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Synthesis, Characterization, and DNA Binding Properties of a Series of Ru, Pt Mixed-Metal Complexes

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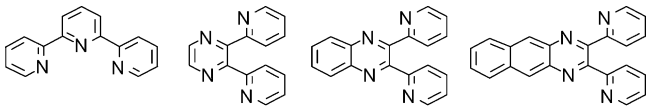
A series of mixed-metal complexes coupling ruthenium light absorbers to platinum reactive metal sites through polyazine bridging ligands have been prepared of the form $[(tpy)RuCl(BL)PtCl_2](PF_6)$ (BL = 2,3-bis(2-pyridyl)-pyrazine (dpp), 2,3-bis(2-pyridyl)quinoxaline (dpq), 2,3-bis(2-pyridyl)benzoquinoxaline (dpb); tpy = 2,2′:6′,2″-terpyridine). These systems possess electron-rich Ru metal centers bound to five polyazine nitrogens and one chloride ligand. This leads to complexes with low-energy Ru → BL charge-transfer bands that are tunable with BL variation occurring at 544, 632, and 682 nm for dpp, dpq, and dpb, respectively. This tuning of the charge-transfer energy results from a stabilization of the BL(π^*) orbitals in this series as evidenced by the cathodic shift in the first reduction of these complexes occurring at –0.50, –0.32, and –0.20 V vs Ag/AgCl, for dpp, dpq, and dpb, respectively. The chlorides bound to the Pt^{II} center are substitutionally labile giving these complexes the ability to covalently bind to DNA. All three title bimetallics, $[(tpy)RuCl(BL)PtCl_2](PF_6)$, avidly bind double-stranded DNA with $t_{1/2}$ = 1–2 min, substantially reducing the migration of DNA through an agarose gel. Details of the synthetic methods, FAB MS data, spectroscopic and electrochemical properties, and DNA binding studies are presented.

Introduction

Polyazine ruthenium complexes have been extensively studied and show interesting photophysical and redox properties. The prototypical $[Ru(bpy)_3]^{2+}$ undergoes both intermolecular excited-state energy and electron transfer (bpy = 2,2′-bipyridine).[1,2] These intermolecular processes are limited by the need for a molecular collision prior to relaxation of the ^{3}MLCT excited state of $[Ru(bpy)_3]^{2+}$ and related chromophores (MLCT = metal to ligand charge transfer). This has led to the rapid development and study of supramolecular polyazine-bridged complexes.[3−7] The coupling of Ru(II) light absorbers (LA) to reactive metal (RM) centers is of particular interest in developing multifunctional supramolecular complexes.

Polyazine bridging ligands (BL) have been widely used as linkers in the construction of polymetallic complexes.[3−7] These BLs serve to connect two metal centers and, by virtue of their π systems, often function as conduits for electron and energy transfer. These polyazine BLs are often good π-acceptors and as such impart interesting and useful spectroscopic and redox properties on polymetallic complexes. One commonly used BL is 2,3-bis(2-pyridyl)pyrazine (dpp).[3−6,8−10] The dpp ligand binds to two metal centers



through a pyridyl and a pyrazine nitrogen and possesses a lower lying π^* orbital than bpy. The π system of this BL has been expanded through the use of the ligands dpq and dpb (dpq = 2,3-bis(2-pyridyl)quinoxaline and dpb =

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2,3-bis(2-pyridyl)benzoquinoxaline).[3,11−14] This extension of the π system leads to BLs that possess lower energy π^* orbitals through the series dpp, dpq, and dpb. This allows for a tuning of BL-localized spectroscopic and electrochemical properties within a supramolecular framework.

The structure and properties of supramolecular complexes are also dictated by the choice of terminal ligands (TL). The most common TL is bpy, but 2,2′:6′,2″-terpyridine (tpy) can also be used. Using tpy as a terminal ligand results in some stereochemical control of supramolecular complexes by eliminating the Δ and Λ isomeric mixtures present in tris-bidentate systems. The tpy ligand is less studied in Ru chemistry as the Ru $\rightarrow$ tpy MLCT excited state in $[Ru(tpy)_2]^{2+}$ is short-lived.[15−17] Polymetallic complexes incorporating the Ru^{II}(tpy) chromophore can have long excited-state lifetimes due to their lower energy MLCT states, which limits thermal population of the deactivating ligand field state.[18−27] Use of a tpy TL with a bidentate BL leaves available the sixth coordination site which can be substituted to tune the properties of such ruthenium chromophores, $[(tpy)RuL(BL)]^{n+}$ (L = monodentate ligand). Related ruthenium chromophores, $[Ru^{IV}(tpy)(bpy)O]^{2+}$ and $[Ru^{III}(tpy)(bpy)OH]^{2+}$, have been shown to site selectively cleave DNA.[28,29]

Mixed-metal complexes coupling Ru(II) to Pt(II) through polyazine bridging ligands have been of recent interest. Systems of the type $[(bpy)_2Ru(BL)PtCl_2]^{2+}$ (BL = dpp,[30−32] dpq,[33] dpb,[32,34,35] or bpm;[36] bpm = 2,2′-bipyrimidine) and dendrimeric systems[37] have shown that coordination of the Pt(II) center to the remote nitrogens of the BL stabilizes the bridging ligand π^* orbitals. This results in a red shift of the BL-based MLCT transitions and BL-based reductions that occur at more positive potentials. The *cis*-$Pt^{II}Cl_2$ moiety has known DNA binding ability and is the basis of a class of anticancer agents, most notably cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$.[38−41] This DNA-binding property of the *cis*-$Pt^{II}Cl_2$ moiety has been shown to be maintained in previous (μ-BL)$PtCl_2$ complexes studied in our laboratory.[32−34] Platinum complexes of polyazine ligands are known that bind to DNA both in intercalative and covalent modes.[42−49]

In this study the $[(tpy)RuCl(BL)PtCl_2]^+$ complexes with BL = dpp, dpq, or dpb have been synthesized and characterized by FAB mass spectral analysis, cyclic voltammetry, and electronic absorption spectroscopy and shown to avidly bind DNA. These complexes represent a class of mixed-metal assemblies of the form TL−LA−BL−RM, where the Cl bound to the Ru center makes possible the extension of the molecular architecture.[50] The variation of the BL within this framework has been used to tune the spectroscopic and redox properties of these complexes.

Experimental Section

Materials. The ligands dpp and tpy were purchased from Aldrich Chemical Co. and used as received. Ruthenium trichloride hydrate and potassium tetrachloroplatinate were received from Johnson Matthey/Alfa-Aesar. Adsorption alumina (80−200) was purchased from Fisher Scientific. The ligands dpq[11] and dpb[12] and the complexes $Ru(tpy)Cl_3$[51] and $PtCl_2(dmso)_2$[52] (dmso = dimethyl sulfoxide) were prepared as previously reported. The supporting electrolyte for the electrochemical studies, tetrabutylammonium hexafluorophosphate (Bu_4NPF_6), was prepared by the metathesis of tetrabutylammonium bromide and potassium hexafluorophosphate. The electrolyte was recrystallized from hot ethanol (twice), dried in a vacuum oven, and stored in a nitrogen-filled glovebox. HPLC-grade acetonitrile was purchased from Mallinckrodt and used for the electrochemical and spectroscopic studies.

Methods. FAB-Mass Spectrometry. Mass spectral analysis was performed by a Fisons VG Quattro I triple-stage quadrapole mass

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spectrometer equipped with a cesium ion gun. The complexes were dissolved in a minimal amount of acetonitrile and introduced into the *m*-nitrobenzyl matrix for analysis.

Electrochemistry. A Bioanalytical Systems, Inc., 100W electrochemical workstation was used to generate cyclic voltammograms. In all cases, 0.1 M Bu_4NPF_6 in acetonitrile served as the supporting electrolyte. The three-electrode system consists of a 1.9 mm platinum disk working electrode, a platinum wire auxiliary electrode, and a Ag/AgCl reference electrode (0.286 V vs NHE). The reference electrode was calibrated against the ferrocene/ferrocenium couple reported as 0.665 V vs NHE in a 0.1 M Bu_4NPF_6/acetonitrile solution.[53] The platinum working electrode was polished between each scan, and the solutions were deoxygenated by bubbling with argon for 20 min.

Electronic Absorption Spectroscopy. Spectra were generated at room temperature in a 1 cm quartz cuvette using a Hewlett-Packard 8452 diode array spectrometer with 2 nm resolution and a spectral range of 190 to 820 nm.

Reactions of Metal Complexes with pBluescript DNA. The reactions of the metal complexes with DNA were conducted using linearized plasmid DNA (pBluescript from Stratagene, 2958 base pairs (bp) and 50.2% GC) and analyzed by gel electrophoresis using a previously published protocol.[33] The concentration of the linearized plasmid DNA solution was determined spectrophotometrically (1 AU = 50 mg of DNA/mL at 258 nm). Concentrations of metal solutions were determined using the extinction coefficients for [(tpy)RuCl(BL)$PtCl_2$](PF_6) ($\epsilon = 1.46 \times 10^4$ M^{-1} cm^{-1} at 544 nm for dpp, 1.00×10^4 M^{-1} cm^{-1} at 632 nm for dpq, and 9.86×10^3 M^{-1} cm^{-1} at 682 nm for dpb). All reactions contain 1 mg of linearized plasmid DNA and 10 mM sodium phosphate, pH 7, in a total volume of 100 mL. The samples were analyzed by electrophoresis in 300 mL agarose gels (0.8% agarose, 89 mM Tris, 89 mM boric acid, pH 8) at 104 V for 1.5 h, with recirculation of the buffer. Gels were then stained in 0.5 μg/mL ethidium bromide for 1 h and photographed with UV illumination. Polaroid prints were scanned using a digital flatbed scanner.

Reactions of Metal Complexes with Calf Thymus DNA. The degree of DNA binding by [(tpy)RuCl(BL)$PtCl_2$](PF_6) was determined by a modification of the method of Barton.[54] Solutions of calf thymus DNA (0.46 mM of DNA base pairs) were incubated with 0.046 mM solutions of [(tpy)RuCl(BL)$PtCl_2$](PF_6) (BL = dpp, dpq, or dpb) at bp:mc ratios of 10:1 at 37 °C in 10 mM phosphate buffer (pH 7). At the appropriate time intervals, a 250 μL aliquot was removed and the DNA precipitated by the addition of 10 μL of NaCl (5 M) and 1.0 mL of EtOH (4 °C). The solution was centrifuged to remove the DNA, and the concentration of unbound metal complex was determined spectroscopically, using controls that lacked DNA. The $t_{1/2}$ values were determined from the hyperbolic regression equation generated by Sigma Plot Scientific Graphing Systems by interpolating the time at which 50% of the complexes were bound.[55]

Synthesis. [(tpy)RuCl(dpp)](PF_6). [(tpy)RuCl(dpp)](PF_6) was prepared by a modification of a previously published method.[56] Ru(tpy)Cl_3 (0.824 g, 1.87 mmol), dpp (0.599 g, 2.56 mmol), and LiCl (0.100 g, 2.38 mmol) were heated under an argon atmosphere for 5 h at reflux in 100 mL of a 2:1 ethanol/deionized water mixture. Addition of 8 mL of triethylamine at the start of the reaction facilitated the reduction of the ruthenium. The reaction mixture changed from brown to maroon and was precipitated by addition to 40 mL of an aqueous, saturated solution of KPF_6. The dark precipitate was collected by vacuum filtration. The product was purified by alumina chromatography prepared with 3:2 (v/v) toluene/acetonitrile. The first colored band to elute (red), following the colorless unreacted dpp ligand, was the desired product and was collected. The column eluant was evaporated under vacuum, dissolved in a minimal amount of CH_3CN, flash precipitated in diethyl ether, and collected by vacuum filtration. Yield: 90% (1.26 g, 1.68 mmol).

[(tpy)RuCl(dpq)](PF_6). [(tpy)RuCl(dpq)](PF_6) was prepared by a modification of the synthesis of [(tpy)RuCl(dpp)](PF_6), substituting dpq (0.727 g, 2.56 mmol) for dpp. The purification was as above with the first colored band (purple) being collected. Yield: 90% (1.35 g, 1.68 mmol).

[(tpy)RuCl(dpb)](PF_6). [(tpy)RuCl(dpb)](PF_6) was prepared by a modification of the synthesis of [(tpy)RuCl(dpp)](PF_6), substituting dpb (0.855 g, 2.56 mmol) for dpp. The purification was as above using a 2:1 (v/v) toluene/acetonitrile eluant. The first intensely colored band (blue), which follows a faint yellow band of unreacted dpb, was collected. Yield: 88% (1.40 g, 1.64 mmol).

General Note on Ru−Pt Bimetallic Complexes. The successful synthesis and purification of the bimetallic complexes depends on the purity of the starting materials and the correct stoichiometry of the reaction mixture. This was due, in part, to the labile nature of the Pt−Cl bonds, which renders absorption chromatography difficult. Therefore, purification relies on the differential solubility of the precursors and products.

[(tpy)RuCl(dpp)$PtCl_2$](PF_6). [(tpy)RuCl(dpp)$PtCl_2$](PF_6) was prepared by heating at reflux in 10 mL of 95% ethanol [(tpy)RuCl(dpp)](PF_6) (0.150 g, 0.200 mmol) and $PtCl_2$(dmso)$_2$ (0.089 g, 0.21 mmol). During the 1 h reaction time, the solution changed from red to purple. Once the reaction mixture had cooled to room temperature, the purple product precipitated and was separated by vacuum filtration on a fine-porosity fritted funnel. The product was washed with two 10 mL portions of ethanol and 10 mL of chloroform. The solid was redissolved in ca. 30 mL of CH_3CN and filtered, and the volume was reduced to 15 mL under vacuum. The product was then flash precipitated by addition to 60 mL of stirring diethyl ether. Yield: 90% (182 mg, 0.180 mmol). FAB-MS (nitrobenzyl alcohol matrix), *m*/*z*: 870, [(tpy)RuCl(dpp)$PtCl_2$]$^+$; 834, [(tpy)RuCl(dpp)PtCl]$^+$; 799, [(tpy)RuCl(dpp)Pt]$^+$; 763, [(tpy)Ru(dpp)Pt]$^+$; 604, [(tpy)RuCl(dpp)]$^+$; 568, [(tpy)Ru(dpp)]$^+$. Anal. Calcd for [(tpy)RuCl(dpp)$PtCl_2$](PF_6)·3H_2O: C, 32.58; H, 2.54; N, 9.17. Found: C, 30.46; H, 2.36; N, 8.31.

[(tpy)RuCl(dpq)$PtCl_2$](PF_6). [(tpy)RuCl(dpq)$PtCl_2$](PF_6) was prepared by a modification of the reaction for [(tpy)RuCl(dpp)$PtCl_2$](PF_6), using [(tpy)RuCl(dpq)](PF_6) (0.160 g, 0.200 mmol). The resulting blue product was obtained in an 85% yield (182 mg, 0.170 mmol). FAB-MS (nitrobenzyl alcohol matrix), *m*/*z*: 920, [(tpy)RuCl(dpq)$PtCl_2$]$^+$; 884, [(tpy)RuCl(dpq)PtCl]$^+$; 848, [(tpy)RuCl(dpq)Pt]$^+$; 811, [(tpy)Ru(dpq)Pt]$^+$; 654, [(tpy)RuCl(dpq)]$^+$; 618, [(tpy)Ru(dpq)]$^+$. Anal. Calcd for [(tpy)RuCl(dpq)$PtCl_2$](PF_6)·3H_2O: C, 35.42; H, 2.61; N, 8.76. Found: C, 35.10; H, 2.60; N, 8.77.

[(tpy)RuCl(dpb)$PtCl_2$](PF_6). [(tpy)RuCl(dpb)$PtCl_2$](PF_6) was prepared by a modification of the reaction for [(tpy)RuCl(dpp)$PtCl_2$](PF_6), using [(tpy)RuCl(dpb)](PF_6) (0.170 g, 0.200 mmol). The resulting green product was obtained in an 80% yield (178 mg, 0.160 mmol). FAB-MS (nitrobenzyl alcohol matrix), *m*/*z*: 970, [(tpy)RuCl(dpb)$PtCl_2$]$^+$; 934, [(tpy)RuCl(dpb)PtCl]$^+$; 899, [(tpy)-

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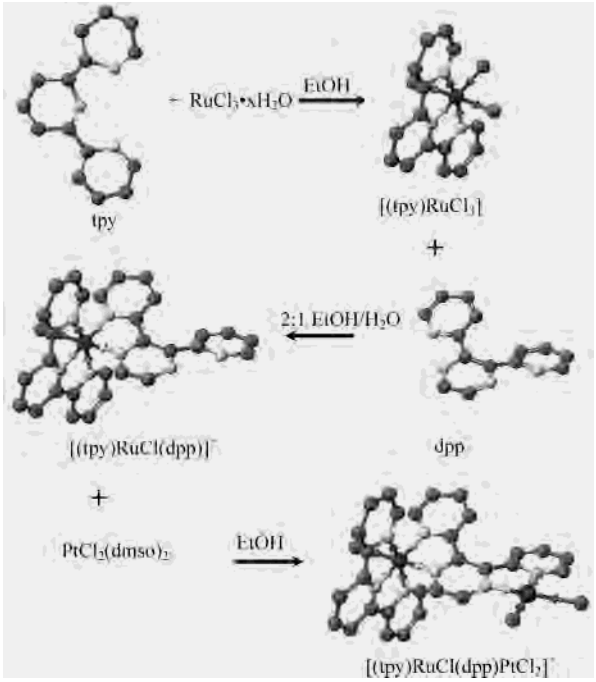
Scheme 1. Building Block Synthetic Method for the Preparation of $[(tpy)RuCl(dpp)PtCl_2](PF_6)$



RuCl(dpb)Pt]$^+$; 862, [(tpy)Ru(dpb)Pt]$^+$; 704,[(tpy)RuCl(dpb)]$^+$; 668, [(tpy)Ru(dpb)]$^+$. Anal. Calcd for $[(tpy)RuCl(dpb)PtCl_2](PF_6)\cdot 3H_2O$: C, 38.00; H, 2.67; N, 8.38. Found: C, 37.81; H, 2.70; N, 8.22.

Results and Discussion

Synthesis. The title bimetallic complexes, $[(tpy)RuCl(BL)PtCl_2](PF_6)$ (BL = dpp, dpq, and dpb), have been prepared in good yield using a building block approach. These complexes are of interest as they couple a tunable light-absorbing ruthenium center to a biologically active, reactive metal *cis*-dichloroplatinum(II) site to form a TL−LA−BL−RM assembly. These particular ruthenium light absorbers have a sixth coordination site, currently occupied with chloride, that is substitutionally labile and will allow for the later attachment of additional units to this molecular architecture, making them synthetically versatile. We have replaced this chloride ligand with CH_3CN and PEt_2Ph to produce a luminescent complex when dpp is used as the BL.[40] Shown in Scheme 1 is our building block synthetic method for $[(tpy)RuCl(dpp)PtCl_2](PF_6)$ which has been shown to be generally applicable by the preparation of the dpq and dpb analogues. This method allows for construction of this molecular architecture by first binding the terminal ligand, tpy, to the ruthenium metal center followed by BL attachment. Attachment of the Pt metal center follows. The Pt reactions occur under mild conditions that do not lead to decomposition of the $[(tpy)RuCl(BL)](PF_6)$ synthons. These mixed-metal $[(tpy)RuCl(BL)PtCl_2](PF_6)$ complexes are not stable under typical adsorption alumina chromatographic methods. Care must be taken to optimize the Pt binding reaction conditions such that Pt binding is nearly quantitative to allow for purification of the mixed-metal system by washing and recrystallization. Due to the AB chelate nature of the dpp, dpq, and dpb BLs, each of these complexes is prepared as cis- and trans-type isomers around the Ru center. These isomers are not separated and are not distinguished by FAB mass spectral analysis, electrochemistry, or electronic absorption spectroscopy.

Table 1. Cyclic Voltammetric Data for a Series of Complexes of the General Formula $[(tpy)RuCl(BL)PtCl_2](PF_6)$ (BL = dpp, dpq, dpb)[a,b]

complex	$E_{1/2}$ (V) $Ru^{II/III}$	$E_{1/2}$ (V) $BL^{0/-}$	$E_{1/2}$ (V) $BL^{-/2-}$	$E_{1/2}$ (V) $tpy^{0/-}$
$[(tpy)RuCl(dpp)](PF_6)$	1.04	−1.07		−1.27
$[(tpy)RuCl(dpq)](PF_6)$	1.06	−0.77		−1.27
$[(tpy)RuCl(dpb)](PF_6)$	1.02	−0.61		−1.25
$[(tpy)RuCl(dpp)PtCl_2](PF_6)$	1.14	−0.50	−1.05	−1.43
$[(tpy)RuCl(dpq)PtCl_2](PF_6)$	1.10	−0.32	−0.91	−1.50
$[(tpy)RuCl(dpb)PtCl_2](PF_6)$	1.12	−0.20	−0.81	−1.51

[a] dpp = 2,3-bis(2-pyridyl)pyrazine, dpq = 2,3-bis(2-pyridyl)quinoxaline, dpb = 2,3-bis(2-pyridyl)benzoquinoxaline, and tpy = 2,2′:6′,2″-terpyridine. [b] Potentials reported in CH_3CN solution with 0.1 M Bu_4NPF_6 and reported vs Ag/AgCl (0.286 V vs NHE).

These complexes have been effectively characterized by FAB mass spectral analysis, and the results are consistent with the formulation of the complexes. For solubility reasons the systems are first dissolved in CH_3CN and then introduced into the *m*-nitrobenzyl alcohol matrix. Molecular ions with loss of the PF_6^- counterion and loss of the coordinated chlorides are observed in the high mass region of the spectrum. Additional peaks are observed for the loss of the Pt metal center and the chloride coordinated to the Ru metal center.

Electrochemistry. Ruthenium(II) polyazine complexes typically display reversible ruthenium-based oxidations and ligand-based reductions, with the dpp, dpq, and dpb bridging ligand reductions occurring positive of tpy-based processes.[3−6,44] This behavior is consistent with the Ru($d\pi$) nature of the highest occupied molecular orbital (HOMO) and the BL(π^*) nature of the LUMO. The BL reductions vary as dpp is replaced by dpq and dpb, becoming easier to reduce within this series. Typically, polymetallic complexes in which the dpp, dpq, or dpb ligand are bridging display two BL-based reductions ($BL^{0/-}$ and $BL^{-/2-}$) prior to the reduction of tpy ($tpy^{0/-}$).[3,5−7,9] The title bimetallic complexes and the previously reported monometallic synthons[44] have been studied by cyclic voltammetry, and these data are summarized in Table 1. Cyclic voltammograms for $[(tpy)RuCl(BL)PtCl_2](PF_6)$ in 0.1 M Bu_4NPF_6 CH_3CN are shown in Figure 1.

The monometallic synthons, $[(tpy)RuCl(BL)](PF_6)$, display a reversible $Ru^{II/III}$ oxidation at 1.02−1.06 V vs Ag/AgCl, consistent with their coordination to five nitrogens of polyazine ligands and one chloride.[44] The first reduction varies in this series from −1.07 to −0.61 V as the BL is varied from dpp to dpq to dpb, consistent with the assignment of this couple at $BL^{0/-}$. The second reduction of these monometallic synthons occurs at relatively constant potential, −1.27 to −1.25 V, consistent with the assignment of this couple as $tpy^{0/-}$.

The title bimetallic complexes, $[(tpy)RuCl(dpp)PtCl_2](PF_6)$, display electrochemistry consistent with their formulation. All three systems have $Ru^{II/III}$ couples that occur at a

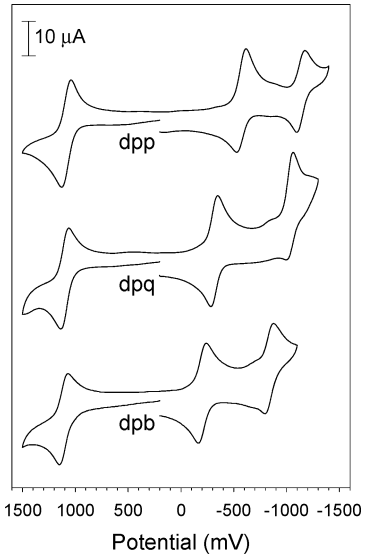


Figure 1. Cyclic voltammograms of $[(tpy)RuCl(BL)PtCl_2](PF_6)$ in 0.1 M Bu_4NPF_6 and reported vs Ag/AgCl (BL = dpp (2,3-bis(2-pyridyl)pyrazine), dpq (2,3-bis(2-pyridyl)quinoxaline), dpb (2,3-bis(2-pyridyl)benzoquinoxaline); tpy = 2,2′:6′,2″-terpyridine).

consistent potential varying only slightly as the BL is changed, 1.14 to 1.12 V, establishing the Ru metal center as the site of localization of the HOMO within this molecular architecture. This $Ru^{II/III}$ oxidation couple occurs at more positive potential in the mixed-metal Ru, Pt complexes relative to the Ru monometallic synthons. This is indicative of a less electron-rich Ru metal center in the bimetallic, Ru, Pt complexes. This results from the addition of the electron-withdrawing Pt center to the remote sites of the BL of the Ru monometallic synthons. Each $[(tpy)RuCl(dpp)PtCl_2](PF_6)$ system displays a first reduction that is BL based. This $BL^{0/-}$ couple varies dramatically as the BL is changed, with a trend similar to the monometallic synthons whereby the substitution of dpp by dpq and dpb shifts this $BL^{0/-}$ couple to more positive potential. This illustrates the role of the BL in this first reduction, establishing it as the localization of the LUMO for these mixed-metal systems. The second reduction in these bimetallic complexes is $BL^{-/2-}$ in nature, in marked contrast to the $tpy^{0/-}$ second reduction for the monometallic synthons. The occurrence of a $BL^{-/2-}$ couple prior to the reduction of tpy terminal ligands is indicative of bimetallic formation in which the BLs are bound to two electropositive metal centers.[3,5−7,9] This results from the stabilization of the BL(π*) orbitals, by coordination to the $Pt^{II}Cl_2$ moiety. The reduction potential for this second couple varies dramatically as the BL is changed, shifting to more positive potentials from dpp to dpq to dpb, consistent with its $BL^{-/2-}$ and inconsistent with a $tpy^{0/-}$ assignment. The third reduction in these bimetallic complexes leads to adsorption to the electrode surface. The potential of this reduction is obtained from square wave voltammetry and varies little as the BL is changed, consistent with the $tpy^{0/-}$ nature of this couple. Scheme 2 summarizes this electrochemical scheme for these Ru, Pt complexes.

Electronic Absorption Spectroscopy. The electronic absorption spectra of the mixed-metal bimetallics are summarized in Table 2 and the spectra shown in Figure 2.

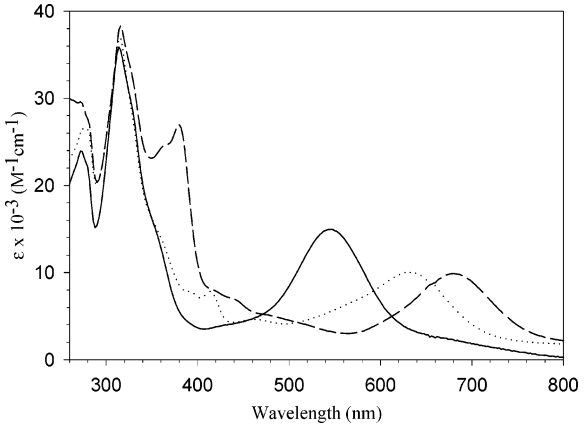


Figure 2. Electronic absorption spectra for $[(tpy)RuCl(BL)PtCl_2](PF_6)$ in CH_3CN at room temperature with BL = dpp (—), dpq (····), or dpb (---) (dpp = 2,3-bis(2-pyridyl)pyrazine, dpq = 2,3-bis(2-pyridyl)quinoxaline, dpb = 2,3-bis(2-pyridyl)benzoquinoxaline; tpy = 2,2′:6′,2″-terpyridine).

Scheme 2. Electrochemical Sequence for $[(tpy)RuCl(BL)PtCl_2](PF_6)$ (BL = dpp, dpq, dpb) with the Synthesized Oxidation State in Boldface

$$[(tpy)Ru^{III}Cl(BL)Pt^{II}Cl_2]^{2+}$$

$$-1e^- \uparrow\downarrow +1e^-$$

$$\mathbf{[(tpy)Ru^{II}Cl(BL)Pt^{II}Cl_2]^{+}}$$

$$-1e^- \uparrow\downarrow +1e^-$$

$$[(tpy)Ru^{II}Cl(BL^{-})Pt^{II}Cl_2]$$

$$-1e^- \uparrow\downarrow +1e^-$$

$$[(tpy)Ru^{II}Cl(BL^{2-})Pt^{II}Cl_2]^{-}$$

$$-1e^- \uparrow\downarrow +1e^-$$

$$[(tpy^{-})Ru^{II}Cl(BL^{2-})Pt^{II}Cl_2]^{2-}$$

The monometallic synthons display $\pi \rightarrow \pi^*$ transitions in the UV with MLCT transitions in the visible to both acceptor ligands with the Ru → BL CT band being lowest in energy.[44] This $[(tpy)RuCl(BL)](PF_6)$ MLCT band shifts to lower energy as the BL is varied, occurring at 510, 570, and 595 nm for BL = dpp, dpq, and dpb, respectively. This red shift is a result of the stabilization of the BL π^* acceptor orbital through this series of complexes, consistent with their electrochemical properties.

The bimetallic complexes, $[(tpy)RuCl(BL)PtCl_2](PF_6)$, display spectroscopic properties similar to the monometallic synthons but modulated by bridge formation. The UV region of the spectrum is dominated by tpy-based $\pi \rightarrow \pi^*$ transitions with the two major features at 272 and 316 nm being present in all three complexes, consistent with their tpy-based assignment. Underlying BL-based transitions likely account for the differences in ϵ in this spectral region. The lowest energy BL-based $\pi \rightarrow \pi^*$ transition shifts as the BL is varied with the dpp and dpq bands appearing as low-energy shoulders on the tpy-based peak at 316 nm. The dpb complex displays a distinct $\pi \rightarrow \pi^*$ transition at 380 nm. The visible region of the spectrum of each $[(tpy)RuCl(BL)PtCl_2](PF_6)$ complex contains intense MLCT transitions to both the tpy and BL. The Ru → tpy CT band occurs as a high-energy shoulder on the more intense Ru → BL CT band and is centered at ca. 460 nm for all three complexes. The Ru →

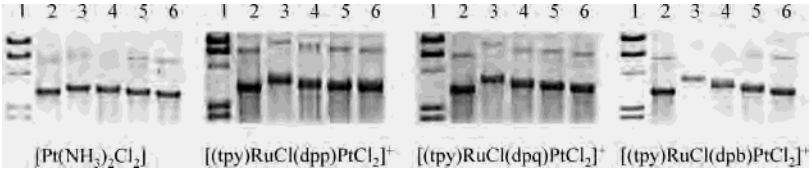


Figure 3. DNA binding study for $[(tpy)RuCl(BL)PtCl_2](PF_6)$ by agarose gel electrophoresis using linearized pBluescript DNA (BL = dpp (2,3-bis(2-pyridyl)pyrazine), dpq = (2,3-bis(2-pyridyl)quinoxaline), dpb = (2,3-bis(2-pyridyl)benzoquinoxaline); tpy = 2,2′:6′,2″-terpyridine). Lane 1 is the molecular weight standard 9.4, 6.6, 4.4, 2.3, and 2.0 kbp, lane 2 is the DNA control, lane 3 is the 5:1 base pairs (bp):metal complex (mc) ratio, lane 4 is the 10:1 bp:mc ratio, lane 5 is the 20:1 bp:mc ratio, and lane 6 is the 100:1 bp:mc ratio.

BL CT band varies in energy as a function of BL occurring at 544 nm for dpp, 632 for dpq, and 682 for dpb consistent with the stabilization of the BL-based π^* orbital through this series. This Ru → BL CT band is significantly red-shifted in the bimetallic complexes relative to the $[(tpy)RuCl(BL)](PF_6)$ monometallic synthons. This is a result of the stabilization of the BL π^* orbitals by coordination of the electropositive Pt(II) metal center consistent with the electrochemical results. These $[(tpy)RuCl(BL)PtCl_2](PF_6)$ systems display Ru → BL CT bands significantly red-shifted from that of the bpy analogues, $[(bpy)_2Ru(BL)PtCl_2](PF_6)_2$.[32] This results from the more electron-rich Ru center in the title bimetallics, which has a five N and one Cl^- coordination environment. This lead to complexes with very low energy transitions, possessing tails into the near-infrared region of the spectrum allowing for monitoring in spectroscopic regions previously inaccessible with known systems. The lowest lying MLCT transition as well as the higher energy (ca. 460 nm) band shift to lower energy as the polarity of the solvent increases, consistent with their MLCT assignment. Spectra in H_2O and $CHCl_3$ are included in the Supporting Information.

DNA Binding. The $[(tpy)RuCl(BL)PtCl_2](PF_6)$ complexes have been studied by gel electrophoresis to explore their ability to bind double-stranded DNA. The Pt(II) site in each of these complexes possesses two labile *cis*-chloride ligands, similar to cisplatin. These labile sites allow these complexes to form covalent adducts with the nitrogen bases of DNA. Figure 3 shows the results of the study of the covalent binding of these complexes and the standard cisplatin to pBluescript DNA.

In this study the metal complexes were combined with pBluescript DNA at varying base pair (bp) to metal complex (mc) ratios. These mixtures were incubated for 4 h at 37 °C with the DNA and binding analyzed by gel electrophoresis. In each case lane 1 is a molecular weight standard and lane 2 is the pBluescript DNA without any metal complex present. Lanes 3−6 are pBluescript DNA incubated with varying bp:mc ratios, 5:1, 10:1, 20:1, and 100:1, respectively. The pBluescript DNA used is 2659 bp, and its incubation with the standard cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, leads to only slight retardation of the migration of the DNA by this assay. Cisplatin is known to bind to DNA and in this assay reduces the rate of migration of this linear 2659 bp DNA slightly with this effect increasing at decreasing bp:mc ratio.[33,36−39] The title bimetallics, $[(tpy)RuCl(BL)PtCl_2](PF_6)$, exhibit avid DNA binding, with the reduction of DNA migration through the gel being much greater for these complexes than the cisplatin control. This more pronounced effect for our mixed-metal complexes has also been observed for related bpy systems[33] and could result from the higher cationic charge or molecular weight of these mixed-metal complexes, a higher degree of binding, or a larger impact on DNA structure upon binding to our complexes relative to cisplatin. All three title bimetallics show a decrease in the rate of DNA migration through the gel as the bp:mc ratio is decreased. This effect is indicative of avid binding of these complexes to DNA.

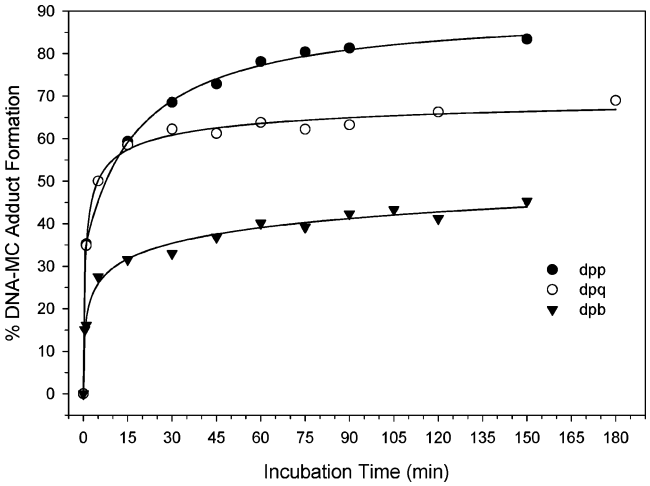


Figure 4. DNA binding assay of $[(tpy)RuCl(BL)PtCl_2](PF_6)$ (BL = dpp, dpq, or dpb) to calf thymus DNA at a 10:1 base pairs:metal complex ratio at 37 °C (dpp = 2,3-bis(2-pyridyl)pyrazine, dpq = 2,3-bis(2-pyridyl)-quinoxaline, dpb = 2,3-bis(2-pyridyl)benzoquinoxaline; tpy = 2,2′:6′,2″-terpyridine).

The degree of binding of the $[(tpy)RuCl(BL)PtCl_2](PF_6)$ complexes to DNA was assayed spectrophotometrically using the method of Barton.[46] Figure 4 shows the result of this study establishing our complexes as covalent binders of DNA. Solutions of calf thymus DNA were incubated at a 10:1 bp:mc ratio at 37 °C. Aliquots of this solution were removed, the DNA precipitated, and the concentration of the remaining metal complex was determined spectrophotometrically. The complexes all bind rapidly to DNA with half-times ($t_{1/2}$) of 1−2 min, significantly lower than that of *cis*-$[Pt(NH_3)_2Cl_2]$ or *cis*-$[Pt(pyridine)_2Cl_2]$. Table 3 summarizes these data. At the 10:1 bp:mc ratio >80% of the $[(tpy)RuCl(dpp)PtCl_2](PF_6)$ complex binds to the DNA. Less of the dpq and dpb bridged complex is able to bind to the DNA,

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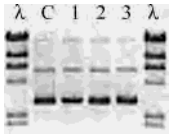

Figure 5. DNA binding study for [(tpy)RuCl(BL)](PF_6) by agarose gel electrophoresis using linearized pBluescript DNA (BL = dpp (2,3-bis(2-pyridyl)pyrazine), dpq = (2,3-bis(2-pyridyl)quinoxaline), dpb = (2,3-bis-(2-pyridyl)benzoquinoxaline); tpy = 2,2′:6′,2″-terpyridine). Lanes λ are molecular weight standards 9.4, 6.6, 4.4, 2.3, and 2.0 kbp, lane C is DNA control, lane 1 is the 5:1 base pairs (bp):metal complex (mc) ratio for BL = dpp, lane 2 is a 5:1 bp:mc ratio for BL = dpq, and lane 3 is a 5:1 bp:mc ratio for BL = dpb.

Table 2. Electronic Absorption Spectroscopy for [(tpy)RuCl(BL)$PtCl_2$](PF_6) in CH_3CN at Room Temperature[a]

complex	λ_{max}^{abs} (nm)	$10^{-3}\epsilon$ (M^{-1} cm^{-1})	assgnt
[(tpy)RuCl(dpp)$PtCl_2$](PF_6)	272	23.9	$\pi \rightarrow \pi^*$ tpy
	316	35.6	$\pi \rightarrow \pi^*$ tpy
	354 (sh)	15.0	$\pi \rightarrow \pi^*$ dpp
	460 (sh)	4.68	Ru → tpy CT
	544	14.6	Ru → dpp CT
[(tpy)RuCl(dpq)$PtCl_2$](PF_6)	272	26.1	$\pi \rightarrow \pi^*$ tpy
	316	36.9	$\pi \rightarrow \pi^*$ tpy
	362 (sh)	13.6	$\pi \rightarrow \pi^*$ dpq
	460 (sh)	4.61	Ru → tpy CT
	632	10.0	Ru → dpq CT
[(tpy)RuCl(dpb)$PtCl_2$](PF_6)	272	29.6	$\pi \rightarrow \pi^*$ tpy
	316	38.3	$\pi \rightarrow \pi^*$ tpy
	380	27.0	$\pi \rightarrow \pi^*$ dpb
	460 (sh)	5.51	Ru → tpy CT
	682	9.86	Ru → dpb CT

[a] BL = dpp (2,3-bis(2-pyridyl)pyrazine), dpq (2,3-bis(2-pyridyl)quinoxaline), or dpb (2,3-bis(2-pyridyl)benzoquinoxaline) and tpy = 2,2′:6′,2″-terpyridine.

Table 3. Summary of DNA Binding Assay of [(tpy)RuCl(BL)$PtCl_2$](PF_6) (BL = dpp, dpq, dpb) to Calf Thymus DNA at a 10:1 Base Pairs:Metal Complex Ratio at 37 °C (dpp = 2,3-Bis(2-pyridyl)Pyrazine, dpq = 2,3-Bis(2-pyridyl)quinoxaline, dpb = 2,3-Bis(2-pyridyl) benzoquinoxaline; tpy = 2,2′:6′,2″-Terpyridine)

metal complex	bp/mc av site size	$t_{1/2}$ (min)
[(tpy)RuCl(dpp)$PtCl_2$]$^+$	12.5	2.5
[(tpy)RuCl(dpq)$PtCl_2$]$^+$	15.0	1
[(tpy)RuCl(dpb)$PtCl_2$]$^+$	22.0	1
cis-[Pt(NH_3)$_2Cl_2$][57]	10.0	240
cis-[Pt(pyridine)$_2Cl_2$][58]	12.8	50
[(tpy)RuCl(bpy)]$^+$ [59]	40.0	16

consistent with their larger molecular size. All of the [(tpy)-RuCl(BL)$PtCl_2$](PF_6) systems occupy sites smaller than [(tpy)RuCl(bpy)]$^+$ (40 bp), consistent with their aforementioned covalent binding through the Pt site.

To explore the nature of this DNA interaction in more detail, the impact of incubation of DNA with the monometallic precursors was explored. Figure 5 shows the results of this study. Incubation of pBluescript DNA with a 5:1 bp:mc ratio of [(tpy)RuCl(BL)](PF_6), BL = dpp, dpq, or dpb, has no impact on the DNA migration. This establishes the role of the $Pt^{II}Cl_2$ moiety on the observed DNA binding for the title [(tpy)RuCl(BL)$PtCl_2$](PF_6) complexes.

Conclusions

A series of mixed-metal complexes of the form [(tpy)-RuCl(BL)$PtCl_2$](PF_6) have been prepared using a building block synthetic methodology. This method has been optimized and shown to be general through the variation of the BL, using dpp, dpq, and dpb. All three title bimetallics display Ru-based oxidations at a constant 1.02−1.04 V vs Ag/AgCl and $BL^{0/-}$ and $BL^{-/2-}$ couple prior to reduction of the tpy TL. These [(tpy)RuCl(BL)$PtCl_2$](PF_6) systems display lowest lying electronic transitions that are Ru(d) → BL(π^*) CT in nature red shifting at the BL(π^*) orbital is stabilized. All three bimetallic complexes avidly bind DNA with $t_{1/2}$ = 1−2 min and occupy binding sties consistent with covalent attachment of the Pt^{II} to DNA. The ruthenium bound Cl^- ligand is substitutionally labile and can be replaced to extend the molecular architecture of these complexes, producing complexes that are emissive in fluid solution.[40] The presence of an efficient LA coupled to a RM center allows these complexes to be applicable in other photochemical energy storage schemes, and their DNA binding ability makes them applicable as spectroscopic probes of metal complex−DNA interactions. In this work the chromophoric property of these complexes is exploited to detect binding to DNA. Work is currently in progress exploring these and related systems.[60]

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Supporting Information Available: Electronic absorption spectra for [(tpy)RuCl(BL)$PtCl_2$](PF_6) in H_2O and CH_3Cl. This material is available free of charge via the Internet at http://pubs.acs.org.

IC025810L

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