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$[Ru(bpy)_2(5\text{-cyanouracil})_2]^{2+}$ as a Potential Light-Activated Dual-Action Therapeutic Agent

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Supporting Information

ABSTRACT: The cation *cis*-$[Ru(bpy)_2(5CNU)_2]^{2+}$ (bpy = 2,2′-bipyridine; 5CNU = 5-cyanouracil) was synthesized and investigated for use as a potential light-activated dual-action therapeutic agent. The complex undergoes efficient photoinduced 5CNU ligand exchange for solvent water molecules, thus simultaneously releasing biologically active 5CNU and generating $[Ru(bpy)_2(H_2O)_2]^{2+}$. The latter binds covalently to ds-DNA, such that photolysis results in the generation of 3 equiv of potential therapeutic agents from a single molecule.

The chemotherapy agent 5-fluorouracil (5FU) has been in use for over 20 years for the treatment of malignancies including colorectal and breast cancers.[1] The mode of action of 5FU is multifaceted and involves its incorporation into DNA and RNA, as well as inhibition of thymidylate synthase, an enzyme important in DNA synthesis and repair.[1] Other derivatives of uracil also exhibit cellular activity including 5-cyanouracil (5CNU), which has been shown to inhibit pyrimidine catabolism *in vivo*.[2] Complexation of 5CNU to a photoactive transition metal complex represents a manner in which this drug may be caged in an inactive state until delivered to its target, at which time it can be released by irradiation with low-energy light.

In addition to the photoinduced release of the caged 5CNU drug, the transition metal portion of the complex itself may be biologically active. It has been shown by us and others that ruthenium and dirhodium complexes hold great potential for photodynamic therapy (PDT).[3−6] In addition, we have also reported photoactive complexes inspired by the mode of action of cisplatin; in these cases, the complex is stable in the dark but undergoes ligand exchange and binds to DNA upon irradiation.[7] These complexes include *cis*-$[Ru(bpy)_2(NH_3)_2]^{2+}$ (bpy = 2,2′-bipyridine) and *cis*-$[Rh_2(O_2CCH_3)_2(CH_3CN)_6]^{2+}$,[7] where a 34-fold increase in toxicity toward Hs27 human skin fibroblasts was reported for the latter upon irradiation with visible light.[7b] It should also be noted that ruthenium complexes are now recognized as promising anticancer drugs, with some being subjected to clinical and preclinical trials.[8]

Nitriles undergo photoinduced ligand exchange more efficiently than other monodentate ligands when bound to ruthenium(II), including pyridine and NH_3.[9,10] As such, complexes with various nitrile ligands are promising PDT candidates. In this vein, we hypothesized that two 5CNU molecules coordinated to ruthenium through their nitrile substituents would efficiently undergo ligand exchange with solvent upon irradiation, thus releasing

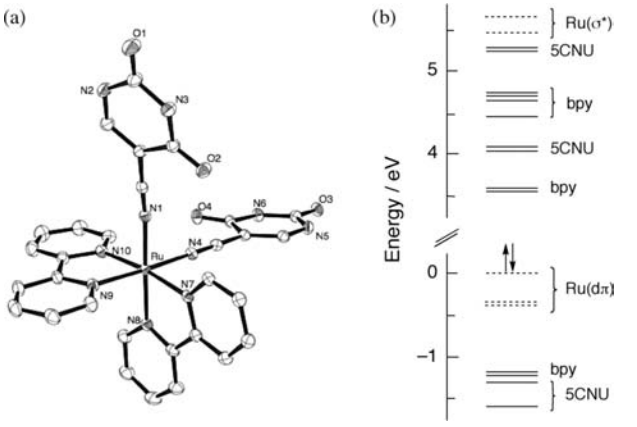

Figure 1. (a) Thermal ellipsoid plot (drawn with 30% probability ellipsoids; hydrogen atoms removed for the sake of clarity) and (b) calculated MO diagram of **1**.

2 equiv of the drug and the diaquaruthenium(II) complex. The present work focuses on the synthesis of the new complex *cis*-$[Ru(bpy)_2(5CNU)_2]^{2+}$ (**1**) and the investigation of its photophysical properties and potential for its use as a dual-action photochemotherapeutic agent.

The reaction of 50 mg (0.1 mmol) of *cis*-$Ru(bpy)_2Cl_2$ with 2.2 equiv of $AgCF_3SO_3$ in 10 mL of methanol at room temperature resulted in the precipitation of AgCl and the formation of red *cis*-$Ru(bpy)_2(CF_3SO_3)_2$. After filtration to remove AgCl, the solution was refluxed with a 5-fold excess of 5CNU for 2 h to generate the yellow complex **1** (see the Supporting Information, SI). The ^{1}H NMR spectrum of **1** is consistent with two 5CNU molecules and two bpy ligands coordinated to the metal center (see the SI), also evidenced in the crystal structure of the product shown in Figure 1a. The Ru−N bond lengths to the 5CNU ligand are 2.034(3) and 2.025(3) Å, while those to the bpy nitrogen atoms positioned trans to 5CNU are 2.044(3) Å. Slightly longer Ru−N bonds were found for the two bpy nitrogen atoms positioned trans to another bpy ligand, 2.061(3) and 2.067(3) Å. These distances are similar to those previously reported for *cis*-$[Ru(bpy)_2(CH_3CN)_2]^{2+}$ (**2**).[11]

The $Ru^{III/II}$ couple of **1** is observed at +1.45 V vs SCE in CH_3CN (0.1 M Bu_4NPF_6), at a potential similar to that measured for **2** at +1.44 V vs SCE,[12,13] and related ruthenium(II)

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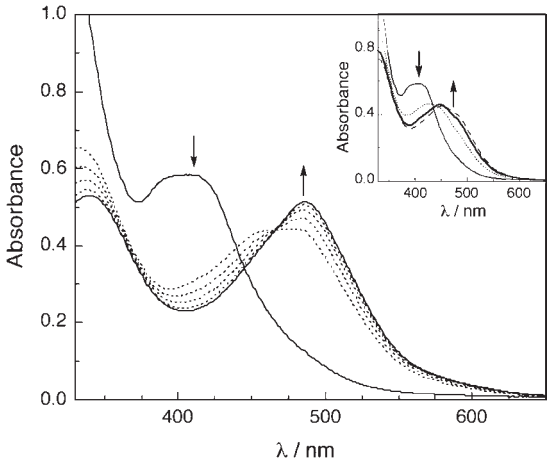

Figure 2. Changes to the electronic absorption spectrum of 77 μM **1** upon irradiation in H_2O at t_{irr} = 0, 5, 7.5, 10, 12.5, and 15 min. Inset: 0, 1, 2, and 3 min ($\lambda_{irr} \geq 395$ nm).

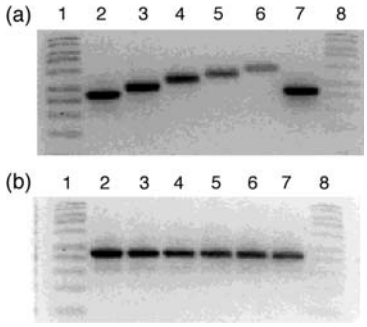

Figure 3. Imaged ethidium bromide stained agarose gel of 50 μM linearized pUC18 plasmid (10 mM phosphate buffer, pH = 8.3) in the presence of various concentrations of **1**: lanes 1 and 8, 1 kb DNA molecular weight standard; lanes 2 and 7, linearized plasmid alone; lanes 3−6, 25, 75, 150, and 250 μM complex (a) irradiated with $\lambda_{irr} \geq 395$ nm (t_{irr} = 15 min) and (b) incubated in the dark for 15 min at 298 K.

complexes.[14,15] A feature consistent with the reduction of one of the bpy ligands in **1** occurs at −1.31 V vs SCE.[15] Two high-energy transitions are observed in the electronic absorption spectrum of the chloride salt of **1** in water at 243 nm (ε = 31 000 M^{-1} cm^{-1}) and 284 nm (ε = 48 300 M^{-1} cm^{-1}) assigned as ligand-centered $\pi\pi^*$ transitions of bpy, with maxima at 240 nm (ε = 17 400 M^{-1} cm^{-1}) and 283 nm (ε = 52 500 M^{-1} cm^{-1}) in **2** in CH_3CN. The shoulder at 315 nm (ε = 21 300 M^{-1} cm^{-1}) in **1** is not present in the absorption spectrum of **2** or in free 5CNU (λ_{max} = 275 nm; ε = 11 700 M^{-1} cm^{-1} in water) and can therefore be assigned to a metal-to-ligand charge-transfer (MLCT) transition from the Ru^{II} center to the 5CNU ligand. The absorption maximum at 410 nm (ε = 7800 M^{-1} cm^{-1}) in **1** (H_2O) is consistent with the Ru → bpy MLCT transition observed at 425 nm (ε = 8590 M^{-1} cm^{-1}) in **2** in CH_3CN.[16]

Density functional theory calculations were performed on **1** in CH_3CN. The minimized structure from the calculations is consistent with the crystal structure, as is evident from the Ru−N bond lengths to the 5CNU ligands calculated to be 2.038 Å and shorter than the other Ru−N bonds in the complex (Table S2 in the SI). Moreover, the calculated orbital energies support the assignments of the electronic absorption transitions (details in the SI). The molecular orbital (MO) diagram calculated for **1** is shown in Figure 1b. The electron density of the three highest occupied molecular orbitals (HOMOs) is localized on the metal center; these MOs are labeled Ru(dπ) in Figure 1b. The lowest unoccupied molecular orbital (LUMO) and LUMO+1 are bpy π^* orbitals, while the electron densities of the LUMO+2 and LUMO+3 are localized on the 5CNU ligands. Accordingly, inspection of Figure 1b reveals that Ru → bpy, followed by Ru → 5CNU, are expected to be the lowest-energy transitions in **1**. It should also be noted that the Ru(σ^*) orbitals lie at signficantly greater energies, ∼5.5 eV above the HOMO, and comprise the LUMO+10 and LUMO+11 (Figure 1b).

Irradiation of the chloride salt of **1** in water with visible light ($\lambda_{irr} \geq 395$ nm) results in spectral changes, with a decrease in the MLCT absorption at 410 nm, as shown in Figure 2. A species with a maximum at ∼450 nm forms within 2 min of irradiation (Figure 2, inset, bold line), attributed to the monoaqua intermediate *cis*-$[Ru(bpy)_2(5CNU)(H_2O)]^{2+}$ (**3**), characterized by mass spectrometry (see the SI) and similar to that previously reported for the photochemistry of **2**.[10] This peak deceases in intensity with continued photolysis with a concomitant increase of the product peak at 490 nm (Figure 2). The peak at 490 nm (ε = 9300 M^{-1} cm^{-1}) is known to correspond to the diaqua complex *cis*-$[Ru(bpy)_2(H_2O)_2]^{2+}$ (**4**),[17] consistent with the photoinduced exchange of each 5CNU ligand with a solvent water molecule. The quantum yield for the disapperance of **1** to generate the monoaqua intermediate **3** was measured to be 0.16 ± 4 (λ_{irr} = 400 nm; see the SI for details), a slighly lower value than that reported for **2** (Φ = 0.21; λ_{irr} = 400 nm).[10] It should be noted that no spectral changes are observed when the sample is kept in the dark under similar experimental conditions.

When **1** (80 μM) is photolyzed for 90 s (see the SI), the majority of the product formed is the monoaqua complex, **3**, with a maximum at ∼450 nm. If that solution is then stored in the dark, no further reactivity is observed (Figure S2 in the SI), a fact that is indicative of the requirement of a photon for the conversion of **3** to the final product, **4**. This experiment clearly supports the conclusion that the formation of the diaqua product **4** from **1** requires the sequential absorption of two photons, one to effect the exchange of each 5CNU ligand.

The photolysis of **1** with visible light was also monitored by ^1H NMR spectroscopy in D_2O. Prior to irradiation, a peak corresponding to the C−H ring proton of the two bound 5CNU molecules is observed at 8.02 ppm. This peak decreases in intensity upon irradiation with the appearance of two new resonances at 8.00 and 8.18 ppm, assigned to the same 5CNU proton in *cis*-$[Ru(bpy)_2(5CNU)(D_2O)]^{2+}$ (**3a**) and free 5CNU in D_2O, respectively. Continued photolysis results in a further decrease of the 5CNU peak corresponding to **3a** and greater intensity of the free 5CNU peak, consistent with the formation of the diaqua product. Spectral changes are also observed for the bpy protons, but overlapping resonances of the reactant, intermediate, and product preclude assignments to be made.

The covalent binding of cisplatin to DNA results in decreased mobility of linearized plasmid in agarose gel electrophoresis.[7,18] Our group has shown that certain complexes are capable of covalently binding to DNA when activated by light.[7] Agarose mobility shift gels were conducted in order to test the ability of **1** to bind to DNA when irradiated. Photolysis of **1** ($\lambda_{irr} \geq 395$ nm; t_{irr} = 15 min) in the presence of linearized pUC18 plasmid results in a decrease of the mobility of ds-DNA as a function of increased complex concentration (Figure 3a).[19] In contrast, when the plasmid

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is incubated in the dark for 15 min, no change in the DNA mobility is observed (Figure 3b). It should also be noted that the monoaqua intermediate, **3**, does not bind to DNA, as is evidenced by the data in Figure S3 (SI). Moreover, control experiments with 5CNU alone revealed that the molecule does not covalently bind to linearized plasmid (Figure S4 in the SI).

The work presented here demonstrates that, upon photolysis of **1** in aqueous media, complex **4** is formed and subsequently covelently binds to DNA. Moreover, 2 equiv of the biologically active 5CNU molecule is released in the process, rendering this type of complex a potential dual-action photochemotherapy agent.

■ ASSOCIATED CONTENT

Supporting Information. Synthesis details, complete crystal structure and crystallographic data, and dark activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Linearization of 100 μM pUC18 or pUC19 plasmids (Bayou Biolabs) was carried out with 50 units of *Sma*I (Invitrogen Life Technologies) restriction enzyme in 100 mM Tris, pH = 7.6, 150 mM NaCl at 37 °C for 1 h, followed by deactivation at 65 °C for 5 min. Additional details can be found in ref 3f.