

## Design Aspects for the Development of Mixed-Metal Supramolecular Complexes Capable of Visible Light Induced Photocleavage of DNA

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Mixed-metal supramolecular complexes that couple ruthenium or osmium based light absorbers to a central rhodium-(III) core have been designed which photocleave DNA upon irradiation with visible light. The complexes  $[\{(bpy)_2Ru(dpp)\}_2RhCl_2](PF_6)_5$ ,  $[\{(bpy)_2Os(dpp)\}_2RhCl_2](PF_6)_5$ , and  $[\{(tpy)RuCl(dpp)\}_2RhCl_2](PF_6)_3$ , where  $bpy = 2,2'$ -bipyridine,  $tpy = 2,2':6',2''$ -terpyridine, and  $dpp = 2,3$ -bis(2-pyridyl)pyrazine, all exhibit intense metal to ligand charge transfer (MLCT) based transitions in the visible but possess lower lying metal to metal charge transfer (MMCT) excited states. These supramolecular complexes with low lying MMCT states photocleave DNA when excited into their intense MLCT transitions. Structurally similar complexes without this low lying MMCT state do not exhibit DNA photocleavage, establishing the role of this MMCT state in the DNA photocleavage event. Design considerations necessary to produce functional DNA photocleavage agents are presented herein.

### Introduction

Much recent emphasis has been placed on developing chemical reagents capable of cleaving DNA. Such systems have promise as structural probes and therapeutic agents, with many transition metal complexes being reported.<sup>1–19</sup> Within

that framework, photochemical approaches are of particular interest because they offer control of the reaction, function through the conversion of light energy to chemical energy, and can be highly targeted.<sup>10–15,20</sup> One popular approach involves the sensitization of molecular oxygen.<sup>5,7,8,21</sup> This approach is limited in some applications due to low oxygen levels or the local depletion of oxygen levels through the photochemical process.

The development of photosensitizers that absorb low energy light, are tunable, and function in the absence of molecular oxygen is of interest. The ability to tune light absorption is advantageous leading to the elimination of interfering absorbance by other chromophores in the system as well as to exploit excitation by a particular light source. A photosensitizer which can be excited with low energy light can avoid the base damage induced by UV-light.<sup>22,23</sup> Oxygen independent systems function under conditions of low oxygen content and often have a different mechanism of photocleavage.<sup>16</sup>

Rhodium and ruthenium complexes have been shown to photocleave DNA. Photolysis at 310 nm of rhodium(III) complexes of phi (9,10-phenanthrenequinone diimine) leads

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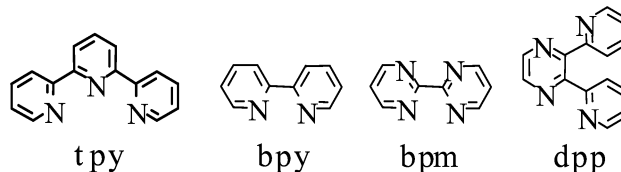
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to hydrogen abstraction from the 3'-carbon of deoxyribose in the sugar phosphate backbone, leading to DNA cleavage.<sup>24</sup> Cleavage selectivity can be modulated by ancillary<sup>25</sup> and active<sup>26</sup> ligand variation or by tethering to DNA.<sup>27–30</sup> [Rh(phi)<sub>2</sub>(phen)]<sup>3+</sup> and [Ru(phi)<sub>2</sub>(phen)]<sup>2+</sup> have recently been shown to stabilize duplex DNA inhibiting transcription.<sup>31</sup> Rh<sub>2</sub>(O<sub>2</sub>CCH<sub>3</sub>)<sub>4</sub>L<sub>2</sub> (L = H<sub>2</sub>O<sup>14</sup> or PPh<sub>3</sub><sup>32</sup>) has exhibited the ability to photocleave DNA when irradiated in the presence of electron acceptors. DNA binding and inhibition of transcription has been shown for Rh<sub>2</sub>(O<sub>2</sub>CCH<sub>3</sub>)<sub>4</sub> and [Rh<sub>2</sub>(O<sub>2</sub>CCH<sub>3</sub>)<sub>2</sub>(phen)<sub>2</sub>]<sup>2+</sup>.<sup>33</sup> Studies have shown site specific oxidative cleavage of DNA using [Ru<sup>IV</sup>(tpy)(bpy)O]<sup>2+</sup> and [Ru<sup>III</sup>(tpy)(bpy)OH]<sup>2+</sup> (tpy = 2,2':6',2''-terpyridine).<sup>34,35</sup> Photoexcitation of ruthenium(II) polypyridyl systems has resulted in oxidative damage to DNA in the presence of an electron acceptor<sup>36–38</sup> and cleavage by oxygen sensitization.<sup>5,7,8</sup> Rh(III) complexes intercalated into DNA serve as electron acceptors for excited Ru chromophores via long-range electron transfer.<sup>39,40</sup>

Interest in the area of supramolecular chemistry of ruthenium(II) polypyridine complexes has resulted from the rich photochemical and electrochemical properties of these complexes.<sup>41–45</sup> Many of these complexes take advantage of the metal to ligand charge transfer (MLCT) properties of ruthenium chromophores incorporating polyazine bridging ligands (BL), focusing on the development of photochemical molecular devices. Within a supramolecular architecture, ligands

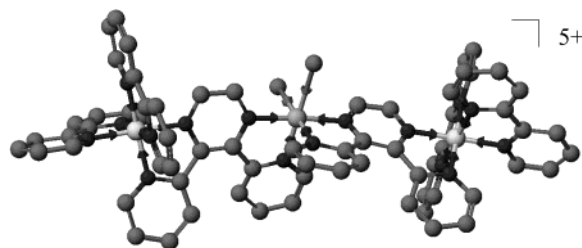
are classified as terminal or bridging, depending on their mode of coordination to the metal center. The bidentate terminal ligand bpy is commonly studied while the analogous tridentate terminal ligand tpy is less well studied (bpy = 2,2'-bipyridine, tpy = 2,2':6',2''-terpyridine). One of the most highly studied polyazine bridging ligands is dpp (2,3-bis(2-pyridyl)pyrazine). This BL often leads to the development



of polymetallic complexes that have emissive MLCT states capable of undergoing intramolecular energy and electron transfer.

Incorporation of rhodium(III) centers into a supramolecular framework containing ruthenium based MLCT absorbers can allow rhodium to function as an intramolecular electron acceptor. A number of such studies have appeared. Interesting systems were studied by Indelli, Scandola, Collin, Sauvage, and Sour that varied the spacer length between ruthenium and rhodium of the form [(tpy)Ru(tpy-(Ph)<sub>n</sub>-tpy)Rh(tpy)]<sup>5+</sup> (*n* = 0, 1, or 2).<sup>46</sup> Systems of the type [(Me<sub>2</sub>phen)<sub>2</sub>Ru<sup>II</sup>(Mebpy-CH<sub>2</sub>CH<sub>2</sub>-Mebpy)Rh<sup>III</sup>(Mebpy)<sub>2</sub>]<sup>5+</sup><sup>46,47</sup> and a dpp bridged system [(bpy)<sub>2</sub>Ru<sup>II</sup>(dpp)Rh<sup>III</sup>(bpy)<sub>2</sub>]<sup>5+</sup><sup>48</sup> have been studied. Endicott et al. have studied Ru<sup>II</sup>,Rh<sup>III</sup> cyanide-bridged complexes.<sup>49</sup> Typically such systems undergo efficient intramolecular electron-transfer quenching of the Ru-based MLCT excited state by the rhodium center.

Trimetallic complexes coupling ruthenium light absorbing centers to reactive metal centers have been of interest as photochemical molecular devices. [{(bpy)<sub>2</sub>Ru(BL)}<sub>2</sub>MCl<sub>2</sub>]<sup>5+</sup> (M = Rh or Ir and BL = 2,3-bis(2-pyridyl)pyrazine (dpp)<sup>50</sup> or 2,2'-bipyrimidine (bpm)<sup>51</sup>) and [{(tpy)RuCl(BL)}<sub>2</sub>RhCl<sub>2</sub>]<sup>3+</sup> (BL = bpm and dpp)<sup>52</sup> show that modification of device components can have dramatic impact on the photophysical and electrochemical properties of these systems. [{(bpy)<sub>2</sub>Ru(dpp)}<sub>2</sub>RhCl<sub>2</sub>]<sup>5+</sup>,



displays a Rh based lowest occupied molecular orbital

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(LUMO) and a ruthenium based highest occupied molecular orbital (HOMO) leading to a lowest lying metal-to-metal charge transfer (MMCT) excited state in this system. The related iridium congener,  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{IrCl}_2^{5+}$ , displays a lowest lying MLCT state due to the higher energy  $d\sigma^*$  orbitals on iridium. Substitution of the bpm BL stabilizes the  $\pi^*$  orbital leading to a system,  $[(\text{bpy})_2\text{Ru}(\text{bpm})]_2\text{RhCl}_2^{5+}$ , with a lowest lying MLCT state.

Recently we communicated a preliminary study of the visible light induced photocleavage of DNA by  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{RhCl}_2^{5+}$ .<sup>53</sup> Herein we report a study of the construction and study of supramolecular mixed-metal complexes that can promote light activated DNA cleavage.

## Experimental Section

**Materials.** The reagents used in the preparation and studies of the complexes are all reagent grade and obtained from Aldrich unless otherwise specified. pUC18 DNA plasmid (2686 bp) was obtained from Bayou Biolabs. Electrophoresis-grade low EEO agarose, tris(hydroxymethyl)aminomethane (Tris), boric acid, and ethidium bromide were obtained from Fisher. *EcoRI* and *HindIII* restriction endonucleases were purchased from Promega. Doubly distilled deionized water, which was used to prepare all aqueous solutions and for all physical measurements, was obtained by passing doubly distilled water through a Milli-Q deionizing system.

**Synthesis.** The complexes  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{RhCl}_2(\text{PF}_6)_5$ ,<sup>50</sup>  $[(\text{tpy})\text{RuCl}(\text{dpp})]_2\text{RhCl}_2(\text{PF}_6)_3$ ,<sup>52</sup> and  $[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}_2(\text{PF}_6)_2$ <sup>54</sup> were synthesized as described previously.

$[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}_2(\text{PF}_6)_5$ . The mixed-metal complex was prepared by heating at reflux for 1 h  $[(\text{bpy})_2\text{Os}(\text{dpp})](\text{PF}_6)_2$  (0.50 g, 49 mmol) and  $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$  (1.0 g, 68 mmol) in 20 mL of 2:1 EtOH:H<sub>2</sub>O mixture. The reaction mixture was cooled to RT and added to 200 mL of a saturated aqueous solution of  $\text{KPF}_6$  to induce precipitation. The resulting mixture was filtered and the purple product collected. This was washed with 0 °C H<sub>2</sub>O (20 mL) followed by diethyl ether (ca. 100 mL). The crude product was dissolved in the minimum volume of  $\text{CH}_3\text{CN}$  (ca. 10 mL), filtered, and flash precipitated in ca. 200 mL of stirring diethyl ether. Yield = 0.49 g (83%).

UV/vis in H<sub>2</sub>O,  $\lambda_{\text{max}}$  ( $\epsilon \times 10^{-4}$  (M<sup>-1</sup> cm<sup>-1</sup>)): 194 nm (9.29), 240 nm (4.28), 334 nm (3.21), 406 nm (1.15), 530 nm (2.22).

FAB-MS ion (*m/z*; relative abundance):  $[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}_2(\text{PF}_6)_4^+$  (2227, 55);  $[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}_2(\text{PF}_6)_3^+$  (2082, 100);  $[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}(\text{PF}_6)_3^+$  (2047, 15);  $[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}_2(\text{PF}_6)_2^+$  (1937, 30);  $[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}(\text{PF}_6)_2^+$  (1902, 10).

**Electronic Absorption Spectroscopy.** Electronic absorption spectra were recorded at room temperature using a Hewlett-Packard 8452A diode array spectrophotometer with a 2 nm resolution. Burdick and Jackson UV grade acetonitrile and doubly distilled deionized water were used as solvents for all measurements. Quartz cuvettes of path length 1 or 0.2 cm were used to measure the UV/visible spectra at room temperature.

**Electrochemistry.** Cyclic voltammograms were recorded using a one-compartment, three electrode cell, Bioanalytical Systems

(BAS), equipped with a platinum wire auxiliary electrode. The working electrode was a 1.9 mm diameter glassy carbon disk from BAS. Potentials were referenced to a Ag/AgCl electrode (0.29 V vs NHE), which was calibrated against the  $\text{FeCp}_2/\text{FeCp}_2^+$  redox couple (0.67 V vs NHE).<sup>55</sup> The supporting electrolyte was 0.1 M  $\text{Bu}_4\text{NPF}_6$ , and the measurements were carried out in Burdick and Jackson UV-grade acetonitrile, which was dried over 3 Å molecular sieves.

**FAB-Mass Spectrometry.** FAB mass spectral analysis was performed by M-Scan Incorporated, West Chester, Pennsylvania, on a VG Analytical ZAB 2-SE high field mass spectrometer using *m*-nitrobenzyl alcohol as a matrix. The trimetallic gave very nice FAB-MS patterns with sequential loss of  $\text{PF}_6$  ion and one chloride being observed. The fragmentation pattern was consistent with the proposed molecular structure.

**Preparation and Purification of pBluescript DNA Plasmid.** The pBluescript (2958 bp, MW = 1952 kDa) DNA plasmid was amplified and purified from *Escherichia coli* strain JM 109 according to the protocol as set out by QIAGEN Inc., 28159 Avenue, Stanford Valencia, CA 91355.

**Preparation of the Molecular Weight Standards.** Molecular weight standards for non-denaturing agarose gel electrophoresis were prepared as described previously.<sup>56</sup>

**DNA Photocleavage Reactions.** The assay of DNA photocleavage was accomplished as previously described.<sup>53</sup> Solutions were 3.5  $\mu\text{M}$  in metal complex, mc, 0.7  $\mu\text{M}$  in DNA base pairs, bp (to achieve a 1:5 mc:bp ratio), and 6.9 mM in phosphate buffer (pH = 7). The ionic strength allowed for ionic association of the cationic metal complexes with DNA. Deoxygenation was accomplished by bubbling with Ar for 30 min prior to the photolysis of the samples in an airtight cell blanketed with Ar. Irradiation used a 1000 W xenon arc lamp, a water IR filter, and 475 nm cutoff filter. Controls included DNA without metal complex incubated, DNA without metal complex photolyzed, and DNA with metal complex incubated. The photolysis of DNA without the metal complex does not lead to any changes in the DNA as detected by gel electrophoresis. Other controls are shown in the gels. All samples were analyzed by electrophoresis in 300 mL agarose gels (0.8% agarose, 90 mM Tris, 90 mM boric acid, pH 8, at an ionic strength of 0.0043 M) at 104 V for 1.5 h, with recirculation of the buffer. Gels were then stained in 0.5  $\mu\text{g/mL}$  ethidium bromide for 1 h and photographed with UV illumination. Polaroid prints were scanned using a MicroTek ScanMaker E6.

## Results and Discussion

Supramolecular complexes can be designed to possess intense MLCT transitions in the visible. Once optically populated, these MLCT states can be designed to be thermodynamically capable of intramolecular electron transfer to a central Rh(III) site. Our design of a Rh site capable of functioning as an electron acceptor is one that possesses low-lying  $d\sigma^*$  orbitals, below the  $\mu$ -BL  $\pi^*$  orbitals, making rhodium the site of localization of the LUMO in the target complexes. In such complexes, <sup>1</sup>MLCT excitation is then followed by conversion to a reactive <sup>3</sup>MMCT state, Figure 1. Preparing a series of complexes with this common feature and comparing their photoreactivity with DNA to that of

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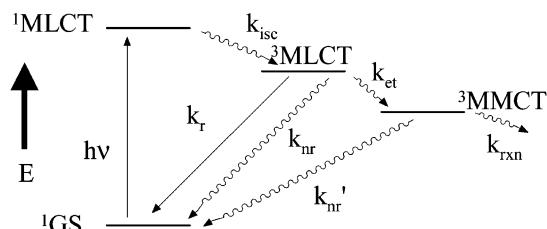
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**Figure 1.** State diagram for DNA photocleavage agents.

**Table 1.** Electrochemical Properties of a Series of Mixed-Metal Trimetallic Complexes Where bpy = 2,2'-Bipyridine, tpy = 2,2':6',2''-Terpyridine, dpp = 2,3-Bis(2-pyridyl)pyrazine, and bpm = 2,2'-Bipyrimidine

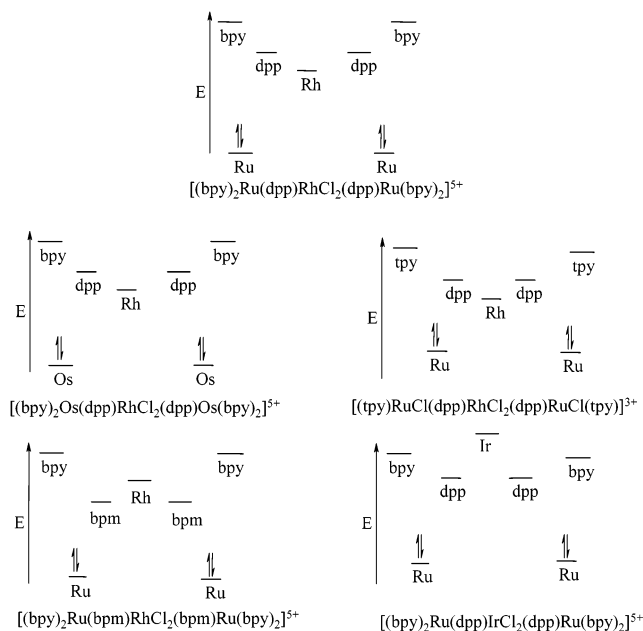
$E_{1/2}$ in V <sup>a</sup>	assignment
1.60	$\{[(bpy)_2Ru(dpp)]_2RhCl_2\}(PF_6)_5^{50}$ 2Ru <sup>III/II</sup>
$E_p^c = -0.39$	Rh <sup>III/I</sup>
-0.79	dpp,dpp/dpp,dpp <sup>-</sup>
-1.02	dpp,dpp <sup>-</sup> /dpp <sup>-</sup> ,dpp <sup>-</sup>
1.70	$\{[(bpy)_2Ru(bpm)]_2RhCl_2\}(PF_6)_5^{51}$ 2Ru <sup>III/II</sup>
-0.13	bpm,bpm/bpm,bpm <sup>-</sup>
-0.26	bpm,bpm <sup>-</sup> /bpm <sup>-</sup> ,bpm <sup>-</sup>
-0.78	Rh <sup>III/I</sup>
1.56	$\{[(bpy)_2Ru(dpp)]_2IrCl_2\}(PF_6)_5^{50}$ 2Ru <sup>III/II</sup>
-0.39	dpp,dpp/dpp,dpp <sup>-</sup>
-0.54	dpp,dpp <sup>-</sup> /dpp <sup>-</sup> ,dpp <sup>-</sup>
1.21	$\{[(bpy)_2Os(dpp)]_2RhCl_2\}(PF_6)_5$ 2Os <sup>III/II</sup>
$E_p^c = -0.39$	Rh <sup>III/I</sup>
-0.76	dpp,dpp/dpp,dpp <sup>-</sup>
-1.00	dpp,dpp <sup>-</sup> /dpp <sup>-</sup> ,dpp <sup>-</sup>
1.12	$\{[(tpy)RuCl(dpp)]_2RhCl_2\}(PF_6)_3^{52}$ 2Ru <sup>III/II</sup>
$E_p^c = -0.47$	Rh <sup>III/I</sup>
-0.87	dpp,dpp/dpp,dpp <sup>-</sup>
-1.20	dpp,dpp <sup>-</sup> /dpp <sup>-</sup> ,dpp <sup>-</sup>

<sup>a</sup> Potentials reported versus the Ag/AgCl (0.29 V vs NHE) reference electrode in 0.1 M Bu<sub>4</sub>NPF<sub>6</sub> CH<sub>3</sub>CN.

close analogues without a low lying MMCT state allow us to establish the role of this MMCT state in the DNA photocleavage reaction. DNA photocleavage agents have been designed that vary light absorbing metals, osmium or ruthenium, as well as terminal ligand, two blys or tpy and chloride.

**Synthesis.** The synthesis of our mixed-metal trimetallic complexes uses a building block approach with the final step being the coupling to the reactive metal center. The complex  $\{[(bpy)_2Os(dpp)]_2RhCl_2\}(PF_6)_5$  represents the first system coupling two osmium light absorbers to a central Rh core. This system is prepared in good yield using a modification of the previously reported method for the preparation of  $\{[(bpy)_2Ru(dpp)]_2RhCl_2\}(PF_6)_5$ .<sup>50</sup> FAB mass spectral analysis, included in the Experimental Section, shows peaks consistent with the molecular ion and successive loss of PF<sub>6</sub><sup>-</sup> counterions and one of the coordinated chlorides.

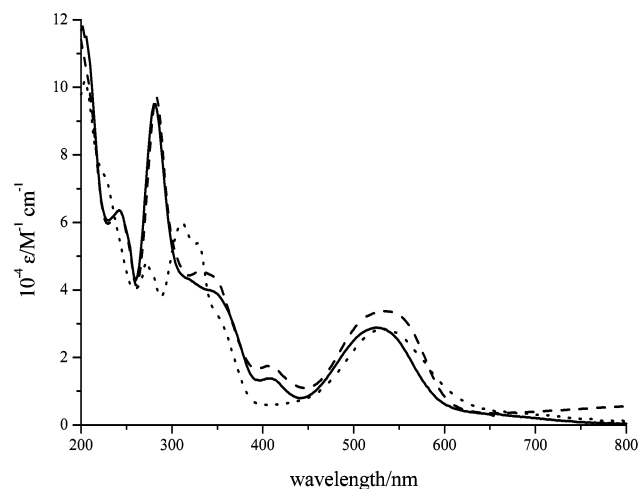
**Electrochemistry.** The electrochemical properties of the osmium based complex,  $\{[(bpy)_2Os(dpp)]_2RhCl_2\}(PF_6)_5$ , as well as the previously reported ruthenium complexes are summarized in Table 1. A cyclic voltammogram of  $\{[(bpy)_2Os(dpp)]_2RhCl_2\}(PF_6)_5$  in 0.1 M Bu<sub>4</sub>NPF<sub>6</sub> is included in the Supporting Information.



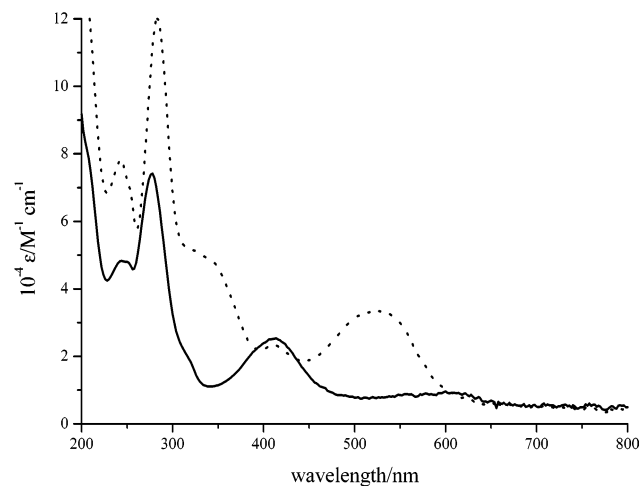
**Figure 2.** The orbital energy diagrams for a series of trimetallic complexes (bpy = 2,2'-bipyridine, tpy = 2,2':6',2''-terpyridine, bpm = 2,2'-bipyrimidine, and dpp = 2,3-bis(2-pyridyl)pyrazine).

All the trimetallic complexes exhibit overlapping terminal metal oxidations consistent with the weak electronic coupling of these Ru or Os metals through the (BL)Rh<sup>III</sup>Cl<sub>2</sub>(BL) core.<sup>50–52</sup> The potential of this couple shifts as a function of the identity of the metal, Ru or Os, and the coordination environment of this metal. For the complexes shown below to photocleave DNA, this couple varies dramatically, showing that DNA photocleavage is independent of this metal redox couple potential within this 1.12–1.60 V vs Ag/AgCl range. The new complex,  $\{[(bpy)_2Os(dpp)]_2RhCl_2\}(PF_6)_5$ , oxidizes at 1.21 V, 390 mV easier than the Ru analogue, consistent with the higher energy dπ orbitals on Os relative to Ru. The complexes  $\{[(bpy)_2Ru(dpp)]_2RhCl_2\}(PF_6)_5$ ,  $\{[(bpy)_2Os(dpp)]_2RhCl_2\}(PF_6)_5$ , and  $\{[(tpy)RuCl(dpp)]_2RhCl_2\}(PF_6)_3$  all possess irreversible first reductions localized on the Rh metal center followed by sequential reduction of the two dpp BLs. Two control systems,  $\{[(bpy)_2Ru(bpm)]_2RhCl_2\}(PF_6)_5$  and  $\{[(bpy)_2Ru(dpp)]_2IrCl_2\}(PF_6)_5$ , have been included that exhibit similar oxidative couples to the active DNA photocleavage agents but possess first reductions localized on the BL.<sup>50,51</sup> This modulation of the site of localization of the LUMO is accomplished in two ways. For  $\{[(bpy)_2Ru(bpm)]_2RhCl_2\}(PF_6)_5$  the use of the bpm BL, being easier to reduce than dpp, lowers the energy of the bpm(π\*) acceptor orbitals below that of the Rh(dσ\*) orbitals. For  $\{[(bpy)_2Ru(dpp)]_2IrCl_2\}(PF_6)_5$  the use of Ir in place of Rh raises the energy of the now Ir(dσ\*) orbitals above that of the dpp(π\*) orbitals. The effect in each case is the same, the localization of the LUMO for these controls on the BL(π\*) orbitals. This variation in the energetics of these trimetallic complexes is illustrated in Figure 2.

Using this series of complexes will allow the probing of the energetic requirements for the generation of a functioning supramolecular system that photocleaves DNA.

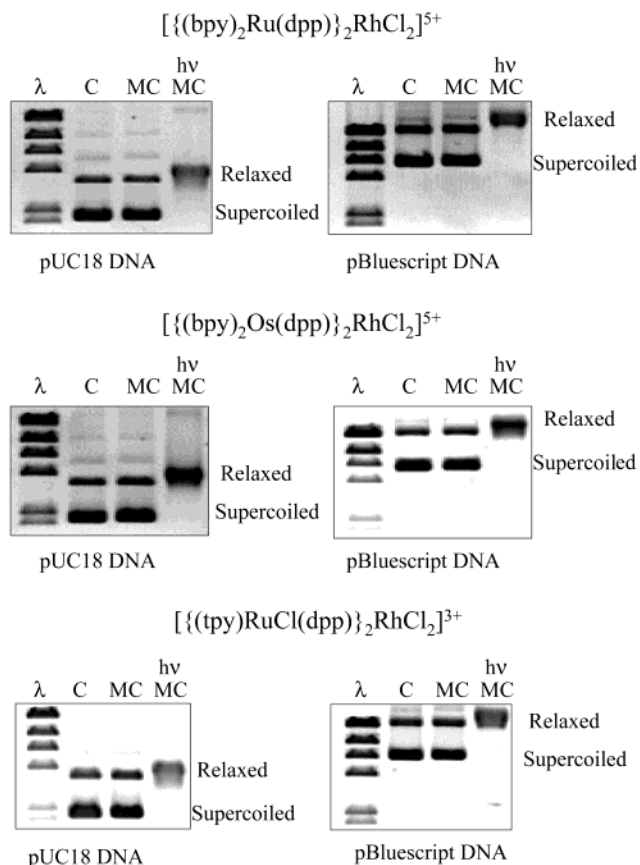


**Figure 3.** Electronic absorption spectra for  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{RhCl}_2(\text{PF}_6)_5$  (—),  $[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}_2(\text{PF}_6)_5$  (---), and  $[(\text{tpy})\text{RuCl}(\text{dpp})]_2\text{RhCl}_2(\text{PF}_6)_3$  (···) in water.



**Figure 4.** Electronic absorption spectra for  $[(\text{bpy})_2\text{Ru}(\text{bpm})]_2\text{RhCl}_2(\text{PF}_6)_5$  (—) and  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{IrCl}_2(\text{PF}_6)_5$  (···) in water.

**Electronic Absorption Spectroscopy.** The electronic absorption spectra of these supramolecular complexes in water are shown in Figure 3. All three complexes possess intense intraligand transitions in the ultraviolet and lowest lying  $\text{Ru}(\text{d}\pi)$  or  $\text{Os}(\text{d}\pi) \rightarrow \text{BL CT}$  bands that occur in the low energy visible. For  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{RhCl}_2^{5+}$  the  $\text{Ru}(\text{d}\pi) \rightarrow \text{dpp}(\pi^*)$  CT transition occurs at 525 nm. The Ru and Os analogues,  $[(\text{bpy})_2\text{M}(\text{dpp})]_2\text{RhCl}_2^{5+}$ , have spectroscopy that is very similar with the Os complex having more intensity in the low energy tail owing to the higher degree of spin-orbit coupling in Os enhancing the  $^3\text{MLCT}$  absorption. All three complexes are efficient light absorbers throughout the ultraviolet and visible. The spectra for the two control systems with BL based LUMOs are shown in Figure 4.<sup>50,51,53</sup> The  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{IrCl}_2^{5+}$  system displays virtually identical spectroscopy to that of the Rh analogue, consistent with the similar molecular architecture and the dominance of the ruthenium chromophores on the observed spectroscopy. The  $[(\text{bpy})_2\text{Ru}(\text{bpm})]_2\text{RhCl}_2^{5+}$  system displays spectroscopy consistent with bpm bridged bis(bipyridine)ruthenium chromophores.<sup>51</sup>



**Figure 5.** Imaged agarose gel showing the photocleavage of pUC18 and pBluescript plasmid by  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{RhCl}_2^{5+}$ ,  $[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}_2^{5+}$ , and  $[(\text{tpy})\text{RuCl}(\text{dpp})]_2\text{RhCl}_2^{3+}$  in the absence of molecular oxygen. Lanes  $\lambda$  are the  $\lambda$  molecular weight standards, lanes C are the plasmid controls, lanes MC are the dark plasmid controls incubated at 37 °C (2 h) in the presence of the metal complex at a 1:5 metal complex:base pair ratio, and lanes hv, MC are the plasmids irradiated at  $\lambda \geq 475$  nm for 20 min in the presence of the metal complex at a 1:5 metal complex:base pair ratio.<sup>53</sup>

**DNA Photocleavage Studies.** Previous studies in our group showed the ability of  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{RhCl}_2^{5+}$  to photocleave DNA when excited by visible light in the presence or absence of oxygen.<sup>53</sup> We also demonstrated that  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{IrCl}_2^{5+}$  and  $[(\text{bpy})_2\text{Ru}(\text{bpm})]_2\text{RhCl}_2^{5+}$  did not exhibit this photocleavage property. To further understand the necessary energetic and structural requirements for DNA photocleavage we have assayed the DNA photocleavage ability of  $[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}_2^{5+}$  and  $[(\text{tpy})\text{RuCl}(\text{dpp})]_2\text{RhCl}_2^{3+}$  as well as continued to study the previously reported  $[(\text{bpy})_2\text{Ru}(\text{dpp})]_2\text{RhCl}_2^{5+}$ . The results of those studies are presented in Figure 5.

The new systems use an osmium light absorbing metal in place of ruthenium,  $[(\text{bpy})_2\text{Os}(\text{dpp})]_2\text{RhCl}_2^{5+}$ , and in the other case change the terminal ligand to tpy and chloride in place of two bpy ligands,  $[(\text{tpy})\text{RuCl}(\text{dpp})]_2\text{RhCl}_2^{3+}$ . This results in substantial changes in the redox properties of the light absorbing metals and the shape of the complexes while maintaining a rhodium based LUMO and a lowest lying MMCT state. In Figure 5, lane  $\lambda$  shows the  $\lambda$  molecular weight standards (23, 9.4, 6.6, 4.4, 2.3, and 2.0 kb top to bottom on each gel). Lane C, the plasmid controls, indicate that pUC18 plasmid and pBluescript plasmids are found

mostly as the supercoiled state with a small amount of nicked, circular DNA. When irradiated ( $\lambda_{\text{irr}} \geq 475$  nm) for 20 min, the plasmid alone does not cleave.<sup>53</sup> When incubated at 37 °C for 2 h in the presence of the metal complex, lanes MC, the plasmid DNA is not cleaved. In the absence of molecular oxygen when either the pUC18 or pBluescript plasmids are irradiated for 20 min ( $\lambda_{\text{irr}} \geq 475$  nm) in the presence of  $[\{(bpy)_2Ru(dpp)\}_2RhCl_2]^{5+}$ ,  $[\{(bpy)_2Os(dpp)\}_2RhCl_2]^{5+}$ , or  $[\{(tpy)RuCl(dpp)\}_2RhCl_2]^{3+}$ , at a 1:5 metal complex to base pair ratio (lanes hv, MC) conversion of the supercoiled DNA to the nicked form is observed. These cleavage reactions are also observed in the presence of molecular oxygen. Previously we have shown that under similar conditions the monometallic synthon,  $[(bpy)_2Ru(dpp)]^{2+}$ , does not photocleave DNA.

To further explore the role of the Rh LUMO, resulting in a MMCT excited state, on the DNA photocleavage the bpm analogue  $[\{(bpy)_2Ru(bpm)\}_2RhCl_2]^{5+}$  and the Ir analogue  $[\{(bpy)_2Ru(dpp)\}_2IrCl_2]^{5+}$ , which contain inaccessible Rh( $d\sigma^*$ ) and Ir( $d\sigma^*$ ) orbitals,<sup>50,51</sup> were previously studied for their ability to photocleave DNA.<sup>53</sup> The Ir analogue has nearly identical electronic absorption spectroscopy to the Rh complex, Figure 4. This allows it to function well as a control system possessing a lowest lying MLCT state. Neither model complex is able to photocleave DNA.

These studies together illustrate the role of the supramolecular architecture, including Rh, on the desired photoreactivity and the generality of this molecular architecture for this application. While the mode of DNA photocleavage is unclear, frank cleavage is observed consistent with reactivity arising from the photogenerated Rh(II) site. It is possible that the excited state reaction associated with the MMCT state precedes the DNA cleavage event. We have looked for evidence of this through monitoring of the spectroscopy of our system photolyzed in the absence of DNA, but see no spectroscopic changes. The DNA cleavage product migrates slightly more slowly through the gel than native nicked plasmid, and similar results have been observed by Turro.<sup>14</sup> This may be suggestive of low levels of photoinduced

binding of the metal complex to DNA. We have explored this possibility using a smaller (30 mer) linearized DNA fragment analyzing photolyzed samples by MADLI mass spectroscopy. We see no evidence of metal complex binding, but low levels of metal binding cannot be ruled out at this time.

These results indicate that we can design varied mixed-metal supramolecular complexes capable of DNA photocleavage and illustrate the necessary energetic requirements for the functioning of such systems. These supramolecular complexes function via a MMCT state, a state previously unexplored for such reactivity. Additionally, similar control systems have been designed without a Rh( $d\sigma^*$ ) based LUMO that do not display this DNA photocleavage behavior. By virtue of their supramolecular architecture, these complexes are amendable to structural variation and the incorporation of additional components. Herein we have shown that variation of the light absorbing metal, from ruthenium to osmium, and variation of the terminal ligand, from two bpps to one tpy and one chloride, are possible while still maintaining the desired photoreactivity, DNA photocleavage. Additionally, photocleavage can occur in the absence of molecular oxygen. This makes this new structural motif for DNA photocleavage of significant interest in photochemical manipulation of DNA. Studies are underway to explore additional supramolecular complexes with this interesting photoreactivity and to probe this reaction in more detail.<sup>57</sup>

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**Supporting Information Available:** A cyclic voltammogram of  $[\{(bpy)_2Os(dpp)\}_2RhCl_2](PF_6)_5$  in 0.1 M  $Bu_4NPF_6$ . This material is available free of charge via the Internet at <http://pubs.acs.org>.

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