

***cis*-[Rh₂(μ-O₂CCH₃)₂(CH₃CN)₆]²⁺ as a Photoactivated Cisplatin Analog**Daniel A. Lutterman,[†] Patty K.-L. Fu,[‡] and Claudia Turro^{*†}

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Cisplatin, *cis*-Pt(NH₃)₂Cl₂, has been a successful antitumor agent against various cancers.^{1–5} Although many related drugs have been developed, their toxicity towards healthy cells and acquired cellular resistance still require improvement.^{1–5} The ligand exchange of the coordinated chloride ions for water molecules in cisplatin produces a diaqua Pt(II) species, *cis*-[Pt(NH₃)₂(H₂O)₂]²⁺, that binds covalently to DNA and disrupts cellular transcription.^{1–5} Limitations of cisplatin and related drugs are their activation through thermal ligand exchange, which can take place in both healthy and cancerous cells, and the cellular resistance developed towards these agents.

Selectivity toward tumor tissue can be achieved through the activation of drugs by light, such that a molecule that has no or low toxicity in the dark becomes highly toxic upon irradiation with low energy light. This mode of drug activation provides a means to localize the action of the drug to the irradiated area and is commonly known as photodynamic therapy (PDT). PDT has been used successfully in the treatment of lung and esophageal cancers,^{6–8} however, a drawback of the PDT drugs currently in use is that their action is mediated by O₂. Since malignant cancers are often hypoxic,^{6–8} new O₂-independent PDT agents must be sought. Recently, *cis*-[Ru(bpy)₂(NH₃)₂]²⁺ was shown to covalently bind to DNA upon irradiation with near-UV light (λ_{irr} ≥ 345 nm).⁹ The present work focuses on the photoinitiated binding of *cis*-[Rh₂(μ-O₂CCH₃)₂(CH₃CN)₆]²⁺ (**1**) (structure shown in Figure 1a) to ds-DNA initiated with visible light (λ_{irr} ≥ 455 nm).

The electronic absorption spectrum of **1** in CH₃CN exhibits peaks with maxima at 363 nm (ε = 420 M⁻¹ cm⁻¹) and 525 nm (ε = 218 M⁻¹ cm⁻¹). A TDDFT calculation on the model complex *cis*-[Rh₂(μ-O₂C₂H₃)₂(CH₃CN)₆]²⁺ (**1a**) in the gas phase predicts transitions at 387 and 501 nm, where the lowest energy transition is predicted to be metal-centered with Rh₂(π*) → Rh₂(σ*) parentage. The transition at higher energy arises from a combination of filled molecular orbitals (MOs) localized on the dirhodium core to orbitals with contributions both from the Rh₂(σ*) MO and from a set of two equatorial RhL_{eq}(σ*) MOs (L_{eq} = O atoms on acetate ligands and N atoms on equatorial CH₃CN ligands). These assignments are in agreement with those of related dirhodium(II,II) paddlewheel complexes, such as Rh₂(μ-O₂CCH₃)₄.^{10,11}

It was previously established that the axial CH₃CN ligands of **1** exchange readily with coordinating solvent molecules (Figure 1a), whereas the equatorial CH₃CN ligands are not labile at room temperature in the dark.¹² The lowest energy electronic transition of **1** shifts from 525 nm in CH₃CN to 555 nm (ε = 160 M⁻¹ cm⁻¹) in H₂O.¹³ This shift is attributed to the exchange of the axial CH₃CN ligands in **1** for solvent H₂O molecules to form *cis*-[Rh₂(μ-O₂CCH₃)₂(CH₃CN)₄(H₂O)₂]²⁺ (**2**), since it is well known that the energies of the Rh₂(σ) and Rh₂(σ*) molecular orbitals (MOs) in dirhodium complexes are dependent on the nature of the axial ligand(s).^{10,11,14} The red shift in the absorption in water is supported

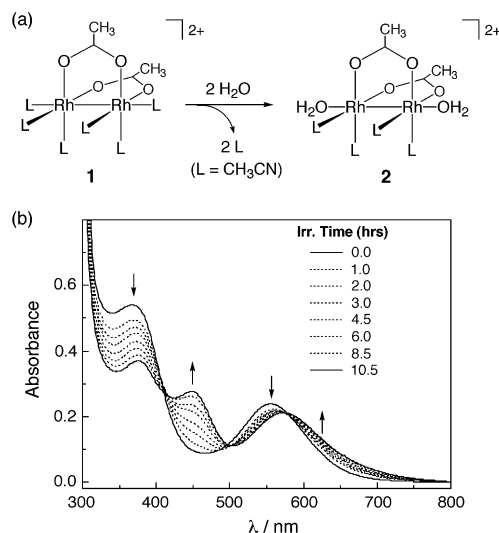


Figure 1. (a) Thermal axial ligand exchange of **1** to generate **2** in aqueous media and (b) photolysis of **2** in H₂O (λ_{irr} ≥ 345 nm).

by gas-phase TDDFT calculations on the two model complexes, **1a** and *cis*-[Rh₂(μ-O₂C₂H₃)₂(CH₃CN)₄(H₂O)₂]²⁺ (**2a**), which predict Rh₂(π*) → Rh₂(σ*) transitions at 501 nm (*f* = 0.0003) and 556 nm (*f* = 0.0004), respectively (*f* = oscillator strength). DFT calculations also show a decrease in the HOMO–LUMO gap of 0.23 eV when the axial ligands are varied from CH₃CN in **1a** to H₂O in **2a**. The ¹H NMR spectrum of **1** in D₂O provides further evidence of the exchange of the two axial CH₃CN ligands in this solvent (Supporting Information).¹³

Following the initial axial ligand exchange of **1** in water to generate **2**, no further spectral changes were noted at room temperature after 5 days or after incubation at 60 °C for 6 h in the dark. Changes in the electronic absorption and ¹H NMR spectra of **2** were observed upon photolysis (λ_{irr} ≥ 455 nm, 5 h) in H₂O and D₂O, respectively.¹⁵ The ¹H NMR spectrum of **2** in D₂O shows the loss of two additional CH₃CN ligands from the complex upon irradiation (λ_{irr} ≥ 455 nm, 1–5 h, Supporting Information), pointing at the dissociation of two equatorial CH₃CN ligands. The changes in the electronic absorption spectrum of **2** observed upon irradiation in H₂O are shown in Figure 1b. The peak at 373 nm decreases in intensity with the concomitant appearance of a band at 450 nm. In addition, the peak at 555 nm shifts to 573 nm (ε = 136 M⁻¹ cm⁻¹) and decreases in intensity. The exchange of two equatorial CH₃CN ligands for H₂O molecules in **2** can give rise to three different isomers of *cis*-[Rh₂(μ-O₂CCH₃)₂(CH₃CN)₂(H₂O)₄]²⁺ (**3a–3c**), complexes with two axial and two equatorial H₂O ligands. Although it is currently unknown if one, two, or all three isomers are generated upon photolysis, TDDFT calculations predict the lowest energy transition of all three species to be red-shifted relative to that in **2** (Supporting Information).

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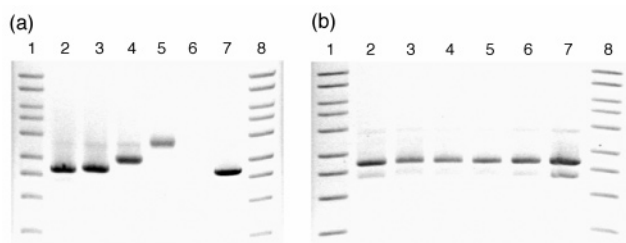


Figure 2. Imaged ethidium bromide stained agarose gel of 50 μ M linearized pUC18 plasmid (10 mM phosphate, pH = 7.5) in the presence of various concentrations of **2** (a) irradiated ($\lambda > 455$ nm) and (b) incubated in the dark at 25 $^{\circ}$ C for 20 min. Lanes 1 and 8: DNA molecular weight standard (1 kb, Sigma). Lanes 2 and 7: linearized plasmid alone. Lanes 3–6: [DNA bp]/[Complex] = 100, 20, 10, 5.

The power dependence of the photolysis of **2** ($\lambda_{\text{irr}} \geq 455$ nm and $\lambda_{\text{irr}} \geq 345$ nm, 1 h) followed by UV-vis and ^1H NMR in H_2O and D_2O , respectively, is consistent with the formation of the product, **3**, via a one-photon process.¹⁶ These results point at the initial photoaquation of one equatorial CH_3CN ligand, followed by the loss of a second equatorial CH_3CN ligand in a dark reaction. The quantum yield for the formation of **3** is dependent on the irradiation wavelength, with $\Phi_{355} = 0.37$ and $\Phi_{509} = 0.09$ at 355 and 509 nm, respectively.¹⁷ Such dependence of the photolysis quantum yield on wavelength has been previously shown for transition metal complexes to be associated with the photochemistry taking place from excited states that lie above the ground state.¹⁸ As discussed above, the excited state of **1** that absorbs at 363 nm has been calculated to possess contributions from $\text{RhL}_{\text{eq}}(\sigma^*)$ molecular orbitals. The set of $\text{RhL}_{\text{eq}}(\sigma^*)$ orbitals are composed of the in-phase and out-of-phase combinations of the $d_{x^2-y^2}$ orbitals of the Rh atoms, each with σ^* character to the equatorial ligands, L_{eq} , including both the bridging acetate and acetonitrile ligands. Placing electron density on the MO with σ^* character of the RhL_{eq} orbitals in the excited state can result in the dissociation of equatorial ligands upon irradiation with light.

Irradiation of **2** in water ($\lambda_{\text{irr}} \geq 455$ nm) in the presence of bpy (bpy = 2,2'-bipyridine) or 9-Et-G (9-Et-G = 9-ethyl guanine) results in the coordination of bpy and 9-Et-G to the dirhodium core (Supporting Information). Similar results were reported for related dirhodium complexes in the presence of bpy in solution upon refluxing over several days,¹⁹ as well as their thermally activated coordination to nucleobases and oligonucleotides.²⁰ Although coordination of the dirhodium complex to ligands and nucleobases in solution is promising, in order for **2** to act as a photo-cisplatin analog, the complex must be able to bind to double-stranded DNA (ds-DNA) upon irradiation.

Photolysis of **2** in the presence of linearized pUC18 plasmid results in decreased mobility of the ds-DNA on an agarose gel (Figure 2a).²¹ In contrast, no change in the mobility was observed for samples of **2** incubated with plasmid in the dark at 25 $^{\circ}$ C for 20 min (Figure 2b) or at 37 $^{\circ}$ C for 4 h (Supporting Information). Such decrease in plasmid mobility on agarose gels has been previously shown to occur upon the covalent binding of cisplatin to ds-DNA owing to the kinking of the DNA induced by the drug.⁹ This effect is also shown in the Supporting Information.

A requirement of a potential PDT agent is low cytotoxicity in the dark and increased toxicity upon irradiation. The cytotoxicity of **2** towards Hs-27 human skin cells increases from an LC_{50} of 410 ± 9 to 12 ± 2 μM when irradiated with visible light (400–

700 nm, 30 min).²² This 34-fold increase in toxicity is significantly greater than the 5-fold increase measured for hematoporphyrin, a key component in the commercially available PDT agent Photofrin, under similar experimental conditions. In addition, the toxicity of **2** towards Hs-27 cells is lower than that of hematoporphyrin ($\text{LC}_{50} = 21 \pm 1$ μM) by a factor of ~ 20 in the dark. Cationic dirhodium complexes with bidentate chelating ligands occupying the four equatorial positions taken up by CH_3CN in **2**, such as *cis*- $[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{phen})_2(\text{H}_2\text{O})_2]^{2+}$, do not exhibit an increase in cytotoxicity towards Hs-27 cells upon irradiation. These properties make **2** a promising photo-cisplatin analog and a potential PDT agent. To our knowledge, **2** is the first metal–metal bonded complex to bind to DNA upon irradiation with visible light.

Acknowledgment. C.T. thanks the National Institutes of Health (RO1 GM64040-01), National Science Foundation (CHE 0503666), and the Ohio Supercomputer Center for partial support of this work.

Supporting Information Available: Gel mobility assays, DFT and TDDFT calculations, photolysis in D_2O followed by NMR, and photolysis in the presence of 9-Et-G and bpy. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA057620Q