PHOTOCHEMICAL AND SPECTROSCOPIC STUDIES OF RU(II) COMPLEXES AS POTENTIAL PHOTODYNAMIC THERAPY AGENTS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

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2010

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ABSTRACT

Cisplatin is an anticancer drug used in the treatment of various cancers. However, cisplatin is toxic towards both healthy and tumor cells alike, resulting in several undesirable side effects. Moreover, some of the most aggressive cancers develop resistance to cisplatin. Photodynamic therapy (PDT) uses light to localize activation of otherwise non-toxic compounds in tumor tissue. Current PDT agents achieve toxicity by the photosensitization of highly reactive singlet oxygen through energy transfer from an excited state. However, this need for the presence of molecular oxygen represents a disadvantage since malignant and drug resistant cells are often hypoxic. To address the drawbacks of cisplatin and PDT drugs as antitumor agents, a combined approach has been made with the development of several photoactive Ru(II) complexes that produced with antitumor activity under irradiation. This blend of cisplatin mimetic metal complexes, inorganic photochemistry and photodynamic therapy has led to the discovery of several photo-activated ruthenium complexes that bind DNA in a manner similar to cisplatin. This new class of compounds is referred to as photo-cisplatin analogs.

A successful PDT agent should possess high molar absorbtivity at long wavelengths where tissue penetration is greatest and there is low absorption by biomolecules. A series of Ru(II) complexes were synthesized with the deprotonated forms of the ligands 8-hydroxyquinoline (quo\(^-\)) and 5-NO\(_2\)-8-hydroxyquinolate (5-NO\(_2\)-
quo⁻) as analogs to the prototypical complex \([\text{Ru(bpy)}_3]^{2+}\) (bpy = 2, 2'–bipyridine) in order to red shift absorption of the new complexes into the optimized PDT window. Electrochemistry, spectroscopy and density functional theory calculations were utilized to investigate the electronic tuning of the occupied \(t_{2g}\)-type orbitals of the metal center with variation in the ligation sphere. The maximum of the lowest energy absorption of complexes containing one, two and three 8-quinolate ligands progressively red shifts from 452 nm in \([\text{Ru(bpy)}_3]^{2+}\) to 510 nm in \([\text{Ru(bpy)}_2(\text{quo})]^+\), 515 nm in \([\text{Ru(bpy)}(\text{quo})_2]\), and 540 nm in \([\text{Ru}(\text{quo})_3]^-\) in water. This bathochromic shift results from the increase in energy of the occupied \(t_{2g}\)-type orbital across the series afforded by coordination of each subsequent quo⁻ ligand to the Ru(II) center. TD-DFT calculations along with electrochemical analysis reveals that the lowest energy transition has contributions in the HOMO from both the quo⁻ ligand and the metal, such that the lowest energy transition is not from an orbital that is purely metal-centered in character as in \([\text{Ru(bpy)}_3]^{2+}\).

The photolabile complex \(\text{cis-}[\text{Ru(phpy})(\text{phen})(\text{CH}_3\text{CN})_2]^+\) (4, phpy⁻ = 2-phenylpyridine, phen = 1,10-phenanthroline) was investigated as a potential photodynamic therapy (PDT) agent. A low energy transition assigned as Ru-phen MLCT was observed with a broad tail extending into the PDT window (600 – 850 nm). Irradiation of 4 with long wavelengths (\(\lambda_{\text{irr}} \geq 630\) nm) resulted in photoinduced ligand exchange of the monodentate acetonitrile ligands with quantum yield (\(\Phi_{\text{Cl}}\)) of 0.25 (\(\lambda_{\text{irr}} = 450\) nm) for the photosubstitution by chloride in \(\text{CH}_2\text{Cl}_2\) to generate \([\text{Ru(phpy)(phen)(CH}_3\text{CN)Cl}]\). This value is similar to that previously reported for the
photosubstitution by chloride in CH$_2$Cl$_2$ in cis-[Ru(bpy)$_2$(CH$_3$CN)$_2$] $^{2+}$ (3, bpy= 2, 2’-bipyridine) $\Phi = 0.31$ ($\lambda_{irr} = 430$ nm). A dependence of the quantum yield of ligand exchange on the wavelength of irradiation was observed for 4. Selective irradiation into the Ru-phen $^1$MLCT ($\lambda_{irr} = 500$ nm) of 4 results in more efficient ligand substitution ($\Phi = 0.25$) as compared to irradiation into the Ru-phpy $^1$MLCT peak ($\lambda_{irr} = 450$ nm; $\Phi =0.08$) for chloride in CH$_2$Cl$_2$. It is generally accepted that ligand dissociation occurs from the M–L ($\sigma^*$) antibonding metal centered $^3$LF state(s). Therefore, a small energy gap between the lowest energy Ru→phen $^3$MLCT state(s) and $^3$LF state is proposed. In addition, the lower quantum yield observed for irradiation in to the Ru→phpy $^3$MLCT is believed to result from the rapid deactivation of the excited species through strong metal-ligand orbital overlap between phpy$^-$ and the metal center.
Dedicated to David.
ACKNOWLEDGEMENTS

The work presented in this dissertation would not have been possible without the valued discussions, contributions and support of my mentors, colleagues, friends and family. I discovered my passion for chemistry at Georgia Southern University in the classroom of Dr. Michele Davis-McGibony. A short time later I joined her research group and began to develop my laboratory skills. Her early inspiration and guidance through the world of science encouraged me to explore areas of chemistry I never had considered. Because of Michele metals and their role in biochemistry became my first research interest.

Soon after graduation from Georgia Southern, I began my graduate career at The Ohio State University in the lab of Dr, Claudia Turro. Claudia’s talents as a scientist and educator have won my immense admiration, respect, and gratitude. She challenges those around her to think critically, be creative and develop as scientists. Her dedication to students is evident, and I am thankful for having benefited from her guidance. Thank you for teaching me how to express my ideas and showing me how to become a stronger scientist and a more thoughtful writer. I couldn’t have asked for a better mentor over the past five years and I will cherish your friendship and guidance always.

I extend my sincere gratitude to all previous Turro group members whose research has laid the foundation for the work presented here. To my Turro group contemporaries,
Scott Burya, Nicole Dickson, Robert Garner, Nick Leed, Yao Liu, Alicia Palmer, Carly Reed, Mark Sgambellone, Yujie Sun, and David Turner, I am indebted for countless discussions and a memorable lab experience. It has been a pleasure working with each of you. Lauren Joyce, your companionship and friendship have enriched my journey. Five years of sharing our workspace, our thoughts, our worries and our triumphs has meant more to me than I can say.

My research has benefited tremendously from the collegiality and generosity of scientific collaborators. The cooperative support of Dr. Judith Gallucci and Dr. Tanya Young made possible the identification of complexes studied in this work. Dr. Randolph Thummel and Maya Ojaimi from the University of Houston kindly have provided assistance in the synthesis of ruthenium complexes.

Finally, I would like to thank my family and friends. You inspired me to go further and higher than I thought I could. Your support for me through this experience has supplied the confidence I needed to succeed, and that means the world to me. Vanessa, it makes me so happy to call you sister. Your love and encouragement are constants in a hectic world. Momma, you are the benchmark for my life. You are the strongest person I know and it is your courage and strength that assures me that I can do anything. I strive daily to make you proud. Mema, every day I miss you more and wish that you had been able to see your first-born grandson pass this milestone. I know that you are here with me and that I have made you proud. To my husband, David Dennis, you are the butter to my bread and breath to my life. I couldn’t imagine life without you in it. If I have had any success at all it is because I have had you at my side. Your love, encouragement and
support has made my life so much richer and I have the greatest honor, to call you my husband and my friend.
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PUBLICATIONS

Sears, R. Bryan, Joyce, Lauren; Maya Ojaimi, Judith Gallucci Thummel, Randolph; Turro, Claudia. “Wavelength Dependent Quantum Yield of Photoinduced Ligand Loss in [Ru(phpy)(phen)(CH$_3$CN)$_2$]” Journal of Inorganic Biochemistry. (manuscript in preparation)

Sears, R. Bryan; Joyce, Lauren; Turro, Claudia. “Electronic Tuning of t$_2$g Orbitals by 8-quinolate Ligands in Ruthenium Complexes Photochem Photobio. 2010.

FIELD OF STUDY

Major Field: Chemistry

Division: Multidisciplinary (Inorganic/Biological)
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapters</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>vi</td>
</tr>
<tr>
<td>Vita</td>
<td>ix</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xiv</td>
</tr>
<tr>
<td><strong>Chapters</strong></td>
<td></td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>References</td>
<td>7</td>
</tr>
<tr>
<td>2. Background</td>
<td>8</td>
</tr>
<tr>
<td>2.1. Photophysical Properties of Ruthenium(II) polypyridyl Complexes</td>
<td>8</td>
</tr>
<tr>
<td>2.2. Photosubstitution reactions in Ruthenium(II) Complexes</td>
<td>13</td>
</tr>
<tr>
<td>2.3 Cisplatin</td>
<td>20</td>
</tr>
<tr>
<td>2.4. Photodynamic Therapy</td>
<td>25</td>
</tr>
<tr>
<td>2.5 Photo-cisplatin analogs</td>
<td>28</td>
</tr>
<tr>
<td>References</td>
<td>34</td>
</tr>
<tr>
<td>3. Experimental Methods</td>
<td>42</td>
</tr>
<tr>
<td>3.1. Materials</td>
<td>42</td>
</tr>
<tr>
<td>3.2. Synthesis</td>
<td>43</td>
</tr>
<tr>
<td>3.3. Instrumentation</td>
<td>52</td>
</tr>
<tr>
<td>3.3 Methods</td>
<td>52</td>
</tr>
<tr>
<td>References</td>
<td>59</td>
</tr>
<tr>
<td>4. Electronic Tuning of Ruthenium Complexes by 8-quinolate Ligands</td>
<td>61</td>
</tr>
<tr>
<td>4.1. Introduction</td>
<td>61</td>
</tr>
<tr>
<td>4.2. Results and Discussion</td>
<td>69</td>
</tr>
<tr>
<td>4.2.1. Electronic Absorption</td>
<td>69</td>
</tr>
<tr>
<td>4.2.2. Electrochemistry</td>
<td>75</td>
</tr>
<tr>
<td>4.2.3. Calculations</td>
<td>79</td>
</tr>
</tbody>
</table>

x
LIST OF TABLES

Table 2.1 Quantum yield of Photoanation, MLCT maxima, emission maxima, and $E_{1/2}^{(II/III)}$ for the series cis-[Ru(bpy)$_2$(X)(Y)]$^{n+}$ in dichloromethane with t-butylammonium chloride ............................................. 16

Table 2.2 Lowest energy absorption maximum ($\lambda_{abs}$), wavelength of irradiation ($\lambda_{irr}$) and Photosubstitution Quantum Yields ($\Phi_{NH_3}$) in amine and for water ($\Phi_L$) in water for [Ru(NH$_3$)$_3$(L)]$^{2+}$ ................................................................. 18

Table 2.3 Absorbance maxima and Quantum Yield of Photosubstitution for cis-[Rh$_2$(μ-O$_2$C$_2$H)$_2$(CH$_3$CN)$_4$(H$_2$O)$_2$]$^{2+}$ in aqueous solution at various irradiation wavelengths ......................................................... 32

Table 2.4 Cytotoxicity for cisplatin, photofrin and Rh$_2$(μ-O$_2$C$_2$H)$_2$(CH$_3$CN)$_6$]$_2$ with Hs-27 skin cells in the dark and irradiated. LC$_{50}$ values given in μM ...... 33

Table 2.1 Absorption maxima and electrochemical potentials for 1 – 5 .............. 70

Table 2.2 Electrochemical and Emission maxima for [Ru(bpy)$_2$]$^{2+}$, [Ru(bpy)$_2$(5-NO$_2$-quo)]$, [Ru(bpy)$_2$(quo)]$, [Ru(bpy)$_2$(l-COO-iqu)]$, [Ru(bpy)$_2$(3-COO-iqu)]$^{n+}$ .................................................. 76

Table 2.3 Lowest Energy Transition (MLCT), potential difference of metal oxidation and ligand reduction ($\Delta E_{\text{redox}}$), and calculated R value from eq. 4.2 ...... 78

Table 4.1 TD-DFT major transitions for [Ru(bpy)$_2$(quo)]$^+$ ........................................ 85

Table 4.2 TD-DFT major transitions for [Ru(bpy)(quo)$_2$] ........................................ 85

Table 4.3 TD-DFT major transitions for [Ru(quo)]$^-$ ............................................. 86

Table 4.4 TD-DFT major transitions for [Ru(bpy)$_2$(5-NO$_2$-quo)]$^+$ ........................... 86
Table 4.8  \([\text{Ru(bpy)}_3]^{2+}\) MO orbital contributions .......................................................... 90
Table 4.9  \(\text{Ru(bpy)}_2(\text{quo})^+\) MO orbital contributions ................................................. 91
Table 4.10 \([\text{Ru(bpy)}(\text{quo})_2]\) MO orbital contributions ......................................................... 91
Table 4.11 \([\text{Ru(quo)}_3]^+\) MO orbital contributions ............................................................ 92
Table 4.12 \([\text{Ru(bpy)}_2(5-\text{NO}_2-\text{quo})]^+\) MO orbital contributions ........................................ 92
Table 5.1  \(\text{IC}_{50}\) values [\(\mu\text{M}\)] for a series of cycloruthenated complexes compared with the antitumor agent cisplatin ................................................................. 104
Table 5.2  Photophysical measurements for complexes 1 – 4 ............................................. 107
Table 5.3  Crystallographic data for \([\text{Ru(phpy)}(\text{phen})(\text{CH}_3\text{CN})_2](\text{PF}_6)\) .................. 113
Table 5.4  Atomic coordinates ( \(x 10^4\)) and equivalent isotropic displacement parameters (Å\(^2 x 10^3\)) for \([\text{Ru(phpy)}(\text{phen})(\text{CH}_3\text{CN})_2](\text{PF}_6). U(\text{eq})\) is defined as one third of the trace of the orthogonalized \(U_{ij}\) tensor……114-115
Table 5.5  Bond lengths (Å) for \([\text{Ru(phpy)}(\text{phen})(\text{CH}_3\text{CN})_2](\text{PF}_6)\) ......................... 116
Table 5.6  Calculated and Experimental Bond Lengths for Complex 4 ................................. 118
Table 5.7  Comparison of \(\text{LC}_{50}\) values for samples protected for light and after irradiation .......................................................... 142
LIST OF FIGURES

Figure 1.1  Schematic diagram showing the activation energies for the reaction of the ground state to products, $E_A^{GS}$, and that for the excited state, $E_A^{ES}$       2

Figure 1.2  Relative energy state diagram showing the $S_0$, $S_1$, and $T_1$ states and the rate constants for various conversion pathways including internal conversion ($k_{nr}^S$), fluorescence ($k_F^S$), excited state singlet reaction ($k_{rea}^S$), intersystem crossing ($k_{ISC}$), thermal deactivation from the triplet state ($k_{nr}^T$), phosphorescence ($k_P^T$), and excited state triplet reaction ($k_{rea}^T$)     4

Figure 2.1  Simplified molecular orbital diagram for [Ru(bpy)$_3$]$^{2+}$                        9

Figure 2.2  Jablonski diagram for the ground and excited state processes of [Ru(bpy)$_3$]$^{2+}$ including fluorescence ($k_F^S$), intersystem crossing (ISC), phosphorescence ($k_P^T$), thermal equilibrium of $^3$MLCT and $^3$LF states ($k_1$, $k_2$) and ligand dissociation                                                                 12

Figure 2.3  Sequential aquation of the chloride ligands of cisplatin resulting in the formation of the active species $cis$-[Pt(NH$_3$)$_2$(OH$_2$)$_2$]$^{2+}$, along with subsequent deprotonation steps                                                        22

Figure 2.4  Schematic representation of the formation of cisplatin–ds-DNA 1,2 intrastrand crosslinks with adjacent guanines (65%) and guanine:adenine (25%)                                                                 23

Figure 2.5  The molecular structure of hematoporphyrin                                                                 26

Figure 2.6  Schematic representation of the production of $^1$O$_2$ by typical sensitizer (S) PDT agents, where $^1$S represents the molecule in its ground state, $^1*$S represents the singlet excited state, and $^3*$S is the triplet excited state of the molecule following intersystem crossing                                                                 27

Figure 3.1  MALDI mass spectroscopy of [Ru(bpy)$_2$(quo)]$^+$                                                      44

Figure 3.2  $^1$H NMR spectra of aromatic region for [Ru(bpy)$_2$(quo)]$^+$                                                 45
Figure 3.3  MALDI mass spectroscopy of [Ru(bpy)(quo)₂] ..............................................46
Figure 3.4  ¹H NMR spectra of aromatic region for [Ru(bpy)(quo)₂] ..........................47
Figure 3.5  MALDI mass spectroscopy of [Ru(quo)₃]⁻ .................................................48
Figure 3.6  ¹H NMR spectra of aliphatic region for [Ru(phpy)(phen)(CH₃CN)]⁺ ......51
Figure 3.7  ¹H NMR spectra of aromatic region for [Ru(phpy)(phen)(CH₃CN)]⁺ ......51
Figure 4.1  Simplified molecular orbital and state diagrams of [Ru(bpy)₃]²⁺ and [Ru(bpy)₂(quo)]⁺ showing the decrease in energy of MLCT transition with the introduction of oxygen in the coordination sphere of ruthenium (II) pseudo octahedral environment .........................................................62
Figure 4.2  Molecular orbital diagram for ruthenium (II) pseudo octahedral environment with non-innocent ligand (NIL) .................................................................65
Figure 4.3  Molecular structure of 2,2'-bipyridine (bpy), 1,10-phenanthroline(phen), 8-hydroxyquinolate (quo⁻), and 5-nitro-8-hydroxyquinolate (5-NO₂-quo⁻) ......................................................................................................................67
Figure 4.4  Electronic absorption (—), excitation (••; λₑₓc = 760) nm and emission (—·; λₑₘ = 500) nm spectra of 2 (30 µM) in 4:1 ethanol/methanol glass at 77 K ........................................................................................................67
Figure 4.5  Electronic Absorption spectrum of [Ru(quo)₃] before (—) and after (—) treatment with NaBH₄ ........................................................................................................71
Figure 4.6  Energy well diagram showing the relative energies of ground state (GS), ¹MLCT, ³MLCT and ³LF in (a) [Ru(bpy)₂(quo)]⁺ and (b) [Ru(bpy)₂(5-NO₂-quo)]⁺ .............................................................................................................74
Figure 4.7  DFT calculated molecular orbital diagrams of 1 – 4 with alignment of the bpy(π*) orbitals due to a similar reduction potential across the series ....80
Figure 4.8  Calculated frontier occupied molecular orbitals of 1 – 4 (isovalue = 0.04).................................................................................................................................81
Figure 4.9  Comparison of the calculated molecular orbital diagrams of 2 and 5 showing selected MOs (isovalue = 0.04) ...............................................................82
Figure 4.10 Calculated and experimental electronic absorption spectra of (a) 2, (b) 3, (c) 4, (d) 5 in acetonitrile .........................................................................................84
Figure 5.1  Scheme showing the photoinduced ligand exchange of 1 – 3 with water where $X = \text{NH}_3$, py, and CH$_3$CN ................................................................. 103

Figure 5.2  Electronic absorption (---), excitation (○○; $\lambda_{\text{em}} = 720$ nm and emission (-----; $\lambda_{\text{exc}} = 500$ nm spectra of 4 (60 µM) in dichloromethane at room temperature ......................................................... 109

Figure 5.3  Temperature dependent emission ($\lambda_{\text{exc}} = 500$ nm of 4 (60 µM) in acetonitrile 0°C to 60°C ............................................................................. 110

Figure 5.4  ORTEP diagram of cationic 4 (solvent, H atoms and the counterion (PF$_6^-$) have been omitted for clarity). Ellipsoids represent a 50% probability .......................................................... 112

Figure 5.5  Calculated molecular orbital diagrams of 4 showing from the bottom HOMO-2, HOMO-1, HOMO, LUMO, and LUMO+1 ........................................ 119

Figure 5.6  DFT calculated frontier molecular orbital of 4. Projected with iso value = 0.004 ................................................................................................................ 120

Figure 5.7  Overlay of absorption spectra and calculated singlet electronic transitions for 4 in CH$_2$Cl$_2$.................................................................................. 121

Figure 5.8  Comparison of shifts in the aliphatic region of $^1$H NMR spectra in CD$_2$Cl$_2$ of [Ru(phpy)(CH$_3$CN)$_4$] and 4 ................................................................. 124

Figure 5.9  Changes in the aliphatic region of the $^1$H NMR spectra during photolysis of 4 in CD$_2$Cl$_2$ with 3 equivalents of TBACl........................................... 125

Figure 5.10  Changes in the aromatic region of the $^1$H NMR spectra during photolysis of 4 in CD$_2$Cl$_2$ with 3 equivalents of TBACl ........................................... 126

Figure 5.11  Changes in the aliphatic region of the $^1$H NMR spectra during photolysis of 4 in CD$_2$Cl$_2$ with 3 equivalents of phen ........................................... 127

Figure 5.12  Changes in the aromatic region of the $^1$H NMR spectra during photolysis of 4 in CD$_2$Cl$_2$ with 3 equivalents of phen ........................................... 128

Figure 5.13  Absorption spectra of 4 (66 µM) upon photolysis ($\lambda_{\text{irr}} \geq 420$ nm) in pyrdine for 0s, 30s, 60s, 90s 120s, 150s, 180s, 210s, 240s, 270s and 300s. .................................................................................. 130
Figure 5.14  (a) Absorption spectra of 4 (66 µM) upon photolysis ($\lambda_{\text{irr}} \geq 420$ nm) in CH$_2$Cl$_2$ with excess TBACl for 0 s, 10 s, 30 s, 40 s, 50 s, 60 s and 120 s. (b) Change in Absorbance vs. Time for 4 at 460 nm (○) and 565 nm (□) .......................................................... 131

Figure 5.15  Absorption spectra of 4 (240 µM) in the presence of excess TBACl protected from light for 0 hr, 12 hr and 24 hr ........................................ 132

Figure 5.16  (a) Changes in the absorption spectra of 4 (154 µM) in CH$_2$Cl$_2$ in the dark before addition of TBACN (---) and after 0 min, 1 min, 2 min, 5 min, 20 min and 30 min (b) photolysis of [Ru(phpy)(phen)(CH$_3$CN)(CN)]$^+$ (154 µM) upon photolysis ($\lambda_{\text{irr}} \geq 420$ nm) in CH$_2$Cl$_2$ with excess TBA•CN for 0 seconds -180 seconds every 10 seconds ........................................... 134

Figure 5.17  (a) Absorption spectra of 4 (100 µM) upon photolysis ($\lambda_{\text{irr}} \geq 420$ nm) in CH$_2$Cl$_2$ with excess bpy and (b) absorption spectra of 4 (44 µM) upon photolysis ($\lambda_{\text{irr}} \geq 420$ nm) in CH$_2$Cl$_2$ with excess phen for 0 s, 10 s, 20 s, 30s, 40 s, 50 s, 60 s, 70 s, 80 s, 90 s and 120 s. ........................................ 135

Figure 5.18  Absorption spectra of 4 (76 µM) upon photolysis ($\lambda_{\text{irr}} \geq 420$ nm) in water for 0 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min and 270 min .......................................................................................... 136

Figure 5.19  Absorption spectra of 4 (66 µM) upon photolysis ($\lambda_{\text{irr}} \geq 630$ nm) in CH$_2$Cl$_2$ with excess TBACl for 0 s, 10 s, 30 s, 40 s, 50 s, 60 s and 120 s. ........................................................................................................ 137

Figure 5.20  Transient absorption spectra of 4 in CH$_2$Cl$_2$ (90 µM) collected at 590 ns (-----□----) and 650 ns (-----■-----) after the laser pulse, respectively .......... 138

Figure 5.21  Ethidium stained agarose gel electrophoresis of 50 µM linearized plasmid (10 mM phosphate buffer, pH 7.5) in the presence of various ratios of (a) cisplatin, incubated for 3 hours (b) 4, $\lambda_{\text{irr}} \geq 420$ nm for 20 min (c) 4, incubated for 24 hrs in the dark. Lanes 1 and 8: DNA molecular weight standard, Lanes 2 and 7: linearized plasmid only; Lanes 3-6 [Complex] : [DNA bp] = 0.5, 1.0, 2.0, 3.0 ...................................................................................... 140

Figure 5.22  Jablonksi diagram showing the kinetics for exciting into (a) the Ru-phpy $^1$MLCT or (b) the Ru-phen $^1$MLCT ........................................................................................................ 145
CHAPTER 1

INTRODUCTION

Photochemical reactions differ from thermal reactions in that they are initiated by the absorption of a photon rather than the application of heat. Irradiation of a molecule with light results in the formation of a high-energy reactive excited state species which can overcome higher activation energy barriers ($E_A$) compared to reactions that are initiated thermally. The differences in the activation barriers for the excited state reaction ($E_{A^{ES}}$) to generate product and that of the corresponding ground state reaction ($E_{A^{GS}}$) are shown in Figure 1.1, showing the clear difference in the relative magnitudes of $E_{A^{ES}}$ and $E_{A^{GS}}$. Therefore, the number of accessible products from photochemical reactions are typically significantly greater than those that are obtainable through thermal activation. Furthermore, photoinitiation reactions allows for direct spatial control resulting in a high degree of reaction localization. This ability to selectively and spatially activate reactions has been useful in a broad number of applications ranging from catalysis to cancer therapies.$^1$–$^6$

The potential deactivation pathways available to a molecule after absorption of a
**Figure 1.1** Schematic diagram showing the activation energies for the reaction of the ground state to products, $E_A^{GS}$, and that for the excited state, $E_A^{ES}$. 
photon are illustrated in Figure 1.2. Typically, the absorption of light \( (I_{\text{abs}}) \) by the singlet ground state \( (S_0) \) results in population of the higher energy singlet excited state \( (S_1) \), which may deactivate to regenerate \( S_0 \) thermally through a process known as internal conversion or radiatively through spin allowed fluorescence. In addition to these deactivation processes, the \( S_1 \) excited state may undergo a photochemical reaction or undergo intersystem crossing (ISC) to form the triplet excited state \( (T_1) \). Because the process of ISC requires a change in multiplicity, it occurs readily in transition metal complexes due to the presence of the heavy-metal atom, which increases spin orbit coupling. Lastly, deactivation of the \( T_1 \) state to the ground state \( S_0 \) can be achieved thermally, radiatively through phosphorescence, or may undergo a chemical reaction.

The overall rate expression for population of the \( S_1 \) excited state as a function of time can be written in terms of each of the possible reaction and deactivation pathways. The rate equation in eq 1.1 can be written in terms of the rate constant of internal conversion \( (k_{nr}^{S}) \), fluorescence \( (k_{F}^{S}) \), intersystem crossing \( (k_{\text{ISC}}) \), and singlet excited state reaction \( (k_{\text{rea}}^{S}) \).\(^7\) By application of the steady state approximation where \( d[S]/dt \approx 0 \) eq 1.1 can be simplified to eq 1.2.\(^7\) The quantum yield for a photochemical process, \( \Phi \), is defined as the moles of product relative to the moles of photons absorbed. Therefore, the quantum yield of each singlet excited state process can be calculated from the rate constants. For example, the quantum yield of the photochemical reaction \( (\Phi_{\text{rea}}) \) from the singlet excited state can be expressed as eq. 1.3 which can be converted to eq 1.4 from 1.2.\(^7\) Similarly, the quantum yield of all other pathways may be written using the corresponding rate constants.\(^7\)
Figure 1.2 Relative energy state diagram showing the $S_0$, $S_1$, and $T_1$ states and the rate constants for various conversion pathways including internal conversion ($k_{nr}^S$), fluorescence ($k_r^F$), excited state singlet reaction ($k_{rea}^S$), intersystem crossing ($k_{ISC}$), thermal deactivation from the triplet state ($k_{nr}^T$), phosphorescence ($k_r^P$), and excited state triplet reaction ($k_{rea}^T$).
A specific photochemical or photophysical event can be achieved by optimizing specific rates to favor the desired process. Of particular interest to this work is the ability to tune the excited state chemical reactivity, such that optimization of this process required the minimization of the rates of competing pathways, such as phosphorescence and fluorescence. The excited state of a molecule can have different electronic and nuclear structures from its ground state precursor, which may result in changes in bond lengths and angles upon exciton, such that the ground and excited state potential energy surfaces are displaced along the nuclear coordinate as depicted in Figure 1.1. The ability to selectively initiate the formation of this highly reactive species remains to be one of the greatest benefits for controlling photochemical reactions.

The work presented here represent attempts to optimize the photochemical and photophysical properties of ruthenium(II) complexes. In particular, this dissertation investigates the effects of various bidentate anionic ligands on the ground state and excited state properties of ruthenium(II) complexes. There is a brief introduction at the
beginning of each chapter that describes the potential photochemical applications related
to the work presented and necessary information in each specific area.
REFERENCES


CHAPTER 2

BACKGROUND

2.1 Photophysical Properties of Ruthenium(II) polypyridyl Complexes

There has been an extensive work on the photophysical properties of polypyridyl Ru(II) complexes.\textsuperscript{1-4} Specifically, the investigation of the complex [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} (bpy = 2,2\textsuperscript{'-}bipyridine) has become a benchmark in the development of the field of inorganic chemistry and is consistently employed as an example to gain understanding of the photophysical properties of many novel ruthenium(II) complexes.\textsuperscript{1-12} Therefore, it is helpful for future topics covered by this work to provide a brief discussion of the photochemical and photophysical properties of [Ru(bpy)\textsubscript{3}]\textsuperscript{2+}.

[Ru(bpy)\textsubscript{3}]\textsuperscript{2+} is a d\textsuperscript{6} complex ruthenium (II) complex with octahedral D\textsubscript{3} microsymmetry.\textsuperscript{1-11} The electron configuration of the ground state and low lying excited states of ruthenium(II) polypyridyl involve the t\textsubscript{2g} and e\textsubscript{g} orbitals that originate from the 4d orbitals of the metal in combination with the \pi-\text{bonding} and \pi*-\text{antibonding} orbitals of the coordinated aromatic ligand. A simplified molecular orbital diagram is provided in Figure 2.1. The filled ground state orbitals (t\textsubscript{2g})\textsuperscript{6} are typical for ruthenium(II) in a strong
Figure 2.1 Simplified molecular orbital diagram for [Ru(bpy)$_3$]$^{2+}$.
ligand field environment. Electron movement from the t_{2g} orbitals to the e_{g} orbitals represent \textit{dd} transitions and are typically weak ($\varepsilon \approx 100 \text{ M}^{-1} \text{ cm}^{-1}$), resulting in ligand field (LF) states with $(t_{2g})^5(e_{g})^1$ electron configuration. Excited states resulting from \textit{dd} transitions commonly give rise to photosubstitution reactions due the population of metal orbitals of M–L(σ*) character. Conversely, the movement of an electron from the $(t_{2g})^6$ set to low lying π* antibonding orbitals of the polypyridyl ligand results in strong ($\varepsilon \approx 20,000 \text{ M}^{-1} \text{ cm}^{-1}$) $d\rightarrow\pi^*$ transition commonly referred to as metal-to-ligand-charge-transfer (MLCT).\textsuperscript{13,14} At higher energies (UV region) ππ* transitions localized on the polypyridyl ligand are observed.

The Jablonski diagram detailing the potential photophysical pathways for [Ru(bpy)$_3$]$^{2+}$ is given in Figure 2.2. The absorption spectrum of [Ru(bpy)$_3$]$^{2+}$ in aqueous solution results in an intense absorption band at 452 nm assigned as $^1$MLCT transition where an electron is promoted from the metal centered t_{2g}-type orbitals into the low lying ligand centered π* orbitals localized on bpy. This transition is often considered as a formal oxidation of the metal center and reduction of the ligand (Ru\textsuperscript{II}–bpy $\rightarrow$ Ru\textsuperscript{III}–bpy$^-$).

It was established in a series of pioneering papers, that the luminescence in [Ru(bpy)$_3$]$^{2+}$ occurs via spin-forbidden charge transfer, as the observed emission is much lower in energy than that of the $^1$MLCT absorption and ligand centered phosphorescence (π*→π).\textsuperscript{15} Furthermore, the large degree of interaction of metal $d\pi$ orbitals with those of the ligand π orbitals results in significant spin-orbit coupling and near quantum efficient intersystem crossing (ISC) from the $^1$MLCT to the $^3$MLCT state(s). The orange phosphorescence of [Ru(bpy)$_3$]$^{2+}$ ($k_P$) is observed at ambient temperatures and is centered
at 620 nm in aqueous solution ($\tau = 0.6 \mu s$).\textsuperscript{1-4}

The temperature dependence of the emission lifetime and quantum yield have revealed the presence of thermally accessibility of non-emissive metal centered $^3$LF state(s) resulting from transitions involving the metal bonding and antibonding orbitals.\textsuperscript{14,16} Population of the M–L antibonding ($\sigma^*$) orbitals has been proposed to result in weakening of one or more Ru-N bonds resulting in ligand dissociation. $[\text{Ru}(\text{bpy})_3]^{2+}$ has previously been shown to undergo photosubstitution reactions via a dissociative mechanism,\textsuperscript{7,14,16} such that cleavage of the Ru-N bond results in formation of a pentacoordinate square-pyramidal species. In the presence of a coordinating anionic monodentate ligand (X) the hexacoordinated monodentate bpy intermediate, $[\text{Ru}(\text{bpy})_2(\eta^1\text{-bpy})(X)]^{2+}$ is formed. The newly formed intermediate may then undergo loss of monodentate bound bpy to form $[\text{Ru}(\text{bpy})X]$. Conversely, the $[\text{Ru}(\text{bpy})_2(\eta^1\text{-bpy})(X)]^{2+}$ intermediate may undergo a process known as self-annealing (or ring closure) to reform $[\text{Ru}(\text{bpy})_3]^{2+}$. Interestingly, the self-annealing process is favored for $[\text{Ru}(\text{bpy})_3]^{2+}$ in aqueous solutions, whereas monodentate ligand substitution is preferred in low polarity solvents.\textsuperscript{7} This effect is presumably caused by the stabilization of the cationic $[\text{Ru}(\text{bpy})_3]^{2+}$ species in aqueous enviroments.\textsuperscript{7} Furthermore, for $[\text{Ru}(\text{bpy})_3]^{2+}$, thermal population of the $^3$LF from the low lying $^3$MLCT state favors rapid non-radiative decay to the ground state.\textsuperscript{14} Therefore, ligand dissociation rates for $[\text{Ru}(\text{bpy})_3]^{2+}$ are extremely low, such that the complex may be thought of as photochemically inert.
Figure 2.2 Jablonski diagram for the ground and excited state processes of $[\text{Ru(bpy)}_3]^{2+}$ including fluorescence ($k_F$), intersystem crossing (ISC), phosphorescence ($k_P$), thermal equilibrium of $^3\text{MLCT}$ and $^3\text{LF}$ states ($k_1$, $k_{-1}$) and ligand dissociation.
2.2 Photosubstitution reactions in Ruthenium(II) Complexes

The chemical stability of the photoexcited states of \([\text{Ru(bpy)}_3]^{2+}\) has prompted investigation of other ruthenium(II) polypyridyl compounds with greater photoreactivity, such as heteroleptic complexes of the type \(\text{cis-[Ru(bpy)}_2\text{L}_2]^{2+}\), \(\text{cis-[Ru(bpy)}_2\text{LX}]^+\) and \(\text{cis-[Ru(bpy)}_2\text{X}_2]\) (where \(\text{L}\) represents monodentate ligands such as \(\text{CH}_3\text{CN}\) and \(\text{py}\); \(\text{X} = \text{Cl}^-, \text{ClO}_4^-, \text{NO}_3^-, \text{NCS}^-, \text{or Br}^-\)). In particular, work involving the series of complexes of the type \(\text{cis-[Ru(bpy)}_2\text{LX}]^+\) and \(\text{cis-[Ru(bpy)}_2\text{X}_2]\) (\(\text{L} = \text{py}, \text{CH}_3\text{CN} \text{and X} = \text{ClO}_4^-, \text{NO}_3^-, \text{NCS}^-, \text{or Br}^-\)) found greater photoreactivity for photosubstitution when \(\text{L} = \text{pyridine in solvents of low polarity, such as dichloromethane}\). \(^{17}\) Furthermore, a mechanism involving a dissociation step from the metal center similar to that observed in \([\text{Ru(bpy)}_3]^{2+}\) was proposed as the quantum yield of monosubstitution for \(\text{cis-[Ru(bpy)}_2(\text{py})_2]^{2+}\) is independent of the identity and concentration of the incoming ligand \(\text{X}\). \(^{17}\)

It was also found that the photosubstitution quantum yields were dependent on the irradiation wavelength for a series of complexes of the congener \([\text{Ru(bpy)}_2\text{XY}]^{n+}\), where \(\text{X} \text{and Y represent monodentate ligands that span the spectrochemical series (Table 2.1)}. \(^{18}\) Photolysis of these complexes in the presence of excess \(t\)-butylammonium chloride revealed a correlation of the lowest charge transfer absorption energy and the quantum yield of photoanation. A linear relationship was observed for the emission energy and quantum yield, as well as with the first reduction potential of the complexes, indicative of a relationship between the energies of the \(^3\text{MLCT}\) and \(^3\text{LF}\) excited states in each complex. \(^{19}\) The dominant role of the \(\text{bpy}\) ligands on the energy of emission and
absorption evident in the dependence of the electrochemical behavior of ruthenium(II) complexes, since in the lowest MLCT state the electron is localized on the ligand resulting in the electronic configuration Ru(III)d⁵–π*(bpy−). This behavior follows a model set forth by Watts and coworkers previously used to describe [Ru(bpy)₃]²⁺, where photosubstitution is a direct consequence of thermal population of the dissociative ³LF excited state from low lying ³MLCT excited states.²⁰ Although, the complete effect of the monodentate ligand σ-bonding character is not clear from the data in Table 2.1, it is evident that increased electron density around the metal center clearly influences the quantum yield of photosubstitution in the series. For example, as monodentate ligands are replaced with stronger field, π-acceptor ligands the electron density at the metal is reduced and consequently the quantum yield of photosubstitution increases. Since photosubstitution is thought to occur via charge transfer type transitions, complexes with π-acceptor ligands result in a more electron deficient of the metal center, such as in the case of ¹MLCT transitions. It was postulated that since a Ru(III) center is required for photochemical reactions in ruthenium polypyridyl complexes. Therefore, delocalization of charge density among the metal and ligand could hinder photosubstitution in complexes with delocalized electron density. As a consequence, when oxidized through excitation into a CT state, the formal charge on the ruthenium atom is less than +3 and would correspond to the decrease in photochemical reactivity.

Several contributions in the late 1960s by Peter Ford and coworkers broadened the fundamental understanding of inorganic photosubstitution reactions with their work on a series of ruthenium(II) pentaamine and tetraamine complexes.²¹ It was shown that
in these systems the specific irradiation wavelength chosen resulted in dramatic
difference in the quantum yields of photosubstitution.\textsuperscript{22-24} This sensitivity to wavelength
further supported the theory that population of the metal centered $^3$LF from the $^3$MLCT
state was required to attain photosubstitution, and resulted in the establishment of the
excited state “tuning” model.\textsuperscript{25} Originally limited to Ru(NH$_3$)$_3$L (where L= nitrogen
donor aromatic heterocycle), the model was extended to many other complexes where the
lowest energy excited state (LEES) is MLCT in character such as [Ru(bpy)$_2$L$_2$]$^{2+}$ (L = 4-
acetylpyridine, 2-cyanopyridine, 4-cyanopyridine).\textsuperscript{26}

According to the model, complexes that are “reactive” readily undergo
photosubstitution and have a LEES that is LF in character, whereas, complexes that are
“unreactive” result from a LEES that is MLCT and requires thermal population of the
$^3$LF excited state for photosubstitution.\textsuperscript{26} Furthermore, ruthenium(II) ammine complexes
containing unsaturated aromatic nitrogen heterocyclic ligands have LF and MLCT
excited states of very similar energy such that the LEES can be tuned with careful
selection of the ligation sphere and solvent.\textsuperscript{27}
Table 2.1 Quantum yield of Photoanation, MLCT maxima, emission maxima, and $E_{1/2}^{(II/III)}$ for the series $cis$-[Ru(bpy)$_2$(X)(Y)]$^{II+}$ in dichloromethane with t-butylammonium chloride.

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>$\Phi$</th>
<th>$\lambda_{\text{obs, max}}$</th>
<th>$\lambda_{\text{em, max}}$</th>
<th>$E_{1/2}^{(II/III)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyridine</td>
<td>Cl</td>
<td>0.04</td>
<td>505</td>
<td>657</td>
<td>0.79</td>
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<td>4-acetylpyridine</td>
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<td>495</td>
<td>643</td>
<td>0.82</td>
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<td>N-methylimidazole</td>
<td>N-methylimidazole</td>
<td>&lt;0.001</td>
<td>483</td>
<td>641</td>
<td>0.94</td>
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<tr>
<td>imidazole</td>
<td>imidazole</td>
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<td>630</td>
<td>1.02</td>
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<td>Cl</td>
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<td>480</td>
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<td>454</td>
<td>584</td>
<td>1.30</td>
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<td>4,4′-bpy</td>
<td>4,4′-bpy</td>
<td>0.29</td>
<td>446</td>
<td>578</td>
<td>1.32</td>
</tr>
<tr>
<td>4-acetylpyridine</td>
<td>4-acetylpyridine</td>
<td>0.29</td>
<td>442</td>
<td>575</td>
<td>1.45</td>
</tr>
<tr>
<td>3-iodopyridine</td>
<td>3-iodopyridine</td>
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<td>575</td>
<td>1.36</td>
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<td>(P(C$_6$H$_5$)$_2$CH$_3$)</td>
<td>(P(C$_6$H$_5$)$_2$CH$_3$)</td>
<td>≈ 0</td>
<td>429</td>
<td>546</td>
<td>1.52</td>
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<tr>
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<td>CH$_3$CN</td>
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<td>425</td>
<td>540</td>
<td>1.44</td>
</tr>
<tr>
<td>CO</td>
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<td>0.05</td>
<td>305</td>
<td>442</td>
<td>&gt;1.90</td>
</tr>
</tbody>
</table>

$^a$Determined in CH$_2$Cl$_2$  $^b$at 77 K in 1:1 methanol-ethanol glass  $^c$Determined in CH$_3$CN with TBAH as the electrolye vs. SCE.
The photophysical properties and photosubstitution of various pentaamine and
tetraamineruthenium(II) complexes of the form \([\text{Ru(NH}_3)_5\text{L}]^{2+}\) \((\text{L} = \text{py, isonicotinamide}
(\text{isn}), \text{pyrazine (pz), 4-acetylpyridine (4-acpy), 2-cyanopyrindine (2-Npy)
and CH}_3\text{CN})\) have been investigated and are presented in Table 2.2.\(^{28,29}\) The dependence of the
quantum yield of ligand exchange on irradiation wavelength is indicative of its sensitivity
of the relative energies of the \(^3\text{LF}\) and \(^3\text{MLCT}\) excited states.\(^{30-32}\) For example, the
energy of the \(^3\text{MLCT}\) excited state is directly related to the energy of the lowest
absorption maximum for ruthenium(II) amine complex. For \([\text{Ru(NH}_3)_5\text{(L)}]^{2+}\), where \(\text{L}\) is
an aromatic nitrogen heterocycle such as pyridine or piperazine, the lowest energy
transition is assigned as \(^1\text{MLCT}.\(^{14}\) The energy of this \(^1\text{MLCT}\) transition is greatly
affected by the addition of electron donating and withdrawing substituents to the nitrogen
heterocycle.\(^{33}\) In the case of \([\text{Ru(NH}_3)_5(4\text{-acpy})]^{2+}\), where the acetyl group increases the
electron donation of the pyridine nitrogen, a destabilization of the charge transfer
processes is observed with an MLCT band at 523 nm.\(^{21}\) Conversely, the electron
withdrawing cyano- substituent in \([\text{Ru(NH}_3)_5(2\text{-NCpy})]^{2+}\) results in a stabilization of the
MLCT with an absorption maximum at 424 nm.\(^{34}\) In the case of \([\text{Ru(NH}_3)_5(\text{isn})]^{2+}\), which
has an MLCT absorption maximum at 479 nm, irradiation directly into or above the
energy of this band results in high quantum yield of photosubstitution.\(^{21}\) However, when
lower irradiation energies were used a dramatic decrease in the ligand exchange quantum
yield is observed. Utilizing the tuning model proposed by Ford, irradiation wavelengths
below the LEES energy results in insufficient population of the \(^3\text{LF}\) states and low
quantum yields of ligand exchange. Therefore, the energy difference between the
Table 2.2 Lowest energy absorption maximum ($\lambda_{abs}$), wavelength of irradiation ($\lambda_{irr}$) and Photosubstitution Quantum Yields ($\Phi_{NH_3}$) in amine and for water ($\Phi_L$) in water for $[\text{Ru(NH}_3)_5\text{L}]^{2+}$.

<table>
<thead>
<tr>
<th>L</th>
<th>$\lambda_{abs}$/ nm</th>
<th>$\lambda_{irr}$/ nm</th>
<th>$\Phi_{NH_3}$</th>
<th>$\Phi_L$</th>
</tr>
</thead>
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<tr>
<td>py</td>
<td>407</td>
<td>405</td>
<td>0.045</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td></td>
<td>449</td>
<td>0.049</td>
<td>0.063</td>
</tr>
<tr>
<td>isn</td>
<td>479</td>
<td>405</td>
<td>0.0045</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>479</td>
<td>0.0011</td>
<td>0.0053</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>0.0004</td>
<td>0.0024</td>
</tr>
<tr>
<td></td>
<td></td>
<td>546</td>
<td>0.0003</td>
<td>0.0007</td>
</tr>
<tr>
<td>4-acpy</td>
<td>523</td>
<td>449</td>
<td>0.0014</td>
<td>0.0086</td>
</tr>
<tr>
<td></td>
<td></td>
<td>520</td>
<td>0.0003</td>
<td>0.0009</td>
</tr>
<tr>
<td>2-NCpy</td>
<td>424</td>
<td>404</td>
<td>0.072</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>436</td>
<td>0.065</td>
<td>0.059</td>
</tr>
<tr>
<td>CH$_3$CN</td>
<td>354</td>
<td>313</td>
<td>0.130</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>366</td>
<td>0.160</td>
<td>0.100</td>
</tr>
</tbody>
</table>
$^3$MLCT and $^3$LF excited states must be large enough in this complex, such that the energy required for population of the dissociative state precludes ligand loss at room temperature. In these systems initial excitation is followed by relatively efficient intersystem crossing to the lowest energy $^3$LF state from which ligand loss can occur. The relative wavelength independence of the quantum yields for the ‘reactive’ complexes implies efficient interconversion to a common state. The opposite effect is observed for [Ru(NH$_3$)$_5$(2-NCpy)]$^{2+}$ and [Ru(NH$_3$)$_5$(CH$_3$CN)]$^{2+}$ which exhibit $^1$MLCT absorption maxima at 424 nm and 354 nm, respectively. In [Ru(NH$_3$)$_5$(2-NCpy)]$^{2+}$ no dependence of quantum yield of photosubstitution on irradiation wavelength is observed, indicating that the $^3$MLCT and $^3$LF excited states are close in energy. Alternatively, initial excitation into the $^1$MLCT manifold is followed by deactivation to both the lowest energy $^3$MLCT and the $^3$LF manifold.

From the examples above and others not cited here, it is evident that careful selection of ligands will greatly affect the energy of this transition, thereby enabling tuning of the relative energies of the $^3$MLCT and $^3$LF states. Altering electron donation and withdrawal abilities of ligands, including the effect of noninnocent ligand behavior, further influence photoreactivity by introduction of variations in the electronic environment surrounding the metal as well as the ancillary ligand. This topic will be further discussed in Chapter 4 and 5.
2.3 Cisplatin

Cisplatin, cis-[Pt(NH$_3$)$_2$Cl$_2$], was first successfully synthesized in 1847 by M. Peyrone.$^{35}$ In 1965 the chemotherapeutic activity of cisplatin was serendipitously discovered when Barnett Rosenberg showed that electrolysis of a platinum electrode, successful inhibition of binary fission in E. coli.$^{36}$ Further examination of several platinum complexes against human sarcomas artificially implanted in rats found that cis-[Pt(NH$_3$)$_2$Cl$_2$] produced the most inhibition of tumor growth.$^{37}$ Later, Hill and coworkers showed cis-[Pt(NH$_3$)$_2$Cl$_2$] to be active against malignant lymphoma, Hodgson’s disease and certain other cancers.$^{38}$ In 1978, cisplatin was approved by the FDA and to date is one of the most successful and potent anticancer drugs available.$^{39}$ Cisplatin alone or in combination with other chemotherapy agents is used in the treatment of 40 – 80% of cancer patients.$^{42}$ Cisplatin has prolific use in treatment of a variety of lung, head and neck, ovarian and bladder cancers.$^{40,41}$ In the case of testicular cancers, since the commercial introduction of cisplatin the cure rate increased dramatically from about 10% to near 90%.$^{42}$

It is interesting to note that the stereoisomer, trans-Pt(NH$_3$)$_2$Cl$_2$ (transplatin), does not exhibit antitumor activity,$^{43}$ which was later determined to be a consequence of this stereoisomer’s inability to form intrastrand crosslinks of double stranded DNA. Later, these observations helped to reveal the mode of action in cisplatin necessary to prevent DNA replication and the source of its anti-tumor activity.$^{43}$ Indeed, toxicity in cisplatin has been found to be highly dependent of hydrolysis, where, exchange of the chloride ligands for water results in the formation of the cationic “active” diaqua species.
cis-[Pt(NH\textsubscript{3})(H\textsubscript{2}O)\textsubscript{2}]\textsuperscript{2+}.\textsuperscript{40,41} A scheme detailing the thermal exchange of the labile chloride ligands in cisplatin is shown in Figure 2.3.\textsuperscript{44} The \textit{in vivo} hydrolysis of cisplatin within cells is facilitated by the low intracellular chloride concentration. Furthermore, upon formation of the diaqua species targets DNA in the nucleus results in inhibition of cellular transcription and, consequently, cell death.\textsuperscript{40,41} The interaction of cisplatin with DNA has been thoroughly investigated.\textsuperscript{44} \textit{Cis-} \text{Pt(NH\textsubscript{3})(H\textsubscript{2}O)\textsubscript{2}]\textsuperscript{2+} has been found to preferentially bind to the N7 position of adjacent guanines in ds-DNA to form GpG 1,2-intrastrand crosslinks (65%). However, intrastrand crosslinks of adjacent guanine:adenine adducts (25%) and other interstrand crosslinks constitute additional adducts (Figure 2.4).\textsuperscript{45} This irreversible covalent interaction of cisplatin with ds-DNA induces a 20° bend in the double helix and consequential unraveling of the duplex.\textsuperscript{44-47} This change in tertiary morphology results in the inability of transcription factors and repair enzymes to recognize the cisplatin-DNA lesion. Unable to repair the damaged DNA, cellular signaling pathways initiate apoptosis, also known as programmed cell death.\textsuperscript{44-47}
Figure 2.3 Sequential aquation of the chloride ligands of cisplatin resulting in the formation of the active species $\text{cis-}[\text{Pt(NH}_3\text{)}_2(\text{OH}_2)_2]^{2+}$, along with subsequent deprotonation steps. (from ref. 44)
Despite the success of cisplatin, several drawbacks still exist, such as drug resistance and a large number of adverse side effects.\textsuperscript{42-47} The latter arise from the indiscriminate activity of cisplatin on healthy and tumor tissue.\textsuperscript{42-47} Derivatives of cisplatin, oxaliplatin and carboplatin, have been synthesized to address this issue, however these analogs have been found to be far less potent than cisplatin.\textsuperscript{42} Cancer cells that are or become resistant to cisplatin often represent some of the most aggressive tumors. These drawbacks have prompted the discovery of several metal complexes with similar mechanism of action and antitumor activity as cisplatin.\textsuperscript{48-50}

Important to the design of new cisplatin-type metal complexes, the geometry, metal oxidation state and nature of the ligands should be considered.\textsuperscript{42} In recent years,

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure24.png}
\caption{Schematic representation of the formation of cisplatin–ds-DNA 1,2 intrastrand crosslinks with adjacent guanines (65\%) and guanine:adenine (25\%).}
\end{figure}
octahedral ruthenium(II)$^{51-55}$, rhodium(III)$^{56-61}$ and square planar nickel(II)$^{62-67}$ complexes have been investigated. As in cisplatin, these mononuclear transition metal complexes have been shown to covalently bind to DNA bases through displacement of labile ligands from the coordination sphere of the metal.$^{68-70}$ The complex A-[Ru(phen)$_2$Cl$_2$] (phen = 1,10-phenanthroline) has previously been reported to preferentially recognize and bind covalently to B-DNA.$^{51}$ Similarly, cis-[Ru(bpy)$_2$Cl$_2$] has been found to exhibit a 1:1 covalent binding to alkylated 6-ketopurines, 9-methylhypoxanthine, and 9-ethylguanine following incubation with DNA bases at 37°C.$^{71}$ The reaction of cis-[Ru(bpy)$_2$(O$_3$SCF$_3$)$_2$] with 9-methylguanine (9-Me-G) has been reported to generate the bis-substituted species, cis-[Ru(bpy)$_2$(9-Me-G)$_2$]$^{2+}$.$^{72,73}$ Furthermore, it has been demonstrated that the water soluble bis(2-phenylazopyridine)ruthenium(II) and related complexes form covalent DNA adducts.$^{74-76}$ These complexes have also been shown to inhibit transcription and possess activity against cisplatin resistant cancer cell lines.$^{76}$ However, these complexes require thermal activation and, therefore, exhibit similar drawbacks to those of cisplatin, including lack of tumor specificity, which result in a large number of negative side effects.$^{77-82}$
2.4 Photodynamic Therapy

Photodynamic Therapy (PDT) matured as a feasible medical technology in the 1980s and is now a viable treatment for many forms of cancer. The premise of PDT consists of the use of light to spatially activate otherwise non-toxic molecules, thereby localizing cytotoxic effects only within the area of irradiation.\(^{83-88}\) The result is a dramatic decrease in the number of negative side effects as toxicity is produced only in the vicinity of the irradiated tissue. Therefore, a vital requirement of PDT agents is that they are nontoxic at the administered dose in the dark, but become toxic only upon irradiation. In addition, successful agents should possess high molar absorbtivity at long wavelengths where tissue penetration is greatest and there is low light absorption by biomolecules. This area of optimal penetration death is known as the photodynamic therapeutic window (650 – 850 nm). Lastly, PDT agents should be readily absorbed by target tissue, which in the case of dense tumor tissue may require a high lipophilicity.

Current PDT agents approved for use by the U.S. Food and Drug Association (FDA) achieve toxicity through photosensitized production of singlet oxygen. The most common PDT agent used in the treatment of cancer is commercially known as Photofrin® and is a mixture of hematoporphyrin (Figure 2.5) and several porphyrin derivatives.\(^{83,84}\) The hydrophobic nature of the porphyrin molecules allows for significant uptake of the PDT agent within cellular tissue. A scheme for the activation of Photofrin is shown in Figure 2.6,\(^{83}\) where excitation of Photofrin® with visible light results in the generation of a short-lived singlet excited state (\(\tau = 1-12\) ns).\(^{89-91}\) The singlet excited state (\(^{1}\text{S}\)) quickly deactivates through intersystem crossing to form a long lived
triplet excited state \( (3^*T) \).\(^{89-91}\)

On mechanism by which the long lived triplet excited state \( (3^*T) \) of Photofrin\(^\circledR \) can then relax to its ground state \( (1S) \) is by energy transfer to the triplet ground state of molecular oxygen, \( ^3O_2 \), to produce the highly reactive singlet oxygen species, \( ^1O_2 \). The \( ^1O_2 \) species generated by photosensitizers like Photofrin\(^\circledR \) can then rapidly react with nearby biomolecules causing irreversible oxidative damage.\(^{83}\) Ultimately, the sensitization of \( ^1O_2 \) species results in cell death either by apoptosis or necrosis.\(^{83}\) However, since the excited states of porphyrins and phthalocyanins are unreactive in the absence of oxygen, cytotoxicity dramatically decreases after all molecular oxygen within the vicinity of the drug is consumed. This mechanism of action is unfortunate as some of

![Figure 2.5: The molecular structure of hematoporphyrin](image)
the most aggressive and drug resistant tumor cells are to be hypoxic,\textsuperscript{92-94} therefore, making treatment of these tumors with photosensitizers, such as Photofrin, unsuccessful. Furthermore, once molecular oxygen in the vicinity is consumed, future treatment at the tumor is limited to slow diffusion of $^3\text{O}_2$ back into the cell.

\textbf{Figure 2.6} Schematic representation of the production of $^1\text{O}_2$ by typical sensitizer (S) PDT agents, where $^1\text{S}$ represents the molecule in its ground state, $^1*\text{S}$ represents the singlet excited state of the sensitizer, and $^3*\text{S}$ is the triplet excited state of the molecule following intersystem crossing.
2.5 Photo-cisplatin Analogs

The success of cisplatin has resulted in the investigation and clinical use of several platinum derivatives. In addition to platinum complexes, a variety of analogous Ru(II), Rh(III) and Ni(II) complexes have been investigated for cisplatin-type cytotoxicity. However, a major drawback of each of these complexes remains to be the thermal activation of ligand displacement for form the cytotoxic diaqua species. Recently, the photochemistry of rhodium (II) and ruthenium(II) complexes excited state ligand exchange has been investigated for their reactivity with biological molecules. This blend of cisplatin-mimetic metal complexes, which brings together inorganic photochemistry and photodynamic therapy has led to the discovery of several photoactivated ruthenium complexes that appear to have cisplatin-type action toward DNA. This new class of compounds is referred to as photo-cisplatin analogs.

The large number of ruthenium(II) amine complex that undergo photosubstitution has resulted in the consideration of these complexes as some of the first photo-cisplatin analogs. The complex cis-[Ru(bpy)₂(NH₃)₂]²⁺, which has a Ru→bpy (MLCT) transition at 345 nm, has been shown to undergo stepwise photoinduced ligand exchange in water of its monodentate amine ligands to form the diaqua cis-[Ru(bpy)₂(H₂O)₂]²⁺ (λᵳᵣᵣ = 400 nm; Φ = 0.018) species in acidic conditions and the cis-[Ru(bpy)₂(OH₂)(OH)]⁺ (λᵳᵣᵣ = 350 nm; Φ = 0.024) at neutral pH. Additionally, no ligand exchange is observed for the complex in the dark under similar experimental conditions. Furthermore, the typical dependence of quantum yield of ligand exchange on the irradiation wavelength was observed for this complex. By monitoring changes to the electronic absorption spectrum,
the photolysis of \( \text{cis-}[\text{Ru(bpy)}_2(\text{NH}_3)_2]^ {2+} \) was found to result in the biphasic formation of \( \text{cis-}[\text{Ru(bpy)}_2(\text{H}_2\text{O})_2]^ {2+} \), indicating that the photosubstitution proceeds through the formation of a mono-aqua intermediate, \( \text{cis-}[\text{Ru(bpy)}_2(\text{NH}_3)(\text{H}_2\text{O})]^ {2+} \). Continued irradiation results in the exchange of the remaining ammine for a water molecule. Furthermore, the photosubstitution reaction was determined to require one photon for the removal of each ammine.

Photolysis of \( \text{cis-}[\text{Ru(bpy)}_2(\text{NH}_3)_2]^ {2+} \) in the presence of one equivalent of free bpy in water results in the formation of the highly emissive \([\text{Ru(bpy)}_3]^ {2+}\), clearly demonstrating the thermal lability of the bound water.\(^{99}\) Furthermore, photolysis of \( \text{cis-}[\text{Ru(bpy)}_2(\text{NH}_3)_2]^ {2+} \) in the presence of short DNA oligomers resulted in covalent binding determined by electrospray spray mass spectrometry (ESMS). An increase in the mass of oligomers possessing adjacent guanine residues were found to have one bound \( \text{Ru(bpy)}_2 \) despite the 15-fold excess of the metal complex. This result suggests, although not definitively, that a 1,2-intrastrand binding mode with preference for adjacent guanine bases similar to cisplatin may be preferred by \( \text{cis-}[\text{Ru(bpy)}_2(\text{OH}_2)_2]^ {2+} \). Additionally, the covalent interaction of \( \text{cis-}[\text{Ru(bpy)}_2(\text{NH}_3)_2]^ {2+} \) with ds-DNA was observed upon photolysis as a decrease in mobility of the linearized DNA by agarose gel electrophoresis. Cisplatin binds ds-DNA covalently and irreversibly inducing a kink in the structure of the duplex at the 1,2-intrastrand crosslink.\(^{46}\) This change in morphology results in a noticeable retardation of mobility for ds-DNA within agarose gels.\(^{46}\) A similar decrease in mobility was observed for ds-DNA irradiated in the presence of \( \text{cis-}[\text{Ru(bpy)}_2(\text{NH}_3)_2]^ {2+} \), which indicates a similar DNA binding to that of cisplatin such
binding was not observed in the dark, however, the ability to covalently interact with ds-DNA upon irradiation was only observed at high-energy wavelengths. Irradiation of cis-[Ru(bpy)$_2$(NH$_3$)$_2$]$^{2+}$ with $\lambda > 450$ nm did not result in photosubstitution, and consequently caused no change in DNA mobility. Since PDT agents must be operative at low energy irradiation (650–850 nm) for tissue penetration, the inability to undergo photosubstitution at longer wavelength represents a significant hindrance for clinical applications of cis-[Ru(bpy)$_2$(NH$_3$)$_2$]$^{2+}$ as a PDT agent.

The analogous complex cis-[Ru(bpy)$_2$(CH$_3$CN)$_2$]$^{2+}$ exhibits a lower energy Ru→bpy (MLCT) transition at 427 nm in water$^{100}$ and its photoaquation quantum yield to form the cis-diaqua species was determined to be 0.22 ($\lambda_{\text{irr}} = 450$ nm). These values are similar for other ruthenium(II) complexes containing acetonitrile ligands, which, were previously shown to have high quantum yields of ligand exchange when compared to other ruthenium(II) polypyridyl complexes. This results in a large production of mono-aqua species upon the absorption of one photon thus, allowing for the investigation of these compounds using ultrafast techniques. Femtosecond time resolved transient absorption measurements revealed the formation of a pentacoordinate intermediate upon excitation with 310 nm, cis-[Ru(bpy)$_2$(CH$_3$CN)]$^{2+}$, which quickly decays to form the monoaquated species or reform the cis-[Ru(bpy)$_2$(CH$_3$CN)$_2$]$^{2+}$ complex. Similar to cis-[Ru(bpy)$_2$(NH$_3$)$_2$]$^{2+}$, photolysis in the presence of ds-DNA has shown retardation of mobility by gel electrophoresis.$^{101}$ Nevertheless, like cis-[Ru(bpy)$_2$(NH$_3$)$_2$]$^{2+}$, cis-[Ru(bpy)$_2$(CH$_3$CN)$_2$]$^{2+}$ is plagued by a dramatic drop in photoreactivity at long irradiation wavelength.
The dinuclear complex, \( \textit{cis} \)-[Rh\(_2\)(\(\mu\)-O\(_2\)CH\(_3\))\(_2\)(CH\(_3\)CN)\(_6\)]\(^{2+}\), is known to undergo facile thermal exchange of the axially bound acetonitrile ligands in coordinating solvents, whereas the equatorially bound acetonitriles are not labile at room temperature in the dark.\(^{102}\) In aqueous environment, the axial acetontiriles are readily exchanged for water and result in the formation of the \( \textit{cis} \)-[Rh\(_2\)(\(\mu\)-O\(_2\)C\(_2\)H)\(_2\)(CH\(_3\)CN)\(_4\)(H\(_2\)O)\(_2\)]\(^{2+}\) species. Upon photolysis, changes in the electronic absorption and \(^1\)H NMR spectra indicate the exchange of two equatorial acetonitrile ligands. Furthermore, power dependence of the photolysis indicate that the formation of the \( \textit{cis} \)-[Rh\(_2\)(\(\mu\)-O\(_2\)C\(_2\)H)\(_2\)(CH\(_3\)CN)\(_2\)(H\(_2\)O)\(_4\)]\(^{2+}\) species is the result of a one-photon process. The quantum yield of photosubstitution in \( \textit{cis} \)-[Rh\(_2\)(\(\mu\)-O\(_2\)CH\(_3\))\(_2\)(CH\(_3\)CN)\(_4\)(H\(_2\)O)\(_2\)]\(^{2+}\) is wavelength dependent indicative that the photochemistry arises from excited states that lie above the lowest energy excited state. Table 2.3 shows the quantum yield of ligand exchange for \( \textit{cis} \)-[Rh\(_2\)(\(\mu\)-O\(_2\)CH\(_3\))\(_2\)(CH\(_3\)CN)\(_4\)(H\(_2\)O)\(_2\)]\(^{2+}\) at various wavelengths of irradiation. In addition, photolysis in the presence of bpy or 9-ethyl guanine has been found to result in their bidentate coordination to the metal core. The photoaquation of \( \textit{cis} \)-[Rh\(_2\)(\(\mu\)-O\(_2\)CH\(_3\))\(_2\)(CH\(_3\)CN)\(_4\)(H\(_2\)O)\(_2\)]\(^{2+}\) and the ability to bind nucleic acids, as well as bidentate ligands, led to its investigation with ds-DNA.
Table 2.3 Absorbance maxima and Quantum Yield of Photosubstitution for \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{C}_2\text{H})_2(\text{CH}_3\text{CN})_4(\text{H}_2\text{O})_2]^{2+} \) in aqueous solution at various irradiation wavelengths.

<table>
<thead>
<tr>
<th>( \lambda_{\text{abs max}} / \text{nm} )</th>
<th>( \lambda_{\text{irr}} / \text{nm} )</th>
<th>( \Phi_{\text{HPO}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>355</td>
<td>355</td>
<td>0.37</td>
</tr>
<tr>
<td>555</td>
<td>509</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Photolysis of \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CH}_3)_2(\text{CH}_3\text{CN})_4(\text{H}_2\text{O})_2]^{2+} \) in the presence of linearized pUC18 plasmid was reported to result in a decrease in gel mobility similar to that of cisplatin, suggesting a comparable binding mode of the complex to the latter. Conversely, \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CH}_3)_2(\text{CH}_3\text{CN})_4(\text{H}_2\text{O})_2]^{2+} \) incubated with ds-DNA in the dark showed no change in mobility. The need for photoactivation of \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CH}_3)_2(\text{CH}_3\text{CN})_4(\text{H}_2\text{O})_2]^{2+} \) to form a covalent DNA adduct is a vital requirement for a potential PDT agent that may mimic cisplatin. The low toxicity of \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CH}_3)_2(\text{CH}_3\text{CN})_6]^{2+} \) in the dark and its increased toxicity upon irradiation was further tested by treating Hs-27 human skin cells with the complex to determine the \( \text{LC}_{50} \) values for cells irradiated and those protected from light. Comparisons of the \( \text{LC}_{50} \) values in the presence of absence of light are shown in Table 2.4. \( \text{Cis-}[\text{Rh}_2(\mu-\text{O}_2\text{C}_2\text{H})_2(\text{CH}_3\text{CN})_6]^2 \) clearly shows a dramatic increase in toxicity with exposure to light resulting in a 34 fold increase in toxicity for cells exposed to light compared to those kept in the dark. In addition, \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{C}_2\text{H})_2(\text{CH}_3\text{CN})_4(\text{H}_2\text{O})_2]^{2+} \) has a much lower toxicity in the dark compared to
the major component of the current FDA approved PDT agent Photofrin, hemataporphyrin.

Even though \( \text{cis-}[\text{Rh}_2(\mu-O_2\text{CH}_3)_2(\text{CH}_3\text{CN})_6]^{2+} \) was shown to have the highest quantum yield of photoaquation of complexes in this series, these high yields are only achieved at higher energy wavelengths. This poor photoinduced ligand exchange at low energy has prompted the work presented here. The goal of this dissertation is to design ruthenium(II) polypyridyl compounds as potential anticancer drugs that address the need for photoactivation at low energy light and to retain activity independent of the presence of oxygen. To this end, the focus is to use metal complexes designed to combine the mode of action of cisplatin with the absorption of light, such that these metal complexes inhibit cellular growth after photolysis but are nontoxic in the absence of light.

Table 2.4 Cytotoxicity for cisplatin, photofrin and \( \text{Rh}_2(\mu-O_2\text{C}_2\text{H})_2(\text{CH}_3\text{CN})_6]^{2+} \) with Hs-27 skin cells in the dark and irradiated. LC\(_{50}\) values given in \( \mu \text{M}. \)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Dark</th>
<th>Irradiated 400 – 700 nm (30 min)</th>
<th>Increase upon irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>131 ±10</td>
<td>110 ± 8</td>
<td>1.2</td>
</tr>
<tr>
<td>Hemataporphyrin</td>
<td>21 ± 1</td>
<td>3.8 ± 0.2</td>
<td>5.5</td>
</tr>
<tr>
<td>( \text{Rh}_2(\mu-O_2\text{CH}_3)_2(\text{CH}_3\text{CN})_6]^{2+} )</td>
<td>410 ± 9</td>
<td>12 ± 2</td>
<td>~34</td>
</tr>
</tbody>
</table>
REFERENCES


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CHAPTER 3

EXPERIMENTAL METHODS

3.1 Materials

RuCl$_3$$\cdot$3H$_2$O, bpy (2,2’-bipyridine), quoH (8-hydroxyquinoline), 5-NO$_2$-quoH (5-NO$_2$-8-hydroxyquinoline), phen (1,10-phenanthroline), phpy$^-$ (2-phenylpyridine), pyridine, LiCl, AgBF$_4$ and NaBH$_4$ were purchased from Aldrich and used without further purification. Sodium chloride, sodium phosphate, sodium hydrogen phosphate, Tris base/HCl, ethidium bromide, gel loading buffer (0.25 % (w/v) bromophenol blue, 0.1 M EDTA, pH = 8.0) and DNA molecular weight standard (10000 kbase), were purchased from Sigma and used as received. The SmaI restriction enzyme was purchased from Invitrogen and used as received. The pUC18 plasmid was purchased from Bayou Biolabs and purified using the Qiaprep spin Miniprep system from Life technology. Water was deionized using a Barnstead Fi-stream filter system to 18 MΩ. Ru(II) complexes were prepared as described below. All reactions were preformed under inert atmosphere and in dry solvent.
3.2. Synthesis

[\textbf{Ru(bpy)}_2\textbf{Cl}_2] \text{ The compound was prepared by modification of a reported method.}^1 0.10 g (0.38 mmol) of RuCl$_3$$\cdot$3H$_2$O, 0.12 g (0.77 mmol) of 2,2´-bipyridine and 1.61 g (37.9 mmol) of LiCl were refluxed in N,N-dimethylformamide (DMF, 10.00 mL) for 8 hours under argon. The starting brown-black solutions turned red-purple, and the mixture was allowed to cool to room temperature, at which time 20 mL of reagent grade acetone:water was added (1:1,v:v). The solution was filtered and the green black solid was stirred in 20 mL of water overnight. The compound was further purified by washing with 3 x 5 mL portions of CH$_2$Cl$_2$ and ether respectively. The compound was dried by suction on a vacuum. Lastly, the compound was purified by separatory funnel with water and CH$_2$Cl$_2$ to remove any remaining [Ru(bpy)$_3$]$^{2+}$. The water layer containing the impurity was removed and the CH$_2$Cl$_2$ layer was collected. Subsequent additions of water to the CH$_2$Cl$_2$ layer were added and this process was repeated until the water layer became colorless. The purified product was isolated by removing the solvent by evaporation. $^1$H NMR in DMSO-d$_6$, $\delta$ (int., mult): 10.02 (2H, d), 8.7 6 (2H, d), 871.56 (2H, d), 8.18 (2H, t), 7.82, 7.78 (4H, t), 7.6 (2H, d), 7.2 (2H, t).

[\textbf{Ru(bpy)}_2(quo)][\textbf{PF}_6] \text{ [Ru(bpy)$_2$Cl$_2$] (0.10 g, 0.16 mmol) was refluxed in 30 mL CH$_2$Cl$_2$ with 0.23 g (1.16 mmol) quoH. The initially purple reaction turned green-brown after \textasciitilde 6 hours and was allowed to reflux for an additional 12 hrs. After cooling, 15 mL of water was added directly to the reaction mixture. The water soluble product [Ru(bpy)$_2$(quo)]$^+$ was isolated in a separatory funnel from water and CH$_2$Cl$_2$. The aqueous layer was }
collected and to it a saturated solution of NH₄PF₆ was added dropwise to precipitate the product, followed by filtration and subsequent washing with water to remove unreacted quinolate ligand (105 mg; 71%). The MALDI and NMR spectrum of [Ru(bpy)₂(quo)]⁺ is shown in figure 3.1 and 3.2. ¹H-NMR (500 MHz; acetone-d₆): δ  9.17 (dd, 1H), 9.02 (m, 5H), 8.79 (d, 4H), 8.47 (m, 4H), 8.03 (m, 1H), 7.95 (m, 4H), 7.76 (m, 2H), 7.46 (m, 1H). MALDI MS m/z = 561 [Ru(bpy)₂(quo)]⁺.

Figure 3.1 MALDI mass spectrometry of [Ru(bpy)₂(quo)]⁺.
[Ru(bpy)(CH₃CN)₄](PF₆)₂ RuCl₃•3H₂O (0.10, 0.38 mmol) g was stirred with 0.05 g (0.38 mmol) bpy in 50 mL of 1.0 N HCl for seven days at room temperature and was protected from light to yield [Ru(bpy)Cl₄]²⁻. The reaction was then dried by rotovaporation and in vacuo over P₄O₁₀ for 3 days. The green solid was then refluxed in acetonitrile for 4 hours to produce the orange [Ru(bpy)(CH₃CN)₄]²⁺ which was evaporated to dryness. The solid was dissolved in water, precipitated with the dropwise addition of a saturated solution of NH₄PF₆, and the resulting [Ru(bpy)(CH₃CN)₄](PF₆)₂ complex was filtered and washed over the filtration frit with cold water (158 mg; 58%).

[Ru(bpy)(quo)]₂ [Ru(bpy)(CH₃CN)₄](PF₆)₂ (0.05 g, 0.07 mmol) was refluxed in CH₂Cl₂ with 0.10 g (0.70 mmol) quoH overnight. The resulting neutral product was extracted by

Figure 3.2 ¹H NMR spectra of aromatic region of [Ru(bpy)₂(quo)]⁺.
successive addition of diethyl ether to the reaction mixture in a separatory funnel. This process was repeated three times and the diethyl ether layers were combined and rotovaped to dryness (30 mg; 78%). The MALDI and NMR spectrum of \([\text{Ru}(\text{bpy})(\text{quo})_2]\) is shown in figure 3.3 and 3.4. \(^1\)H NMR (acetone-\(d_6\)): \(\delta\) 9.14 (dd, 1H), 8.95 (td, 5H), 8.70 (dt, 4H), 8.42 (td, 4H), 7.88 (m, 4H), 7.74 (m, 1H). MALDI MS \(m/z = 544\) \([\text{Ru}(\text{bpy})(\text{quo})_2]^+\).

![MALDI mass spectrometry of [Ru(bpy)(quo)_2]](image)

**Figure 3.3** MALDI mass spectrometry of \([\text{Ru}(\text{bpy})(\text{quo})_2]\).
[Ru(quo)_3] of RuCl_3•3H_2O (0.1 g, 0.38 mmol) was dissolved in 25 mL ethanol and 5 mL of water with 0.166 g (1.15 mmol) 8-hydroxyquinoline. The mixture was refluxed overnight and a green precipitate formed, and after cooling the suspension was filtered. The solid was washed with water on the filter frit to remove excess quinolate ligand (164mg; 80%). MALDI MS m/z = 537 [Ru(quo)_3]^+ (Figure 3.5).

Na[Ru(quo)_3] Reduction of 0.10 g of [Ru(quo)_3] was achieved by the addition of 0.030 g (0.76 mmol) NaBH_4 in dry acetonitrile under inert atmosphere. Electrochemical and electronic absorption measurements confirmed the presence of the anionic complex [Ru(quo)_3]^-.
A procedure similar to that described above for [Ru(bpy)$_2$(quo)]$^+$ was followed. The initially purple reaction mixture in CH$_2$Cl$_2$ turned pink after 8 hours and was allowed to reflux for an additional 12 hrs. Purification by separatory funnel from water and CH$_2$Cl$_2$ yielded the [Ru(bpy)$_2$(5-NO$_2$-quo)](PF$_6$) product (133 mg; 72%). $^1$H-NMR (500 MHz; acetone-$d_6$): $\delta$ 9.29 (dd, 2H), 8.48 (m, 6H), 7.95 (m, 6H), 7.69 (dd, 1H), 7.40 (m, 5H), 6.77 (dd, 1H). MALDI MS m/z = 606 [Ru(bpy)$_2$(5-NO$_2$-quo)]$^+$. 

**Figure 3.5** MALDI mass spectroscopy of [Ru(quo)$_3$]$^+$. 

[Ru(bpy)$_2$(5-NO$_2$-quo)](PF$_6$)
$[\text{Ru(bpy)}_2(\text{NH}_3)_2](\text{PF}_6)_2$ The compound was prepared by a modification of a reported method.$^{3}$ Cis-Ru(bpy)$_2$Cl$_2$ (0.63 mg; 1.0 mmol) was stirred in a solution of anhydrous methanol with 0.39 g (2.0 mmol) AgBF$_4$ for 1 hr, followed by filtration to remove the solid AgCl. Solvent evaporation resulted in the formation of a red/purple solid that was filtered and washed with ether and dichloromethane. A 2.0 M NH$_3$ solution (10 mL) was added to the solid and the mixture was stirred at room temperature for 1 hr, after which time the product was passed through a Sephadex SP C-25 column and eluted with 0.2 M HCl. The brown eluent was evaporated to dryness and the solid was precipitated from acetone/ether with 40 % yield. $^1$H NMR in DMSO-$d_6$, $\delta$ (int., mult): 9.0 (2H, d), 8.6 (2H, d), 8.4 (2H, d), 8.0 (2H, t), 7.6 (4H, t), 7.3 (2H, d), 7.0 (2H, t), 2.9 (NH$_3$). ESMS m/z = 445, $[\text{Ru(bpy)}_2(\text{NH}_3)_2]^+$.

$[\text{Ru(bpy)}_2(\text{CH}_3\text{CN})_2](\text{PF}_6)_2$ The compound was prepared by a modification of a reported method.$^4$ A solution of cis-[Ru(bpy)$_2$Cl$_2$] (0.484 g, 1.0 mmol) was refluxed for 1.5 hr in 30 mL of a 1:1 (v/v) mixture of acetonitrile and water. The solution was then filtered while still hot and reheated to the point of boiling on a hot plate. A saturated aqueous solution of NH$_4$PF$_6$ (5 mL) was added, the reaction mixture was allowed to cool slowly to room temperature, and was then placed in an ice bath. The precipitate was collected by filtration, washed with 30 mL of cold water, and was air-dried to afford a red solid with 70 % yield. $^1$H NMR in DMSO-$d_6$, $\delta$ (int., mult): 9.3(2H, d), 8.6 (2H, d), 8.4 (2H, d), 8.242 (2H, t), 7.9 (2H, t), 7.8 (2H, t), 7.6(2H, d), 7.3 (2H, t), 2.3 (3H,s). ESMS m/z = 641, $[\text{Ru(bpy)}_2(\text{CH}_3\text{CN})_2](\text{PF}_6)_2$.
[Ru(phpy)(CH$_3$CN)$_4$]PF$_6$ [Ru(phpy)(CH$_3$CN)$_4$](PF$_6$) was prepared by cycloruthenation of [$\eta^6$-C$_6$H$_6$]RuCl($\mu$-Cl)$_2$ as previously described.\(^5\) [$\eta^6$-C$_6$H$_6$]RuCl($\mu$-Cl)$_2$ (1.5 g, 3.0 mmol), KPF$_6$ (2.22 g, 12.0 mmol), NaOH (0.48 g, 6.02 mmol) and phenylpyridine (6.02 mmol) were stirred in 50 mL CH$_3$CN at 45 – 50°C for 20 hrs. The resulting yellow slurry was evaporated to dryness under reduced pressure, and the residue was purified by column chromatography on Al$_2$O$_3$ using CH$_3$CN as the eluent. Bright yellow crystals were obtained by diffusion of ethyl ether into a concentrated solution of the yellow solid in a mixture of CH$_2$Cl$_2$;CH$_3$CN (1:1 v:v). $^1$H NMR (acetonitrile-d$_3$), $^\delta$ (int., mult): 8.89 (1H, d), 7.95 (1H, dd), 7.86 (1H, d), 7.72 (1H, td), 7.70 ((1H, d), 7.15 (1H, td), 7.07 (1H, td), 6.95 (1H, td), 2.49 (3H, s), 2.13 (3H, s), 1.94 (6H, s). ESMS (m/z): 419 (5 %) [M + H]$^+$, 379 (63 %) [(M + H)-MeCN]$^+$, 338 (37 %) [(M + H) – 2MeCN]$^+$, 297 (45 %) [(M + H) - 3 MeCN]$^+$, 256 [(M + H) - 4 MeCN]$^+$.

[Ru(phen)(phpy)(MeCN)$_2$]PF$_6$ Under argon and shielded from light, [Ru(phpy)(CH$_3$CN)$_4$]PF$_6$ (612 mg, 1.08 mmol) and 1,10-phenanthroline (195 mg, 1.08 mmol) were stirred in dry MeCN (50 mL) at room temperature for 15 h. The solvent was evaporated, redissolved in acetonitrile and eluted from an alumina column with CH$_2$Cl$_2$. The reddish band was concentrated and crystallized from ether to provide cis-[Ru(phpy)(phen)(CH$_3$CN)$_2$]$^+$ as red-brownish crystals (482 mg, 67%). The $^1$H NMR spectra of cis-[Ru(phpy)(phen)(CH$_3$CN)$_2$]$^+$ in CD$_3$CN is given in Figure 3.6 and 3.7. $^1$H NMR : $^\delta$ 9.77 (dd, 1H, $J$ = 5.1, 1.2 Hz), 8.70 (dd, 1H, $J$ = 8.4, 1.2 Hz), 8.35 (dd, 1H, $J$ = 7.5, 0.6 Hz), 8.23 (dd, 1H, $J$ = 8.1, 5.1 Hz), 8.15 (dd, 1H, $J$ = 5.1, 0.9 Hz), 8.11 (d, 1H, $J$ =
= 8.7 Hz), 8.06 (dd, 1H, J = 8.4, 1.2 Hz), 7.91 (d, 1H, J = 9.0 Hz), 7.81 (d, 1H, J = 7.2 Hz), 7.75 (d, 1H, J = 7.8 Hz), 7.38 (m, 2H), 7.31 (td, 1H, J = 7.2, 1.5 Hz), 7.24 (dd, 1H, J = 8.1, 1.5 Hz), 7.08 (td, 1H, J = 8.2, 1.5 Hz), 6.54 (td, 1H, J = 7.4, 1.5 Hz), 2.23 (s, 3H, CH₃), 2.05 (s, 3H, CH₃).

**Figure 3.6** ¹H NMR spectra of aliphatic region for [Ru(phpy)(phen)(CH₃CN)₂]⁺.

**Figure 3.7** ¹H NMR spectra of aromatic region for [Ru(phpy)(phen)(CH₃CN)₂]⁺.
3.3 Instrumentation.

$^1$H-NMR spectra were recorded on a Bruker DRX-500 spectrometer and MALDI-TOF mass spectrometry was performed on a Bruker Reflex III mass spectrometer with 2,5-dihydroxybenzoic acid as the matrix. The electrochemistry measurements were conducted on a BAS Systems CV-50W instrument with a single-compartment three-electrode cell. The working electrode was a 1.0 mm diameter Pt disk (BAS) with a Pt wire auxiliary electrode and a Ag/Ag$^+$ pseudo-reference electrode. Absorption measurements were performed on a Hewlett-Packard diode array spectrometer (HP8453). Emission spectra were collected on a SPEX FluoroMax-2 spectrometer with DataMax-Std software.

3.4 Methods

Solutions were deoxygenated with argon by passing argon gas through a syringe needle into the reaction vessel that was sealed from the atmosphere using a rubber septum. The pressure developed during this time was allowed to escape through a needle outlet placed at the top of the reaction vessel in another rubber septum.

All photophysical measurements were performed in a $1 \times 1$ cm quartz cuvette equipped with a rubber septum, and the solutions were bubbled with Ar for 15 min prior to each measurement.$^6$ Quantum yield measurements were taken relative to potassium ferrioxalate, by a previously reported method. Quantum yields measurements were calculated from absorption changes collected at several irradiation wavelengths, $\lambda_{\text{irr}} = \ldots$
355, 400, 450 and 500 nm. The wavelength of irradiation was controlled using a bandpass filter with maximum transmittance at 355 nm (10LF10, Newport), 400 nm (6L217, Newport), 450 nm (10BF10-450, Oriel) or 500 nm (10BF10-500, Oriel) each with a 10 nm bandwidth. The photon flux was first determined by calculating the moles of Fe$^{2+}$, $n_{Fe^{2+}}$, produced when a solution of K$_3$Fe(C$_2$O$_4$)$_3$ in aqueous sulphuric acid was irradiated by light. The Fe$^{3+}$ ions are reduced to Fe$^{2+}$ via the following reaction:

$$[Fe_3+(C_2O_4)_3]^{3-} + hv \rightarrow [Fe_2+(C_2O_4)_3]^{2-} + C_2O_4^-$$

The product Fe(C$_2$O$_4$)$_2$ does not absorb incident light and the Fe$^{2+}$ ions can be spectrophotometrically determined as a red colored complex with 1,10-phenanthroline which absorbs at $\lambda_{\text{max}} = 510$ nm. The moles of Fe$^{2+}$ produced upon irradiation is therefore calculated according to the following equation:

$$n_{Fe^{2+}} = \frac{6.023 \times 10^{20} \cdot V_1 \cdot V_3 \cdot A}{V_2 \cdot l \cdot \varepsilon}$$

(3.1)

where $V_1$ is the volume of actinometer solution irradiated (2 ml), $V_2$ is the volume of aliquot taken for analysis (1 mL), $V_3$ is the final volume to which aliquot $V_2$ is diluted (10 ml), $\varepsilon$ is the molar extinction coefficient of potassium ferrioxalate ($1.15 \times 10^4$ M$^{-1}$cm$^{-1}$), and $A$ is the absorbance of potassium ferrioxalate at 510 nm. K$_3$Fe(C$_2$O$_4$)$_3$ was used as the standard due to its wide spectral range of known quantum yields. The quanta absorbed by the actinometer ($n_a$) can be calculated using equation 3.2:

$$n_{Fe^{2+}} = \frac{n_{Fe^{2+}}}{\Phi_\lambda}$$

(3.2)
In eq 3.2, $\Phi_\lambda$ is the quantum yield at 350 nm, 1.21. Since the photon flux is the number of photons absorbed per second, a plot of $n_a$ versus irradiation time results in a straight line, the slope of which is equal to the photon flux. The changes in the absorption resulting from formation of each Ru(II) complex was used to determine the concentration of the product at a given time a the selected irradiation wavelength. Since the quanta absorbed at a given irradiation time is known ($n_a$), equation 3.3 can then be applied to calculate the quantum yield where the slope of a plot of $n_{Ru^{2+}}$ vs. photon absorbed is the quantum yield of the reaction.

$$\Phi_\lambda = \frac{(n_{Ru^{2+}})_t}{(n_a)_t}$$  \hspace{1cm} (3.3)

Solutions for electrochemical measurements were prepared in distilled acetonitrile with 0.1 M tetrabutylammonium hexafluorophosphate as the electrolyte. The Ru(III/II) couple was measured using the cyclic voltammetry mode at a 100 mV scan rate. At the end of each experiment, a small amount of ferrocene (Fc) was added as an internal standard and was used as a reference for calculating the oxidation and reduction potentials of each complex with $E_{1/2}(Fc^{+/0}) = 0.39$ V vs SCE.\textsuperscript{7}

Emission spectra of samples in methanol:ethanol (1:4 v/v) were obtained by degassing the solution with nitrogen for 20 minutes prior to each experiment. Spectra at 77 K were recorded using a liquid nitrogen Dewar with quartz windows and the sample
was either a solid or in a 1:4 mixture of nethanol:ethanol in a quartz NMR tube inside the dewar.

The transient absorption signal was measured following sample excitation with the 532 nm output from a frequency doubled Spectra-Physics GCR-150-10 Nd:YAG laser (fwhm ~ 8 ns, ~5 mJ/pulse). The output from a 150 W Xe arc lamp (USHIO) powered by a PTI PS-220 power supply (in a PTI housing with f/4 focusing lens), pulsed with electronics built in-house, was focused onto the sample at a 90° with respect to the laser beam with an f/4 lens. The lamp light and the laser beam each pass through computer-controlled Uniblitz shutters with Uniblitz (model D122) drivers prior to reaching the sample. The white light transmitted by the sample was collimated and focused with two fused silica plano-convex lenses (f/4, 1 in diameter) onto the entrance slit of a Spex H-20 single monochromator (1200 gr/mm grating blazed at 500 nm), and the signal was detected utilizing a Hamamatsu R928 photomultiplier tube (modified in house to accommodate high light intensity applications) powered by a Stanford Research PS325 power supply. The signal was digitized on a Tektronics 400 MHz oscilloscope (TDS 380) triggered by the signal of a photodiode produced by each laser pulse reaching the sample (~5% laser light was split by a quartz plate). A PowerMac G4 (Apple) equipped with a National Instruments GPIB interface (NI-488.2) and a National Instruments data acquisition board (PCI-1200) was programmed with Labview 4.1 software to control the data acquisition by the oscilloscope and the PMT voltage. The timing of the triggering of the laser oscillator and Q-switch, the lamp pulser, and various shutters was accomplished using a digital delay generator (SRS DG535), whose action
was coupled to the acquisition cycles of the computer through AND-gate circuitry. Attenuated scattered laser light yielded an overall instrument response function with fwhm = 12.5 ns. Emission lifetimes greater than 100 ns were measured on the same instrument with 90° excitation/detection geometry.\(^8\)

Diffraction-quality crystals of \(\text{cis-[Ru(phpy)(phen)(CH}_3\text{CN)_(2)]}^+\) were formed by layering a solution of concentrated complex in CHCl\(_3\) with diethyl ether followed by storage of the covered solution at –5°C for one week to yield dark brownish-red crystals. Examination of the diffraction pattern was performed with a Nonius Kappa CCD diffractometer,\(^9\) and data was collected at 150 K using an Oxford Cryosystems Cryostream Cooler. The data collection strategy was set up to measure a hemisphere of reciprocal space with a redundancy factor of 3.5, constituting that 90% of reflections were measured at least 3.5 times. Phi and omega scans with a frame width of 1.0° were used. Data integration was done with Denzo, and scaling and merging of the data was done with Scalepack.\(^9\) The location of the Ru atom was obtained by the Patterson method in SHELXS-97.\(^9\) The remaining atoms were located by standard Fourier methods. Full-matrix least-squares refinements based on F\(^2\) were performed in SHELXL-97, as incorporated in the WinGX package.\(^9\)

The molecular and electronic structure calculations of 1 – 4 were performed using the Gaussian03 (G03) program package.\(^10\) The B3LYP functional with the 6-31G* basis set was used for hydrogen, carbon, nitrogen, and oxygen and the Stuttgart/Dresden (SDD) energy-consistent pseudo-potentials for ruthenium.\(^54\)–\(^57\) All geometry optimizations were performed in C1 symmetry with subsequent frequency analysis to show that the
structures are at the local minima on the potential energy surface, and the solvent was modeled using the polarizable continuum model (PCM). The orbital analysis was completed with GaussView 3.0. The vertical singlet transition energies of the complexes were computed at the time-dependent density functional theory (TD-DFT) level in acetonitrile within G03 using the ground-state optimized structure of each complex.

Gel mobility experiments were carried out using 100 µM pUC18 plasmid DNA linearized with 10 units of Sma I in 100 mM Tris, pH = 7.6, 100 mM MgCl₂, 150 mM NaCl at 37 °C for 1 hr, followed by deactivation at 65°C for 5 min. The concentration of plasmid was spectrochemically determined using an extinction coefficient of 6 600 M⁻¹ cm⁻¹ at 260 nm. The ratio of DNA base pairs (bp) to metal complex (mc), (bp):(mc), varied from 100 to 0.5. The control solutions containing cisplatin were incubated at 40 °C for 4 hrs in 5 mM phosphate buffer, pH = 7.2 before adding 3 µL of loading dye was added to each sample. Control samples containing molecular weight standard and the linearized plasmid were loaded onto the gel ass soon as they were prepared. The solutions were loaded onto a 0.8 % agarose gel containing 0.09 M Tris, 0.002 M disodium salt of EDTA and 0.09 M boric acid, pH 7.5. The gel electrophoresis was conducted at 95 V for 1 hr. The gels were stained with 0.5 µg / mL EtBr for 30 min and were submerged in a water bath for 15 min to remove excess EtBr before imaging on a Gel Doc 2000 (Biorad) transilluminator equipped with Quality One software. The Ru(II) complexes were irradiated at various wavelengths and lengths of time at 25 °C before loading each sample onto the gel.

Phototoxicity and cytotoxicity were determined for H1299 lung cancer cells
grown to 80% confluence in 96 well plates. Cells were dosed with various concentration of complex for 6 hrs followed by washing with 150\(\mu\)L of PBS buffer. 200 \(\mu\)L of growth medium were added to the cells and allowed to incubated for 72 hours. Irradiation of the samples was performed with blue filter light (455 nm) provide on a Maestro CRI. Samples were either incubated in the dark or irradiated for 20 minutes. Cell death was quantified by counting grids of cells stained with 100\(\mu\)L of a 1% methylene blue incubated for 1 hr at room temperature followed by measurements of optical density with a microplate spectrophotometer (Bio-Teck Instrument). LC\(_{50}\) values were determined by lethal concentration required to produce cell death in 50% of the population. Determination of the LC\(_{50}\) was determined in triplicate.
REFERENCES


CHAPTER 4

“ELECTRONIC TUNING OF RUTHENIUM COMPLEXES BY 8-QUINOLATE LIGANDS”


4.1 Introduction

Ruthenium coordination compounds containing polypyridyl ligands have long been the benchmark for understanding the photophysical properties of transition metal complexes.\(^1\) In particular, the photophysical properties and dynamics of \([\text{Ru} (\text{bpy})_3]^{2+}\) (bpy = 2,2'-bipyridine) have been investigated extensively including the effect of structural modifications to the bidentate ligands in the coordination sphere of the complex on the ground and excited state redox chemistry.\(^2\)\(^{-}\)\(^27\) For example, the presence of oxygen in the coordination environment of ruthenium dramatically affects the energy of the lowest energy transitions assigned as metal–to–ligand charge transfer (MLCT).\(^28\)\(^{-}\)\(^38\) Figure 4.1 compares the simplified molecular orbital and state diagrams of \([\text{Ru} (\text{bpy})_3]^{2+}\) and \([\text{Ru} (\text{bpy})_2(\text{quo})]^+(\text{quo}^- = \text{quinolate})\) The introduction of oxygen in the coordination
sphere, in the form of the quo⁻ ligand, raises the energy of the metal centered bonding ($d\pi$) orbitals and lowers that of the metal antibonding ($d\sigma^*$) orbitals. It is important to note that the energy of the bpy antibonding ($\pi^*$) orbitals are unaffected by the new ligand. The result is a decrease in the lowest energy MLCT transitions upon introduction of one oxygen into the coordination sphere.

It has also been shown that, with careful selection of ligands, it is possible to tune the redox chemistry of the metal center. The low energy MLCT transitions of ruthenium complexes are of particular interest for applications that include their use in dye-sensitized solar energy conversion, photocatalytic C-H bond activation, and photodynamic therapy (PDT). Thus, the investigation of ligands that alter the ground state of the ruthenium complex becomes important for these applications.

**Figure 4.1** Simplified molecular orbital and state diagrams of [Ru(bpy)$_3$]$^{2+}$ and [Ru(bpy)$_2$(quo)]$^+$ showing the decrease in energy of MLCT transition with the introduction of oxygen in the coordination sphere of ruthenium (II) pseudo octahedral environment.
and excited state redox properties of ruthenium complexes is crucial for the design of new systems with improved properties.

Transition metal complexes with redox-active, non-innocent ligands, have recently received increased interest because of their applications in stoichiometric or catalytic molecular transformations.\textsuperscript{31} The distinction between innocent and non-innocent ligand behavior was first made by Jorgensen in 1966 with the statement that “ligands are innocent when they allow oxidation states of the central atoms to be defined.”\textsuperscript{53} In general, the term non-innocent is applied to ligands that have significant interaction with the metal center, directly influencing the metal redox chemistry and making the oxidation state of the metal ambiguous.\textsuperscript{31,32} Furthermore, complexes with non-innocent ligands result in extensive electron delocalization of both the metal and ligand. Because of the nature of the interaction between the ligand and the metal orbitals, modulation of the electron distribution can be easily achieved by alterations to both the ancillary and non-innocent ligands. Greater non-innocent behavior is observed when there is more overlap with metal d\pi orbitals. Therefore, the redox active ligand allows for the ability to tune, and at times increase, the range of redox chemistry available for metal complexes. This broadening of redox chemistry in turn increases the scope of reactions that these complexes can catalyze.

Ruthenium complexes with heteroatom N,O donor non-innocent ligands (NIL) have been of particular interest. For the case of Ru-(NIL) complexes this effect arises from significant overlap of the Ru(d\pi) set and NIL(\pi) frontier orbitals resulting in extensive delocalization of electron density over the metal-ligand \pi-network (Figure 4.2).
This Ru-NIL electron delocalization was previous described as an aromatic 5-membered ring composed of the metal center, heteroatoms and their α-carbons.\textsuperscript{54-56} In complexes containing N,O-coordinated non-innocent ligands the highest occupied molecular orbitals (HOMOs) possess contributions from both the metal and ligand. In addition, the lowest unoccupied molecular orbital (LUMO) can be NIL centered with additional contribution from the metal orbitals. As previously discussed, the lowest energy electronic transition in ruthenium polypyridyl complexes is MLCT. However, the high degree of electron distribution arising from the mixed contribution of the HOMO and LUMO results in a transition that is not purely from a metal centered orbital to that of the ligand.
Figure 4.2 Molecular orbital diagram for ruthenium (II) pseudo octahedral environment with non-innocent ligand (NIL).
The strong binding of the N,O 8-quinolate ligand to metals has long been known, and transition metal complexes of these ligands and their derivatives have been used in a variety of applications.\textsuperscript{31-52} The sensing of closed-shell transition metal and heavy ions by the resulting strongly luminescent chelated tris-homoleptic species represents an example of such applications.\textsuperscript{50} In addition, the electroluminescence of Al(quo)\textsubscript{3} and Ga(quo)\textsubscript{3} has proven useful in organic light emitting diodes, OLEDs.\textsuperscript{41,52} Although the complexation of quo\textsuperscript{-} derivatives with many metals has been extensively investigated, there are a limited number of reports for ruthenium complexes.\textsuperscript{30-38} Of these, little is known about the effect of the ligand on redox and optical properties of these complexes.

Previous work focused on [Ru(L)\textsubscript{2}(quo)]\textsuperscript{+}, where L = bpy, phen, dmphen (4,7-dimethyl-1,10-phenanthroline) and tmphen (3,4,7,8-tetramethyl-1,10-phenanthroline).\textsuperscript{30} The molecular structures of these ligands are shown in Figure 4.3. The coordination of the π-donor oxygen atom to the metal results in an increase in energy of the metal-centered highest occupied molecular orbital (HOMO) relative to that in [Ru(bpy)\textsubscript{3}]\textsuperscript{2+}, such that the MLCT transition shifts to lower energy and a cathodic shift of the Ru(III/II) couple is observed.\textsuperscript{30} For example, the MLCT absorption of [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} at 452 nm shifts to 515 nm in [Ru(bpy)\textsubscript{2}(quo)]\textsuperscript{+} in water.\textsuperscript{30} In addition, it was found that the higher energy metal-centered HOMO in the latter makes this complex easier to oxidize than [Ru(bpy)\textsubscript{3}]\textsuperscript{2+}.

The MLCT transition in metal complexes is commonly expressed as a formal oxidation of the metal center and corresponding reduction of the ligand as described by eq 4.1
Figure 4.3 Molecular structure of 2,2’-bipyridine (bpy), 1,10-phenanthroline (phen), 8-quinolate (quo\(^-\)), and 5-nitro-8-quinolate (5-NO\(_2\)-quo\(^-\)).
\[ \text{Ru}^{\text{II}} - \text{L} + h\nu \rightarrow \left[ \text{Ru}^{\text{III}} - \text{L}^+ \right]^* \]  \hspace{1cm} (4.1)

Therefore, the lowest spin allowed MLCT transition can be related to the redox behavior of a metal complex as described by the relationship in eq. 4.2

\[ h\nu(\text{MLCT}) = \Delta E_{\text{redox}} + R \]  \hspace{1cm} (4.2)

where \( \Delta E_{\text{redox}} \) represents the difference between the metal and ligand first reduction potentials and \( R \) represents the reorganization energy and various solvent terms.\(^{31}\) For typical Ru polypyridyl complexes the value of \( R \) is on the order of 0.2 eV. Although, this simplified picture is useful for most Ru(II) complexes, the presence of multiple quinolate ligands results in a large degree of mixing between the metal and ligand frontier orbitals complicating traditional electronic structure predictions. It has previously been reported that the presence of NILs can result in a value for \( R \) as high as 1.0 eV.\(^{32}\) Therefore, the relationship between the MLCT transition and \( \Delta E_{\text{redox}} \) can deviate greatly with increasing NIL behavior.

The present work focuses on the investigation of a series of Ru(II) 8-quinolate complexes that includes \([\text{Ru(bpy)}_2(\text{quo})]^+\) (2), \([\text{Ru(bpy)}(\text{quo})_2]\) (3), \([\text{Ru(quo)}_3]^-\) (4) and \([\text{Ru(bpy)}_3(5-\text{NO}_2-\text{quo})]^+\) (5), where the structures of the ligands are shown in Figure 4.3. In order to investigate the ground state effects of the number of oxygen atoms on the ligation sphere of the metal, spectroscopic and electrochemical measurements were compared to those of the parent complex \([\text{Ru(bpy)}_3]^{2+}\) (1). TD-DFT calculations were used to aid in the understanding of the electronic and electrochemical behavior of the complexes.
4.2 RESULTS AND DISCUSSION

4.2.1 Electronic Absorption

The electronic absorption maxima and extinction coefficients of 1 – 5 in acetonitrile are listed in Table 4.1, together with electrochemical data, and representative absorption, excitation and emission spectra of 2 are shown in Figure 4.4. The differences in the ligand-centered (LC) absorption maxima in the 240 – 310 nm range among the complexes results from variations of the bidentate ligand in the coordination sphere. The \( \pi\pi^* \) transition of free bpy ligand appears at 290 nm in CH$_3$CN, which shifts to 286 nm when coordinated to Ru(II).\(^1\) The free protonated form of the quo\(^-\) bidentate ligand, quoiH, absorbs at 310 nm in acetonitrile, which is apparent in 2 and 3 as a shoulder of the bpy \( \pi\pi^* \) band at 335 and 370 nm, respectively. Similarly, the free 5-NO$_2$-quoiH ligand absorbs at 353 nm in acetonitrile, and this peak shifts to 348 nm in 5 in the same solvent. The homoleptic [Ru(quoi)$_3$]$^-$ (4) complex exhibits a ligand-centered \( \pi\pi^* \) transition at 260 nm, consistent with the analogous Al(quoi)$_3$ and Ga(quoi)$_3$ systems with reported maxima at 260 nm and 265 nm, respectively.\(^52,55\) It is interesting to note that 4 is oxidized by oxygen in air to generate the corresponding Ru(III) species, [Ru(quoi)$_3$], which is easily reduced by treatment with NaBH$_4$ (Figure 4.5).

A progressive red shift in the lowest energy absorption maximum is observed from 1 to 3. The lowest energy transition for Ru(II) complexes with bpy ligands are well
### Table 4.1. Absorption maxima and electrochemical potentials for 1 – 5.

<table>
<thead>
<tr>
<th>Complex</th>
<th>L₁</th>
<th>L₂</th>
<th>L₃</th>
<th>( \lambda_{\text{abs}}/\text{nm} (\varepsilon / \times 10^3 \text{ M}^{-1}\text{cm}^{-1})^{a} )</th>
<th>( E_{1/2}^{b} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>bpy</td>
<td>bpy</td>
<td>bpy</td>
<td>243 (85.9), 286 (19.4), 452 (14.4)</td>
<td>+1.29, -1.58</td>
</tr>
<tr>
<td>2</td>
<td>bpy</td>
<td>bpy</td>
<td>quo⁻</td>
<td>250 (45.5), 335 (11.1), 400 (9.7), 510 (11.0)</td>
<td>+0.91, -1.58</td>
</tr>
<tr>
<td>3</td>
<td>bpy</td>
<td>quo⁻</td>
<td>quo⁻</td>
<td>310 (41.2), 370 (22.0), 458 (15.4), 515 (12.1)</td>
<td>+0.87, -1.58</td>
</tr>
<tr>
<td>4</td>
<td>quo⁻</td>
<td>quo⁻</td>
<td>quo⁻</td>
<td>212 (39.6), 260 (33.3), 540 (7.32)</td>
<td>+0.33, -1.77</td>
</tr>
<tr>
<td>5</td>
<td>bpy</td>
<td>bpy</td>
<td>5-NO₂-quo⁻</td>
<td>244 (32.3), 293 (48.3), 464 (16.3) 496 (17.8)</td>
<td>+1.17, -1.48</td>
</tr>
</tbody>
</table>

\(^{a}\)In acetonitrile. \(^{b}\)In acetonitrile with 0.1 M tetrabutylammonium hexafluorophosphate vs. SCE.
Figure 4.4  Electronic absorption (—), excitation (••; \( \lambda_{\text{em}} = 760 \) nm) and emission (— —; \( \lambda_{\text{exc}} = 500 \) nm) spectra of 2 (30 \( \mu \)M) in 4:1 ethanol/methanol glass at 77 K.
Figure 4.5 Electronic Absorption spectrum of [Ru(quo)$_3$] before (——) and after (—) treatment with NaBH$_4$.

known to be $^1$MLCT in character, with maximum at 452 nm for 1 in CH$_3$CN.$^2$ Therefore, the transitions at 510 nm and 515 nm for 2 and 3, respectively, are assigned as $^1$MLCT (Ru→bpy). It is apparent that there is a red-shift in the Ru→bpy MLCT transition with the successive coordination of each quo$^-$ ligand from 1 to 3. In 4, where bpy is not present and three π-donor oxygen atoms are present in the coordination sphere, the peak at 540 nm is assigned as $^1$MLCT (Ru→quo$^-$). Interestingly, the lowest energy transition in 5 shifts to shorter wavelength with maximum at 496 nm compared to 2. As will be discussed in more detail below, this peak is assigned as $^1$MLCT (Ru→NO$_2$-quo$^-$), since the NO$_2$-quo$^-$ ligand is easier to reduce than bpy (Table 4.1). TD-DFT calculations are
also consistent with the assignment of this transition in 5 as $^1\text{MLCT (Ru→NO}_2\text{-quo}^-)$ and is discussed in detail later. The blue shift in the $^1\text{MLCT (Ru→NO}_2\text{-quo}^-)$ peak in 5 compared to that corresponding to $^1\text{MLCT (Ru→quo}^-)$ in 4 is counterintuitive, since the NO$_2$-quo$^-$ ligand is easier to reduce than quo$^-$ (Table 4.1). The lower energy transition in 4 compared to 5 is attributed to a higher energy Ru(II) t$_{2g}$-type HOMO in the former arising from the presence of three $\pi$-donor oxygen atoms in this complex, in contrast to the presence of only one such atom in the latter.

The absorption, excitation, and emission spectra of 2 are shown in Figure 4.4. The complex exhibits weak luminescence with maximum at 767 nm in CH$_3$CN at room temperature, and an increase in emission intensity is observed at 77 K in a EtOH/MeOH glass (4:1 v/v). This finding is consistent with the thermal accessibility of nonemissive low-lying ligand-field (LF) dd state(s) from the lowest energy $^3\text{MLCT state that is typical of Ru(II) complexes, including 1.}^2$ In addition, the emission maximum observed for 2 is similar to those reported previously for related Ru(II) complexes containing quo$^-$, which range from 757 to 783 nm for [Ru(L)$_2$(quo)]$^+$ (L = phen, dmphen, tmphen) in water.$^{30}$ The excitation spectrum of 2 overlays well with the absorption spectrum (Figure 4.4), indicating that the luminescence does not arise from an impurity in the sample. Emission was not detected at 298 K in CH$_3$CN or at 77 K in EtOH/MeOH glasses for 3, 4 or 5. The lowest energy excited state in 3 is expected to be $^3\text{MLCT(Ru→bpy)}$, it is likely that the LF state(s) lie at lower energy than in 2 based on the shift of the $^1\text{MLCT absorption peak to lower energy resulting from a higher energy t}_{2g}$-type Ru(II) HOMO (Figure 4.6).
In 4 and 5, the lowest $^3\text{MLCT}$ state is expected to be $\text{Ru}\rightarrow\text{(quo}^-\text{)}$ and $\text{Ru}\rightarrow\text{(NO}_2\text{-quo}^-\text{)}$, respectively, and these states appears to be non-emissive in these complexes.

![Energy well diagram](image_url)

**Figure 4.6** Energy well diagram showing the relative energies of ground state (GS), $^1\text{MLCT}$, $^3\text{MLCT}$ and $^3\text{LF}$ in (a) $[\text{Ru(bpy)}_2\text{(quo)}]^{\text{+}}$ and (b) $[\text{Ru(quo)}_2\text{(bpy)}]$. 


4.2.2 Electrochemistry

The electrochemical oxidation potential of each complex, $E_{1/2}$, is presented in Table 4.1 in CH$_3$CN. In general, 2 – 5, which contain quinolate ligands, are more easily oxidized than 1, showing a reversible one electron oxidation in the range of +1.17 to +0.33 V vs SCE assigned to the Ru(III/II) couple. This trend is consistent with the previously reported [Ru(bpy)$_2$(quo)]$^+$ and [Ru(phen)$_2$(quo)]$^+$, with Ru(III/II) couples at +0.94 V and +0.87 V vs SCE, respectively. Complexes 2, 3 and 5 are not oxidized by O$_2$ in air ($E_{O_2/O_2^-} = +0.43$ V vs SCE) consistent with their oxidation potentials (Table 4.1). The Ru(III/II) couple at +0.33 V for 4 makes the reduction of O$_2$ by the reduced Ru(II) complex favorable, such that the Ru(III) oxidation state of the complex is present under aerobic conditions. The ease of oxidation of 4 is attributed to the higher energy ruthenium centered HOMO with the addition of three π–donor oxygen atoms to the coordination sphere in the series. This trend has also been observed in the isoquinoline complexes [Ru(bpy)$_2$(1-COO-iQu)]$^+$ (1-COO-iQu$^- = $ isoquinoline-1-carboxylate) and [Ru(bpy)$_2$(3-COO-iQu)]$^+$ (3-COO-iQu$^- = $ isoquinoline-3-carboxylate), with a Ru(III/II) reduction potentials of +0.86 V and +0.85 V vs SCE respectively (Table 4.2). Furthermore, a comparison of the oxidation potentials of 2 and 5 reflect the electron withdrawing nature of the 5-NO$_2$-quo$^-$ ligand, where the decrease in π–electron donation from the oxygen atom in 5-NO$_2$-quo$^-$ shifts the Ru$_{III/II}$ couple of 5 (+1.17 vs. SCE) to a more positive potential as compared to 2 (+0.91 vs SCE). The first ligand-centered reduction potentials for 1 – 3 were found to be –1.58 V vs SCE, corresponding to the well-known
Table 4.2 Electrochemical and Emission maxima for $[\text{Ru(bpy)}_2]^{2+}, [\text{Ru(bpy)}_2(5\text{-NO}_2\text{-quo})]^+, [\text{Ru(bpy)}_2(\text{quo})]^+, [\text{Ru(bpy)}_2(1\text{-COO-iqu})]^+, [\text{Ru(bpy)}_2(3\text{-COO-iqu})]^+$.

<table>
<thead>
<tr>
<th>L</th>
<th>$\lambda_{\text{abs}}$/nm ($\epsilon$ / x10$^3$ M$^{-1}$ cm$^{-1}$)$^a$</th>
<th>$\lambda_{\text{em}}$/ nm$^a$</th>
<th>$E_{00}$/ eV$^b$</th>
<th>$E_{1/2}$/ V$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>bpy</td>
<td>243 (37.9), 286 (117), 452 (19.6)</td>
<td>610</td>
<td>2.10</td>
<td>+1.26</td>
</tr>
<tr>
<td>5-NO$_2$-quo$^-$</td>
<td>244 (32.3), 293 (48.3), 496 (17.8)</td>
<td>—</td>
<td>—</td>
<td>+0.94</td>
</tr>
<tr>
<td>quo$^-$</td>
<td>245 (66.2), 290 (86.5), 460 (15.8)</td>
<td>ii</td>
<td>1.85</td>
<td>+0.60</td>
</tr>
<tr>
<td>1-COO-iqu$^-$</td>
<td>239 (27.6), 290 (33.4), 480 (7.86)</td>
<td>764</td>
<td>2.10</td>
<td>+0.74</td>
</tr>
<tr>
<td>3-COO-iqu$^-$</td>
<td>238 (30.0), 290 (35.9), 470 (5.77)</td>
<td>697</td>
<td>2.17</td>
<td>+0.75</td>
</tr>
</tbody>
</table>

$^a$ In acetonitrile $^b$ At 77 K in EtOH/MeOH glass (4:1 v/v) $^c$ In acetonitrile with 0.1 M tetrabutylammonium hexafluorophosphate vs. SCE
The reduction of the quinolate ligand was observed at a more negative potential, –1.77 V vs SCE, in 4,31 consistent with the assignment of the \(^1\text{MLCT}\) transitions in 2 and 3 and the \(^3\text{MLCT}\) emission in 2 as arising from Ru\(\rightarrow\)bpy. Two reduction potentials were measured for 5, one at –1.48 V vs SCE and –1.59 V vs SCE, corresponding to the reduction of the NO\(_2\)-quo\(^-\) and one of the bpy ligands, respectively. This finding is consistent with the assignment of the \(^1\text{MLCT}\) absorption peak as Ru\(\rightarrow\)NO\(_2\)-quo\(^-\) in 5.

Comparison of the redox potential difference and lowest energy optical transition is presented in Table 4.3. MLCT transitions are defined as promotion of an electron from a ground state metal orbital to antibonding ligand \(\pi^*\). This process is often described as formal oxidation of the metal center and reduction of the ligand. As described in more detail previously, this results in a direct relationship between the maximum of the MLCT optical transition, expressed in eV, and the electrochemical difference of the metal oxidation and ligand reduction potentials, given by eq. 4.2. Often, the deviation between these values, R (eq 4.2), is used to express the degree of non-innocent ligand behavior present in a given complexes. For ruthenium polypyridyl complexes, this value is typically on the order of 0.1 – 0.2 eV. As is apparent from Table 4.3, the R values for 2 – 5 fall well within this range, with the exception of 4 which has an R value of 0.20 eV perhaps indicating significant orbital mixing in this complex.
Table 4.3 Lowest Energy Transition (MLCT), potential difference of metal oxidation and ligand reduction ($\Delta E_{\text{redox}}$), and calculated R value from eq. 4.2.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\lambda_{\text{MLCT}}$/ nm&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MLCT/eV</th>
<th>$\Delta E_{\text{redox}}$/V</th>
<th>R&lt;sub&gt;calc&lt;/sub&gt;/eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ru(bpy)&lt;sub&gt;3&lt;/sub&gt;]&lt;sup&gt;2+&lt;/sup&gt; (1)</td>
<td>452</td>
<td>2.74</td>
<td>2.87</td>
<td>0.13</td>
</tr>
<tr>
<td>[Ru(bpy)&lt;sub&gt;2&lt;/sub&gt;(quo)]&lt;sup&gt;+&lt;/sup&gt; (2)</td>
<td>510</td>
<td>2.43</td>
<td>2.49</td>
<td>0.06</td>
</tr>
<tr>
<td>[Ru(bpy)(quo)&lt;sub&gt;2&lt;/sub&gt;] (3)</td>
<td>515</td>
<td>2.41</td>
<td>2.45</td>
<td>0.04</td>
</tr>
<tr>
<td>[Ru(quo)&lt;sub&gt;3&lt;/sub&gt;]&lt;sup&gt;−&lt;/sup&gt; (4)</td>
<td>540</td>
<td>2.30</td>
<td>2.10</td>
<td>-0.20</td>
</tr>
<tr>
<td>[Ru(bpy)&lt;sub&gt;3&lt;/sub&gt;(5-NO&lt;sub&gt;2&lt;/sub&gt;-quo)]&lt;sup&gt;+&lt;/sup&gt; (5)</td>
<td>496</td>
<td>2.50</td>
<td>2.65</td>
<td>0.15</td>
</tr>
</tbody>
</table>

<sup>a</sup> Lowest energy absorbance maxima measured in acetonitrile  
<sup>b</sup> In acetonitrile with 0.1 M tetrabutylammonium hexafluoro-phosphate vs. SCE
4.2.3 Calculations

The calculated molecular orbital (MO) diagrams of 1 – 4 are shown in Figure 4.7, where the energy of the bpy-based LUMOs of 1 – 3 are set at the same energy since they are expected to be similar based on their near identical bpy-localized reduction potentials (Table 4.1). Likewise, the LUMO of 4 is set at the same energy as the LUMO+1 of 3 because they are both quo⁻ based π* orbitals, and are expected to lie at similar energies. Furthermore, the HOMO of 1 was set at 0.00 eV, and a gradual increase in the energy of this orbital is noted from 1 to 4 (1.02 eV), consistent with the decreased ligand field splitting expected for the stepwise increase in number of oxygen atoms in the coordination sphere across the series. The same trend is observed experimentally, with a net shift in the oxidation potentials of 1 and 4 of 0.96 V, where 4 is the most easily oxidized complex in the series with $E_{1/2}(\text{[Ru]}^{III/II}) = +0.33$ V vs. SCE. The MOs associated with the HOMO and HOMO-1 of 1 – 4 are displayed in Figure 4.8. These orbitals show that electron density is shared between the metal center and the quo⁻ ligands in 2 – 4.

Figure 4.9 shows the frontier orbitals and the MO diagrams of 2 and 5. The LUMO of 5 is calculated to be a π* orbital centered on the 5-NO₂-quo⁻ ligand instead of the bpy π* LUMO of 2. This finding is reasonable since the former is more easily reduced than the latter. The t₂g set is lower in energy in 5 compared to 2 because of the electron withdrawing nature of the nitro substituent on the 5-NO₂-quo⁻ ligand which, decreases the π-donating ability of the quinolate oxygen and therefore decreases the π* character of
Figure 4.7 Calculated molecular orbital diagrams of 1 – 4.
Figure 4.8 Calculated frontier occupied molecular orbitals of 1 – 4 (isovalue = 0.04).
Figure 4.9 Comparison of the calculated molecular orbital diagrams of 2 and 5 showing selected MOs (isovalue = 0.04).
the t_{2g} set. This result is also consistent with the more positive oxidation potential of 5 compared to that of 2 (Table 4.1).

Time-dependent DFT (TD-DFT) calculations are in good agreement with the experimental electronic transitions. Figure 4.10 shows the experimental absorption spectra of 2 – 5 overlaid with the calculated transitions. It is evident from Figure 4.10 that the peaks with high oscillator strength are predicted fairly well by the calculations, and can therefore be useful in their assignments. Tables 4.4 – 4.7 list the calculated orbital contributions for 2 – 5. The lowest energy vertical singlet transition for 1 is calculated at 473 nm ($f = 0.0017$), which together with various other transitions of substantial oscillator strength ($\lambda = 436$ nm, $f = 0.0136$), contribute to the broad absorption peak with maximum at 452 nm observed experimentally. Likewise, the lowest lying transitions of 2 – 5 posses contributions from a number of orbitals, some of which are depicted in Figure 4.8. For 2 – 5 there are low-lying singlet transitions from the HOMO to the ligand $\pi^*$ orbitals, where the HOMO has contributions from both the metal and quinolate ligand. In 2 and 3 transitions calculated at 513 nm ($f = 0.0528$, HOMO-1→LUMO+1) and 540 nm ($f = 0.1074$, HOMO-2→LUMO), respectively, correspond to movement of electron density from the Ru(II) to bpy $\pi^*$ orbitals. In 4 the calculated transition at 513 nm ($f = 0.2268$) has significant contributions from movement of electron density from the Ru-based HOMO, HOMO-1 and HOMO-2 to the LUMO, LUMO+1, and LUMO+2, which are unoccupied quo` $\pi^*$ orbitals. The transition maxima calculated for 5 at 501 nm ($f = 0.1552$) and at 461 nm ($f = 0.1369$) are from the metal-based HOMO-1 and HOMO-2 to
Figure 4.10 Calculated and experimental electronic absorption spectra of (a) 2, (b) 3, (c) 4, (d) 5 in acetonitrile.
Table 4.4 TD-DFT major transitions for [Ru(bpy)₂(quo)]⁺

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>f (&gt;0.02)</th>
<th>Major Transition (&gt;10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>606.96</td>
<td>0.0266</td>
<td>HOMO → LUMO+1 (72%)</td>
</tr>
<tr>
<td>516.98</td>
<td>0.0517</td>
<td>HOMO-1 → LUMO+1 (69%)</td>
</tr>
<tr>
<td>480.31</td>
<td>0.0477</td>
<td>HOMO → LUMO+2 (49%)</td>
</tr>
<tr>
<td>469.99</td>
<td>0.0836</td>
<td>HOMO → LUMO+2 (70%)</td>
</tr>
<tr>
<td>454.88</td>
<td>0.0311</td>
<td>HOMO-2 → LUMO (26%), HOMO-2 → LUMO+1 (27%)</td>
</tr>
<tr>
<td>411.54</td>
<td>0.0258</td>
<td>HOMO-1 → LUMO+2 (73%)</td>
</tr>
<tr>
<td>389.95</td>
<td>0.0286</td>
<td>HOMO → LUMO+6 (76%)</td>
</tr>
<tr>
<td>380.77</td>
<td>0.0239</td>
<td>HOMO-1 → LUMO+3 (89%)</td>
</tr>
<tr>
<td>350.70</td>
<td>0.0919</td>
<td>HOMO-1 → LUMO+5 (30%), HOMO-1 → LUMO+6 (32%)</td>
</tr>
</tbody>
</table>

Table 4.5 TD-DFT major transitions for [Ru(bpy)(quo)₂]

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>f (&gt;0.02)</th>
<th>Major Transition (&gt;10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>529.03</td>
<td>0.0525</td>
<td>HOMO → LUMO+1 (89%)</td>
</tr>
<tr>
<td>505.68</td>
<td>0.0368</td>
<td>HOMO → LUMO+2 (69%)</td>
</tr>
<tr>
<td>490.71</td>
<td>0.0280</td>
<td>HOMO-1 → LUMO+1 (78%)</td>
</tr>
<tr>
<td>467.47</td>
<td>0.0402</td>
<td>HOMO-1 → LUMO+2 (71%)</td>
</tr>
<tr>
<td>437.66</td>
<td>0.0438</td>
<td>HOMO-2 → LUMO+2 (51%)</td>
</tr>
<tr>
<td>364.32</td>
<td>0.0907</td>
<td>HOMO-3 → LUMO+2 (43%)</td>
</tr>
<tr>
<td>360.00</td>
<td>0.0235</td>
<td>HOMO-3 → LUMO+2 (32%)</td>
</tr>
<tr>
<td>348.92</td>
<td>0.0302</td>
<td>HOMO → LUMO+6 (85%)</td>
</tr>
</tbody>
</table>
**Table 4.6** TD-DFT major transitions for [Ru(quo)$_3$]$^\text{–}$

<table>
<thead>
<tr>
<th>$\lambda$ (nm)</th>
<th>f (&gt;0.03)</th>
<th>Major Transition (&gt;10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>521.44</td>
<td>0.2347</td>
<td>HOMO-1$\rightarrow$LUMO+2(17%), HOMO$\rightarrow$LUMO+1(16%)</td>
</tr>
<tr>
<td>521.18</td>
<td>0.2336</td>
<td>HOMO-2$\rightarrow$LUMO+2(-11%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HOMO-1$\rightarrow$LUMO+1(16%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HOMO-1$\rightarrow$LUMO+2(13%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HOMO$\rightarrow$LUMO(11%), HOMO$\rightarrow$LUMO+1(13%)</td>
</tr>
<tr>
<td>364.83</td>
<td>0.0313</td>
<td>HOMO$\rightarrow$LUMO+5(46%)</td>
</tr>
</tbody>
</table>

**Table 4.7** TD-DFT major transitions for [Ru(quo)$_5$(5-NO$_2$-quo)]$^+$

<table>
<thead>
<tr>
<th>$\lambda$ (nm)</th>
<th>f (&gt;0.02)</th>
<th>Major Transition (&gt;10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>534.41</td>
<td>0.0362</td>
<td>HOMO$\rightarrow$LUMO(35%), HOMO$\rightarrow$LUMO+2(36%)</td>
</tr>
<tr>
<td>502.04</td>
<td>0.0809</td>
<td>HOMO-1$\rightarrow$LUMO+2(30%) , HOMO$\rightarrow$LUMO+2(20%)</td>
</tr>
<tr>
<td>494.13</td>
<td>0.1150</td>
<td>HOMO-1$\rightarrow$LUMO(59%)</td>
</tr>
<tr>
<td>471.90</td>
<td>0.0319</td>
<td>HOMO-2$\rightarrow$LUMO(17%), HOMO-2$\rightarrow$LUMO+2(15%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HOMO-1$\rightarrow$LUMO+2(24%)</td>
</tr>
<tr>
<td>461.16</td>
<td>0.1104</td>
<td>HOMO-2$\rightarrow$LUMO(48%)</td>
</tr>
<tr>
<td>445.52</td>
<td>0.0753</td>
<td>HOMO-2$\rightarrow$LUMO+2(60%)</td>
</tr>
<tr>
<td>426.26</td>
<td>0.0340</td>
<td>HOMO-2$\rightarrow$LUMO+1(57%)</td>
</tr>
<tr>
<td>361.31</td>
<td>0.0358</td>
<td>HOMO-3$\rightarrow$LUMO(26%), HOMO-3$\rightarrow$LUMO+2(28%)</td>
</tr>
<tr>
<td>352.23</td>
<td>0.0336</td>
<td>HOMO$\rightarrow$LUMO+7(54%)</td>
</tr>
</tbody>
</table>

86
the LUMO, respectively, where the latter is a 5–NO₂-quo⁻ π*orbital. As a result of the stabilization of the HOMO in 5, the lowest-lying singlet transition in this complex is higher in energy than that of 2, in agreement with the experimentally determined lowest energy transitions of the complexes listed in Table 4.1. In addition to the low energy Ru-5-NO₂-quo⁻ MLCT transitions, 5 possesses some calculated transitions of very low oscillator strength, with Ru(dπ)→bpy(π*) character at higher energy (λ = 358 nm, f = 0.0136; λ = 361 nm, f = 0.0089).

The assignment of electronic transitions is often aided by TD-DFT calculations. Analysis of the oscillator strength in conjunction with the orbital character through use of orbital projections allows for assignment of transitions type. In the case of metal complexes involving innocent ligands, the distinction of metal centered and ligand centered orbitals is clear from molecular orbital projections. For example, in [Ru(bpy)₃]²⁺ the lowest energy transition is calculated at 436 nm (f = 0.0136, HOMO-2→LUMO), where significant metal character is calculated for the HOMO-2 and predominant bpy in character for the LUMO. In the case of [Ru(bpy)₃]²⁺, molecular orbital projections show electron distribution clearly localized on either the metal or the ligand orbitals. However, the presence of non-innocent ligands, such as quo⁻ and 5-NO₂-quo⁻, results in a large degree of electron delocalization on the metal and ligand. Thus, it is necessary to examine the electron distribution from a given molecular orbital closely for accurate assignment. For this reason, the percent contributions for each calculated transition were examined in conjunction with the analysis of electron density distributions.
The percent orbital character of each orbital was calculated with GaussSum 1.05 and GaussView 3.0 and the resulting values for 2 – 5 are presented in Table 4.8 – 4.12. As is typical of ruthenium polypyridyl complexes, the HOMO, HOMO-1, and HOMO-2 of 1 are largely metal in character (>76% Ru). It is clear from examination of the orbital contribution that the amount ligand character of the HOMO is dramatically larger in the quinolate series of complexes when compared to 1. For complex 2, the HOMOs has a calculated 78% quo character making the ligand contribution to this orbital predominant. A similar increase in ligand character is observed throughout the series with the addition of each oxygen atom to the coordination sphere.

Upon closer analysis of the percent orbital character and TD-DFT calculated transitions, detailed assignment can be made of the electronic absorption spectra for the series. The calculated absorption for complex 2 at 516 nm \( (f = 0.0517) \) is assigned as HOMO-1→LUMO+1. Analysis of the orbital character of the HOMO-1 and LUMO+1 reveals 77% ruthenium and 93% bpy character, respectively. This transition represents a shift in electron density from the metal centered orbitals to those with ligand character and is assigned as MLCT(Ru→bpy). Complex 3, which has calculated transition at 529 \( (f = 0.0525) \), was determined to be the result of electron density movement from HOMO→LUMO+1. The HOMO of 3 was determined to have electron density shared on the quoi ligand (63%) and metal orbitals (32%). The LUMO+1 of 3 was found to have significant electron density localized on the quinolate ligand (62%). Therefore the transition from HOMO→LUMO+1 can be assigned as ML-LCT. Likewise, complex 4 has HOMO and HOMO-1 character of 55% quo\(^-\) (\( \pi \)) and 45% ruthenium (d\( \pi \)). The
LUMO+1 and LUMO+2 is overwhelmingly quinolate character (>91%). The transition for 4 at 521 nm ($f = 0.2347$) is assigned as HOMO-1→LUMO+1 and HOMO-1→LUMO+2. Because of the high degree of electron density distribution in the HOMOs of 4 this electronic transition is assigned as ML-LCT.

Lastly it is useful to compare the spectral assignments for the lowest energy electronic transitions of 2 and 5. As discussed above, the lowest energy transition for 2 is calculated at 516 nm and is assigned as MLCT(Ru→bpy). Interestingly, the calculated lowest energy transition of 5 is at 502 nm ($f = 0.089$) and is assigned as HOMO-1 (77% ruthenium)→LUMO+2 (94% 5-NO$_2$-quo$^-$). Therefore, this transition has been assigned as MLCT(Ru→5-NO$_2$-quo$^-$). This change in the lowest energy assignment is the result of the electron withdrawing nature of the nitrate substituent on the quinolate ligand, reducing the ability of the oxygen atom to provide electron density to the metal.
**Table 4.8** [Ru(bpy)$_3$]$^{2+}$ (1) MO orbital contributions.

<table>
<thead>
<tr>
<th>Orbital</th>
<th>% Ru</th>
<th>% bpy</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUMO+5</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>LUMO+4</td>
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<td>97</td>
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<tr>
<td>LUMO+3</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>LUMO+2</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td>LUMO+1</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td>LUMO</td>
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<td>99</td>
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<tr>
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<td>17</td>
</tr>
<tr>
<td>HOMO-1</td>
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<td>24</td>
</tr>
<tr>
<td>HOMO-2</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>HOMO-3</td>
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</tr>
<tr>
<td>HOMO-4</td>
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<td>100</td>
</tr>
<tr>
<td>HOMO-5</td>
<td>2</td>
<td>98</td>
</tr>
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</table>
Table 4.9 [Ru(bpy)$_2$(quo)]$^+$ (2) MO orbital contributions.

<table>
<thead>
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<th>Orbital</th>
<th>% Ru</th>
<th>% bpy</th>
<th>% quo</th>
</tr>
</thead>
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<td>3</td>
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</tr>
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Table 4.10 [Ru(bpy)(quo)$_2$] (3) MO orbital contributions.

<table>
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<th>Orbital</th>
<th>% Ru</th>
<th>% bpy</th>
<th>% quo</th>
</tr>
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<tr>
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<td>8</td>
<td>16</td>
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<td>LUMO+9</td>
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<td>9</td>
<td>10</td>
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<td>LUMO+8</td>
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<td>3</td>
</tr>
<tr>
<td>LUMO+7</td>
<td>80</td>
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<td>19</td>
</tr>
<tr>
<td>LUMO+6</td>
<td>19</td>
<td>3</td>
<td>78</td>
</tr>
<tr>
<td>LUMO+5</td>
<td>3</td>
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<td>96</td>
</tr>
<tr>
<td>LUMO+4</td>
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<td>73</td>
<td>22</td>
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<tr>
<td>LUMO+3</td>
<td>5</td>
<td>20</td>
<td>75</td>
</tr>
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<td>LUMO+2</td>
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<td>32</td>
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### Table 4.11 $[\text{Ru}(\text{quo})_3]^+$ (4) MO orbital contributions.

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### Table 4.12 $[\text{Ru}(\text{bpy})_2(5-\text{NO}_2\text{-quo})]^+$ (5) MO orbital contributions.

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4.3 Conclusion

A series of Ru-quinolate complexes have been synthesized and electronic properties reported. A progressive red shift in the lowest energy absorption maxima assigned as $^1$MLCT (Ru→bpy) was observed in complexes 1 – 3. This is direct result of the increasing energy of the metal centered $t_{2g}$-type HOMO with the stepwise addition of oxygen atoms to the coordination sphere from 1 to 3. In complex 4, where three π-donor oxygen atoms are present in the coordination sphere, the transition at 540 nm was assigned as MLCT (Ru→quo⁻). Interestingly, the lowest energy transition in 5 was found to shift to shorter wavelength with maximum at 490 nm due to the electron withdrawing nitro-subsistent when compared to 3. This shift in energy was attributed to a decrease in nucleophilicity of the oxygen atom, resulting in a decreased ability of the oxygen atom to act as a π-donor ligand to the metal..

Accordingly, a shift in the oxidation potential for the complex was observed from 1 to 4. In general, 2 – 5 which contain quinolate ligands, are more easily oxidized than 1 showing a reversible one electron oxidation in the range of +1.17 to +0.33 V assigned to the Ru(III/II) couple. Complexes 2, 3 and 5 are not oxidized by O₂ in air ($E^{O_2/O_2^-} = +0.43$ V) consistent with their oxidation potentials. However, the Ru(III/II) couple at +0.33 V for 4 occurs well above oxidative potential of oxygen such that the Ru(III) oxidation state of the complex is present under aerobic conditions but is easily reduced by a mild reducing agent. The ease of oxidation of 4 is attributed to the higher energy ruthenium centered HOMO that results from the presence of three π-donor oxygen atoms in the coordination sphere of the metal.
With the aid of DFT calculations, a gradual increase in the relative energy of the HOMO’s from 0.00 eV in 1 to 1.02 eV in 4, was noted across the series, consistent with the decreased ligand field splitting expected as the number of oxygen atoms in the coordination sphere is increased from zero in 1 to three in 4. Furthermore, these calculations revealed HOMO character having contribution from both metal and quinolate ligand. TD-DFT calculations in conjunction with percent orbital contribution calculations allowed for assignment of experimental electronic transitions. The strong degree of electron delocalization in the HOMO, HOMO-1 and HOMO-2 in the series revealed the lowest energy transition in 1 – 4 to be ML-LCT in character. This interaction, s observed when non-innocent ligands are present and is reported here for ruthenium quinolates. These findings provides valuable insight into the effect of the metal ligand interactions of N,O-bidentate anionic ligands on the photophysical and redox properties of the corresponding Ru(II) complexes.
REFERENCES


CHAPTER 5

“Photoinduced Ligand Exchange in cis-[Ru(phpy)(phen)(CH$_3$CN)$_2$]$^{2+}$ with Low Energy Irradiation”

5.1 Introduction

The anticancer agent cisplatin, $\textit{cis}$-Pt(NH$_3$)$_2$Cl$_2$, has had remarkable success for treatment of a variety of cancers.$^{1-7}$ Toxicity in cisplatin derives from the formation of the diaqua species, $\textit{cis}$-[Pt(NH$_3$)$_2$(OH)$_2$]$^{2+}$, upon cellular uptake by thermal ligand exchange.$^5$ The diaqua species has been shown to preferentially bind adjacent guanine residues resulting in GpG (65 %) and ApG (25%) intrastrand crosslinks, leading to the inhibition of cellular transcription and resulting in cell death.$^7$ However, a major drawback of cisplatin is its nonspecific action on both normal and tumor tissue.$^2-7$ Consequently, a large number of adverse side effects occur with the therapeutic use of cisplatin.$^6,7$ Furthermore, some of the most aggressive acquire resistance to cisplatin after long periods of treatment.$^{1-7}$

These drawbacks have led to the investigation of analogous metal complexes with labile coordinated ligands including octahedral Ru(II)$^{8-12}$ and Rh(III)$^{13-19}$ as well as square planar Ni(II)$^{20-24}$ complexes. In particular, $\Lambda$-$\textit{cis}$-Ru(phen)$_2$Cl$_2$ (phen = 1,10-
phenanthroline) has been shown to undergo thermal ligand exchange of its labile chloride ligands and to preferentially bind to B-DNA. Similarly, bis(2-phenylazopyridine)ruthenium(II) and other related complexes have been shown to produce anti-tumor activity in cisplatin-resistant cell lines through inhibition of cellular transcription. In some cases, the toxicity of these compounds has been linked to their ability to covalently bind DNA. However, like cisplatin, thermal ligand exchange in these complexes is operative and occurs in both healthy tissue and tumors. Therefore, a means of localizing anti-tumor activity for cisplatin analogs is highly desired in order to reduce these harmful side effects.

The treatment known as photodynamic therapy (PDT) provides a means of localizing anti-tumor activity by the irradiation of otherwise non-toxic compounds with visible or near infrared light to produce a phototoxic species. Current PDT agents, such as the FDA approved drug Photofrin®, achieve phototoxicity by undergoing efficient energy transfer from their long-lived excited state to molecular oxygen, resulting in the production of singlet oxygen, $^1\text{O}_2$, a reactive species that has been shown to cause oxidative damage to biomolecules. Therefore, the success of these PDT agents is highly dependent on the concentration of oxygen within the vicinity of the drug. This feature represents a drawback, since some of the most malignant tumors are hypoxic. In addition, the depth of penetration of light in tissue is highly wavelength dependent, with optimal penetration taking place in the PDT window (650 - 850 nm). Unfortunately, irradiation of Photofrin® within this PDT window results in low quantum
yields of $^1$O$_2$ production ($\Phi = 0.17$). Therefore, novel PDT agents that can be activated with low energy light and have oxygen-independent toxicity are highly desirable.

The photoinduced ligand exchange of Ru(II) complexes was initially published by Ford and coworkers, and the photosubstitution of pentaammine and tetrammine Ru(II) complexes is well established.$^{47-50}$ It is accepted that the quantum yield of ligand photosubstitution is highly dependent on the wavelength of irradiation.$^{51-53}$ This sensitivity to wavelength in polypyridine complexes has been attributed to the relative energies of the metal-to-ligand charge transfer (MLCT) and metal centered ligand field (LF) states.$^{47-53}$ Photoinduced ligand exchange in these complexes has been shown to occur via a dissociative mechanism through thermally accessible $^3$LF state(s) from the lowest-energy $^3$MLCT state. Therefore, the relative energy gap between the $^3$MLCT and $^3$LF states has a dramatic effect on the efficiency of ligand exchange in these compounds.

The photochemistry of a series of ruthenium polypyridyl complexes including $cis$-[Ru(bpy)$_3$(NH$_3$)$_2$] (1), $cis$-[Ru(bpy)$_2$(py)$_2$] (2) and $cis$-[Ru(bpy)$_2$(CH$_3$CN)$_2$] (3) have been shown to undergo ligand exchange when irradiated in the ultraviolet and visible spectral regions.$^{46}$ Sequential photoaquation is observed in 1 – 3, ultimately resulting in the formation of $cis$-[Ru(bpy)$_2$(H$_2$O)$_2$]$^{2+}$ (Figure 5.1). Furthermore, gel electrophoresis of irradiated 1 – 3 in the presence of linearized ds-DNA results in a decrease in mobility similar to that observed for cisplatin bound to DNA. Thus, these polypyridyl Ru(II) complexes can be thought of as photoactivated cisplatin analogs which do not bind DNA in the dark. Interestingly, 3 exhibits significantly more efficient photoaquation under low energy irradiation ($\lambda_{irr} = 450$ nm; $\Phi = 0.22$) when compared to 1 ($\lambda_{irr} = 450$ nm; $\Phi < 0.005$)
103 and 2 (λ<sub>irr</sub> = 436 nm; Φ = 0.006). Nevertheless, efficient ligand exchange and DNA binding for 1 – 3 only occurs at wavelengths outside the desired PDT window (λ<sub>irr</sub> ≤ 500 nm).

![Diagram showing photoinduced ligand exchange](image)

**Figure 5.1** Scheme showing the photoinduced ligand exchange of 1 – 3 with water where X = NH₃, py, and CH₃CN.

Recently, attention has been given to a series of cyclometalated ruthenium(II) complexes and their biological activity.⁵⁴-⁶³ Pfeffer et al. have shown that the complex cis-[Ru(phen)(phpy)(CH₃CN)]₂ (4; phpy = 2-phenylpyridine) inhibits the growth of various tumors implanted in mice and does not cause severe side effects on the liver, kidneys or the neuronal sensory system.⁵⁷,⁶²,⁶³ The results of toxicity studies for a series of these ruthenium cyclometalated complexes with several tumor cell lines are listed in Table 5.1.⁶¹ From these studies, complex 4 clearly exhibits the greatest inhibition of cellular growth with an IC₅₀ value of 1.9 ±0.2 μM towards the A-172 cell line. Further
analysis of the cell cycle profile of tumor cells dosed with 4 revealed significant induction of sub-G1 phase cell arrest and induction of apoptosis.\textsuperscript{57}

**Table 5.1** IC\textsubscript{50} values [\(\mu\text{M}\)] for a series of cycloruthenated complexes compared with the antitumor agent cisplatin.

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<th>Tumor cell line</th>
<th>IC\textsubscript{50} ((\mu\text{M}))</th>
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<td></td>
<td>A-172</td>
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<tr>
<td>cisplatin</td>
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<td>[Ru(phpy)(CH\textsubscript{3}CN)\textsubscript{4}]\textsuperscript{+}</td>
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</tr>
<tr>
<td>[Ru(phpy)(P(CH\textsubscript{3})\textsubscript{2}Ph)(CH\textsubscript{3}CN)\textsubscript{3}]\textsuperscript{+}</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>[Ru(phpy)(P(Ph)\textsubscript{3})(CH\textsubscript{3}CN)\textsubscript{2}]\textsuperscript{+}</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>[Ru(phpy)(phen)(CH\textsubscript{3}CN)\textsubscript{2}]\textsuperscript{+}</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>[Ru(phpy)(4,4\textsuperscript{-}dm-bpy)(CH\textsubscript{3}CN)\textsubscript{2}]\textsuperscript{+}</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>[Ru(phen)\textsubscript{2}(CH\textsubscript{3}CN)\textsubscript{2}]\textsuperscript{2+}</td>
<td>&gt;50</td>
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</table>

\textsuperscript{a}Ref. 61 \textsuperscript{b}IC\textsubscript{50} values of solutions exposed to light were two times higher than those kept in the dark (IC\textsubscript{50} values here are for solutions protected from light)

In addition, similar reduction in tumor size was reported for xenografted mouse models dosed with 4 and cisplatin.\textsuperscript{57} A study of glioblastoma cells implanted in nude mice revealed a decrease in tumor volume by 45\% for both 4 and cisplatin. Furthermore, a decrease in adverse side effects was observed for 4 when compared to cisplatin. Evaluation of the chronic toxicity for mice periodically injected with 4 and cisplatin revealed that after 3 weeks cisplatin reduced body weights in mice by about 25\%, whereas 4 had no significant effect.\textsuperscript{57} Indeed, analysis of blood markers showed that
cisplatin induced variations in uric acid, aspartate aminotransferase, alanine aminotransferase, \( \alpha \)-amylase, glucose, bicarbonate, and iron, corresponding to alterations in hepatic and renal functions.\(^{57}\) Similar effects were not observed for these blood markers in mice treated with 4, indicating a decrease in the number of adverse side effects as compared to cisplatin.\(^{57}\) Later, Pfeffer published work focusing on the combination treatment of 4 with ionizing radiation (IR).\(^{62}\) This work showed that the combination of these two therapeutics resulted in extended inhibition of cellular proliferation for several tumor cell lines.

As a side note in these initial reports, 4 was found to produce IC\(_{50}\) two-fold higher with exposure to ambient light as compared to solutions kept in the dark.\(^{61}\) Despite the degree of photoactivity exhibited by 4, in the work by Pfeffer et al. samples were protected from light, but the photophysical properties of this complex were not investigated. The photolability of 4 along with the increased cytotoxicity upon exposure to light have prompted investigation of this complex as a potential photoactivated cisplatin analog. Furthermore, the structural similarity of 4 to 3 indicates the former may exhibit efficient photochemistry similar to that observed for 3. In addition, the covalent binding of the \( \sigma \)-donating carbanion of the phpy\(^{-}\) ligand to the metal was expected to red shift the lowest MLCT transition, a desired feature for a potential PDT agent. Spectroscopic measurements and TD-DFT calculations were performed with 4 and its photoinduced ligand exchange was investigated.
5.2 RESULTS AND DISCUSSION

5.2.1 Spectral Properties

The electronic absorption maxima and molar extinction coefficients of \( 1 - 4 \) in dichloromethane are listed in Table 5.2. A peak corresponding to the ligand centered \( \pi\pi^* \) transition of phen is observed at 265 nm, as is the case in \([\text{Ru(phen)}_3]^{2+}\) and similar phen containing ruthenium (II) complexes.\(^{66}\) A shoulder at 290 nm is consistent with the \( \pi\pi^* \) transition of the phpy ligand and is also observed in \([\text{Ru(phpy)}(\text{CH}_3\text{CN})_4]^{+}\). Similar to the previously reported compounds \( 1 - 3 \), \( 4 \) exhibits low energy absorption maxima in the range of 370 nm to 550 nm corresponding to the metal-to-ligand charge transfer (MLCT) from the ruthenium center to each of the bidentate ligands. Specifically, the \( \text{Ru} \rightarrow \text{phpy}^- \) MLCT band is observed with an absorption maximum of 395 nm, similar to that of \([\text{Ru(phpy)}(\text{CH}_3\text{CN})_4]^{+}\) reported at 380 nm.\(^{64,65}\) As \([\text{Ru(phpy)}(\text{CH}_3\text{CN})_4]^{+}\) does not exhibit any absorbance beyond 380 nm the peaks observed at 460 nm and 490 nm are assigned as \( \text{Ru} \rightarrow \text{phen} \) MLCT transitions. It is worthwhile to note that the lowest energy \( \text{Ru} \rightarrow \text{phen} \) MLCT peak has a broad tail from 540 – 660 nm with \( \varepsilon = 1 \, 200 \, \text{M}^{-1} \, \text{cm}^{-1} \) at 600 nm. This extension of absorbance into the red region of the visible spectrum has proven useful for excitation at low energy, a desirable characteristic of potential PDT agents.

The emission spectrum of \( 4 \) is shown in Figure 5.2 in dichloromethane. At room temperature \( 4 \) exhibits a strong luminescence centered at 720 nm (\( \tau = 60 \, \text{ns} \)).
Table 5.2 Photophysical measurements for complexes 1 – 4

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\lambda_{abs}$ / nm ($\varepsilon / 10^3$ M$^{-1}$ cm$^{-1}$) $^a$</th>
<th>$\lambda_{irr}$ / nm</th>
<th>$\Phi^b$</th>
<th>$\Phi_{py}$</th>
<th>$\Phi_{phen}^b$</th>
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<td>2</td>
<td>244 (22.4), 289 (47.1), 338 (12.2), 457 (7.7)</td>
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</tr>
<tr>
<td>3</td>
<td>243 (19.9), 283 (57.2), 425 (8.9)</td>
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<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>290 (28.0), 395 (6.3), 460 (7.8), 490 (7.4)</td>
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<td>0.10</td>
<td>0.03</td>
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</tr>
<tr>
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<td></td>
<td>500</td>
<td>0.08</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ in dicholormethane  
$^b$ quantum yield of photoaquation 1 – 3 and photoanation 4
Comparison of the excitation spectrum of 4 with its electronic absorption spectrum shows good agreement, indicating that the emission is not due to a highly luminescent impurity. In addition this shows that the two MLCTs (Ru(II)→phpy and Ru(II)→phen) are in high communication with one another. Furthermore, no dependence was found for excitation into either MLCT on the emission of 4. In order to probe the relative energy of the low lying $^3$dd orbitals, the temperature dependence of the emission carried out in acetonitrile from 0°C to 60°C and is shown in Figure 5.3. The use of acetonitrile as a solvent is important as it ensures that the decrease in emission is not a result of the photochemical ligand exchange to generate a non-emissive photoproduct. A typical decrease in emission was observed with increasing temperature indicating that the $^3$dd states of 4 are thermally accessible from the lowest lying $^3$MLCT state, as is often the case in emissive Ru(II) polypyridyl complexes.
Figure 5.2  Electronic absorption (—), excitation (○ ○; $\lambda_{em} = 720$) nm and emission (—; $\lambda_{exc} = 500$) nm spectra of 4 (60 $\mu$M) in dichloromethane at room temperature.
Figure 5.3  Temperature dependence of the emission of 4 (60 μM) in acetonitrile 0ºC to 60ºC ($\lambda_{exc} = 500$ nm).
5.2.2 X-Ray Diffraction Structural Characterization

The crystal structure of 4 has been previously reported, however, the low resolution of the structure left the orientation of the phpy$^-$ and phen ligand unclear. A higher resolution X-ray crystal structure is presented in this work and the ORTEP diagram is given in Figure 5.4. Crystallographic details and atomic coordinates of the brown reddish crystal are given in Tables 5.3 and 5.4, respectively. The structure of the ruthenancycle features an octahedral Ru(II) surrounded by one sp$^2$ carbon, three pyridine moieties and two acetonitriles. As described previously, the monodentate acetonitrile ligands are in a cis configuration similar to 1 – 3. In order to unambiguously determine which of the two possible phpy$^-$ binding sites contained the pyridyl nitrogen atom, all non-hydrogen atoms were initially labeled as carbon atoms. Isotropic refinement indicated that the atom located trans to the acetonitrile ligand is likely to be the nitrogen atom, as its Uiso value (0.018 Å$^2$) was significantly smaller than the 0.023 Å$^2$ value for C17. Therefore, the former was relabeled as N5. Further analysis of bond length revealed that the ruthenium nitrogen bond (Ru-N2) bound in the trans position to C17 was longer (2.154(2) Å) than the Ru-N1 and Ru-N5 bonds trans to pyridine nitrogen atoms, 2.062(2) Å and 2.059(2) Å, respectively (Table 5.5). Further examination of the bond lengths in the crystal structure shows two non-equivalent monodentate acetonitrile ligands. The σ-donation from pyridyl nitrogen of the electron rich phpy$^-$ ligand strongly disfavors electron flow along the Ru-N3 bond causing a longer bond (2.025(3) Å). This is in contrast to the much shorter Ru-N4 bond of the acetonitrile located trans to phen (2.016(3) Å).
Figure 5.4 ORTEP diagram of cationic 4 (solvent, H atoms and the counterion (PF$_6$) have been omitted for clarity). Ellipsoids represent a 50% probability.
<table>
<thead>
<tr>
<th>Property</th>
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Table 5.4. Atomic coordinates ( x $10^4$) and equivalent isotropic displacement parameters (Å$^2$ x $10^3$) for [Ru(phpy)(phen)(CH$_3$CN)$_2$](PF$_6$). U(eq) is defined as one third of the trace of the orthogonalized $U^{ij}$ tensor.

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### Table 5.4. (continued)

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*Occupancy factor is 0.475(4)

**Occupancy factor is 0.455(3)
Table 5.5. Bond lengths (Å) for [Ru(phpy)(phen)(CH₃CN)₂](PF₆) as determined from X-Ray crystallography.

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5.2.3 Electronic Structure Calculations

Electronic structure density functional theory (DFT) and time dependent DFT (TD-DFT) calculations were performed on complex 4. Table 5.6 lists the calculated bond lengths of the minimized lowest energy singlet ground state geometry of, which are compared to those determined in the crystal structure. The calculated Ru-N bond lengths between the metal center and the acetonitrile ligands (N4 and N5) are both within 0.004 Å of those determined crystallographically. All other calculated Ru-N and Ru-C bond lengths are within 0.07 Å longer of those determined in the crystal structure.

Figure 5.5 shows a molecular orbital diagram of 4, and electron density projections of selected orbitals are shown in Figure 5.6. As expected, the HOMO, HOMO−1, and HOMO−2 are ruthenium-based orbitals that constitute a t2g-type set. However, noticeable electron density within the HOMO of 4 is apparent on the phpy ligand. The LUMO and LUMO+1 are ligand-based π* orbitals with electron density localized on the phen ligand. Ligand π* character is also present in the LUMO+2 and LUMO+3, however it is localized on the phpy ligand (Figure 5.6). At significantly higher energy, 2.3 eV above the LUMO, there are a variety of orbitals possessing ruthenium-based antibonding character, including the LUMO+7 and LUMO+10 to LUMO+15.

The identities of the MOs were used in the assignment of electronic transitions calculated by TD-DFT in dichloromethane, treating the solvent using the conductor-like polarizable continuum medium model. The experimental absorption spectrum in dichloromethane is overlaid with the calculated electronic transitions is shown Figure 5.7.
Maxima calculated at 352 nm \((f = 0.0471)\) and 360 nm \((f = 0.0513)\) are assigned as \(^1\text{MLCT}\) to the phpy\(^-\) ligand owing to a large contribution from the HOMO\(\rightarrow\)LUMO+3 and HOMO–2\(\rightarrow\)LUMO+2 transitions, respectively. These calculated transitions can be correlated to the experimental peak with a maximum measured at 395 nm. The experimental absorption maxima at 460 and 490 nm can be associated with transitions calculated at 429 nm \((f = 0.0428)\) and 443 nm \((f = 0.1318)\) which are assigned as \(^1\text{MLCT}\) to the phen ligand because of their major contributions from HOMO–2\(\rightarrow\)LUMO+1 and HOMO–2\(\rightarrow\)LUMO, respectively. These assignments are consistent with those predicted from the comparisons of the electronic absorption data among various complexes.

**Table 5.6** Calculated and Experimental Bond Lengths for complex 4.

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<th>Calculated L (Å)</th>
<th>Experimental L (Å)</th>
<th>Δ</th>
</tr>
</thead>
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<td>2.062(2)</td>
<td>0.0417</td>
</tr>
<tr>
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<td>2.2234</td>
<td>2.154(2)</td>
<td>0.0692</td>
</tr>
<tr>
<td>Ru-N3</td>
<td>2.0289</td>
<td>2.025(3)</td>
<td>0.0036</td>
</tr>
<tr>
<td>Ru-N4</td>
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<td>2.016(3)</td>
<td>0.0032</td>
</tr>
<tr>
<td>Ru-N5</td>
<td>2.1068</td>
<td>2.059(2)</td>
<td>0.0476</td>
</tr>
<tr>
<td>Ru-C17</td>
<td>2.0470</td>
<td>2.029(3)</td>
<td>0.0177</td>
</tr>
</tbody>
</table>
Figure 5.5 Calculated molecular orbital diagram of 4.
Figure 5.6 Calculated frontier molecular orbitals of 4 (isovalue = 0.004).
Figure 5.7 Overlay of absorption spectrum (45 μM) and calculated singlet electronic transitions of 4 in CH₂Cl₂.
5.2.4 Identification of Photoproducts by NMR.

The $^1$H–NMR spectrum of $[\text{Ru}(\text{phpy})(\text{phen})(\text{CH}_3\text{CN})_2]^+$ in $\text{CD}_2\text{Cl}_2$ is shown in Figure 5.8a. Two resonances integrating to 3H each were observed for the non-equivalent ruthenium-bound CH$_3$CN ligands known from the crystal structure. One peak is observed at 2.16 ppm, corresponding to CH$_3$CN bound trans to N5-phpy. The second at 2.40 ppm corresponding to CH$_3$CN bond trans to N1-phen. Assignment of the aliphatic protons bound trans to phpym$^-$ was confirmed by comparison with $[\text{Ru}(\text{phpy})(\text{CH}_3\text{CN})_4]^+$, shown in Figure 5.8b, which displays a singlet at 2.16 ppm. Peak assignments for the aromatic region for 4 was achieved by comparison with $[\text{Ru}(\text{phpy})(\text{CH}_3\text{CN})_4]^+$ and $[\text{Ru}(\text{phen})_2(\text{CH}_3\text{CN})_2]^{2+}$ and matched well with those found in the literature.$^{65,68}$

The photolysis of 4 monitored by $^1$H–NMR spectroscopy in $\text{CD}_2\text{Cl}_2$ with three-fold excess $t$-butylammonium chloride (TBACl) $\lambda_{\text{irr}} = 450 \pm 10$ nm is shown in Figure 5.9. Upon irradiation, decrease in intensity of the peak associated with the two bound acetonitrile ligands along with an increase of a new signal at 1.95 ppm, which corresponds to free acetonitrile within 45 minutes of irradiation. Additionally, the formation of an intermediate is observed at with a peak at 2.11 ppm. The location of this peak suggest formation of the mono-chloro intermediate. Furthermore, the upfield shift of the intermediate peak with respect to the peak corresponding to the acetonitrile ligand bound trans to phpym suggest that ligand loss occurs preferentially from CH$_3$CN trans to N1-phen. Integration of bound and free acetonitrile showed stoichiometric conversion to the bis-substituted photoproduct cis-$[\text{Ru}(\text{phpy})(\text{phen})\text{Cl}_2]^-$. After 30 minutes of irradiation (not shown). Changes in the aromatic region (Figure 5.10) were more difficult to
identify, but the growth of new peaks was observed throughout the photolysis indicating the change in the local environment around the bidentate ligands.

The photolysis of cis-[Ru(phpy)(phen)(CH\textsubscript{3}CN)\textsubscript{2}]\textsuperscript{1+} in the presence of three-fold excess phenanthroline was also followed by \textsuperscript{1}HNMR (\(\lambda_{\text{irr}} = 450 \pm 10\) nm), and the spectral changes as a function of irradiation time are shown in Figure 5.11 and 5.12. Similar decrease of the signal for the metal bound CH\textsubscript{3}CN peaks was observed, coincident with the growth of the peak at 1.95 ppm corresponding to free acetonitrile (Figure 5.11). In addition, the resonances of aromatic protons corresponding to the free phenanthroline ligand at 9.40 ppm and 8.35 ppm decreased during photolysis (Figure 5.12). Simultaneously, the appearance of the ruthenium bound phenanthroline multiplets were observed at 6.85 ppm and 6.65 ppm. Interestingly, the accumulation of the monosubstituted species was not observed in the aliphatic region of the spectra. This behavior is believed to arise from the chelate effect of the phen ligand, where formation of the monosubstituted Ru(phpy)(phen)(\(\eta^{1}\)-phen)(CH\textsubscript{3}CN) intermediate promotes the second CH\textsubscript{3}CN ligand to exchange thermally to generate bidentate coordination of phenanthroline. Complete conversion of cis-[Ru(phpy)(phen)(CH\textsubscript{3}CN)\textsubscript{2}]\textsuperscript{1+} to the cis-[Ru(phpy)(phen)\textsubscript{2}]\textsuperscript{1+} photoproduct is observed in approximately 30 minutes. In addition, neither reaction with TBACl or phen proceeds in the dark under similar conditions after 1 hour.
Figure 5.8 Comparison of shifts in the aliphatic region of the $^1$H NMR spectra of (a) 4 and (b) $[\text{Ru}(\text{phpy})(\text{CH}_3\text{CN})_4]^+$ in CD$_2$Cl$_2$. 
**Figure 5.9** Changes in the aliphatic region of the $^1$H NMR spectra during photolysis of 4 (510 μM) in CD$_2$Cl$_2$ with 3 equivalents of TBACl.
Figure 5.10 Changes in the aromatic region of the $^1$H NMR spectra during photolysis of 4 (510 μM) in CD$_2$Cl$_2$ with 3 equivalents of TBACl.
Figure 5.11 Changes in the aliphatic region of the $^1$H NMR spectra during photolysis of 4 (520 $\mu$M) in CD$_2$Cl$_2$ with 3 equivalents of phen.
Figure 5.12 Changes in the aromatic region of the $^1$H NMR spectra during photolysis of 4 (520 μM) in CD$_2$Cl$_2$ with 3 equivalents of phen.
5.2.5 Photoinduced Ligand Exchange followed by Electronic Absorption Spectroscopy.

Previous studies involving the photolysis of 1 – 3 in water under inert atmosphere have shown sequential exchange of two monodentate ligands with water as the solvent to yield [Ru(bpy)₂(H₂O)₂]²⁺. Likewise, irradiation (λ ≥ 420 nm) of 4 in pyridine under argon results in exchange of two monodentate ligands with solvent molecules to generate [Ru(phpy)(phen)(py)₂]⁺ (Figure 5.13). The photolysis of 100 μM [Ru(phpy)(phen)(CH₃CN)₂]⁺ in the presence of 200 μM t-butylammonium chloride (TBACl) in CH₂Cl₂ under argon is shown in Figure 5.14a (λₘₚ ≥ 420 nm). The spectral features of the absorption profile indicate the formation of bis-substituted cis-[Ru(phpy)(phen)Cl₂]⁻. Changes in the absorption spectra represent a decrease in the reactant, observed at 460 nm and formation of the photoproduct cis-[Ru(phpy)(phen)(CH₃CN)Cl] monitored at 565 nm (t = 0 – 50 s). Two distinct isosbestic points are observed at 350 nm and 500 nm during photolysis indicating the formation of a single photoproduct. Longer irradiation time resulted in no further change in the absorption spectrum as shown in Figure 5.14b. The quantum yield of ligand exchange, Φ, was measured at several wavelengths (Table 5.2), and the values were relatively unaffected by the presence of air. In addition, no changes to the absorption spectra were observed when the sample was kept in the dark under similar conditions for 24 hr (Figure 5.15).
Figure 5.13 Absorption spectra of 4 (66 µM) upon photolysis ($\lambda_{irr} \geq 420$ nm) in pyridine at irradiation time, $t_{irr} = 0, 30, 60, 90, 120, 150, 180, 210, 240, 270$ and $300$ s.
Figure 5.14 (a) Absorption spectra of 4 (100 μM) upon photolysis (λ_{irr} ≥ 420 nm) in CH₂Cl₂ with excess TBACl at irradiation time, t_{irr} = 0, 10, 30, 40, 50, 60 and 120 s. (b) Change in Absorbance vs. Time for 4 at 460 nm (○) and 565 nm (□).
The dark reaction and photolysis of 154 μM 4 in the presence of 30 mM t-butyrammonium cyanide (TBACN) is shown in Figure 5.16. Upon addition of TBACN in the dark, an immediate increase in absorption centered at 690 nm is observed (Figure 15.5a). This increase in absorption continues until 20 mins, but then no additional spectral changes are observed up to 30 minutes of reaction time. The changes in the absorption spectra, including the increase in absorption at low energy, is believed to be the result of the thermal substitution of a single acetonitrile ligand by one CN⁻ to form [Ru(phpy)(phen)(CH₃CN)(CN)]⁺. Photolysis of the monosubstituted

Figure 5.15 Changes to the absorption spectra of 4 (240 μM) in the presence of 20-fold excess TBACl protected from light at times: 0 hr, 12 hr and 24 hr.
[Ru(phpy)(phen)(CH$_3$CN)(CN)] complex with $\lambda_{\text{air}} \geq 420$ nm is shown in Figure 5.15b. Upon irradiation, a new absorption band appears at 530 nm concomittant with a decrease in the $^1$MLCT absorption at 400 nm and 460 nm, indicating the formation of [Ru(phpy)(phen)(CN)$_2$]$^–$. No further changes in the absorption spectra were observed for longer periods of irradiation. The dark thermal monosubstitution reaction of 4 in the presence of a strong ligand, such as CN$^–$, clearly indicates ligand exchange of the bound acetonitriles are not equivalent. The thermal exchange of one monodentate ligand in 4 represents a limitation for its use as a PDT agent as these complexes must be inert in the absence of light.

To ensure the formation of the bis-substituted product, photolysis with excess bidentate ligands (phen, bpy) was performed. In case of both bidentate ligands, substitution of the acetonitrile ligands of the complex was observed during photolysis (Figure 5.17). Irradiation ($\lambda_{\text{air}} \geq 420$ nm) under argon with 40 $\mu$M $cis$-[Ru(phpy)(phen)(CH$_3$CN)$_2$]$^+$ in the presence of 800 $\mu$M phen resulted in the formation of $cis$-[Ru(phpy)(phen)$_2$]$^+$ confirmed by the characteristic absorption with a maximum at 550 nm ($\epsilon = 8$ 200 M$^{-1}$ cm$^{-1}$) (Figure 5.17b). As before, samples under similar condition kept in the dark for 24 hr showed no change in the absorption spectra.

An understanding of the photochemistry in aqueous environments is important for the application of potential PDT agents, thus the photolysis of 4 in water was conducted and is displayed in Figure 5.18. Irradiation of the sample resulted in a slower but detectable photoexchange of the monodentate ligand. Two isosbestic points are apparent at 405 nm and 490 nm indicating the formation of the di-substituted species.
Figure 5.16 (a) Absorption spectrum of 4 (154 µM) in CH$_2$Cl$_2$ and changes that take place 0 min, 1 min, 2 min, 5 min, 20 min and 30 min after the addition of TBACN (---) and (b) photolysis of the solution containing the product from part (a) at irradiation times of 0-180 seconds in 10 sec intervals (λ$_{irr}$ ≈ 420 nm).
Figure 5.17 (a) Absorption spectra of 4 (100 µM) upon photolysis ($\lambda_{\text{irr}} \geq 420$ nm) in CH$_2$Cl$_2$ with 20 eq. bpy and (b) absorption spectra of 4 (44 µM) upon photolysis ($\lambda_{\text{irr}} \geq 420$ nm) in CH$_2$Cl$_2$ with 20 eq. phen at irradiation times = 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 120 s.

135
Analysis of the time frame of photolysis reveals slower photosubstitution in aqueous environment compared to substitution in CH₂Cl₂. It should be noted that PF₆⁻ salt of the complex had low solubility in water and exchange of the PF₆⁻ ion for chloride was not successful.

Figure 5.18 Absorption spectra of 4 (76 µM) upon photolysis (λ_irr ≥ 420 nm) in water at irradiation times = 0, 90, 120, 150, 180, 210, 240 and 270 min.

Analysis of the time frame of photolysis reveals slower photosubstitution in aqueous environment compared to substitution in CH₂Cl₂. It should be noted that PF₆⁻ salt of the complex had low solubility in water and exchange of the PF₆⁻ ion for chloride was not successful.

Similar to 1 – 3, a dependence of the energy of irradiation and the quantum yield of ligand exchange was observed in complex 4. Interestingly, selective irradiation into the Ru→phen MLCT peak (λ_irr = 450 ± 10 nm) in CH₂Cl₂ in the presence of 20 equivalents of chloride resulted in a larger quantum yield of product formation [Ru(phpy)(phen)(CH₃CN)Cl] (Φ = 0.25) as compared to irradiation in the Ru→phpy ᵃ MLCT absorption (λ_irr = 400 ± 10 nm; Φ = 0.08). Interestingly, complex 4 exhibits
significant photosubstitution under low energy irradiation ($\lambda_{\text{irr}} = 500 \pm 10$ nm; $\Phi = 0.08$). This low energy photoinduced ligand exchange was further investigated by irradiating at several wavelengths extending into the far visible spectrum. Figure 5.19 shows the photosubstitution of 4 in the presence of excess TBACl irradiated with $\lambda \geq 630$ nm. Although lower energy wavelengths required longer irradiation times, significant ligand exchange was observed within 20 minutes. The ability of mononuclear ruthenium polypyridyl complexes to undergo photoinduced ligand exchange under irradiation within the PDT window has not been previously reported and represent the practicality of these complexes to applied clinically.

**Figure 5.19** Changes to the absorption spectrum of 4 (66 µM) upon photolysis ($\lambda_{\text{irr}} \geq 630$ nm) in CH$_2$Cl$_2$ with excess TBACl at irradiation times, $t_{\text{irr}} = 0, 2, 4, 6, 10, 14$ and 18 min..
5.2.6 Transient Absorption

The transient absorption spectra of 90 µM 4 in dichloromethane following nanosecond excitation (λ_{exc} = 355 nm; fwhm = 8 ns) is shown in Figure 5.20. A typical bleach of the 1MLCT state is observed at 455 nm and 500 nm, which is coincident with characteristic formation of peaks associated with the 3MLCT state, with positive absorption at 365 nm corresponding to formation of the phen⁻ and a broad absorption at 550-620 nm. Although within the temporal response of the instrument, the transient absorption signal at 365 nm could be fitted to a monoexponential decay resulting in a τ = 55 ns. Similarly, the decay of the broad transient absorption at 600 nm could be fit monoexponentially to give τ = 50 ns. The presence of excess chloride ligand (1.8 mM) did not affect the lifetime of the 3MLCT excited state. Furthermore, these values are in agreement with the lifetime of emission at 720 nm (τ = 60 ns).

![Figure 5.20](image-url)

**Figure 5.20** Transient absorption spectra of 4 in CH₂Cl₂ (90 µM) collected at 20 ns (---○---) and 60 ns (----■----) after the laser pulse, respectively.
5.2.7 Biological Activity and Phototoxicity

The biological target and source of cisplatin’s antitumor activity is the covalent link that it forms with ds-DNA. This covalent binding of cisplatin has been shown to result in the decreased mobility of ds-DNA by agarose gel electrophoresis. The decrease in mobility is a consequence of a change in morphology as the result of the 20° bend in double helical DNA produced upon formation of the cisplatin-DNA adduct, which results in change in tertiary structure for the linearized DNA resulting in fewer intercalation sites and a decrease in EtBr emission. Furthermore, a relationship between the reduction in mobility and relative concentration of metal complex [mc] to base pairs [bp] has been established. Figure 5.21a shows the decrease in mobility of ds-DNA in the presence of cisplatin were [DNA bp]:[mc] was varied from 100:1 to 5:1. The visualization of the DNA in agarose gels is achieved by staining with Ethidium Bromide (EtBr) which becomes emissive upon intercalation. It is apparent from Figure 5.21a that the greatest decrease in the emission of EtBr is observed for the highest [DNA bp]:[mc] ratio in lane 6.

Comparison of mobility retardation for ruthenium(II) complexes bound to ds-DNA has been shown to indicate a binding mode similar to that of cisplatin. Since DNA can be a target for potential antitumor agents, comparison of changes in mobility often are used to screen complexes for biological activity. For this reason, mobility changes for complex 4 in the presence and absence of light are presented as an indicator of 4 as a photoactivated cisplatin analog. It is worthwhile to note that the [DNA bp:mc] ratio used for mobility gels with 4 differ from those used for cisplatin. When the range of...
Figure 5.21 Ethidium stained agarose gel electrophoresis of 50 µM linearized plasmid (10 mM phosphate buffer, pH 7.5) in the presence of various ratios of (a) cisplatin, incubated for 3 hours Lanes 3-6 [Complex] : [DNA bp] = 100, 20, 10, 5. (b) 4, λ_{irr} \geq 420 \text{ nm} for 20 \text{ min} (c) 4, incubated for 24 hrs in the dark. Lanes 1 and 8: DNA molecular weight standard, Lanes 2 and 7: linearized plasmid only; Lanes 3-6 [DNA bp] : [mc] = 0.5, 1.0, 2.0, 3.0.
[DNA bp:mc] was used for cisplatin were applied for mobility studies of irradiated 4 and ds-DNA, no mobility was observed such that the linearized DNA remained in the loading wells after electrophoresis. A variety of conditions were attempted and the range of 0.5:1 to 3:1 were found to produce the necessary separation of the irradiated complex with ds-DNA. Irradiation of 50 μM linearized ds-DNA in the presence of increasing ratios of [DNA bp]: [4] are shown in Figure 5.21b. Within this range, a decrease in the mobility was observed in Lanes 3-6. Moreover, the need for photoactivaiton of 4, is evident by comparison of mobility in irradiated samples and those kept in the dark (Figure 21(c)). With the differences in complex to DNA raios in mind, a comparison of gel mobility [DNA bp] : [cisplatin] (Figure 5.21a) shows that 4 represents a lower change in mobility. However, like cisplatin, complex 4 demonstrages a dramatic decrease in the emission intensity of the EtBr stain, which can be correlated with a decrease in intercalation sites for the duplex. This decrease in emission is most prevalent in lane 6 containing the highest concentration of ratio of 4. Although the decrease in mobility for 4 is not as great as that for cisplatin, the behavior of the photoactivated species with DNA indicates that is forming a covalent interaction that results in change in the tertiary structure of ds-DNA.

The success of a potential PDT agent requires that toxicity is increased dramatically upon irradiation. In order to investigate the phototoxicity, complex 4 was dosed to Hs-27 skins cells and H2119 lung cancer cells at various concentrations and LC$_{50}$ values were determined for samples irradiated and those protected from light. Table 5.7
Table 5.7 Comparison of LC$_{50}$ values for samples protected for light and after irradiation.

<table>
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<th>Compound</th>
<th>LC$_{50}$/µM</th>
<th>Dark</th>
<th>Irradiated</th>
<th>Increase upon Irradiation</th>
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<tr>
<td>cisplatin</td>
<td></td>
<td>131 ± 10</td>
<td>110 ± 8$^a$</td>
<td>1.2</td>
</tr>
<tr>
<td>Photofrin</td>
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<td>21 ± 1</td>
<td>3.8 ± 0.2$^a$</td>
<td>5.5</td>
</tr>
<tr>
<td>$[\text{Rh}_2(\text{OAc})_2(\text{CH}_3\text{CN})_6]^{2+}$</td>
<td></td>
<td>410 ± 9</td>
<td>12 ± 2$^a$</td>
<td>34</td>
</tr>
<tr>
<td>$\text{cis-}[\text{Ru(bpy)}_2(\text{NH}_3)_2]^{2+}$</td>
<td></td>
<td>221</td>
<td>152$^a$</td>
<td>1.5</td>
</tr>
<tr>
<td>$\text{cis-}[\text{Ru(phpy)(phen)(CH}_3\text{CN)}_2]^+$</td>
<td></td>
<td>2.7</td>
<td>7.1$^b$</td>
<td>2.6</td>
</tr>
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</table>

$^a$ 400 – 700 nm irradiation (30 min) $^b$ 435 – 480 nm irradiation (30 min)
compares the LC\textsubscript{50} values of cisplatin, Photofrin, \textit{cis}-[Rh\textsubscript{2}(OAc)\textsubscript{2}(CH\textsubscript{3}CN)\textsubscript{6}]\textsuperscript{2+} \textit{cis}-[Ru(bpy)\textsubscript{2}(NH\textsubscript{3})\textsubscript{2}]\textsuperscript{2+}, and \textit{cis}-[Ru(phpy)(phen)(CH\textsubscript{3}CN)\textsubscript{2}]\textsuperscript{+}. Cisplatin, which is not photoactive, shows little change in toxicity upon irradiation. Whereas, the currently used PDT agent, Photofrin\textsuperscript{®}, produces a 5.5 increase in toxicity upon exposure to visible light. [Rh\textsubscript{2}(OAc)\textsubscript{2}(CH\textsubscript{3}CN)\textsubscript{6}]\textsuperscript{2+} exhibits the greatest change in toxicity upon irradiation with an increase of LC\textsubscript{50} of \textasciitilde34 fold as compared to cells kept in the dark. Lastly, complex 4 exhibited a 3-fold increase in toxicity under irradiation compared to cells protected from light. In addition, 4 exhibits higher toxicity than the current PDT agent under irradiation. The small change in toxicity observed for cells irradiated and those protected from light is likely a result of the ability of 4 to thermally exchange of one acetonitrile ligand in the dark in the presence of a strong ligand. This high toxicity in the dark is unfortunate, as a vital requirement of potential PDT agents is low toxicity in the absence of light.
5.3 Conclusions

It is generally accepted that the dissociation of photolabile ligands takes place from $^3\text{LF}$ state(s), which lies slightly above the low-lying $^3\text{MLCT}$ state and has M–L $\sigma^*$ character. It was recently found that the relative quantum yield of ligand exchange was dependent on the energy gap between the dissociative metal centered $^3\text{LF}$ state(s) and the $^3\text{MLCT}$ state. A potential energy surface depicting the excited states manifolds of 4 is given in Figure 5.22. As discussed before, two distinct MLCT states exist for 4 and, therefore, a difference in the energy gap between the $^3\text{LF}$ and Ru→phen or Ru→phpy $^3\text{MLCT}$ state is expected. The larger quantum yield observed for selective irradiation of the Ru→phen MLCT absorption maximum can be indicative of close proximity, both in geometry and energy, of the Ru→phen $^3\text{MLCT}$ and the $^3\text{LF}$ state. A greater change in geometry is expected along the shorter ruthenium acetonitrile bond located trans to phen ligand upon CT excitation. This distortion in the Ru(II)-N5 bond can result in the preferential dissociation of this ligand.

Furthermore, lower quantum yields are observed for irradiation at the higher energy Ru→phpy $^3\text{MLCT}$ maximum, indicating the poor communication of this and the $^3\text{LF}$ state(s). The lower quantum yield observed for irradiation into the Ru→phpy $^1\text{MLCT}$ transition can be explained by fast population of the corresponding $^3\text{MLCT}/\text{LC(phpy)}$ state, followed by $^3\text{MLCT(phen)}$, without population of the $^3\text{LF}$ states.
Figure 5.22 Potential Energy Surface diagram showing pathways upon excitation into the (a) Ru-phpy $^1$MLCT/LC or (b) Ru-phen $^1$MLCT.
has been determined to result from the promotion of an electron from the HOMO, which
have significant metal character (>68%). This is in contrast to Ru-phpy transitions
assignment at higher energies resulting from HOMO→LUMO+2 transitions, where there
is extensive Ru(dπ) and ligand orbital mixing resulting in HOMOs with both ruthenium
(53%) and phpy (42%) character. This larger degree of electronic mixing of the HOMO
results in the Ru→phpy MLCT to be more ligand centered (LC) in character is
designated as such in Figure 5.22.

These effects on orbital mixing have been previously described in the
photochemical reactivity of the series of complexes of the type [Ru(tpy)(L)(DMSO)],
where tpy = 2,2’:6’,2’’-terpyridine; L = 2,2’-bipyridine (bpy), N,N,N’,N’-
tetramethylethylene diamine (tmen), acetylacetonate (acac), oxalate (ox), or malonate
(mal) has been reported by Rack et al. It was found that when L is a π-acceptor (bpy)
or σ-donor ligand (tmen) photoinduced isomerization of the DMSO ligand occurred with
relatively high quantum yields when compared to complexes containing π-donating
ligands (acac, ox, mal) which resulted in low to no photoisomerization. DFT calculations
for these complexes containing π-donating ligands revealed extensive mixing of the
Ru(dπ) orbitals with the filled ligand orbitals to result in delocalization of electron
density of the HOMOs with shared character on the metal and ligand. This is in contrast
to complexes containing either π-acceptor (bpy) or σ-donor (tmen) ligands where the
HOMOs was found to be mostly ruthenium in character, 78.9% and 83.2% respectively,
similar to that of [Ru(bpy)3]2+ (80.8%). It was postulated that since a Ru(III) center is
required for isomerization, delocalization of charge density among the metal and ligand
could hinder isomerization in complexes with delocalized electron density.\textsuperscript{59} As a consequence, when oxidized through excitation into a CT state, the formal charge on the ruthenium atom is less than +3 and would correspond to the decrease in photochemical reactivity. Similarly for \textit{4}, excitation into the Ru-phpy MLCT/LC band results in a less than +3 formal charge on the ruthenium center, thus lower photosubstitution is observed for this transition. However, irradiation into the Ru-phen results in a localization of the electron density from the highly metallic HOMO-2 to the ligand base LUMO+2. The result is a formal charge separated species that results in ligand dissociation.

Lastly, photoactivation of \textit{4} in the presence of linearized DNA has been shown to result a decrease in mobility indicating an interaction similar to that of cisplatin. Furthermore, the investigation into the phototoxicity of \textit{4} revealed a ~3-fold increase upon irradiation when compared to samples kept in the dark. This increase in toxicity upon irradiation is similar to that of the current PDT agent Photofrin, which increases by a factor of 5.5 upon irradiation. Complex \textit{4} has also been shown to undergo efficient photosubstitution with low energy irradiation. Therefore, this represents the first photo-cisplatin analog with activity within the desired photodynamic therapeutic window, demonstrating the potential of \textit{4} as a viable PDT agent suitable for \textit{in vivo} application.
REFERENCES


CHAPTER 6

CONCLUSION

6.1 Introduction

Cisplatin is an anticancer drug used in the treatment of various cancers. However, cisplatin is toxic towards both healthy and tumor cells alike, resulting in several undesirable side effects. Moreover, some of the most aggressive cancers develop resistance to cisplatin. Photodynamic therapy (PDT) uses light to localize activation of otherwise non-toxic compounds in tumor tissue. Current PDT agents achieve toxicity by the photosensitization of highly reactive singlet oxygen through energy transfer from an excited state. However, this need for the presence of molecular oxygen represents a disadvantage since malignant and drug resistant cells are often hypoxic. To address the drawbacks of cisplatin and PDT drugs as antitumor agents, a combined approach has been made with the development of several photoactive Ru(II) complexes that produced with antitumor activity under irradiation. This blend of cisplatin mimetic metal complexes, inorganic photochemistry and photodynamic therapy has led to the discovery
of several photo-activated ruthenium complexes that bind DNA in a manner similar to cisplatin. This new class of compounds is referred to as photo-cisplatin analogs.

6.2 Ruthenium Complexes with Quinolato Ligands

A successful PDT agent should possess high molar absorbptivity at long wavelengths where tissue penetration is greatest and there is low absorption by biomolecules. A series of Ru-quinolate complexes have been synthesized and electronic properties reported. A progressive red shift in the lowest energy absorption maxima assigned as $^1$MLCT (Ru$\rightarrow$bpy) was observed in complexes 1–3. This is direct result of the increasing energy of the metal centered $t_2g$-type HOMO with the stepwise addition of oxygen atoms to the coordination sphere from 1 to 4. In complex 4, where three π-donor oxygen atoms are present in the coordination sphere, the transition at 540 nm was assigned as MLCT (Ru$\rightarrow$quo$^-$). Interestingly, the lowest energy transition in 5 was found to shift to shorter wavelength with maximum at 490 nm due to the electron withdrawing nitro-subsistent when compared to 3. This shift in energy was attributed to a decrease in nucleophilicity of the oxygen atom, resulting in an increased orbital overlap of the ligand π* and metal dπ orbital.

Accordingly, a shift in ease of oxidation for the complex was observed from 1 to 4. In general, 2–5 which contain quinolate ligands, are more easily oxidized than 1 showing a reversible one electron oxidation in the range of +1.17 to −0.33 V assigned to the Ru(III/II) couple. Complexes 2, 3 and 5 are not oxidized by O$_2$ in air ($E^{\text{O}_2/O_2^-}$ = +0.43
V) consistent with their oxidation potentials. However, the Ru(III/II) couple at +0.33 V for 4 occurs well above oxidative potential of oxygen such that the Ru(III) oxidation state of the complex is present under aerobic conditions but is easily reduced by a mild reducing agent. The ease of oxidation of 4 is attributed to the higher energy ruthenium centered HOMO with the addition of three π-donor oxygen atoms to the coordination sphere in the series.

With the aid of DFT calculations, a gradual increase in the relative energy of the HOMO’s from 0.00 eV in 1 to 1.02 eV in 4, was noted across the series, consistent with the decreased ligand field splitting expected for the increase in number of oxygen atoms in the coordination sphere from zero in 1 to three in 4. Furthermore, these calculations revealed HOMO character having contribution from both metal and quinolate ligand. TD-DFT calculations in conjunction with percent orbital contribution calculations allowed for assignment of experimental electronic transitions. The strong degree of electron delocalization in the HOMO, HOMO-1 and HOMO-2 in the series revealed the lowest energy transition in 1 – 4 be ML-LCT in character. This interaction, s observed when non-innocent ligands are present and is reported here for ruthenium quinolates. These findings provides valuable insight into the effect of the metal ligand interactions of N,O-bidentate anionic ligands on the photophysical and redox properties of the corresponding Ru(II) complexes.
6.3 Ruthenium Complexes with Photoinduced Ligand Exchange

The feasibility of ruthenium(II) photocisplatin analogs relies on effective ligand exchange under low irradiation energy. To this end the complex cis-[Ru(phpy)(phen)(CH$_3$CN)$_2$] was investigated for its ability to undergo ligand exchange. It is generally accepted that dissociation of photolabile ligands takes place from $^3$LF state(s), which lies slightly above the low-lying $^3$MLCT. Recently, it was found that the relative quantum yield of ligand loss was dependent on the energetic gap between the dissociative metal centered $^3$LF state(s) and the $^3$MLCT. An energy well diagram depicting the excited states of 4 is given in Figure 18. As discussed before, two distinct MLCT states exist for 4 and, therefore, a difference in the energy gap between the $^3$LF and Ru→phen $^3$MLCT or Ru→phpy $^3$MLCT state is expected. Furthermore, dissociation from the $^3$LF state results in population of the metal centered anti-bonding orbitals ($\alpha^*_M$). This results in metal-ligand bond destabilization, elongation and ligand dissociation. Under this model, the larger quantum yield observed for selective irradiation at the Ru→phen MLCT absorption maximum indicates close proximity, both in geometry and energy, of the Ru→phen $^3$MLCT and the $^3$LF state. A greater change in geometry is expected along the shorter ruthenium acetonitrile bond located trans to phen ligand upon CT excitation. This distortion in the Ru(II)-N5 bond results in a more dramatic elongation of this ruthenium acetonitrile bond and preferential ligand dissociation. The lower quantum yield of ligand loss observed for irradiation into the Ru-phpy MLCT is thought to caused by rapid deactivation of the $^3$MLCT state through strong covalent mixing of metal and ligand based orbitals.
Lower quantum yields are observed for irradiation at the higher energy Ru→phpy $^3$MLCT maximum, indicating the poor communication between the two $^3$MLCT states.

The lower quantum yield observed for irradiation into the Ru→phpy $^3$MLCT transition is thought to be caused by the strong metal-ligand orbital mixing along the ruthenium $d\pi$ and php$\pi^*$ allowing for rapid thermal deactivation of the Ru→phpy $^3$MLCT excited state. In addition, assignment of the Ru-phen MLCT electronic transition has been determined to result from the promotion of an electron from the HOMOs, which have significant metal character (>68%). This is in contrast to Ru-phen transitions assignment at higher energies resulting from HOMO→LUMO+2 transitions, where there is extensive Ru($d\pi$) and ligand orbital mixing resulting in HOMOs with both ruthenium (53%) and php (42%) character.

These effects on orbital mixing have previously been seen in the photochemical reactivity for the series of complexes of the type [Ru(tpy)(L)(DMSO)], where tpy = 2,2’:6’,2”-terpyridine; L = 2,2’-bipyridine (bpy), N,N,N’,N”-tetramethylethylene diamine (tmen), acetylacetonate (acac), oxalate (ox), or malonate (mal) has been reported by Rack et al. It was found that when L is a $\pi$-acceptor (bpy) or $\sigma$-donor ligand (tmen) photoinduced isomerization of the DMSO ligand occurred with realityitly high quantum yields. Conversely, complexes containing $\pi$-donating ligands (acac, ox, mal) resulted in low to no photoisomerization to occur. DFT calculations for these complexes containing $\pi$-donating ligands revealed extensive mixing of the Ru($d\pi$) orbitals with the filled ligand orbitals to result in delocalization of electron density of the HOMOs with shared character on the metal and ligand. This is in contrast to complexes containing either $\pi$-
acceptor (bpy) or σ-donor (tmen) ligands where the HOMOs was found to be mostly ruthenium in character, 78.9% and 83.2% respectively, similar to that of \([\text{Ru(bpy)}_3]^{2+}\) (80.8%). It was postulated that since a Ru(III) center is required for isomerization, delocalization of charge density among the metal and ligand could hinder isomerization in complexes with delocalized electron density. As a consequence, when oxidized through excitation into a CT state, the formal charge on the ruthenium atom is less than +3 and would correspond to the decrease in photochemical reactivity. Similarly for 4, excitation into the Ru-phpy MLCT band results in a less than +3 formal charge on the ruthenium center, thus lower photosubstitution is observed for this transition. However, irradiation into the Ru-phen results in a localization of the electron density from the highly metallic HOMO-2 to the ligand base LUMO+2. The result is a formal charge separated species that results in ligand dissociation.

Lastly, photoactivation of 4 in the presence of linearized DNA has been shown to result a decrease in mobility indicating an interaction similar to that of cisplatin. Furthermore, investigations into the phototoxicity of 4 revealed a 3-fold increase upon irradiation when compared to samples kept in the dark. This increase in toxicity upon irradiation is similar to that of the current PDT agent Photofrin, which increases by a factor of 5.5 upon irradiation. Lastly, 4 has been shown to undergo efficient photosubstitution with low energy irradiation. Therefore, this represents the first photosensitive analog with activity within the desired photodynamic therapeutic window, demonstrating the potential of 4 as a viable PDT agent suitable for in vivo application.
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178


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