 0.98 equivalents of guanosine and 2.8 equivalents of AgNO}_3 \text{ in water was stirred in the dark at room temperature for 3 days. Reaction mixture was filtered to remove precipitated AgCl, and orange filtrate was rotovapped to dryness. Acetone was added to precipitate orange product (75% yield). Anal. Calcd for C}_{14}H_{27}O_{14}N_{11}ClAu: C, 20.87; H, 3.38; N, 19.12%. Found: C, 20.96; H, 3.02; N, 18.57%. } \varepsilon_{201\text{nm}} = 54554 \text{ M}^{-1} \text{cm}^{-1}, \varepsilon_{250\text{nm}} = 15846 \text{ M}^{-1} \text{cm}^{-1}, \varepsilon_{366\text{nm}} = 884 \text{ M}^{-1} \text{cm}^{-1}. \]

\[ \text{1H-NMR in D}_2\text{O: } \delta 8.70 (s, 1H), 8.37 (s, 0.59H), 6.01 (d, 0.96H), 5.95 (d, 0.34H), 4.71 (t, 1.75H), 4.39 (t, 1.37H), 4.26 (m, 1.51H), 3.88 (m, 3.51H), 3.70 (s, 1.05H), 3.60 (t, 4.15H), 2.99 (broad s, 4.13H). \]

\[ \text{[Au(dien)(5’GMP)](NO}_3\text{)}_2 \cdot (\text{H}_2\text{O})_3 \text{ (4): A solution of [AuCl(dien)]Cl}_2 \text{ (103.0mg, 0.253mmol) was activated with 2.8 equiv. of AgNO}_3 \text{ (120.7mg, 0.711mmol) in 3mL water overnight in the dark while stirring at room temperature. The solution was filtered to remove precipitated AgCl; the resulting solution was colorless. 0.98 equivalents of 5’GMP (90.4mg, 0.249mmol) in 2mL H}_2\text{O was slowly added and the reaction mixture was left to stir at room temperature for one day. The solution turned light orange. At the end of day, a small amount of solid precipitated. The reaction mixture was filtered and the remaining solution was lyophilized. NMR showed 13% free 5’GMP present. The solid was re-dissolved in 5mL D}_2\text{O and solution was left to stir for two days, after which free 5’GMP was still present (analyzed by NMR). The reaction mixture was heated overnight at 40-45°C. The solution was evaporated to dryness and the solid was dried in the dessicator. No free ligand was present. } \text{1H-NMR in D}_2\text{O: } \delta 8.76 (s, 1H), 6.06 (d, 1.06H), 4.39 (m, 6.91H), 3.54 (t, 1.2H), 3.45 (t, 2.64H), 2.44 (broad s, 3.34H).} \]
Anal. Calcd. for C$_{14}$H$_{32}$AuN$_{10}$O$_{17}$P:  C, 20.01; H, 3.84; N, 16.67%.  Found:  C, 20.18; H, 3.5; N, 16.24%.

**This synthesis was difficult to reproduce. Though NMR did not show any impurities, elemental analysis showed C, H, and N% to be very low (could be presence of salts such as NaCl, HCl, etc). For the majority of attempts, however, there was always an excess of free 5’GMP present and it was difficult to separate the ligand from the product because both are extremely soluble in water. It is likely that the 5’GMP ligand acquired H$_2$O over time, which would alter the molecular weight.

Size exclusion chromatography was performed in an attempt to separate the product from free ligand based on size/molecular weight. In theory, the larger Au-complex should exit the column first and the smaller components such as free ligand and other impurities should move through the column slower allowing separation. The column was prepared using Sephadex G-10 medium (40-120 µ particle size) and packed with water. A 10 mL solution of the crude product (approx. 18 mM) was passed through the column and collected in fractions. All fractions were lyophilized and analyzed by NMR. The third fraction contained no free 5’GMP ligand, however the amount of solid was very small.

The Sephadex G-10 has an exclusion limit of 700 MW. The exclusion limit is defined as the molecular weight at which molecules are too large to be trapped in the stationary phase. The molecular weight of [Au(dien)(5’GMP)]$_{2}^{2+}$ is ~660 g/mol, suggesting that the Sephadex G-10 was not the optimal medium to use. In order to obtain better separation, a medium with a smaller exclusion limit should be used. Water was the only eluent used in the initial attempt. In order to provide better separation, a mixed solvent system may need to be used.
The following compounds are additional \([\text{Au(dien)(N-heterocycle)}]^3+\) compounds that were either not successful, have not yet received suitable elemental analysis, or were only performed on a small NMR scale and have yet to be scaled up and isolated.

**[Au(dien)(5’CMP)](NO\textsubscript{3})\textsubscript{3} (5):** A solution of [AuCl(dien)]Cl\textsubscript{2} was prepared in 10 mL H\textsubscript{2}O. 0.98 equivalents of 5’CMP and 2.8 equivalents of AgNO\textsubscript{3} were added and reaction mixture was stirred at room temperature in the dark for 3 days. Precipitated AgCl was removed via vacuum filtration and the remaining filtrate was rotovapped to dryness, resulting in an orange film on the glass. Acetone was added to precipitate orange solid. \(^1\text{H-NMR in D}_2\text{O}: \delta 8.14 (d, 0.50H), 8.09 (d, 1.00H), 6.28 (d, 0.86H), 6.22 (d, 0.59H), 5.98 (t, 1.08H), 4.19 (broad m, 8.47H), 3.51 (broad m, 10.88H), 2.96 (broad s, 2.30H), 2.42 (broad s, 3.77H). Anal. Calcd. for C\textsubscript{13}H\textsubscript{27}AuN\textsubscript{9}O\textsubscript{17}: C, 19.29; H, 3.36; N, 15.58%. Found: C, 16.48; H, 2.64; N, 14.44%.

**[Au(dien)(1-MetCyt)](NO\textsubscript{3})\textsubscript{3} (6a):** A solution of [AuCl(dien)]Cl\textsubscript{2} (33.6mg, 0.083mmol) was dissolved in 2mL H\textsubscript{2}O. 0.98 equivalents of 1-MetCyt (10.3mg, 0.082mmol) was dissolved in 1mL H\textsubscript{2}O and added to the [AuCl(dien)]Cl\textsubscript{2} solution. The solution changed from yellow to orange. 2.8 equivalents of AgNO\textsubscript{3} (40.0mg, 0.236mmol) was added to the reaction mixture. The reaction was left to stir at room temperature in the dark for 3 days. The mixture was filtered to remove AgCl, and the remaining light yellow solution was rotovapped to dryness, resulting in a nearly invisible film. Acetone was added and sides of flask were scratched to remove a light yellow solid. Acetone was decanted and solid was dried in dessicator. \(^1\text{H-NMR in D}_2\text{O}: \delta 7.81 (d, 0.80H), 7.70 (d, 0.75H), 6.13 (d, 1.00H), 3.61 (broad s, 7.38H), 3.44 (s, 0.62H), 3.43 (s, 0.90H), 3.11 (broad s, 3.19H). Anal. Calcd. for C\textsubscript{9}H\textsubscript{20}AuN\textsubscript{9}O\textsubscript{10}: C, 17.68; H, 3.3; N, 20.62%. Found: C, 17.37; H, 3.41; N, 13.51%. The presence of a broad peak at 3.61ppm and the fact that the final product is yellow indicates the presence of free [AuCl(dien)]Cl\textsubscript{2}. 

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Elemental analysis of the free 1-MetCyt ligand and a AgCl test both show that excess chloride is present. If the 1-MetCyt ligand is in fact a chloride salt, it is likely that the N3 site, that to with gold(III) will bind, is protonated. It was thought that adding 1 equivalent of NaOH would neutralize the ligand and deprotonate this site. The initial results are promising; the final product is orange, similar to all other AuN₄ compounds and the NMR contains a triplet in the dien region rather than a broad singlet so it is thought that there is no free dien present. Elemental analysis of this product indicates the produce is very impure, most likely due to the presence of salt impurities in the free ligand. To obtain a pure final product, the 1-methylcytosine ligand must first be purified. A new graduate student in the lab, James Beaton, is taking over the project and is currently working on ligand purification/synthesis.

[Au(dien)(1-MetCyt)]Cl₃ (6b): A solution of [AuCl(dien)]Cl₂ (107.6mg, 0.265mmol) was dissolved in 2mL H₂O. 0.98 equivalents of 1-MetCyt (32.5mg, 0.260mmol) was dissolved in 2mL H₂O. The solution of 1-MetCyt was added to the [AuCl(dien)]Cl₂ solution and the reaction mixture was left to stir overnight at room temperature. Solution immediately turned from yellow to dark orange. Overnight a small amount of black solid precipitated. Solution was filtered and rotovapped to dryness, resulting in an orange solid. Solid was dried in dessicator and analyzed by NMR. ¹H-NMR in D₂O: δ 7.78 (d, 1.00H), 6.10 (d, 1.00H), 3.79 (broad d, 1.38H), 3.57 (t, 4.63H), 3.43 (s, 2.60H), 3.41 (s, 0.40H), 3.27 (m, 1.64H), 3.05 (broad s, 4.25). Anal. Calcd. for C₉H₂₀AuN₉O₁₀: C, 20.33; H, 3.79; N, 15.81%. Found: C, 15.52; H, 3.08; N, 18.57%

It is possible that the [Au(dien)(1-MetCyt)]Cl₃ synthesis was successful with a few impurities (peaks at 3.79 and 3.27ppm), as indicated by elemental analysis. The absence of free 1-MetCyt and the presence of the sharp triplet at 3.57ppm are also indicative of a successful
reaction. This reaction should be attempted again with an extra purification step, perhaps recrystallization with water/acetone.

\[\text{[Au(dien)(quinolone)]Cl}_3 \ (7): \ 0.98 \text{ equivalents of quinolone (10.68 } \mu \text{L) was added to a solution of [AuCl(dien)]Cl}_2 \ (37.5 \text{mg in 1 mL H}_2\text{O}). \text{ The reaction was stirred at room temperature for 30 minutes. Reaction turned light orange over time. Reaction was evaporated to dryness. }^1\text{H-NMR in D}_2\text{O: } \delta \ 8.95 \text{ (d, 1.00H), 8.83 (d, 1.11H), 8.12 (m, 2.16H), 8.01 (t, 1.07H), 7.82 (m, 2.20H), 3.46 (t, 4.12H), 2.66 (broad s, 3.56H).}\]

\[\text{[Au(dien)(isoquinoline)]Cl}_3 \ (8): \text{ Reaction was performed on a small scale and followed by NMR. A solution of [AuCl(dien)]Cl}_2 \ (4.8 \text{mg in 700 } \mu \text{L D}_2\text{O}) \text{ was prepared. 0.98 equivalents (1.36 } \mu \text{L) isoquinonline was added and reaction was followed by NMR. }^1\text{H-NMR in D}_2\text{O: } \delta \ 9.57 \text{ (s, 1.00H), 8.42 (m, 2.99H), 8.17 (m, 2.15H), 7.98 (m, 1.09H), 3.48 (t, 6.52H), 2.78 (broad s, 5.12H), 2.39 (broad s, 0.59H).}\]

** The NMR spectra of the Au(III)-quinoline and iso-quinoline species suggest that the reactions were successful. It would be interesting to scale-up the reaction and isolate a product and determine the K_a with free N-acetyletryptophan. Though the pKa’s of the free ligand are are 5.0 and 5.4 respectively (both lower than DMAP), it is still expected that they would pi-stack efficiently with N-AcTrp due to the extended π-system into another 6-membered ring. This could, however, also aid in binding to DNA, therefore it would also be necessary to perform an EtBr assay.

\[\text{[Au(dien)(pyridine)](NO}_3)_3 \ (9): \ 0.98 \text{ equivalents of pyridine (5.69 } \mu \text{L) was added to a solution of [AuCl(dien)]Cl}_2 \ (29.3 \text{mg in 1 mL H}_2\text{O}). \text{ Solution immediately turned from yellow to orange. 2.8 equivalents of AgNO}_3 \ (34.1 \text{mg) was added to the reaction mixture. Reaction was stirred at room temperature in the dark overnight. Solution was filtered to remove AgCl; filtrate was}\]
yellow. Reaction mixture was evaporated to dryness and acetone was added to precipitate a sticky yellow solid. $^1$H-NMR in D$_2$O: δ 8.79 (t, 2.00H), 8.64 (t, 0.73H), 8.29 (t, 0.29H), 8.09 (t, 1.52H), 7.87 (t, 0.56H), 3.60 (broad s/t, 7.30H), 3.10 (broad s, 1.64H), 2.60 (broad s, 1.02H).

To add both steric hindrance and lipophilicity to the Au(III) compounds, Me$_4$dien (1,1,7,7-tetramethyldehtylenetriamine) was used in place of dien. In theory, this would allow for a decrease in the rate of reactivity, as well as aid in the drugs’ entrance into cells. It is still of interest to synthesize this on a larger scale and expand the series of Au(III)-N-heterocycle compounds.

[Au(Me$_4$dien)Cl]PF$_6$: This procedure was adapted from the literature [1]. One hundred µL of Me$_4$dien was added to 300 µL of ice cold ether and left to stir. HAuCl$_4$, 34.5 mg, was dissolved in 100 µL ice cold ether. While stirring on ice, the HAuCl$_4$ solution was slowly added dropwise to the Me$_4$dien solution. An orange paste immediately formed. (The paper being followed did not specify how long to leave reaction stirring on ice. It was noticed that if the reaction mixture was left to stir for a long period of time that the orange paste turned brown, therefore, reaction was left to stir for only one minute. Ether was immediately decanted. The orange paste was washed three times with 200 µL ether. Cold water, 300 µL, was added to the orange paste; solid did not completely dissolve. Extraction was performed with ten 600 µL portions of cold ether. Aqueous solution was frozen with liquid nitrogen and subsequently thawed under running water with “vigorous agitation”. NH$_4$PF$_6$, 21.1 mg, was added and mixture was re-frozen. Solid immediately formed upon addition of salt. Once thawed, the solid was isolated and dried in dessicator. The resulting solid was orange in color. $^1$H-NMR in D$_2$O: δ 3.50 (t, 3.98H), 3.21 (s, 12H), 2.92 (broad s, 4.70H ). Anal. Calcd. for C$_8$H$_{21}$AuClF$_6$N$_3$P : C, 17.94; H, 3.76; N, 7.84%. 

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Found: C, 15.41; H, 3.12; N, 7.28%. If two –Cl’s are present, the elemental analysis fits better: Anal. Calcd. for C\textsubscript{8}H\textsubscript{21}AuCl\textsubscript{3}F\textsubscript{6}N\textsubscript{3}P (%): C, 15.81; H, 3.48; N, 6.92%. Found (%): C, 15.41 (-0.40%); H, 3.12 (-0.36%); N, 7.28 (+0.36%).

2,2’,2”-terpyridine (terpy) is another well known chelating ligand used for Au(III) compounds. [AuCl(terpy)]Cl\textsubscript{2} was initially synthesized to compare to that of [AuCl(dien)]\textsuperscript{2+}, however it was decided not to continue any further due to the increased binding to DNA (see Table 7.1).

[AuCl(terpy)]Cl\textsubscript{2} • 2H\textsubscript{2}O: This compound was synthesized using a previously reported method [2]. The yield was 73%. NMR and elemental analysis results were consistent with previous reports. \textsuperscript{1}H-NMR in D\textsubscript{2}O: δ 9.28 (d, 2.00H), 8.88 (m, 1.11H), 8.72 (m, 6.47H ), 8.15 (m, 2.25H). Anal. Calcd. for AuCl\textsubscript{15}N\textsubscript{3}H\textsubscript{11}Cl\textsubscript{3}•2H\textsubscript{2}O: C, 31.46; H, 2.64; N, 7.34%. Found: C, 31.46; H, 2.35; N, 7.11%

**Crystallization was attempted for many of the compounds above using water/acetone, however crystals were never obtained. For most attempts, no solid ever precipitated and if any solid did come out of solution it was a very small amount and sticky; never crystals. As noted above, most compounds seem to have one or two waters present. These waters could be a key part to their crystal structure, which could be why it is difficult to precipitate.

Crystallization should, however, continue to be attempted. We could try other solvents such as methanol and ether. There are also other techniques we could use. We could simply dissolve the solids in minimal amount of water or any other suitable solvent and place in the fridge undisturbed. It is best to do this in an NMR tube or a type of vial with a large surface area
for the crystals to form/grow. We could also use a slow cooling technique in which we suspend
the solid in a solvent with a boiling point of 30-90 °C, such as methanol. The heat should aid in
dissolving the compound. Once the compound is fully dissolved, the solution is then cooled
slowly with the hope of precipitating crystals. Solvent diffusion is another technique in which a
solution is placed in a sample vial and a second less dense solvent is carefully dripped down the
side of the vial. Crystals form at the boundary where the solvents slowly diffuse [3].

6.3 Experimental

IR: For all IR measurements, KBr pellets were used. To prepare a KBr pellet, approximately
100 mg KBr was weighed and placed in a mortar. 2% of the sample was added and the
KBr/sample mixture was ground to a fine powder. It is important to work fast as KBr is
hygroscopic, absorbing water in the air, which can result in a high background in the IR
spectrum. Once the mixture was ground to a fine power, a pellet was formed using a pellet press,
and the pellet was quickly analyzed by both far and near IR.

Melting point experiments: DNA melting point experiments were carried out on a JASCO V-
550 UV/VIS spectrophotometer. All measurements were carried out in 10 mM NaClO₄ buffer,
pH 7.5. Calf thymus DNA was dissolved in the buffer and the DNA concentration was
determined by absorption measurements at 260nm. A titration of DNA was performed to
evaluate the effects of increasing drug concentration on the melting point of the DNA. DNA was
incubated for 1 hour with r₁ (ratio of drug to DNA) values of 0.01, 0.03, 0.05, 0.075, and 0.1, the
DNA concentration always being 100 µM. The temperature ranged from 41.5 to 95°C. Each
experiment was done in duplicate.
Fluorescence Spectroscopy:

- Basic fluorescence spectra of compounds: A 5 µM solution was prepared in water and the samples were excited at 280 nm.
- Fluorescence quenching assay: Methods were adapted from those previously published [4]. A 3 mL solution of N-AcTrp (5 µM) in water was titrated with aliquots of the corresponding quenching Au-compound (7.5 mM) in the range [quencher]/[N-AcTrp]) 1-10.

NMR Spectroscopy: $^1$H NMR spectra were obtained on a Varian Mercury-300MHz Spectrometer; chemical shifts were referenced to the residual D$_2$O peak at 4.8 ppm. 2D NMR spectra were obtained on a Bruker AVANCEIII 600MHz instrument.

- Reactions with small molecules: 0.006-0.009 mmol solutions of Au-compounds were added to a solution containing 1 equivalent of N-Acetylcysteine or N-Acetylmethionine. The reaction was followed hourly by NMR.
- pH dependency: 10-12 mM solutions of Au-compounds were prepared in D$_2$O. pH was adjusted using 1M DNO$_3$ and 1M NaOD; 0.1M solutions were used for tighter pH ranges. Readings of the pH meter for D$_2$O solutions were not corrected for deuterium isotope effects and are designated as pH* values.
- Temperature dependency: 10-12 mM solutions of Au-compounds were prepared in D$_2$O. Temperature was adjusted every ~10 °C and the $^1$H NMR spectra was recorded.
- 2D NMR: [Au(dien)(9-EtG)](NO$_3$)$_3$ was analyzed by 2D COSY NMR and [AuCl(N-Medien)]Cl$_2$ was analyzed by 2D HMBC NMR. Both samples were prepared in D$_2$O at approximately 50 mM concentration.
**Zinc Finger Preparation:** All proteins were purchased from GenScript. Each peptide was dissolved in degassed deionized water to a final concentration of 0.2 mg/mL. pH was adjusted to 7.2-7.4 using a NH₄OH solution. 1.3 equivalents of zinc acetate was added to free peptide solution to make zinc finger. For both mutant peptides, the zinc finger was incubated at 37 °C for 2 hours to ensure complete formation of zinc finger. Other peptides, Cys₃His HIV NCp7(F2) and Sp1(F3), form zinc finger immediately; no incubation was necessary.

**CD:** CD spectra were obtained using a JASCO J-600 Spectropolarimeter (Jasco Corp., Tokyo, Japan). Each spectrum was recorded in the wavelength range of 190–240 nm in a 1 mm cuvette path length at room temperature under N₂ gas. Spectra were baseline-corrected and smoothed. A CD spectrum of both free peptide and zinc finger were run before drug was added. 1.3 equivalents of drug was added to zinc finger and CD was recorded at various time points.

**PAR Assay:** To obtain a calibration curve, a 50 µM PAR solution was titrated with 0.1, 0.5, 1, and 2 molar equivalents of zinc (from 10 mM Zinc Acetate solution) and the intensity at 500 nm was measured. For the ZF/PAR/Au reaction, a solution containing 30 µM ZF (mutant Cys₃His) and 50 µM PAR was prepared. 1.3 equivalents of [Au(dien)(9-EtG)](NO₃)₃ was added and the reaction was followed by UV/VIS every 10 minutes for 1 hour.

**Mass Spectrometry with NCp7(F2) and Sp1(F3):** Experiments were performed using the same parameters and procedures outlined in Chapter 3. Experiments performed using apo-peptide, however, were run without ammonium acetate. Apo-peptide samples were diluted as follows: 20 µL concentrated sample in 200 µL methanol.

**Mass Spectrometry with full NCp7 Zinc Finger:** Experiments were performed using the same instrumentation and parameters as described in Chapter 5.

A. Reaction of NC with [Au(dien)(DMAP)]³⁺ and [Au(dien)(9-EtG)]³⁺
1:1 drug:ZF reaction: A solution of Au(III) complex (7.5 \( \mu \)M in 1 \( \mu \)L H\(_2\)O) was added to a solution of NC (7.5 \( \mu \)M in 1 \( \mu \)L H\(_2\)O) and volume was brought up to 10 \( \mu \)L with 7\( \mu \)L 150 \( \mu \)M ammonium acetate and 1 \( \mu \)L isopropyl alcohol. The solution was analyzed immediately in positive ion mode.

0.5:1 drug:ZF reaction: A solution of Au(III) complex (7.5 \( \mu \)M in 1 \( \mu \)L H\(_2\)O) was added to a solution of NC (7.5 \( \mu \)M in 2 \( \mu \)L H\(_2\)O) and volume was brought up to 10 \( \mu \)L with 6\( \mu \)L 150 \( \mu \)M ammonium acetate and 1 \( \mu \)L isopropyl alcohol. The solution was analyzed immediately in positive ion mode.

B. Competitive binding studies

SL2-RNA (7.5 \( \mu \)M in 1\( \mu \)L H\(_2\)O) and either [Au(dien)(9-EtG)]\(^{3+}\) or [Au(dien)(DMAP)]\(^{3+}\) (7.5 \( \mu \)M in 1 \( \mu \)L H\(_2\)O) were added simultaneously to a solution of NCp7 (7.5 \( \mu \)M in 2 \( \mu \)L H\(_2\)O). Volume was brought up to 10 \( \mu \)L with 5 \( \mu \)L 150 \( \mu \)M ammonium acetate and 1 \( \mu \)L isopropyl alcohol. The solution was analyzed initially and after 15 minutes incubation at room temperature in negative ion mode.

**EMSA Gel Shift Assay:** As a control experiment, \(^{32}\)P end-labeled SL2 RNA (2 nM) was incubated with varying concentrations of NCp7 in binding buffer (50 mM Tris-HCl, 40 mM MgCl\(_2\), 200 mM NaCl, 0.1 mM ZnCl\(_2\), 5% glycerol, and 1% BME) for 1 hr at 30\( ^\circ \)C. In all subsequent reactions, 250 nM NCp7 was incubated with 2 nM SL2/SL3 for 30 minutes in binding buffer, followed by the addition of increasing concentrations of Au(III)-metal complex with an addition 1 hr incubation period. Experiments performed in low zinc buffer were performed in the same manner. Low zinc buffer components are: 50 mM Tris-HCl, 40 mM MgCl\(_2\), 200 mM NaCl, 5% glycerol, and 1% BME (There is still 10 \( \mu \)M zinc in the final reaction
because the protein itself is diluted in 10 mM Tris-HCl (pH8.0), 25 mM NaCl, 5 mM MgCl$_2$, 1 mM ZnCl$_2$, 1 mM BME, and 0.01% PEG).

**MTT Assay:** Human colon carcinoma cells HCT116 and human ovarian carcinoma cells A2780 were cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS). Cells were routinely passaged at 80-90% confluency. For the MTT assay, cells were plated at a density of 5,000 cells per well in a 96-well plate and incubated overnight at 37°C in a 5 % CO$_2$ atmosphere. Cells were treated with varying drug concentrations (50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 µM) for 72 hours. After drug removal, cells were incubated with MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) (0.5 mg/ml in media) for 3 hours. The excess MTT was removed, the dye dissolved in 100 µl of DMSO, and absorbance was read on a Bio-Tek uQuant Microplate Spectrophotometer at 570nm.

**Au recovery:** [5] Due to the high cost of HAuCl$_4$ it is useful to be able to recycle the Au waste we produce in the lab and synthesize chloroauric acid ourselves. To do so, all gold waste was evaporated down to dryness. The resulting solid (51.57 g) was dissolved in aqua regia (200 mL of 3:1 HCl:HNO$_3$). Solution was heated at 70 °C for ~15 minutes followed by stirring at room temperature for ~1 hour. The solution was filtered and filtrate was reduced to ~50 mL. The filtrate was diluted with H$_2$O, and HAuCl$_4$ was extracted with butyl diglyme. The extract was washed with 1.5 M HCl three times. Oxalic acid, 5 wt%, was added and solution was heated at 70-80 °C for 4-5 hours; brown/black solid immediately formed. The solution was filtered to remove solid. Ether was added to the filtrate and the mixture was placed in the fridge to precipitate solid. The mixture was filtered and the solid was washed with 4M HCl, water, and methanol. At this point, the solid is metallic gold. This is where gold recovery stopped,
however in order to produce HAuCl$_4$ we would simply need to dissolve the metallic gold in aqua regia (see equation 1 below) and carefully evaporate the solution.

$$\text{Au} + \text{HNO}_3 + 4 \text{HCl} \rightarrow \text{HAuCl}_4 + \text{NO} + 2 \text{H}_2\text{O} \quad (1)$$

6.4 Results and Discussion:

6.4.1 Characterization of select Au(III) compounds:

6.4.1.1 IR Characterization

The main purpose for performing IR was to observe the absence of the distinct Au-Cl band upon binding of N-donors. IR was performed for [Au(dien)(9-EtG)][NO$_3$]$_3$, [Au(dien)(guanosine)][NO$_3$]$_3$, and [AuCl(dien)]Cl$_2$ (See Figure 6.1 for structures). The far IR spectra (500 – 100 nm) is shown in Figure 6.2. The strong band at 360 nm indicates the presence of a Au-Cl bond, which is not present in either the 9-EtG or guanosine compound. This is a

![Figure 6.2. Far-IR spectra of [AuCl(dien)]Cl$_2$, [Au(dien)(gua)][NO$_3$]$_3$, [Au(dien)(9-EtG)][NO$_3$]$_3$.](image.png)
useful technique, in addition to NMR, to determine the presence of free starting material, 
\([\text{AuCl(dien)}]\text{Cl}_2\).

**6.4.1.2 Structural information using 2D NMR**

The two-dimensional COSY (correlation spectroscopy) NMR spectrum of \([\text{Au(dien)(9-EtG)}]^{3+}\) shows the \(^1\text{H}\) spectra along both axes (Figure 6.3). The peaks on the diagonal correspond to the peaks in a regular 1D-\(^1\text{H}\)-NMR experiment, while the cross peaks indicate couplings between pairs of nuclei. Unfortunately, from this spectrum, we do not gain any structural information we did not already know; the protons on the ethyl group of the 9-EtG ligand couple with each other, as do those on the tridentate dien ligand.

![Figure 6.3. 2D COSY NMR spectrum of [Au(dien)(9-EtG)](NO₃)₃.](image_url)

An HMBC (heteronuclear multiple-bond correlation) 2D NMR was performed for \([\text{AuCl(N-Medien)}]\text{Cl}_2\) (Figure 6.4). This type of NMR detects correlations between different nuclei, in this case \(^1\text{H}\) and \(^{13}\text{C}\), over 2-4 bond lengths. This experiment was performed not necessarily to learn more about the structure of the compound, but to aid in identifying which
hydrogens each peak represented on the $^1$H NMR spectrum. The $^{13}$C NMR spectrum has three distinct peaks, which one would expect based on the structure. The $^1$H NMR spectrum, however, has 4 peaks, and it is unclear which N-Medien hydrogens are which peaks. Due to integration, it is clear which peak is the methyl group in both the $^{13}$C and $^1$H spectra. The cross-peaks indicate that the $^1$H NMR peaks farthest downfield represent the “B” hydrogens in the labeled structure in Figure 6.4. This multiplet integrates as 4 so all “B” protons are in a similar environment. The “A” protons, however, are split into two 2H multiplets differing by ~ 0.45 ppm. The 2D HMBC spectrum does not show any coupling to these protons, other than the corresponding $^{13}$C peak, so it is still difficult to determine which peaks are which hydrogens specifically.

Figure 6.4. 2D HMBC spectrum of [AuCl(N-Medien)]Cl$_2$. 

Figure 6.4. 2D HMBC spectrum of [AuCl(N-Medien)]Cl$_2$. 

Figure 6.4. 2D HMBC spectrum of [AuCl(N-Medien)]Cl$_2$. 

6.4.1.3 Temperature and pH dependency experiments with \([\text{Au}(\text{dien})(5\text{’CMP})]^3+\) and \([\text{Au}(\text{dien})(1\text{-MetCyt})]^3+\) compounds

Temperature dependency experiments by NMR are a useful technique in determining if rotamers are present in solution. For example, where at 20°C two peaks are present in the $^{195}\text{Pt}$ NMR spectrum for \([\text{Pt}(\text{dien})(1\text{MetCyt})]^2+\), only one broad peak is present at 80°C. This coalescence of peaks indicates rotamers are present in solution due to hindered rotation around the Pt-N3 axis [6]. Similarly, the low field $^1\text{H}$ NMR spectrum in d$_7$-DMF showed coalescence of the secondary dien-NH amine protons at 40°C. The X-ray crystal structure of \([\text{Pd}(\text{dien})(\text{cytosine})]^2+\) provides evidence for the presence of rotomers, with the cytosine ligand being perpendicular to the dien [6].

$^1\text{H}$ NMR temperature dependency studies were performed for both \([\text{Au}(\text{dien})(5\text{’CMP})]^3+\)

![Figure 6.5](image.png)

**Figure 6.5.** Temperature dependency experiment of \([\text{Au}(\text{dien})(5\text{’CMP})]^3+\). Peaks marked with * indicate those that coalesce upon increase of temperature.
and [Au(dien)(1-MetCyt)]^{3+}. Though both compounds are impure according to their elemental analysis results, both NMR spectra appeared to show no evident impurities. NMR of [Au(dien)(5‘CMP)]^{3+} showed splitting of the H6 doublets, similar to what is seen for the H8 singlet in [Au(dien)(9-EtG)](NO₃)₃. Unlike for the 9-EtG complex, upon heating the sample these peaks coalesce (Figure 6.5), indicating this is not due to pH dependency as is the case for [Au(dien)(9-EtG)]^{2+}, but rather due to the presence of rotamers in solution. Upon cooling the solution, the NMR spectrum returned to its initial state.

Similar results were expected for the [Au(dien)(1-MetCyt)]^{3+} complex due to the similarities in the cytosine structures. However, unlike the 5‘CMP complex, there was no peak coalescence upon temperature increase, rather the doublet furthest upfield decreased in intensity, until the peak was no longer present at 70°C (Figure 6.6). It is unclear as to why increasing the temperature would cause peaks to disappear, as the typical result is peak coalescence. However, the fact that the peaks did not coalesce indicates the peak splitting is likely not due to rotamers in solution, but more likely a result of pH dependency.

Figure 6.7 shows the change in NMR spectra over a pH range for [Au(dien)(1-MetCyt)]^{3+}. As the pH* is increased from 3.0 to 4.8, the H6 doublet most downfield (most likely pertaining to the protonated compound) disappears, and one doublet is present (most likely the deprotonated form). As pH* is increased to 7.4, a new doublet appears upfield, which may represent the deprotonated form of the NH₂ on the 1-methylcytosine ligand. H5 appears to show some pH dependency as well, but it is not as clear as the H6.

Lippert et al. have previously reported pKₐ studies with Pt(II)-1-MetCyt compounds. Under extremely basic conditions, the exocyclic amino group of [Pt(dien)(1-MetCyt)]^{2+} becomes deprotonated with a pKₐ of 13.5 in D₂O [7, 8]. If the upfield doublet that appears at pH*>6.5 in
fact represents the deprotonation of the NH$_2$ on the Au-complex, the pK$_a$ can roughly be estimated to be \(~7.4\). This extreme reduction in pK$_a$ values between Au and Pt is also seen for the

![Figure 6.6. NMR spectra of [Au(dien)(1-MetCyt)]$^{3+}$ at varying temperatures.](image)

![Figure 6.7. NMR spectra of [Au(dien)(1-MetCyt)]$^{3+}$ at varying pH* values.](image)
9-EtG complex, where the pKa of N1H of 9-EtG is reduced from 9.57 for Pt-dien to 8.17 for Au-dien [9]. Lippert et al. have also reported that at alkaline pH values, Pt(II)-1-MetCyt complexes can undergo cytosine deamination, forming the 1-methyluracilate complex. With the dien complex, specifically, a competing reaction also takes place where Pt(II) migrates from the N3 position to the exocyclic N4 position, thus producing syn and ani rotamers (Figure 6.8A) [7, 8]. These rotamers, however, were distinguished by significant shifts in the H5 NMR peaks, which in the case of the [Au(dien)(1-MetCyt)]^{3+} complex, the H5 peaks do not shift a lot. However, the NMR spectra in Figure 6.7 appear to be very similar to an experiment by Lippert et al. where a solution of [Pt(NH₃)₃(1-MetCyt)] was monitored at alkaline pH over time, showing the shift from the 1-MetCyt to 1-MetUracilate complex (Figure 6.8B) [7]. Due to this similarity, it would be worth while to investigate this further once a pure [Au(dien)(1-MetCyt)]^{3+} compound is synthesized.

**Figure 6.8.** (A) Scheme showing both deamination and metal migration for [Pt(dien)(1-MetCyt)]^{2+} and (B) NMR spectra of [Pt(NH₃)₃(1-MetCyt)]^{2+} at pD=12.7 taken at different time points. [7]
6.4.2 Reactivity with biomolecules

6.4.2.1 DNA Melting Point Experiments

The primary goal of this thesis is to synthesize Au-metal complexes that interact with zinc fingers, in turn inhibiting DNA binding. Because our primary target is the zinc finger itself, it is important that our compounds do not favor binding with DNA. To analyze the degree of binding to DNA, melting point studies of Calf Thymus (CT) DNA in the presence of metal-complex were performed. Table 6.1 shows the ΔTm relative to the calf thymus DNA titrations for both the activated and non-activated forms of [AuCl(dien)]^{3+} and [AuCl(terpy)]^{3+} as well as various other Au(III) compounds synthesized.

| Table 6.1. ΔTm values relative to the CT-DNA titrations for various Au(III)-complexes. |
|---|---|---|---|---|---|
| r_i | 0.01 | 0.03 | 0.05 | 0.075 | 0.1 |
| [AuCl(dien)]Cl_2 | 2.2 | 0.3 | 2 | 4.45 | 6.6 |
| [Au(NO_3)(dien)](NO_3)_2 | 1.8 | 3.8 | 2.85 | 6.35 | 6.1 |
| [AuCl(terpy)]Cl_2 | 2.8 | 4.3 | 7.9 | 10.35 | 12.6 |
| [Au(NO_3)(terpy)](NO_3)_2 | 3.7 | 5.3 | 11.2 | N/A | N/A |
| [Au(dien)(DMAP)]Cl_3 | 2.226 | 2.856 | 4.704 | 5.922 | 7.014 |
| [Au(dien)(2amino4picoline)]Cl_3 | 0.294 | 3.528 | 1.596 | 1.344 | 1.848 |
| [Au(dien)(4aminoquinaldine)]Cl_3 | 0.882 | 2.856 | 3.738 | 6.804 | 7.014 |
| [Au(dien)(5’GMP)](NO_3)_2 | -0.05 | 0.35 | 1.4 | 2.7 | 4 |
| [Au(dien)(9-EtG)](NO_3)_3 | 0.882 | 1.932 | 2.106 | 4.116 | 3.906 |
| Triplatin NC | 3.8 | 12.1 | 17.8 | --- | --- |

Though all compounds induce stabilization of DNA, some induce less than others. The compounds showing the least amount of stabilization are those where X = 2-amino-4-picoline, 5’GMP and 9-EtG. When compared to the ΔTm values of Triplatin NC, a known Pt-compound that binds strongly to DNA, all values for [Au(dien)X]^{3+} are not very significant which suggests DNA is not the primary target for these compounds and there will be little competitive binding.
between the drug and DNA. Both the activated and non-activated forms of $[\text{AuCl(terpy)}]^ {2+}$ bind strongly to DNA; $r_i$ values of 0.075 and 0.1 for the activated species did not cause any DNA denaturation. This suggests that the terpy ligand is intercalating with the DNA base pairs and binding very strongly. Because DNA seems to be a target for the Au(III)-terpy compounds, synthesis of $[\text{Au(terpy)(N-nucleobase)}]^ {3+}$ compounds was not continued.

### 6.4.2.2 Fluorescence quenching assay with N-AcTrp

Fluorescence spectroscopy can be used to monitor changes in the $\pi$-cloud of the indole ring of the N-AcTrp due to noncovalent interactions with the planar metallated ligand. Changes in the emission intensity of N-AcTrp upon complex formation with ligand can be used to calculate binding constants for the binding of the small molecules.

Fluorescence studies were performed to observe the $\pi-\pi$ stacking interactions between N-AcTrp and $[\text{Au(dien)(5`GMP)}]^ {2+}$ and $[\text{Au(dien)(guanosine)}]^ {3+}$, Table 6.2 (K$_a$ values for other Au(III) complexes reported in Chapter 2). For the 5’GMP compounds, the typical trend is observed. Both the Pt(II) and Au(III) complexes have a higher K$_a$ value than that of free ligand, and the Au(III) complex $\pi$-stacks more efficiently than the Pt(II) complex. The K$_a$ of free guanosine could not be calculated due to its insolubility in the aqueous buffer. Unlike all the other complexes studied, in the case of guanosine, the Pt(II) complex $\pi$-stacks better than the Au(III) complex. It is interesting to compare the differences in K$_a$ values between the 9-EtG, guanosine, and 5’GMP complexes. The K$_a$ values of Au(III) metal complexes are 16.7, 11.0, and $19.7 \times 10^3$ M$^{-1}$, respectively. All three ligands are very similar in structure, with guanosine adding a pentose sugar ribose, and the 5’GMP adding both the ribose sugar and a phosphate group. It is evident that the presence of a phosphate group enhances the $\pi$-stacking, whereas when only the sugar is present the stacking is decreased. A K$_a$ value for $[\text{Au(dien)(5`GMP)}]^ {2+}$
was calculated to be almost 4 times that of free N-AcTrp. This assay should be repeated, however, because only one trial was performed and the fluorescence was performed by titrating 10-100 equivalents of drug, resulting in extremely low intensity values. It is likely that these low values resulted in an inaccurate $K_a$ value and 1-10 molar equivalents should be used. Nonetheless, it is likely that the Au(III)-5’GMP compound has a significantly higher $K_a$ for the ZF when compared to that of free NAcTrp. This large enhancement in π-stacking due to the phosphate group was also seen for the Pt(II) complex when reacted with the Cys$_3$His HIV-NCp7(F2) zinc finger protein [10].

Table 6.2. Association Constants Obtained from Eadie-Hofstee Plots of N-AcTrp with Different Au(III) Quenchers

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_a \times 10^3$ M$^{-1}$</th>
<th>St. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>free 5’GMP</td>
<td>3.1</td>
<td>0.3</td>
</tr>
<tr>
<td>[Pt(dien)(5’GMP)]</td>
<td>6.9 (12.4)</td>
<td>0.2</td>
</tr>
<tr>
<td>[Au(dien)(5’GMP)]$^{2+}$</td>
<td>19.7 (74.6)</td>
<td>0.1</td>
</tr>
<tr>
<td>free guanosine</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>[Pt(dien)(guanosine)]</td>
<td>13.1</td>
<td>1.29</td>
</tr>
<tr>
<td>[Au(dien)(guanosine)]$^{3+}$</td>
<td>11.0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* Values in parenthesis are $K_a$ values calculated for the C-terminal finger of HIV-NCp7

Calculation of $K_a$ values was attempted for both the 2-amino-4-picoline and 4-aminoquinaldine compounds, however when excited at 280 nm, both of these compounds fluoresce in the same region as that of N-AcTrp, Figure 6.9. Therefore, when the Au(III) compounds were titrated into the solution of N-AcTrp, rather than solely observing a quenching effect from π-stacking, the fluorescence intensity was altered from the fluorescence of the Au compounds themselves. Various methods were attempted to correct for this increase in fluorescence from the Au compounds, such as exciting at another wavelength or trying to subtract out the fluorescence of the Au compounds so the only quenching observed would be
from the π-stacking, however none proved to be successful.

6.4.2.3 Reactivity with amino acids by NMR

The reaction between \([\text{Pd}(\text{dien})(\text{nucleobase})]^2+\) complexes and N-Acetyltryptophan is pH dependent; at pH values above 5, the nucleobase ligand is substituted by the amino acid [4]. It was of interest to see if this same trend occurred for Au(III) complexes. \([\text{Au}(\text{dien})(9\text{-EtG})]^3+\) was reacted with N-AcTrp in a 1:1 ratio; if this reaction were to take place, this would mean the \(K_a\) values calculated for \([\text{Au}(\text{dien})(9\text{-EtG})]^3+\) with free N-AcTrp would be inaccurate. A 1:1 reaction was followed over time at room temperature, resulting in no apparent reaction between the two compounds (Figure 6.10). It is important to note that the Pd(II) complex only reacted with N-AcTrp at pH values above 5. The pH of the Au(III) reaction was not recorded, and must further be investigated in order to definitively say that \([\text{Au}(\text{dien})(9\text{-EtG})]^3+\) does not react with N-AcTrp.

Figure 6.9. Fluorescence spectra of \([\text{Au}(\text{dien})(2\text{-amino-4-picoline})]\text{Cl}_3\) and \([\text{Au}(\text{dien})(4\text{-aminoquinaldine})]\text{Cl}_3\) (excitation wavelength = 280nm, emission scan = 300-450 nm).
[Au(dien)(5’GMP)]^{2+} was reacted with 1 equivalent of N-Acetyltryptophan and NMR spectra were recorded at various pH* values (pH* refers to the reading of the pH meter in deuterated solvent). pH* ranged from approximately 3.7 (the initial pH*) up to 12.64 (Figure 6.11). At initial pH* 4.76 there was no reaction between the Au(III)5’GMP complex and the N-AcTrp. At pH* 5.99, however, there are new peaks seen slightly upfield from those representing bound 5’GMP, suggesting that the 5’GMP ligand may begin to dissociate at pH*>6. As the pH* increases to 8.23 these upfield peaks become more prominent, and at pH* 12.64, the 5’GMP ligand is completely free; no Au-bound 5’GMP is present. It does not seem, however, that the N-Acetyltryptophan is replacing the 5’GMP as is the case for Pd-nucleobase compounds, as there is no clear shift in the N-AcTrp peaks on the NMR spectra.

It is possible that this trend is a property of the [Au(dien)GMP]^{2+} complex, and not in fact due to the reaction with N-Acetyltryptophan, therefore a pH dependency experiment was performed with only [Au(dien)(GMP)]^{2+} (Figure 6.12). Similar peaks for the aromatic region of

**Figure 6.10.** NMR spectra of a 1:1 reaction of [Au(dien)(9-EtG)]^{3+} and N-AcTrp over time.
[Au(dien)](GMP)]^{2+} \text{ were seen as those observed in the reaction with N-AcTrp (Figure 6.11). Most likely, the shift in peaks that is seen when the Au-complex is reacted with N-Acetyltryptophan is not evidence of the nucleobase being substituted by the amino acid but}
rather due to the pH dependency of the compound itself. Similar pH dependency was later seen for $[\text{Au(dien)}(9\text{-EtG})]^3^+ \text{ and } [\text{Au(dien)}(\text{DMAP})]^3^+$ compounds, where at low pH* values the center nitrogen on the dien ligand was protonated, and as the pH* was increased, it became deprotonated [9]. Like the 9-EtG, the 5’GMP ligand appears to dissociate at high pH* values.

A)

![NMR spectra of 1:1 reaction of $[\text{Au(dien)}(5’\text{GMP})]^2^+$ with N-AcMet (A) and N-AcCys (B) over time. Only the aromatic region is shown in (B) showing the 5’GMP ligand dissociates over time.]

**Figure 6.13.** NMR spectra of 1:1 reaction of $[\text{Au(dien)}(5’\text{GMP})]^2^+$ with N-AcMet (A) and N-AcCys (B) over time. Only the aromatic region is shown in (B) showing the 5’GMP ligand dissociates over time.
The reaction of \([\text{Au(dien})(5'\text{GMP})]^2^+\) with N-AcMet and N-AcCys was also followed over time by NMR (Figure 6.13). It is important to know how the Au(III) compounds interact with the amino acids on a small scale to help gauge how the compound will interact with the intact zinc finger protein. As seen with other AuN₄ compounds in Chapter 3, \([\text{Au(dien)}(5'\text{GMP})]^2^+\) does not react readily with N-AcMet. Due to the ‘soft’ nature of the Au(III) metal center, it is more likely that the Au(III) complex would interact with the sulfur containing amino acids due to sulfur being a ‘soft’ base, however the presence of a methyl group on the sulfur atom in N-AcMet slows down the reaction tremendously when compared to that of N-AcCys. A small peak corresponding to free 5’GMP was present with \([\text{Au(dien)}(5'\text{GMP})]^2^+\), however this is likely an impurity of the compound itself; the S-methyl peak of N-AcMet does not shift over time indicating no reaction. Upon immediate mixing of \([\text{Au(dien)}(5'\text{GMP})]^2^+\) with N-AcCys, however, a large peak at 7.9 ppm corresponding to free 5’GMP was present and the solution was cloudy. A white solid formed over time, and at 4 hours only free 5’GMP was present. Though all AuN₄ compounds react readily with N-AcCys, \([\text{Au(dien)}(5'\text{GMP})]^2^+\) is the only compound that results in loss of ligand over time (refer to Chapter 3 for studies with \([\text{Au(dien)}(\text{DMAP/9-EtG})]^3^+\) compounds and N-AcCys).

\([\text{Au(N-Medien)(DMAP)}]\text{Cl}_3\) was reacted with 2.5 equivalents of N-AcMet, Figure 6.14. Unlike the reactions highlighted in Chapter 3 for \([\text{Au(dien)}(\text{N-heterocycle compounds})]^3^+\) and N-AcMet where no reaction is observed, \([\text{Au(N-Medien)(DMAP)}]\text{Cl}_3\) appears to react slightly with N-AcMet, indicated by an upfield shift in the S-CH₃ peak. This peak shifts slightly from 2.13 to 2.24 ppm with a second S-CH₃ peak appearing at 2.54, as seen previously with the AuN₃Cl species (Chapter 3). The S-CH₂ multiplet also shifts slightly upfield indicating sulfur binding to gold. Peaks corresponding to the DMAP ligand shift upon reaction with N-AcMet, however
there is no indication of free DMAP. Similarly, the N-Medien peaks shift slightly upfield, however there is no indication of free N-Medien ligand. An upfield shift in the N-Me peak suggests sulfur binding resulting in more electron density on the gold metal center and therefore a shielding effect on the N-Me group. With both the DMAP and N-Medien ligands remaining bound, this suggests a ring opening mechanism similar to what has been previously reported in the literature [11-12], where the NH$_2$-Au bond breaks and the nucleophilic sulfur binds, Figure

**Figure 6.14.** NMR spectra of [Au(N-Medien)(DMAP)]Cl$_3$ + NAcMet over time. N-Medien region shown in (A) and aromatic region shown in (B).
In Chapter 2 we report that the presence of the DMAP ligand stabilizes the N-Medien ligand and prevents ring opening. However, it may be possible that the further decrease in pH (pH ~3.0 after addition of N-AcMet) as well as the presence of the nucleophilic sulfur atom induces this ring-opening mechanism.

![Figure 6.15. Suggested structure of product from reaction of [Au(N-Medien)(DMAP)]^{3+} and N-AcMet.](image)

It is clear the methyl group on the center nitrogen of the dien ligand affects the reactivity of the complex. Previous CV studies show a less negative peak potential for [Au(N-Medien)(DMAP)]Cl$_3$ when compared to the non-methylated analogue, indicating the Au(III) metal center is less stable. This decrease in stability may be cause for a greater reactivity and susceptibility to reduction by the nucleophilic sulfur.

### 6.4.3 Reactivity with Cys$_3$His and Cys$_2$His$_2$ mutant zinc finger proteins

Cys$_2$His$_2$ and Cys$_3$His mutant peptides containing the same sequence of the C-terminal NCp7 zinc finger with a methionine deleted from the sequence (compare Figure 3.1 and Figure 6.16) were studied. It was originally thought that eliminating the methionine would eliminate any competitive metal binding on the backbone.
6.4.3.1 CD experiments

CD experiments were performed to determine the change in structure, if any, of the zinc finger protein upon reaction with the Au(III) complexes. As indicated in Figure 6.17, the Au(III) complexes react extremely fast with the Cys$_3$His mutant peptides, all resulting in immediate zinc ejection and complete loss of ordered structure. It is thought that this may be due to the fact that the coordination sphere is constricted and therefore more susceptible to loss of zinc. As expected, the Cys$_2$His$_2$ mutant zinc finger reacts slower than that of the Cys$_3$His due to the fewer number of reactive cysteine residues. As seen for the non-mutated NCp7(F2) and Sp1(F3) peptides, data shown in Chapter 3, the 9-EtG species appears to react slightly slower than the chloride species and, as a result, cause less zinc ejection. Due to the extremely fast reactivity of the Cys$_3$His mutant zinc finger, further studies with this peptide were not performed.

6.4.3.2 Fluorescence experiments

As seen for the non-mutated peptides, a $K_a$ value was not able to be calculated for the Au(III) complexes due to the rapid Zn ejection; a large amount of quenching is observed for all Au(III) compounds, including the –Cl species, indicating the decrease in fluorescence is likely to result from a change in peptide conformation upon zinc ejection. Fluorescence time studies, Figure 6.18, clearly show the difference in reactivity between the Cys$_3$His and Cys$_2$His$_2$ peptides.

Figure 6.16. Model mutant peptide; X = (His)$_2$; (His)(Cys).
For the Cys$_3$His mutant, all compounds appear to react in a similar manner and on a similar time scale (Figure 6.18A). All compounds react fast, with an immediate sharp drop in fluorescence from 0 to 6 minutes, after which the fluorescence intensity no longer decreases. For the Cys$_2$His$_2$, there is an initial decrease in fluorescence, the DMAP species decreasing the most (Figure 6.18B). This is likely due to the fact that the DMAP species has a higher $K_a$ than [Au(dien)(9-EtG)]$^{3+}$, therefore this initial decrease in fluorescence is due both to initial recognition through $\pi$-stacking, followed by immediate zinc ejection. After this initial decrease in fluorescence

**Figure 6.17.** CD Spectra of Au(III) compounds reacted with Cys$_3$His (A) and Cys$_2$His$_2$ (B) mutant zinc finger peptides. Reactions are 1:1 and spectra were recorded at time 0.
intensity, the signal remains constant for each compound, perhaps suggesting no more zinc is being ejected and/or the tryptophan residue in the Cys$\_2$His$\_2$ mutant peptide does not change significantly in conformation when zinc is ejected.

6.4.3.3 PAR Zn ejection Assay with Cys$\_3$His mutant peptide

PAR assays (PAR = 4-(2-pyridylazo)resorcinol) are often performed to quantitatively measure the amount of free zinc present in a solution. Free PAR has a maximum absorption at
~410nm; when bound to zinc, forming the complex Zn(PAR)_2, the maximum absorption is at ~500 nm. This technique is often used to monitor the amount of zinc ejection over a period of time. This method, however, was not successful in these experiments. As a control, increasing concentrations of Zn acetate were titrated into a 50 µM solution of PAR. Though a suitable calibration curve was observed, the absorbance intensity decreased over time, suggesting the Zn(PAR)_2 complex was not stable in solution. When a solution containing both ZF and PAR was analyzed by UV/VIS, a large peak at 500 nm was present, suggesting that the PAR chelates zinc from the zinc finger (this was also seen for TSQ – a fluorescence method used to monitor zinc ejection – by another member of the group). [Au(dien)(9-EtG)](NO_3)_3 (1.3 equivalents) was added to the ZF/PAR solution and the reaction was monitored over time. The peak at 500 nm (that indicating Zn bound to PAR) decreased over time. If zinc is being ejected from the zinc finger, as indicated by CD experiments, this peak should increase over time. Due to these results and the fact that the PAR appears to chelate the Zn from this particular peptide sequence, this technique was not further optimized.

6.4.4 ESI-MS studies with NCp7(F2) and Sp1(F3) zinc finger and apo-peptides

The ESI-MS studies reported in Chapter 3 were also performed for [Au(N-Medien)(DMAP)]^{3+} with the Cys_His NCp7 C-terminal zinc finger, Figure 6.19. Unlike what was seen for both [Au(dien)(9-EtG)]^{3+} and [Au(dien)(DMAP)]^{3+}, intact Au(III)-complex was not seen for the reaction of NCp7(F2) and [Au(N-Medien)(DMAP)]^{3+}. Compared to its non-methylated analogue, [Au(N-Medien)(DMAP)]^{3+} reacts more rapidly, resulting in primarily apo-species (740.9975 m/z, 3^+) as well as Au/F (806.3173 m/z, 3^+) and Au_2/F (872.3093 m/z, 3^+); no peaks pertaining to intact NCp7(F2) were present. This increase of reactivity may be due to the
decrease in stability of the Au(III) metal center as indicated from CV experiments (Chapter 2) for the N-methyldien compounds.

ESI-MS studies were also performed with apo-peptide (both Cys3His and Cys2His2) and [Au(dien)(DMAP)]^{3+}. For the reaction with apo-NCp7(F2), intact [Au(dien)(DMAP)]^{3+} was not observed, however a large peak pertaining to [AuCl(dien)]^{2+} was present. The primary species present was apo-peptide (741.3292 m/z, 3^+) at "time 0" with no formation of Au/F, Figure 6.20A. Similar to that seen for the reaction with the NCp7(F2) zinc finger, the 24 hour spectra was difficult to interpret, with multiple unidentifiable peaks present (Figure 6.20B). There is a large peak present at 605.1428 m/z (4^+) indicating the presence of Au/F. This peak was not present at the 24 hour time point for the reaction with NCp7(F2) zinc finger.

**Figure 6.19.** ESI-MS spectrum of [Au(N-Medien)(DMAP)]^{3+} + NCp7(F2) at initial time point.
Figure 6.20. ESI-MS spectra of NCp7(F2) apo-peptide + [Au(dien)(DMAP)]$^{3+}$ at initial (A) and 24 hr (B) time points.

Figure 6.21. ESI-MS spectra of Sp1(F3) apo-peptide + [Au(dien)(DMAP)]$^{3+}$ at initial (A) and 24 hr (B) time points.
ESI-MS studies with apo-Sp1(F3) and [Au(dien)(DMAP)]^{3+} were similar to that performed with zinc finger. Initial time points showed the main species to be apo-peptide, with no formation of Au/F, Figure 6.21A. Unlike the zinc finger reaction, however, no intact Au(III) compound was present; only [AuCl(dien)]^{2+} was visible. The rate of reactivity with apo-peptide was somewhat slower than that of the zinc finger, with less Au/F present after 24 hours. Figure 6.21B shows the primary species to still be apo-peptide.

6.4.5 Reactivity with the full HIV-NCp7 Zinc Finger Protein

Chapter 5 discusses the reactivity of [Au(dien)(9-EtG)]^{3+} with the full NCp7 zinc finger and SL2-RNA. This section continues those studies and expands to that of [Au(dien)(DMAP)]Cl_{3}.

6.4.5.1 FT-ICRMS Experiments

In collaboration with Drs. Daniele Fabris and John Mangrum from SUNY-Albany, FT-ICRMS experiments were performed with the full NCp7 zinc finger protein, SL2-RNA, and Au(III) complexes including [Au(dien)(DMAP)]^{3+} and [Au(dien)(9-EtG)]^{3+}.

In addition to those experiments already described (Chapter 5), [Au(dien)(9-EtG)]^{3+} and SL2-RNA were added simultaneously to a solution of NCp7 in a 1:1:2 ratio, respectively, to mimic competitive binding conditions. At time 0 (MS was run immediately after mixing reagents), the NCp7/RNA complex appeared to be the only species present (Figure 6.22). After incubating for 15 minutes, a new peak pertaining to Au_{2}F was present. In agreement with experiments in Chapter 5 of this dissertation, this suggests that over time, [Au(dien)(9-EtG)]^{3+} can disrupt RNA/NCp7 complex formation.

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A 1:1 and 1:0.5 reaction of NCp7 and [Au(dien)(DMAP)]$^{3+}$ was performed to determine if the DMAP ligand decreased the rate of reactivity when compared to the 9-EtG. The $K_a$ value for [Au(dien)(DMAP)]$^{3+}$ with free N-AcTrp is 25.5 M$^{-1}$ compared to a lower $K_a$ of 16.7 for the 9-EtG complex, therefore it is expected that the Au(III)-DMAP complex would be more likely to π-stack with the Trp residue in the C-terminal finger of NCp7, therefore slowing down the covalent binding and resulting Zn ejection/Au replacement. Many species are present in the 1:1

![Figure 6.22. FT-ICRMS spectra (negative ion mode) of competitive binding study. [Au(dien)(9-EtG)]$^{3+}$ and SL2-RNA were added simultaneously to a solution of NCp7 and the reaction was monitored over time. Top spectrum was recorded immediately and bottom spectrum was recorded after 15 minutes incubation.](image-url)
Figure 6.23. FT-ICRMS spectrum of 1:1 reaction of $[\text{Au(dien})(\text{DMAP})]^3^+$ and NCp7.

Figure 6.24. FT-ICRMS spectra of 0.5:1 reaction of $[\text{Au(dien})(\text{DMAP})]^3^+$ (A) and $[\text{Au(dien)}(9-\text{EtG})]^3^+$ (B) with NCp7.
reaction, including AuF, Au$_2$F, and Au$_3$F which we also see for the Au(III)-9-EtG complex. However, we do see the presence of intact NCp7 (two Zn ions present) as well as NCp7 with 1 Zn and 1 Au atom bound (Figure 6.23). The presence of these species supports the theory that the DMAP ligand would slow down the reaction with NCp7. The 1:0.5 reaction of [Au(dien)(DMAP)]$^{3+}$ : NCp7 shows only the presence of intact ZF (Figure 6.24A). In the presence of the 9-EtG complex, however, peaks representing NCp7 with 1Zn and 1Au bound as well as Au$_2$F and Au$_3$F are present (Figure 6.24B), again showing the DMAP ligand slows down the reaction with the NCp7 zinc finger protein.

6.4.5.2 EMSA Gel Assays

In Chapter 5, EMSA gels (performed by Erica Peterson) were used to determine whether the Au(III)/Pt(II) drugs were inhibiting the formation of the NCp7/RNA complex. In the case of [Au(dien)(9-EtG)]$^{3+}$ the gel showed no complex inhibition. This was contrary to the MS results, which showed free RNA after addition of drug to pre-formed NCp7/RNA complex. The following studies expand on those previously discussed.

In order to mimic the MS experiment more closely, NCp7 was incubated with SL2-RNA for 30 minutes followed by the addition of Au(III)-drug. As shown in Figure 6.25A/B, no inhibition was observed. NCp7 also binds strongly to SL3, with a $K_d = 28 \pm 3$ nM [13]. An EMSA gel experiment was performed with [Au(dien)(DMAP)]$^{3+}$ and SL3, Figure 6.25C, however like that of SL2, no inhibition was observed.

It is possible that the binding buffer for NCp7 and SL2 contains something that is interfering with the reaction, therefore similar experiments were performed in buffer with low concentration of ZnCl$_2$, Figure 6.26. Inhibition of the NCp7/SL2 complex was observed in the presence of [Au(dien)(DMAP)]$^{3+}$ and [Au(dien)(9-EtG)]$^{3+}$, with IC$_{50}$ values of 2 mM and
Figure 6.25. Effect of \([\text{Au(dien)}(\text{N-heterocycle})]^{3+}\) on SL2 RNA-NCp7 protein interaction, \([\text{Au(dien)}(\text{9-ETG})]^{3+}\) (A) and \([\text{Au(dien)}(\text{DMAP})]^{3+}\) (B). NCp7 was incubated with NC for 30 minutes followed by addition of drug for 1 h. Lane 1 contains SL2 only, Lane 2 SL2 and NCp7 only; Lanes 3-9 contain NC, SL2 and 500, 250, 125, 62.5, 31.3, 15.6, and 7.8 µM of I respectively. (C) Similar study as shown in (B), with SL2 instead of SL2.

Figure 6.26. Effect of low-zinc containing buffer on EMSA gel assay. (A) NCp7/RNA + increasing concentrations of \([\text{Au(dien)}(\text{DMAP})]^{3+}\). (B) NCp7/RNA + increasing concentrations of \([\text{Au(dien)}(\text{9-ETG})]^{3+}\); red box indicates presence of high aggregate species in the sample well. IC\(_{50}\) graphs are shown for both drugs.
456 µM, respectively. In the case of \([\text{Au(dien)(9-EtG)})^{3+}\) a large band shifts to the top of the sample well, indicating formation of higher aggregate species. It was determined that this species was due to a SL2-drug species, as this band was the same intensity with or without NCp7, Figure 6.27.

![Figure 6.27](image)

**Figure 6.27.** High aggregate species is due to drug:SL2 complex. Lane 1: SL2, Lane 2: NCp7/SL2, Lane 3: NC/1 mM \([\text{Au(dien)(9-EtG)})^{3+}\) + SL2, Lane 4: 1 mM \([\text{Au(dien)(9-EtG)})^{3+}\) + SL2.

It is difficult to directly compare the results of the FTICR-MS experiments to those of the EMSA gels; the parameters for each experiment are quite different from one another. The MS experiments were performed in water and we have shown that the buffers used for the EMSA gels may contain elements that interfere with the reaction. The relative concentrations used are also quite different. For MS studies, the NCp7/RNA complex was formed in a 2:1 ratio, whereas for the EMSA gel the final concentration of NCp7 was 100 nM compared to 2 nM final concentration of SL2-RNA. This large excess of NCp7 could be a key factor towards preventing complex inhibition by the Au-complexes.
6.4.6 MTT Cytotoxicity Assay

An MTT assay was used to determine the IC$_{50}$ values of the Au(III) metal compounds in both HCT116 (colorectal carcinoma) and A2780 (ovarian cancer) cell lines, Table 6.3. Trials indicated with a * are those performed with fresh drug solution prepared immediately before the assay was performed. All other trials were performed with a drug solution that had been stored in the freezer over a period of time. When a drug solution that has been frozen over time is used, the IC$_{50}$ values are much lower than when a fresh solution is used. It is possible that these compounds are not stable over time, or perhaps the difference in reactivity is related to their pH dependency [9].

| Table 6.3. IC$_{50}$ values for Au(III) compounds in HCT116 and A2780 cell lines. |
|---------------------------------|----------|----------|----------|----------|----------|----------|
|                                | HCT116   | A2780    |
| Compounds Name                 | Trial 1* | Trial 2  | Trial 3  | Trial 4* | Trial 1  | Trial 2*  |
| [AuCl(dien)]$_2^{2+}$           | >50      | 20.6     | 10.6     | >50      | 4.65     | 19.8      |
| [Au(dien)(DMAP)]$_3^{3+}$       | >50      | 11.2     | 10.3     | >50      | 3.65     | 17.3      |
| [Au(dien)(2am4pic)]$_3^{3+}$    | >50      | 22.1     | 12.6     | >50      | 5        | 21.4      |

Recently the cytotoxicity experiments were repeated for [AuCl(dien)]Cl$_2$ and [Au(dien)(DMAP)Cl$_3$ and the IC$_{50}$ values for [Au(dien)(9-EtG)](NO$_3$)$_3$, [AuCl(N-Medien)]Cl$_2$ and [Au(N-Medien)(DMAP)]Cl$_3$ were also calculated, Table 6.4. Higher concentrations of drug were used in order to calculate a more exact IC$_{50}$ value. In these experiments, a fresh solution was used for each trial. All values are higher than those in Table 6.3. The reported IC$_{50}$ value for [AuCl(dien)]Cl$_2$ in the literature is $8.2 \pm 0.93$ µM in A2780/S cell lines [14]. The closest values we get to those in the literature are from assays performed with drug solutions that have been frozen (A2780 Trial 1, Table 6.3). It is unclear as to why we are getting much higher values with
fresh solutions than reported in the literature. Further experiments need to be performed to determine the stability of these compounds in solution over time as well as the effect of temperature on their stability.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>HCT116</th>
<th>A2780</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
<td>[AuCl(dien)]Cl₂</td>
<td>186</td>
<td>154</td>
</tr>
<tr>
<td>[AuCl(N-Medien)]Cl₂</td>
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<td>160</td>
</tr>
<tr>
<td>[Au(dien)(DMAP)]Cl₃</td>
<td>160</td>
<td>185</td>
</tr>
<tr>
<td>[Au(dien)(9-EtG)][NO₃]₃</td>
<td>163</td>
<td>180</td>
</tr>
<tr>
<td>[Au(N-Medien)(DMAP)]Cl₃</td>
<td>112</td>
<td>---</td>
</tr>
</tbody>
</table>

6.4.7 HIV testing

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<th>Compound</th>
<th>Virus</th>
<th>IC₉₀ µM*</th>
<th>IC₅₀ µM*</th>
<th>TC₅₀ µM†</th>
<th>Therapeutic Index ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Au(dien)(5’GMP)]²⁺</td>
<td>Ba-L a</td>
<td>&gt;100</td>
<td>54.1</td>
<td>&gt;100</td>
<td>&gt;1.85</td>
</tr>
<tr>
<td></td>
<td>NL4-3 b</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>91US001 c</td>
<td>91.8</td>
<td>50.8</td>
<td>&gt;100</td>
<td>&gt;1.97</td>
</tr>
<tr>
<td>Azidothymidine</td>
<td>Ba-L a</td>
<td>909 nM</td>
<td>11.2 nM</td>
<td>&gt;1,000nM</td>
<td>&gt;89.1</td>
</tr>
<tr>
<td></td>
<td>NL4-3 b</td>
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<td>29.3 nM</td>
<td>&gt;1,000nM</td>
<td>&gt;34.2</td>
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<tr>
<td></td>
<td>91US001 c</td>
<td>37.1 nM</td>
<td>2.55 nM</td>
<td>&gt;1,000nM</td>
<td>&gt;392</td>
</tr>
</tbody>
</table>

a CCR5-tropic, Group M Subtype B, lab-adapted isolate
b CXCR4-tropic, Group M Subtype B, molecular clone
c CCR5-tropic, Group M Subtype B, clinical isolate
*IC₅₀ and IC₉₀ = 50% and 90% inhibition of virus replication, respectively
†TC₅₀ = 50% cytotoxicity
‡Therapeutic Index = TC/IC

[Au(dien)(5’GMP)]²⁺ was sent to the Southern Research Institute for HIV testing. [Au(dien)(5’GMP)]²⁺ inhibited both HIV-1 Ba-L and HIV-1 91US001 with IC₅₀ values in the 50
µM range; but it also appeared to significantly enhance the replication of HIV-1 NL4-3 in the 10-32 µM range, Table 6.5 and Figure 6.28.

**Figure 6.28.** HIV-testing results from the Southern Research Institute for [Au(dien)(5′GMP)]^{2+} with three HIV isolates.
The following compounds have been sent out for similar testing: [AuCl(dien)]^{2+}, [Au(dien)(9-EtG)]^{3+}, [Au(dien)(DMAP)]^{3+}, and [Au(N-Medien)(DMAP)]^{3+}. We are currently awaiting the results.

6.5 Conclusions

Multiple Au(III)-dien-N-heterocycle compounds have been synthesized in addition to those published [9]. Successful compounds were characterized by NMR, UV/VIS, and elemental analysis. Preliminary studies showed that the 5’GMP and 1-MetCyt compounds were pH dependent as previously seen for the 9-EtG and DMAP analogs [9]. Further studies will need to be performed once a pure [Au(dien)(1-MetCyt)]^{3+} compound is synthesized, however preliminary studies show possibility of rotamers in solution as previously seen for the Pt(II)-1-MetCyt compound [6]. Further stability studies should be performed for all Au(III)-dien-N-heterocycle compounds synthesized; cytotoxicity studies showed varying results for fresh vs. frozen solutions. Fresh solutions gave IC_{50} values above 100 µM, however when solutions that had been frozen for a period of days/weeks were used, IC_{50} values were reduced to approximately 10-20 µM.

DNA proved not to be a primary target for all of the Au-dien compounds studied, however the Au-terpy compounds appeared to bind strongly and thus further study of these compounds was not continued. All Au(III) compounds studied π-stack effectively with N-AcTrp. As seen previously, Au(III) compounds appear to π-stack more efficiently than the isostructural/isoelectronic Pt(II) analogs, except in the case of [Au(dien)(guanosine)]^{3+} which has a slightly lower K_a value than [Pt(dien)(guanosine)]^{2+}. The AuN_4 coordination sphere slowed
down reactivity with NAcMet when compared to that of AuN₃Cl, however the compounds are still highly reactive with N-AcCys.

Studies with mutant zinc finger peptides showed that all Au(III) compound studied were highly reactive with both the Cys₃His and Cys₂His₂ zinc fingers, resulting in immediate zinc ejection. It is possible that removing the methionine from the sequence altered the zinc coordination sphere in such a way that caused the peptide to be more reactive, however it was evident that the Cys₂His₂ was slower to react, which is expected due to the fewer number of reactive sulfur containing cysteine residues.

Studies with the full intact NCp7 zinc finger showed [Au(dien)(DMAP)]³⁺ to be less reactive than the 9-EtG compound, with intact zinc finger and Zn/Au-finger species be present. EMSA gel assays investigating the possibility of NCp7/RNA complex inhibition were performed. It is likely the binding buffer used has some effect on the reaction taking place, as removing the ZnCl₂ allowed for an IC₅₀ value of 2mM and 456 μM to be calculated for [Au(dien)(DMAP)]³⁺ and [Au(dien)(9-EtG)]³⁺, respectively. The micromolar IC₅₀ value of the Au(III)-9-EtG complex is within the range of other reported NCp7/RNA antagonists in the literature [15]. It is possible that this HIV nucleocapsid-nucleic acid antagonism may result in a loss of viral activity. Antiviral studies at the Southern Research Institute are currently underway.

6.6 References


Chapter 7. General Conclusions and Future Directions

Zinc finger proteins exhibit a variety of functions, including transcription, DNA repair, and apoptosis [1]. Displacement of the central zinc ion, along with mutation of coordinated amino acids can result in a loss of biological function. There has been an increasing interest to synthesize complexes that selectively target zinc finger proteins, in turn inhibiting DNA/ZF interactions and therefore resulting in loss of protein function, Figure 7.1. Au(I) and Au(III) compounds including auranofin and multinuclear compounds have been designed to target zinc finger proteins such as the Cys$_2$His$_2$ containing Sp1 transcription factor and the Cys$_3$His containing PARP-1 zinc finger proteins. In all cases, zinc is immediately ejected and replaced by either Au(I) or Au(III) ions [2-6]. Au(I) and Au(III) have a high affinity for protein thiols due to their ‘soft’ nature. This can significantly reduce the cellular activity and selectivity of these drugs, as they may easily react with other sulfur containing proteins such as human serum albumin (HSA).

Figure 7.1. Graphical representation of ZF/DNA antagonism.
The HIV nucleocapsid zinc finger, NCp7, is of particular interest in this work. NCp7 is involved in multiple steps of the HIV life cycle, thus making it a desirable drug target. Recognition of NCp7 with DNA/RNA occurs via \( \pi \)-stacking interactions between guanine in the DNA/RNA and the tryptophan in the C-terminal zinc finger of the NCp7 protein, F2. Mutation of this tryptophan residue significantly reduces the nucleic acid chaperone activity of NCp7, which correlates to inhibition of viral replication [7].

Electrophilic attack by \([\text{MCl(dien)}]^n+\) (M = Pt(II), Pd(II), Au(III); dien = diethylenetriamine) on NCp7(F2) results in conformational changes and ejection of Zn. For the Au analog, as indicated by electrospray ionization mass spectrometry (ESI-MS) experiments, all ligands were displaced and ‘goldfingers’ were formed [8]. To slow down this reaction and make the drugs more selective, the Cl\(^-\) was replaced with a more substitution-inert nitrogen donor. Platinated nucleobases such as \([\text{Pt(dien)(9-EtG)}]^2+\) (9-EtG = 9-ethylguanine) were shown to \( \pi \)-stack effectively in a non-covalent manner with tryptophan of the C-terminal finger of HIV Nucleocapsid, NCp7(F2) [9]. In this work, this concept was expanded to Au(III) complexes as Au(III) is isoelectronic and isostructural with Pt(II).

Novel Au\(^{\text{III}}\)(dien)(N-heterocycle) compounds were synthesized and characterized. Previously reported studies show \([\text{AuCl(dien)}]\text{Cl}_2\) to exhibit pH dependency with the central nitrogen on the tridentate dien ligand deprotonated at a \( pK_a \) of 4.0 [10]. The acidity of the dien ligand is affected by the nature of the fourth ligand as a leaving group; \([\text{Au(dien)(9-EtG)}]^3+\) reduces the \( pK_a \) to 3.3 and \([\text{Au(dien)(DMAP)}]^3+\) (DMAP = 4-dimethylaminopyridine) increases the \( pK_a \) to 4.7, with no evidence of hydrolysis at basic pH. The metal center of \([\text{Au(dien)(N-heterocycle)}]^3+\) compounds was shown to be more stable to reduction than when Cl\(^-\) is present, with consequences for reactivity with biomolecules: specifically, significant enhancement of \( \pi-\pi \)
stacking interactions with tryptophan was observed relative to isostructural and isoelectronic platinum(II) and palladium-(II) compounds.

NMR studies show the presence of a more inert N-donor as the leaving group slows down the reaction with the sulfur-containing amino acid N-acetylmethionine (N-AcMet). All Au(III) compounds react readily with N-acetylcysteine (N-AcCys), however lack of N-heterocycle ligand dissociation indicates, to our knowledge, the first long-lived N-heterocycle-Au-S species in solution.

Of the Au(III) compounds studied, [Au(dien)(DMAP)]^{3+} appears to be the least reactive, with ESI-MS studies showing presence of intact NCp7(F2) ZF at initial reaction times. Reactivity of the Cys₃His NCp7 zinc finger was compared to the Cys₂His₂ Sp1(F3) zinc finger. CD experiments showed a slower rate of reactivity for Sp1(F3) which is to be expected due to the fewer number of nucleophilic cysteines. Interestingly, ESI-MS studies show no presence of intact zinc finger for experiments performed with Sp1(F3). This suggests the mode of action of these compounds is dependent on the nature of the zinc binding core. It is also important to note that NCp7 contains a tryptophan amino acid and the Sp1(F3) does not, indicating the possible importance of this key residue in slowing down the reactivity of these Au(III) compounds.

Mass spectrometry studies were expanded to that of the full HIV NCp7 zinc finger. [Au(dien)(9-EtG)]^{3+} reacts quickly with NCp7, resulting in immediate zinc ejection and replacement with up to three gold ions. Similar to studies with the C-terminal finger, [Au(dien)(DMAP)]^{3+} appears to react slower indicated by the presence of intact NCp7. Addition of [Au(dien)(9-EtG)]^{3+} to preformed NC-SL2 complex results in release of free RNA; based on EMSA (electrophoretic mobility shift assay) studies, [Au(dien)(9-EtG)]^{3+} disrupts the NCp7-RNA complex with an IC₅₀ of ~450 uM.
Though [Au(dien)(DMAP)]\(^{3+}\) was shown to be more stable and less reactive than the parent [AuCl(dien)]\(^{2+}\) species, it is still too reactive to be considered a selective compound to any degree. However, through the extensive characterization of the [Au(dien)(N-heterocycle)]\(^{3+}\) compounds, we now have a better understanding of the chemistry and can work towards synthesizing more stable compounds. A compound of particular interest, as discussed in Chapter 6, is [Au(dien)(1-MetCyt)]\(^{3+}\) (1-MetCyt = 1-methylcytosine). Previous studies on the Pt(II) analogue show an increased K\(_a\) value with tryptophan compared to that of 9-EtG [11]. It is possible that the hindered rotation around the M-N\(_3\) axis could reduce the rate of reactivity. Once a pure product is achieved, the solution chemistry of the Au(III)-1-MetCyt complex will need to be investigated further due to likely pH dependency and possible metal migration and deamination [12,13]. Using accumulated Pt(II) data as a guide, other planar nucleobase ligands such as xanthosine may also be investigated due to their high π-stacking ability.

Due to the severe pH dependency of the center nitrogen on the dien ligand when bound to Au(III), we are investigating the use of other possible chelating ligands. Expanding from the Au(III)(N-Medien) species, methyl groups can be added to the primary amines, ultimately having a pentamethylated dien species. The presence of methyl groups may aid in eliminating the pH sensitivity of the Au(III) compounds. The \(^1\)H NMR spectrum of the [Pt(Me\(_4\)dien)(9-EtG)]\(^{2+}\) compound (recent unpublished work by Samantha Tsotsoros) shows two H(8) signals indicating restricted rotation around the Pt-purine bond; this is also confirmed by molecular modeling. The shorter Au(III) bond should enhance this effect.

Another chelate ligand of interest is based on an early Au(III) compound, [AuCl\(_2\)(dampa)] (dampa = 2\(\prime\)[(dimethylamino)methyl]phenyl), Figure 7.2, made as a cisplatin analog incorporating an anionic phenyl donor in a 5-membered chelate ring [14,15]. The
unpublished bis(substituted)

compound, Figure 7.2, is an interesting C-based analog of dien. The chemistry of [AuCl₂(dampa)] causes slow substitution of Cl- by N-donor ligands; pyridine only displaces the Au-Cl bond upon activation [16]. Au-NH₃ compounds have been reported [17], therefore we will likely prepare [AuCl(NH₃)(dampa)]⁺ for incorporation of the N-heterocycle ligand, the first N-donor ligands being 9-EtG and DMAP for comparison with the completed work. This slow substitution rate will ideally translate to a slower rate of reactivity.

References


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APPENDIX
Appendix A.

Metallated DNA-Protein Crosslinks as Probes for Zinc Finger Conformation and Reactivity – A Review

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*Both authors contributed equally

Department of Chemistry, Virginia Commonwealth University, Richmond, VA, USA

Encyclopedia of Metalloproteins: Springer-Verlag Berlin Heidelberg, 2013

Abstract:

This entry describes the interactions of zinc finger peptides with metallated DNA and small molecule platinum-metal probes. The interactions have biological consequences from sequence-specific inhibition of zinc finger function to hijacking of transcription factors by binding to platinated DNA. Small molecule interactions have chemical consequences because the formal analogy between alkylation and metallation suggests the possibility of electrophilic attack of the cysteine and histidine residues with eventual zinc ion ejection and loss of tertiary structure and consequently biological function. These approaches create new opportunities to investigate the chemical properties of zinc fingers and may possibly produce new leads for disease intervention where zinc finger function is implicated.
Introduction:

In biology, zinc serves either a structural or catalytic purpose. When zinc acts in a catalytic manner, it is bound to a water molecule that is converted to a hydroxyl ligand. Structural zinc is characterized by coordination to four amino acids, generally histidine and cysteine. These types of zinc sites include zinc fingers (ZF), which compose 2-3% of the human genome [1]. The most common role of zinc fingers in biology is in the binding of DNA to transcription factors and they are also involved in RNA packaging, transcriptional activation, regulation of apoptosis and protein folding and assembly.

Zinc fingers are defined by the number and type of amino acids coordinated to the central zinc atom (Figure A1). Analysis of Cys$_2$His$_2$ zinc finger proteins has allowed for elucidation of DNA recognition mechanisms and their role in transcriptional regulation. The Cys$_3$His motif is involved in RNA and DNA recognition, cell-cycle control and cellular signaling pathways. The third common zinc Cys$_4$ finger motif is found in human DNA repair enzymes and many nuclear localized systems [2].

Figure A1: The three main coordination environments of zinc fingers: Cys$_2$His$_2$, Cys$_3$His and Cys$_4$.

Coordination to zinc enhances the nucleophilicity of the Zn-Cys bond, allowing for attack by small electrophiles. The chemical reactivity is dependent on the nature of the zinc coordination sphere (Cys$_2$His$_2$; Cys$_3$His, Cys$_4$) [2]. The interaction of zinc finger proteins with
DNA and RNA can be inhibited upon chemical modification of the zinc binding ligands. There is a formal analogy between alkylation and metallation, where in the latter case chemical modification refers to electrophilic attack of the cysteine and histidine residues by Pt and Co agents respectively (Figure A2).

**Figure A2:** General schematic of electrophilic agent attack on a zinc finger. Platinum agents attack at cysteine residues, while cobalt agents attack at histidine residues. $X=(\text{Cys})(\text{His})$ or $\text{Cys}_2$.

In both cases, however, eventual loss of zinc is seen and the proteins lose tertiary structure and therefore loss of biological function. Tethering of the electrophilic agent to a sequence-specific DNA may allow for targeting and differentiation between different zinc fingers and enhancement of selectivity. In this review, the approaches to the use of small molecule metal-based electrophiles and formation of DNA-protein crosslinks as probes for zinc finger conformation and reactivity will be discussed. These approaches create new opportunities to investigate the chemical properties of zinc fingers and may possibly produce new leads for disease intervention where zinc finger function is implicated.

**Targeting of Zinc Fingers by Cobalt Schiff Base Complexes**

Cobalt(III) Schiff-base (Co-sb) complexes may disrupt the structure of a zinc finger peptide by axial ligation of the Co(III) ion to the nitrogen of the imidazole ring of a histidine
residue [3]. The reaction of Co(III)-sb with histidine is a dissociative ligand exchange process, with loss of the labile axial NH$_3$ ligands by H$_2$O facilitating histidine binding. Following this recognition, the axial NH$_3$ ligand is displaced by water. The reaction of Co(III)-sb at the zinc finger site irreversibly inhibits protein activity as a result of zinc ejection due to the binding of cobalt to histidine [4]. Zinc ejection causes loss of tertiary structure and therefore loss of function.

Initial studies showed that cobalt complexes inhibit binding of the human transcription factor Sp1, to its consensus sequence. DNA-coupled conjugates of the cobalt complexes selectively inhibited Sp1 in the presence of several other transcription factors. The Snail family of Cys$_2$His$_2$ zinc finger transcription factors, Slug, Snail, and Sip1, are emerging as anti-cancer drug targets as they are implicated in tumor metastasis through the regulation of epithelial-to-mesenchymal (EMT) transitions. Slug, Snail, and Sip1 bind to the Ebox consensus sequence CAGGTG in the promoter region of target genes with high specificity to mediate transcriptional repression. To improve specificity for the Snail family of transcription factors, an

Figure A3: Scheme for zinc finger-specific inactivation using Co(III)-sb-DNA conjugates. Co(III)-Ebox is recognized by the Snail family of transcription factors. Loss of axial ligands and subsequent binding of histidine results in eventual ejection of Zn$^{2+}$ and transcription factor inactivation.
oligonucleotide containing the DNA consensus sequence was conjugated to the Co(III) complex yielding Co(III)-Ebox (Figure A3) [4].

No significant changes in the structure of DNA occurred upon conjugation. Through addition of the Ebox DNA sequence to the Co(III) metal complex, peptide-binding specificity was improved 150-fold over Co(III)-sb. Co(III)-Ebox was found to effectively inhibit the Slug, Snail and Sip1 zinc finger transcription factors from binding to their DNA targets with eventual loss of Zn$^{2+}$ [4]. Studies demonstrate that neither the oligo, or the Co(III) Schiff base complex alone, are sufficient for transcription factor inactivation at concentrations where the conjugated complex mediates inhibition.

Subsequently, a Co(III) Schiff base DNA-conjugate has been designed to target the Gli C$_2$H$_2$ transcription factors in the Hedgehog (Hh) pathway, which has been implicated in the formation and development of certain cancers such as medulloblastomas and basal cell carcinomas [5]. In order to test the specificity and efficacy of Co(III)-DNA complex, the authors tested the Ci consensus sequence alone, Co(III)-sb, Co(III)-Ci, and Co(III)-Mut (one base pair mutation). The complete structure of Co(III)-Ci was found to be the most effective at inhibiting Gli family ZF transcription factors. The mutation of one base pair in Co(III)-CiMut inhibited its specific and potent activity. The activity of Co(III)-Ci was specific, as no other zinc finger transcription factor tested was inhibited by the addition of the complex [5]. These results show that subtle changes in the oligomer attached to Co(III) can modulate the specificity and activity of these Co(III)-DNA conjugates both in vitro and in vivo.

The studies performed using Co(III)-DNA complexes demonstrate the development of a versatile class of specific and potent complexes that may be used to study zinc finger proteins and may prove valuable as an experimental tool and as anti-cancer therapeutics.
Pt Complexes as DNA/Protein Crosslinkers

The structure and nature of DNA adducts of platinum complexes may be manipulated to facilitate ternary DNA-Pt-protein crosslinks. Table A1 summarizes the compounds studied. One of the first examples is the use of trans-diaminedichloroplatinum(II) (TDDP). In order to map the section of HIV1 RNA that was recognized by the nucleocapsid NC protein, TDDP was used as a crosslinking agent. After digestion of a crosslinked solution of HIV1 RNA and NC protein, the crosslink was shown to form between positions 315-324 of the RNA. This suggests the possible recognition site for the NC protein on HIV1 RNA [6].

Various mononuclear and dinuclear platinum complexes have been analyzed for their crosslinking abilities to zinc finger containing proteins such as the DNA repair proteins, UvrA and UvrB, and Sp1. The repair of the DNA adducts of the anticancer agent cis-diaminedichloroplatinum(II) (cisplatin, CDDP) was examined with the bacterial UvrABC complex [7]. A sterically hindered analog, exo-[N-2-methylamino-2,2,1-bicyclohepane]dichloroplatinum(II) (BCH) was compared for its protein crosslinking abilities. UvrAB proteins recognized the mono and diadducts of BCH with a higher affinity than those of cisplatin. Analysis of the crosslinks showed the involvement of UvrB in the ternary nucleo-protein complexes. In general, UvrB has a greater affinity for the adducts formed by BCH over those formed by cisplatin [7].

Dinuclear platinum complexes bind to DNA in a manner that is unique from cisplatin and other mononuclear complexes, the main adducts being long-range (Pt,Pt) interstrand crosslinks [8]. Dinuclear complexes may be formally bi, tri or tetrafunctional (depending on number of substitution-labile chlorides present – see Table A1). Even in the presence of a CDDP-like cis
Table A1. Various metal compounds form crosslinks with zinc finger proteins and DNA

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>DNA Studied</th>
<th>Zn Finger Studied</th>
<th>Crosslink</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDP</td>
<td>![CDDP Structure]</td>
<td>Plasmid pSP65</td>
<td>UvrAB</td>
<td>DNA monoadduct crosslink formation of UvrB</td>
<td>[6]</td>
</tr>
<tr>
<td>TDDP</td>
<td>![TDDP Structure]</td>
<td>$^{32}$P HIV-1 RNA (311-415)</td>
<td>HIV1 NC</td>
<td>Crosslink formation between RNA (between positions 315-324) and HIV1 NC</td>
<td>[5]</td>
</tr>
<tr>
<td>(Pt-Pt)-2,2/c,c</td>
<td>![Pt-Pt-2,2/c,c Structure]</td>
<td>49 bp duplex</td>
<td>UvrA</td>
<td>Crosslink formation between platinated DNA and UvrB</td>
<td>[8]</td>
</tr>
<tr>
<td>(Pt-Pt)-1,1/t,t</td>
<td>![Pt-Pt-1,1/t,t Structure]</td>
<td>49 bp duplex</td>
<td>UvrA</td>
<td>Crosslink formation between platinated DNA and UvrB</td>
<td>[7,8]</td>
</tr>
<tr>
<td>(Pt-Pt)-1,2/t,c</td>
<td>![Pt-Pt-1,2/t,c Structure]</td>
<td>Plasmids pSP73KB and pUC19</td>
<td>Sp1</td>
<td>Crosslink formation between platinated DNA and Sp1</td>
<td>[7]</td>
</tr>
<tr>
<td>(Pt-Pt)-1,2/c,c</td>
<td>![Pt-Pt-1,2/c,c Structure]</td>
<td>Plasmids pSP73KB and pUC19</td>
<td>Sp1</td>
<td>Crosslink formation between platinated DNA and Sp1</td>
<td>[7]</td>
</tr>
<tr>
<td>(Pt-Ru)</td>
<td>![Pt-Ru Structure]</td>
<td>49 bp duplex</td>
<td>UvrA</td>
<td>Crosslink formation between platinated DNA and UvrB</td>
<td>[8]</td>
</tr>
</tbody>
</table>
-[PtCl$_2$(amine)$_2$] coordination sphere the (Pt,Pt)-interstrand crosslinks form preferentially (Figure A4).

Thus, upon bifunctional binding to DNA, the third Cl$^-$ site can then bind to a protein, preferentially at Cys, His, or Met residues [8]. Platinum complexes are known to bind in the major groove of DNA and the ability of the dinuclear complexes to crosslink UvrA and UvrB suggests that the proteins contact the major groove of the DNA helix within 4-4.5Å [9]. The flexibility of the dinuclear platinum adducts may be important in their crosslinking to Sp1 due to the DNA bending induced upon Sp1 binding [8].

The results of these platinum crosslinking studies suggest that the formation of metal-mediated DNA-protein complexes may play a role in the cytotoxic properties of these compounds due to the irreversible crosslinking, and therefore sequestering, of repair proteins or transcription factors to DNA. The differences between mononuclear and dinuclear platinum complexes may also explain the different cytotoxic properties of the two classes of anticancer drugs. The differences in DNA adduct structure between mononuclear and dinuclear complexes may also be used to probe protein recognition of structurally different DNA crosslinks [8].
Interaction of Platinum Molecules with Zinc Fingers:

The demonstration of zinc finger crosslink formation by platinated DNA is fundamentally an example of coordination compounds acting as electrophiles towards the peptide cysteine residues. Small molecule platinum-metal agents have therefore been explored for their ability to target the HIV nucleocapsid retroviral protein NCp7. Figure A5 shows the structures of the metal complexes studied as well as that of NCp7 nucleocapsid protein. Again, functionality can be studied by use of structurally distinct compounds such as CDDP, trans-[PtCl(9-EtGua)(pyr)$_2$]$^+$, [PtCl(terpy)]$^+$ and [PtCl(dien)]$^+$ [10]. Further, the reactivity of the central metal ion may be altered by use of the general structure [MCl(chelate)] (M = Pt(II), Pd(II), or Au(III) and chelate = dien or terpy). Interestingly, both Pt(II) and Pd(II)-dien species showed evidence of adduct formation on ZF2 by replacement of the M-Cl bond with zinc-bound thiolate. Eventual loss of the dien ligand, as well as zinc ejection, was observed. Due to the strong thiol affinity of gold drugs, both Au(III) compounds reacted extremely fast, producing

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**Figure A5.** Structures of HIV NCp7 nucleocapsid protein (ZF; C-terminal finger, ZF2, shown in dashed box) and the platinum-metal complexes studied for molecular recognition and electrophilic attack.
only “gold fingers”. For all terpy compounds, metal exchange occurred, producing the product [Zn(terpy)]^{2+}. These results show that by modifying both the metal and ligand of the reacting compound, the chemical reactivity of zinc finger proteins can be altered [2].

A major challenge for all small molecule electrophiles is selectivity. To enhance the selectivity of these drugs, it is important to understand the details of the zinc finger – DNA interaction. The recognition of DNA or RNA by the HIV nucleocapsid protein NCp7 is dominated primarily by π-stacking between the purine nucleic acid bases and the planar aromatic amino acid residues, especially between guanine and tryptophan [2]. Fluorescence quenching studies have shown that metallation of nucleobase compounds significantly enhances the π - π stacking interactions with L-tryptophan. Metallation modifies frontier orbital properties, lowering the π - acceptor LUMO of the metallated nucleobase, thus improving the overlap towards the π - donor HOMO of the tryptophan. Study of the biologically relevant C-terminal peptide (ZF2) of the HIV-NCp7 zinc knuckle showed that [Pt(dien)(9EtGua)]^{2+} and [Pt(dien)(5′GMP)] quench tryptophan fluorescence with $K_a$ association constants of $7.5 \times 10^3$ and $12.4 \times 10^3$ M$^{-1}$ respectively. Though there is little difference between their binding with the simple amino acid tryptophan, the extra phosphate group on the 5′GMP causes a significant increase in binding with ZF2 [11]. ESI-MS of both [Pt(dien)9EtGua]$^{2+}$ and [Pt(dien)(5′GMP)] with ZF2 showed formation of a 1:1 adduct between the peptide and Pt-complex. It is most likely that this adduct formation is facilitated by the π - π stacking interactions between the tryptophan and the platinated nucleobase. Incubation of cis-[Pt(NH$_3$)$_2$(Guo)$_2$]$^{2+}$ with ZF2 showed significantly less formation of the 1:1 adduct, possibly due to steric hinderance from the mutually cis-oriented purines [11].
The monofunctional \textit{trans}-[PtCl(9-EtGua)(pyr)\textsubscript{2}]\textsuperscript{+} has high reactivity towards sulfur over nitrogen, and it has also been shown to cause Zn ejection in model chelates (see below). In the presence of a 1:1 stoichiometric ratio of \textit{trans}-[PtCl(9-EtGua)(pyr)\textsubscript{2}]\textsuperscript{+}, the ESI-MS of ZF2 showed numerous peaks consistent with multiple adduct formation. The peak corresponding to the adduct ([ZF2(Pt[pyr])\textsubscript{2}-Zn] shows two [Pt(pyr)]\textsubscript{2} units bound to the protein with resultant loss of Zn. CD experiments revealed changes in the three-dimensional structure of the protein, consistent with the loss of tertiary structure due to Zn ejection. Therefore, the proposed mechanism for attack of Pt-nucleobase compounds on zinc fingers consists of two steps: 1) non-covalent recognition through \(\pi-\pi\) stacking of tryptophan with platinated nucleobase and 2) covalent interaction with Pt-S bond formation followed by zinc ejection [11].

\textbf{Platinated DNA Affects Zinc Finger Conformation:}

To expand on the studies of platinum-nucleobase complex - zinc finger interactions, the interaction of Pt(dien)(6-mer) (6-mer = d(5'-TACGCC-3')) with the C-terminal finger of the HIV NCp7 zinc finger, ZF2, was studied by NMR Spectroscopy. In this case the Pt(dien) moiety was bound to the single guanine residue (Figure A6-a) [10]. The solution structures of ZF2, the 6-mer/ZF2, and Pt(dien)6mer/ZF2 adducts were calculated from NOESY-derived distance constraints. For the 6-mer/ZF2 structure, stacking with tryptophan was observed as well as showed that there was an interaction between Trp37 and the ribose protons of the carbohydrate moiety of Gua4, confirming the carbohydrate-aromatic ring interaction as a key recognition site between NCp7 protein and oligonucleotides [10]. The NMR spectrum of ZF2 in the presence of Pt(dien)6-mer showed weakening of the Trp37-Gua4 contact attributed by the steric effects caused by platination of Gua4. The aromatic ring of the tryptophan residue changes orientation (Figure A6-b), causing the DNA to be in a completely different position than when platinum is
Molecular dynamics calculations of the DNA-protein interactions showed that the π-stacking between Trp37 and Gua4 as well as the hydrogen bonding between the pentose and phosphate oxygen(s) of Cyt5 and Gua4 both help stabilize the interactions between the 6-mer and ZF2. Once platinum is bound, the 6-mer is less flexible and stays in one stable conformation on the surface of the zinc finger. The Gua4–Trp37 interaction is disrupted, resulting in hydrogen bonding interactions between Cyt6 and Cys49 and Thr50. Additionally, the backbone CH group is close to N3 of Cyt6. With the addition of this third hydrogen bond, the bonding mode imitates the three intermolecular Cyt–Gua base pair hydrogen bonds (Figure A6-c) [10].

In conclusion, these results show for the first time structural characterization of platinated single-stranded DNA interacting with a zinc finger protein, resulting in conformational change of the peptide. These results further demonstrate the feasibility of using DNA-tethered coordination compounds to target specific zinc finger proteins [10].
Small Molecule Models for Platinated DNA-Zinc Finger Interactions:

To provide a small molecule model for the reactions of platinum complexes with ZFs, the chelate N,N’-bis(2-mercaptoethyl)-1,4-diazacycloheptanezinc(II), [Zn(bme-dach)]₂, was studied. Structures for model metal/DNA/protein crosslinks and zinc ejection were deduced from ESI-MS spectra of this [Zn(bme-dach)]₂ with trans-[PtCl(9-EtGua)(pyr)₂]⁺, a model for a monofunctional adduct of platinum on DNA. The schematic of the reaction is shown in Figure A7. The intermediate species [Zn(bme-dach)-Pt(9-EtGua)(pyr)₂]⁺ was observed and after 20 hours was identified as the major species present. This is considered to be the first heterodinuclear Pt,Zn monothiolate bridged species to be identified using biologically relevant species. MS experiments showed the final product to be [Pt(bme-dach)]⁺, giving proof of Zn ejection followed by platinum replacement [12]. Similar experiments were performed for [Pt(dien)Cl]Cl and [Pt(terpy)Cl]Cl. Results showed formation of mono- and dithiolate bridged intermediates as well as metal exchange and multimetallic aggregate species [2].

![Figure A7. Reaction pathway of [Zn(bme-dach)]₂ with trans-[PtCl(9-EtGua)(pyr)₂]⁺ with formation of monothiolate bridged and metal exchanged species [13].](image-url)
represent suitable models for the molecular structure of ternary DNA–protein complexes involving zinc finger proteins discussed above [12].

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• Spell, Sarah R.; Tsotsoros, Samantha D.; Mangrum, John B.; Peterson, Erica J.; Fabris, Daniele; Farrell, Nicholas P. A new class of HIV nucleocapsid protein (NCp7)-nucleic acid antagonists. To be submitted to Journal of Inorganic Biochemistry.

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