Supplementary Material (ESI) for Chemical Communications

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For

A Photosensitive {Ru-NO}^{6} Nitrosyl bearing Dansyl Chromophore: Novel NO Donor with a Fluorometric On/Off Switch

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Experimental Section

Chemical Reagents and Syntheses. Dansyl-chloride, imidazole, $CsCO_3$, and other starting materials were obtained from Sigma-Aldrich Chemical Co. except for $AgBF_4$, which was obtained from Alfa-Aesar. All solvents were purchased from Fisher Sciences and distilled prior to use: Et_2O from Na; MeCN and CH_2Cl_2 from CaH_2. NanoPure H_2O was used to prepare all aqueous solutions. The ligands H_2Me_2bpb and dansyl-imidazole (ds-Im) were synthesized as previously described.

[(Me_2bpb)Ru(NO)(Ds-im)]BF_4 (1). A batch of 0.140 g (0.277 mmol) of [(Me_2bpb)Ru(NO)Cl] was stirred in 40 mL of MeCN and treated with 1 equiv of $AgBF_4$ (0.054 g, 0.277 mmol). Upon heating to reflux temperature, the greenish-brown solution generated a beige precipitate was observed (AgCl) and the solution held at this temperature for 12 h. Next, 3 equiv of dansyl-imidazole (0.250 g, 0.831 mmol) in 5 mL of MeCN were added to the hot solution and continue refluxing for only 1 h. Upon cooling, the resulting orange-red solution was concentrated to ~5 mL, and stored at –20 °C overnight to precipitate trace amounts of AgCl and unreacted starting material (chloride adduct). After filtration, the solvent was removed in vacuo, and the residue dissolved in ~5 mL of CH_2Cl_2. Addition of Et_2O and subsequent cooling to –20 °C overnight affords the product as a pure greenish-red solid. The nitrosyl-dye conjugate **1** was washed with several small portions of Et_2O and dried in vacuo. Yield: 60 mg (26%). Anal. calcd. for $C_{35}H_{31}N_8O_5SRuBF_4$ (**1**): C 48.65, H 3.62, N 12.98; found: C 48.50, H 3.68, N 12.95. Selected IR frequencies (KBr disk, cm^{-1}): 1868 (s, ν_{NO}), 1639 (vs, ν_{CO}), 1597 (vs), 1566 (w), 1483 (s), 1376 (m), 1354 (m), 1184 (m), 1170 (w), 1155 (w), 1070 (vs), 795 (m), 762 (m), 685 (m), 634 (s), 586 (m), 487 (w). Electronic absorption spectrum in MeCN, λ_{max} in nm (ε in M^{-1} cm^{-1}): 385 (4 950), 300 (9 530), 272 (15 790). ^{1}H NMR in CD_3CN, δ from TMS: 9.01 (d 2H), 8.68 (d 1H), 8.40 (s 2H), 8.35 (d 1H), 8.23 (t 2H), 8.00 (d 2H), 7.88 (t 2H), 7.79 (s 1H), 7.60 (t 1H), 7.48 (m 2H), 7.29 (d 1H), 7.26 (s 1H), 6.56 (s 1H), 2.89 (s 6H).

[(Me_2bpb)Ru(NO)(im)]BF_4 (2). A slurry of 0.175 g (0.342 mmol) of [(Me_2bpb)Ru(NO)Cl] in 20 mL MeCN was mixed with a 5 mL solution of 0.067 g (0.342 mmol) of $AgBF_4$ in MeCN. This green-brown solution was refluxed for 16 hrs, which resulted in a turbid green-red solution. A 5 mL solution of MeCN containing 3 equiv of imidazole (0.070 g, 1.026 mmol) was added and reflux continued for several hrs. The solution was cooled to ambient temperature and filtered over Celite to remove AgCl. The dark red solution was concentrated to ~10 mL and placed at –20 ºC overnight to precipitate trace impurities. The next day, 5 mL of Et_2O was added to the filtrate and the solution kept at –20 ºC for several days to afford **2** as a dark red crystalline solid. Yield: 143 mg (68 %). Anal. calcd. for $C_{23}H_{20}N_7O_3RuBF_4$ (**2**): C 43.83, H 3.20, N 15.56; found: C 44.50, H 3.08, N 14.95. Single crystals suitable for X-ray diffraction were obtained from an MeCN:Et_2O (1:1) mixture of **2** at ambient temperature, which afforded dark red blocks. Selected IR frequencies (KBr disk, cm^{-1}): 1872 (vs, ν_{NO}), 1642 (s, ν_{CO}), 1590 (vs), 1483 (m), 1400 (w), 1388 (w), 1360 (m), 1074 (s), 1031 (s), 756 (m), 684 (m), 661 (m). Electronic absorption spectrum in MeCN, λ_{max} in nm (ε in M^{-1} cm^{-1}): 550 (500), 394 (7 160), 302 (13 110), 277 (20 330). ^{1}H NMR in CD_3CN, δ from TMS: 9.14 (d 2H), 8.45 (s 2H), 8.32 (m 2H), 8.18 (d 2H), 7.92 (m 2H), 7.38 (s 1H), 6.84 (s 1H), 6.50 (s 1H), 2.33 (s 6H).

Physical Measurements. Electronic absorption spectra were recorded on a Varian Cary 50 spectrophotometer, while fluorescence emission spectra were monitored using a Perkin-Elmer LS50B Fluorescence/Luminescence Spectrometer. IR spectra were obtained on a Perkin-Elmer Spectrum One FTIR spectrometer, while 1H-NMR spectra were scanned using Varian 600 MHz instrumentation. X-band EPR spectra of photolyzed ($\lambda \geq 400$ nm, 0.4 W) solutions of **1** and **2** were obtained using a Bruker 500 ELEXSYS spectrometer at 125 K, and concaminant photorelease of NO was monitored using the *in*NO Nitric Oxide Monitoring System (Innovative Instruments, Inc.) fitted with the *amino*-2000 electrode.

Photolysis Experiments. Quantitative photolyses of **1** and **2** in MeCN or aqueous solutions with 400 nm monochromatic light were performed using an Apex Illuminator

(150 W Xenon lamp) equipped with a Cornerstone 130 1/8M monochromator, while qualitative samples (EPR, biological studies) were illuminated with an IL 410 Illumination System from Electro-FiberOptics Corp (halogen lamp) equipped with a $\lambda \geq$ 400 nm cutoff filter (measured light intensity = 0.4 W).

Cell Culture and Staining Protocols. The breast cancer cell line (MDA-MB-231) was a kind gift from Dr. Mina Bissell. Cell growth media and FBS were obtained from Gibco or Gemini bioproducts, respectively. Microscope chamber slides were purchased from Nunc Lab-Tek, while reagents for preparing the PBS supplemented with 1 mM Ca^{2+}/Mg^{2+} (PBS-Ca/Mg) were procured from Sigma-Aldrich. Briefly, cells were grown in DMEM/5% FBS supplemented with penicillin/streptomycin. Cells at ~70% confluence in 8-well microscope chamber slides were washed 3 times with PBS-Ca/Mg and fixed with 4% paraformaldehyde (PFA) for 20 min at room temperature. The cells were then treated with a solution containing 4 μL of 10 mM stock solution of **1** in MeCN diluted with 196 μL of PBS-Ca/Mg. After 1 h incubation at 37°C, cells were washed 3 times with PBS-Ca/Mg. Light-exposed samples were illuminated with 1 min of visible light ($\lambda \geq$ 400 nm, ~0.3 W measured intensity) or kept in the dark (control). Lastly, all samples were stained with 10 μM DAPI for 15 min (to visualize nuclei) and washed with PBS-Ca/Mg and H_2O. The slides were mounted with Fluoromount-G (Southern Biotech) under glass cover slips, and sealed with nail varnish. Fluorescence microscopy images were visualized with an Axiovert 200 fluorescence microscope (Zeiss) coupled to an AttoArc 2 HBO 100 W light source (Zeiss), and captured by a CCD camera controlled by SPOT Advanced software v4.0.9. Exposure times were chosen to best reflect the image as observed through microscope lens: blue (DAPI): 500 ms; Green (nitrosyl-dye conjugate **1**): 3-7 s.

DAPI = 4′-6-Diamidino-2-phenylindole; FBS= Fetal bovine serum; PBS= Phosphate buffer saline.

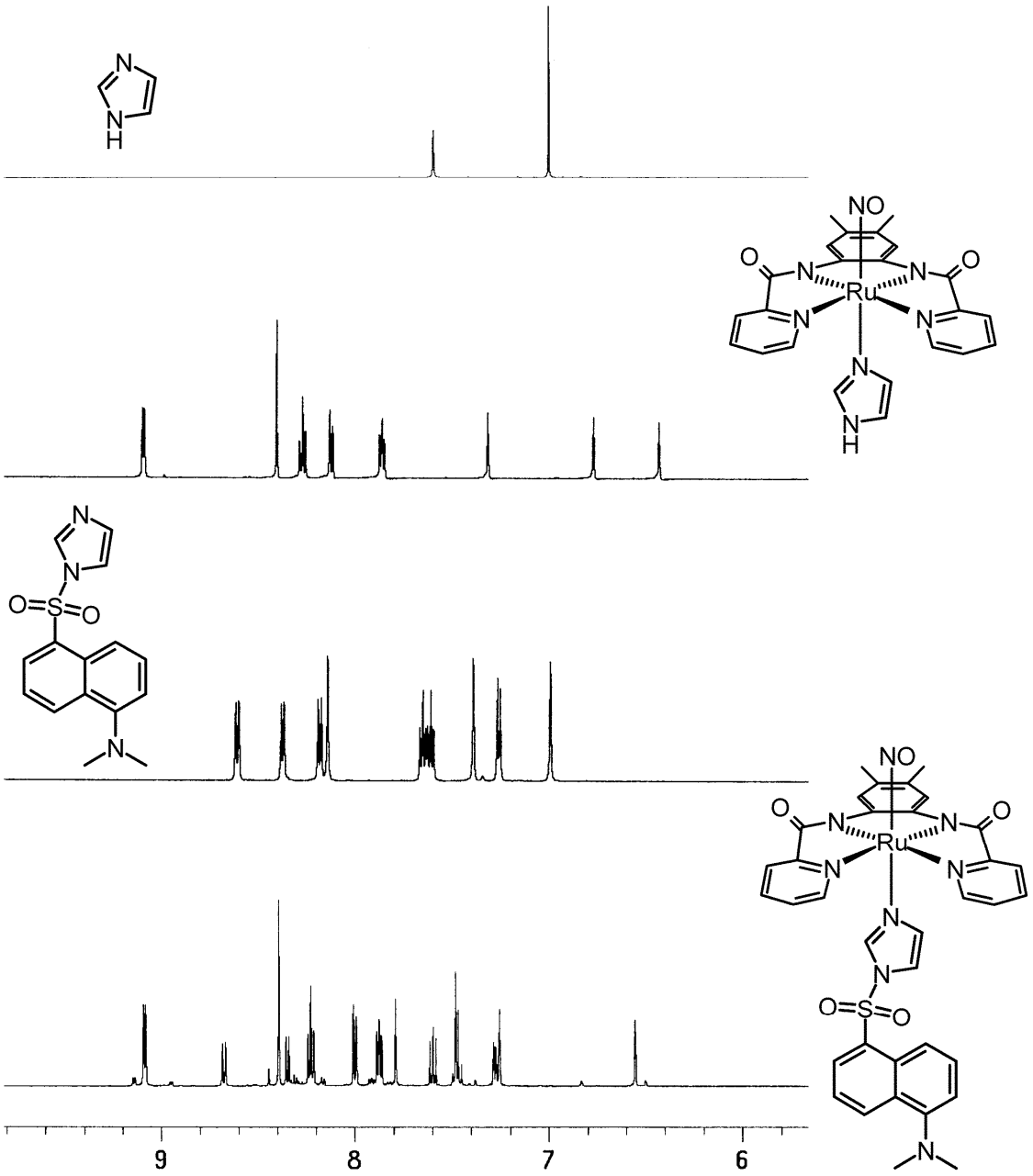

Fig. S1. ^{1}H-NMR spectrum in CD_3CN of the ligands and metal complexes utilized in this work (from top to bottom): (*i*) imidozole only, (im); (*ii*) the non-fluorescent nitrosyl $[(Me_2bpb)Ru(NO)(im)]BF_4$ (**2**), (*iii*) the unbound ligand dansyl-Imidazole (Ds-im); (*iv*) the nitrosyl-fluorophore conjugate $[(Me_2bpb)Ru(NO)(Ds\text{-}im)]BF_4$ (**1**). Instrument parameters: 600 MHz Varian spectrometer; 298 K.

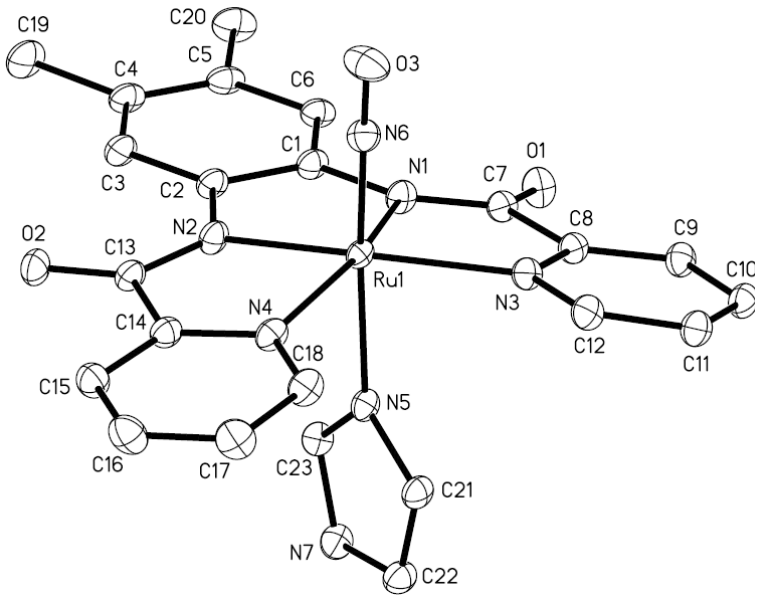

Fig. S2. Thermal ellipsoid plot (50% probability level) of $[(Me_2bpb)Ru(NO)(im)]^+$, the cation of **2**. Selected bond distances and bond angles: Ru–N1 = 1.9944(16); Ru–N2 = 1.9938(15); Ru–N3 = 2.1212(16); Ru–N4 = 2.1242(17); Ru–N5 = 2.0910(16); Ru–N6 = 1.7577(17); Ru–N6–O3 = 174.38(17). The structure has ca. 6% disorder in the orientation of the complex, but only the disordered position of the Ru was included in the model. This disorder was indicated by diffuse scattering in the diffraction pattern. The BF_4 group is disordered into two orientations and also involves a partially occupied molecule of CH_3CN. Hydrogen atoms were not added into the structure factor calculation for the latter molecule of CH_3CN.

Spectroscopic properties of 2:

The IR spectrum of **2** (ν_{NO} = 1872 cm^{-1}; ν_{CO} = 1642 cm^{-1}) is nearly identical to that of **1** with the notable exception of the absence of characteristic ν_{SO} stretches near 1180 cm^{-1} (as observed in **1**). The ^{1}H-NMR spectrum of **2** confirms the bound imidazole moiety, whose symmetry (in CD_3CN solution) is clearly broken upon binding the metal center (see Figure S1). X-ray quality crystals of **2** were grown at room temperature using a dilute solution in MeCN/Et_2O mixture (1:1). The nearly linear Ru–N–O moiety

(174.38(17)°), and its bond distances (Ru–N(O) = 1.7577(17) Å; N–O = 1.143(2) Å) are typical of photoactive $\{Ru\text{-}NO\}^6$ nitrosyls. The bond distances from the ruthenium center to the carboxamido-*N* donors (Ru–N1 = 1.9938(15) Å) are similar to those observed in other nitrosyls derived from deprotonated bpb^{2-} ligands (reference 16b of the text). The $Ru–N_{py}$ distances (Ru–N3 = 2.1212(16) Å) are also within the expected range.

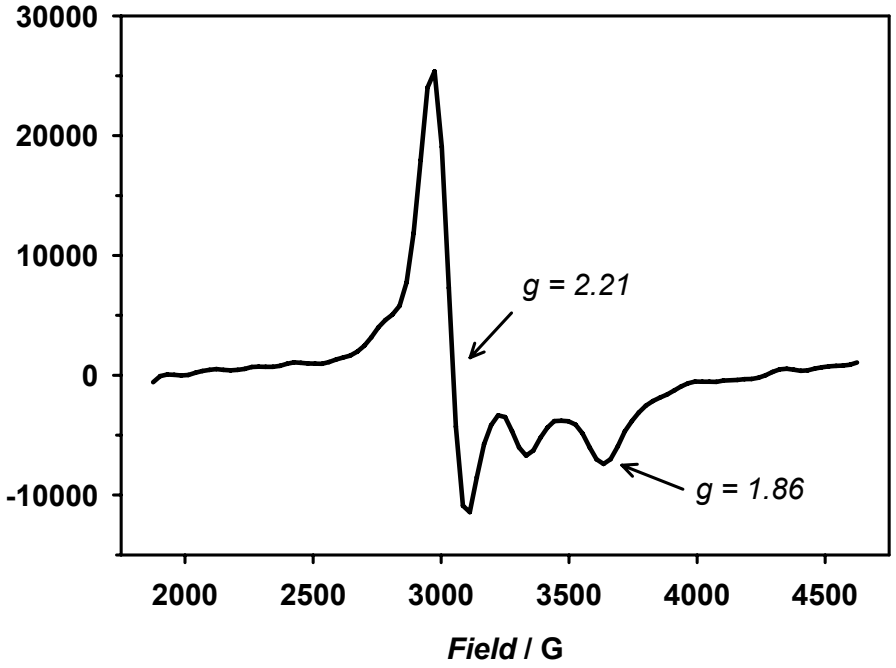

Fig. S3. X-band EPR spectrum of a photolyzed solution of **1** in H_2O/glycerol (3:1) glass (125 K). The g values of the primary signals are indicated. Instrument settings: microwave frequency, 9.4 GHz; microwave power, 1 mW; frequency modulation, 100 kHz; modulation amplitude, 2 G.

Table S1. Crystal data and structure solution parameters for $[(Me_2bpb)Ru(NO)(im)]BF_4$, complex **2**. Diffraction data was collected on a Bruker Apex 2 diffractometer, and the crystal structure solved using direct methods in SHELXS-97 software package (Sheldrick, 1997). Additional structural parameters are available in the cif file in ESI.

empirical formula	$C_{23.75}H_{21.25}N_{7.38}O_3RuBF_4$
FW	645.86
crystal color	dark red block
crystal size (mm)	0.33 × 0.27 × 0.16
T (K)	90(2)
wavelength (Å)	0.7103
crystal system	triclinic
space group	$P\bar{1}$
a (Å)	8.9006(4)
b (Å)	10.4858(5)
c (Å)	14.2498(7)
α (deg)	102.537(3)
β (deg)	104.160(3)
γ (deg)	93.973(3)
V (Å^3)	1248.21(10)
Z	2
d_{calc} (g/cm^3)	1.718
μ (mm^{-1})	0.702
Reflections collected	24557
Independent reflections	8239 [R(int) = 0.0190]
GOF[a] on F^2	1.085
final R indices	$R1$ = 0.0330
[$I>2\sigma(I)$]	w$R2$ = 0.0882
R indices[b]	$R1$ = 0.0371
all data[c]	w$R2$ = 0.0924

[a] GOF = $[\Sigma[w(F_o^2 - F_c^2)^2]/M - N)]^{1/2}$ (M = number of reflections, N = number of parameters refined). [b] $R1 = \Sigma| |F_o| - |F_c| | / \Sigma |F_o|$; [c] $wR2 = [\Sigma[w(F_o^2 - F_c^2)^2]/ \Sigma[w(F_o^2)^2]]^{1/2}$