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Communication

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Selective Release of Aromatic Heterocycles from Ruthenium Tris(2-pyridylmethyl)amine with Visible Light

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Supporting Information

ABSTRACT: Three complexes of the general formula $[Ru(TPA)L_2](PF_6)_2$ [TPA = tris(2-pyridylmethyl)amine], where L = pyridine (**1**), nicotinamide (**2**), and imidazole (**3**), were prepared and characterized spectroscopically. X-ray crystallographic data were obtained for **1** and **3**. Complexes **1**−**3** show strong absorption in the visible region and selective release of heterocycles upon irradiation with visible light. Time-dependent density functional theory calculations are consistent with the presence of singlet metal-to-ligand charge-transfer bands in the visible region in **1**−**3**. Caged heterocycles **1**−**3** are highly stable in solution in the dark, including in cell growth media. Cell viability data show no signs of toxicity of **1**−**3** against PC-3 cells at concentrations up to 100 μM under light and dark conditions, consistent with Ru(TPA) acting as a nontoxic and effective photocaging group for aromatic heterocycles.

Nitrogen-containing aromatic heterocycles such as pyridines and imidazoles are a ubiquitous class of functional groups found in many biomolecules, natural products, and drugs.[1] Their prevalence in bioactive compounds makes heterocycles attractive targets for photocaging applications, where spatiotemporal control over biological activity can be achieved.[2] Despite their prevalence, only a few studies have reported photocaging of these groups in bioactive compounds, mostly focusing on ruthenium-based caging groups derived from bi- or tridentate ligands such as 2,2′-bipyridine (bpy) and 2,2′:6′,2″-terpyridine.[3−9] In this Communication, we report that ruthenium complexes derived from the tetradentate ligand tris(2-pyridylmethyl)amine (TPA) show selective release of pyridine and imidazole heterocycles upon irradiation with visible light. Furthermore, Ru(TPA) complexes are highly stable in cell growth media and well tolerated by cells under light and dark conditions.

Three complexes were synthesized for this study starting from the tetradentate ligand TPA. Treatment of TPA with *cis*-$[Ru(Me_2SO)_4Cl_2]$ in methanol at 80 °C, followed by concentration of the reaction mixture, afforded a 2:1 mixture of $[Ru(TPA)(Me_2SO)Cl]Cl$ isomers as previously described.[10] Complexes of the general formula $[Ru(TPA)L_2](PF_6)_2$, where L = pyridine (py; **1**), nicotinamide (NA; **2**), also known as niacinamide, and imidazole (Im, **3**), were prepared by heating $[Ru(TPA)(Me_2SO)Cl]Cl$ in 1:1 py/H_2O for **1**, or in the presence of 10 equiv of NA in 3:5 acetone/H_2O for **2**, or in the presence of 10 equiv of Im in 1:1 EtOH/H_2O for **3** at 80−100 °C for **2** or **3**, respectively. Precipitation with NH_4PF_6, followed by filtration, afforded **1**−**3** in 75−89% yield from TPA.

Complexes **1**−**3** were characterized by electronic absorption, ^{1}H NMR, COSY, NOESY, and IR spectroscopies, electrospray ionization mass spectrometry, and elemental analysis. Complexes with caged pyridines **1** and **2** exhibit maxima at 355 nm (ε = 10800 M^{-1} cm^{-1}) and 385 nm (ε = 11200 M^{-1} cm^{-1}), respectively, with strong shoulders in the visible region that stretch to approximately 450 nm. Imidazole complex **3** features an absorption maximum at 425 nm (ε = 10300 M^{-1} cm^{-1}). On the basis of time-dependent density functional theory calculations, absorption for **1** and **3** at λ > 400 nm results from several overlapping singlet metal-to-ligand charge-transfer (MLCT) transitions that are dπ(Ru) to π^*(pyridine of TPA) in nature (Figures S19 and S20). ^{1}H NMR spectra of **1**−**3** indicate two distinct monodentate heterocycles (py, NA, and Im) in each complex, one positioned *cis* and one *trans* to the basic nitrogen atom of TPA (see Figures 1 and S1−S3 for spectra), where *cis* donors show resonances upfield with respect to *trans* donors due to shielding by adjacent pyridine groups of TPA.[11] These assignments were confirmed by COSY and NOESY analyses. Mass spectra for **1**−**3** show ion clusters with major peaks at m/z

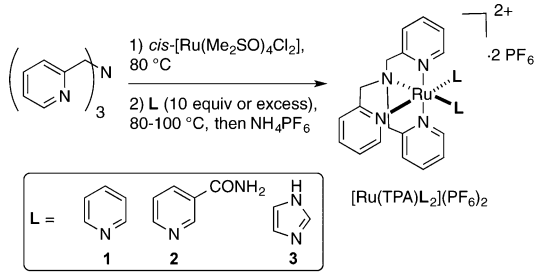

Figure 1. Synthesis of caged heterocycle complexes of the general formula $[Ru(TPA)L_2](PF_6)_2$, where L = py (**1**), NA (**2**), and Im (**3**).

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values consistent with the cations $\{[Ru(TPA)L_2](PF_6)\}^+$, where L = py, NA, and Im (Figures S7–S9).

In addition to spectral characterization, diffusion of diethyl ether into a solution of **1** or **3** in acetone afforded crystals suitable for X-ray crystallographic analysis. Select data for **1** and **3** are described in Figure 2; full tables can be found in the Supporting Information. In both cases, Ru–N bond distances to the N1 donor, *trans* to the basic nitrogen N3, are slightly longer than distances to the N6 donor *cis* to N3.

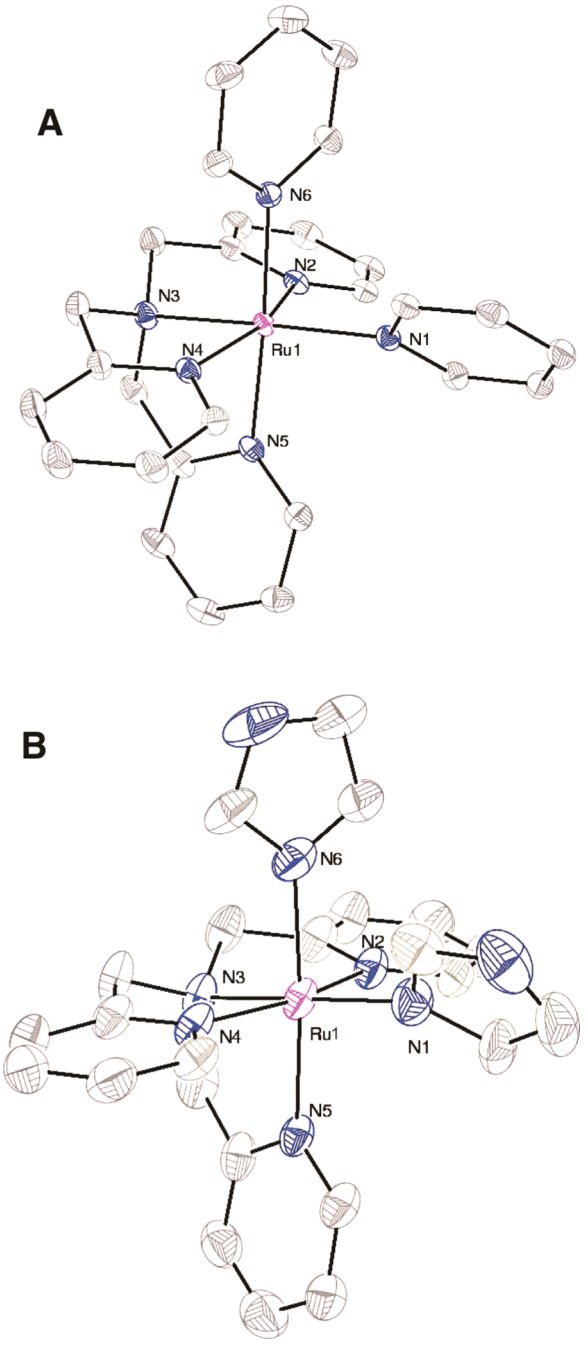

Figure 2. ORTEP diagrams of the dications **1** (A) and **3** (B). Thermal ellipsoids are shown at 50% probability. Hydrogen atoms are omitted for clarity. Selected bond distances (Å) for **1**: Ru–N1, 2.114(2); Ru–N6, 2.108(2). Selected bond distances (Å) for **3**: Ru–N1, 2.121(7); Ru–N6, 2.103(8).

Irradiation of **1**–**3** with visible light promotes dissociation of the monodentate ligands. The spectral changes of **1** in CH_2Cl_2 in the presence of 10 mM Bu_4NCl as a function of the irradiation time are shown in Figure 3 ($\lambda_{irr} \geq 395$ nm), and those of **2** and **3**

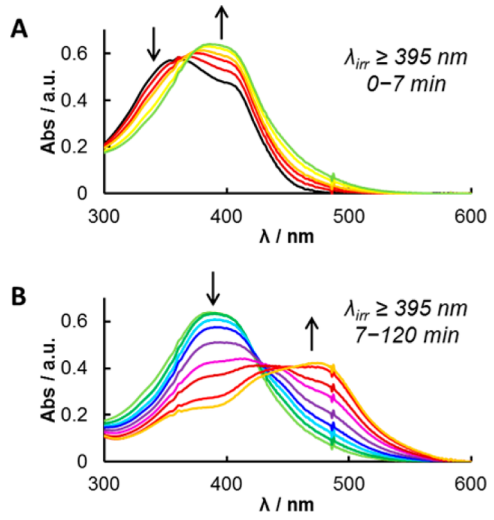

Figure 3. Electronic absorption spectra of **1** in CH_2Cl_2 with 10 mM Bu_4NCl irradiated with $\lambda \geq 395$ nm for 0–7 min (A) and 7–120 min (B).

are provided in Figures S10 and S11. It is evident in Figure 3A that the MLCT absorption of **1** at 380 nm shifts to 392 nm upon substitution of one py ligand with Cl^-, forming $[Ru(TPA)(py)Cl]^+$ within approximately 7 min. This initial step is followed by the formation of $[Ru(TPA)Cl_2]$ with λ_{max} = 475 nm through photosubstitution of the second py ligand by Cl^- over a period of 2 h (Figure 3B). Similar trends are observed for **2** and **3**, as shown in Figures S10 and S11. The quantum yields for the first ligand exchange process with λ_{irr} = 400 nm (Φ_{400}) are $9.7(8) \times 10^{-3}$, $9.1(8) \times 10^{-3}$, and $5.3(3) \times 10^{-4}$ for complexes **1**–**3**, respectively. The agreement in Φ_{400} for **1** and **2** is expected because of the similar nature of the dissociating ligands. The lower Φ_{400} for substitution of Im versus py is consistent with the trend observed for the photoanation of $[Ru(bpy)_2L_2]^{2+}$ (L = py or Im).[12]

In order to gain insight into the selectivity of heterocycle release, solutions of complexes **1**–**3** were also irradiated in D_2O (10% acetone-d_6), followed by ^1H NMR spectroscopy ($\lambda_{irr} > 395$ nm). In each case, formation of a single photoproduct was observed after irradiation. Over the time course of irradiation, downfield resonances for *cis* monodentate donors decrease in intensity, with new resonances appearing for free corresponding monodentate ligand py, NA, and Im, as confirmed by doping samples after irradiation with free ligand. These data are consistent with caged nitrile complexes derived from Ru(TPA), which also show selective release of nitrile donors *cis* to the basic nitrogen of TPA.[11,13]

In addition to photochemical release, complexes **1**–**3** show exceptional stability in solution in the dark, making Ru(TPA) an attractive caging group for aromatic heterocycles used in biological applications. When monitored spectrophotometrically in dimethyl sulfoxide (DMSO) at 23 °C over the course of 24 h, complexes **1**–**3** show no sign of decomposition. In addition, **1**–**3** exhibit exceptional stability when incubated in Dulbecco's modified Eagle's medium (pH 7.2) at 37 °C over 24 h, making them appropriate for long-term experiments in cell culture.

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These data are consistent with related ruthenium complexes containing monodentate pyridine donors, which were shown to be stable to thermal ligand exchange and aquation in aqueous media.[14]

In order for Ru(TPA) to be appropriate as a photoreactive chemical tool for biological studies, complexes and their photochemical byproducts should be nontoxic and well tolerated by cells. As an initial step to probe for toxicity, complexes **1**−**3** were evaluated against PC-3 cells, a prostate cancer cell line that is particularly susceptible to toxic metal complexes such as cisplatin.[15,16] After PC-3 cells were treated with **1**−**3** over a broad concentration range (10 nM to 100 μM) and the cells were left in the dark or irradiated for 40 min with visible light (λ_{irr} > 395 nm), the effects on the cell viability were measured using MTT assay after 48 h. Data for **2** are shown in Figure 4, and data for **1** and **3**

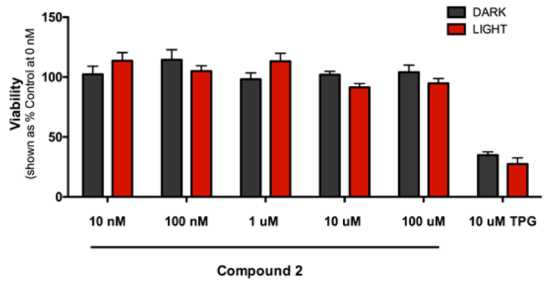

Figure 4. Cytotoxicity of **2** in PC-3 prostate cancer cells. Cells were incubated in the presence of **2** (10 nM to 100 μM) for 30 min and left in the dark (black bars) or irradiated (red bars) for 40 min with a 250 W tungsten halogen lamp (λ_{irr} > 395 nm). For comparison, cells were treated with the known cytotoxic agent TPG. The cell viability was determined by MTT assay after 48 h and is reported relative to control with only the vehicle (1% DMSO) added. Error bars represent the standard error of the mean of quadruple wells, and data are representative of three independent experiments.

are provided in Figures S21 and S22. The cytotoxic compound thapsigargin (TPG; 10 μM) was used as a positive control. Compounds **1**−**3** showed no visual signs of toxicity, such as contraction or membrane blebbing, and did not affect the viability outside the range of error, as judged by MTT assay, even at the highest concentration tested (100 μM), which is over 3 orders of magnitude higher than the IC_{50} for cisplatin against PC-3 cells.[15] Taken together, these data support the idea that caged heterocycles derived from Ru(TPA) could be used to garner spatiotemporal control over biological activity in cell-based assays, without producing side effects due to toxicity from the metal complex cage.

In conclusion, we report that Ru(TPA) functions as an effective caging group for aromatic, nitrogen-based heterocycles. Pyridine and imidazole complexes derived from Ru(TPA) are stable and nontoxic and show selective release of bound heterocyclic monodentate ligands upon irradiation with visible light. These data support the further development of ruthenium complexes derived from this family of higher-denticity ligands as caging groups for aromatic heterocycles and light-activated tool compounds for chemical biology.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge via the Internet at The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.5b02600.

X-ray crystallographic data for **1** in CIF format (CIF)
X-ray crystallographic data for **3** in CIF format (CIF)
Experimental procedures for the preparation of **1**−**3**, characterization data for **1**−**3**, experimental procedures for photochemical and biological studies, and cell viability data for **1** and **3** (PDF)

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Notes

The authors declare no competing financial interest.

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