Dirhodium Complexes as Possible Dual-Binding Photodynamic Therapy Agents

Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation "with Research Distinction in Chemistry" in the undergraduate colleges of The Ohio State University

by

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May 2012

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Abstract

A new type of dirhodium paddlewheel complexes were synthesized that contain ligands with extended π-systems. These ligands are expected to increase the affinity of these complexes for DNA because they are known to intercalate between the DNA bases in related systems. In addition, the new complexes possess equatorial acetonitrile ligands that exchange for water molecules upon photolysis, which produces an activated form of the complex that is able to bind covalently to DNA. These features lead to dual-binding through covalent binding to DNA upon irradiation and intercalation of the extended π-system ligand between the DNA bases. Currently, there are no reports of photoactivated dual-binding agents. These complexes possess features desired of photodynamic therapy agents, primarily because they are inactive in the dark, and readily bind to DNA when irradiated.

The dirhodium bis-amidate complexes cis-H,H-$[Rh_2(HNOCCH_3)_2(bpy)(CH_3CN)_4][BF_4]_2$ (bpy = 2,2´-bipyridine) and cis-H,H-$[Rh_2(HNOCCH_3)_2(dpq)(CH_3CN)_4][BF_4]_2$ (dpq = dipyrido[3,2-f:2',3'-h]-quinoxaline) were successfully synthesized from cis-H,H-$[Rh_2(HNOCCH_3)_2(CH_3CN)_6][BF_4]_2$ with the corresponding bidentate ligand. The bpy complex was prepared in 7 days, and because it cannot intercalate between the DNA bases, it is used as a control for no intercalation, but it was shown to bind covalently to DNA upon irradiation. The dpq complex was synthesized successfully in 1 day. Studies

of this complex are underway and its intercalation ability has not yet been proven. The synthesis of related complexes with other bidentate ligands is ongoing.

The preparation of complexes possessing acetate bridging ligands instead of amidates were attempted from the reaction of cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6]^{2+}$ and bpy. Cis-$[Rh_2(CH_3COO)_2(bpy)(CH_3CN)_4]^{2+}$ was not successfully synthesized, but it is still of interest, since its precursor cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6]^{2+}$ binds to DNA upon irradiation and it is inactive under dark conditions.

Dedication

I dedicate this thesis to my mother Analida de Espinosa Martinez and family. This work is also dedicated to David Eschenbach and my closest friends.

Acknowledgements

I owe much gratitude to my advisor Professor Claudia Turro for her guidance and time invested in this research. Professor Claudia's advice has been extremely helpful in my undergraduate studies and has served to prepare me for my graduate career in the chemistry field. I especially thank Scott Burya for sharing his expertise in dirhodium core complexes chemistry and for playing an important role in the development of my laboratory skills. I also thank Alycia Palmer for her assistance in the characterization of complexes by mass spectrometry and the rest of my lab mates who are constantly providing support, and who are involved in my everyday work. I also express my gratitude to The National Secretariat for Science and Technology (SENACYT) of the Republic of Panama for funding my undergraduate studies in chemistry and biochemistry at The Ohio State University with the "Undergraduate Studies of Excellence" Scholarship. Finally, I thank Dr. Etilvia Arjona, whose advice and work assisted in my transfer from the University of Panama to The Ohio State University.

Vita

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Major Field: Chemistry

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Chapter 1: Introduction and Background

Photodynamic therapy (PDT) achieves high level of selectivity for malignant cells, resulting in low levels systemic toxicity and low levels of invasiveness, through the use of light activated cytotoxins[1]. PDT has been used to treat lung, bladder and ovarian cancer quite effectively[2]. PDT uses organic drugs, which are oxygen dependent. Photofrin®, an FDA-approved chemotherapy PDT agent, it is excited from the ground singlet state which decays to form an excited triplet state following irradiation. When the excited triplet state relaxes to the singlet ground state, it transfers energy to triplet molecular oxygen, $^3O_2$, producing singlet oxygen, $^1O_2$. Singlet oxygen is toxic and causes damage to biomolecules, resulting in cell death[3]. The mechanism of action of Photofrin® is shown in **Figure 1**.

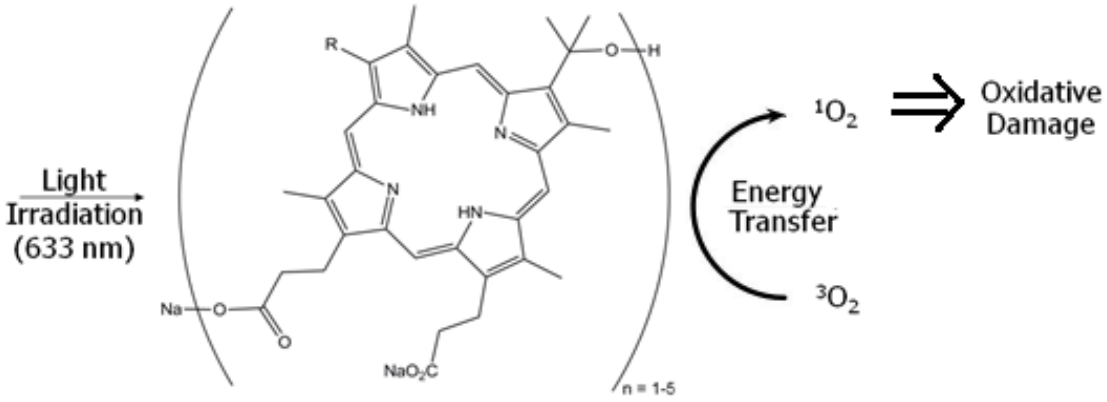

Figure 1. Mechanism of action of Photofrin®.

Because of the requirement of oxygen in tissue to generate the damaging species, organic PDT agents are not operable in hypoxic tissues. Tumors, because of the accelerated cell growth and metabolic demands, have a low concentration of molecular oxygen[4]. This is one of the main drawbacks of organic PDT agents. Transition metal complexes have now received more attention because they have the potential to operate via a different mode of action than organic PDT agents.

Another FDA-approved chemotherapy agent is Cisplatin®, cis-diamminedichloroplatinum(II). Cisplatin® undergoes thermal ligand exchange, in which one or both of the chlorides are exchanged for water to form the activated complexes cis-$[Pt(NH_3)_2(H_2O)Cl]^+$ and cis-$[Pt(NH_3)_2(H_2O)_2]^+$ which bind irreversibly to DNA[5]. These activated complexes bind to purines in DNA, most commonly guanine, forming 1,2-intrastrand adducts that kink DNA and inhibit the activity of DNA polymerases. The 1,2-intrastrand adducts formed with the platinum complex are unable to be repaired by excision repair systems in the cell, so the cell is targeted to death. The mechanism of action of Cisplatin® is shown in **Figure 2.** The main disadvantage of Cisplatin is that, as mentioned, is thermally activated and it causes damage to both healthy and malignant cells.

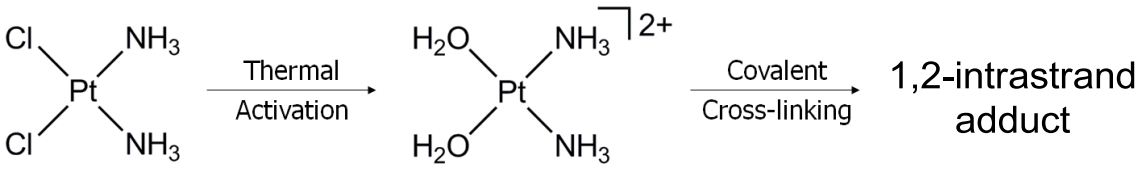

Figure 2. Mechanism of action of Cisplatin®

Only two analogs of Cisplatin® have been approved by the FDA: Carboplatin® (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)) and Oxaliplatin® ([(1*R*,2*R*)-cyclohexane-1,2-diamine](ethanedioato-*O*,*O'*)platinum(II)), shown in **Figure 3**.

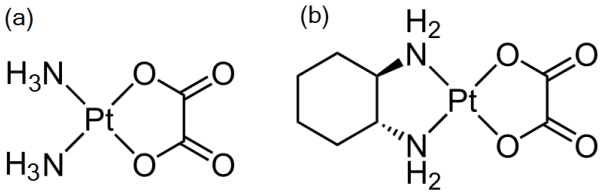

Figure 3. FDA-approved Cisplatin® analogs **(a)** Carboplatin® and **(b)** Oxaliplatin®

Dual-Binding action refers to the ability of a complex to be able to both bind covalently to DNA by coordination and to intercalate extended π-system ligands in between the DNA bases. Such dual binding has been shown to be important in platinum complexes for activity towards Cisplatin-resistant cell lines[6]. It has been shown that one dirhodium bis-acetate complex also has this dual-binding ability[7]. Cis-[Rh_2(dap)(μ-O_2CCH_3)$_2$(η^1-O_2CCH_3)-(CH_3OH)](O_2CCH_3) (dap = 1,12-diazaperylene), shown in **Figure 4**, was determined by 2D-NOESY NMR spectroscopy to covalently bind to adenine N7 and to intercalate between adjacent adenines (A) of a 12-mer duplex. The increase of distance between A6H8 and A7H8 was observed as the correlation between these two protons decreased significantly after intercalation of the dap ligand. The covalent binding was shown by ^{1}H NMR Spectroscopy, where one of the rhodium atoms bind to the A6 residue of the d(CTCTCAACTTCC) 12-mer studied.

The drawback of this dual-binding complex is that is thermally activated because the equatorial CH_3OH and η^1-CH_3COO^- ligands readily exchange. This is undesired, since toxicity should be controlled and the complex should only be activated to act on malignant cells.

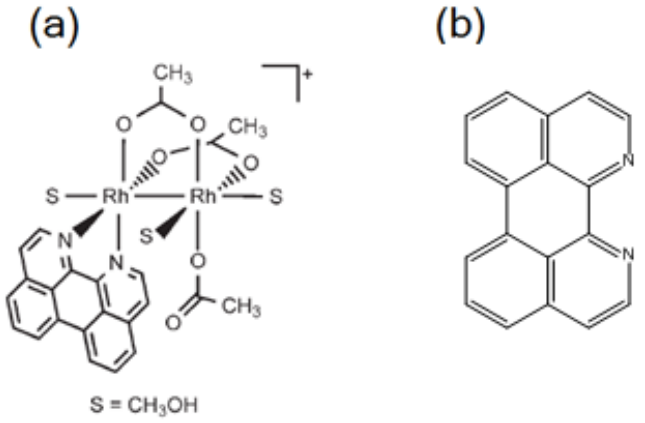

Figure 4. (a) Dual-binding complex cis-$[Rh_2(dap)(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)\text{-}(CH_3OH)](O_2CCH_3)$ **(b)** dap ligand (dap = 1,12-diazaperylene)

The dirhodium core complexes studied presented in this work are expected to be dual-binding photodynamic therapy agents. The dual-action in these complexes is also intercalation and covalent binding; however, in this case covalent binding is triggered by irradiation of the compounds. Currently, there are no reports of dual-binding photoactivated complexes. In addition, PDT agents that have novel DNA binding modes may be active against cisplatin-resistant cell lines[6], so the study of these complexes may lead to new drugs whose mode of action differs significantly from those currently available.

The main considerations for successful PDT agents are[8]:

- Complexes should be activated with irradiation in the "PDT Window" (600-850 nm), because this range results in greater tissue penetration.
- The photolysis process should be rapid and efficient, with high quantum yield (Φ) of the desired reaction.
- PDT agents must be inactive in the dark, but the photo-products should bind DNA readily.

The types of complex explored in this research are shown in **Figure 5** and the ligands of interest are shown in **Figure 6.**

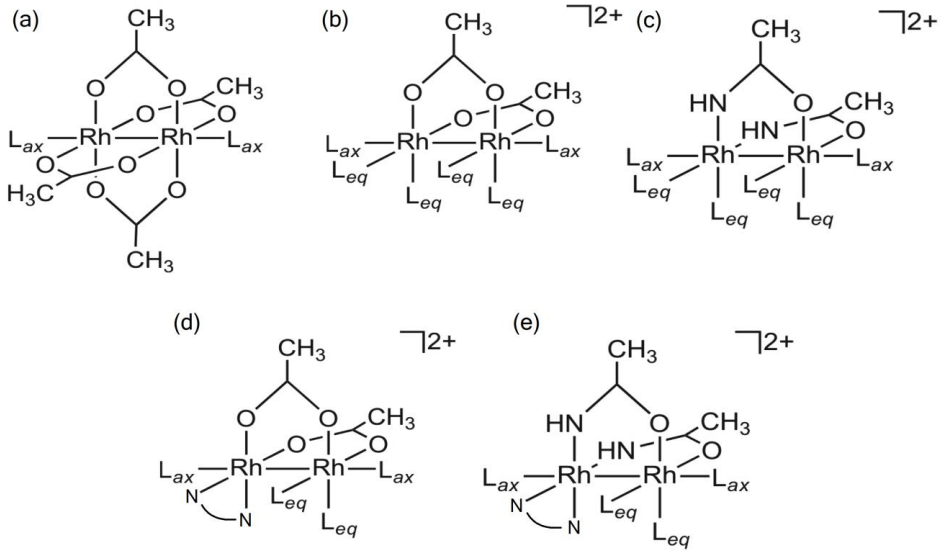

Figure 5. Complexes involved in this research: **(a)** $Rh_2(CH_3COO)_4$ **(b)** cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6]^{2+}$ **(c)** H,H (H,H = cis-H,H-$[Rh_2(HNOCCH3)_2(CH3CN)_6]^{2+}$ **(d)** cis-$[Rh_2(OOCCH_3)2(N\text{-}N)(CH_3CN)4]^{2+}$ where N-N = bpy, dpq, dppz, dppn **(e)** H,H-(N-N) where N-N = bpy, dpq, dppz. All L_{ax} and L_{eq} represent axially and equatorially bound CH_3CN, respectively.

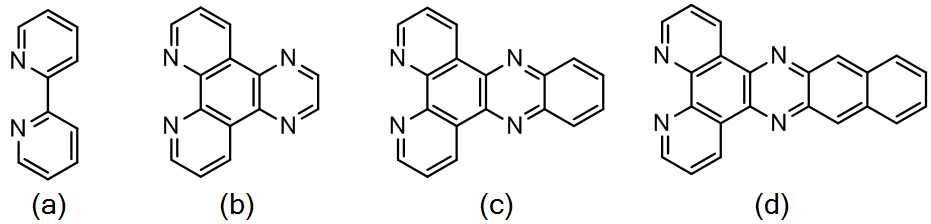

Figure 6. Ligands of interest: **(a)** bpy (bpy = 2,2'-bipyridine) **(b)** dpq (dpq = dipyrido[3,2-f:2',3'-h]-quinoxaline) **(c)** dppz (dppz = dipyrido[3,2-a:2',3'-c]phenazine) **(d)** dppn (dppn = benzo[i]-dipyrido[3,2-a:2',3'-c]phenazine)

The precursors of the complexes synthesized in this study have shown to have properties desired for PDT agents. The complex cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6]^{2+}$ was shown to be inactive in the dark and to bind to DNA upon irradiation[9].

To generate dual-binding agents, two of the Rh-CH_3CN_{eq} bound a rhodium atom are exchanged for a bidentate ligand to form cis-$[Rh_2(CH_3COO)_2(N\text{-}N)(CH_3CN)_4]^{2+}$, where N-N = dqp, dppz, dppn. The extended π-systems in the dpq, dppz, and dppn ligands are expected to intercalate between the DNA bases, which will increase the affinity of the dirhodium complexes for DNA. The photoactivated binding is initiated by the exchange of the remaining two Rh-CH_3CN_{eq} ligands for water molecules upon irradiation, followed by the facile thermal exchange water ligand(s) for DNA bases.

Chapter 2: Dirhodium Bis-Amidate Complexes

2.1 Materials

Reagents: 1,10-phenanthroline-5,6-dione, ethylenediamine, *o*-phenylenediamine, bpy (bpy = 2,2'-bipyridine) were purchased from Sigma-Aldrich. **Solvents:** methanol, acetonitrile, diethyl ether, all purchased from Sigma-Aldrich, dichloromethane purchased from Mallinckrodt Chemicals. **^{1}H NMR solvents:** acetonitrile-d_3 and deuterium oxide all purchased from Sigma-Aldrich. All reagents and solvents were used as purchased.

The complex H,H (H,H = *cis*-H,H-$[Rh_2(HNOCCH_3)_2(CH_3CN)_6][BF_4]_2$) was synthesized by Scott Burya[10].

2.2 Instrumentation

Electrospray ionization mass spectrometry (ESI-MS) data were acquired on a Bruker MicroTOF spectrometer and ^{1}H NMR spectra were collected using a Bruker 400 MHz Avance III or a Bruker 250 MHz DPX.

2.3 Synthesis and Characterization

2.3.1 Ligand Synthesis

2.3.1.1 Synthesis of dipyrido[3,2-f:2',3'-h]-quinoxaline (dpq)

The ligand dpq (dpq = dipyrido[3,2-f:2',3'-h]-quinoxaline) was synthesized from 1,10-phenanthroline-5,6-dione and ethylenediamine by a published procedure[11]. A solution of ethylenediamine (9.3 mg, 1.55 mmol) and 1,10-phenanthroline-5,6-dione (194.4 mg, 0.925 mmol) in ethanol (65 mL) was stirred at 40˚C for 3 h and then at room temperature overnight. The solvent was evaporated under reduced pressure, and the resulting solid was recrystallized from methanol to yield dpq (178.3 mg, 0.77 mmol, 83% yield). The product was characterized by ^{1}H NMR (250 MHz, acetonitrile-d_3). The NMR spectrum is shown in **Figure 7**.

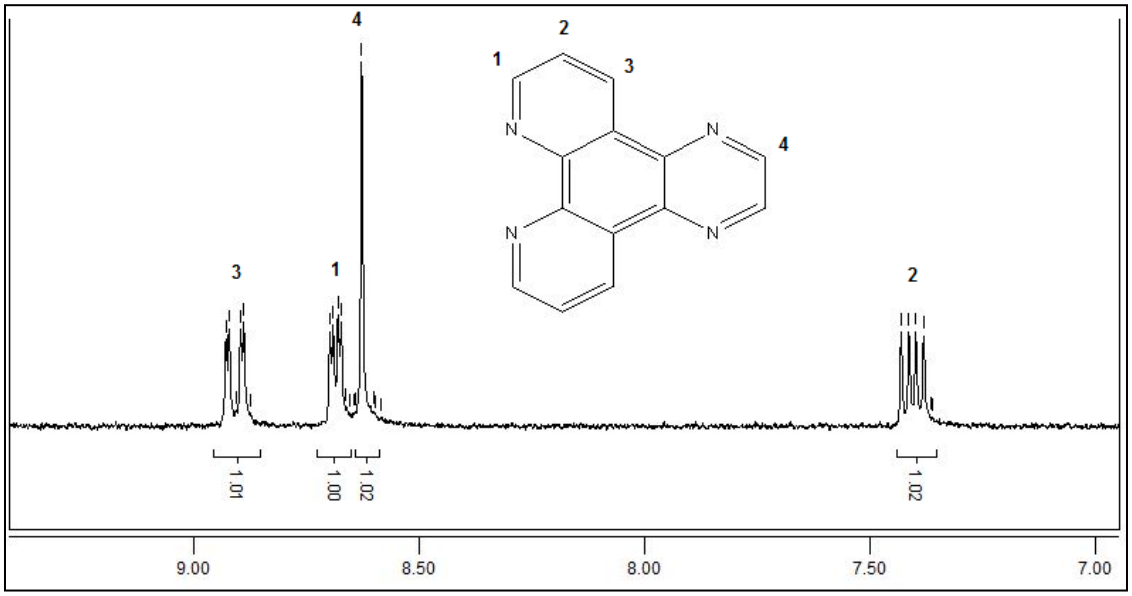

Figure 7. ^{1}H NMR spectrum of dpq (250 MHz, acetonitrile-d_3)

2.3.1.1 Synthesis of dipyrido[3,2-a:2',3'-c]phenazine (dppz)

The ligand dppz (dppz = dipyrido[3,2-a:2',3'-c]phenazine) was synthesized from 1,10-phenanthroline-5,6-dione and *o*-phenylenediamine by a published procedure[12]. A solution of *o*-phenylenediamine (66.1 mg, 0.61 mmol) and 1,10-phenanthroline-5,6-dione (65.6 mg, 0.31 mmol) in ethanol (15 mL) was boiled for 5 min. The solution was then cooled down slowly in an ice bath and the resulting solid was filtered and washed with cold ethanol (3 x 5mL). The resulting solid was recrystallized from ethanol to yield dppz (67.2 mg, 0.24 mmol, 76% yield). The product was characterized by ^{1}H NMR (250 MHz, acetonitrile-d_3). The NMR spectrum is shown in **Figure 8**.

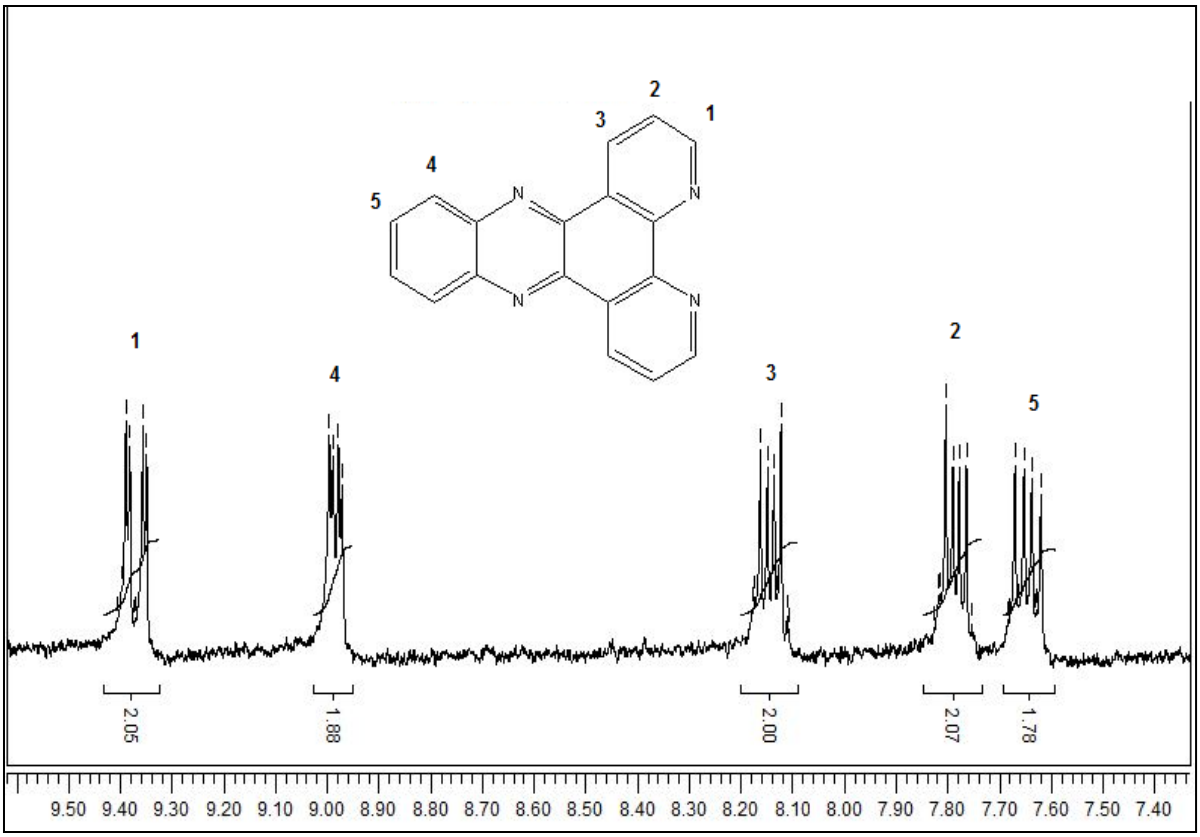

Figure 8. ^{1}H NMR spectrum of dppz (250 MHz, acetonitrile-d_3)

2.3.2 Synthesis of Dirhodium Bis-Amidate Complexes

2.3.2.1 Synthesis of cis-H,H-[$Rh_2(HNOCCH_3)_2(bpy)(CH_3CN)_4][BF_4]_2$

The complex H,H-bpy (H,H-bpy = cis-H,H-[$Rh_2(HNOCCH_3)_2(bpy)(CH_3CN)_4][BF_4]_2$) was synthesized from H,H (H,H = cis-H,H-[$Rh_2(HNOCCH_3)_2(CH_3CN)_6][BF_4]_2$) and bpy (bpy = 2,2'-bipyridine). In order to determine the ideal reaction time, H,H (10.0 mg, 13.5 µmol) was dissolved in acetonitrile (2.5 mL) and stirred covered from light with aluminum foil. A solution of bpy (11.8 mg, 75.6 µmol) in acetonitrile (1.0 mL) was added to the H,H solution and the mixture was stirred covered from light at room temperature. After 24 h, a 0.875 mL aliquot was taken from the reaction mixture and excess diethyl ether was added to the aliquot and a precipitate formed. The mixture was sonicated and then centrifugated, and the supernatant was discarded with a pipette. The precipitate was washed by adding excess diethyl ether, then sonicating and centrifugating the mixture. The supernatant was discarded and the resulting solid was redissolved in a minimal volume of acetonitrile. The solution was sonicated and centrifugated. The precipitate was then discarded and the supernatant was dried under a stream of air. The same procedure was also repeated after 3 days, 7 days and 8. The resulting solids from each aliquot were studied by ^{1}H NMR (400 MHz, deuterium oxide) to determine the purity of the product at each reaction time. The NMR spectra are shown in **Figure 9**, which indicate that after 7 days, only one product is observed in the ^{1}H NMR spectrum, consistent with H,H-bpy.

After the ideal time for the synthesis of H,H-bpy was determined, H,H (20 mg, 27.0 µmol) was dissolved in acetonitrile (5.0 mL) and covered from light. A solution of bpy

(23.6 mg, 151.2 µmol) in acetonitrile (2.0 mL) was added to the H,H solution and was allowed to react in the dark at room temperature for 7 days. Excess diethyl ether was added to the solution and a precipitate formed. The mixture was sonicated and then centrifugated.

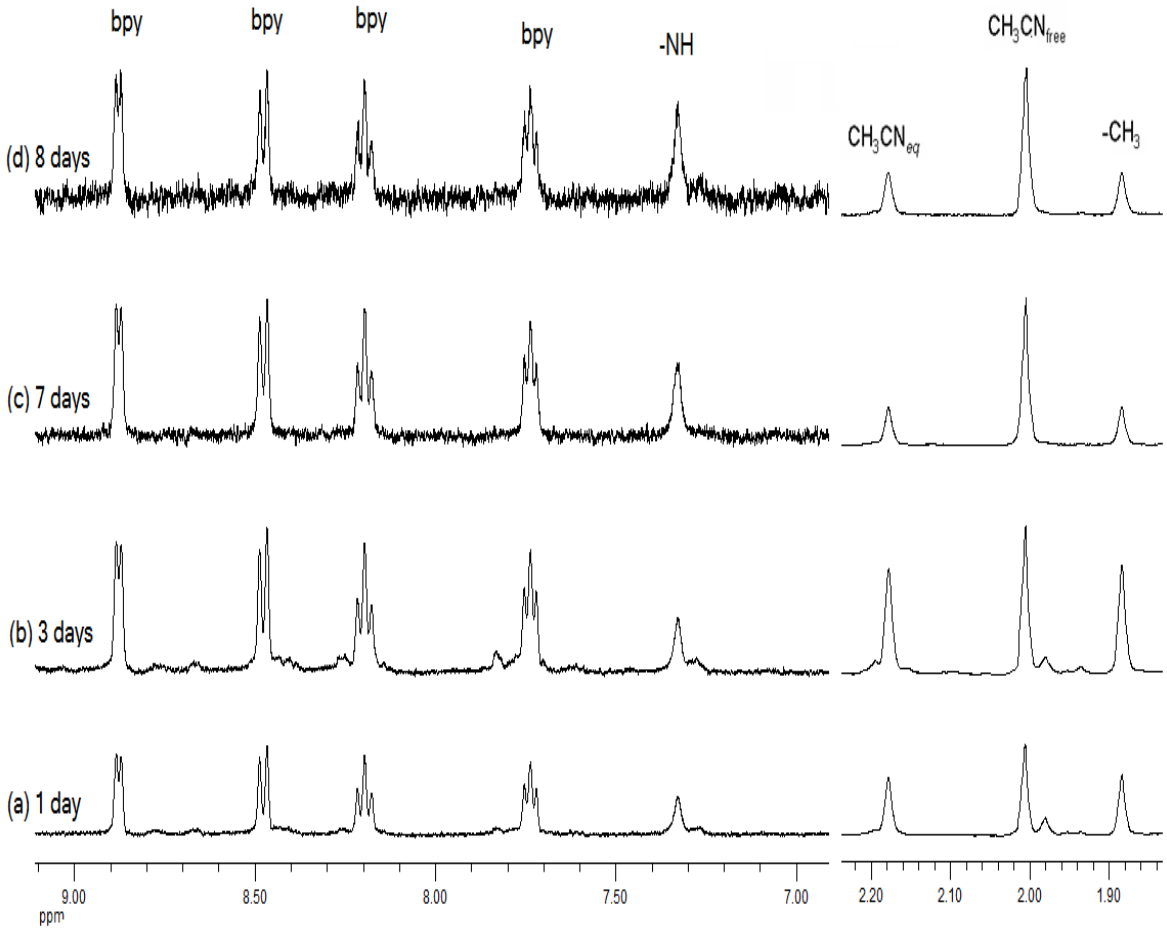

Figure 9. ^{1}H NMR (400 MHz, deuterium oxide) spectra of reaction of H,H with bpy after **(a)** 1 day, **(b)** 3 days, **(c)** 7 days, **(d)** 8 days.

The supernatant was discarded with a pipette, and the precipitate was washed by adding excess diethyl ether, then sonicating and centrifugating the mixture. The supernatant was discarded and the resulting solid was redissolved in a minimal volume of acetonitrile. The solution was sonicated and centrifugated. The precipitate was discarded and the supernatant was dried under a stream of air to yield H,H-bpy (13.7 mg, 16.8 µmol, 62 % yield). The product was characterized by ^{1}H NMR spectroscopy (400 MHz, deuterium oxide) and spectrum is shown in **Figure 10**.

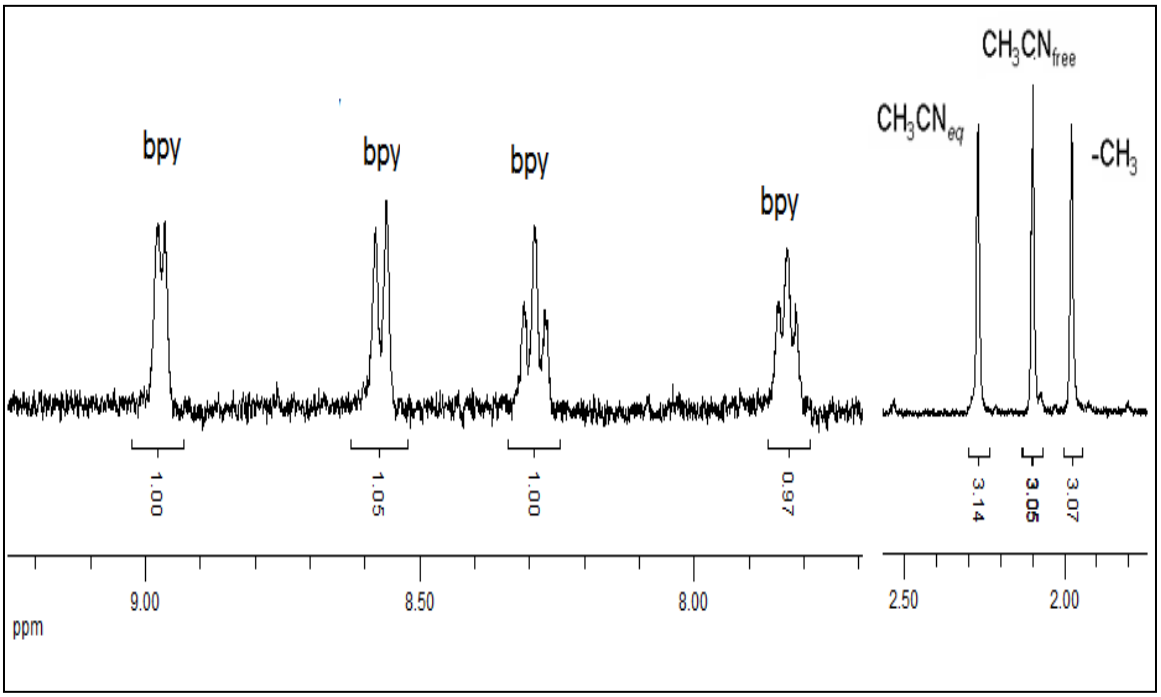

Figure 10. ^{1}H NMR (400 MHz, deuterium oxide) of H,H-bpy. The –NH peak is not seen in the spectrum.

The H,H-bpy product was also characterized by ESI-MS. The mass spectrum of the product is shown in **Figure 11.** The parent ion, $[Rh_2(HNOCCH_3)_2(bpy)(CH_3CN)_2]^{2+}$, was observed at *m/z* = 280.0, and the peak observed at *m/z* = 259.5 corresponds to the loss of one acetonitrile ligand, $[Rh_2(HNOCCH_3)_2(bpy)(CH_3CN)]^{2+}$. The identities of the other peaks remain unknown.

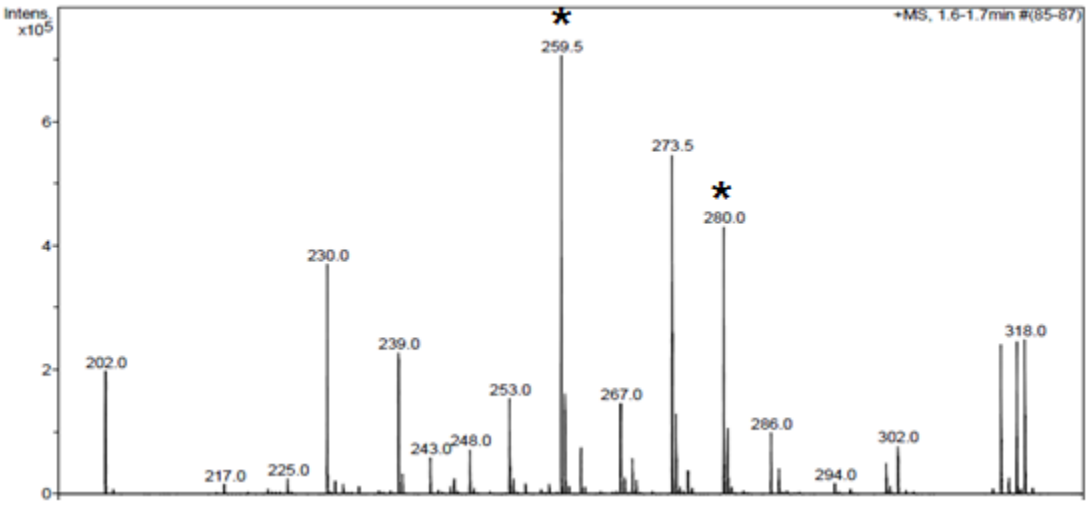

Figure 11. ESI-MS spectrum of H,H-bpy complex.

2.3.2.2 Synthesis of cis-H,H-[Rh$_2$(HNOCCH$_3$)$_2$(dpq)(CH$_3$CN)$_4$][BF$_4$]$_2$

The complex H,H-dpq (H,H-dpq = *cis*-H,H-$[Rh_2(HNOCCH_3)_2(dpq)(CH_3CN)_4][BF_4]_2$) was synthesized from H,H and dpq. In order to determine the ideal reaction time, H,H (10.0 mg, 13.5 µmol) was dissolved in acetonitrile (2.5 mL) and stirred covered from light with aluminum foil. A suspension of dpq (4.7 mg, 20.3 µmol) in acetonitrile (1.0 mL) was added to the H,H solution and the mixture was stirred covered from light at room temperature. After 24 h, a 0.875 mL aliquot was taken from the reaction mixture.

Excess diethyl ether was added to the aliquot and a precipitate formed. The mixture was sonicated and then centrifugated. The supernatant was discarded with a pipette. The precipitate was washed by adding excess diethyl ether, then sonicating and centrifugating the mixture. The supernatant was discarded and the resulting solid was redissolved in a minimal volume of acetonitrile. The solution was sonicated and centrifugated. The precipitate was discarded and the supernatant was dried under a stream of air. The same procedure was repeated after 3 days and 7 days the reaction was started. The resulting solids from aliquot were studied by ^{1}H NMR (400 MHz, deuterium oxide) to determine the purity of the product at each reaction time. The NMR spectra are shown in **Figure 12**. All the reactions showed the formation of H,H-dpq, however, in the ^{1}H NMR spectra, undesired products and impurities were also observed.

In order to determine the optimal method to obtain a clean product, the reaction was repeated as described above, but washing with excess dichloromethane after washing with diethyl ether and before dissolving in minimal acetonitrile. This process was performed on the reaction after 1 day and after 3 days. The ^{1}H NMR (400 MHz, deuterium oxide) spectra of these reactions are shown in **Figure 13**. After 1 day, after washing with dichloromethane, only one product was observed in the ^{1}H NMR spectrum. After 3 and 7 days, H,H-dpq was the primary product observed, however, vestiges of H,H-$(dpq)_2$ were also observed after the dichloromethane wash.

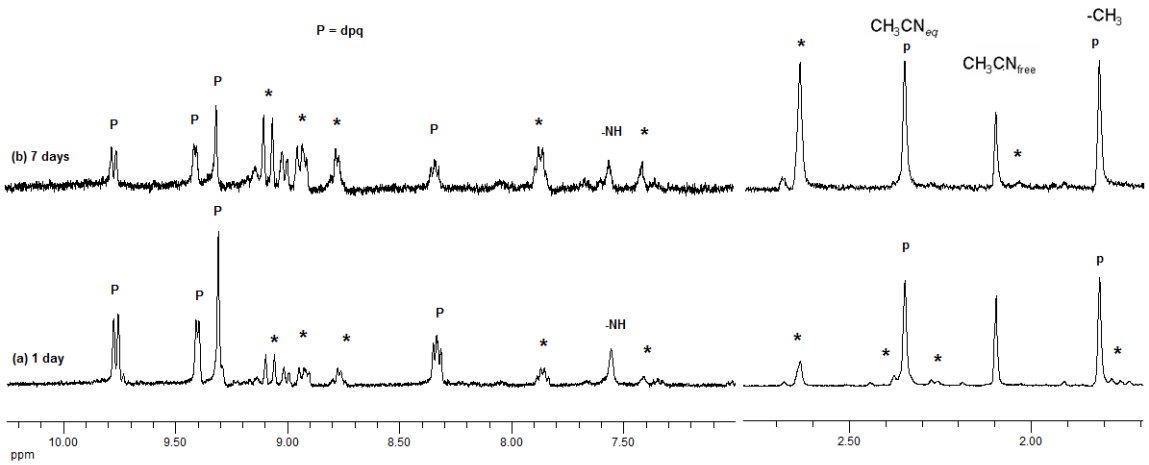

Figure 12. ^{1}H NMR spectra of H,H reaction with dpq after **(a)** 1 day, **(b)** 7 days without washing with dichloromethane. The resonances marked with **P** correspond to the desired product, H,H-dpq complex. The resonances marked with an asterisk **(*)** correspond to impurities (undesired products, unreacted starting material).

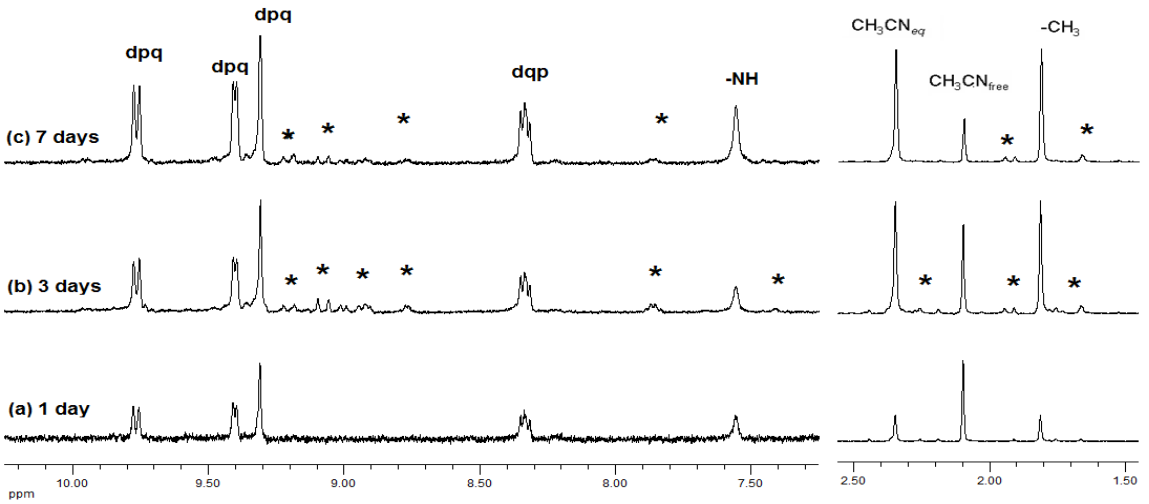

Figure 13. ^{1}H NMR spectra of H,H reaction with dpq after **(a)** 1 day, **(b)** 3 **(c)** 7 days washing with dichloromethane during the workup. The resonances marked with an asterisk **(*)** correspond to impurities (undesired products, unreacted starting material).

A comparison of the reaction after 1 day with and without dichloromethane wash is shown in **Figure 14.**

After 1 day, the desired product H,H-dpq is formed, however, H,H-$(dpq)_2$ is also observed by ^{1}H NMR spectroscopy. Washing with dichloromethane removes H,H-$(dpq)_2$, however, it also dissolves some of the H,H-dpq complex. The dichloromethane wash reduces the yield of H,H-dpq, but makes it possible to obtain a clean product.

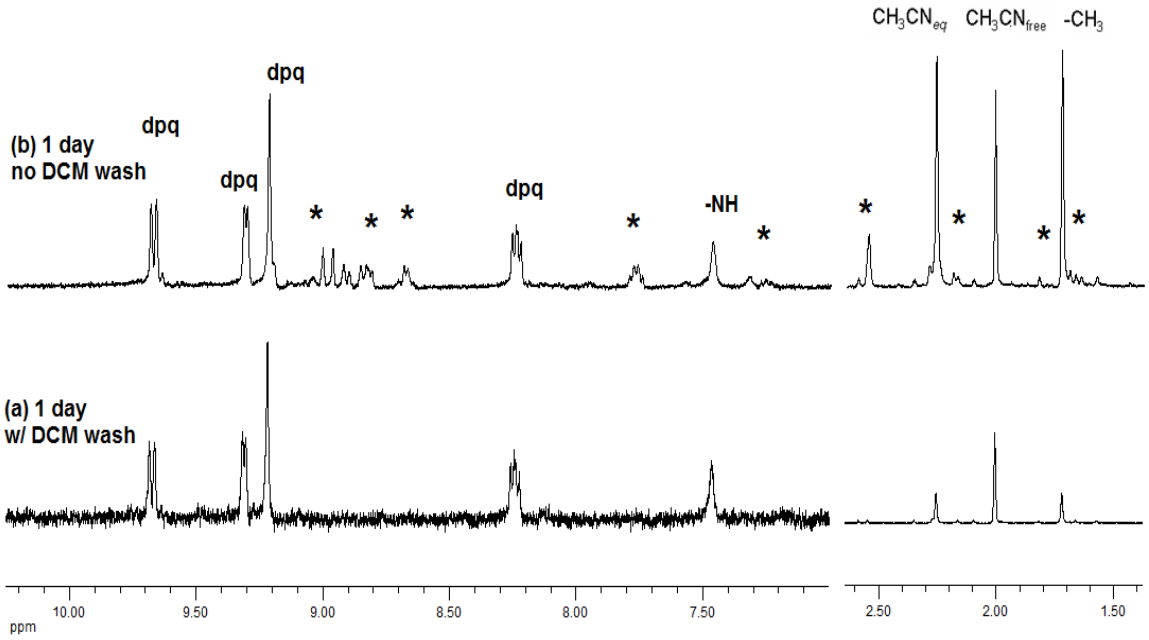

Figure 14. ^{1}H NMR spectra of H,H reaction with dpq after 1 day **(a)** washing with dichloromethane during workup, and **(b)** not washing with dichloromethane during workup. The resonances marked with an asterisk **(*)** correspond to impurities (undesired products, unreacted starting material).

In order to determine the optimal ratio of dpq ligand to H,H complex, a solution of H,H (5.0 mg, 6.75 µmol) in acetonitrile (1.25 mL) was stirred covered from light at room temperature. A suspension of dpq (1.6 mg, 6.90 µmol) in acetonitrile (0.5 mL) was added to the H,H solution and reacted at room temperature, covered from light. After 1 day and 3 days, an aliquot of 0.875 mL was taken and treated as described above, without washing with dichloromethane. The ^{1}H NMR (400 MHz, deuterium oxide) of the reactions are shown in **Figure 15**, comparing the conditions to the previous reaction with different dpq ligand to H,H complex ratio.

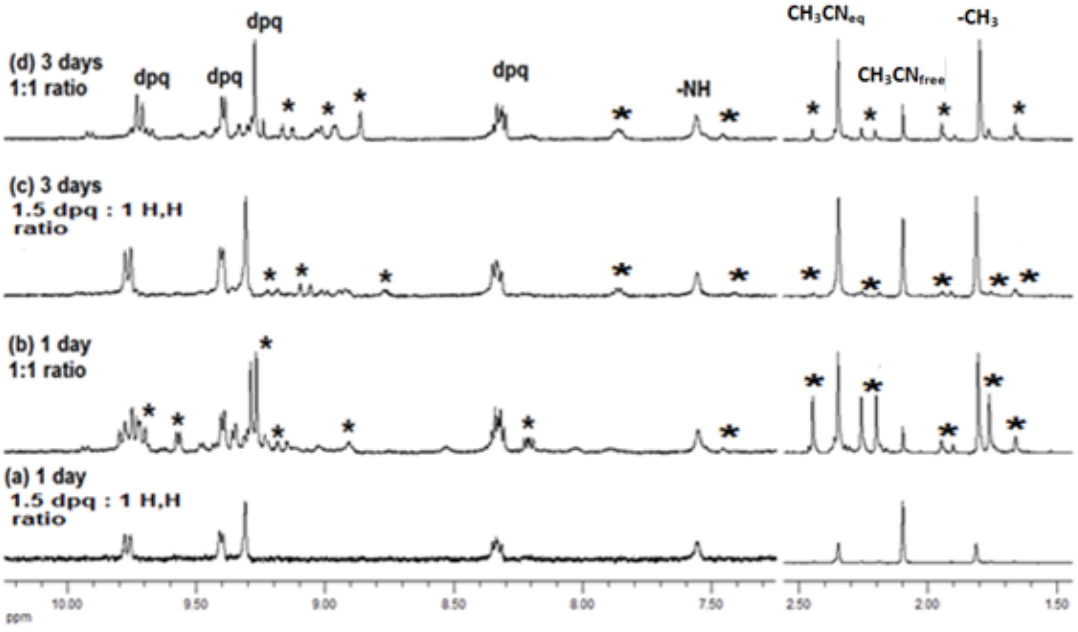

Figure 15. ^{1}H NMR spectra of H,H reaction with dpq after 1 day and 3 days. **(a) and (c)** reacting H,H with excess dpq. **(b) and (d)** reacting H,H with bpy in 1:1 ratio. The resonances marked with an asterisk **(*)** correspond to impurities (undesired products, unreacted starting material).

After the ideal conditions for the synthesis of H,H-dpq were determined, H,H (20 mg, 27.0 µmol) was dissolved in acetonitrile (5.0 mL) and covered from light. A suspension of dpq (9.7 mg, 40.5 µmol) in acetonitrile (2.0 mL) was added to the H,H solution and reacted in the dark at room temperature for 7 days. Excess diethyl ether was added to the solution and a precipitate formed. The mixture was sonicated and then centrifugated. The supernatant was discarded with a pipette. The precipitate was washed by adding excess diethyl ether, then sonicating and centrifugating the mixture. The precipitate was then washed with excess dichloromethane, sonicating and centrifugating after adding the respective solvent. The supernatant was discarded and the resulting solid was redissolved in a minimal volume of acetonitrile. The solution was sonicated and centrifugated. The precipitate was discarded and the supernatant was dried under a stream of air to yield H,H-dpq (11.3 mg, 10.9 µmol, 40 % yield). The product was characterized by ^{1}H NMR (400 MHz, deuterium oxide). The NMR spectrum is shown in **Figure 16**.

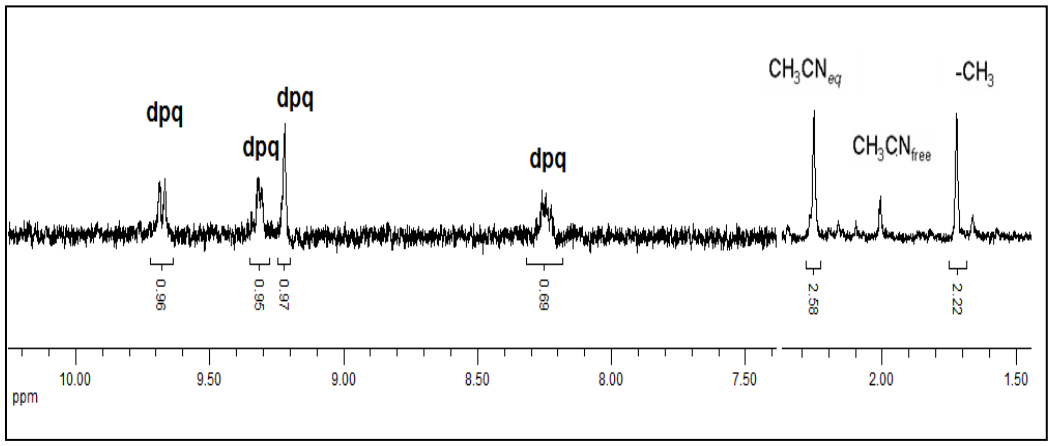

Figure 16. ^{1}H NMR spectrum of H,H-dpq complex (400 MHz, deuterium oxide). The –NH peak is not seen in the spectrum.

The product was also characterized by ESI-MS. The mass spectrum of the product is shown in **Figure 17.** The parent ion (and base peak), $[Rh_2(HNOCCH_3)_2(dpq)(CH_3CN)_2]^{2+}$, was observed at *m/z* = 318.0. The identities of the other peaks are unknown.

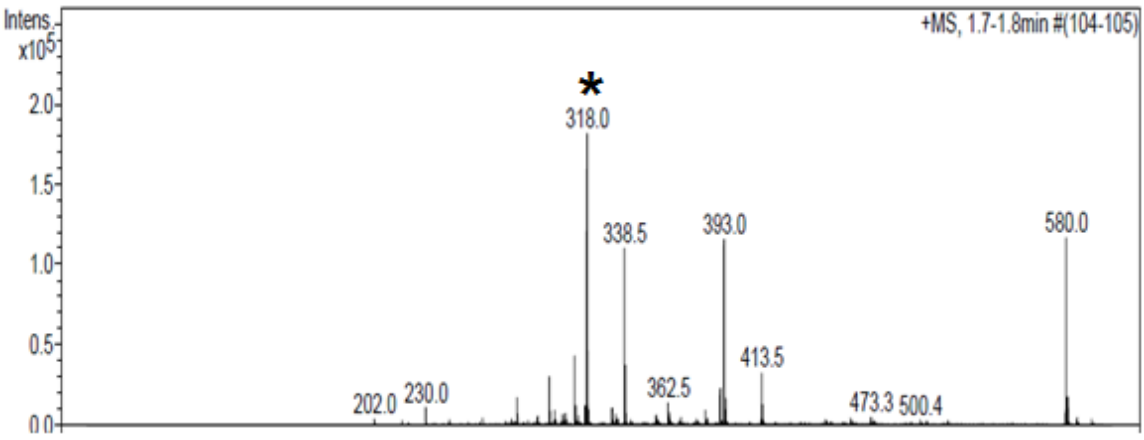

Figure 17. ESI-MS spectrum of H,H-dpq complex.

2.4 Discussion

The dirhodium H,H-bpy and H,H-dpq complexes were synthesized from the reaction of the H,H complex with excess of each respective ligand. The substitution of two coordinated acetonitrile molecules for the bidentate N-N ligands takes places *trans* to the nitrogen atoms of the amidate bridge. This can be rationalized by studying the bond lengths of the H,H complex. The Rh-CH_3CN_{ax} bond lengths are 2.242(4) Å on average, and the Rh-$CH_3CN_{eq}{}^{O}$ and Rh-$CH_3CN_{eq}{}^{N}$ bonds are on average, 2.004(4) and 2.026(4) Å[10]. The longer Rh-CH_3CN axial bonds are expected, since axial acetonitrile ligands undergo thermal exchange readily in water[9,10]. Now, comparing the equatorial acetonitrile

ligands that can be substituted for a bidentate N-N ligand, the Rh-$CH_3CN_{eq}^{N}$ are longer than the Rh-$CH_3CN_{eq}^{O}$, which may be related to the preference for substitution *trans* to the nitrogen atoms of the amidate bridge.

It is important to mention that the thermal exchange of the Rh-CH_3CN_{ax} for water in solution do not activate the complex for DNA binding. This is an advantage of this new type of complexes over Cisplatin, which is thermally activated to bind DNA, causing low selectivity and affecting both regular and malignant cells.

Photolysis followed by the changes to the electronic absorption spectrum in water of the precursor complexes H,H and H,H-bpy over 60 min, $\lambda_{irr} \geq 495$ nm, show that both of these species undergo ligand exchange[10,13]. In the case of the H,H complex, both Rh-$CH_3CN_{eq}^{N}$ and Rh-$CH_3CN_{eq}^{O}$ exchanged upon irradiation in water to form *cis*-H,H-$[Rh_2(HNOCCH_3)_2(CH_3CN)_3(H_2O)_3]^{2+}$. In the case of the H,H-bpy complex, only the Rh-$CH_3CN_{eq}^{O}$ ligands are available for exchange upon irradiation to form *cis*-H,H-$[Rh_2(HNOCCH_3)_2(bpy)(CH_3CN)(H_2O)_3]^{2+}$. This type of exchange triggered by irradiation is critical for DNA binding. In order to covalently bind to DNA, the Rh-CH_3CN_{eq} ligands need to exchanged for solvent water molecules so that covalent binding to DNA occurs where the water is incorporated after photolysis, followed by replacement by a nucleobase of the DNA double helix.

Regarding the dual-binding action of the dirhodium bis-amidate complexes, a DNA binding gel assay of the H,H-bpy complex shows no change in mobility of DNA in the dark control, and reduced mobility of DNA upon irradiation for 30 min, $\lambda_{irr} \geq$ 395 nm[13].

This suggests that *cis*-H,H-$[Rh_2(HNOCCH_3)_2(N\text{-}N)(CH_3CN)_4]^{2+}$ (N-N = bpy, dpq, dppz) can bind covalently to DNA only upon irradiation and cannot bind covalently in the dark, a desired feature of PDT agents.

The H,H-bpy is a control for the second mode of action of the dual-binding agents: intercalation. The π-system of the bpy ligand is not extended enough to intercalate in between the DNA bases. Regarding the H,H-dpq complex, DNA binding assays are underway. The synthesis of H,H-dppz is underway in order to perform the same type of DNA studies mentioned above.

Chapter 3: Dirhodium Bis-Acetate Complexes

3.1 Materials

Reagents: rhodium (III) chloride trihydrate was purchased from Pressure Chemicals Co. 2,3-naphthalenediamine was purchased from Spectrum. 1,10-phenanthroline-5,6-dione, sodium acetate trihydrate, triethyloxonium tetrafluoroborate (TEO), bpy (bpy = 2,2'-bipyridine) were all purchased from Sigma-Aldrich. **Solvents:** ethanol (200 proof) was purchased from Decon Labs. Methanol, diethyl ether, and acetonitrile were all purchased from Sigma-Aldrich. Glacial acetic acid and dichloromethane were purchased from Mallinckrodt Chemicals. **^{1}H NMR solvents:** methanol-d_4, acetonitrile-d_3, chloroform-d, deuterium oxide were all purchased from Sigma-Aldrich.

The ligand dpq (dpq = dipyrido[3,2-f:2',3'-h]-quinoxaline) was previously synthesized by the method described in chapter 2.

3.2 Synthesis and Characterization

3.2.1 Ligand Synthesis

3.2.1.1 Synthesis of benzo[i]-dipyrido[3,2-a:2',3'-c]phenazine (dppn)

The ligand dppn (dppn = benzo[i]-dipyrido[3,2-a:2',3'-c]phenazine) was synthesized from 1,10-phenanthroline-5,6-dione and 2,3-naphthalenediamine by a modified published procedure[12]. A solution of 2,3-naphthalenediamine (112.0 mg, 0.71 mmol) and 1,10-phenanthroline-5,6-dione (72.0 mg, 0.34 mmol) in ethanol (8.0 mL) was boiled for 15 min. The solution was then cooled down slowly in an ice bath and the resulting solid was filtered and washed with cold ethanol (3 x 5mL) to yield dppn (70.1 mg, 0.21 mmol, 62 % yield) as an orange solid. The product was characterized by ^{1}H NMR (250 MHz, chloroform-d). The NMR spectrum is shown in **Figure 18**.

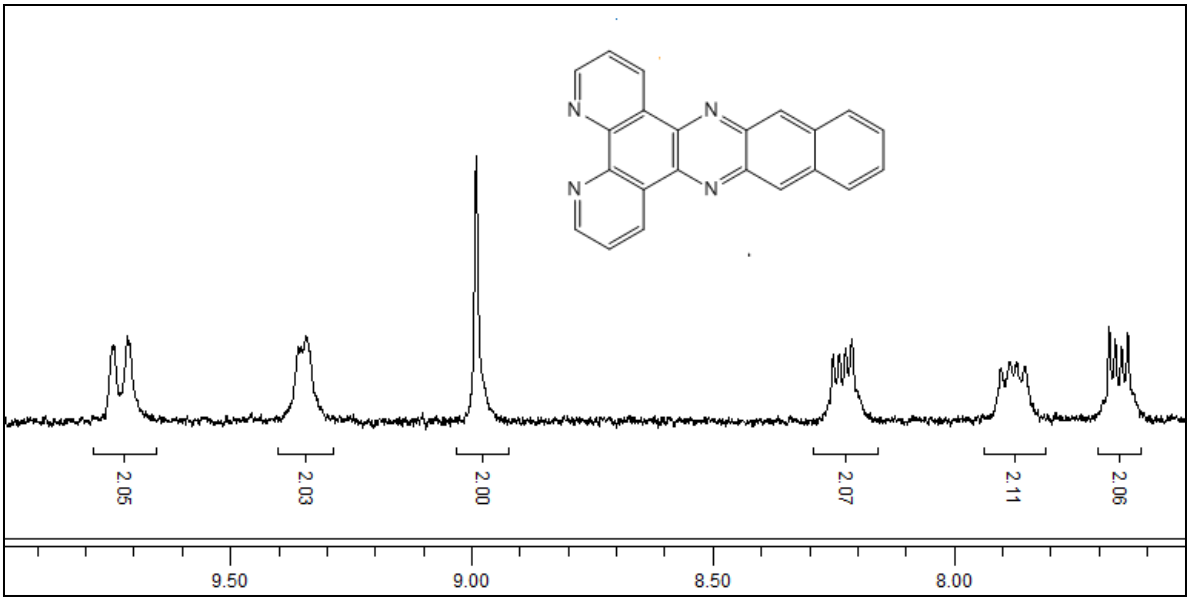

Figure 18. ^{1}H NMR spectrum of dppn (250 MHz, chloroform-d)

3.2.2 Synthesis of Dirhodium Bis-Acetate Complexes

3.2.2.1 Synthesis of $Rh_2(CH_3COO)_4$

$Rh_2(CH_3COO)_4$ was synthesized from $RhCl_3 \cdot 3H_2O$, sodium acetate trihydrate and glacial acetic acid by a published procedure[14]. A mixture of $RhCl_3 \cdot 3H_2O$ (91.5 mg, 0.35 mmol), $CH_3COONa \cdot 3H_2O$ (183.8 mg, 1.36 mmol) and glacial acetic acid (10 mL) was refluxed for 1 h in ethanol (200 proof, 10 mL). The solvent was then evaporated under reduced pressure and the resulting solid was transferred to a centrifugation vial with methanol. The solution was then cooled down in the freezer. The mixture was centrifugated and the supernatant was removed with a pipette and discarded. The precipitate was dried in the oven at 80˚C overnight to yield $Rh_2(CH_3COO)_4$ (51.8 mg, 0.12 mmol, 68 % yield) as an emerald green solid. The product was characterized by ^{1}H NMR (250 MHz, methanol-d_4). The NMR spectrum is shown in **Figure 19**.

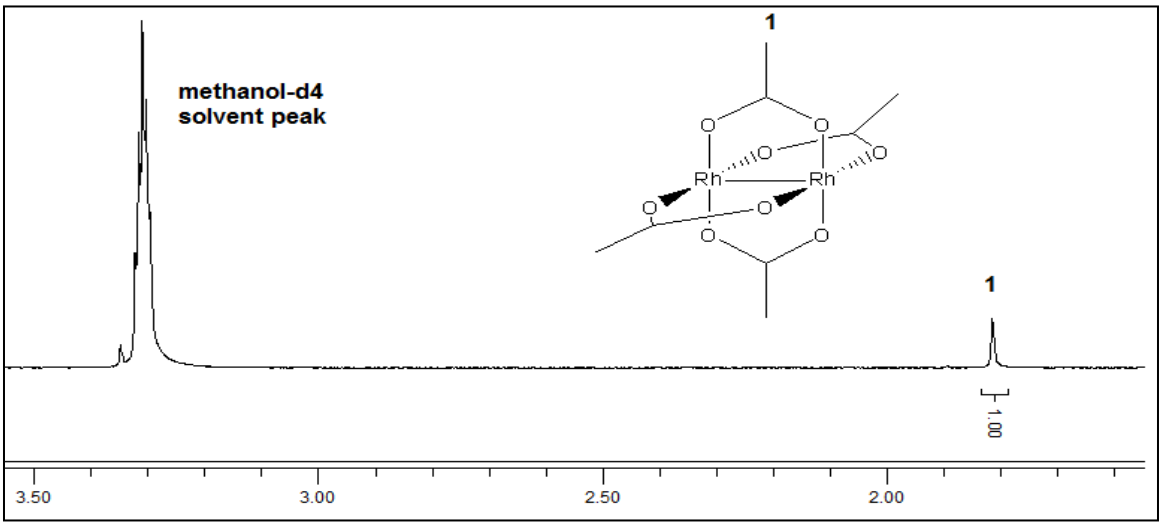

Figure 19. ^{1}H NMR spectrum of $Rh_2(OOCCH_3)_4$. (250 MHz, methanol-d_4)

3.2.2.2 Synthesis of cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6][BF_4]_2$

Cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6][BF_4]_2$ was synthesized from $Rh_2(CH_3COO)_4$ and triethyloxonium tetrafluoroborate (TEO) by a modified published procedure[15]. A mixture of $Rh_2(CH_3COO)_4$ (19.8 mg, 0.044 mmol) and triethyloxonium tetrafluoroborate (1M, 175 µL, 0.175 mmol) was reacted at 45˚C for 5 h in acetonitrile (1.0 mL). The solvent was then evaporated under a stream of air and resulting solid was washed with dichloromethane (3 x 5 mL) and with diethyl ether (2 x 5 mL), centrifugating and removing the supernatant with a pipette after each wash. The resulting solid was dissolved in a minimal volume of acetonitrile and transferred to a vial. The solvent was evaporated under a steam of air to yield cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6][BF_4]_2$ (23.8 mg, 0.032 mmol, 72 % yield) as an bright purple solid. The product was characterized by ^1H NMR (250 MHz, deuterium oxide). The NMR spectrum is shown in **Figure 20**.

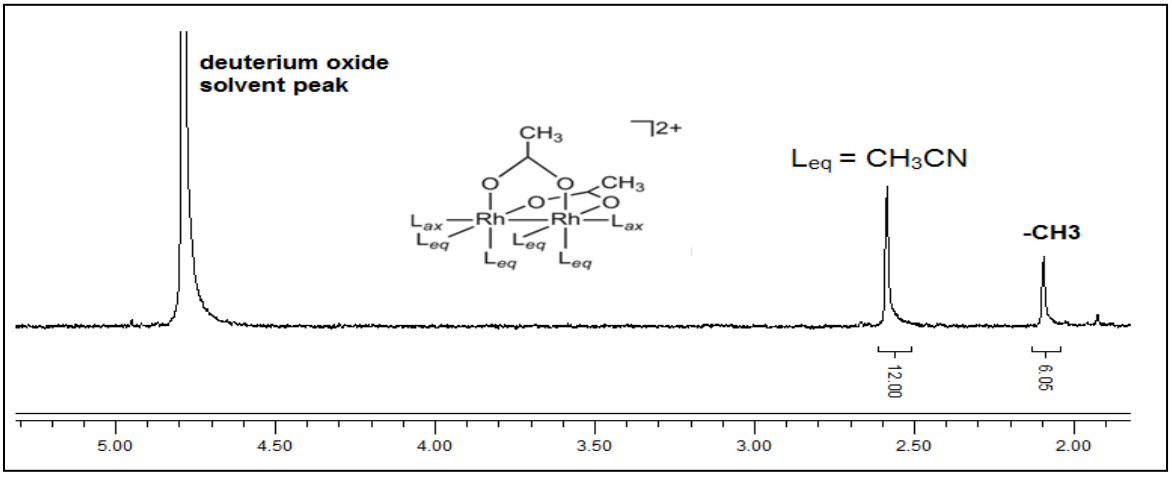

Figure 20. ^1H NMR spectrum cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6][BF_4]_2$ (250 MHz, deuterium oxide)

The product was also characterized by ESI-MS. The mass spectrum of the product is shown in **Figure 21.** The base peak, $[Rh_2(CH_3COO)_2(CH_3CN)_3F]^+$, was observed at *m/z* = 466.0, and the peak at *m/z* = 429.9 corresponds to $[Rh_2(CH_3COO)_2(CH_3CN)_2F]^+$. The identity of the other peaks remains unknown.

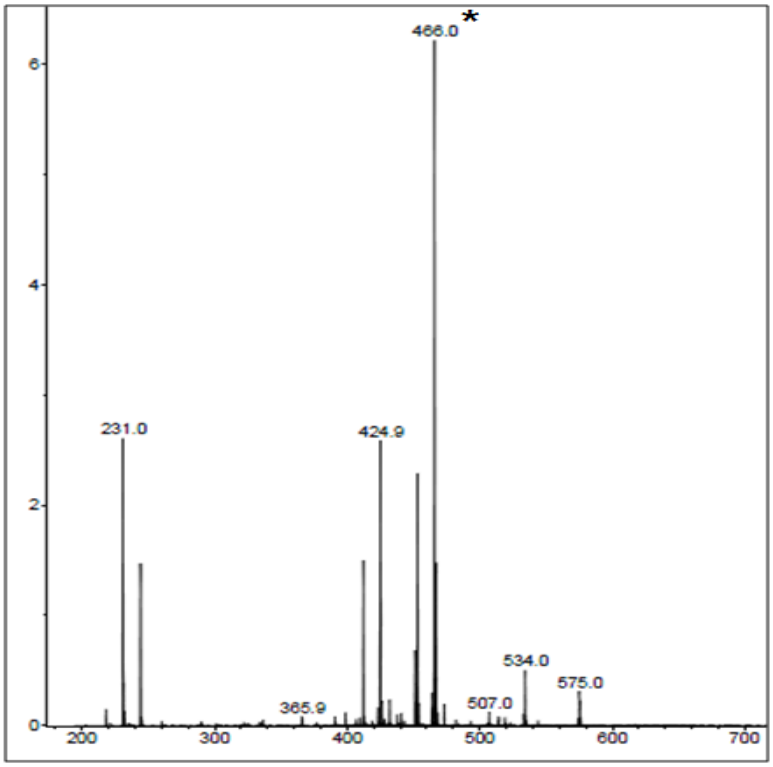

Figure 21. ESI-MS spectrum of cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6][BF_4]_2$. The base peak ion corresponds to the formula $[Rh_2(CH_3COO)_2(CH_3CN)_3F]^+$, seen at m/z = 466.0

3.2.2.3 Synthesis of cis-$[Rh_2(CH_3COO)_2(bpy)(CH_3CN)_4][BF_4]_2$

The synthesis of cis-$[Rh_2(CH_3COO)_2(bpy)(CH_3CN)_4][BF_4]_2$ was attempted according a published procedure[16]. The complex cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6][BF_4]_2$ (2.5 mg, 3.4 µmol) was reacted with bpy (0.6 mg, 3.8 µmol) in acetonitrile (1.0 mL) at room temperature for 24 h. The reaction was treated with excess diethyl ether and a precipitate

formed. The mixture was centrifugated and the supernatant was removed with a pipette and discarded. The precipitate was washed again with diethyl ether, centrifugated and the solvent was removed and discarded. The resulting solid was transferred to a vial with a minimal volume of acetonitrile and a ^{1}H NMR (250 MHz, deuterium oxide) spectrum was collected, which showed that the reaction was unsuccessful. The NMR spectrum showed the presence of multiple products and impurities including unreacted bpy and starting dirhodium complex, bis-bpy adduct, bpy bound in the axial position, etc among other unknown species.

Because cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6][BF_4]_2$ is photoactive[9], the complex was reacted with bpy in the dark under conditions similar conditions to those described. This reaction was also unsuccessful. In another attempt to synthesize the cis-$[Rh_2(CH_3COO)_2(bpy)(CH_3CN)_4][BF_4]_2$ complex, a solution of cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6][BF_4]_2$ (10 mg, 13.4 µmol) and bpy (2.2 mg, 13.7 µmol) in acetonitrile (2 mL) was irradiated with 455 nm light for 4 h, monitoring the reaction by ^{1}H NMR (400 MHz, deuterium oxide) after 0.5 h, 1 h, 2 h, and 4 h. The NMR spectra again showed a complex mixture of products.

Lastly, a solution of cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6][BF_4]_2$ (10 mg, 13.4 µmol) and bpy (2.2 mg, 13.7 µmol) was refluxed for 24 h in acetonitrile (5 mL). A similar reaction was performed in methanol, a weaker coordination ligand, refluxing for 7 days. After inspection by ^{1}H NMR (400 MHz, deuterium oxide) spectroscopy, both reactions failed to yield primarily cis-$[Rh_2(CH_3COO)_2(bpy)(CH_3CN)_4][BF_4]_2$.

3.3 Discussion

Like the H,H complex, cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6]^{2+}$ also undergoes thermal exchange of the Rh-CH_3CN_{ax} ligands and covalent binding is not triggered by this exchange[9]. Photolysis studies by electronic absorption showed that two Rh-CH_3CN_{eq} bound to the same rhodium atom are exchanged for water[9], which generates cis-$[Rh_2(CH_3COO)_2(CH_3CN)_2(H_2O)_4]^{2+}$, the activated complex that binds to DNA covalently. DNA binding assay of the cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6]^{2+}$ complex showed no decrease in mobility of DNA in the dark, and binding upon irradiation. Moreover, a 34-fold increase in cell death upon irradiation was observed with Hs-27 human skin cells[9].

The attempt to exchange two Rh-CH_3CN_{eq} ligands in cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6]^{2+}$ for bidentate bpy has been so far unsuccessful. It has been shown that the formation of the cis-$[Rh_2(CH_3COO)_2(bpy)(CH_3CN)_4]^{2+}$ is photoinduced in the reaction of cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6]^{2+}$ and bpy in 1:1 ratio[17]. However, these studies did not involve the isolation of the mono-bpy dirhodium bis-acetate complex and were primarily performed at low concentration.

Future studies of dirhodium bis-acetate complexes will involve the synthesis of cis-$[Rh_2(CH_3COO)_2(N\text{-}N)(CH_3CN)_4]^{2+}$ (N-N = bpy, dpq, dppz, dppn) and the photolysis studies of these compounds to determine if they can be activated upon irradiation by the exchange of the remaining Rh-CH_3CN_{eq} ligands. In addition, it has to be determined if the dpq, dppz, and dppn analogs of the series can intercalate between the DNA bases to achieve dual-binding.

Chapter 4: Conclusions

Preliminary experiments have shown that dirhodium bis-amidate complexes have a promising potential as dual-binding photodynamic therapy agents. The successfully synthesized model complex H,H-bpy was shown to bind to DNA upon irradiation. This indicates that the other complexes of the series, H,H-dpq and H,H-dppz have high possibilities of also binding to DNA upon activation by light. Future work regarding these complexes will include the study of the intercalating ability of the dpq and dppz ligands bound to the dirhodium core. It has yet to be shown whether H,H-dpq and H,H-dppz are inactive in the dark, since the increased affinity for DNA due to the extended π-system may affect the activity of the complexes.

Regarding the dirhodium bis-acetate complexes, future work will involve the synthesis of the complexes of the series cis-$[Rh_2(CH_3COO)_2(N\text{-}N)(CH_3CN)_4]^{2+}$, where N-N = bpy (the model complex for no intercalation), dpq, dppz, and dppn. Since the precursor molecule cis-$[Rh_2(CH_3COO)_2(CH_3CN)_6]^{2+}$ has shown desired photodynamic therapy agent features, these complexes may also have a strong potential as photoactivated dual-binding agents.

Both types of complexes, dirhodium bis-acetate and dirhodium bis-amidate complexes have the potential to be the next generation photodynamic therapy agents, which may

represent a breakthrough for transition metal complexes as new drugs for treating malignant conditions based on their novel mode of action.

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