RUTHENIUM(II) POLYPYRIDYL COMPLEXES AS POTENTIAL ANTICANCER DRUGS: SYNTHESIS, CHARACTERIZATION, CELL PERMEABILITY AND LOCALLIZATION

by

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ABSTRACT

RUTHENIUM(II) POLYPYRIDYL COMPLEXES AS POTENTIAL ANTICANCER DRUGS:
SYNTHESIS, CHARACTERIZATION, CELL PERMEABILITY
AND LOCALLIZATION

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Research on biological activities of ruthenium polypyridyl complexes (RPCs) continues to attract interest as these complexes have shown promising anticancer activity both *in vitro* and *in vivo*.[1-3] The mononuclear RPC, $[(phen)_2Ru(tatpp)]^{2+}$ (MP) and related dinuclear complex $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ (P) have been shown to both intercalate with DNA and shown potentiated DNA cleavage under anaerobic and reducing conditions[4,5], as well as shown good selectivity and cytotoxicity towards malignant cell lines *in vitro* and tumors *in vivo*.[5] Both complexes contain the redox-active tatpp ligand which is thought to be an essential component for the observed biological activities. This thesis is focused on developing improvements to the stereoselective syntheses of the chiral complexes, ΔΔ-P and Δ-MP. It is also investigated the synthesis of a new analogue $[(phen)_2Ru(tadbp)]^{2+}$ (B) and its chiral form Δ-B which contains a

modified tatpp ligand that is only capable of binding one Ru ion. Moreover this thesis explores the ability of these large complexes to traverse the cell membranes and get into cells and cell nuclei by isolating treated cells and nuclei and determining their ruthenium content by graphite furnace atomic absorption method (GFAAS). The GFAAS data show that the two examined complexes ΔΔ-P and Δ-MP are able to quickly penetrate into cancer cells (H-358) and concentrate in nuclei, which is postulated due to their high binding affinity to DNA.

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LIST OF ABBREVIATIONS

A	Adenine
AcOH	Acetic acid
BLM	Bleomycin
bp	Base-pair
bpy	2,2´-Bipyridine
CD_3CN	Deuterated acetonitrile
CDDP	*cis*-Diamminedichloroplatinum(II)
Cisplatin	*cis*-Diamminedichloroplatinum(II)
CN	Cyanide
cpdppz	12-Cyano-12,13-dihydro-11*H*-cyclopenta[*b*]dipyrido[3,2-*h*:2′3′-*j*]phenazine-12-carbonyl
CV	Cyclic Voltammetry
d	Doublet
DIP	4,7-Diphenyl-1,10-phenanthroline
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
dppn	Benzo[*i*]dipyrido [3,2-*a*:2',3'-*c*]phenazine
dppz	Dipyrido[3,2-a: 2',3'-c]phenazine
dpq	2,3-Bis(2-pyridyl)pyrazine
ee%	Enantiomeric Excess

EI-MS	Electron Impact Ionization Mass Spectrometry
eq	Equivalent
EtOH	Ethanol
EDTA	Ethylenediaminetetraacetic Acid
Fc	Ferrocene
Fig	Figure
G	Guanine
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
GI_{50}	Causes 50% growth inhibition concentration
GSH	Glutathione
H-358	Human non-small cell lung cancer cells
HCC-2998	Human colon carcinoma cells
His	Histidine
HPLC	High-performance liquid chromatography
IC_{50}	Half maximal inhibitory concentration
Im	Imidazole
In	Indazole
MeCN	Acetonitrile
MeOH	Methanol
MP	$[(phen)_2Ru(tatpp)]^{2+}$
MTD	Maximum tolerated dose
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NAMI-A	[ImH][trans-$RuCl_4$(DMSO)(Im)]
NCI	National Cancer Institute
NHE	Normal hydrogen electrode
NMR	Nuclear Magnetic Resonance
P	$[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$
phen	1,10- Phenanthroline
pO_2	Partial pressure of oxygen
rac	Racemic
RPCs	Ruthenium Polypyridyl complexes
RT	Room temperature
s	Singlet
SHE	Standard hydrogen electrode
t	Triplet
T/C	Tumor growth value
tadbp	9,11,20,22-Tetraazadipyrido[3,2-a: 2',3']dibenzo[3",2"-l:2"',3"'-n]pentacene
tatpp	9,11,20,22-Tetraaza tetrapyrido[3,2-a:2'3'-c:3'',2''-1:2''',3''']-pentacene
TEA	Triethylamine
TMS	Tetramethylsilane
tpphz	Tetrapyrido[3,2-a: 2′,3′-c: 3″,2″-h: 2″,3"'-j]phenazine
tpy	2,2´:6´,2"-Terpyridine
UV	Ultraviolet

CHAPTER 1

RUTHENIUM(II) POLYPYRIDYL COMPLEXES AS POTENTIAL ANTICANCER AGENTS

1.1 Introduction of cancer and chemotherapy

1.1.1 Cancer

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells.[6] The rapid increase of cancer cases represents a real challenge for health systems. Cancer can be treated with surgery, radiation, chemotherapy, hormone therapy, and targeted therapy.[7] Treatment varies based on the types of cancer and the cancer stages. Some types of cancer require a combination of surgery, radiation, and chemotherapy.[8]

1.1.2 Chemotherapy

A chemotherapeutic agent is a term used to identify compounds that are effective at killing or slowing growth of cancer cells in a human being while causing as little as possible deleterious side-effects.[8] They are also referred to as antineoplastic agents. Chemotherapy is frequently used in combination with surgery or radiotherapy as these two other treatments are only effective at removing or killing tumor cells where they are known to be. Due to the limitations in cancer detection technologies, this often means that metastases that are microscopic or small are not detected and therefore not treated by surgery or radiotherapy, resulting in an eventually return of the observable cancer.[9] While the tumor is microscopic or small, it is often more susceptible to chemotherapy and the number of malignant cells is not too large and the chemotherapeutic agents can effectively kill most or all of cancer cells, ideally leading to a cure.[10] Most chemotherapeutic agents function by damaging rapidly dividing cells.[7] Once a significant amount of cellular damage occurs, many cells will become apoptotic and start the programmed cell death.[11]

Since the 1940s, over 175 antineoplastic agents have been developed and are available in the United State, Europe and Japan.[8] Chemotherapy drugs can be classified into five major groups. These are: 1) alkylating agents: methyl hydrazines and platinum coordination complexes; 2) natural products: plant products and microorganism products; 3) antimetabolites: folate antagonists and purine antagonists; 4) miscellaneous: hydroxyurea and Imatinib mesylate; and 5) hormones and antagonists: corticosteroids and estrogens. [9]

1.1.2.1 *cis*-Diamminedichloroplatinum(II)

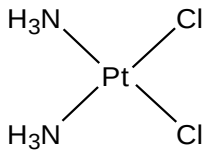


Figure. 1.1 Structure of *cis*-diamminedichloroplatinum(II) (CDDP) (Trade name: Cisplatin™).

cis-Diamminedichloroplatinum(II) (CDDP) (Figure. 1.1), has been a cornerstone in modern chemotherapy,[12] since its discovery in 1965,[13] its identification in 1969[14,15] and its clinical application in the early 1970s.[16] CDDP, usually in combination with other drugs, is used for the initial management of several major solid tumors classes,[17] such as the lung, head-and-neck, and colon. It is also used as a second-line treatment against most other advanced cancers such as cancers of the breast, prostate as well as against glioblastomas, and pleural mesotheliomas.[8]

The antitumor properties of CDDP are attributed to the kinetics of chloride ligand displacement reactions leading to DNA crosslinking activities. In the CDDP-DNA adducts, there are 60-65% are intrastrand GG diadducts and 25-30% are AG diadducts (Figure. 1.2).[18] CDDP induced DNA crosslinking is known to inhibit replication, transcription, and other nuclear functions, and can arrest cancer cell proliferation and tumor growth.[19] A number of additional properties of CDDP are discovered, including activation of signal transduction pathways leading

to apoptosis.[20] The antitumor selectivity of CDDP is beyond fast-dividing cells as it has been shown to invoke the host's immune system to anticipate causing tumor cell death.[8,21] The trans diamminedichloroplatinum(II) isomer also binds DNA, but is clinically ineffective. The mechanism(s) involving steric interactions of platinum species, perhaps with cellular macromoleeules such as DNA or RNA, might explain the ineffeciency.[17]

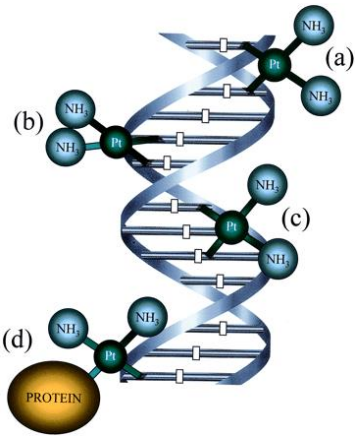


Figure. 1.2 Main adducts formed in the interaction of CDDP with DNA. (a), interstrand cross-link; (b), 1,2-intrastrand cross-link; (c), 1,3-intrastrand cross-link; and (d), protein-DNA cross-link.[21]

One of the major drawbacks in the use of CDDP in clinical applications is that the tumors can develop resistance to CDDP.[22] CDDP resistance develops from the inactivation of its activity inside the cytoplasm and from faster repair of DNA lesions.[23] Other limitations of CDDP are its inherently low solubility and a number of side-effects. One of the major concerns of side-effects is Nephrotoxicity. The others include neurotoxicity, nausea and vomiting, ototoxicity, and etc.[7,24]

1.1.2.2 Metal-based chemotherapy

The discovery of the anticancer activity of CDDP sparked intense interests and researches to find other metal-based antineoplastic agents that not only have the same or

better efficacy as CDDP, but also can overcome some of the side-effects and resistance of seen with CDDP. The generated interests are in molecules containing other heavy metals, particularly those of groups 8, 9, and 10, many of which show similar substitutional kinetics to platinum. When developing new metal based chemotherapies, one must consider the inherent toxicity of the metal complexes and the ability of a body to clear the drugs as accumulation of metal ions.[25]

The Ni(II)-salphen (Figure. 1.3.) complex meets the main requirements for quadruplex-stabilizing molecules, and induces a high degree of quadruplex DNA-stabilization and telomerase inhibition.[26,27] The anticancer activity of gold(III) complexes has also been investigated,[28] due to its isoelectronic configuration with platinum(II) and the same tetracoordinate square-planar geometries as CDDP. There are many distinct classes of metal-based drugs with antitumor activity in experimental models; unfortunately none has yet achieved full clinical use.[29]

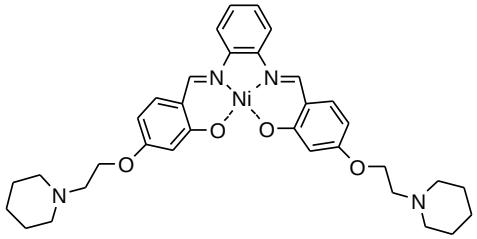


Figure. 1.3 Structure of Ni(II)-salphen complex.

1.1.3 Ruthenium complexes

The thermodynamic stability of ruthenium in several different oxidation states, the nature of its redox couples, and the relative ease for preparation of mixed-ligand complexes, all make ruthenium complexes particularly attractive targets of study.[25,30,31]

1.1.3.1 Ruthenium drugs with labile ligands

Metal coordination compounds with 'slow' metal-ligand exchange rates, comparable to those of cell division processes, often appear to be highly active in killing cancer cell lines.[32] A classical example is CDDP. Its ligand-exchange kinetics are in the order of minutes to hours rather than microseconds to seconds (as for many other coordination compounds), thereby giving platinum high kinetic stability and preventing rapid equilibration reactions.[17] Ru(II) and Ru(III) have similar ligand exchange kinetics to those of Pt(II).[32] The ruthenium complex [ImH][trans-$RuCl_4$(DMSO)(Im)] (NAMI-A) (Im = imidazole, DMSO = dimethylsulfoxide) (Figure. 1. 4) is one example of ruthenium complexes with chloride ligands which has been in clinical trials for anticancer activity.[33] This ruthenium complex is one of the few drugs that have shown promising results for treatment of late stage lung cancer inhibiting further development and spread of metastatic tumors.[33,34]

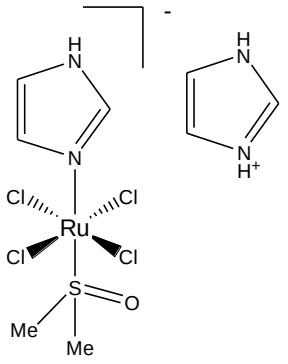


Figure. 1.4 Structure of [ImH][trans-$RuCl_4$(DMSO)(Im)] (NAMI-A).

1.1.3.2 Ruthenium polypyridyl complexes (RPCs)

Polypyridyl ligands, as known as polypyridines, are compounds that contain more than one pyridine unit, such as 2,2'-bipyridine, and 1,10-phenanthroline. When these ligands are bound to a metal center, they have been found to have some interesting photophysical properties as well as redox activities. It has been well documented that some of these metal

complexes are fairly stable.[35] Well-known Ru(II) complexes with polypyridyl ligands have been extensively tested for DNA-binding, antibacterial activity, antitumor cytotoxic properties and etc.[36-38] Much of the early studies concerning Ru(II) complexes and its biological activities were started by Dwyer and co-workers in the 1940s to 1960s.[35,39-42] Their attention was focused on complexes, such as $[Ru(phen)_3](ClO_4)_2$ and $[Ru(bpy)_3](ClO_4)_2$ (Figure. 1.5), [39,42] which laid the ground work for this study. The results of toxicity for these complexes to mice are summarized in table 1.1.[39]

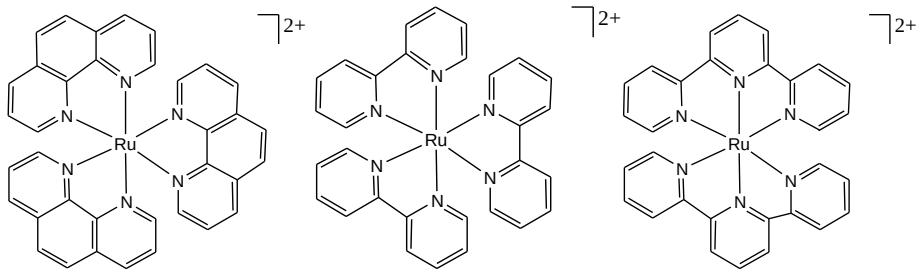


Figure. 1.5 Structures of $[Ru(phen)_3]^{2+}$, $[Ru(bpy)_3]^{2+}$ and $[Ru(trpy)_2]^{2+}$.

Table 1.1 Toxic doses of selected ruthenium complexes for mice.
(The compounds were dissolved in saline and given by intraperitoneal injection. The results are given in the table as the approximate minimum lethal dose, which is recorded as dose (mg) per kg of subject body weight.)

Compound	Optical form and toxic dose	
	(+)* mg/kg	(-)* mg/kg
$[Ru(phen)_3](ClO_4)_2$	9.2	>18.4
$[Ru(bpy)_3]I_2$	15.7~16.8	15.7~16.8
$[Ru(trpy)_2](ClO_4)_2$	>3	>3
$[Ru(trpy)_2]I_2$	>3	>3

* (+) and (-) refer to sign of rotation in either the Na_D line or Hg_{5461} line.

When large amounts of these complexes (five times the toxic intraperitoneal dose) are delivered intraperitoneally into mice, they cause paralysis and death by respiratory failure. It

accumulates appreciably in kidney and liver, but the amounts found in other tissues are below that in blood.[40] The effect of complex ions on transmission at a neuromuscular junction was evaluated by Dwyer et al.[40] Their results show that the neuromuscular blocking activity of the complex cations on nerve-muscular junction possesses curare-like activity and the complexes which are coordinately saturated are potent inhibitors of acetylcholinesterase. ^{106}Ru(II) perchlorate passes unchanged through the animal according to the radioactive labeled ruthenium experiment,[43] which indicates that the active entity is Ru(II) cation as a whole. After intraperitoneal administration, the (+) and (±) forms of the complex appear at a faster rate in blood and urine than the (-) form. The (+) form penetrates tissues at a greater rate than the (-) form. Moreover the (+) form is more rapidly absorbed than the (-) form; the higher toxicity of the former is to be expected.[43]

Tris(3,4,7,8-tetramethyl-1,10-phenanthroline) ruthenium(II) dichloride and acetyl-acetonatobis (3,4,7,8-tetramethyl-1,10-phenanthroline) ruthenium(II) dichloride were tested by Dwyer and coworkers for the inhibition of Landschutz ascites tumor growth.[41] The doses and number of animals used, their changes in weight, and the T/C values are given in Table 1.2.

These results suggest that tris(3,4,7,8-tetramethyl-1,10-phenanthroline) ruthenium(II) dichloride is a less active inhibitor of tumor growth than acetylacetonatobis (3,4,7,8-tetramethyl-1,10-phenanthroline) ruthenium(II) dichloride. Although a single dose of 3.5 mg/kg dose not inhibit tumor growth, a dose of 4.0-4.5 mg/kg produces slight inhibition whereas a dose of 5.0 mg/kg causes quite strong inhibition without significant weight loss.

Table 1.2 Effect of Ru chelates on Landschutz Ascites Tumor growth in the mouse (A single dose of the complex in aqueous solution was administered intraperitoneally on the following day of inoculated tumor cells. The animals were killed seven days after tumor inoculation and the tumor cells removed from the peritoneal cavity by repeated washing with sterile saline.)

Compound (in water)	Dose (mg/kg)(0.3 to 0.5 mL)	Number of daily doses	Number of mice	Mean weight change (g)	Tumor growth (T/C)
Controls	water	1 to 4	40	2	-
Tris(3,4,7,8-tetramethyl-1,10-phenanthroline) ruthenium(II) dichloride	10	1	5	-1	0.43
		2	5	-2	0.13
		3	5	-2	0.34
		4	5	-3	0.19
	3.5	1	5	1	1.08
Acetyl acetonatobis (3,4,7,8-tetramethyl-1,10-phenanthroline) ruthenium(II) dichloride	4	1	20	1	0.76
	4.5	1	10	1	0.54
	5	1	15	0	0.12
		2	5	-1	0.04
		3	5	-1	0.04
		4	5	-3	0.03

In addition, *in vitro* experiment shows that acetylacetonate bis(3,4,7,8-tetramethyl-1,10-phenanthroline) Ru(II) dichloride is lethal *in vitro* to cultured ascites P-388 mouse lymphocytic leukemia cells at a concentration of $3x10^{-7}$ M.[44] 1,10-phenanthroline chelates are generally more potent than corresponding 2,2'-bipyriding chelates, and heterolepitic (acetylacetonato) monovalent chelates of both series are more active than the corresponding identical-ligand divalent chelates.[44]

1.1.3.3 RPCs with DNA

It has been shown that by replacing the phen ligand with a larger planar aromatic ligand such as dppz and cpdppz (Figure. 1.6), RPCs can lead to much higher DNA binding affinities which fully intercalate into the DNA base-pairs,[45,46] and can be as probes for DNA.[47] The complex $[(phen)_2Ru(dppz)]^{2+}$ has been shown to bind by the intercalation as well as

electrostatics force. The binding constants for Δ- and Λ-$[(phen)_2Ru(dppz)]^{2+}$ are $3.2x10^6$ M^{-1} and $1.7x10^6$ M^{-1} respectively. They are approximately three orders of magnitude higher than those with no intercalation.[36] The dimer $[(phen)_2Ru(\mu\text{-}c4(cpdppz)_2)Ru(phen)_2]^{4+}$, shown in Figure. 1.6, has a DNA binding constant on the order of $10x10^{12}$ M^{-1}. This complex was reported to have promising antitumor activity against platinum resistant tumor types *in vitro*.[48]

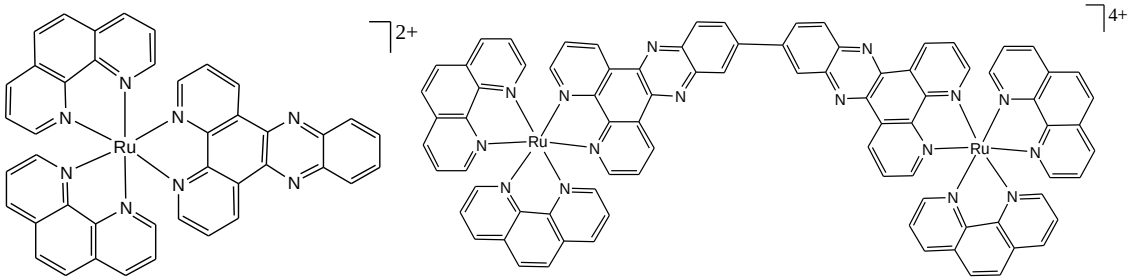


Figure. 1.6 Structure of $[(phen)_2Ru(dppz)]^{2+}$ and $[(phen)_2Ru(\mu\text{-}c4(cpdppz)_2Ru(phen)_2]^{4+}$.

1.1.4 Previous research work in the MacDonnell group

The MacDonnell group have investigated the biological properties of the ruthenium(II) polypyridyl complexes, $[(phen)_2Ru(tatpp)Ru(phen)_2]Cl_4$,[49] (P), and $[(phen)_2Ru(tatpp)]Cl_2$, (MP) which are shown in Figure. 1.7.[50] It has been shown that both P and MP bind DNA via intercalation and can cleave DNA under anaerobic and reducing conditions.[4] In particular, they show good cytotoxicity against colon and breast cancer tumor cell lines which suggests that these complexes may be promising antineoplastic agents against cancer cells under hypoxic stress.

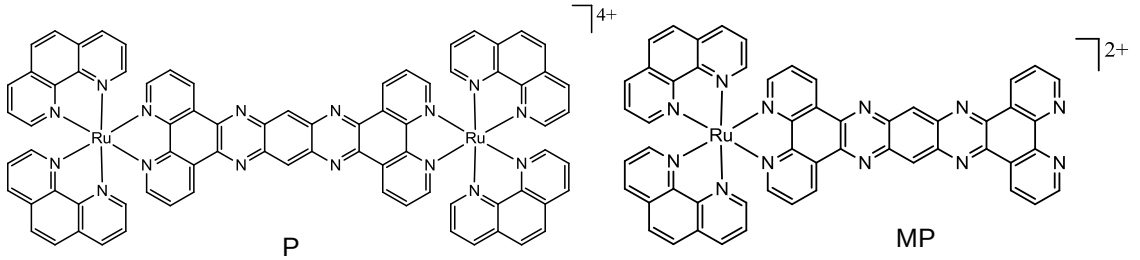


Figure. 1.7 Structure of $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ (P), and $[(phen)_2Ru(tatpp)]^{2+}$ (MP).

1.1.4.1 DNA cleavage assay

To quickly screen whether or not the RPCs cleave DNA, a simple cleavage assay using plasmid DNA (pUC 18-2686 bp) was employed. The plasmid pUC 18 exists in three different topological configurations: supercoiled DNA (form I), circular DNA (form II) and linear DNA (form III). These three forms of DNA can be separated by agarose gel electrophoresis and visualized under UV light after staining the gel with ethidium bromide. Single-strand (ss) cleavage converts supercoiled (form I) to circular DNA (Form II). In comparison, double-strand (ds) cleavage converts supercoiled DNA to linear DNA (Form III). DNA-cleavage ability can be semi-quantitatively assessed by the assay.[51]

Complex P is shown to cleave DNA with a reducing agent glutathione (GSH), and this cleavage activity is enhanced under anaerobic conditions (Figure. 1.8).[4,5] Since Fe-BLM can cause single strand nicks under anaerobic conditions, and it requires O_2 for double strand cleavage activity.[5] Fe-BLM is used as a positive control to show the concentration level of oxygen in the glove box.

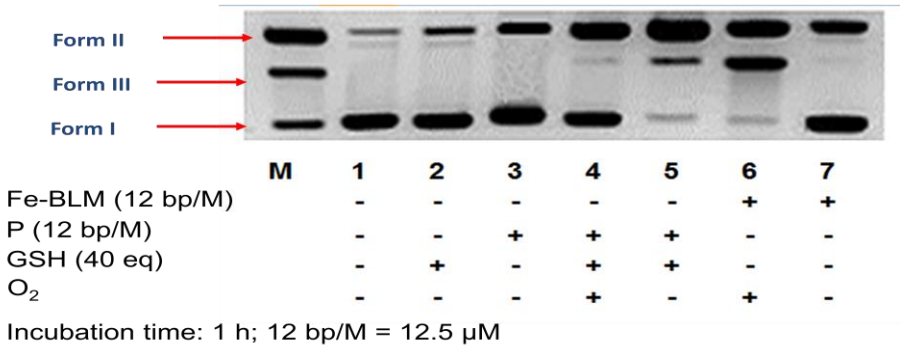


Figure. 1.8 DNA cleavage activities of complexes Fe-BLM and P as present of GSH under normoxic and anaerobic conditions in plasmid DNA assay.

1.1.4.2 Cytotoxicity of ruthenium complexes

The cytotoxicity study was conducted by Abhishek Yadav.[4] The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to study the effect on

cell viability after incubation for up to 96 hours in the presence of a series of complexes including chiral complexes. MP has been found to be modestly cytotoxic (GI_{50} is in the 0.1 to 10 μM range.) against a wide range of cancer cell lines.

With this background, there are several questions that need to be addressed: 1) Is there a structure related anticancer ability for the series of RPCs? 2) How do the complexes distribute in cells or in the nuclei? 3) Is there a correlation between the drug uptakes and the cytotoxicity? Towards these ends, to answer these questions, to synthesize a series of analogues of ruthenium complexes is the first step.

CHAPTER 2

SYNTHESIS OF NOVEL COMPLEX $[(PHEN)_2RU(TADBP)](PF_6)_2$ AND CHIRAL DI/MONO-NUCLEAR COMPLEXES

2.1 Introduction

There are numerous reports on the synthesis of RPCs containing large planar aromatic intercalating ligands such as dpq, dppz, dppn, and tatpp (Figure. 2.1). Ulrich Schatzschneider et al. did systematic evaluation of a series of ruthenium complexes with aromatic bidentate ligands (N-N=bpy, phen, dpq, dppz, and dppn) to study the influence of ligand surface area on cellular uptake efficiency and cytotoxicity.[37] They reported that cellular uptake efficiency and cytotoxicity are increased with the size of the aromatic surface of the N-N ligand.

The MacDonnell group has been exploring the chemistry of ruthenium-tatpp complexes as potential anti-tumor agents.[5,49,50,52-57] These complexes show good *in vivo* anti-tumor growth properties, in mouse tumor model studies and show some selectivity towards cancer cells under hypoxic stress. Compared to dppz and dppn, the tatpp ligand has a larger and more extended aromatic plane, which leads to facile reduction of the tatpp ligand at modest reduction potentials (E_{red} ≈ 0.0 to -0.10 V vs. NHE). It is postulated that this 'redox-activity' of the tatpp ligand is essential for the observed hypoxia selectivity, as the reduced species is involved with the DNA cleavage activity of the complexes. At present there are two ruthenium-tatpp complexes under investigation, $[(phen)_2Ru(tatpp)]^{2+}$ (MP) and $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ (P). Both P and MP have been shown to intercalate with DNA[5,51,58,59] and cause DNA cleavage under anaerobic and reducing conditions.[5]

Given the activity of the MP complex, one question we sought to answer was the role of the open chelated coordination site. It is unclear if this is required or if it could be replaced with

a non-chelating analogue as in complex $[(phen)_2Ru(tadbp)](PF_6)_2$ (where tadbp = 9, 11, 20, 22-tetraazadipyrido[3, 2-a: 2', 3']dibenzo[3'', 2''-l: 2''', 3'''-n]pentacene) which is shown in Figure 2.2. In this chapter, we explore the synthesis of the monotopic tadbp ligand and the related ruthenium(II) complex as well as the synthetic routes to MP and P, which still required some optimization.

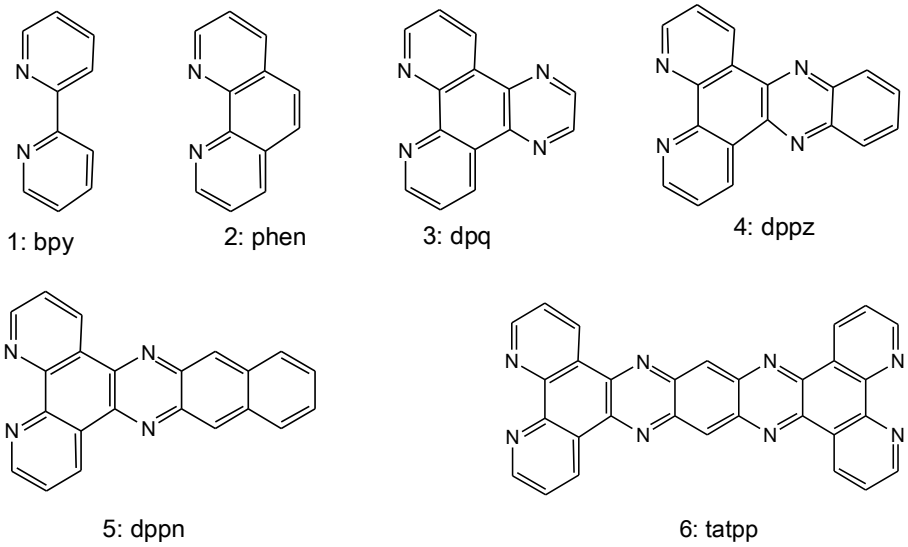


Figure. 2.1 Polypyridyl ligands and abbreviations.

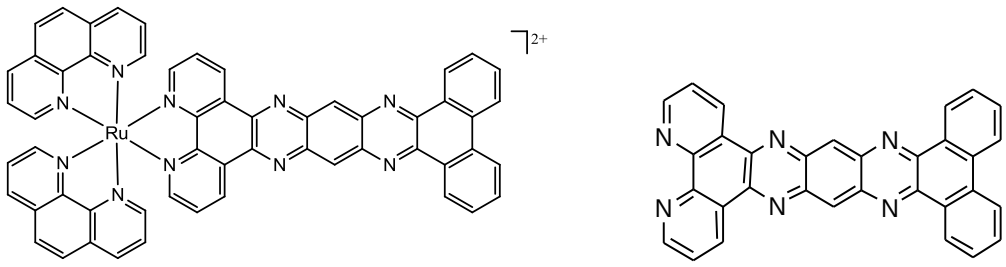


Figure. 2.2 The structure of complex $[(phen)_2Ru(tadbp)]^{2+}$ and tadbp ligand.

2.2 Results and Discussion

The synthetic route taken for the synthesis of $[(phen)_2Ru(tadbp)]^{2+}$ is shown in Figure. 2.3, which is very similar to that used to prepare MP.

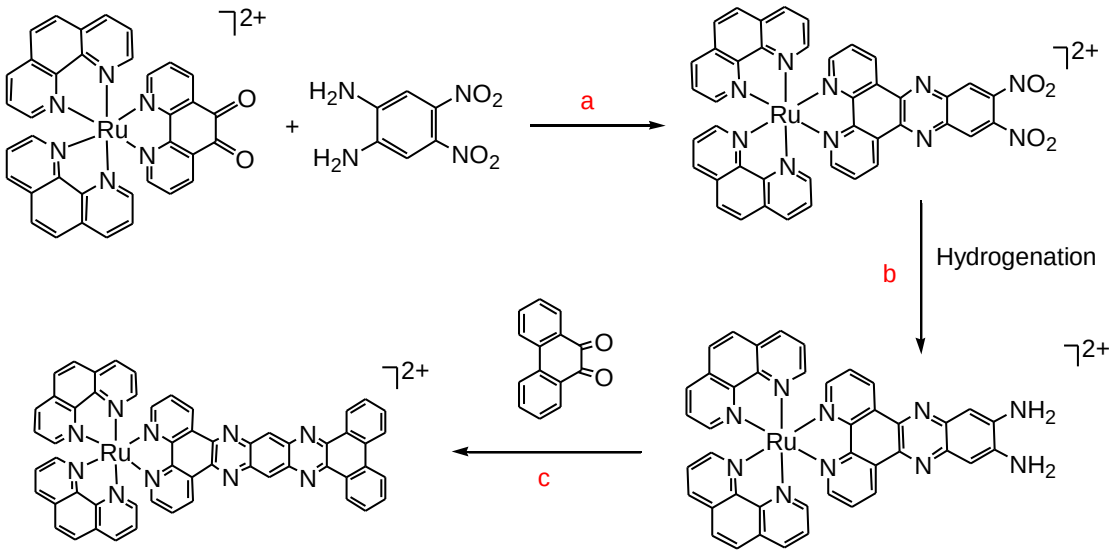


Figure. 2.3 Scheme for synthesis of $[(phen)_2Ru(tadbp)]^{2+}$.

2.2.1 Synthesis of complex $[(phen)_2Ru(diaminodppz)](PF_6)_2$

In order to increase the yield, different reducing agents and reduction methods were studied for the conversion of nitro groups to amino groups. Our investigation indicates that a pure product can be obtained when one of the two methods is used. The two methods are H-Cube hydrogenation using a H-Cube Midi^{TM} and two-cycle hydrogenation with a Parr shaker hydrogenator.

Although the original preparation of $[(phen)_2Ru(diaminodppz)]^{2+}$ was reported by Kim[60] in this lab, we had difficulty getting the reduction reaction to go smoothly and in high yield using that procedure. The procedure reported by Kim is shown as Figure. 2.4, which states the method to convert nitro groups to amino groups using 10% palladium on carbon at 60 ºC for 24 h, under 5 atm (73.5 psi) hydrogen pressure with ethanol in a high pressure reactor. The

product is purified by a column chromatography (alumina treated with a solution of 10% TEA in n-hexane) using a solution of NH_4PF_6 in CH_3CN (10 mg/mL) as an eluent. The yield of $[(phen)_2Ru(diaminodppz)]^{2+}$ by this procedure is 77%.

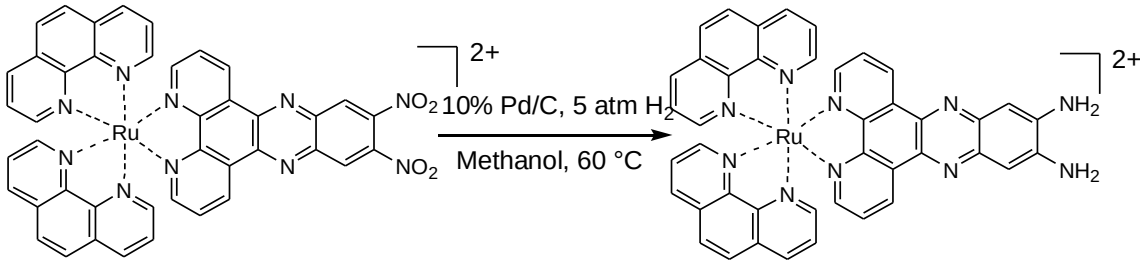


Figure. 2.4 Scheme for synthesis of $[(phen)_2Ru(diaminodppz)]^{2+}$ in a High Pressure Reactor.

Herein we report on the results of several alternative methods for reduction of the dinitro species.

Method 1: H-Cube hydrogenation

One promising method explored was based on a hydrogenation apparatus, known as the “H-Cube”; which is short for a H-Cube brand Continuous-flow Hydrogenation Reactor. It uniquely combines a high pressure, high temperature solution-based hydrogenation in a continuous flow reactor using a disposable catalyst cartridge system. It allows fast and cost-efficient hydrogenation with often superior yield when compares to conventional methods. Typical operating conditions are hydrogenation of a 0.15 mM solution (100 mg) of the dinitro ruthenium complex in MeCN at 40 ºC and 40 bar H_2, using a 10% Pd/C catalyst and a flow rate of 2 mL/min. It is important that the reaction solution is relatively dilute and filtered before hydrogenation in the H-Cube system to avoid precipitation in the system, which can damage the equipment. The yields and purity of the product (based on NMR analysis) for the different runs, using the H-Cube system are presented in Table 2.1. Unfortunately the hydrogen generation

system of the H-Cube broke and despite two attempts to fix it, it still remains unusable. Alternative methods were needed as this instrumentation proved unreliable.

Table 2.1 H-Cube hydrogenation of $[(phen)_2Ru(dinitrodppz)]^{2+}$

Concentration (mM)	Catalyst cartridge	Flow rate (mL/min)	Purity (%)	yield (%)
0.50	10 g	7	60	--
0.15	10 g	7	100	92
0.15	70 X 4 mm	2	100	92

Method 2: Two-cycle hydrogenation in a Parr shaker hydrogenator

We also explored the use of a Parr shaker/hydrogenator for the reduction reaction. Using typical hydrogenation conditions in which 100 mg $[(phen)_2Ru(dinitrodppz)]Cl_2$ in 60 mL methanol was hydrogenated under 60 psi hydrogen pressure at room temperature for 24 hours over 100 mg 10% Pd/C. We found that the reaction would not go to completion, as shown in ^1H NMR spectrum in Figure 2.5, even if the reaction time was extended, the pressure increased, or the reaction heated. These data are collected in Table 2.2. However, we found that if the reaction mixture solution was filtered, the intermediate was isolated and added back to the hydrogenator for a second cycle with new catalyst. We could drive the reduction reaction to completion, as listed in Table 2.2. The ^1H NMR of one of other intermediates is shown in the Appendix-1.

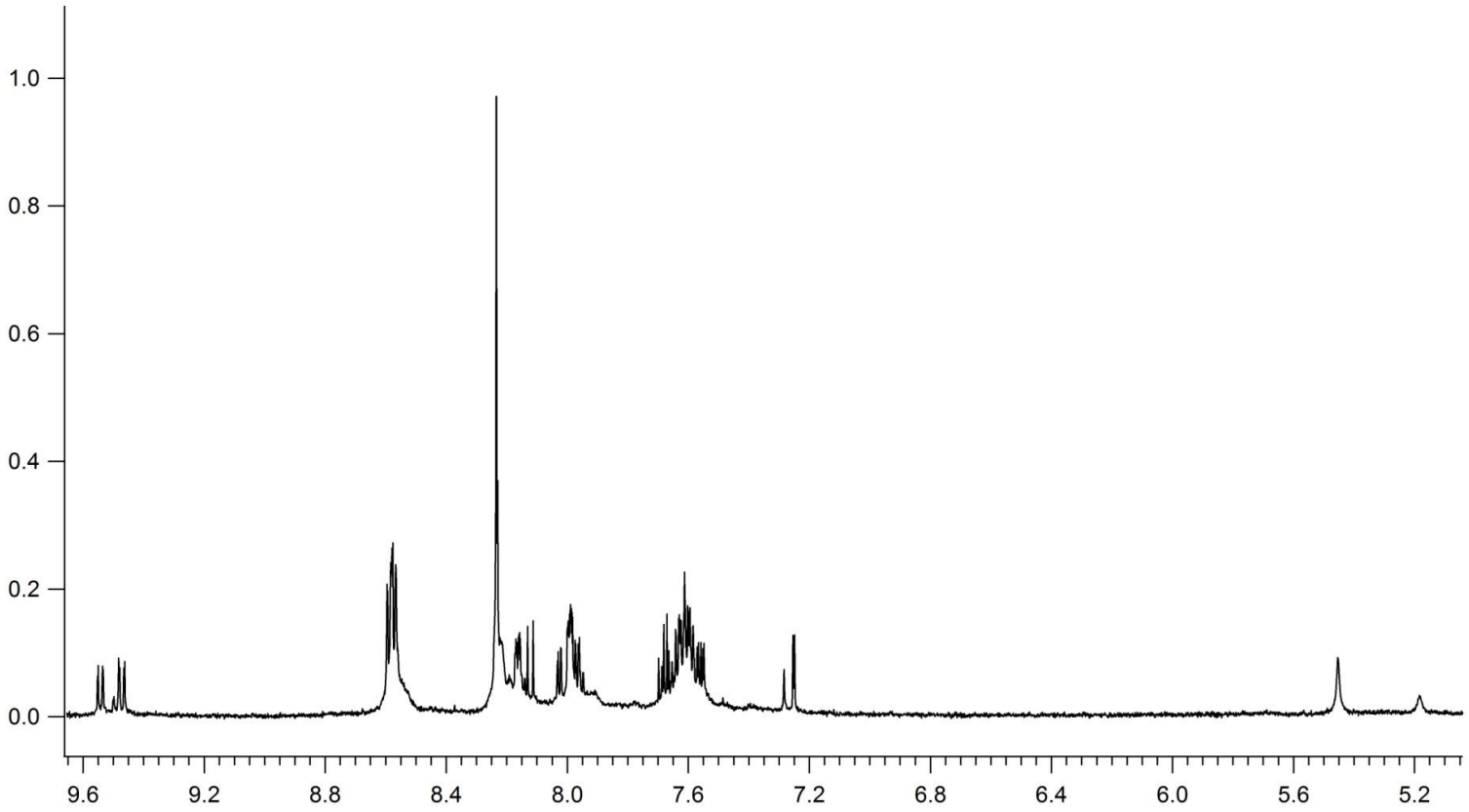


Figure. 2.5 ^{1}H NMR spectrum (500 MHz, Solvent: MeCN-D_3) of intermediate of reduction of $[(phen)_2Ru(dinitrodppz)]^{2+}$.

Table 2.2 The conditions study of hydrogenation of $[(phen)_2Ru(dinitrodppz)]^{2+}$ (100 mg) in a high pressure reactor

Solvent	Temp (ºC)	Pressure (psi)	Time (h)	Cycles	Result
MeCN	RT	60	24	1	Incomplete
MeCN	40	140	24	1	Incomplete
MeCN	60	140	24	1	Incomplete
EtOH	60	140	24	1	Incomplete
MeOH	60	140	24	1	Incomplete
MeOH	RT	60	48	1	Incomplete
MeOH	RT	140	48	1	Incomplete
MeOH+AcOH (100 mL+5 mL)	RT	60	24	1	Incomplete
MeOH	RT	60	24	2	Complete

Method 3: Reduction with hydrazine and various catalysts

Previous reports of hydrazine based reductions of nitro groups suggested a low pressure and 'hydrogen-free' method of driving this reduction reaction. We examined this low pressure (1 atm), no H_2 method using several different catalysts. A typical reaction was conducted as follows.

$[(phen)_2Ru(dinitrodppz)]Cl_2$ (50 mg 0.06 mmol) was dissolved into 30 mL of solvent and then 50 mg of catalyst was added into a 2 neck flask. After degassing by N_2 for 15 minutes, hydrazine (0.5 – 3 mL) was added dropwise and the solution was refluxed for 3 hours under N_2. The solution was then filtered through a pad of Celite, followed by washing with a large amount of solvent. The collected solution was rotovapped to 5 mL. An aqueous solution of ammonium hexafluorophosphate (40 mg 0.24 mmol, dissolved in 5 mL of DI water) was added to form a

precipitate which was isolated by filtration and washed with 5mL of DI water. The product was dried in a vacuum oven overnight and characterized by ^{1}H NMR. The conditions of different reactions are summarized in Table 2.3. Unfortunately, the results indicate that a completed reduction product cannot be obtained with the hydrazine co-catalysts system.

Table 2.3 The conditions study of hydrazine- (Pd/C, PtO_2 and Pt /Alumina) co-catalysts reduction of $[(phen)_2Ru(dinitrodppz)]Cl_2$ (50 mg)

Solvent	Catalyst	Hydrazine (mL)	Cycle	Result
EtOH	Pd/C	0.5	1	Incomplete
EtOH	Pd/C	3.0	1	Incomplete
EtOH	Pd/C	3.0	2	Incomplete
EtOH	PtO_2	3.0	1	Incomplete
EtOH	PtO_2	3.0	2	Incomplete
EtOH	Pt /Alumina	3.0	1	Incomplete
EtOH	Pt /Alumina	3.0	2	Incomplete
MeOH	Pd/C	3.0	1	Incomplete
MeOH	Pd/C	3.0	2	Incomplete

Of the three methods explores, the H-cube was definitely the best; however the reliability of the instrument was poor and required us to explore other methods. Hydrazine-based reduction schemes failed to yield clean product, but hydrogenation in a Parr shaker reactor is an acceptable method, although it sometimes required a second cycle of hydrogenation to complete the reduction.

2.2.2 Synthesis of complex $[(phen)_2Ru(tadbp)](PF_6)_2$

The Schiff-base coupling reaction between $[(phen)_2Ru(dadppz)](PF_6)_2$ and 1,10-phenanthracene-10,11-dione was proposed for the synthesis of the analogue complex $[(phen)_2Ru(tadbp)](PF_6)_2$. Unfortunately the reaction was not straightforward and required some optimization to drive the reaction to completion. Table 2.4 lists the various solvent combinations tried and their effect of the product purity. Ultimately, it was found that a EtOH: MeCN: AcOH (2: 1: 1) mixture with a 12 hours reflux gave the desired product in pure form. Notably, it was found that longer reaction time can hurt the reaction and lead to some product decomposition as seen for the reaction done in EtOH: MeCN: AcOH (2:1:1) solvent for 24 hours reflux. The complex was characterized by ^{1}H NMR spectroscopy and is shown in Figure. 2.6. The EI-MS is shown in Figure A-2.

Table 2.4 The conditions and results of reaction for synthesis of $[(phen)_2Ru(tadbp)](PF_6)_2$.

Solvent	Time (h)	Result
MeCN	12	No reaction
MeCN + H_2O (1:1)	12	Further purification needed
MeCN + H_2O + AcOH (1:1:0.1)	12	Further purification needed
EtOH + AcOH (1:0.1)	12	Further purification needed
AcOH	12	Further purification needed
EtOH: MeCN: AcOH(2:1:1)	12	Complete
EtOH: MeCN: AcOH(2:1:1)	24	Further purification needed

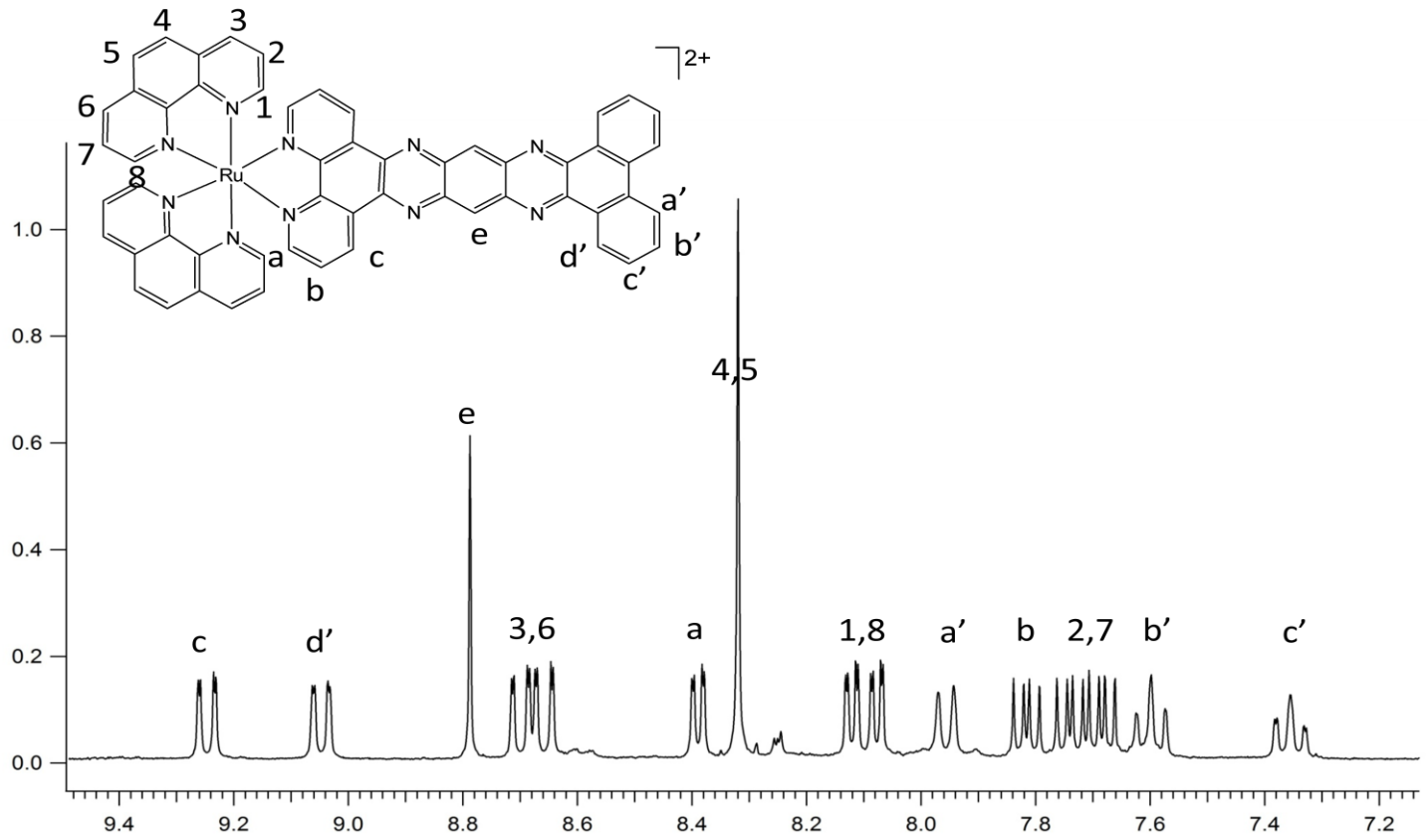


Figure. 2.6 ^{1}H NMR spectrum (500 MHz, Solvent: MeCN-D_3) of $[(phen)_2Ru(tadbp)](PF_6)_2$.

The UV-Vis spectra of the complex $[(phen)_2Ru(tadbp)](PF_6)_2$ (Figure. 2.7) are very similar to the complex of $[(phen)_2Ru(tatpp)](PF_6)_2$.[54] The spectrum of the parent complex has an intense band centered at 330 nm and a broad peak in the region of 435 ~ 450 nm. Addition of triethylamine results in the partial reduction to $[(phen)_2Ru^{II}(tadbp^{\cdot-})]^{\cdot+}$ and visible irradiation completes this process, as well as driving the reaction further to the doubly-reduced species $[(phen)_2Ru^{II}(tadbpH^{-})]^{+}$. This photochemical behavior is identical to that seen for MP under similar conditions and reflects the similarity of their structures and electronic properties. For the singly reduced species $[(phen)_2Ru^{II}(tadbp^{\cdot-})]^{\cdot+}$, we observe two new absorption bands in the near-IR at 850 nm (weak) and 955 nm (strong), and a discernible band at 400 nm. Meanwhile, a partial bleaching of the absorption at 330 nm happens. With continued irradiation, the near IR bands are bleached with a corresponding peak growth at 650 nm, which is characteristic of the doubly-reduced species $[(phen)_2Ru^{II}(tadbpH^{-})]^{+}$. The fact that the tatbp complex has similar photochemical properties to MP suggests it will have similar biological properties in terms of DNA cleavage.

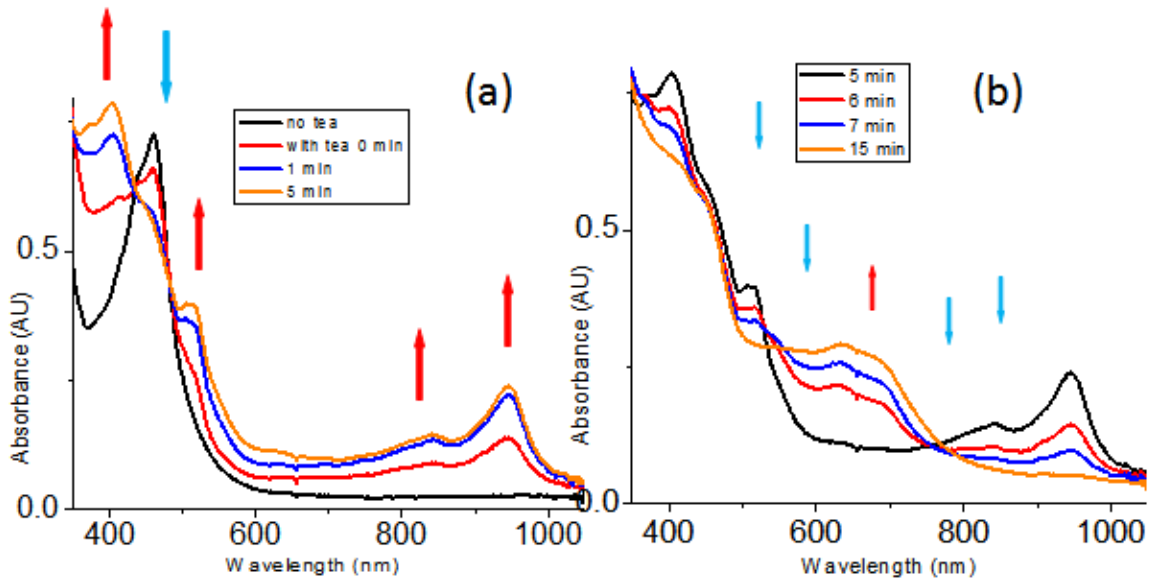


Figure. 2.7 UV-Vis spectra of $[(phen)_2Ru(tadbp)]^{2+}$ (17 μM) in MeCN. (a) Absorption spectra of B (17 μM without TEA, black line) after addition of 50 mM TEA (red line) and then; radiated by 430 nm LED light for 1 minute (blue line) and 5 minutes (orange line). (b) Absorption spectra of B after 430 nm radiation for 5 minutes (black line), 6 minutes (red line), 7 minutes (blue line), and 15 minutes (orange line).

2.3 Synthesis of ΔΔ-[(phen)$_2$Ru(tatpp)Ru(phen)$_2$]$^{4+}$, Δ-[(phen)$_2$Ru(tatpp)]$^{2+}$ and Δ-[(phen)$_2$Ru(tadbp)]$^{2+}$

2.3.1 Stereoisomers of RPCs

The D_3 symmetric tris-bidentate octahedral complexes e.g. [Ru(phen)$_3$]$^{2+}$ (Figure. 2.8) have a helical structure in which the three bidentate ligands lie along the threads of a right or left-handed screw, meaning the complexes exist as enantiomers. The two mirror-image forms are: a “right-handed enantiomer (designated as Δ) and a “left handed” enantiomer (designated as Λ).

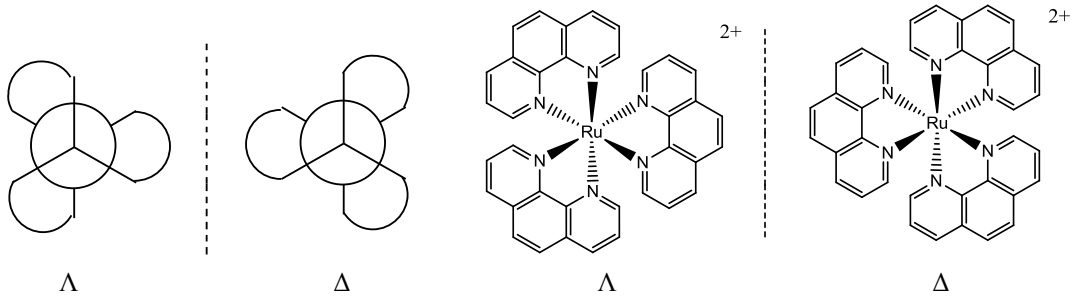


Figure. 2.8 Definition and structures of Λ and Δ-[Ru(phen)$_3$]$^{2+}$.

When two such metal centers are bridged by a symmetric ligand such as tatpp then the complex may adopt one of two diastereoisomeric forms: ΔΔ/ΛΛ (a pair of enantiomers) or ΔΛ/ΛΔ (meso) as is shown in Figure 2.9.

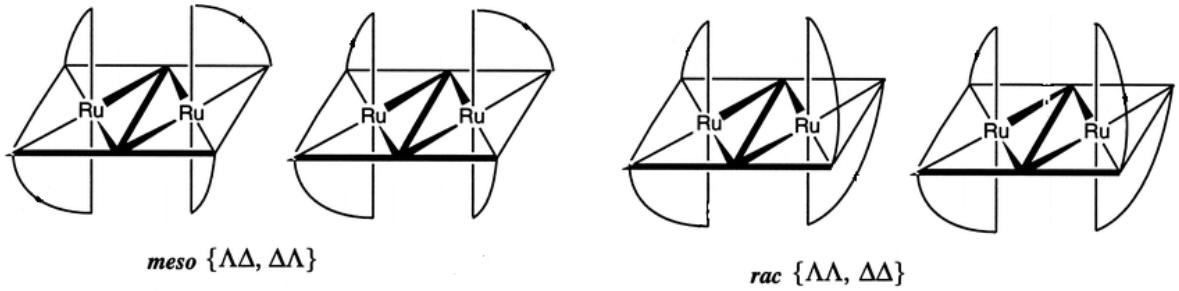


Figure. 2.9 Stereoisomeric forms of $[(Ru(pp)_2)_2(\mu\text{-BL})]^{n+}$.
Where pp is a symmetrical bidentate ligand (C_{2v} point group symmetry) such as bpy, and BL is a symmetrical (C_{2v}) bridging ligand such as 2,2′-bipyrimidine (bpm).

Hua and Von Zelewsky established the convenient resolutions of rac-$[Ru(phen)_2(py)_2]^{2+}$ and rac-$[Ru(bpy)_2(py)_2]^{2+}$ by conventional diastereoisomer formation using a chiral material arsenyl-(+)-tartrate and O,O'-dibenzoyltartrate anions respectively.[61-64] Chiral precursors can be used to synthesize a single stereoisomeric form of the complexes derivatives or diastereomers. The MacDonnell lab pioneered the use of enantiopure coordinatively saturated complexes, such as $[(phen)_2Ru(phendione)]^{2+}$ and $[(phen)_2Ru(phen\text{-}5,6\text{-}diamine)]^{2+}$[36], to directly prepare chiral derivatives and multimetallic assemblies.[65,66] In this study, we explore the use of enantiopure Δ-$[(phen)_2Ru(phendione)]^{2+}$ and Λ-$[(phen)_2Ru(phendione)]^{2+}$ as chiral building blocks to prepare ΔΔ-P and related stereoisomers in pure form.

2.3.2 Resolution of Δ-$[(phen)_2Ru(phendione)](PF_6)_2$

Hua and von Zelewsky established the convenient resolution of rac-$[Ru(phen)_2(py)_2]^{2+}$ and rac-$[Ru(bpy)_2(py)_2]^{2+}$ by conventional diastereoisomer formation using the chiral arsenyl-(+)-tartrate and O,O'-dibenzoyltartrate anions, respectively.[61-64] Hiort[36] and Barton[67,68] reported a method to seperate Λ and Δ forms of $[(phen)_2Ru(phendione)]^{2+}$ complexes.[36] Subsequently, Kim and MacDonnell developed a more efficient procedure using sodium arsenyl -L-(+)-tartrate to precipitate predominantly the Λ form complex, leaving behind a supernatant enriched in the Δ

form. Addition of sodium arsenyl-D-(-)-tartrate to this supernatant precipitated Δ-$[(phen)_2Ru(phendione)]^{2+}$. At this point Λ form is not pure enough. However, the ratio of Λ to Δ form in a racemic complex isn't always fifty-fifty. Sometimes Δ form is pure enough to continue to the next step. Their method is modified and optimized in this thesis. Instead of adding different form of D or L-arsenyl tartrate, one form of arsenyl tartrate is used for a Λ or Δ form resolution cycle. Normally, one resolution cycle needs two times resolution. The supernatant enriched with other form was kept for resolution. During the siting time, more precipitation was formed. More ruthenium complex can be recovered and living more enantiomeric richer complex in the solution.

The enantiomeric excess (ee%) value of separated complexes was determined by separation on a HPLC-LARIHC-RN™ column with a mobile phase of acetonitrile/methanol/triethylamine/acetic acid 10/90/0.2/0.3 (by volume). The result shows that the ee% value is between 65%~87% via the first run of chiral resolution and the ee% value is improved via repeating the chiral resolution step for one more time (ee% = 99%, Figure. 2.10).

Figure. 2.10 Separation of racemic /Δ-[(phen)$_2$Ru(phendione)](PF$_6$)$_2$ on the HPLC-LARIHC-RN™ Column with a mobile phase of acetonitrile/methanol/triethylamine/acetic acid 10/90/0.2/0.3 (by volume).

Since there are multiple metathesis reactions involved in the isolation and purification of these complex salts, it is not uncommon to have residual salts, such as tetrabutylammonium hexafluorophosphate (which has similar solubility with the complexes) contaminating the products. Therefore we found that limiting the amount of added salt in a metathesis reaction and washing the resulting precipitate with copious amounts of washing solvent to be important to isolating pure products.

2.3.3 Synthesis of Δ-[(phen)$_2$Ru(diaminodppz)](PF$_6$)$_2$

The procedures to synthesize Δ-[(phen)$_2$Ru(dinitrodppz)](PF$_6$)$_2$ are as same as synthesis of the racemic form, however, the reduction of this nitro complex was more difficult

than observed with the racemic complex, presumably due to differing solubility properties. The two-cycle hydrogenation method using a Parr shaker hydrogenator did not work. We screened the reaction for better reaction conditions and catalysts as shown in Table 2.5 and ultimately found that we could complete the reduction using PtO_2 as the catalyst. Notably, this worked so well that only a single hydrogenation cycle was required.

Table 2.5 The conditions and results of hydrogenation reaction of Δ-$[(phen)_2Ru(dinitrodppz)]Cl_2$ (50 mg) in a Parr shaker hydrogenator

Solvent	Volume (mL)	Catalyst	Cycles	Result
EtOH	35	Pd/C	1	Incomplete
MeOH	35	Pd/C	1	Incomplete
MeOH	35	Pd/C	2	Incomplete
MeOH	35	Pd/C	3	Incomplete
MeOH	60	Pd/C	1	Incomplete
MeOH	35	Pt /Alumina	1	Incomplete
MeOH	35	Pt /Alumina	2	Incomplete
MeOH	60	PtO_2	1	Complete

2.3.4 Synthesis of Δ-MP, Δ-$[(phen)_2Ru(tadbp)]^{2+}$ and ΔΔ-P

Δ-MP and Δ-$[(phen)_2Ru(tadbp)]^{2+}$ were synthesized from Δ-$[(phen)_2Ru(diaminodppz)](PF_6)_2$, which is shown in Figure. 2.11, using the same method as used in the synthesis of racemic complexes. The complexes were characterized by EI-MS and ^1H NMR spectroscopy in CD_3-CN.

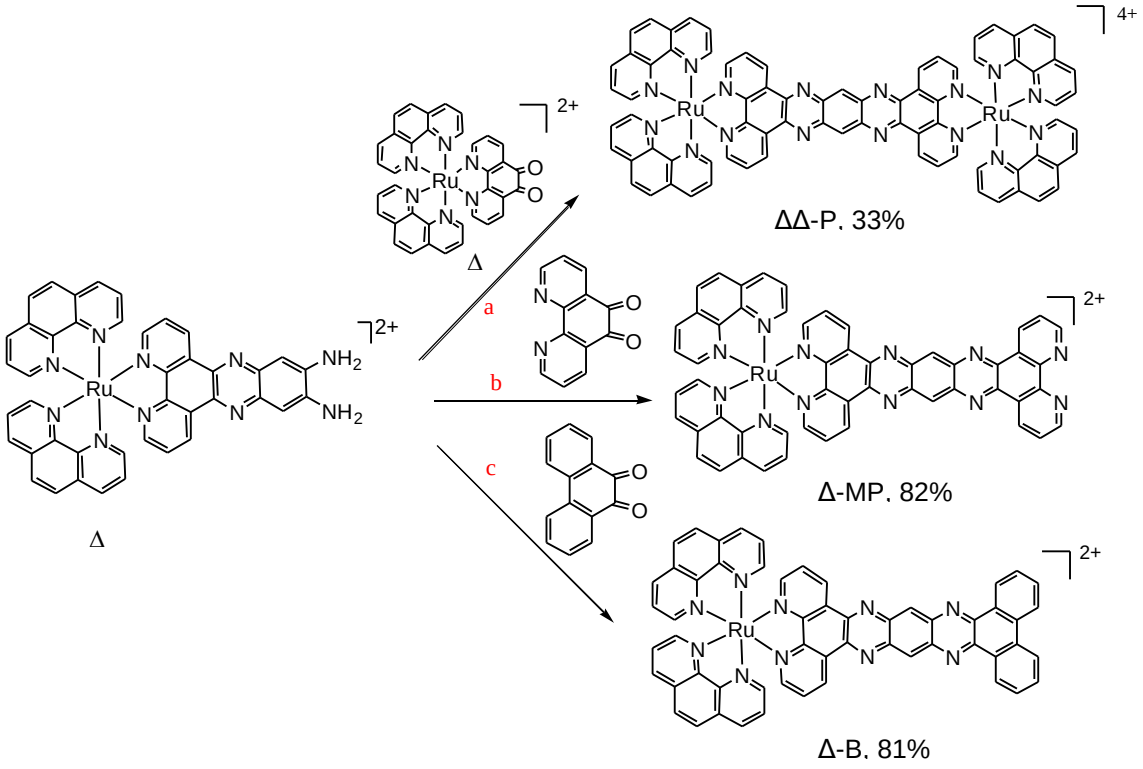


Figure. 2.11 Scheme of synthesis of ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ (ΔΔ-P), Δ-$[(phen)_2Ru(tatpp)]^{2+}$ (Δ-MP) and Δ-$[(phen)_2Ru(tadbp)]^{2+}$ (Δ-B).

a: Synthesis of ΔΔ-P by reacting Δ-$[(phen)_2Ru(diaminodppz)]^{2+}$ with Δ-$[(phen)_2Ru(phendione)]^{2+}$; b: synthesis of Δ-MP by reacting Δ-$[(phen)_2Ru(diaminodppz)]^{2+}$ with 1,10-phenanthroline-5,6-dione (phendione); and c: synthesis of Δ-B by reacting Δ-$[(phen)_2Ru(diaminodppz)]^{2+}$ with 9,10-phenanthraquinone.

Δ-MP can also be synthesized by a single step reaction between Δ-$[(phen)_2Ru(phendione)](PF_6)_2$ and 1.2 equivalents of 11,12-diamino-dipyrido[3,2-*a*:2',3'-*c*]phenazine, which was reported by Yadav[5]. An alternative pathway is reported in this thesis. The reaction is started with $[(phen)_2Ru(diaminodppz)](PF_6)_2$ (product from nitro group complex hydrogenation) and 1,10-phenanthroline -5,6-dione (phendione) in 60% yield.

Slightly different methods for the synthesis of ΔΔ-P were reported by Kim[60] and Yadav,[5] respectively. Table 2.6 shows a comparison of reaction conditions of these two methods. Both methods use water as a component of the solvent. In addition, in order to get pure product,

further purification is needed for both methods. But both methods required further purification to obtain a pure product.

Table 2.6 Reaction conditions of synthesis of ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2](PF_6)_4$

Author	$[(phen)_2Ru(diaminodppz)]$: $[(phen)_2Ru(phendione)]$ (mg)	Solvent (mL)	Reaction time (h)	Further purification
Kim	(20:30)	H_2O:MeCN = 10:10	12	Metathesis (10% NH_4PF_6 in ethanolic water)
Yadav	(20:20)	H_2O:MeCN: AcOH = 10:10:1	24	Diethyl ether precipitation

A modified synthesis and purification procedure of ΔΔ P is reported in this thesis. With an improved reaction solvent system and a short silica column, the pure complex was obtained and characterized by ^{1}H NMR spectroscopy in CD_3CN.

2.4 Summary and Conclusion

This study shows that the heteroleptic RPCs can be synthesized by ligand displacement and coupling reactions. Hydrogenation of nitro group is the key step of the whole procedure. H-Cube hydrogenation is most convenient and efficient way to reduce the nitro RPCs, compared with other methods studied in this thesis. The alternated way is two-cycle hydrogenation. Although super-addition of catalyst is not very rare in catalytic reactions, two-cycle hydrogenation is not simply adding more catalyst. The second time of hydrogenation finally converts the intermediate ruthenium complex to the amino ruthenium complex. Palladium on carbon is a conventional catalyst for hydrogenation reactions. PtO_2 was found efficient for hydrogenation of the chiral Δ--$[(phen)_2Ru(dinitrodppz)]^{2+}$. An analogue complex

$[(phen)_2Ru(tadbp)](PF_6)_2$ of MP and its enantiomerically pure form Δ-$[(phen)_2Ru(tadbp)](PF_6)_2$ were synthesized. Technical modifications and optimizations of some reaction procedures are involved to improve the repeatability and product yield.

2.5 Experimental

2.5.1 Reagents and Materials

All reagents were used without further purification. $RuCl_3 \cdot xH_2O$ (99.9%) was purchased from Alfa Aesar. 1,10-phenantroline (99%+) and ammonium hexafluorophosphate (95%+) were purchased from Sigma-Aldrich.

1,10-phenanthroline-5,6-dione (phendione) was prepared by the bromine-catalyzed oxidation of 1,10-phenanthroline with a mixture of concentrated sulfuric and nitric acid.[65] 4,5-dinitro-benzene-1,2-diamine and dichloro-bis(1,10-phenanthroline-N^1,N^{10}) ruthenium (II) were synthesized according to the literature.[2,69,70] $[(phen)_2Ru(phendione)](PF_6)_2$,[71] $[(phen)_2Ru(dinitrodppz)](PF_6)_2$,[60] 11,12-dinitro-dipyrido[3,2-*a*:2',3'-*c*]phenazine,[72] sodium arsenyl tartrate,[73] Δ-$[(phen)_2Ru(phendione)](PF_6)_2$[60] and 4,5-dinitro-1,2-penylenediamine[72] were prepared by literature procedures.

2.5.2 Instrumentation

^1H NMR (500 MHz and 300 MHz) and ^{13}C NMR (500 MHz) spectra were obtained on a JEOL Eclipse Plus 500 or a 300 MHz NMR spectrometer using CD_3CN or d^6-DMSO as a solvent. Chemical shifts (δ values) are given in parts per million and referenced to TMS. UV-Vis spectra were obtained using an Agilent 8453 UV-Visible spectrophotometer. ESI-MS spectra were obtained on a Thermo LCQ Deca XP quadrupole ion trap instrument equipped with a conventional ESI source (Thermo-Fisher Scientific, West Palm Beach, FL). The determination of enantiomeric purity is done by a HPLC system using chiral stationary phases as described in the literature.[74,75]

Hydrogenation reaction was carried out by employing the following equipments: a ThalesNano H-Cube Midi^{TM} Scale-up Hydrogenation Reactor, a 3900 Shaker Hydrogenation Apparatus (Parr instrument company) and a model 4848 High Pressure Reactor.

2.5.3 Synthesis

$[(phen)_2Ru(diaminodppz)](PF_6)_2$

Method 1: H-Cube hydrogenation

$[(phen)_2Ru(dinitrodppz)](PF_6)_2$ (100 mg 0.09 mmol) was dissolved in 600 mL of acetonitrile and filtered through a nylon filter (pore sizes of 0.2 μm). The H-Cube was fitted with a THS 01131 CatCart (10% Pd/C, 70 X 4 mm). The reaction conditions are: hydrogen pressure 40 bar, temperature 40 ºC, flow rate 2 mL/min. The effluent from the H Cube was collected and rotovapped to an approximate volume of 10 mL. Addition of diethyl ether resulted in the formation of a precipitate which was isolated by filtration. The compound was dried in a vacuum oven at 40 ºC overnight. The ^{1}H NMR spectrum of this complex is identical to that previously reported in a literature.[60] Typical yield was 92%.

Method 2: Two-Cycle Hydrogenation in a 3900 Parr Shaker Hydrogenation Apparatus

$[(phen)_2Ru(dinitrodppz)]Cl_2$ (100 mg 0.11 mmol) was dissolved in 60 mL of methanol and transferred into a glass pressure reaction vessel. After degassing the solution by bubbling N_2 for 5 minutes, 100 mg of 10% Pd/C was added and the vessel fitted into a shaker-hydrogenator. After charging the vessel with hydrogen at 60 psi, the mixture was allowed to shake and react for 24 hours at room temperature. After relieving the pressure, the resulting solution was filtered through a pad of Celite to remove the Pd/C catalyst. The Celite pad was washed with another 60 mL of methanol. All the filtrate was rotovapped to approximately 10 mL. Addition of diethyl ether resulted in the formation of a precipitate which was isolated by filtration. The compound was dried in a vacuum oven at 40 ºC overnight. The ^{1}H NMR spectrum

of this product is shown in Figure. 2.5, reveals that the reduction reaction is incomplete after one cycle of reduction.

The product from the initial hydrogenation (80 mg) was dissolved into 50 mL of methanol, and hydrogenated as before in the presence of 80 mg of 10% Pd/C catalyst. After relieving the pressure, the resulting solution was treated with aforementioned procedures. In this case, the product was isolated by addition of an aqueous solution of ammonium hexafluorophosphate (50 mg 0.30 mmol) to the solution after a rotovap procedure. The product was filtrated and washed with 5 mL DI water. The compound was dried in a vacuum oven at 40 ºC overnight. Typical yield was 79%. The ^{1}H NMR spectrum of this complex is identical to that previously reported in a literature.[60]

Δ-$[(phen)_2Ru(phendione)]^{2+}$

1.0 g *rac*-$[(phen)_2Ru(phendione)]Cl_2$ was dissolved in 25 mL hot water and then 30 mL hot solution containing 2 g sodium arsenyl -D-(-)-tartrate was added. The solution was kept heating and stirred rigorously about 5 min at 80 ºC. Then the solution was chilled for 3 hours (put into a fridge to stay between 2-8 °C). The precipitate was flitted and washed with cold water and then suspend in 50 mL hot 2 M nitric acid solution. The precipitate was dissolved and precipitated as PF_6 salt by addition of an aqueous solution of ammonium hexafluorophosphate (50 mg 0.306 mmol, dissolved in minimum amount of DI water). The product was isolated by filtration and washed with large amount of DI water (until the filtrate is neutral). The compound was dried in a vacuum oven for overnight (crud product 0.4 ~ 0.6 g) and was determined the enantiomeric excess (ee%) value by HPLC. Second resolution is necessary to get greater than 99% ee value. The procedure for second resolution is same as above. Final yield was 22%.

$[(phen)_2Ru(tatpp)](PF_6)_2$ and Δ-$[(phen)_2Ru(tatpp)](PF_6)_2$

$[(phen)_2Ru(diaminodppz)](PF_6)_2$ (100 mg 0.09 mmol) and 1,10-phenanthroline -5,6-dione (phendione) (24 mg 0.11 mmol) were dissolved in a mixed solvent (50 mL of ethanol, 25 mL of acetonitrile and 25 mL of acetic acid) in a 250 mL round bottom flask. The reactant mixture was refluxed for 12 hours under N_2(g) protection. The solution was rotovapped to approximately 30 mL, and followed by addition of an aqueous solution of ammonium hexafluorophosphate (50 mg 0.31 mmol, dissolved in minimum amount of DI water) to form a precipitate which was isolated by filtration and washed with 5 mL of DI water and 20 mL of ethanol. The compound was dried in a vacuum oven at 60 ºC overnight (82% yield). The complex contains a large conjugated aromatic planer which forms a π-π stacking structure, making NMR peaks broad. Addition of zinc tetrafluoroborate hydrate can avoid this issue. The ^{1}H NMR of this compound is identical to that previously reported in a literature.[49] The Δ-$[(phen)_2Ru(tatpp)](PF_6)_2$ was prepared in a similar way by starting with Δ-$[(phen)_2Ru(diaminodppz)](PF_6)_2$.

ΔΔ-P

Δ-$[(phen)_2Ru(diaminodppz)](PF_6)_2$ (100 mg 0.09 mmol) and Δ-$[(phen)_2Ru(phendione)](PF_6)_2$ (90 mg 0.09 mmol) were dissolved in a mixed solvent (50 mL of EtOH, 25 mL of MeCN, and 25 mL of AcOH) in a 250 mL round bottom flask and the solution was refluxed for 12 hours under N_2. The resulting solution was rotovapped to approximately 30 mL total volume. Addition of an aqueous solution of ammonium hexafluorophosphate (50 mg 0.30 mmol, dissolved in minimum amount of DI water) resulted in the formation of a precipitate which was isolated by filtration and washed with 5 mL of DI water. The compound was dried in a vacuum oven at 60 ºC overnight. Crude yield 98 mg (53%). To remove the unreacted reactants, further purification (silica column chromatography) was applied.

Silica column chromatography

Silica gel 40-63 μm (230-400 mesh) was used to make a 3 cm by 0.5 cm glass column. The crude product (6 mg in 1 mL) was loaded and eluted with MeCN. The fraction with dark brown color was collected. After the eluant was colorless, the column was stripped by elution with NH_4PF_6 saturated MeCN. A second dark brown fraction was collected. The combined fractions were precipitated using the ether precipitation method and washed with plenty of DI water. The compound was dried in a vacuum oven at 60 ºC overnight. Yield for this step was 62% and overall yield was 33%. The ^{1}H NMR spectrum of this complex is identical to that previously reported in a literature.[60]

$[(phen)_2Ru(tadbp)](PF_6)_2$ and Δ-$[(phen)_2Ru(tadbp)](PF_6)_2$

$[(phen)_2Ru(diaminodppz)](PF_6)_2$ (100 mg 0.09 mmol) and 9,10-phenanthraquinone (23 mg 0.11 mmol) were dissolved in a mixed solvent (50 mL of EtOH, 25 mL of MeCN, and 25 mL of AcOH) and the solution was refluxed for 12 hours under N_2. The resulting solution was rotovapped to approximately 30 mL total volume. Addition of an aqueous solution of ammonium hexafluorophosphate (50 mg 0.30 mmol, dissolved in minimum about of DI water) resulted in a precipitate which was isolated by filtration and washed with 5 mL of DI water, 20 mL of EtOH and 10 mL of chloroform. The compound was dried in a vacuum oven at 60 ºC overnight. Typical yield was 83%. ^{1}H NMR of this compound (500 MHz, MeCN-D_3) is shown as Figure. 2.6. (δ) 9.25 (d, *J* = 8.26 Hz, 2H), 9.05 (d, *J* = 8.26 Hz, 2H), 8.79 (s, 2H), 8.67 (dd, 4H), 8.39 (d, *J* = 5.50 Hz, 2H), 8.32 (s, 4H), 8.12 (d, *J* = 5.16 Hz, 2H), 8.08 (d, *J* = 3.78 Hz, 2H), 7.96 (d, *J* = 8.26 Hz, 2H), 7.83 (dd, 2H), 7.75 (dd, 2H). 7.70 (dd, 2H). 7.61(t, *J* = 7.91 Hz, 2H) 7.37 (t, *J* = 8.26 Hz, 2H).

The Δ-$[(phen)_2Ru(tadbp)](PF_6)_2$ was prepared in a similar way by starting with Δ-$[(phen)_2Ru(diaminodppz)](PF_6)_2$.

Δ-$[(phen)_2Ru(diaminodppz)](PF_6)_2$

$[(phen)_2Ru(dinitrodppz)]Cl_2$ (50 mg 0.05mmol) was dissolved in 60 mL of methanol. After degassing the solution by bubbling N_2 though it for 5 minutes, 50 mg of PtO_2 was added and the vessel fitted into the shaker-hydrogenator. After charging the vessel with hydrogen at 60 psi, the mixture was allowed to shake and react for 24 hours at room temperature. After relieving the pressure, the resulting solution was filtered through a pad of Celite to remove the Pd/C catalyst. The Celite pad was washed with 60 mL of methanol and the combined methanol fractions were rotovapped to approximately 10 mL total volume. The product was isolated by addition of an aqueous solution of ammonium hexafluorophosphate (50 mg 0.30 mmol) which resulted in formation of a precipitate which was isolated by filtration and washed with 5 mL of DI water. The compound was dried in a vacuum oven at 40 ºC overnight. Typical yield was 79%. The ^1H NMR spectrum of this complex is identical to that previously reported in a literature.[60]

CHAPTER 3

PERMEABILITY AND LOCALLIZATION OF RUTHENIUM-TATPP COMPLEXS IN H358 CULTURED MALIGANT CELLS

3.1 Introduction

The biological activities of RPCs against cancer cells *in vitro* and *in vivo* has been investigated since the late 1950s[39-41]. It has been known for over 20 years now that RPCs containing large planar aromatic ligands such as dppz and tpphz bind DNA tightly due to a combination of intercalation and electrostatics.[76-79] It is postulated that their activity in cells is due to DNA binding and a number of studies have been conducted to show the RPCs not only enter the cell, but that they accumulate in the nucleus. The results of these studies are often inconsistent and contradictory with some claiming passive diffusion occurs into the cytoplasm, but no extensively into the nucleus,[68,80,81] whereas others see active transport into the cytoplasm and accumulation in the nuclei.[82] For many, the ability of RPCs to even passively cross the cell membrane was considered questionable given that they are high molecular weight complexes and carry a positive charge as either divalent or tetravalent cations. In this chapter, we examined the ability of Δ-MP and ΔΔ-P to enter H358 cancer cells and the cell nucleus using fractions of these cells collected after different incubation periods. As ruthenium is completely xenobiotic, all ruthenium found can be attributed to that obtained by uptake of RPCs.

3.2 Experimental

3.2.1 Reagents and Materials

Fetal Bovine Serum (Inactivated, USA origin, sterile-filtered, cell culture tested, cell culture tested), L-Glutamine solution (200 mM, BioXtra, solution, sterile-filtered, suitable for cell culture), Penicillin–Streptomycin (Solution stabilized, sterile-filtered, suitable for cell culture, with 10,000 units penicillin and 10 mg streptomycin/mL), RPMI-1640 Medium (500 mL With L-glutamine and sodium bicarbonate, liquid, sterile-filtered, cell culture tested), Trypsin-EDTA solution (0.12% trypsin, 0.02% EDTA, trypsin gamma irradiated by SER-TAIN Process, without phenol red, in Dulbecco's Phosphate Buffered Saline) and 25% tetramethylammonium hydroxide were purchased from Sigma-Aldrich.

All reagents were used without further purification or processing. MTT, CDDP and DMSO were purchased from Sigma-Aldrich. Carbon Dioxide Gas (USP) and Nitrogen Gas (USP) were purchased from Metroplex Service Welding Supply, INC.

Cancer Cell lines:

H-358 non-small lung adenocarcinoma cell lines were purchased from ATCC.

3.2.2 Instrumentation

Cancer cell lines were cultured in the following incubators: a VWR symphony^{TM} Water-Jacketed CO_2 Incubator (Model 6.5 W).

The ruthenium content was tested by a Thermo Electron Corporation GF 95Z Zeeman Furnace with M series AA-spectrometer.

3.2.3 Cancer cell lines culture

H-358 cancer cell lines were maintained in culture in RPMI-1640 with 10% FBS at 37°C with 5% CO_2 in humidified atmosphere. The culture medium was supplemented with 2 mM L-glutamine and 400 IU/mL penicillin.[83]

The culture and drug treatment were conducted in the laboratory of Dr. S. Mandal, a collaborator on this project at UTA. The H358 cells were treated with 5 μM ΔΔ-P and Δ-MP for three different time periods (1 hour, 24 hours, and 72 hours). After RPCs treatments, the whole cells and nuclei were collected respectively; one for whole cell (wc) analysis and other for isolation nuclear fraction (nf) analysis. H358 cells were maintained in RPMI 1640 medium with 10% fetal bovine serum, 100 units/mL penicillin. For the whole cell, each dish was rinsed 3X with pre-warmed PBS, and on removal of the last rinse, addition of 2 mL of trypsin-EDTA solution was used to harvest the cells. The samples of the whole cells were stored at -80 °C. To prepare dry samples for GFAAS, each sample was transferred to a glass vial (the weight of the empty glass vial was recorded). The sample preparation procedures of GFAAS are stated in GFAAS experiment part.

In order to obtain cellular organelles, in which they retain most of their original biochemical properties, mild detergents and following by fractionation of cellular components by differential centrifugation are usually employed.[84] For the nuclei pellet: treated and untreated cells were harvested by centrifugation at 500 g, resuspended in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES-KOH)-treated buffer (20 mM Tris/HCl, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol (DTT) and 0.2 mM phenylmethanesulfonyl fluoride (PMSF))[85], incubated on ice for 10 min and then centrifuged at 3500 g for 5 min.[86] The pellet contains enriched nuclei. The samples of the nuclei fractions were stored at -80 °C. To prepare dry samples for GFAAS, each sample was transferred to a glass vial (the weight of the empty glass vial was recorded). The sample preparation procedures of GFAAS are stated in GFAAS experiment part.

3.2.4 GFAAS experiment

The ruthenium content of the samples was tested by a Thermo Electron Corporation GF 95Z Zeeman Furnace with M series AA-spectrometer and a specific ruthenium emission lamp (Hollow cathode lamp). A standard curve for ruthenium concentration was determined using a custom grade ruthenium standard of 1009 ±10 µg/mL in 3.3% HCl (Inorganic Ventures Inc., Christiansburg, VA) as a stock solution. The ruthenium content standard curve is shown graphically in Figure 3.1.

The procedure for preparation of ruthenium content analysis samples are as bellow:

1). The whole cell or nuclei samples were transferred from cryotubes to glass vials. The samples were dried at 100 ºC. The mass of samples were measured and recorded. A typical mass was 2 ~ 3 mg for the whole cells and 2 ~ 3 mg for the nuclei fractions.

2). The dried samples were digested using 100 µL 25% tetramethylammonium hydroxide. The solution was pipetted up and down so that all the dried samples were all collected as one suspension and most of the dried samples were redissolved. Keep the sample at 2-8 °C for 24 h. After all samples were dissolved, mark this solution as stock solution.

3). 10 µL of stock solution was diluted to 500 µL in a cryotube using Millipore water.

4). The ruthenium content was determined by GFAAS as follows: 10 mL aliquots of the sample solutions were introduced into the graphite furnace and atomized at a temperature of 25000 °C. For each sample 6 measurements were performed. The furnace was continuously purged with argon gas at a flow rate of 3.0 L min^{-1} and the atomized sample analyzed at a wavelength of 349.4 nm. The results are collected in Table 3.1 and shown graphically in Figure 3.2. The data are an average of 6 measurements and the standard deviations are shown on the bar graphs in Figure 3.2.

3.3 Results and Discussion

An initial study of localization of Δ-MP and ΔΔ-P by determining ruthenium content in H358 was conducted using GFAAS. As shown in Figure 3.1, the standard curve of ruthenium content was obtained at 349.4 nm, which in this wavelength the samples were analyzed. The standard curve at 379.9 nm is shown in appendix A-4. (This standard curve at different absorption wavelength is as a supplemental reference.)

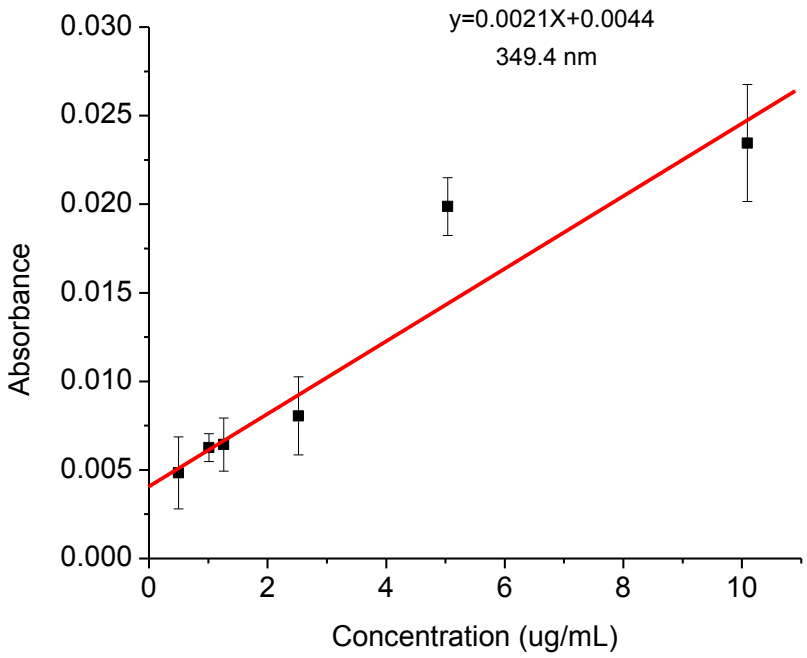


Figure. 3.1 Ruthenium content test standard cure by GFAAS at 349.4nm. (Ruthenium custom-grade standard 1009 ±10 µg/mL solution in 3.3% HCl)

Table 3.1. Ruthenium Content in the whole cell (wc) and nuclear fraction (nf) of RPC treated H358 cells at various incubation times.

Treatment time (h)	RPC	wc or nf	Ru contant (μg/mg)	[RPC][a] nmol/mg	% RPC[b] in nf
1	Δ-MP	wc	9.3±1.1	92	1.7
		nf	1.6±2.2	16	
1	ΔΔ-P	wc	9.7±4.8	48	1.1
		nf	1.1±0.8	5.4	
24	Δ-MP	wc	11.9±2.6	120	9.4
		nf	11.2±2.0	110	
24	ΔΔ-P	wc	7.5±0.8	37	10.3
		nf	7.7±2.8	38	
72	Δ-MP	wc	13.5±1.5	130	8.0
		nf	10.8±0.6	110	
72	ΔΔ-P	wc	6.7±1.5	33	9.0
		nf	6.0±1.1	30	

a) the [RPC] for ΔΔ-P was half the measured [Ru] as there are 2 Ru/ ΔΔ-P. b) percent Ru in the nucleus was calculated from $\%Ru_{nf} = (1-([RPC]_{wc} - ([RPC]_{nf}*0.1))/[RPC]_{wc})*100\%$ which assumes the nucleus is 10% by mass of the dried cells.

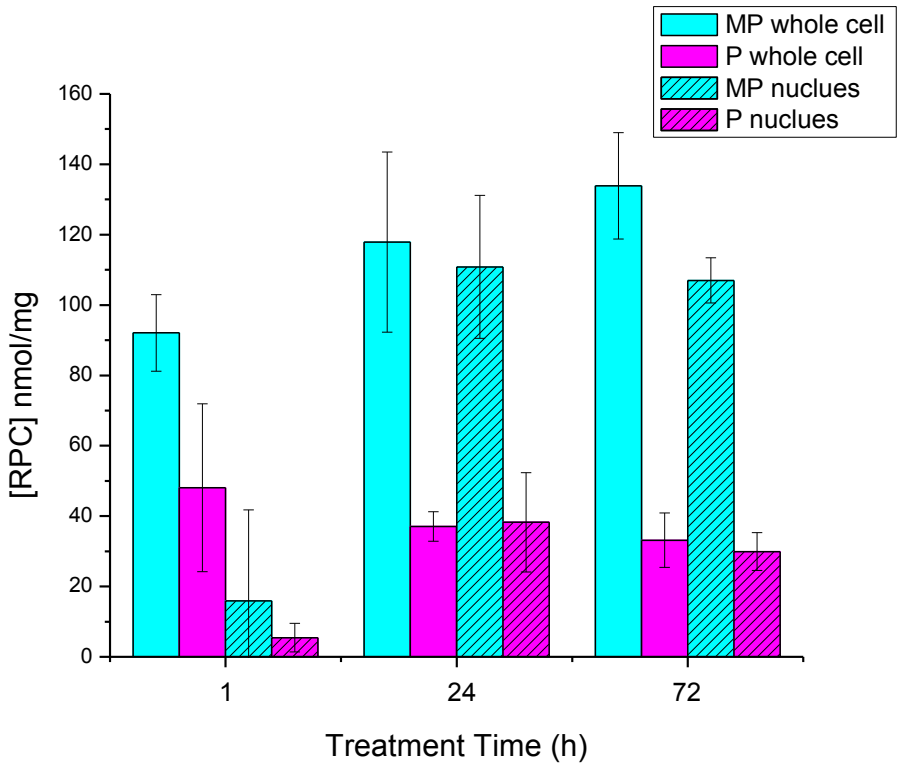


Figure. 3.2 Comparison of ruthenium uptake in H-358 whole cells and isolated nuclei after 1 h, 24 h, and 72 h, incubation with 5 μM Δ-MP and ΔΔ-P

As seen Figure 3.2, there is appreciable uptake of ruthenium in the wc after just 1 h incubation time. In terms of [RPC], the uptake for ΔΔ-P is approximately half that of Δ-MP in both the wc and nf. After 24 h incubation, the [RPC] of Δ-MP in the whole cells has increased by 30% from the 1 h timepoint. Significantly, the [RPC] in the nf is now essentially the same as that of the whole cells for both Δ-MP and ΔΔ-P, indicating that the drug is almost uniformly distributed throughout the cell after 24 h. This situation is essentially unchanged even after 72 h incubation periods, indicating the RPC uptake has level-ed and reached equilibrium.

There are several notable items that can be said from this data. First, both complexes are quick to enter the cell, establishing peak concentrations in the nf around 24 h. The whole cell [RPC] does increase upon 24 h incubation times, but not significantly thereafter. Using

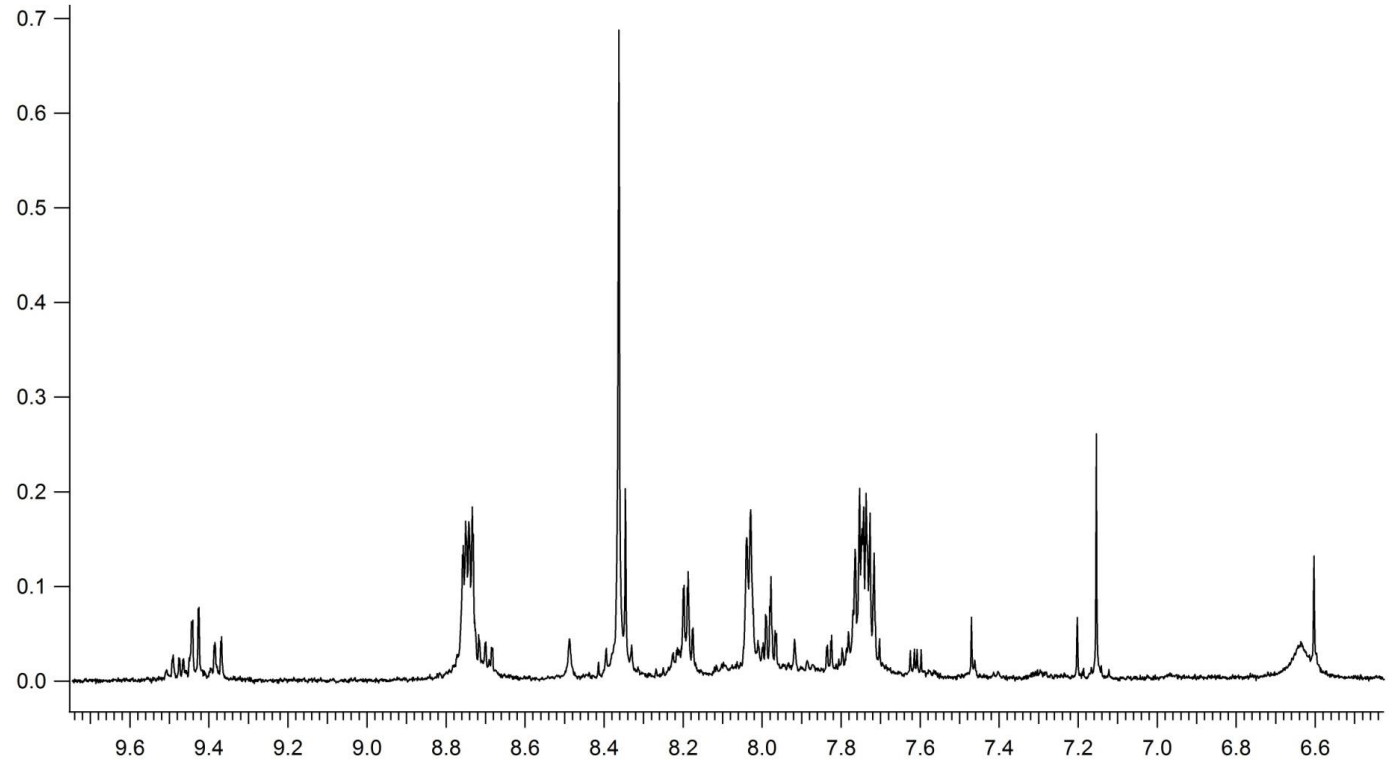
estimates of the nucleus being approximately 10% of the cell mass,[87] we can approximate the [RPC] is essentially the same in the nf as the wc.

3.4 Conclusion

In the ruthenium content experiments, the H-358 cancer cell line was employed to study the ruthenium complex uptake by the whole cell and nucleus. GFAA data revealed that both RPC saturate the nucleus of the cells with RPCs in under 24 h incubation time, with the maximal concentration of ΔΔ-P being approximately half that of Δ-MP. Upon longer incubation times, the [RPC] continues to increase with most of this ending up in the cytoplasm, such that the RPC distribution approximates the nucleus/whole cell volume ratio of roughly 10%. We presume that the additional charge on ΔΔ-P is responsible for the decreased uptake relative to Δ-MP. From all these data, it is clear that these RPCs have little difficult entering both the cells and their nuclei quickly and remain there for substantial periods of time.

APPENDIX A

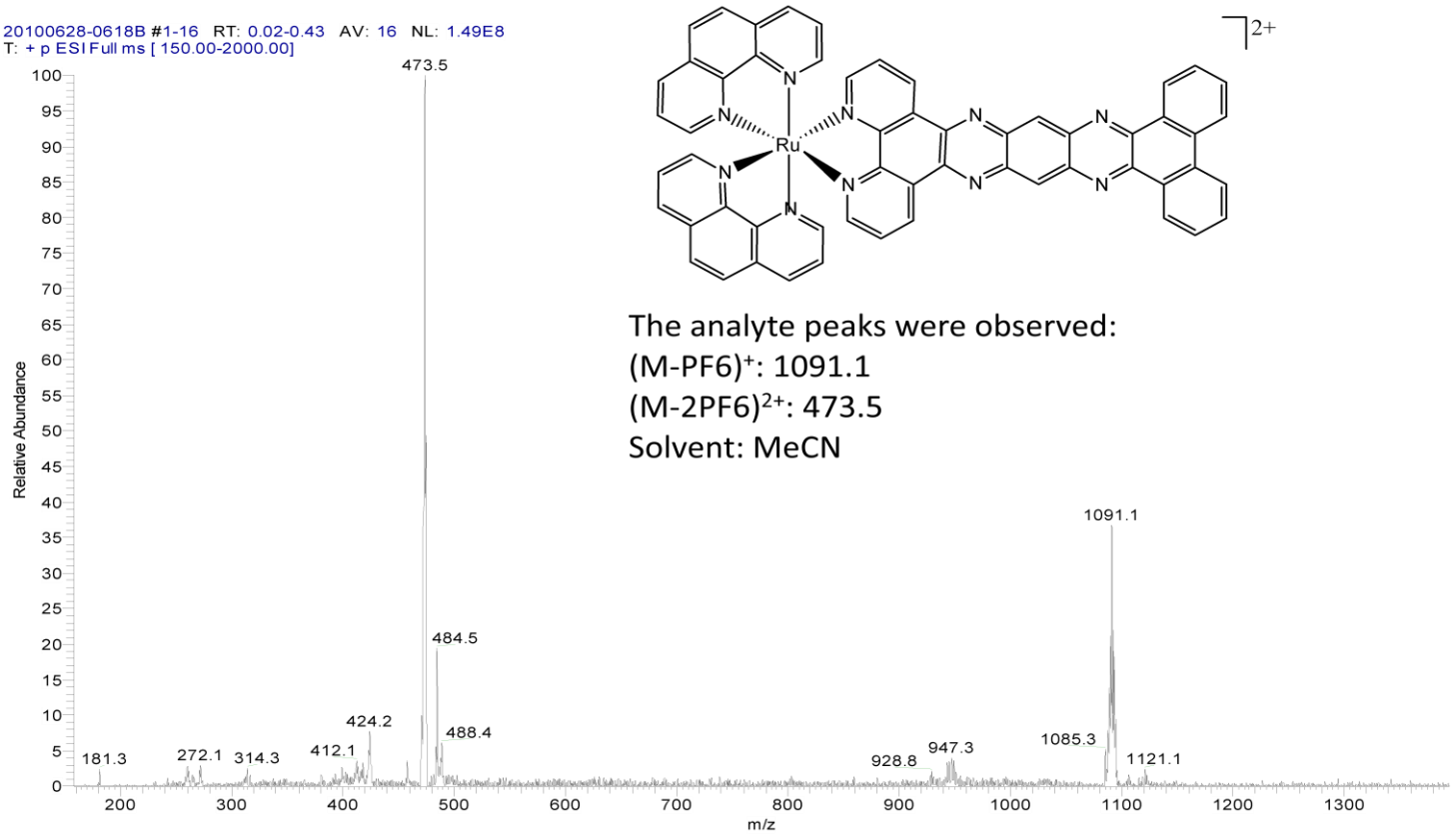
^{1}H NMR FOR INTERMEDIATE BY $[(PHEN)_2RU(DINITRODPPZ)]^{2+}$ REDUCTION REACTION.
(500 MHZ, SOLVENT: $MeCN\text{-}D_3$)



A: ^{1}H NMR for intermediate by $[(phen)_2Ru(dinitrodppz)]^{2+}$ reduction reaction. (500 MHz, Solvent: MeCN-D_3)

APPENDIX B

MASS SPECTROSCOPY OF $[(PHEN)_2RU(TADBP)](PF_6)_4$

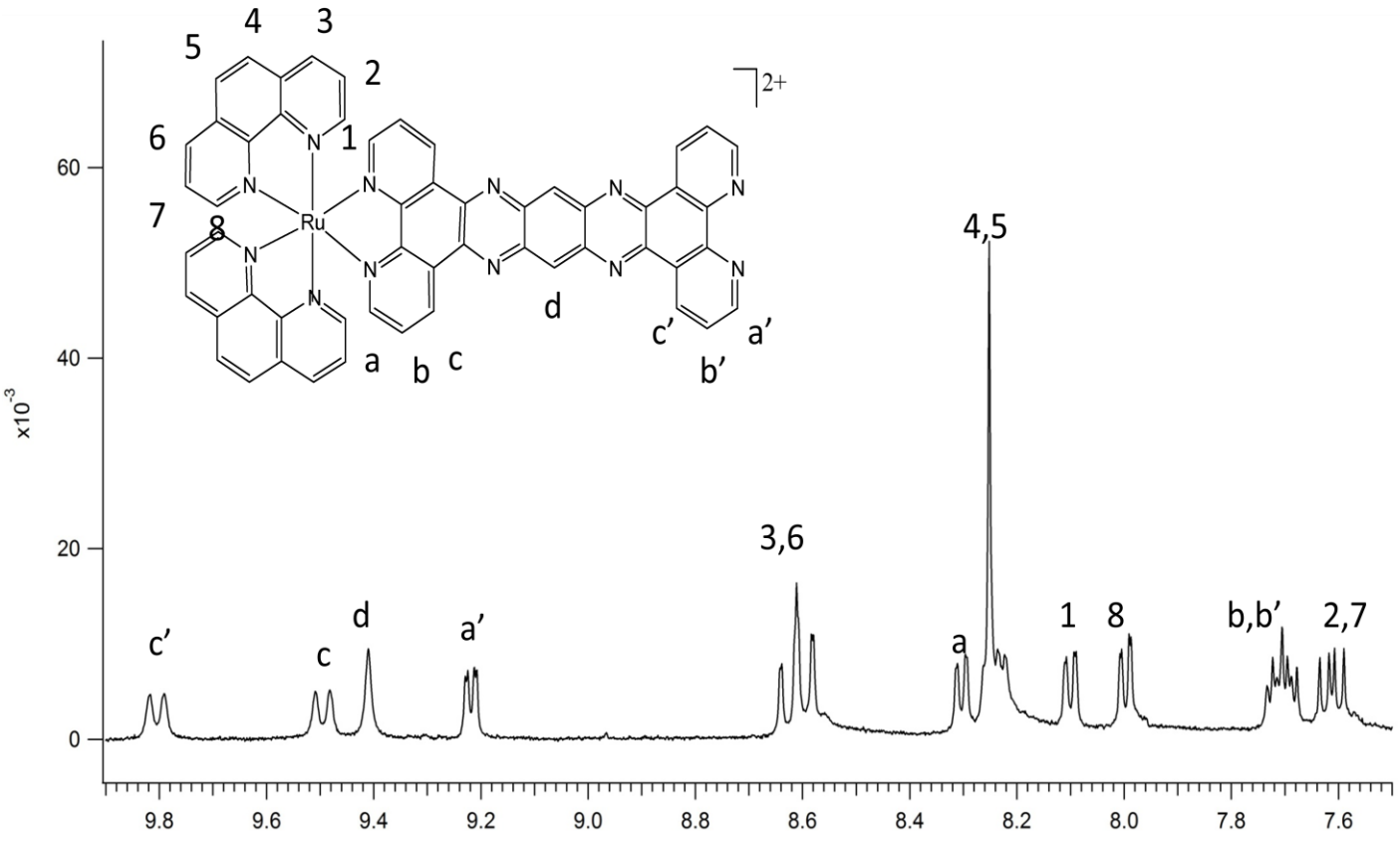


The analyte peaks were observed:
$(M\text{-}PF6)^{+}$: 1091.1
$(M\text{-}2PF6)^{2+}$: 473.5
Solvent: MeCN

B: Mass spectroscopy of $[(phen)_2Ru(tadbp)](PF_6)_4$

APPENDIX C

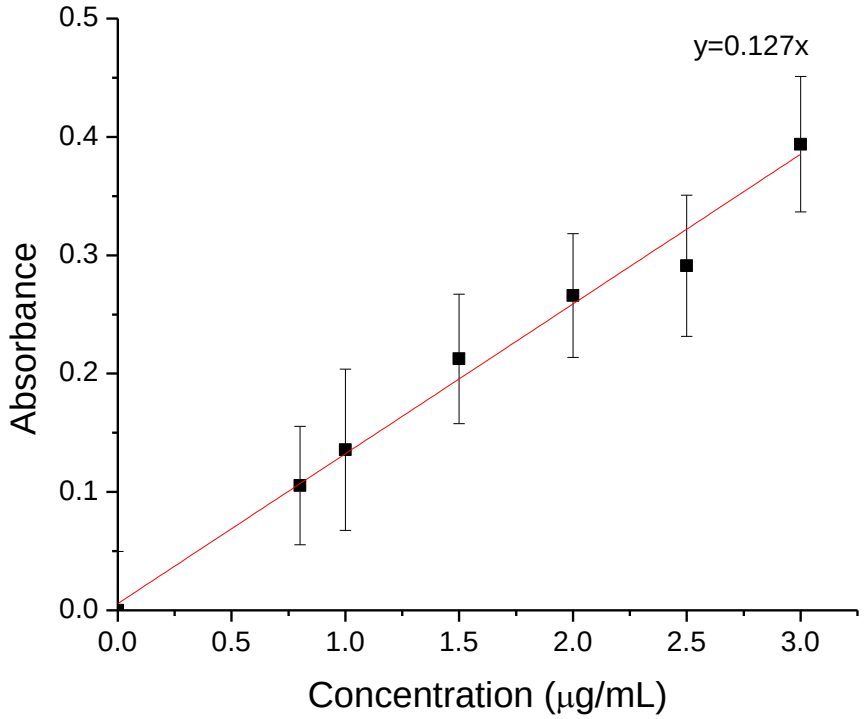
^{1}H NMR SPECTRUM OF [$(PHEN)_2RU(TADBP)$]$(PF_6)_4$. (500 MHZ, SOLVENT: MeCN-D_3)



C: ^{1}H NMR spectrum (500 MHz, Solvent: MeCN-D_3) of $[(phen)_2Ru(tatpp)](PF_6)_2$

APPENDIX D

RUTHENIUM CONTENT TEST STANDARD CURE BY GFAAS AT 379.9 NM.



D: Ruthenium content test standard cure by GFAAS at 379.9nm. (Ruthenium custom-grade standard 1009 ±10 µg/mL solution in 3.3% HCl)

REFERENCES

(1) Mazumder, U. K.; Gupta, M.; Karki, S. S.; Bhattacharya, S.; Rathinasamy, S.; Thangavel, S. *Chemical & Pharmaceutical Bulletin* **2004**, *52*, 178-185.

(2) Thota, S.; Karki, S. S.; Jayaveera, K. N.; Balzarini, J.; De Clercq, E. *J. Enzyme Inhib. Med. Chem.* **2010**, *25*, 513-519.

(3) Liu, Y.-J.; Liang, Z.-H.; Li, Z.-Z.; Yao, J.-H.; Huang, H.-L. *DNA Cell Biol.* **2011**, *30*, 829-838.

(4) Abhishek Yadav; Thamara Janaratne; Arthi Krishnan; Singhal, S. S.; Sanjay Awasthi; MacDonnell, F. M. *Manuscript in preparetion* **2011**.

(5) Janaratne, T. K.; Yadav, A.; Ongeri, F.; MacDonnell, F. M. *Inorg. Chem.* **2007**, *46*, 3420-3422.

(6) Enwonwu, C. O.; Meeks, V. I. *Crit. Rev. Oral Biol. M* **1995**, *6*, 5-17.

(7) Foye, W. O. *Cancer Chemotherapeutic Agents*; ACS Professional Reference Book, 1995.

(8) Missailidis, S. *Anticancer Therapeutics*; Wiley-Blackwell A John Wiley & Sons, Ltd., Publication, 2008.

(9) Salmon, S. E.; Sartorelli, A. C. Cancer Chemotherapy; Appleton-Lange, 1998.

(10) Judith McKay; Schacher, T. *The Chemotherapy Survival Guide: Everything You Need to Know to Get Through Treatment*; New Harbinger Publications, 2009.

(11) Elmore, S. *Toxicol. Pathol.* **2007**, *35*, 495-516.

(12) Mir, O.; Ropert, S.; Goldwasser, F. *Lancet Oncol.* **2009**, *10*, 304.

(13) Rosenberg, B.; Van Camp, L.; Krigas, T. *Nature* **1965**, *205*, 698-699.

(14) Rosenberg, B.; Van Camp, L.; Grimley, E. B.; Thomson, A. J. *J. Biol. Chem.* **1967**, *242*, 1347-1352.

(15) Rosenberg, B.; Vancamp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, *222*, 385-386.

(16) Rosenberg, B. *Adv. Exp. Med. Biol.* **1977**, *91*, 129-150.

(17) Lippert, B. *Cisplatin: chemistry and biochemistry of a leading anticancer drug*; Wiley-VCH, 1999.

(18) Brabec, V.; Kasparkova, J. *Drug Resist. Update* **2005**, *8*, 131-146.

(19) Cohen, S. M.; Lippard, S. J. *Prog. Nucleic Acid Res. Mol. Biol.* **2001**, *67*, 93-130.

(20) Basu, A.; Krishnamurthy, S. *J. Nucleic Acids* **2010**, *2010*, 1-16.

(21) Gonzalez, V. M.; Fuertes, M. A.; Alonso, C.; Perez, J. M. *Mol. Pharmacol.* **2001**, *59*, 657-663.

(22) Dempke, W.; Voigt, W.; Grothey, A.; Hill, B. T.; Schmoll, H. J. *Anticancer Drugs* **2000**, *11*, 225-236.

(23) Kartalou, M.; Essigmann, J. M. *Mutat. Res.* **2001**, *478*, 23-43.

(24) Carter, S. k.; Bakowski, M. T.; Hellmann, K. *Chemotherapy of Cancer*; Wiley Medical, 1987.

(25) Kostova, I. *Recent Pat Anticancer Drug Discov* **2006**, *1*, 1-22.

(26) Bruijnincx, P. C. A.; Sadler, P. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 197-206.

(27) Reed, J. E.; Arnal, A. A.; Neidle, S.; Vilar, R. *J. Am. Chem. Soc.* **2006**, *128*, 5992-5993.

(28) Milacic, V.; Fregona, D.; Dou, Q. P. *Histology and Histopathology* **2008**, *23*, 101-108.

(29) Hannon, M. J. *Pure Appl. Chem.* **2007**, *79*, 2243-2261.

(30) Alessio, E.; Mestroni, G.; Bergamo, A.; Sava, G. In *Metal Ions in Biolgical Systems, Vol 42: Metal Complexes in Tumor Diagnosis and as Anticancer Agents*; Marcel Dekker: New York, **2004**; Vol. 42, p 323-351.

(31) Alessio, E.; Mestroni, G.; Bergamo, A.; Sava, G. *Current Topics in Medicinal Chemistry* **2004**, *4*, 1525-1535.

(32) Reedijk, J. *Platinum Metals Rev.* **2008**, *52*, 2-11.

(33) Levina, A.; Mitra, A.; Lay, P. A. *Metallomics* **2009**, *1*, 458-470.

(34) Bratsos, L.; Jedner, S.; Gianferrara, T.; Alessio, E. *Chimia* **2007**, *61*, 692-697.

(35) Brandt, W. W.; Dwyer, F. P.; Gyarfas, E. D. *Chem. Rev.* **1954**, *54*, 959-1017.

(36) Hiort, C.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc.* **1993**, *115*, 3448-3454.

(37) Schatzschneider, U.; Niesel, J.; Ott, I.; Gust, R.; Alborzinia, H.; Wolfl, S. *Chem. Med. Chem.* **2008**, *3*, 1104-1109.

(38) Chen, T.; Liu, Y.; Zheng, W.-J.; Liu, J.; Wong, Y.-S. *Inorg Chem* **2010**, *49*, 6366-8.

(39) Dwyer, F. P.; Gyarfas, E. C.; Rogers, W. P.; Koch, J. H. *Nature* **1952**, *170*, 190-1.

(40) Dwyer, F. P.; Wright, R. D.; Gyarfas, E. C.; Shulman, A. *Nature* **1957**, *179*, 425-6.

(41) Dwyer, F. P.; Mayhew, E.; Roe, E. M. F.; Shulman, A. *Br. J. Cancer* **1965**, *19*, 195-195.

(42) Dwyer, F. P.; Reid, I. K.; Shulman, A.; Laycock, G. M.; Dixson, S. *Australian Journal of Experimental Biology and Medical Science* **1969**, *47*, 203-203.

(43) Koch, C. J. *Cancer Res.* **1993**, *53*, 3992-3997.

(44) Shulman, A.; Laycock, G. M.; Bradley, T. R. *Chem.-Biol Interact.* **1977**, *16*, 89-99.

(45) Kumar, C. V.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 5518-5523.

(46) Friedman, A. E.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *Nucleic Acids Res.* **1991**, *19*, 2595-2602.

(47) Gill, M. R.; Thomas, J. A. *Chem. Soc. Rev.* **2012**, *41*, 3179-3192.

(48) Lincoln, P.; Norden, B. *Chem. Commun.* **1996**, 2145-2146.

(49) Konduri, R., University of Texas at Arlington, 2003.

(50) Tacconi, N. R. d.; Chitakunye, R.; Macdonnell, F. M.; Lezna, R. O. *J Phys Chem A* **2008**, *112*, 497-507.

(51) Yadav, A. Thesis, University of Texas at Arlington 2008.

(52) Liu, Y.; Peterson, D. A.; Kimura, H.; Schubert, D. *J. Neurochem.* **1997**, *69*, 581-593.

(53) Wouters, K. L.; de Tacconi, N. R.; Konduri, R.; Lezna, R. O.; MacDonnell, F. M. *Photosynth. Res.* **2006**, *87*, 41-55.

(54) de Tacconi, N. R.; Lezna, R. O.; Chitakunye, R.; MacDonnell, F. M. *Inorg. Chem.* **2008**, *47*, 8847-8858.

(55) Flamigni, L.; Encinas, S.; Barigelletti, F.; MacDonnell, F. M.; Kim, K. J.; Puntoriero, F.; Campagna, S. *Chem. Comm.* **2000**, 1185-1186.

(56) de Tacconi, N. R.; Lezna, R. O.; Konduri, R.; Ongeri, F.; Rajeshwar, K.; MacDonnell, F. M. *Chem.-Eur. J.* **2005**, *11*, 4327-4339.

(57) Singh, S.; de Tacconi, N. R.; Boston, D.; MacDonnell, F. M. *Dalton Trans* **2010**, *39*, 11180-7.

(58) Eriksson, M.; Mehmedovic, M.; Westman, G.; Akerman, B. *Electrophoresis* **2005**, *26*, 524-532.

(59) Rajput, C.; Rutkaite, R.; Swanson, L.; Haq, I.; Thomas, J. A. *Chem. Eur. J.* **2006**, *12*, 4611-4619.

(60) Kim, M.-J.; Thesis, University of Texas at Arlington, 2000.

(61) Hua, X.; Von Zelewsky, A. *Inorg. Chem.* **1991**, *30*, 3796-3798.

(62) Hua, X.; Lappin, A. G. *Inorg. Chem.* **1995**, *34*, 992-994.

(63) Hua, X.; von Zelewsky, A. *Inorg. Chem.* **1995**, *34*, 5791-5797.

(64) Kolp, B.; Viebrock, H.; von Zelewsky, A.; Abeln, D. *Inorg. Chem.* **2001**, *40*, 1196-1198.

(65) Bodige, S.; Torres, A. S.; Maloney, D. J.; Tate, D.; Kinsel, G. R.; Walker, A. K.; MacDonnell, F. M. *J. Am. Chem. Soc.* **1997**, *119*, 10364-10369.

(66) MacDonnell, F. M.; Bodige, S. *Inorg. Chem.* **1996**, *35*, 5758-&.

(67) Barton, J. K.; Danishefsky, A.; Goldberg, J. *J. Am. Chem. Soc.* **1984**, *106*, 2172-2176.

(68) Puckett, C. A.; Barton, J. K. *J. Am. Chem. Soc.* **2006**, *129*, 46-47.

(69) Guo, W.; Obare, S. O. *Tetrahedron Lett.* **2008**, *49*, 4933-4936.

(70) Yu, M.; Lin, H.; Lin, H.-K. *Chem. J. Chinese U* **2007**, *28*, 83-86.

(71) Alston, J. R.; Kobayashi, S.; Younts, T. J.; Poler, J. C. *Polyhedron* **2010**, *29*, 2696-2702.

(72) Kleineweischede, A.; Mattay, J. *Eur. J. Org. Chem.* **2006**, 947-957.

(73) Schlessinger, G. *Inorg. Synth.* **1970**, *12*, 267-269.

(74) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry*; 5th ed.; Springer Science, 2007.

(75) Nishiki, M.; Miyataka, H.; Niino, Y.; Mitsuo, N.; Satoh, T. *Tetrahedron Lett.* **1982**, *23*, 193-196.

(76) Jenkins, Y.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry* **1992**, *31*, 10809-10816.

(77) Lutterman, D. A.; Chouai, A.; Liu, Y.; Sun, Y.; Stewart, C. D.; Dunbar, K. R.; Turro, C. *J. Am. Chem. Soc.* **2008**, *130*, 1163-1170.

(78) Bhat, S. S.; Kumbhar, A. S.; Kumbhar, A. A.; Khan, A. *Chemistry* **2012**, *18*, 16383-16392.

(79) Very, T.; Despax, S.; Hebraud, P.; Monari, A.; Assfeld, X. *Phys Chem Chem Phys* **2012**, *14*, 12496-12504.

(80) Puckett, C. A.; Barton, J. K. *J. Am. Chem. Soc.* **2009**, *131*, 8738-8739.

(81) Puckett, C. A.; Barton, J. K. *Biochem.* **2008**, *47*, 11711-11716.

(82) Gill, M. R.; Garcia-Lara, J.; Foster, S. J.; Smythe, C.; Battaglia, G.; Thomas, J. A. *Nat Chem* **2009**, *1*, 662-667.

(83) Basu, I.; Locker, J.; Cassera, M. B.; Belbin, T. J.; Merino, E. F.; Dong, X.; Hemeon, I.; Evans, G. B.; Guha, C.; Schramm, V. L. *J. Biol. Chem.* **2010**, *286*, 4902-4911.

(84) De Duve, C.; Beaufay, H. *J. Cell. Biol.* **1981**, *91*, 293-299.

(85) Andrews, N. C.; Faller, D. V. *Nucleic Acids Res.* **1991**, *19*, 2499.

(86) Ansari, K. I.; Hussain, I.; Das, H. K.; Mandal, S. S. *FEBS J* **2009**, *276*, 3299-3307.

(87) Bruce Alberts; Alexander Johnson; Julian Lewis; Martin Raff; Keith Roberts; Walter., P. *Molecular Biology of the Cell*; 4 th ed.; Garland Science, 2002.

BIOGRAPHICAL INFORMATION

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