Syntheses and anti-cancer evaluation of optically pure gold (I) phosphine complexes

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**SYNTHESES AND ANTI-CANCER EVALUATION OF OPTICALLY
PURE GOLD(I) PHOSPHINE COMPLEXES**

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

2015

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List of Abbreviations

δ	chemical shift (ppm)
$^{\circ}\text{C}$	degree centigrade
Bu	butyl
calcd.	calculated
Cisplatin	Cis-diaminedichloroplatinum(II)
aq	Aqueous
Ar	aryl (substituted aromatic ring)
<i>n</i> Bu	<i>n</i> -butyl
<i>t</i> Bu	<i>tert</i> -butyl
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dppe	1,2-bis(diphenylphosphino)ethane
ee	enantiomeric excess
eq	equatorial
d	doublet
Et	ethyl
g	gram
equiv	equivalent

h	hour
Hz	Herz
IC ₅₀	Concentration at which the cell colony forming ability is reduced by 50%
GC-MS	Gas Chromatograph Mass Spectrometer
IR	infrared
H	hydrogen
m	multiplet
Me	methyl
mg	milligram
mp	melting point
NMR	Nuclear magnetic resonance
<i>J</i>	coupling constants
Ph	phenyl
ppm	part per million
q	quartet
RNA	ribonucleic acid
rt	room temperature
MHz	megahertz

min	minute(s)
mL	milliliter
mmol	millimole
mol %	mole percent
s	singlet
t	triplet
tert	tertiary
v/v	volume to volume ratio
$[\alpha]_D$	Specific optical rotation measured at the sodium D line (589nm)
$[\alpha]_{436}$	Specific optical rotation measured at the wave length of 436nm
Å	angstrom
sat	saturated
Tf	trifluoromethanesulfonyl
THF	tetrahydrofuran
TLC	thin layer chromatography
SAR	structure-activity relationship

Chapter 1

General Introduction

Chapter 1

General Introduction

1.1 Cancer and Cancer Therapy

Cancer represents a collection of diseases which are caused by certain changes to cells function-controlling genes. The most common character of cancer cells is that they divide without stopping and spread into other tissues of the human body. Currently, there are more than a hundred type of cancers that have been defined, caused either by inheritance or certain environmental exposure.

Cancer can be generally divided into the following types: Carcinoma, which is formed by epithelial cells inside and outside the human body; Sarcoma, formed by bone cells and soft tissues including muscle; Leukemia, formed by the bone marrow; Lymphoma, formed by lymphocytes cells; Multiple myeloma, from plasma cells; Melanoma, which begins in melanocytes forming cells and other types of cancers like breast cancer, *etc.* The complexity and variety of cancer diseases demand different and specialized treatments.

Commonly used cancer treatment includes surgery, radiation therapy, chemotherapy, immunotherapy, precision medicine, targeted therapy, hormone therapy and stem cell transplant. Although much progress have been made to date, lots of problems remain unsolved. The greatest challenge is how to reduce the side effect accompanied by all kinds of therapies, which requires the discovery of a clear cancer cell related-target. For chemotherapy the development of a new generation of anti-cancer

drugs that only inhibits cancer cell growth is highly desirable. Besides, it has been proven that the genomic characteristics of single cancer cell differs from each other even in the same tumors, which leads to the resistance of cancer therapy and raises the possibility of cancer relapse. These facts urges researchers to optimize the current cancer therapy methods on one hand, and also to develop new cancer therapies that are more efficient and with high selectivity between cancer cells and normal cells in our human body, so as to provide faster and safer treatments.[1]

1.2 Metal Complexes in Chemotherapy

Metal complexes have various applications in a wide spectrum of pathological disorders including chemotherapy. In 1965, the inhibition of *Escherichia coli* bacteria by *cis*-[PtCl₂(NH₃)₂] (cisplatin) was first described by Barnett Rosenberg.[2] Researchers moved on and found cisplatin to be highly effective at inhibiting cancer cell lines. Nowadays, cisplatin and its carboplatin and oxaliplatin derivates are used to treat various types of cancers.[3]

The mechanism of cisplatin has been well studied. Firstly, one of the chloride ligands is slowly displaced by water molecules within the cell, where the resulting aqua ligand [PtCl(H₂O)(NH₃)₂]⁺ readily binds to guanine (DNA) giving [PtCl(guanine-DNA)(NH₃)₂]⁺. Additional cross-linkage can further occur via displacement of the remaining chloride ligand, typically by another guanine. In this way,

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cisplatin crosslinks DNA in several different patterns. Apoptosis is then initiated upon inability of the cell to repair the ‘damaged’ DNA. [4]

Scheme 1.1 Chemical structure of cisplatin and platinum-based anticancer drugs.

On top of platinum anticancer agents, ruthenium complexes with oxidation state +2 and +3 have been found to have anticancer activity especially against metastatic cancers. Similar with cisplatin, ruthenium complexes also have the ability to bind DNA so as to stop cancer growth. Na *trans*-[Ru(Im)(Me₂SO)Cl₄] (NAMI) and (ImH)*trans*-[Ru(Im)(Me₂SO)Cl₄] (NAMI-A) (Scheme 1.2) are currently being tested in clinical applications.[5a] Mechanistic research shows that both NAMI and NAMI-A can reduce the angiogenesis activity in tumor growth which will lead the cancer cells to apoptosis process.[5b]

Scheme 1.2 Chemical structure of ruthenium complexes NAMI and NAMI-A.

Gallium complexes have attracted much attention on their anticancer property. In the early 1980s, gallium salts (gallium nitrate for example) have already been proved to be cytotoxic against lymphoma.[6] Complex tetrakis(1-octanol) tris(5-aminosalicylate) gallium(III) was effective in inhibition of several cancer cell lines. The mechanism of gallium complexes' cytotoxicity is not clear yet, but evidence have shown that these complexes have multiple effects on membrane permeability, the cytoskeleton, mitochondria and enzymes which support cancer cell growth.[7]

Scheme 1.3 Chemical structure of anticancer gallium complexes

Another metal used in cancer treatment is rhenium, and radioactive $^{186/188}\text{Re}$ compounds have been developed as an radiation therapy agent. Besides, rhenium complexes are also applied in cell imaging as luminescent probes.[8] Recently, researchers have found that the Re complexes are also promising candidates for chemotherapy. Numerous Re(I) tricarbonyl bisimine complexes have been synthesized and identified with anticancer activity. (Scheme 1.4) Although Rh complexes' cytotoxicity mechanism have not been clearly identified, the current distribution research shows that

Re(I) complexes never stay in the cell nucleus, but accumulate in the cytoplasm instead,[9] thus demonstrating that Re(I) complexes have totally different cytotoxicity mechanism with cisplatin and its derivatives.

Scheme 1.4 Re(I) tricarbonyl bisimine complexes with anticancer activity.

Titanocene dichloride (TiCp_2Cl_2) and its derivatives are also found to have potent anticancer activity against various sorts of human cancer cell lines especially in renal carcinoma. Except titanium, a series of metallocenes with generally +4 oxidation state transition metals have been identified to have anticancer activity (Scheme 1.5). The cytotoxicity of titanocene dichloride and vanadocene dichloride complexes have been well studied, both complexes inhibiting DNA and RNA syntheses by accumulating in the nucleus of cancer cells. It is not hard to illustrate that its similar structure with cisplatin lead to their similarity in bioproperty.[10]

Scheme 1.5 The structure of metallocenes with anticancer activity.

M=Ti, V, Cr, Mn, Zr, Nb, Mo, Tc, Hf, Ta, W, Re

More and more transition metal complexes were also found to have cytotoxicity against cancer, which have indeed proved the potential of transition metal complexes as anti-cancer agents.

1.3 The medicinal applications of gold: past, present and future

Chrysotherapy gold based treatment can be found throughout the history of human civilization. In the early 2500 BC, the Chinese applied gold based drugs for different diseases. Gold based treatments were also developed in the 17th century in Europe to cure ailments including melancholy, fainting, fevers, *etc.* The mixture of gold chloride and sodium chloride $\text{Na}[\text{AuCl}_4]$ was applied in syphilis treatment during the 19th century.[11]

Medicinal research on gold based drugs continued until the 20th century when Robert Koch discovered the bacteriostasis of $\text{K}[\text{Au}(\text{CN})_2]$ against tubercle bacillus in 1890, gold therapy was introduced for the tuberculosis treatments later in the 1920s. Although gold therapy was soon proved ineffective towards tuberculosis, gold compounds were found to be very effective against rheumatoid arthritis. A lot of results have indicated that gold compounds are also very effective in treatment of other types of rheumatic diseases like juvenile arthritis, discoid lupus erythematosus, palindromic rheumatism, *etc.* The medicinal application of gold drugs was also developed in inflammatory skin disorders therapy.[12] In 1985, the gold phosphine complex auranofin

was introduced as a major treatment against rheumatoid arthritis.[13] Numerous research has been done related with the cytotoxicity, metabolism and structure-property-relationship of auranofin from then on. Besides rheumatoid arthritis, auranofin is found to be a very potent inhibitor against *entamoeba histolytica*. It also processes potent toxicity against tuberculosis. Currently, auranofin is under investigation against HIV infection.[14]

Scheme 1.6 Molecule structures of auranofin and chloro(triethylphosphine)gold

1.3.1 The linear two-coordinated gold(I) diphosphine complexes

Former chrysotherapy research of auranofin has also led to further study on its anticancer activity. Auranofin and Et_3PAuCl (Scheme 1.6) has been shown to have toxicity against B16 melanoma cells and P388 leukemia cells as well as human cancer cell lines via *in vitro* screening.[15] The cytotoxicity mechanism research indicates that both complexes target mitochondria in cells and act as inhibitors of thioredoxin reductase (TrxR), which has been proved to play a key role in cancer cells survival.[16] Once thioredoxin reductase is inhibited, the hydrogen peroxide formed in the mitochondria respiratory chain cannot be reduced, which makes hydrogen peroxide accumulate within

the mitochondria. Furthermore, the thioredoxin oxidized by proteins cannot be reduced either as gold complex has blocked the thioredoxin system. Thus, hydrogen peroxide and oxidized thioredoxin, such as peroxiredoxin will continuously increase within cells, which will lead to the opening of PT (permeability transition) pore and the increasing in the outer membrane permeability. As a result, apoptotic factors will be released and cell line will enter the suicide program.[17] (Scheme 1.7)

Scheme 1.7 The cell apoptosis mechanism induced by auranofin

In 1987, Mirabelli group reported an anticancer investigation of bis(diphenylphosphino)ethane (dppe) gold(I) complex and its derivatives. A series of free diphosphine ligands, which have similar structure with dppe possess cytotoxicity against P388 leukemia, turned out to be much more potent when coordinated with gold(I) metal. The author also screened other transition metals including Ag(I), Pt(II), Pd(II), Ni(II) and Rh(I) instead of gold(I), but anticancer activity was found to be absent. The fact indicates the combination of gold(I)-phosphine is necessary to maintain the anticancer activity of this series of complexes.[18]

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The structure-property-relationship of dppe-gold(I) derivatives have been discussed over 30 years until now when numerous linear two-coordinate gold(I) complexes with anticancer effect have been discovered (Scheme 1.8).[19] Among the dppe derivatives, replacement of phenyl group on P atom by ethyl or benzyl group resulted in a loss in anticancer activity; while cyclohexyl or heterocyclic rings will afford better anticancer performance. Besides, the length of the carbon skeleton of diphosphine ligands is also important: for free diphosphine ligands, a longer carbon chain will give a higher anticancer activity; but for gold(I) coordinated complexes, the shorter carbon chain molecule turned out to be more potent against cancer cell lines. It also deserves to be noticed that the gold(I) ligands *trans* to diphosphine also affect the complexes' biological property, but not as crucial as the former factors.

Scheme 1.8 Dppe gold(I) based complexes with anticancer property

On the other hand, Berners-Price's group have pointed out that the anticancer activity of gold(I) diphosphine complexes are highly related with the

lipophilic-hydrophilic balance.[19a] The reason may lie in the target of the gold(I) phosphine complexes — mitochondrial. Lipophilic cations have proven to accumulate more easily in mitochondria due to their lipophilic-cationic character, thus showing a selective cytotoxicity against cancer cells whose membranes are hyperpolarized.[20]

1.3.2 Tetrahedral gold(I) diphosphine complexes

The anticancer activity of tetrahedral gold(I) phosphine complex $[\text{Au}(\text{dppe})_2]^+$ was firstly reported by Berner-Price in 1986. The complex showed both *in vivo* anticancer activity against ip P388 leukemia in mice and *in vitro* cytotoxicity against M5076 reticulum cell sarcoma and several other cancer cell lines.[21] It has been proven that $[\text{Au}(\text{dppe})_2]\text{Cl}$ inhibited the uptake of thymidine, uridine, leucine into DNA, which as a result caused DNA single-stranded to break and produce a significant number of DNA-protein cross-links against L1210 cell line within 1 hour at low drug concentrations. Research also shows that the complex has even greater effect on protein biosynthesis. Unfortunately, the pre-clinical trials of $[\text{Au}(\text{dppe})_2]\text{Cl}$ was abandoned since severe hepatotoxicity was observed in dogs.

However, researchers still have great interest upon tetrahedral gold(I) phosphine complexes as its significant cytotoxicity against a wide spectrum of cancer cell lines. Mckeage's group have synthesized and evaluated the anticancer property of a series tetrahedral gold(I) phosphine complexes (Scheme 1.9). Interestingly, the

$[\text{Au}(\text{R}_2\text{P}(\text{CH}_2)_n\text{PR}_2)_2]$ type complexes retains their anticancer activity when Au(I) is replaced with Ag(I) or Cu(I), which is very different from linear two-coordinate gold(I) phosphine complexes. The phenyl groups on the P atom turned out to maintain the highest potency instead of other substituent groups including pyridyl group and shorter carbon back skeleton of diphosphine ligands also gives better anticancer property. [22]

Scheme 1.9 Tetrahedral gold(I) diphosphine complexes with anticancer property

Compared with neutral two-coordinated gold(I) phosphine complexes, the bioactive portion of tetrahedral gold(I) phosphine complexes bears positive charges (usually 1+ or 2+), which indicates that the mechanism of drug absorption by cell lines and drug cytotoxicity will not be the same with these two types of complexes. Besides, the gold centre of tetrahedral gold(I) complexes may not be the most active site in the molecule when entering the *in vivo* system as it is tetra-coordinated and sterically hindered. There are also some similarities: the target for both type of gold(I) complexes is the mitochondria; the *in vitro* cytotoxicity of tetrahedral gold(I) complexes are also highly related with the lipophilicity-hydrophilicity balance—the more lipophilic molecule turns

out to have more potency against cancer cells but poor selectivity between cancers and normal cells.

1.3.3 Gold(I) N-heterocyclic carbene (NHC) complexes

More recently, new types of gold(I) complexes exhibiting anticancer activity has been developed using N-heterocyclic carbene (NHC) ligands. NHC ligands have similar electronic properties with phosphines and are relatively easier to be prepared, NHCs are also more stable since they have higher σ donating ability to the metal centers and are also better π acceptors. Several gold(I) NHC complexes have shown significant anticancer activity (Scheme 1.10). Bioproperty study indicates that this type of gold(I) complexes have the tendency to induce mitochondrial membrane permeabilization at low drug concentrations.[23] In addition, gold(I) NHC complexes have been proven to react with selenocysteine and cysteine, which indicates the possible inhibition effect against thioredoxin reductase inside cells' mitochondrial. Thus the gold(I) NHC complexes may have similar anticancer mechanism with other types of gold(I) phosphine complexes. What is also worth to notice is that NHC ligands themselves have not been detected with any anticancer activity, thus the main function of NHC ligands may lies in the stabilization of gold(I) canters and undergo ligand exchange reactions when the drug is absorbed by cancer cells.

Scheme 1.10 Gold(I) N-heterocyclic carbene complexes with anticancer property **1.3.4 Gold(III) complexes**

In last decade, gold(III) complexes were found to have comparable anticancer activity with cisplatin derivatives against a column of human cancer cell lines when tested *in vitro*, such as gold(III) polyamines, gold(III) porphyrins, gold(III) polypyridines and gold(III) dithiocarbamate complexes.[24] Mononuclear gold(III) complexes have attracted a lot of attention in the chemotherapy research considering similar electronic properties with Au(III) and Pt(II), and also the tendency of forming square planar complexes. However, numerous reports have indicated that the biological activities and anti-proliferative mechanism of gold(III) complexes are totally different with that of cisplatin anticancer drugs. DNA or RNA have been proven not to be the primary targets of gold(III) complexes. The problem lies in the mechanism research is that most gold(III) complexes are not stable under physiological conditions and the gold(III) metal centre will be immediately reduced to gold(I). As a result, it is challenging to determine the actual oxidation state of gold that lead to the anticancer activity. Recently, Che's group

has described several cyclometallated gold(III) complexes which the oxidation state of gold has been shown to be very stable under physiological conditions.[25] The proposed mechanism is the selective inhibition of thioredoxin reductase inside the mitochondria. The inhibition activity of mammalian cytosolic thioredoxin was also reported but no evidence showed that the +3 oxidation state was maintained during the tests.

Scheme 1.11 Gold(III) complexes with anticancer property

1.3.5 Gold nanoparticle-based drug carrier

The application of gold in cancer therapy is not only limited to chemotherapy. In recent decades, gold nanoparticles (GNPs) have been explored as a promising candidate as effective anticancer drug carriers with great advantages of reducing side effect during cancer therapy.[26] GNPs' sizes arrange from 1 to 150 nm, and possess unique properties. The GNP-conjugated drugs have higher perfusion rate against cancer target, which can low down the toxicity against normal cells and tissues.

The targeting approaches of the GNP conjugated drugs towards cancer targets have been well studied, which is mainly through three routes: passive targeting, active

targeting, or a combination of both. Active targeting is mainly by pre-conjugating various probes or targeting agents like antibodies, small molecules or peptides with GNPs to raise up the perfusion rate against the cancer target. While passive targeting takes advantage of the ERP effect to deposit anticancer drugs in cancer cells. Presently, there are various GNPs under investigation of cancer drug delivery sorted by their different sizes and shapes which are shown in the following table (table 1.1).

Table 1.1 The NGPs under cancer therapy investigation. (Reproduced with from Elsevier via Rightslink)

Name	Ingredient	Target
Aurimmune	PEG-Thiol GNP modified with TNF- α (~27 nm)	Solid tumor
AuroShell	Silica nanoparticales coated with Gold (~150 nm)	Solid tumor
Verigene	Gold	Genetics

1.4 Synthesis of Phosphines

Phosphines are very important organophosphorus compounds which are widely used in metal complex catalysis and organocatalysis. Chiral phosphines have especially attracted much research interest in the preparation of organometallic complexes as catalysts for asymmetric synthesis.[27] The challenges in phosphine syntheses is mainly

because of their highly oxidizable nature. The tendency for free organo phosphines to form their corresponding phosphine oxide depends on their steric and electronic property. For example, PH_3 is a gas at room temperature and reacts vigorously with air to form phosphorus pentoxide; while PPh_3 appears to be solid and remain stable in air. Commonly used synthetic methods of phosphines will be introduced in this part, including the asymmetric synthesis of P-chiral phosphines.

1.4.1 Synthesis of Phosphines via Substitution Reactions

1.4.1.1 Alkylation Reactions of Phosphine

The substitution reactions of phosphine and alkyl halides with support of activated carbon or Pt, Pd or Au based catalysts under high temperatures produce alkylphosphonium halides salts. Major form of products depend on different phosphine-alkyl halides ratio.[28]

Scheme 1.12 The substitution reaction of phosphines and alkyl halides

When the molar number of phosphine equals that of alkyl halides, the

substitution reaction will give mixed products including mono-, di- and trialkylphosphonium halides where the monoalkylphosphonium is mostly the major product. Alkylphosphonium halides salts can be easily transformed to their corresponding phosphines via reaction with aqueous NaOH solution.

Besides alkyl halides, substitution reactions of alcohols, esters and amines with phosphines were also well reported.[29]

Scheme 1.13 The substitution reaction of phosphines and alcohols, esters and amines

The alkylation with alcohols and ethers requires the presence of aluminosilicates or zeolites and high temperature, giving products mostly as mono- or diorganylphosphines. While for the alkylation with amines will give mixed products as primary, secondary and tertiary phosphines with copper chromite or other group I B/VIII B metals as catalysts.

1.4.1.2 Reactions of Phosphides and Organic Halides

Phosphines can be transferred into nucleophilic phosphide ions which react with organic halides to afford primary and secondary phosphines. The strongly basic conditions required in the reactions can be achieved by either alkali metals in organic solvent (or liquid NH_3) or alkali metal hydroxides in polar non-hydroxylic solvents.[30]

Under 1:1 alkali metal-phosphine molar ratio, substitution reactions occurs with alkyl halides, aryl halides, sodium halocarboxylates, aminoalkyl halides. Metal-phosphide salts can produce relatively pure phosphines with high yield due to disubstituted metal phosphide formation is not favored except with lithium.

Scheme 1.14 Substitution reaction of phosphides with organyl halides.

Through controlling the ratio of butyl lithium and phosphine, it is possible to

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prepare lithium phosphide, dilithium phosphide and trilithium phosphide in their isolated form. Furthermore, tertiary phosphines with three different substituent groups can be synthesized stepwise by this method.

Scheme 1.15 Substitution reactions of Lithium phosphides with organyl halides.

Similar reactions between sodium phosphide salts and dihaloalkanes in liquid NH_3 produce cyclic phosphines or primary alkylenediphosphines depending on the carbon chain length of dihaloalkanes.

Scheme 1.16 Substitution reactions of phosphides and dihaloalkanes.

Except using alkali metal, strong bases like KOH can also produce phosphides

by deprotonation of phosphine.[31]

Scheme 1.17 Substitution reactions in the presence of strong base.

In contrast with metal phosphides, substitution reactions of phosphines and dihaloalkanes under strong basic condition give products in a different way. Reactions of 1,3-dibromopropane or 1,3-dibromobutane afford no cyclic phosphines, but only produce primary alkyldiphosphines. While reaction with 1,4-dibromopentane under KOH condition produce predominantly the cyclic product.

Scheme 1.18 Substitution reactions of 1,3-dibromopropane and 1,4-dibromopentane.

1.4.2 Synthesis of Phosphines via Addition Reactions

1.4.2.1 Addition Reactions of C=C bond and C≡C bond

In the presence of a strong base, phosphines undergo nucleophilic addition

reactions on C=C bond of alkenes containing strong electron-withdrawing group at one of the sp^2 carbon.[32] The resultant products can be primary, secondary, tertiary phosphines or a mixture, depending on different reaction conditions and the (electronic and steric) properties of alkenes.

Scheme 1.19 Nucleophilic addition reactions of C=C bond.

Under similar conditions, phosphines can undergo nucleophilic addition with functionalized alkynes.[33] Under mild conditions, the reaction resulted in product with stereoselectivity which is tris(*Z*-2-organylvinyl)phosphines in relatively high yield.

Scheme 1.20 Nucleophilic addition reactions of phosphines to C≡C bond.

Instead of using strong bases, acid catalyzed electrophilic addition of phosphines to various alkenes have been reported.[34] Although the mechanism remains unclear, acid catalyzed addition reactions of phosphines is applicable to linear and cyclic alkenes without activation groups.

Scheme 1.21 Electrophilic addition reactions of phosphines to C=C bond.

Since the 1950s, the photochemical preparation of tertiary phosphines has been continuously reported.[35] Photochemical reactions of phosphines with unsaturated compounds showed different mechanisms with nucleophilic reactions as shown in Scheme 1.22. The radical addition of C=C bond is accompanied with polymerization as side-reactions and sometimes even become the major reaction instead.

R = H, Alkyl, substituted alkyl

Scheme 1.22 Radical addition reactions of phosphines to C=C bond.

1.4.2.2 Addition Reactions of C=O bond and C=N bond

Primary and secondary phosphines are typical nucleophiles, which react with carbonyl and imino compounds in acid catalyzed condition.[36] The major form of product depends on the number of hydrogen atoms connected with phosphorus atom.

Scheme 1.23 Addition reactions of phosphines to aldehyde and ketone.

Phosphines are able to add to imino compounds to afford amino substituted phosphines in the presence of an acid catalyst under mild conditions.

Scheme 1.23 Addition reactions of phosphines to imino compounds.

In the presence of triethylamine (NEt_3) phosphines also can proceed via addition reaction to isocyanates' $\text{C}=\text{N}$ bond by nucleophilic attack under mild conditions. This method is used to synthesize carbamoyl- and thiocarbamoyl-phosphines.

Scheme 1.24 Addition reactions of phosphines to isocyanates.

1.4.3 Preparation of Chiral Phosphines via Asymmetric Synthesis

The preparation of optically pure phosphines via asymmetric synthesis also have been well studied. Some synthetic routes transform non-phosphorus chiral

compounds' chirality to non-chiral phosphines, the commonly used chiral inducers include amino acids, tartaric acid, terpenes, *etc.*

Scheme 1.25 Stereoselective transformations between phosphine and (-)-ephedrine.

However, this type of synthetic procedures usually requires many steps which is not quite efficient and cause loss in the yield. The reactants are also limited by each step, in some cases the starting phosphine compound is required to be chiral itself. In recent years, our group have developed simple and efficient asymmetric synthesis of chiral phosphines by palladium(II) promoted or catalyzed hydrophosphination reactions.[38] A spectrum of optically pure tertiary phosphines bearing C* or P* or both chiral centers have been successfully synthesized from simple secondary phosphines like Ph₂PH under mild conditions assisted by palladium(II) complexes. Unsaturated substrates functionalized with unprotected groups such as aldehyde, keto, ester, cyano and alcohol can be prepared directly through this method, which will be discussed greater detail in chapters 2 and 3.

1.5 Scope and Summary of Thesis Research

Although cisplatin and its derivative complexes such as carboplatin have been introduced into clinical therapy against a variety of cancer, platinum(II) based anticancer drugs are not always effective against some type of cancers especially with gastrointestinal and breast cancers.[39] Moreover, cisplatin and its derivatives produce undesirable side-effects including nephrotoxicity and hematotoxicity when applied to *in vivo* system, which is considered to be another drawback in chemotherapy.[40]

Gold(I) phosphine complexes with significant anticancer property is believed to be very promising as a new class of anticancer drugs. With totally different cytotoxic mechanism with Pt(II) anticancer drugs, gold(I) phosphine complexes probably acts on a different spectrum of cancer targets compared with the cisplatin family, and have the possibility to overcome the side effects introduced in the current chemotherapeutics.

On the other hand, among all the synthetic and natural small molecule drugs currently in use, more than half of them contain one or more chiral centers. The enantiomeric drugs may have profoundly different behaviors in biological systems which depends on their different shapes in 3-dimensional space as well as the homochiral nature of biological system. However, the effect of anti-cancer reagents' chirality on their cytotoxicity and selectivity against cancer cells and normal cells haven't been studied much.

In this thesis, a series of enantiopure gold(I) phosphine complexes bearing one or

two chiral centers have been synthesized efficiently through a hydrophosphination reaction promoted by chiral palladium(II) catalyst or template. Especially, the chiral dinuclear gold(I) diphosphine complexes synthesized in this project displays excellent cytotoxicity against the cancer cell lines and also had almost no effect on the normal human cell lines.

The activity-structure relationship of gold(I) based anticancer complexes is explored with these 2 factors in mind: (1) The effect in variations in the type and number of substituents in the gold(I) phosphine complexes on their cytotoxicity and selectivity against human breast cancer cell lines MDA-MB-231 and human mammary epithelial cell lines MCF10A. (2) The cytotoxicity and anticancer property of gold(I) phosphine complexes with opposing chirality is also evaluated based on in vitro screening results.

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Chapter 2

Asymmetric Synthesis and Anticancer Evaluation of Functionalized Chiral Gold(I) monophosphine Complexes

**Asymmetric Synthesis and Anticancer Evaluation of Functionalized Chiral Gold(I)
Monophosphine Complexes**

2.1 Introduction

Organometallic complexes have been synthesized and applied in medicinal therapy of different diseases over the centuries. In 1965, *cis*-diamminedichloro-platinum(II) (cisplatin) was found to possess anti-cancer activities against several types of cancer cell-lines. Subsequently cisplatin and its derivative complexes such as carboplatin have been introduced into clinical therapy against a variety of cancers till today. However, cisplatin and its derivatives are not highly effective against some types of cancers especially in gastrointestinal and breast cancers. Moreover, cisplatin and its derivatives produce undesirable side-effects including nephrotoxicity and hematotoxicity when applied to in vivo system. Besides, other transition metal complexes including Ru(III), Ga(0), Fe(II), Co(II) were also found to have cytotoxicity against cancer, which have indeed proved the potential of transition metal complexes as anti-cancer agents.[1]

At the same time, the anti-cancer activities of gold(I) phosphine complexes have been discovered and attracted lots of scientists' interest since the anti-rheumatic agent, auranofin, have shown interesting cytotoxicity against some cancer cell lines. Among the gold(I) phosphine complexes synthesized, $[\text{Au}(\text{dppe})_2]\text{Cl}$ (dppe:

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1,2-bis(diphenylphosphino)ethane) and $\text{Au}_2(\text{dppe})\text{Cl}_2$ have shown very good anti-cancer properties against a spectrum of different cancer cell lines. Some evidence have shown that unlike platinum anti-cancer drugs which interact with DNA chains directly, gold(I) phosphine complexes mainly targets the mitochondria and may also can break DNA bonds as well as DNA-protein cross-links in cancer cells. However, the clinical application of these complexes was not successful because of their serious hepatotoxicity in animal model. The problem potentially lies in the high lipophilicity of the molecule which leads to a non-selective cytotoxicity against both cancer cells and normal cells. In 1999, some improvement have been made by the group of Berners-Price who found that by modifying the lipophilicity of the molecule, gold(I) phosphine complexes may have better anti-cancer selectivity and cytotoxicity.

On the other hand, among all the synthetic and natural small molecule drugs currently in use, more than half contain one or more chiral centers. Enantiomeric drugs may have profoundly different effects in biological systems which depend on their different shapes in 3-dimensional space as well as the homochiral nature in biological systems. However, the effect of the reagents' chirality on their cytotoxicity and selectivity against cancer cells and normal cells haven't been well studied.[2]

This chapter focuses on the development of functionalized optically pure gold(I) mono-phosphine complexes as potential anticancer materials. The introduction of selected functional groups serves to fine-tune control of the drug properties. Similarly, the

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involvement of molecular chirality provides a potential handle to establish the structure-activity relationship thus improving on drug efficiency. Based on these drug design concepts, our group have previously reported on the study of a chiral phosphanorbornene-gold complex in the treatment of K36 lymphoma cells which are naturally tumorigenic in syngenic AKR mice and non-immunogenic to host mice.[3]

In this chapter, the optically pure mono-phosphines were synthesized via a palladium(II) catalyzed asymmetric *phospha*-Michael condensation of diphenylphosphine with *N*-enoylbenzotriazoles. The catalysis work was conducted in collaboration with Mr. Renta Jonathan Chew who will report in greater detail in his Ph.D. thesis.

A novel series of amide and esters functionalized chiral gold(I) mono-phosphine complexes were synthesized. According to the *in vitro* result against human breast cancer cell line MDA-MB-231 which do not respond well to typical cisplatin treatments,[4] the synthesized chiral gold(I) mono-phosphine complexes possesses promising anticancer activity. The complexes' cytotoxicity against normal human skin cell line HaCaT is also evaluated at the same time in order to discuss the drugs' selectivity and possible side effects. The *in vitro* screening results indicated that the benzotriazole and esters functionalized gold(I) mono-phosphine complexes possess promising anticancer activity.

2.2 Results and Discussion

2.2.1 Design and synthesis

Numerous heterocyclic compounds were discovered and developed as a main class of pharmacologically active agents. Nitrogen heterocycles can be found in many kinds of natural and synthetic drugs, especially exhibit diverse biological and pharmacological activities.

Benzotriazole-based heterocycles are typical examples which possessed different bio-properties in combination with various of molecule skeletons. Alizapride is an antiemetic agent containing the benzotriazole group and is mainly used in the treatment of vomiting and nausea; while vorozole is another benzotriazole containing agent which acts as a non-steroidal aromatase inhibitor (Figure 2.1).[5]

Figure 2.1 The structures of biologically active benzotriazole containing compounds.

Besides, there are also several benzotriazole compounds have been discovered with the ability to inhibit some types of kinases. In 2006, a class of benzotriazole ester compounds (Figure 2.1) was reported as mechanism-based inactivators of SARS-3CL^{pro}

which plays an important role for viral replication of severe acute respiratory syndrome (SARS).[6] Some benzotriazole containing molecules was reported by Philipp's group to possesses light activated DNA cleaving property.[7] For the above reasons, the benzotriazole group is also introduced to enantiopure gold(I) mono-phosphine complexes so as to evaluate its effect on gold complexes' anticancer efficiency.

On the other hand, the introduction of ester groups into the original drug molecules, has led to improved pharmaceutical performance including greater drug efficiencies and depression of side effects. Heroin is a derivate medicine of morphine which blocks deep aching pain. Replacing the hydroxyl groups with methyl ester group, heroin becomes much more potent than morphine mainly due the change in solubility of drug molecule which make heroin pass through the blood-brain barrier much faster than morphine.

Figure 2.2 The molecule structures of morphine and heroin.

When comparing cisplatin with its ester group substituted derivatives, carboplatin and oxaliplatin, a lot of reports also points out that carboplatin and oxaliplatin have much less side effects and higher tolerance dose especially for older patients in the clinical chemotherapy without loss in their anticancer efficiency.[8]

Figure 2.3 The molecule structures cisplatin, carboplatin and oxaliplatin.

Similar phenomenon is also observed among gold complexes possessing anticancer activities. As mentioned in Chapter 1, auranofin and Et_3PAuCl (Figure 2.4) have been reported about their toxicity against B16 melanoma cells and P388 leukemia cells as well as several human cancer cell lines. According to *in vitro* screening results, auranofin bearing several methyl ester groups is more potent in inhibiting cancer cells growth than Et_3PAuCl .

Figure 2.4 Molecule structures of auranofin and chloro(triethylphosphine)gold

Despite the diversity of function patterns and mechanisms, the bioactivity of different molecules have indeed influenced by introduction of ester groups which is a

result of change in solubility, stability and other crucial properties of compounds. Thus, it is also necessary to conduct a more systematic study related to the 'ester group effect' against gold-based anticancer activity.

In order to study the anticancer activity of ester and amide functionalized chiral gold complexes, four neutral mono-phosphine gold(I) complexes **1~4** have been designed and synthesized (Figure 2.5). The complexes were chosen to illustrate whether amide and esters functional groups would offer gold phosphine complexes with higher anticancer activity and lower cytotoxicity.

Figure 2.5 The molecule structures of chiral gold(I) mono-phosphine complexes

However, a synthetic challenge would be associated with the synthesis of desired air-sensitive functionalized chiral phosphine ligands. Our group has recently reported that optically pure mono-phosphine ligands which could be prepared efficiently by the palladium(II) catalyzed asymmetric *phospha*-Michael additions with diarylphosphines.[9] Catalytic asymmetric hydrophosphination of α,β -unsaturated mono esters and amides are

rarely reported which is mainly due to poor activation at the electrophilic site.[10] In order to overcome the limitation, substrates with sufficient activating analog bound to the enoyl portion while being as a good leaving group to be replaced by strong nucleophiles after the hydrophosphination reactions are necessary.

After screening of several potential substrates, *N*-enoylbenzotriazole was proven to be the most appropriate candidate mainly because of its ease of preparation and low cost of starting materials. This substrate also gives a good chance for the synthesis of chiral phosphine ligands bearing different ester functional groups due to benzotriazole has been reported as a good leaving group in acyl substitution reactions.[11]

Scheme 2.1 Hydrophosphination of compound **6** with diphenylphosphine (**7**).

As shown in Scheme 2.1, the hydrophosphination reaction was catalyzed by (S)-**5**, Bt-substituted **6** and equimolar of diphenylphosphine reacted in 3.6 ml of degassed chloroform under presence of 1 equivalent triethylamine for 6 hours. The yield of chiral mono-phosphine **8** was determined by the ³¹P{¹H} NMR spectrum of the crude product.

In order to determine the enantiomeric excess (*ee*), the obtained monophosphine **8** were allowed to react with enantiopure dimeric complex (*R*)/(*S*)-**9** (≥ 0.5 equiv.) in chloroform to form derivatives **10**. Enantiomeric excess (*ee* %) was then determined from the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of the integral ratios of respective diastereomers (Scheme 2.2), which is calculated to be 96%. Value obtained by such calculation could also be backed up by conventional HPLC analyses. Making use of this high yield and *ee* hydrophosphination reaction, it is possible to synthesize the desired benzotriazole containing chiral monophosphine ligands efficiently with no waste of starting material under mild conditions.

Scheme 2.2 The enantiomeric excess determination of product **8**.

As shown in Scheme 2.3, diastereomeric adducts **10** are stable and can be observed by NMR, however, purification of **10** on silica gel unexpectedly led to hydrolysis with concomitant elimination of the benzotriazole group. The resultant

carboxylate functionality binds to palladium metal centre to form a neutral P,O-chelated complex **11**, which the absolute configuration has been determined by X-ray diffraction.

Scheme 2.3 The hydrolysis of product **10** due to flash chromatography.

Scheme 2.4 The *ee* determination ³¹P{¹H} NMR spectrum of product **8**

The desired ester(s) substituted chiral mono-phosphine ligands were prepared efficiently by treating **8** with sodium alkoxides, alcohol and chloroform via a simple one pot substitution reactions without loss of optical purity. The target enantiopure monophosphine gold(I) complexes could be synthesized in high yields from the simple coordination reaction between 1 equivalent of [ClAuSMe₂] and the corresponding

optically pure monophosphines. Different with their free ligand, complexes **1~4** are air-stable and can be crystallized quickly from various solvent systems. The crystal X-ray diffraction data showed the absolute configuration of complex (*R*)-**2**, and the gold(I) atom possessed linear two-coordinate structure. In solid state, the complexes' chemical structure and optical property remained unchanged after being restored for long periods which is a very important characteristic as anticancer drug candidates.

Scheme 2.5 The synthesis of chiral gold(I) mono-phosphine complex (*R*)-**2**

Figure 2.6 The molecule structure and absolute stereochemistry of (*R*)-**2**

2.2.2 Cytotoxicity Evaluation against Breast Cancer

To examine the cytotoxicity of the chiral gold(I) diphosphine complexes **1-4**, these complexes were screened against a human cell line panel consisting of MDA-MB231 (human breast cancer cells). MDA-MB231 cell line is a typically triple-negative cancer, which refers to cancer that is negative for estrogen receptor (ER-), progesterone receptors (PR-), and HER2 receptors (HER2-). A triple-negative cancer does not respond to hormonal therapy or therapy which targets HER2 receptors. Flow cytometry was introduced to evaluate cell viability quantitatively after dying the cell with propidium iodide (PI). PI is membrane impermeable and generally excluded from viable cells, PI can bind to DNA with a stoichiometry of one PI molecule per 4 to 5 base pairs of DNA without sequence preference. It is used to differentiate necrotic, apoptotic and normal cells and identify dead cells in population. Eventually, the dose-response curve (Figure 2.7) was illustrated to calculate the IC_{50} value of complexes **1-4** against breast cancer cell lines MDA-MB-231 (Table 2.1).

The death population of breast cancer cells under each drug concentration was collected after 17 hours treatment. Dose-response curve and IC_{50} of chiral gold(I) monophosphine complexes **1-4** was illustrated by GraphPad Prism (5.0). The nonlinear regression curve fit model was chosen using the log(inhibitor) vs. response-variable response equation.

Figure 2.7 The dose-response curve of complexes **1-4** against MDA-MB231 cell line

Complex	IC_{50} [nM]
(<i>R</i>)- 1	536.1
(<i>R</i>)- 2	427.4
(<i>R</i>)- 3	738.8
(<i>R</i>)- 4	500.7
Cisplatin	No effect detected

Table 2.1 The IC_{50} of complexes **1-4** against MDA-MB231 cell line

The *in vitro* test results showed that the optically pure gold(I)-diphosphine complexes **1-4** have IC_{50} varies from 0.43 to 0.74 μM ($1\mu\text{M}=1000\text{nM}$) against

MDA-MB-231 cell lines which is much more lower than cisplatin which IC_{50} is 7.8 μM against the same cancer cell lines after 72 hours treatment. Compared to other known gold(I) complexes with anticancer activity including Ph_3PAuCl and Auranofin, the IC_{50} of complexes **1-4** is also much more lower. **1**, **2** and **4** bearing benzotriazole group or methyl ester groups turned out to be more efficient in inhibiting the growth of cancer cells than complex **3** with an ethyl ester group. The cytotoxicity of gold(I)-diphosphine complexes is improved when amide and ester groups introduced in the drug molecule. In this aspect, gold(I)-diphosphine complexes is thought to become more efficient when the molecule is more hydrophilic.

2.2.3 Selectivity against breast cancer and normal cell lines

The IC_{50} evaluations of monophosphine gold(I) complexes **1-4** have shown that they are highly effective against the breast cancer cell line MDA-MB231. In order to examine their potential in clinical applications, the cytotoxicity of complexes **1-4** against both breast cancer cells MDA-MB231 and normal human keratinocyte cells HaCaT have been studied and compared under similar experimental conditions (drug concentration, treatment time, *etc.*). As shown in Figure 2.8, the *in vitro* test results indicated the cytotoxicity against normal skin cell line is much more lower than the breast cancer cells. Complexes **1** and **2** bearing the benzotriazole group and methyl ester group turned out to possess better selectivity between cancer cells and normal cells, as the drug concentration

increases, the breast cancer cells have been inhibited by 20% to 80%, while the normal cells death percentage is not more than 20% even under the highest drug concentration. Complexes **3** and **4** also have obvious selectivity between cancer and normal cell lines, however under 2000nM drug treatment, the death percentage of normal cell line reached 30 to 40% which is relatively higher compared to complexes **1** and **2**. These results therefore demonstrate that the functionalities on phosphine ligands have substantial effects on both the cytotoxicity and selectivity of gold(I) complexes' anticancer activity.

Figure 2.8 MDA-MB231 and HaCaT cell death percentage after treatment with complexes **1-4**.

2.3 Conclusion

In this chapter the enantiomeric pure gold(I) monophosphine complexes **1-4** were designed and synthesized successfully through palladium catalyzed asymmetric *phospha*-Michael condensation reactions. The anti-cancer activities of each complex was evaluated by *in vitro* tests against human breast cancer cell line MDA-MB-231 and normal human keratinocyte cells HaCaT. Complexes **1** and **2** bearing benzotriazole group and methyl ester group showed both promising cytotoxicity and selectivity.

2.4 Experimental Section

Material and Methods

General Methods. All air sensitive manipulations were performed under a positive pressure of nitrogen using Schlenk techniques. Solvents were degassed prior to use when necessary. Methanol (AR), toluene (AR) and hexane (AR) were purchased from Avantor Performance Materials. Chloroform (AR) was purchased from Fischer Scientific, dichloromethane (AR) from Alfa Aesar and ethyl acetate (AR) from Merck. Acetone (AR) was purchased from QReC. Solvents were used directly without further purification. Low Temp PAIRSTIRRER PSL-1800 machine was used for controlling low temperatures for reactions. Silica plug filtration was conducted on Merck silica gel Geduran Si 60 (0.040-0.063mm).

NMR spectra were recorded on Bruker ACF 400 spectrometer. ¹H NMR spectra chemical

shifts were reported in δ ppm relative to tetramethylsilane ($\delta = 0.00$ ppm). Multiplicities were given as: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The number of protons (n) for a given resonance was indicated by nH while coupling constants were reported as J value in Hertz (Hz). ^{13}C NMR spectra chemical shifts were recorded relative to solvent resonance (CDCl_3 : $\delta = 77.16$ ppm). $^{31}\text{P}\{^1\text{H}\}$ NMR spectra chemical shifts are referenced to an external standard of 85% H_3PO_4 . Optical rotations of monophosphine products were measured as soon as possible without inert gas protection in the specified solution using a 0.1 dm cell with an Atago AP-300 automatic polarimeter. Chiral palladacycles (*S*)-**5**, (*R*)-**5** [13], (*S*)-**9**, (*R*)-**9** [14] and substituted *N*-enoylbenzotriazoles **1** [15] were prepared according to literature methods.

Caution! Perchlorate salts of metal complexes are potentially explosive and should be handled with care.

(A) General procedure for preparation of chiral tertiary monophosphines via asymmetric hydrophosphination of *N*-enoylbenzotriazoles

All the procedures were conducted under nitrogen protection, to a solution of Ph_2PH (11.2 mg, 0.060 mmol, 1 equiv.) in degassed chloroform (3.2 mL) and acetone (0.4 mL) was added (*S*)-**5** (0.8 mg, 0.003 mmol, 5 mol%) and stirred at room temperature under nitrogen environment until complete dissolution before cooling to -80°C . Subsequently, *N*-enoylbenzotriazole **6** (0.060 mmol, 1 equiv.) was added and the walls of the setup washed with chloroform (0.5 mL). Triethylamine (6.1 mg, 0.060 mmol, 2 equiv.) in CHCl_3 (0.5 mL) was then added dropwise over a period of 5 minutes. The solution was stirred at -80°C and the reaction monitored by $^{31}\text{P}\{^1\text{H}\}$ NMR. Upon completion, the setup was moved to ambient temperature and the reaction mixture subjected to vacuum with heating to remove any volatiles. The dried mixture was re-dissolved in chloroform (8 mL) and filtered through a silica plug using a Pasteur pipette fixed on a separate nitrogen filled 2-neck Schenk flask to remove (*S*)-**5** and phosphine oxides (if any). Solvent was removed from the eluent under reduced pressure to afford chiral tertiary phosphine **8** as the pure product.

(B) General procedure for transformation of asymmetric hydrophosphination product into its ester equivalent.

All the procedures were conducted under nitrogen protection, (*R*)-**8** was obtained in accordance with **General Procedure (1)**, except then the reaction mixture was not passed through a silica plug. The vacuum-dried reaction mixture was re-dissolved in CHCl_3 (1.7 mL) and MeOH (4.2 mL), followed by addition of NaOMe (3.20 mg, 0.060 mmol, 1 equiv.) and stirred at room temperature for 1 hour to afford (*R*)-**12**.

(C) General procedure for preparation of chiral gold(I) monophosphine complexes

Compounds (*R*)-**8** and (*R*)-**12** were obtained in accordance with **General Procedures (A)** and **(B)** respectively. Dichloromethane (10 mL) was added to the reaction mixture, followed by ClAuSMe_2 (17.67 mg, 0.060 mmol, 1 equiv.) and stirred at room temperature for 1 hour in the dark. The crude product was purified by column chromatography (Hexane : dichloromethane = 1:2) and crystallized in chloroform and pentane to afford

colorless crystal.

(4) Synthesis and Characterization

(R)-8 was prepared according to **General Procedure (A)** (93% yield, 97% *ee*):

$[\alpha]_D^{24} = +129.8^\circ$ [*c* 0.3, CHCl₃]. $^{31}\text{P}\{^1\text{H}\}$ (CDCl₃, 162 MHz): δ 0.61; ^1H (CDCl₃, 400 MHz): δ 3.65-3.71 (m, 1H), 4.15-4.23 (m, 1H), 4.42-4.46 (m, 1H), 7.12-8.15 (m, 19H); ^{13}C (CDCl₃, 101 MHz): δ 39.67 (d, 1C, $^2J_{\text{CP}} = 24$ Hz), 40.80 (d, 1C, $^1J_{\text{CP}} = 13$ Hz), 114.55-146.22 (m, 24C), 170.70 (d, 1C, $^3J_{\text{CP}} = 15$ Hz).

(S)-8 was prepared according to **General Procedure (A)**, except that catalyst **(R)-5** was

employed (95% yield, 98% *ee*): $[\alpha]_D^{26} = -149.2^\circ$ [*c* 0.7, CHCl₃]. $^{31}\text{P}\{^1\text{H}\}$ (CDCl₃, 162 MHz): δ 0.61; ^1H (CDCl₃, 400 MHz): δ 3.53-3.59 (m, 1H), 4.03-4.11 (m, 1H), 4.30-4.34 (m, 1H), 7.00-8.03 (m, 19H); ^{13}C (CDCl₃, 101 MHz): δ 39.66 (d, 1C, $^2J_{\text{CP}} = 25$ Hz), 40.78 (d, 1C, $^1J_{\text{CP}} = 14$ Hz), 114.54-146.20 (m, 24C), 170.69 (d, 1C, $^3J_{\text{CP}} = 15$ Hz).

(R)-12c was prepared according to **General Procedure (A)** (91% yield, 99% *ee*): $[\alpha]_D^{24} = +58.6^\circ$ [*c* 0.7, CHCl₃]. ³¹P{¹H} (CDCl₃, 162 MHz): δ 1.53; ¹H (CDCl₃, 400 MHz): δ 3.58-3.64 (m, 1H), 3.76 (s, 3H), 4.05-4.13 (m, 1H), 4.36-4.40 (m, 1H), 7.08-8.02 (m, 18H); ¹³C (CDCl₃, 101 MHz): δ 39.30 (d, 1C, ²*J*_{CP} = 24 Hz), 41.02 (d, 1C, ¹*J*_{CP} = 15 Hz), 52.11 (s, 1C), 114.48-146.22 (m, 24C), 166.97 (s, 1C), 170.38 (d, 1C, ³*J*_{CP} = 14 Hz).

(R)-1 was prepared according to **General Procedure (C)** (40% yield): $[\alpha]_D^{25} = +76.9^\circ$ [*c* 0.6, CHCl₃]. ³¹P{¹H} (CDCl₃, 162 MHz): δ 45.08; ¹H (CDCl₃, 400 MHz): δ 3.75-3.82 (m, 1H), 4.42-4.51 (m, 1H), 4.77-4.85 (m, 1H), 7.17-8.09 (m, 19H); ¹³C (CDCl₃, 101 MHz): δ 38.33 (d, 1C, ²*J*_{CP} = 13 Hz), 40.79 (d, 1C, ¹*J*_{CP} = 36 Hz), 114.34-146.28 (m, 24C), 168.65 (d, 1C, ³*J*_{CP} = 20 Hz).

(R)-2 was prepared according to **General Procedure (C)** (43% yield): $[\alpha]_{\text{D}}^{25} = +60.6^\circ$ [*c* 0.7, CHCl₃]. $^{31}\text{P}\{^1\text{H}\}$ (CDCl₃, 162 MHz): δ 44.55; ^1H (CDCl₃, 400 MHz): δ 2.80-2.88 (m, 1H), 3.17-3.26 (m, 1H), 3.50 (s, 3H), 4.37-4.44 (m, 1H), 7.19-8.00 (m, 15H); ^{13}C (CDCl₃, 101 MHz): δ 37.08 (d, 1C, $^2J_{\text{CP}} = 11$ Hz), 41.09 (d, 1C, $^1J_{\text{CP}} = 36$ Hz), 52.24 (s, 1C), 127.02-134.73 (m, 18C), 170.66 (d, 1C, $^3J_{\text{CP}} = 21$ Hz).

(R)-3 was prepared according to **General Procedure (C)** (36% yield): $[\alpha]_{\text{D}}^{25} = +125.0^\circ$ [*c* 1.3, CHCl₃]. $^{31}\text{P}\{^1\text{H}\}$ (CDCl₃, 162 MHz): δ 44.64; ^1H (CDCl₃, 400 MHz): δ 1.05 (t, 3H, $^3J_{\text{HH}} = 7$ Hz) 2.79-2.86 (m, 1H), 3.16-3.25 (m, 1H), 3.93 (q, 2H, $^3J_{\text{HH}} = 7$ Hz), 4.36-4.43 (m, 1H), 7.18-8.00 (m, 15H); ^{13}C (CDCl₃, 101 MHz): δ 14.05 (s, 1C), 37.31 (d, 1C, $^2J_{\text{CP}} = 12$ Hz), 41.16 (d, 1C, $^1J_{\text{CP}} = 36$ Hz), 61.24 (s, 1C), 127.05-134.74 (m, 18C), 170.18 (d, 1C, $^3J_{\text{CP}} = 20$ Hz).

(R)-4 was prepared according to **General Procedure (C)** (49% yield): $[\alpha]_{\text{D}}^{25} = +131.6^{\circ}$ [*c* 0.8, CHCl₃]. $^{31}\text{P}\{^1\text{H}\}$ (CDCl₃, 162 MHz): δ 44.79; ^1H (CDCl₃, 400 MHz): δ 2.82-2.90 (m, 1H), 3.18-3.27 (m, 1H), 3.50 (s, 3H), 3.88 (s, 3H), 4.43-4.51 (m, 1H), 7.24-7.98 (m, 14H); ^{13}C (CDCl₃, 101 MHz): δ 36.87 (d, 1C, $^2J_{\text{CP}} = 11$ Hz), 41.23 (d, 1C, $^1J_{\text{CP}} = 35$ Hz), 52.34 (s, 1C), 52.36 (s, 1C), 126.61-140.17 (m, 18C), 166.60 (s, 1C), 170.39 (d, 1C, $^3J_{\text{CP}} = 20$ Hz).

(5) Single Crystal X-ray Diffraction Data

Figure 2.9 Molecular structure and absolute stereochemistry of the derivative complex (S,R)-11 with 50% thermal ellipsoids shown. Hydrogen atoms except those on the chiral centre are omitted for clarity.

Table 2.2 Crystal data and structure refinement for (*S,R*)-11

Identification code	leung867s	
Chemical formula	C ₃₅ H ₃₄ NO ₂ PPd	
Formula weight	638.00 g/mol	
Temperature	103(2) K	
Wavelength	0.71073 Å	
Crystal size	0.060 x 0.140 x 0.160 mm	
Crystal habit	colorless plate	
Crystal system	orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 9.4258(6) Å	$\alpha = 90^\circ$
	b = 15.4512(8) Å	$\beta = 90^\circ$
	c = 19.8253(12) Å	$\gamma = 90^\circ$
Volume	2887.4(3) Å ³	
Z	4	
Density (calculated)	1.468 g/cm ³	
Absorption coefficient	0.731 mm ⁻¹	
F(000)	1312	
Theta range for data collection	3.34 to 31.06 °	

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Index ranges	-8<=h<=13, -22<=k<=20, -28<=l<=28
Reflections collected	27112
Independent reflections	9226 [R(int) = 0.1121]
Coverage of independent reflections	99.6%
Absorption correction	multi-scan
Max. and min. transmission	0.9570 and 0.8920
Refinement method	Full-matrix least-squares on F ²
Refinement program	SHELXL-2014/6 (Sheldrick, 2014)
Function minimized	$\Sigma w(F_o^2 - F_c^2)^2$
Data / restraints / parameters	9226 / 0 / 364
Goodness-of-fit on F ²	0.990
Final R indices	6951 data; I>2σ(I); R1=0.0573, wR2=0.1035
	all data R1 = 0.0833, wR2 = 0.1170
Weighting scheme	$w=1/[\sigma^2(F_o^2)+(0.0171P)^2]$ where $P=(F_o^2+2F_c^2)/3$
Absolute structure parameter	0.0(0)
Largest diff. peak and hole	0.896 and -0.699 eÅ ⁻³
R.M.S. deviation from mean	0.127 eÅ ⁻³

Figure 2.10 Molecular structure and absolute stereochemistry of the derivative complex (*R*)-**2** with 50% thermal ellipsoids shown. Hydrogen atoms except those on the chiral centre are omitted for clarity.

Table 2.3 Crystal data and structure refinement for (*R*)-**2**

Identification code	leung874s
Chemical formula	$C_{22}H_{21}AuClO_2P$
Formula weight	580.77 g/mol
Temperature	103(2) K
Wavelength	0.71073 Å
Crystal size	0.100 x 0.200 x 0.420 mm
Crystal habit	colorless block
Crystal system	orthorhombic

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Space group	P 21 21 21	
Unit cell dimensions	$a = 8.5024(3) \text{ \AA}$	$\alpha = 90^\circ$
	$b = 13.3425(5) \text{ \AA}$	$\beta = 90^\circ$
	$c = 17.9639(7) \text{ \AA}$	$\gamma = 90^\circ$
Volume	$2037.88(13) \text{ \AA}^3$	
Z	4	
Density (calculated)	1.893 g/cm^3	
Absorption coefficient	7.443 mm^{-1}	
F(000)	1120	
Theta range for data collection	2.65 to 33.19°	
Index ranges	$-13 \leq h \leq 12$, $-18 \leq k \leq 20$, $-25 \leq l \leq 27$	
Reflections collected	16905	
Independent reflections	7699 [R(int) = 0.0868]	
Coverage of independent reflections	99.3%	
Absorption correction	multi-scan	
Max. and min. transmission	0.5230 and 0.1460	
Refinement method	Full-matrix least-squares on F^2	
Refinement program	SHELXL-2014/6 (Sheldrick, 2014)	
Function minimized	$\Sigma w(F_o^2 - F_c^2)^2$	
Data / restraints / parameters	7699 / 204 / 245	

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Goodness-of-fit on F2	0.967
$\Delta/\sigma_{\max}$	0.001
Final R indices	5821 data; $I > 2\sigma(I)$; $R1=0.0576$, $wR2= 0.1035$
	all data $R1 = 0.0851$, $wR2 = 0.1127$
Weighting scheme	$w=1/[\sigma^2(F_o^2)]$
	where $P=(F_o^2+2F_c^2)/3$
Absolute structure parameter	0.0(0)
Largest diff. peak and hole	2.143 and -3.322 eÅ ⁻³
R.M.S. deviation from mean	0.275 eÅ ⁻³

(6) Cell culture and cytotoxicity assay

MDA-MB-231 (human breast adenocarcinoma) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Cells were cultured in a 37 °C, 5% CO₂ and 75% humidified incubator. All the drug treatment experiments were performed in serum-free DMEM medium.

The cytotoxicity of the novel gold(I) monophosphine complexes **1-4** and the control drugs on various cell lines was assessed by propidium iodide (PI) staining coupled with flow cytometry analysis (BD Accuri C6). Briefly, 2.0×10^4 cells were seeded into each well of a 24-well multiplate and allowed to attach overnight in 1 ml complete medium. Next, cells were exposed to different concentrations (including 0 nM drug

concentration as the reference) of each of the drugs for 17 h. After treatment, the cells were harvested by trypsinization, washed with phosphate-buffered saline and subjected to PI staining for FACS analysis. PI-positive cells were considered apoptotic. All experiments were performed in triplicates. Vehicle (solvent DMSO) controls were performed accordingly.

Drug concentration (nM)	(R)-1			(R)-2		
Test No.	1	2	3	1	2	3
100	37.4%	24.9%	33.5%	39.2%	21.0%	15.3%
500	51.4%	47.8%	55.2%	61.3%	43.3%	55.1%
1000	81.5%	66.0%	74.3%	72.1%	69.5%	59.9%
1500	73.5%	77.5%	65.4%	72.2%	73.2%	70.7%
2000	77.5%	82.0%	79.3%	74.9%	77.7%	71.5%
	(R)-3			(R)-4		
Test No.	1	2	3	1	2	3
100	57.9%	37.3%	60.4%	37.9%	35.7%	40.2%
500	56.1%	44.7%	57.1%	56.1%	62.6%	53.2%
1000	77.3%	80.6%	61.3%	77.3%	77.9%	67.1%
1500	74.4%	73.5%	78.3%	74.5%	75.4%	66.8%
2000	74.5%	77.3%	69.5%	78.2%	82.6%	77.0%

Table 2.4 Percentage cell death of MDA-MB231 after treatment of complexes **1-4**

Figure 2.11 The dose-response curve of complexes 1-4 against MDA-MB231 cell line

Drug concentration (nM)	(R)-1			(R)-2		
Test No.	1	2	3	1	2	3
100	9.8	9.5	9.9	13.7	12	14
500	12.7	12.7	12.5	17.2	19.5	16.4
1000	12.8	12.9	12.9	18.1	17.5	19.9
1500	13.3	13.1	13.5	22.3	23	20.5
2000	14.8	15	14.5	23	20.3	20.7
	(R)-3			(R)-4		
Test No.	1	2	3	1	2	3
100	15.2	14.9	15.5	17.2	16.9	18.8
500	17.9	19.5	18	19.2	19.5	18.4
1000	18.5	15.5	19.9	20.5	19.3	21.8
1500	20.3	20.5	21.7	22.8	20.5	23.2
2000	35	34.2	36.1	33.5	34.7	33.9

Table 2.5 Percentage cell death of HaCaT after treatment of complexes 1-4

2.5 References

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Chapter 3

Asymmetric Synthesis and Anticancer Evaluation of
Functionalized Chiral Digold Diphosphine Complexes

**Asymmetric Synthesis and Anticancer Evaluation of Functionalized Chiral Digold
Diphosphine Complexes**

3.1 Introduction

Platinum based complexes such as cisplatin and carboplatin are considerably the most powerful and efficient anticancer drugs we have for chemotherapy today. They are used almost universally in the treatment of testicular, ovarian and several other forms of cancer diseases. Despite significant success in the platinum dominated cancer-therapies, there, however remains a continuous and intense effort to search for alternative drugs that can deal efficiently with cancers which are resistant to these well established chemotherapeutic reagents.

Among the numerous approaches, the development of gold(I) phosphine complexes for cancer therapy has received notable attention, which is discussed in detail in Chapter 1. Both gold(I) and gold(III) complexes exhibit anticancer activity. Noticeably, gold(III) complexes commonly requires diamino-ligands to stabilize its high oxidation state. Moreover, it has been reported that some free phosphines themselves are anti-tumor agents. As such, this project generally targets to gold(I) complexes which have more affinity with phosphine ligands exhibit improved anti-cancer performance.[1] In general, phosphine-gold(I) complexes show a wide spectrum of biological activities, unlike platinum anticancer drugs which interact with DNA chains directly, gold(I) phosphine

complexes primarily target the mitochondrial and may also break DNA bonds as well as DNA-protein cross-links in cancer cells.[2] Many evidence have shown that specific mitochondrial functions that are different from normal cells, are very important for the survival of cancer cells.[3] Hence, biological studies of gold-phosphine compounds contribute to multi-pronged approaches toward developing new cancer therapies, providing much needed alternatives to ongoing anticancer research.

In Chapter 2, we observed that when an ester group was introduced into the carbon skeleton of the structurally rigid phosphine ligands, the resulting phosphine-gold complexes showed high cytotoxicity toward the cancerous cells. As such, we are interested in the further development of functionalized chiral gold-phosphine complexes against other forms of cancers, particularly breast cancer that do not respond well to typical cisplatin treatments.[4] Herein we report the biological effects of a novel series of methyl and ethyl-esters substituted chiral phosphine gold complexes in the treatments of the human breast cancer cell lines MDA-MB-231.

In this chapter, two chiral (-)-diphosphine-digold(I) complexes containing mono- and di-methylester substituted diphosphine ligands have been prepared and structurally characterized. Both complexes are highly potent against breast cancer cell line MDA-MB-231 but showed much lower cytotoxicity against the normal human breast epithelial cells MCF10A. When compared with its mono-substituted analogue, the di-methylester substituted complex caused markedly lower and relatively insignificant

damage to the normal breast cells. The analogous mono- and di-ethylester substituted complexes with the same stereochemistry exhibited similar anti-cancer properties but with noticeably higher cytotoxicity against the MCF10A cells. The enantiomeric complex (+)-diphosphine-digold(I) complexes containing the di-methylester substituted diphosphine ligand exhibited clearly different biological properties from its (-)-enantiomer. Furthermore, a structurally similar diphosphine-digold(I) complex but in the absence of an ester substituent, indiscriminately killed both cancerous and healthy cells. The current study thus revealed that the introduction of multi-esters, particularly methylesters, is an efficient approach to suppress the side-effects and to improve the efficiency of potential gold-based anti-cancer reagents. When combined with the biological observations, the chirality of gold complexes may serve as a sensitive probe for future mechanistic studies. This part of work has been published as a full paper in European Journal of Medicinal Chemistry.[5]

3.2 Results and Discussion

3.2.1 Design and synthesis

In order to further study of anti-cancer properties of ester-functionalized chiral gold(I) phosphine complexes, five neutral diphosphine digold complexes **13-17** have been designed and synthesized (Figure 3.1). The complexes were selected to illustrate: (1) whether an ester substituent would offer a higher drug activity against cancer cells and

lower cytotoxicity to normal human cells, (2) if the composition of the ester group(s) would affect on drug efficiency and, (3) whether complexation with breast cancer cell were sensitive to the stereochemistry of the phosphine gold complexes. The preparation of the desired air-sensitive functionalized chiral diphosphines would be the main synthetic challenge. We have recently reported that diester-substituted tertiary diphosphine ligands which could be prepared efficiently through chiral palladium template controlled asymmetric hydrophosphination reaction between diphosphine and the respective diester-substituted alkynes.[6] The mono-ester or phenyl substituted diphosphine ligands were prepared analogous to the asymmetric P-H addition reaction using corresponding monosubstituted vinyl diphenylphosphine as the starting materials.[7] All the desired ligands were obtained efficiently by adopting these established synthetic strategies. The desired diphosphine digold complexes could be prepared in high yields from a simple coordination reaction between 2 mol of $[\text{ClAuSMe}_2]$ and the corresponding enantiomerically pure diphosphines. Different from their free ligands, complexes **13-17** were air-stable and could be readily crystallized from various solvent systems. In the solid state, the complexes remained unchanged after being kept for one year, which is considered as an important property as anticancer drug candidates.

Figure 3.1 Molecular structures of optically pure gold(I)-diphosphine complexes **13-17**

According to the structural designs, the diester- substituents in both complexes (SS)-**13** and (SS)-**14** were located on adjacent chiral carbon centers. Figure 3.2 depicted the molecular structure and absolute stereochemistry of (SS)-**13** [8]. As expected, the complex contained two linear P-Au-Cl units. The conformation analysis revealed that the two adjacent ester groups on the P-C-C-P linkage project into different directions due to the intra-molecular steric interactions. Such an uncompromised staggered orientation *ipso facto* separated the two P-Au-Cl fragments effectively on the two sides of the molecules. Therefore, the commonly observed intra-molecular auriophilic gold-gold bond [9] was not observed between the two gold atoms despite the fact that all the three bonds in the P-C-C-P linkage were single and rotatable bonds. From a stereochemical viewpoint, the formation of a gold-gold bond within a diphosphine complex would generate a

six-membered ring structure which might render serious steric hindrances when the Au-Au unit was approaching the living cells.

Figure 3.2 Molecular structure and absolute stereochemistry of (*SS*)-**13**

Interestingly, the optically pure forms of the mono-substituted diphosphine complexes **15-17** were found to be more soluble in most organic solvents. These unsymmetrical di-gold complexes could not be induced to form single crystals that were suitable for structural analyses. Nevertheless, when a solution of (*S*)-**15** was mixed with its enantiomeric counterpart (*R*)-**15**, single crystals of the mono-ester complex were readily obtained. The crystallographic studies revealed that the chiral complex packed as a pair of enantiomers in the same crystal lattice. Importantly, structural analysis confirmed that the two P-Au-Cl units linked by the same diphosphine ligand project into

different directions (Figure 3.3).[10] No intra-molecular gold-gold bond was formed in **15** despite the fact that the diphosphine ligand was less sterically demanding than those observed in (*SS*)-**13**. Clearly the two P-Au-Cl units in the mono-substituted complexes were able to interact independently with the living cells in the subsequent biological experiments.

Figure 3.3 Molecular structure of ($\pm$)-**15**

3.2.2 Cytotoxicity evaluation

A preliminary evaluation of the cytotoxicity of complexes **13-17** was conducted using propidium iodide staining coupled with flow cytometry. In these experiments, the human metastatic breast cell line MDA-MB-231 was used. The cancerous cells were treated separately with each gold complex for up to 17 h before they were harvested and

stained with propidium iodide. The stained cells were subjected to flow cytometry and the cell viabilities were analyzed quantitatively. Cisplatin was used as a referencing treatment control. The resulting dose-response curves for the gold complexes were shown in Figure 3.4 and the corresponding IC_{50} values were presented in Table 3.1. All the gold complexes displayed high anti-cancer activities with their IC_{50} values ranging from 0.32 to 1.41 μM against breast cancer cells. Detailed analysis revealed that diester-substituted complexes **13** and **14** were more efficient in inducing cancer cell death than their mono-substituted counterparts, **15** and **16**. On the other hand, the only gold compound **17**, which did not contain any ester functionality in the molecular structure, displayed the lowest cytotoxicity as compared with the four other similar phosphine-gold complexes. These cytotoxicity results therefore suggested that when more ester functionalities were incorporated into phosphine ligands, the resulting gold complexes tend to exhibit enhanced anti-cancer activity. It was noteworthy that under the same biological evaluation conditions, cisplatin did not show any significant in-vitro anti-cancer activity. It was also important to note that, prior to complexation with the gold(I) metal ion, the free phosphine ligands did not show any detectable biological activity, especially at concentrations below 2.0 μM .

Figure 3.4 The dose-response curve of complexes **13-17** against MDA-MB231 cell line.

Complex	IC ₅₀ [μ M]
(RR)-13	0.56 $\pm$ 0.211
(SS)-13	0.44 $\pm$ 0.093
(SS)-14	0.32 $\pm$ 0.100
(S)-15	1.16 $\pm$ 0.169
(S)-16	1.20 $\pm$ 0.103
(S)-17	1.41 $\pm$ 0.239
Cisplatin	No effect detected

Table 3.1 Cytotoxicity (IC₅₀: [μ M]) of the phosphine-gold complexes against the MDA-MB231 cell line.

3.2.3 Functional group effects on selectivity

The IC₅₀ determinations confirmed that the gold complexes were highly active against the breast cancer line MDA-MB231. In order to examine their potential clinical

values, the biological effects of these gold compounds on both breast cancer cells and normal human breast epithelial cells MCF10A were studied and compared under similar experimental conditions. These epithelium cells were obtained from healthy mammary gland. The in vitro test results were shown in Figure 3.5. As deduced from their IC_{50} values, the diester-substituted gold complexes (SS)-**13** and (SS)-**14** showed high potency against the cancerous cells. In addition, both complexes exhibited significantly low cytotoxicity against the healthy human cells (Figure 3.5a, b). Between these two complexes, the diethylester complex (SS)-**14** consistently exhibited higher activity than its dimethylester counterpart (SS)-**13** with both the healthy and cancerous cells. For example, when a 0.5 μ M solution of (SS)-**14** was used, 85.5% of the cancerous cells and 2.8% of the healthy cells were killed (Figure 3.5b). Under the same condition, a diluted solution of (SS)-**13** killed 67.7% and 1.8% of the cancerous and healthy cells, respectively (Figure 3.5a). Similarly, a more concentrated solution (2 μ M) of (SS)-**14** killed 96.3 and 8.6% of the cancerous and the healthy cells respectively, while a solution of (SS)-**13** killed slightly lesser breast cancer cells (87.1%) and mammary gland cells (3.1%). According to the observed cytotoxicity against healthy cells, (SS)-**13** will probably exhibit lesser side effects than (SS)-**14** when they are applied to in vivo animal tests. Nevertheless, these biological tests indicate that both complexes are potentially useful drug candidates and will be subjected to further evaluations in the near future.

Comparatively, both the mono-ester substituted complexes (*S*)-**15** and (*S*)-**16** clearly exhibited lower cytotoxicity to cancer cells as compared to their corresponding diester counterparts, particularly at lower concentration range (Figure 3.5c, d). Interestingly, similar to their di-substituted analogues, the methyl-ester substituted complex (*S*)-**15** showed significantly lower cytotoxicity against healthy mammary cells than their ethyl-ester counterpart (*S*)-**16**. For example, a 2 μ M solution of (*S*)-**15** only killed 5% of the mammary gland cells but a solution of (*S*)-**16** with the same concentration killed 21.4% of the healthy cells.

The importance of incorporating ester groups in the gold complexes can be further confirmed when complex (*S*)-**17** was employed in the biological tests. With no ester functionalities in the phosphine skeleton of (*S*)-**17**, Figure 3.5e showed that this complex is significantly less potent against cancerous cells. Furthermore, it also exhibited markedly higher toxicity against healthy cells. Moreover, the complex eliminated more mammary cells than the breast cancer cells at lower concentrations. These biological tests therefore demonstrate that the incorporation of ester functionalities in phosphine ligands is an efficient approach to control both the cytotoxicity and selectivity in these potential anti-cancer gold reagents.

Figure 3.5 MDA-MB231 and MCF10A cell death percentages after treatments with complexes **13-17**.

3.2.4 Effect of chirality on drug efficiency

The presence of the stable chiral carbon centers in the gold complexes allows for the study of their stereochemistry effects in the biological tests. As complex (*SS*)-**13** demonstrated high potency against breast cancer cells with minimal damage to healthy cells, the corresponding enantiomer (*RR*)-**13** was prepared and examined. Figure 3.5f revealed that the cytotoxicity of this (*RR*)-enantiomer against the breast cancer cells was comparable with that of (*SS*)-**13**. However, the (*RR*)-enantiomer was markedly more toxic against healthy mammary gland cells: A 2 μ M solution of (*RR*)-**13** killed 30.1% of healthy cells but with the same concentration, a solution of (*SS*)-**13** only killed 3.1% the same cells (Figure 3.6). Clearly, stereochemistry does indeed play an important role in the biological properties of these phosphine-gold complexes.

Figure 3.6 MCF10A cell death percentages after treatments with enantiomeric complexes (*RR*)-**13** and (*SS*)-**13**.

3.3 Conclusion

The current study demonstrated that phosphine-gold complexes are biologically highly active when they are in contact with breast cancer and healthy mammary gland cells. Complementing established mechanisms of gold(I) phosphine complexes, incorporating ester functional groups into the carbon skeleton of the phosphine ligand can dramatically enhanced their selectivity toward cancerous cells. Significantly higher selectivity can be achieved when more ester groups are introduced. The chemical compositions of the ester substituents also play a significant role in the bioselectivity. When compared with a gold complex that bears an ethylester substituent, the methylester analogue is noticeably less toxic to the healthy mammary gland cells while both complexes are capable of efficiency killing the cancerous cells. Thus, the employment of the methylester functional group may strategically reduce the potential side effects in further cancer research. Furthermore, the bioselectivity is sensitive to the chirality of the gold complexes as (*SS*)-**13** has little effect on the healthy cells when compared with its *RR* enantiomer under the same experimental condition. Such an observation may bear heavily on the future SAR investigations.

3.4 Experimental Section

Material and Methods

General. All air-sensitive syntheses were performed under a positive pressure of nitrogen using a standard Schlenk line. Solvents were degassed prior to use when necessary. Column chromatography was conducted on silica gel 60 (Merck). NMR spectra were recorded on Bruker ACF 400 Hz spectrometers. Chemical shifts are referenced to the chloroform-d solvent signals ($\delta = 7.26$ for ^1H and $\delta = 77.16$ for ^{13}C NMR spectra). Optical rotations were measured on the specified solution in a 0.1 dm cell with a Atago AP-300 polarimeter. The diphosphine ligands were prepared according to the literature methods. [11]

(1) General procedures for the synthesis of optically pure diphosphine-gold(I) complexes 13-17

A dichloromethane solution (30 mL) containing the selected diphosphine ligand (0.1 mmol) was treated with chloro(dimethyl sulfide)gold(I) (63.0 mg, 0.211 mmol) for 3 hours in a dark environment at ambient temperature. The solvent and dimethyl sulfide was removed in vacuo. The optically pure gold(I)-diphosphine complex was crystallized from chloroform and diethyl ether as a colorless crystal (60-65 % yield).

Scheme 3.1 General preparation procedure of complexes **13-17**

(SS)-13. $[\alpha]_D^{24} = -83.2^\circ$ ($c=0.02$, CH_2Cl_2). ^{31}P NMR (162 MHz, CDCl_3) δ 37.78 (s, 2P, -P[-CH(COOCH₃)-]-). ^1H NMR (400 MHz, CDCl_3) δ 7.71-7.44 (m, 20H, -C₆H₅), 4.46 (d, 2H, $^2J_{\text{HP}} = 2$ Hz, -CH(-P)-), 3.18 (s, 6H, -OCH₃). ^{13}C NMR (101 MHz, CDCl_3) δ 167.88 (t, 2C, $^2J_{\text{CP}} = 3.5$ Hz, -C(O)-), 135.39-125.51 (m, 24C, -C₆H₅), 52.92 (s, 2C, -OCH₃), 47.74 (dd, 2C, $^1J_{\text{PC}} = 14.1$ Hz, $^2J_{\text{PC}} = 18.2$ Hz, -CH(COOCH₃)-). LCMS (ESI) for $\text{C}_{30}\text{H}_{28}\text{Au}_2\text{ClO}_4\text{P}_2$ $[\text{M}-\text{Cl}]^+$: calcd, 943.05 (100.0%), 945.05 (32.0%), 944.05 (21.6%); found, 943.02 (100.0%), 945.08 (32.0%), 944.08 (21.6%). Complex **(RR)-13** was

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prepared similarly from the corresponding (*RR*)-diphosphine ligand. The enantiomeric complex exhibited identical spectroscopic properties except for the optical rotation, which was recorded as $[\alpha]_D^{24} = +84.0^\circ$ ($c=0.02$, CH_2Cl_2).

(SS)-14

(SS)-14. $[\alpha]_D^{24} = -93.8^\circ$ ($c=0.05$, CH_2Cl_2). ^{31}P NMR (162 MHz, CDCl_3) δ 37.53 (s, 2P, $-P[-\text{CH}(\text{COOC}_2\text{H}_5)-]$). ^1H NMR (400 MHz, CDCl_3) δ 7.75-7.40 (m, 20H, $-\text{C}_6\text{H}_5$), 4.44 (d, 2H, $^2J_{\text{HP}} = 2.0$ Hz, $-\text{CH}(-\text{P})-$), 3.76-3.68 (m, 2H, $-\text{OCH}_2-$), 3.52-3.43 (m, 2H, $-\text{OCH}_2-$), 0.88 (t, 6H, $J_{\text{HH}} = 7.0$ Hz, $-\text{CH}_3$). ^{13}C NMR (101 MHz, CDCl_3) δ 167.42 (t, 2C, $^2J_{\text{CP}} = 3.5$ Hz, $-\text{C}(\text{O})-$), 135.49-125.80 (m, 24C, $-\text{C}_6\text{H}_5$), 62.70 (s, 2C, $-\text{OCH}_2-$), 47.73 (dd, 2C, $^1J_{\text{PC}} = 14.1$ Hz, $^2J_{\text{PC}} = 17.2$ Hz, $-\text{CH}(\text{COOC}_2\text{H}_5)-$), 13.59 (s, 2C, $-\text{CH}_3$). LCMS (ESI) for $\text{C}_{32}\text{H}_{32}\text{Au}_2\text{ClO}_4\text{P}_2$ $[\text{M}-\text{Cl}]^+$: calcd, 971.08 (100.0%), 973.08 (32.0%), 972.08 (23.8%); found, 971.09 (100.0%), 973.08 (32.0%), 972.11 (23.8%).

(S)-15

(S)-15. $[\alpha]_D^{24} = -43.7^\circ$ ($c=0.04$, CH_2Cl_2). ^{31}P NMR (162 MHz, CDCl_3) δ 44.63 (d, 1P, $^3J_{\text{PP}} = 50.2$ Hz, $-P[-\text{CH}(\text{COOCH}_3)-]$), δ 29.96 (d, 1P, $^3J_{\text{PP}} = 50.2$ Hz, $-P(-\text{CH}_2)-$). ^1H NMR

(400 MHz, CDCl₃) δ 7.83-7.45 (m, 20H, -C₆H₅), 4.02-3.93 (m, 1H, -CH(-P)-), 3.50-3.40 (m, 1H, -CH₂(-P)-), 3.10 (s, 3H, -OCH₃), 2.64-2.43 (m, 1H, -CH₂(-P)-). ¹³C NMR (101 MHz, CDCl₃) δ 168.11 (s, 1C, -C(O)-), 134.29-124.74 (m, 24C, -C₆H₅), 52.83 (s, 1C, -OCH₃), 42.41 (dd, 1C, ¹J_{PC} = 27.3 Hz, ²J_{PC} = 6.1 Hz, -CH(COOCH₃)-), 28.92 (dd, 1C, ¹J_{PC} = 8.1 Hz, ²J_{PC} = 39.4 Hz, -OCH₂-). LCMS (ESI) for C₂₈H₂₆Au₂ClO₂P₂ [M-Cl]⁺: calcd, 885.04 (100.0%), 887.04 (32.0%), 886.05 (19.5%); found, 885.12 (100.0%), 887.11 (32.0%), 886.15 (19.5%).

(S)-**16**

(S)-**16**. [α]_D²⁴ = -30.0° (c=0.03, CHCl₃). ³¹P NMR (162 MHz, CDCl₃) δ 44.95 (d, 1P, ³J_{PP} = 50.2 Hz, -P[-CH(COOC₂H₅)-]), 30.29 (d, 1P, ³J_{PP} = 50.2 Hz, -P(-CH₂-)). ¹H NMR (400 MHz, CDCl₃) δ 7.71-7.42 (m, 20H, -C₆H₅), 4.04-3.94 (m, 1H, -CH(-P)-), 3.53-3.42 (m, 3H, -CH₂(-P)-, -OCH₂-), 2.56-2.45 (m, 1H, -CH₂(-P)-), 0.70 (t, 3H, J_{HH} = 7.2 Hz, -CH₃). ¹³C NMR (101 MHz, CDCl₃) δ 167.78 (s, 1C, -C(O)-), 134.54-125.06 (m, 24C, -C₆H₅), 62.61 (s, 1C, -OCH₂-), 42.31 (dd, 1C, ¹J_{PC} = 6.1 Hz, ²J_{PC} = 27.3 Hz, -CH(COOC₂H₅)-), 28.77 (dd, 1C, ¹J_{PC} = 7.6 Hz, ²J_{PC} = 235.4 Hz, -CH₂-), 13.43 (s, 1C, -CH₃). LCMS (ESI) for C₂₉H₂₈Au₂ClO₂P₂ [M-Cl]⁺: calcd, 899.06 (100.0%), 901.06 (32.0%), 900.06 (20.5%); found, 899.11 (100.0%), 901.12 (32.0%), 900.16 (20.5%).

(S)-17

(S)-17. $[\alpha]_{\text{D}}^{24} = -67.4^{\circ}$ ($c=0.02$, CH_2Cl_2). ^{31}P NMR (162 MHz, CDCl_3) δ 46.83 (d, 1P, $^3J_{\text{PP}} = 53.5$ Hz, $-P[-\text{CH}(\text{Ph})-]$), 29.31 (d, 1P, $^3J_{\text{PP}} = 53.5$ Hz, $-P(-\text{CH}_2-)$). ^1H NMR (400 MHz, CDCl_3) δ 7.47-7.00 (m, 25H, $-\text{C}_6\text{H}_5$), 4.25-4.16 (m, 1H, $-\text{CH}(-\text{P})-$), 3.44-3.33 (m, 1H, $-\text{CH}_2(-\text{P})-$), 2.81-2.72 (m, 1H, $-\text{CH}_2(-\text{P})-$). ^{13}C NMR (101 MHz, CDCl_3) δ 132.70-126.04 (m, 30C, $-\text{C}_6\text{H}_5$), 42.70 (dd, 1C, $^1J_{\text{PC}} = 5.6$ Hz, $^2J_{\text{PC}} = 32.3$ Hz, $-\text{CH}(\text{Ph})-$), 31.76 (dd, 1C, $^1J_{\text{PC}} = 11.6$ Hz, $^2J_{\text{PC}} = 36.4$ Hz, $-\text{CH}_2-$). LCMS (ESI) for $\text{C}_{32}\text{H}_{28}\text{Au}_2\text{ClP}_2$ $[\text{M}-\text{Cl}]^+$: calcd, 903.07 (100.0%), 904.07 (34.6%), 905.07 (32.0%); found, 903.10 (100.0%), 904.13 (34.6%), 905.11 (32.0%).

(2) Single Crystal X-ray Diffraction Data

a. X-ray crystallography data for complex (SS)-13

Table 3.2 Crystal data and structure refinement for (SS)-13.

Identification code	(SS)-13	
Empirical formula	C ₃₀ H ₂₈ Au ₂ Cl ₂ O ₄ P ₂	
Formula weight	979.30	
Temperature	103(2) K	
Wavelength	0.71073 Å	
Crystal system	Tetragonal	
Space group	P4(1)2(1)2	
Unit cell dimensions	$a = 13.7241(3)$ Å	$= 90^\circ$.
	$b = 13.7241(3)$ Å	$= 90^\circ$.

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	$c = 16.3516(4) \text{ \AA}$	$= 90^\circ$
Volume	$3079.84(12) \text{ \AA}^3$	
Z	4	
Density (calculated)	2.112 Mg/m^3	
Absorption coefficient	9.828 mm^{-1}	
F(000)	1848	
Crystal size	$0.16 \times 0.14 \times 0.12 \text{ mm}^3$	
Theta range for data collection	$1.94 \text{ to } 31.02^\circ$	
Index ranges	$-19 \leq h \leq 18, -19 \leq k \leq 19, -21 \leq l \leq 23$	
Reflections collected	22926	
Independent reflections	4907 [$R(\text{int}) = 0.0663$]	
Completeness to $\theta = 31.02^\circ$	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.3851 and 0.3023	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4907 / 0 / 182	
Goodness-of-fit on F^2	1.054	
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0303, wR2 = 0.0674$	
R indices (all data)	$R1 = 0.0409, wR2 = 0.0944$	
Absolute structure parameter	-0.004(12)	
Largest diff. peak and hole	$0.794 \text{ and } -1.101 \text{ e.\AA}^{-3}$	

b. X-ray crystallography data for complex ($\pm$)-15

Crystal Structure Report for complex (±)-15

A colorless plate-like specimen of $C_{29}H_{27}Au_2Cl_5O_2P_2$, approximate dimensions 0.040 mm x 0.200 mm x 0.240 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured.

The integration of the data using a triclinic unit cell yielded a total of 80983 reflections to a maximum θ angle of 32.68° (0.66 Å resolution), of which 23881 were independent (average redundancy 3.391, completeness = 98.9%, $R_{\text{int}} = 15.42\%$, $R_{\text{sig}} = 18.37\%$) and 12367 (51.79%) were greater than $2\sigma(F^2)$. The final cell constants of $a = 11.4039(7)$ Å, $b = 16.2996(10)$ Å, $c = 19.1657(13)$ Å, $\alpha = 103.695(4)^\circ$, $\beta = 98.238(4)^\circ$, $\gamma = 103.751(4)^\circ$, volume = $3286.0(4)$ Å³, are based upon the refinement of the XYZ-centroids of reflections above $20\sigma(I)$. Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.380.

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The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.2100 and 0.7040.

The final anisotropic full-matrix least-squares refinement on F^2 with 846 variables converged at $R1 = 6.72\%$, for the observed data and $wR2 = 17.02\%$ for all data. The goodness-of-fit was 0.972. The largest peak in the final difference electron density synthesis was $2.834 \text{ e}^-/\text{\AA}^3$ and the largest hole was $-4.102 \text{ e}^-/\text{\AA}^3$ with an RMS deviation of $0.334 \text{ e}^-/\text{\AA}^3$. On the basis of the final model, the calculated density was 2.103 g/cm^3 and $F(000)$, 1960 e^- .

Table 3.3 Sample and crystal data for ($\pm$)-15.

Identification code	($\pm$)-15	
Chemical formula	$\text{C}_{29}\text{H}_{27}\text{Au}_2\text{Cl}_5\text{O}_2\text{P}_2$	
Formula weight	1040.63 g/mol	
Temperature	103(2) K	
Wavelength	0.71073 $\text{\AA}$	
Crystal size	0.040 x 0.200 x 0.240 mm	
Crystal habit	colorless plate	
Crystal system	triclinic	
Space group	P -1	
Unit cell dimensions	$a = 11.4039(7) \text{ \AA}$	$\alpha = 103.695(4)^\circ$
	$b = 16.2996(10) \text{ \AA}$	$\beta = 98.238(4)^\circ$
	$c = 19.1657(13) \text{ \AA}$	$\gamma = 103.751(4)^\circ$
Volume	$3286.0(4) \text{ \AA}^3$	

Z	4
Density (calculated)	2.103 g/cm ³
Absorption coefficient	9.449 mm ⁻¹
F(000)	1960

Table 3.4 Data collection and structure refinement for (±)-**15**.

Theta range for data collection	2.51 to 32.68 °
Index ranges	-14<=h<=17, -24<=k<=24, -29<=l<=28
Reflections collected	80983
Independent reflections	23881 [R(int) = 0.1542]
Absorption correction	multi-scan
Max. and min. transmission	0.7040 and 0.2100
Refinement method	Full-matrix least-squares on F ²
Refinement program	SHELXL-2014/6 (Sheldrick, 2014)
Function minimized	$\Sigma w(F_o^2 - F_c^2)^2$
Data / restraints / parameters	23881 / 668 / 846
Goodness-of-fit on F ²	0.972
$\Delta/\sigma_{\max}$	0.002
Final R indices	12367 data; I>2σ(I) all data
	R1 = 0.0672, wR2 = 0.1313 R1 = 0.1567, wR2 = 0.1702
Weighting scheme	$w=1/[\sigma^2(F_o^2)+(0.0391P)^2]$ where $P=(F_o^2+2F_c^2)/3$

Largest diff. peak and hole	2.834 and -4.102 eÅ ⁻³
R.M.S. deviation from mean	0.334 eÅ ⁻³

(3) Cell culture

MDA-MB-231 (human breast adenocarcinoma) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). MCF10A cells (normal human mammary epithelial cells) were growth in DMEM/F12 supplemented with 5% horse serum, hydrocortisone (0.5 µg/ml), cholera toxin (100 ng/ml), insulin (10 µg/ml), and EGF (20 ng/ml). All cells were cultured in a 37 °C, 5% CO₂ and 75% humidified incubator. All the drug treatment experiments were performed in serum-free DMEM medium.

(4) Cytotoxicity assay

The cytotoxicity of the novel diphosphine-digold complexes **13-17** and the control drugs on various cell lines was assessed by propidium iodide (PI) staining coupled with flow cytometry analysis (BD Accuri C6). Briefly, 2.0×10^4 cells were seeded into each well of a 24-well multiplate and allowed to attach overnight in 1ml complete medium. Next, cells were exposed to different concentrations (including 0 nM drug concentration as the reference) of each of the drugs for 17 hours. After treatment, the cells were harvested by trypsinization, washed with phosphate-buffered saline and subjected to

PI staining for FACS analysis. PI-positive cells were considered apoptotic. All experiments were performed in triplicates. Vehicle (solvent DMSO) controls were performed accordingly.

(5) *In vitro* screening results for complexes 13-17

Drug concentration (nM)	(RR)-13			(SS)-13			(SS)-14		
Test No.	1	2	3	1	2	3	1	2	3
100	29.9	33.6	17.1	44.5	23.6	37.7	79.5	43.3	32
500	63.4	50.3	53.6	59.5	66.3	77.3	92.8	89.7	73.9
1000	90.7	74.7	88	91.2	80.5	89	97.3	92.5	93.4
1500	93.8	90.5	87.7	86.5	83.7	91.7	96.5	95.3	96.1
2000	93.6	95.9	90.6	85.4	85.5	90.4	98.5	93.8	96.7
	(S)-15			(S)-16			(S)-17		
Test No.	1	2	3	1	2	3	1	2	3
100	5.3	3.3	4.7	3.7	2	5.5	3.2	5.5	4.3
500	9.6	13.9	21.5	6.5	11.6	9.8	7.9	10.1	11.6
1000	30.9	35.6	43.9	40.1	37.5	44.3	11.8	15.3	12.8
1500	86.2	65.5	77.8	62.8	73.2	65.7	36.4	46.4	29.8
2000	86.6	88	90.5	82.7	89.4	80.5	53.7	56.4	47.9

Table 3.5 Percentage cell death of MDA-MB231 after treatment of complexes **13-17**

Drug concentration (nM)	(RR)-13			(SS)-13			(SS)-14		
Test No.	1	2	3	1	2	3	1	2	3
100	1.5	2.2	1.4	2.7	1.3	2.3	3.4	2.1	2.7
500	3.7	4	2.9	1.6	2.2	1.7	1	3.3	4.2
1000	15.4	16.7	13.2	2.6	3	3.3	9.6	5.6	7.7
1500	27.1	27.3	28.5	4.1	3.9	4.5	6.1	5.5	4.5
2000	30.5	32.1	27.6	3.1	2.7	3.5	8.4	9.5	8
	(S)-15			(S)-16			(S)-17		
Test No.	1	2	3	1	2	3	1	2	3
100	1.8	2.3	1.2	1.6	2.5	2	23.6	17.5	20.3
500	1.1	2.5	1.9	3.4	4.4	2.7	29.3	25.6	31.9
1000	2.2	3.6	2.3	10.5	11.7	9	42.5	40.9	37.7
1500	5.2	4.3	5.6	14.6	15.4	13.3	31.9	43	35.4
2000	5.3	5.1	4.7	21.2	19.6	23.3	35.1	37.5	30

Table 3.6 Percentage cell death of MCF10A after treatment of complexes **13-17**

Propidium iodide (PI)

3.5 Reference

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Conclusion

This thesis has discussed asymmetric synthesis of enantiomeric pure gold(I) phosphine complexes and studied their cytotoxicity and selectivity against human cancer and normal cell lines. Chapter 1 introduces types of cancer therapies and the medical applications of organometallic complexes including gold and other transition metals. The traditional synthesis of phosphines is also included. Chapter 2 describes the asymmetric syntheses of optically pure gold(I) monophosphine complexes with amide and ester substituents through efficient Pd(II) catalyzed hydrophosphination reactions. The *in vitro* screening showed promising anticancer activity against human breast cancer cells, the complexes bearing benzotriazole group or methyl ester group showed considerable selectivity between cancer cells and normal cells, however the candidates are still not ideal enough to suppress the side effects possible brought by the chemotherapy. In Chapter 3, a series of optically pure digold diphosphine complexes with one or two ester functional group(s) in the carbon skeleton were synthesized through Pd(II) promoted hydrophosphination reactions. The mono- and dimethylester substituted diphosphine candidates are highly potent against breast cancer cells but showed much more lower cytotoxicity against the normal human breast epithelial cells. When compared with its mono-substituted analogue the di-methylester substituted complex caused markedly lower and relatively insignificant damage to the normal breast cells. The enantiomeric complex (+)-diphosphine-digold(I) complexes containing the dimethylester substituted

diphosphine ligand exhibited clearly different biological properties from its (-)-enantiomer. Furthermore, a structurally similar diphosphine-digold(I) complex but in the absence of an ester substituent, indiscriminately killed both cancerous and healthy cells.

The current study thus revealed that the introduction of multi-esters, particularly *methylesters*, is an efficient approach to suppress the side effects and to improve the efficiency of potential gold-based anticancer reagents. When combined with the biological observations, the chirality of gold complexes may serve as a sensitive probe for the future mechanistic studies. The thesis has demonstrated that gold(I) phosphine complexes are biologically highly active when they are in contact with breast cancer and healthy mammary gland cells. Complementing established mechanisms of gold(I) phosphine complexes, incorporating ester functional groups and changing complexes' chirality can dramatically enhance their selectivity and anticancer activity. This research hopefully will make contribute to the future SAR (structure-activity relationship) investigations.