Ru(II), Os(II), AND Rh$_2$(II,II) COMPLEXES AS POTENTIAL PHOTODYNAMIC THERAPY AGENTS

DISSERTATION

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ABSTRACT

Photodynamic therapy (PDT) has been used in medical practice for more than a decade in the US with the clinical approval of Photofrin®. PDT is a cancer therapy that operates with greater selectivity than conventional chemotherapy treatments. The selectivity of the treatment lies in the combination of a photosensitizer (the PDT agent) and visible light irradiation. The localization of irradiation allows for specificity by selectively irradiating the tissue of interest, while leaving healthy cells undamaged. In this manner, the cytotoxic agents are only generated in the irradiated area.

Although PDT has many benefits, drawbacks remain. These include the depth of skin penetration achievable by the irradiation wavelengths required by current drugs, the level of cytotoxicity experienced in the dark, the hypoxic nature of tumor tissue diminishing the effect of cytotoxic reactive oxygen species (ROS), and the general tolerance to current agents. Metal-based complexes can offer unique solutions to a number of these drawbacks. In this document, the photophysical properties and DNA interactions of dirhodium, ruthenium, and osmium complexes are investigated for their abilities to address some of the shortcomings of current cancer photochemotherapies.

A series of dirhodium(II,II) complexes of the type \( \text{cis-[Rh}_2(\mu-O_2CCH_3)_2(dppn)(L)]^{2+} \), where dppn = benzo[\(i\)]dipyrido[3,2-a:2’,3’-h]quinoxaline and \( L = 2,2’\)-bipyridine (bpy), 1,10-phenanthroline (phen), dipyrido[3,2-f:2’3’-h]quinoxaline
(dpq), dipyrido[3,2-a:2',3'-c]phenazine (dppz), and dppn, was synthesized and its photophysical properties investigated. The ability of the complexes to bind and photocleave DNA was also probed, along with their toxicity toward human skin cells in the dark and when irradiated with visible light. Nanosecond time-resolved absorption measurements established that the lowest energy excited state in the series is dppn-localized \( ^3\pi\pi^* \) in DMSO. All complexes except the bis-dppn complex photocleave DNA efficiently via a mechanism that is mostly mediated by reactive oxygen species. The DNA photocleavage by the bis-dppn complex is significantly lower than that measured for the others, however, it exhibits the largest increase in toxicity upon irradiation within the series. These results are discussed in Chapter 3, in terms of the known ability of the complexes to transverse cellular membranes, their toxicity in the dark, and their photophysical properties.

In Chapter 4 a discussion of three new complexes \([\text{Ru(bpy)}_2(\text{dpqp})]^2+\) (dpqp = pyrazino[2',3':5,6]pyrazino-[2,3-f][1,10]phenanthroline), \([\text{Ru(bpy)}_2(\text{dppn})]^2+\), and \([\text{Os(bpy)}_2(\text{dppn})]^2+\) is presented. These complexes provide improvement to the drawbacks of current PDT agents by utilizing longer lifetimes, dual mechanisms of reactivity, and longer wavelengths of absorption, respectively. \([\text{Ru(bpy)}_2(\text{dpqp})]^2+\) exhibits strong luminescence in water at room temperature, a behavior that is strikingly different from that of the related non-emissive “DNA light-switch” prototype \([\text{Ru(bpy)}_2(\text{dppz})]^2+\) under similar conditions. The combination of its strong DNA binding affinity and relatively long-lived triplet metal-to-ligand charge-transfer (\(^3\text{MLCT}\)) excited state in an aqueous solution results in more efficient DNA photocleavage by \([\text{Ru(bpy)}_2(\text{dpqp})]^2+\) than
[Ru(bpy)$_2$(dppz)]$^{2+}$. Irradiation of [Ru(bpy)$_2$(dppn)]$^{2+}$ with visible light results in nearly complete DNA cleavage within 30 s ($\lambda_{\text{irr}} \geq 455$ nm), likely from the combined action of guanine oxidation from the $^3$MLCT state and from population of the $^3\pi\pi^*$, which is able to efficiently sensitize the photoproduction of $^1O_2$. [Os(bpy)$_2$(dppn)]$^{2+}$ generates $^1O_2$ with a quantum yield of 0.42 upon irradiation from its low-lying $^3\pi\pi^*$ excited state, which together with its $^3$MLCT absorption, results in efficient DNA cleavage with irradiation at $\lambda_{\text{irr}} \geq 645$ nm.

The new complexes $cis$-[Rh$_2$(µ-O$_2$CCH$_3$)$_2$(C$_6$H$_5$CN)$_4$]$^{2+}$] and $cis$-[Rh$_2$(µ-O$_2$CCH$_3$)$_2$(4F-C$_6$H$_5$CN)$_4$]$^{2+}$ were synthesized and studied as potential photo-cisplatin analogs. Theoretical calculations were performed to assist in the understanding of the electronic structures of the complexes. Both complexes were inert to ligand exchange in the dark in H$_2$O, CH$_3$CN, and CH$_2$Cl$_2$. $cis$-[Rh$_2$(µ-O$_2$CCH$_3$)$_2$(4F-C$_6$H$_5$CN)$_4$]$^{2+}$, however, showed photosubstitution of its four equatorial 4F-C$_6$H$_5$CN ligands for CH$_3$CN with $\lambda_{\text{irr}} \geq 455$ nm in 25 min, whereas $t_{\text{irr}} > 2$ hr was required for $cis$-[Rh$_2$(µ-O$_2$CCH$_3$)$_2$(C$_6$H$_5$CN)$_4$]$^{2+}$]. The photosubstitution of the complexes with H$_2$O was also investigated to assess their biological viability. Both complexes exhibit reduced ligand substitution in water due to their hydrophobicity and the $\pi$-stacking of the benzonitrile ligands. The photoinduced DNA binding of $cis$-[Rh$_2$(µ-O$_2$CCH$_3$)$_2$(C$_6$H$_5$CN)$_4$]$^{2+}$] was investigated by gel electrophoresis and compared to $cis$-[Rh$_2$(µ-O$_2$CCH$_3$)$_2$(CH$_3$CN)$_6$]$^{2+}$], a complex known to covalently bind DNA upon irradiation. These results are discussed in Chapter 5.
Dedicated to my parents and my husband, Mike.
I would like to express sincere gratitude to my PhD advisor, Professor Claudia Turro. Thank you for teaching me so much about how to think critically, how to write scientifically, and for your encouragement over the past five years. Your instruction and advice have been invaluable to me. Thank you also to Prof. Terry Gustafson and Prof. Yiyung Wu for serving on my committees here at OSU. A special thank you to Dr. Yao Liu and Dr. Danny Lutterman for all of their time and effort in helping me to understand the fundamental aspects of Turro group research and for their instruction of lab techniques and instrumentation in my first years in the group. I am grateful to all the Turro group members for their countless discussions over research projects and their always willingly offered insight whenever research questions would arise. A special thank you to Dave, Yujie, Robert, Nicole, Carly and Bryan for always dropping whatever you might be working on to help me in your own areas of expertise.

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“DNA Photocleavage by an Osmium(II) Complex in the PDT Window” Yujie Sun, Lauren E. Joyce, Nicole M. Dickson, and Claudia Turro *Chemical Communications* 2010, 46, 6759-6761.


“Efficient DNA Photocleavage by [Ru(bpy)₂(dppn)]^{2+}, With Visible Light” Yujie Sun, Lauren E. Joyce, Nicole Dickson, and Claudia Turro *Chemical Communications* 2010, 46, 2426-2428.

FIELDS OF STUDY

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LIST OF ABBREVIATIONS

ax.................................................................axial
bpy.................................................................2,2'-bipyridine
DFT............................................................density functional theory
DPBF..........................................................diphenylisobenzofuran
dppn............................................................benzo[i]dipyrido[3,2-a:2',3'-h]quinoxaline
dppz............................................................dipyrido[3,2-a:2',3'-c]phenazine
dpq..............................................................dipyrido[3,2-f:2',3'-h]quinoxaline
dpqpp......................................................pyrazino[2',3':5,6]pyrazino-[2,3-f][1,10]phenanthroline
EnT.............................................................energy transfer
eq.................................................................equatorial
ES.................................................................excited state
f.................................................................oscillator strength
fl.................................................................fluorescence
FPT.............................................................freeze-pump-thaw
GMP......................................................guanosine monophosphate
GS..............................................................ground state
hat...............................................................1,4,5,8,9,12-hexaazatriphenylene
HOMO.......................................................highest occupied molecular orbital
ic...............................................................internal conversion
isc............................................................intersystem crossing
Kb.............................................................binding constant
kq.............................................................quenching rate constant
LC............................................................ligand centered
LC_{50}......................................................50% lethal concentration
LF............................................................ligand field
LUMO...................................................lowest unoccupied molecular orbital
MC............................................................metal centered
MLCT.......................................................metal-to-ligand charge transfer
NHE..........................................................normal hydrogen electrode
nr...............................................................non-radiative
PCM......................................................polarizable continuum model
PDT..........................................................photodynamic therapy
ph...........................................................phosphorescence
phen........................................................1,10-phenanthroline
PSS.................................................................polystyrene sulfonate
pydppn................................................3-(pyrid-2'-yl)-4,5,9,16-tetraaza-dibenzo[a,c]naphthacene
ROS..............................................................reactive oxygen species
s..............................................................base pair binding site size
SOD.................................................................superoxide dismutase
TAE.....................................................................tris-acetate-EDTA buffer
TBE.................................................................tris-borate-EDTA buffer
$^{1}\text{O}_2$..............................................................singlet oxygen
$^{3}\text{O}_2$..............................................................triplet oxygen
$\Phi$.......................................................................quantum yield
$\Delta_0$..................................................................ligand field splitting
CHAPTER 1

INTRODUCTION

Commonly used chemotherapy agents and radiotherapy pose a variety of problems in that the side effects associated with these treatments are particularly harmful to the patients, such as the nephrotoxicity and ototoxicity which occur with the widely used drug cisplatin.\(^1\) Additionally, with radiotherapy further malignancies can often occur resulting in health effects as damaging as the cancer itself.\(^2\) The use of nontoxic drugs that become highly cytotoxic only under irradiation with visible light is a field known as photodynamic therapy (PDT), which represents an alternative to the unavoidable and costly side effects of typical cancer therapies. PDT is selective to tumors because the localized irradiation of a photosensitizer limits the cytotoxicity to only the malignancies and surrounding areas, rather than also afflicting all healthy cells.

Currently used PDT agents include Photofrin\textsuperscript{®} and Levulan\textsuperscript{®} clinically approved in the US in 1995 and 2000, respectively. Shown in Figure 1.1, Photofrin\textsuperscript{®} is a mixture of hematoporphyrin derivatives and Levulan\textsuperscript{®} is 5-aminolevulinic acid.\(^2-3\) The use of both of these compounds, in combination with light, ultimately results in cytotoxicity, but as opposed to Photofrin\textsuperscript{®}, Levulan\textsuperscript{®} is a prodrug, not a photosensitizer. Levulan\textsuperscript{®} is a
promoter for accumulation of protoporphyrin IX, a naturally occurring photosensitizer, which then behaves in a manner similar to Photofrin\textsuperscript{®} 3-4.

![Figure 1.1](image)

**Figure 1.1** Molecular structures of PDT agents Photofrin\textsuperscript{®} and Levulan\textsuperscript{®}.

Figure 1.2 presents a generalized Jablonski diagram illustrating kinetic pathways possible for a typical photosensitizer. Upon initial excitation from a singlet ground state (\(^1\)GS) to the Frank-Condon, singlet excited state (\(^1\)ES) there is vibrational relaxation down to the lowest energy vibrational level of that state (internal conversion, ic). Non-radiative intersystem crossing (isc) competes with fluorescence (fl) and non-radiative relaxation (nr) to yield the lowest energy triplet excited state (\(^3\)ES). The enhanced spin-orbit coupling associated with heavy metal complexes allows for intersystem crossing to have a quantum yield close to unity, whereas in non-metallic porphyrins the population of the \(^3\)ES can approach unity as with uroporphyrin III (0.93),

\textsuperscript{6} but is more commonly
limited\textsuperscript{5-7} as with hematoporphyrin in H\textsubscript{2}O (0.83).\textsuperscript{6} Deactivation from this state can then occur by non-radiative relaxation to the \textsuperscript{1}GS, radiative relaxation (phosphorescence, ph), energy transfer (EnT) to generate \textsuperscript{1}O\textsubscript{2} from ground state triplet oxygen, or by a reaction with a biomolecule.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{General Jablonski diagram illustrating the kinetic pathways available to a photosensitizer. Type I and II mechanisms for cell death are also shown.}
\end{figure}

In general, PDT agents are known to be operative by two mechanisms, known as Type I and Type II.\textsuperscript{8-10} As shown in Figure 1.2, a Type I mechanism involves cell death by the direct damage to DNA or other biomolecules by the excited photosensitizer. For example, hydrogen atom abstraction from the DNA sugar backbone or electron transfer from a DNA base afforded by the excited state of the photosensitizer resulting in the
formation of highly reactive species.\textsuperscript{11} Oxidation of DNA is often possible because the excited state of the photosensitizer has greater oxidizing power than the ground state complex.\textsuperscript{8}

Most PDT agents, however, follow a Type II mechanism (Figure 1.2), where the excited photosensitizer is able to transfer energy to another species which is ultimately responsible for cell death. In most cases the acceptor of the energy transfer is molecular oxygen (\textsuperscript{3}O\textsubscript{2}).\textsuperscript{8,12} Upon collisional energy transfer from a triplet excited state of a photosensitizer, singlet oxygen (\textsuperscript{1}O\textsubscript{2}) is formed. \textsuperscript{1}O\textsubscript{2} is a reactive oxygen species (ROS) that is able to undergo a number of cellular reactions, including oxidation of lipids and proteins, membrane damage, destruction of organelles, and activation or inhibition of cellular functions.\textsuperscript{13} These processes ultimately induce cell death by apoptosis or necrosis.\textsuperscript{3,8} For efficient energy transfer to oxygen the energy of the \textsuperscript{3}ES must be > 94 kJ/mol (0.97 eV), the energy of \textsuperscript{1}O\textsubscript{2}. Not unlike a Type I mechanism, the target of the ROS is often DNA. Although there is still no clear mechanism, it is known that \textsuperscript{1}O\textsubscript{2} selectively modifies deoxyguanosine.\textsuperscript{11}

In addition to production of ROS there are other factors, such as DNA binding affinity, which can contribute to the effectiveness of a PDT agent. There are multiple modes of binding for complexes and DNA. Major and minor grooves are present in the DNA structure, which alternate along the twisted secondary structure of the double helix (Figure 1.3). Cations, such as Ru(bpy)\textsubscript{3}\textsuperscript{2+} (bpy = 2,2'-bipyridine) for example, can bind very weakly by electrostatic attraction to the negatively charged phosphodiester backbone along the grooves of DNA.\textsuperscript{14} Major or minor groove binding is dependent on the shape
and size of the complex. Minor groove binding often involves hydrogen bonding, as in Hoechst 33342. Two other strong binding modes associated with DNA include covalent binding and intercalation.\textsuperscript{15} Intercalation is a $\pi$-bonding interaction which occurs when a small planar heteroaromatic molecule or ligand inserts itself into the DNA stack between adjacent base pairs. Under intercalation, the DNA must untwist and lengthen to accommodate the inserted moiety.\textsuperscript{16} Covalent binding can occur between Lewis-acid metallic complexes and the nucleophilic bases of DNA.\textsuperscript{15} These binding modes are discussed in further detail, with respect to the pertinent complexes in Chapters 3 – 5; Chapters 3 and 4 include complexes with varied ability to intercalate, whereas Chapter 5 consists of studies on complexes which are expected to covalently bind to DNA upon photoinduced ligand exchange.

\textbf{Figure 1.3} Schematic illustration of binding modes accessible to DNA.
Despite the non-metallic nature of both the clinically approved PDT agents, there are some metal-centered complexes undergoing clinical trials. The use of transition metal complexes for biological applications has been an active area of research because their properties and reactivity are easily tuned with simple changes of the ligands and metals. Metal-containing compounds that have been studied as second-generation photosensitizers can be grouped into two categories, metallopolypyrrolic compounds and transition-metal complexes. With the addition of an appropriate closed-shell metal to the well studied polypyrrolic compounds, metallopolypyrrolic complexes essentially maintain the properties of the parent polypyrrolic ring but gain an increased production of due to the greater efficiency in triplet excited state population with the enhanced spin-orbit coupling and intersystem crossing. The majority of transition metal complexes proposed for PDT induce cytotoxicity by generation, similar to the first-generation porphyrins. Similarly, a series of dirhodium(II,II) complexes which produce and possess photocytotoxic properties is presented in Chapter 3.

Although Photofrin offers selectivity benefits over traditional chemotherapy, it is still beset by many drawbacks that have fueled research for new, superior PDT complexes. Prolonged cutaneous sensitivity to light is a major issue that can be circumvented through development of agents that are readily cleared biologically. Other characteristics of an effective PDT agent include a long-lived excited state to enable energy transfer to oxygen, to produce greater yields, specificity for tumor tissue, and low toxicity in the dark with high toxicity under irradiation. In addition, complexes that have large absorption coefficients in the range 650 – 850 nm are desirable because
their absorption falls within the “phototherapeutic window”, the range where light penetration through tissue is greatest.\(^2\) Tumors are often hypoxic, such that a \(^1\)O\(_2\) mediated mechanism is not viable for all cancers. Therefore, complexes with properties which allow for a Type I mechanism are also of value. Chapter 4 details the reactivity of two Ru(II) complexes and one Os(II) complex that show promise as PDT agents because of their ability to achieve some of the above mentioned desirable characteristics.

With regard to oxygen-independent mechanisms, photoactive complexes modeled after the widely used chemotherapy agent, cisplatin \(\text{cis-}[\text{Pt(NH}_3)_2\text{Cl}_2]\), and related derivatives, have been established.\(^{18,19}\) Cisplatin has shown tremendous success as a metallic antitumor complex for the treatment of ovarian, testicular, head, neck, esophageal, and small-cell lung cancers and has hence been the driving force behind much of the research on metal-containing anticancer drugs.\(^1,20-21\) Figure 1.4a illustrates the mechanism of cisplatin, a thermally active complex, that is able to undergo the sequential exchange of two chloride ligands for two water molecules under physiological conditions. The diaqua species, \(\text{cis-}[\text{Pt(NH}_3)_2(\text{OH}_2)_2]^{2+}\), can then bind covalently to DNA. It is known that cisplatin-DNA binding predominantly occurs at intrastrand d(GpG) sites (Figure 1.4b) and that these crosslinks ultimately cause inhibition of transcription and DNA replication.\(^1\) Like most currently available anticancer drugs, there are drawbacks associated with cisplatin (notably its lack of malignant cell specificity) which warrant the search for improved complexes. In Chapter 5, a series of dirhodium(II,II) complexes with photolabile benzonitrile ligands are presented as photo-cisplatin analogs.
Figure 1.4 (a) Stepwise exchange of chloride ligands for water in cisplatin (b) illustration of a cisplatin induced d(GpG) crosslink in DNA.\textsuperscript{22}
References

1. Reedijk, J. PNAS 2003, 100, 3611.


CHAPTER 2

EXPERIMENTAL METHODS

2.1 Materials

Sodium chloride, sodium phosphate, gel loading buffer (0.05% (w/v) bromophenol blue, 40% (w/v) sucrose, 0.1 M EDTA (pH = 8.0), 0.5% (w/v) sodium lauryl sulfate), Trizma® base, Tris/HCl, ethidium bromide, sodium acetate, benzonitrile, and 4-fluorobenzonitrile were purchased from Sigma Aldrich and used as received. Triethylhexonium tetrafluoroborate 1 M in CH₂Cl₂ was purchased from Acros Organics and used as received. RuCl₃·3H₂O was purchased from Strem and used as received, and glacial acetic acid was purchased from Fisher. Calf thymus DNA was purchased from Sigma and was dialyzed against 5 mM Tris buffer (50 mM NaCl, pH = 7.5) three times over a period of 48 h until \( A_{260}/A_{280} > 1.8 \), where \( A_{260} \) and \( A_{280} \) represent the absorbance at 260 and 280 nm, respectively.¹ The pUC18 plasmid was purchased from Bayou Biolabs and purified using the QIAprep Spin Miniprep Spin System from Qiagen. Methanol and dichloromethane were dried over CaH₂, and distilled under a nitrogen atmosphere prior to use, and 1,3-diphenylisobenzofuran (DPBF) and polystyrene sulfonate (PSS) were purchased from Aldrich and used without further purification. [Ru(bpy)₃]PF₆₂,² [Ru(bpy)₂(dpzp)]PF₆₂,³ [Ru(bpy)₂(dpqq)]PF₆₂,⁴ [Ru(bpy)₂(dppn)]PF₆₂,⁵
[Os(bpy)$_2$(dppn)](PF$_6$)$_2$ and Rh$_2$(μ-O$_2$CCH)$_3$$_4$ were prepared according to literature methods. cis-[Rh$_2$(μ-O$_2$CCH)$_3$$_2$(bpy)(dppn)](O$_2$CCH)$_2$, cis-[Rh$_2$(μ-O$_2$CCH)$_3$$_2$(phen)(dppn)](O$_2$CCH)$_2$, cis-[Rh$_2$(μ-O$_2$CCH)$_3$$_2$(dpq)(dppn)](O$_2$CCH)$_2$, and cis-[Rh$_2$(μ-O$_2$CCH)$_3$$_2$(dppz)(dppn)](O$_2$CCH)$_2$ were synthesized in a similar manner according to the literature methods. Generally, a suspension of dppn and cis-[Rh$_2$(μ-O$_2$CCH)$_3$$_2$(η$^1$-O$_2$CCH)(L)(CH$_3$OH)](O$_2$CCH)$_2$, (L = bpy, phen, dpq, dppz, dppn) was refluxed under N$_2$ in CH$_3$CN for 24 hours yielding the corresponding product as a red precipitate.

cis-[Rh$_2$(μ-O$_2$CCH)$_3$$_2$(NCC$_6$H$_5$)$_4$](BF$_4$)$_2$: [5.2](BF$_4$)$_2$ was synthesized in a manner similar to that reported for cis-[Rh$_2$(μ-O$_2$CCH)$_3$$_2$(CH$_3$CN)$_6$](BF$_4$)$_2$. A suspension of Rh$_2$(μ-O$_2$CCH)$_3$$_4$ (37 mg, 84 µmol) in neat benzonitrile (8 mL) was stirred under N$_2$ for 20 min before a 4-fold excess 1 M Et$_3$O$^+$(BF$_4$) in CH$_2$Cl$_2$ (0.67 mL) was added via syringe. The round bottom flask was protected from light by aluminum foil while it stirred under N$_2$, over moderate heat (not to reflux) for 4 hrs, upon which the solution turned a magenta purple color. To the solution 8 mL MeOH was added. Purification was accomplished by extraction of the unreacted benzonitrile with cyclohexane 3 times, in which the product remained in the colored methanol layer. The methanol layer was then dried down to a sticky purple solid and characterized by NMR (Figure 2.1). $^1$H NMR (CD$_3$OD) $\delta$ (splitting, integration) 7.72 (d, 4 H), 7.61 (t, 2 H), 7.38 (t, 4 H), 2.32 (s, 3 H). The quintet at 3.31 and singlet at 4.78 are attributed to the CD$_3$OD resonances.
Figure 2.1 ¹H NMR spectrum of cis-[Rh₂(µ-O₂CCH₃)₂(NCC₆H₅)₄](BF₄)₂ in CD₃OD showing integrations of product peaks.

cis-[Rh₂(µ-O₂CCH₃)₂(4F-C₆H₄CN)₄](BF₄)₂: A suspension of Rh₂(µ-O₂CCH₃)₄ (26 mg, 59 µmols) and 6-fold excess 4-fluoro-C₆H₄CN (172 mg, 1.4 mmol) was stirred under N₂ in dry methanol (20 mL) for 20 min before a 4-fold excess 1 M Et₃O⁺(BF₄) in CH₂Cl₂ (0.47 mL) was added via syringe. The round bottom flask was protected from light by aluminum foil while it was stirred under N₂ over moderate heat (not to reflux) for 24 hrs, during which time the solution turned a magenta purple color. The solution was condensed to ~3 mL and Et₂O was added to precipitate the product as a purple sticky solid which was characterized by NMR (Figure 2.2). ¹H NMR ((CD₃)₂CO) δ (splitting,
integration) 8.00 (q, 4 H), 7.20 (t, 4 H), 2.25 (s, 3 H). The quintet at 2.05 ppm is attributed to the (CD₃)₂CO resonance.

**Figure 2.2** $^1$H NMR spectrum of cis-[Rh₂(μ-O₂CCH₃)₂(4F-C₆H₄CN)₄](BF₄)₂ in (CD₃)₂CO showing integrations of the product.

### 2.2 Instrumentation

$^1$H NMR spectra were collected on a 400 MHz Bruker system, and chemical shifts were referenced to the residual solvent peak using Advanced Chemistry Development
NMR Processor Academic Edition software. Steady-state absorption spectra were recorded on a HP diode array spectrometer (HP8453) with HP8453 WinSystem software. Corrected steady-state emission and excitation spectra were measured on a SPEX Fluoromax-2 luminescence spectrometer with a 90° optical geometry equipped with a 150 W xenon arc lamp as the source. The home-built transient absorption instrument for measurements on the nanosecond and microsecond timescales was previously reported, with the exception of a PowerMac G4 (Apple) equipped with a National Instruments GPIB interface (NI-488.2) and a National Instruments data acquisition board (PCI-1200) programmed with Labview 4.1 software to control the data acquisition by the oscilloscope and the PMT voltage.¹⁰ Excitation was accomplished using a frequency-tripled (355 nm) of frequency doubled (532 nm) Spectra-Physics GCR-150 Nd:YAG laser (fwhm ~ 8 ns) as the excitation source.

The relative changes in viscosity were measured on a Cannon-Manning semi-micro viscometer. The viscometer was immersed in a constant temperature water bath controlled by a Neslab (model RTE-100) circulator set to 25 °C. A 150 W Xe arc lamp in a PTI housing (Milliarc Compact Lamp Housing) powered by an LPS-220 power supply (PTI) with an LPS-221 igniter (PTI) was used for the DNA photocleavage and photolysis experiments. The irradiation wavelength was selected by placing long-pass colored glass filters (Melles Griot) and a 10 cm water cell in the light path. The ethidium bromide stained agarose gels were imaged using a Gel Doc 2000 transilluminator (BioRad) equipped with Quantity One (v. 4.1.1) software.
2.3 Methods

The DNA photocleavage experiments were carried out using 10 µL or 20 µL total sample volume in 0.5 mL transparent Eppendorf tubes containing 100 µM pUC18 plasmid and 20 µM of each metal complex. Irradiation of the solutions was performed either in air or after six freeze-pump-thaw cycles in quartz tubes equipped with a Kontes stopcock (using ~3-fold greater solution volume). Following irradiation, 4 µL of DNA gel loading buffer was added to each sample. The electrophoresis was carried out using a 1% agarose gel stained with 0.5 mg/L ethidium bromide in 1X TAE buffer (40 mM tris-acetate, 1 mM EDTA, pH ~ 8.2); other conditions are specified as needed. Using Quantity One software, background corrected intensity values of both form I and form II bands for each lane were calculated. The sum of the total intensity of both forms was taken as the total sample intensity and percent photocleavage of each sample was calculated to be the percent intensity of form II.

Plasmid linearization was accomplished by incubating 50 units of Sma I with 10 µg of pUC18 DNA and 10 µL of REact 4 buffer (Invitrogen) at 30 °C for 1 hr followed by 10 min at 65 °C. The linearized DNA was subsequently separated from the enzyme using a QIAquick gel extraction kit. The concentration of plasmid DNA was determined from its absorption at 260 nm using an extinction coefficient of 6,600 M⁻¹cm⁻¹ in accordance with the Qiagen protocol. The DNA mobility experiments were carried out using 20 µL total sample volume in 0.5 mL transparent Eppendorf tubes containing 20 µM linearized pUC18 plasmid, 10 mM sodium phosphate buffer, and the concentration of each metal complex was varied. Following irradiation or dark incubation, 4 µL of
DNA gel loading buffer was added to each sample. The electrophoresis was carried out in 1 x TBE buffer (TBE = tris-borate/EDTA, 0.09 M tris-borate, 0.002 M EDTA, pH = 8.3) using a 0.75% agarose gel, run at 92 V for 1 hr. Staining was performed after electrophoresis by soaking the gel in a 0.5 µg/mL aqueous ethidium bromide solution followed by washing in water for 30 min prior to imaging.

The relative change in viscosity was measured using sonicated herring sperm DNA (1 mM) in 5 mM Tris-HCl, pH 7.5, 20 mM NaCl, and increasing the concentration of each complex. The data are presented as \( \left( \frac{\eta}{\eta_0} \right)^{1/3} \) versus \([M]/[DNA]\) where \( \eta = t_1 - t_2 \) represents the viscosity of DNA in the presence of the complex, \( \eta_0 = t_n - t_0 \) represents the viscosity of DNA in the absence of the complex, \( t_0 \) is the flow time of buffer alone, \( t_1 - t_2 \) is the flow time of DNA alone, and \( t_n \) is the flow time of DNA and complex.

The binding constant of each metal complex to calf thymus DNA was determined by optical titration at room temperature using 5 µM complex, and the calf thymus DNA concentration was varied from 0 to 100 µM (5 mM Tris/HCl, 50 mM NaCl, pH = 7.5). The dilution of metal complex concentration at the end of each titration was negligible. The DNA binding constant, \( K_b \), was obtained from fits of the titration data to eq 2.1 \(^{11-12}\)

\[
\frac{\varepsilon_a - \varepsilon_f}{\varepsilon_b - \varepsilon_f} = \frac{b - (b^2 - 2K_b^2C_t[DNA]_t/s)^{1/2}}{2K_bC_t} \quad (2.1)
\]
where \( b = 1 + K_b C_t + K_b [\text{DNA}]_t / 2s \), \( C_t \) and \([\text{DNA}]_t\) represent the total complex and DNA concentrations, respectively, \( s \) is base pair binding site size, and \( \epsilon_a \), \( \epsilon_f \), and \( \epsilon_b \) represent the molar extinction coefficients of the apparent, free, and bound metal complexes, respectively. The value of \( \epsilon_b \) was determined from the plateau of the DNA titration, where addition of DNA did not result in further changes to the absorption spectrum.

Photophysical measurements were performed in a 1 \( \times \) 1 cm quartz cuvette equipped with a rubber septum, and the solutions were bubbled with argon for 15 min prior to each measurement, unless otherwise noted. For determination of \( \Phi_{1O_2} \), aerated solutions with concentrations adjusted to obtain absorption of 0.05 at the excitation wavelength (\( \lambda_{exc} = 475 \) nm) were used. The quantum yield of singlet oxygen produced by each complex was measured relative to \([\text{Ru(bpy)}_3]^{2+}\) as the standard, with known \( \Phi_{1O_2} \) (0.81 in CH\(_3\)OH),\(^\text{13}\) by monitoring the fluorescence quenching of DPBF, a known \( ^1\text{O}_2 \) target.\(^\text{14}\) Under these conditions, the plot of fluorescence intensity of DPBF versus irradiation time yields a slope that can be compared relative to that of the standard and used to calculate \( \Phi_{1O_2} \) for each complex.

Human skin fibroblasts (Hs-27) were obtained from the American Type Culture Collection, cell line CRL-1634 (Manassas). Cells were cultured in Dulbecco’s modified Eagle medium, containing 10% fetal bovine serum (Invitrogen Life Technologies), 50 \( \mu \text{g/mL} \) gentamicin, 4.5 mg/mL glucose, and 4 mM L-glutamine (Invitrogen Life Technology). Cell cultures were incubated in a humidified atmosphere containing 5% CO\(_2\) at 37 \( ^\circ \)C. For assessing the cytotoxicity and photocytotoxicity of different
compounds, subconfluent (50% - 80% confluent) monolayers of Hs-27 in 60 mm culture dishes were used. The monolayers were washed twice with phosphate-buffered saline (PBS) to ensure that the culture dishes were free of any culture medium, after which time fresh PBS containing different concentrations of each compound was added to cover the fibroblasts. The cells were irradiated through the PBS buffer, which does not absorb light in the visible region. After irradiation, the cells were removed from the dishes by trypsinization, seeded into 24-well culture dishes, and incubated for 2 to 4 days or until the untreated control group reached confluence. N-lauroyl sarcosine (200 µL, 40 mM) was then added to each well and the cells were allowed to lyse for at least 15 min. Quantitative determination of the protein content in each well was undertaken using Peterson's Modification of the Micro-Lowry Method (Sigma reagent kit), where the lysate was treated with 200 µL Lowry reagent for 20 min and then with 100 µL Folin-Ciocalteu phenol reagent for 30 min or until color developed. A portion of the contents (200 µL) of each well was transferred to a 96-well plate for absorbance determination using a multiwell plate reader (Dynatech Laboratory). The absorbance at 630 nm was monitored, which is proportional to the total protein content and the number of cells in each well.

The molecular and electronic structure determinations in Chapter 5 were performed with density functional theory (DFT) using the Gaussian 03 (G03) program package. The B3LYP functional together with the 6-31G* basis set were used for H, C, N, F, and O, along with the Stuttgart/Dresden (SDD) energy-consistent pseudopotentials for Rh. All geometry optimizations were performed in C1 symmetry
with subsequent frequency analysis to show that the structures are local minima on the potential energy surface. In the present work, solvent effects were modeled on the gas phase optimized structures using the polarizable continuum model (PCM). 23-24
2.4 References


5. Sun, Yujie Ru(II) and Os(II) Polypyridyl Complexes as Luminescence Sensors and PDT Agents. Diss. The Ohio State University, 2010. Columbus, Ohio.


CHAPTER 3

PHOTOPHYSICAL PROPERTIES, DNA PHOTOCLEAVAGE, AND PHOTOCYTOTOXICITY OF A SERIES OF DPPN DIRHODIUM(II,II) COMPLEXES


3.1 Background

Dirhodium(II,II) tetracarboxylate paddlewheel complexes have been shown to exhibit anticancer properties and are known to be potent inhibitors of DNA transcription and protein synthesis.\(^1\) There are numerous reports that focus on the elucidation of the nature of DNA binding of dirhodium complexes.\(^2\) There are two unique binding positions on the dirhodium core noted as axial \((ax)\) and equatorial \((eq)\), shown in Figure 3.1. It is known that in coordinating solvents the labile axial ligands readily exchange with solvent whereas the equatorial ligands have greater stability with regard to thermal exchange.\(^3\) There is a greater affinity for adenine binding in dirhodium tetracarboxylates, contrary to the guanine selective covalent interaction of square planar cisplatin. It is believed that this preference stems from the favorable hydrogen bonding interaction of the carboxylate oxygen with the exocyclic adenine \(\text{NH}_2(6)\) amino group, as opposed to the repulsive interaction between the carboxylate oxygen and guanine O6.\(^2\) Despite the evidence for dirhodium-DNA covalent binding under certain reaction conditions, the axial binding of
Rh\textsubscript{2}(\mu-O\textsubscript{2}CCH\textsubscript{2})\textsubscript{4} to DNA in buffer at room temperature results in a very weak interaction with binding constant, $K_b = 4.6 \times 10^2$ M\textsuperscript{-1}.\textsuperscript{1a} Furthermore, studies relating the intercalation of ligands with extended π-systems to DNA photocleavage show evidence of enhanced reactivity in complexes that bind strongly to the double helix.\textsuperscript{4} It is therefore desirable to synthesize new dirhodium complexes with stronger DNA binding, such that their anticancer properties may be enhanced.

**Figure 3.1** Molecular structure of Rh\textsubscript{2}(\mu-O\textsubscript{2}CR)\textsubscript{4}(L)\textsubscript{2} (R = CH\textsubscript{3}) illustrating the axial and equatorial ligand positions available on each metal atom.

Rh\textsubscript{2}(II,II) complexes possessing ligands with extended π-systems exhibit activity toward HeLa and COLO-316 cancer cell lines, making these types of compounds useful as potential antitumor agents.\textsuperscript{5-6} Specifically, dirhodium(II,II) complexes based on the dipyrido[3,2-a:2’,3’-c]phenazine (dppz) ligand were previously shown to exhibit an increase in toxicity upon irradiation similar to that of hematoporphyrin.\textsuperscript{7-8} In addition, the DNA photocleavage by $cis$-[Rh\textsubscript{2}(\mu-O\textsubscript{2}CCH\textsubscript{3})\textsubscript{2}(dppz)\textsubscript{2}]\textsuperscript{2+} and $cis$-[Rh\textsubscript{2}(\mu-
O$_2$CCH$_3$)$_2$(bpy)(dppz)$_2^{2+}$ (bpy = 2,2'-bipyridine) remained active in the absence of oxygen, making these complexes potential candidates for oxygen-independent PDT.

When oxygen is available, $^{1}$O$_2$ generated by the photoactivated drugs can cause oxidative damage to biomolecules including proteins, nucleic acids, and membrane lipids. One particular drawback of these systems is that in the absence of oxygen the excited states themselves are not reactive. Therefore, when oxygen in the vicinity of the photosensitizer is consumed, the drug becomes inactive until a replenished supply of O$_2$ can diffuse into the cells or region of the tumor. In addition, the most aggressive and drug-resistant tumors are hypoxic, thereby rendering these PDT drugs less effective toward them. Therefore, the discovery of new PDT agents whose action does not depend on the availability of oxygen is a highly desirable goal.

The work presented in this chapter focuses on a related series of complexes, cis-[Rh$_2$(µ-O$_2$CCH$_3$)$_2$(dppn)(L))]$_2^{2+}$, where dppn = benzo[i]-dipyrido[3,2-a:2',3'-c]phenazine and L = bpy (3.1), phen (1,10-phenanthroline, 3.2), dpq (dipyrido[3,2-f:2',3'-h]quinoxaline, 3.3), dppz (dipyrido[3,2-a:2',3'-c]phenazine, 3.4), and dppn (3.5). A schematic representation of the molecular structures of the complexes and ligands is depicted in Figure 3.2. The photophysical properties of the complexes and their ability to bind and photocleave DNA were probed, along with their toxicity toward human skin cells in the dark and when irradiated with visible light. The known ability of 3.1 – 3.5 to transverse cellular membranes, their toxicity in the dark, and their photophysical properties were used to explain the observed results.
**Figure 3.2** Molecular structures of the dirhodium complexes showing numbering scheme and ligand structures.

<table>
<thead>
<tr>
<th>Complex</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>bpy</td>
</tr>
<tr>
<td>3.2</td>
<td>phen</td>
</tr>
<tr>
<td>3.3</td>
<td>dpq</td>
</tr>
<tr>
<td>3.4</td>
<td>dppz</td>
</tr>
<tr>
<td>3.5</td>
<td>dppn</td>
</tr>
</tbody>
</table>

\[ \text{cis-[Rh}_2(\mu-O_2CCH_3)(dppn)(L)]^{2+} \]
3.2 Results

3.2.1 Photophysical Properties

The electronic absorption maxima of 3.1 – 3.5 are listed in Table 3.1 and the corresponding spectra in water are shown in Figure 3.3. Complexes 3.1 – 3.5 exhibit ligand-centered (LC) transitions with maxima in the 250 to 430 nm range in water. By comparison, the free dppn ligand exhibits absorption maxima at 318 nm (65,000 M⁻¹ cm⁻¹), 390 nm (9,400 M⁻¹ cm⁻¹), and 414 nm (12,500 M⁻¹ cm⁻¹) in CHCl₃. Similarly, the related tridentate ligand, 3-(pyrid-2'-yl)-4,5,9,16-tetraazadibenzo[a,c]naphthacene (pydppn), exhibits comparable LC transitions at 329 nm (66,000 M⁻¹ cm⁻¹), 398 nm (13,000 M⁻¹ cm⁻¹), and 421 nm (17,000 M⁻¹ cm⁻¹) in CHCl₃. Absorption maxima in the ranges 259 – 272 and 316 – 329 nm are observed in 3.1 – 3.5, which can be associated with ligand-centered transitions in each complex. For example, from comparison to the spectral features of the free dppn ligand, the absorption features observed at 316 – 329 nm in 3.1 – 3.5 are assigned to transitions of dppn. A metal-to-ligand charge transfer (MLCT) transition is observed in the related complexes cis-[Rh₂(µ-O₂CCH₃)₂(bpy)₂]²⁺, cis-[Rh₂(µ-O₂CCH₃)₂(bpy)(dppz)]²⁺, and cis-[Rh₂(µ-O₂CCH₃)₂(dppz)₂]²⁺ with maxima at 408 nm (ε = 2,920 M⁻¹ cm⁻¹), 432 nm (3,100 M⁻¹ cm⁻¹), and 434 nm (5,460 M⁻¹ cm⁻¹), respectively, which is also expected to be present in 3.1 – 3.5. It is likely that this MLCT peak is indeed present, but is obscured by the more intense dppn features in this LC spectral region. In addition, complexes 3.1 – 3.5 exhibit a significantly weaker metal-centered (MC) RhRhπ*→RhRhσ* transition with maxima at ~600 – 621 nm. This MC transition is also observed in cis-[Rh₂(µ-O₂CCH₃)₂(bpy)₂]²⁺ and Rh₂(µ-O₂CCH₃)₄ at 512
nm (ε = 380 M⁻¹ cm⁻¹)²⁰ and 585 nm (ε = 241 M⁻¹ cm⁻¹), respectively.²¹ As was previously reported for the related complexes *cis*-\([\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{bpy})(\text{dppz})]^{2+}\) and *cis*-\([\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{dppz})_2]^{2+}\) complexes 3.1 – 3.5 are non-emissive at 298 K in water.

Table 3.1 Absorption Maxima, Sensitized Singlet Oxygen Quantum Yields, and Transient Absorption Lifetimes of 3.1 – 3.5.

<table>
<thead>
<tr>
<th>Complex</th>
<th>(\lambda_{\text{abs}} / \text{nm} (\varepsilon \times 10^{-3} / \text{M}^{-1}\text{cm}^{-1})^a)</th>
<th>(\Phi_{1\text{O}_2}^b)</th>
<th>(\tau / \mu\text{s}^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>259 (38.5), 270 (37.9), 323 (45.4), 405 (9.4), 600 (1.6)</td>
<td>0.7</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>261 (60.5), 325 (59.7), 406 (10.7), 618 (1.6)</td>
<td>0.9</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>259 (57.1), 318 (39.6), 409 (7.9), 609 (1.1)</td>
<td>0.8</td>
<td>2.4</td>
</tr>
<tr>
<td>4</td>
<td>272 (36.7), 329 (25.3), 390 (13.6), 430 (9.9), ~620 (3.8)</td>
<td>0.4</td>
<td>3.5</td>
</tr>
<tr>
<td>5</td>
<td>260 (60.5), 316 (67.0), 410 (13.2), 621 (2.0)</td>
<td>0.4</td>
<td>4.1</td>
</tr>
</tbody>
</table>

²⁰In water. ²¹In methanol (\(\lambda_{\text{irr}} = 450 \text{ nm}\)). ²²In deareated DMSO (\(\lambda_{\text{exc}} = 355 \text{ nm}, \text{fwhm} \sim 8 \text{ ns}\)).
Figure 3.3 Electronic absorption spectra of 5 µM of (a) 3.1, (b) 3.2, (c) 3.3, (d) 3.4, and (e) 3.5 in H₂O.
Although 3.1 – 3.5 are not emissive, the complexes exhibit a long-lived excited state observed by transient absorption spectroscopy. The transient absorption spectra of 3.1 – 3.5 are shown in Figure 3.4 in deaerated DMSO (λ_{exc} = 355 nm), where it is apparent that all the complexes possess similar spectral features, with a peak at ~430 nm and broad bands with maxima at 520 – 540 nm, with monoexponential decay lifetimes ranging from 2.4 to 4.1 μs (Table 3.1). A similar transient absorption signal was observed for 25 μM free dppn ligand in deoxygenated CHCl₃ (λ_{exc} = 355 nm), with a broad maximum at 540 nm and τ = 13.0 μs (Figure 3.4f). An increase in the transient absorption signal at λ < 420 nm is also observed for free dppn. The apparent shift in the maximum of this peak to lower energies in 3.1 – 3.5 can be explained by the overlap of the ground state absorption bleach with maxima at ~410 nm contributing to the transient absorption signal. On the basis of the comparison of the maxima and lifetimes of the transient absorption signals of 3.1 – 3.5 to those measured for the free dppn ligand, the excited state observed for the dirhodium complexes is assigned as arising from the \( ^3\pi\pi^* \) centered on the dppn ligand.
Figure 3.4 Transient absorption spectra of (a) 3.1, (b) 3.2, (c) 3.3, (d) 3.4, (e) 3.5, (f) free dppn in DMSO collected between 20-80 ns after the excitation pulse ($\lambda_{\text{exc}} = 355$ nm, fwhm $\sim$ 8 ns).
Since the mechanism of action of PDT agents currently in use relies on the production of \( ^1\text{O}_2 \), the quantum yield of photosensitized \( ^1\text{O}_2 \) production (\( \Phi_{^1\text{O}_2} \)) for 3.1 – 3.5 was measured in methanol (Table 3.1). For the sake of comparison, it is noted that Photofrin® has \( \Phi_{^1\text{O}_2} = 0.17 \). It is interesting to note that \( \Phi_{^1\text{O}_2} \) for 3.1 – 3.3 is \( \sim \)2-fold greater than those for 3.4 and 3.5. This trend is unexpected, since the excited state lifetimes of 3.4 and 3.5 are longer than those of 3.1 – 3.3, which should result in greater photosensitization of \( ^1\text{O}_2 \) in the former. A possible explanation for the difference in \( \Phi_{^1\text{O}_2} \) is the aggregation of the more hydrophobic 3.4 and 3.5 in alcohols and water, a trend that has been reported for cationic complexes of Ru(II) and Rh(II,II) possessing ligands with extended \( \pi \)-systems.\(^7\-^8\)

### 3.2.2 DNA Binding and Photocleavage

Prior relative viscosity experiments revealed that, at low concentrations of complex (up to 60 \( \mu \)M) and DNA (200 \( \mu \)M DNA), 3.1 and 3.2 intercalate between the DNA bases effectively, but 3.3 – 3.5 do not.\(^5\) By using greater concentrations of the probe (up to 300 \( \mu \)M) and 1 mM DNA, some intercalation by 3.3 and 3.4 is also apparent (Figure 3.5). Relative viscosity measurements of 3.5 at high concentration were not possible due to the lower solubility of this complex. Owing to the juxtaposed nature of the two dppn ligands on the complex and the results from the relative viscosity measurements at low concentrations, it is believed that 3.5 does not intercalate between the DNA bases. A lack of change in relative viscosity of DNA solutions upon increased
concentrations of complex was previously reported for the related complexes \( \text{cis-}[\text{Rh}_2(\mu-O_2\text{CCH}_3)_2(\text{bpy})(\text{dppz})]^2+ \) and \( \text{cis-}[\text{Rh}_2(\mu-O_2\text{CCH}_3)_2(\text{dppz})_2]^2+ \), which are not intercalators.\(^7\)\(^8\)

**Figure 3.5** Plot of relative viscosity changes upon increasing the concentration of (○) ethidium bromide, (▼) 3.1, (◊) 3.2, (■) 3.3, (□) 3.4, (♦) Ru(bpy)_3^{2+}, in the presence of 1 mM DNA.

Changes in the absorption spectra of 3.1 – 3.5 upon addition of DNA were also observed and fit to yield binding constants, \( K_b \), and base pair binding site size, \( s \). These hypochromic and bathochromic shifts are often associated with intercalation of the
complexes, however, aggregation of cationic hydrophobic complexes enhanced by the presence of the polyanionic DNA backbone has a similar effect.\textsuperscript{7,23-24} In order to distinguish between these two mechanisms, the changes in absorption of 3.1 – 3.5 were also recorded in the presence of a similar concentration of the random-coil polyanion polystyrene sulfonate (PSS). Shown in Figure 3.6, a comparison of the absorption spectra recorded in the presence of 100 µM DNA (bases) and 100 µM PSS reveals that 3.1 – 3.3 indeed intercalate between the DNA bases, whereas the absorption shifts observed for 3.4 and 3.5 are a result of enhanced aggregation effected by the polyanion. Complex 3.3 exhibits absorption changes due to contributions from both intercalation and aggregation. The shifts in the absorption of 3.1 and 3.2 as a function of DNA concentration were used to estimate their DNA binding constants, resulting in values of $1.2 \times 10^6$ M$^{-1}$ and $1.0 \times 10^5$ M$^{-1}$, respectively (Figure 3.7). The values are consistent with those of other intercalators.\textsuperscript{23-24}
Figure 3.6 Electronic absorption spectra of (a) 3.1, (b) 3.2, (c) 3.3, (d) 3.4, and (e) 3.5 alone (−), as well as in the presence of 100 µM DNA (−−−) and 100 µM PSS (−−).
**Figure 3.7** Fits to the changes in electronic absorption upon DNA titration of (a) 3.1 and (b) 3.2, showing the binding constants, $K_b$, and binding site size, $s$.

Figure 3.8 compares the photocleavage of 100 µM pUC18 plasmid DNA in air in the presence of 3.1–3.5. The plasmid-only control is shown in lane 1, illustrating the mobility of both the major supercoiled form (form I) and the trace nicked, circular form (form II) present in the samples. It is clear from Figure 3.8 that none of the complexes cleave DNA in the dark, but 3.1–3.4 efficiently generate single-strand breaks (SSBs, form II) upon irradiation with visible light ($\lambda_{\text{irr}} \geq 375$ nm) for 20 min with percent formation of form II ranging from 64% to 68% (Table 3.2). Irradiation of 3.5 in air results in significantly lower DNA photocleavage, 43%, compared to 3.1–3.4 under similar irradiation conditions (Figure 3.8a, lane 11). The DNA photocleavage is associated with the formation of reactive oxygen species, as illustrated by the DNA photocleavage of 3.1–3.4 under various experimental conditions shown in Figure 3.9. In Figure 3.9a–3.9d lanes 3 shows greater photocleavage in D$_2$O compared to H$_2$O (lanes 2), indicative that $^1$O$_2$ plays a role in the reactivity, since the lifetime of $^1$O$_2$ is 3-fold.
greater in D$_2$O than H$_2$O.$^{25}$ The addition of known radical and reactive oxygen species scavengers, sodium azide (lanes 3) and superoxide dismutase (SOD, lanes 5), as well as subjecting the sample to six freeze-pump-thaw (FPT) cycles (lanes 4), thereby rendering the sample devoid of oxygen, result in decreased cleavage.$^{25}$ As was previously reported for related dirhodium complexes, there appears to be a dominant O$_2$-dependent pathway and minor O$_2$-independent reactivity.$^{7}$ The DNA photocleavage by 3.5 is relatively invariant in D$_2$O, after FPT cycles, and in the presence of azide and SOD, results that support a DNA damage mechanism that is independent of oxygen. Since the quantum yields of photosensitized $^1$O$_2$ are similar for 3.4 and 3.5, the difference in DNA photocleavage ability of the two complexes can be attributed to the ability of 3.4 to partially intercalate between the DNA bases, thus generating $^1$O$_2$ within the local DNA environment. Given that 3.5 does not intercalate between the DNA bases, $^1$O$_2$ produced upon irradiation is likely not in the vicinity of the duplex.
Figure 3.8 Ethidium bromide stained agarose gel with 100 µM pUC18 plasmid and 20 µM complex (5 mM Tris, pH = 7.5, 50 mM NaCl). Photocleavage by $\geq 375$ nm, 20 min), where lanes 1, 2, 4, and 6 are dark controls and lanes 3, 5, and 7 are irradiated; lane 1, plasmid only; lanes 2 and 3, 3.1; lanes 4 and 5, 3.2; lanes 6 and 7, 3.3; lanes 8 and 9, 3.4; lanes 10 and 11, 3.5.
Figure 3.9 Ethidium bromide stained agarose gels with 100 µM pUC18 plasmid and 20 µM complex (5 mM Tris, pH = 7.5, 50 mM NaCl, $\lambda_{irr} \geq 385$ nm, 12 min). Photocleavage by (a) 3.1, (b) 3.2, (c) 3.3, (d) 3.4, all with; lane 1, plasmid only, dark, lane 2, irr. in air; lane 3, irr. in D$_2$O; lane 4, irr. with 2 mM NaN$_3$; lane 5, irr. after six freeze-pump-thaw cycles; lane 6, irr. with 2 units SOD.
Figure 3.10 Ethidium bromide stained agarose gel with 100 µM pUC18 plasmid and 20 µM complex (5 mM Tris, pH = 7.5, 50 mM NaCl, λ_{irr} ≥ 385 nm, 12 min). Photocleavage by 3.5, lane 1, plasmid only, dark, lane 2, irr. in air; lane 3, irr. in D_2O; lane 4, irr. with 2 mM NaN_3; lane 5, irr. after six freeze-pump-thaw cycles; lane 6, irr. with 2 units SOD.
Table 3.2 DNA Photocleavage, LC\textsubscript{50} Values Irradiated and in the Dark, and Partition Coefficients for 3.1 – 3.5.

<table>
<thead>
<tr>
<th>Complex</th>
<th>% Form II\textsuperscript{a}</th>
<th>LC\textsubscript{50} / µM\textsuperscript{a}</th>
<th>Dark</th>
<th>Irradiated\textsuperscript{b}</th>
<th>Increase</th>
<th>Log(P)\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>64</td>
<td>200 ± 20</td>
<td>30 ± 5</td>
<td>6.7</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>68</td>
<td>178 ± 17</td>
<td>22 ± 4</td>
<td>8.1</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>3.3</td>
<td>66</td>
<td>51 ± 5</td>
<td>9 ± 3</td>
<td>5.7</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>66</td>
<td>355 ± 18</td>
<td>17 ± 3</td>
<td>21</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>43</td>
<td>384 ± 24</td>
<td>16 ± 4</td>
<td>24</td>
<td>1.02</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}See text. \textsuperscript{b}\lambda_{irr} = 400 – 700 nm, 30 min. \textsuperscript{c}From ref. 5, ± 0.03.

3.2.3 Toxicity and Phototoxicity

The toxicity of 3.1 – 3.5 toward two cancer cell lines, HeLa and COLO-316, was previously investigated in the dark.\textsuperscript{5} The data revealed that 3.1 – 3.4 induce apoptosis, whereas 3.5 does not. It is believed that, owing to its hydrophobicity, 3.5 does not transverse the cellular membrane as well as the other complexes. Furthermore, the toxicity of complexes 3.1 and 3.5 in the dark toward Hs-27 human skin fibroblasts was previously examined in relation to the partition coefficient of each complex,\textsuperscript{26} also known to be related to the ability of molecules to transverse cellular membranes.\textsuperscript{5} A similar result is observed with the Hs-27 cell line presented here, where complexes 3.4 and 3.5 exhibit significantly lower toxicity in the dark, with LC\textsubscript{50} (LC\textsubscript{50} = concentration required
to kill 50% of the cells) values of 355 and 384 µM, respectively, compared to 51 – 200 µM for 3.1 – 3.3 (Table 3.2). For the Hs-27 cell line, it appears that complexes 3.4 and 3.5 do not transverse the cellular membrane as well as 3.1 – 3.3. This trend is supported by the partition coefficients (P) previously reported, also listed in Table 3.2 as log(P), which show that 3.4 and 3.5 are significantly more hydrophobic than 3.1 – 3.3. Although the log(P) value for 3.4 is closer to those of 3.1 – 3.3 than that of 3.5, it has been previously noted that a linear progression of permeability with ring additions is not followed in this series of complexes possibly due to the π-stacking of the diimine ligands in solution.5

In addition to the ability to enter cells, photocytotoxicity also depends on the reactivity of each complex when excited with light. The LC50 values for 3.1 – 3.5 irradiated for 30 min with visible light show that the most and least phototoxic compounds are 3.3 and 3.1, respectively, and 3.2, 3.4, and 3.5 exhibit similar toxicities. It is possible that the more hydrophobic complexes 3.4 and 3.5 are bound within the cellular membrane, and 1O2 sensitization initiates reactivity toward phospholipids, ultimately resulting in cell death.27 In contrast, complexes 3.1 – 3.3 are able to penetrate the cellular membrane and can, therefore, damage intracellular components through the formation of reactive oxygen species. Work is currently underway to elucidate the differences in mechanism for these systems.

The values of the increase in toxicity toward Hs-27 of 3.1 – 3.5 upon irradiation are listed in Table 3.2, ranging from 5.7- to 8.1-fold for 3.1 – 3.3. These values are similar to the 5.5-fold increase previously measured by us for hematoporphyrin, a key
component of the PDT drug Photofrin®, under similar experimental conditions, with LC$_{50}^{\text{dark}}$ = 22 ± 1 µM and LC$_{50}^{\text{irr}}$ = 3.8 ± 0.2 µM. In contrast, the increase in toxicity of 3.4 and 3.5 when irradiated was measured to be 21 and 24, respectively, making them potential candidates for PDT. It should be noted that this greater increase in 3.4 and 3.5 compared to hematoporphyrin reflects the lower toxicity of the complexes in the dark, a desirable property for a PDT agent.

### 3.3 Conclusions

The photophysical properties of the series of complexes $\text{cis-}[\text{Rh}_2(\mu-O_2\text{CCH}_3)_2(dppn)(L)]^{2+}$ ($L = \text{bpy (3.1), phen (3.2), dpq (3.3), dppz (3.4), and dppn (3.5)}$) were investigated, and it was found that the lowest energy excited state in 3.1 – 3.5 is dppn-localized $^3\pi\pi^*$ with lifetimes of 2.4 – 4.1 µs in DMSO. Complexes 3.1 – 3.4 photocleave DNA efficiently via a mechanism mediated by reactive oxygen species, whereas the lower reactivity of 3.5 toward DNA is independent of the presence of oxygen. It should be noted, however, that all complexes also exhibit a photocleavage pathway that is independent of oxygen. Therefore, a direct comparison of the DNA photocleavage efficiency and quantum yield of photosensitized $^1\text{O}_2$ production is not possible. The toxicity of 3.1 – 3.5 toward Hs-27 human skin fibroblasts is significantly enhanced upon irradiation with visible light as compared to those measured in the dark, with a 21- and 24-fold increase in toxicity for 3.4 and 3.5, respectively. This increase is significantly greater than that measured under similar experimental conditions for hematoporphyrin (5.5-fold). The dramatic increase in toxicity upon irradiation of 3.4 and
3.5 makes them potentially useful as PDT agents. Experiments designed to gain further understanding of the mechanism of photoinduced cell death in the presence of these complexes are currently underway.
3.4 References


CHAPTER 4

INTERACTIONS OF RUTHENIUM(II) AND OSMIUM(II) POLYPYRIDYL COMPLEXES WITH DNA

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4.1 Background

Unlike the rhodium complexes discussed in the previous chapter, ruthenium and osmium complexes have significantly lower documented anticancer activity.\(^1\) The photophysics of ruthenium and osmium, however, have been thoroughly examined and complexes with polypyridine ligands, including derivatives of Ru(bpy)\(_3\)\(^{2+}\) (4.1; bpy = 2,2’-bipyridine), are known to possess versatile excited-state properties (Figure 4.1).\(^2\text{-}^7\)

The nature of the ligands in ruthenium and osmium polypyridyl complexes allow for intense ligand centered (LC) transitions in the UV region.\(^1\text{-}^8\) Metal-to-ligand charge transfer (MLCT) transitions are also present in these complexes, with absorption features that extend well into the visible region.\(^8\) The reactivity of these complexes, however,
often stems from the low energy excited states, particularly the \( ^3\)MLCT and triplet ligand field (\(^3\)LF), also referred to as \(^3\)dd-transitions.\(^2\) The \(^3\)MLCT state is typically emissive and when its lifetime is sufficiently long, bimolecular reactivity is commonly observed, including energy transfer and electron transfer.\(^2\) The \(^3\)LF state, however, quickly undergoes non-radiative deactivation and, in some cases is also linked to ligand dissociation, depending on its energy and its thermal accessibility from other states.\(^9\) Figure 4.2 schematically compares the reactive pathways in ruthenium and osmium d\(^6\) complexes. The potential energy surface of the \(^3\)LF excited state is significantly displaced along the nuclear coordinates with respect to that of the \(^1\)GS resulting in some vibrational overlap with the ground state,\(^2\) such that lowering the energy of the \(^3\)LF state results in greater rates of non-radiative (nr) decay (Figure 4.2). The \(^3\)LF state can lie above or below the \(^3\)MLCT and \(^3\)LC states, also depicted in Figure 4.2. In the case of osmium complexes the larger ligand field splitting, \(\Delta_o\), causes a higher energy \(^3\)LF state, such that it is thermally inaccessible from the \(^3\)MLCT state.\(^10-11\) Because of the relatively smaller \(\Delta_o\) in ruthenium complexes, the \(^3\)LF state can play a prominent role in excited state dynamics. When the \(^3\)LF state is close in energy to the \(^3\)MLCT state, the lifetimes are very short and emission is typically very weak or not observed.\(^2\) As the \(^3\)MLCT – \(^3\)LF energy gap is increased there is competition between phosphorescence (ph), non-radiative decay from the \(^3\)MLCT, and thermal population of the \(^3\)LF state. The ligation sphere around the Ru(II) metal center can have a profound effect on the energies of the low-lying excited states, \(^3\)LF, \(^3\)MLCT, and \(^3\)\(\pi\pi^*\), thereby also affecting their photophysical properties and photochemistry.
Figure 4.1 Molecular structure of the ruthenium and osmium complexes showing numbering scheme and ligand structure.
When the ligation sphere around the metal center is tuned appropriately, the low-lying reactive excited states can be exploited to yield properties that may be useful for a number of biological applications.\textsuperscript{4-7,12} Specifically, complexes with planar aromatic ligands have been investigated extensively as probes of double-stranded DNA (ds-DNA).\textsuperscript{13} Ligand variation about the metal center can be used to attain different ds-DNA binding modes and preference for particular binding sites or base sequence, with concomitant changes in the photophysical properties of the complex.\textsuperscript{4-7,12}
To illustrate this point, the luminescence of Ru(bpy)$_2$(dppz)$_{2+}$ (4.2, dppz = dipyrido[3,2-a:2´,3´-c]phenazine), a well known derivative of 4.1 can be examined as a function of environment, including DNA. The emission maximum and intensity, as well as lifetime, of 4.1 is relatively invariant to solvent, whereas 4.2 is highly emissive in organic solvents but exhibits negligible luminescence in water.\textsuperscript{12d} In the presence of ds-DNA, however, 4.2 exhibits greatly enhanced emission, a photophysical characteristic known as the “DNA light-switch”. The $^3$MLCT excited states of 4.2 are localized on $\pi^*$ orbitals of the dppz ligand, that are located proximal or distal to the metal, $^3$MLCT$^{\text{prox}}$ (bpy) and $^3$MLCT$^{\text{dis}}$ (phenazine), respectively. Temperature dependence studies on the luminescence of 4.2 have shown that the lowest energy excited state is the non-emissive $^3$MLCT$^{\text{dis}}$ state, whereas the luminescent $^3$MLCT$^{\text{prox}}$ state lies at a higher energy.\textsuperscript{14-15} The coupled nature between these two $^3$MLCT excited states and the thermal accessibility of the bright $^3$MLCT$^{\text{prox}}$ in various solvent environments has produced a more clear description of the light switch effect. In addition, other ruthenium complexes with dppz derivatives have been extensively studied to explain further the mechanism and to discover new DNA probes.\textsuperscript{12b,16}

In addition to the numerous photophysical studies of ruthenium(II) complexes as DNA probes, there are also reports devoted to the potential application of 4.2 and related complexes for PDT.\textsuperscript{17-18} The relatively short lifetime of 4.2, however, results in a low quantum yield of sensitized $^1$O$_2$, making it less useful as a potential PDT agent. In efforts aimed at improving PDT agents there have been studies centered on achieving higher quantum yields of $^1$O$_2$ production relative to Photofrin\textsuperscript{©},\textsuperscript{19-20} as well as tuning the energy
of the $^3$MLCT excited state to attain photoinduced redox chemistry.\textsuperscript{21} The extension of the absorption of the complexes into the phototherapeutic window (650 – 850 nm) is also a desirable goal, as this is the wavelength range where light penetration through tissue is deepest.\textsuperscript{8}

The work presented here focuses on optimizing the binding of PDT complexes to DNA, increasing the yield of cytotoxic reactive oxygen species (ROS), achieving dual reactivity, and extending the wavelength of irradiation into the phototherapeutic window. The molecular structures of Ru(bpy)$_2$(dpqp)$^{2+}$ (4.3, dpqp = pyrazino[2´,3´:5,6]pyrazino[2,3-f][1,10]phenanthroline), Ru(bpy)$_2$(dppn)$^{2+}$ (4.4), and Os(bpy)$_2$(dppn)$^{2+}$ (4.5) are shown in Figure 4.1, which possess extended polyaromatic ligands capable of achieving high DNA binding constants through intercalation. These complexes were investigated to evaluate their potential as PDT agents with improved properties.

4.2 Results

4.2.1 Photophysical Properties

The electronic absorption maxima, molar extinction coefficients, and emission properties of 4.1 – 4.5 are listed in Table 4.1. The maximum at 287 nm in 4.1 is a well known $\pi\pi^*$ transition of the bpy ligands.\textsuperscript{2} Likewise, 4.3 – 4.5 exhibit strong absorption at $\lambda < 300$ nm attributed to bpy ($\pi\pi^*$). The maximum at 365 nm in 4.3 in water is not present in 4.1, but is present at 360 nm in the free ligand and is similar to that observed for the dppz $^1\pi\pi^*$ transitions at 359 and 370 nm in 4.2, therefore this peak is assigned as a $^1\pi\pi^*$ transition of the dpqp ligand in 4.3. Likewise, 4.4 and 4.5 exhibit dppn based $^1\pi\pi^*$
transitions that are red-shifted relative to those of dppz owing to its extended conjugation. These dppn-centered peaks are observed at 390 and 414 nm in 4.4 and at 390 and 412 nm in 4.5 in water.\footnote{2,22} The assignment of these transitions is supported by the $^1\pi\pi^*$ transitions of the free dppn ligand with maxima at 390 and 414 nm in CHCl$_3$. The low energy maximum at 457 nm and shoulder at 430 nm in 4.3 are similar to that at 450 nm in 4.1 and are therefore assigned to the $^1$MLCT absorption. Although a $^1$MLCT transition is also present at 444 nm in 4.4, it overlaps with the LC dppn transitions. Given the similar ligand based orbitals on 4.4 and 4.5, the higher energy $t_{2g}$-type orbitals on the Os(II) metal center relative to Ru(II) result in the red-shift of the $^1$MLCT transition in 4.5 to 470 nm.\footnote{10-11,23} Additionally, a further broad low-energy absorption centered at 650 nm in 4.5 is assigned as arising from $^3$MLCT transitions, typically forbidden but known to appear with the large spin-orbit coupling associated with the heavier Os(II).\footnote{23–25}

Table 4.1 Photophysical Properties and Sensitized Singlet Oxygen Quantum Yield for 4.1 – 4.5.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\lambda_{\text{abs}}$ / nm (ε $\times 10^{-3}$/ M$^{-1}$ cm$^{-1}$)$^a$</th>
<th>$\lambda_{\text{em}}$ / nm ($\Phi_{\text{em}}$)$^b$</th>
<th>$\Phi_{1^{1}\text{O}_2}$)$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>287 (62.9), 450 (13.0)</td>
<td>619 (0.062)</td>
<td>0.81$^e$</td>
</tr>
<tr>
<td>4.2</td>
<td>359 (17.5), 370 (17.2), 445 (16.3)</td>
<td>629 (0.083)</td>
<td>0.16</td>
</tr>
<tr>
<td>4.3</td>
<td>365 (16.4), 430 (10.4), 457 (12.3)</td>
<td>617 (0.039)$^c$</td>
<td>0.76</td>
</tr>
<tr>
<td>4.4</td>
<td>390 (16.4), 414 (18.5), 444 (13.5)</td>
<td>617 (0.003)</td>
<td>0.88</td>
</tr>
<tr>
<td>4.5</td>
<td>390 (16.4), 412 (18.5), 470 (17.4), 650 (3.3)</td>
<td>–</td>
<td>0.43</td>
</tr>
</tbody>
</table>

$^a$In water. $^b$In CH$_3$CN, at 298 K. $^c$In H$_2$O, at 298 K. $^d$In CH$_3$OH. $^e$From ref. 26
Complexes 4.3 and 4.4 emit with a maximum at 617 nm with quantum yields of $\Phi = 0.039$ in water and $\Phi = 0.003$ in CH$_3$CN, respectively. The luminescence in these complexes is assigned as arising from the $^3$MLCT state based on the spacing of vibronic features observed in each complex at 77 K, which are typical of Ru(II) polypyridyl complexes, and similar to that of 4.1. For 4.3, this assignment is further supported by the well matched lifetimes of the steady-state emission to those observed for characteristic bpy radical anion transient absorption at 370 nm and bleach at 450 nm consistent with the $^3$MLCT state of these and related complexes, including 4.1. In contrast, the transient absorption spectra for 4.4 and 4.5 show strong absorption features at 540 nm in CH$_3$CN with $\tau = 33 \pm 5$ µs and $\tau \sim 79$ ns, respectively. The maximum of these transients is similar to that of the free dppn ligand, with $\tau = 18 \pm 2$ µs, and thus these features can be characterized as dppn – $^3\pi\pi^*$ in character.

The quantum yields of sensitized $^1$O$_2$ were measured for 4.2 – 4.5 in CH$_3$OH (Table 4.1), using 1,3-diphenyl-isobenzofuran as a trapping agent and 4.1 as the standard ($\Phi_{^1{O}_2} = 0.81$). Consistent with the longer lifetime of the low-energy emissive $^3$MLCT state of 4.3 ($\tau = 582$ ns, in H$_2$O) as compared to the dominant non-radiative decay observed in 4.2, the $\Phi_{^1{O}_2}$ is significantly greater in the former. Likewise, the lifetime associated with the $^3\pi\pi^*$ low-energy excited state allows for production of $^1$O$_2$ with $\Phi_{^1{O}_2} = 0.88$. Although the lifetime of the $^3\pi\pi^*$ state of 4.5 is relatively short, the low energy of this state is close to that of $^1$O$_2$, resulting in $\Phi_{^1{O}_2} = 0.43$.  

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4.2.2 DNA Binding and Photocleavage

In contrast to photophysical similarities that exist between 4.1 and 4.3 – 4.5, the complexes exhibit distinctly different interactions and reactivity with DNA. As mentioned previously, intercalation is possible with the presence of an extended, planar ligand, and both dpqp and dppn fulfill these requirements. The changes of the electronic absorption spectrum of 4.3 as a function of calf-thymus DNA concentration were used to estimate the DNA binding constant, $K_b$. Hypochromic and bathochromic shifts were observed for 10.4 $\mu\text{M}$ 4.3 in the presence of up to 71.5 $\mu\text{M}$ ct-DNA, resulting in $K_b = 2.0 \times 10^6$ M$^{-1}$ ($s = 1.62$). Similarly for 4.4, when dppn ligands are in the coordination sphere of Ru(II) complexes, binding constants are known to be in the range of $10^6$ M$^{-1}$.22 Binding constants of this magnitude are typically associated with intercalation as opposed to electrostatic binding, as is the case for 4.1, with $K_b = 700$ M$^{-1}$.29 In addition, the DNA binding constant reported for 4.2, a known intercalator, is also in the range $10^6 – 10^7$ M$^{-1}$.12 As mentioned previously, monitoring changes in the relative viscosity of DNA solutions with increasing concentration of complex is a reliable method to assign an intercalative binding mode. Complex 4.1, known to bind weakly to DNA, shows no change in viscosity, whereas 4.4 and 4.5 both exhibit a pronounced increase in relative DNA viscosity. Therefore, 4.4 and 4.5 intercalate between the DNA bases.

Ethidium bromide stained agarose gels comparing the photocleavage of 4.1 – 4.4 are shown in Figure 4.3. In both gels, lane 1 shows the migration of pUC18 alone, kept in the dark as a control, which is composed mostly of unreacted supercoiled plasmid (form I). Without irradiation, no apparent DNA cleavage is observed in lanes 2, 4, and 6 for
complexes 4.1, 4.2, and 4.3, respectively (Figure 4.3a). Lane 7 shows the DNA photocleavage of 4.3, indicating greater reactivity when compared to 4.2 under similar experimental conditions (Figure 4.3a). Likewise, in Figure 4.3b no apparent DNA cleavage is observed in lanes 2, 4, and 6 for complexes 4.1, 4.2, and 4.4, respectively, when kept in the dark. Lane 7 shows the DNA photocleavage of 4.4, where again greater reactivity is seen relative to that of 4.2 under similar experimental conditions. It is noteworthy that 4.4 shows a comparatively enhanced degree of photocleavage and under a shorter irradiation time than 4.3. The explanation for this disparity will be elaborated upon in the discussion. In both cases, the photocleavage is consistent with the greater quantum yield of sensitized \(^1\)O\(_2\) production of 4.3 and 4.4 (\(\Phi_{1O_2} = 0.76\) and \(\Phi_{1O_2} = 0.88\), respectively) relative to that of 4.2 (\(\Phi_{1O_2} = 0.16\)) measured in CH\(_3\)OH.

Figure 4.4 presents a comparison of the DNA photocleavage between 4.1, 4.4, and 4.5. Given the common dppn ligand present in 4.4 and 4.5 their direct comparison is relevant to emphasize the observed differences in DNA photocleavage. Again, it can be seen that the pUC18 control in lane 1 remains intact in the dark, showing only trace circular nicked impurity (form II). Lanes 2, 4 and 6 for complexes 4.1, 4.4, and 4.5, respectively, kept in the dark show no photocleavage. A comparison of the lanes containing similarly irradiated samples (3, 5, and 7), show that the only photocleavage arises from 4.5 in lane 7 (\(\lambda_{irr} \geq 645\) nm, \(t_{irr} = 1.5\) h). In 4.1 and 4.4, there is negligible absorption at 645 nm whereas in 4.5 the \(^3\)MLCT absorption maximum is at 650 nm. For this reason 4.5 is the only complex capable of sensitizing \(^1\)O\(_2\) under these conditions.
Figure 4.3 Ethidium bromide stained agarose gels of the photocleavage of 100 µM pUC18 plasmid (5 mM Tris, pH = 7.5, 50 mM NaCl) and 20 µM of the chloride salt of each complex in air: lane 1, plasmid only; lanes 2 – 3, 4.1; lanes 4 – 5, 4.2; lanes 6 – 7, (a) 4.3 and (b) 4.4; lanes 1, 2, 4, and 6: dark; lanes 3, 5, and 7: irradiated ($\lambda_{irr} \geq 455$ nm, $t_{irr} = (a) 15$ min and (b) 30 s).
The oxygen dependence of the DNA photocleavage by 4.3 – 4.5 was further investigated and the results are shown in Figures 4.5 – 4.7. In Figure 4.5, lanes 3 and 4 show greater DNA photocleavage by 4.3 in D₂O compared to H₂O, in agreement with the 3-fold longer lifetime of $^1\text{O}_2$ in the former. Additionally, negligible DNA cleavage by 4.3 was observed under deoxygenated conditions (lane 5). These findings suggest that the DNA photocleavage of 4.3 is primarily mediated by $^1\text{O}_2$. Similarly, Figure 4.6 displays the DNA photocleavage of 4.4 in D₂O (lane 3), which shows only a slight enhancement compared to that in H₂O (lane 2). It is also apparent in Figure 4.6 that the deaerated...
sample exhibits only a small amount of DNA photocleavage (lane 4), and that the presence of NaN₃ (lane 5), a $^1$O₂ and •OH scavenger, and SOD (lane 6) have a small effect on the photocleavage of 4.4. The data presented in Figure 4.6 exclude the sole participation of $^1$O₂ and other ROS in the DNA cleavage mechanism. In addition, the quantum yields for $^1$O₂ generation of 4.1 and 4.4 are similar (Table 4.1), however, no photocleavage by 4.1 is observed in 30 s (Figure 4.6). This observation can be explained, in part, by the low DNA binding constant of 4.1. Again, lanes 3 and 4 in Figure 4.7a, and lanes 2 and 3 in Figure 4.7b exhibit greater DNA cleavage in D₂O compared to H₂O, consistent with the involvement of $^1$O₂ in the mechanism. Similar to 4.3, no DNA photocleavage was obtained under deoxygenated conditions (lane 5, Figure 4.7a). In addition, the presence of SOD (lane 6, Figure 4.7a) does not affect the DNA photocleavage of 4.5 compared to that in H₂O (lane 3, Figure 4.7a). In the presence of NaN₃, the DNA photocleavage of 4.5 is reduced (lane 4, Figure 4.7b). Overall, these results are consistent with DNA damage by 4.5 under irradiation mediated by sensitized $^1$O₂.
Figure 4.5 Ethidium bromide stained agarose gel of the photocleavage of 100 μM pUC18 plasmid by 20 μM [4.3]Cl₂ (lanes 3 – 5, λirr ≥ 395 nm, 10 min) in 5 mM Tris, pH = 7.4, 50 mM NaCl: lane 1, plasmid only, dark; lane 2, dark; lane 3, irradiated in H₂O buffer in air; lane 4, irradiated in D₂O buffer in air; lane 5, irradiated in H₂O buffer after six freeze-pump-thaw cycles.
Figure 4.6 Ethidium bromide stained agarose gel of the photocleavage of 100 μM pUC18 plasmid by 20 μM [4.4]Cl₂ (lanes 2 – 6, λ_{irr} ≥ 475 nm, 50 sec) in 5 mM Tris, pH = 7.4, 50 mM NaCl: lane 1, plasmid only, dark; lane 2, irradiated in H₂O buffer in air; lane 3, irradiated in D₂O buffer in air; lane 4, irradiated in H₂O buffer after six freeze-pump-thaw cycles; lane 5, irradiated in H₂O buffer with 2 mM NaN₃; lane 6, irradiated in H₂O buffer with 2 units SOD.
Figure 4.7 Ethidium bromide stained agarose gel of the photocleavage of 100 μM pUC18 plasmid by 20 μM [4.5]Cl₂ in 5 mM Tris, pH = 7.4, 50 mM NaCl at (a) t_{irr} = 60 min, λ_{irr} ≥ 495 nm; lane 1, plasmid only, dark; lane 2, dark; lane 3, in air; lane 4, in D₂O; lane 5, six freeze-pump-thaw cycles; lane 6, 2 units superoxide dismutase. (b) t_{irr} = 60 min, λ_{irr} ≥ 455 nm; lane 1, plasmid only, dark; lane 2, in air; lane 3, in D₂O; and lane 4, 50 mM NaN₃.
Because of the unusually efficient DNA photocleavage by \textit{4.4} in 30 seconds of irradiation ($\lambda_{\text{irr}} \geq 450$ nm), the wavelength dependence of photocleavage of \textit{4.4} was assessed. Shown in Figure 4.8, the photocleavage of 100 $\mu$M plasmid with 20 $\mu$M \textit{4.4} under various wavelengths of irradiation ranging from $\lambda \geq 475$ nm to $\lambda \geq 590$ nm indicate that \textit{4.4} is capable of inducing significant DNA cleavage up to $\lambda_{\text{irr}} \geq 550$ nm.

![Ethidium bromide stained agarose gel of the photocleavage of 100 $\mu$M plasmid by 20 $\mu$M \textit{4.4}Cl\textsubscript{2} in air ($t_{\text{irr}} = 5$ min, 5 mM Tris, pH = 7.5, 50 mM NaCl) at various irradiation wavelengths: lane 1, dark; lane 2, $\lambda \geq 475$ nm; lane 3, $\lambda \geq 495$ nm; lane 4, $\lambda \geq 515$ nm; lane 5, $\lambda \geq 530$ nm; lane 6, $\lambda \geq 550$ nm; lane 7, $\lambda \geq 570$ nm; lane 8, $\lambda \geq 590$ nm.](image)

**Figure 4.8** Ethidium bromide stained agarose gel of the photocleavage of 100 $\mu$M pUC18 plasmid by 20 $\mu$M \textit{4.4}Cl\textsubscript{2} in air ($t_{\text{irr}} = 5$ min, 5 mM Tris, pH = 7.5, 50 mM NaCl) at various irradiation wavelengths: lane 1, dark; lane 2, $\lambda \geq 475$ nm; lane 3, $\lambda \geq 495$ nm; lane 4, $\lambda \geq 515$ nm; lane 5, $\lambda \geq 530$ nm; lane 6, $\lambda \geq 550$ nm; lane 7, $\lambda \geq 570$ nm; lane 8, $\lambda \geq 590$ nm.
4.3 Discussion

4.3.1 Ru(bpy)$_2$(dpqp)$_2^{2+}$

Synthesized as a photophysically interesting analog of 4.2, 4.3 was found to be highly luminescent in H$_2$O even in the absence of DNA. Theoretical calculations of the ordering of the LUMOs of 4.1 – 4.3, validated with electrochemical measurements, show the presence of a low-lying $^3$MLCT$^{dis}$ state centered on the distal portion of the dpqp ligand as the lowest energy excited state of the complex. A $^3$MLCT$^{prox}$ state centered on an ancillary bpy ligand or the proximal, bpy-like portion of the dpqp ligand is also calculated for 4.3, similar to that described above for 4.2. The strong aqueous luminescence with a relatively long lifetime (582 ns) make possible the efficient energy transfer to $^3$O$_2$.

Although 4.1 and 4.3 both exhibit similarly high quantum yields for the production of sensitized $^1$O$_2$, negligible DNA photocleavage is observed under either set of conditions (Figure 4.3, lane 3 in each panel) by the former. The difference in DNA photocleavage between 4.1 and 4.3 can be explained by the weak electrostatic DNA binding of the former and strong intercalative binding of the latter.

4.3.2 Ru(bpy)$_2$(dppn)$_2^{2+}$

The photophysical properties of 4.4 show that although it is only weakly emissive, ($\Phi_{em} = 0.003$) from the $^3$MLCT excited state, the lowest energy excited state is the dppn-centered long-lived $^3\pi\pi^*$. The energy of the $^3$MLCT and the dppn $^3\pi\pi^*$ excited states
result in an arrangement suitable for dual reactivity of 4.4 with regard to DNA damage. Complex 4.4 exhibits the most efficient photocleavage in the series, attained within 30 sec of irradiation. Although this efficiency was attributed in part to the 0.88 quantum yield of $^1$O$_2$ production, similar Ru complexes containing the related pydppn (3-(pyrid-2′-yl)-4,5,9,16-tetraaza-dibenzo[a,c]naphthacene) ligand have near unity $\Phi_{1}O_2$ and strong DNA intercalation yet they do not show DNA photocleavage within the same irradiation time.$^{20}$

A possible explanation for the very efficient DNA photocleavage of 4.3 is the additional involvement of the $^3$MLCT excited state. Using the first reduction potential ($E_{1/2}([\text{Ru}]^{2++}) = -0.46$ V vs. NHE) and the $E_{00} \sim 2.1$ eV, an excited state reduction potential, $E_{\text{red}}^*$, of $\sim 1.64$ V vs. NHE can be estimated for 4.4, making it a strong oxidizing agent. With +1.29 V vs. NHE as the oxidation potential of guanine in water at pH = 7,$^{31}$ a favorable driving force of -0.35 V is calculated for the generation of G$^+$ from the $^3$MLCT state of 4.4. Guanine oxidation by the excited states of Ru(II) complexes has been previously shown to result in DNA cleavage and adduct formation, but these cases do not rival the high reactivity of 4.4.$^{5a,32}$ For example, although DNA photocleavage from guanine oxidation was reported for [Ru(hat)$_3$]$^{2+}$ (hat = 1,4,5,8,9,12-hexaazatriphenylene), a complex with similar excited state reduction potential as that of 4.4, 80% DNA damage required 3 min irradiation at 436 nm.$^{32b}$ Stern–Volmer plots of the changes in the emission lifetime (670 ns) of 4.4 as a function of GMP concentration in 50 mM deaerated Tris buffer (pH=7.5) resulted in $k_q = 2.1 \times 10^8$ M$^{-1}$s$^{-1}$. As expected,
no quenching of the lifetime of 4.1 by GMP is observed, since with $E_{\text{red}}^* = 0.93$ V vs. NHE guanine oxidation is thermodynamically unfavorable.

4.3.3 Os(bpy)$_2$(dppn)$^{2+}$

The variation in properties between 4.4 and 4.5 can be explained by the fundamental differences between the Ru(II) and Os(II) metal centers of each complex, respectively. It is clear from the presence of the very low-energy $^3\text{MLCT}$ absorption ($\lambda \sim 650$ nm) in 4.5 that the larger spin-orbit coupling of the heavier Os(II) relaxes the rules for the usually spin-forbidden triplet to singlet transition which also results in the faster decay of the $^3\pi\pi^*$ excited state relative to that of 4.4. The measured lifetimes of the $^3\pi\pi^*$ excited states for 4.4 and 4.5 are 33 $\mu$s and $\sim79$ ns, respectively. Likewise, because of the low-energy absorption, extending into the PDT window, 4.5 can produce $^1\text{O}_2$ with longer wavelength irradiation than those accessible to 4.1 – 4.4. However, longer irradiation times are required due to the shorter lifetime of the excited state, which results in a significantly smaller $\Phi_{^1\text{O}_2}$ (0.43). It is also clear from the results of the oxygen dependence photocleavage gels that the mechanism of DNA cleavage can be attributed to $^1\text{O}_2$, with no contribution from guanine oxidation, as is the case for 4.4. The lower energy of the $^3\text{MLCT}$, ($E_{00} = 1.39$ eV) of 4.5 excludes an excited state reduction potential positive enough for the favorable oxidation of guanine.
4.4 Conclusions

The DNA interactions of the related complexes 4.3 – 4.5 were investigated and it was found that all three photocleave DNA efficiently. Significant drawbacks to current PDT treatments are addressed by each complex. The unique luminescence in water and long-lived $^3\text{MLCT}$ excited-state lifetime of 4.3 as compared to 4.2 allow for energy transfer to $^3\text{O}_2$. In this instance 4.3 shows improvement over Photofrin® with its greatly increased production of the cytotoxic species, $^1\text{O}_2$. Likewise, a greater $\Phi_1\text{O}_2$ is generated upon photoexcitation by 4.4. The energy of the $^3\text{MLCT}$ excited state is well suited to not only transfer energy to $^3\text{O}_2$, but also to oxidize directly guanine bases giving this complex a highly efficient, dual photocleavage mechanism. In addition, it was shown that 4.4 exhibits efficient photocleavage at wavelengths up to 550 nm. In contrast to 4.3 and 4.4, 4.5 significantly improves upon a different quality required for effective PDT, the need for reactivity in the phototherapeutic window of irradiation. The switch from ruthenium in 4.4 to osmium in 4.5 shifts the energy of the $^3\text{MLCT}$ absorption to low enough energies to allow for 86% DNA photocleavage well into the phototherapeutic window, with $\lambda_{\text{irr}} \geq 645$ nm.

Complexes 4.3 – 4.5 produce an increase in the relative viscosity of DNA solutions with increasing complex concentrations. The results of these experiments illustrate that, in addition to the photophysical properties that enhance the PDT potential of this series, the complexes bind strongly to DNA through intercalation of their respective extended planar aromatic ligand. The strong binding associated with
intercalating species provides this series with improved photocleavage relative to the control complex 4.1 due to the close proximity of the photogenerated ROS with DNA.
4.5 References


29. Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K.


CHAPTER 5

PHOTOREACTIVITY AND THEORETICAL CALCULATIONS OF \textit{cis}-\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{C}_6\text{H}_5\text{CN})_4^{2+} \text{ AND } \textit{cis}-\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(4\text{F-C}_6\text{H}_5\text{CN})_4^{2+}

5.1 Background

Since the highly cytotoxic nature of cisplatin (Figure 5.1) was first established, the complex has been the standard in research related to metal complex cancer therapies.\textsuperscript{1-3} As discussed in Chapter 1, cisplatin binds to DNA in both healthy and malignant cells after the thermal exchange of two chloride ligands for water molecules. There has been extensive investigation into improved cisplatin derivatives that address the issues of toxic side effects and acquired resistance of the parent complex.\textsuperscript{4-6} For instance, the carboplatin was designed with less labile chelating carboxylate ligands in place of chloride ions to reduce its reactivity, and therefore its toxicity, relative to cisplatin.\textsuperscript{7-8} Carboplatin is currently used clinically and its structure is shown in Figure 5.1.

More recently, however, research on light-activated cisplatin analogs has emerged because of the improvement in selectivity of photoactivated systems.\textsuperscript{6,9-12} Many mononuclear transition metal complexes have been developed with the goal of utilizing photolabile ligands to produce a reactive species that can directly interact with DNA or other biomolecules.\textsuperscript{6,9-12} For instance octahedral Pt(IV) complexes that undergo
photoinitiated loss of two ligands to yield active square planar Pt(II) products have been
developed,\textsuperscript{6,13} as well as a number of Ru(II) and Os(II) arene complexes which operate by
photoinduced ligand dissociation.\textsuperscript{6,14} In addition, Ru(bpy)$_2$(NH$_3$)$_2$$^{2+}$ (bpy = 2,2'-
bipyridine) (Figure 5.1) has been shown to exchange the two NH$_3$ ligands for H$_2$O ($\lambda_{irr}$ = 400 nm), such that in the presence of plasmid DNA it is able to covalently bind the
duplex and inhibit its mobility in agarose gel, similar to that reported for cisplatin.\textsuperscript{12}

Along with the mononuclear metal photoactive complexes, it was recently shown
that $\textit{cis}$-[Rh$_2$(μ-O$_2$CCH$_3$)$_2$(CH$_3$CN)$_4$(H$_2$O)$_2$$^{2+}$] (5.1) (Figure 5.1) also has the ability to
bind covalently to DNA.\textsuperscript{11} It is known that the axial ligands of 5.1 (as detailed in Chapter
3) are labile in the dark, in coordinating solvent.\textsuperscript{15} The equatorial ligands, however, are
inert to substitution in the dark and were shown to be photo labile under visible light
irradiation, where $^1$H NMR spectroscopy showed that two equatorial ligands were
substituted by H$_2$O molecules.\textsuperscript{11}

To probe further the ability of metal-metal bonded complexes to behave as
photoactivated cisplatin analogs, derivatives of 5.1 where the acetonitrile groups were
replaced with para-substituted benzonitrile ligands were synthesized. It has been
previously shown that variation of the electron donating/withdrawing ability of the para
substituent in benzonitrile ligands imparts an electronic effect on nickel coordination
complexes.\textsuperscript{16} In order to investigate this effect on the photochemical properties of
dirhodium complexes, photophysical characterization and theoretical calculations were
undertaken on two new complexes, $\textit{cis}$-[Rh$_2$(μ-O$_2$CCH$_3$)$_2$(NCC$_6$H$_5$)$_4$(L)$_2$](BF$_4$)$_2$ (5.2) and
$\textit{cis}$-[Rh$_2$(μ-O$_2$CCH$_3$)$_2$(4F-NCC$_6$H$_5$)$_4$(L)$_2$](BF$_4$)$_2$ (5.3), where L = coordinating solvent
(Figure 5.1). The results are presented along with their DNA interactions and are compared to findings for 5.1.

\[ \text{Figure 5.1} \] Molecular structures of (a) cisplatin (b) carboplatin (c) cis-[Ru(bpy)\_2(NH\_3)\_2]^{2+} (d) cis-[Rh\_2(\mu-O\_2CCH\_3)\_2(CH\_3CN)\_4(H\_2O)\_2]^{2+} (5.1) and (e) cis-[Rh\_2(\mu-O\_2CCH\_3)\_2(N)\_4(L)\_2]^{2+} (5.2 and 5.3).

5.2 Results and Discussion

5.2.1 Photophysical Properties

The low-energy electronic absorption spectra of 5.2 and 5.3 in water and acetonitrile are shown in Figure 5.2, and their ligand structure, maxima and molar
absorptivity are listed in Table 5.1. Complexes 5.2 and 5.3 possess two very similar low-energy absorption peaks with maxima at 370 and 555 nm and 374 and 547 nm, respectively in water, which are typical of complexes of this type.\textsuperscript{17-18} For example, 5.1 exhibits a maximum at 373 nm assigned as Rh$_2$(π*)→RhL(σ*) and a second maximum at 555 nm assigned as a Rh$_2$(π*)→Rh$_2$(σ*) transition.\textsuperscript{11} The lower energy assignment of Rh$_2$(π*)→Rh$_2$(σ*) has also been made for the absorption maximum occurring between 550 – 600 nm for Rh$_2$(μ-O$_2$CCH$_3$)$_4$(H$_2$O)$_2$, and likewise the same assignments are made for the maxima in 5.2 and 5.3.\textsuperscript{19} The benzonitrile ligands alone have broad high energy ππ* transitions at 270 and 263 nm for benzonitrile and 4-fluorobenzonitrile in CH$_3$CN,\textsuperscript{20} respectively, which tail out to ~300 nm. These transitions are intense and are expected to be present at similar energies in 5.2 and 5.3, such that the Rh$_2$(π*)→RhL(σ*) peak at ~370 nm appears only as a shoulder (Figure 5.2).

**Table 5.1** Equatorial Ligand Structures and Electronic Absorption for 5.1 – 5.3.

<table>
<thead>
<tr>
<th>Complex</th>
<th>L$_\text{eq}$</th>
<th>λ$_\text{abs}$/ nm (ε/ M$^{-1}$cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1$^{a,c}$</td>
<td>N≡C–CH$_3$</td>
<td>363 (420), 555 (160)</td>
</tr>
<tr>
<td>5.2$^{a}$</td>
<td>N=C–</td>
<td>370 (1,019), 555 (204)</td>
</tr>
<tr>
<td>5.3$^{b}$</td>
<td>N=C–F</td>
<td>370 (491), 520 (134)</td>
</tr>
</tbody>
</table>

$^a$In water. $^b$In CH$_3$CN. $^c$From ref. 11.
A shift in the maximum of the lowest energy transition is observed with different coordinating solvent in the axial positions of 5.2 and 5.3, such as its blue shift in CH$_3$CN as compared to H$_2$O (Figure 5.2). This finding further supports the idea that this transition involves the Rh$_2$(σ*) orbitals which have significant overlap with the out-of-phase linear combinations of the σ-bonded axial ligands.$^{17-18,21}$ Similar to other bridged acetate dirhodium complexes such as 5.1$^{11}$ and the Rh$_2$ series in Chapter 3,$^{22}$ 5.2 and 5.3 are non-emissive at 298 K in water.

![Figure 5.2](image)

**Figure 5.2** Electronic absorption spectra of (a) 5.2 and (b) 5.3 in CH$_3$CN (——) and H$_2$O (—).

### 5.2.2 Photolysis and DNA Binding

The photolysis of 5.2 and 5.3 under various conditions was monitored to study the ability of the complexes to undergo photo induced ligand exchange. Figure 5.3 shows the
changes in electronic absorption spectra of 5.2 and 5.3 upon irradiation over 22 min in CH\textsubscript{3}CN (\(\lambda_{\text{irr}} \geq 455\) nm). The variation in efficiency of photosubstitution is apparent by the significantly faster spectral changes in 5.3 as compared to 5.2. For 5.2 very slight changes can be seen with a decrease of the maximum at 525 nm and a slight increase in absorption at ~412 nm. At long irradiation times all spectral features decrease before any new distinct maximum appears at 412 nm or other wavelengths. A similar growth of a new peak with maximum at 404 nm for 5.3 is observed.

To evaluate structurally the changes that result in the observed change in the electronic absorption spectra, photolysis in CD\textsubscript{3}CN was monitored by \(^1\)H NMR over a similar time window. The changes to the aromatic region of the \(^1\)H NMR spectra are shown for the photolysis of 5.2 and 5.3 in Figures 5.4 and 5.5, respectively (\(\lambda_{\text{irr}} \geq 455\) nm). Through the integration of the aromatic multiplets, the ratio of equatorial ligands and free benzonitrile can be determined. For 5.2, after 20 min ~1.5 of the equatorial benzonitrile ligands have been substituted. Only in the last \(^1\)H NMR spectrum plotted of 5.2 after 140 min irradiation is disappearance of all the bound benzonitrile ligands apparent. Complex 5.3 however, shows complete exchange of all four equatorial 4-fluorobenzonitrile ligands for CD\textsubscript{3}CN in 20 min. All equatorial ligands exhibit the same chemical shift for their aromatic protons regardless of neighboring L\textsubscript{eq} because the through-space distance among the ligands is large enough to negate a difference in their local environment. In addition, it is apparent that coordination of two different nitriles on the same metal has little effect on the position of the aromatic shifts of benzonitrile and 4-
fluorobenzonitrile. It should be noted that upon prolonged irradiation both complexes photodegrade.

**Figure 5.3** Changes to the electronic absorption spectra of (a) 0.42 mM 5.2 and (b) 0.75 mM 5.3 over 22 min of irradiation in CH$_3$CN ($\lambda_{\text{irr}} \geq 455$ nm).
Figure 5.4 Changes to $^1$H NMR spectrum of 0.25 mM 5.2 in CD$_3$CN over 140 min ($\lambda_{\text{irr}} \geq 455$ nm), where (a) and (b) are resonances of the bound and free benzonitrile, respectively. The changes to the integrations over (a)* and (b)* are shown on the right.
Figure 5.5 Changes to $^1$H NMR spectrum of 0.75 mM 5.3 in CD$_3$CN over 20 min of irradiation ($\lambda_{irr} \geq 455$ nm), where (a) and (b) are resonances of the bound and free 4-fluorobenzonitrile ligands, respectively. The changes to the total integrations over (a) and (b) are shown on the right.
To probe the biological viability of these photolabile complexes, their irradiation in aqueous conditions was examined. Figure 5.6 shows the changes in electronic absorption spectra of 5.2 and 5.3 in H₂O upon photolysis over 15 and 11.5 hours, respectively (λ irr ≥ 345 nm). It should be noted that 5.2 and 5.3 were stable in the dark, at room temperature for 7 days in H₂O. It is qualitatively evident from the photolysis experiments that the 4-fluorobenzonitrile ligand is slightly more labile under irradiation than the benzonitrile. It is apparent that the hypochromic and bathochromic shift of the 550 nm maximum coupled with the growth of a new lower energy peak at ~560 nm is faster in 5.3, whereas the slower photochemistry of 5.2 precludes the observation of the new low energy peak during the same irradiation time. Similar spectral changes were observed in the photolysis of 5.1 in H₂O in addition to a more dramatic growth of a new peak with maximum at 450 nm.¹¹
Figure 5.6 Changes to the electronic absorption upon irradiation ($\lambda_{\text{irr}} \geq 345$ nm) of (a) 0.78 mM 5.2 over 15 hours and (b) 0.95 mM 5.3 over 11.5 hours. Insets show magnified view of changes to the low energy peak.
The diminished amount of photosubstitution in H$_2$O as compared to CH$_3$CN observed in 5.2 and 5.3 may be due to the hydrophobicity of the ligands. The benzonitrile ligands alone are both hydrophobic making the exchange with water unfavorable. However, in the related mononuclear Ru(II) complexes *cis-*-[Ru(bpy)$_2$(NCC$_6$H$_5$)$_2$](Cl)$_2$ and *cis-*-[Ru(bpy)$_2$(4F-NCC$_6$H$_4$)$_2$](Cl)$_2$, the benzonitrile ligands are exchanged with H$_2$O under $\lambda_{irr} = 400$ nm with quantum yields of $\Phi = 0.17 \pm 0.01$ and $\Phi = 0.22 \pm 0.02$, respectively.$^{23}$ The difference between these complexes and the dirhodium systems, 5.2 and 5.3, lies in the orientation of the monodentate ligands with respect to one another when bound to the metal center. In the mononuclear Ru complexes, the two benzonitrile ligands are in a *cis*-configuration where their phenyl rings are oriented perpendicular to one another in space. On the Rh$_2$ center, however, they are bound to adjacent metals in the same orientation and thus allow for stabilizing $\pi$-interactions between the phenyl rings. It is the ability of the ligands to $\pi$-stack in 5.2 and 5.3 in water that is believed to retain a photolyzed ligand in close enough proximity (within the solvent cage) to the metal, such that it can efficiently re-coordinate to the metal.

It has been shown by agarose gel electrophoresis that the photo induced exchange of two equatorial CH$_3$CN ligands in 5.1 is sufficient to facilitate the covalent binding of the complex to ds-DNA.$^{11}$ Although the spectral changes of 5.2 in H$_2$O are small, it was of interest to investigate the possibility for binding to ds-DNA. Figure 5.7 shows images of gels run with linearized plasmid in the presence of 5.1 and 5.2. In Figure 5.7a the decreased mobility of the DNA with increasing concentrations of 5.1, under $\lambda_{irr} \geq 455$ nm is observed as compared to the samples kept in the dark, shown in Figure 5.7b. In
contrast, Figure 5.7c shows the same mobility between all samples, under $\lambda_{\text{irr}} \geq 385$ nm for 20 min. This similarity to the dark control experiments is evidence of a non-covalent interaction between 5.2 and ds-DNA when under these irradiation conditions. It is still unknown whether the improved lability of 5.3 would show different results from 5.2. Current studies are underway to elucidate the ability of 5.3 to bind covalently ds-DNA.

Figure 5.7 Imaged ethidium bromide stained agarose gel of 50 μM (a) and 20 μM (b) linearized pUC18 plasmid (10 mM phosphate, pH = 7.5) in the presence of various concentrations of (a) 5.1 irradiated ($\lambda_{\text{irr}} \geq 455$ nm, $t_{\text{irr}} = 20$ min), (b) 5.1 incubated in the dark at 25 °C for 20 min, and (c) 5.2 irradiated ($\lambda_{\text{irr}} \geq 385$ nm, $t_{\text{irr}} = 20$ min), where complex concentration increases over lanes 3-6. Lanes 1 and 8: DNA molecular weight standard (1kb, Sigma). Lanes 2 and 7: linearized plasmid alone. Lanes 3-6: (a and b) [DNA bp]/[Complex] = 100, 20, 10, 5 and (c) [Complex] (μM) = 10, 20, 40,100.
5.2.3 Electronic Structure Calculations

The electronic structures of 5.1 – 5.3 were calculated in water and CH$_3$CN using the polarizable continuum medium model for solvent, and the resulting molecular orbital diagrams are compared in Figure 5.8. Both the HOMO and LUMO of 5.1 – 5.3 (Figure 5.9) show electron density centered on the Rh-Rh inter-nuclear axis. The electron density is localized on the metals in the LUMOs, whereas it is shared between the metal centers and equatorial ligands in the HOMOs of 5.2 and 5.3. Therefore, the LUMOs are expected to lie at similar energies and were set at the same energy in Figure 5.8 at 0.0 eV. As expected, 5.1 – 5.3 have Rh$_2$(π*) character in their HOMO and HOMO-1 and Rh$_2$(σ*) character in their LUMOs. Below the metal-metal based HOMOs there are CH$_3$CN or benzonitrile-type ligand π-bonding orbitals which are calculated to be higher in energy for 5.2 and 5.3 as compared to 5.1. This difference can be explained by the delocalization of the π-system of the phenyl rings on these complexes onto the nitrile portion of the ligand, which effectively lowers the ligand orbitals such that their electronic overlap with the metal based orbitals is smaller, resulting in lower interaction. Likewise, in all three complexes, the first three LUMOs are the Rh$_2$ antibonding orbitals, however, the LUMO+5 and LUMO+6 differ between the acetonitrile and benzonitrile complexes. In those with benzonitrile ligands, these orbitals are antibonding between a rhodium and its two ligated benzonitrile ligands with much more equal distribution over the metal and ligands than that calculated for any RhL antibonding orbitals of 5.1.
Figure 5.8 Calculated molecular orbital diagram of 5.1 – 5.3.
Figure 5.9 Molecular orbital electron density projections of HOMO-6 – LUMO+6 for 5.1 – 5.3 (isovalue = 0.04 au).
Figure 5.9 continued
To aid in the confirmation of electronic transition assignments of 5.2 and 5.3, TD-DFT calculations were performed. Figure 5.10 shows that there is good correlation of wavelength and oscillator strength between the predicted and experimental transitions of 5.2 and 5.3 in H$_2$O. The lowest energy transitions for 5.2 and 5.3 are calculated at 540 and 561 nm and 542 and 563 nm in H$_2$O, respectively, which are in good agreement with the experimental results discussed above. Additionally, the calculated orbital contributions to these transitions support the assignments described previously based on analogous complexes. Specifically, the Rh$_2$(π*)→Rh$_2$(σ*) assignments for the lowest energy peak and electron movement to orbitals with antibonding Rh$_2$L character (L = benzonitrile) in the transitions calculated at 398 and 401 nm for 5.2 and 5.3, respectively.

\[ \text{Figure 5.10} \] Experimental electronic absorption (——) and calculated transitions (—) of (a) 5.2 and (b) 5.3, in H$_2$O.
Table 5.2 Singlet Electronic Transitions ($\lambda > 340$ nm) of 5.2.

<table>
<thead>
<tr>
<th>No.</th>
<th>$\lambda$ (nm)</th>
<th>$f$</th>
<th>Major Transitions (Contributions &gt;10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>561.1375</td>
<td>0.0008</td>
<td>HOMO-$\rightarrow$LUMO (78%)</td>
</tr>
<tr>
<td>2</td>
<td>540.3266</td>
<td>0.0005</td>
<td>H-9-$\rightarrow$LUMO (10%), H-1-$\rightarrow$LUMO (68%)</td>
</tr>
<tr>
<td>3</td>
<td>433.2809</td>
<td>0.0002</td>
<td>H-8-$\rightarrow$LUMO (15%), H-3-$\rightarrow$LUMO (71%)</td>
</tr>
<tr>
<td>4</td>
<td>398.2121</td>
<td>0.0449</td>
<td>H-14-$\rightarrow$LUMO (-21%), H-2-$\rightarrow$LUMO (39%)</td>
</tr>
<tr>
<td>5</td>
<td>389.5295</td>
<td>0.0004</td>
<td>H-8-$\rightarrow$L+1 (14%), H-3-$\rightarrow$L+1 (35%)</td>
</tr>
<tr>
<td>6</td>
<td>387.7751</td>
<td>0.0015</td>
<td>H-13-$\rightarrow$LUMO (17%), H-8-$\rightarrow$LUMO (20%), HOMO-$\rightarrow$L+2 (13%)</td>
</tr>
<tr>
<td>7</td>
<td>377.7331</td>
<td>0.0008</td>
<td>H-12-$\rightarrow$L+1 (-19%), H-3-$\rightarrow$L+2 (27%)</td>
</tr>
<tr>
<td>8</td>
<td>372.7589</td>
<td>0.0080</td>
<td>HOMO-$\rightarrow$L+1 (61%)</td>
</tr>
<tr>
<td>9</td>
<td>362.1326</td>
<td>0.0010</td>
<td>H-9-$\rightarrow$L+1 (10%), H-1-$\rightarrow$L+1 (51%)</td>
</tr>
<tr>
<td>10</td>
<td>353.3698</td>
<td>0.0006</td>
<td>H-12-$\rightarrow$LUMO (35%), H-9-$\rightarrow$LUMO (37%)</td>
</tr>
<tr>
<td>11</td>
<td>340.5761</td>
<td>0.0025</td>
<td>H-2-$\rightarrow$LUMO (11%), H-1-$\rightarrow$L+2 (32%)</td>
</tr>
</tbody>
</table>
Table 5.3 Singlet Electronic Transitions ($\lambda > 340$ nm) of 5.3.

<table>
<thead>
<tr>
<th>No.</th>
<th>$\lambda$ (nm)</th>
<th>$f$</th>
<th>Major Transitions (Contributions $&gt;$10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>563.3558</td>
<td>0.0008</td>
<td>H-7$\rightarrow$LUMO (11%), HOMO$\rightarrow$LUMO (72%)</td>
</tr>
<tr>
<td>2</td>
<td>542.4542</td>
<td>0.0004</td>
<td>H-6$\rightarrow$LUMO (10%), H-1$\rightarrow$LUMO (62%)</td>
</tr>
<tr>
<td>3</td>
<td>434.0089</td>
<td>0.0003</td>
<td>H-4$\rightarrow$LUMO (27%), H-3$\rightarrow$LUMO (56%)</td>
</tr>
<tr>
<td>4</td>
<td>401.5264</td>
<td>0.0459</td>
<td>H-14$\rightarrow$LUMO (-22%), H-2$\rightarrow$LUMO (43%)</td>
</tr>
<tr>
<td>5</td>
<td>391.238</td>
<td>0.0002</td>
<td>H-13$\rightarrow$LUMO (16%), H-4$\rightarrow$LUMO (17%), HOMO$\rightarrow$L+2 (11%)</td>
</tr>
<tr>
<td>6</td>
<td>389.5172</td>
<td>0.0012</td>
<td>H-4$\rightarrow$L+1 (24%), H-3$\rightarrow$L+1 (24%)</td>
</tr>
<tr>
<td>7</td>
<td>378.8296</td>
<td>0.0011</td>
<td>H-12$\rightarrow$L+1 (21%), H-4$\rightarrow$L+2 (15%), H-3$\rightarrow$L+2 (21%)</td>
</tr>
<tr>
<td>8</td>
<td>374.9685</td>
<td>0.0092</td>
<td>HOMO$\rightarrow$L+1 (57%)</td>
</tr>
<tr>
<td>9</td>
<td>363.7368</td>
<td>0.0011</td>
<td>H-1$\rightarrow$L+1 (45%)</td>
</tr>
<tr>
<td>10</td>
<td>355.9569</td>
<td>0.0002</td>
<td>H-12$\rightarrow$LUMO (33%), H-6$\rightarrow$LUMO (12%), H-5$\rightarrow$LUMO (26%)</td>
</tr>
<tr>
<td>11</td>
<td>343.6822</td>
<td>0.0007</td>
<td>H-2$\rightarrow$LUMO (11%), H-1$\rightarrow$L+2 (31%)</td>
</tr>
</tbody>
</table>
5.3 Conclusions

Derivatives of \( \text{cis-}[\text{Rh}_2(\mu\text{-O}2\text{CCH}_3)_2(\text{CH}_3\text{CN})_4(\text{H}_2\text{O})_2]^{2+} \) (5.1) where benzonitrile and 4-fluorobenzonitrile were used in place of the equatorial acetonitrile ligands were studied for their ability to undergo photoinduced ligand exchange under various conditions. It was shown that the electronic absorption spectra of 5.2 and 5.3 closely resemble that of 5.1. The equatorial ligand substitutions of 5.2 and 5.3, however, not only vary from that of 5.1, but also have appreciable differences between one another. In solvents with favorable solubility of the ligands, such as CH\(_3\)CN, ligand exchange is facile for all four equatorial ligands on 5.3, but occurs on a much slower time scale for 5.2. This observation is reasonable given the electron withdrawing nature of the 4-fluorobenzonitrile, making it more labile.

In aqueous solutions the ability of the ligands to diffuse away from the complex appears hindered such that ligand-metal re-association within the solvent cage predominates over ligand exchange, which is supported by the calculated optimized geometries of 5.2 and 5.3 that point to a \( \pi \)-stacked orientation of the ligands. Further studies of the ligand exchange quantum yields of 5.2 and 5.3 in H\(_2\)O need to be completed alongside the photolysis changes to their \(^1\text{H} \) NMR spectra in H\(_2\)O. This data will allow for a clear comparison of both the efficiency of photosubstitution and the respective photoproducts generated, such that they can be compared to those obtained for 5.1. Although 5.2 lacks the ligand lability necessary for covalent binding with DNA, the greater lability of 5.3 makes it more promising as a potential PDT agent.
5.4 References


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CHAPTER 6

CONCLUSIONS

The photophysical and photochemical properties of transition metal complexes can be tuned through the variation of the ligation sphere and identity of the central metal. Rh$_2$(II,II), Ru(II) and Os(II) complexes containing the extended polypyridine ligand, dppn, showed both increased binding to ds-DNA, and achieved increased energy transfer to $^3$O$_2$, owing to their lowest energy $^3\pi\pi^*$ excited state. Additionally, Rh$_2$(II,II) benzonitrile complexes were shown to have tunable photoinduced ligand exchange properties as a function of the presence of an electron withdrawing group in the monodentate ligand.

Investigation into a series of complexes cis-[Rh$_2$(μ-O$_2$CCH$_3$)$_2$(dppn)(L)]$^{2+}$ with varied lengths of the second polypyridyl ligand, L showed that the lowest energy excited state in all the complexes is dppn-localized $^3\pi\pi^*$ with lifetimes of 2.4 – 4.1 μs in DMSO. The complexes photocleave DNA efficiently via a mechanism that is both mediated by reactive oxygen species and partially independent of the presence of oxygen. The bis-dppn complex, however, photocleaves DNA with greater oxygen independence than the rest of the series. In addition, this complex exhibits a lower reactivity toward DNA in general, likely due to the inability of the complex to intercalate. The toxicity of the series
toward Hs-27 human skin fibroblasts is enhanced significantly upon irradiation with visible light as compared to those measured in the dark, with up to a 24-fold increase in toxicity for the bis-dppn complex. This increase is significantly greater than that measured under similar experimental conditions for hematoporphyrin, under similar experimental conditions. The dramatic increases in toxicity upon irradiation make this series potentially useful for photodynamic therapy.

The DNA interactions of Ru(bpy)$_2$(dpqp)$^{2+}$, Ru(bpy)$_2$(dppn)$^{2+}$, and Os(bpy)$_2$(dppn)$^{2+}$ were investigated, and each complex photocleaves DNA with high efficiency. The three complexes address significant drawbacks to current PDT treatments, each by their unique photochemical properties. The aqueous luminescence and long-lived $^3$MLCT excited state lifetime of Ru(bpy)$_2$(dpqp)$^{2+}$ allow for energy transfer to $^3$O$_2$ with improved production of $^1$O$_2$ over Photofrin®. Likewise, a greater $\Phi$$_1$O$_2$ was observed with Ru(bpy)$_2$(dppn)$^{2+}$ upon photoexcitation, in addition to the ability of the complex to directly oxidize guanine bases. With regard to improvement of irradiation wavelength into the phototherapeutic window (650 – 850 nm), Os(bpy)$_2$(dppn)$^{2+}$ shifts the $^3$MLCT state to low enough energies to allow for 86% photocleavage with $\lambda_{\text{irr}} \geq 645$ nm. An increase in the relative viscosity of DNA solution with increasing complex concentrations is apparent for all three complexes, illustrating that in addition to the photophysical properties that enhance PDT, the complexes are also capable of intercalation. The strong DNA binding of these complexes provides this series with improved DNA photocleavage relative to Ru(bpy)$_3^{2+}$. The latter interacts weakly with DNA such that ROS produced by the complex are not in close proximity to DNA.
Derivatives of \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{CH}_3\text{CN})_4(\text{H}_2\text{O})]^2^+ \) , where benzonitrile and 4-fluorobenzonitrile were used in place of acetonitrile were studied for their ability to undergo photoinduced ligand exchange under various conditions. It was shown that in solvents with favorable solubility of the ligands, such as \( \text{CH}_3\text{CN} \), the exchange of four equatorial 4F-C\(_6\)H\(_4\)CN ligands for four \( \text{CH}_3\text{CN} \) ligands can occur in 25 min, whereas the same exchange occurs on a much slower time scale for \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{C}_6\text{H}_5\text{CN})_4]^2^+ \). This observation is reasonable given the electron withdrawing nature of the 4-fluorobenzonitrile.

Calculated optimized geometries of the complexes show a parallel orientation of the equatorial ligands which appears favorable for \( \pi \)-stacking interactions. These structural features are believed to influence the aqueous photolysis results which show reduced spectral changes in comparison to organic solvents. The inability of \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{C}_6\text{H}_5\text{CN})_4(\text{H}_2\text{O})]^2^+ \) to bind ds-DNA covalently in the same way of \( \text{cis-}[\text{Rh}_2(\mu-\text{O}_2\text{CCH}_3)_2(\text{CH}_3\text{CN})_4(\text{H}_2\text{O})]^2^+ \) is attributed to the reduction in photoinduced ligand exchange. These studies provide a framework for further investigation into the effect of the equatorial ligand on the photoactivated ligand exchange properties of dirhodium complexes.

The work described here shows that many shortcomings associated with typical PDT agents can be diminished through thoughtful design of transition metal complexes. The use of a highly conjugated ligand in a variety of coordination spheres, as well as the utilization of both second and third row transition metals resulted in favorable properties of the complexes making them potential PDT agents. Investigation into the lability of
new systematically designed equatorial ligands for Rh$_2$ complexes resulted in a better understanding of how to control the efficiency of photoinduced ligand labilization. This information will enable the logical design of superior metal chemotherapy agents in aspects related to toxicity, binding to biomolecules, and membrane translocation.
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Chapter 2 References


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