THE VERSATILITY OF PALLADIUM SYSTEMS:

FROM PALLADACYCLES TO CYCLIC PEPTIDES AND BEYOND

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By

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ABSTRACT

This work started with the aim to synthesize monomeric ring-opened analogues of the palladacycles [(κ^2-iminoisoidoline)PdCl(PCy_3)] incorporating iminoisoindoline and phosphine ligands, and evaluate their catalytic activities in C-C coupling reactions compared to their monomeric palladacyclic precursors. This project further evolved to investigate cyclic peptides derived from flaxseed oil as alternative ligands in palladium coordination chemistry for applications in C-C coupling reactions. During the course of our studies, we observed that cyclic peptides derived from flaxseed oil had a high affinity for first row transition metals.

In the first project, ring-opened analogues of iminoisoindoline-based palladacycles [(κ^2-iminoisoidoline)PdCl(PCy_3)] were synthesized, characterized and investigated for catalytic activities in Suzuki C-C coupling reactions. Iminoisoindoline-based monomeric palladacycles synthesized in our group showed reasonable activity in Suzuki coupling reactions of aryl halides and phenyl boronic acids in general. However, when deactivating groups were involved on the coupling substrates, or the steric hindrance of the coupling substrates were increased, the catalytic activity decreased drastically. The objective of this work was thus to synthesize monomeric ring-opened iminoisoindoline-based palladium complexes derived from the analogous monomeric palladacycles and compare their activity in Suzuki coupling reactions to that of the initial palladacycles. This work represents the only known example of direct ring-opening of a well-defined monomeric palladacycle into its ring-opened analogue to date. Catalytic activity of the ring-opened palladium complexes were evaluated with Suzuki coupling reactions, and the catalytic activity remained outstanding even with deactivating groups

or increased steric hindrance on the substrates. Moreover, this catalyst was even active in the presence of water.

The second project is a study of the coordination chemistry of cyclic peptides derived from flax oil with palladium precursors and further applied to other analogous transition metals complexes. Saskatchewan has the highest contribution to national flax production, and flax is an affordable crop with a high nutrition value. The bitter flavor originates from the cyclic peptides in the flaxseed oil and is the reason why flaxseed oil is not widely commercialized as a cooking oil. Very few examples from literature report the interaction of cyclic peptides with transition metals, and no study of this particular class of cyclic peptides derived from flax oil with transition metals has been reported. With that being said, we investigated the interaction between this naturally occurring ligand and transition metal precursors such as palladium, to determine if a coordination complex could form. We successfully observed new cyclic peptide based metal complexes forming with palladium and platinum used as precursors, with relatively harsh reaction conditions being required. In contrast, Cu and Fe-based complexes incorporating CLA (Cyclolinopeptide A) formed virtually instantaneously upon the introduction of CLA to the metal under mild conditions. Moreover, it was surprising to observe fast reduction of Fe(III) and Cu(II) species to their corresponding Fe(II) and Cu(I) analogues, with no such examples in literature reported before.

LIST OF ABBREVIATIONS

Abbreviation

^{1}H NMR.....Proton Nuclear Magnetic Resonance

^{13}C NMR.....Carbon 13 Nuclear Magnetic Resonance

^{31}P NMR.....Phosphorus 31 Nuclear Magnetic Resonance

C-C.....Carbon-Carbon

CD.....Circular Dichroism

CLA.....Cyclolinopeptide A

Cy.....Cyclohexyl

dba.....dibenzylideneacetone

TFA……....Trifluoroacetic acid

EPR.....Electron Paramagnetic Resonance

ESI.....Electrospray Ionization

MS.....Mass Spectrometry

NMR.....Nuclear Magnetic Resonance

XANES.....X-Ray Absorption Near Edge Structure

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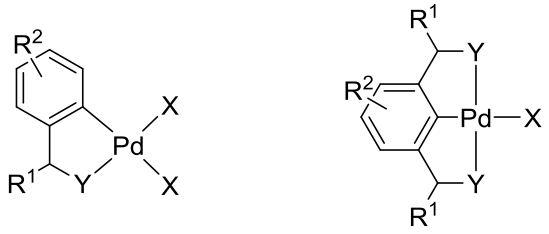
Chapter 1. Introduction

1.1 Palladacycles

1.1.1 Definition of Palladacycles

In general, a palladacycle (Figure 1.1) can be defined as any palladium compound containing one palladium–carbon bond intramolecularly stabilized by one or two neutral donor atoms (Y), where the organic moiety acts as a C-anionic four-electron donor ligand or as a C-anionic six-electron donor ligand.[1]



Y = NR_2, =NR, PR_2, AsR_2, SR, SeR, etc.
R^1, R^2 = alkyl, aryl, etc.
X = Cl, Br, I, OTf, OAc, solvent, etc.

Figure 1.1. Structural definition of a palladacycle

It is important to note that in order to be referred as palladacyclic species there should be at least one Pd-C bond in the cyclic structure where a palladium atom is incorporated into. (Figure 1.2).[1]

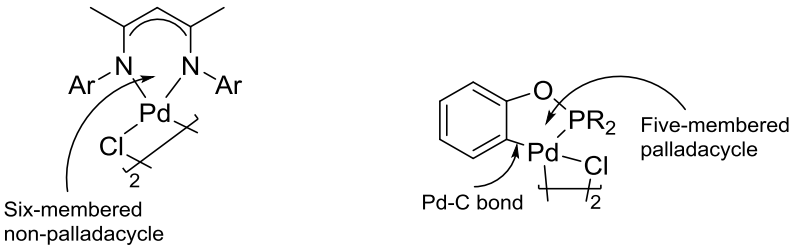
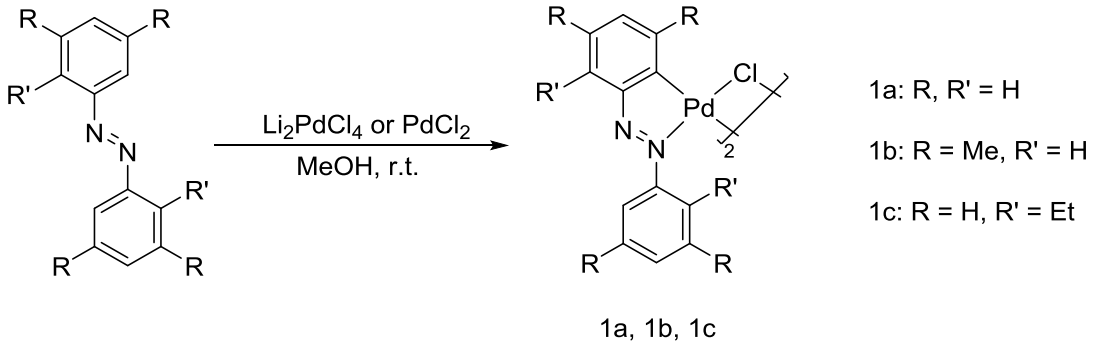


Figure 1.2. Examples of a non-palladacyclic and a palladacyclic complex

1.1.2 Evolution of Palladacycles

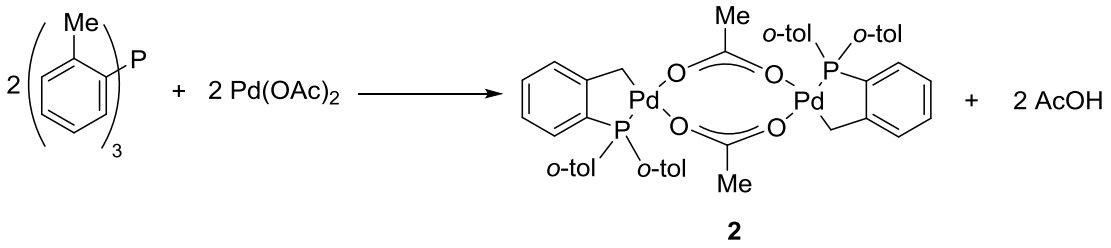
It has been exactly fifty years since the discovery of palladacycles. In 1965, Cope and his coworkers carried out reactions of azobenene or its derivatives with Li_2PdCl_4 or $PdCl_2$, to afford the first isolated, well-characterized palladacycles **1a, 1b** and **1c** (Scheme 1.1). [2]



Scheme 1.1. First palladacycles made in 1965 by Cope and coworkers

Until 1995, palladacycles were considered only to be intermediates in catalytic reactions.[3, 4] It was Herrmann *et al.* who isolated cyclopalladated tri-*o*-tolyl-phosphine complex **2** (Scheme 1.2) and were the first to test it as a catalyst precursor for Pd-catalyzed Heck and other cross-coupling reactions. The Herrmann complex is air, moisture, and thermally stable, as well as commercially available.[5] The discovery of the Herrmann Complex raised high expectations for this class of compounds, as these species could activate more economic substrates than those applied for coupling reactions thus far (aryl iodides or aryl triflates), such as aryl chlorides, hence, potentially enabling industrial application of these cross-coupling reactions mediated by palladacycle catalysts.[6] Since then, palladacycles have been ubiquitous in catalytic transformations, playing significant roles as catalyst precursors or active

intermediates.[7-13]



2

Scheme 1.2. The first palladacycle (**2**) used as a pre-catalyst in carbon-carbon (C-C) cross-coupling reactions, widely known as "the Herrmann Complex"

1.2. Application of palladacycles in Suzuki-Miyaura Carbon-Carbon cross-coupling reactions

1.2.1 Brief introduction of Suzuki reaction

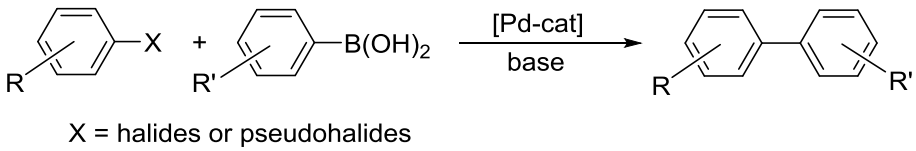
To avoid redundancy, Suzuki-Miyaura Carbon-Carbon cross-coupling reactions will be abbreviated as Suzuki reactions in this thesis. Collectively, Pd-catalyzed coupling reactions represent some of the most powerful and versatile tools available to synthetic organic chemists. Their widespread popularity stems from the fact that they are generally tolerant to a large number of functional groups, which allows them to be employed in a wide range of applications.[14] In fact, no other transition metals can offer such versatile methods for C-C bond formations as Pd.[15] Palladacycles, as a class of palladium-based complexes, weren't investigated as a pre-catalyst in C-C cross coupling reactions until Herrmann *et al.* evaluated the catalytic activity of **2** in 1995,[5] and since then, these compounds have experienced a renaissance that has been fundamental in the recent development of homogeneous catalysis, particularly in the case of C-C cross coupling reactions. Other important

areas where palladacycles have found recent applications include their use as mesogenic[16-23] and photoluminescent[23-33] agents, as well as biological applications for cancer treatment. [34, 35]

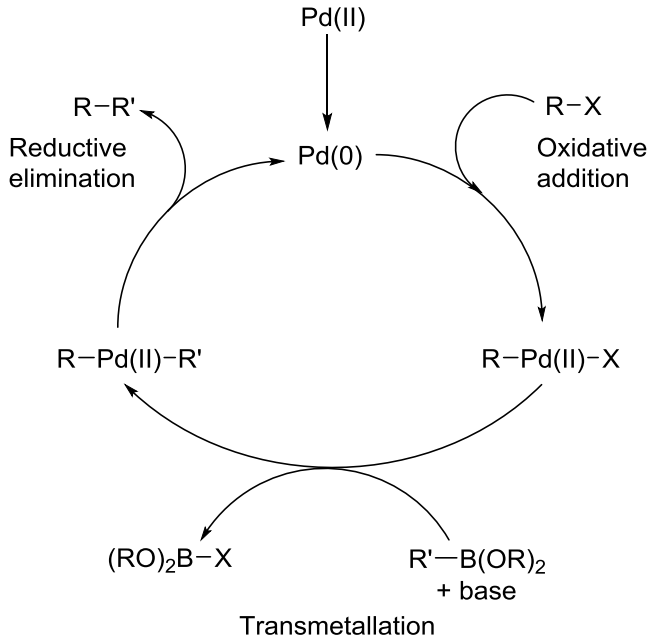
Arguably, among palladacycle-catalyzed cross coupling reactions, the Suzuki reaction of aryl and vinyl halides/triflates with boronic acids is emerging as a favorite,[36-40] and it has been applied industrially to the production of compounds such as losartan, [41] a Merck® antihypertensive drug. This popularity is attributable to a variety of factors, and the following six advantages account for the Suzuki reaction being the most popular among palladium-catalyzed cross-coupling reactions.

1. Commercial availability of a large number of boronic acids
2. Stability of boronic acids to heat (up to 110 ^{o}C), air, and moisture
3. Tolerance to a broad range of functional groups
4. Mild reaction conditions
5. Low toxicity (but not non-toxic)
6. Easy separation of inorganic boronic byproducts from reaction mixture

A general equation of Suzuki reaction is shown in Scheme 1.3, and its general mechanism is shown in Scheme 1.4.[1]



Scheme 1.3. A general expression of Suzuki reaction



Scheme 1.4. General mechanism for Suzuki reaction

1.2.2 Obstacles of coupling aryl chloride substrates in Suzuki reactions

The Suzuki reaction is an extremely powerful method for the formation of biaryl compounds.[36, 39, 40] However, for many years a major limitation of the Suzuki reaction has been the poor reactivity of aryl chlorides, which from the standpoints of cost and availability are more attractive substrates than the corresponding bromides, iodides, and triflates. Traditional palladium/triarylphosphine catalysts are only effective for the coupling of certain activated aryl chlorides (for example, heteroaryl chlorides and substrates that bear electron-withdrawing groups), but not for aryl

chlorides in general. The low reactivity of chlorides is usually attributed to the strength of the C-Cl bond (bond dissociation energies for Ph-X: Cl: 96 kcal/mol; Br: 81 kcal/mol; I: 65 kcal/mol).[42, 43] Consequently, there has been considerable attention focused on the development of catalysts that are able to promote the coupling of aryl chloride substrates. Their reluctant oxidative addition to Pd(0) centers is usually the rate limiting step in Pd-catalyzed coupling reactions.[42, 44]

Major breakthrough has been achieved by a number of research groups in last two decades: catalysts based on bulky, electron-rich phosphines (and carbenes) have proven to be particularly effective and versatile.[14, 45] Rather than exhaustively list all the reported palladacycles for aryl chloride coupling reactions, this work aims to provide insights about the catalyst design philosophy based on varying the bulk and electron-donating ability of phosphines.

1.2.3. Phosphine-based palladacycles

1.2.3.1 Brief introduction of phosphines

There are many phosphine-involved Pd-based catalytic systems that can be used to catalyze Suzuki reactions, and in synthetic laboratories those are typically employed by *in situ* formation from an appropriate Pd(0) or Pd(II) precursor and a triarylphosphine.[11] The role of electron-rich phosphines in the mechanism of Suzuki reaction is to accceleate the rate limiting oxidative addition step of aryl halides; the role of the bulkiness of a phosphine assists facile reductive elimination, which is the last step in the catalytic cycle of a Suzuki reaction. The most intuitive method to tell if

one particular phosphine is a strong electron donating ligand is to check the pKa value of the conjugate acids of this very phosphine; the higher the pKa, the stronger a Lewis base this phosphine is. The pKa values of commonly used phosphines are: $P(tBu)_3$ (11.4), PCy_3 (9.7), $P(nBu)_3$ (8.4), PPh_3 (2.7).[15] Triphenylphosphine is the most affordable phosphine, and it is air stable; however, its relatively low electron-donating ability makes the resulting Pd complexes less active, especially when aryl chloride substrates are used. Tricyclohexylphosphine is more expensive than triphenylphosphine and air sensitive, however, its much higher pKa makes it arguably the most popular phosphine to be used in palladium-based catalytic systems for Suzuki reactions.[1, 11, 15] Tri-*tert*-butylphosphine is supposed to be the most ideal bulky, electron donating phosphine based on its structure and pKa value (11.4), and in many cases it has proven to be highly active in Pd-based systems for Suzuki reactions; however, it is extremely air sensitive and based on what literature had indicated only research groups with world-class expertise in air-sensitive operation such as the Fu group from CalTech (The relevant work by the Fu group was performed when the Fu group was in MIT) grew quite fond of it, making it less practical for average research groups to utilize its potential, not to mention industrial application.[11, 15, 45, 46]

Since 1998, dialkyl(*o*-biphenyl)phosphine pioneered by the Buchwald group from MIT had drawn great attention as these phosphines-involved Pd-based catalytic systems showed very promising activity in Suzuki reactions, even at room temperature.[47] The efficiency of these dialkyl(*o*-biphenyl)phosphine ligands was attributed to their relatively high basicity, which would facilitate oxidative addition;

their size and the fact that the secondary ring of the biphenyl function may coordinate to the palladium centre(s). After one decade of development of dialkyl(*o*-biphenyl)phosphines, Buchwald *et al.* have made a portfolio of dialkyl(*o*-biphenyl)phosphines; moreover, with numerous experiments the efficacy of phosphine could be predicted by the functional group on the phosphine (Figure 1.3), which is remarkable because in general, roles of phosphines are not entirely understood and their performance is not always predictable.[49] Buchwald and co-workers have thoroughly studied dialkylbiarylphosphines in Pd-catalyzed C-C coupling reactions from 1998 to 2008.[47, 48]

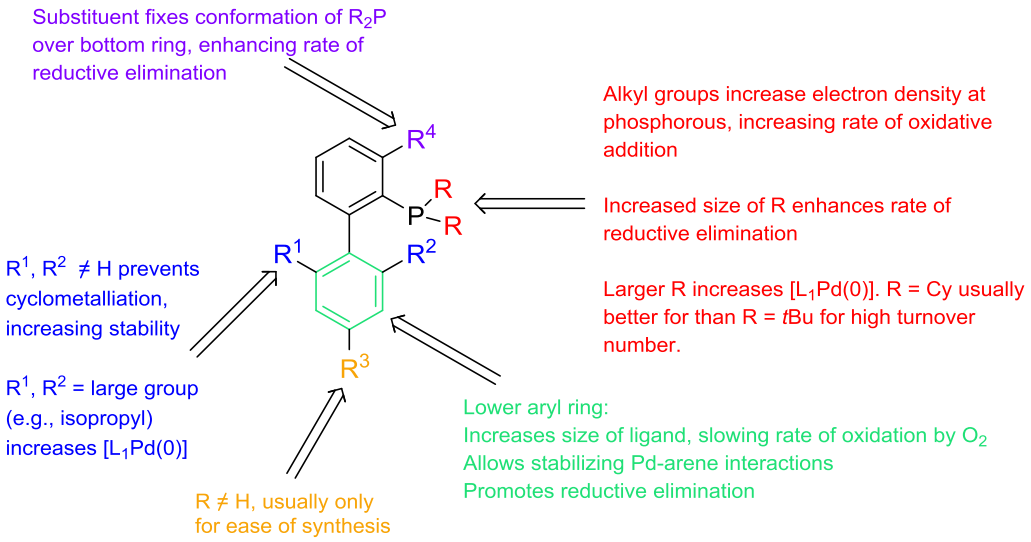


Figure 1.3. Structural features of the dialkylbiarylphosphines and their impact on the efficacy of catalysts using these ligands. This figure is adapted from reference 48.

1.2.3.2 Phosphine adducts of palladacycles

As mentioned in chapter 1.2.3.1, “classical” methods of employing phosphines

in Pd-based C-C coupling reactions involved *in sit*u formation of the active catalyst from an appropriate Pd(II) or Pd(0) precursor and a triarylphosphine. Unfortunately, while useful, these classical catalyst systems suffer from two major limitations: first, in general, they need to be used in high loadings, typically a few mol% Pd. Second, they show little or no activity with aryl chloride substrates. High loading of Pd catalysts makes their application in fine chemical or pharmaceutical industries less appealing because the palladium contamination of the product must be in the low ppm region, and the resulting nessary product clean-up is expensive. This, coupled with the high price of not only the palladium but often the ligands, can make the whole process prohibitevly expensive. [11]

Therefore, *in situ* formation of the active catalyst is not the best idea to fully utilize the potential of Pd-based complexes, that is why well-defined palladacycles with triarylphosphine ligands have become a popular topic of research. Well-defined palladacycles have several advantages than the *in situ* formed Pd catalysts:

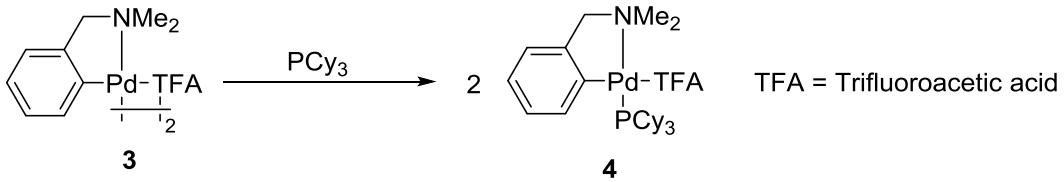
1. With rigid ring structure, palladacycles could potentially be more air and thermally stable than commerically available commonly used Pd precursors such as $PdCl_2$, $Pd(OAc)_2$, $Pd(PPh)_4$, $Pd_2(dba)_3$.

2. Loading of palladacycles is significantly reduced compared to the *in situ* formation of the active Pd catalyst, making it more appealing in low ppm Pd(0) residue applications.

3. Development of the palladacycles and evaluation of their catalytic performance benefits chemical understanding of how the structure and functional

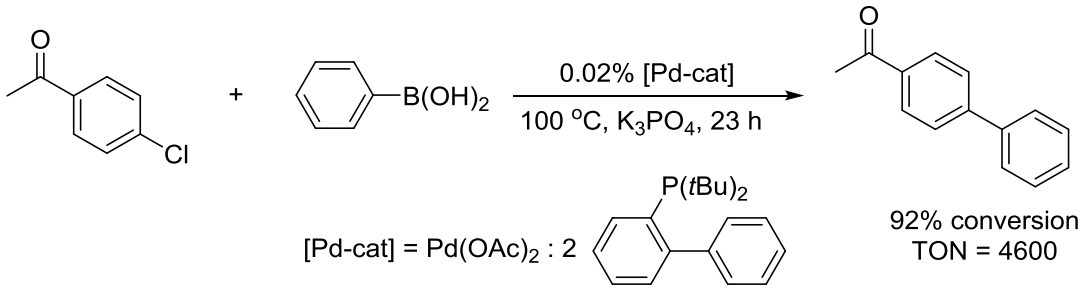
goups on the palladacycles would tune their catalytic efficacy, while *in situ* formation of the active Pd catalyst provides limited insights for the catalysis as the only thing can be altered in the catalytic design is the ratio between the Pd precursor and the triarylphosphine.

Therefore, we reason that palladacycles can act as an air-stable, a robust and clean source for the highly efficient delivery of low-coordinate Pd(0) phosphine species, and the design of the Pd pre-catalyst tend to be palladacycles incorporating triarylphosphine ligands. In 2001 Bedford and co-workers pioneered N-based palladacycle **4** (Scheme 1.5) which is extremely active for Suzuki coupling reactions and is the highest reactivity record holder in terms of turn over number (TON, TON = $\frac{mol\ product}{mol\ catalyst}$) for coupling aryl chlorides, and it even surpassed the previous highest record holder from the Buchwald group by 20 times.[50] It is worth mentioning that the catalytic system from the Buchwald group was still using the *in situ* method (Scheme 1.6)[51], and this sharp contrast is a good demostration that development of well-defined palladacycle is the future of catalyst design. Selected reactions highlighting the catalytic performance of complex **4** are shown in Table 1.1.



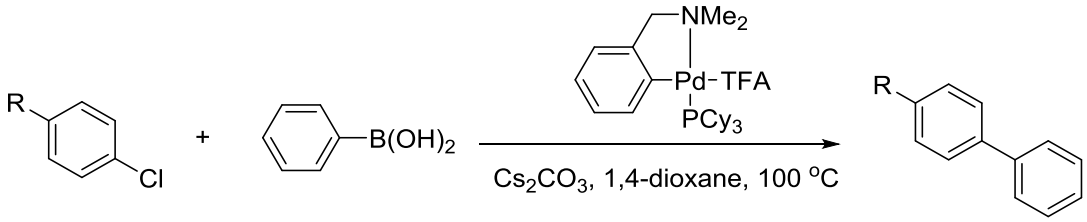
3 **4**

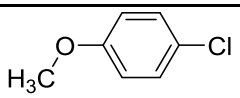
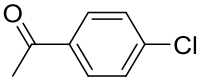
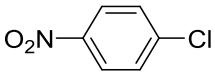
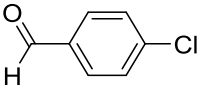
Scheme 1.5. Synthesis of super active N-based palladacycle by Bedford group.[50]



Scheme 1.6. Buchwald's *in situ* Pd-dialkyl(*o*-biphenyl)phosphine catalytic system as the previous highest TON holder for coupling aryl chlorides.[51]

Table 1.1. Select results of aryl chloride coupling from Bedford's highly active palladacycle **4**.



Entry	Substrate	Cat %	Conversion (%)	TON
1		0.01	80	8000
2		0.01	100	10000
3		0.01	100	10000
4		0.001	99	99000

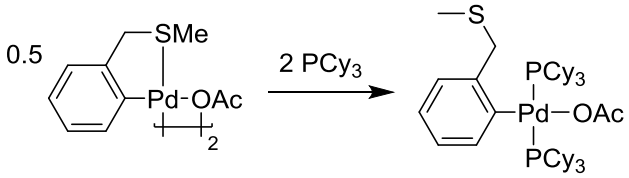
As can be seen from Table 1.1, palladacycle **4** is highly active for activited substrates with electron-withdrawing groups *para* to the chloride that quantitative

conversion was always observed even at extreme low loading. Impressively palladacyle **4** is also very active even at very low loading for deactivited groups with electron-donating group *para* to the chloride, which is difficult for the coupling reaction because of the increased electron density on the Ar-Cl bond. It is noticable that caesium carbonate as the base and 1,4-dioxane as the solvent is the best combination for the Bedford system, and subsquently became the standard in evaluating Pd-based systems in C-C coupling reeactions.[52-55] Bedford's system addressed several highlights for the design of a highly active Pd-based catalytic system for coupling aryl chlorides: (i) a well-defined palladacycle incorporating tricyclohexylphosphine ligands; (ii) N-based palladacycles (which seems to be a more promising choice than S-based or O-based palladacycles based on literature); (iii) a monomeric palladacycle is more preferable than a dimeric palladacycle (dimeric palladacycle **3** is less active than monomeric palladacycle **4**[50] and thus coupling results using **3** is not shown in Table 1.1).

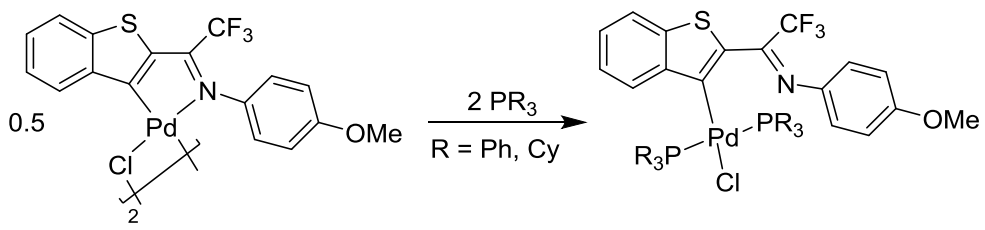
1.3 Ring-opening of monomeric palladacycles

The use of phosphine-based compounds to split dimeric palladacyclic complexes has been known since the 1970s.[56] One more achievement that wasn't mentioned in chapters 1.2.3.2 about Bedford's palladacycle **4** is that it is the very first time the catalytic activity of a resultant monomeric palladacycle was evaluated in a C-C coupling reaction and a direct comparison was made between the monomeric palladacycle and its dimeric precursor to prove that improved activity could be

achieved by splitting a dimeric palladacycle into its monomeric analogues using PCy_3. Surprisingly, very few palladacycles have been ring-opened with Lewis bases to yield their well-defined acyclic analogues. The Bedford group has converted a dimeric palladacycle to its monomeric acyclic analogues using a thioether-based palladacycle; however, in their system the monomeric monophosphine ligated palladacyclic species could not be formed (Scheme 1.7). The acyclic bisphosphine palladium complex was not reported to be investigated in C-C coupling reactions. The Likhar group has reported that a chloride-bridged dimeric benzothiophene-based palladacycle reacts with PPh_3 or PCy_3 to yield the acyclic bisphosphine analogue. As in the Bedford example, the monomeric monophosphine precursor could not be synthesized. Turnover numbers up to 184 were reported for the Suzuki coupling of 4-chloroanisole with phenylboronic acid for the ring-opened benzothiophene-based palladacycle (Scheme 1.8). The chloride-bridged dimeric benzothiophene-based palladacycle was not investigated in Suzuki coupling reactions.[58]



Scheme 1.7. Synthesis of a ring-opened thioether-based palladium complex.[50,57]



Scheme 1.8. Synthesis of a ring-opened benzothiophene-based palladium complex.[58]

1.4 Previous Work in Foley Group

Previous work by Dr. Jackson Chitanda from the Foley group reported a synthetic routine of *N, N'*-diaryliminoisoindoline-based dimeric palladacycle and its corresponding monomeric palladacyclic splitted by PCy_3. Both dimeric and monomeric palladacycle were fully characterized, and the catalytic efficacy were evaluated for the dimeric palladacycle. The catalytic efficacy of the monomeric palladacycle were not explored, and ring-opening of monomeric palldacycle was not attempted. [52-55]

1.5 Research objectives

Our goal was to perform a direct comparison of catalytic activity of a well-defined monomeric palladacycle and its well-defined ring-opened analogue, which has not been reported yet. By destabilizing a monomeric palladacycle through the ring-opening process using PCy_3, we anticipate a significant increased activity for the Suzuki coupling reactions of aryl chlorides. Moreover, we expect the improved catalytic activity for the ring-opened analogue of a monomeric palladacycle extends to aryl chlorides with deactivated groups, as well as the coupling of bulky substrates.

1.6 Cyclicpeptide A

1.6.1 Brief introduction of plant cyclopeptides

Plant cyclopeptides are defined as cyclic compounds formed mainly with the peptide bonds of 2-37 amino acid residues and discovered in higher plants.[59] About 455 cyclopeptides have been discovered from higher plants during the past half century, belonging to 26 families, 64 genera, and 120 species. On the basis of their structural skeletons and distribution in plants, Zhou *et al.* proposed the systematic structural classification of plant cyclopeptides which are divided into two classes, five subclasses, and eight types (Figure 1.4).[59]

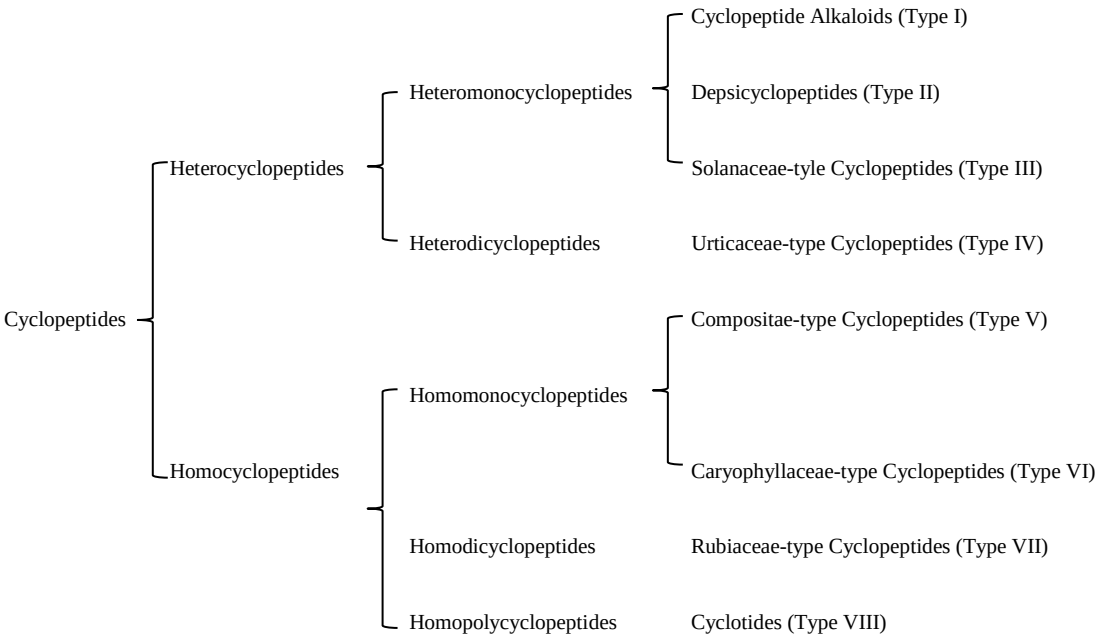


Figure 1.4. Classification of plant cyclopeptides based on structural differences. This figure is adapted from reference 59.

1.6.2 Cyclolinopeptide A from flaxseed oil

1.6.2.1 Brief introduction of flaxseed and flaxseed oil

Flaxseed is one of the oldest cultivated crops and continues to be widely grown for oil, fiber and food.[60] The average worldwide flaxseed production between 2007 and 2011 was 1,862,449 tons.[61] Saskatchewan is always the highest contributor for flaxseed production. For example in 2014/2015 Saskatchewan's flaxseed production alone was 501.2 thousand tons, which was more than 60% of the total Canadian domestic production.[62] Increasing demand for edible oil sources with significant percentages of omega-3 fatty acids is resulting in consumption of flaxseed as a functional food.[63]

1.6.2.2 Cyclolinopeptide A from flaxseed oil

It is worth mentioning that the very first plant cyclopeptide, named cyclolinopeptide A, was isolated and determined from flaxseed by Kaufmann and Tobschirbel[64] in 1959, and thus laid the foundation of plant cyclopeptides research. Cyclolinopeptide A (CLA) is under the category of Caryophyllaceae-type cyclopeptide (Type VI). The "lino" from the name is simply because of flax's binomial name *Linum usitatissimum*; letter A reflects the chronological order of this particular cyclopeptide being discovered, which in this case means being the first. There are complications with respect to the nomenclature of cyclolinopeptides as more were discovered from 1999 to 2014 by different research groups and there hasn't been a unified agreement in this area; Reaney *et al.* in 2015 summarized the

literature names of the cyclolinopeptides and proposed their new nomenclature system to avoid confusion.[63] The only cyclopeptide that will be discussed in depth through this thesis is cyclolinopeptide A and since it is the very first discovered cyclolinopeptide, nomenclature would not be an issue.

1.6.3 Applications of CLA

Since first being isolated from linseed by Kaufmann and Tobschirbel, [64] it only took five years for Professor Weygand to present the synthesis of CLA through classical solution methods, which was the only way known at that time for such "large" peptides.[65, 66] In the early 1970's CLA has been the object of intensive structural studies, with Naider *et al.* for Circular Dichroism (CD) studies, Brewster *el al.* for NMR studies, and Tenelli *et al.* for conformational energy calculations.[67-69] Likely reasons for motivation of intensive structural studies are CLA being the first isolated plant cyclopeptide, as well as the presence of two Pro residues which suggests that the accessible conformational space might be constrained compared to known medium-sized peptides.[70] The amino acid sequence of CLA was determined as *c*(Pro-Pro-Phe-Phe-Leu-Ile-Ile-Leu-Val) from early structural studies.

In 1987 Di Blasio *et al.* were first to successfully obtain a crystal structure from X-ray studies of CLA.[71] The author concluded that the solid state and solution conformations of CLA are essentially identical. Soon there had been several reports of CLA being crystallized from different solvent mixtures, leading to three different polymorphs: an orthorhombic form, grown from isopropanol–water, a monoclinic

form, grown from N,N-dimethylformamide (DMF)–isopropanol, and a triclinic form, grown from acetone–benzene. Remarkably, in spite of the different environments in which the crystals were grown, the cyclolinopeptide A molecules in all crystal forms show the same conformation.[71-73] A high resolution image of the crystal structure of CLA (Figure 1.5) was provided by Saskatchewan Structural Science Centre (SSSC) in their relative recent report of CLA's X-ray study.[74]

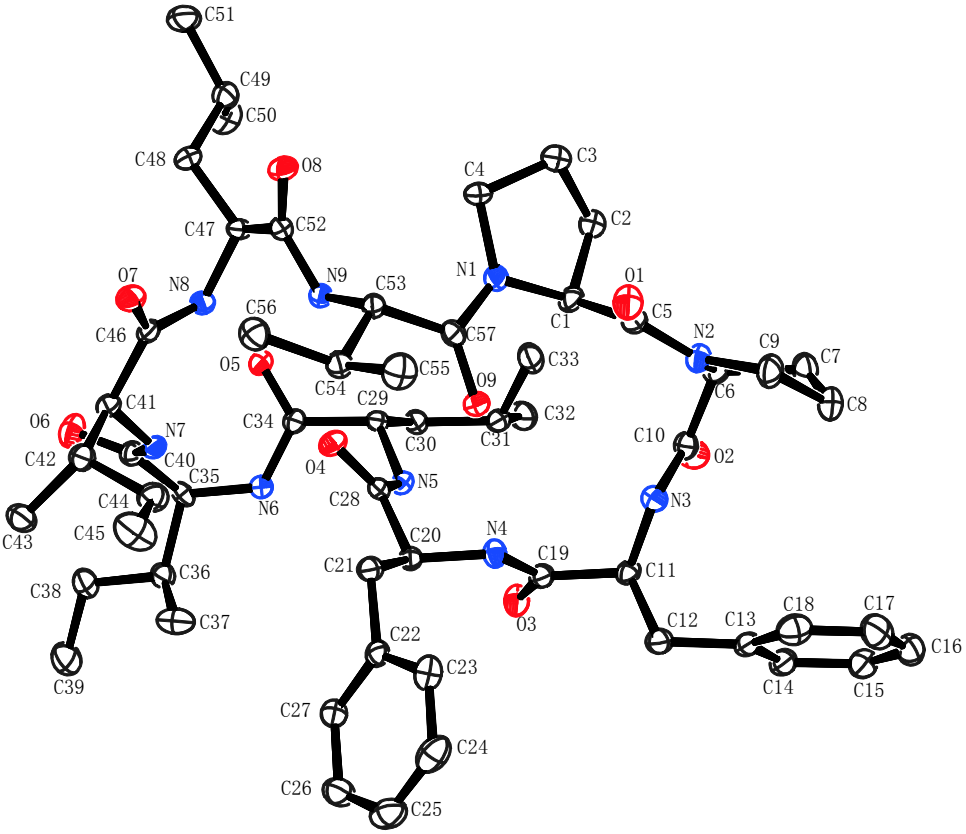


Figure 1.5. Crystal structure of CLA. This figure is adapted from reference 74.

The elucidation of the 3D structure of CLA resulted in significant research interest towards its immunosuppressive studies since its Pro-Pro-Phe-Phe sequence is

shared with Antamanide (abbreviated as AA, shown in Figure 1.6),[75] a homodetic cyclodecapeptide *c*(Pro-Pro-Phe-Phe-Val-Pro-Pro-Ala-Phe-Phe) isolated from the poisonous mushroom *Amanita phalloides*. Antamanide, one of the most extensively investigated cyclic peptides, received huge scientific attention because of its strong antidote activity against phallotoxins, a family of extremely toxic bicyclic heptapeptides isolated from the same source as AA itself.[76]

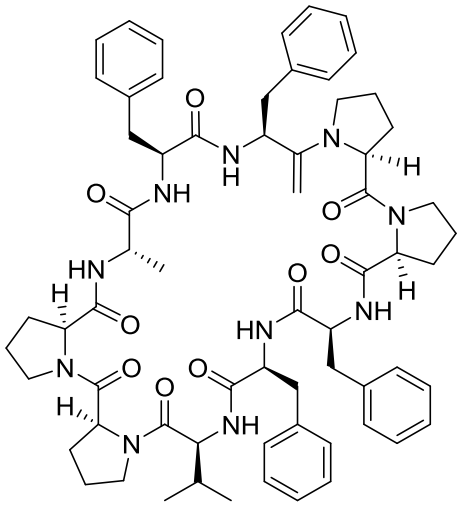


Figure 1.6. Representation of Antamanide

Siemion *et al.* were the first to report CLA's strong immunosuppressive activity in 1991.[77] The influence of CLA on the humoral response was determined by the plaque forming cell test and the influence on the cellular immune response by the delayed-type hypersensitivity test. The effect of CLA in this test was comparable with that exerted by a known powerful immunosuppressant – cyclosporine A (Figure 1.7).[78] However, in the test of inhibiting calcium dependent activation of T lymphocytes, the dosage of CLA required to complete inhibition was ten times as high

as that of cyclosporine A.[79, 80]

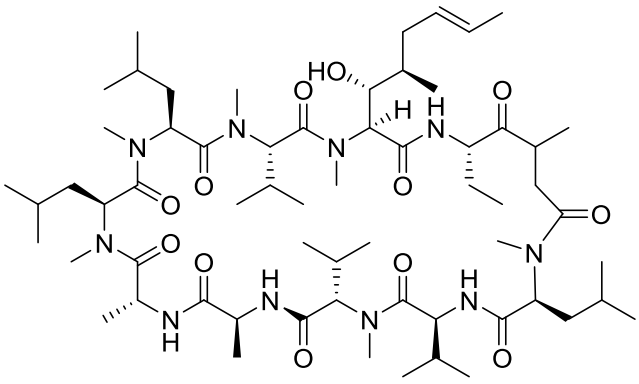


Figure 1.7. Representation of Cyclosporin A.[79]

As it had been mentioned above, CLA can be considered as an analogue of a cyclic decapeptide, Antamanide, which is also known for its ability to form complexes with metal ions of IA and IIA groups.[81] In the case of CLA, this tendency for metal ions complexation is strongly reduced.[82] It has been found that CLA binds with K^+, Na^+, Ca^{2+}, Mg^{2+}, and Ba^{2+} from NMR and CD (Circular Dichroism) studies, while binding affinity to Ba^{2+} being the strongest. It was indicated by CD data that depending on the concentration, two types of such complexes are present in acetonitrile solution: 1 : 2 sandwiches and 1 : 1 equimolar complexes. NMR data were consistent with an equimolar form. Addition of bivalent metal ions to the CLA solution induces drastic structural changes. In the equimolar Ba^{2+}/CLA complex the peptide backbone contains all-trans peptide bonds and the global shape of the complexed peptide can be described as a bowl with a polar concave side hosting Ba^{2+} and the opposite convex side predominantly apolar.[83] To the best of our knowledge,

no application of resulting metal ion complex binding with CLA has been reported.

1.6.4 Research objectives

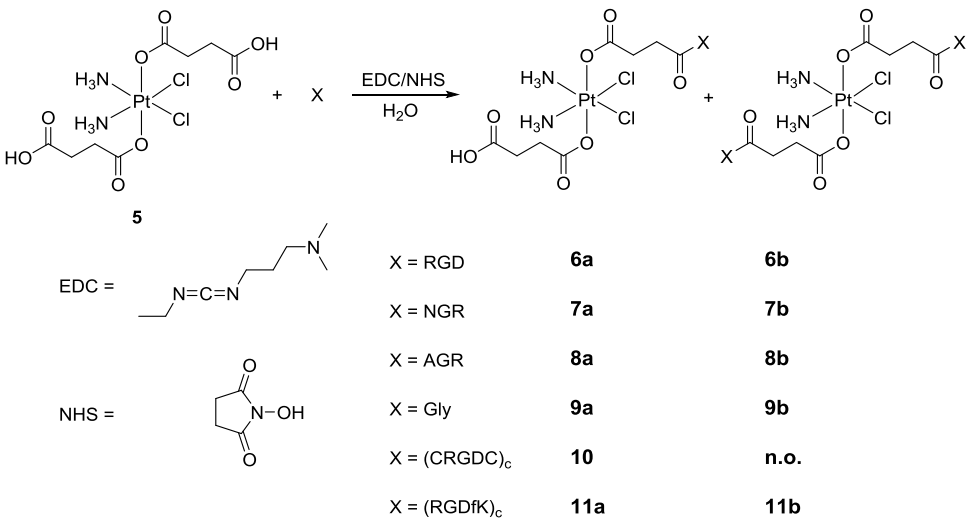
As mentioned previously, the applications of CLA to date have been limited. There were a few reports of its immunosuppressive properties and ion binding properties with few selected ions. As a matter of fact, a SciFinder® search performed on April 15, 2015 showed only 190 articles contained the concept of cyclolinopeptides; the majority of the articles were based on the structural studies of CLA, the synthesis of CLA derivatives, and immunosuppressive studies of CLA and its derivatives. Very few articles got cited more than 10 times, even for review articles, and there is no example showcasing CLA coordinating with transition metals. As organometallic chemists, we believe that there is much of CLA being unexplored: we would envision CLA as a naturally occurring, non-toxic, large-sized ligand that could allow transition metals to possibly have multiple binding sites on CLA. It is highly anticipated that we could make a well-defined Pd complex incorporating CLA and commercially available Pd precursors, and the catalytic activity of certain C-C coupling reactions of this well-defined Pd complex could be significantly improved than the Pd precursors alone. It is also envisaged that if a Pt complex incorporating CLA and Pt precursors could be successfully synthesized, this Pt complex should exhibit interesting properties in cytotoxicity studies as Pt-based complex had shown promising results in cancer treatments. If we explore further, first row transition metals could also be great candidates for investigation of their binding abilities to

CLA because first row transition metals could potentially suggest wider range of applications with their affordable prices.

1.6.5 Noticeable examples of peptides (preferably cyclic) coordinating with transition metals

Rather than exhaustively list every single example showcasing cyclic/linear peptides with similar number/sequence of amino acid residues as CLA coordinating with transition metals from literature, this section emphasize on those that were reasonably well cited, preferably with a crystal structure, as well as provided insights and enlightenments for organometallic chemists to design their own transition metal based complexes using CLA.

Among articles containing the concept of cyclolinopeptides, the one got most cited (106 citations) is by the Lippard group from MIT. They showcased Pt-based complex incorporating peptides with promising cancer therapeutic properties (Scheme 1.9).[84] The peptides involved were linear peptides with fewer amino acid residues than CLA; however, the structure of their Pt-based precursor inspired us in the design of our Pt-based complex using CLA, which will be described in detail in Chapters 3.3.1.



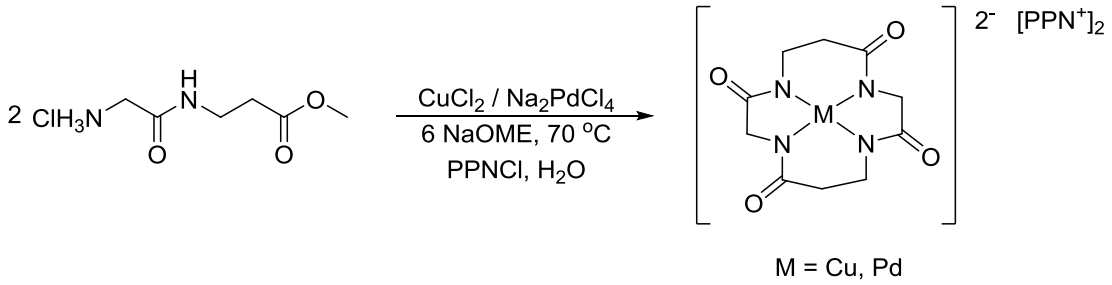
Scheme 1.9. Synthesis of Lippard's Pt-peptide complex for cancer treatment.

This scheme is adapted from reference 84.

In Pt-themed peptide related research, other references could only enlighten us to some extend: there is one great example demonstrating efficient liquid-phase synthesis of cyclic metallopeptides from linear peptides bearing functional platinum complexes, and the ^{1}H NMR spectrum indicated a much more symmetrical species after the cyclization.[85] We didn't find any reported crystal structure for Pt-based complexes. The majority of characterization methods involved MS (Mass Spectrometry), NMR, and EPR (Electron Paramagnetic Resonance).

While no X-ray structure of a Pt complex incorporating cyclic peptide(s) has been reported, in Pd-themed peptide related research last least one class of Pd complex has been reported with crystal structure successfully obtained with smaller peptides.[86, 87] The scope of this reaction extended to Ni and Cu, and the crystal structure of the Cu analogue was successfully obtained four years later by another

research group (Scheme 1.10).[88] Characterization of this type of reaction is very comprehensive, possibly because of the small size of peptides involved in the reaction.



Scheme 1.10. Synthesize of Pd or Cu based complex incorporating small peptides. This scheme is adapted from reference 88.

Contemporaneously, crystal structure of Cu incorporating the larger sized peptide, asciacyclamide, was also successfully obtained. This structure has two copper ions separated by a bridging carbonate anions embedded in the saddle-shaped ascidiacyclamide ligand. The copper ions in the complex are further coordinated to three nitrogen donors, and a deprotonated amide, and to two water molecules (Figure 1.8).[89] The affinity of the ascidiacyclamide-copper complex for carbonate is especially interesting and has suggested that these metal cyclic peptide conjugates could be involved in the activation and transport of CO_2 *in vivo* for specific biochemical processes.

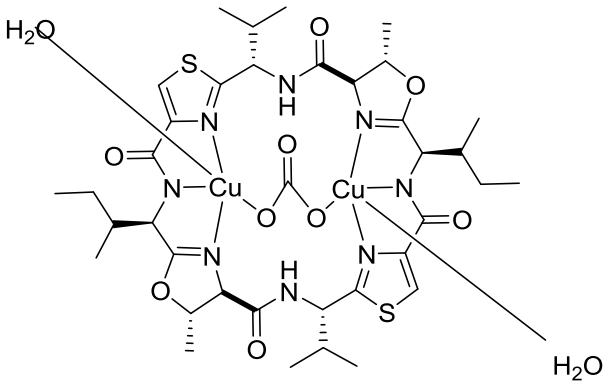
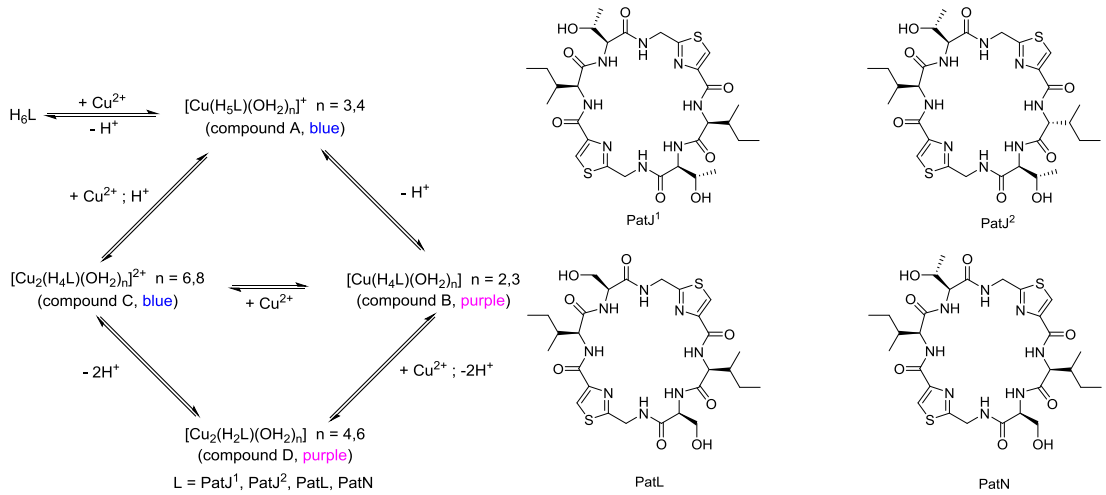


Figure 1.8. Bis-copper(II) complex of asciacyclamide.

Figure is adapted from reference 89.

One advantage of copper coordination chemistry is that without the crystal structure, EPR and colour changes (UV/Vis) of the copper complexes could still provide valuable insights about the reaction progress/mechanism. Comba *et al.* fully utilize these two characterization methods for patellamide A derivatives and their copper (II) complexes (Scheme 1.11).[90]



Scheme 1.11. Cu(II) complex incorporating patellamide A derivatives.

This scheme is adapted from reference 90.

To summarize, almost no examples from the literature in the area of transition metal complexes incorporating peptides (preferably cyclic) showed application in organometallic chemistry. In the meantime, very few examples successfully obtained crystal structure, and it might just be the nature of transition metal complexes incorporating peptides being unfavorable to crystalize from solution. Without a crystal structure, all the alternative characterization methods such as MS, EPR, UV-Vis are promising but provide limited insights as to where exactly the transition metals are coordinated to the in peptides, also these alternative characterization methods possess higher potential risk of being misinterpreted. Due to the inherent poor crystallinity of larger cyclolinopeptides, we anticipate it would be difficult to obtain crystal structures of CLA coordination complexes with transition metals.

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Chapter 2. Ring-opened palladacycles for applications in C-C coupling reactions

2.1 Abstract

The metallocyclic ring in a monomeric iminoisoindoline-based palladacycle [(κ^2-iminoisoidoline)PdCl(PCy_3)] (**3**) can be ring opened by coordination of phosphine to form the *trans* bis(phosphine) iminoisoindoline-based palladium(II) complex [(η^1-iminoisoidoline)PdCl(PCy_3)$_2$] (**4**) where the η^1-iminoisoindoline is attached only by an Ar-Pd bond. The ring-opened analogue showed significantly higher coupling activity in Suzuki coupling reactions with aryl chlorides than any iminoisoindoline-based palladacycle studied to date. These results are compared to existing monomeric palladacycles and reported acyclic analogues.

2.2 Introduction

2.2.1 Iminoisoindolines

N,*N*′-Diaryliminoisoindolines have the general structure shown in Figure 2.1. They are easily synthesized in one step by reaction of phthalaldehyde with two equivalents of a primary arylamine.

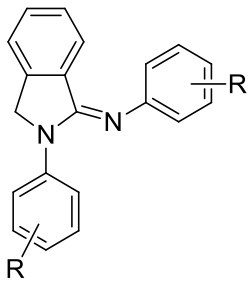
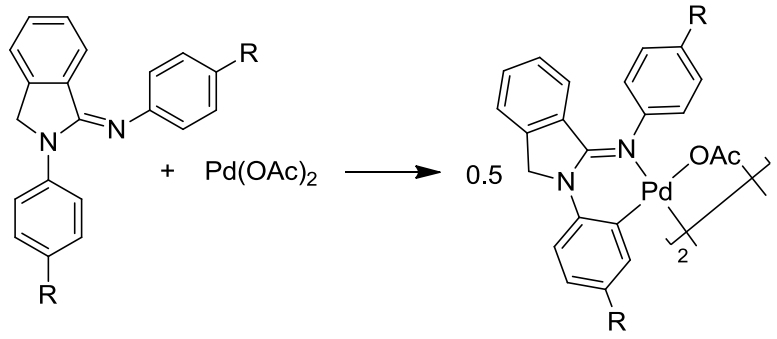


Figure 2.1. General structure of *N*,*N*′-diaryliminoisoindolines

The synthesis of *N*,*N*′-diaryliminoisoindolines was first reported in 1909 and in the last century only 24 papers have been published concerning their synthesis and applications,[1] the most comprehensive of which have been published by the Takahashi group.[2-4] Despite their ease of synthesis, diaryliminoisoindolines have never previously been investigated as ligands prior to the Foley group's initial studies.[5-8] A likely reason for the lack of interest in diaryliminoisoindolines is simply that they are not naturally occurring products and thus have been of limited interest to organic/bioorganic disciplines. They are, however, well suited to coordinate to palladium via the imine nitrogen and to undergo subsequent C-H activation via orthopalladation to form the corresponding palladacycles (Scheme 2.1). The Foley group has previously reported the synthesis of a series of air and moisture-stable *N*,*N*′-Diaryliminoisoindoline-based palladacycles. These complexes were investigated as pre-catalysts in the activation of aryl chlorides for the formation of biphenyls and cinnamates in Suzuki and Heck coupling reactions. *N*,*N*′-Diaryliminoisoindolines have also recently been investigated by the Karminski-Zamola group for their antiproliferative activity.[9]



Scheme 2.1. General reaction for formation of iminoisoindoline-based palladacycles

2.2.2 Palladacycles

The definition of palladacyle could be found at Figure 1.1 in Chapters 1.1.1.[10] Since their first application in catalysis in 1995, palladacycles have rapidly emerged as dominant precatalysts for a large variety of coupling reactions due to their ease of preparation, air and moisture stability, low loading and high activity.[10-19] The active catalyst in palladacyclic mediated coupling reactions likely involves the reduction of the Pd(II) precursor to Pd(0) by initial arylation of the Pd(II) species by arylboronic acid to form the arylated palladacycle. The palladacycle then undergoes a ring-opening process followed by reductive elimination of the arylated ligand.[20-24] Despite the presumed formation of nanoparticles and elimination of the ligand forming the palladacycle, the nature of the palladacycle precatalysts still has a dramatic effect on the activity of the system in coupling reactions. Studies have suggested that while Pd(0) nanoparticles are formed in coupling reactions involving palladacycles, the nanoparticles themselves are not the active species, but represent the catalyst resting state from which low-coordinate monomeric homogeneous catalysts can be formed via complexation Pd(0) to free ligands in solution.[25-27] If this supposition holds true in our systems, on way to increase catalyst activity would be to preform a ring-opened analogue to a given palladacycle and evaluate the destabilized system in coupling reactions, wherein the rate of ligand elimination to form Pd(0) species should greatly increase.

The objective of this work was thus to synthesize a monomeric ring-opened iminoisoindoline-based palladium complex derived from the analogous monomeric

palladacycle and compared its activity in Suzuki coupling reactions to that of the initial palladacycle. Monomeric phosphine-ligated palladacycles have been demonstrated to be considerably more active for Suzuki coupling reactions than their dimeric chloride or acetate bridged palladacyclic precursors. In particular, PCy_3 adducts of monomeric palladacycles have shown increased activity for the coupling of arylchlorides.[12-19, 28-30] Surprisingly, very few palladacycles have been ring-opened with Lewis bases to yield their well-defined acyclic analogues. The only examples that came even close to this idea were discussed in depth in chapter 1.3 with scheme 1.7 by the Bedford group and scheme 1.8 by the Likhar group.[28-30]

In this work, a monomeric iminoisoindoline-based palladacycle incorporating PCy_3 was investigated as a pre-catalyst in the Suzuki coupling reaction with the activated aryl chlorides 4-chlorobenzylaldehyde and deactivated aryl chlorides 4-chloroanisole. The synthesis of ring-opened palladium analogue incorporating an η^1-iminoisoindoline and two equivalents of PCy_3 was explored. The resulting ring-opened acyclic palladium complex was fully characterized by multi-nuclear NMR spectroscopy, mass spectrometry and X-ray crystallography. The new acyclic palladium complex was found to be highly active in Suzuki coupling reactions involving aryl chlorides even in the presence of water.

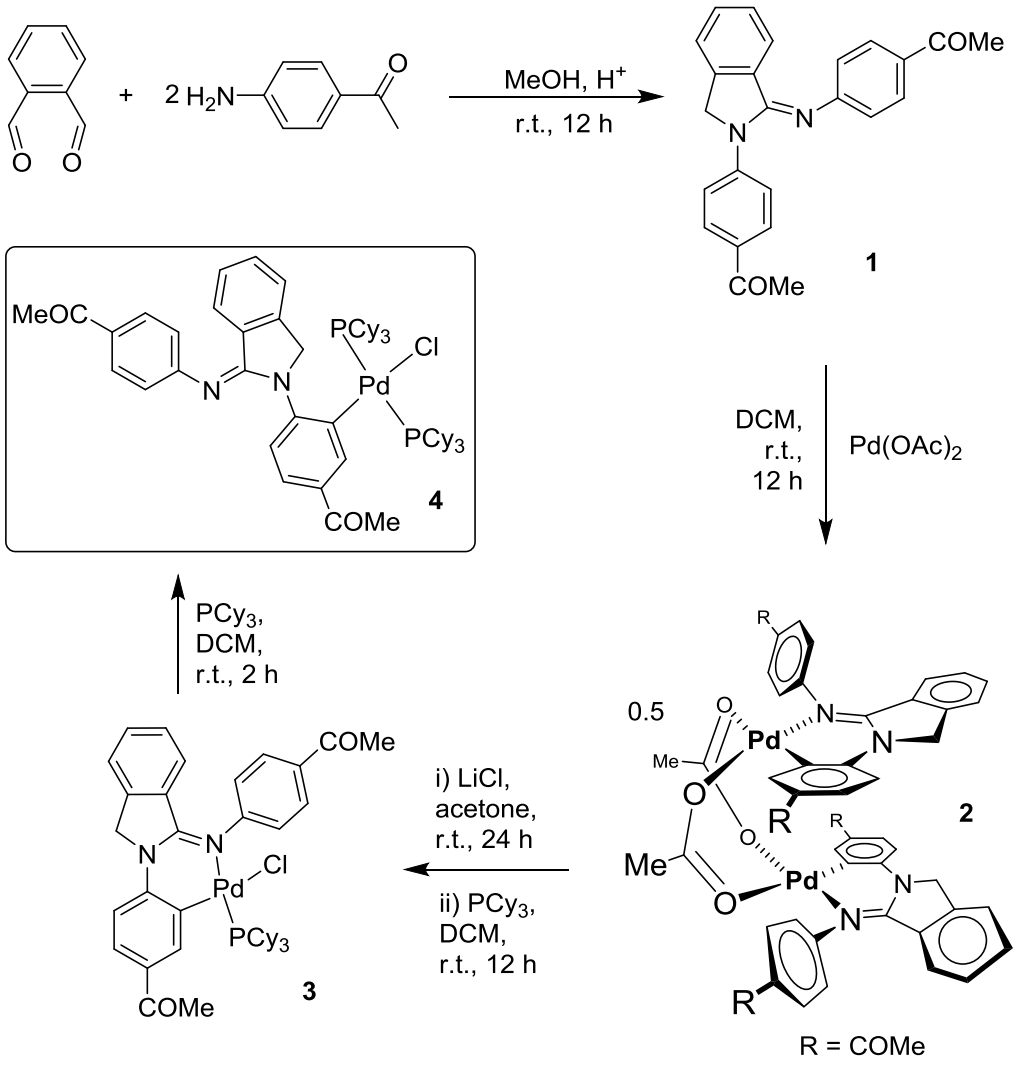
To the best of our knowledge, this work represents the only example of a conversion of a monomeric palladacycle to its ring-opened analogue, allowing for a direct comparison between the two species in C-C coupling reactions.

2.3 Results and discussion

2.3.1 Cyclopalladation and subsequent ring-opening of the metallocycle

Following procedures previously published by our group, reaction of phthalaldehyde with two equivalents of aminoacetophenone resulted in formation of iminoisoindoline **1** (Scheme 2.2). *Para*-substituted iminoisoindoline **1** was chosen because the corresponding dimeric palladacycle **2** reported turnover numbers (TONs) up to 100 for the coupling reaction of 4-chlorobenzaldehyde with phenylboronic acid.[6] As well, the acyl groups provide an effective NMR handle to monitor the formation of subsequent complexes. While a variety of *para*-substituted *N,N'*-diaryliminoisoindolines have now been reported, we have been unsuccessful in synthesizing the analogous meta-substituted *N,N'*-diaryliminoisoindolines by reaction of phthalaldehyde with *meta*-substituted aniline derivatives with such compounds remaining unknown in the literature.

Subsequent reaction of **1** with $Pd(OAc)_2$ in dichloromethane at room temperature resulted in formation of the acetato-bridged, dimeric palladacyclic complex **2** with the general formula, $[(\kappa^2\text{-iminoisoindoline})Pd(\mu\text{-OAc})]_2$ (Scheme 2.2). The previously reported solid state structures of these complexes show that they adopt a characteristic closed book conformation where the two iminoisoindoline ligands stacked on top of the other in an *anti* configuration.[6]



Scheme 2.2. Synthesis of a ring-opened iminoisoindoline-based palladacycle from commercially available starting materials

Dimeric palladacycle **2** was then reacted with an excess of LiCl in acetone resulting in an insoluble solid that is presumably the analogous chloride-bridge dimer (structural characterization was not performed due to the intractability of the solid). Subsequent reaction of the presumed chloride-bridged dimeric palladacycle with one equivalent of PCy_3 in DCM affords the corresponding monomeric palladacycle **3** with the general formula [(κ^2-iminoisoindoline)PdCl(PCy_3)]. Treatment of [(κ^2-

iminoisoindoline)PdCl(PCy_3)] **3** with another equivalent of PCy_3 in DCM yielded the acyclic [(η^1-iminoisoindoline)PdCl(PCy_3)$_2$] complex **4** as a yellow solid in quantitative yield from **3**.

A characteristic indication of the formation of a monomeric species with iminoisoindolines is the observed ^{1}H NMR resonance for the CH_2 protons of the iminoisoindoline ring. In dimeric complexes, the methylene protons are diastereotopic resulting in formation of two doublets. Complex **4** shows a singlet at 6.36 ppm for two protons indicating the methylene protons are now chemically equivalent and the new complex has an average C_s symmetry in solution. This is a downfield shift from the analogous ^{1}H NMR resonance in **3** at 5.21 ppm. The free iminoisoindoline ligand **1** also shows a singlet which is considerably upfield relative to complex **4** at 5.01 ppm. The ^{31}P NMR spectrum of **4** shows a singlet at 19 ppm consistent with only one isomer being present in solution and a *trans* orientation of the two PCy_3 ligands. The analogous ^{31}P chemical shift for the monomeric palladacycle **3** appears at 37 ppm.

Complex **4** is stable at room temperature under an inert atmosphere for at least four weeks, however, upon exposure to air at room temperature it slowly decomposes to form [(κ^2-iminoisoindoline)PdCl(PCy_3)] **3** along with formation of Cy_3P=O due to reaction with ambient O_2 as evidenced by ^{1}H and ^{31}P NMR studies. The reaction with O_2 is slow with 30% decomposition observed after 3 days exposure to ambient atmosphere. This is in contrast to the Likhar group's benzothiophene-based acyclic diphosphine analogue which is reported to be air and moisture stable (Scheme 1.8 in chapter 1.3).[30]

2.3.2 Crystal structure of [(η^1-iminoisoindoline)PdCl(PCy_3)$_2$] (4)

Suitable crystals of **4** were grown by slow evaporation from a 50:50 dichloromethane/hexane solution at room temperature. An ORTEP plot for complex **4** is shown in Figure 2.3 and included in its caption are selected bond distances and angles. The crystal structure of **4** exhibits a monomeric species with a distorted square-planar coordination geometry around the palladium atom and a *trans* disposition of the two PCy_3 groups. The Pd-C bond distance is 2.008(4) Å which is essentially identical to that of the palladacyclic [(κ^2-iminoisoindoline)PdCl(PCy_3)] type complexes like **3** which all exhibit Pd–$C_{palladate}$ bond distances at 2.00(1) Å (we have previously reported the crystal structure of a complex very similar to **3**, but with H in place of the COMe groups on the aromatic rings). The two Pd-P bond distances in **4** are 2.390(1) and 2.369(1) Å which are longer than the corresponding Pd-P distance of 2.290(1) Å in [(κ^2-iminoisoindoline)PdCl(PCy_3)] type complexes consistent with a *trans* influence of zero in **4** versus the strong *trans* influence of PCy_3 in [(κ^2-iminoisoindoline)PdCl(PCy_3)].

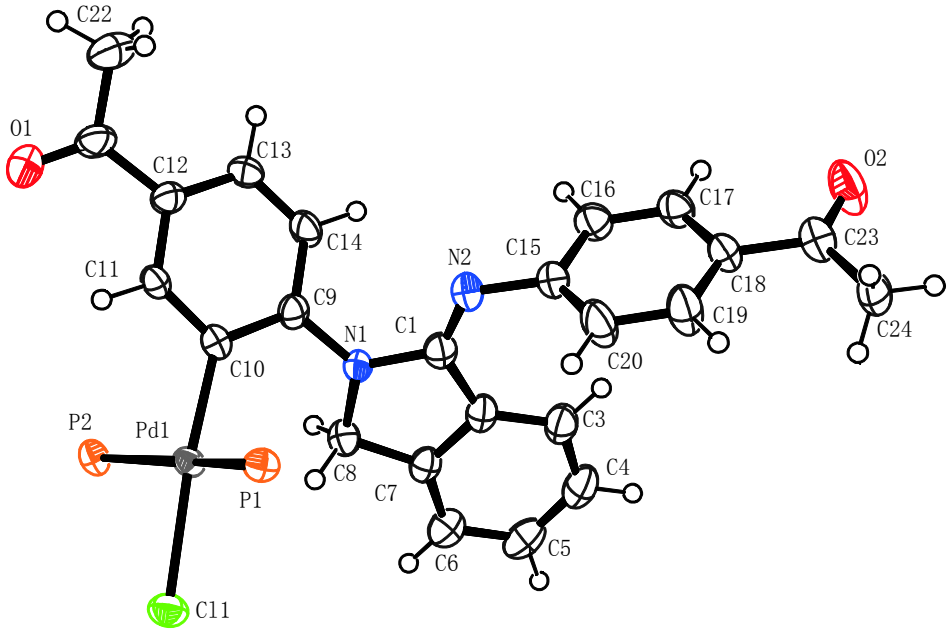


Figure 2.2. ORTEP plot of **4** at the 50% probability level. The cyclohexyl groups on the PCy_3 ligands have been omitted for clarity. Selected bond lengths [Å] and angles [°]: Pd(1)-C(10) = 2.008(4), Pd(1)-P(1) = 2.3687(11), Pd(1)-P(2) = 2.3901(10), Pd(1)-Cl(1) = 2.4022(10), C(10)-Pd(1)-P(1) = 91.30(11), C(10)-Pd(1)-P(2) = 91.40(11), P(1)-Pd(1)-P(2) = 166.68(4), C(10)-Pd(1)-Cl(1) = 174.69(10), P(1)-Pd(1)-Cl(1) = 88.51(4), P(2)-Pd(1)-Cl(1) = 89.99(4).

2.3.3 Catalytic studies

Suzuki coupling reactions of alkyl chlorides with phenylboronic acid provide a simple way to screen new precatalysts for C-C coupling activity and compare the results to that reported in the literature with other palladium-based systems. Suzuki reactions involving the coupling of simple aryl iodides or bromides have become somewhat trivial with many precatalysts reporting high TONs in the hundreds of

thousands or even in the millions. For example, Bedford's group has reported that a phosphinite-based palladacycle is active for the Suzuki coupling of 4-bromoacetophenone with a TON of 475 million.[13] As far as we know, this represents the world record in terms of absolute TON for any Suzuki reaction. A TON of 910,000 is also achieved for the coupling of the electronically deactivated substrate 4-bromoanisole. However, the same catalyst reports a TON of only 6 for the coupling of aryl chloride 4-chloroanisole.[31]

The coupling of aryl chlorides remains challenging, with most palladacycles reporting TONs of only up to 100 for activated aryl chlorides. For example, within our own iminoisoindoline-based palladacycles, dimeric palladacycle **2** exhibits TONs of 100 for the coupling of 4-chlorobenzylaldehyde with phenylboronic acid. The TON falls down to only 20 when deactivated aryl chloride 4-chloroanisole is used. That being said, there are of course exceptional catalysts in the literature, however they remain a very rare breed. For example, one of the best Pd-based catalysts for Suzuki reactions involving aryl chlorides is a $[(NHC)PdCl_2]_2$ system from Nolan's group which requires only 0.1% catalyst loading with 1 h reaction times at ambient temperature for deactivated aryl chlorides.[32, 33] The most active catalyst that we know in terms of absolute TON for the Suzuki coupling of 4-chloroanisole is a long-lived, π-acidic, phosphite-based palladacycle from Bedford's group which achieved TONs of up to 128,000 after 2 days of reaction time.[34] 4-Chloroanisole is often used as a benchmark coupling substrate as it is difficult to couple due to its electronically deactivated nature resulting in a significant resistance to undergoing oxidative

addition reactions.

Catalytic activity of precatalysts **2-4** was initially evaluated in the Suzuki coupling reaction of phenylboronic acid with 4-chlorobenzaldehyde to produce 1,1'-biphenyl-4-carboxaldehyde (Table 2.1). Suzuki coupling reaction conditions consisted of 1,4-dioxane as solvent, Cs_2CO_3 as base and a reaction temperature of 80 °C. These reaction conditions are commonly applied to new precatalysts and allow for direct comparison of previously published results in the general literature. No attempt to optimize the nature of the base or solvent was performed (these conditions often end up being the best conditions in systems where solvent and base optimization was undertaken).

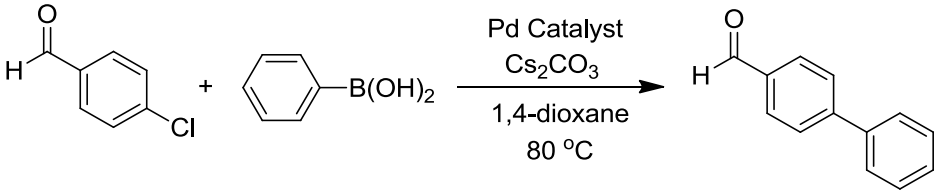
Compound **3** showed 85% conversion after 4 h at 1.0mol% Pd loading with complete conversion observed after 24 h. A maximum TON of 3800 was achieved with 0.01% catalyst loading although % conversion greatly decreased with decreasing mol % Pd (Table 2.1). Acyclic [(η^1-Iminoisoindoline)PdCl(PCy_3)$_2$] **4** was considerably faster than **3**, achieving quantitative yields for the coupling of 4-chlorobenzaldehyde after only 2 h at 1.0% Pd loading. Acyclic complex **4** also achieved higher TONs than palladacycle **3** with TONs up to 4700 being observed after 16 h. Precatalyst **4** was active when water was added to the reaction mixture, achieving quantitative yields at 1.0% catalyst loading however longer reaction times of 4 h were required. Complex **4** was even active when water was used as the only solvent for the reaction, achieving quantitative yields in 17 h. Complex **3** was inactive in the presence of water. These TONs are up to 50 times higher than those observed

for dimeric palladacycle **2** (Table 2.1).

Table 2.1. Suzuki coupling of 4-chlorobenzaldehyde with phenylboronic acid.[a]

[a]Reaction conditions: 1.0 mmol aryl halides, 1.5 mmol phenylboronic acid, 2.5 mmol Cs_2CO_3, 5 mL of dioxane.

[b]Determined by ^1H NMR in reference to dodecahydrotriphenylene as internal standard.



Entry	Catalyst (mol % Pd)	Time (h)	Conversion (%)[b]	TON (mol product/mol Pd)
1	**2** (1.0)	24	100	100
2	**3** (1.0)	4	85	85
3	**3** (1.0)	24	100	100
4	**3** (0.1)	24	87	870
5	**3** (0.01)	24	38	3800
6	**4** (1.0)	2	100	100
7	**4** (0.02)	16	93	4700
8	**4** (1.0)[c]	4	100	100
	[c](in presence of 1 mL water)			
9	**4** (1.0)[d]	17	100	100
	[d](water as solvent)			

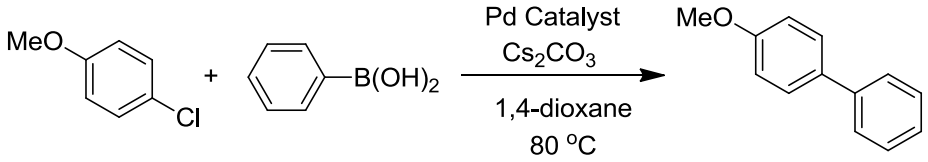
Catalytic activity of acyclic precatalyst **4** was next evaluated in the challenging coupling reaction of electronically deactivated 4-chloroanisole (Table

2.2). TONs of up to 1900 were achieved with 0.02 % Pd loading. This result is two orders of magnitude better than that for dimeric palladacycle **2** which exhibits a TON of only 20 and an order of magnitude better than monomeric palladacycle **3**.

Table 2.2. Suzuki coupling of 4-chloroanisole with phenylboronic acid.[a]

[a]Reaction conditions: 1.0 mmol aryl halides, 1.5 mmol phenylboronic acid, 2.5 mmol Cs_2CO_3, 5 mL of dioxane.

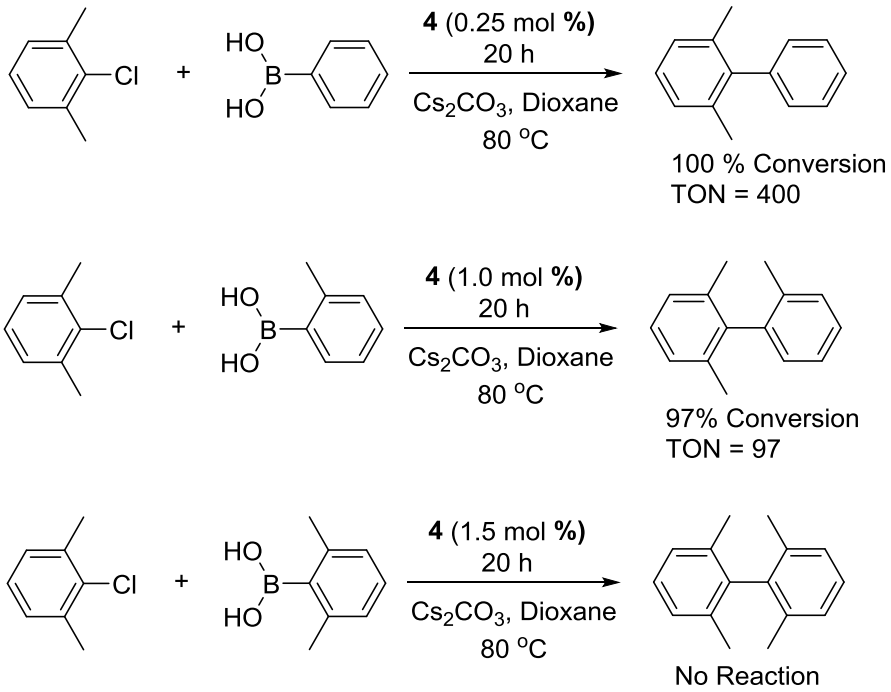
[b]Determined by ^1H NMR in reference to dodecahydrotriphenylene as internal standard.



Entry	Catalyst (mol % Pd)	Time (h)	Conversion (%)[b]	TON (mol product/mol Pd)
1	**2** (1.0)	24	20	20
2	**3** (0.1)	24	18	180
3	**4** (0.1)	24	96	960
4	**4** (0.02)	24	38	1900

Aryl chlorides with substituents on the *ortho* positions are also challenging substrates to couple with arylboronic acids. The steric hindrance inhibits Pd-Ar bond formation and subsequent formation of the new C-C bond in the final coupled product. We initially investigated acyclic complex **4** in the coupling of 2-chloro-1,3-dimethylbenzene with phenylboronic acid. At 0.25 % catalyst loading, quantitative yields were obtained after 24 h with a TON of 400 (Scheme 2.3). We then extended

this chemistry to include *ortho* substituents on the arylboronic acid. The coupling reaction of 2-chloro-1,3-dimethylbenzene with *o*-tolylboronic acid also showed good activity with a TON of 97. However extending the coupling of bulky substrates to 2,6-dimethylphenylboronic acid was unsuccessful and indicated the limit to which bulky substrates could be coupled with complex **4**.



Scheme 2.3. Reaction of *ortho* substituted aryl chlorides with arylboronic acids

2.4 Conclusions

Monomeric iminoisoindoline-based palladacycle **3** can be ring-opened by reaction with PCy_3 to form the acyclic **4**. By destabilizing through inhibiting chelating effect of the κ^2-iminoisoidoline ligand in **3**, we anticipated an increased activity for the Suzuki coupling reactions of aryl chlorides. While we have no evidence for nanoparticle formation, the reaction rate for complex **4** dramatically increased as did overall TON relative to that of palladacycle **3**. This increase in activity is especially

pronounced when compared to dimeric palladacycle **2**, where **4** is more active by up to two orders of magnitude. [(η^1-iminoisoidoline)PdCl(PCy_3)$_2$] (**4**) demonstrated excellent activity for the coupling of activated and deactivated aryl chlorides and was even active when water was used as the sole reaction medium in contrast to its precursors to **3** which demonstrated no activity in water. Complex **4** also showed good activities in the coupling of bulky substrates. One of the main features when using palladacycles such as [(κ^2-iminoisoidoline)PdCl(PCy_3)] (**3**) as precatalysts is that they are usually air and moisture stable and can be stored under ambient atmosphere. While the acyclic analogue **4** was more reactive, the disadvantage of using the ring-opened complex was its reactivity with O_2 necessitating storage under inert atmosphere.

2.5 Experimental

2.5.1 General

Unless otherwise stated, all reactions were performed under N_2 using standard Schlenk techniques or in a N_2-filled drybox. All reaction temperatures for catalytic reactions refer to the temperature of pre-equilibrated oil or sand baths. ^{1}H and ^{13}C {^{1}H} NMR spectra were recorded on a Bruker 500 MHz Avance spectrometer. Chemical shifts for ^{1}H and ^{13}C NMR are reported in ppm in reference to the residual ^{1}H resonances of $CDCl_3$ (^{1}H: δ 7.26; ^{13}C: δ 77.23). Coupling constants are given in Hz. Elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer. $Pd(OAc)_2$ was purchased from PMO Pty Ltd, Australia. All other chemicals

were purchased from the Sigma-Aldrich Chemical Company and used as received. The syntheses of ligand **1** and palladacycles **2** and **3** have previously been reported.[5-8] Their synthesis has been included below due to minor changes in their preparation and improved yield as a result of this research.

2.5.2 Synthesis of iminoisoindoline (1)

Phthalaldehyde (2.31 g, 17.2 mmol), 4'-aminoacetophenone (4.37 g, 36.1 mmol), formic acid (0.05 mL) and methanol (30 mL) were combined in a 100 mL Schlenk flask equipped with a stir bar. The solution was stirred for 12 h at room temperature during which the iminoisoindoline precipitates out of solution. The resulting precipitate was isolated by filtration, washed with methanol (3 × 20 mL) and then dried under vacuum to obtain a yellow solid in 90% yield (5.70 g). ^{1}H NMR ($CDCl_3$, ppm): δ 8.11 (d, J =8.4, 2H, Ar), 8.01 (d, J = 8.8, 2H, Ar), 7.99 (d, J = 8.3, 2H, Ar), 7.50 (d, J = 7.5, 1H, Ar), 7.45 (m, 1H, Ar), 7.11 (m, 1H, Ar), 7.05 (d, J = 8.3, 2H, Ar), 6.78 (d, J = 7.6, 1H, Ar), 5.01 (s, 2H, CH_2), 2.63 (s, 3H, COCH_3), 2.59 (s, 3H, COCH_3).

2.5.3 Synthesis of [Pd(iminoisoindoline)(μ-OAc)]$_2$ (2)

Iminoisoindoline **1** (0.650 g, 1.77 mmol) and $Pd(OAc)_2$ (0.436 g, 1.94 mmol, 1.1 equiv) were dissolved in dichloromethane (30 mL) in a 100 mL Schlenk flask equipped with a stir bar. After 12 h of stirring at ambient temperature, the reaction mixture was filtered through Celite to remove palladium black. The filtrate was

concentrated and hexane (30 mL) was added to precipitate the desired palladacycle. The resulting pale brown precipitate was isolated, washed with cold hexane (3 × 10mL), and dried under vacuum to obtain **2** in 42% yield (0.437 g). ^{1}H NMR ($CDCl_3$, ppm): (*anti* and *syn* isomers are observed in solution with the *anti* isomer being the dominant form); *anti-isomer:* δ 8.26 (s, 2H, Ar), 7.85 (d, *J* = 8.0, 2H, Ar), 7.53 (m, 4H, Ar), 7.38 (m, 4H, Ar), 7.10 (d, *J* = 8.2, 2H, Ar), 7.04 (m, 2H, Ar), 6.36 (d, *J* = 8.5, 2H, Ar), 5.95 (d, *J* = 8.0, 2H, Ar), 5.77 (d, *J* = 8.1, 2H, Ar), 4.76 (d, *J* = 16.8, 2H, C*H*$_2$), 3.82 (d, *J* = 16.8, 2H, C*H*$_2$), 2.58 (s, 6H, COC*H*$_3$), 2.56 (s, 6H, COC*H*$_3$), 1.66 (s, 6H, C*H*$_3$COO).

2.5.4 Synthesis of [(κ^2-iminoisoidoline)PdCl(PCy$_3$)] (3)

A flask was charged with [Pd(iminoisoindoline)(μ-OAc)]$_2$ (**2**) (0.193 g, 0.181 mmol) and excess LiCl (0.153 g, 3.62 mmol) in acetone (20 mL) under air and the mixture was stirred for 24 h. The resulting yellow precipitate was isolated, washed with water (3 x 10 mL) then with acetone (3 x 10 mL) and dried under vacuum. The resulting yellow powder then suspended in dichloromethane and reacted with equimolar amounts of PCy_3 at room temperature. Over the course of 12 h with stirring, the yellow suspension gradually became a homogeneous solution. The resulting solution was filtered through celite and the solvent was removed under vacuum to result in **3** as a yellow powder in quantitative yields. ^{1}H NMR ($CDCl_3$, ppm) *δ* 8.19 (s, 1H, Ar), 7.92 (d, *J* = 8.7, 2H, Ar), 7.68 (d, *J* = 8.4, 1H, Ar), 7.61-7.45 (m, 4H, Ar), 7.11 (m, 1H, Ar), 6.96 (d, *J* = 8.5, 1H, Ar), 6.48 (d, *J* = 8.1, 1H, Ar), 5.21

(s, 2H, –CH_2–), 2.61 (s, 3H, –C(=O)CH_3), 2.56 (s, 3H, –C(=O)CH_3), 2.22 (m, 3H, PCy_3), 1.85 (m, 6H, PCy_3), 1.71 (m, 6H, PCy_3), 1.60 (m, 9H, PCy_3), 1.19 (m, 3H, PCy_3), 1.00 (m, 6H, PCy_3). ^{31}P NMR ($CDCl_3$, ppm) δ 37.31.

2.5.5 Synthesis of [(η^1-iminoisoidoline)PdCl(PCy_3)$_2$] (4)

In a 100 mL Schlenk flask equipped with a stir bar, [(κ^2-iminoisoidoline)PdCl(PCy_3)] (**3**) (0.607 g, 0.770 mmol) was reacted with equimolar amounts of PCy_3 in dichloromethane (10 mL). The reaction mixture was allowed to stir for 2 h following which solvent was removed under vacuum resulting in formation of **4** as a yellow powder in quantitative yields. Crystals were obtained by slow evaporation from a 50:50 dichloromethane/hexane solution. ^{1}H NMR ($CDCl_3$, ppm) δ 8.39 (s, 1H, Ar), 7.92 (d, J = 8.4, 2H, Ar), 7.87 (d, J = 8.4, 1H, Ar), 7.52 (d, J = 8.5, 1H, Ar), 7.42-7.35 (m, 2H, Ar), 7.05 (m, 1H, Ar), 6.89 (d, J = 8.3, 2H, Ar), 6.73 (d, J = 8.0, 1H, Ar), 6.36 (s, 2H, –CH_2–), 2.60 (s, 3H, –C(=O)CH_3), 2.52 (s, 3H, –C(=O)CH_3), 2.16 (m, 6H, PCy_3), 1.96 (m, 12H, PCy_3), 1.68 (m, 6H, PCy_3), 1.55 (m, 24H, PCy_3), 1.03 (m, 18H, PCy_3). ^{13}C NMR ($CDCl_3$, ppm): δ 198.34 (−C(=O)CH_3), 197.61 (−C(=O)CH_3), 156.89 (C=N–), 153.98, 149.89, 145.72, 141.56, 131.41, 131.22, 130.93, 130.46, 130.39, 129.35, 127.56, 126.40, 126.32, 123.62, 122.96, 122.22, 121.38, 121.12, 57.74 (–CH$_2$–), 31.54 (PCy_3), 31.25 (PCy_3), 30.27 (–C(=O)CH$_3$), 30.10 ((–C(=O)CH$_3$), 28.18 (PCy_3), 28.03 (PCy_3), 27.82 (PCy_3), 26.64 ((PCy_3). ^{31}P NMR ($CDCl_3$, ppm) δ 19.02. Elemental analysis (%) calcd. for $C_{60}H_{85}ClN_2O_2P_2Pd$: C 67.34, H 8.01, N 2.62; found: C 66.87, H 7.70, N 2.86. HRMS

m/z calcd for $C_{60}H_{85}ClN_2O_2P_2Pd$: 1068.4810 [M], 753.2801 $[M\text{-}PCy_3\text{-}Cl]^+$; found 753.2898 $[M{-}PCy_3{-}Cl]^+$.

2.5.6 General procedure for Suzuki coupling reactions

In a typical run, a 40 mL vial equipped with a stir bar was charged with aryl chloride (1.00 mmol), cesium carbonate (2.50 mmol), arylboronic acid (1.50 mmol) and the internal standard (dodecahydrotriphenylene, 0.10 mmol). Under nitrogen, the mixture was dissolved in 1,4-dioxane (5 mL) and a known mol % catalyst was introduced. If the catalyst loading is less than 1%, dilution or multi-step dilution for the catalyst was performed other than direct weighing. The dilution was always prepared freshly and consumed in the same day to minimize the potential degradation of the catalyst in a diluted solution. All trials with catalysts from the diluted solution were performed three times to ensure the reproducibility. The reaction mixture was sealed with a septum and moved to a pre-heated oil bath at 80 °C. After the specific time, a syringe purged with nitrogen was used to take aliquots from the reaction mixture to monitor the conversion of the starting material. Solvent was removed under vacuum, and the residue was dissolved in $CDCl_3$ for ^1H NMR analysis. Percent conversions were determined by ^1H NMR against dodecahydrotriphenylene as the internal standard. We found that percent conversions could also be determined against the remaining aryl halide by comparison of the aldehyde protons as only one product is formed (provided the aryl halide is not volatile).

2.5.7 X-ray structure determination of complex 4

Data was collected at -100 °C on a Nonius Kappa CCD diffractometer, using the COLLECT program.[35] Cell refinement and data reductions used the programs DENZO and SCALEPACK.[36] SIR97[37] was used to solve the structures and SHELXL97[38] was used to refine the structures. ORTEP-3 for Windows[39] was used for molecular graphics and PLATON[40] was used to prepare material for publication. H atoms were placed in calculated positions with U_{iso} constrained to be 1.5 times U_{eq} of the carrier atom for methyl protons and 1.2 times U_{eq} of the carrier atom for all other hydrogen atoms.

Crystal data. $C_{60}H_{85}ClN_2O_2P_2Pd$, FW = 1070.11, monoclinic, space group $P2_1/c$, a = 10.9284(3) Å, b = 25.7450(3) Å, c = 20.7944(6) Å, β = 101.9280(9)°, Z = 4, ρ_{calc}: 1.242 Mg/m^3, 76884 collected reflections, 10123 unique (R_{int} = 0.1272), Final R indices [I> 2σ(I)] were R1 = 0.0521, wR2 = 0.0895.

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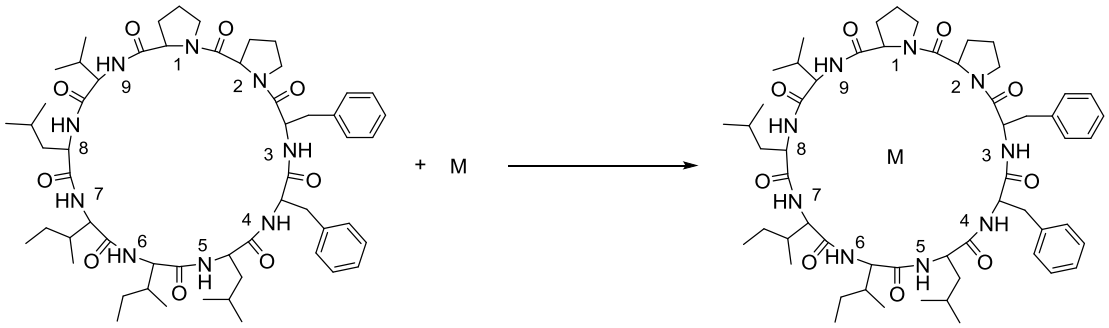
Chapter 3. Cyclinopeptides as selective ligands for light transition metals

Disclaimer

The cyclolinopeptide project was a collaborative work with Pramodkumar Jadhav who was a postdoctoral researcher in the Foley group between July 2013 and June 2014. Dr. Jadhav's work was mainly focused on synthesis of platinum and iron based complexes utilizing cyclolinopeptide A (CLA) and/or cyclolinopeptide B (CLB), while my work only involved CLA because of the scarce supply and lower purity of CLB. Dr. Jadhav noticed that CLA and CLB behaved identically when binding with the transition metal tested, and thus in some of the contexts in this chapter the plural form of "cyclolinopeptide" appeared. Any work in this thesis involving platinum and iron were done by Dr. Jadhav, and presenting results from his work was only meant to serve a better purpose of providing a comprehensive perspective of cyclolinopeptide's metal binding properties. I do, however, have no intention to give any impression that those works were performed by me. Dr. Jadhav's characterization of Pt and Fe complex is very comprehensive, involving NMR, IR, UV-Vis, EPR, MS, and Raman spectroscopy; however, none of Dr. Jadhav's spectroscopic or spectrometric data is presented according to the Departmental Thesis Writing Policy. XANES (X-Ray Absorption Near Edge Structure) experiments were performed by Robert Bauer under the supervision by Dr. Ramaswami Sammynaiken, and results were interpreted by Dr. Sammynaiken.

To avoid redundancy, I just refer "Dr. Jadhav" as "we" when presenting Dr. Jadhav's work.

3.1 Abstract



M = Cu(I), Cu(II), Fe(II), Fe(III), Pd(II)

Cyclolinopeptides are a group of naturally occurring Caryophyllaceae-type cyclopeptide (Type VI) comprised of eight or nine amino acid residues found in flaxseed (*Linum usitatissimum*) oil. In this work, we investigated cyclolinopeptides as a new class of ligand for the formation of transition metal complexes. It was discovered that these cyclic peptides possess particularly high affinity for the formation of first row transition metal complexes versus their heavier congeners. In particular, we have observed unusual reaction phenomena with regards to stabilization of copper and iron species. Synthesis and applications of these complexes along with attempts to make heavier transition metal analogues will be discussed.

3.2 Introduction

3.2.1 Facts about flax

Flax comes from the blue-flowered plant crop grown mainly in the cool, northern climate of the western Canadian prairies. Canada is the largest flax producer and exporter in the world and Saskatchewan is the major contributor to Canada's flax production.[1] Nutritious value is the major contributor for flax's increasing demand,

especially the significant percentages of omega-3 fatty acids found in flaxseed.[2]

Table 3.1. Production of flax by province in Canada, unit is 10^3 tons

Year	Manitoba	Saskatchewan	Alberta	Western Canada Total
2000/2001	205.7	469.9	17.8	693.4
2001/2002	199.4	495.3	20.3	715.0
2002/2003	214.6	444.5	20.3	697.4
2003/2004	195.6	533.4	25.4	754.4
2004/2005	132.1	355.6	29.2	516.9
2005/2006	147.3	881.4	53.3	1,082.0
2006/2007	193.0	759.5	36.3	988.8
2007/2008	105.4	511.8	16.3	633.5
2008/2009	161.3	666.8	33.0	861.1
2009/2010	193.0	708.7	28.4	930.1
2010/2011	81.3	311.2	30.5	423
2011/2012	54.6	289.6	54.7	398.9
2012/2013	66	381	41.9	488.9
2013/2014	48.3	492.8	73.7	614.8
2014/2015	99.0	501.2	216.0	816.2

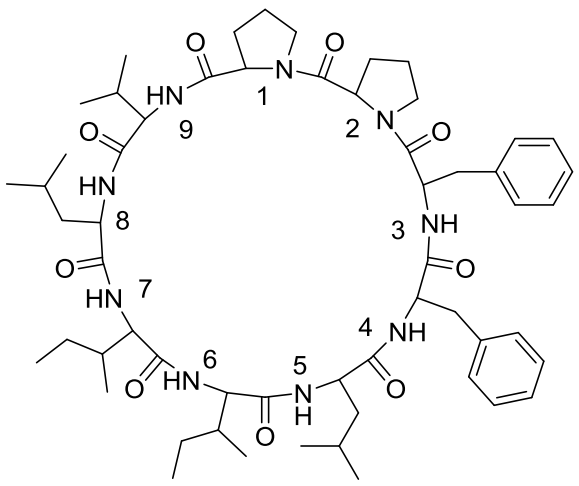
3.2.2 CLA

Cyclolinopeptides are a class of cyclic peptides comprised of eight to nine amino acid residues found in flaxseed oil. Cyclopeptides are under the category of Caryophyllaceae-type cyclopeptide, and cyclopeptide is defined as cyclic compounds

formed mainly with the peptide bonds of 2-37 amino acid residues and discovered in higher plants.[3] Methods of extracting cyclolinopeptides from flax, as well as separating them each as a pure substance were successfully developed by Reaney *et al*.[4-6]

Cyclolinopeptide A (CLA) was the first cyclopeptide discovered back in 1959, making it also the first cyclolinopeptide discovered.[7] Likely reasons for CLA being first cyclolinopeptides discovered are (i) CLA is the most abundant among all, (ii) CLA is the easiest cyclolinopeptide to be separated and isolated.[6]

CLA has been fully characterized for the past half century, and the sequence of CLA was determined as *c*(Pro-Pro-Phe-Phe-Leu-Ile-Ile-Leu-Val) from early structural studies in the 1970s.[8-10] The crystal structure of CLA was first reported by Di Blasio *et al.* in 1987,[11] followed by several other research groups successfully obtained crystal structures in other solvent conditions.[12, 13] Figure 3.1 showed a representation of CLA that will be used in this thesis. CLA's crystal structure could be found at Figure 1.5 in Chapters 1.6.3.



CLA

Figure 3.1. Representation of CLA that is used in this thesis

One major research motivation for CLA is its similar structure to Antamanide (Figure 1.6 in Chapters 1.6.3), a homodetic cyclodecapeptide isolated from the poisonous mushroom *Amanita phalloides*, which possesses strong immunosuppressive activities.[15, 16] Siemion *et al.* were the first to report CLA's strong immunosuppressive activity in 1991.[17] Their follow up study concluded that CLA's immunosuppressive activity was to some extent competitive to another well-known immunosuppresor: Cyclosporin A (Figure 1.7 in Chapters 1.6.3).[18-20]

Other than immunosuppressive activities, CLA was also found being able to bind with K+, Na^{+}, Ca^{2+}, Mg^{2+}, and Ba^{2+} from NMR and CD studies, with a binding affinity to Ba^{2+} being the strongest.[21] However, this tendency for metal ion complexation is strongly reduced compared to Antamanide, [22, 23] and to the best of our knowledge no application of the resulting metal ion complex binding with CLA has been reported.

A Sci-Finder® search performed on April 15, 2015 showed only 190 articles contained the concept of cyclolinopeptides, with most entries involved in CLA's structural studies, immunosuppressive studies, as well as structural and immunosuppressive studies on synthesized CLA derivatives. Very few articles got cited more than 10 times, even for review articles, and there is no example showcasing CLA coordinating with transition metals. As organometallic chemists we believe there is much of CLA chemistry that remains unexplored. We envisage CLA as a naturally occurring, low-toxic, large-sized ligand for transition metals. The objective here is to explore its coordination ability, study the structure of the resulting

metal-CLA complexes, and evaluate its catalytic activities if a well-defined system can be successfully obtained and characterized. Thus the objective of this project is more fundamental in nature consisting of exploring the coordination chemistry of CLA to transition metals and subsequently studying their potential reactivity in organometallic reactions.

3.2.3 Transition metal complexes incorporating peptides (preferably cyclic)

As mentioned previously, there has been no report of transition metal complexes incorporating CLA or any other cyclolinopeptides to date. There are however several examples of transition metal complexes incorporating peptides. These examples are reasonably well cited, and they offered certain enlightenment to our own choice of transition metal precursors, as well as choice of characterization methods for this project.

The Lippard group from MIT showcased Pt-based complexes incorporating small linear peptides with promising cancer therapeutic properties (Scheme 1.9 in chapters 1.6.5),[24] and their structure of Pt precursor inspired us to achieve significant breakthroughs in our own Pt-based complexes incorporating CLA (more details in 3.3.1).

Other examples from Pt-themed peptide related research appeared to be much less useful; moreover, there has been no report of crystal structures in this area. At least there was one reported crystal structure of a Pd-themed peptide related research, and in this study the success of obtaining crystal extends to a copper precursor for the

same reaction conditions (Scheme 1.10 in Chapters 1.6.5).[25-27]

In Cu-themed peptide related research, the crystal structure of Cu incorporating a larger sized peptide (asciacyclamide) was also reported.[28] Interestingly, two copper atoms were inside of the ring to accommodate a larger sized cyclic peptide (Figure 1.8 in Chapters 1.6.5).

One advantage of copper coordination chemistry is that even without a crystal structure, EPR and colour changes (UV/Vis) of the copper complexes could still provide valuable insights about the reaction mechanism. Comba *et al.* fully utilize these two characterization methods for patellamide A derivatives and their corresponding Cu(II) complexes. (Scheme 1.11 in chapters 1.6.5.)[29]

3.3 Results and Discussions

3.3.1 Cyclolinopeptide complexes of precious metals

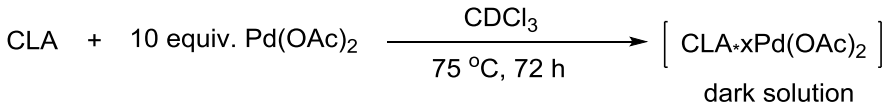
It is worth addressing some points at the beginning of this section. The quantity of CLA provided for this project is only one gram, and it has been shared between Dr. Jadhav and me. To maximize number of trails, almost all the reaction of CLA with transition metal precursors were at NMR tube scales to keep the consumption of CLA at approximately ten milligrams for each experiment. Since those were NMR tube scale reaction, and ^{1}H NMR spectroscopy is an affordable and readily available method of characterization, ^{1}H NMR spectroscopy has always been involved as one of the characterization methods. Because of the low reaction scale, in many cases the reactions took place in deuterated solvents to make the subsequent

NMR characterization more convenient. ^{1}H NMR spectroscopy unfortunately could not provide us with critical structural assignment of the resulting transition metal complex because of the complicated and broad features exhibited in the ^{1}H spectrum of CLA. However, change in the ^{1}H NMR spectrum could potentially provide valuable insights: Shionoya *et al.* noticed that the ^{1}H NMR spectrum became much more symmetrical when their Pt-based complex incorporating cyclic peptide was formed.[30] ^{1}H NMR spectroscopy could also provide preliminary assessment if the oxidation state of the transition metal had changed after complexation: for example, if the ^{1}H spectrum of a Cu^{2+} precursor is very sharp after the introduction of CLA, it could indicate that the paramagnetic Cu^{2+} got reduced to a diamagnetic Cu^{+} complex.

CLA was screened with common palladium precursors to explore its coordination properties to palladium. Palladium acetate ($Pd(OAc)_2$) was initially selected because of its good availability, relative affordable price, distinct NMR signals, as well as good solubility in a variety of organic solvents. In addition, $Pd(OAc)_2$ has good thermal stability to a certain degree, at least in the conditions of our test: we performed a controlled test by promoting the temperature of $Pd(OAc)_2$ (in $CDCl_3$) at 80 ^{o}C for 48 h and observed no sign of decomposition.

CLA was first reacted at room temperature with $Pd(OAc)_2$ under a variety of stoichiometric conditions (1:2, 1:1, 2:1). No colour change had been observed, nor was any change observed by ^{1}H NMR spectroscopy. Significant colour change of the reaction mixture would occur only when excess equivalents of $Pd(OAc)_2$ were used under elevated temperatures and prolonged reaction times (Scheme 3.1). Peaks

between 2.5 to 1.5 ppm in ^{1}H NMR became much sharper, indicating that some interaction should have happened. However, ESI-MS was attempted of the resulting dark coloured solution but was unsuccessful, neither was the attempt of growing crystals out of the solution.



Scheme 3.1. Reaction of CLA with $Pd(OAc)_2$

It was envisaged that by deprotonating the NH proton(s) on CLA, CLA would have negatively charged site(s) and thus more accessible for coordination with Pd^{2+} precursors. NaO*t*Bu was first used because of its affordability and strong basic property; moreover, it is an non-nucleophilic base that shoud not interfere with the process of transition metal binding to CLA. Multiple ratios of NaO*t*Bu to CLA (2:1, 4:1, 8:1) was studied, but even at eight equivalents of NaO*t*Bu no deprotonation of the NH proton that appear at ~7 ppm region on ^{1}H spectrum were observed. $Pd(OAc)_2$ was introduced after the addition of the base, and reaction at neither room temperature nor at an elevate temperature could result in ESI-MS signals to be observed.

*t*BuLi was also used as the base as it is arguably the strongest commercially available base. Immediate deprotonation of the NH proton was observed from the disappearance of the peaks at around 7 ppm region in ^{1}H spectrum. This resulting substance was monitored by ^{1}H NMR 12 h after the initial addition of *t*BuLi; the CLA was reprotonated after 12 h. As a result, $Pd(OAc)_2$ was introduced twenty

minutes after the addition of *t*BuLi, however, reaction at neither room temperature nor at an elevate temperature could allow ESI-MS be successfully obtained. Solvents attempted for reactions of $Pd(OAc)_2$ with CLA include $CDCl_3$, C_6D_6 and DMSO-d_6.

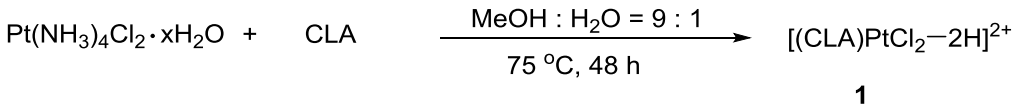
Other commercially available Pd precursors such as $PdCl_2$ and $Pd(MeCN)_2Cl_2$ suffer from solubility issues: with $PdCl_2$ being insoluble in all $CDCl_3$, C_6D_6 and DMSO-d_6 and $Pd(MeCN)_2Cl_2$ is only soluble in DMSO-d_6. Despite the solubility problem, tests with or without different types of base, as well as at room temperature or at an elevated temperature were performed. ESI-MS could not be successfully obtained either.

It was also envisaged that if a Pd(0) precursor was used, deprotonation of CLA wouldn't be necessary to reduce the energy barrier for CLA to coordinate on the Pd atom. However, commonly used commercially available Pd(0) precursor $Pd_2(dba)_3$ decomposed upon contact with CLA.

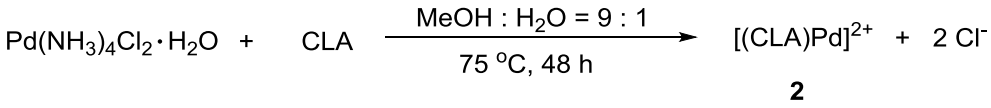
In the meantime, we were working on screening platinum-based precursors for reactions with cyclolinopeptides A because platinum, in the same group as palladium, shares many chemical similarities; moreover, platinum complexes tent to have interesting cytotoxicity properties, particular for cancer therapy. We anticipated that once we successfully synthesized well-defined [(CLA)Pt] complexes, our collaborators in the College of Pharmacy could perform cytotoxicity studies on them.

Initial screening tests with Pt/CLA combinations suffered similar inclusive results as palladium analogues, however work done by the Lippard group inspired us with the choice of platinum precursors.[24] Commercially available $Pt(NH_3)_4Cl_2 \cdot xH_2O$

happened to share some structural similarities as Lippard's Pt precursor, and a preliminary reaction studying $Pt(NH_3)_4Cl_2 \cdot xH_2O$ with CLA was highly promising (Scheme 3.2). Inspiration was raised to use the palladium analogue of this platinum precursor and replicate the exact same reaction conditions (Scheme 3.3).



Scheme 3.2. Reaction of $Pt(NH_3)_4Cl_2 \cdot xH_2O$ with CLA gives product **1** as characterized by ESI-MS



Scheme 3.3. Reaction of $Pd(NH_3)_4Cl_2 \cdot H_2O$ with CLA using exact same condition as Scheme 5.1 gives product **2** as characterized by ESI-MS

CLA is highly soluble in methanol but its solubility decreased drastically in water; neither $Pt(NH_3)_4Cl_2 \cdot xH_2O$ nor $Pd(NH_3)_4Cl_2 \cdot H_2O$ are soluble in methanol. Therefore, a MeOH : H_2O = 9 : 1 solution was found to be most feasible to solve the solubility problems. Both $Pt(NH_3)_4Cl_2 \cdot xH_2O$ and $Pd(NH_3)_4Cl_2 \cdot H_2O$ are white powders in solid form and colourless when dissolved by MeOH : H_2O = 9 : 1 solution; the final Pt and Pd products share the same physical properties as their corresponding precursors. This final air and moisture stable palladium complex **2** could be characterized by ESI-MS (Figures 3.2 − 3.4) and matches exactly with the calculated

isotopic pattern of the chemical formula $[C_{57}H_{85}N_9O_9Pd]^{2+}$

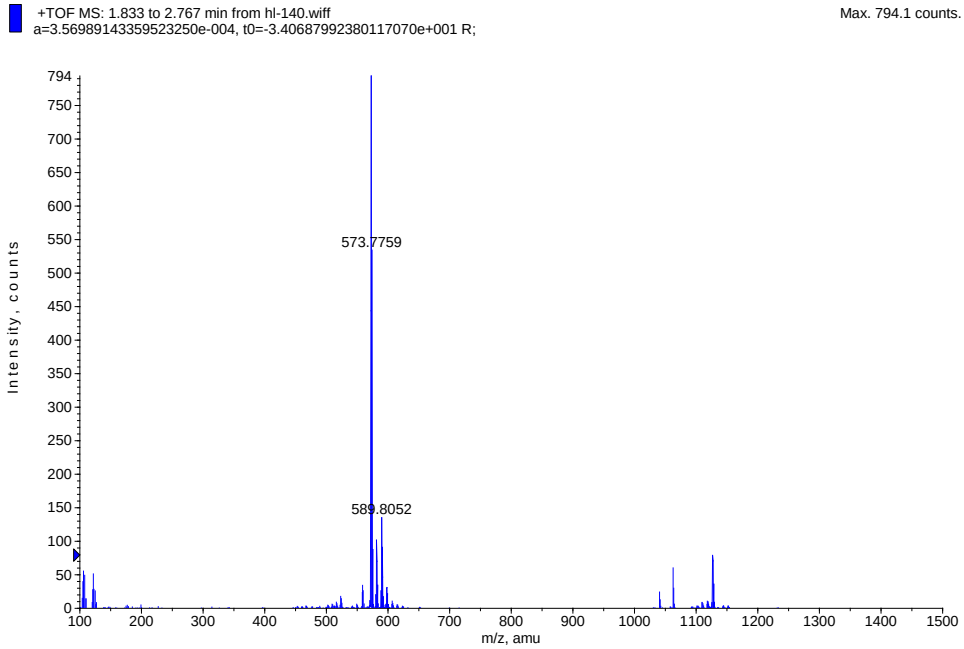


Figure 3.2. Full scope of the ESI-MS plot of **2**

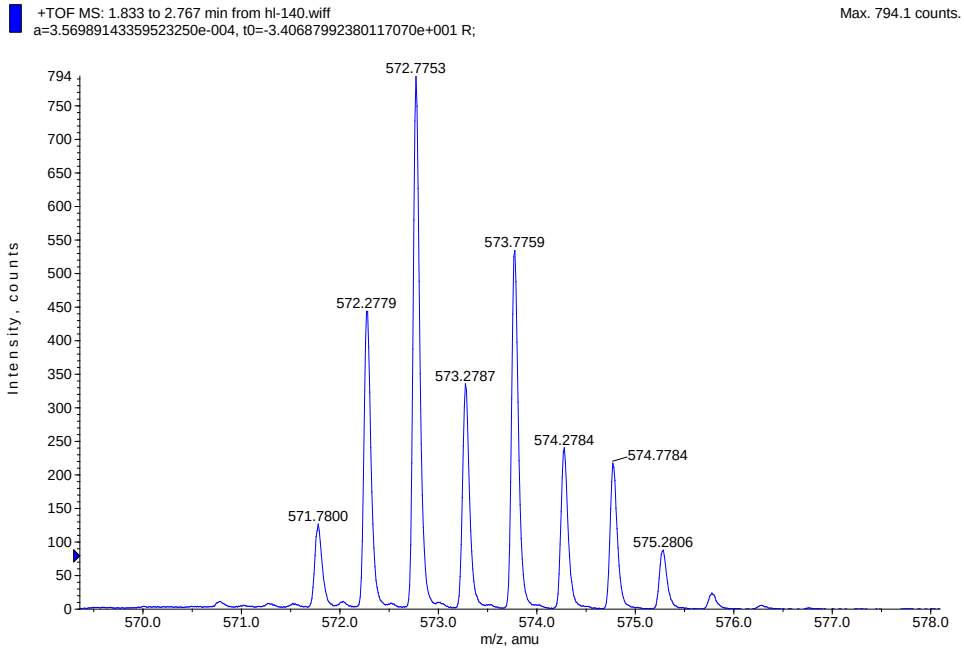


Figure 3.3. Major peak expanded for **2**

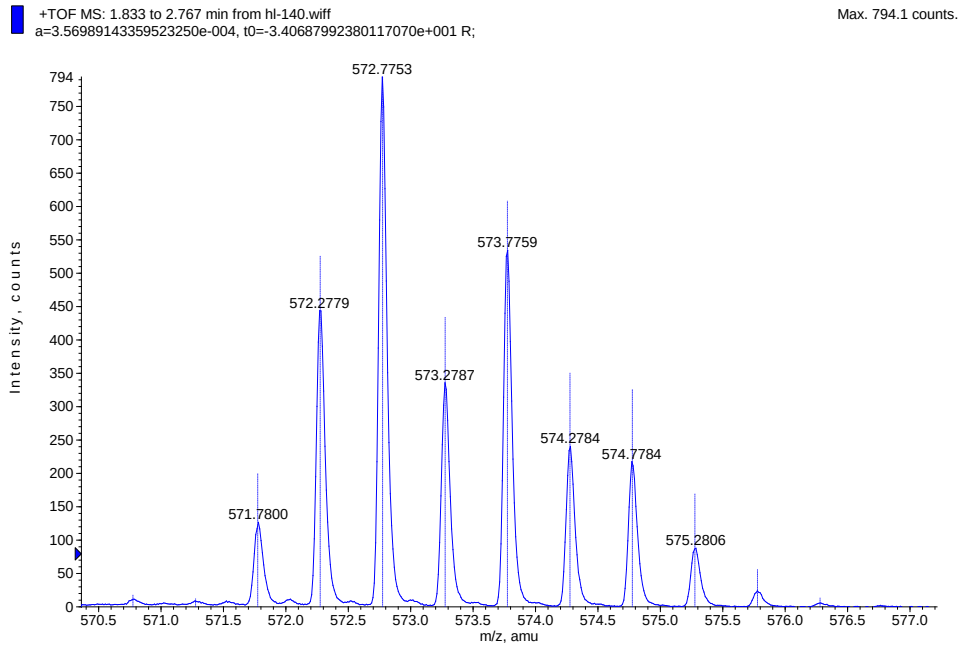
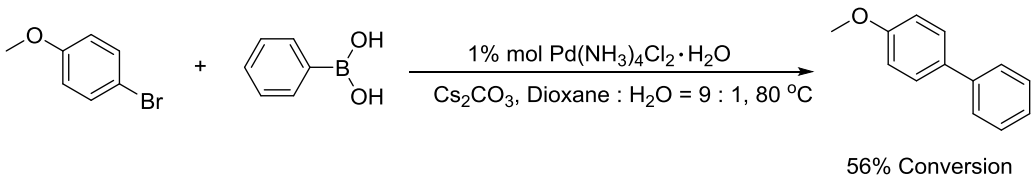


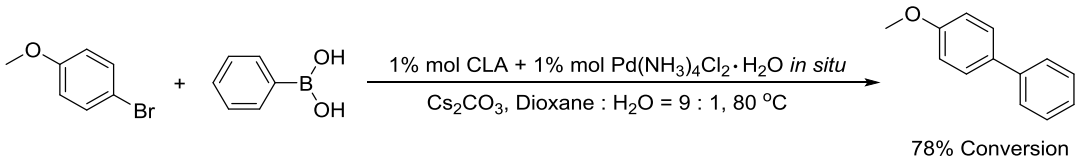
Figure 3.4. Figure 3.3 with calculated isotopic pattern of **2** superimposed on actual data for **2** (Dashed lines represent calculated isotope pattern for **2**)

As the case in Scheme 3.2, the ESI-MS plot showed the major peak of **1** fit in the description of chemical formula $[(CLA)PtCl_2 - 2H]^{2+}$ (comparing to the $[(CLA)Pd]^{2+}$ of **2**), the difference of the two chlorine atoms remaines unexplained. It was also noticed that despite varying the stoichiometry of CLA and $Pt(NH_3)_4Cl_2$ (1:2, 1:1, 2:1) in Scheme 3.2, the final product remained as $[(CLA)PtCl_2 - 2H]^{2+}$, consistent with platinum was coordinated to multiple sites within the CLA ring and not just on the outside.

Catalytic activity of $[(CLA)Pd]^{2+}$ complex was evaluated in Suzuki coupling reactions using 4-bromoanisole and phenylboronic acid (Scheme 3.4 and 3.5). Compared to the palladium precursor $Pd(NH_3)_4Cl_2 \cdot H_2O$, the catalytic activity of $[(CLA)Pd]^{2+}$ was slightly improved.



Scheme 3.4. Evaluation of catalytic activity of $Pd(NH_3)_4Cl_2 \cdot H_2O$ using Suzuki coupling reactions



Scheme 3.5. Evaluation of catalytic activity of **2** formed *in situ* using Suzuki coupling reactions

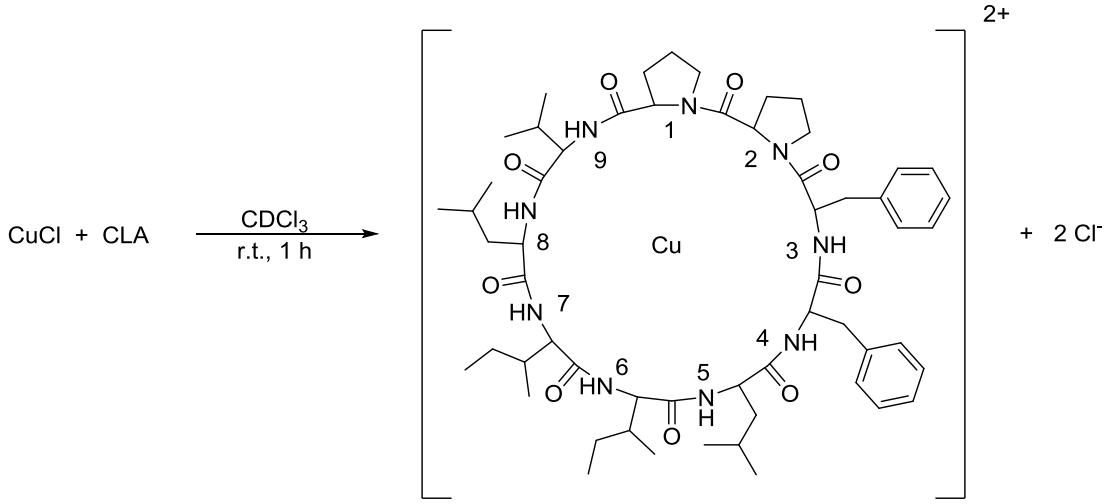
The reactions with palladium and platinum complexes all require long reaction times, high temperature and the formed complexes were not well-defined species. We spent tremendous efforts optimizing the reaction conditions as well as searching literature references. It appears to be the nature of the Pd/Pt complexes being not best suited for the CLA coordination studies. As mentioned previously, to the best of our knowledge, there is no crystal structure reported of Pt complex incorporating linear or cyclic peptides, and only one example of Pd in that matter. On the other hand, several examples of Cu complex incorporating cyclic peptides have been reported, from a smaller cyclic tetrapeptide to a bigger cyclic octapeptide. It seems reasonable to start exploring CLA's interaction with first row transition metals starting with copper.

3.3.2 Cyclolinopeptide complexes of first row transition metals

3.3.2.1 Cyclolinopeptide complexes incorporating copper

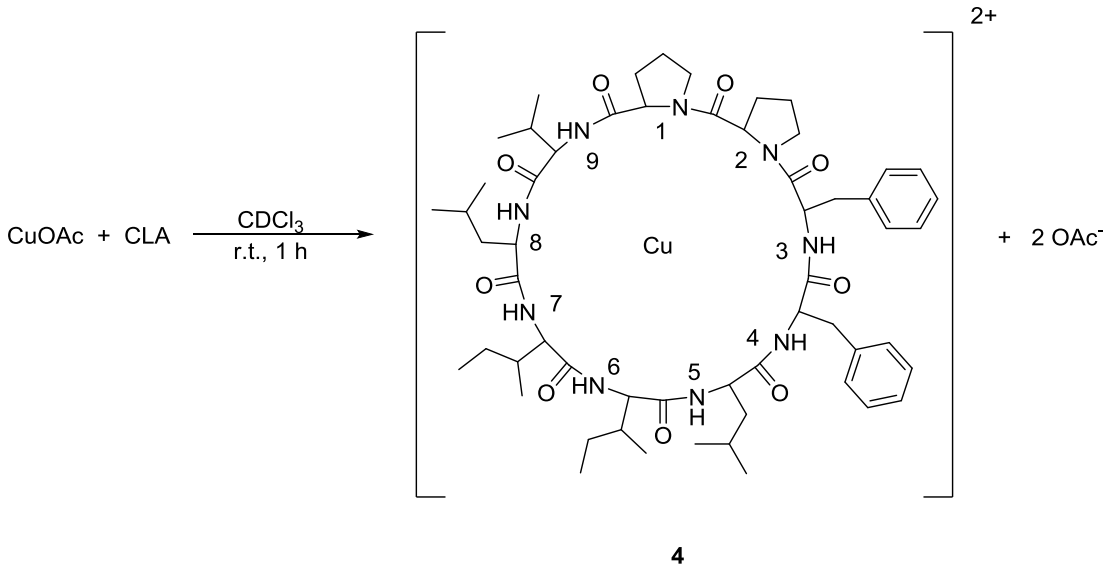
The Foley group has expertise in copper chemistry, and our understanding of copper chemistry allowed us to choose copper as a possibly better candidate with CLA because its light transition metal character such as smaller atom size might promote coordination with CLA.

A preliminary study of Cu(I) precursors with CLA on NMR tube scale reactions was performed (Scheme 3.6 and 3.7). CuCl and CuOAc are both insoluble in $CDCl_3$. Introduction of 1 equiv. of CLA in the presence of 1 equiv. of CuCl or CuOAc in $CDCl_3$ resulted in immediate solubilization of the copper precursor and formation of a homogeneous solution.



3

Scheme 3.6. Reaction of CLA with CuCl in an NMR tube scale reaction



Scheme 3.7. Reaction of CLA with CuOAc in an NMR tube scale reaction

^{1}H NMR spectra were successfully obtained with no broadening, indicating that the formed complexes could very likely be Cu(I) complexes. ESI-MS plots were also successfully obtained, and it was a significant breakthrough by the characterization with ESI-MS that clean peaks with an exact match to the isotopic pattern of the chemical formula $[(C_{57}H_{85}N_9O_9)Cu]^{2+}$ could be observed. ESI-MS plots (Figure 3.5) using CuOAc as the copper precursor (Scheme 3.6 and 3.7) was shown with major peak expansion (Figure 3.6) and was compared to calculated isotopic pattern (Figure 3.7). This experiment demonstrated that copper could be a more suited metal to react with CLA as the reaction conditions were very mild and the resulting ESI-MS plots were as expected.

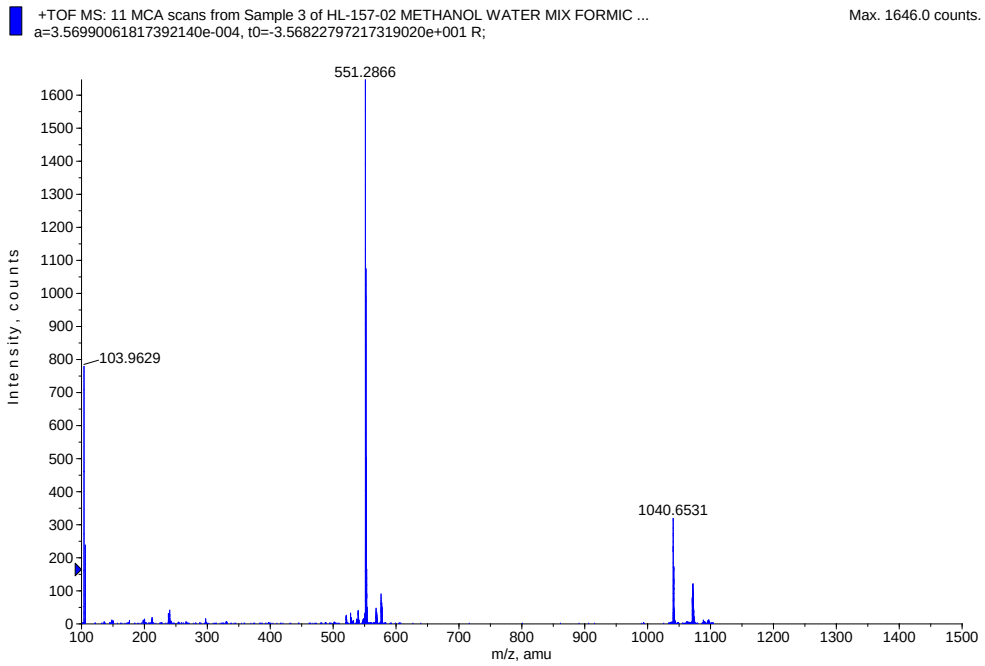


Figure 3.5. Full scope of the ESI-MS spectrum of product **4**

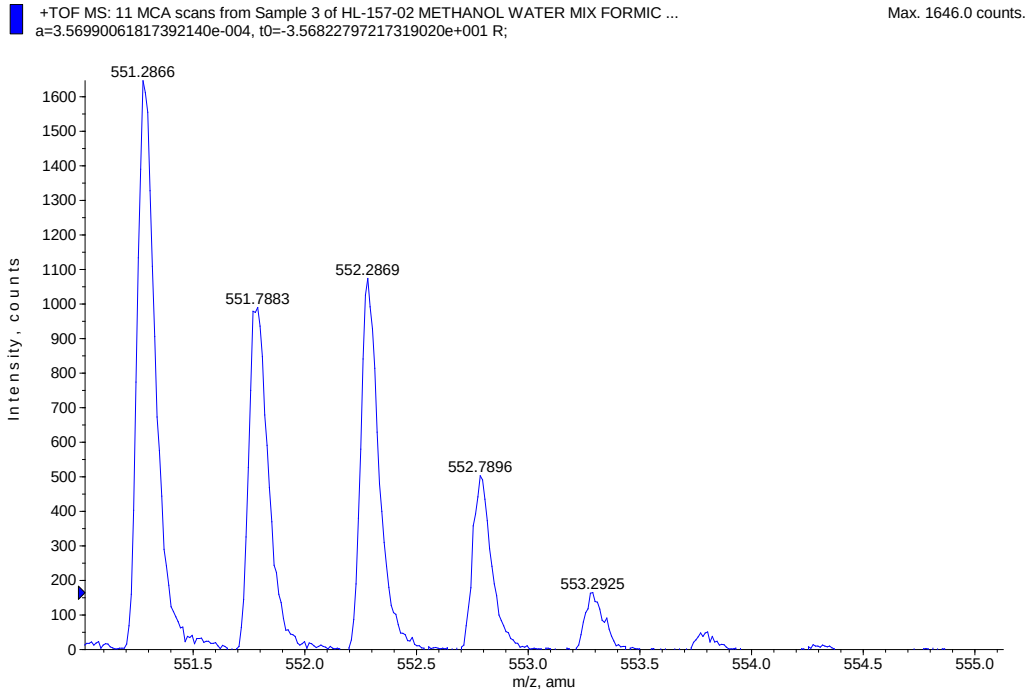


Figure 3.6. Major peak of **4** expanded from Figure 3.5

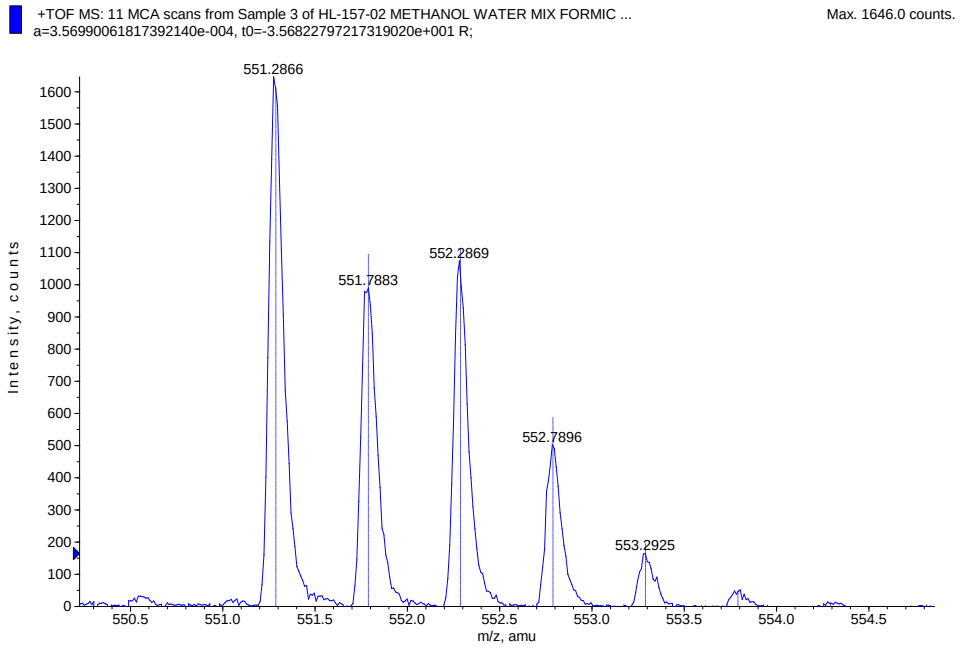
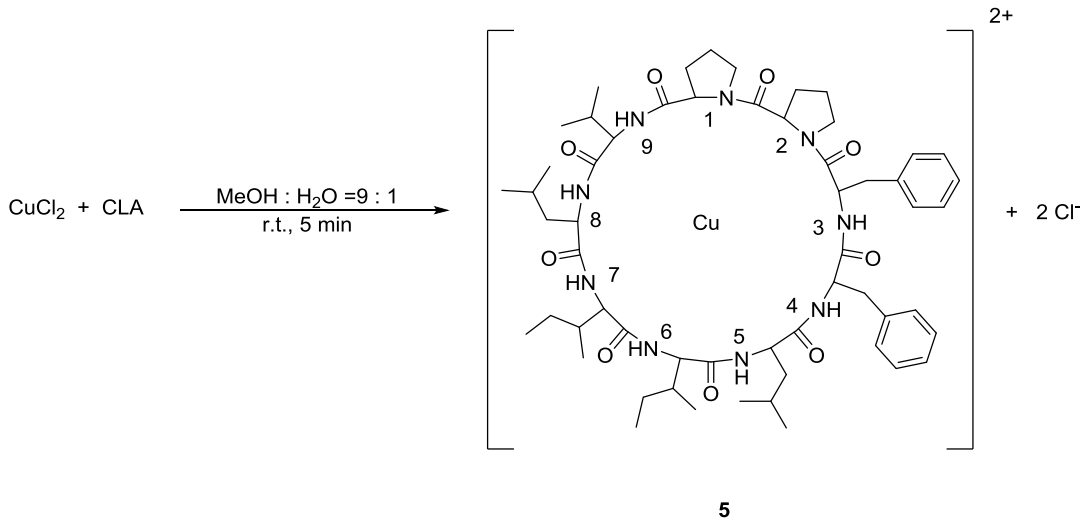


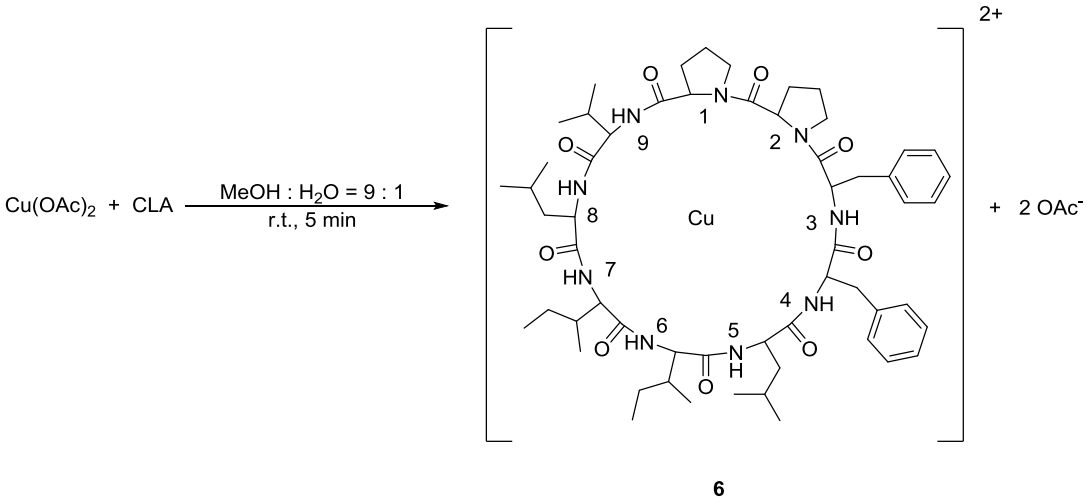
Figure 3.7. Figure 3.6 superimposed with calculated isotopic pattern of $[(C_{57}H_{85}N_9O_9)Cu]^{2+}$

With to the success with Cu(I) precursors, analogues reactions of Cu(II) precursors were performed. Reactions with Cu(II) precursors took place virtually instantaneously under mild conditions (Scheme 3.8 and 3.9), with a colour change from blue/green to a pale yellow colour that was identical to that of the Cu(I) experiments (Scheme 3.6 and 3.7).



5

Scheme 3.8. Reaction of CLA with $CuCl_2$ in mild conditions



6

Scheme 3.9. Reaction of CLA with $Cu(OAc)_2$ in mild conditions

ESI-MS spectrum also confirmed the feasibility of this reaction, with only one peak showing as the desire peak (Figure 3.8). The expanded region (Figure 3.9) looked identical to the case using Cu(I) as the copper precursor (Figure 3.7); also, the isotopic pattern matched exactly as the calculated one using the chemical formula chemical formula $[(C_{57}H_{85}N_9O_9)Cu]^{2+}$, which is interpreted as $[(CLA)Cu]^{2+}$. To avoid

redundancy in the following context the major peak expansion from ESI-MS plots will be omitted; major peak expansion superimposed with calculated isotopic pattern will be kept to serve the same purpose.

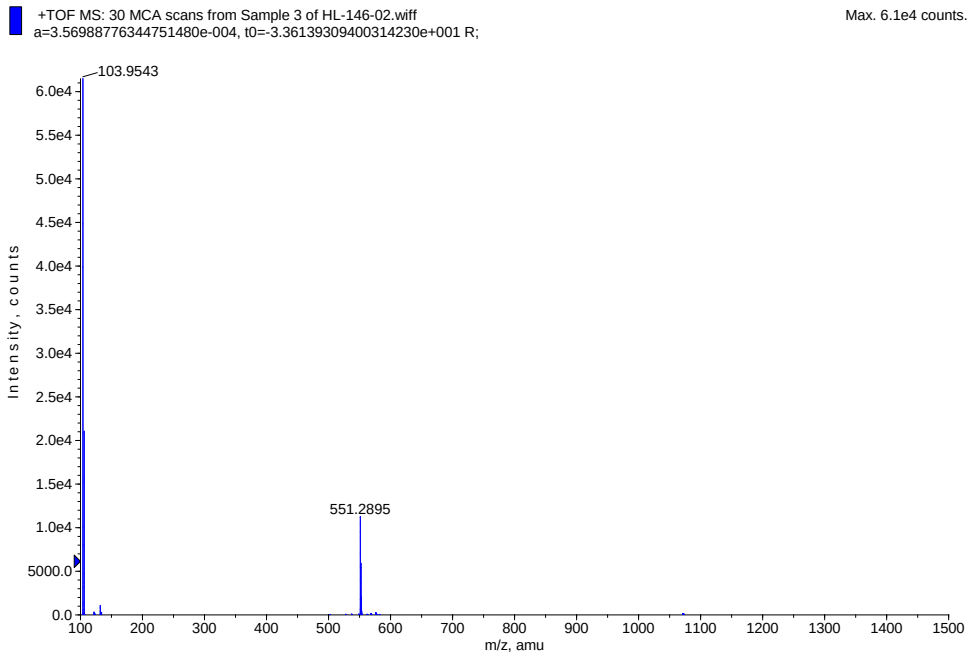


Figure 3.8. Full scope of the ESI-MS spectrum of product **6** in Scheme 8.2

It is worth mentioning that for Figure 3.5 there is still excess CLA appearing at m/z=1040 region while for Figure 3.8 the plot is remarkably clean. Weighing Cu(I) precursors in a glovebox at very low loading (~ 1mg) resulted the issue of unreliable reading of its mass because of electrostatics, while this issue does not apply to weighing Cu(II) precursors in open atmosphere.

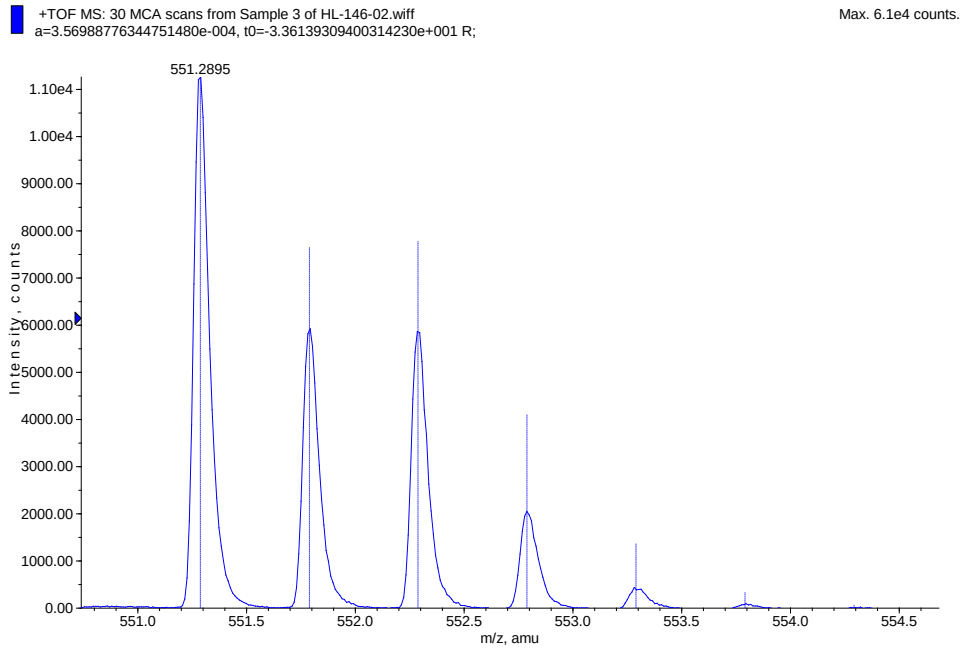


Figure 3.9. Major peak from Figure 3.8 superimposed with calculated isotopic pattern of $[(C_{57}H_{85}N_9O_9)Cu]^{2+}$

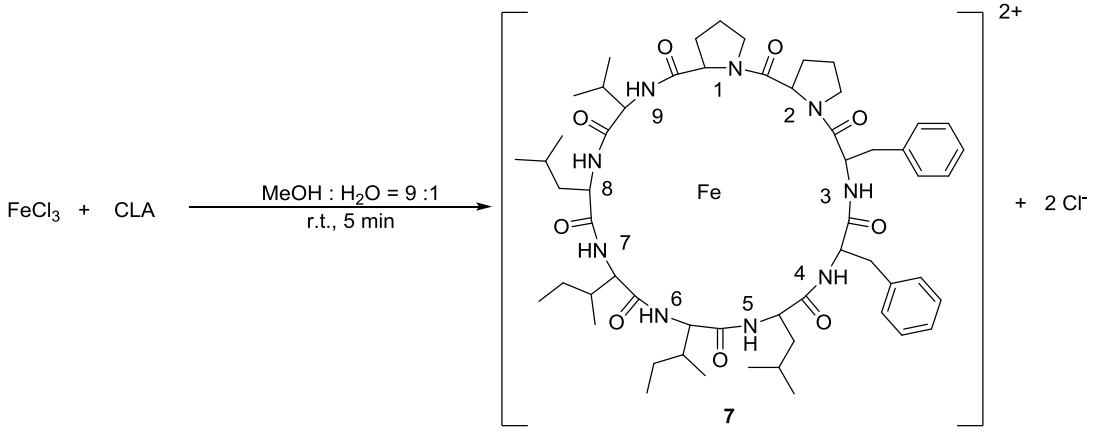
Moreover, the products from Scheme 3.8 and Scheme 3.9 resulted in ^{1}H NMR spectra with no peak broadening, indicating that the formed copper complexes are most likely diamagnetic; if so, along with the observed colour changes of the reaction mixture, it is reasonable to speculate that CLA reduced the Cu(II) precursors to Cu(I) during coordination and keep the oxidation state of copper as Cu(I) in this complex.

To summarize, significant breakthroughs in Cu-themed coordination chemistry with CLA has been accomplished particularly in terms of utilizing ESI-MS as the characterization method. It appears that CLA has a significantly higher affinity to Cu-based precursors compared to Pt and Pd-based precursors, with reactions all taking place in mild conditions and virtually no time. Moreover, redox reaction could have taken place based on the observation of colour fading from blue/green (Cu(II)

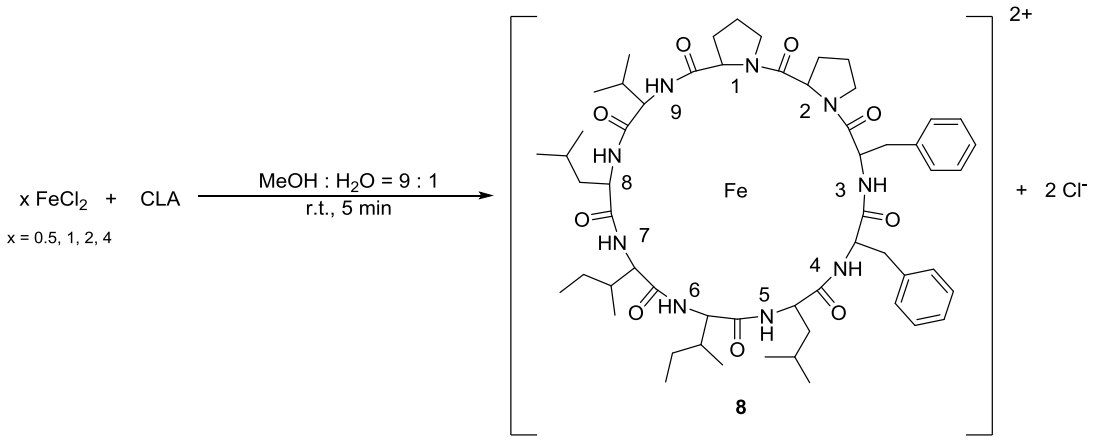
precursors) to pale yellow (possible Cu(I) complexes incorporating CLA). We were motivated to investigate the scope this redox phenomenon to other first row transition metals and iron appeared as a good candidate because iron also has more than one commonly observed oxidation states; moreover, Fe^{2+} is not as air sensitive as Cu^{+}.

3.3.2.2 Cyclolinopeptide complexes incorporating iron

$FeCl_3$ was tested with CLA under the same mild reaction conditions as Cu(II) precursors to explore if any significant colour change would occur. As with Cu(II), reaction of Fe(III) with CLA resulted in rapid colour fading and production of NMR spectra consistent with a diamagnetic species (Scheme 3.10). These evidences all indicated that CLA has reducing ability during coordination with light transition metals. Moreover, when Fe(II) was used as the precursor to react with CLA under a variety of stoichiometric conditions (Fe:CLA = 1:1, 2:1, 4:1, 1:2), only one peak consistent with $[(CLA)Fe]^{2+}$ was observed, consistent with iron being coordinated to multiple sites within the CLA ring and not just on the outside (Scheme 3.11).



Scheme 3.10. Reaction of CLA with $FeCl_3$ in mild conditions



Scheme 3.11. Reactions of CLA with $FeCl_2$ in mild conditions under a variety of stoichiometric ratios

3.3.2.3 XANES of [(CLA)Cu] and [(CLA)Fe] complexes

X-Ray Absorption Near Edge Structure (XANES) were employed to determine the oxidation state of copper in complex **6** from Scheme 3.9, complex **7** from Scheme 3.10 and complex **8** from Scheme 3.11 by Robert Bauer from the Department of Physics. The experiments were conducted at 95 K to minimize the photo reduction of Cu(II) to Cu(I) during the X-ray absorption. Preliminary results from XANES showed that the oxidation state of copper is Cu(I) and the oxidation state of iron is Fe(II) in these complexes. Figure 3.10 and 3.11 are provided by Dr. Sammynaiken and CPA is a preferred naming than CLA by Dr. Sammynaiken, thus no change was attempted in these figures.

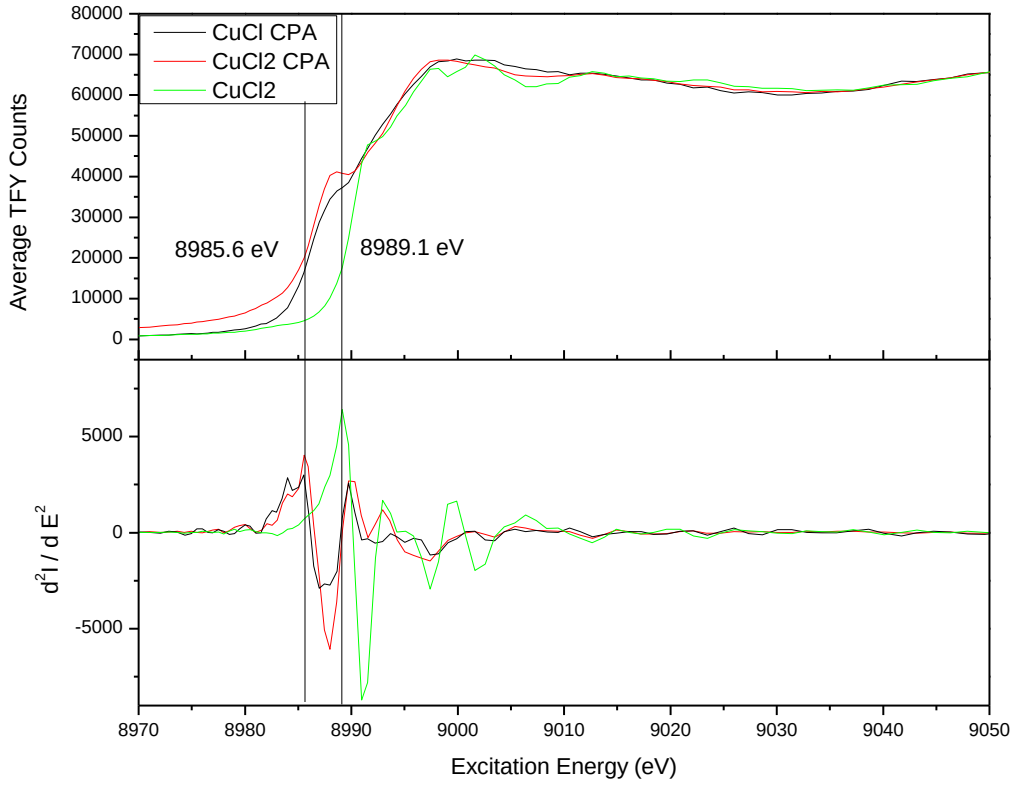


Figure 3.10. Copper K-edge XANES of [Cu(II)CLA] and [Cu(I)CLA] complexes and $CuCl_2$ standard Copper complexes

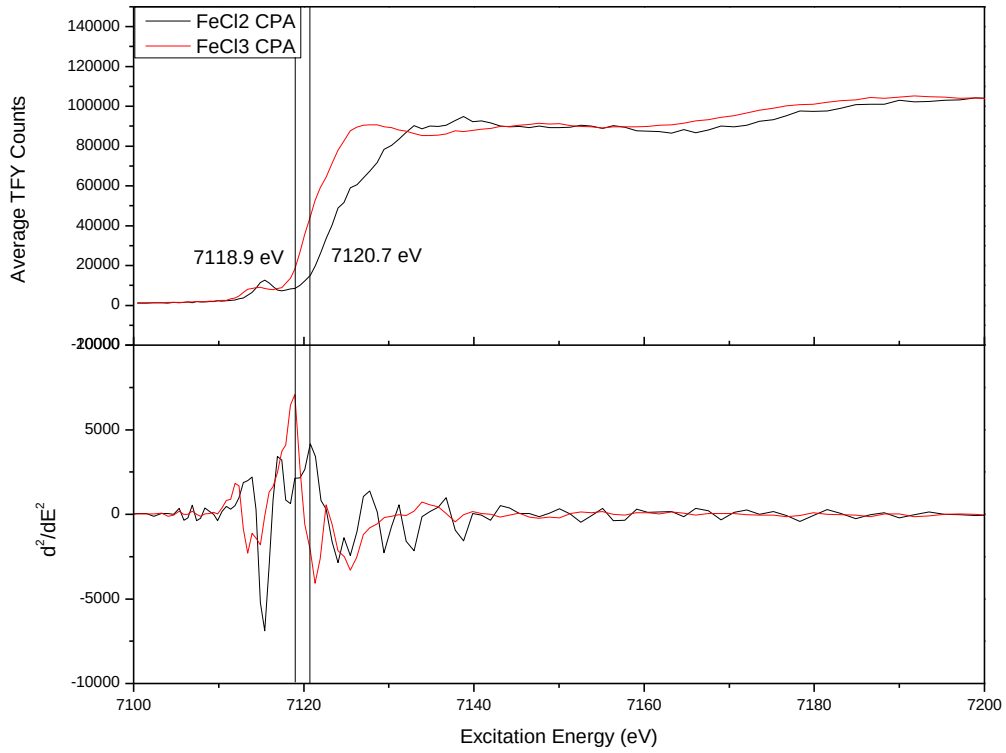


Figure 3.11. Iron K-edge XANES of [Fe(II)CLA] and [Fe(III)CLA] complexes

The copper shows a significant shift in the edge feature when complexed with cyclolinopeptide A. The result may indicate a change in the oxidation state of the copper from Cu(II) to Cu(I). An energy shift of ~ 5 eV is sufficient to justify this statement.[31] Figure 8.1 shows a shift of 2.9 eV of the peak positions, and 3.5 eV of the edge onset. This shift is close to 5 eV but not enough to definitively state that there is a change in the oxidation state. If the complexes are Cu(I); the intensity of the edge peak with respect to the other edge features seems to indicate that both have a coordination number of 3, and are either in a T-shaped arrangement or trigonal

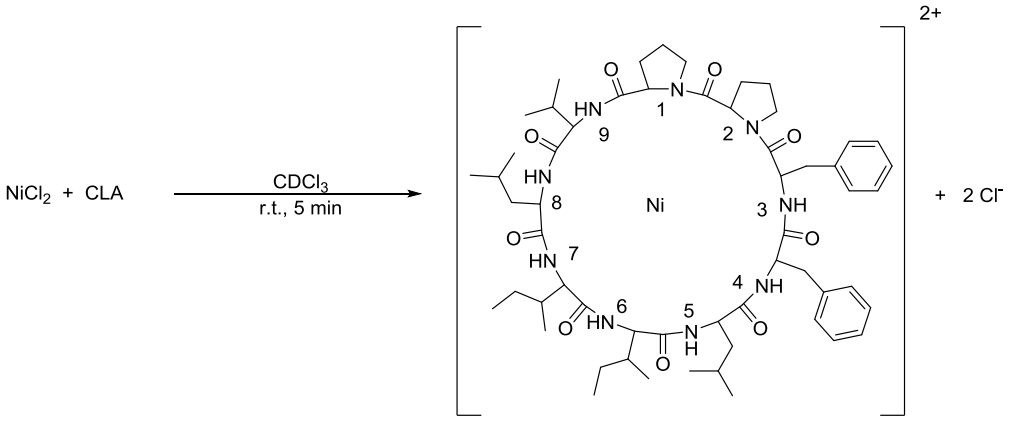
planar.[32, 33] Due to the differences in the features T-shaped seems more likely two different bond angles. More analysis by XANES is required to better conclude the oxidation state of copper.

The Iron also shows a shift the edge onset of 1.8 eV; between the two complexed oxidation states of iron with the cyclolinopeptide A. The shift may indicate that the Fe(III) when complexed has a lower affinity for its electrons converting a percentage of the iron to a lower oxidation state Fe(II) complex. The pre-edge features indicate that the Fe(II) has changed oxidation state having a higher percentage of Fe(II) than Fe(III) complex. The Fe(II) pre-edge forbidden transition between the 1s and 3d states are indicated by peaks less than ~ 7112.1 eV and Fe(III) greater than ~7113.5 eV.[34] Figure 8.2 shows that the pre-edge features for both the iron complexes indicates that it is most likely a mixture of the two iron oxidation states; as described in the paper by Berry *et al*.[35] Further XANES experiments are required to verify this. If both oxidation states are presented in the complex, then the complex from Fe(II) looks like it has the T_d geometry while the Fe(III) complex has the O_h geometry. If the iron is in the complexes is Fe(II); the complex from Fe(II) is likely C_{4v} geometry and Fe(III) is still likely O_h.[33]

3.3.2.4 Cyclolinopeptide complexes incorporating nickel

After extensive reactions with palladium precursors and easily successful reactions with copper precursors, screening test with nickel was highly anticipated since it is in the same group as palladium and only one atomic number less than

copper. Preliminary test with $NiCl_2$ were conducted under the same mild conditions as reactions with Cu(I) precursors. $NiCl_2$ is insoluble in $CDCl_3$, but the suspension of $NiCl_2$ in $CDCl_3$ was easily dissolved with the introduction of CLA, consistent with coordination taking place virtually instantaneously (Scheme 3.12).



9

Scheme 3.12. Reaction of CLA with $NiCl_2$

ESI-MS experiments showed that product **9** also fits in the general $[(CLA)M]^{2+}$ format, with $[(CLA)Ni]^{2+}$ as the major product peak.

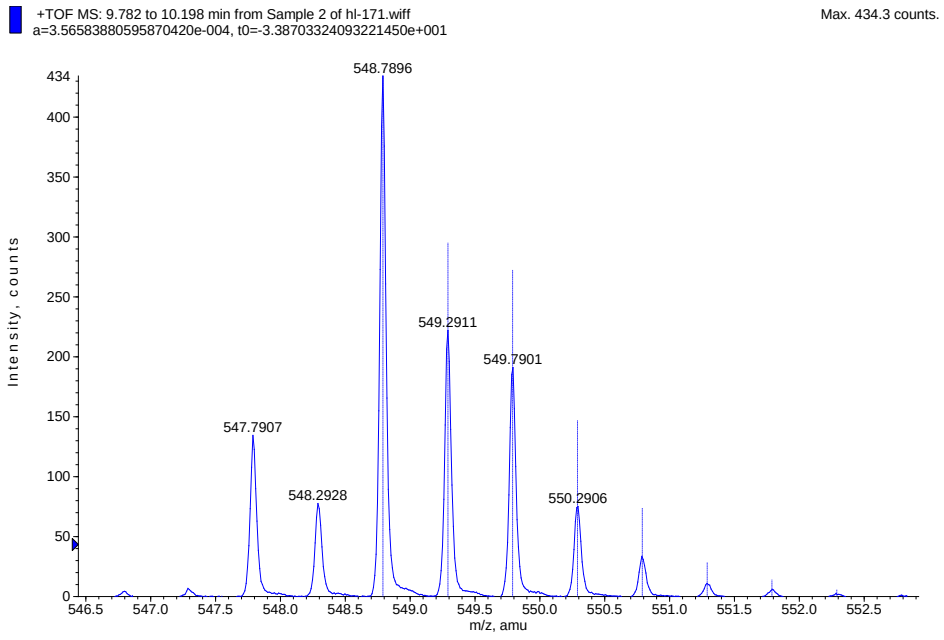


Figure 3.12. Major ESI-MS peak of product **9** superimposed with calculated isotopic pattern of $[(CLA)Ni]^{2+}$

The left side of Figure 9 shows that the experimental isotopic splitting pattern does not match perfectly with the calculated isotopic splitting pattern. One possible explanation is that a second complex **10** with the chemical formula $[(CLA)Ni{-}2H]^{2+}$ coexists with the major product **9**. Calculated isotopic pattern for **10** was plotted on the ESI-MS plot (Figure 3.13). The ratio between product **9** and product **10** is roughly 3:1 after calculation by Analyst® QS software.

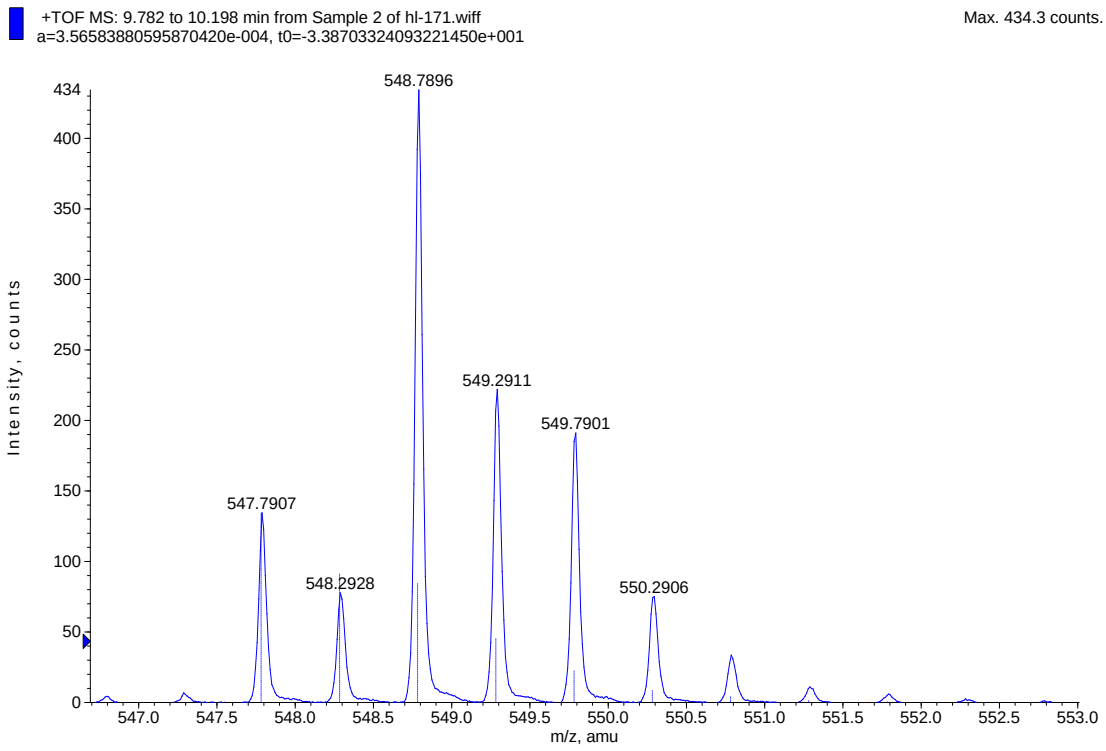


Figure 3.13. Calculated isotopic pattern of $[(CLA)Ni{-}2H]^{2+}$ (product **10**) overlapping with ESI-MS plot from Scheme 3.12.

3.4 Conclusions

In conclusion, cyclolinopeptides are a new class of ligand that are completely isolated from natural products and can bind with light transition metals with ease. Initial results suggest that cyclolinopeptide has the ability to reduce Cu(II) to Cu(I) and Fe(III) to Fe(II) during the coordination process. With other transition metals such as nickel and cobalt, more than one product could be formed. It appears CLA prefers to bind light transition metals over their heavier metal analogues, with palladium and platinum precursor all require prolonged heating. The nature of the coordination remains unknown, and attempts to grow crystals for X-ray diffraction studies have been unsuccessful to date.

3.5 Experimental

Unless otherwise stated, all NMR tube reactions were performed under N_2 using standard Schlenk techniques or in a N_2-filled drybox. All reaction temperatures refer to the temperature of pre-equilibrated oil or sand baths. ^{1}H NMR spectra were recorded on a Bruker 500 MHz Avance spectrometer. High resolution HPLC-MS was performed on an Agilent HPLC 1100 series directly connected to QSTAR XL Systems (Mass Spectrometer Hybrid Quadrupole-TOF LC-MS/MS, Applied Biosystems, Toronto, Canada) and electrospray ionization (ESI) was used. $Pd(OAc)_2$ was purchased from Precious Metals Online (PMO) Pty Ltd, Australia. All other chemicals were purchased from the Sigma-Aldrich Chemical Company and used as received.

3.5.1 Synthesis of $[(CLA)PtCl_2]^{2+}$ (1)

CLA (0.010 g, 0.0096 mmol) and tetraamineplatinum(II) chloride hydrate ($Pt(NH_3)_4Cl_2 \cdot xH_2O$) (0.003 g, 0.001 mmol) were combined in a small vial, 2 mL of MeOH : H_2O = 9:1 solution was introduced to the vial. The vial was sealed and heated at 75 ^oC for two days. ESI-MS *m/z* calcd for $[C_{57}H_{83}N_9O_9PtCl_2]^{2+}$: 651.265 $[M]^{2+}$; found 651.2596 $[M]^{2+}$.

3.5.2 Synthesis of $[(CLA)Pd]^{2+}$ (2)

CLA (0.012 g, 0.012 mmol) and $Pd(NH_3)_4Cl_2 \cdot H_2O$ (0.003 g, 0.01 mmol) were combined in a small vial, 2 mL of MeOH : H_2O = 9 : 1 solution was introduced to the vial. The vial was sealed and heated at 75 ^oC for two days. ESI-MS *m/z* calcd for $[C_{57}H_{85}N_9O_9Pd]^{2+}$: 572.775 $[M]^{2+}$; found 572.7753 $[M]^{2+}$.

3.5.3 Synthesis of $[(CLA)Cu]^{2+}$ (3)

CuCl (0.002 g, 0.02 mmol) was suspended in 2mL of $CDCl_3$ in a small vial under inert atmosphere at room temperature. CLA (0.012 g, 0.012 mmol) was introduced to the suspension and homogenation of the reaction mixture was observed overnight. ESI-MS *m/z* calcd for $[C_{57}H_{85}N_9O_9Cu]^{2+}$: 551.289 $[M]^{2+}$; found 551.2866 $[M]^{2+}$.

3.5.4 Synthesis of $[(CLA)Cu]^{2+}$ (4)

CuOAc (0.0020 g, 0.020 mmol) was suspended in 2mL of $CDCl_3$ in a small

vial under inert atmosphere at room temperature. CLA (0.012 g, 0.012 mmol) was introduced to the suspension and homogenation of the reaction mixture was observed overnight. ESI-MS *m/z* calcd for $[C_{57}H_{85}N_9O_9Cu]^{2+}$: 551.289 $[M]^{2+}$; found 551.2866 $[M]^{2+}$.

3.5.5 Synthesis of $[(CLA)Cu]^{2+}$ (5)

CLA (0.011 g, 0.011 mmol) and $CuCl_2$ (0.002 g, 0.015 mmol) were combined in a small vial, 2 mL of MeOH : H_2O = 9:1 solution was introduced to the vial. ESI-MS *m/z* calcd for $[C_{57}H_{85}N_9O_9Cu]^{2+}$: 551.289 $[M]^{2+}$; found 551.2895 $[M]^{2+}$.

3.5.6 Synthesis of $[(CLA)Cu]^{2+}$ (6)

CLA (0.011 g, 0.011 mmol) and $Cu(OAc)_2$ (0.002 g, 0.01 mmol) were combined in a small vial, 2 mL of MeOH : H_2O = 9 : 1 solution was introduced to the vial. ESI-MS *m/z* calcd for $[C_{57}H_{85}N_9O_9Cu]^{2+}$: 551.289 $[M]^{2+}$; found 551.2895 $[M]^{2+}$.

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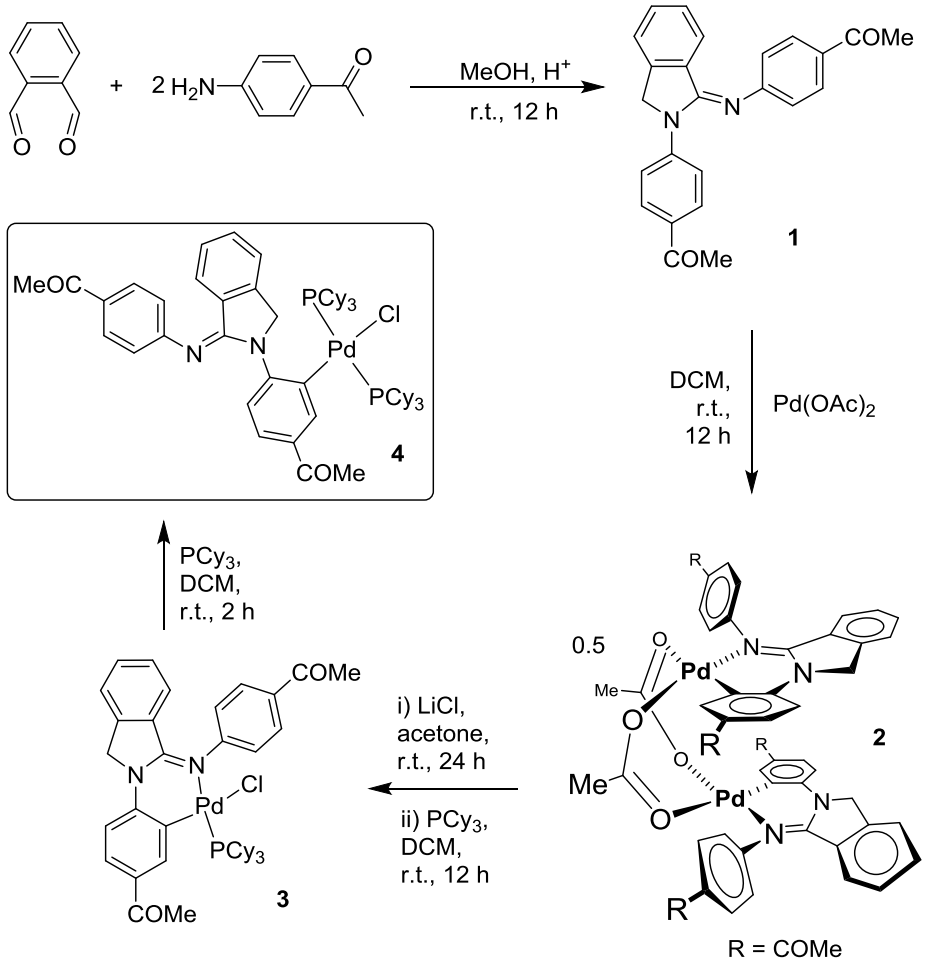
Chapter 4. Summary and conclusion

The object of part 1 of this M.Sc. thesis was to ring-open a well-defined monomeric palladacycle with a strong Lewis base to obtain a well-defined acyclic analogue of the palladacycle. The object subsequently extended to perform a direct comparison in Suzuki reactions coupling aryl chloride and phenylboronic acid to observe a significant improvement in the catalytic activity from the monomeric palladacycle to the ring-opened analogue. Follow the scheme shown in Scheme 4.1, the monomeric palladacycle **3** and its corresponding ring-opened analogue **4** were successfully synthesized, characterized and their catalytic activity were evaluated in Suzuki reactions. During this work, the catalytic efficacy of the monomeric palladacycle [(κ^2-iminoisoidoline)PdCl(PCy_3)] (**3**) was evaluated and found to be more active than the dimeric palladacycle **2**. The palladacycle **3** was subsequently ring-opened by the strong Lewis base PCy_3 to form an acyclic species [(η^1-iminoisoidoline)PdCl(PCy_3)$_2$] (**4**). Complex **4** is characterized by MS, NMR and X-ray studies, all of which were consistent with the anticipated acyclic structure. By destabilizing the κ^2-iminoisoidoline in **3**, it was anticipated that an increased activity for Suzuki coupling reactions of aryl chlorides would be observed. The reaction rate for complex **4** dramatically increased in terms of overall TON relative to that of palladacycle **3**. This activity enhancement is particularly phenomenal when compared to the dimeric palladacycle **2**, where **4** is up to two orders of magnitude more active. [(η^1-iminoisoidoline)PdCl(PCy_3)$_2$] (**4**) exhibited remarkable activity for the coupling of activated and deactivated aryl chlorides; moreover, [(η^1-

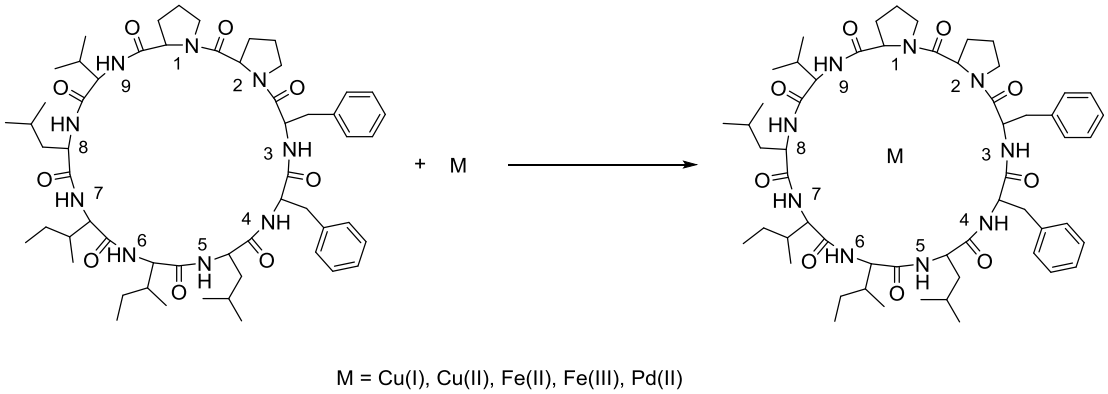
iminoisoidoline)PdCl(PCy_3)$_2$] (**4**) was even active when water was used as the sole reaction medium in contrast to the palladacyclic precursors **2** or **3** which demonstrated no activity when water was present. Furthermore, Complex **4** demonstrated great activity in the coupling of bulky substrates. One of the main advantages of using palladacycles such as [(κ^2-iminoisoidoline)PdCl(PCy_3)] (**3**) as precatalysts is that they tolerate air better than complex **4**. While the acyclic analogue **4** was more reactive, the disadvantage of using the ring-opened complex was its reactivity with O_2 necessitating storage under inert atmosphere.



Scheme 4.1. Synthesis of palladacycle [(κ^2-iminoisoidoline)PdCl(PCy_3)] (**3**) and acyclic species [(η^1-iminoisoidoline)PdCl(PCy_3)$_2$] (**4**)

The other part of this M.Sc. work was to focus on coordination chemistry of palladium with CLA. The initial research motivation of this project was to utilize CLA isolated from flaxseed oil as a new class of ligand and explored its coordination chemistry with palladium, as well as evaluating if the presence of CLA would improve the catalytic activity of palladium precursors in Suzuki C-C coupling reactions. The reactions of CLA with Pd-based, as well as Pt-based precursors, all required harsh reaction conditions, such as elevated reaction temperatures and prolonged reaction times. Among all the Pd precursors such as $Pd(OAc)_2$, $PdCl_2$, $Pd(MeCN)_2Cl_2$, $Pd_2(dba)_3$ and $Pd(NH_3)_4Cl_2$, only $Pd(NH_3)_4Cl_2 \cdot H_2O$ produced appreciable amount of products are characterized by ESI-MS. While the catalytic activity of [(CLA)Pd] complexes in Suzuki reactions did improve for the coupling of aryl bromides with arylboronic acids versus its $Pd(NH_3)_4Cl_2 \cdot H_2O$ precursor, the improvements are too small to conclude that CLA played any significant role in the catalytic mechanism to improve the catalytic efficacy of the resulting [(CLA)Pd] complex. Investigation of cytotoxic activities of $[(CLA)PtCl_2-2H]$ is still ongoing from our collaborators in College of Pharmacy. In contrast, Cu and Fe-based complexes incorporating CLA formed virtually instantaneously upon the introduction of CLA to the metal under mild conditions, and the ESI-MS showed little to no fragmentation being observed. It would be great if the crystal structure studies of the resulting complexes were successful to allow us to have a better perspective about how exactly transition metals are coordinated to CLA; however, due to the inherent poor crystallinity of CLA, it would be difficult in nature to obtain crystal structures of

CLA coordination complexes with transition metals. Moreover, it is interesting to observe the potential redox ability of CLA to reduce Cu(II) to Cu(I) and Fe(III) to Fe(II) as evidenced by colour change, ^{1}H NMR spectroscopy, as well as XANES. While more work needs to be done to fully assure this supposition, this work represents the first example of a cyclic peptide exhibiting redox properties to the best of our knowledge.



Scheme 4.2. Coordination chemistry of CLA with select transition metals