CHAPTER 2: PROTOCOLS[ρ]

2.1: INTRODUCTION

Any doctoral thesis functions primarily as the synthesis and exposition of the writer's research. However, many theses, especially those from experimental laboratories, moonlight in a second, almost equally important role: reference work for future researchers. Indeed, these pages offer a singular opportunity to pass on the details of fundamental experimental protocols, unhastened and unabridged by the exigencies of publication.

In this chapter, a set of experimental procedures essential to the study of metalloinsertors is described.[1] Guidelines for both syntheses and nucleic acid experiments are addressed; however, procedures specific to a single line of investigation (e.g. the synthesis of eilatin or crystal structure refinement) are included in the pertinent chapter.

2.2: INSTRUMENTATION AND MATERIALS

All reagents were obtained from commercial sources and used as received without further purification. NMR spectra were recorded on a Varian 400 MHz spectrometer. Mass spectrometry was performed at either the Caltech mass spectrometry facility or in the Beckman Institute Protein/Peptide Micro Analytical Laboratory (PPMAL). Absorption spectra were recorded on a Beckman DU 7400 spectrophotometer, and circular dichroism spectra were recorded on a Jasco J-500A spectropolarimeter. Unless otherwise noted, all reactions were performed under ambient conditions.

[ρ] Parts of this chapter were adapted from Zeglis, B. M.; Barton, J. K. DNA base mismatch detection with bulky rhodium metallointercalators: synthesis and applications. *Nature Protocols.* **2007**, *2(2)*, 357−371.

Oligonucleotides were synthesized on an ABI 3400 DNA synthesizer and purified in duplicate (DMT-off and DMT-on) before use. HPLC purifications were performed on an HP1100 high-pressure liquid chromatography system equipped with a diode array detector using a Varian DynaMax C18 semipreparative column. Irradiations were performed using an Oriel Instruments solar simulator (320−440 nm). All PAGE experiments described employed denaturing 20% polyacrylamide gels (SequaGel, National Diagnostics) and were performed according to published procedures. Further, gels were developed using Molecular Dynamics phosphorimaging screens and a Molecular Dynamics Storm 820 phosphorimager and were subsequently visualized and quantified with Molecular Dynamics ImageQuant software. Further instrumentation and materials specific to individual investigations (e.g. X-ray irradiation sources, etc.) will be addressed in the relevant chapters.

2.3: Synthetic Protocols

2.3.1: Synthesis of *ortho*-quinone ligand precursors

2.3.1.1: Synthesis of chrysene-5,6-quinone

Chrysene-5,6-quinone was produced by the method of Graebe and Honigsburger with minor modifications.[2] A 250 mL round bottom flask was charged with 10.0 g (44 mmol) chrysene and 110 mL glacial acetic acid (**Figure 2.1**). Subsequently, 46 g sodium dichromate were added slowly with stirring to the reaction mixture, and the resultant slurry was heated to reflux. The reaction was then monitored until white solid could no longer be seen in the refluxing suspension (approximately 24−36 h). At this point, heating was stopped, the mixture was poured into 100 mL boiling water, and the product

was hot filtered through a medium glass frit. The resultant orange precipitate was washed three times with 100 mL boiling water and subsequently recrystallized from hot ethanol to yield the orange, crystalline product (85% yield).

^{1}H-NMR (CD_2Cl_2): 9.39 ppm (d, 1H); 8.16 ppm (m, 4H); 7.92 (d, 1H); 7.77 ppm (t of d, 2H); 7.57 ppm (t of d, 2H).

ESI-MS (m/z): 257 $[M+H]^+$

2.3.1.2: SYNTHESIS OF 3,4-BENZO[A]PHENAZINE QUINONE

The 3,4-benzo[a]-phenazine quinone ligand precursor was synthesized according to the procedure published by Junicke et al.[3, 4] A 250 mL round bottom flask was charged with 4.5 g (20 mmol) 2,3-dichloro-1,4-napthoquinone and 2.0 g (20 mmol) *o*-phenylene diamine in 125 mL pyridine (**Figure 2.2**). The solution was brought to reflux and allowed to stir with heating for 1 h. The reaction mixture was then allowed to cool to room temperature and was subsequently filtered on a medium glass frit to yield a brown-red solid (the intermediate, 6-pyridinium-benzo[a]phenazine-5-olate) in quantitative yield.

The red-brown solid was then freed from excess pyridine via rotary evaporation. After weighing, the solid was placed in a second round-bottom flask and dissolved in 10 mL glacial acetic acid and 1 mL concentrated nitric acid. Water (0.66 mL/g) was added accordingly. The resultant mixture was heated in a boiling water bath for 1 h (the reaction is complete when only yellow-orange precipitate remains). After heating, the product was precipitated by pouring the solution into 100 mL cold water, collected via filtration on a medium glass frit, and washed three times with ethanol (50 mL) and three times with

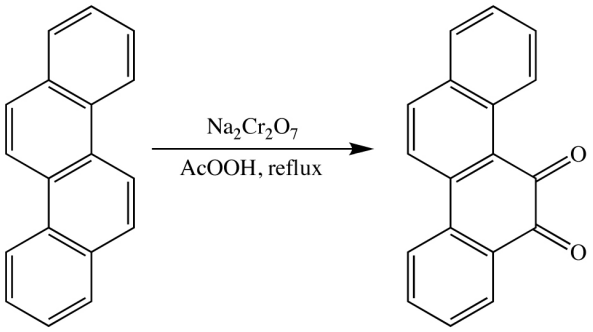


Figure 2.1: Synthesis of chrysene-5,6-quinone. Chrysene is oxidized to chrysene-5,6-quinone with sodium dichromate.

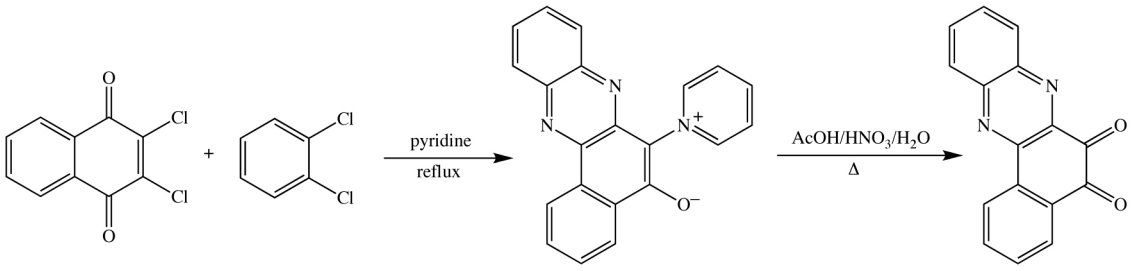


Figure 2.2: Synthesis of 3.4-benzo[a]phenazine quinone. 2,3-dichloro-1,4-naphthoquinone is reacted with *ortho*-phenylene diamine in pyridine to produce a zwitterionic intermediate (6-pyridinium-benzo[a]phenazine-5-olate) that is subsequently oxidized by nitric acid to form the desired product.

diethyl ether (50 mL). A yellow-green powder results (>75% yield) that can be re-crystallized from 7:3 chloroform:ethyl acetate (vol:vol).

^{1}H-NMR ($CDCl_3$): 8.78 ppm (d, 1H); 8.24 ppm (d, 1H); 8.21 ppm (d, 1H); 8.15 ppm (d, 1H); 8.01 ppm (t, 1H); 7.94 ppm (t, 1H); 7.90 ppm (t, 1H); 7.73 ppm (t, 1H).

ESI-MS: 261 $[M+H]^+$

2.3.2: SYNTHESIS OF BISDIPYRIDYL COMPLEXES

2.3.2.1: SYNTHESIS OF $[Rh(bpy)_2Cl_2]Cl$

[$Rh(bpy)_2Cl_2]^+$ was prepared by analogy to the method of Gillard and co-workers with minor modifications.[5, 6] A 50 mL round-bottom flask was charged with 0.64 g $RhCl_3$ (2.8 mmol) and 50 mg hydrazine monohydrochloride in 12.5 mL deionized water. A solution of 0.85 g (5.6 mmol) bipyridine in 12.5 mL ethanol was then added, and the resultant solution was deoxygenated via the freeze-pump-thaw technique. The degassed mixture was then brought to reflux under argon and heated until all the materials have dissolved (approximately 20 minutes) and formed a yellow-orange solution. While still hot, the resultant solution was filtered through a medium glass frit and immediately placed in a refrigerator to chill at 4 ºC overnight to promote crystallization. The next morning, the resulting yellow crystals (0.95 g, 70% yield) were collected by filtration and dried under vacuum. A synthetic scheme for this reaction and those described in 2.3.4−2.3.9 are shown in **Figure 2.3**.

^{1}H-NMR (d_6-DMSO): 9.71 ppm (d, 2H); 9.0 ppm (d, 2H); 8.91 ppm (d, 2H); 8.63 ppm (t, 2H); 8.33 ppm (t, 2H); 8.17 ppm (t, 2H); 7.82 ppm (d, 2H); 7.59 ppm (t, 2H).

ESI-MS: 486 $[M]^+$

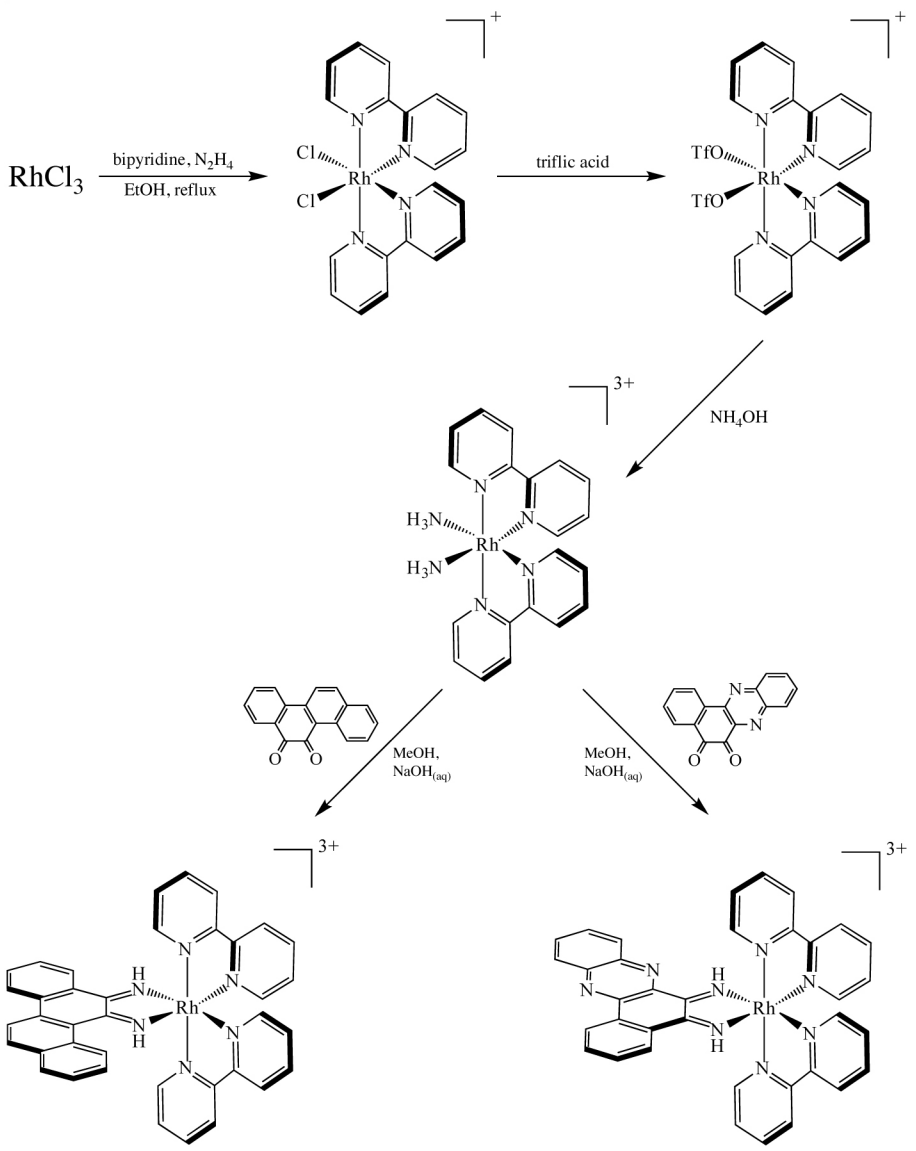


Figure 2.3: Scheme for the synthesis of *rac*-Rh(bpy)$_2$(chrysi)$^{3+}$ and *rac*-Rh(bpy)$_2$(phzi)$^{3+}$

2.3.2.2: SYNTHESIS OF $[Rh(bpy)_2(OTf)_2]OTf$

$[Rh(bpy)_2(OTf)_2]OTf$ and $[Rh(bpy)_2(NH_3)_2](X)_3$ were prepared by the method of Gidney and coworkers with minor modifications.[7] A 50-mL Schlenk flask was charged with 500 mg $Rh(bpy)_2(Cl)_2^+$ (~ 1 mmol) and deoxygenated by evacuating it and refilling with $Ar_{(g)}$ three times. Subsequently, 5 g triflic acid (excess) were added carefully to the reaction vessel under positive argon pressure (caution: triflic acid is very reactive, pyrophoric, and, to make matters worse, eats through gloves). After the addition of HOTf, the reaction vessel was closed with a rubber septum, the septum was pierced with a 16-gauge needle, and the flask was purged with argon for 60 seconds. The dark red reaction mixture was allowed to stir for 16 h with periodic $Ar_{(g)}$ purges to remove HCl generated by the reaction. After 16 h, the reaction mixture was added dropwise to 300 mL vigorously stirring diethyl ether cooled to -78 ºC. The resultant yellowish precipitate was collected via filtration on a Buchner funnel, washed with cold diethyl ether, and used as promptly as possible.

2.3.2.3: SYNTHESIS OF $[Rh(bpy)_2(NH_3)_2](X)_3$

In a 250-mL round-bottom flask fitted with a reflux condenser, 500 mg $Rh(bpy)_2(OTf)_2^+$ (0.6 mmol) were suspended in 50 mL concentrated NH_4OH. The suspension was stirred, brought to reflux, and heated until all of the material went into solution (15 min); over the course of heating, the insoluble, singly charged complex is converted to the more soluble, triply-charged complex. Depending on the desired counter-ion, the product can be isolated one of two ways: (a) if the PF_6^- salt is desired, excess NH_4PF_6 should be added to the solution, and the reaction mixture should be

cooled overnight to facilitate precipitation; (b) if the OTf$^-$ counter-ion is desired, the NH_4OH should simply be removed by rotary evaporation at room temperature. Recoveries are best using the evaporation method and range from 80−100% depending on mechanical losses.

^{1}H NMR (d_6-acetone, PF_6^- salt): 9.45 ppm (d, 2H); 9.05 ppm (d, 2H); 8.89 ppm (d, 2H); 8.79 ppm (split t, 2H); 8.45 ppm (split t, 2H); 8.30 ppm (split t, 2H); 8.05 ppm (d, 2H); 7.74 ppm (split t, 2H); 5.06 ppm (broad s, 6H).

ESI-MS: 449 $[M-2H]^+$

2.3.2.4: METALLATION BY CONDENSATION

The metallation of the intercalating and inserting ligands (*i.e.* phi, chrysi, phzi) is accomplished via the condensation of the ligand *ortho*-quinones onto a metal *cis*-diammine complex (**Figure 2.4**).[6] This clean and facile reaction is based on the pioneering work of Sargeson on inter- and intramolecular condensation reactions.[8−13] Indeed, Schiff base condensations have been shown to be possible with coordinated ligands with retention of configuration at the metal center. Two alternative methods exist: (1) the metallation of the diamine followed by the air-mediated oxidation of the ligand to the corresponding diimine[14−16] and (2) the *in situ* deprotection and subsequent metallation of a trimethylsilylimine variant of the ligand of interest.[17] Both strategies, however, are severely limited by product yield and the requirement for cumbersome anaerobic conditions.

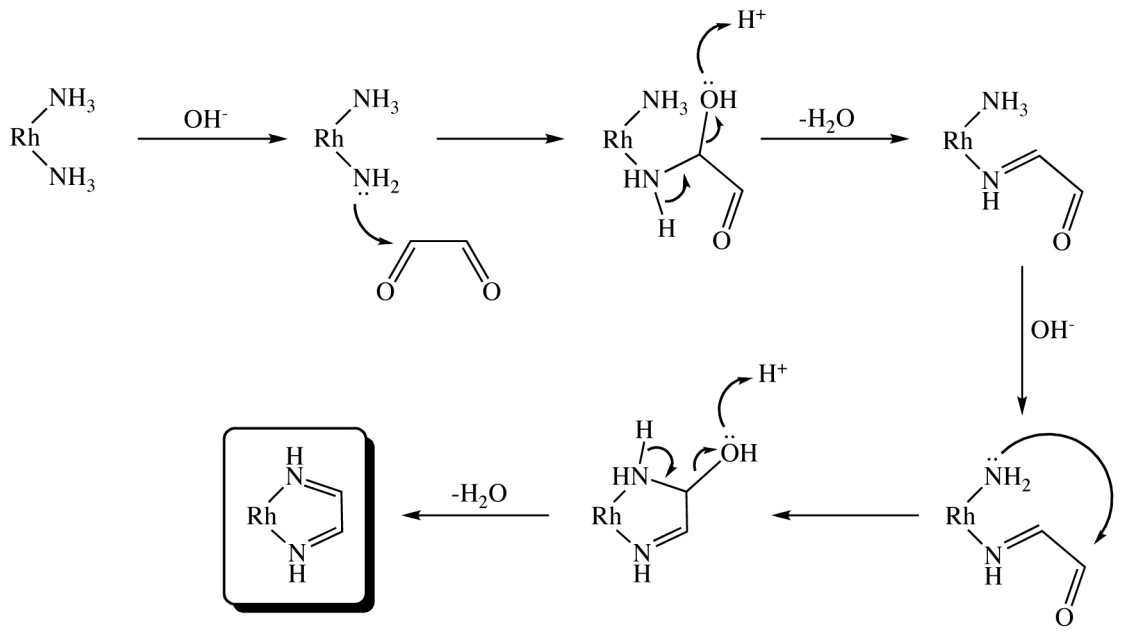


Figure 2.4: *O*-quinone condensation mechanism. One of the ammines in a *cis*-diammine complex is deprotonated by a base and attacks the quinone carbon to form a hemiaminal intermediate. This structure can then dehydrate to form an imine. This reaction is then followed by an identical, intramolecular condensation to form the desired diimine ligand.

In the condensation reaction, one of the ammines in a *cis*-diammine complex is deprotonated by a base and attacks the quinone carbon to form a hemiaminal intermediate, a structure that quickly and cleanly dehydrates to form an imine. This reaction is then followed by an identical, intramolecular condensation to form the desired diimine ligand. Many metal *cis*-ammine complexes can be employed for this reaction. However, one must pay particular attention to the pKa values for the coordinated ammines in question.[18, 19] *Cis*-ammine complexes of Rh(III) work well, because the pKa of the metal-bound ammines is approximately 10 and are thus readily deprotonated by aqueous NaOH. The pKa values of ammines in analogous Ru(II) complexes, in contrast, are higher and mostly likely require a stronger base to make the reaction proceed.

2.3.2.5: SYNTHESIS OF $[Rh(bpy)_2(chrysi)](Cl)_3$

In a 100-mL round-bottom flask, 195 mg $[Rh(bpy)_2(NH_3)_2](PF_6)_3$ (0.2 mmol) and 57 mg chrysene-5,6-quinone (0.22 mmol) were dissolved in 50 mL MeCN with rapid stirring under ambient conditions.[20, 21] After 10 min, 2 mL aqueous sodium hydroxide (0.4 M) were added, and the reaction vessel was closed to prevent evaporation. After 3 h, the reaction was halted by bringing the pH of the solution to 7.0 by adding a stoichiometric amount of $HCl_{(aq)}$. By this point, the reaction should have changed color dramatically from orange/yellow to dark red; the reaction can also be monitored by TLC using silica F plates in a solvent system of 3:1:1 MeCN/H_2O/MeOH (vol/vol/vol) with 0.1 M KNO_3. After neutralization, the MeCN and H_2O were removed *in vacuo* by rotary evaporation.

The resulting solid was re-dissolved in a minimum volume of water and purified via cation-exchange chromatography using Sephadex SP-C25 ion exchange resin. Four inches of resin pre-equilibrated with 0.05 M $MgCl_2$ were poured into a 1−1.5 inch diameter column and subsequently washed with copious (500 mL) deionized H_2O. The rhodium complex was loaded onto the column simply by passing the aqueous Rh solution through the resin (the rhodium complex will 'stick' to the top of the column, forming a thin, dark red band). The complex was then eluted by slowly increasing the [$MgCl_2$] in the eluent in 500-mL batches, starting with 0.05 M $MgCl_2$ and increasing in increments of 0.05 M until the red band of the metal complex has passed through the column. The resultant eluted solution was concentrated on a reverse-phase cartridge primed with MeOH and eluted with 1:1:0.001 H_2O/MeCN/TFA (vol/vol/vol). Finally, the solvent was removed by lyophilization to yield the product as a dark red powder (25 mg, 75%). The complex can be further purified via reverse-phase high-performance liquid chromatography using an HP1100 HPLC system, a Varian DynaMax C18 semipreparative column, and an elution gradient of 85:15 to 40:60 H_2O (0.1% TFA):MeCN (0.1% TFA) over 60 min.

^{1}H-NMR (d_4-methanol): 8.94 ppm (t, 2H); 8.86 ppm (t, 2H); 8.80 ppm (d, 1H); 8.77 ppm (d, 1H); 8.56 ppm (split t, 2H); 8.44 ppm (m, 5H); 8.40 ppm (d, 1H); 8.15 ppm (m, 1H); 8.03 ppm (m, 1H); 7.95 ppm (m, 3H); 7.86 ppm (d, 1H); 7.81 ppm (d, 1H); 7.64 ppm (m, 5H).

ESI-MS: 671 $[M-2H]^+$

UV-Vis (H_2O, pH 7.0, **Figure 2.5**): λ_{max} 302 nm (ε = 57,000 M^{-1}), 315 nm (ε = 52,200 M^{-1}), 391 nm (ε = 10,600 M^{-1}).

2.3.2.6: SYNTHESIS OF $[Rh(bpy)_2(phzi)](Cl)_3$

In a 100-mL round-bottom flask, 100 mg $[Rh(bpy)_2(NH_3)_2](PF_6)_3$ (0.1 mmol) and 35 mg 3,2-benzo[a]phenazine quinone (0.125 mmol) were dissolved in 50 mL MeCN with rapid stirring under ambient conditions.[3] After 10 min, 2 mL aqueous sodium hydroxide (0.4 M) were added, and the reaction vessel was closed to prevent evaporation. After 3 h, the reaction was halted by bringing the pH of the solution to 7.0 by adding a stoichiometric amount of $HCl_{(aq)}$. By this point, the reaction should have changed color dramatically from orange-yellow to dark brown-yellow; the reaction can also be monitored by TLC using silica F plates in a solvent system of 3:1:1 MeCN/H_2O/MeOH (vol/vol/vol) with 0.1 M KNO_3. After neutralization, the MeCN and H_2O were removed *in vacuo* by rotary evaporation.

The resulting solid was re-dissolved in a minimum volume of water and purified via cation-exchange chromatography using Sephadex SP-C25 ion exchange resin. Four inches of resin pre-equilibrated with 0.05 M $MgCl_2$ were poured into a 1−1.5 inch diameter column and subsequently washed with copious (500 mL) deionized H_2O. The rhodium complex was loaded onto the column simply by passing the aqueous Rh solution through the resin (the rhodium complex will ‘stick’ to the top of the column, forming a thin, dark brown band). The complex was then eluted by slowly increasing the [$MgCl_2$] in the eluent in 500-mL batches, starting with 0.05 M $MgCl_2$ and increasing in increments of 0.05 M until the red band of the metal complex has passed through the column. The resultant eluted solution was concentrated on a reverse-phase cartridge primed with MeOH and eluted with 1:1:0.001 H_2O/MeCN/TFA (vol/vol/vol). Finally, the solvent was removed by lyophilization to yield the product as a brownish-yellow powder (20 mg,

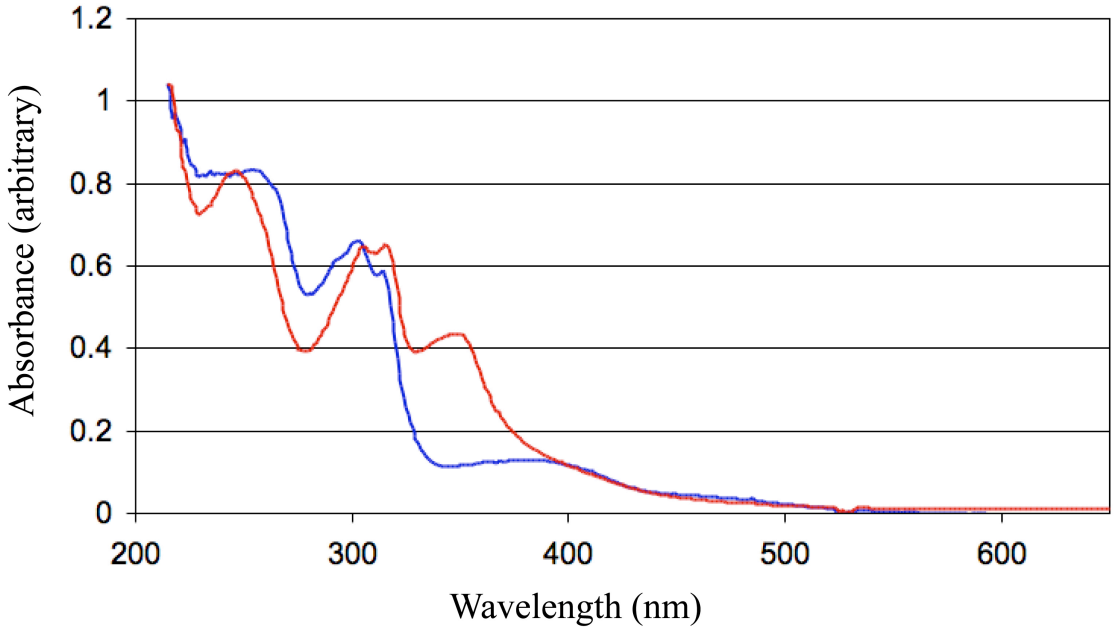


Figure 2.5: UV-Vis spectra of $Rh(bpy)_2(chrysi)^{3+}$ and $Rh(bpy)_2(phzi)^{3+}$. Extinction coefficients for $Rh(bpy)_2(chrysi)^{3+}$ (blue): 302 nm ($\varepsilon = 57{,}000\ M^{-1}$), 315 nm ($\varepsilon = 52{,}200\ M^{-1}$), 391 nm ($\varepsilon = 10{,}600\ M^{-1}$). Extinction coefficients for $Rh(bpy)_2(phzi)^{3+}$ (red): 304 nm ($\varepsilon = 65{,}800\ M^{-1}$), 314 nm ($\varepsilon = 67{,}300\ M^{-1}$), 343 nm ($\varepsilon = 39{,}300\ M^{-1}$). Both spectra were taken in H_2O at pH 7.0.

60%). The complex can be further purified via reverse-phase high-performance liquid chromatography using an HP1100 HPLC system, a Varian DynaMax C18 semipreparative column, and an elution gradient of 85:15 to 40:60 H_2O (0.1% TFA):MeCN (0.1% TFA) over 60 min.

^{1}H-NMR (d_6-DMSO): 14.88 ppm (s, 1H); 14.70 ppm (s, 1H); 9.02 ppm (m, 4H); 9.02 ppm (m, 4H); 8.91 ppm (d, 2H); 8.72 ppm (d, 1H); 8.60 ppm (t, 2H); 8.54 ppm (d, 1H); 8.47 ppm (t, 2H); 8.32 ppm (d, 1H); 8.20 ppm (d, 1H); 8.11 ppm (t, 1H); 8.02 (m, 3H); 7.94 (t, 1H); 7.84 (t, 1H); 7.75 (m, 3H); 7.69 (d, 1H).

ESI-MS: 671 $[M-2H]^+$

UV-Vis (H_2O, pH 7.0, **Figure 2.5**): λ_{max} 304 nm (ε = 65,800 M^{-1}), 314 nm (ε = 67,300 M^{-1}), 343 nm (ε = 39,300 M^{-1}).

2.3.2.7: SYNTHESIS OF $[Rh(bpy)_2(phi)](Cl)_3$

In a 100-mL round-bottom flask, 70 mg $[Rh(bpy)_2(NH_3)_2](Cl)_3$ (0.77 mmol) and 20 mg 9,10-phenanthrenequinone (0.97 mmol) were dissolved in 50 mL MeCN with rapid stirring under ambient conditions.[22] After 10 min, 2 mL aqueous sodium hydroxide (0.4 M) were added, and the reaction vessel was closed to prevent evaporation. After 3 h, the reaction was halted by bringing the pH of the solution to 7.0 by adding a stoichiometric amount of $HCl_{(aq)}$. By this point, the reaction should have changed color dramatically from orange/yellow to dark red; the reaction can also be monitored by TLC using silica F plates in a solvent system of 3:1:1 MeCN/H_2O/MeOH (vol/vol/vol) with 0.1 M KNO_3. After neutralization, the MeCN and H_2O were removed *in vacuo* by rotary evaporation.

The resulting solid was re-dissolved in a minimum volume of water and purified via cation-exchange chromatography using Sephadex SP-C25 ion exchange resin. Four inches of resin pre-equilibrated with 0.05 M $MgCl_2$ were poured into a 1−1.5 inch diameter column and subsequently washed with copious (500 mL) deionized H_2O. The rhodium complex was loaded onto the column simply by passing the aqueous Rh solution through the resin (the rhodium complex will 'stick' to the top of the column, forming a thin, dark orange band). The complex was then eluted by slowly increasing the [$MgCl_2$] in the eluent in 500-mL batches, starting with 0.05 M $MgCl_2$ and increasing in increments of 0.05 M until the red band of the metal complex has passed through the column. The resultant eluted solution was concentrated on a reverse-phase cartridge primed with MeOH and eluted with 1:1:0.001 H_2O/MeCN/TFA (vol/vol/vol). Finally, the solvent was removed by lyophilization to yield the product as a red-orange powder. The complex can be further purified via reverse-phase high-performance liquid chromatography using an HP1100 HPLC system, a Varian DynaMax C18 semipreparative column, and an elution gradient of 85:15 to 40:60 H_2O (0.1% TFA):MeCN (0.1% TFA) over 60 min.

^{1}H-NMR (D_2O): 8.72 ppm (d, 2H); 8.65 ppm (d, 2H); 8.55 ppm (d, 2H); 8.50 ppm (t, 2H); 8.35 ppm (t, 2H); 8.25 ppm (two overlapping t, 4H); 7.75 ppm (two overlapping t, 4H); 7.70 ppm (d, 2H); 7.60 ppm (t, 2H); 7.55 ppm (t, 2H).

ESI-MS: 619 $[M-2H]^+$

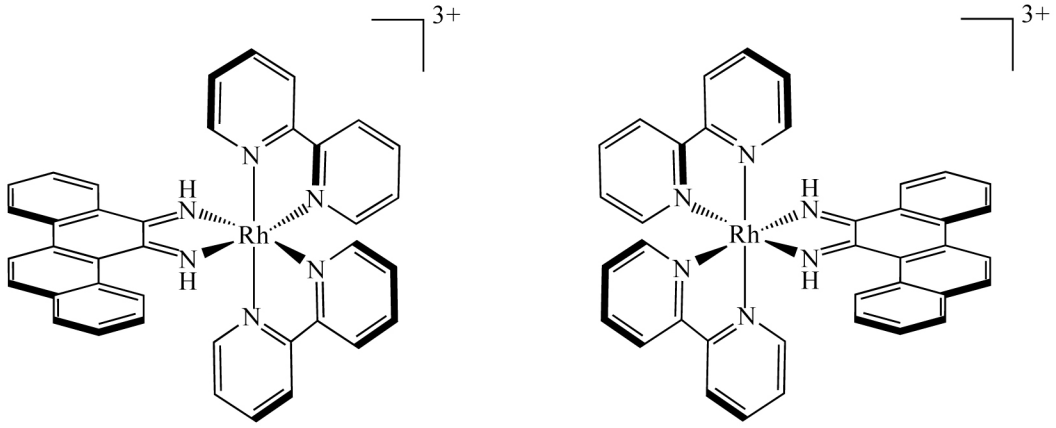
UV-Vis (H_2O, pH 7.0): λ_{max} 301 nm, 313 nm, 362 nm (ε = 19,400 M^{-1}).

2.3.3: Enantiomeric Separation of Δ- and Λ-Rh(bpy)$_2$(chrysi)$^{3+}$

Because metalloinsertors bind DNA enantiospecifically, in many cases it has been advantageous to separate the two enantiomers of a complex (**Figure 2.6**). Cation exchange chromatography with a chiral eluent is employed.[23, 24]

In order to separate the enantiomers, one very long column (1.7 m x 1.5 cm) and two smaller columns (0.5 m x 1.5 cm, referred to as guard columns) were filled with Sephadex SP-C25 ion exchange resin equilibrated with water. All three columns were subsequently eluted with 0.15 M (+)-KSb-tartrate. One of the guard columns was then set aside, and the other guard column and the long column were arranged in series with a peristaltic pump as shown in **Figure 2.7**. *Rac*-Rh(bpy)$_2$(chrysi)$^{3+}$ (1 g) dissolved in 5 mL water was then loaded carefully on top of the large column to form a very small band. The pump was turned on and allowed to run continuously, with the chiral eluent in a closed loop. After approximately 1 d, separation became apparent. The fast band is the Λ-enantiomer, while the slower band is the Δ-enantiomer. The first guard column was detached after it 'caught' the first band and was replaced with the second guard column. The second guard column was detached after it 'caught' the second band.

The two guard columns were then washed with 0.05 M $MgCl_2$ to remove the remaining (+)-KSb-tartrate. With careful attention paid to the segregation of the two solutions, the compounds were removed from the guard columns by washing the columns with 0.5 M $MgCl_2$. Each solution was then concentrated on a 5 g C18 cartridge (Waters), washed with copious water, eluted with 1:1:0.001 MeCN/H_2O/TFA (vol/vol/vol), and lyophilized to dryness to yield a red powder (~300 mg for each enantiomer).



Δ-Rh(bpy)$_2$(chrysi)$^{3+}$ Λ-Rh(bpy)$_2$(chrysi)$^{3+}$

Figure 2.6: Structures of Δ- AND Λ-Rh(bpy)$_2$(chrysi)$^{3+}$

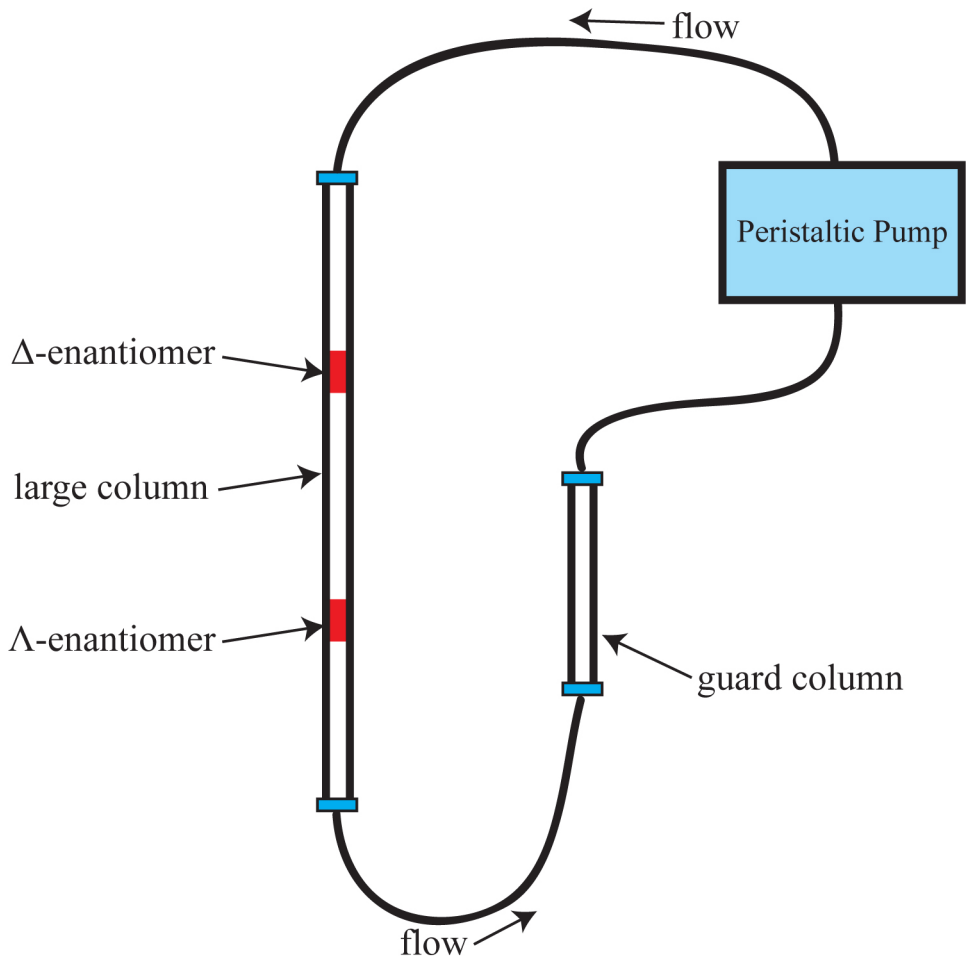


Figure 2.7: Schematic for enantiomer separation procedure. Two columns filled with cation exchange resin and equilibrated with 0.15 M (+)-KSb-tartrate are placed in-line with a peristaltic pump. As the eluent is cycled through the system over the course of a few days, the enantiomers separate, traveling at different speeds through the column due to their diastereomeric interactions with the chiral eluent.

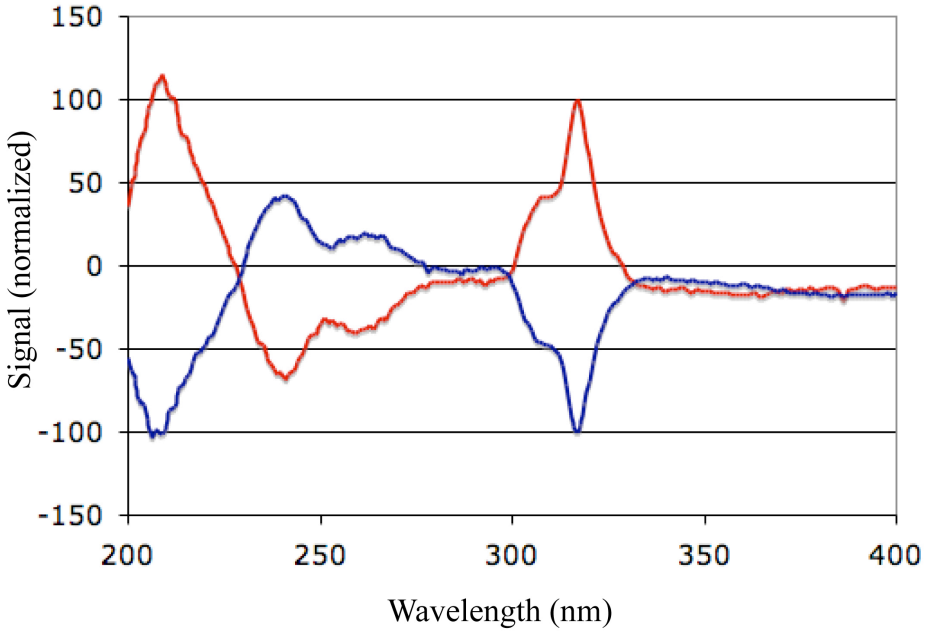


Figure 2.8: Circular dichroism spectra of Δ- and Λ-Rh(bpy)$_2$(chrysi)$^{3+}$. The spectra for the two enantiomers are shown in blue (Δ) and red (Λ). Δε values for Δ-Rh(bpy)$_2$(chrysi)$^{3+}$: 233 (34), 264 (26), 286 (-12), 308 (-42), 318 (-100), 341 (6). Δε values for Λ-Rh(bpy)$_2$(chrysi)$^{3+}$: 233 (-34), 264 (-26), 286 (12), 308 (42), 318 (100), 341 (-6)

Circular dichroism was employed to ascertain the enantiopurity of each solution (**Figure 2.8**). Δε values for Δ-$Rh(bpy)_2(chrysi)^{3+}$: 233 (34), 264 (26), 286 (-12), 308 (-42), 318 (-100), 341 (6). Δε values for Λ-$Rh(bpy)_2(chrysi)^{3+}$: 233 (-34), 264 (-26), 286 (12), 308 (42), 318 (100), 341 (-6).

2.3.4: SYNTHESIS OF TRISHETEROLEPTIC METALLOINSERTORS

2.3.4.1: SYNTHESIS OF $[Rh(phen)Cl_4](HPhen^+)$

The phenanthrolium salt of $[Rh(phen)Cl_4]^-$ was made according to the method of Broomhead and coworkers with minor modifications.[25, 26] Rhodium chloride hydrate (0.6 g) was added to concentrated hydrochloric acid (20 mL) in a 500 mL round-bottom flask. The solid was completely dissolved by refluxing the mixture for 3 h in an oil bath. Phenanthroline hydrate (1 g) was added, and the solution was heated for an additional 20 min during which boiling distilled water (300 mL) was added in 100 mL increments. The resultant solution was chilled at 4 °C overnight to promote crystallization and filtered in the morning to yield the product as a yellow crystalline solid. A synthetic scheme for this reaction and those described in 2.3.4.1−2.3.4.5 are shown in **Figure 2.9**.

2.3.4.2: SYNTHESIS OF $[Rh(phen)Cl_4](H_3O^+)$

The phenanthrolium salt was converted to the hydronium salt according to the method of McKenzie and co-workers.[27] The isolated phenanthrolium crystals (above) were added to fresh, concentrated hydrochloric acid (60 mL in a 100 mL round-bottom flask) and dissolved by refluxing the mixture for 5 h. The resulting solution was chilled

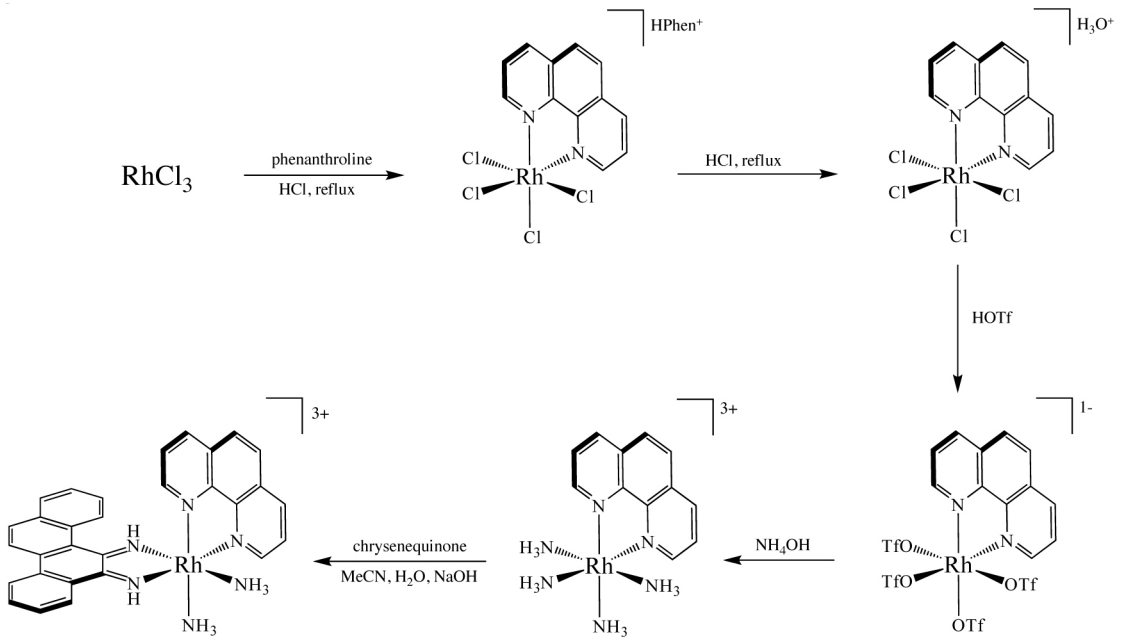


Figure 2.9: Synthetic strategy for trisheteroleptic metalloinsertors. The complexes are assembled sequentially. First, the phenanthroline ligand is added, followed by the stepwise conversion of the chloride ligands to ammines. A single chrysene-5,6-quinone is condensed onto the metal, leaving $Rh(phen)(chrysi)(NH_3)_2^{3+}$, the final intermediate on the way to the trisheteroleptic metalloinsertor molecules

at 4 °C overnight to promote crystallization and filtered in the morning to yield the product as an orange crystalline solid (needles, 0.61 g, 91%).

^{1}H-NMR (d_6-DMSO): 9.2 ppm (d, 2H); 8.83 ppm (d, 2H); 8.29 ppm (s, 2H); 8.15 ppm (d of d, 2H).

2.3.4.3: Synthesis of $[Rh(phen)(OTf)_4](H_3O^+)$

The chloride complex was then converted to the extremely synthetically useful triflate complex.[28–30] A 50 mL Schlenk flask was charged with the hydronium salt (100 mg), evacuated, and filled with $Ar_{(g)}$. Under positive argon pressure, triflic acid (5 g, excess) was added carefully to the reaction vessel using a glass Pasteur pipette (caution: triflic acid is very reactive, pyrophoric, and, to make matters worse, eats through gloves). After the addition of HOTf, the reaction vessel was closed with a rubber septum, the septum was pierced with a 16-gauge needle, and the flask was purged with argon for 60 seconds. The dark red reaction mixture was allowed to stir for 16 h with periodic $Ar_{(g)}$ purges to remove HCl generated by the reaction. After 16 h, the reaction mixture was added dropwise to 300 mL vigorously stirring diethyl either cooled to -78 ºC. The resultant brownish precipitate was collected via filtration on a Buchner funnel, washed with cold diethyl ether, and used as promptly as possible.

2.3.4.4: Synthesis of $[Rh(phen)(NH_3)_4](OTf)_3$

The triflate complex (600 mg) was added to 50 mL concentrated ammonium hydroxide (28−30%) in a round-bottom flask. The reaction was placed in an oil bath and

refluxed for 15 min. The resulting solution was evaporated to dryness, yielding a quantitative recovery of the tetra-ammine product (beige powder).

^{1}H-NMR (d_6-DMSO): 9.23 ppm (d, 2H); 9.02 ppm (d, 2H); 8.39 (s, 2H); 8.26 (d of d, 2H); 5.02 (broad s, 2H); 4.02 (broad s, 2H).

ESI-MS: 349 $[M-2H]^{+}$

This transformation can also be achieved by an alternative method, the reflux of the triflate precursor in condensed liquid ammonia. This method, however, can be somewhat dangerous for obvious reasons.

2.3.4.5: SYNTHESIS OF $[Rh(phen)(chrysi)(X)_2](Cl)_3$

In a 250-mL round-bottom flask, $Rh(phen)(NH_3)_4^{3+}$ (220 mg, 0.276 mmol) was combined with chrysene-5,6-quinone (67 mg, 0.261 mmol) in acetonitrile (100 mL) and an aqueous solution of sodium hydroxide (40 mL, 0.4 M). The reaction was capped to prevent evaporation and stirred overnight. Over the course of the reaction, the mixture changed colors from orange to dark red. After 16 h, the reaction was stopped by neutralization with hydrochloric acid; the reaction mixture was then anion-exchanged on a Sephadex QAE-25 column that had been pre-equilibrated with 0.05 M $MgCl_2$. The solution was concentrated on a reversed phase C-18 cartridge, washed, eluted, and lyophilized to dryness. The resultant red-orange powder was used in subsequent reactions without purification or further characterization.

2.3.4.6: SYNTHESIS OF [RH(PHEN)(CHRYSI)(BPY)](Cl)$_3$

In a 100-mL round-bottom flask, Rh(phen)(chrysi)(X)$_2$ (150 mg, 0.22 mmol) was combined with 2,2'-dipyridyl (150 mg, 0.8 mmol) in a 50/50 mixture of ethanol and deionized water (50 mL total volume) (**Figure 2.10**). The reaction was stirred at reflux overnight in an oil bath. The mixture was then allowed to cool, diluted with 200 mL H_2O, and purified by cation exchange chromatography.

Four inches of resin pre-equilibrated with 0.05 M $MgCl_2$ were poured into a 1−1.5 inch diameter column and subsequently washed with copious (500 mL) deionized H_2O. The rhodium complex was loaded onto the column simply by passing the aqueous Rh solution through the resin (the rhodium complex will 'stick' to the top of the column, forming a thin, dark orange band). The complex was then eluted by slowly increasing the [$MgCl_2$] in the eluent in 500-mL batches, starting with 0.05 M $MgCl_2$ and increasing in increments of 0.05 M until the red band of the metal complex has passed through the column. The resultant eluted solution was concentrated on a reverse-phase cartridge primed with MeOH and eluted with 1:1:0.001 H_2O/MeCN/TFA (vol/vol/vol). Finally, the solvent was removed by lyophilization to yield the product as a red-orange powder. The complex can be further purified via reversed phase high-performance liquid chromatography using an HP1100 HPLC system, a Varian DynaMax C18 semipreparative column, and an elution gradient of 85:15 to 40:60 H_2O (0.1% TFA):MeCN (0.1% TFA) over 60 min.

ESI-MS: 693 $[M-2H]^+$

UV-Vis (H_2O, pH 7.0): λ_{max} 267 nm (ε = 68,000 M^{-1}), 301 nm (ε = 40,000 M^{-1}), 313 nm (ε = 30,400 M^{-1}), 389 nm (ε = 19,400 M^{-1}).

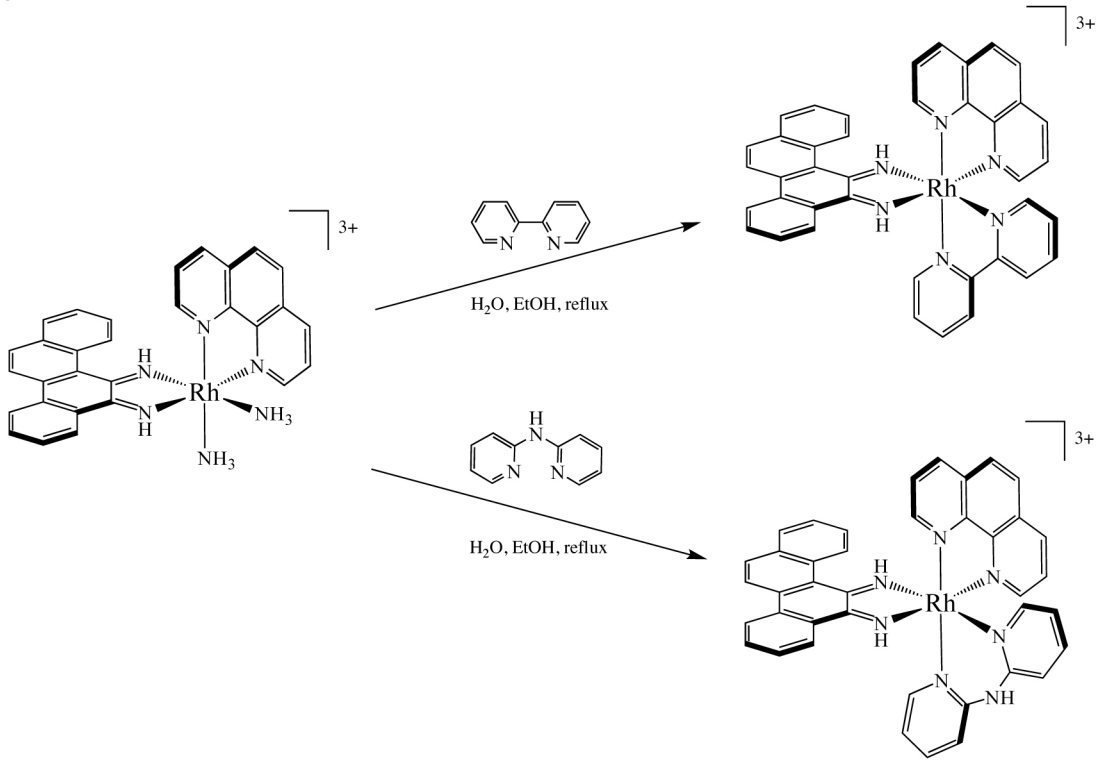


Figure 2.10: Metallation of bpy and HDPA ligands. In the final step of the synthesis of trisheteroleptic metalloinsertors, the dipyridyl ligand [2,2'-bipyridine (bpy) or 2,2'-dipyridylamine (HDPA)] is metallated via reflux in a 1:1 mixture of H_2O:EtOH.

2.3.4.7: SYNTHESIS OF [RH(PHEN)(CHRYSI)(HDPA)](Cl)$_3$

In a 100-mL round-bottom flask, Rh(phen)(chrysi)(X)$_2$ (150 mg, 0.22 mmol) was combined with 2,2'-dipyridylamine (150 mg, 0.75 mmol) in a 50/50 mixture of ethanol and deionized water (50 mL total volume) (**Figure 2.10**). The reaction was stirred at reflux overnight in an oil bath. The mixture was then allowed to cool, diluted with 200 mL H_2O, and purified by cation exchange chromatography.

Four inches of resin pre-equilibrated with 0.05 M $MgCl_2$ were poured into a 1−1.5 inch diameter column and subsequently washed with copious (500 mL) deionized H_2O. The rhodium complex was loaded onto the column simply by passing the aqueous Rh solution through the resin (the rhodium complex will 'stick' to the top of the column, forming a thin, dark orange band). The complex was then eluted by slowly increasing the [$MgCl_2$] in the eluent in 500-mL batches, starting with 0.05 M $MgCl_2$ and increasing in increments of 0..05 M until the red band of the metal complex has passed through the column. The resultant eluted solution was concentrated on a reverse-phase cartridge primed with MeOH and eluted with 1:1:0.001 H_2O/MeCN/TFA (vol/vol/vol). Finally, the solvent was removed by lyophilization to yield the product as a red-orange powder. The complex can be further purified via reverse-phase high-performance liquid chromatography using an HP1100 HPLC system, a Varian DynaMax C18 semipreparative column, and an elution gradient of 85:15 to 40:60 H_2O (0.1% TFA):MeCN (0.1% TFA) over 60 min.

ESI-MS: 708 $[M-2H]^+$, 354 $[M-H]^{2+}$

UV-Vis (H_2O, pH 7.0): λ_{max} 269 nm (ε = 66,400 M^{-1}), 301 nm (ε = 31,100 M^{-1}), 408 nm (ε = 6,500 M^{-1}).

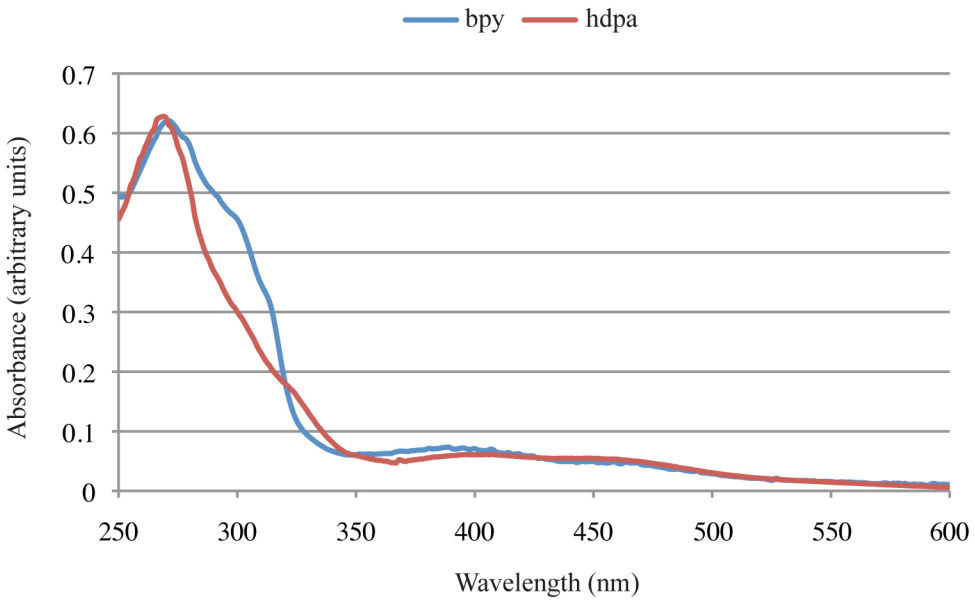


Figure 2.11: UV-Vis spectra of trisheteroleptic Rh(phen)(chrysi)(L)$^{3+}$ complexes. For L = bpy (blue), λ_{max} 267 nm (ε = 68,000 M^{-1}), 301 nm (ε = 40,000 M^{-1}), 313 nm (ε = 30,400 M^{-1}), 389 nm (ε = 19,400 M^{-1}). For L = HDPA (red), λ_{max} 269 nm (ε = 66,400 M^{-1}), 301 nm (ε = 31,100 M^{-1}), 408 nm (ε = 6,500 M^{-1})

2.3.5: SYNTHESIS OF LINKER-MODIFIED DIPYRIDYL LIGANDS

The trisheteroleptic metalloinsertor constructs discussed in Chapter 1 are employed most often in the design of mismatch-specific, bifunctional conjugates. These molecules typically contain two subunits: a mismatch-specific rhodium metalloinsertor and a second, non-specific moiety on which we seek to confer mismatch-specificity. The two subunits, of course, need to be covalently linked in some manner. This duty falls to special linker-modified dipridyl ligands that have been developed in our laboratory over the past five years.[31−33]

The topics of bifunctional conjugates and linker-modified ligands will be addressed in more detail in Chapter 7. However, the synthetic protocols for many of these ligands will be included here in the interest of centralization. It is important to note that in all syntheses involving bipyridine ligands, all glassware should be washed rigorously with a 0.1 M EDTA solution in order to eliminate free iron. Also, it is advisable to soak all silica (for columns and TLC) in a solution of 1:10 NEt_3:hexanes to eliminate smearing during analytical and preparative chromatography

2.3.5.1: SYNTHESIS OF 4-(7-BROMOHEPTYL)-4'-METHYL-2,2'-BIPYRIDINE (^{BR}BPY)

A 50 mL Schlenk flask was flame-dried and subjected to three rounds of evacuation and re-filling with $Ar_{(g)}$. The flask was then charged by syringe with 3.9 mL (28 mmol) diisopropylamine, and 20 mL THF (dry and under argon, Fluka) were transferred into the flask via cannula.[1] The flask was cooled to -78 °C in a dry ice/acetone bath, followed by the dropwise addition of 13.5 mL (27 mmol) M BuLi. The resultant light yellow LDA solution was kept at -78 °C as the second reaction vessel was prepared.

[1] Cannula means “little reed” in Latin.

A 500 mL, three-necked round-bottom flask was flame-dried, charged with 5 g (27 mmol) 4,4'-dimethyl-2,2'-bipyridine, and subjected to three rounds of evacuation and refilling with $Ar_{(g)}$. 200 mL THF (dry and under argon, Fluka) were transferred into the flask via cannula, and the reaction mixture was cooled to -78 °C in a dry ice/acetone bath. The LDA solution was then transferred into the 500 mL round-bottom flask via cannula, and the resultant dark brown reaction mixture was allowed to stir at -78 °C for 1 h. After 1 h, 29.9 mL (40 g, 135 mmol, 5 equiv.) dibromohexane were added to the reaction via syringe. The reaction mixture was immediately transferred to a dry ice bath (i.e. no acetone) and allowed to warm slowly to room temperature over the next 16 h. During this time, the reaction changed colors dramatically from brown to dark green to green to dark yellow and, finally, to light yellow.

Once at room temperature, the reaction vessel was opened to air, and H_2O (150 mL) was added to the reaction mixture to quench any remaining LDA. The pH of the mixture was adjusted to ~10 with saturated $NaHCO_{3(aq)}$. The basified reaction mixture was then extracted once with 50 mL Et_2O and subsequently with 50 mL increments of CH_2Cl_2 until the organic layer no longer stains red when spotted on a TLC plate (silica) and dipped in an Fe(II) solution. At this point (~ 250 mL total volume organic layer), the organic layer was extracted with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the final product as a yellow oil.

The crude product was purified via column chromatography (SiO_2 pretreated with 1:10 NEt_3:hexanes) with a solvent system of 1:1 EtOAc:Hexanes. In this solvent system, the product has an $R_f = .80$, and the unreacted starting material has an $R_f = .60$. The purified product, nicknamed ^{Br}bpy, is a white solid (8.1 g) (**Figure 2.12**).

^{1}H-NMR (CD_2Cl_2): 8.50 ppm (split d, 2H); 8.27 ppm (s, 2H); 7.14 ppm (d, 2H); 3.4 ppm (t, 2H); 2.69 ppm (t, 2H); 2.42 ppm (s, 3H); 1.8 ppm (m, 2H); 1.7 ppm (m, 2H); 1.37 ppm (m, 6H).

ESI-MS: 346, 348 $[M+H]^+$

2.3.5.2: SYNTHESIS OF 4-(7-PHTHALIMIDOHEPTYL)-4'-METHYL-2,2'-BIPYRIDINE (^{PHTH}BPY)

The bromide-terminated linker was converted to an amine-terminated linker by an adapted Gabriel amine synthesis.[34, 35] In a 250 mL round-bottom flask, ^{Br}bpy (0.35 g) was combined with potassium phthalimide (0.225 g) in 20 mL DMF and heated to 130 °C for 12 h. After cooling to room temperature, water (100 mL) was added, and the reaction mixture was brought to ~ pH 10 with saturated $NaHCO_{3(aq)}$. This solution was then extracted 3 times with 75 mL CH_2Cl_2, washed once with brine, dried over $MgSO_4$, and evaporated to dryness to yield a white solid (500 mg, > 95%, ^{Phth}bpy) that was pure by TLC (SiO_2, 1:1 EtOAc:Hex).

^{1}H-NMR ($CDCl_3$): 8.53 ppm (m, 2H); 8.21 ppm (m, 2H); 7.83 ppm (m, 2H); 7.69 ppm (m, 2H); 7.12 ppm (m, 2H); 3.67 ppm (t, 2H); 2.67 ppm (t, 2H); 2.42 ppm (s, 3H); 1.66 ppm (m, 4H); 1.37 ppm (m, 6H).

ESI-MS: 414 $[M+H]^+$

2.3.5.3: SYNTHESIS OF 4-(7-AMINOHEPTYL)-4'-METHYL-2,2'-BIPYRIDINE (^{NH2}BPY)

In a 250 mL round-bottom flask, ^{Phth}bpy (180 mg, 0.44 mmol) was dissolved in EtOH (75 mL) by heating to 70 °C for 1 h. After 1 h, hydrazine monohydrate (0.1 mL, 2.0 mmol) was added, and the reaction mixture was stirred at 70 °C for 16 h. After cooling to room temperature, the solvent was removed *in vacuo*. The residue was taken up in 50 mL 1 M hydrochloric acid. The acid phase was extracted twice with 50 mL $CHCl_3$ to eliminate any residual phthalimide products. The combined organic phases were washed with 50 mL 1 M HCl to prevent inadvertent product loss. The pH of the combined aqueous layers was then adjusted to ~10 with saturated $NaHCO_3$ solution, and the newly basic aqueous layer was extracted 4 times with 50 mL $CHCl_3$, washed with brine, dried over $MgSO_4$, and dried *in vacuo* to yield a white solid that was pure by NMR (150 mg, 90% yield, nicknamed ^{NH2}bpy).

^{1}H-NMR (CD_2Cl_2): 8.51 ppm (m, 2H); 8.25 ppm (m, 2H); 7.12 ppm (m, 2H); 2.70 ppm (m, 4H); 2.43 ppm (s, 3H); 1.85 ppm (m, 4H); 1.39 ppm (m, 6H).

ESI-MS: 284 $[M+H]^+$

2.3.5.4: SYNTHESIS OF 4-(3-(1,3-DIOXOLAN-2-YL)PROPYL)-4'-METHYL-2,2'-BIPYRIDINE (^{DO}BPY)

A 50 mL Schlenk tube was flame-dried and subjected to three rounds of evacuation and re-filling with $Ar_{(g)}$. The flask was then charged by syringe with 7.4 mL (5.35 g, 53 mmol) diisopropylamine, and 25 mL THF (dry and under argon, Fluka) were transferred into the flask via cannula.

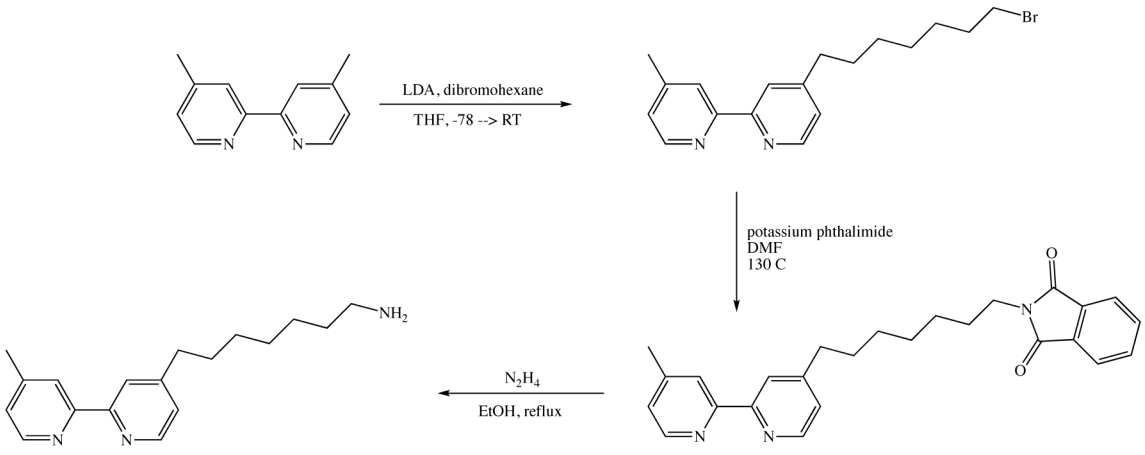


Figure 2.12. Synthetic route to ^{NH2}bpy. 4,4'-Dimethylbipyridine is first mono-alkylated with dibromohexane and LDA. This bromide is then converted to an amine through the via the Gabriel amine synthesis.

The flask was cooled to -78 °C in a dry ice/acetone bath, followed by the dropwise addition of 26 mL (53 mmol) M BuLi. The resultant light yellow LDA solution was kept at -78 °C as the second reaction vessel was prepared.

A 500 mL, three-necked round-bottom flask was flame-dried, charged with 10 g (54 mmol) 4,4'-dimethyl-2,2'-bipyridine, and subjected to three rounds of evacuation and refilling with $Ar_{(g)}$. 200 mL THF (dry and under argon, Fluka) was transferred into the flask via cannula, and the reaction mixture was then cooled to -78 °C in a dry ice/acetone bath. The LDA solution was then transferred into the 500 mL round-bottom flask via cannula, and the resultant dark brown reaction mixture was allowed to stir at -78 °C for 1 h. After 1 h, 30 mL (roughly 5 equiv.) 2-(3-bromopropyl)-1,3-dioxolane were added to the reaction via syringe. The reaction mixture was immediately transferred to a dry ice bath (*i.e.* no acetone) and allowed to warm slowly to room temperature over the next 16 h. During this time, the reaction changed colors dramatically from brown to dark green to green to dark yellow and, finally, to light yellow.

Once at room temperature, the reaction vessel was opened to air, and H_2O (150 mL) was added to the reaction mixture to quench any remaining LDA. The pH of the mixture was adjusted to ~10 with saturated $NaHCO_{3(aq)}$. The basified reaction mixture was then extracted once with 50 mL Et_2O and subsequently with 50 mL increments of CH_2Cl_2 until the organic layer no longer stains red when spotted on a TLC plate (silica) and dipped in an Fe(II) solution. At this point (~ 250 mL total volume organic layer), the organic layer was extracted with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the final product as a yellow oil.

The crude product was purified via column chromatography (SiO_2 pretreated with 1:10 NEt_3:hexanes) with a solvent system of 3:7 EtOAc:Hexanes. In this solvent system, the purified product, nicknamed ^{DO}bpy is a white solid (10.2 g) (**Figure 2.13**).

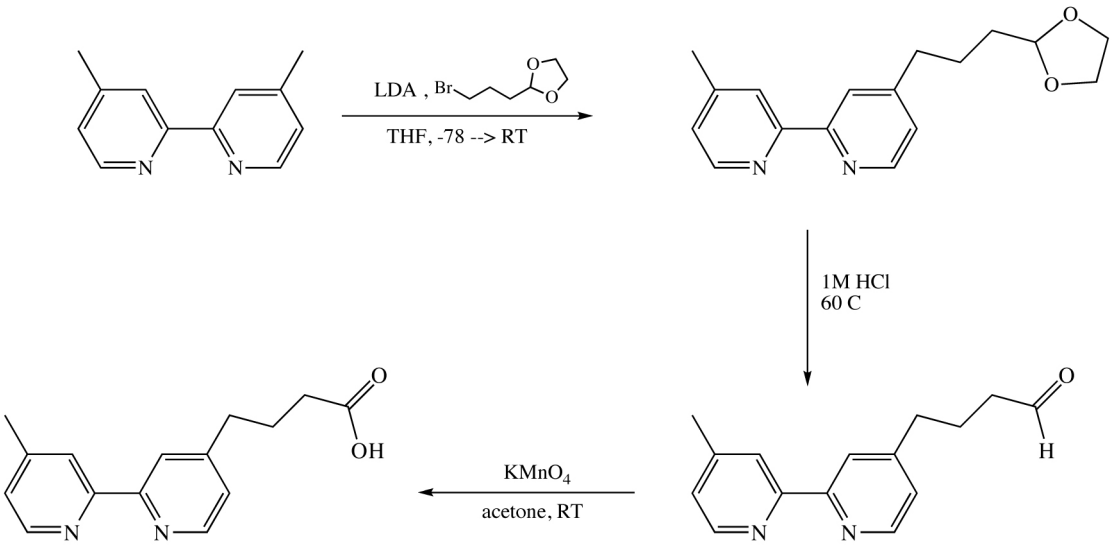
^{1}H-NMR ($CDCl_3$): 8.50 ppm (m, 2H); 8.20 ppm (m, 2H); 7.11 ppm (m, 2H); 4.87 ppm (t, 1H); 3.8−3.9 (m, 4H); 2.74 ppm (t, 2H); 2.42 ppm (s, 3H); 1.83 ppm (m, 2H); 1.73 (2H).

ESI-MS: 285.0 $[M+H]^+$

2.3.5.5: SYNTHESIS OF 4-(4'-METHYL-2,2'-BIPYRIDIN-4-YL)BUTANOIC ACID (BPY')

In a 250 mL round-bottom flask, combined 1.3 g ^{DO}bpy and 125 mL 1M HCl. Heated the resultant solution to 60 °C for 3 h. After 3 h, the reaction mixture was allowed to cool to room temperature, and its pH was adjusted to ~10 with saturated $NaHCO_{3(aq)}$. The basified reaction mixture was then extracted four times with 100 mL CH_2Cl_2, until the organic layer no longer stains red when spotted on a TLC plate (silica) and dipped in an Fe(II) solution. At this point (~ 400 mL total volume organic layer), the organic layer was extracted with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the aldehyde intermediate as a clear oil (0.95 g, quantitative, **Figure 2.13**). ESI-MS (m/z): 241 $[M+H]^+$.

In a 250 mL round-bottom flask, the aldehyde intermediate (0.95 g, 3.9 mmol) and $KMnO_4$ (616 mg, 3.9 mmol) were dissolved in 100 mL acetone and the solution was stirred for 3 h. After 3 h, the acetone was removed under vacuum, the residue was taken up in H_2O (200 mL), and the resultant solution was heated to 90 °C for 2 more h. After 2



2.13: Synthetic route to bpy'. Dimethylbipyridine is first monoalkylated with 2-(3-bromopropyl)-1,3-dioxolane and LDA. This dioxolane is then deprotected to yield an aldehyde intermediate that is subsequently oxidized with $KMnO_4$ to give the final carboxylic acid product.

h, the solution was filtered through an EDTA-soaked pad (the EDTA is to remove Fe contamination common in Celite). The pH of the solution was adjusted to 4.8 (the isoelectric point of the molecule), and the aqueous layer was extracted with CH_2Cl_2, until the organic layer no longer stained red when spotted on a TLC plate (silica) and dipped in an Fe(II) solution. At this point (~ 400 mL total volume organic layer), the organic layer was extracted with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the carboxylic acid (bpy') as a white solid (0.80 g, 80%).

^{1}H-NMR ($CDCl_3$): 8.55 ppm (m, 2H); 8.24 ppm (d, 2H); 7.2 ppm (m, 2H); 2.80 ppm (t, 2H); 2.42 ppm (m, 2H); 2.05 ppm (m, 2H).

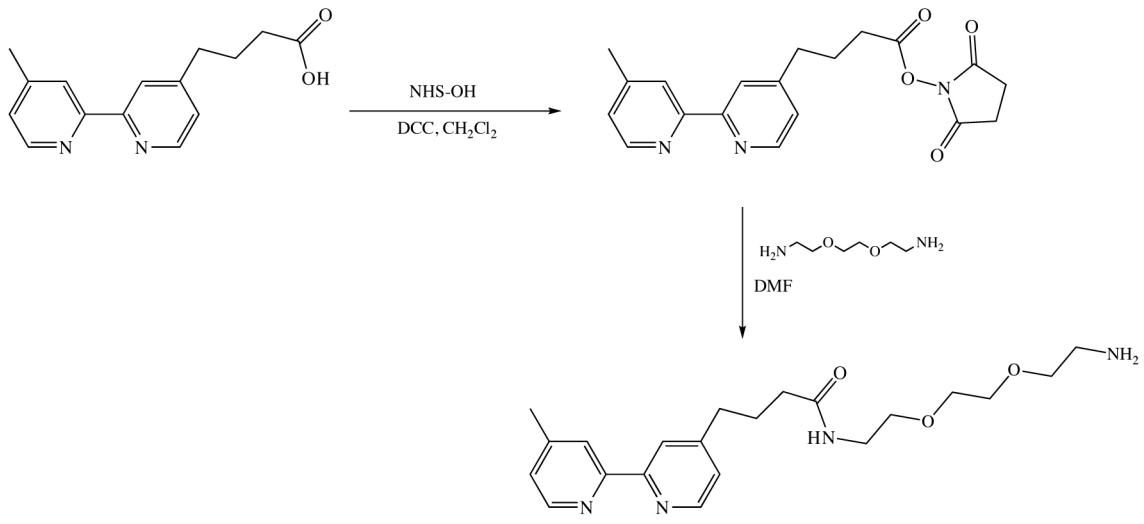
ESI-MS: 257.0 $[M+H]^+$

2.3.5.6: SYNTHESIS OF *N*-(2-(2-(2-AMINOETHOXY)ETHOXY)ETHYL)-4-(4'-METHYL-2,2'-BIPYRIDIN-4-YL)BUTANAMIDE (^{PEG}BPY)

Bpy' (100 mg, 1.9 mmol), DCC (732 mg, 2.8 mmol), and NHS-OH (400 mg, 2.8 mmol) were dissolved in 100 mL CH_2Cl_2 in a 250 mL round-bottom flask and stirred for 2 h at room temperature (**Figure 2.14)**. After 2 h, a precipitate had become apparent; the reaction was then placed in the cold room overnight to facilitate precipitation. In the morning, the solution was filtered and reduced *in vacuo* to yield the pure N-succinimidyl ester product as a clear oil (1 g, 1.8 mmol).

^{1}H-NMR ($CDCl_3$): 8.55 ppm (dd, 2H); 8.26 ppm (d, 2H); 7.21 ppm (d, 2H); 2.86 ppm (m, 6H); 2.68 ppm (t, 2H); 2.46 ppm (s, 3H) ; 2.17 ppm (t, 2H).

ESI-MS: 354.0 $[M+H]^+$



2.14: Synthetic route to ^{PEG}bpy. Bpy' is converted to a ligand bearing an ethylene-glycol-based linker through an N-hydroxysuccinimidyl ester intermediate.

Next, 100 g of the NHS-ester were dissolved in 3 mL DMF and added to a solution of 2 mL (excess) 2,2'-(ethylenedioxy)bis(ethylamine) in 1 mL DMF. After two h, 0.05 mL DIEA were added to ensure reaction. The reaction mixture was stirred for 16 h at room temperature. After 16 h, the reaction mixture was concentrated *in vacuo*, taken up in CH_2Cl_2, extracted twice with a saturated $NaHCO_3$ solution, dried over $MgSO_4$, and re-concentrated *in vacuo*. The final product (^{PEG}bpy) was obtained pure as a clear oil.

^{1}H-NMR ($CDCl_3$): 8.5 ppm (m, 2H); 8.24 ppm (m, 2H); 7.14 ppm (m, 2H); 3.9−3.6 ppm (m, 4H); 3.5−3.3 pm (m, 10H); 2.43 ppm (s, 3H); 2.25−2.15 ppm (m, 2H); 2.15−2.05 (m, 2H).

ESI-MS: 387.0 $[M+H]^+$

2.3.5.7: SYNTHESIS OF *N*-(6-BROMOHEXYL)-*N*-(PYRIDIN-2-YL)PYRIDIN-2-AMINE (^{BR}DPA)

Modified dipyridylamine ligands were synthesized by analogy to the methods of Krasinski and co-workers.[36] In a flame-dried, 250 mL round-bottom flask fitted with a reflux condenser, 2,2'-dipyridylamine (HDPA, 5 g, 29 mmol) was dissolved in THF (50 mL). The solution was purged with $Ar_{(g)}$ for 15 min. After 15 min, NaH (1.08 g, 29 mmol) was added slowly to the reaction mixture, and the solution was again purged with $Ar_{(g)}$ for 15 min. After 15 min, 10 g dibromohexane (42 mmol, 1.3 equiv.) were added. The solution was again purged for 10 min and brought to reflux at 70 °C for 16 h.

The next morning, the solution was allowed to cool to room temperature. Once at room temperature, the reaction vessel was opened to air, and H_2O (150 mL) was added to the reaction mixture to quench any remaining NaH. The pH of the mixture was adjusted

to ~10 with saturated $NaHCO_{3(aq)}$. The basified reaction mixture was then extracted with CH_2Cl_2 (5 x 50 mL). After the extractions, the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the final, crude product as a reddish-yellow oil.

The crude product was purified via column chromatography (SiO_2 pre-treated with 1:10 NEt_3:hexanes) with a solvent system of 1:1 EtOAc:Hexanes. Purification yielded the final product (nicknamed ^{Br}DPA) as a white solid (2.5 g, 25%) (**Figure 2.15**).

^{1}H-NMR ($CDCl_3$): 8.3 ppm (d, 2H); 7.4 ppm (d of d, 2H); 7.03 ppm (d, 2H); 6.84 ppm (d, 2H); 4.1 ppm (t, 2H); 3.38 ppm (m, 2H); 1.8 ppm (m, 2H); 1.6 ppm (m, 2H); 1.5−1.4 ppm (m, 6H).

ESI-MS: 334.0 and 336.0 $[M+H]^+$

2.3.5.8: SYNTHESIS OF *N*-(6-PHTHALIMIDOHEXYL)-*N*-(PYRIDIN-2-YL)PYRIDIN-2-AMINE (^{PHTH}DPA)

The bromide-terminated linker was converted to an amine-terminated linker by an adapted Gabriel amine synthesis.[34, 35] In a 250 mL round-bottom flask, ^{Br}DPA (1 g, 3 mmol) was combined with potassium phthalimide (1.53 g, 9 mmol) in 50 mL DMF and heated to 130 °C for 12 h. After cooling to room temperature, water (100 mL) was added, and the reaction mixture was brought to ~ pH 10 with saturated $NaHCO_{3(aq)}$. This solution was then extracted 3 times with 75 mL CH_2Cl_2, washed once with brine, dried over $MgSO_4$, and evaporated to dryness to yield a white solid (500 mg, > 95%, nicknamed ^{Phth}DPA) that was pure by TLC (SiO_2, 1:1 EtOAc:Hex).

^{1}H-NMR ($CDCl_3$): 8.28 ppm (m, 2H); 7.8 ppm (d, 2H); 7.65 ppm (d, 2H); 7.4 ppm (m, 2H); 7.05 ppm (m, 2H); 6.8 ppm (m, 2H); 4.1 ppm (t, 2H); 3.6 ppm (m, 2H); 1.8 ppm (m, 4H); 1.6 ppm (m, 6H).

ESI-MS: 401 $[M+H]^+$

2.3.5.9: SYNTHESIS OF N^1,N^1-DI(PYRIDIN-2-YL)HEXANE-1,6-DIAMINE (^{NH2}DPA)

In a 250 mL round-bottom flask, ^{Phth}DPA (1 g, 2.5 mmol) was dissolved in EtOH (100 mL) by heating to 80 °C for 1 h. After 1 h, hydrazine monohydrate (0.1 mL, 2.0 mmol) was added, and the reaction mixture was stirred at 70 °C for 16 h. After cooling to room temperature, the solvent was removed *in vacuo*. The residue was taken up in 50 mL 1 M hydrochloric acid. The acid phase was extracted twice with 50 mL $CHCl_3$ to eliminate any residual phthalimide products. The combined organic phases were washed with 50 mL 1 M HCl to prevent inadvertent product loss. The pH of the combined aqueous layers was then adjusted to ~10 with saturated $NaHCO_3$ solution, and the newly basic aqueous layer was extracted four times with 50 mL $CHCl_3$, washed with brine, dried over $MgSO_4$, and dried *in vacuo* to yield a white solid that was pure by NMR (650 mg, 90% yield, nicknamed ^{NH2}DPA).

^{1}H-NMR ($CDCl_3$): 8.25 ppm (d, 2H); 7.4 ppm (d of d, 2H); 7.01 ppm (d, 2H); 6.75 ppm (d, 2H); 4.05 ppm (t, 2H); 2.60 ppm (m, 2H); 1.8 ppm (m, 2H); 1.6 ppm (m, 2H); 1.4−1.3 ppm (m, 6H).

ESI-MS: 271 $[M+H]^+$

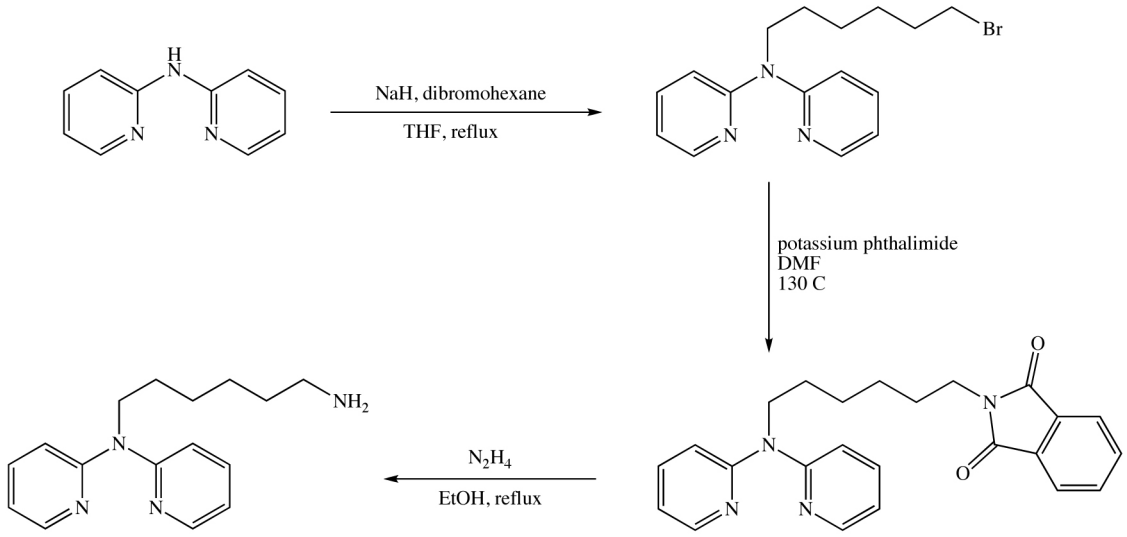


Figure 2.15: Synthetic route to ^{NH2}DPA. 2,2'-dipyridylamine (HDPA) is first monoalkylated with dibromohexane and NaH. This bromide is then converted to an amine through the well-known Gabriel amine synthesis.

2.3.5.10: SYNTHESIS OF 7-(DIPYRIDIN-2-YLAMINO)HEPTANENITRILE (^{CN}DPA)

In a 250 mL round-bottom flask, ^{Br}DPA (1 g, 3 mmol) was combined with potassium cyanide (400 mg, 6 mmol) in 100 mL DMSO and heated to 90 °C for 12 h. After cooling to room temperature, water (100 mL) was added, and the reaction mixture was brought to ~ pH 10 with saturated $NaHCO_{3(aq)}$. This solution was then extracted three times with 75 mL CH_2Cl_2, washed once with brine, dried over $MgSO_4$, and evaporated to dryness to yield a white solid (600 mg, 70%, nicknamed ^{CN}DPA) that was pure by TLC (SiO_2, 1:1 EtOAc:Hex) (**Figure 2.16**).

^{1}H-NMR ($CDCl_3$): 8.25 ppm (d, 2H); 7.5 ppm (d of d, 2H); 7.09 ppm (d, 2H); 6.89 ppm (d, 2H); 4.15 ppm (t, 2H); 3.44 ppm (m, 2H); 1.8 ppm (m, 2H); 1.6 ppm (m, 2H); 1.5−1.4 ppm (m, 6H).

ESI-MS: 281 $[M+H]^+$

2.3.5.11: SYNTHESIS OF 7-(DIPYRIDIN-2-YLAMINO)HEPTANOIC ACID (DPA')

In a 100 mL round-bottom flask, ^{CN}DPA (800 mg, 2.8 mmol) was dissolved in a mixture of 20 mL concentrated HCl and 5 mL concentrated H_2SO_4 and refluxed at 70 °C overnight. After 16 h, H_2O (100 mL) was added to the reaction mixture, and the pH was adjusted to 4.0 with NaOH(s). The aqueous layer was extracted with CH_2Cl_2 (4 x 50 mL). At this point (~ 200 mL total volume CH_2Cl_2), the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the carboxylic acid (DPA') as a white solid (600 mg, 80%).

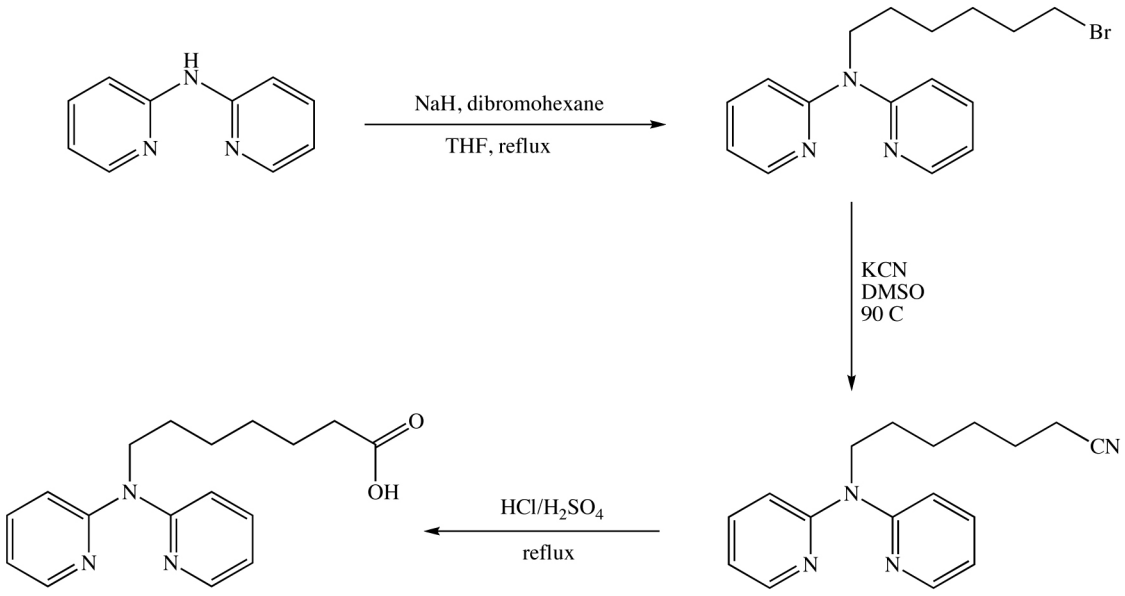


Figure 2.16: Synthetic route to DPA'. 2,2'-dipyridylamine (HDPA) is first monoalkylated with dibromohexane and NaH. This bromide is then converted to the carboxylic acid through a cyano intermediate.

^{1}H-NMR ($CDCl_3$): 8.21 ppm (d, 2H); 7.44 ppm (d of d, 2H); 7.2 ppm (d, 2H); 7.01 ppm (d, 2H); 4.21 ppm (t, 2H); 3.54 ppm (m, 2H); 1.8−1.6 ppm (m, 4H); 1.5−1.4 ppm (m, 6H).

ESI-MS: 300 $[M+H]^+$

2.3.5.12: EXAMPLE METALLATION: SYNTHESIS OF RH(PHEN)(CHRYSI)(BPY')$^{3+}$

In a 100-mL round-bottom flask, Rh(phen)(chrysi)$(X)_2$ (150 mg, 0.22 mmol) was combined with bpy' (150 mg, 0.6 mmol) in a 50/50 mixture of ethanol and deionized water (50 mL total volume) (**Figure 2.17**). The reaction was stirred at reflux overnight in an oil bath. The mixture was then allowed to cool, diluted with 200 mL H_2O, and purified by cation exchange chromatography.

Four inches of resin pre-equilibrated with 0.05 M $MgCl_2$ were poured into a 1−1.5 inch diameter column and subsequently washed with copious (500 mL) deionized H_2O. The rhodium complex was loaded onto the column simply by passing the aqueous Rh solution through the resin (the rhodium complex will 'stick' to the top of the column, forming a thin, dark orange band). The complex was then eluted by slowly increasing the $[MgCl_2]$ in the eluent in 500-mL batches, starting with 0.05 M $MgCl_2$ and increasing in increments of 0.05 M until the red band of the metal complex has passed through the column. The resultant eluted solution was concentrated on a reverse-phase cartridge primed with MeOH and eluted with 1:1:0.001 H_2O/MeCN/TFA (vol/vol/vol). Finally, the solvent was removed by lyophilization to yield the product as a red-orange powder. The complex can be further purified via reverse-phase high-performance liquid chromatography using an HP1100 HPLC system, a Varian DynaMax C18

semipreparative column, and an elution gradient of 85:15 to 40:60 H_2O (0.1% TFA):MeCN (0.1% TFA) over 60 min.

ESI-MS: 793 $[M-2H]^+$

UV-Vis (H_2O, pH 7.0): λ_{max} 267 nm (ε = 68,000 M^{-1}), 301 nm (ε = 40,000 M^{-1}), 313 nm (ε = 30,400 M^{-1}), 389 nm (ε = 19,400 M^{-1}).

2.3.5.13: EXAMPLE METALLATION: SYNTHESIS OF RH(PHEN)(CHRYSI)(DPA')$^{3+}$

In a 100-mL round-bottom flask, Rh(phen)(chrysi)$(X)_2$ (150 mg, 0.22 mmol) was combined with DPA' (150 mg, 0.5 mmol) in a 50/50 mixture of ethanol and deionized water (50 mL total volume) (**Figure 2.17**). The reaction was stirred at reflux overnight in an oil bath. The mixture was then allowed to cool, diluted with 200 mL H_2O, and purified by cation exchange chromatography.

Four inches of resin pre-equilibrated with 0.05 M $MgCl_2$ were poured into a 1−1.5 inch diameter column and subsequently washed with copious (500 mL) deionized H_2O. The rhodium complex was loaded onto the column simply by passing the aqueous Rh solution through the resin (the rhodium complex will 'stick' to the top of the column, forming a thin, dark orange band). The complex was then eluted by slowly increasing the $[MgCl_2]$ in the eluent in 500-mL batches, starting with 0.05 M $MgCl_2$ and increasing in increments of 0.05 M until the red band of the metal complex has passed through the column. The resultant eluted solution was concentrated on a reverse-phase cartridge primed with MeOH and eluted with 1:1:0.001 H_2O/MeCN/TFA (vol/vol/vol). Finally, the

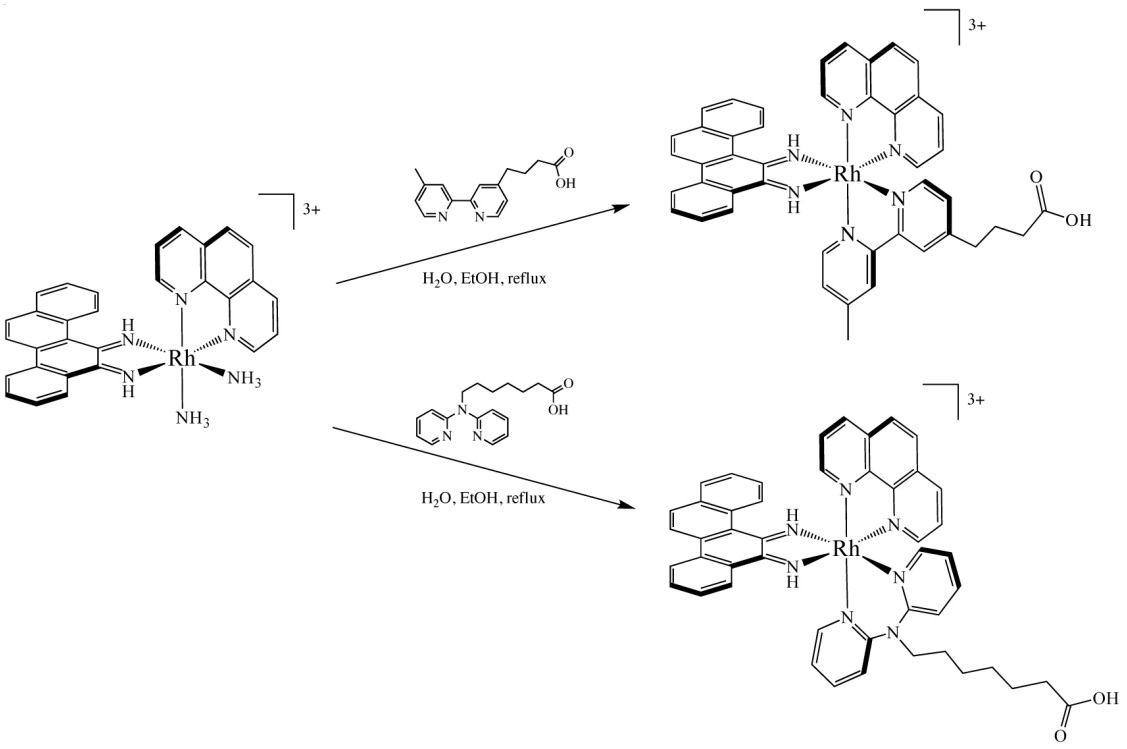


Figure 2.17: Metallation of bpy' and DPA' ligands. In each case, the linker-modified ligand [4-(4'-methyl-2,2'-bipyridin-4-yl)butanoic acid (bpy') or 7-(dipyridin-2-ylamino)heptanoic acid (DPA')] is metallated via reflux in a 1:1 mixture of H_2O:EtOH.

solvent was removed by lyophilization to yield the product as a red-orange powder. The complex can be further purified via reverse-phase high-performance liquid chromatography using an HP1100 HPLC system, a Varian DynaMax C18 semipreparative column, and an elution gradient of 85:15 to 40:60 H_2O (0.1% TFA):MeCN (0.1% TFA) over 60 min.

ESI-MS: 836 $[M-2H]^+$

UV-Vis (H_2O, pH 7.0): λ_{max} 269 nm (ε = 66,400 M^{-1}), 301 nm (ε = 31,100 M^{-1}), 408 nm (ε = 6,500 M^{-1}).

2.3.6: SYNTHESIS OF HDPA-BASED METALLOINSERTORS

2.3.6.1: SYNTHESIS OF $[Rh(chrysi)(NH_3)_4](Cl)_3$

$[Rh(NH_3)_6](PF_6)_3$ (200 mg, 0.3 mmol), chrysene-5,6-quinone (81 mg, 0.3 mmol), MeCN (100 mL) and NaOH (1 mL, 1 M) were combined in a 250-mL round-bottom flask and stirred for 18 h.[6] After 18 h, the reaction was stopped by neutralization with hydrochloric acid. The acetonitrile was removed *in vacuo*, and the residue was re-dissolved in 100 mL H_2O. The product was then anion-exchanged on a Sephadex QAE-25 column that had been pre-equilibrated with 0.05 M $MgCl_2$. Subsequently, the resulting solution was concentrated on a reverse phase C-18 cartridge, washed, eluted, and lyophilized to dryness. The resultant powder was used in subsequent reaction steps without further purification or characterization.

2.3.6.2: SYNTHESIS OF $[Rh(chrysi)(HDPA)_2](Cl)_3$

In a 100-mL round-bottom flask, $[Rh(chrysi)(NH_3)_4](Cl_3)$ (30 mg, 0.002 mmol) was combined with HDPA (7 mg, 0.004 mmol, 2 equiv.) in a 50/50 mixture of ethanol and deionized water (50 mL total volume) (**Figure 2.18**). The reaction was stirred at reflux overnight in an oil bath. The mixture was then allowed to cool, diluted with 200 mL H_2O, and purified by cation exchange chromatography.

Four inches of resin pre-equilibrated with 0.05 M $MgCl_2$ were poured into a 1−1.5 inch diameter column and subsequently washed with copious (500 mL) deionized H_2O. The rhodium complex was loaded onto the column simply by passing the aqueous Rh solution through the resin (the rhodium complex will 'stick' to the top of the column, forming a thin, dark orange band). The complex was then eluted by slowly increasing the $[MgCl_2]$ in the eluent in 500-mL batches, starting with 0.05 M $MgCl_2$ and increasing in increments of 0.05 M until the red band of the metal complex has passed through the column. The resultant eluted solution was concentrated on a reverse-phase cartridge primed with MeOH and eluted with 1:1:0.001 H_2O/MeCN/TFA (vol/vol/vol). Finally, the solvent was removed by lyophilization to yield the product as a red-orange powder. The complex can be further purified via reverse-phase high-performance liquid chromatography using an HP1100 HPLC system, a Varian DynaMax C18 semipreparative column, and an elution gradient of 85:15 to 40:60 H_2O (0.1% TFA):MeCN (0.1% TFA) over 60 min.

ESI-MS: 699 $[M-2H]^+$

UV-Vis (H_2O, pH 7.0): λ_{max} 287 nm (ε = 42,100 M^{-1}), 321 nm (ε = 23,200 M^{-1}), 442 nm (ε = 8,700 M^{-1}) (**Figure 2.19**).

2.3.6.3: SYNTHESIS OF [RH(HDPA)(Cl)$_4$]K

[Rh(HDPA)(Cl)$_4$]K was synthesized according to the method of Lee, et al.[37] $RhCl_3$ (263 mg, 1.1 mmol) and KCl (75 mg, 1.2 mmol) were dissolved in MeOH (10 mL) and heated to reflux for 2 h. After 2 h, a red-orange precipitate had formed in the reaction vessel. At this point, added HDPA (180 mg, 1.1 mmol) and continued reflux for 4 more h. During this time, a yellow precipitate formed. After 4 h, the solution was allowed to cool to room temperature, and the precipitate was collected by vacuum-filtration and dried under vacuum (300 mg, 66%).

ESI-MS (negative ion mode): 452 $[M-H+K]^-$

2.3.6.4: SYNTHESIS OF [RH(HDPA)(OTF)$_4$]K

A 50-mL Schlenk flask was charged with 300 mg [Rh(HDPA)(Cl)$_4$]K and deoxygenated by evacuating it and refilling with $Ar_{(g)}$ three times. Subsequently, 5 g triflic acid (excess) was added carefully to the reaction vessel under positive argon pressure (caution: triflic acid is very reactive and pyrophoric). After the addition of HOTf, the reaction vessel was closed with a rubber septum, the septum was pierced with a 16-gauge needle, and the flask was purged with argon for 60 seconds. The dark red reaction mixture was allowed to stir for 16 h with periodic $Ar_{(g)}$ purges to remove HCl generated by the reaction. After 16 h, the reaction mixture was added dropwise to 300 mL vigorously stirring diethyl either cooled to -78 ºC. The resultant yellowish precipitate was collected via filtration on a Buchner funnel, washed with cold diethyl ether, and used as promptly as possible.

2.3.6.5: SYNTHESIS OF $[Rh(HDPA)(NH_3)_4](X)_3$

In a 250-mL round-bottom flask fitted with a reflux condenser, 400 mg $[Rh(HDPA)(OTf)_4]K$ was suspended in 50 mL concentrated NH_4OH. The suspension was stirred, brought to reflux, and heated until all of the material went into solution (15 min); over the course of heating, the insoluble, singly charged complex is converted to the more soluble, triply-charged complex. Depending on the desired counter-ion, the product can be isolated one of two ways: (a) if the PF_6^- salt is desired, excess NH_4PF_6 should be added to the solution, and the reaction mixture should be cooled overnight to facilitate precipitation; (b) if the OTf^- counter-ion is desired, the NH_4OH should simply be removed by rotary evaporation at room temperature. Recoveries are best using the evaporation method and range from 80−100% depending on mechanical losses.

ESI-MS: 341 $[M-2H]^+$

2.3.6.6: SYNTHESIS OF $[Rh(HDPA)(CHRYSI)(NH_3)_2](Cl)_3$

In a 250-mL round-bottom flask, $Rh(HDPA)(NH_3)_4^{3+}$ (40 mg, 0.02 mmol) was combined with chrysene-5,6-quinone (7 mg, 0.02 mmol) in acetonitrile (30 mL) and an aqueous solution of sodium hydroxide (10 mL, 0.4 M). The reaction was capped to prevent evaporation and stirred overnight. After 16 h, the reaction was stopped by neutralization with hydrochloric acid; the reaction mixture was then anion-exchanged on a Sephadex QAE-25 column that had been pre-equilibrated with 0.05 M $MgCl_2$. The solution was concentrated on a reverse phase C-18 cartridge, washed, eluted, and lyophilized to dryness. The complex was further purified via reverse-phase high-performance liquid chromatography using an HP1100 HPLC system, a Varian DynaMax

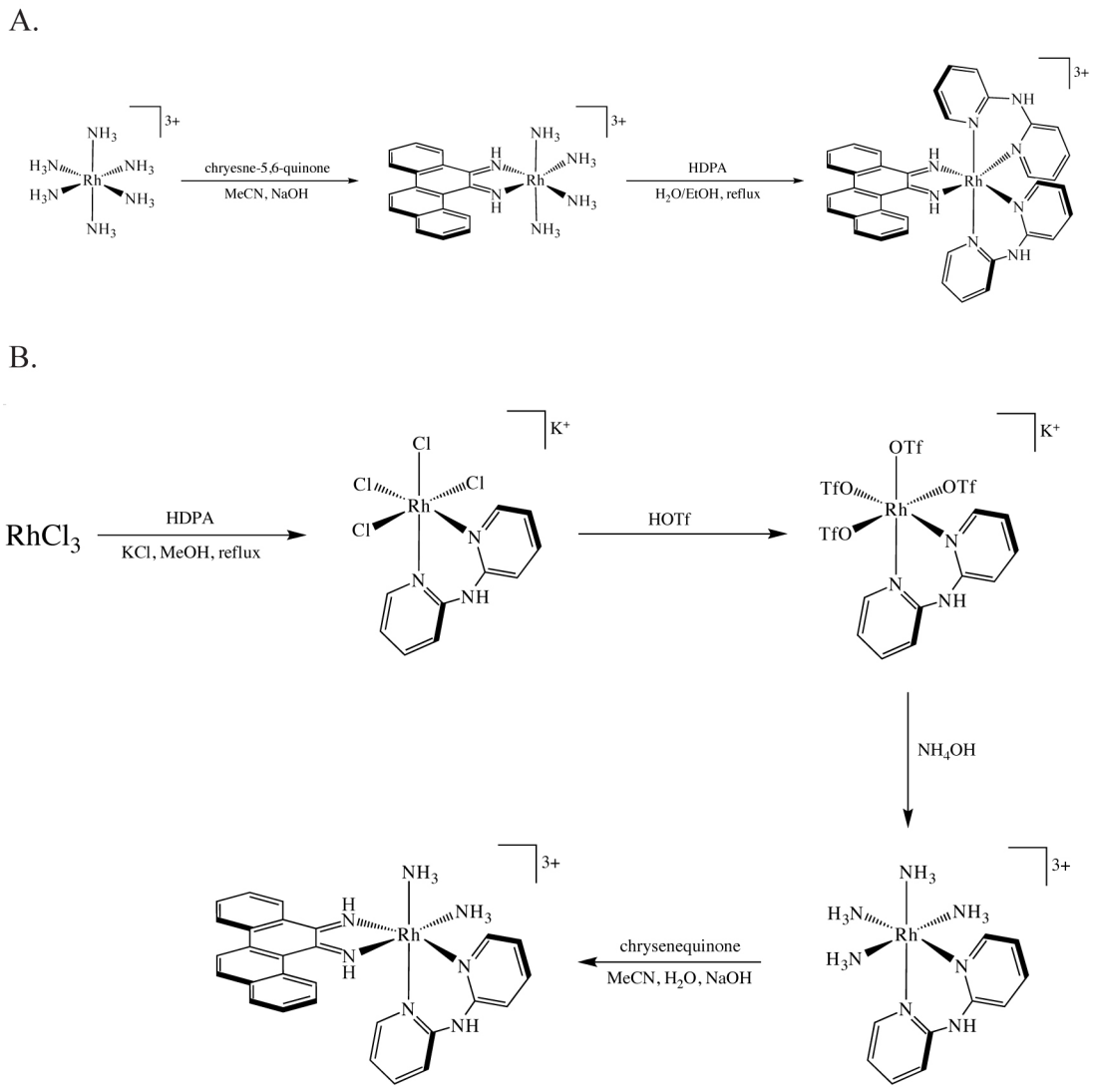


Figure 2.18: Synthesis of HDPA-based metalloinsertors. (A) $Rh(HDPA)_2(chrysi)^{3+}$ is synthesized in two steps from $Rh(NH_3)_6^{3+}$; (B) $Rh(chrysi)(HDPA)(NH_3)_2^{3+}$ is synthesized stepwise from $RhCl_3$.

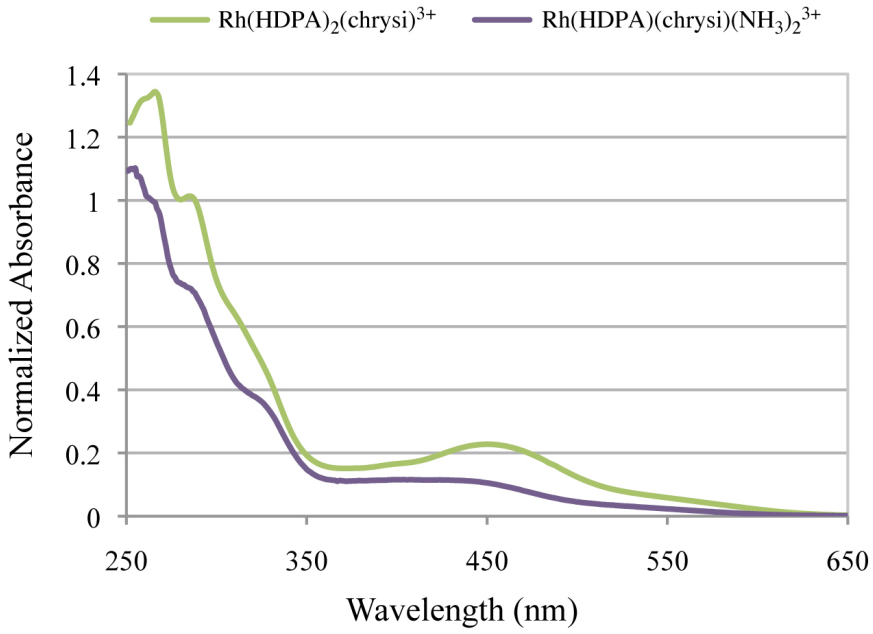


Figure 2.19: UV-Vis spectra of $Rh(HDPA)_2(chrysi)^{3+}$ and $Rh(chrysi)(HDPA)(NH_3)_2^{3+}$. For $Rh(HDPA)_2(chrysi)^{3+}$ (green): λ_{max} 287 nm (ε = 42,100 M^{-1}), 321 nm (ε = 23,200 M^{-1}), 442 nm (ε = 8,700 M^{-1}). For $Rh(HDPA)(chrysi)(NH_3)_2^{3+}$ (purple): λ_{max} 261 nm (ε = 42,645 M^{-1}), 281 nm (ε = 30,850 M^{-1}), 321 nm (ε = 15,900 M^{-1}), 404 nm (ε = 4,800 M^{-1})

C18 semipreparative column, and an elution gradient of 85:15 to 40:60 H_2O (0.1% TFA):MeCN (0.1% TFA) over 60 min.

ESI-MS: 564 $[M-2H]^+$

UV-Vis (H_2O, pH 7.0): λ_{max} 261 nm (ε = 42,645 M^{-1}), 281 nm (ε = 30,850 M^{-1}), 321 nm (ε = 15,900 M^{-1}), 404 nm (ε = 4,800 M^{-1}) (**Figure 2.19**).

2.4: NUCLEIC ACID PROTOCOLS

The nature of synthesis demands the precise reporting of individual reactions as they were performed. This type of writing is not as useful in describing nucleic acid experiments; in this case, the reader is far more likely to be referencing a protocol in order to repeat the *type* of experiment rather than the specific experimental details. Therefore, in this section, generalized protocols will be reported in order to simply the landscape for the future reader.

2.4.1: DNA SYNTHESIS AND PURIFICATION[38]

Oligonucleotides were synthesized using standard phosphoramidites on an ABI 3400 DNA synthesizer (reagents from ABI or Glen Research). The synthesizer was set to leave the DMT protecting group on the DNA and leave the DNA on the beads. After the synthesis run was complete, the columns were taken off the synthesizer and dried on a lyophilizer for 10 min. After 10 min, the columns were opened, and the beads were poured into 1.7 mL microcentrifuge tubes. The beads were suspended in 1.0 mL concentrated NH_4OH and heated to 60 °C for 12 h. After 12 h, the tubes were allowed to cool to room temperature, and the NH_4OH solution was separated from the beads, filtered through a microfilterfuge tube, and evaporated to dryness in a speedvac.

After evaporation, the DMT-protected DNA was taken up in 500 mL H_2O, filtered through a microfilterfuge tube, and purified via reverse phase HPLC with a Varian DynaMax^{TM} C18 semi-preparative column and gradient of 5:95 to 45:55 MeCN:50 mM NH_4OAc (aq) over 30 min. The product-containing fraction was collected in a 15 mL centrifuge tube and lyophilized to dryness. Next, in order to remove the DMT group, the dry DNA was taken up in 300 μL 85% acetic acid, vortexed to ensure complete dissolution, centrifuged, and allowed to sit for 15 min (at this point, the solution may turn a slight orange/pink color if the DNA is concentrated enough). After 15 min, 1.0 mL EtOH is added to precipitate the DNA, and the resultant suspension was moved to a 1.5 mL microcentrifuge tube and centrifuged. The supernatant was then discarded, the solid was further dried in a speedvac.

The dried DMT-off DNA was resuspended in 500 μL H_2O and subjected to a second round of HPLC with a gradient of 2:98 to 17:83 MeCN:50 mM NH_4OAc (aq) over 30 min. The product-containing fraction was collected in a 15 mL centrifuge tube and lyophilized to dryness. After evaporation, the purified DNA was taken up in 200 μL buffer (10 mM Tris·HCl, pH 7.1), quantified by UV-Vis, and stored at -20 °C.

2.4.2: DNA RADIOLABELING AND PURIFICATION[39]

In a 1.7 mL microcentrifuge tube, 15 μL H_2O, 1 μL oligonucleotide (100 μM stock), 2 μL polynucleotide kinase buffer (10X, Roche Biosciences), 1 μL polynucleotide kinase (Roche Biosciences), and 1 μL γ-^{32}P-ATP (MP Biomedicals, 5 mCi stock) were combined. The solutions were then heated at 37 °C for 2 h. After 2 h, 80 μL water was added to the reaction, and the solutions were filtered through Micro Bio-Spin 6

chromatography columns (centrifugation should not exceed 3,000 rpm) and dried *in vacuo*.

After evaporation, the labeling reactions were taken up in 10 μL denaturing formamide loading dye (80% formamide, 10 mM NaOH, 0.025% xylene cyanol, and 0.025% bromophenol blue in 1X TBE buffer), loaded on a 20% denaturing PAGE gel, and electrophoresed for 60−90 min at 90 W with 1X TBE as the running buffer. After electrophoresis, one of the gel plates was removed, leaving the gel affixed to the other plate. The gel was visualized via X-ray, and the parts of the gel that correspond to the full-length, labeled DNA were cut out with a clean razor blade. Using tweezers, the cut out gel pieces were placed into a clean centrifuge tube, 1 mL 100 mM triethylammonium acetate (pH 7.0) was added to the tube, and the tube was incubated overnight at 37 °C.

The next day, the DNA-containing triethylammonium solution was removed and placed in a clean 1.7 mL microcentrifuge tube. The solvent was then removed via speedvac. After evaporation, the samples were re-dissolved in 100 μL water and purified using a Micro Bio-Spin 6 chromatography column (centrifugation should not exceed 3,000 rpm). The solvent was again removed via speedvac. After evaporation, the purified, labeled DNA was taken up in 50 μL buffer (10 mM Tris·HCl, pH 7.1) and stored at 4 °C

2.4.3: MAXAM-GILBERT SEQUENCING REACTIONS[39]

In a small microcentrifuge tube, 32 μL water, 2 μL calf thymus DNA (4 mM stock), and a minimum volume of water containing radiolabeled DNA (corresponding to >1,000,000 counts) were combined. This solution was then divided into two tubes (~18 μL each) for the A+G and C+T sequencing reactions.

For the A+G reaction, 2 μL piperidine formate (formed by mixing 5 μL piperidine, 75 μL formic acid, and 20 μL water) were added to the radiolabeled DNA solution, and the tube was vortexed, centrifuged, and incubated at 37 °C for 30 min. After 30 min, 240 μL hyrdrazine stop solution (0.3 M sodium acetate pH 7.0, 0.1 mM EDTA pH 8.0, 100 μg/mL yeast tRNA) were added to the solution to terminate the reaction.

For the C+T reaction, 30 μL hydrazine monohydrate were added to the radiolabeled DNA solution, and the tube was vortexed, centrifuged, and incubated at 37 °C for 30 min. After 30 min, 200 μL hyrdrazine stop solution (0.3 M sodium acetate pH 7.0, 0.1 mM EDTA pH 8.0, 100 μg/mL yeast tRNA) were added to the solution to terminate the reaction. From this point on, both reaction tubes were treated identically.

Ethanol (750 μL, 200 proof) was added to each reaction, and the tubes were mixed thoroughly and placed in dry ice for 15 min. After 15 min, the tubes were centrifuged for 12 min at 14,000 rpm at 4 °C. After centrifugation, the supernatant was removed carefully to ensure that the radiolabeled pellet (as checked by Geiger counter) was not lost.

A second ethanol precipitation followed the first. Each pellet was taken up in 100 μL H_2O, and 50 μL 7.5 M NH_4OAc was added to each tube, followed by thorough mixing and centrifugation. Ethanol (750 μL, 200 proof) was then added to each reaction, and the tubes were mixed thoroughly and placed in dry ice for 15 min. After 15 min, the tubes were centrifuged for 12 min at 14,000 rpm at 4 °C. After centrifugation, the supernatant was removed carefully to ensure that the radiolabeled pellet (as checked by Geiger counter) was not lost. Samples were dried on a speedvac for 30 min to remove any residual EtOH.

After removing the EtOH, both samples were then dissolved in 100 μL 10% piperidine, vortexed, centrifuged, and heated to 90 °C for 30 min. After allowing the reactions to cool to room temperature, the piperidine was removed *in vacuo*. Once dry, the samples were taken up again in 100 μL water, vortexed, centrifuged, and then dried again *in vacuo.* This cycle was repeated three more times to yield the completed samples.

2.4.4: ELECTROPHORESIS EXPERIMENTS

2.4.4.1: RECOGNITION AND PHOTOCLEAVAGE[24]

Radiolabeled DNA duplexes were prepared to a final concentration of 2 μM by combining 2 μL forward strand (100 μM stock), 2 μL complementary strand (100 μM stock), 4 μL radiolabeled strand (from **2.4.2**, ~50 nM stock), and 92 μL buffer (*e. g.* 40 mM NaCl, 20 mM NaPi, pH 7.1), heating the solution to 90 °C for 5 min, and then allowing the solution to cool to room temperature slowly over the course of 60 min. Duplexes either containing or lacking a central defect (mismatch, abasic site, single base bulge, etc.) were prepared.

For the recognition experiments, 10 μL metalloinsertor stock solution [e. g. 2 μM $Rh(bpy)_2(chrysi)^{3+}$ or $Rh(bpy)_2(phzi)^{3+}$] were combined with 10 μL DNA stock solution in a 1.7 mL microcentrifuge tube. Dark and light control samples were also prepared and, of course, lacked the appropriate solution components. Because metalloinsertor photocleavage is single-stranded, each duplex was interrogated twice, once with each of the two strands radioactively labeled. Samples were irradiated with an Oriel Instruments solar simulator (320−440 nm). Irradiations were performed in open,

Vertically-oriented 1.7 mL microcentrifuge tubes in a Lucite sample holder. After irradiation, samples were incubated at 60 °C for 30 min to degrade any metastable products and then dried under vacuum. Dried samples were redissolved in denaturing formamide loading dye (see above for components) and electrophoresed on a 20% denaturing polyacrylamide gel for 60−90 min at 90 Watts. Images of the gels were obtained via phosphorimagery (Molecular Dynamics) and quantified using ImageQuant software (**Figure 2.20**).

2.4.4.2: BINDING CONSTANT TITRATIONS

Photocleavage titrations are performed to determine the binding constants of metalloinsertors for defect sites of interest.[40] Procedurally, these experiments are very similar to the recognition and photocleavage experiments described above.

In order to perform a binding constant titration, a series of solutions were created in 1.7 mL microcentrifuge tubes, each combining 10 μL radiolabeled duplex DNA stock solution (constant concentration, for example, 1 μM) with 10 μL metalloinsertor stock solution (variable concentrations). The gradient of metalloinsertor concentrations was centered on the approximated dissociation constant of the complex in question; for example, a concentration gradient of 100 nM, 200 nM, 400 nM, 600 nM, 800 nM, 1 μM, 2 μM, 4 μM, 6 μM, 8 μM, 10 μM and 15 μM was employed for a metalloinsertor with an

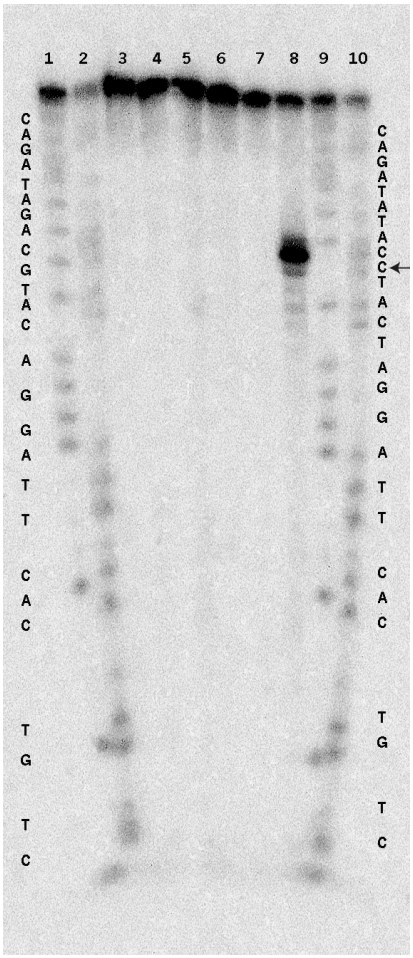


Figure 2.20: Sample mismatch photocleavage gel. 20% polyacrylamide gel showing mismatch-specific photocleavage by Δ-Rh(bpy)$_2$(chrysi)$^{3+}$ of the oligonucleotide 5'-A CTG TCA CTT AGG ATC AT***C*** CAT ATA GAC GCG-3', where *C* denotes the site of a central CC mismatch. Conditions employed: 1 μM duplex DNA, 50 mM NaCl/10mM NaPi pH 7.1 buffer, 1 μM Rh complex when applicable, irradiations performed on an Oriel Instruments solar simulator (320 nm−440 nm) for 15 min. Lanes 1 and 2: matched DNA, sequencing reactions. Lane 3: matched DNA, light control. Lane 4: matched DNA, dark control. Lane 5: matched DNA irradiated with Δ-Rh(bpy)$_2$(chrysi)$^{3+}$. Lane 6: mismatched DNA, light control. Lane 7: mismatched DNA, dark control. Lane 8: mismatched DNA irradiated with Δ-Rh(bpy)$_2$(chrysi)$^{3+}$. Lanes 9 and 10: mismatched DNA, sequencing reactions.

anticipated specific dissociation constant of approximately 1 μM. Dark and light control samples were also prepared and, of course, lacked the appropriate solution components.

Irradiations were performed for 15 min in open, vertically-oriented 1.7 mL microcentrifuge tubes (in Lucite sample holders) using an Oriel Instruments solar simulator (320−440 nm). After irradiation, samples were incubated at 60 °C for 30 min to degrade any metastable products and then dried under vacuum. Dried samples were redissolved in denaturing formamide loading dye and electrophoresed on a 20% denaturing polyacrylamide gel for 60−90 min at 90 Watts. Images of the gels were obtained via phosphorimagery (Molecular Dynamics). The fraction cleaved at the defect site was quantitated using ImageQuant software, expressed as a fraction of the total parent DNA, and fit to a single site, one parameter binding model. The mid-point of the resultant sigmoid (the log of [rhodium complex] at the inflection point of the curve) is the apparent dissociation constant, and the apparent dissociation constant minus half the DNA concentration used in the titration yields the true dissociation constant. The apparent and true binding constants can be determined by taking the inverse of the above values.

2.4.4.3: COMPETITION TITRATIONS

When site recognition via direct methods such as photoactivated strand scission fails, competition titrations provide an alternative means to determine the specificity of a complex. Two types of competition experiments exist: those employing non-specific metallointercalators and those using site-specific metalloinsertors. The former cannot readily provide quantitative data but yields valuable qualitative information on the

relative affinity of the molecule of interest for different sites in the DNA duplex. The latter allows for the interrogation of only a single site in the duplex, but it can provide important quantitative data about the system.[41−43]

For a competition experiment with a non-specific metallointercalator, samples of radiolabeled, duplex DNA (10 μL, 1 μM) in buffer (e. g. 50 mM NaCl, 10 mM NaPi, pH 7.1) were incubated with a saturating amount of a non-specific metallointercalator (for example, 5 μL $Rh(bpy)_2(phi)^{3+}$ solution, with a concentration that provides a saturating amount of 1 rhodium molecule/4 base pairs) and irradiated for 20 min using an Oriel Instruments solar simulator (320−440 nm) in the presence of variable concentrations of the metal complex of interest (5 μL for a total sample volume of 20 μL). Dark, light, and other control samples were also prepared and, of course, lacked the appropriate solution components. After preparation, the samples were dried on a speedvac, taken up in denaturing formamide dye, and electrophoresed on a 20% denaturing polyacrylamide gel for 60−90 min at 90 Watts. Images of the gels were obtained via phosphorimagery (Molecular Dynamics) and quantified using ImageQuant software. In the absence of metalloinsertor, the non-specific metallointercalator will indiscriminately cleave at all base pairs in the duplex, including the mismatch site. If the complex in question specifically binds the mismatch, the intensity of the metallointercalator photocleavage band at that site will decrease upon its addition. This photocleavage attenuation is the indicator of specific binding.

For a competition experiment with a site-specific metalloinsertor, samples of radiolabeled, duplex DNA (10 μL, 1 μM) in buffer (e. g. 50 mM NaCl, 10 mM NaPi, pH 7.1) were incubated with a site-specific metalloinsertor with a known affinity for the site

of interest (for example, 5 μL of a $Rh(bpy)_2(chrysi)^{3+}$ solution, with a concentration that results in 50% of the target site bound) and irradiated for 20 min using an Oriel Instruments solar simulator (320−440 nm) in the presence of variable concentrations of the molecule of interest (5 μL for a total sample volume of 20 μL, with concentrations spread across the relevant concentration regime). Dark, light, and other control samples were also prepared and, of course, lacked the appropriate solution components. After preparation, the samples were dried on a speedvac, taken up in denaturing formamide dye, and electrophoresed on a 20% denaturing polyacrylamide gel for 60−90 min at 90 Watts. Images of the gels were obtained via phosphorimagery (Molecular Dynamics). The fraction cleaved at the defect site was quantitated using ImageQuant software, expressed as a fraction of the total parent DNA, and plotted against the concentration of the metal complex of interest. The binding and dissociation constants of the non-photocleaving complex were calculated by solving simultaneous equilibria involving the DNA, the site-specific metalloinsertor, and the metal complex of interest in Mathematica 6.0.

2.4.5: MALDI-TOF ANALYSIS OF CLEAVAGE FRAGMENTS[44]

For mass spectrometry analysis of photocleavage products, solutions of duplex were incubated with solutions of the molecule of interest and irradiated as described above in section **2.4.4.1**. After irradiation and incubation, the samples were dried *in vacuo*, resuspended in 10 μL water, desalted using 10 μL OMIX C18 tips (Varian), dried *in vacuo* again, and resuspended in 2 μL H_2O. Light and dark controls were also prepared. Mass spectrometry was performed using a Voyager DE-PRO MALDI-TOF

instrument with a 337 nm nitrogen laser source (Applied Biosystems). A 4-hydroxypicolinic acid matrix was employed. All mass spectra were internally calibrated using the mass of the parent oligonucleotide.

2.5: REFERENCES

1. Zeglis, B. M.; Barton, J. K. *Nature Protocols* **2007**, *2(2)*, 357−371.
2. Graebe, C.; Honigsberger, F. *Justus Liebigs Annalen Der Chemie* **1900**, *311* (1/3), 257−275.
3. Junicke, H.; Hart, J. R.; Kisko, J. L.; Glebov, O.; Kirsch, I. R.; Barton, J. K. *Proceedings of the National Academy of Sciences U. S. A.* **2003**, *100*, 3737−3742.
4. Vanallan, J. A.; Reynolds, G. A. *Journal of Organic Chemistry* **1963**, *28* (4), 1019−1022.
5. Gillard, R. D.; Osborn, J. A.; Wilkinson, G. *Journal of the Chemical Society* **1965**, *(JUL)*, 4107−4110.
6. Murner, H.; Jackson, B. A.; Barton, J. K. *Inorganic Chemistry* **1998**, *37* (12), 3007−3012.
7. Gidney, P. M.; Gillard, R. D.; Heaton, B. T. *Journal of the Chemical Society-Dalton Transactions* **1972**, (23), 2621−2628.
8. Creaser, I. I.; Geue, R. J.; Harrowfield, J. M.; Herlt, A. J.; Sargeson, A. M.; Snow, M. R.; Springborg, J. *Journal of the American Chemical Society* **1982**, *104* (22), 6016−6025.

9. Creaser, I. I.; Harrowfield, J. M.; Herlt, A. J.; Sargeson, A. M.; Springborg, J.; Geue, R. J.; Snow, M. R. *Journal of the American Chemical Society* **1977**, *99* (9), 3181−3182.

10. Geue, R. J.; Hohn, A.; Ralph, S. F.; Sargeson, A. M.; Willis, A. C. *Journal of the Chemical Society-Chemical Communications* **1994**, (12), 1513−1515.

11. Geue, R. J.; Korybutdaszkiewicz, B.; Sargeson, A. M. *Journal of the Chemical Society-Chemical Communications* **1993**, (18), 1454−1456.

12. Harrowfield, J. M.; Herlt, A. J.; Lay, P. A.; Sargeson, A. M.; Bond, A. M.; Mulac, W. A.; Sullivan, J. C. *Journal of the American Chemical Society* **1983**, *105* (16), 5503−5505.

13. Harrowfield, J. M.; Robertson, G. B.; Sargeson, A. M.; Whimp, P. O. *Journal of the Chemical Society-Chemical Communications* **1975**, (4), 109−111.

14. Krotz, A. H.; Kuo, L. Y.; Barton, J. K. *Inorganic Chemistry* **1993**, *32* (26), 5963−5974.

15. Pyle, A. M.; Chiang, M. Y.; Barton, J. K. *Inorganic Chemistry* **1990**, *29* (22), 4487−4495.

16. Belser, P.; Vonzelewsky, A.; Zehnder, M. *Inorganic Chemistry* **1981**, *20* (9), 3098−3103.

17. Schlosser, K.; Hoyer, E. *Zeitschrift Fur Anorganische Und Allgemeine Chemie* **1972**, *387* (1), 91−95.

18. Palmer, J. W.; Basolo, F. *Journal of Physical Chemistry* **1960**, *64* (6), 778−780.

19. Palmer, J. W.; Basolo, F. *Journal of Inorganic & Nuclear Chemistry* **1960**, *15* (3−4), 279−286.

20. Jackson, B. A.; Barton, J. K. *Journal of the American Chemical Society* **1997**, *119* (52), 12986−12987.

21. Jackson, B. A.; Henling, L. M.; Barton, J. K. *Inorganic Chemistry* **1999**, *38* (26), 6218−6224.

22. Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *Journal of the American Chemical Society* **1992**, *114* (7), 2303−2312.

23. Keene, F. R. *Coordination Chemistry Reviews* **1997**, *166*, 121−159.

24. Jackson, B. A.; Barton, J. K. *Biochemistry* **2000**, *39* (20), 6176−6182.

25. Broomhead, J. A.; Grumley, W. *Inorganic Chemistry* **1971**, *10* (9), 2002−2009.

26. Broomhead, J. A.; Kane, M. *Inorganic Chemistry* **1971**, *10* (1), 85−87.

27. McKenzie, E. D.; Plowman, R. A. *Journal of Inorganic & Nuclear Chemistry* **1970**, *32* (1), 199−205.

28. Dixon, N. E.; Lawrance, G. A.; Lay, P. A.; Sargeson, A. M. *Inorganic Chemistry* **1984**, *23* (19), 2940−2947.

29. Dixon, N. E.; Lawrance, G. A.; Lay, P. A.; Sargeson, A. M.; Taube, H. *Inorganic Syntheses* **1990**, *28*, 70−76.

30. Dixon, N. E.; Lawrance, G. A.; Lay, P. A.; Sargeson, A. M. *Inorganic Chemistry* **1983**, *22* (5), 846−847.

31. Petitjean, A.; Barton, J. K. *Journal of the American Chemical Society* **2004**, *126* (45), 14728−14729.

32. Schatzschneider, U.; Barton, J. K. *Journal of the American Chemical Society* **2004**, *126* (28), 8630−8631.

33. Lim, M. H.; Lau, I. H.; Barton, J. K. *Inorganic Chemistry* **2007**, *46*, 9528−9530.

34. Gibson, M. S.; Bradshaw, R. W. *Angewandte Chemie−International Edition* **1968**, *7* (12), 919−930.

35. Gabriel, S. *Chemische Berichte* **1887**, *20*, 2224−2237.

36. Krasinski, A.; Radic, Z.; Manetsch, R.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. *Journal of the American Chemical Society* **2005**, *127*, 6686−6692.

37. Lee, J. R.; Lung, J. W.; Huang, J. Chinese Chemical Society **2003**, *50* (227−232).

38. Zeglis, B. M.; Pierre, V. C.; Barton, J. K. Chemical Communications **2007**, 4565−4579.

39. Sambrook, J.; Russell, D. Molecular Cloning: A Laboratory Manual. 3rd Edition ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 2001.

40. Ernst, R. J.; Song, H.; Barton, J. K. Journal of the American Chemical Society **2009**, *131* (6), 2359−2366.

41. Jackson, B. A. California Institute of Technology, Pasadena, 2001.

42. Hart, J. R. Synthesis and applications of bulky rhodium(III) intercalators for the recognition of DNA mismatches. California Institute of Technology, Pasadena, CA, 2006.

43. Garbett, N. C.; Chaires, J. B. Binding: A polemic and rough guide. In Biophysical Tools for Biologists: Vol. 1 in Vitro Techniques, Elsevier Academic Press Inc: San Diego, 2008; Vol. 84, pp 3−23.

44. Brunner, J.; Barton, J. K. Journal of the American Chemical Society **2006**, *128* (21), 6772−6773.