INCREASING LIPOPHILICITY OF REDOX ACTIVE RUTHENIUM COMPLEXES AS A MEANS TO ENHANCE CYTOTOXICITY AND REDUCE ANIMAL TOXICITY

by

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July 17, 2012

ABSTRACT

INCREASING LIPOPHILICITY OF REDOX ACTIVE RUTHENIUM COMPLEXES AS A MEANS TO ENHANCE CYTOTOXICITY AND REDUCE ANIMAL TOXICITY

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The dinuclear and monomeric ruthenium(II) polypyridyl complexes $[(phen)_2Ru(tatpp)Ru(phen)_2]Cl_4$ (**P**) and monomer $[(phen)_2Ru(tatpp)]Cl_2$ (**MP**) are promising candidates for anticancer drug development in terms of the observed anti-tumor activity in mouse models. These complexes contain the redox-active tatpp bridging ligand which seems to be the critical component for biological activity. Ruthenium complexes containing the tatpp ligand have been shown to cleave DNA with an inverse dependence on the $[O_2]$, exhibit selective and good cytotoxicity towards a number of cultured malignant cell lines, and have tolerable acute toxicity in mice. Significantly, the animal toxicity of **P** and **MP** is significantly less than simple ruthenium polypyridyl complexes, such as $[Ru(1,10\text{-phenanthroline})_3]^{2+}$ which may be due to the enhanced lipophilicity of these complexes.

This thesis is a direct test of the following hypothesis. We postulate that by increasing the lipophilicity of **P** and **MP** we can further mollify their acute toxicity and enhance their cytotoxicity towards malignant cancer cells. Chapters 1 and 2 of this thesis develop this hypothesis in terms of a review of the prior literature and our synthetic approach to construct such complexes.

In Chapter 2, the details of the synthesis and characterization of four new lipophilic ruthenium-tatpp complexes based on the **P** and **MP** structures. These are

$(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][PF_6]_4$ (**P_{Ph2}**), (Ph_2phen, 4,7-diphenyl1-1,10-phenanthroline), $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2][PF_6]_4$ (**P_{Me4}**), (Me_4phen, 3,4,7,8 tetramethyl-1,10-phenanthroline), $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$ (**MP_{Me4}**), $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$ (**MP_{Ph2}**). All of these can be metathesized to their chloride salt, which is the preferred form for water solubility and biological testing.

Chapter 3 presents the effect of these structural changes on the biological activity of the novel complexes in terms of the maximum tolerable dose (MTD) observed in mice, the IC_{50} values against malignant cell line, H358, and the ability of these complexes to cleave DNA, in vitro. In order to quantify the increase in lipophilicity, the partition coefficients (*log P*) were determined for the ruthenium complexes via the shake-flask method in PBS at pH 7.4 and octanol as well as in deionized water and octanol. It was found that the lipophilicity of these complexes increased as the lipophilic ancillary ligands changes from phen to Ph_2phen and Me_4phen ligands. The ability of these complexes to cleave DNA was maintained even with these ligand modifications. The cytotoxicity study against H358 cell line have revealed that the most promising activity was shown by **P_{Me4}** and **P_{Ph2}** with an IC_{50} value of about 10 μM. The lipophilic ruthenium complexes **P_{Ph2}**, **P_{Me4}**, **MP_{Ph2}**, **MP_{Me4}** showed no acute animal toxicity in a screen of the MTD in Balb/c mice with doses up to 80 mg drug/Kg mouse.

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CHAPTER 1

RUTHENIUM(II) POLYPYRIDAL COMPLEXES ENHANCE CELLULAR UP TAKEN BY INCREASING LIPOPHILICITY

1.1 Cancer Facts

Cancer is a major health problem where mutant cells divide and spread abnormally without control. In 2010, the National Institutes of Health reported that each day 3,400 people in the United States are diagnosed with cancer, and another 1,500 die from the disease.[1] In fact, cancer is the cause of almost 13% of all deaths in United States of America. Moreover, developing countries have more than 70% of all cancer deaths.[2] Deaths from cancer worldwide are projected to continue increasing, with an expected 12 million deaths in 2030. Cancer affects people of all ages and races and continues to challenge scientists to develop treatments for different types of melanoma. It has been estimated that half of cancer cases can be prevented by adoption of a healthy life style, avoidance of tobacco and alcohol use, and reduced exposure to sunlight.[3]

1.2 Biological Activity of Lipophilic Ruthenium(II) Polypyridyl Complexes

Cisplatin, a platinum metal complex, is one of the most widely used anticancer drugs. It has been utilized in the treatment of various types of cancers; however, it is not effective against all cancers and many tumor-types develop a resistance to this agent. It also has a number of undesirable side effects that limit its use.[4,5] Despite over 30 years of intensive research in platinum-based drugs only two analogues have been accepted for clinical use.[6] Ruthenium(II) complexes display similar chemical substitution kinetics to that of Pt(II) complexes but as a d^6 transition metal will favor 6 coordination and therefore have unique geometries relative to Pt(II).[7] Recently there has been an increased amount of attention on ruthenium complexes as an

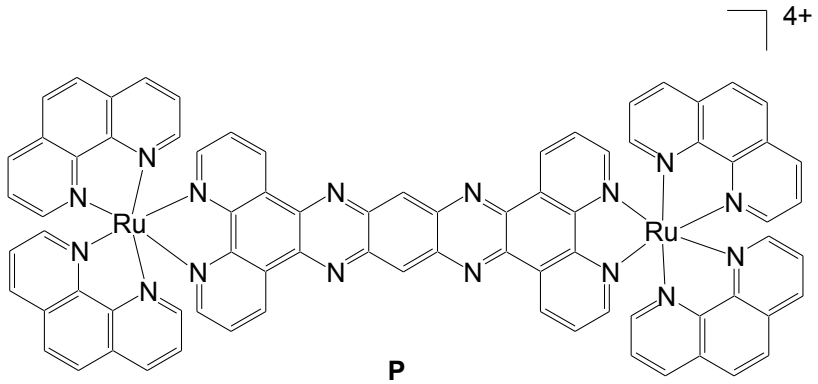
alternative anticancer metallopharmaceutical to cisplatin.[8,9] Ruthenium complexes have shown potential as anticancer drugs.[8] Some of the octahedral ruthenium metal complexes that have shown a promising cytotoxicity toward cancer cells in vitro or vivo are NAMI-A (ImH[*trans*-ImDMSORuCl$_4$]) and KP1019 (indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III)]) but some of the toxic side effects were discovered for NAMI-during the first clinical study.[10,11]

More recently, there has been a renewed interest in the anticancer properties of ruthenium(II) polypyridyl complexes (RPCs), which are known for their advantageous for cellular uptake in vivo,[12] stability,[13] and interesting biological activity.[13] These cations such as the parent complexes; [Ru(2,2'-bipyridine)$_3$]$^{2+}$ and [Ru(1,10-phenanthroline)$_3$]$^{2+}$ were chemically stable, Coordinatively saturated, substitutionaly inert, and biological active.[12] These complexes exhibited enzyme inhibitory activities and toxicity in mice.[14,12]

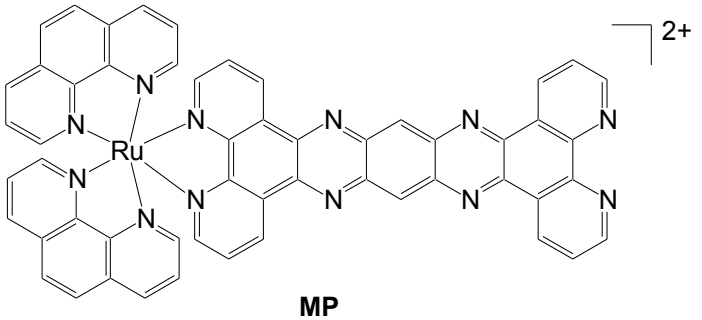
Dwyer and coworkers also reported the neurotoxicity or curare-like behavior of these complexes in vivo. Ultimately, they showed that these complexes are competitative inhibitors of acetylcholinesterase (AChE).[12] Furthermore, they found that the capability of these compounds to inhibit AChE depends on many aspects such as the charge, size, enantiomeric forms and the properties of the ligands. The high toxicity or low toxicity of the ruthenium complexes in mice is related to the ability of the complexes to penetrate the cells; which is dependent on the lipid/water partition (lipophilicity).[12]

More recently, the MacDonnell group has reported on the unusual DNA cleavage activity of the metallointercallator [(phen)$_2$Ru(tatpp)Ru(phen)$_2$]$^{4+}$ (**P**) which also displays promising anti-tumor activity in vivo (mouse animal study).[15] Both the dinuclear ruthenium(II) polypyridyl complex, [(phen)$_2$Ru(tatpp)Ru(phen)$_2$]$^{+4}$ and it's mononuclear analogue, [(phen)$_2$Ru(tatpp)]$^{+2}$ (**MP**), shown in Figure 1.1, show promising anti-cancer activity in vitro and in vivo and much of this activity is attributed to the redox active tatpp ligand present in these complexes. [8,4] The terminal ligands seem to be one area in which changes can be made to the complex which is unlikely to alter the reactivity of the tatpp ligand. In **P** and **MP**, phen and bpy

have been examined and it is seen that the type of the terminal ligand used can dramatically affect the biological-activity of the complex.[16]



P

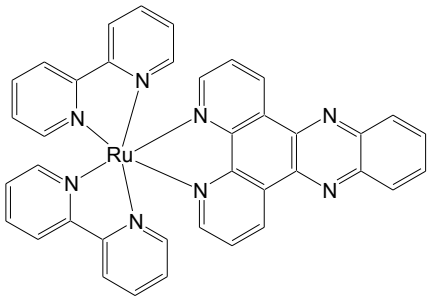


MP

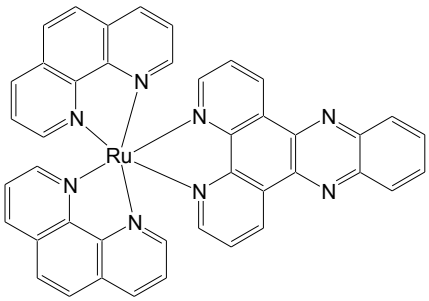
Figure 1.1: The chemical structure of **P** = $[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$, **MP** = $[(phen)_2Ru(tatpp)]^{2+}$, (where phen = 1,10-phenanthroline)

Previous research with analogues of $[Ru(phen)_3]^{2+}$ has revealed that use of lipophilic ancillary ligands in the synthesis of ruthenium(II) polypyridyl complexes can increase their uptake by cells and potency.[17] Lipophilicity is an important factor that can affect the biological activity on most therapeutic compounds.[17] Another study with $[(phen)_2Ru(dppz)]^{2+}$ (where dppz is dipyrido[3,2-*a*:2′,3′-*c*]phenazine) has shown that cellular uptake is correlated to the structure and the lipophilicity of the compounds.[18] Substitution of the 1,10-phenanthroline with lipophilic 4,7-diphenyl-1,10-phenanthroline, shown in Figure 1.2, was shown to exhibit enhanced cellular

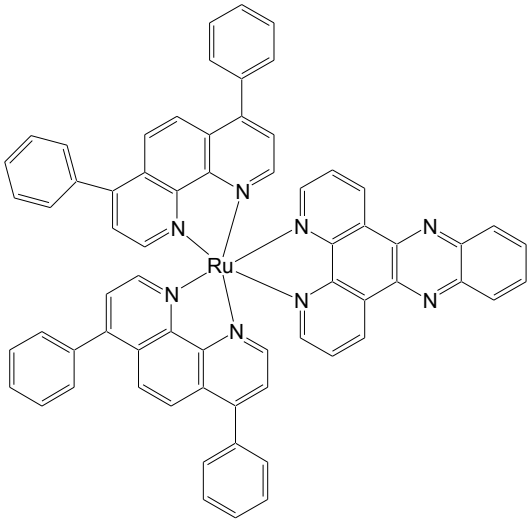
uptake of the complex. In 2008, Barton *et al*. examined the mechanism of cellular entry of luminescent ruthenium(II) polypyridyl complexes into HeLa cells where the cellular uptake was tracked and measured by confocal microscopy and flow cytometry. They have reported that the more lipophilic ruthenium(II) complex , $[(Ph_2phen)_2Ru(dppz)]^{2+}$, was transported more rapidly inside the cell compared to $[(phen)_2Ru(dppz)]^{2+}$ and $[(bpy)_2Ru(dppz)]^{2+}$.[18]



$[(bpy)_2Ru(dppz)]^{2+}$



$(phen)_2Ru(dppz)]^{2+}$



$[(Ph_2phen)_2Ru(dppz)]^{2+}$

Figure 1.2: The chemical structures of dppz complexes of Ru(II)

This transportation was very much correlated to the lipophilicity of these compounds and not with the size or overall charge. This study's outcome was in agreement with reports on cisplatin analogues, where the complexes with the highest lipophilicity displayed the maximum cellular uptake. Hence, the poor uptake into the cell membrane is due to the hydrophilicity of the complexes.[18,19,20,21] The enhanced cytotoxicity of $[(Ph_2phen)_2Ru(dppz)]^{2+}$ towards HeLa cells over $[(phen)_2Ru(dppz)]^{2+}$ or $[(bpy)_2Ru(dppz)]^{2+}$ was postulated to be due to the increased lipophilc character.[22]

Zava *et al*. reported that the more lipophilic ruthenium polypyridyl complexes appeared to induce cell death by targeting the plasma membrane, not the nuclear DNA.[23] In their experiment, different concentrations of $[Ru(L)_3]^{2+}$ complexes (where L = bpy, [2,2'-Bipyridine]-4,4'-diamine, $N^4,N^4,N^{4'},N^{4'}$-tetraethyl, [2,2'-Bipyridine]-4,4'-dicarboxylic acid, 4,4'-diethyl ester, [4,4′-dimethoxy-2,2′-bipyridine, 2,2'-Bipyridine, 4,4'-dimethyl) (shown in Figure 1.3) were evaluated for their effect on ovarian cancer cell growth using the MTT assay (MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).[23]

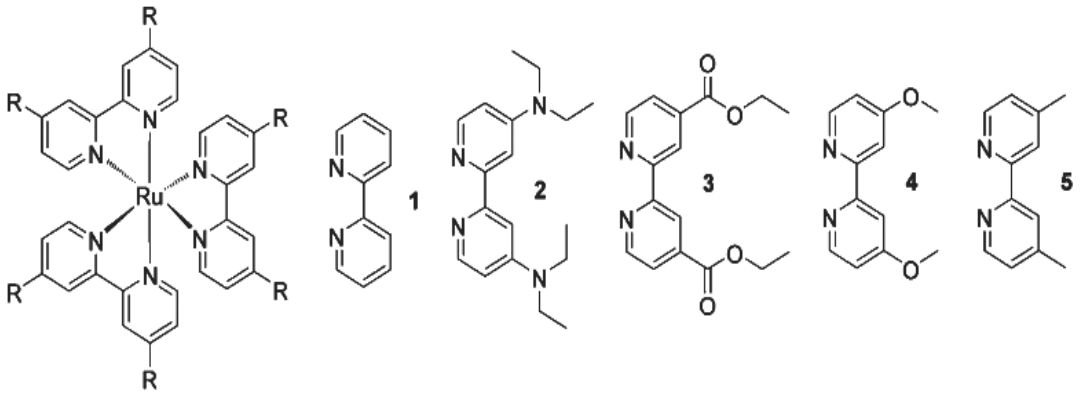
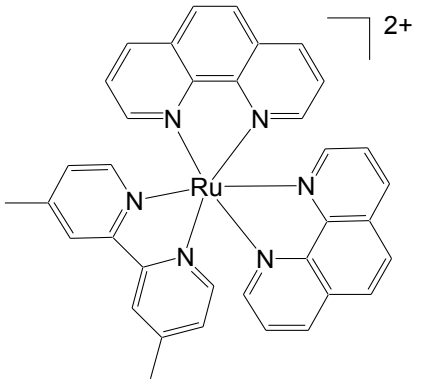


Figure 1.3: The chemical structures of Ru(II) tris(bpy) complexes

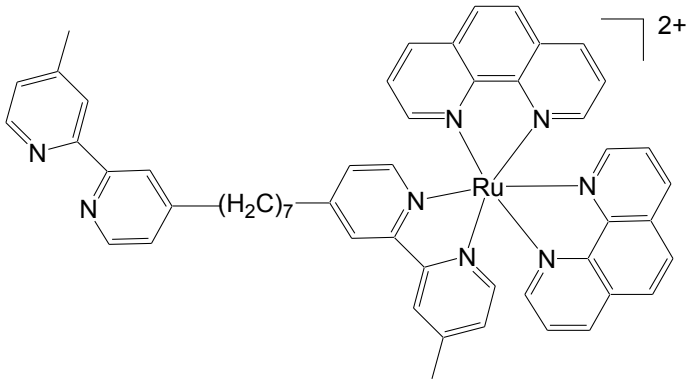
One of the five different compounds was highly cytotoxic (less than 1μm) toward A2780 cell line. The high cytotoxicity of this tris-(4,4′-dimethoxy-2,2′-bipyridine) ruthenium complex was explained by its high lipophilicity and its ability to bind to the plasma membrane of the cell. The lipophilicity of the five complexes was determined by using the partition coefficient (*log* $P_{o/w}$) experiment. The study showed that as the lipophilicity of the bipyridine ligand of the ruthenium(II) complexes increased, the cytotoxicity increased significantly.[23]

In general, the higher the lipophilicity of a drug, the stronger its binding to protein and the better its volume of distribution.[24,25] In 1978, Watanabe *et al*. demonstrated that the volume of distribution is increased by increasing the lipophilicity of drugs, when administering fifteen basic drugs to animals such as dogs.[26]

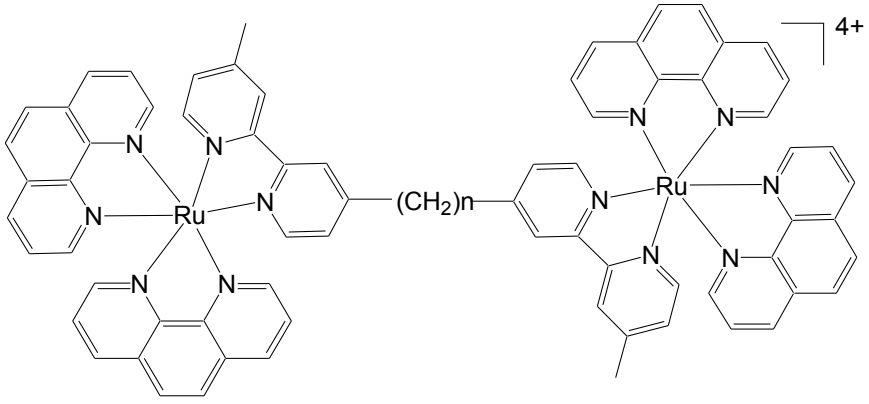
Pisani *et al*. recently described the behavior of lipophilic ruthenium(II) polypyridyl cations, as chemotherapeutic agents and their ability to target the mitochondria of L1210 cells and damage it.[27] The dinuclear ruthenium(II) complexes $[\{Ru\text{-}(phen_2)_2\}\{\mu\text{-}bb_n\}]^{4+}$ (phen = 1,10-phenanthroline) with flexible bridging ligands such as bb2 {1,2-bis[4(4'-methyl-2,2'-bipyridyl)]ethane}, bb5 {1,5-bis[4(4'-methyl-2,2'-bipyridyl)]pentane}, bb7 {1,7-bis[4(4'-methyl-2,2'-bipyridyl)]heptane}, and bb10 {1,10-bis4(4'-methyl-2,2'-bipyridyl)]decane} (Rubbn; where bbn=1,n-bis[4(4’-methyl-2,2’-bipyridyl)]-nane (n=2, 5, 7, 10, 12 or 16)) and their corresponding mononuclear complexes (shown in Figure 1.4) were synthesized and used in this experiment to study the uptake mechanism and cellular localization.[27] The accumulation of the metal complexes in the mitochondria has a vast influence on their cytotoxicity; which is related to the nature of the ligand associated with the complex. The outcomes of this experiment demonstrated that lipophilic dinuclear ruthenium(II) complexes have a high cytotoxicity when they enter the cell by passive diffusion and poison the mitochondria, resulting in cell death by apoptosis.[27]



$[Ru(phen)_2(4,4'\text{-dimethyl-bipyridine})]^{2+}$



$[Ru(phen)_2\{bis\text{-}[4(4'\text{-methyl-2,2'-bipyridyl})\text{-}1,7\text{-heptane}\}]^{2+}$



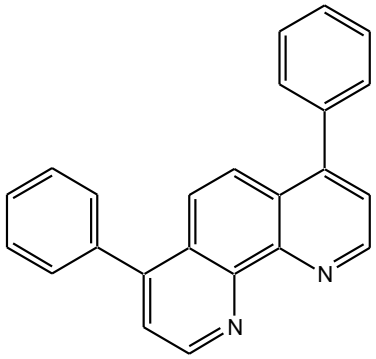
$[\{Ru(phen)_2\}_2\{m\text{-bbn}\}]^{4+}$

Figure 1.4: The chemical structures of dinuclear and mononuclear Ru(II) complexes (where bbn=1,n-bis[4(4'-methyl-2,2'-bipyridyl)]-nane (n=2, 5, 7, 10, 12 or 16)

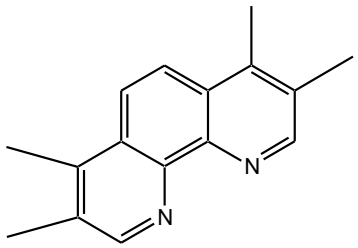
Earlier studies in our lab by Yadav and Janaratne have shown that ruthenium(II) polypyridyl complexes containing the redox-active tatpp ligand include: **P** and **MP** are potent chemotherapeutic agents as they exhibited DNA cleavage activity, high cytotoxicity and low animal toxicity.[28,29] The maximum tolerable dose (MTD) for **P** and **MP** was found to be ~ 65 mg/Kg compared to 6.6 mg/Kg for the parent complex $[Ru(phen)_3]^{2+}$.[29] The cytotoxicity study against non-small cell lung cancer (NSCLC) H358 (Human Caucasian Bronchioalveolar Carcinoma) and H226 (Lung Squamous Carcinoma) cancer lines have revealed that the most promising activity was shown by **P** and **MP** compared to $[Ru(phen)_3]^{2+}$. [29] The types of ancillary ligands that surround the metal center play an important role in the biological activity of these RPCs.

1.3 Scope of Thesis

It is postulated that using lipophilic ancillary ligands such as 4,7-diphenyl-1,10-phenanthroline (Ph_2phen) and 3,4,7,8-tetramethylphen-1,10-phenanthroline (Me4phen), as shown in Figure 1.5, to synthesize mononuclear and dinuclear cationic ruthenium-tatpp complexes including: $[(Ph_2phen)_2Ru(tatpp)]Cl_2$, $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2]Cl_4$, $[(Ph_2phen)_2Ru(tatpp)]Cl_2$, $[(Me_4phen)_2Ru(tatpp)]Cl_2$, $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2]Cl_4$, $[Ru(Me_4phen)_3]Cl_2$ will enhance their cellular uptake and therefore efficacy in terms of cytotoxicity. It is also postulated that the increased lipophilicity will reduce their acute animal toxicity as the toxicity of RPCs is primarily thought to be associated with their peak blood concentration. More hydrophilic complexes build up concentration rapidly in blood after intraperitoneal (ip) injection due to rapid perfusion through tissue and this blood concentration, if it reaches some critical level, leads to wide scale AChE inhibition and associated neurotoxicity and potentially death.[12]



4,7-diphenyl-1,10-phenanthroline



3,4,7,8-tetramethylphen,1,10-phenanthroline

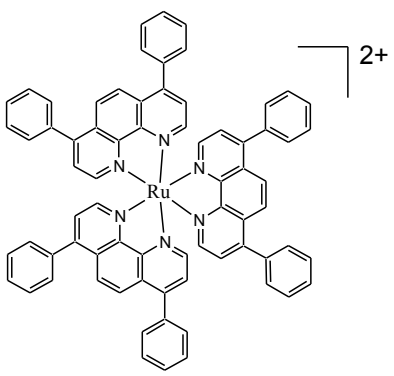
Figure 1.5: The chemical structure of lipophilic ancillary ligands

CHAPTER 2

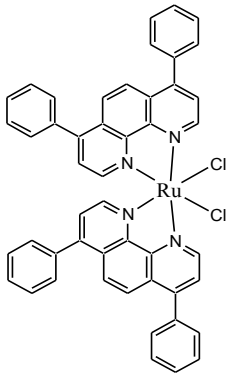
SYNTHESIS OF LIPOPHILIC RUTHENIUM POLYPYRIDYL COMPLEXES

2.1 Introduction

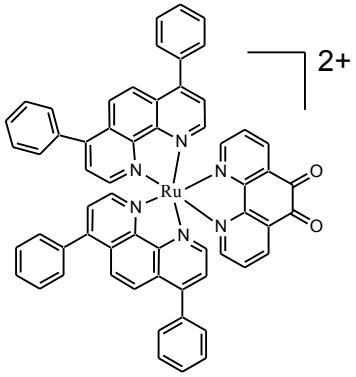
As presented in Chapter 1, the terminal ligands in ruthenium(II) polypyridyl complexes can play an important role in the biological activity of the complex. Substituting the 1,10-phenanthroline ligand with more lipophilic ligands such as 4,7-diphenyl-1,10-phenanthroline or 3,4,7,8-tetramethylphen-1,10-phenanthroline can significantly enhance the cellular uptake in dppz-based complexes.[18] In this chapter, we present the synthesis and characterization of several new lipophilic ruthenium complexes including; $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][PF_6]_4$, $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$, $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2][PF_6]_4$, and $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$,which are shown in Figure 2.1-2.2. These complexes were generally prepared in a manner similar to the phen complexes, except that the appropriate substituted phen ligand was used in the synthesis. For comparison, we have also prepared the homoleptic complexes $[Ru(Ph_2phen)_3][PF6]_2$ and $[Ru(Me_4phen)_3][PF6]_2$ shown in Figure 2.1 and 2.2 using a modified procedure that was reported by Wrighton *et al*.[30]



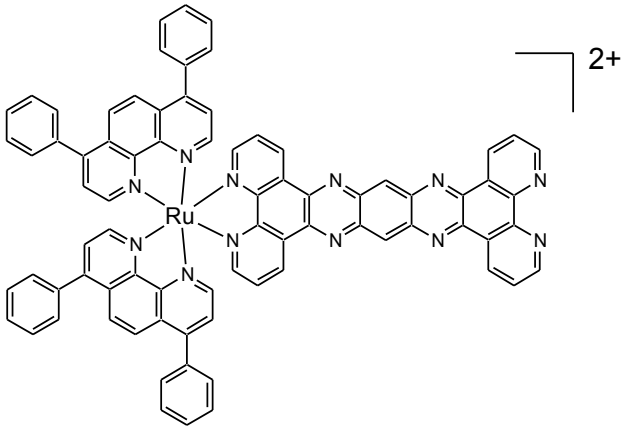
$[Ru(Ph_2phen)_3]^{2+}$



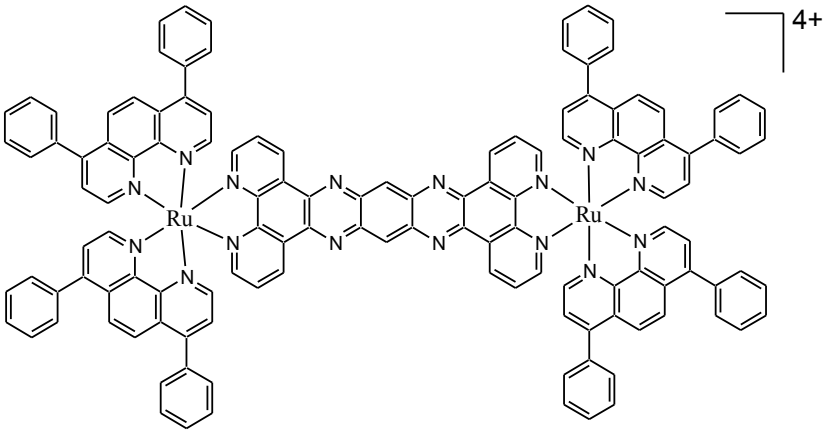
$Ru(Ph_2phen)_2Cl_2$



$[Ru(Ph_2phen)_2phendione]^{2+}$

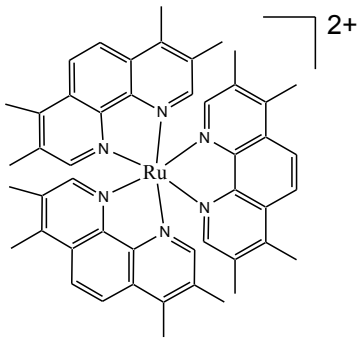


$[(Ph_2phen)_2Ru(tatpp)]^{2+}$

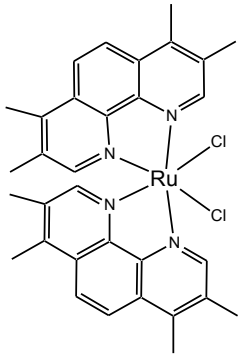


$[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2]^{4+}$

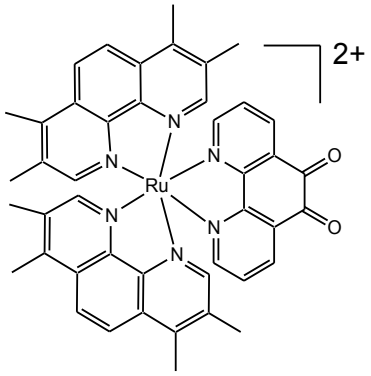
Figure 2.1: The chemical structures of lipophilic Ru(II) Ph_2phen complexes



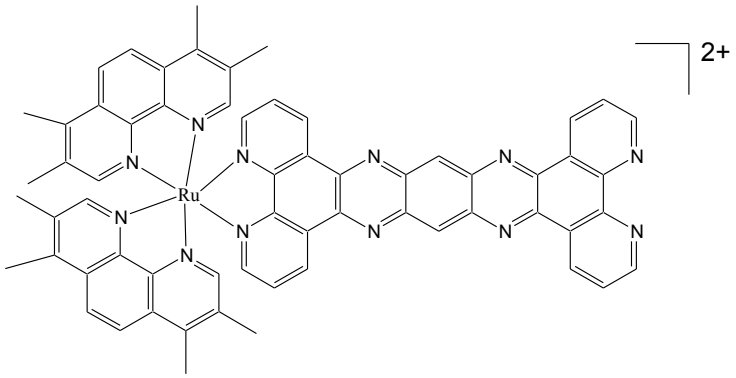
$[Ru(Me_4phen)_3]^{2+}$



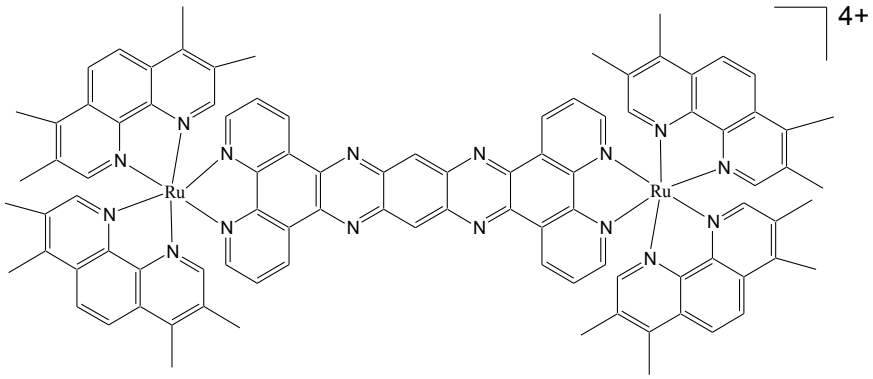
$Ru(Me_4phen)_2Cl_2$



$[Ru(Me_4phen)_2phendione]^{2+}$



$[(Me_4phen)_2Ru(tatpp)]^{2+}$



$[(Me4phen)_2Ru(tatpp)Ru(Me4phen)_2]^{4+}$

Figure 2.2: The chemical structures of lipophilic Ru(II) Me_4phen complexes

2.2 Experimental Section

2.2.1 Chemicals

All of the reagents and solvents used were of reagent grade and were used as received unless otherwise noted. 1,10-Phenanthroline-5,6-dione (phendione) was synthesized based on literature procedures,[31] ruthenium(III) chloride trihydrate (Pressure Chemical Co) was used as received, tetrabutyl ammonium chloride hydrate, 4,7-diphenyl-1,10-phenanthroline (Ph_2phen), 3,4,7,8-tetramethylphen,1,10phenanthroline (Me_4phen) , ammonium hexafluorophosphate, N,N-diethylformamide, ethanol, lithium chloride, chloroform, acetonitrile (Aldrich) were used as received. 4,5-dinitro-o-phenylene-diamine and 9,11,20,22-tetraazatetrapyrido[3,2-a:2',3'-c:3'',2''-l:2''',3'''-n]-pentacene (tatpp) were prepared as described in the literature.[32,33] 11,12-diaminodipyrido[3,2-*a*:2',3'-*c*]phenazine (dadppz) was synthesized as previously described.[34]

2.3 Instrumentation

^{1}H NMR spectra were obtained on JEOL Eclipse Plus 300 or 500 MHz Spectrometers using either CD_3CN, $(CD_3)_2CO$ and CD_3Cl as the solvent, and referenced to the residual 1H signals in the solvent using TMS as the standard for zero ppm.

2.4 Synthesis

2.4.1 Synthesis of $[Ru(Ph_2phen)_3][PF_6]_2$

This complex was prepared by using a modified procedure that was reported by Wrighton *et al.*[30] Ph_2phen ligand (0.28 g, 0.84 mmol) and $RuCl_3{\cdot}3H_2O$ (0.034 g, 0.13 mmol) were dissolved in 20 mL of ethanol. After refluxing for 14 h, the mixture was cooled to room temperature and the product was precipitated by adding an excess amount of aqueous ammonium hexafluorophosphate (NH_4PF_6). The precipitate was filtered and washed with ethanol followed by washing with copious amount of water. Yield = 83 %. ^{1}H NMR (CD_3CN) δ = 7.59-7.62 (m,

30H, H_{Ph}), 7.64 (d, 6H, J_{HH} = 5.7Hz, H_3, H_8), 8.20 (s, 6H, H_5, H_6), 8.25 (d, 6H, J_{HH} = 5.1Hz, H_2, H_9). ESI-MS (m/z): 548.95 $[[Ru(Ph_2phen)_3]^{2+}$-2PF6$]^{2+}$.

2.4.2 Synthesis of $[Ru(Me_4phen)_3][PF_6]_2$

This complex was prepared by using a modified procedure that was reported by Wrighton *et al*.[30] Me_4phen ligand (0.19 g, 0.80 mmol) and $RuCl_3{\cdot}3H_2O$ (0.034g, 0.13 mmol) were added to 20 mL of ethanol. After refluxing for 14 h, the mixture was cooled down to room temperature and the product was precipitated by adding an excess amount of aqueous NH_4PF_6. The precipitate was washed with ethanol followed by washing with copious amount of water. Yield = 93%. ^{1}H NMR (CD_3CN) δ = 2.18 (s, 18H, C*H*$_3$), 2.73 (s, 18H, C*H*$_3$), 7.6 (s, 6H, H_5, H_6), 8.33 (s, 6H, H_2, H_9). ESI-MS (m/z): 404.85 $[[Ru\ (Me_4phen)_3]^{2+}$-2$PF_6]^{2+}$.

2.4.3 Synthesis of $[Ru(Ph_2phen)_2Cl_2]$

This complex was prepared in analogous fashion to $Ru(bpy)_2Cl_2$ reported by Sullivan *et al*. with slight modification.[35] Ph_2phen ligand (0.56 g, 1.68 mmol), $RuCl_3{\cdot}3H_2O$ (0.2 g, 0.76 mmol) and LiCl (0.11g, 2.6 mmol) were dissolved with 20 mL of dimethylformamide (DMF). The solution was refluxed overnight for 14 h under nitrogen. The mixture was allowed to cool to room temperature and the dark purple product was precipitated by adding water (~30 mL). The precipitate was then washed with copious amounts of water. A yield of 94% was obtained.

2.4.4 Synthesis of $[(Ph_2phen)_2Ru(phendione)]Cl_2$

This complex was prepared in an analogous fashion to $[Ru(phen)_2phendione](PF_6)_2{\cdot}5H_2O$.[36] A mixture of $Ru(Ph_2phen)_2Cl_2$ (0.05 g, 0.06 mmol) and phendione (0.013 g 0.06 mmol) was dissolved in 50 mL of ethanol and refluxed for 5 h. After cooling the product was precipitated by addition of aqueous NH_4PF_6. The product was filtered and washed with ethanol (20 mL) followed by washing with water. Yield = 90%. Anal. Calcd for $C_{60}H_{38}F_{12}N_6O_2P_2Ru$: C, 56.92; H,

3.03; N, 6.63; Found C, 57.42; H, 2.66; N, 6.50. ^{1}H NMR (CD_3CN) δ = 7.54-7.64(m, 36H, H_{Ph}), 7.78 (d, 2H, J_{HH} = 6.0Hz, H_c), 8.11(d, 2H, J_{HH} = 6.0Hz, H_c), 8.19 (d, 2H, H_3, H_9), 8.21(s, 2H, H_5, H_6), 8.26(d, 2H, J_{HH} = 6.0Hz, H_2, H_9) 8.41(d, J_{HH} = 6.0Hz, 3.0Hz, H_a). ESI-MS (m/z): 1121.20 $[[Ru(Ph_2phen)_2(phendione)]^{2+}\text{-}PF_6]^+$, 488.33 $[Ru(Ph_2phen)_2(phendione)]^{2+}\text{-}2PF_6]^{2+}$.

2.4.5 Synthesis of $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][PF_6]_4$

A mixture of tatpp (0.1 g, 0.21 mmol) and $Ru(Ph_2phen)_2Cl_2$ (0.42 g, 0.5 mmol) was suspended in 15 mL of ethanol and 15 mL of water and refluxed for 7 days under N_2. The mixture was then stored at 4°C for 12 h and filtered. The addition of aqueous NH_4PF_6 resulted in a precipitate, which was isolated by filtration and washed with 10 mL of water (3x) and 10 mL of ethanol (3x). The crude product was further purified by repeated metatheses between the Cl^- and PF_6^- salts. The Cl^- salt was prepared from the PF_6^- salt by adding a concentrated solution of n-tetrabutylammonium chloride in acetone to a concentrated solution of the $[\mathbf{P_{Ph2}}][PF_6]_4$ salt in acetone. The resulting precipitate was filtered and washed with acetone and diethyl ether subsequently. The hexafluorophosphate salt was prepared from the $[\mathbf{P_{Ph2}}]Cl_4$ by dissolving the complex in a minimum amount of water and adding a concentrated solution of ammonium hexafluorophosphate. The resulting precipitate was filtered and washed with water, ethanol, and diethyl ether. The final product yield was 41%. Anal. Calcd for $C_{126}H_{78}F_{24}N_{16}P_4Ru_2$: C, 58.25; H, 3.03; N, 8.63; Found C, 57.18; H, 2.46; N, 8.67. ^{1}H NMR (CD_3COCD_3) δ = 7.61-7.66(m, 40H, Ph), 7.78-7.80 (dd, 8H, J_{HH} = 5.0Hz, 10.0Hz, H_2,H_5), 8.06, (dd, 4H, J_{HH} = 5.0Hz, 10.0Hz, H_b), 8.34 (s, 8H), 8.64 (d, 4H J_{HH} = 10.0Hz, H_a), 8.74 (d, 8H J_{HH} = 5.0Hz, H_1,H_6), 9.28 (s, 4H, H_d), 9.79 (d, 4H, J_{HH} = 10.0Hz, H_c). ESI-MS (m/z): 712.47 $[[\mathbf{P_{Ph}}]^{3+}\text{-}3PF_6]^{3+}$, 504.61 $[[\mathbf{P_{Ph}}]^{4+}\text{-}2PF_6]^{4+}$.

2.4.6 Synthesis of $[(Ph_2phen)_2Ru(tatpp)][PF_6]_4$

Method 1:

$[Ru(Ph_2phen)_2(phendione)]Cl_2$ (0.14 g, 0.13 mmol) and dadppz (0.04 g, 0.13 mmol) were dissolved in 50 mL mixture of glacial acetic acid and absolute ethanol (10:90). The solution was heated to reflux for 12 h and then cooled down to room temperature. The addition of aqueous NH_4PF_6 resulted in a precipitate, which was isolated by filtration and washed with water and dried under vacuum. Yield = 60%. Anal. Calculated for $C_{78}H_{46}F_{12}N_{12}P_2Ru$: C, 60.74; H, 3.01; N, 10.90. Anal. Found: C, 59.16; H, 2.94; N, 10.87. ^{1}H NMR (500MHz), (CD_3CN), $[\mathbf{MP_{Ph}}Zn]^{2+}$ (one to three fold molar excess $Zn(BF_4)_2$ was added). δ = 7.57-7.60 (m, Ph), 7.88 (dd, dd, J_{HH} = 5.0Hz, 1.8Hz $H_{b'}$), 8.15 (s, H_6), 8.18 (d, J_{HH} = 10.0Hz, H_3, H_4), 8.23 (d, J_{HH} = 5.0Hz, H_6), 8.24 (d, J_{HH} = 10.0Hz, H_2, H_5), 8.32 (d, J_{HH} = 8.0Hz, 3.5Hz, H_1), 8.38 (d, J_{HH} = 5.0Hz, $H_{a'}$), 9.23, (d, 2H, J_{HH} = 5.0Hz, H_a), 9.62 (s, H_d), 9.71 (d, , J_{HH} = 8.0Hz, $H_{c'}$), 9.94 (d, J_{HH} = 7.4Hz, H_c) . ESI-MS (m/z): 1397 $[[\mathbf{MP_{Ph}}]^{2+}\text{-}PF_6]^+$, 626 $[[\mathbf{MP_{Ph}}]^{2+}\text{-}2PF_6]^{2+}$.

Method 2: This method involves the following 3 steps.

Step 1: Synthesis of $[(Ph_2phen)_2Ru(dndppz)][PF_6]_2$

$[(Ph_2phen)_2Ru(phendione)]Cl_2$ (0.14 g, 0.13 mmol) and 4,5-dinitro-1,2-phenylenediamine (0.026 g, 0.13 mmol) were dissolved in mixture of 5 mL of glacial acetic acid and 50 mL of absolute ethanol in 100 mL round bottomed flask. The solution was refluxed overnight and then cooled down to room temperature. Product was isolated upon the addition of aqueous NH_4PF_6, filtered and washed with water and dried in the vacuum at 60^{o}C. Yield = 85%. This complex was changed to Cl^- salt and used in the following step.

Step 2: Synthesis of $[(Ph_2phen)_2Ru(dadppz)][PF_6]_2$

A mixture of $[(Ph_2phen)_2Ru(dndppz)]Cl_2$ (0.1 g, 0.082 mmol) and 10% Pd/C (0.05g) in 50 mL of ethanol was carried out at room temperature at 5 atm of H_2(g) for 24 h. The reaction mixture was filtered through a pad of Celite and the solvent volume was reduced to 5 mL under the reduced pressure. To the concentrated filtrate was added a concentrated aqueous solution of NH_4PF_6 which precipitated the product. The product was filtered and washed with water. Yield = 75%. This complex was changed to Cl^- salt and used in the following step.

Step 3: Synthesis of $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$

$(Ph_2phen)_2Ru(dadppz)]Cl_2$ (100 mg, 0.087 mmol) and phendione (0.018 g 0.069 mmol) were dissolved in mixture of 5 mL of glacial acetic acid and 50 mL of absolute ethanol in 100 mL round bottomed flask. The solution was refluxed overnight and then cooled down to room temperature. Product was isolated upon the addition of aqueous NH_4PF_6, filtered and washed with water and dried in the vacuum at 60^{o}C. Yield = 60%. Both methods have the same characterization results for NMR, MS, and CHN as shown above in method 1.

2.4.7 Synthesis of $[Ru(Me_4phen)_2Cl_2]$

This complex was prepared in analogous fashion to $Ru(bpy)_2Cl_2$ reported by Sullivan *et al*. with slight modification.[35] Me_4phen ligand (0.56 g, 2.37 mmol), $RuCl_3{\cdot}3H_2O$ (0.1g, 0.38 mmol) and LiCl (0.11g, 2.6 mmol) were dissolved into 20 mL of dimethylformamide (DMF). The solution was refluxed overnight for 14 h under nitrogen. The mixture was allowed to cool down to room temperature and the product was precipitated by adding water (~30 mL). The precipitate was then washed with copious amounts of water. Yield = 98%.

2.4.8 Synthesis of $[(Me_4phen)_2Ru(phendione)]Cl_2$

This complex was prepared in an analogous fashion to $[Ru(phen)_2phendione](PF_6)_2.5H_2O$.[36] A mixture of $Ru(Me_4phen)_2Cl_2$ (0.2 g, 0.85 mmol) and phendione (0.062 g, 0.3 mmol) was dissolved in 50 mL of ethanol and refluxed for 5 h. After cooling the product was precipitated out with an excess amount of aqueous NH_4PF_6. The product was filtered and washed with ethanol followed by washing with water. Yield = 71%. Anal. Calcd for $C_{44}H_{38}F_{12}N_6O_2P_2Ru$: C, 49.21; H, 3.57; N, 7.83; Found C, 48.42; H, 3.32; N, 7.53. ^{1}H NMR (CD_3CN) δ = 2.36 (br. s, CH_3), 2.77 (d), 7.42 (dd, 2H, J_{HH} = 6.0Hz, 3.0Hz, H_b), 7.60 (s, 2H, H_5), 7.79 (dd, 2H, J_{HH} = 6.0Hz, 3.0Hz H_c), 7.90 (s, 2H, H_6), 8.36 (d, J_{HH} = 3.0Hz, 4H, H_2, H_9), 8.43 (dd, 2H, J_{HH} = 6.0Hz, 3.0Hz, H_a).

ESI-MS (m/z): 929.27 $[[(Me_4phen)_2Ru(phendione)]^{2+}\text{-}PF_6]^+$, 392.87 $[[(Me_4phen)_2Ru(phendione)]^{2+}\text{-}2PF6]^{2+}$.

2.4.9 Synthesis of $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2][PF_6]_4$

A mixture of tatpp (0.1 g, 0.021 mmol) and $Ru(Me_4phen)_2Cl_2$ (0.32 g, 1.36 mmol) was suspended in 15 mL of ethanol and 15 mL of water and refluxed for 7 days under N_2. The mixture was then stored at 4°C for 12 h and filtered. The addition of aqueous NH_4PF_6 resulted in a precipitate, which was isolated by filtration and washed with 10 mL of water (3x) and 10 mL of ethanol (3x). The crude product was further purified by repeated metatheses between the Cl- and PF_6^- salts. The Cl^- salt was prepared from the PF_6^- salt by adding a concentrated solution of n-tetrabutylammonium chloride in acetone to a concentrated solution of the $[\mathbf{P_{Me4}}][PF_6]_4$. The resulting precipitate was filtered out and washed with acetone diethyl ether. The hexafluorophosphate salt was prepared from the chloride form by dissolving the complex $[\mathbf{P_{Me4}}]Cl_4$ in a minimum amount of water and adding a concentrated solution of ammonium hexafluorophosphate. The resulting precipitate was filtered out and washed with water, ethanol, and diethyl ether. Yield = 34%. Anal. Calcd for $C_{94}H_{78}F_{24}N_{16}P_4Ru_2$: C, 51.00; H, 3.55; N, 10.12; Found C, 50.71; H, 3.34; N, 9.74. ^{1}H NMR (CD_3CN) δ = 2.23 (s, 24H, CH_3), 2.77 (d, 24H, CH_3), 7.71 (s, 4H, H_4), 7.74 (dd, 4H, J_{HH} = 3.5Hz, 10.0Hz, H_b), 7.87 (s, 4H, H_1), 8.04 (d, 4H J_{HH} = 5.0Hz, H_a), 8.38 (s, 8H, H_3,H_4), 9.62 (d, 4H, J_{HH} = 10.0Hz, H_c), 9.65 (s, 2H, H_d),. ESI-MS (m/z): 409 $[[\mathbf{P_{me4}}]^{4+}\text{-}4PF_6]^{4+}$.

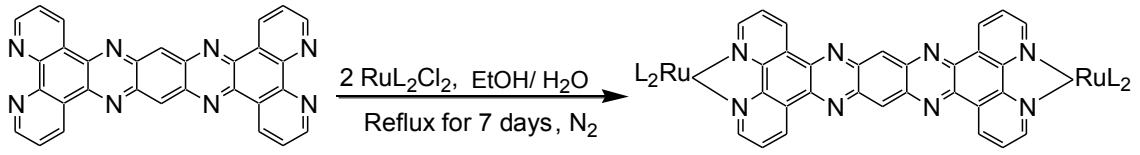
2.4.10 Synthesis of $[(Me_4phen)_2Ru(tatpp)][PF_6]_4$

$[(Me_4phen)_2Ru(phendione)][Cl_2]$ (0.1 g, 0.13 mmol) and dadppz (0.04, 0.13 mmol) were dissolved in mixture of 5 mL of glacial acetic acid and 50 mL of absolute ethanol in 100 mL round bottomed flask. The solution was refluxed overnight and then cooled down to room temperature. The addition of aqueous NH_4PF_6 to the solution resulted in a precipitate, which

was isolated by filtration and washed with water and dried under vacuum. Yield = 71 %. ^{1}H NMR (CD_3CN) δ = 2.23 (s, 12H, CH_3), 2.77 (d, 12H, CH_3), 7.73 (s, 4H, H_4), 7.75 (dd, 4H, J_{HH} = 4.5Hz, 9.8Hz, $H_{b'}$), 7.91 (s, 4H, H_1), 8.05 (d, 2H J_{HH} = 9.0Hz, $H_{a'}$), 8.32 (dd, 2H, J_{HH} = 5.0Hz, 10.0Hz, H_b), 8.38 (s, 4H, H_3,H_4), 9.25 (d, 2H, J_{HH} = 8.0Hz, H_a), 9.60 (d, 2H, J_{HH} = 9.5Hz, $H_{c'}$), 9.62 (s, 2H, H_d), 9.95 (d, 2H, J_{HH} = 10.0Hz, H_c). ESI-MS (m/z): 529.60 $[[\mathbf{MP_{Me4}}]^{2+}\text{-}2PF_6]^{2+}$.

2.5 Results and Discussion

The complete synthetic route followed for preparation of the ruthenium(II) dimer complexes $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][PF_6]_4$, $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2]$ $][PF_6]_4$ (Ph_2phen = 4,7-diphenyl-1,10-phenanthroline, Me_4phen = 3,4,7,8-tetramethyl,1,10-phenanthroline) and ruthenium(II) monomer $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$, $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$ is shown in Scheme 2.1 and 2.2. Complex $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][PF_6]_4$ and $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2]$ $][PF_6]_4$ were synthesized using tatpp [33] and corresponding $[Ru(Ph_2phen)_2]Cl_2$ [35] or $[Ru(Me_4phen)_2]Cl_2$ [35] in 1:2 molar ratio, by refluxing for 7 days in water and ethanol (1:1).



Tatpp

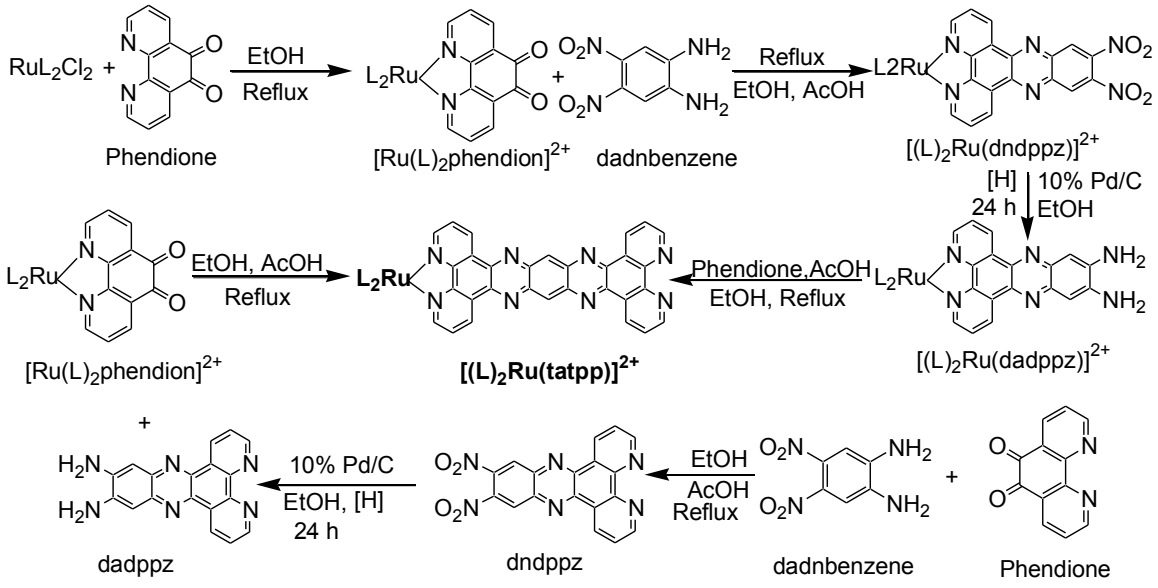
$[(L)_2Ru(tatpp)Ru(L)_2]^{4+}$

L = Ph_2phen or Me_4phen

Scheme 2.1: Synthetic route for Ru(II) dinuclear complexes

The pure dinuclear compounds $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][PF_6]_4$ and $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2]$ $][PF_6]_4$ were obtained by repeated metatheses between the hexafluoride salt and the chloride salt. Ruthenium(II) mononuclear complexes $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$, $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$ were synthesized via two different

routes as shown in Scheme 2.2. The condensation reaction between 11,12-diaminodipyrido[3,2-a:2',3'-c]phenazine [37] and $[Ph_2Ru(phendione)]^{+2}$ or $[Me_4Ru(phendione)]^{+2}$ proceeds to give the desired complexes $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$, $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$. In another route $[(Ph_2phen)_2Ru(phendione)]^{2+}$ or $[(Me_4phen)_2Ru(phendione)]^{2+}$ coupled with 1,2-diamino-4,5-dinitrobenzene to obtain $[(Ph_2phen)_2Ru(dinitro\text{-}dppz)]^{+2}$ $[(Me_4phen)_2Ru(dinitro\text{-}dppz)]^{+2}$ respectively, which are further reduced to $[(Ph_2phen)_2Ru(diamino\text{-}dppz)]^{2+}$ or $[(Ph_2phen)_2Ru(diamino\text{-}dppz)]^{+2}$ using H_2 atm over Pd/C as a catalyst. In the last step $[(Ph_2phen)_2Ru\ (diamino\text{-}dppz)]^{+2}$ or $[(Ph_2phen)_2Ru(diamino\text{-}dppz)]^{+2}$ was coupled with one equivalent of phendione in glacial acetic acid and ethanol (1:1) to obtain mononuclear ruthenium complexes $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$, and $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$ respectively.



Scheme 2.2: Synthetic route for Ru(II) mononuclear complexes

^{1}H NMR of ruthenium(II) dinuclear $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][PF_6]_4$, $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2][PF_6]_4$ and ruthenium(II) monomer $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$, $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$ complexes were taken in CD_3COCD_3. The ^{1}H NMR of $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][PF_6]_4$ and $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2][PF_6]_4$ in CD_3COCD_3 shows characteristic AMX splitting pattern for the aromatic H_a, H_b, and H_c in the 7-10 ppm region similar to $[(phen)_2Ru(tatpp)Ru(phen)_2][PF_6]_4$ [33] and $[(phen)_2Ru(tatpq)Ru(phen)_2][PF_6]_4$ [33] . The H_c proton of tatpp ligand is observed at most downfield 9.79 ppm and 9.62 ppm for $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][PF_6]_4$ and $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2][PF_6]_4$ respectively due to its proximity to the pyrazine nitrogen lone pairs, with agreement to the H_c proton of $[(phen)_2Ru(tatpp)Ru(phen)_2][PF_6]_4$ at 9.79 ppm. Phenyl protons in $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][PF_6]_4$ were observed as a multiplet between 7.61-7.66 ppm.

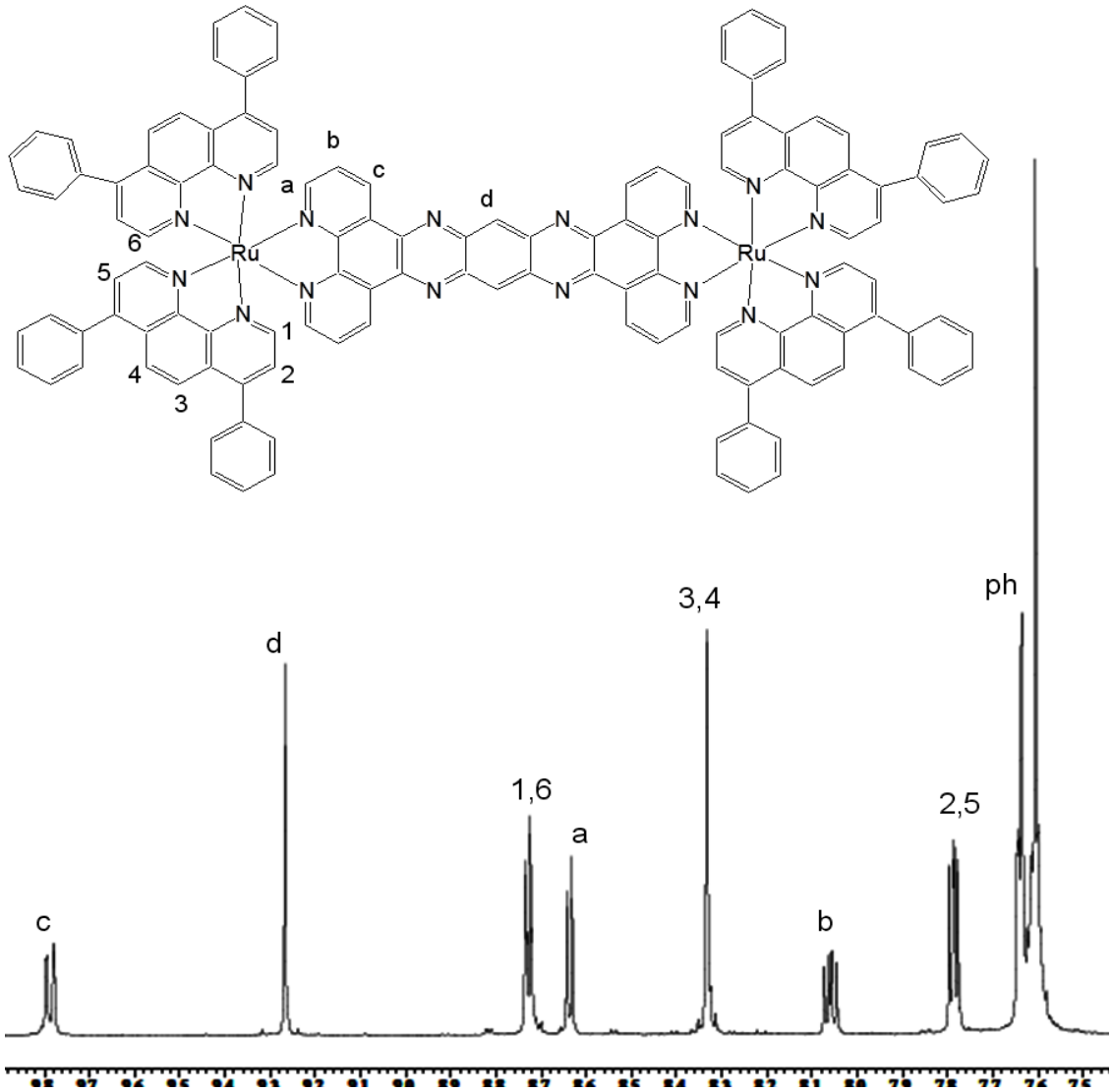


Figure 2.3: ^{1}H NMR spectrum of $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2]^{4+}$

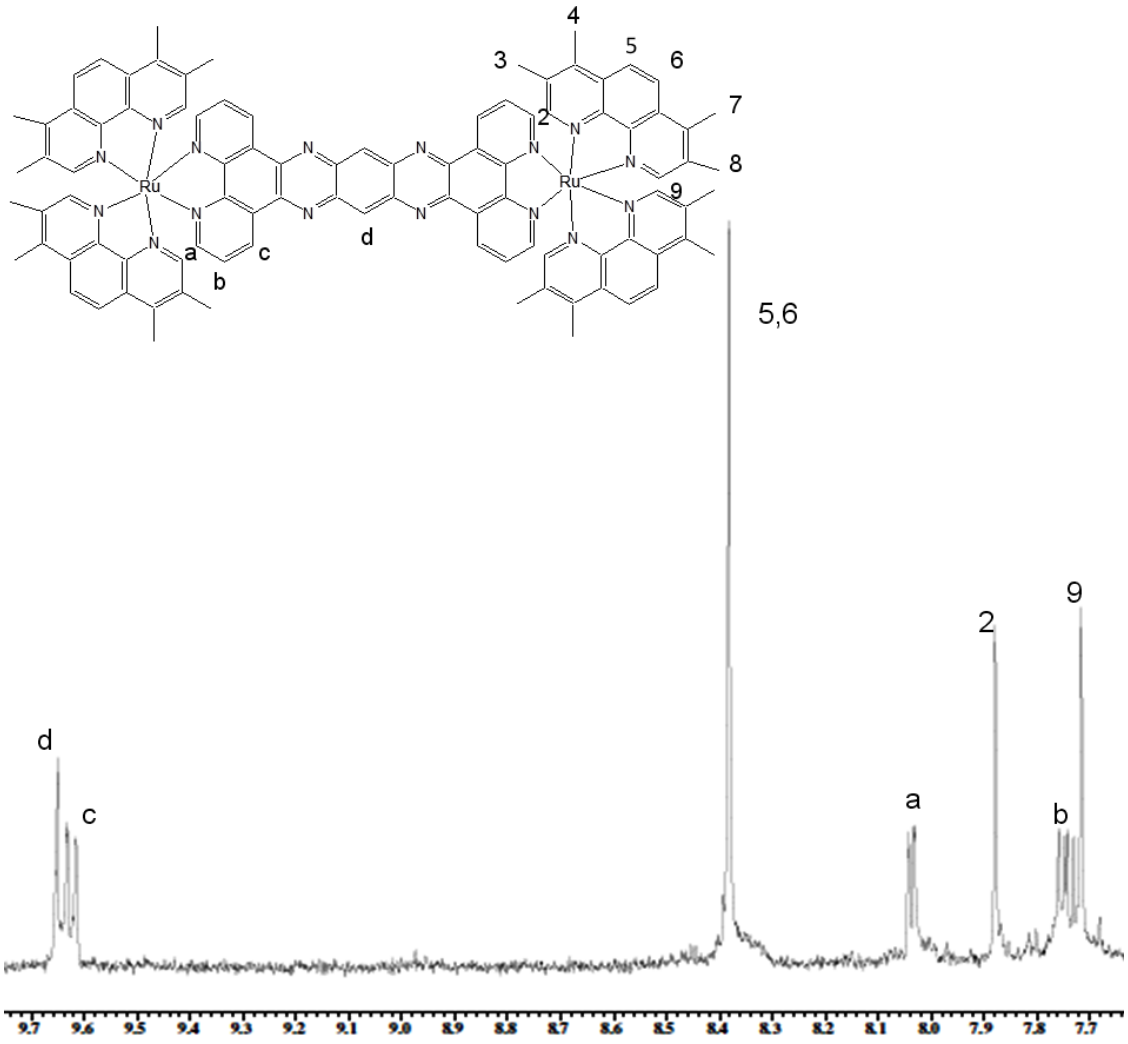


Figure 2.4: ^{1}H NMR spectrum of $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2]^{4+}$, (Downfield region)

In both the dinuclear complexes a sharp singlet is observed between 8.34-8.38 ppm for H_4 and H_5 is slightly upfield with $[(phen)_2Ru(tatpp)Ru(phen)_2][PF_6]_4$.[33] Methyl protons in $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2][PF_6]_4$ were observed as a broad singlet at 2.23 ppm (24 hydrogen atoms), and a doublet at 2.77 ppm (24 hydrogen atoms) Figure 2.5.

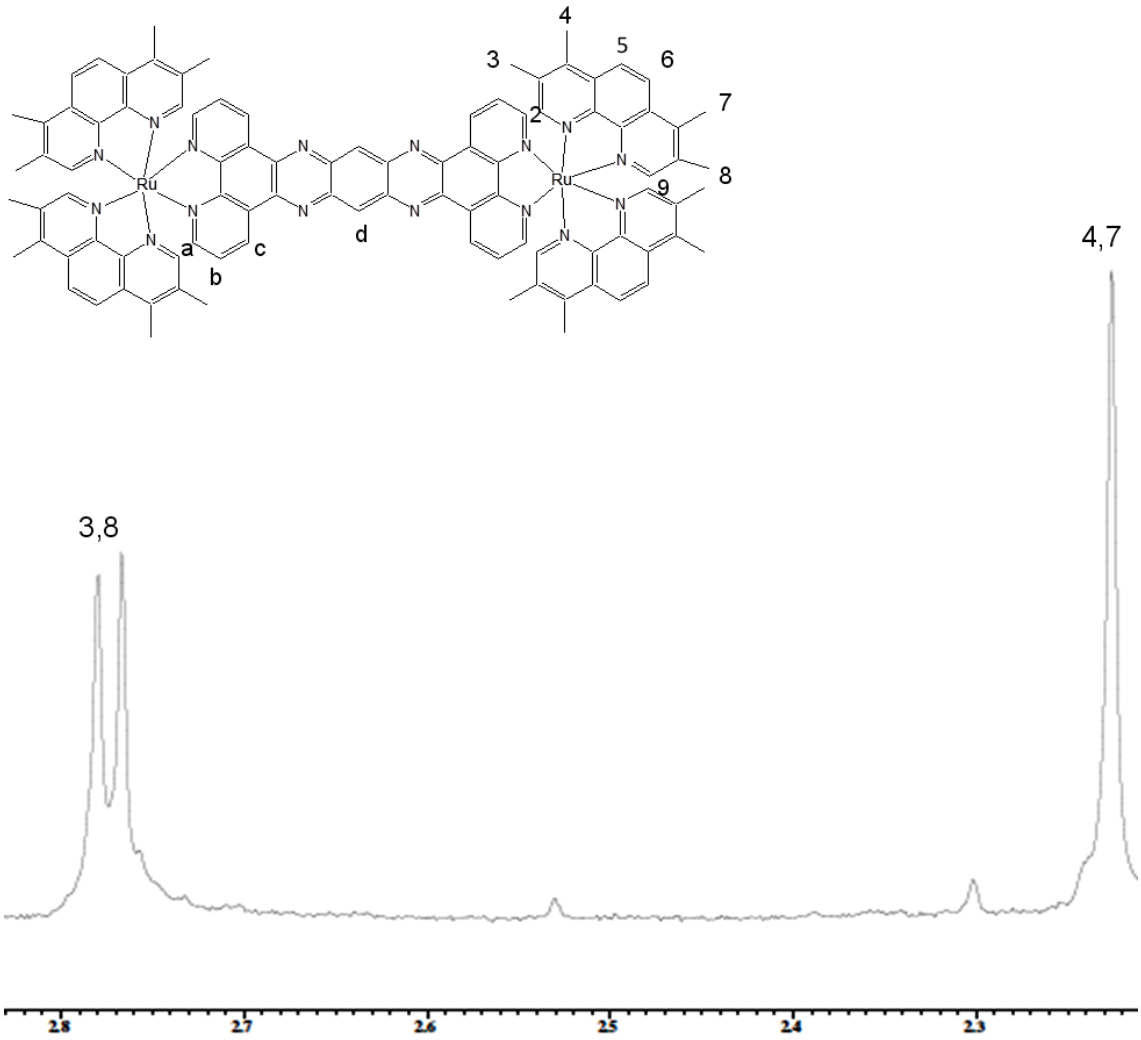


Figure 2.5: ^{1}H NMR spectrum of $[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2]^{4+}$, (Upfield region)

^{1}H NMR of mononuclear ruthenium(II) is more complex than their dinuclear analogue. $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$ and $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$ complexes do not show well resolved proton NMR, presumably because of aggregation via stacking of the tatpp ligands, which has been reported for the phen and bpy complexes.[38] Well resolved proton NMR can be obtained by coordination of a Zn(II) to the free end of the bridging ligand, which apparently helps to break the aggregation as the NMR signals become noticeably sharper. In a typical

NMR experiment, a one to three fold molar excess of zinc(II) tetraflouroborate was added to the NMR sample to saturate the coordination site.

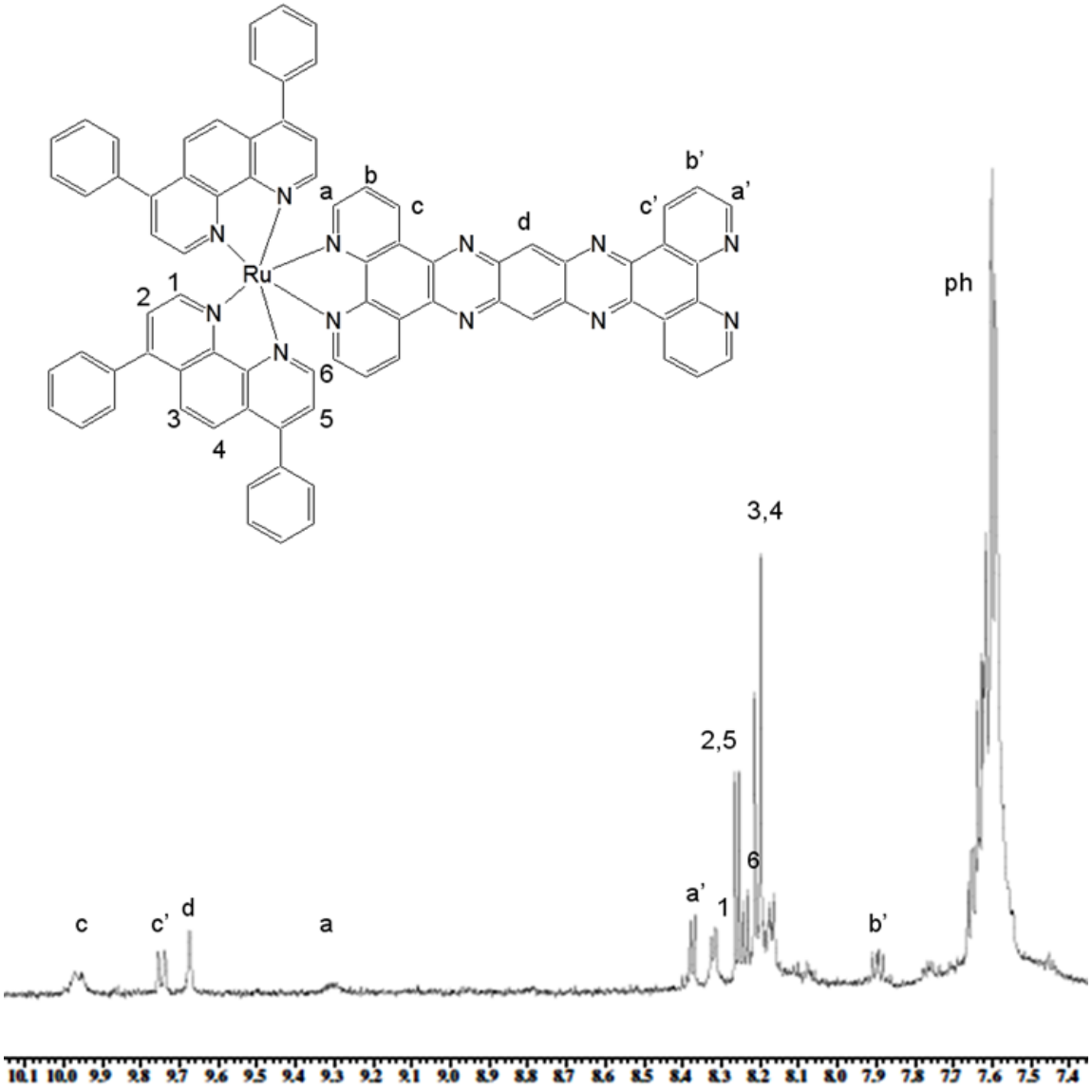


Figure 2.6: ^{1}H NMR spectrum of $[(Ph_2phen)_2Ru(tatpp)]^{2+}$ in the absence of $Zn(BF_4)_2$

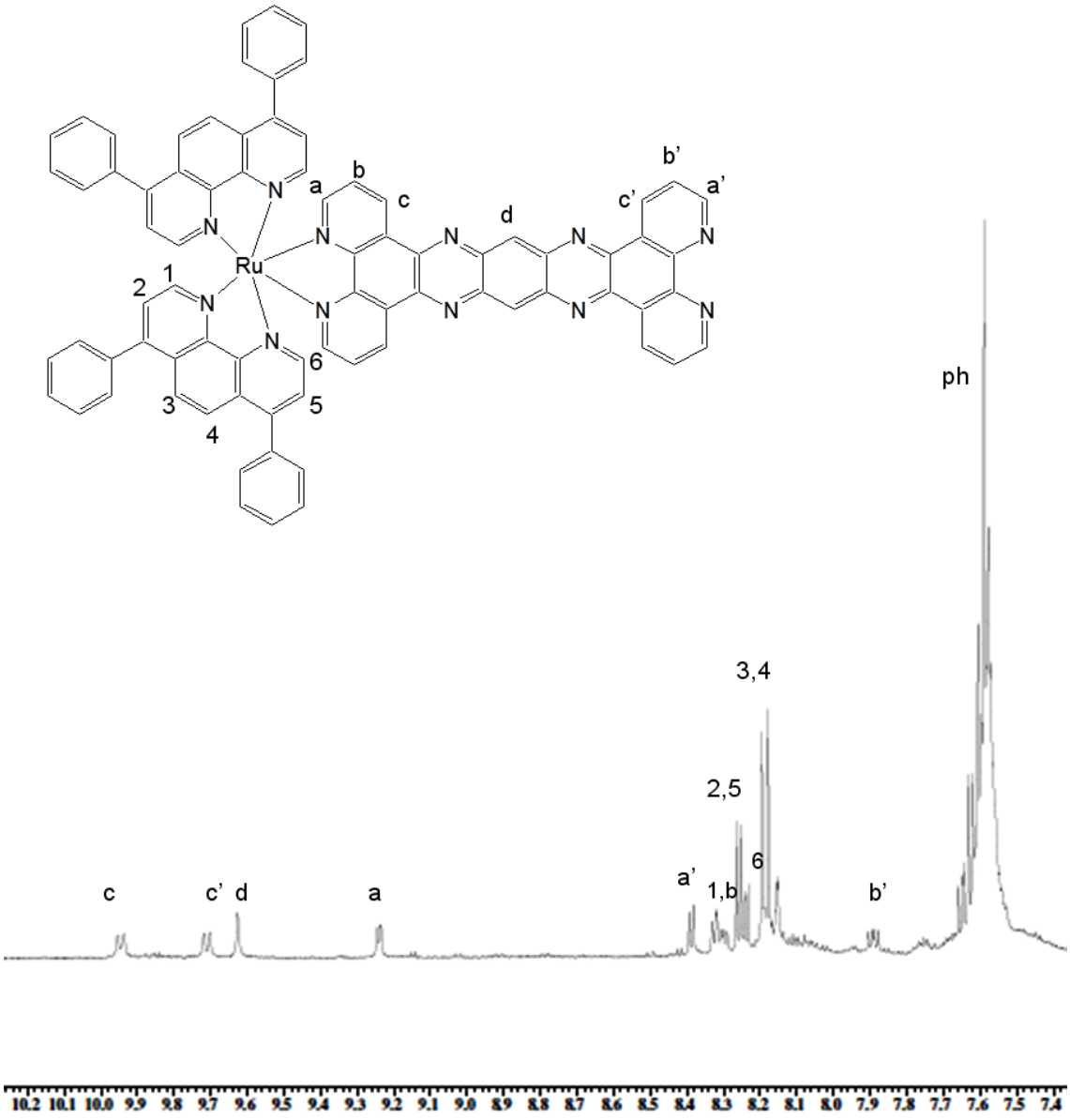


Figure 2.7: ^{1}H NMR Spectrum of $[(Ph_2phen)_2Ru(tatpp)]^{2+}$ with excess $Zn(BF_4)_2$

The peaks have been assigned comparing the ^{1}HNMR spectra of $[(phen)_2Ru(tatpp)Ru][PF_6]_2$ to that of the related tatpp dimer $[(phen)_2Ru(tatpp)Ru(phen)_2][PF_6]_4$ spectra. In these mononulcear complexes $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$ and $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$, the protons of the

ligand tatpp are of particular interest as they show two different AMX coupled sets related to the two different ends of the ligand and a singlet for the central 'benzene' protons.

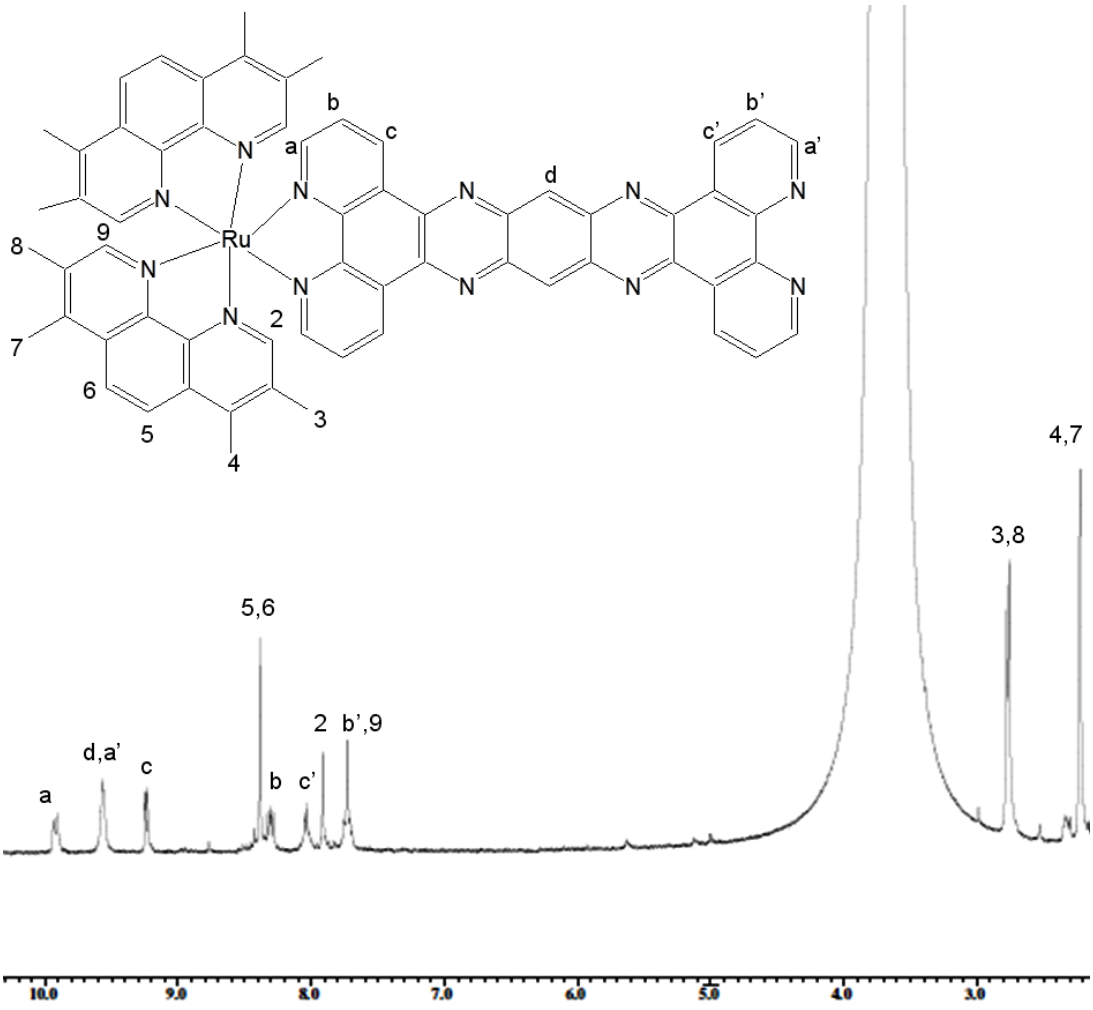


Figure 2.8: ^{1}H NMR spectrum of $[(Me_4phen)_2Ru(tatpp)]^{2+}$ with excess $Zn(BF_4)_2$, (Full NMR)

In complex $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$ the AMX set closest to the ruthenium metal (H_a, H_b, H_c) are observed at 9.23, 8.32, and 9.94 ppm whilst those on the non-coordinated end ($H_{a'}$,$H_{b'}$, $H_{c'}$) are observed at 8.38, 7.88, and 9.71 ppm. A similar downfield shift for H_a(9.25), H_b(8.32), H_c(9.95) in $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$ is observed compared to $H_{a'}$(8.05), $H_{b'}$(7.73), $H_{c'}$(9.60).

The central benzene proton (H_d) is observed at 9.62 ppm in $[(Ph_2phen)_2Ru(tatpp)][PF_6]_2$ and 9.62ppm in $[(Me_4phen)_2Ru(tatpp)][PF_6]_2$ compared to 9.50 ppm in the analogue $[(phen)_2Ru(tatpp)][PF_6]_2$ in the same solvent.

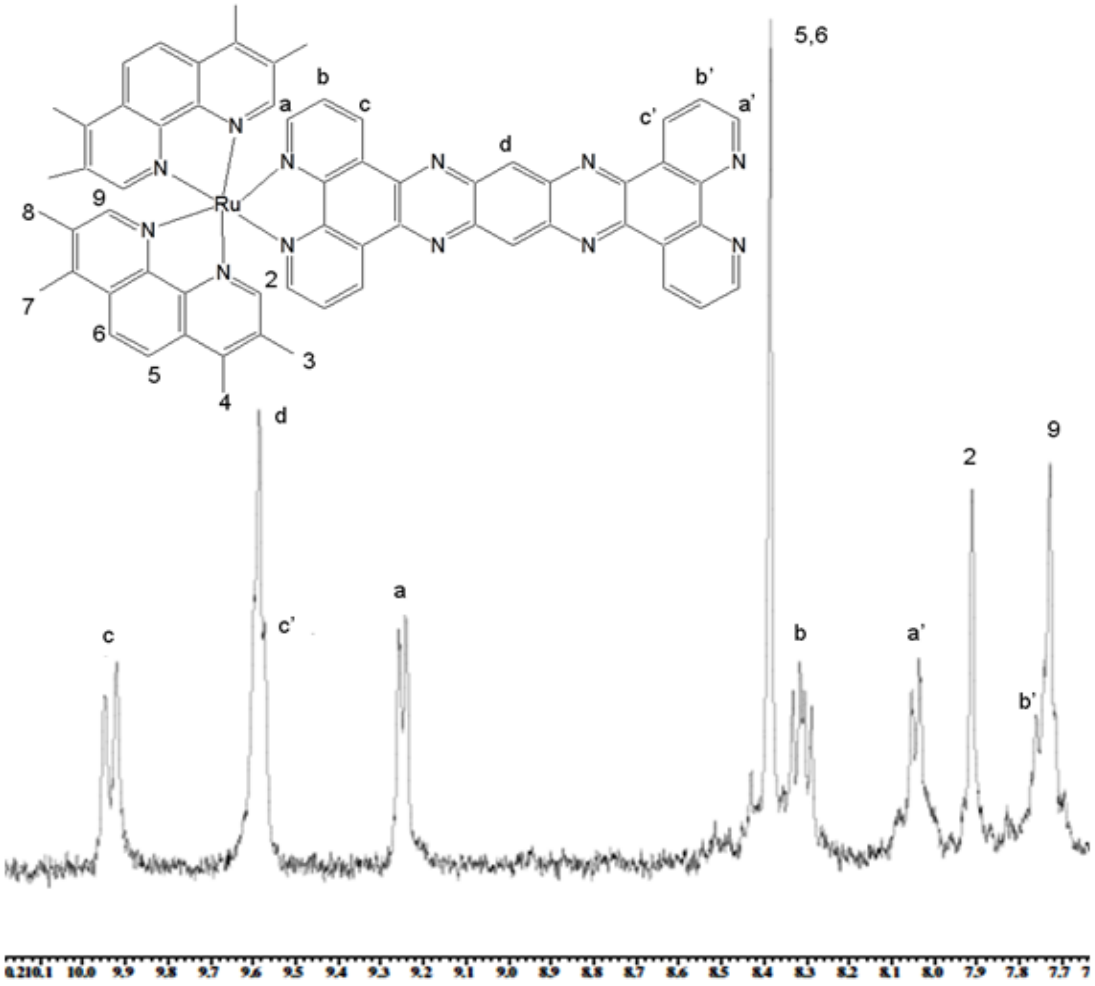


Figure 2.9: ^1H NMR Spectrum of $[(Me_4phen)_2Ru(tatpp)]^{2+}$ with excess $Zn(BF_4)_2$, (Expended down field region)

CHAPTER 3

THE EFFECT OF RUTHENIUM COMPLEXES POLYPYRIDYL LIPOPHILICITY ON THE DNA CLEAVAGE, CYTOTOXICITY AND ANIMAL TOXICITY

3.1 Introduction

Lipophilicity is defined as having an affinity for lipids where the molecules will be attracted to a lipophilic environment. Usually, lipophilicity is measured quantitatively by examining the partitioning of a compound between two immiscible liquid phases such as water and octanol. The most common method of measuring lipophilicity, the *n*-octanol–water partition coefficient (*log* $P_{O/W}$) was proposed by Fujiti *et al*. in 1964.[39] *Log P* measures the ratio of concentrations of a compound in the two phases of a mixture at equilibrium and the log value is obtained.[40]

As the lipophilicity or alternatively, the hydrophobicity of the compound increases, the *log P* value increases. Lipophilicity can be an important parameter for any potential drug candidate. Lipophilicity is an important parameter for any potential drug candidate.[17] The affinity of the drug to their receptor target is just one factor among many affecting a drugs action.[41] Other important factors that can be dependent on a drugs lipophilicity include the ability to cross the cell membrane, the ability to get into the bloodstream, and the rate of metabolism and/or clearance from the body via excretion.

In earlier studies with ruthenium polypyridyl complexes, Yadav established that larger more lipophilic ruthenium complexes were less acutely toxic to mice than the smaller, more hydrophilic ones.[29] These data were consistent with an early study by Dwyer and coworkers, that postulated that the difference in toxicity observed between Δ-$[Ru(phen)_3]^{2+}$ and Λ-$[Ru(phen)_3]^{2+}$ was related to the latter ones faster perfusion through tissue and build-up in the

bloodstream.[12] Λ-$[Ru(phen)_3]^{2+}$ was more acutely toxic even though Δ-$[Ru(phen)_3]^{2+}$ was the stronger inhibitor of acetylcholinesterase, which was attributed to the enantiomers faster build-up in the blood (peak blood concentration).[12] Given this data, we postulated that complexes with enhanced lipophilicity would be better tolerated by mice as they would be slower to be absorbed into the bloodstream. Dwyer and coworkers had also showed in other studies that the cytotoxicity of $[Ru(Me_4phen)_3]^{2+}$ was higher than that seen for $[Ru(phen)_3]^{2+}$ suggesting that more lipophilic complexes may be more cytotoxic towards malignant cells.[13]

In this chapter, we have quantified the lipophilicity of our tatpp complexes plus several related control complexes and examined the role of lipophilicity in modifying the complexes ability to cleave DNA, cytotoxicity towards malignant cultured cells, and acute toxicity towards mice. Our hypothesis is that increasing the lipophilicity of ruthenium polypyridyl complexes will not affect their DNA cleavage activity, will enhance their cytotoxicity (by aiding transfer across the cell membrane), and will reduce their acute toxicity by slowing their perfusion into the bloodstream.

3.2 Chemicals

The following ruthenium polypyridyl complexes were used in this experiment: [**MP**]Cl_2, [**P**]Cl_4, [$\mathbf{P_{Ph2}}$]Cl_4, [$\mathbf{MP_{Ph2}}$]Cl_2, [$\mathbf{P_{Me4}}$]Cl_4, [$\mathbf{MP_{Me4}}$]Cl_2, $[Ru(phen)_3]Cl_2$, $[Ru(Ph_2phen)_3]Cl_2$, $[Ru(Me_4phen)_3]Cl_2$. These complexes were synthesized in the laboratory as described previously in Chapter 2. Phosphate buffered saline (PBS) (10X) was purchased from Bio-Rad. Tris Cl, EDTA (ethylenediamintetraacetic acid), Tris-acetate, agarose, ethidium bromide, dimethyl sulfoxide (DMSO) and glutathione (GSH) were used as received from Sigma Aldrich. Supercoiled plasmid pUC18 was obtained from Bayou Biolabs. Millipore water was used to prepare all buffers. RPMI-1640 medium, 10% fetal bovine serum (FBS), Trypan blue solution, sodium bicarbonate, trypsin-EDTA (1X), FBS heat inactivated, 1.1% penicillin/streptomycin, vitamin solution (1X), and vitamin solution (100X) were purchased also from Sigma.

3.3 Cell Lines and Cultures

The cell line H358 (human non-small cell lung cancer -bronchioalveolar) line was obtained from the NCI-Frederick Cancer DCTD Tumor/Cell Line Repository sources; Dr. Gazdar (NCI-H358M). The NSCLC cell lines were cultured in RPMI-1640 medium with 10% Fetal Calf Serum at 37°C in a humidified atmosphere of 5% CO_2.

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3.4 Instrumentation

The agarose gels were analyzed using AlphaImager 2000 gel analysis system and electrophoresis was performed by FotoDyne Foto/Force 250 electrophoresis system. A single chamber count was performed on a hemacytometer.

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3.5 Experimental

3.5.1 Determination of the Partition Coefficient (*log* $P_{o/w}$)

The lipophilicity of $[Ru(Ph_2phen)_3]Cl_2$ compound was determined by using the shake-flask method with octanol and PBS at pH of 7.4. The solute (33.3×10^{-5} M) was dissolved first in the octanol and PBS, then the two saturated phases were shaken for 30 minutes at room temperature and set forth to equilibrate for 24 hours. After this period of time, the absorbance of the compound in each solvent was measured with a Hewlett-Packard HP8453A spectrophotometer. The corresponding concentration of the solute in each solvent was determined for the absorbance at the maximum wavelength of the spectra and used in calculating the partition coefficient. The partition coefficient (*P*) is the ratio of the equilibrium concentration of the dissolved compound in two phases:

$$log\ P_{o/w} = log\ ([solute]octanol\ /[solute]water)$$

The *log P* was also obtained for biphasic solution of DI water and n-octanol using the same general procedure as above.

3.5.2 DNA Cleavage Assay

A typical 1% agarose gel DNA cleavage experiment was performed in Eppendorf tubes at a total volume of 40 µL of phosphate buffer (4 mM Na_3PO_4 and 50 mM NaCl) at pH 7.35 containing 4 µL supercoiled pUC18 DNA (1 µg/1 µL, 0.154 mM DNA base pairs) and other constituents as elucidated in Table 3.1. The stock solutions for all ruthenium metal complexes were prepared by using 2% DMSO and Millipore water.

3.5.2.1 Preparation of DNA Cleavage Assay:

As listed in Table 3.1, Eppendorf tubes were filled with DNA, GSH, phosphate buffer, and the ruthenium(II) complexes successively. There were two control samples, both without the ruthenium complex; one with DNA and buffer, and one with DNA, GSH and buffer. All samples were prepared to have the same amount of DNA (4 µL) and the same total volume (40 µL). For each ruthenium complex analyzed, one Eppendorf tube contained the metal complex without GSH, and one tube contained the metal complex and GSH. Once the solution was made up the final concentrations of [DNA] = 0.154 mM, [GSH] = 0.513 mM, and [Ru complex] = 0.0128 mM.

Table 3.1: Preparation of DNA cleavage assay samples

Tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DNA	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL
GSH		8 µL		8 µL		8 µL		8 µL		8 µL		8 µL		8 µL
$[(phen)_2Ru(tatpp)Ru(phen)_2]^{4+}$			8 µL	8 µL										
$[(phen)_2Ru(tatpp)]^{2+}$					8 µL	8 µL								
$[(Dipphen)_2Ru(tatpp)Ru(Dipphen)_2]^{4+}$							8 µL	8 µL						
$[(Dipphen)_2Ru(tatpp)]^{2+}$									8 µL	8 µL				
$[(Me4phen)_2Ru(tatpp)Ru(Me_4phen)_2]^{4+}$											8 µL	8 µL		
$[(Me4phen)_2Ru(tatpp)]^{2+}$													8 µL	8 µL
Buffer	36 µL	28 µL	28 µL	20 µL	28 µL	20 µL	28 µL	20 µL	28 µL	20 µL	28 µL	20 µL	28 µL	20 µL
Total Volume	**40 µL**	**40 µL**	**40 µL**	**40 µL**	**40 µL**	**40 µL**	**40 µL**	**40 µL**	**40 µL**	**40 µL**	**40 µL**	**40 µL**	**40 µL**	**40 µL**

3.5.2.2 DNA Cleavage Reaction

Once the samples were prepared in the Eppendorf tubes they were left to incubate for 12 hours at room temperature in a dark place. The cleavage reaction was stopped by adding 3 µL sodium acetate and 80 µL ethanol to precipitate the DNA in each tube. The solutions were then kept in a -20°C refrigerator overnight. The samples were then centrifuged at 4°C at 13,000 rpm for 30 minutes. After centrifugation, the samples were vacuum dried for 30 – 60 minutes. Thereafter, 80 µL of Tris-HCl EDTA buffer (40 mM Tris-Cl, 1 mM EDTA, pH 8.0) and 12 µL loading buffer (30% glycerol in distilled water with 0.1% w/v bromophenol blue) were added to all the samples. The samples were mixed thoroughly and 6 µL of each was loaded into a well of the prepared 1% agarose gel containing 0.4 g of agarose, 40 mL of Tris-Cl EDTA buffer, and 4.0 µL ethidium bromide. The gel was subjected to electrophoresis at 60 V for 2 hours using TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). The same cleavage reaction was performed under anaerobic conditions to determine if DNA cleavage could be enhanced.

3.5.3 Cytotoxicity Assay

The effect of the following lipophilic ruthenium(II) polypyridyl complexes: [$\mathbf{P_{Ph2}}$]Cl_4, [$\mathbf{MP_{Ph2}}$]Cl_2, [$\mathbf{P_{Me4}}$]Cl_4, and [$\mathbf{MP_{Me4}}$]Cl_2 on the growth of a population of H358 cells was measured by conducting the Trypan blue assay. A hemocytometer was used to measure cell density by counting cells that were resistant to staining with Trypan blue. The cells were plated into each well of a 96-well flat-bottomed microtiter plate for 24 hours before adding a medium containing various concentrations of lipophilic ruthenium polypyridyl complexes including 0.01 µM, 0.1 µM, 1.0 µM, 10 µM, and 100 µM. Trypan blue was added to each well after 96 hours of incubation and were allowed to incubate for an additional 6 hours at room temperature. The IC_{50} of the lipophilic ruthenium complexes was measured and defined as the concentration of the complexes that inhibit cell growth by 50%.

3.5.4 Animal Toxicity

Animal study was carried out according to the protocol approved by the Institutional Animal Care and Use Committee (IACUC A08.018, approved 2/20/08). Male Balb/c mice, twelve to fourteen weeks of age, were obtained from Dr. Liping Tang's laboratory. The animals were housed in a temperature controlled room and allowed to acclimate before treatment. The following lipophilic ruthenium polypyridyl complexes, [**MP**]Cl_2, [**P**]Cl_4, [$\mathbf{P_{Ph2}}$]Cl_4, [$\mathbf{MP_{Ph2}}$]Cl_2, [$\mathbf{P_{Me4}}$]Cl_4, [$\mathbf{MP_{Me4}}$]Cl_2, were screened for acute toxicity by intraperitoneal injection where three mice were designated for each complex. One group of four mice was used as control. Stock solutions of ruthenium complexes were prepared using PBS buffer, pH 7.4 and 2% DMSO. At first a single mouse (~ 27 g) was given a single dose (90 µL) of 6.0 mg/mL (20 mg/Kg) and monitored for 24 hours to observe any toxic symptoms or death. When the mice survived the dosage, two additional mice per group were taken and the doses were escalated to 12 mg/mL (40 mg drug/Kg mouse) and monitored for 24 h. Once the mice survived the previous dosage, the doses were escalated to 18 mg/mL (60 mg/Kg) and monitored for 24 h. The last dose that the mice were given after they survived the 18 mg/mL dosage after 48 h was 24 mg/mL (80 mg/Kg) and they were monitored for 24 h.

3.6 Results and Discussion

3.6.1 Lipophilicity of Ruthenium Polypyridyl Complexes

The lipophilicity, *log* $P_{O/W}$, values for the following ruthenium polypyridyl compounds, [$\mathbf{MP_{Ph2}}$]Cl_2, [$Ru(Ph_2phen)_3$]Cl_2, [$\mathbf{P_{Ph2}}$]Cl_4, [$\mathbf{MP_{Me4}}$]Cl_2, [$Ru(Me_4phen)_3$)]Cl_2, [$\mathbf{P_{Me4}}$]Cl_4, [**MP**]Cl_2, [**P**]Cl_4, [$Ru(phen)_3$)]Cl_2 were measured under two sets of conditions. The biphasic mixture was either a mixture of water and n-octanol or a mixture of PBS buffer (pH 7.4) and n-octanol. *Log P* values obtained for both methods are listed in Table 3.2 where the compounds are listed in order of decreasing lipophilicity in the octanol/PBS system.

In general, the *log P* values in the water/octanol system are lower than those in the PBS/octanol system, which is most likely due to the lower ionic strength of the pure water system. Nonetheless, the general trends are the same in both systems. Complexes with lower bidentate cations and Ph_2phen ligands are the most lipophilic, followed by the tetradentate cations complexes with Ph_2phen ligands. Next are the bidentate cations complexes with Me_4phen ligands, followed by the tetradentate cations complexes with Me_4phen ligands. The same holds for the phen derivatives with the homoleptic $[Ru(phen)_3]^{2+}$ being the most hydrophilic of all those tested.

Our results of the lipophilicity trend agrees with the results of Barton *et al*., where they have found that the complex containing the Me_4phen ligand is intermediate in lipophilicity amid the complexes containing Ph_2phen and phen ligands. Ph_2phen had shown a much higher lipophilicity character than phen.[42] We hypothesize that the permeability of the ruthenium polypyridyl complexes into the cell may have a strong correlation with the lipophilicity of the compounds and that will have important effects on the biological activity.[43]

Table 3.2 Measuring lipophilicity of different Ru(II) complexes

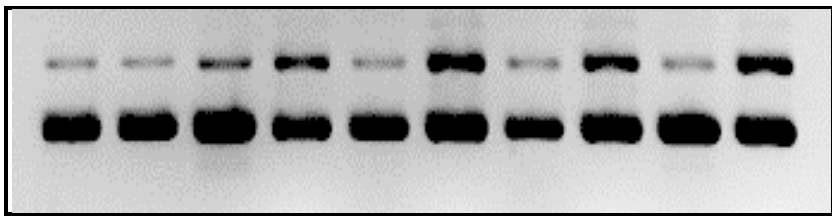
Ruthenium Complexes	**log P, PBS (pH 7.4)**	**log P, DI water**
$[(Ph_2phen)_2Ru(tatpp)][Cl_2]$	2.3	1.6
$[Ru\ (Ph_2phen)_3][Cl_2]$	1.9	1.4
$[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2][Cl_4]$	1.7	0.46
$[Ru\ (Me_4phen)_3][Cl_2]$	1.6	-0.9
$[(Me_4phen)_2Ru(tatpp)][Cl_2]$	1.5	-0.6
$[(Me_4phen)_2Ru(tatpp)Ru(Me_4phen)_2][Cl_4]$	1.0	-1.4
$[(phen)_2Ru(tatpp)][Cl_2]$	-0.4	-1.3
$[(phen)_2Ru(tatpp)Ru(phen)_2][Cl_4]$	-0.6	-1.0
$[Ru\ (phen)_3][Cl_2]$	-1.1	-1.5

3.6.2 DNA Cleavage of Ruthenium Polypyridyl Complexes

The ability of the tatpp complexes to cleave DNA was examined using DNA plasmid cleavage assay under aerobic and anaerobic conditions. Prior work had established that **P** and **MP** were DNA cleavage agents in the presence of GSH and that they showed potentiated DNA cleavage under anaerobic conditions.[28,29] The results of DNA cleavage assay with all the following ruthenium(II) polypyridyl compounds, $\mathbf{P_{Ph2}}$, $\mathbf{MP_{Ph2}}$, $\mathbf{P_{Me4}}$, $\mathbf{MP_{Me4}}$ are shown in Figure 3.1 through 3.4.

Figures 3.1 and 3.2 show the extent of DNA cleavage after 12 h incubation time under aerobic conditions. The data show that all of the complexes cleave DNA in the presence of GSH but not without GSH. For comparison purposes, **P** and **MP** were included as they have been previously shown to cleave DNA when GSH is present and serve as positive controls. The experiment demonstrates that changes to the terminal phenanthroline ligands have little to no effect on the DNA cleavage activity, which has been attributed to the tatpp ligand.

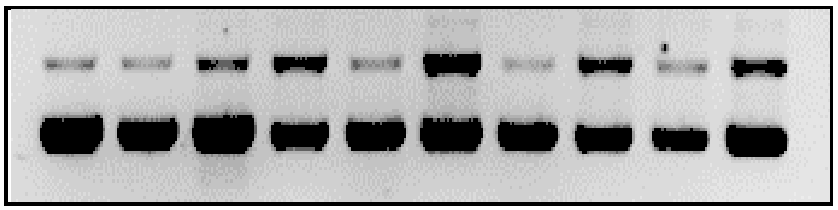
In Figure 3.3, the DNA cleavage activity of all three dinuclear Ru(II) complexes **P**, $\mathbf{P_{Ph2}}$ and $\mathbf{P_{Me4}}$ were examined and compared under anaerobic conditions. As can be seen, all three complexes show DNA cleavage activity only when GSH is present. In Figure 3.4, the same experiment was conducted on the three mononuclear complexes, **MP**, $\mathbf{MP_{Me4}}$ and $\mathbf{MP_{Ph2}}$ with qualitatively similar results, except that the extent of DNA cleavage was considerably greater for identical reaction times. Thus it is clear that while all the tatpp complexes are active under both aerobic and anaerobic conditions, the mononuclear Ru(II) complexes demonstrate greater activity.



Lane	1	2	3	4	5	6	7	8	9	10
DNA	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM
GSH		513 µM		513 µM		513 µM		513 µM		513 µM
$[P]^{4+}$			12.8 µM	12.8 µM						
$[MP]^{2+}$					12.8 µM	12.8 µM				
$[P_{Ph2}]^{4+}$							12.8 µM	12.8 µM		
$[MP_{Ph2}]^{2+}$									12.8 µM	12.8 µM

Figure 3.1: DNA cleavage by **P**, **MP**, $\mathbf{P_{Ph2}}$ and $\mathbf{MP_{Ph2}}$ under aerobic conditions

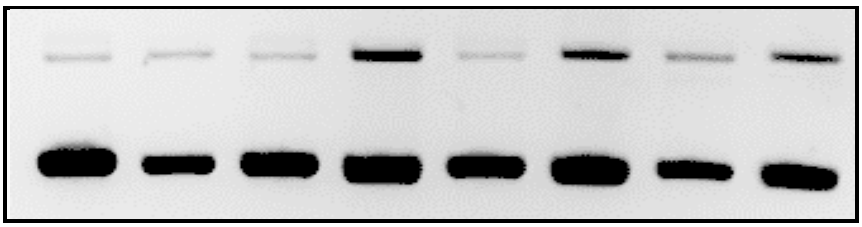
DNA cleavage assay exhibiting conversion of supercoiled pUC18 plasmid DNA (0.154 mM bp) to circular DNA upon treatment with 0.0128 mM of ruthenium complexes **P**, **MP**, $\mathbf{P_{Ph2}}$ and $\mathbf{MP_{Ph2}}$ with and without 0.513 mM GSH under aerobic conditions at 20°C for 12 h in phosphate buffer (4 mM Na_3PO_4 and 50 mM NaCl) at pH 7.35.



Lane	1	2	3	4	5	6	7	8	9	10
DNA	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM
GSH		513 µM		513 µM		513 µM		513 µM		513 µM
$[P]^{4+}$			12.8 µM	12.8 µM						
$[MP]^{2+}$					12.8 µM	12.8 µM				
$[P_{Me4}]^{4+}$							12.8 µM	12.8 µM		
$[MP_{Me4}]^{2+}$									12.8 µM	12.8 µM

Figure 3.2: DNA cleavage by **P**, **MP**, $\mathbf{P_{Me4}}$ and $\mathbf{MP_{Me4}}$ under aerobic conditions

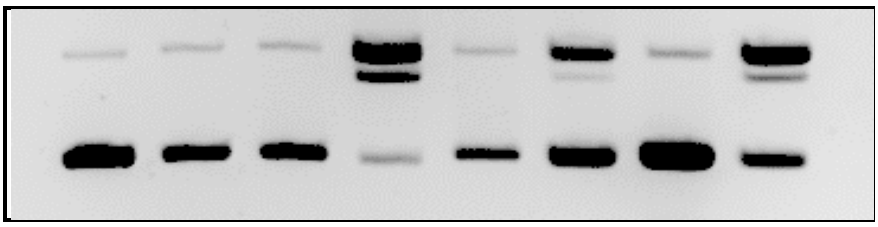
DNA cleavage assay exhibiting conversion of supercoiled pUC18 plasmid DNA (0.154 mM bp) to circular DNA upon treatment with 0.0128 mM of ruthenium complexes **P**, **MP**, $\mathbf{P_{Me4}}$ and $\mathbf{MP_{Me4}}$ with and without 0.513 mM GSH under aerobic conditions at 20°C for 12 h in phosphate buffer (4 mM Na_3PO_4 and 50 mM NaCl) at pH 7.35.



Lane	1	2	3	4	5	6	7	8
DNA	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM
GSH		513 µM		513 µM		513 µM		513 µM
$[P]^{4+}$			12.8 µM	12.8 µM				
$[P_{Ph2}]^{4+}$					12.8 µM	12.8 µM		
$[P_{Me4}]^{4+}$							12.8 µM	12.8 µM

Figure 3.3: DNA cleavage by **P**, $\mathbf{P_{Ph2}}$ and $\mathbf{P_{Me4}}$ under anaerobic conditions

DNA cleavage assay exhibiting conversion of supercoiled pUC18 plasmid DNA (0.154 mM bp) to circular DNA upon treatment with 0.0128 mM of ruthenium complexes **P**, $\mathbf{P_{Ph2}}$ and $\mathbf{P_{Me4}}$ with and without 0.513 mM GSH under anaerobic conditions at 20°C for 12 h in phosphate buffer (4 mM Na_3PO_4 and 50 mM NaCl) at pH 7.35.



Lane	1	2	3	4	5	6	7	8
DNA	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM	154 µM
GSH		513 µM		513 µM		513 µM		513 µM
$[MP]^{2+}$			12.8 µM	12.8 µM				
$[MP_{Ph2}]^{2+}$					12.8 µM	12.8 µM		
$[MP_{Me4}]^{2+}$							12.8 µM	12.8 µM

Figure 3.4: DNA cleavage by **MP**, **MP_{Ph2}** and **MP_{Me4}** under anaerobic conditions

DNA cleavage assay exhibiting conversion of supercoiled pUC18 plasmid DNA (0.154 mM bp) to circular DNA upon treatment with 0.0128 mM of ruthenium complexes **MP**, **MP_{Ph2}** and **MP_{Me4}** with and without 0.513 mM GSH under anaerobic conditions at 20°C for 12 h in phosphate buffer (4 mM Na_3PO_4 and 50 mM NaCl) at pH 7.35.

3.6.3 Cytotoxicity in Cancer Cells

The cytotoxicity of the lipophilic complexes relative to each other was examined in cultured H358 cells. As seen in Figure 3.4, their cytotoxicity is similar but not identical. The IC_{50} of dinuclear complexes, $\mathbf{P_{Ph2}}$ and $\mathbf{P_{Me4}}$ is approximately 10 μM while the IC_{50} of $\mathbf{MP_{Ph2}}$ and $\mathbf{MP_{Me4}}$ is approximately 70 μM. Thus in contrast to the DNA cleavage data, the dinuclear complexes are the more cytotoxic. **P** and **MP** containing redox-active tatpp bridging ligand have shown high cytotoxicity toward H358 malignant cell line with an IC_{50} values about 15 μM and 13 μM respectively.[29] $\mathbf{P_{Ph2}}$ and $\mathbf{P_{Me4}}$ have shown enhanced cytotoxicity against cancerous cell line compared to **P** and **MP**. This result supports our hypothesis that as the lipophilicity of **P** and **MP** increase, the cytotoxicity increases.

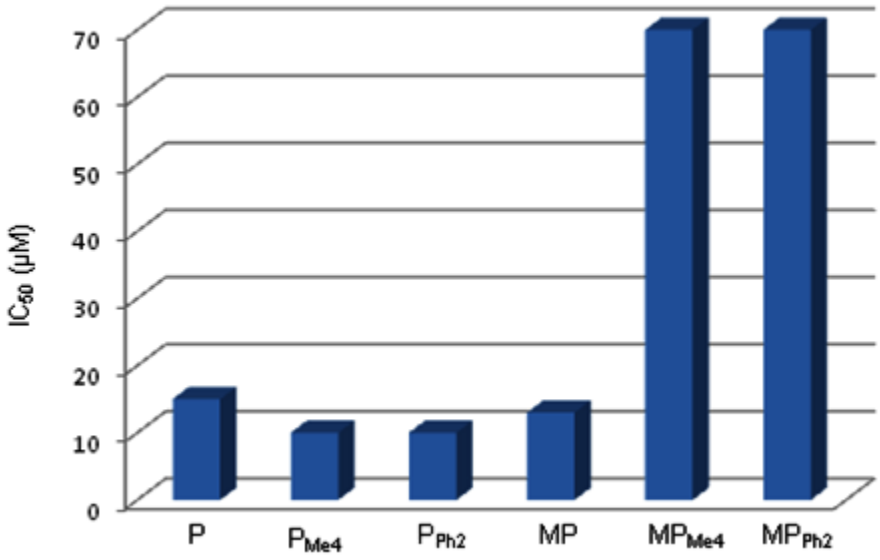


Figure 3.5: Cytotoxicity towards non-small cell lung carcinoma cell line (H358)

3.6.4 Animal Toxicity of Lipophilic Ruthenium Polypyridyl Complexes

The maximum tolerable dose (MTD) of these tatpp complexes was examined in Balb/c mice. The data in Table 3.3 clearly supports our hypothesis that as the lipophilicity of the ruthenium polypyridyl complexes such as **P** and **MP** increase, the animal toxicity decreases. In 2008, Yadav have studied the maximum tolerable dose MTD (mg/Kg) for **P** and **MP** complexes where it was found to be 67 mg/Kg for C57 BL/6 male mice.[29] Our results show that $\mathbf{P_{Ph2}}$, $\mathbf{P_{Me4}}$, $\mathbf{MP_{Me4}}$, $\mathbf{MP_{Ph2}}$ and **P** have higher MTD than **MP** complex in Balb/c mice. There were signs of systemic toxicity including sickness and morbidity by **MP** after treatment with 40 mg/Kg where the animal was sacrificed. The difference in activity between $\mathbf{MP_{Ph2}}$, $\mathbf{MP_{Me4}}$, and **MP** cations complexes is most likely due to difference in penetration. $\mathbf{MP_{Ph2}}$ and $\mathbf{MP_{Me4}}$ are significantly more lipophilic than **MP** as shown before in the partition coefficient experiment. This data suggests that these lipophilic ruthenium polypyridyl complexes are not toxic for animals after the dosage of 80 mg/Kg.

Table 3.3: Maximum tolerable dose (mg/Kg) for Ru(II) polypyridyl complexes administered to Balb/c mice

Compound	Maximum tolerable dose (mg/Kg)
P	>80 mg/Kg
MP	40 mg/Kg
$\mathbf{P_{Ph2}}$	>80 mg/Kg
$\mathbf{MP_{Ph2}}$	>80 mg/Kg
$\mathbf{P_{Me4}}$	>80 mg/Kg
$\mathbf{MP_{Me4}}$	> 80 mg/Kg

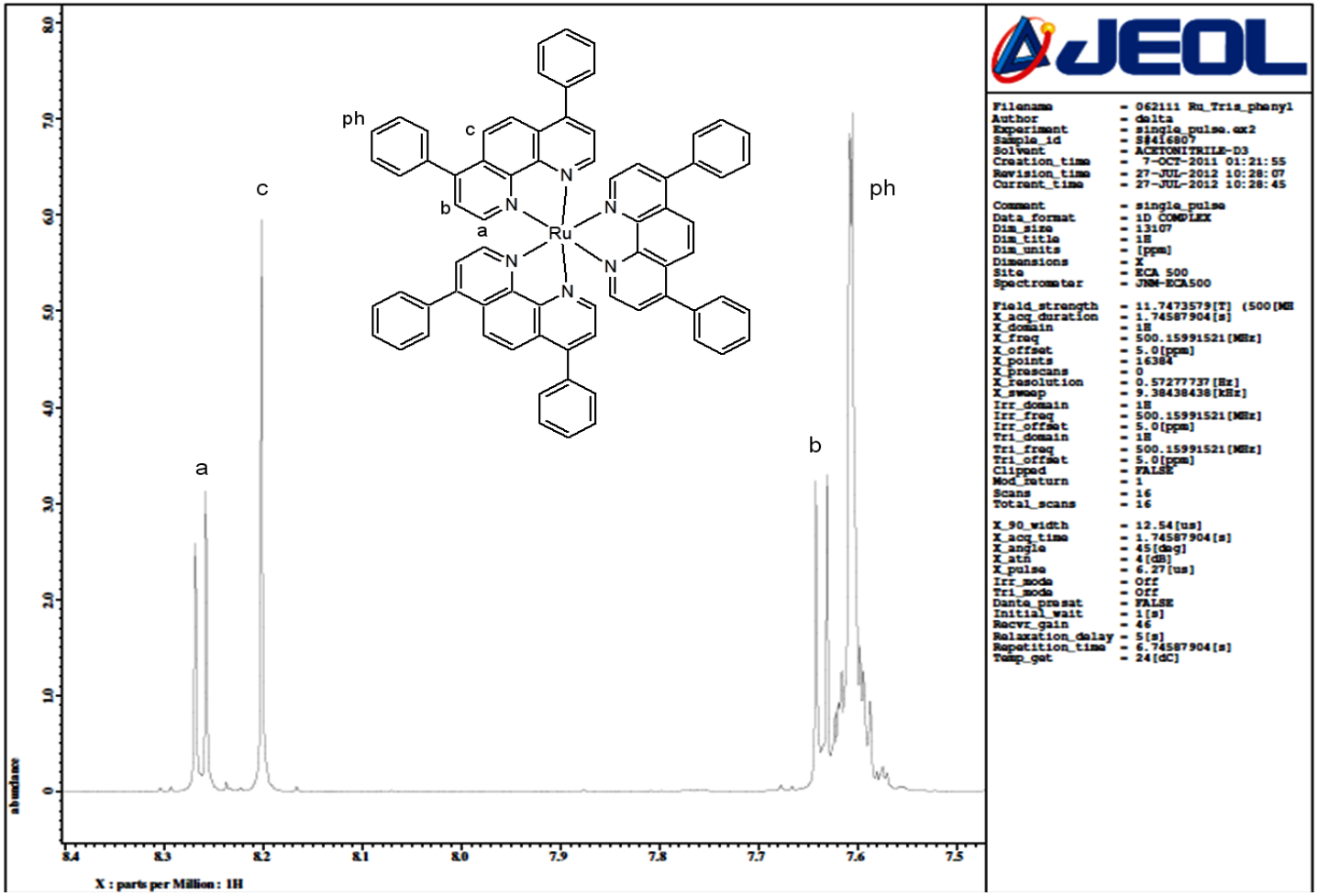
3.7 Conclusions

We have investigated novel lipophilic ruthenium-tatpp polypyridyl complexes. These complexes; $\mathbf{P_{Ph2}}$, $\mathbf{P_{Me4}}$, $\mathbf{MP_{Ph2}}$, and $\mathbf{MP_{Me4}}$ were synthesized and characterized based on the **P** and **MP** structures. It has been found that, these lipophilic complexes have DNA cleavage activity under aerobic and anaerobic conditions. The cytotoxicity study against H358 cell line have revealed that the most promising activity was shown by $\mathbf{P_{Me4}}$ and $\mathbf{P_{Ph2}}$ with an IC_{50} value of about 10 μM. The animal toxicity of theses RPCs decreased as the lipophilicity of the ancillary ligands increased. It was found that these lipophilic RPCs are not toxic for animals after the dosage of 80 mg/Kg and that may due to a relatively slower rate of diffusion of these RPCs into the blood stream. From these data, it is clear that the combination of ruthenium-tatpp complexes and the lipophilic ancillary phenanthroline ligands had beneficial effect by increasing cytotoxicity and decreasing animal toxicity.

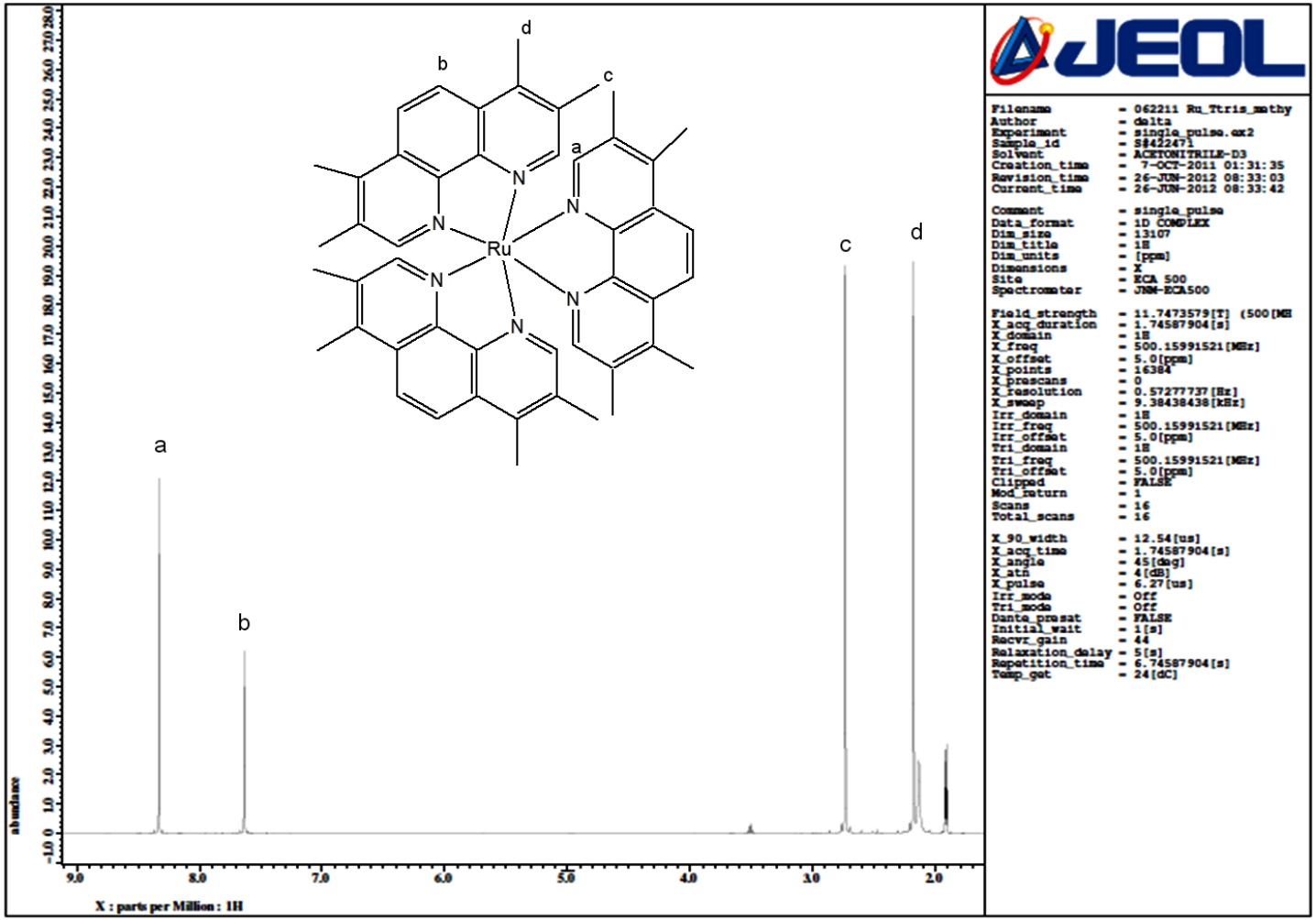
In further anticancer activity studies, the cytotoxicity of these novel lipophilic ruthenium - tatpp polypyridyl complexes will be determined against different human melanoma cell lines. As well, these complexes will be examined for their capability to slow or stop tumor progression in xenograft human carcinoma model in nude mice.

APPENDIX A

^{1}H NMR of Ruthenium Polypyridyl Complexes



^{1}H NMR $[Ru(Ph_2phen)_3]^{2+}$



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Solvent	= ACETONITRILE-D3
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Revision_time	= 26-JUN-2012 08:33:03
Current_time	= 26-JUN-2012 08:33:42
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Dim_title	= 1H
Dim_units	= [ppm]
Dimensions	= X
Site	= ECA 500
Spectrometer	= JNM-ECA500
Field_strength	= 11.7473579[T] (500[MH
X_acq_duration	= 1.74587904[s]
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X_freq	= 500.15991521[MHz]
X_offset	= 5.0[ppm]
X_points	= 16384
X_prescans	= 0
X_resolution	= 0.57277737[Hz]
X_sweep	= 9.38438438[kHz]
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Irr_freq	= 500.15991521[MHz]
Irr_offset	= 5.0[ppm]
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Tri_freq	= 500.15991521[MHz]
Tri_offset	= 5.0[ppm]
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X_atn	= 4[dB]
X_pulse	= 6.27[us]
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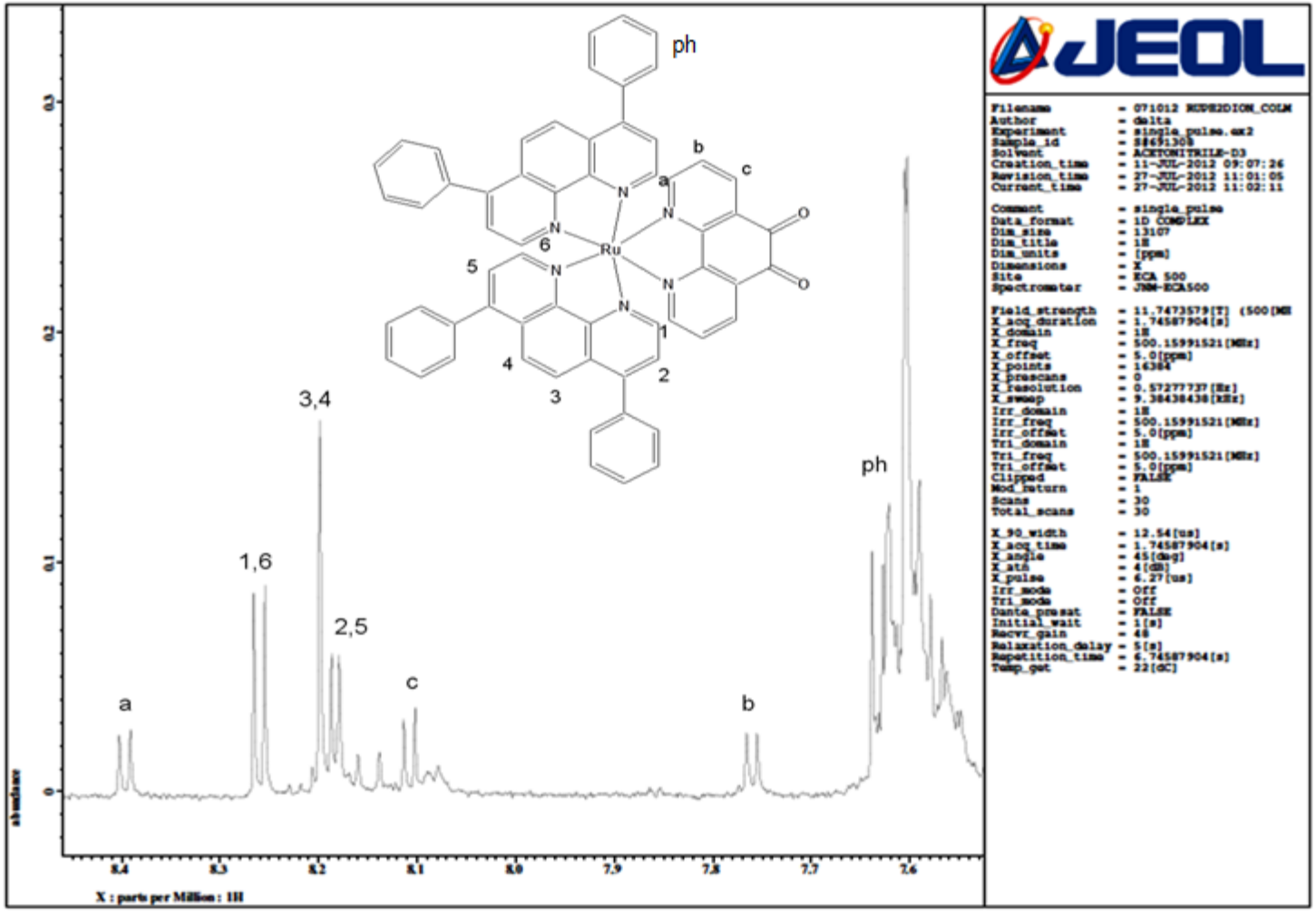
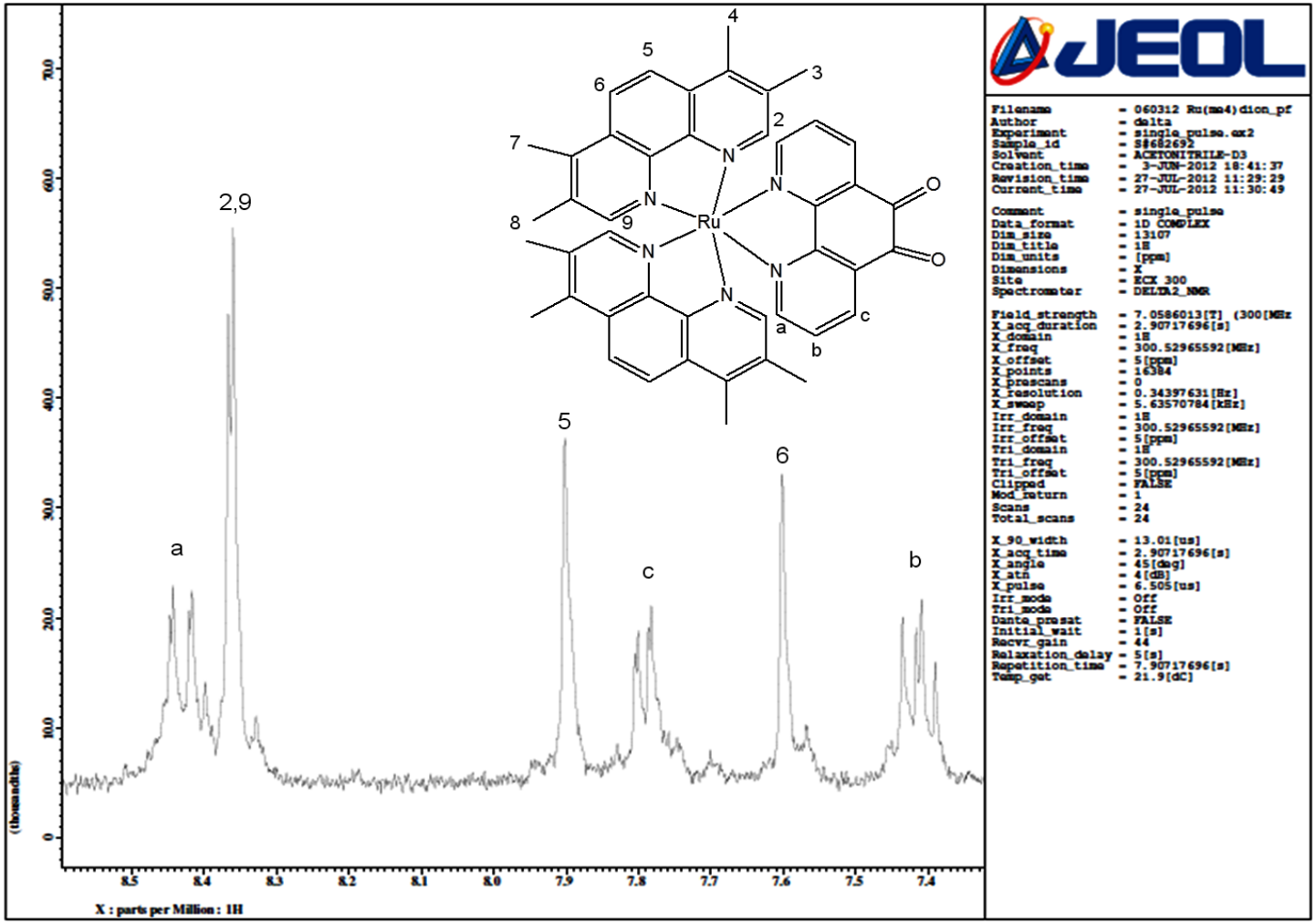
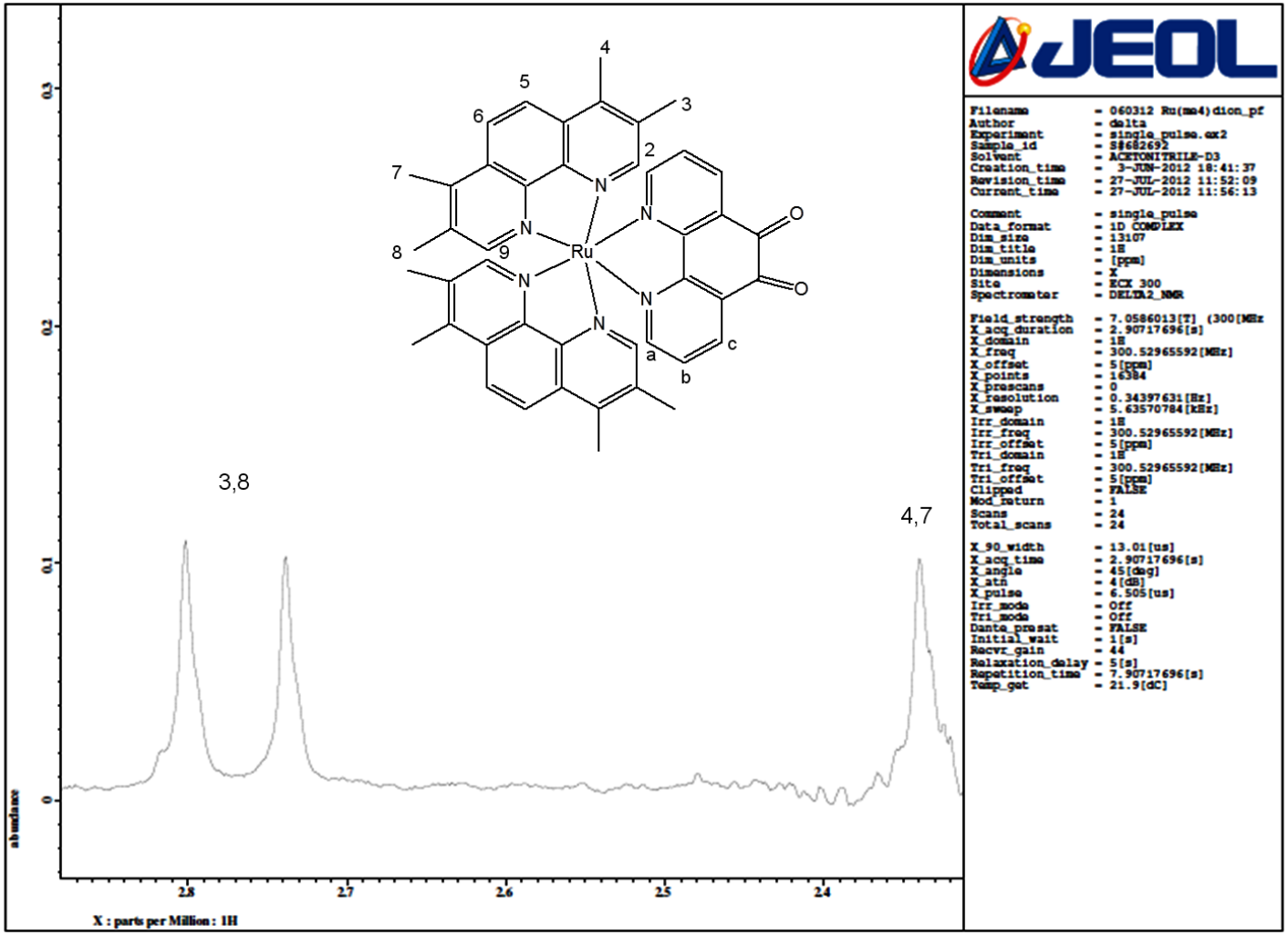
^{1}H NMR of $[Ru(Me_4phen)_3]^{2+}$



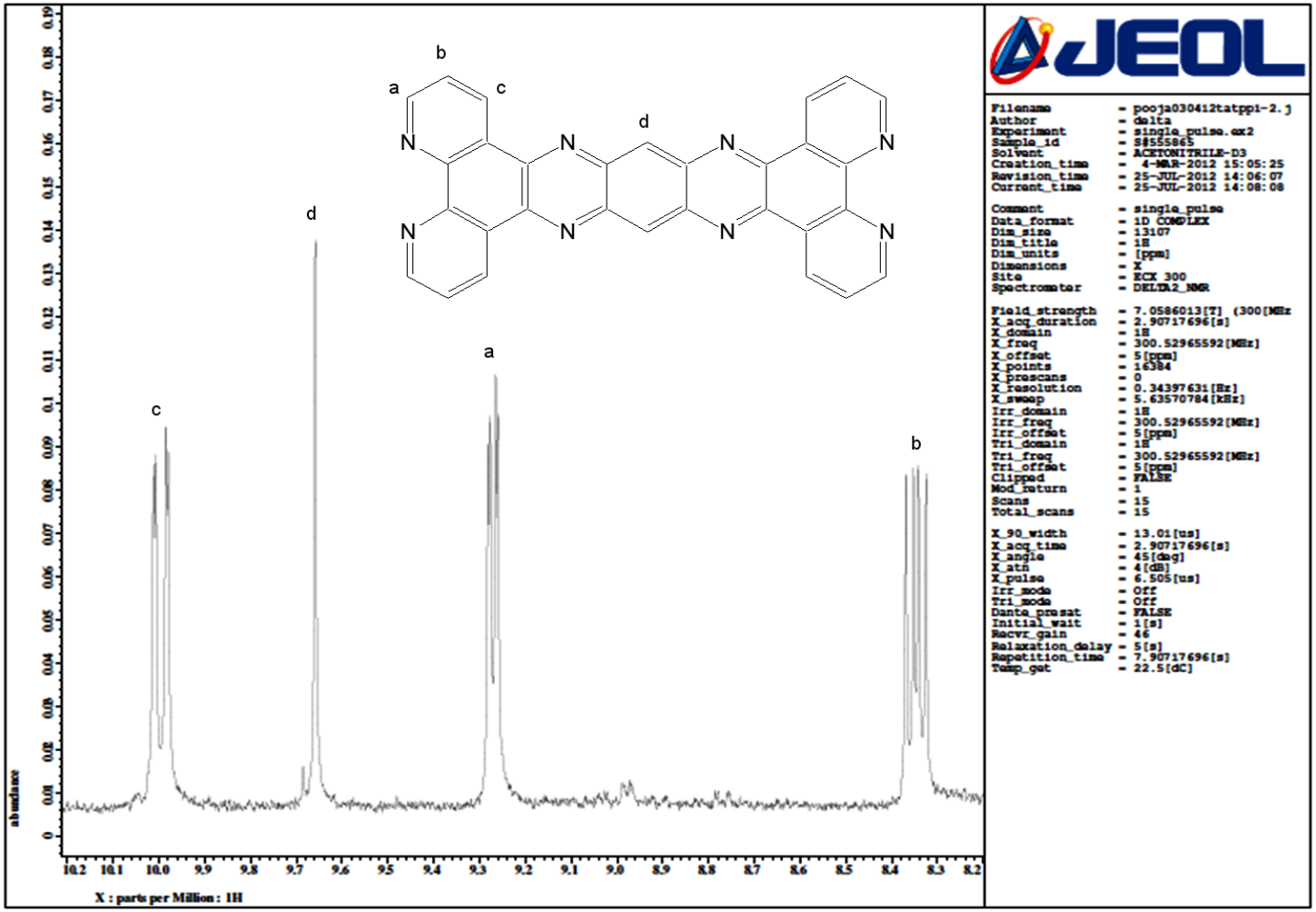
^{1}H NMR of [Ru(Ph$_2$phen)$_2$phendione]$^{2+}$



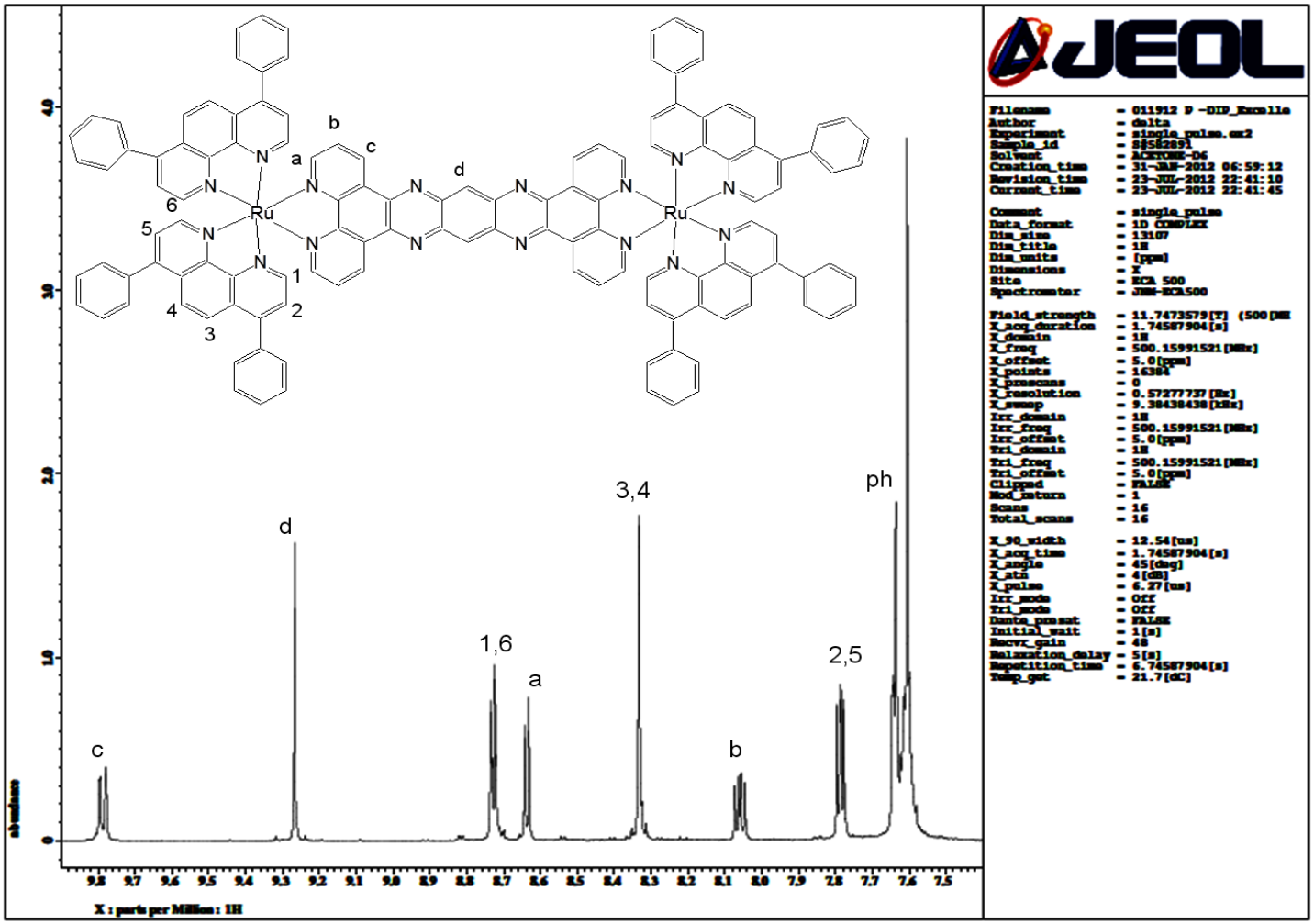
^{1}H NMR of [Ru(Me$_4$phen)$_2$phendione]$^{2+}$, (Downfield region)



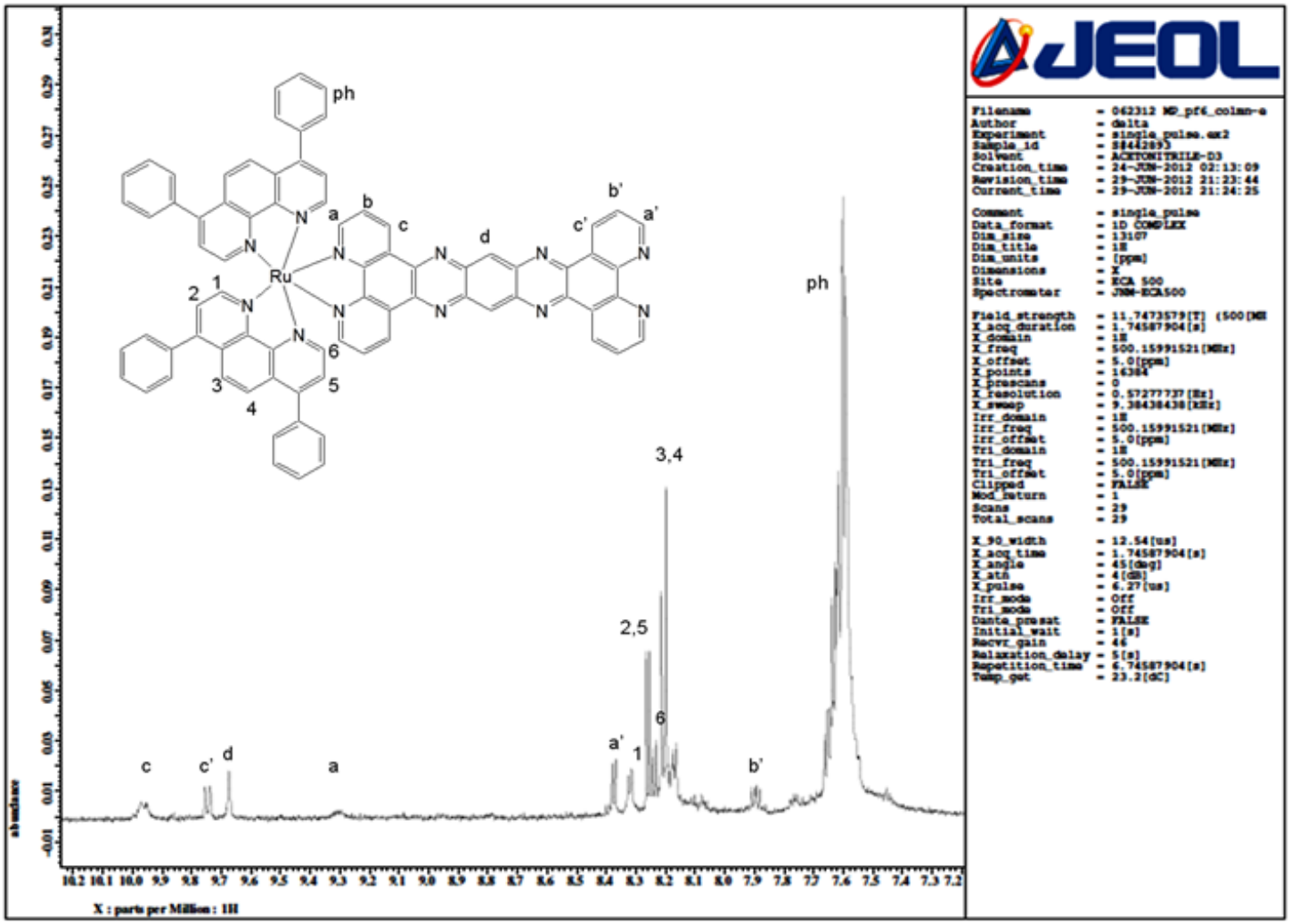
^{1}H NMR of [Ru (Me_4phen)$_2$phendione]$^{2+}$, (Upfield region)



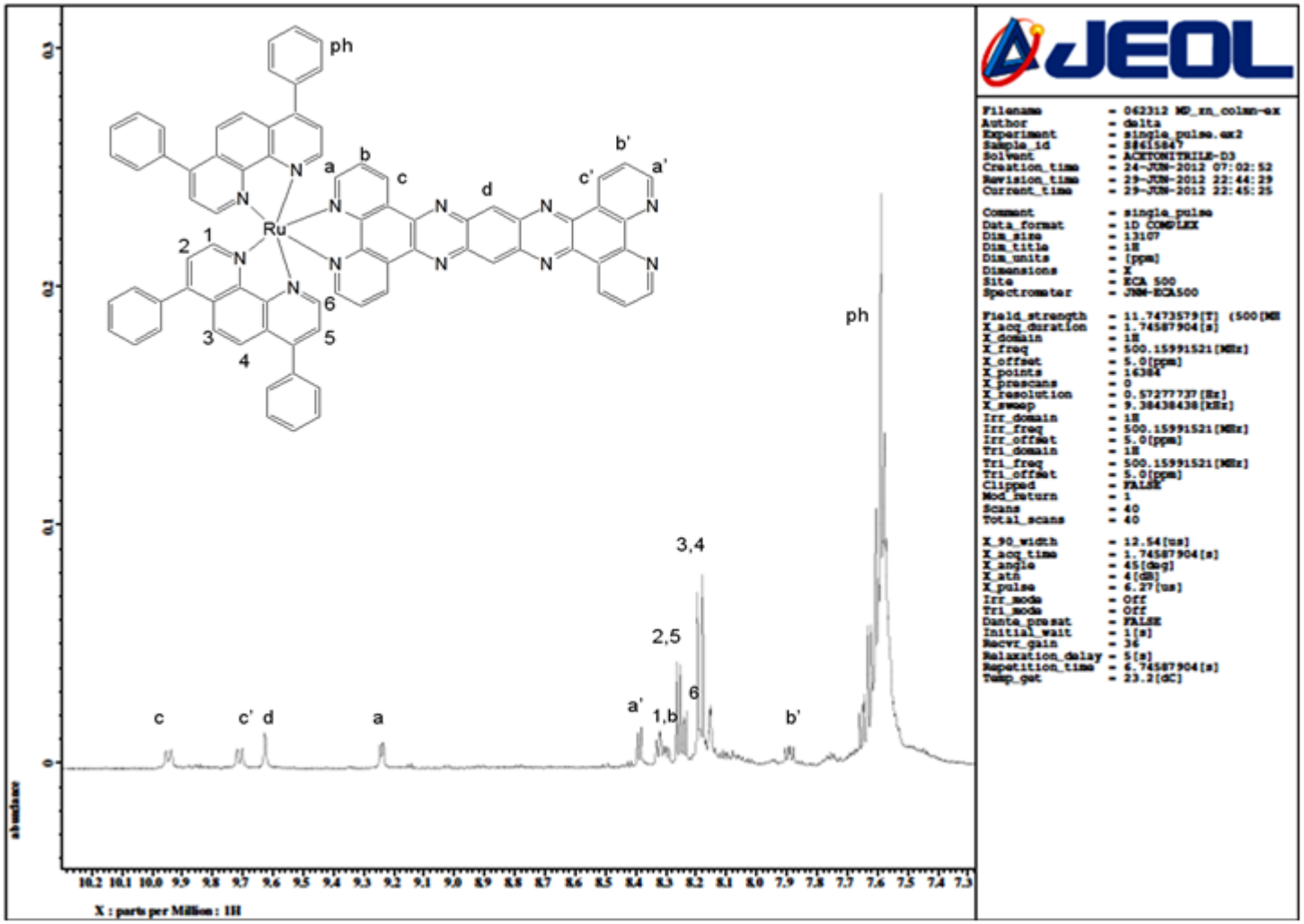
^{1}H NMR of Tatpp



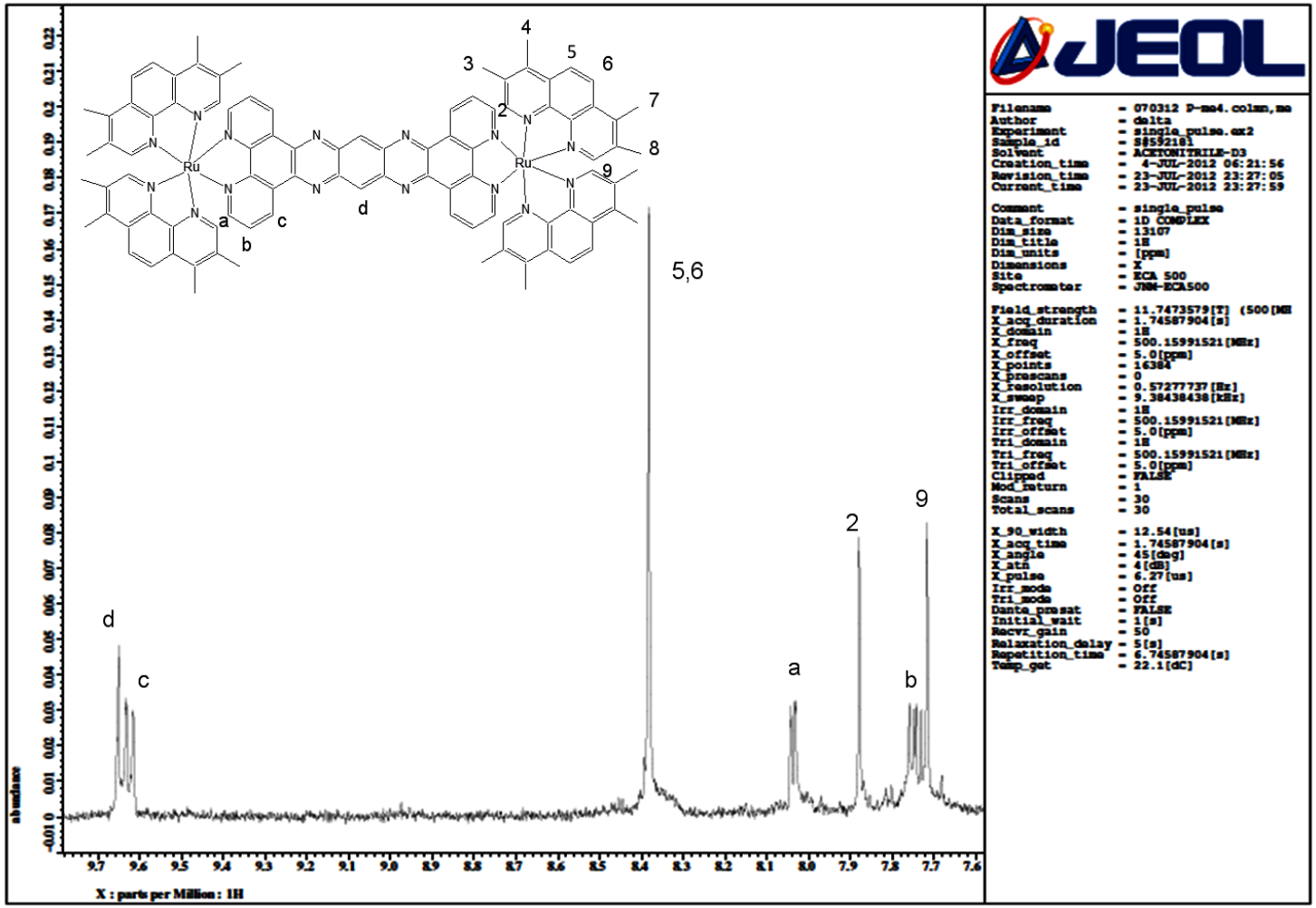
^{1}H NMR of $[(Ph_2phen)_2Ru(tatpp)Ru(Ph_2phen)_2]^{4+}$



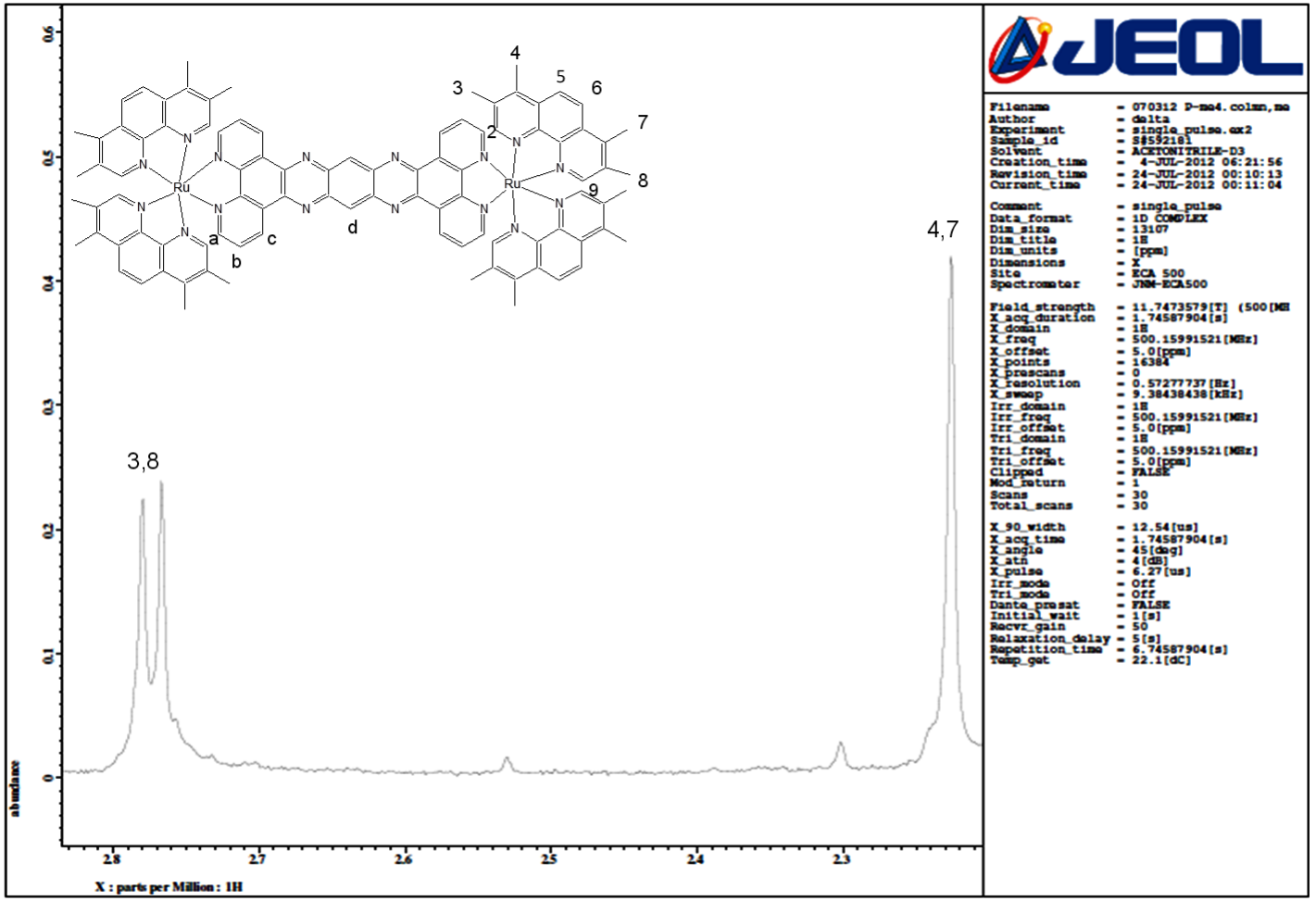
^{1}H NMR of [(Ph$_2$phen)$_2$Ru(tatpp)]$^{2+}$



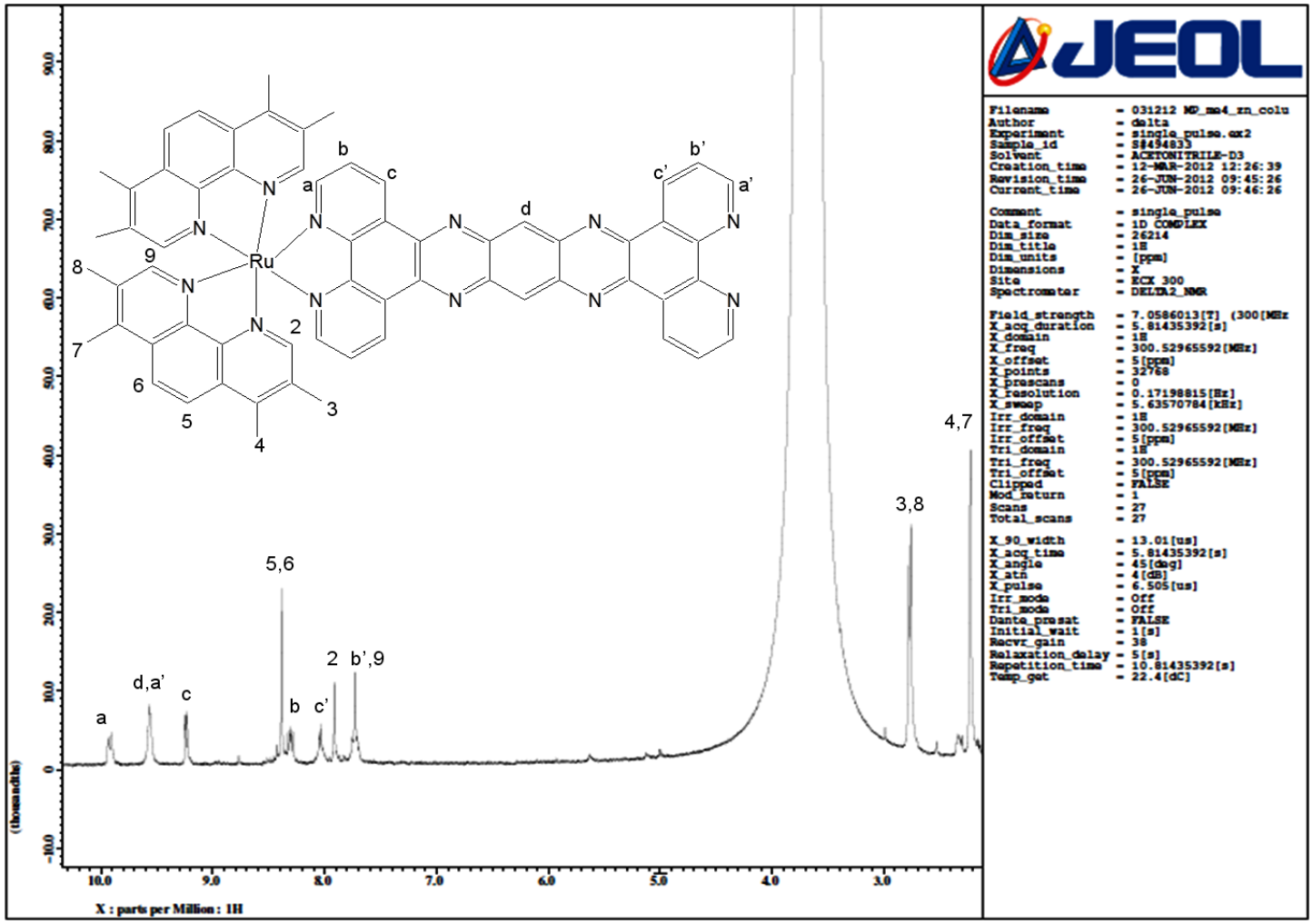
^{1}H NMR of $[(Ph_2phen)_2Ru(tatpp)]^{2+}$ with excess $Zn(BF_4)_2$



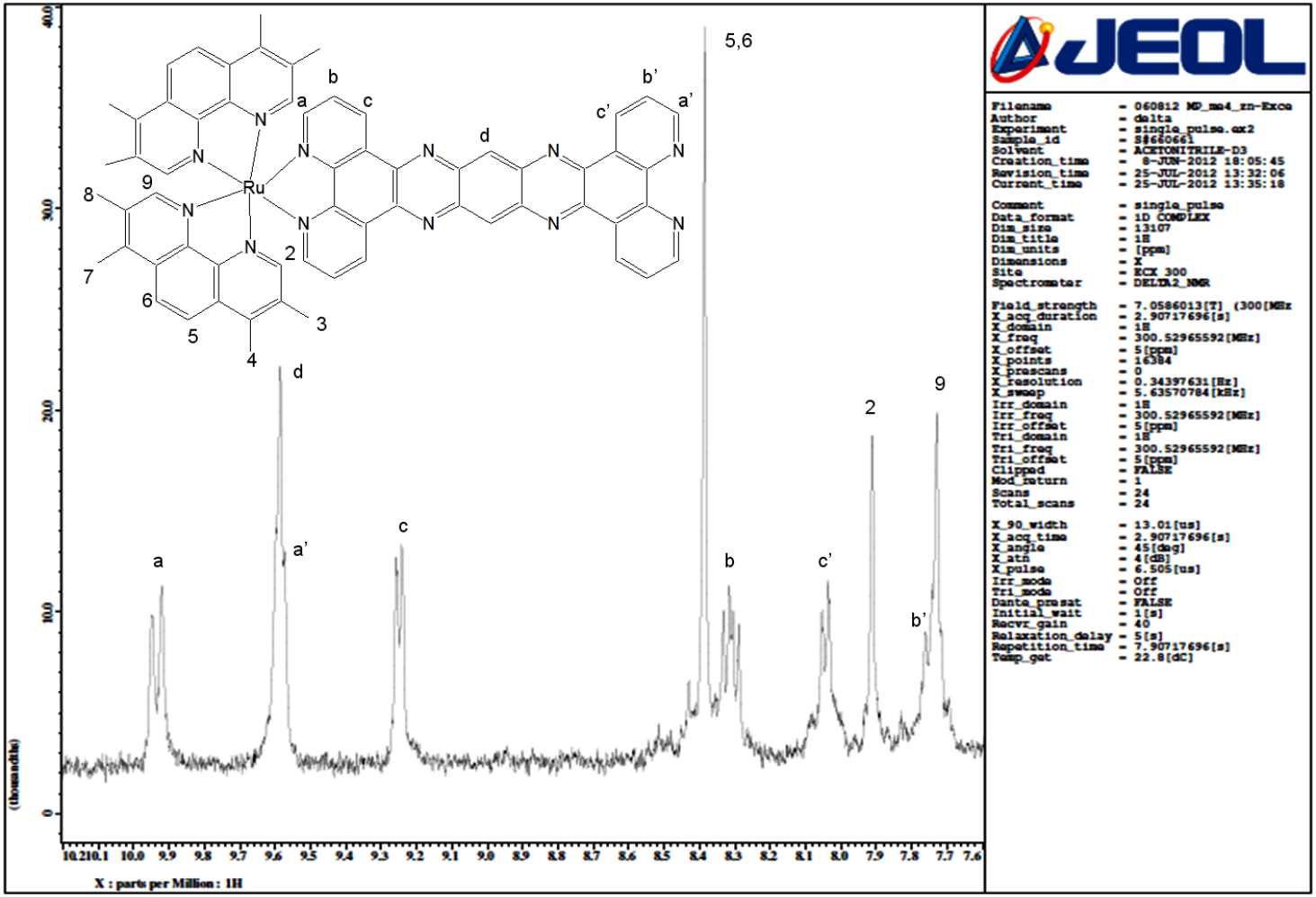
^{1}H NMR of [(Me4phen)$_2$Ru(tatpp)Ru(Me4phen)$_2$]$^{4+}$, (Downfield region)



^{1}H NMR of $[(Me4phen)_2Ru(tatpp)Ru(Me4phen)_2]^{4+}$, (Upfield Region)



^{1}H NMR of $[(Me4phen)_2Ru(tatpp)]^{2+}$ with excess $Zn(BF_4)_2$



^{1}H NMR of $[(Me4phen)_2Ru(tatpp)]^{2+}$ with excess $Zn(BF_4)_2$

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BIOGRAPHICAL INFORMATION

Nagham Alatrash was born and grew up in Syria. After finishing high school in Syria, she moved to the United States and attended the University of Texas Pan American majoring in Chemistry with a minor in Biology. She was a 2008 LSAMP scholar and was involved in undergraduate research under the supervision of Dr. Hassan Ahmad. With Dr. Ahmad, she investigated the effect of green tea polyphenols in cancer prevention. She continued her research in the Spring of 2009 as a Biochemistry Research Assistant and served as a lab manager in Dr. Ahmad's research laboratory.

Nagham received her B.S. degree from the University of Texas Pan American in May 2009. In the summer of 2010, she worked as a Cardiothoracic Research Assistant at Rhode Island Hospital. Nagham started her graduate studies in Fall 2010 in the Department of Chemistry at University of Texas at Arlington. Her research advisor was Prof. Frederick MacDonnell.

Nagham Alatrash graduated from University of Texas at Arlington with a Master in Chemistry in 2012.