RUTHENIUM(II) POLYPYRIDYL COMPLEXES AS POTENTIAL ANTICANCER DRUGS: SYNTHESIS, CHARACTERIZATION, CELL PERMEABILITY AND INVESTIGATION OF THEIR HYPOXIA-SELECTIVITY

by

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ABSTRACT

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Supervising Professor: Dr. Frederick M. MacDonnell

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

binding one Ru ion. We hypothesize that the new complex B will retain the promising biological activity of the related MP complex, but will be more lipophilic and less reactive with extraneous metal ions than MP.

Moreover this thesis explores the ability of these large complexes to transverse the cell membrane and get into cell and the cell nuclei by isolating treated cells and nuclei and determining their ruthenium content by atomic absorption method. The data show that the two complexes examined ΔΔ-P and Δ-MP, are able to quickly pass into cancer cells (H-358) and are observed to build up in the nuclei, which were postulated due to their high affinity for binding to DNA.

Finally, the hypothesis that some of these complexes may be more cytotoxic towards cell under hypoxic stress was examined. HCC-2998 cells treated with ΔΔ-P and Δ-MP under normoxic and hypoxic conditions for a period of 4 hour showed similar responses to the dosing of these drugs. While this is not the definitive study on this hypothesis, it does suggest that there is little to no enhancement of the cytotoxicity of these RPCs due to hypoxia.

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LIST OF ABBREVIATIONS

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
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
GI_{50}	Causes 50% growth inhibition concentration
GSH	Glutathione
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	Tumour 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
WHO	World Health Organization

CHAPTER 1

RUTHENIUM(II) POLYPYRIDYL COMPLEXES AS POTENTIAL ANTI-CANCER AGENTS

1.1 Introduction of cancer and chemotherapy

1.1.1 Cancer

Cancer is a group of diseases characterized by uncontrolled growth and the spread of abnormal cells; absence of apoptosis cell death. Cancer is caused by both external factors (such as tobacco, chemicals) and internal factors (inherited mutations, hormones). These factors may act together or in sequence to initiate or promote carcinogenesis. Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths (around 13% of all deaths) in 2008 (WHO Media centre). The rapid increase in the global cancer burden represents a real challenge for health systems worldwide.

Cancer can be treated with surgery, radiation, chemotherapy, hormone therapy, biological therapy, and targeted therapy.[3] Treatment varies based on the type of cancer and its stage. The stage of a cancer refers to how much it has grown and whether the tumor has spread from its original location. If the cancer is confined to one location and has not spread, the most common treatments are surgery. If surgery cannot remove all of the tumors, the options for treatment include radiation, chemotherapy, or both. Some cancers require a combination of surgery, radiation, and chemotherapy.[4]

1.1.2 Chemotherapy

Chemotherapeutic agent is the term used to identify the drugs that against cancer, also as antineoplastic agents. Before chemotherapy, the most common treatment for cancer is

surgery. But most case diagnosis of testicular cancer still meant a death sentence.

Some of these chemotherapeutic agents are designed to damage rapidly diving cells due to the inherent nature of cancer cells, which is hallmarked by their frequent and uncontrolled cellular division. The first chemotherapeutic treatments used this principle to induce apoptosis of these frequently dividing cells in the blood and lymphatic system. Since the 1940s, 175 antineoplastic agents have been developed and are commercially available in the United State, Europe and Japan.[4]

Chemotherapy drugs can be classified into five major groups. These are:[5]

1. Alkylating Agents

   Methyl Hydrazines
   Platinum Coordination complexes: Cisplatin, Carboplatin, Oxaliplatin

2. Natural Products

   Plant Products
   Microorganism Products

3. Antimetabolites

   Folate Antagonists: Methotrexate
   Purine antagonists

4. Miscellaneous

   Hydroxyurea
   Imatinib Mesylate

5. Hormones and Antagonists

   Corticosteroids: Prednisone, Dexamethasone
   Estrogens: Ethinyloestradiol

1.1.2.1 Cisplatin

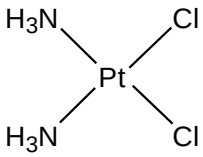


Fig. 1.1 Structure of *cis*-diamminedichloroplatinum(II) (CDDP) (trade name Cisplatin).

Cisplatin (*cis*-diamminedichloroplatinum(II) (CDDP)) (Fig. 1.1), since its discovery in 1965, its identification in 1969 and its clinical application in the early 1970s; continues to be a cornerstone in modern chemotherapy. Cisplatin is remarkable for its lack of myelosuppression. Now over 25 years after the description of its anticancer activity, cisplatin is still the first-line chemotherapeutic treatment and used in the initial management of several major solid tumors attests to its therapeutic importance.[6] Cisplatin, usually in combination with other drugs, is being used for treatment of the lung, head-and-neck, stomach, colon, uterus and as second-line treatment against most other advanced cancers such as cancers of the breast, pancreas, liver, kidney, prostate as well as against glioblastomas, metastatic melanomas, and peritoneal or pleural mesotheliomas.[4]

Cisplatin reacts directly with thiol groups and imidazole (His) residues (such as glutathione). Intracellular levels of glutathione have been linked to cisplatin detoxification. The antitumor properties of cisplatin are attributed to the kinetics of the chloride ligand displacement reactions leading to DNA crosslinking activities (Fig. 1.2). DNA crosslink inhibit replication, transcription and other nuclear functions and arrest cancer cell proliferation and tumour growth. A number of additional properties of cisplatin are now emerging, including activation of signal transduction pathways leading to apoptosis. Cisplatin shows the selectivity to cancer cell beyond only target to fast-dividing cell, it invoke the host's immune system to anticipate causing tumor cell death.[4, 7] In comparison to it's geometric isomer transplatin, the nature of the adducts formed in the *in vivo* reaction between DNA and transplatin is not yet completely elucidated, and

in vitro there wre some controversies. Sterical constraints preclude formation of 1,2-intrastrand cross-links in DNA which might explain the clinical inefficiency of transplatin.[6]

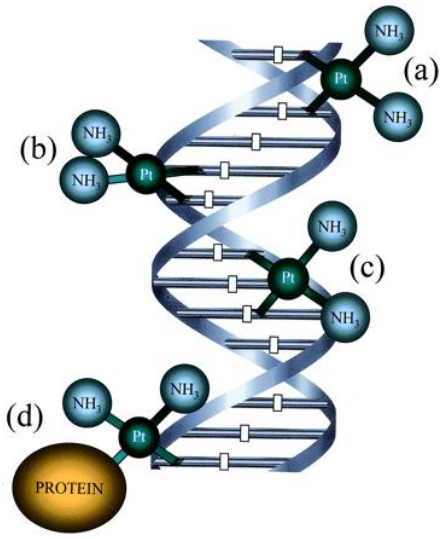


Fig. 1.2 Main adducts formed in the interaction of cisplatin with DNA. (a), interstrand cross-link; (b), 1,2-intrastrand cross-link; (c), 1,3-intrastrand cross-link; and (d), protein-DNA cross-link.[7]

One of the major drawbacks in the use of cisplatin in clinical applications has been the development of cisplatin resistant tumours. Cisplatin resistance develops from the inactivation of its activity inside the cytoplasm and from faster repair of DNA lesions. Several studies have been done to identify the expression of genes related to cisplatin resistance. Another limitation of cisplatin is its' inherent solubility.

Cisplatin has a number of side-effects that can limit its use. Nephrotoxicity (kidney damage) is a major concern. This is why IV treatment with cisplatin (such as Platinol-AQ) is formulated with diuretics such as mannitol. Neurotoxicity (nerve damage) can be anticipated by performing nerve conduction studies before and after treatment. Nausea and vomiting: cisplatin is one of the most emetogenic chemotherapy agents. Ototoxicity (hearing loss): unfortunately there is at present no effective treatment to prevent this side effect, which may be severe. Electrolyte disturbance: Cisplatin can cause hypomagnesaemia, hypokalemia and

hypocalcaemia. The hypocalcaemia seems to occur in those with low serum magnesium secondary to cisplatin, so it is not primarily due to the Cisplatin. This agent can also cause profound bone marrow suppression (Myelotoxicity).[3, 8]

1.1.2.2 Metal-based chemotherapy

The field of metal-based antineoplastic agents is initiated by cisplatin, a metal coordination compound containing no organic units and which is currently one of the leading drugs used against cancer. The discovery of the anticancer activity of cisplatin sparked intense interest and research to find other metal-based antineoplastic agents that not only has the same efficacy as cisplatin but can overcome some of the side-effects and resistance of existing chemotherapy. This has generated interest in molecules containing other heavy metals of Groups 8, 9, 10 of the periodic table, which have similar properties to platinum. These metals may differ in oxidation state, ligand affinity, and substitution kinetics. When developing new metal based chemotherapies, one must consider the inherent toxicity of the metal complexes produced. Accumulation of metal ions in the body can lead to deleterious effects. Thus bio-distribution and clearance of the metal complexes as well as its pharmacological specificity are to be considered.[9]

Pt(IV) prodrug is being investigated as an agent which has new modes of interaction with the classical target DNA.[10-12] Sadler et al. are developing nontoxic photolabile diam(m)ino platinum(IV) diazido complexes which can inhibit the growth of human bladder cancer cells upon irradiation with light.[13, 14] The Ni(II)-salphen (Fig. 1.3.) complex incorporates the main requirements for quadruplex-stabilizing molecules, induces a high degree of quadruplex DNA-stabilization and telomerase inhibition.[15, 16] Gold(III) is isoelectronic with platinum(II) and tetracoordinate gold(III) complexes have the same square-planar geometries as cisplatin; the anticancer activity of gold(III) compounds has been investigated. Previous studies suggested that, in contrast to cisplatin, gold complexes target proteins but not DNA.[17] Moreover, the study

and explore of complexes of iron, cobalt and other transition metals on anticancer activities build up a solid basic bio-chemistry knowledge. There are now many distinct classes of metal-based drugs with antitumour activity in experimental models. Unfortunately, none has as yet achieved full clinical use.

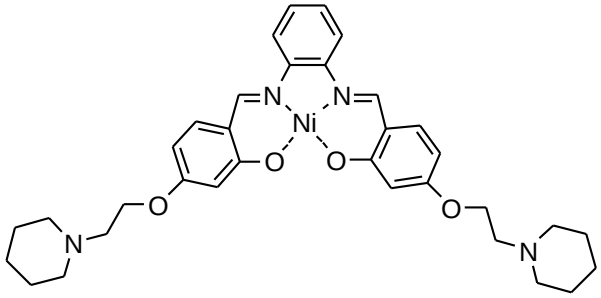


Fig. 1.3 Structure of Ni(II)-salphen complex.

1.1.3 Ruthenium complexes

Generally speaking, the chemistry of ruthenium complexes, with special attention to their electron-transfer properties, has been receiving continuous attention over several decades. Ruthenium offers a wide range of oxidation states which are accessible chemically and electrochemically (From oxidation state -2 in $[Ru(CO)_4]^{2-}$ to +8 in RuO_4). Therefore, the complexes of ruthenium are redox-active and their application as redox reagents in different chemical reactions is of much current interest. The thermodynamic stability of ruthenium in several different oxidation states, the nature of its redox couples, and the relative ease for preparation mixed-ligand complexes, all make ruthenium complexes particularly attractive targets of study.[9, 18, 19]

Well-known Ru(II) complexes with polypyridyl ligands have been extensively tested for their DNA-binding, antibacterial activity, antitumour cytotoxic properties and etc. Much of the early studies concerning Ru(II) complexes and it's biological activities was started by Dwyer and co-workers in the 1940s to 1960s.[20-23] Given this historical context, the achievement by Dwyer

and co-workers has enlightened our research work as well as others. Moreover, an excellent example of this point is the ruthenium complex [ImH][trans-$RuCl_4$(DMSO)(Im)] (NAMI-A) (Im = imidazole, DMSO = dimethylsulfoxide) (Fig. 1. 4), which has successfully completed Phase I clinical trials. NAMI-A acts by inhibiting a membrane protein kinase C, which triggers a series of events that eventually cause apoptosis. This ruthenium complex is one of the few drugs that have shown promising results for the treatment of late stage lung cancer inhibiting further development and spread of metastatic tumors.[24, 25]

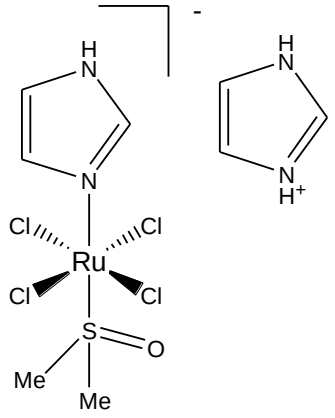


Fig. 1.4 Structure of [ImH][trans-$RuCl_4$(DMSO)(Im)] (NAMI-A).

1.1.3.1 Ruthenium drugs with labile ligands

Compare with cisplatin and NAMI-A, although, the two complexes have different coordination number; the different metals are all coordinated with chloride as labile ligand. Once in the bloodstream, cisplatin can then diffuse across cell membranes into the cytoplasm, which can then undergo chloride dissociation and replacement with an aqua ligand to form cationic platinum complexes, such as $[Pt(NH_3)_2(OH)Cl]^+$. These complexes are formed when a water molecule attacks the platinum metal center, thus eliminating a chloride ion which acts as a non-coordinating anion. The cell essentially traps the cisplatin by transforming it into a cationic component of a neutral molecule. After losing two Cl^- anions, cisplatin-aqua species reacts with

DNA.[6] The key factor explaining why platinum can function as an antineoplastic drug likely relates to its ligand-exchange kinetics, which are in the order of minutes to days rather than microseconds to seconds (as for many other coordination compounds), thereby giving platinum high kinetic stability and preventing rapid equilibration reactions. Ru(II) and Ru(III) have similar ligand exchange kinetics to those of Pt(II). NAMI-A, [ImH][trans-$RuCl_4$(DMSO)(Im)], is a Ru(III) complex with DMSO and imidazole coordinated to the ruthenium. NAMI-A has been shown to possess antitumor and more importantly antimetastatic activities in preclinical studies.[26]

The proposed mechanisms of action of NAMI-A include: (1) interaction with the cell cycle regulation culminating in transient accumulation of cells in the G2/M phase,[27] (2) inhibition of matrix metalloproteinases, (3) increasing extracellular matrix around tumor vasculature, thereby preventing neoplastic cells from invading nearby tissues and blood vessels,[28] and (4) binding through coordination to nucleic acids, therefore, having a direct effect on tumor cell DNA.[29,30]

KP1019, [InH][trans-$RuCl_4(In)_2$] (In = indazole), is a stable Ru(III) complex containing two indazole heterocycles coordinated to the metal center via nitrogen atoms (Fig. 1.5). Unlike NAMI-A, KP1019 is thought to possess direct cytotoxic activity promoting apoptosis in a number of cancer cell lines as well as in a range of tumor models (especially colorectal cancers).[31, 32] Besides NAMI-A and KP1019, a large number of other ruthenium complexes have been prepared and tested for antitumor activity in cultured tumor cells and animal models is RM175 (Fig. 1.5).[19, 33, 34]

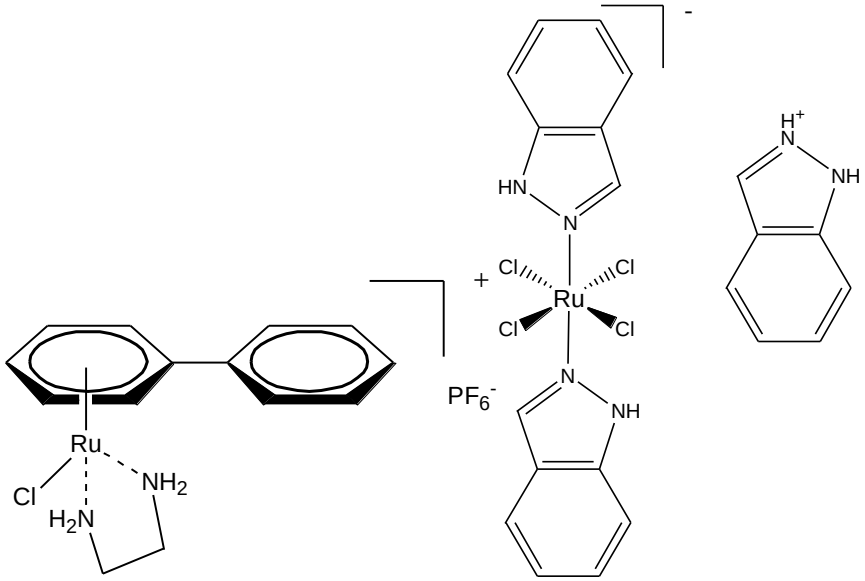


Fig. 1.5 Structure of RM175 and KP1019.

Other classes of ruthenium complexes have been extensively studied, such as Ru(III) polyaminecarboxylato complexes (Fig. 1.6).[9, 18, 25] These complexes are characterized by unusually rapid ligand exchange of chloro to aqua. They also have a propensity for oxidation to Ru(IV) (and possibly Ru(V)) species under biologically relevant conditions (by aerial dioxygen or H_2O_2 at pH = 5 - 8). Therefore, likely modes of anticancer action of these complexes include rapid ligand-exchange reactions with active centers of enzymes (such as cysteine residues of proteases or protein tyrosine phosphatases), as well as induction of cellular oxidative stress. In addition, the complex its analogues can affect cellular signaling by acting as NO scavengers.[35, 36]

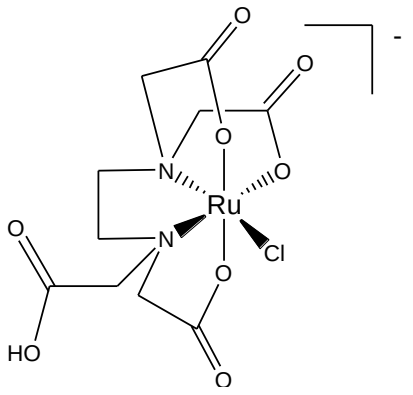


Fig. 1.6 Structure of $[Ru^{III}(pac)Cl]^-$ complex (pac = edta (ethylenediaminetetraacetate)).

Unlike Pt-complexes, binding to extracellular proteins is thought to cause the ruthenium complexes which have labile ligands such as chloro or carboxylate loss of biological activity.

1.1.3.2 Ruthenium polypyridyl complexes

Polypyridyl ligands, otherwise known as polypyridines, are compounds that contain more than one pyridine unit, such as 2,2'-bipyridine, 1,10-phenanthroline, and 2,2';6'2''-terpyridine. When these ligands are bound to a metal center, they have been found to have some interesting photophysical properties as well as redox activity. It has been well documented that some of these metal complexes are fairly stable complexes.[21] One well known polypyridyl complex is $[Ru(bpy)_3]$.

The Dwyer group began reporting his research on the biological activities metal polypyridyl complex as far back as the late 1940's. A lot of his attention began to focus on Ru polypyridyl complexes such as: $[Ru(phen)_3](ClO_4)_2$ and $[Ru(bpy)_3](ClO_4)_2$ (Fig. 1.7) in 1950s[20, 37] which laid the ground work for this study. The bactericidal properties and their actions on enzymes have been discussed by Dwyer et al.[20, 37] The result of toxicity to mice is shown as table 1.1.[20]

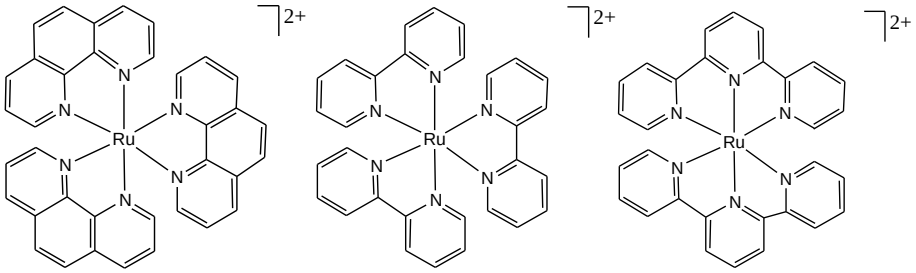


Fig. 1.7 Structures of $[Ru(phen)_3]^{2+}$; $[Ru(bpy)_3]^{2+}$ and $[Ru(trpy)_2]^{2+}$.

Table 1.1 Ruthenium complexes toxic dose 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.)

Compound	Optical form and toxic dose	
	(+) mgm/kgm	(-) mgm/kgm
$[Ru(phen)_3](ClO_4)_2$	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

When large amounts of these complexes (five times the toxic intraperitoneal dose) were delivered intraperitoneally into mice, they caused paralysis and death by respiratory failure. The effect of complex ions on transmission at a neuromuscular junction was evaluated by Dwyer et al.[22] The result shows that the neuromuscular blocking activity of the complex cations on both the toad Sartorius and rate diaphragm nerve-muscular junction and the complexes which are coordinately saturated and of high chemical stability are potent inhibitors of acetylcholinesterase and possess curare-like activity.

The radioactive labeled ruthenium experiment shows that the complex administered intraperitoneally did not change in the tissue of mice and rats. The complex was excreted

mainly in the urine. No traces of it could be found in nervous tissues. It accumulated appreciably in kidney and liver; but the amounts found in other tissues were below that in blood. After intraperitoneal administration the (+) and (±) forms of the complex appeared at a faster rate in blood and urine than the (-) form. The (+) form penetrated tissues at a greater rate than the (-) form. Moreover the (+) form was more rapidly absorbed than the (-) form; the higher toxicity of the former is to be expected.

Tris(3,4,7,8-tetramethyl-1,10-phenanthroline) ruthenium(II) dichloride (Ru 1) and acetylacetonatobis (3,4,7,8-tetramethyl-1,10-phenanthroline) ruthenium(II) dichloride (Ru 2) were tested by Dwyer and coworkers for the inhibition of Landschutz ascites tumor growth. The doses and number of animals used, their changes in weight, and the T/C values are given in Table 1.2.

Table 1.2 Effect of Ru chelates on Landschutz Ascites Tumour growth in the mouse (A single dose of the complex in aqueous solution was administered intraperitoneally on the following day of inoculated tumour cells. The animals were killed seven days after tumour inoculation and the tumour 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)	Tumour growth (T/C)
Controls	water	1 to 4	40	2	-
Ru 1	10	1	5	-1	0.43
		2	5	-2	0.13
		3	5	-2	0.34
		4	5	-3	0.19
Ru 2	3.5	1	5	1	1.08
	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

These results suggest that Ru 1 was a less active inhibitor of tumour growth than Ru 2. Although a single dose of 3.5 mg/kg did not inhibit tumour growth; a dose of 4.0-4.5 mg/kg produced slight inhibition whereas 5.0 mg/kg caused quite strong inhibition without significant weight loss.

In addition, *in vitro* experiments show that fully coordinated 1,10-phenanthroline and 2,2'-bipyridine chelates of Ru(II) are lethal *in vitro* to cultured and ascites P-388 mouse lymphocytic leukaemic cells. 1,10-phenanthroline chelates are generally more potent than corresponding 2,2'-bipyriding compounds, and heterolepitic (acetylacetonato) monovalent chelates of both series are more active than the corresponding identical-ligand divalent chelates. Lethal potency is greatest for Ru(II) chelates containing highly alkylated ligands.[38]

1.1.3.3 Ruthenium polypyridyl complexes with DNA

It has been shown that by replacing the phen ligand with larger planar aromatic ligand such as dppz and cpdppz (Fig. 1.8) can lead to much higher DNA binding affinities and which fully intercalate into the DNA base-pairs.[39, 40] The complex $[(phen)_2Ru(dppz)]^{2+}$ has been shown to bind by intercalation as well as electrostatics. The binding constants for Δ- and Λ-$[(phen)_2Ru(dppz)]^{2+}$ are $3.2x10^6$ M^{-1} and $1.7x10^6$ M^{-1} respectively which is approximately three orders of magnitude higher than those with no intercalation.[41] The dimer $[(phen)_2Ru(\mu\text{-}c4(cpdppz)_2)Ru(phen)_2]^{4+}$ has a DNA binding constant on the order of $10x10^{12}$ M^{-1}. This complex is reported to have promising antitumor activity against platinum resistant tumor types *in vitro*.[42]

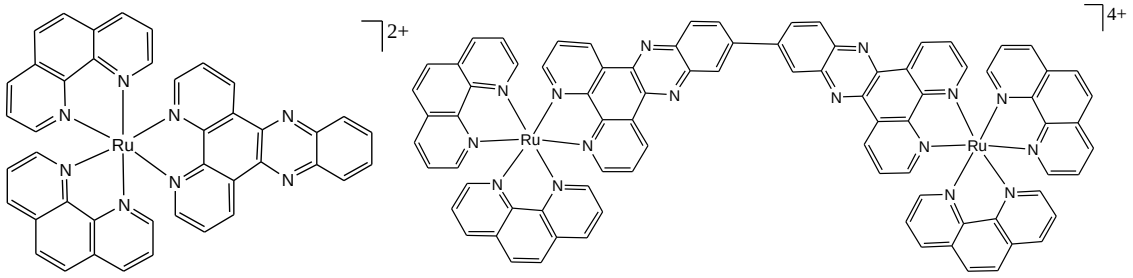
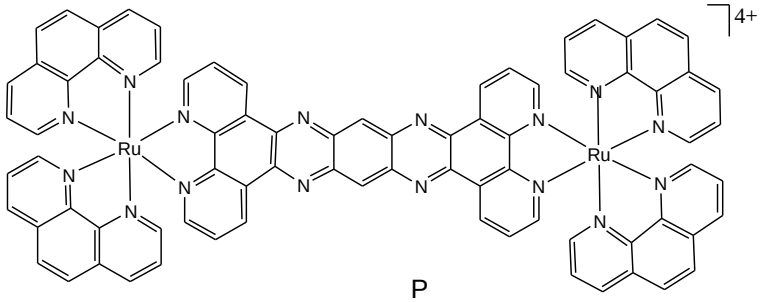


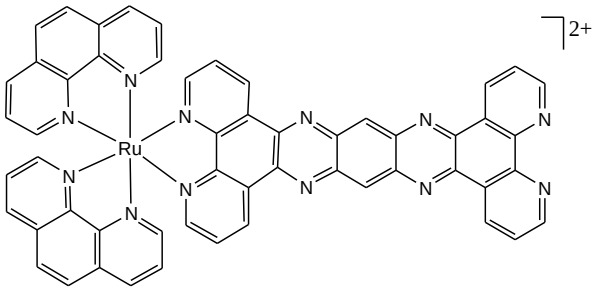
Fig. 1.8 Structure of $[(phen)_2Ru(dppz)]^{2+}$ and $[(phen)_2Ru(\mu\text{-}c4(cpdppz)_2Ru(phen)_2]^{4+}$.

1.1.4 Previous research work in the MacDonnell group

The MacDonnell group investigated the biological properties of the ruthenium(II) polypyridyl complexes, $[(phen)_2Ru(tatpp)Ru(phen)_2]Cl_4$,[43] [1], and $[(phen)_2Ru(tatpp)]Cl_2$, [2] which are shown in Fig. 1.9.[44] Ruthenium(II) compounds containing extended planar aromatic ligands have been extensively investigated as probes for DNA.[45,46,47] It has been shown that [1] and [2] both bind DNA via intercalation and can cleave DNA under reducing conditions. They have an unusual inverse dependence on the oxygen concentration, meaning that they cleave DNA better as the $[O_2]$ is lowered.[2,1] These two compounds have also been found to be modestly cytotoxic against a wide range of cancer cell lines (screened in the NCI-60 DTP Human Tumor Cell Line Screen). In particular, the mononuclear ruthenium complex [2] shows good cytotoxicity against colon and breast cancer tumor cell lines. This cytotoxicity coupled with the low oxygen potentiated DNA cleavage activity suggest these complexes may be promising antineoplastic agents against cells under hypoxic stress.



P



MP

Fig. 1.9 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 ruthenium polypyridyl complexes cleave DNA, was to use a simple cleavage assay using plasmid DNA (pUC 18-2686 bp). pUC 18 exists in three different topological confirmation: Supercoiled DNA (form I), circular DNA (form II) and linear DNA (form III) which can be separated by agarose gel electrophoresis and visualized under UV light after staining the gel with ethidium bromide. (Fig. 1.10)

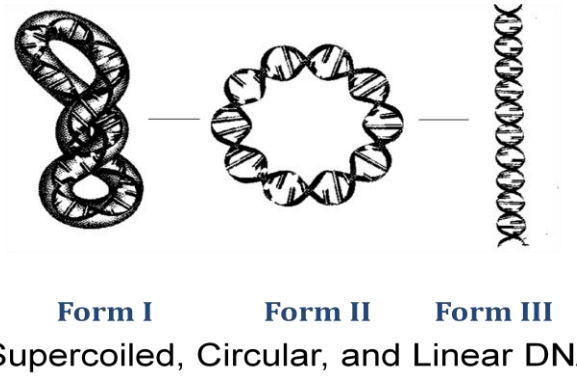


Fig. 1.10 Topoisomers of plasmid DNA: form I (supercoiled DNA), II (circular DNA), and III (linear DNA).

The DNA cleavage assay was conducted by Dr. Thamara Janaratne in the MacDonnell group. The results show that the $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ (P) cut DNA with glutathione (GSH) as a reducing agent, and is enhance under anaerobic conditions compared to normoxic conditions (Fig. 1.11). Fe-BLM was used as a positive control to show that the concentration of oxygen in the glove box was low, because while Fe-BLM can cause single strand nicks under anaerobic conditions, it required O_2 for double strand cleavage activity.[2]

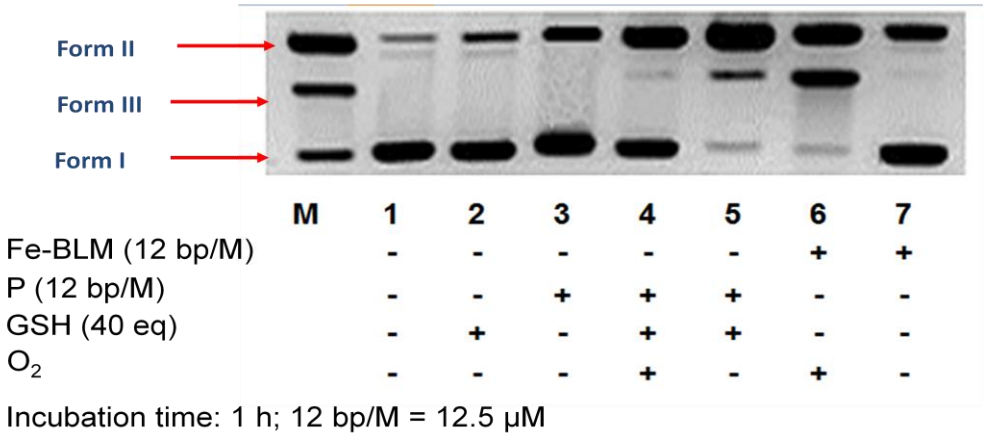


Fig. 1.11 DNA cleavage activities of complexes Fe-BLM and P as present of GSH under normoxic and anaerobic conditions in plasmid DNA assay.

1.1.4.2 Redox ability of Ruthenium polypyridyl complexes

Unlike the complexes with liable ligands, the ruthenium polypyridyl complexes are very stable and will not undergo ligand dissociation under the condition be investigated. These complexes have the ability to cleavage DNA in the presence of a reducing agent like glutathione. This shows that the redox ability of those complexes is the key point of bio-activities. Table 1.3 shows reduction potential of some different ruthenium polypyridyl complexes.[44]

Table 1.3 First reduction potentials of ruthenium(II) polypyridyl complexes

	Complexes	^{1}E/V
1	$[(phen)_2Ru(tatpp)Ru(phen)_2](PF_6)_4$	-0.023[a]
2	$[(phen)_2Ru(tatpp)](PF_6)_2$	-0.103 (0.5)[a]/-0.253 (0.5)[a]
3	$[(phen)_2Ru(p\text{-}CN\text{-}dppz)](PF_6)_2$	-0.180[b]
4	$[(phen)_2Ru(tadbp)](PF_6)_2$	-0.183[c]
5	$[(phen)_2Ru(o\text{-}CN\text{-}dppz)](PF_6)_2$	-0.260[b]
6	$[(phen)_2Ru(p\text{-}Br\text{-}dppz)](PF_6)_2$	-0.490[b]
7	$[(phen)_2Ru(o\text{-}Br\text{-}dppz)](PF_6)_2$	-0.530[b]
8	$[(phen)_2Ru(dppz)](PF_6)_2$	-0.720[b]
9	$[Ru(phen)_3](PF_6)_2$	-1.050[b]

a: Electrochemical Data (vs. SHE) for Complexes (as the PF_6^- Salts) in Acetonitrile at Complex Concentration of 20 μM.[44]
b: From cyclic-voltammetry at 50 mV/s in Dry DMF; Reduction potentials reported vs. NHE (Fc^+/Fc internal reference).[48]
c: The first reduction potentials determined by CV (obtained at a glassy carbon disk electrode (0.018 cm^2) in acetonitrile/0.1 M n-Bu_4NPF_6 at 293 K (vs. NHE)

Ru(II) complexes with polypyridyl ligands have been extensively synthesized and tested for their various properties such as photophysical, electrochemical activity and cytotoxicity. Reliable methods of synthesizing stable complexes with predictable structures; the ability to tune reduction potentials all make ruthenium(II) polypyridyl complexes so special.

1.1.4.3 Cytotoxicity of ruthenium complexes

The cytotoxicity study was conducted by Abhishek Yadav.[1] H-358 and H-226 human lung cancer cell were chosen to be studied based on the fact that complexes P and MP have shown good cytotoxicity against lung cancer tumor cell lines. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (cell sensitivity assay) was used to study the effect on cell viability after incubation for up to 96 hour in the presence of a series of complexes including chiral pure complexes. IC_{50} (half maximal inhibitory concentration) values are listed in Table 1.4.

Table 1.4 IC_{50} (μM) values of different ruthenium(II) polypyridyl complexes.

Complex	NSCLC-H-358	NSCLC- H-226
rac-$[Ru(phen)_3]^{2+}$	87 ± 4	93 ± 6
Δ-$[Ru(phen)_3]^{2+}$	65 ± 4	61 ± 5
Λ-$[Ru(phen)_3]^{2+}$	94 ± 6	116 ± 7
rac-$[(phen)_2Ru(tatpp)]^{2+}$	13 ± 2	12 ± 2
Δ-$[(phen)_2Ru(tatpp)]^{2+}$	9 ± 1	7 ± 1.
Λ-$[(phen)_2Ru(tatpp)]^{2+}$	14± 1	13 ± 2
mix-$[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$	15 ± 1	16 ± 1
ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$	9 ± 1	9 ± 1
ΛΛ-$[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$	17 ± 1	17 ± 1
ΔΛ-$[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$	15 ± 1	19 ± 1

Chosen cytotoxicity data screened in the NCI-60 DTP Human Tumor Cell Line Screen is show as below table 1.5. The complex $[(phen)_2Ru(tatpp)]^{2+}$ has also been found to be modestly cytotoxic (GI_{50} (GI_{50}: half growth inhibition concentration) values in the 0.1 to 10 μM range) against a wide range of cancer cell lines.

Table 1.5 Selected results from the NCI-60 DTP Human Tumor Cell Line Screen of ruthenium complex of Δ-MP.

Panel	Cell Line	GI_{50}[a] (μM)
Leukemia	CCRF-CEM	7.96
	K-562	6.12
Non Small Cell Lung Cancer	A549/ATCC	3.46
	HOP-62	0.43
	NCI-H23	1.18
Colon Cancer	COLO 205	2.41
	HCC-2998	0.40
	HCT-116	3.14
	HCT-15	2.90

Most Sensitive Cell Lines (GI_{50} < 10 μM)

With this background, there are several questions that need to be addressed: 1) Is the *in vitro* DNA-cleavage activity responsible for cell cytotoxicity, 2) Do hypoxic conditions in live cells leads to even more effective DNA cleavage by these compounds? 3) Is there a correlation between the ligand reduction potential and the cytotoxicity? Towards these ends, a research

program to answer these questions have begun and synthesis of series of analogue of ruthenium complexes is the first step.

1.2 Introduction of synthesis of ruthenium polypyridyl complexes

Since the orange-red trisbipyridine ruthenium(II) chloride was firstly prepared by heating a mixture of ruthenium(III) chloride with bipyridine at 250 ºC.[49] The studies are not limited by homo-ligand complexes. New heteroleptic (mixed-ligands) ruthenium(II) polypyridyl complexes have been synthesized, such as bis(2,2′-bipyridine)(dipyrido[3,2-a : 2',3'-c]phenazine-N^4N^5)ruthenium(II).[50] This synthesis method opened a door for more novel polypyridyl complexes. Schiff base reaction (condensations with ammonia and primary amines: formation of imines) is widely used in extending the aromatic polypyridyl conjugated plane. In the synthesis of polypyridyl complexes, aromatic amines are commonly used. In most cases nitro-group compounds are the precursor of those aromatic amines. In order to reduce the nitro-compounds to amines, high pressure hydrogenation reduction often be employed.

1.2.1 Schiff base reaction

Normally under acidic conditions, imines are usually formed when nucleophilic amines attack the electrophilic C of the carbonyl group in aldehydes or ketones. After undergoing condensation, the O of the carbonyl is replace with the N forming a C=N. Like amines, imines are basic; a substituted imine is also called a Schiff base. The mechanism of imine formation begins with a protonation of the oxygen atom on the carbonyl group. Then the reaction is followed by nucleophilic addition of the amine to carbon of the protonated carbonyl group. Deprotonation of the nitrogen atom gives an unstable intermediate called a carbinolamine. The carbinolamine reacts to form an imine by the loss of water and formation of a double bond: dehydration. This dehydration follows the same mechanism as the hydroxyl group converts it to a good leaving group, and it leaves as water. The resulting cation is stabilized by a resonance

structure with all octets filled and the positive charge on nitrogen. Loss of a proton gives the imine (Fig. 1.12).

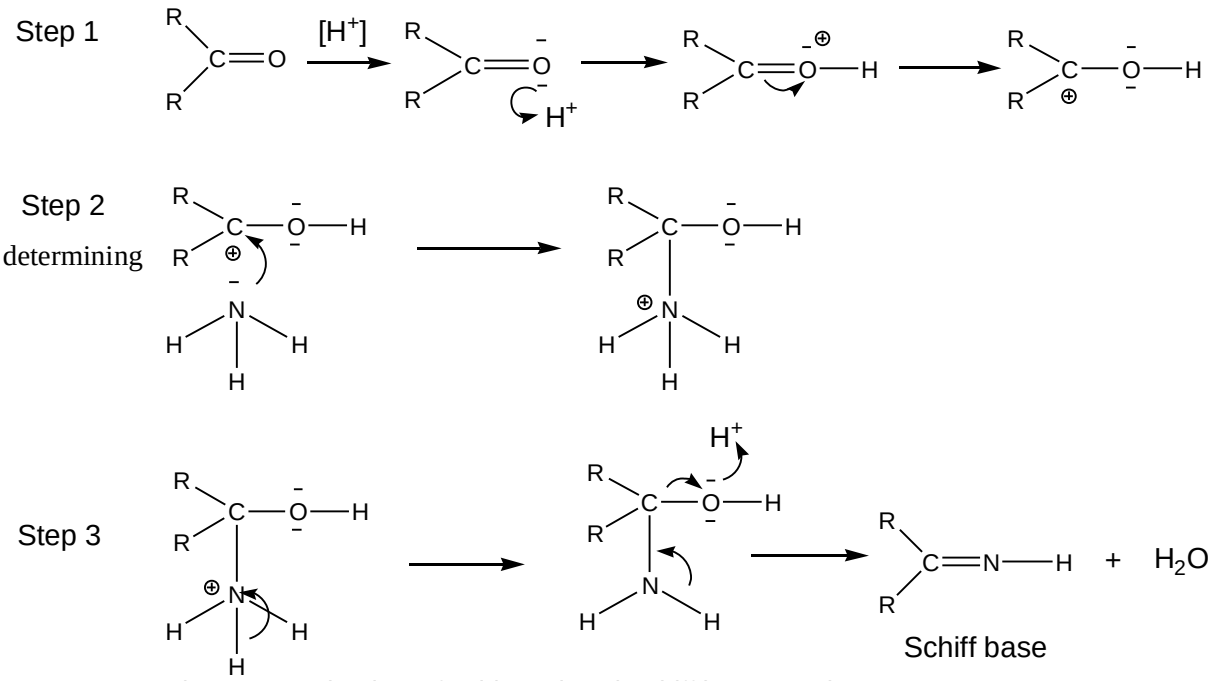


Fig. 1.12 Mechanism of acid-catalyzed Schiff base reaction.

The proper pH is crucial for the formation of imine (pKa = 25). The rate increases in the pH range 0-4.5, where decomposition of the cation intermediate is rate controlling.[51] If the solution is too acidic, however, the amine becomes protonated and less nucleophilic, inhibiting the attack of amine.

1.2.2 Reduction of nitro-group

Reduction of aromatic nitro compounds gives various nitrogen compounds, such as amines, imines, and oximes, where the N-O bonds are cleaved. This is one of the basic reactions of nitro compounds. The sequence of nitration and reduction is the most important method for preparation of aromatic amines. A large number of reducing agents have been used for the reduction of nitro groups.[52-54] The catalytic hydrogenation of aromatic nitro compounds to

amines has been recognized as one of the simplest procedures.[55] The procedures for the reduction reactions are described in the series of books. Most common reagents are: Fe + AcOH,[56] Zn + NaOH, Fe + HCl,[57] Sn + HCl,[33] H_2-Raney Ni,[58] H_2-PtO_2,[59] H_2-Pd/C,[60] and N_2H_4-Pd/C.[61] Sodium sulfide and polysulfides are also effective for this transformation.[62] The combination of sodium borohydride with cobalt(II), copper(II) and rhodium(III) halides has been used to reduce functional groups such as nitro, nitriles, amides, and olefins, which are inert to $NaBH_4$ itself.[63] Aromatic nitro compounds are reduced to amines with formic acid and triethylamine with Pd/C.[64]

Under reducing conditions, nitro aromatic compounds may react by a variety of pathways which are summarized in Fig. 1.13. In most cases, the major process is reduction of the nitro functional group to the corresponding amine group (Fig. 1.13, reactions I-III). Formally, this process consists of a series of two-electron additions, proceeding through nitroso and hydroxylamino intermediates. However, the reduction potentials for reactions I and II are very similar, so polarography performed on acid to neutral aqueous solutions gives only two waves: the first corresponding to a four-electro reduction for formation of the hydroxylamine, and the second corresponding to a two-electron reduction of the hydroxylamine to the amine.[65]

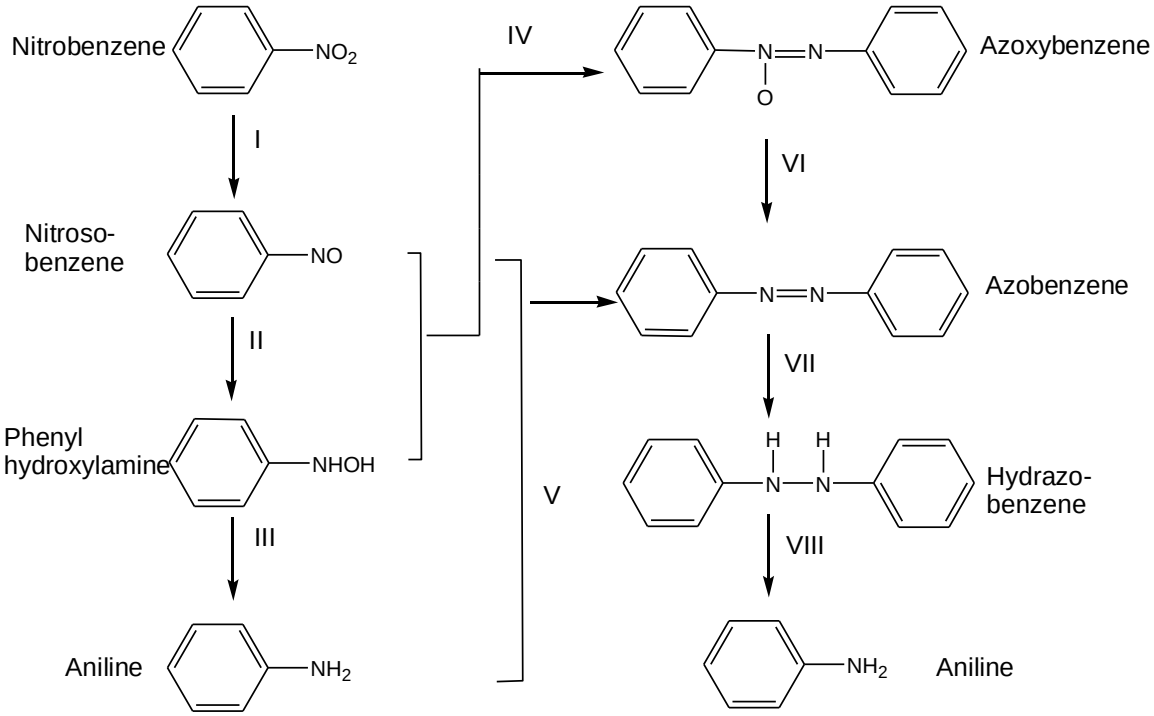


Fig. 1.13 Scheme of proposed pathways for reduction of nitro aromatic compound.

CHAPTER 2

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

2.1 Introduction

The synthesis and preparation of ruthenium polypyridyl complexes are increasingly attracting the interest of researchers due to their unique useful photophysical, electronic, pharmacological and photocatalytic properties[66-70] from the fact that they exhibit multiple stable oxidation states and attractive excited-state. Large amount of polypyridyl ligands are synthesized not only limited on 2,2′-bipyridine and 1,10-phenanthroline but also to some varies large conjugated planers to explore the unusual acceptor ligand characteristics, for example: dpq, dppz, dppn, tatpp (Fig. 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.[71] Ruthenium complexes of the dppz and tpphz ligand complexes are known to act as molecular light switches which luminesce upon intercalation into DNA.[66]

In the MacDonnell group, Mahn-jong Kim and Abhishek Yadav synthesized $[(phen)_2Ru(tatpp)Ru(phen)_2]Cl_4$,[43] and $[(phen)_2Ru(tatpp)]Cl_2$ respectively, and they did a lot of research work on those di/mono-nuclear ruthenium polypyridyl complexes.[2, 44, 72-78] Compare with dppz and dppn, the tatpp ligand has larger area and more conjugated planes. However, they have similar photochemical and electrochemical properties. The dinuclear ruthenium(II) complexes, $[(bpy)_2Ru(tatpp)Ru(bpy)_2]^{4+}$ and $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ are photochemically

reactive and in the presence of sacrificial donors both the dinuclear complexes undergo multiple "tatpp" ligand-based reductions upon visible light irradiation.[74] More recently, $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ has been shown to both intercalate with DNA and cause DNA cleavage under anaerobic, reducing conditions.[2]

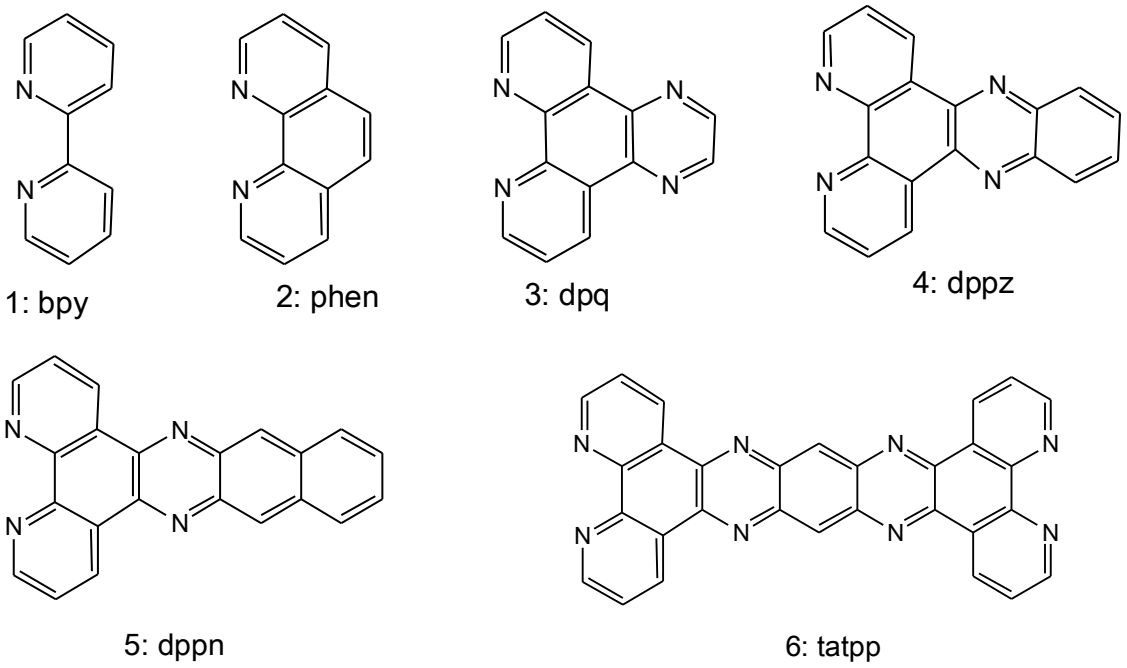


Fig. 2.1 Polypyridyl ligands and abbreviations.

In order to study the influence of reduction potentials of ruthenium(II) polypyridyl complexes with different ligands on cellular uptake efficiency and cytotoxicity, the novel complex $[(phen)_2Ru(tadbp)](PF_6)_2$ (tadbp = 9, 11, 20, 22-tetraazadipyrido[3, 2-a: 2', 3']dibenzo[3'', 2''-l: 2''', 3'''-n]pentacene) is designed and synthesized (Fig. 2.2). The difference between tatpp and tadbp ligands is that the tadbp only has one bidentate coordination site which can bind with ruthenium. That's why tadbp can only form mononuclear ruthenium complex.

The multiple steps synthesis involes ligand exchange, schiff base reaction (such as steps a and c) and nitro-group reduction (such as step b in Fig. 2.3). The synthesis process is

the scheme in Fig. 2.3. Since the two complexes $[(phen)_2Ru(tadbp)](PF_6)_2$ and $[(phen)_2Ru(tatpp)]$ $(PF_6)_2$ are so similar, basic principle of synthesis are similar. Although the synthesis methods of complexes $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ and $[(phen)_2Ru(tatpp)]^{2+}$ had been reported,[79-81] certain critical steps are hard to repeat. Some steps have very low yield. In this thesis, modified synthesis methods and new synthesis methods are reported. For example: Hydrogenation reaction step b in the scheme 2.3 is critical for this whole synthesis process. However the method using the high temperature and high pressure steel reactor reported in Kim's thesis is hard to repeat and has very low yield due to purification of product using column chromatography. The method reported here using H-cube hydrogenation reactor has multiple advantages: time efficiency, low requirement for activity of solvents and high yield (no further purification needed).

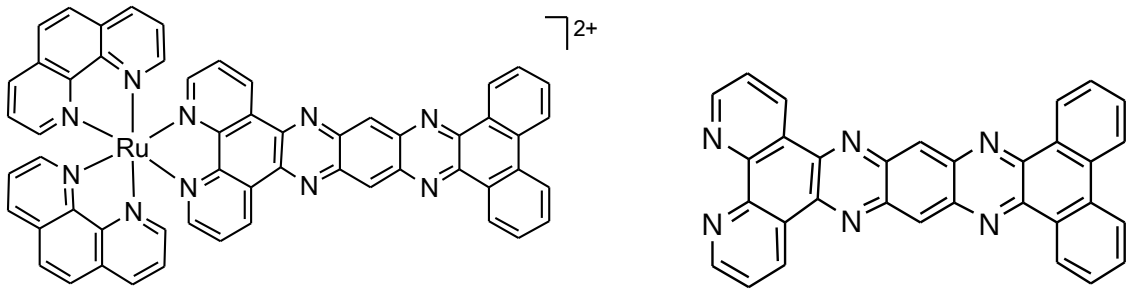


Fig. 2.2 The structure of complex $[(phen)_2Ru(tadbp)]^{2+}$ and tadbp ligand.

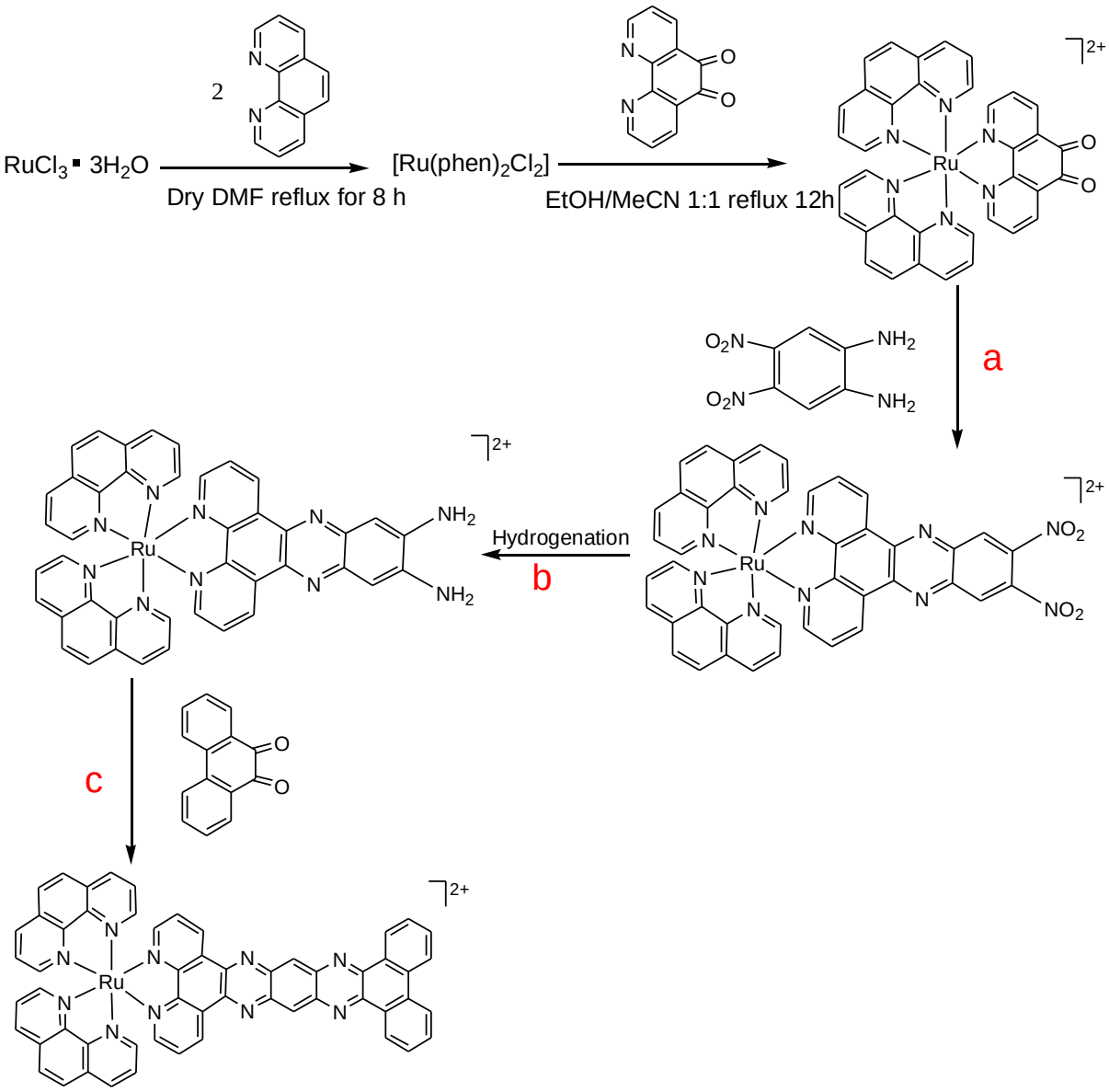


Fig. 2.3 Scheme for synthesis of $[(phen)_2Ru(tadbp)]^{2+}$. Step a and c are Schiff base reactions; b is nitro-group reduction reaction.

2.2 Results and Discussion

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

The $[(phen)_2Ru(dinitrodppz)](PF_6)_2$ complex is easily synthesized by a single step reaction of 1.2 equivalents of 1,2-diamino-4,5-dinitrobenzene and $[(phen)_2Ru(phendione)](PF_6)_2$ in the presence of acetic acid as outlined in scheme 2.3.b (Fig. 2.3). This is a typical Schiff base reaction (condensations with ammonia and primary amines: formation of imines). According to the method reported by Mahn-Jong Kim[79] (Fig. 2.4 a), $[(phen)_2Ru(phendione)]Cl_2$ is used which means before reaction the salt exchange step (from PF_6 salt to Cl salt) is needed. The method mentioned here is modified to avoid the salt exchange step (Fig. 2.4 b). The product is characterized by ^1H NMR spectroscopy in CD_3CN which is identical to that previously reported in the literature.[79]

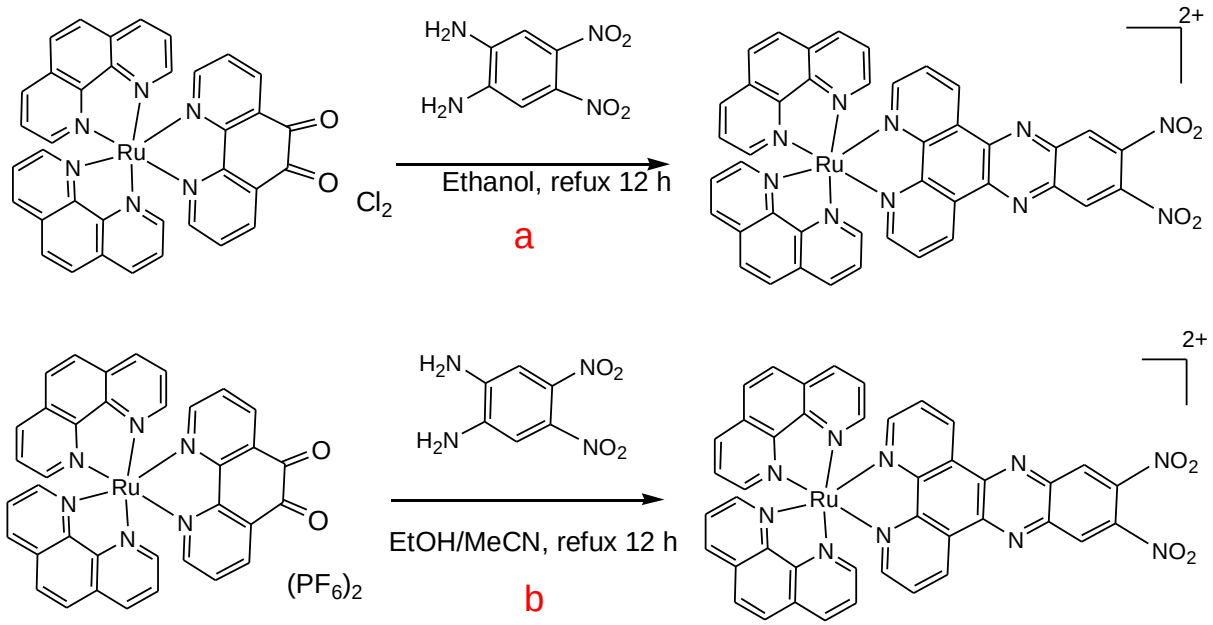


Fig. 2.4 Two schemes for synthesis of $[(phen)_2Ru(dinitrodppz)]^{2+}$. a) is the method reported by Mahn-Jong Kim[79]; b) is the method described in this thesis.

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

According to Kim's method,[79] the column chromatography (alumina treated with a solution of 10% TEA in n-hexane) was employed to purify the product using a solution of NH_4PF_6 in CH_3CN (10 mg/mL) as an eluent (Fig 2.5).

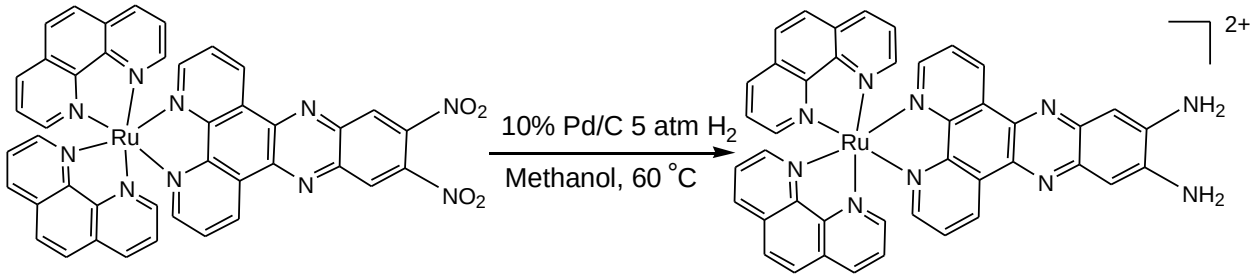


Fig. 2.5 Scheme for synthesis of $[(phen)_2Ru(diaminodppz)]^{2+}$ in High Pressure Reactor.

In order to avoid the chromatography, different reducing agents and reduction methods have been studied for the conversion of nitro groups to amino groups. Pure $[(phen)_2Ru(diaminodppz)](PF_6)_2$ can be obtained when one of the two methods are used. They are H-Cube hydrogenation using an H-Cube Midi™ and 2-step hydrogenation in a parr shaker hydrogenator.

Method 1: H-Cube hydrogenation

H-Cube is short term for H-Cube Continuous-flow Hydrogenation Reactor. It uniquely combines continuous-flow with endogenous hydrogen generation and a disposable catalyst cartridge system. It allows fast and cost-efficient hydrogenation with superior yield when compared to conventional methods. This is a very convenient method: 1) Acetonitrile can be used as a solvent. More options of solvent can be used; moreover further salt exchange can be avoided. Generally speaking methanol and ethanol are two most common and active solvents

for hydrogenation reaction, in order to dissolve in those two solvents the Cl salt complexes are needed. 2) The method is fast and the pure product can be formed directly. The synthesis scheme shows as Fig 2.6. The product is characterized by ^{1}H NMR spectroscopy in CD_3CN which is identical to that previously reported in the literature.[79]

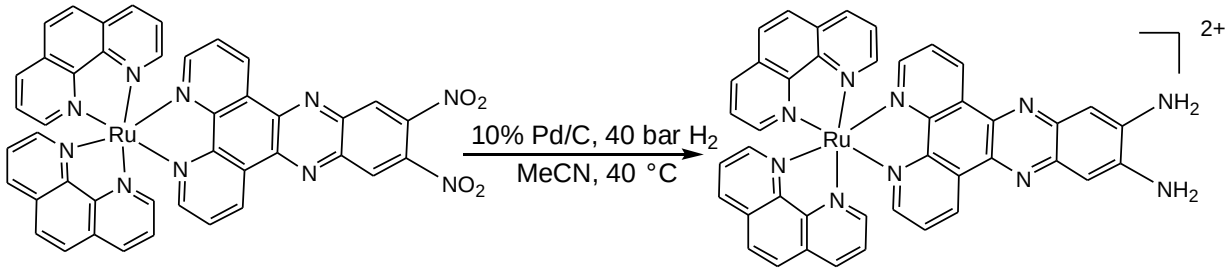


Fig. 2.6 Scheme of synthesis $[(phen)_2Ru(diaminodppz)]^{2+}$ by H-Cube Midi™.

In addition, the method has some requirements for the concentration of react solution and after reaction cleaning since using catalysis column. The reaction solution must be very dilute and filtered before go through the HPLC system. After hydrogenation, a long time period of cleaning of catalysis column is needed.

Method 2: 2-cycle hydrogenation in a parr shaker hydrogenator

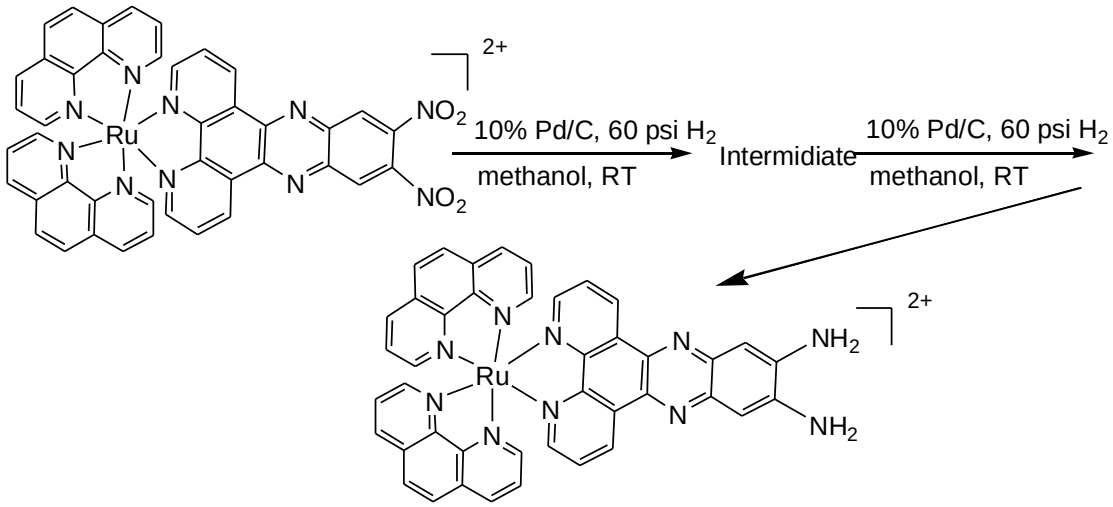


Fig. 2.7 Scheme for synthesis of $[(phen)_2Ru(diaminodppz)]^{2+}$ in a parr shaker hydrogenator (2-cycle).

Parr Shaker type hydrogenator is used; however the ^1H NMR (Fig. 2.8) of the product for first hydrogenation indicates that it is a reaction intermediate. Based on the mechanism of reduction of nitro aromatic compound mentioned before, in order to get the pure $[(phen)_2Ru(diaminodppz)](PF_6)_2$ further reduction is needed (Fig. 2.7).

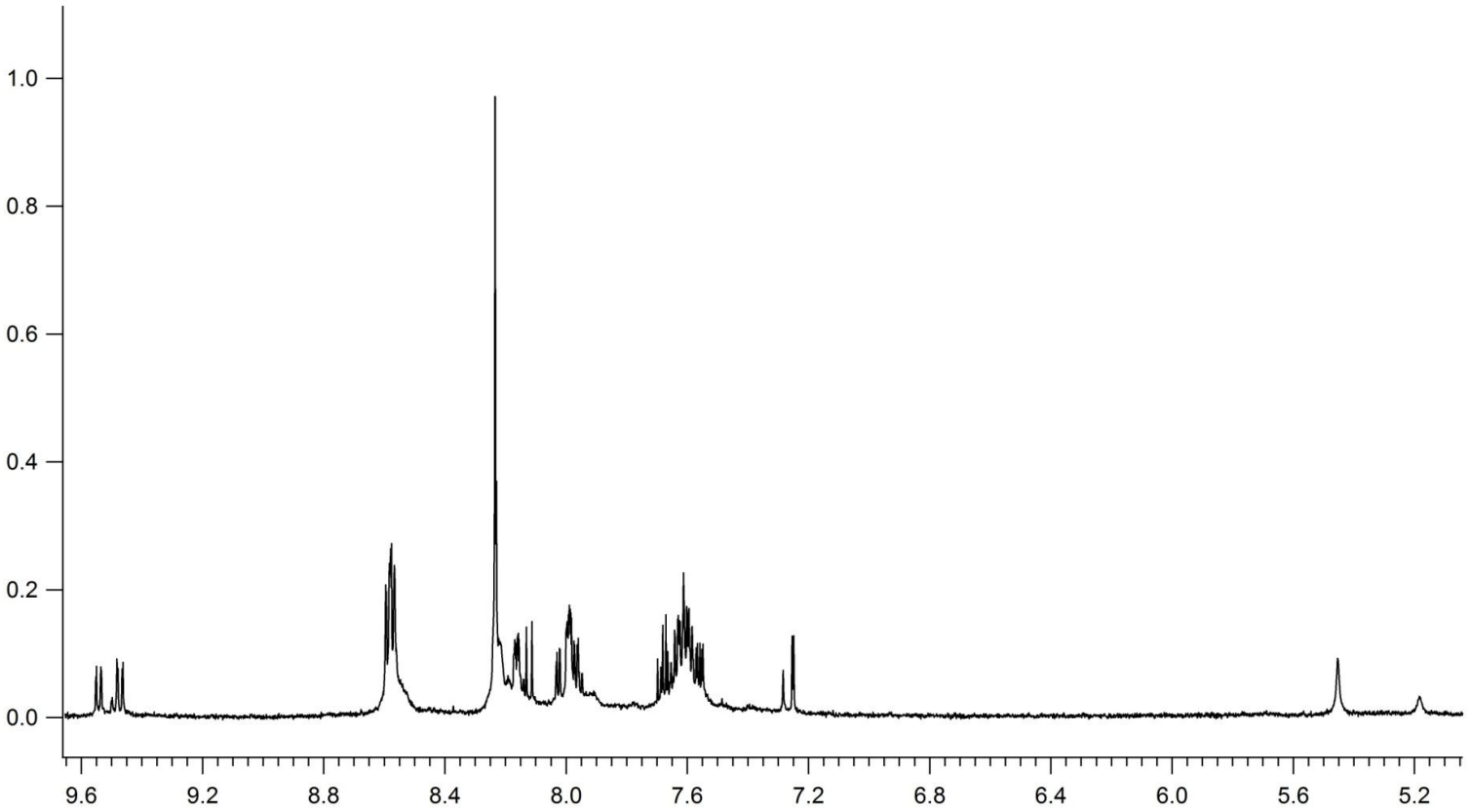


Fig. 2.8 ^{1}H NMR spectra (500 MHz, Solvent: MeCN-D_3) of intermediate of reduction of $[(phen)_2Ru(dinitrodppz)]^{2+}$.

Different methods and conditions are tried to make $[(phen)_2Ru(diaminodppz)](PF_6)_2$. In most cases, only reduction intermediates are formed. The ^1H NMRs of other intermediate is shown in the Appendix-1.

Method 1: Hydrogenation by high pressure reactor.

$[(phen)_2Ru(dinitrodppz)](PF_6)_2/Cl_2$ (100 mg) is dissolved into 100 mL solvent and 100 mg 10% Pd/C is added in to the solution in a reactor. The conditions are listed in table 2.1. (Cycle: repeat of the same procedure redoing the reduction reaction.) The reaction is kept under a certain pressure. The resulting solution is filtered through celite, followed by washing with a large amount of solvent and the filtrate is rotovapped to 10 mL. An aqueous solution of ammonium hexafluorophosphate (50 mg, 0.31 mmol, dissolved in 10 mL of DI water) is added in to the resulting solution to form a precipitate which is isolated by filtration and washed with 5 mL DI water. The compound is dried in a vacuum oven overnight and characterized by ^1H NMR (the results are shown as table 2.1).

Table 2.1 The conditions and results of hydrogenation of $[(phen)_2Ru(dinitrodppz)]^{2+}$ (100 mg) by 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

Table 2.1- continued

MeOH+AcOH (100 mL+5 mL)	RT	60	24	1	Incomplete
MeOH	RT	60	24	2	Complete

Method 2: Hydrazine- (Pd/C, PtO_2 and Pt /Alumina) co-catalysts reduction

$[(phen)_2Ru(dinitrodppz)]Cl_2$ (50 mg 0.06 mmol) is dissolved into 30 mL solvent and then 50 mg catalyst is added into the 2 neck 100 mL flask. After degassing by N_2 for 15 min the solution is refluxed for 3 hour under N_2; followed by addition of hydrazine dropwise. The solution is refluxed for 2 more hours. The resulting solution is filtered through celite, followed by washing with a large amount of solvent and rotovapped to 5 mL. An aqueous solution of ammonium hexafluorophosphate (40 mg 0.24 mmol, dissolved in 5 mL of DI water) is added to form a precipitate, which is isolated by filtration and washed with 5mL DI water. The compound is dried in a vacuum oven overnight and characterized by ^1H NMR (the results are shown as table 2.2). The conditions of different reactions are listed as table 2.2. (Cycle: repeat same procedure.)

Table 2.2 The conditions and results 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

Table 2.2- continued

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

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

In order to explore the correlation of the reduction potential and cytotoxicity of complexes, the novel analogue complex $[(phen)_2Ru(tadbp)](PF_6)_2$ (Fig. 2.2) is synthesized by changing the coordinated ligands as Fig. 2.9 scheme. This reaction is a Schiff base reaction; however, one of the reactant is a large complex, the reaction is not easy as reaction (a) in Fig. 2.3. Many conditions for this Schiff base reaction are tested. The best condition is described in experiment part. Following table 2.3 compares different conditions and the results.

Table 2.3 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 (1:1) + AcOH (5 mL)	12	Further purification needed
EtOH + AcOH (5 mL)	12	Further purification needed

Table 2.3- continued

AcOH	12	Further purification needed
EtOH: MeCN: AcOH(2:1:1)	12	Complete
EtOH: MeCN: AcOH(2:1:1)	24	Further purification needed

According to the results, protic solvent is crucial for this reaction. Although water is one of the protic solvents, but itself is one of the products, which shift the equilibrium in an unfavorable direction according to Le Chantlier's principle. This indicates that it is preferable to use ethanol instead of water as part of the solvent system. The better solvent conditions is modified from the method reported by Kim and Yadav.[80,79] Longer reaction time does not have a desirable effect on the reaction conditions.

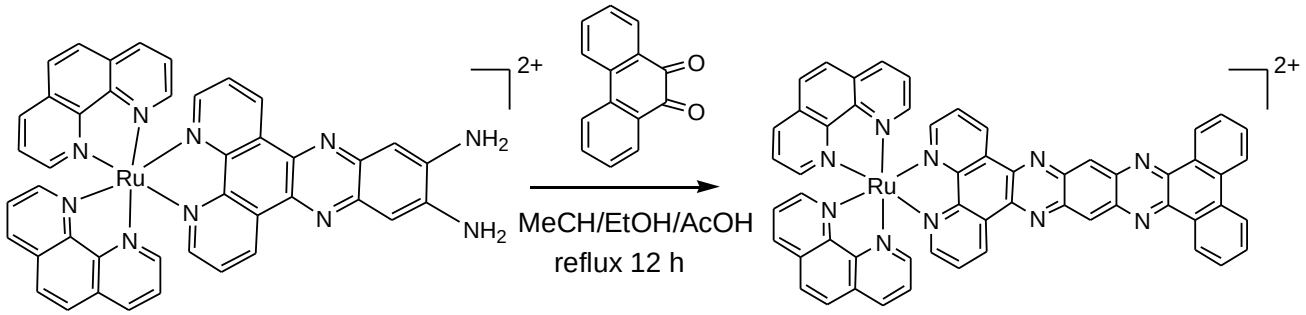


Fig. 2.9 Scheme for synthesis of novel complex $[(phen)_2Ru(tadbp)](PF_6)_2$ (B).

The new complex is characterized by ^1H NMR spectroscopy (500 MHz, Solvent: MeCN-D_3) which is shown as Fig. 2.10 and EI-MS (A-2)

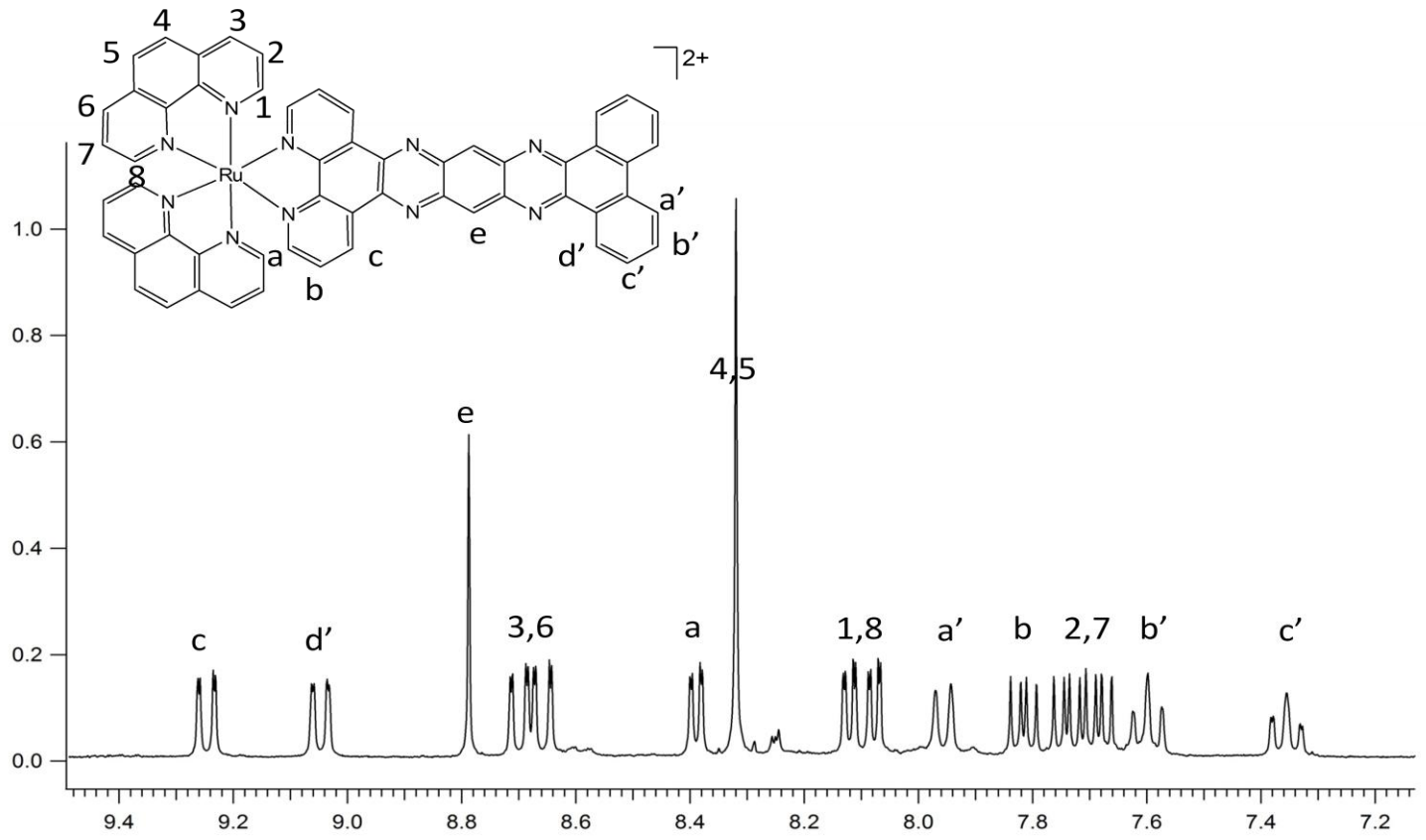
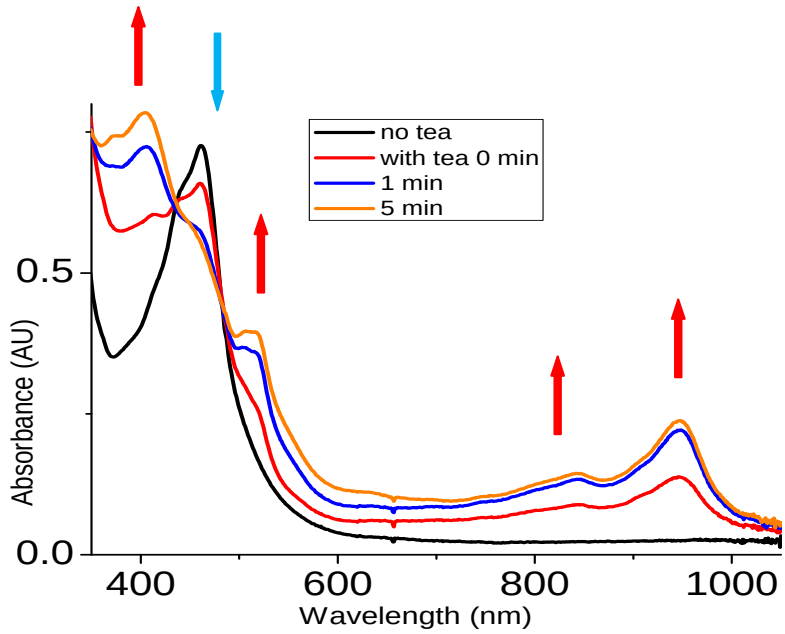


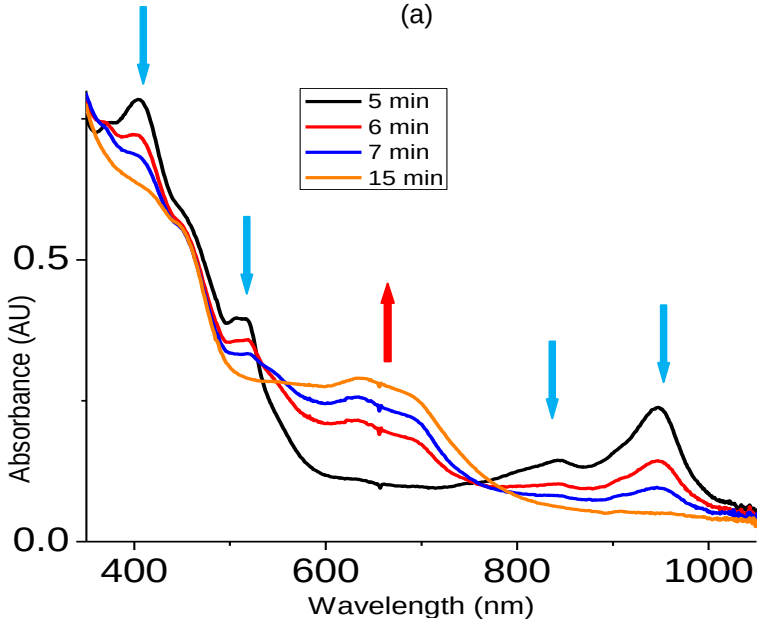
Fig. 2.10 ^{1}H NMR spectra (500 MHz, Solvent: MeCN-D_3) of $[(phen)_2Ru(tadbp)](PF_6)_2$.

UV-vis properties of $[(phen)_2Ru(tadbp)](PF_6)_2$

For the complex $[(phen)_2Ru(tadbp)](PF_6)_2$, the UV-Vis spectra (Fig. 2.11) is very similar to the $[(phen)_2Ru(tatpp)](PF_6)_2$.[74] The spectra of parent complex has an intense band located at 330 nm and a broad in the region of 435~ 450 nm. The spectra of the species $([(phen)_2Ru(tadbp)]^{\cdot+}$ formed by add triethylamine (TEA) into the solution of complex shows two new absorption bands in the near-IR at 850 nm (weak) and 955 nm (strong), and a discernible band at 400 nm; as well as a partial bleaching of the absorption at 330 nm. At 430 nm radiation, the corresponding change of spectra is due to the formation of $H[(phen)_2Ru(tadbp)]^+$ and $H_2[(phen)_2Ru(tadbp)]^{2+}$.



(a)



(b)

Fig. 2.11 UV-Vis spectra of $[(phen)_2Ru(tadbp)]^{2+}$ in MeCN (17 μM).(a) Absorption spectra of B (17 μM without TEA, black line) after addition of 0.05 M TEA (red line) and then; radiated by 430 nm LED light for 1 min (blue line) and 5 min (orange line). (b) Absorption spectra of B after 430 nm radiation for 5 min (black line), 6 min (red line), 7 min (blue line), and 15 min (orange line).

2.3 Synthesis of ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$, Δ-$[(phen)_2Ru(tatpp)]^{2+}$ and Δ-$[(phen)_2Ru(tadbp)]^{2+}$

2.3.1 Stereoisomers of ruthenium polypyridyl complexes

Enantiomers of Tris-bidentate Mononuclear Ru complexes

The D_3 symmetric tris-bidentate octahedral complex such as $[Ru(phen)_3]^{2+}$ (Fig. 2.12), has a helical structure in which the three bidentate ligands lie along the threads of a screw. It has no symmetry plane because its two halves are not mirror images, but it does have a threefold symmetry axis, which passes through the Ru(II) ion and is nearly perpendicular to the plane of the figure. The complexes can exist in two nonidentical mirror-image forms: a “right-handed enantiomer (designated as Δ) in which the three ligands screw would advance into the page as you rotate it to the right (clockwise) about the threefold axis, and a “left handed” enantiomer (designated as Λ) in which the ligands screw would advance into the page as you rotate it to the left (counterclockwise) about the threefold axis.

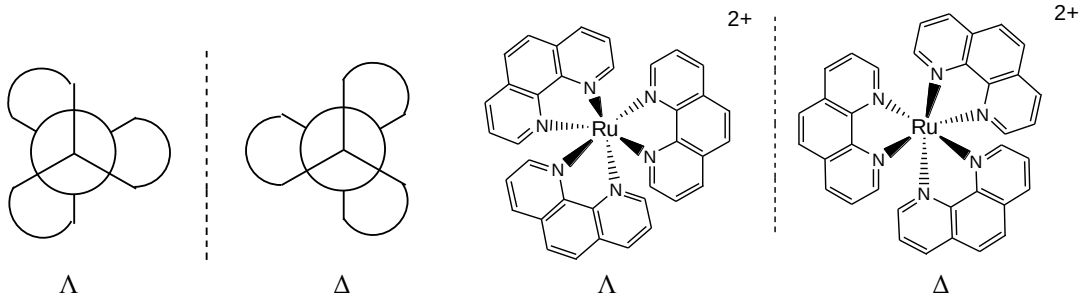


Fig. 2.12 Definition and structures of Λ and Δ-$[Ru(phen)_3]^{2+}$.

The enantiomers have identical properties except for their reactions with other chiral substances and their effect on plane-polarized light. They are also labeled as (+) or (-),

depending on the direction on the direction of rotation of the plane of polarization. A mixture of the (+) and (-) isomers is called a racemic mixture.

Stereoisomers of dinuclear ruthenium complexes

Ligand-bridged dinuclear ruthenium complexes represent the simplest examples of the multinuclear assemblies (Fig. 2.13). The individual metal centers are tris(bidentate) in nature, each may inherently possess right- or left-handed chirality (Δ and Λ respectively). In principle, a dinuclear species may therefore exist in two diastereoisomeric forms: ΔΔ/ΛΛ (a pair of enantiomers) or ΔΛ/ΛΔ (meso) where the bridge is relatively rigid. The stereoisomerism has a profound effect on shape and on the electronic interactions within the molecule.

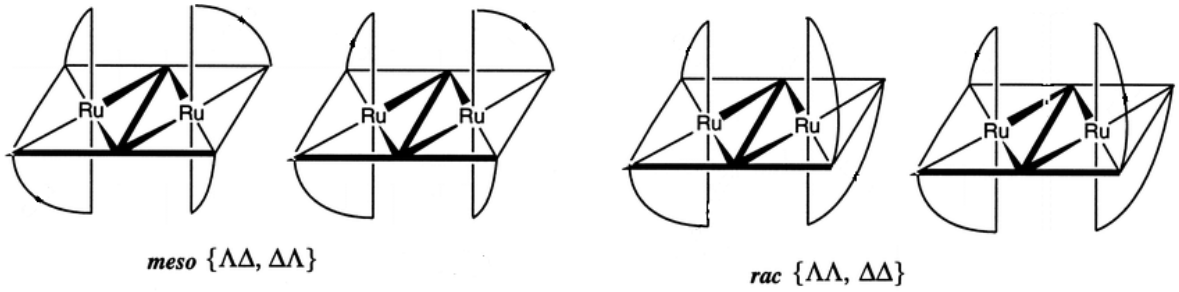


Fig. 2.13 Stereoisomeric forms of $[(Ru(pp)_2)_2(\mu\text{-}BL)]^{n+}$.
Where pp is a symmetrical bidentate ligand (C_{2v} point group symmetry) such as bpy, and BL is a symmetrical (C_{2v}) bridging ligand such as 2,2′-bipyrimidine (bpm).

Stereospecific synthesis of ruthenium complexes

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.[82-85] The chiral precursors were used to synthesize the more complicated chiral ruthenium complexes. A number of studies have been reported using condensation reactions of chiral monomers containing the 1,10-phenanthroline-5,6-diione ligand, e.g. $[(phen)_2Ru(phendione)]^{2+}$[86], as the precursor to form bridged complexes

of predetermined stereochemistry.[87, 88] In this thesis, the method is applied for the synthesis of homochiral mono/dinuclear complexes using Δ-$[(phen)_2Ru(phendione)]^{2+}$ as the enantiomerically pure chiral building block.

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

The method by Mahn-Jong Kim[79] using sodium arsenyl -L-(+)-tartrate to separate the Λ form complex first and then using sodium arsenyl -D-(-)-tartrate to get Δ form from the filtrate. However both Λ and Δ forms are not pure enough to continue to the next step. The method used in this thesis is modified. The separated complex was determined the enantiomeric excess (ee%) value by separation on the HPLC-LARIHC-RN™ column with a mobile phase of acetonitrile/methanol/triethylamine/acetic acid 10/90/0.2/0.3 (by volume). The result showed that the ee% value is between 65%~87% via fist run chiral resolution and the ee% value was improved via repeating the chiral resolution step above one more times (ee% = 99%, Fig. 2.14).

Fig. 2.14 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).

Multiple and large scale salt exchange reactions evolved in this procedure. It is easy to have some residual salts formed, such as tetrabutylammonium chloride (which has similar solubility with the complexes). Washing with a lot of solvent is critical to avoid this situation during this procedure. If only has small amount of tetrabutylammonium chloride, ether precipitation can be used to remove the tetrabutylammonium chloride.

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

The procedure to make Δ-[(phen)$_2$Ru(diaminodppz)](PF$_6$)$_2$ is same as racemic form (describe previous). However Δ-[(phen)$_2$Ru(dinitrodppz)](PF$_6$)$_2$ seems resistant from reduction. The 2 cycle hydrogenation in a parr shaker hydrogenator which is a successful way to make

racemic $[(phen)_2Ru(diaminodppz)](PF_6)_2$ does not work for the reduction of Δ-$[(phen)_2Ru(dinitrodppz)](PF_6)_2$. Here table 2.4 lists the conditions tested using parr shaker hydrogenator. The general procedure is: $[(phen)_2Ru(dinitrodppz]]Cl_2$ is dissolved in the solvent. Catalyst is added to the solution and keeping reaction at 60 psi for 24 hour. The resulting solution is filtered through celite, followed by washing with plenty of ethanol. The resulting filtrate is rotovapped and then precipitated out by adding an aqueous solution of ammonium hexafluorophosphate which is isolated by filtration and washed with DI water. The compound is dried in a vacuum oven overnight.

Table 2.4 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 ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$, Δ-$[(phen)_2Ru(tatpp)]^{2+}$ and Δ-$[(phen)_2Ru(tadbp)]^{2+}$

Δ-$[(phen)_2Ru(tatpp)]^{2+}$ and Δ-$[(phen)_2Ru(tadbp)]^{2+}$ these two complexes can be made from Δ-$[(phen)_2Ru(diaminodppz)](PF_6)_2$ (Fig. 2.15) and the procedures are same as racemic $[(phen)_2Ru(tatpp)]^{2+}$ and $[(phen)_2Ru(tadbp)]^{2+}$ described in experiment part. The complexes are characterized by EI-MS and ^1H NMR spectroscopy in CD_3-CN.

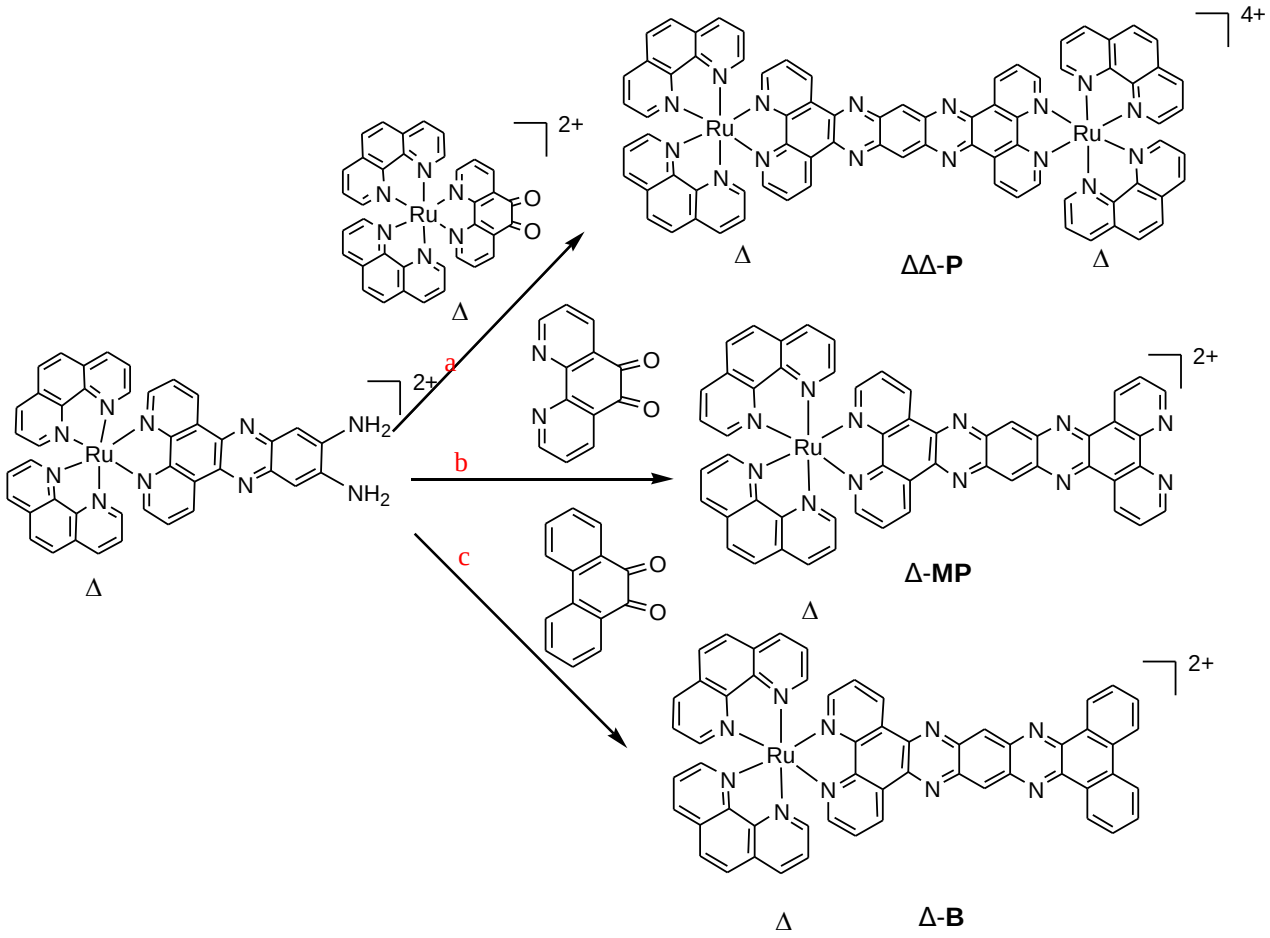


Fig. 2.15 Scheme of synthesis of ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$ (ΔΔ-P), Δ-$[(phen)_2Ru(tatpp)]^{2+}$ (Δ-MP) and Δ-$[(phen)_2Ru(tadbp)]^{2+}$ (Δ-B).

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.

There are another pathway to make Δ-$[(phen)_2Ru(tatpp)](PF_6)_2$[80] which is a single step by reaction Δ-$[(phen)_2Ru(phdione)](PF_6)_2$ and 1.2 equivalents of 11,12-diamino-dipyrido[3,2-*a*:2',3'-*c*]phenazine (shown as Fig. 2.16).

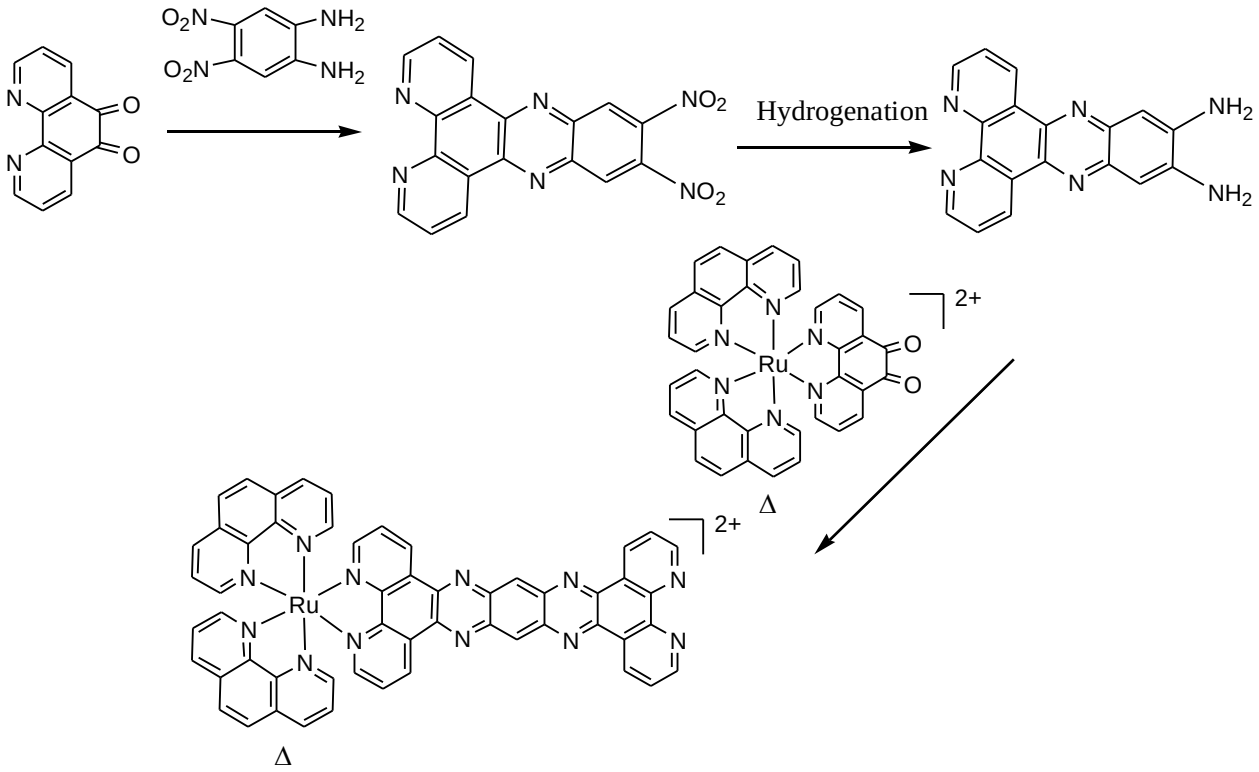


Fig. 2.16 Scheme of alter pathway of synthesis of Δ-$[(phen)_2Ru(tatpp)](PF_6)_2$.

2.3.5 Synthesis of ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2](PF_6)_4$

ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2](PF_6)_4$ is made from a single step by reaction of one equivalent of Δ-$[(phen)_2Ru(diaminodppz)](PF_6)_2$ and Δ-$[(phen)_2Ru(phendione)](PF_6)_2$. Although

this should be a simple Schiff base condensation reaction. The reaction often gives impure product or incomplete reactions due to a competing side reaction that has been described by Kim.[79] In order to get rid of the impurities (the unreacted starting materials) silica pipette column purification with silica is employed to obtain pure sample. The pure complex is characterized by ^{1}H NMR spectroscopy in CD_3-CN.

2.4 Summary and Conclusion

The study shows that the heteroleptic (mixed-ligands) ruthenium polypyridyl complexes can be synthesized by ligand displacement reactions. The most important is that the Schiff base reaction which can extend fully aromatic bridging plan can help make new functional structures. The complexes may also be prepared stereospecifically using some enantiomerically pure building blocks, such as Δ--$[(phen)_2Ru(phendione)](PF_6)_2$. A novel polypyridyl complex $[(phen)_2Ru(tadbp)](PF_6)_2$ and its enantiomerically pure form Δ-$[(phen)_2Ru(tadbp)](PF_6)_2$ are synthesized and the synthesis procedures are described in this thesis. However the synthesis procedures are similar to those that Kim[79] and Yadav[80] reported in their thesis. Due to poor repeatability and high yield requirement; modifications of some reaction procedures are needed.

2.5 Experimental

2.5.1 Reagents and Materials

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

1,10-phenanthroline-5,6-dione (phendione) is prepared by the bromine-catalyzed oxidation of 1,10-phenanthroline with a mixture of concentrated sulfuric and nitric acid.[87] 4,5-Dinitro-benzene-1,2-diamine and dichloro-bis(1,10-phenanthroline-N^1,N^{10}) are synthesized according to reported procedures.[89-91] $[(phen)_2Ru(phendione)](PF_6)_2$,[92]

$[(phen)_2Ru(dinitrodppz)](PF_6)_2$,[79] 11,12-dinitro-dipyrido[3,2-*a*:2',3'-*c*]phenazine,[93] sodium arsenyl tartrate,[94] Δ-$[(phen)_2Ru(phendione)](PF_6)_2$[79] and 4,5-dinitro-1,2-penylenediamine[93] are prepared by literature procedures.

2.5.2 Instrumentation

^1H NMR (500 MHz and 300 MHz) and ^{13}C NMR (500 MHz) spectra are obtained on the JEOL Eclipse Plus 500 or 300 MHz NMR spectrometer using CD_3CN and d^6-DMSO as the solvent. Chemical shifts (δ values) are given in parts per million and referenced to TMS. UV-Vis spectra are obtained using an Agilent 8453 UV-Visible spectrophotometer. ESI-MS spectra are obtained on a Thermo LCQ Deca XP quadrupole ion trap instrument equipped with a conventional ESI source (Thermo-Fisher Scientific, West Palm Beach, FL) in all ESI-MS experiments. Cyclic- and differential pulse voltammetric experiments are performed on a CH Instruments model CHI 620A electrochemical analyzer. Tetrabutylammonium hexafluorophosphate is used as the supporting electrolyte. The working and the auxiliary (counter) electrodes employed are glassy carbon and platinum; the reference electrode chosen is a saturated silver chloride electrode (Ag/AgCl). Ferrocene is chosen as the standard. The determination of enantiomeric purity is done by HPLC system using chiral stationary phases as described in the literature.[51, 63]

Hydrogenation reaction is carried by employing as following equipments: ThalesNano H-Cube Midi™ Scale-up Hydrogenation Reactor, Parr instrument company model 3900 Shaker Hydrogenation Apparatus and model 4848 High Pressure Reactor.

2.5.3 Synthesis

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

Since the synthesis method mentioned by Kim[79], used ethanol as the solvent making it necessary to prepare the chloride salt of $[(phen)_2Ru(phendione)]^{2+}$[79] first (the PF_6 salt is formed

by adding NH_4PF_6 to precipitate out the product). In order to avoid a step of salt exchange, in this thesis modification of solvent system is made. Instead of mono-solvent ethanol, the mix solvent system (ethanol: acetonitrile = 1: 1) is applied.

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

The method using 10% Pd/C in a methanol system and heating to 60 ºC under 5 atm of $H_2(g)$ for 18 h in a parr 4848 high pressure reactor mentioned by Kim[79]. However the reduction reaction is not completed, further column chromatography purification (alumina treated with a solution of 10% TEA in n-hexane with an eluent NH_4PF_6 in CH_3CN) is needed. New methods and new equipments are expored in this thesis to increase the yield.

Method 1: H-Cube hydrogenation

$[(phen)_2Ru(dinitrodppz)](PF_6)_2$ (100 mg 0.09 mmol) is dissolved in 600 mL acetonitrile and filtered through a nylon filter with pores of 0.2 µm. The H-Cube is fitted with a THS 01131 CatCart (10% Pd/C, 70 mm long) and operated using the following: pressure 40 bar, temperature 40 ºC, flow rate 2 mL/min. The effluent from the H Cube is collected and rotovapped to an approximate volume of 10 mL. Addition of diethyl ether resulted in the formation of a precipitate which is isolated by filtration. The compound is dried in a vacuum oven at 40 ºC overnight. The ^1H NMR spectra of this complex is identical to that previously reported in the literature.[79]

Method 2: Hydrogenation in a 3900 Shaker Hydrogenation Apparatus

$[(phen)_2Ru(dinitrodppz)]Cl_2$ (100 mg 0.11 mmol) is dissolved in 60 mL methanol and placed in a glass pressure reaction vessel. After degassing the solution by bubbling N_2 though it for 5 min, 100 mg 10% Pd/C is added and the vessel fitted into the shaker-hydrogenator. After charging the vessel with hydrogen at 60 psi the mixture is allowed to shake and react for 24

hour at room temperature. After relieving the pressure, the resulting solution is filtered through a pad of Celite to remove the Pd/C catalyst. The Celite pad is washed with another 60 mL of methanol and the combined methanol fractions are rotovapped to approximately 10 mL total volume. Addition of diethyl ether resulted in the formation of a precipitate which is isolated by filtration. The compound is dried in a vacuum oven at 40 ºC overnight. The ^{1}H NMR spectrum of this product, shown in Fig. 2.7, reveals that the reduction reaction is incomplete at this stage.

The product from the initial hydrogenation (80 mg) is dissolved into 50 mL methanol, and hydrogenated as before in the presence of 80 mg 10% Pd/C catalyst. After relieving the pressure, the resulting solution is filtered through a pad of Celite to remove the Pd/C catalyst. The Celite pad is washed with another 60 mL of methanol and the combined methanol fractions are rotovapped to approximately 10 mL total volume. In this case, the product is isolate by addition of an aqueous solution of ammonium hexafluorophosphate (50 mg 0.30 mmol) which resulted in formation of a precipitate which is isolated by filtration and washed with 5 mL DI water. The compound is dried in a vacuum oven at 40 ºC overnight. The ^{1}H NMR spectra of this complex is identical to that previously reported in the literature.[79]

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

The synthesis mothod mentioned by Yadav[80] is react 11, 12- diaminodipyridophenazine with $[(phen)_2Ru(phendione)](PF_6)_2$ (Fig. 2.17). In this thesis, an alternated pathway is reported shown as reaction b in Fig. 2.16.

$[(phen)_2Ru(diaminodppz)](PF_6)_2$ (100 mg 0.09 mmol) and 1,10-phenanthroline -5,6-dione (phendione) (24 mg 0.11 mmol) are dissolved in a mixed solvent (50 mL ethanol, 25 mL acetonitrile and 25 mL acetic acid) in a 250 mL round bottom flask and refluxed for 12 hour under N_2(g). The resulting reaction solution is cooled to room temperature. And then the solution is rotovapped to approximately 30 mL total volume, 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 is isolated by filtration and washed with 5 mL DI water and 20 mL ethanol. The compound is dried in a vacuum oven at 60 ºC overnight. Because the large conjugated aromatic planer in the structure of $[(phen)_2Ru(tatpp)](PF_6)_2$, the molecules easily form a π-π stacking structure which broadens NMR peaks. In order to avoid the π-π stacking, zinc tetrafluoroborate hydrate ($Zn(BF_4)_2 \cdot xH_2O$) is added and the ^1H NMR of this compound is identical to that previously reported in the literature.[81]

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

ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2](PF_6)_4$

Different methods for the synthesis of this complex are reported by Kim[79] and Yadav[80] respectively. Table 2.4 shows the comparesion of reaction conditions of these two methods. Both methods use water as part of the solvent. As mentioned above, water is one of the products of this Schiff base reaction, which shift the equilibrium in an unfavorable direction according to Le Chantlier's principle. In addition, in order to get pure product, further purification is needed for both methods. But both purification methods are not efficient enough to get pure product.

Table 2.5 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

Modification of solvent system and further purification approach is made in this thesis. $[(phen)_2Ru(diaminodppz)](PF_6)_2$ (100 mg 0.09 mmol) and $[(phen)_2Ru(phendione)](PF_6)_2$ (90 mg 0.09 mmol) are dissolved in a mixed solvent (50 mL EtOH, 25 mL MeCN, and 25 mL AcOH) in a 250 mL round bottom flask and the solution is refluxed for 12 hour under N_2; and the reaction flask is covered with aluminum foil. The resulting solution is cooled to room temperature and is 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 is isolated by filtration and washed with 5 mL DI water. The compound is dried in a vacuum oven at 60 ºC overnight. To remove the unreacted reactants, further purification (silica column chromatography) is applied.

Silica column chromatography

Silica is used (1 inch) as the solid phase for column chromatography in a glass Pasteur Pipet (5.75") which is eluted with MeCN. 1 mL sample solution (6 mg complex is dissolved in 1 mL MeCN) is loaded for each pipette column. According to different colors collect different components. After the eluant is colorless eluted with NH_4PF_6 saturated MeCN. The complex (in the dark brown eluant) is precipitated out using the ether precipitation method and washed with plenty of DI water. The compound is dried in a vacuum oven at 60 ºC overnight. The ^1H NMR spectra of this complex is identical to that previously reported in the literature.[79]

$[(phen)_2Ru(tadbp)](PF_6)_2$ 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) are dissolved in a mixed solvent (50 mL EtOH, 25 mL MeCN, and 25 mL AcOH) in a 250 mL round bottom flask and the solution is refluxed for 12 hour under N_2. The resulting solution is cooled to room temperature and then 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 is isolated by filtration

and washed with 5 mL DI water, 20 mL EtOH and 10 mL chloroform. The compound is dried in a vacuum oven at 60 ºC overnight. ^{1}H NMR of this compound (500 MHz, MeCN-D_3) is shown as Fig. 2.11. (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-$[(phen)_2Ru(tadbp)](PF_6)_2$ is prepared in a similar way by starting with Δ-$[(phen)_2Ru(diaminodppz)](PF_6)_2$.

Δ-$[(phen)_2Ru(diaminodppz)](PF_6)_2$

As mentioned above Δ-$[(phen)_2Ru(dinitrodppz)](PF_6)_2$ seems resistant to reduction. The 2 cycle hydrogenation in a parr shaker hydrogenator which is the successful way to make racemic $[(phen)_2Ru(diaminodppz)](PF_6)_2$ is not a good method for reduction of Δ-$[(phen)_2Ru(dinitrodppz)](PF_6)_2$. A new catalyst PtO_2 is employed to successfully reduce the chiral form of Δ-$[(phen)_2Ru(dinitrodppz)](PF_6)_2$.

$[(phen)_2Ru(dinitrodppz)]Cl_2$ (100 mg 0.11mmol) is dissolved in the methanol. After degassing the solution by bubbling N_2 though it for 5 min, 80 mg PtO_2 is added and the vessel fitted into the shaker-hydrogenator. After charging the vessel with hydrogen at 60 psi, the mixture is allowed to shake and react for 24 hour at room temperature. After relieving the pressure, the resulting solution is filtered through a pad of Celite to remove the Pd/C catalyst. The Celite pad is washed with another 60 mL of methanol and the combined methanol fractions are rotovapped to approximately 10 mL total volume. In this case, the product is isolate by addition of an aqueous solution of ammonium hexafluorophosphate (50 mg 0.30 mmol) which resulted in formation of a precipitate which is isolated by filtration and washed with 5 mL DI water. The compound is dried in a vacuum oven at 40 ºC overnight. The ^{1}H NMR spectra of this complex is identical to that previously reported in the literature.[79]

CHAPTER 3
STUDIES OF BIOACTIVITY OF RU(II) POLYPYRIDYL COMPLEXES BASED ON STRUCTURES

3.1 Introduction

The biological activity of RPCs against cancer cells in cyto and in animals has been under investigation since the late 1950's[20, 22, 23]. For example, it was shown by Dwyer and co-workers that these complexes are non-toxic when taken orally but are potent neurotoxins when injected into an animal. RPCs containing the dppz and tpphz ligands are known to act as molecular light switches which luminesce upon intercalation into DNA.[66] To be effective, these compounds must reach the desired location inside the cell. There are several routs across the cell membrane. Based on different mechanism, the effects of uptake rate and the intracellular distribution are different. The ability of these RPCs to enter cells was questioned as they are both high molecular weight complexes and charged as either divalent or tetravalent cations.

As mentioned before, P and MP[43] bind DNA via intercalation and cleave DNA under reducing conditions. These two compounds have also been found to exhibit low micromolar cytotoxicity against a wide range of cancer cell lines in the NCI 60 cell line panel and are active tumor regression agents *in vivo*. In their DNA cleaving activity, they have an unusual inverse dependence on the oxygen concentration, meaning that they cleave DNA better as the [O_2] is lowered[1, 2]. These findings suggest that these two complexes may show enhances cytotoxicity towards cells under hypoxic stress. We have completed a preliminary investigation of this hypothesis and show that there is little difference in their observed cytotoxicity towards HCC-2998 cells incubated under normoxic (20% O_2) or hypoxic (1% O_2) conditions.[10]

3.1.1 Cancer cell lines culture and cytotoxicity

In the 50 years since the publication of the first human cancer cell line, HeLa[95], thousands of cell lines have been derived. These have provided tools to study in depth the biochemistry and simplified the drug screen procedure, although some caution is required in interpreting data obtained by studying cells *in vitro*.

Cytotoxicity assays are widely used especially in the field of drug development to assess the toxicity of anticancer agents. There are a number of different assays which designed to evaluate cellular drug sensitivity and they generally fall into two groups: those that measure cell survival and those that measure cytotoxicity. The cell survival assay requires a measure of the ability of cells to proliferate and this is usually an estimate of the ability of individual cells to form colonies. The cytotoxicity assays include methods such as trypan blue dye exclusion[96] and tetrazolium dye (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)) colormetric methodassess the structural integrity and metabolic function of the cells after drug exposure. Trypan Blue is a vital dye. The reactivity of trypan blue is based on the fact that the chromopore is negatively charged and does not interact with the cell unless the membrane is damaged. Therefore, all the cells which exclude the dye are viable. Cell viability is calculated as the number of viable cells divided by the total number of cells within the grids on the hemacytometer. The IC_{50} (half maximal inhibitory concentration) is very common metric used to indicate the drug sensitivity. A drug's IC_{50} is easily determined by MTT assay with values below 10 μM generally being considered significant.

The American National Cancer Institute (NCI) as well as the pharmaceutical industry has developed rapid-throughput microtitration assays to screen new cytotoxic agents. These assays measure the effect of a drug on the growth of a population of cells and the endpoint is an estimate of cell number. Use of a tetrazolium dye (MTT) as an indirect measure of cell number was first reported in the early 1980s[97]. The NCI evaluated MTT dye reduction as a possible endpoint in a rapid screening assay[98]. The mechanism of this assay is: cells in the

exponential phase of growth are exposed to a cytotoxic drug. Surviving cell numbers are determined indirectly by MTT dye reduction. MTT is a yellow water-soluble tetrazolium dye that is reduced by live cells to a purple formazan product that is insoluble in aqueous solutions[72]. The amount of MTT-formazan produced can be determined spectrophotometrically once solubilized in a suitable solvent.

3.1.2 DNA metal-intercalators and cellular uptake

The biological significance of the interaction between metal ions and nucleic acids has become a well-established fact. The effect of platinum-based chemotherapeutic drugs probably originates from their attack on DNA. Metal ions can interact with nucleic acids in two distinct modes of binding: diffuse binding and site binding, both of which are important for the structure and function of nucleic acids.[99] Moreover, DNA has a strong affinity for many heterocyclic aromatic dyes, such as acridine and its derivatives. Intercalation was first proposed by L. S. Lerman which explained the source of this affinity, and this mode of DNA binding[100]. The covalent attachment of organic intercalators to transition metal coordination complexes, yielding metallointercalators, can lead to novel DNA interactions that influence biological activity. Metallointercalation has been extended into three dimensions by Barton and others using octahedral Rh, Ru, or Os complexes containing multi-heterocyclic aromatic ligands such as phen, phi or dppz, dpq, and dppn.[101-103] There are two kinds of noncovalent interactions between metallointercalators and DNA: intercalation and insertion.[101] Rhodium and ruthenium intercalators served as the first tethered probes of long-range oxidative DNA damage.[45, 104]

Understanding how metal-based probes and therapeutics gain entry to the cell is important for their practical development, as the import mechanism has implications for cell-type specificity, the rate of uptake, and their intracellular fate. The pathways into the cell include passive diffusion, transport proteins, and endocytosis.[99, 105] The uptake properties of metal complexes which are luminescent can be examined by fluorometry, confocal microscopy[106] and

flow cytometry.[107] For non-luminescent complexes, inductively couples plasma mass spectrometry (ICP-MS),[108, 109] atomic absorption spectroscopy (AAS)[110], and UV-visible absorption spectroscopy[111] are used. A series of dipyridophenazine (dppz) complexes of Ru(II) were synthesized for systematic study of cellular uptake by Barton el at.[107] Because that the complexes are actually transported in to cellular interior rather than associating solely at the membrane surface by confocal microscopy.[112] For $[(DIP)_2Ru(dppz)]^{2+}$, intense luminescence in the interior is apparent within 2 h. For $[(phen)_2Ru(dppz)]^{2+}$ and $[(bpy)_2Ru(dppz)]^{2+}$, microscopy experiment also show uptake into the cellular interior but, consistent with the flow cytometry data, on a slower time scale (≥ 4 h for phen). For all complexes, greatest luminescence is evident in the cytoplasm, likely associated with the mitochondria and endoplasmic reticulum.[113]

3.1.3 Tumor hypoxia

As a solid tumor grows to reach a diameter greater than a few millimeters, it often outgrows its blood supply, leaving portions of the tumor with regions where the oxygen concentration is significantly lower than in healthy tissues (hypoxia). Hypoxic tumor cells are usually resistant to radiotherapy and chemotherapy. Compared with the oxygen concentrations present in most normal tissues (where mean venous pO_2 usually exceeds 40 mm Hg), malignant tumors have lower oxygen concentrations: many areas of a tumor have pO_2 values < 10 mm Hg. Such low oxygen concentrations are well known to cause radiation resistance. The oxygen concentration experiment using microelectrodes with multicell tumor spheroids *in vitro* were carried on by Robert M. Sutherland et.al. The result shows that most human solid tumors are heterogeneously oxygenated and contain hypoxic regions. For example, the median pO_2 measured with electrodes for human breast tumors is 29 mm Hg, whereas in normal breast the median pO_2 is 68 mm Hg (Fig. 3.1).[114, 115]

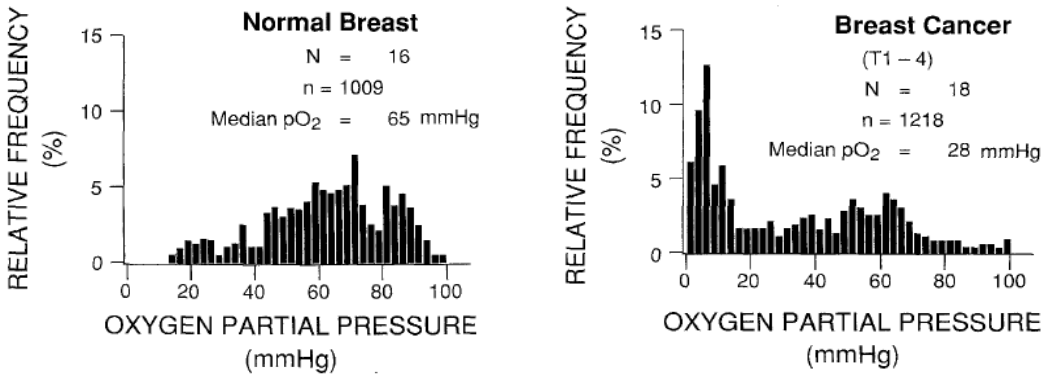


Fig. 3.1 Oxygen partial pressure (pO_2) distributions for normal breast and breast cancers (clinical stages T1-4). N = number of patients; n = number of pO_2 measurements made with needle electrodes using computerized pO_2 histography.

The overriding problem in the development of drugs effective against solid tumors is to differentiate between neoplastic cells and normal tissues. Some antineoplastic agents, such as cisplatin target at fast growing cell. However they don't have the ability to tell the difference between cancer cells and normal fast growing cells such as hair follicles. Although the hypoxic cells in the solid tumors resistant to conventional antineoplastic agents and radiation therapy, at the same time the hypoxic microenvironment offers an opportunity to differentiate between neoplastic cells and normal tissues.

SR 4233 (3-amino-1,2,4-benzotriazine 1,4-dioxide, WIN 59075, tirapazamine), a lead compound is a new class of bioreductive antineoplastic agents, the benzotriazine di-N-oxides. Because the N-oxidation of tertiary aliphatic amines decreases the electron density on the nitrogen and the reduction of N-oxides is oxygen inhibited.

Several groups have investigated the mechanism for the selective hypoxic toxicity of SR 4233, and there is general agreement on the broad outlines of the mechanism. Fig. 3.2 shows a diagram of the proposed mechanism for the selective cytotoxicity. It shows that the damaging species is an oxidizing radical which is 'back-oxidized' to the parent drug in the

presence of oxygen. This oxidizing radical abstracts hydrogen from cellular targets leaving an oxidized target molecule along with the stable 2-electron reduction product, SR 4317.[116-119]

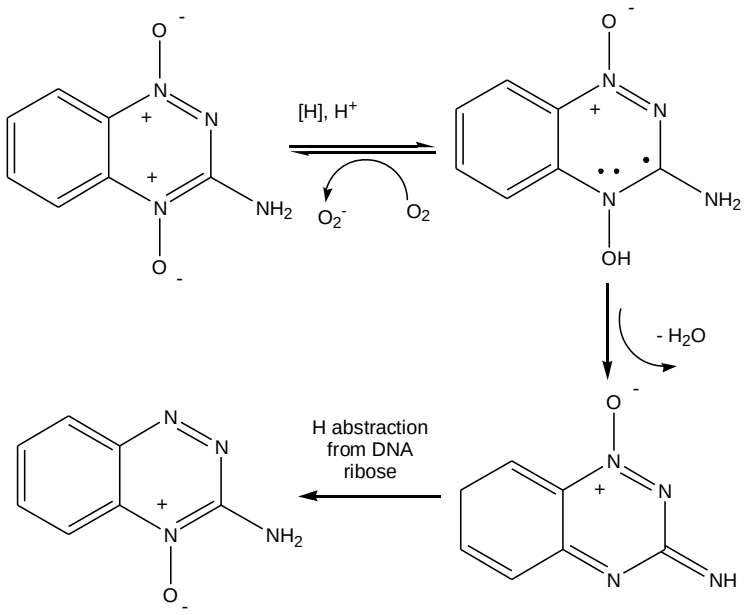


Fig. 3.2 Schematic representation of the mechanism of cell killing by SR 4233 under hypoxic conditions.

The research did by Elaine M. Zeman et al. shows that the cytotoxicity of SR-4233 as a function of time for three of the seven different cell lines (hamster, mouse, and human). Cells were exposed to the drug under both normoxic and hypoxic conditions. Drug concentrations were chosen so as to be able to match the cytotoxicity of the drug under the two conditions as closely as possible. For all the cell lines tested, a hypoxic cytotoxicity ratio, or a ratio of drug concentration under normoxic to hypoxic conditions giving equivalent cytotoxicity, could be calculated from these data. Typically, this ratio is approximately 75-200 for the rodent lines and 15-50 for the human lines tested.[116]

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), DMEM Medium (500 mL Dulbecco's Modified Eagle's Medium - high glucose With 4500 mg/L glucose, L-glutamine, sodium pyruvate, 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) are purchased from Sigma-Aldrich.

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

Cancer Cell lines:

HCC-2998 colorectal carcinoma cell line is purchased from DTP, DCTC Tumor Refository.

MCF-7 breast cancer cell line, CCL-228 colon carcinoma cell line and H-358 non-small lung adenocarcinoma cell line are purchased from ATCC.

3.2.2 Instrumentation

Cancer cell lines are cultured in the following incubators: VWR symphony^{TM} Water-Jacketed CO_2 Incubator (Model 6.5 W), New Brunswick Scientific Galaxy CO_2 Incubator (Model 14S, with 1-19% O_2 control).

96-well plates for MTT assay are read from BMG Labtech FLUOstar Omega Microplate Readers.

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

3.2.3 Cancer cell lines culture

HCC-2998, and MCF-7

The cell lines are maintained in culture in RPMI-1640 with 10% FBS at 37°C with 5% CO_2 in a humidified atmosphere. The culture medium is supplemented with L-glutamine 2 mM, penicillin 400 IU/mL.[120, 121]

CCL-228

The cell line is maintained in culture in DMEM with 10% FBS at 37°C with 5% CO_2 in a humidified atmosphere. The culture medium is supplemented with L-glutamine 2 mM, penicillin 400 IU/mL.[122]

3.2.4 Drug sensitivity assay

Living cells density can demonstrate the degree of the drug sensitivity. In this chapter, two ways to measure the living cell density are employed. 1) Using a hemocytometer to count dye excluding cells resistant to staining with trypan blue. 2) MTT assay. Place approximate 2 x 10^4 cells into each well of a 96-well flat-bottomed microtiter plate. After 24 hour incubation, the cells were treated with varying concentrations of ruthenium polypyridyl complexes. After certain hour's incubation, 20 μL of 5 mg/mL MTT is introduced to each well and incubated for 2 hour at room temperature. Finally, the medium is decanted gently, and then add 100 μL DMSO into each well sitting for 8 hour to dissolve the purple formazan dye. Absorbance values read by FLUOstar Omega Microplate Reader at 560 nm are directly output to an excel spreadsheet to calculate the IC_{50} (μM).

3.3 Results and Discussion

3.3.1 Ruthenium content

Cellular uptake experiment:

Many biological and biophysical methods are available to study the cellular uptake. According to different compounds, different methods, model systems and techniques have been utilized. The most common method to evaluate certain compounds' (which have carbonyl group) uptake is by coupling a peptide to a fluorophore and measuring the fluorescence of treated cells. It is a convenient method and has been used to study both localization and amount of uptake. Drawbacks to this method are that uptake does not always correlate with bioavailability.[123] If the compounds have certain properties such as luminance or radioactivity, the direct methods which are confocal microscopy technique or radioactivity meter method can be used to determine the distribution and uptake of these compounds. For coordinatively saturated and substitutionally inert ruthenium(II) polypyridyl complexes, the most common method is using atomic absorption spectrometry to measure the content of ruthenium.[71]

Cell culture and drug treatment were conducted in the laboratory of Dr. Sanjay Awasthi (University of North Texas Health Science Center). H-358 cancer cell lines are cultured at 37 °C with 5% CO_2 in a humidified atmosphere in an incubator. The cancer cell lines are treated with 10 μM ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2]Cl_4$ (ΔΔ-P)) for five different time periods (2 min, 2 h, 24 h, 48 h, 72 h), and then washed by PBS.

The whole cell is collected and dried at 100 ºC. The resulting powder is digestion with 25% tetramethylammonium hydroxide. Before analysis of ruthenium content, a calibration curve is build using ruthenium custom-grade standard 1009 ±10 μg/mL solution in 3.3% HCl by GFAAS. The Absorptions are determined using ruthenium lamp at 379.9 nm (A-4).Each sample of H-358 cancer cell line (contains 2.2 x 10^4 cells) are treated with 10 μM ΔΔ-P at different time periods. Each sample digested by 80 μL 25% tetramethylammonium hydroxide. The Ru contents are determined by GFAAS (Table 3.1). From table 3.1 we can see that the ruthenium content is mainly accumulated along the time. For some Ru(III) complexes, cellular entry is

mediated by he iron transport protein transferrin, such as the drug candidate KP1019 (binds transferrin with displacement of a chloride ligand, then enters the cell by clathrin-mediated endocytosis).[124] Compare with a Ru(II) complex containing the lipophilic DIP ligand enters by passive diffusion in a membrane-potential dependent manner.[125] The complex of ΔΔ-P which has large lipophilic portion may passively diffuse across the plasma membrane and accumulate inside cells driven by both the plasma and mitochondrial membrane potentials.

Table 3.1 Ruthenium content uptake results of ΔΔ-P (10 μM) in H-538 cancer cell lines

Sample	Treatment time of ΔΔ-P	# of cells	Ru content (ng/1 x 10^6 cells)
control	0	2.2 x 10^6	0.0000
#1	2 min	2.2 x 10^6	0.0050
#2	2 h	2.2 x 10^6	0.1681
#3	24 h	3.4 x 10^6	0.5371
#4	48 h	4.0 x 10^6	0.9125
#5	72 h	3.6 x 10^6	2.2444

Comparison of cellular and nuclear uptake

Cell culture and drug treatment were conducted in the laboratory of Dr. S. Mandal, a collaborator on this project at UTA. H-358 cancer cell line is cultured at 37 °C with 5% CO_2 in a humidified atmosphere in an incubator. The cancer cells are treated with 5 μM ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2]Cl_4$ (ΔΔ-P) and Δ-$[(phen)_2Ru(tatpp)]Cl_2$ (Δ-MP) for three different time periods (1 h, 24 h, and 72 h). Whole cell and nucleus are collected respectively. The samples are dried in the oven at 100-110 ºC, and weighted. The dried samples are digested by 100 μL 25% tetramethylammonium hydroxide. The GFAAS determine method is as above, and

349.9nm absorbance is collected. The results of cellular and nuclear uptake are shown as Fig. 3.3.

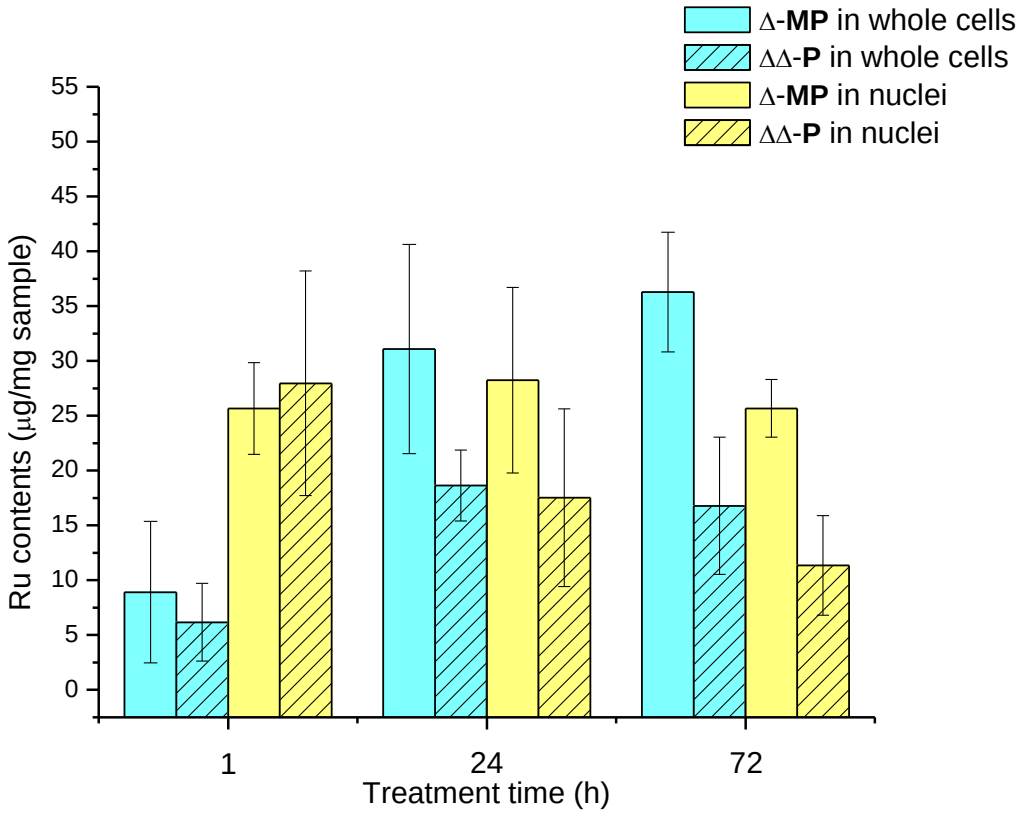


Fig. 3.3 Comparison of ruthenium uptake in H-358 whole cells and isolated nuclei after 1 hr, 24 h, and 72 h, incubation with 5 μM Δ-MP and ΔΔ-P

The ruthenium content of Δ-MP is also accumulated along the time in the whole. Comparing with the content of ΔΔ-P in the whole cell, the value seems reaches a plateau around 24 hour. Moreover, compare the rate of entry and the amount; Δ-MP is taken up more and more readily inside cells versus ΔΔ-P since its large bulk. Nuclear uptake for Δ-MP seems keeps a constant, however, the uptake for ΔΔ-P is decreasing. It seems that the nuclear uptake of Δ-MP get saturated within every short time (around 1 hour), although the cellular uptake is

increase along the time. However the lifetime of ΔΔ-P is short, it decayed after 1 hour incubation.

3.3.2 Study of hypoxic effect on cell

The CCL-228 and MCF-7 cancer cells are grown under 1% O_2, and then are compared with the cells under normal condition (37 °C with 5% CO_2 in a humidified atmosphere) to calculate the percent growth using MTT assay. The results are shown in Fig. 3.4. Each cell line responded to the hypoxic conditions differently. The relative percent growth shows that the growth of cells in hypoxic conditions is slow. Moreover, the cells are unhealthy under hypoxic conditions which affect both cell shape and adhesives. However, after re-incubating under normal conditions, the cells can become normal. Therefore, the incubation period can be designed which the conditions are alternated between hypoxic and normal conditions to avoid overestimation of cytotoxicity of our complexes.

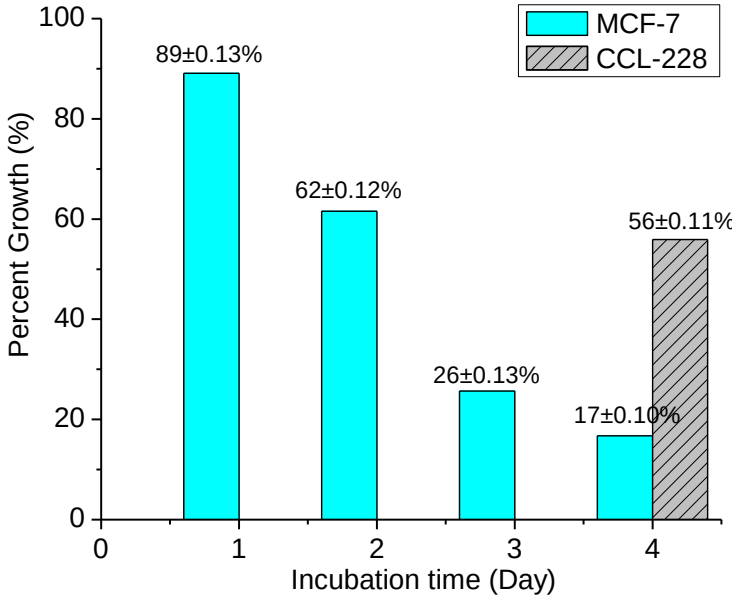


Fig. 3.4 Percent growth of MCF-7 and CCL-228 Cancer Cells under 1% O_2 condition for 4 days.

The CCL-228 and MCF-7 cancer cells are grown under hypoxic conditions (1% O_2, 37 °C with 5% CO_2 in a humidified atmosphere) and then are compared with the cells under normal conditions (37 °C with 5% CO_2 in a humidified atmosphere) to obtain the relative percent growth. According to this result, the hypoxic condition can seriously affect the growth of cell lines during long period time expose. However, the effect is not significantly noticed within 12 hour. The cytotoxicity study under hypoxic conditions is designed only expose to the 1% O_2 concentration with 4 to 6 hour.

In order to compare the drug sensitivity for cancer cell under difference O_2 concentration, certain cell lines and ruthenium complexes are prescreened. Table 3.2 lists the drug sensitivity results for four complexes: $[(phen)_2Ru(tatpp)]Cl_2$, $[(phen)_2Ru(pbtp_\alpha)]Cl_2$, $[(phen)_2Ru(p\text{-}CN\text{-}dppz)]Cl_2$, $[(phen)_2Ru(o\text{-}CN\text{-}dppz)]Cl_2$ on two cancer cell lines (MCF-7 and CCL-228).

Table 3.2 IC_{50} (μM) results of ruthenium complexes for MCF-7 and CCL-228 cancer cell lines

Complexes	MCF-7	CCL-228
$[(phen)_2Ru(tatpp)]Cl_2$	33±7	>100
$[(phen)_2Ru(pbtp_\alpha)]Cl_2$	N/A	>100
$[(phen)_2Ru(p\text{-}CN\text{-}dppz)]Cl_2$	76±6	N/A
$[(phen)_2Ru(o\text{-}CN\text{-}dppz)]Cl_2$	85±6	N/A

The results from the prescreen show complex $[(phen)_2Ru(tatpp)]Cl_2$ is more sensitive to MCF-7 than other complexes. According to the first reduction potentrials, it shows the complex which has higher first reduction potentrials is more cytotoxic aganst the cancer cell lines.

Hypoxic-selectivity experiment

HCC-2998 cell line (a highly differentiated human colon carcinoma cell line) is chosen to be treated with 3 different complexes: Δ-$[(phen)_2Ru(tatpp)]Cl_2$ (Δ-MP), $[(phen)_2Ru(dppz)]Cl_2$ and cisplatin under normoxic conditions (19%-20% O_2, 37 °C with 5% CO_2 in a humidified atmosphere) and hypoxic conditions (1% O_2, 37 °C with 5% CO_2 in a humidified atmosphere). HCC-2998 cell line is sensitive to ruthenium polypyridyl complex according to NCI-60 DTP Human Tumor Cell Line Screen which GI_{50} is 0.40 μM. The cancer cells are exposed to 5 different drug concentrations (10, 1, 0.1, 0.01 and 0.001 μM). After 4 hour of incubation, the cell density is calculated by hemocytometer to count dye excluding cells resistant to staining with trypan blue. Table 3.3 shows the results of hypoxic selectivity of 3 complexes.

Table 3.3 Results of living cell numbers of HCC-2998 cancer cell lines treat with 5 different concentration (0.001, 0.01, 0.1, 1 and 10 μM) of Δ-MP, $[(phen)_2Ru(dppz)]Cl_2$ and cisplatin for 4 hour under normoxic and hypoxic (1% O_2) conditions

	Cisplatin (# of cell /10^5)		Δ-MP (# of cell /10^5)		$[(phen)_2Ru(dppz)]Cl_2$ (# of cell /10^5)	
Concentration (μM)	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
0.001	2.24	2.22	2.22	2.18	2.30	2.28
0.01	2.22	2.14	2.18	2.14	2.28	2.24
0.1	2.22	2.08	2.14	2.12	2.24	2.22
1	2.16	2.04	2.04	2.02	2.20	2.18
10	2.06	1.98	2.00	1.96	2.16	2.14

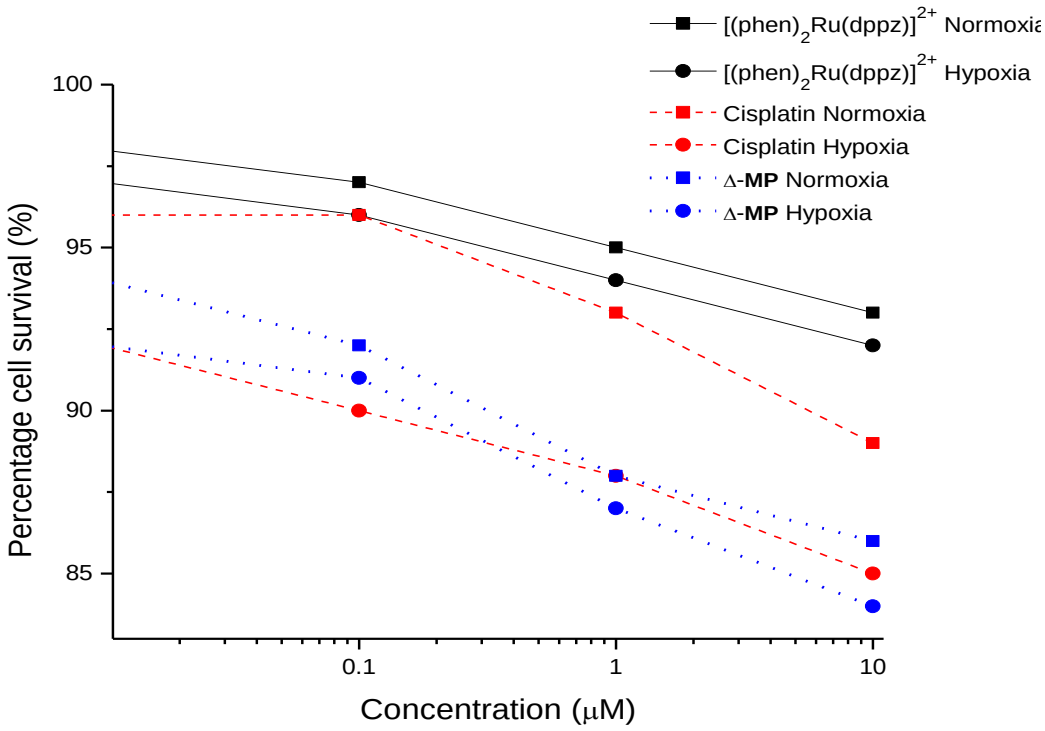


Fig. 3.5 Percentage cell survival of HCC-2998 cancer cell lines treat with 5 different concentration (0.001, 0.01, 0.1, 1 and 10 μM) of Δ-MP, $[(phen)_2Ru(dppz)]Cl_2$ and cisplatin for 4 hour under normoxic and hypoxic (1% O_2) conditions.

The difference of cell density under normoxic and hypoxic conditions is not significant, as well as the drugs concentrations effect is not remarkable. There are two possible reasons: 1) the treatment concentrations are too low to show the distinguishable difference, and 2) the time for expose to the complexes and hypoxic conditions is too short. In Fig. 3.5, the difference of hypoxic effect is scaled up by comparing the percentage of cell survival (since the initial number of cells is 2.32×10^5). Compare with Δ-MP and $[(phen)_2Ru(dppz)]Cl_2$, Δ-MP has higher cytotoxicity against HCC-2998 cell lines both under normoxic and hypoxic conditions. However the cisplatin shows the positive effect on the hypoxic selectivity. Proliferation of cancer cells is inhibited by low oxygen tensions.[114] It is commonly believed that the potency of DNA-damaging

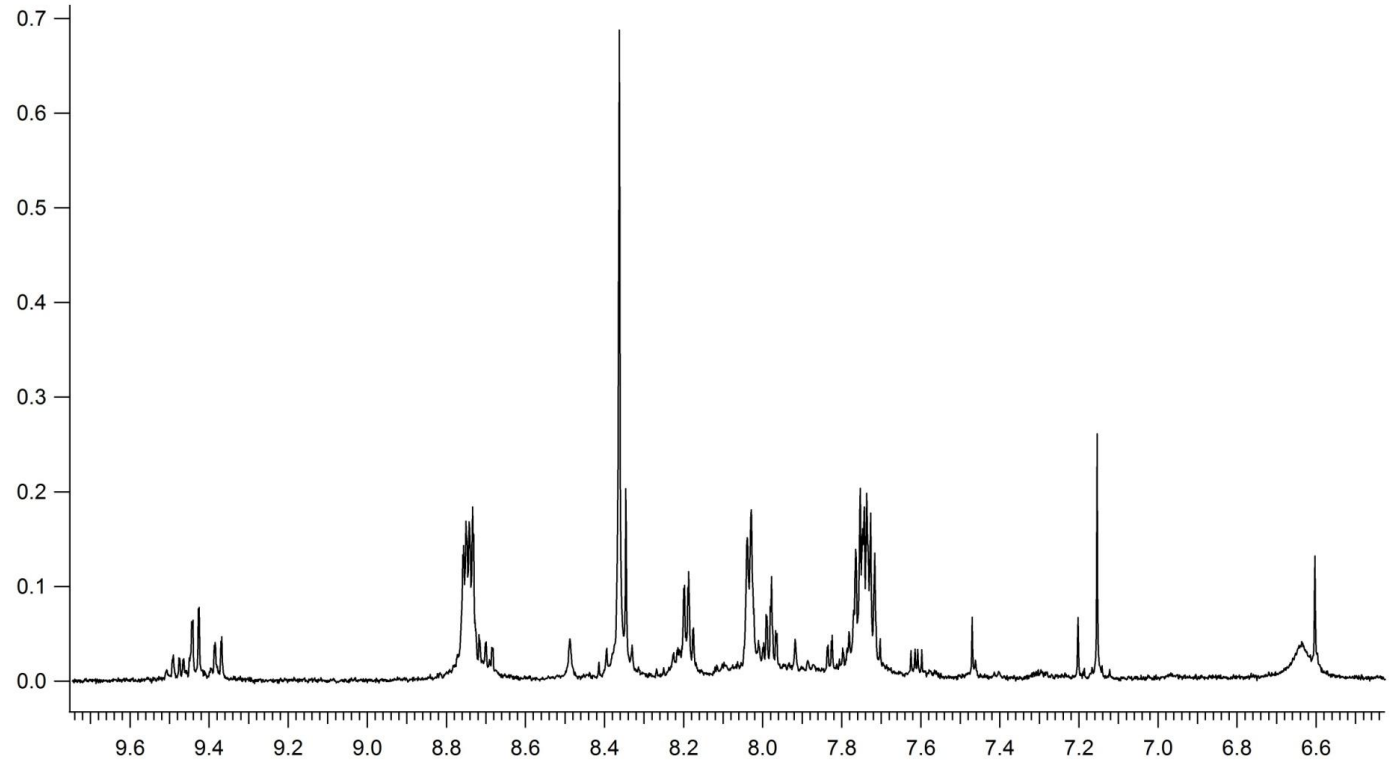
agents, like cisplatin, to cause apoptosis is strongly dependent on the growth rate. Thus, a reduction of growth rate by hypoxia could be an important mechanism leading to resistance to cisplatin.[126] Accorring to the hypoxia induced resistance of cisplatin in solid tumor, the results showing above is interesting and further expirement will be introduced to elaborate more details of hypoxia experiment in cyto.

3.4 Discussion and Conclusion

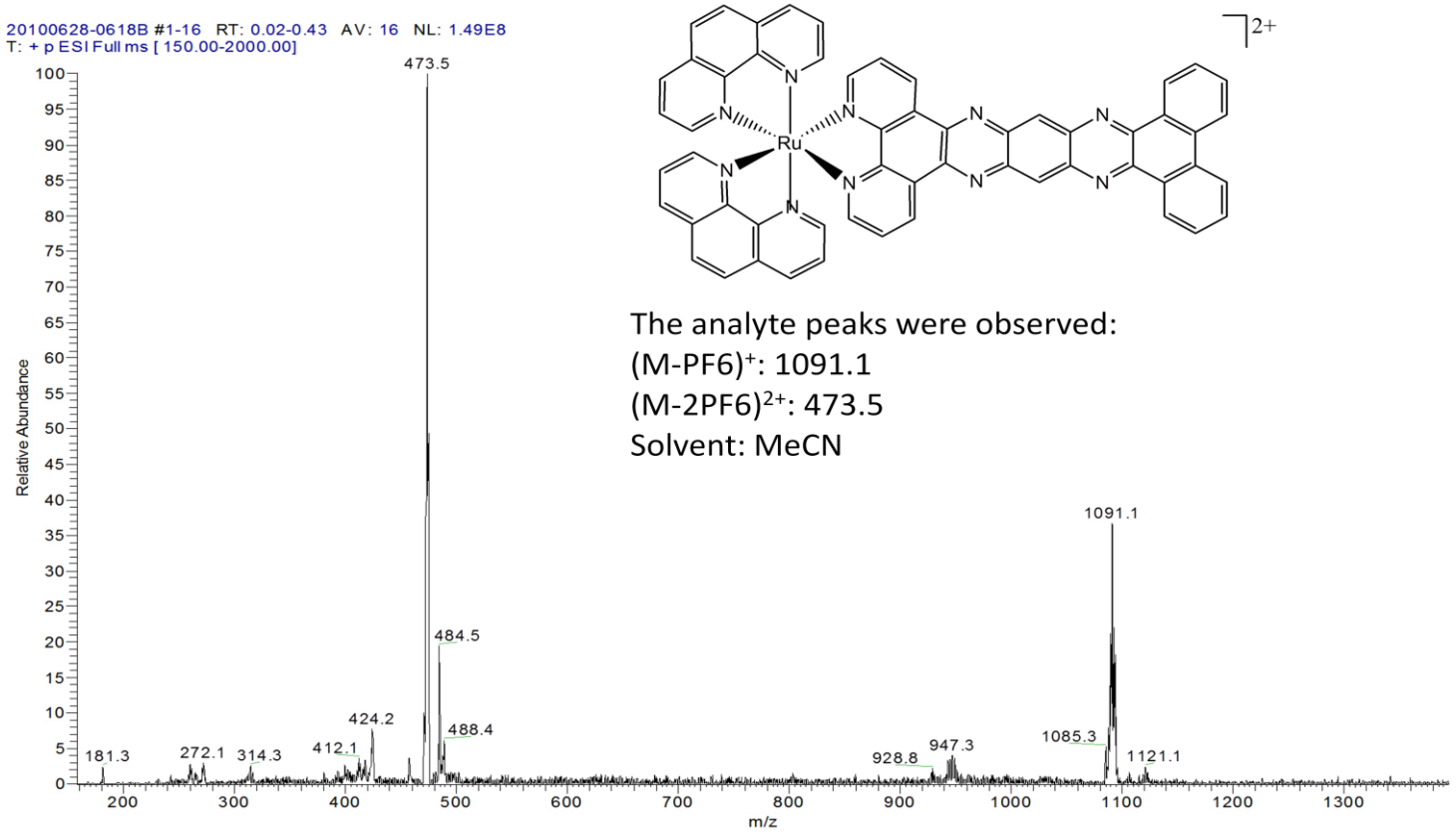
In the ruthenium content experiments, different complexes are employed into the H-358 cancer cell lines. The compression between ruthenium uptake by whole cell and nucleus is dressed by series of experiments. The results show that although ΔΔ-$[(phen)_2Ru(tatpp)Ru(phen)_2]Cl_4$ (ΔΔ-P) and Δ-$[(phen)_2Ru(tatpp)]Cl_2$ (Δ-MP) are analogues, but they have different cellular and nuclear uptake pattern: 1) Δ MP is accumulated along the time in the whole cell; 2) ΔΔ-P in the whole cell seems has a maximum value around 24 hour; 3) Nuclear uptake for Δ-MP keeps constant, however, the uptake for ΔΔ-P is decreasing along the time period. For the cytotoxicity screen test, it shows the complex which has higher first reduction potentrials is more cytotoxic aganst the cancer cell lines. Cytotoxicity studies under normoxic and hypoxic conditions (1% O_2) which shows that Δ-$[(phen)_2Ru(tatpp)]Cl_2$ (Δ-MP) is more sensitive to the cancer cell lines, give the base line to further hypoxic-selectivity research.

APPENDIX

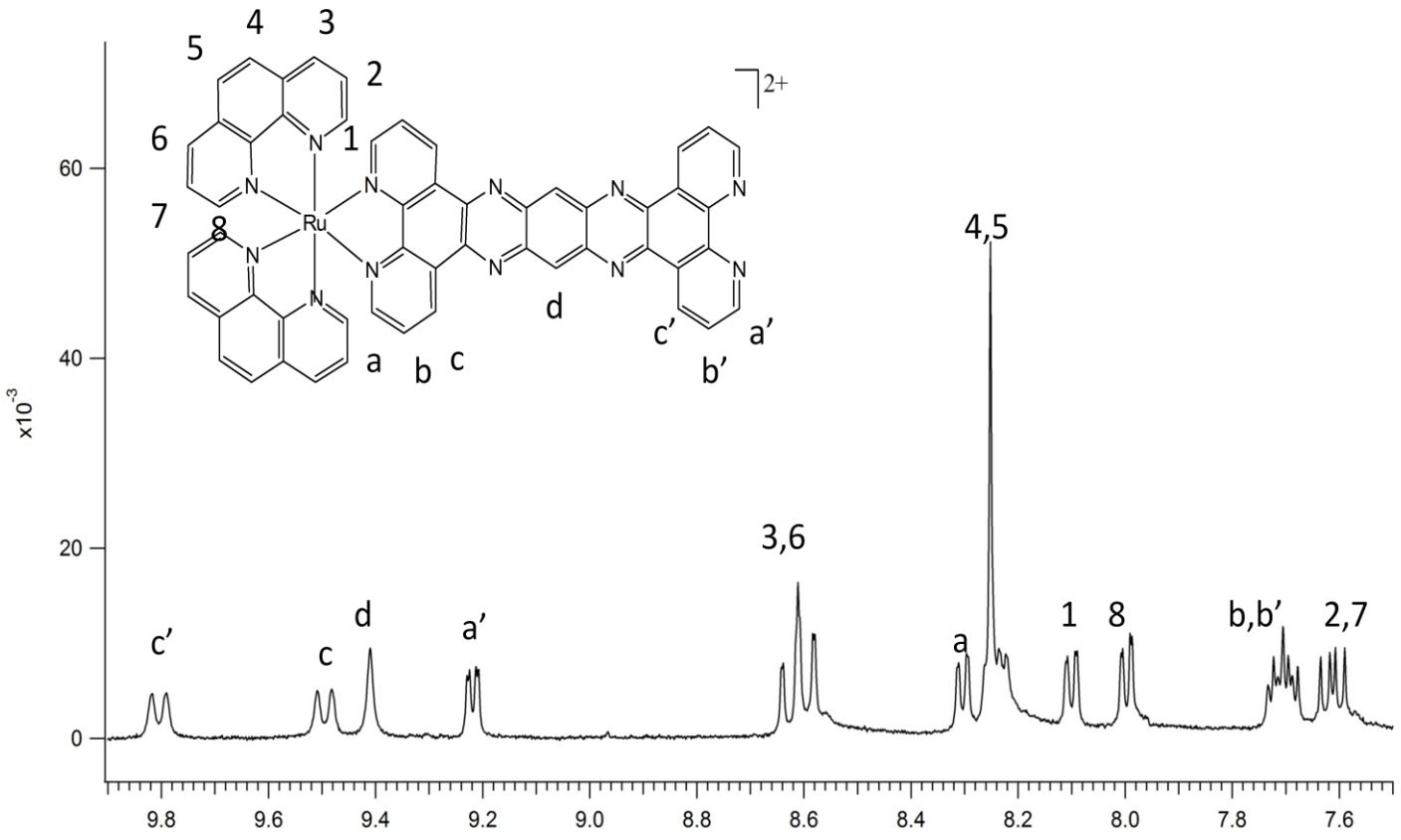
CHARACTERIZATION OF RUTHENIUM POLYPYRIDYL COMPLEXES (NMR & MS)



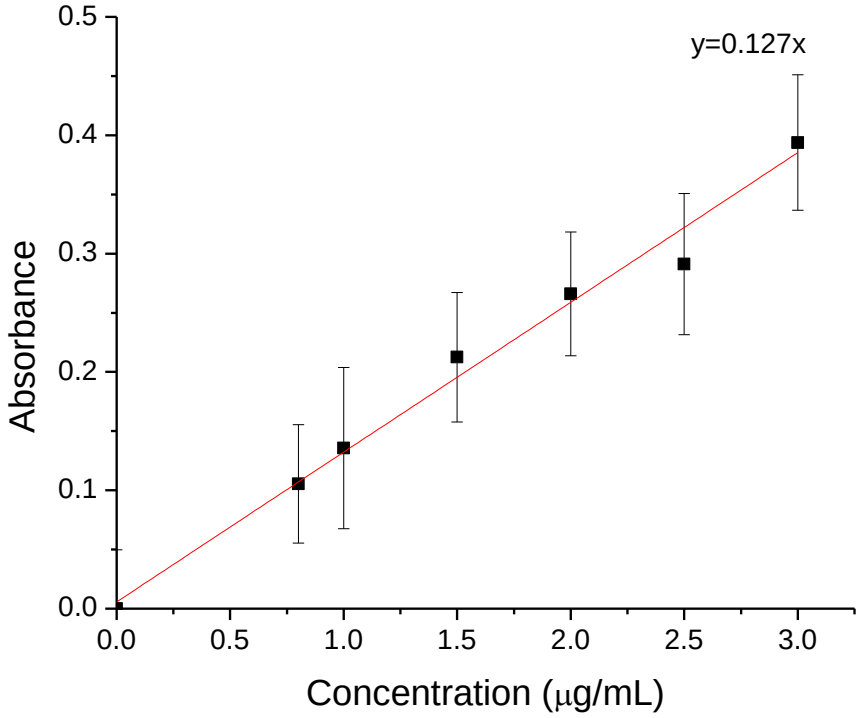
A-1: ^{1}H NMR for intermediate by $[(phen)_2Ru(dinitrodppz)]^{2+}$ reduction reaction. (500 MHz, Solvent: MeCN-D_3)



A-2: Mass spectroscopy of $[(phen)_2Ru(tadbp)](PF_6)_4$



A-3: ^{1}H NMR spectra (500 MHz, Solvent: MeCN-D_3) of $[(phen)_2Ru(tatpp)](PF_6)_2$



A-4: Ruthenium content test standard cure by GFAAS at 379.9nm. (Ruthenium custom-grade standard 1009 ±10 µg/mL solution in 3.3% HCl)

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BIOGRAPHICAL INFORMATION

Yanling Chen was born in Kunming, China. She received her Bachelor's Degree in Applied Chemistry from Beijing Normal University in 2005. From 2005-2006, she did research on photocatalytic TiO_2 nano-crystal fabrication and applications as a Research Assistance under the supervision of Dr. Jinfang Zhi, a professor at Technical Institute of Physics and Chemistry, the Chinese Academy of Sciences.

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