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Nitric Oxide Production by Visible Light Irradiation of Aqueous Solution of Nitrosyl Ruthenium Complexes

Marília Gama Sauaia,[†] Renata Galvão de Lima,[†] Antonio Claudio Tedesco,[†,‡] and Roberto Santana da Silva*[,†,‡]

Departamento de Química da Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. dos Bandeirantes 3900, 14040-901, Ribeirão Preto, São Paulo, Brazil, and Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café s/n, 14040-903, Ribeirão Preto, São Paulo, Brazil

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$[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)](PF_6)_5$ (L is NH_3, py, or 4-acpy) was prepared with good yields in a straightforward way by mixing an equimolar ratio of *cis*-$[Ru(NO_2)(bpy)_2(NO)](PF_6)_2$, sodium azide ($NaN_3$), and *trans*-$[RuL(NH_3)_4(pz)]$ $(PF_6)_2$ in acetone. These binuclear compounds display $\nu(NO)$ at ca. 1945 cm^{-1}, indicating that the nitrosyl group exhibits a sufficiently high degree of nitrosonium ion (NO^+). The electronic spectrum of the $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ complex in aqueous solution displays the bands in the ultraviolet and visible regions typical of intraligand and metal-to-ligand charge transfers, respectively. Cyclic voltammograms of the binuclear complexes in acetonitrile give evidence of three one-electron redox processes consisting of one oxidation due to the $Ru^{2+/3+}$ redox couple and two reductions concerning the nitrosyl ligand. Flash photolysis of the $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ complex is capable of releasing nitric oxide (NO) upon irradiation at 355 and 532 nm. NO production was detected and quantified by an amperometric technique with a selective electrode (NOmeter). The irradiation at 532 nm leads to NO release as a consequence of a photoinduced electron transfer. All species exhibit similar photochemical behavior, a feature that makes their study extremely important for their future application in the upgrade of photodynamic therapy in living organisms.

Introduction

Nitric oxide (NO) has been postulated as being a prime substance to numerous biological processes, including vasodilatation and neurotransmission, and it can also act as a causative agent in different pathophysiological mechanisms.[1−6] NO also appears to be critical for the tumoricidal activity of the immune system,[7−12] once it can kill a variety of tumor cells by triggering the genetically programmed series of events termed apoptosis. This theory has opened up the possibility of designing new drugs that can deliver NO into tissues and to the bloodstream in a sustained and controlled manner. One way to develop NO-delivering agents is to combine compounds that are thermodynamically stable in physiological conditions but labile under light stimulation. The potential use of these pro-drugs should enhance the efficiency of some clinical therapies, such as photodynamic therapy (PDT).

In PDT, a drug sensitive to light (photosensitizer) and a source of visible light should be combined to form intracellular lethal agents. Presently, the use of PDT is restricted to

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[*] To whom correspondence should be addressed.

[†] Departmento de Química da Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo.

[‡] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo.

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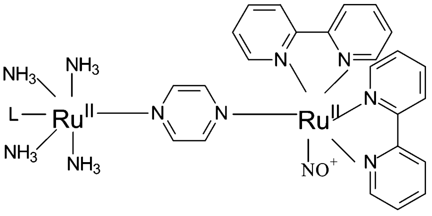
the formation of oxygen reactive species that will act as tumoricidal agents. Although this clinical treatment has met with success in the case of many types of cancer, there are limitations due to the hypoxic characteristic of the tumor cells.[12−16] One way to solve this problem is to increase the generation of other damaging species, like NO, in the cell milieu.[11] The role of NO in cancer therapy depends on a variety of intracellular conditions, but it is recognized that high levels of NO are generally tumoricidal and its antitumoral activity includes inhibition of tumor cell proliferation, promotion, and differentiation.[1]

To increase the intracellular levels of NO for application in PDT, it is necessary to use a photosensitive compound that displays absorption bands in the visible region and that can produce NO only upon stimulation with light. *S*-nitrosoglutathione (GSNO)[17] and *S*-nitroso-*N*-acetylcysteine-zinc phthalocyanine (NacySNO-ZnPC)[11] are some examples of compounds capable of releasing NO after a luminous stimulus in the 320−440 nm range. Metalonitrosyl complexes have also gained much attention due to their ability to generate NO upon exposure to light.[18−29] In one of the pioneering studies in this field, Ford et al. analyzed the photolabilization of NO from $Na_2[Fe(CN)_5(NO)]$, Roussin's black salt ($NH_4[Fe_4S_3(NO)_7]$), Roussin's red salt ($Na_2[Fe_2S_2(NO)_4]$),[30−31] and [Ru(P)(ONO)(NO)],[32−34] where P is a porphyrin ring. Similar results were obtained by flash-photolysis studies of $[Ru(NO)Cl_3]$ and $K_2[Ru(NO)Cl_5]$,[35] in which relaxation of the rabbit aortic ring was observed after photolabilization of NO in the ultraviolet region. The effect of the electron density under the metal center seems to be crucial for the control of the amount of NO produced by light stimulation. Tfouni et al. have studied the trans effect of the ligand, L, in *trans*-$[RuL(NH_3)_4(NO)]^{3+}$ complexes, where L is phosphine or a pyridine-derivative ligand,[5] and found some dependence of the NO quantum yield on the π-acceptor characteristic of L. More recently, da Silva and co-workers have described the photochemistry of $[Ru^{II}L_5(NO)]^{n+}$ (L can be a σ-donor or π-acceptor ligand) and reported photolabilization of NO by light irradiation at 355 nm.[18−20,26,27] In those systems, the increase of NO quantum yield apparently resulted from an increase of the π-acceptor character of the ligand L.



L = NH_3, pyridine or 4-acetylpyridine

Figure 1. Schematic representation of the $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ complex.

Unfortunately, the majority of these complexes can only deliver NO upon ultraviolet light irradiation. Considerable effort has been directed toward the synthesis of complexes that can generate NO by visible light irradiation. One possibility is to use polynuclear systems that can be controlled by appropriate modification of their molecular topology and to develop compounds capable of delivering NO upon light irradiation. Very recently, we have described a nitrosyl binuclear ruthenium complex that undergoes NO release by a photoinduced electron-transfer mechanism.[21] We now propose to control the irradiation wavelength of the visible light changing the ligand L in the $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)](PF_6)_5$ species, where L = NH_3, 4-acetilpyridine (4-acpy), or pyridine (py) (Figure 1), as an NO delivery agent. Here we show the synthesis, spectroscopical, electrochemical and photochemical studies of these binuclear nitrosyl ruthenium complex. Ligand L in the $\{[Ru(NH_3)_4L]^{2+}\}$ fragment undergoes a change to control the energy of the metal−ligand charge transfer (MLCT) band in the visible region.

Experimental Section

2.1. Apparatus. Ultraviolet−visible (UV−vis) spectra were recorded on a Hitachi U-3501 and Genesys-2 apparatus from Spectronic. Infrared (IR) spectra were recorded on a Protegé 460 series FT-IR spectrophotometer, using solid samples pressed in KBr pellets. The pH measurements were carried out using a 430 pH meter from Corning.

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The HPLC system consisted of a Shimadzu (Kyoto, Japan) liquid chromatograph equipped with an LC-10AD pump, Rheodyne injector model 7125, SPD-10AV UV−vis detector, and C-R6A integrator. A CLC−ODS column (250 × 4.6 mm i.d.; Shimadzu, Japan) with 5-μm particle size linked to an octadecyl chain was used. Isocratic elution with 25:75 methanol/water containing 0.1% HTFA, pH = 3.0, was used at a constant flow rate of 1.0 mL min^{-1}, at room temperature ($T = 30.0 \pm 0.1$ °C).

Electrochemical studies were performed in acetonitrile with a potentiostat−galvanostat AUTOLAB PGSTAT 30 model. A conventional three-electrode cell containing a Pt wire auxiliary electrode, Ag/AgCl reference electrode, and Pt working electrode was employed. The supporting electrolyte was Bu_4NPF_6 (TBAP) (0.1 M), and ferrocene was employed as internal standard.[36]

Photolysis of the binuclear complexes in acetate buffer solution at pH 4.5 and ionic strength of 0.1 M adjusted with $NaBF_4$ was performed using a laser flash photolysis apparatus consisting of a Continuum Q-switched Nd:YAG laser (Continuum, Santa Clara, CA) with excitation provided by a third (355 nm) and second (532 nm) harmonic. The pulse length was 8 ns, the beam diameter incident on the sample was 6 mm, and the repetition rate was 10 Hz. The pulse energy was typically 10 mJ/pulse, as measured by a Field Master power-meter with an L-30 V head. NO release was detected and measured with an ISO-NOP (NOmeter) from World Precision Instruments that directly detects NO concentration by an amperometric technique. The sensitivity of this apparatus ranges from 1 nM to 20 μM, with a 2-mm sensor. The output of the sensor was recorded with an IBM PC computer linked to a DUO-18 acquisition board from WPI.

2.2. Chemicals and Reagents. $RuCl_3 \cdot nH_2O$, 2,2′-bipyridine (bpy), pyridine (py), 4-acetylpyridine (4-acpy), pyrazine (pz), 1,10′-phenantroline, and Reinecke's salt—$(NH_4)[Cr(NH_3)_2(NCS)_4] \cdot H_2O$—were purchased as high purity reagents from Aldrich Chemicals. Potassium ferrioxalate ($K_3[Fe(C_2O_4)_3] \cdot 3H_2O$) was purchased from Fisher Scientific Co. and used as supplied. Acetone was obtained from Carlo Erba, acetonitrile from Mallinckrodt, and methanol from Merck. Doubly distilled H_2O was used in all experiments.

2.3. Laser Flash Photolysis Experiments. The sample (3.0 mL) was placed in a quartz square cuvette with an optical path length of 1 cm containing a magnetic stirring bar. It was routinely thermostatized at 25.0 ± 0.1 °C. The sample solution was stirred continuously and irradiated for defined periods of time.

Two kinds of analyses were carried out during the flash photolysis experiments. First, the UV−vis spectrum of the sample was recorded after each irradiated period. Second, the NO measurements were accomplished with the selective electrode (NOmeter) placed in the cuvette, but positioned outside the light path to avoid any photoelectric interference.

2.4. NOmeter Calibration. The calibration curve of the NO sensor electrode was constructed by several dilutions of a known volume of a saturated nitric oxide solution in 10.0 mL of a previously degassed acetate buffer solution (pH = 4.5), where the NOmeter electrode was adapted. The current value was recorded in nÅ after each volume addition. The NO concentration was calculated according to the reported NO molar fraction solubility (2.1×10^{-3} M at 25.0 ± 0.1 °C).[37]

2.5. Quantum Yield Measurements. Light intensities were determined before each photolysis experiment by the chemical actinometry procedure. The actinometers used were potassium ferrioxalate at $\lambda_{irr} = 355$ nm and Reinecke's salt at $\lambda_{irr} = 532$ nm.[38]

Nitric oxide quantum yields (ϕ_t) were calculated on the basis of the NO concentrations obtained by the NOmeter measurement. The calculated values were plotted versus t. These plots were linear for the first 40% of the reaction, with a negative slope. The extrapolated quantum yield at $t = 0$ (y intercept) was taken as ϕ_{NO} for the photolabilization of NO from $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ complexes. Evaluation of ϕ_{NO} at $t = 0$ eliminates possible complications resulting from photoreactions of primary products.[19−20] All the reported data are the average of three independent experiments, and the error is the standard deviation.

2.6. Syntheses of the Complexes. The mononuclear complexes *cis*-$[Ru(NO_2)(bpy)_2(NO)](PF_6)_2$[39] and *trans*-$[RuL(NH_3)_4(pz)](PF_6)_2$, where L = NH_3,[40] py,[41] or 4-acpy,[42] were prepared according to methods previously described in the literature. The purity of these complexes was tested by comparison to the UV−visible spectrum as previously published and also by high performance liquid chromatograph (HPLC). The peak retention times (t_r) for *trans*-$[RuL(NH_3)_4(pz)](PF_6)_2$ were 4.8, 4.4, and 4.2 min for L = 4-acpy, py, and NH_3, respectively.

2.6.1. *cis*-$[Ru(bpy)_2(pz)(NO)](PF_6)_3$. The *cis*-$[Ru(NO_2)(bpy)_2(NO)](PF_6)_2$ salt (0.150 g, 1.92×10^{-4} mol) was dissolved in acetone (20 mL). An equimolar amount of NaN_3 (0.013 g) was then dissolved in methanol (5 mL) and added dropwise to the above solution. After 10 min, pyrazine (0.256 g, 3.2×10^{-3} mol) previously dissolved in acetone (5 mL) was added to the stirred solution and the reaction was allowed to proceed for 15 h. The mixture was concentrated under vacuum almost to dry and NH_4PF_6 (0.300 g) dissolved in water (3.0 mL) was added. The *cis*-$[Ru(NO_2)(bpy)_2(pz)](PF_6)$ salt was obtained as a brown precipitate, which was collected by filtration and washed with ether (10 mL). The salt was then dissolved in acetonitrile (20 mL), and 1 mL of concentrated HPF_6 was added to the stirred solution. After 15 min, the solution was concentrated by rotary evaporation until dry. The NH_4PF_6 (1 g) previously dissolved in 2.5 mL of H_2O was then added to produce a precipitate. The solution was filtered off, and the solid was collected and air-dried. Yield 71.2%. Anal. Calcd for $C_{24}H_{20}N_7OP_3F_{18}Ru$: C, 30.08; H, 2.09; N, 10.23. Found: C, 30.44; H, 2.01; N, 10.53.

2.6.2. $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)](PF_6)_5$ (L = NH_3, py, or 4-acpy). These binuclear complexes were synthesized from *cis*-$[Ru(NO_2)(bpy)_2(NO)](PF_6)_2$ and *trans*-$[RuL(NH_3)_4(pz)](PF_6)_2$ in equimolar proportion. NaN_3 (0.013 g; 1.92×10^{-4} mol) dissolved in methanol (5 mL) was added dropwise to *cis*-$[Ru(NO_2)(bpy)_2(NO)](PF_6)_2$ (0.150 g; 1.92×10^{-4} mol) dissolved in acetone (15 mL). The mixture was kept under stirring, and after 10 min, a solution of *trans*-$[RuL(NH_3)_4(pz)](PF_6)_2$ (1.92×10^{-4} mol) in acetone (10 mL) was added and the reaction was allowed to proceed for 22 h at room temperature. After adding concentrated HPF_6 (1 mL) and ethanol (200 mL) to the ruthenium solution, a dark red precipitate was collected by filtration. The $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)](PF_6)_5$ salt was then dissolved in acetone and reprecipitated by addition of ethanol. Yield 47.9% for L = NH_3. Anal. Calcd for $C_{24}H_{35}N_{12}OP_5F_{30}Ru_2$: C, 20.08; H, 2.44; N, 11.72. Found: C, 19.98; H, 2.50; N, 11.56. Yield 54.3% for L = py Anal. Calcd for $C_{29}H_{37}N_{12}OP_5F_{30}Ru_2$: C, 23.28; H, 2.47; N, 11.23. Found: C, 23.32; H, 2.51; N, 10.95. Yield 51.5% for L = 4-acpy

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Table 1. Electronic Spectral Data of Some Ruthenium Complexes in 0.1 M HCl

complex	λ, nm (log ϵ, M cm^{-1})
$[Ru(NH_3)_5(pz)Ru(bpy)_2(NO)]^{5+}$	286 (4.46), 408 (3.70), 486 (3.87), 530 (3.98)
$[Ru(py)(NH_3)_4(pz)Ru(bpy)_2(NO)]^{5+}$	292 (4.40), 400 (3.70), 556 (4.27)
$[Ru(4\text{-acpy})(NH_3)_4(pz)Ru(bpy)_2(NO)]^{5+}$	290 (4.44), 560 (4.24)
cis-$[Ru(bpy)_2(pz)(NO)]^{3+}$	300 (4.26), 338 (3.74), 432 (3.34)

Table 2. Electrochemical Data, in Acetonitrile, of Ruthenium Complexes[a]

complex	$E_{1/2}$ (NO$^{+/0}$)	$E_{(2c)}$(NO$^{0/-}$)	$E_{1/2}$ (Ru$_A$$^{2+/3+}$)
$[Ru(NH_3)_5(pz)Ru(bpy)_2(NO)]^{5+}$	0.12	−0.81	0.40
$[Ru(py)(NH_3)_4(pz)Ru(bpy)_2(NO)]^{5+}$	0.17	−0.71	0.50
$[Ru(4\text{-acpy})(NH_3)_4(pz)Ru(bpy)_2(NO)]^{5+}$	0.20	−0.68	0.59
$[Ru(NH_3)_5(pzH)]^{3+}$	–	–	0.43
trans-$[Ru(py)(NH_3)_4(pzH)]^{3+}$	–	–	0.53
trans-$[Ru(4\text{-acpy})(NH_3)_4(pzH)]^{3+}$	–	–	0.59
cis-$[Ru(bpy)_2(pz)(NO)]^{3+}$	0.22	−0.65	–

[a] Numeric values are relative to the internal standard Fc$^{+/0}$.

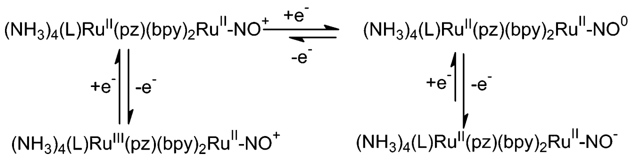
Anal. Calcd for $C_{31}H_{39}N_{12}O_2P_5F_{30}Ru_2$: C, 24.20; H, 2.54; N, 10.92. Found: C, 24.04; H, 2.43; N, 11.22.

3. Results and Discussion

The binuclear ruthenium complexes were synthesized from the corresponding *trans*-tetraamin(L)(pyrazine)ruthenium(II) and acetonebis(bipyridine)nitroruthenium(II) starting complexes. The resulting mixture of *trans*-$[RuL(NH_3)_4pz]^{2+}$ and *cis*-$[Ru(NO_2)(acetone)(bpy)_2]^+$ developed a reddish-orange color with an electronic absorption spectrum showing a broad band around 530 (L = NH$_3$) and 560 nm (L = py or 4-acpy). The purities of the binuclear and *cis*-$[Ru(bpy)_2(pz)(NO)](PF_6)_3$ complexes were analyzed by elemental analysis, which confirmed the proposed formula. The other tetraamin(pyrazine)ruthenium(II) complexes were isolated as hexafluorophosphate salts, and their electronic spectra were compared to the published results.[39,41−42]

Absorption data for *cis*-$[Ru(bpy)_2(pz)(NO)]^{3+}$ and $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$, L = NH$_3$, py, or 4-acpy, are collected in Table 1. The electronic spectrum of *cis*-$[Ru(bpy)_2(pz)(NO)]^{3+}$ complex is very similar to the *cis*-$[Ru(bpy)_2L(NO)]^{3+}$ species (L = py, 4-pic, and 4-acpy) previously published.[43] The high-energy absorbance in the ultraviolet region was attributed as an intraligand band due to the $\pi-\pi^*$ transition associated with the bound ligand, although a shoulder at 338 nm was characterized as a MLCT band due to a $d_\pi(Ru^{II})-\pi^*(NO^+)$ transition. The band at 432 nm, which is not observed in the electronic spectrum of *cis*-$[Ru(bpy)_2L(NO)]^{3+}$ species,[43] was tentatively assigned as a MLCT involved in a $d_\pi(Ru^{II})-\pi^*(pz)$ transition. The electronic spectra of the binuclear ruthenium complexes also show similarities with the *cis*-$[Ru(bpy)_2(pz)(NO)]^{3+}$ complex but with an extra band in the visible region. The maximum wavelength of the lower-energy broad absorption is dependent on the solvent and was characterized as a MLCT attributed to the transition of $d_\pi(Ru^{II})$ orbital to π-antibonding orbital originated from the unsaturated ligands. Those MLCT bands are widely accepted as the electronic transition between the metal ion d orbital and the LUMO of the ligand, as observed in similar pyridine-substituted ruthenium(II) complexes.[43] This band, which is observed in all the electronic spectrum of $Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ species, was mainly attributed to the $d_\pi(Ru^{II})-\pi^*(pz)$ transition by comparison to the electronic spectrum of *trans*-$[RuL(NH_3)_4(pzH)]^{3+}$ complexes (L = py and 4-acpy).[42] The second MLCT band, which is usually found near the ultraviolet region in the electronic spectrum of the *trans*-$[RuL(NH_3)_4(pz)]^{2+}$ complex,[42] almost disappears in the electronic spectrum of $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ in aqueous solution. The symmetry of the binuclear species probably restricts the electron transition between the orbital centered in the metal and the π^* orbital centered in the pyridine derivative ligand, as previously observed by Tfouni and Bento.[42]

Scheme 1



Cyclic voltammograms of the binuclear complexes in acetonitrile exhibit three one-electron redox processes, which consist of one oxidation and two reductions with respect to the starting redox state of $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$, in the potential region −1.2 to 0.8 V vs ferrocene/ferrocenium couple. Voltammetric half-wave potentials for this electrochemical process are given in Table 2.

The oxidation was assigned as being due to the metal-centered Ru(II)/Ru(III) couples of the $\{[Ru^{II}L(NH_3)_4(pz)]^{2+}\}$ fragment, with a mixed-valence Ru(III)−Ru(II) being generated after this electrochemical oxidation. The assignment of the oxidation potential of these binuclear complexes can be inferred from the reasonable agreement of these values with the oxidation potentials of the corresponding *trans*-$[RuL(NH_3)_4(pz)]^{2+}$ species.[42] The $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ complex undergoes two reduction processes, as described in Scheme 1. These processes are similar to those previously observed for *cis*-$[RuL(bpy)_2NO]^{3+}$ (L = 4-pic, py, or 4-acpy).[43]

UV−vis spectroelectrochemical experiments were performed in acetonitrile at 25.0 ± 0.1 °C using an OTTLE cell. The resulting spectrum of the $[Ru^{II}L(NH_3)_4(pz)Ru^{II}$-

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(43) Sauaia, M. G.; da Silva, R. S. *Trans. Metal Chem.* **2003**, *28*, 254.

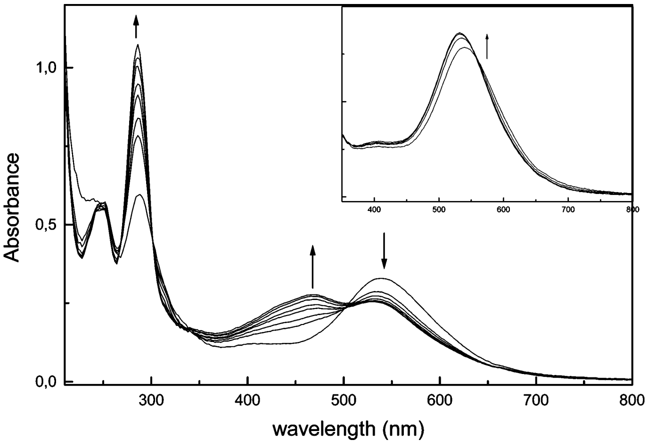


Figure 2. Changes in the UV−visible spectrum of the $[\text{Ru}^{\text{II}}(\text{py})(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ complex during electrolysis at −1.00 V vs $\text{Fc}^{+/0}$ in acetonitrile/0.1 M Bu_4NPF_6. Inset: electrolysis of $[\text{Ru}^{\text{II}}(\text{py})(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ at 0.00 V vs $\text{Fc}^{+/0}$.

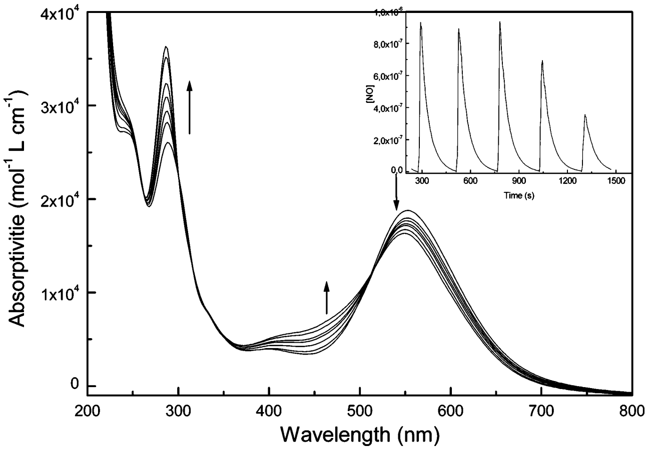


Figure 3. UV−visible spectra of the $[\text{Ru}^{\text{II}}(\text{4-acpy})(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ complex in acetate buffer solution (pH = 4.5) during flash-photolysis at $\lambda_{\text{irr}} = 355$ nm. Inset: chronoamperogram of NO release as measured by the NOmeter.

Table 3. Quantum Yields Data (ϕ_{NO}) in mol einstein^{-1} for $[\text{RuL}(\text{NH}_3)_4(\text{pz})\text{Ru}(\text{bpy})_2((\text{NO})]^{5+}$ in Acetate Buffer Solution (pH = 4.5)

L	$\lambda_{\text{irr}} = 355$ nm	$\lambda_{\text{irr}} = 532$ nm
NH_3	0.08 ± 0.01	0.025 ± 0.002
py	0.05 ± 0.01	0.038 ± 0.002
4-acpy	0.07 ± 0.01	0.036 ± 0.002

$(\text{bpy})_2(\text{NO}^0)]^{4+}$ complex was found to be almost reversible when a potential at 0.00 V vs $\text{Fc}^{+/0}$ was applied for 10 min. During the reduction process, the lowest-energy band shifts around 30 nm to higher energy, indicating that the generated $\{[\text{Ru}^{\text{II}}\text{-NO}^0]\}$ complex has little effect on the back-bonding of $\{[\text{Ru}^{\text{II}}\text{-pz}]\}$ in the $[\text{RuL}(\text{NH}_3)_4(\text{pz})\text{Ru}(\text{bpy})_2(\text{NO})]^{4+}$ complex. The monoreduction of $[\text{Ru}^{\text{II}}\text{L}(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{4+}$ shifts the MLCT band hypsochromically by nearly 100 nm, but the intensity of this band shows very little modulation (Figure 2). The process is not totally reversible, indicating that the full reduction process is accompanied by nitroxyl release. Each $[\text{Ru}^{\text{II}}\text{L}(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ species was also oxidized in a one-electron step, and these couples were monitored by UV−vis spectroelectrochemistry again. When L = py or 4-acpy, the oxidation is an irreversible process and probably involves loss of the pyrazine bound to Ru(II) in the $\{[\text{Ru}^{\text{II}}\text{L}(\text{NH}_3)_4(\text{pz})]\}$ fragment once the MLCT band is not regenerated (Figure 3) when a potential of 0.10 V vs $\text{Fc}^{+/0}$ is applied to the oxidized binuclear compound.

Flash-photolysis of the $[\text{Ru}^{\text{II}}\text{L}(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ species was performed upon light irradiation at 355 and 532 nm in an acetate buffer solution, pH 4.5, to avoid the electrophilic attack of hydroxide ion as observed for the *cis*-$[\text{RuL}(\text{bpy}_2)(\text{NO})]^{3+}$ complex.[58] The quantum yields observed for NO release from $[\text{Ru}^{\text{II}}\text{L}(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$, obtained in situ by amperometric detection, are collected in Table 3. The signal recorded by the NOmeter quickly increases when photolysis was initiated but decreases after the light was turned off, owing to NO consumption.[18−19] The resulting optical spectral changes observed during photolysis at 355 nm is consistent with substitution of NO by a water molecule, as observed in Figure 3 for the $[\text{Ru}^{\text{II}}(\text{4-acpy})(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ complex, whose spectrum is representative of the photolysis of all the $[\text{Ru}^{\text{II}}\text{L}(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ complexes studied herein. The electronic spectrum also exhibits an isosbestic point at 510 nm, which demonstrates that a simple reaction mechanism is going on during light irradiation. The MLCT band attributed to the $d\pi(\text{Ru})-\pi^*(\text{pz})$ transition in the $[\text{Ru}^{\text{II}}\text{L}(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ complex slightly decreases. This should reflect the low withdrawing effect of the $\{\text{Ru}^{\text{III}}(\text{bpy})_2(\text{H}_2\text{O})\}$ fragment on the $d\pi(\text{Ru})-\pi^*(\text{pz})$ back-bonding from $[\text{Ru}^{\text{II}}\text{L}(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{H}_2\text{O})]^{5+}$, generated by light irradiation of the nitrosyl binuclear complex, once is virtually unaffected. Taking into account that the photolabilization of NO at 355 nm in the *cis*-$[\text{RuL}(\text{bpy})_2(\text{NO})]^{3+}$ complex concerns light irradiation of the MLCT band, characterized as a $d\pi(\text{Ru})-\pi^*$ (NO) transition,[18−19] we can infer a similar photochemical pathway for the photolysis of $[\text{Ru}^{\text{II}}\text{L}(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ in aqueous solution (Scheme 2).

Flash-photolysis of the $[\text{Ru}^{\text{II}}\text{L}(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ complex upon light irradiation at 532 nm gives evidence of NO release but with a different photochemical pathway from the one observed when an aqueous solution of these complexes was irradiated at 355 nm. The MLCT band of

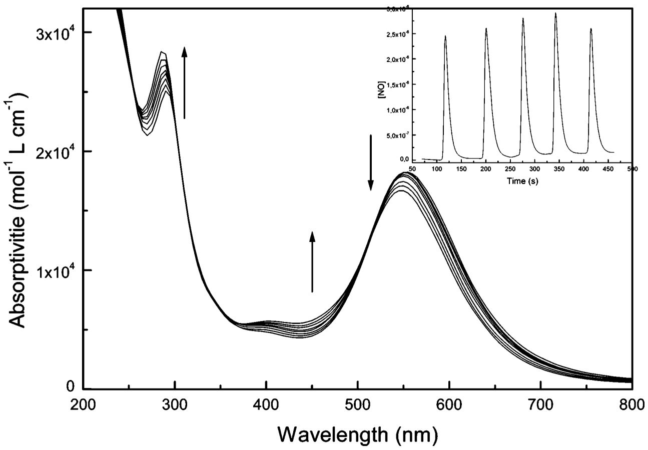
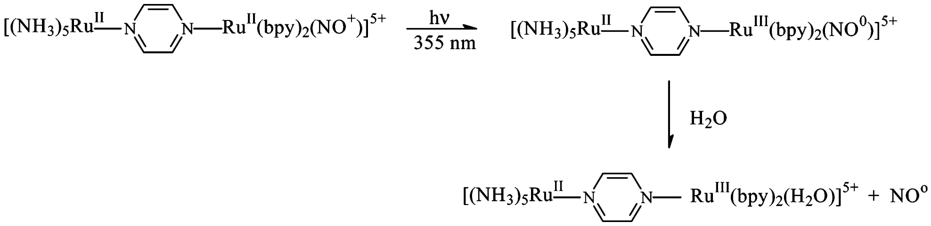
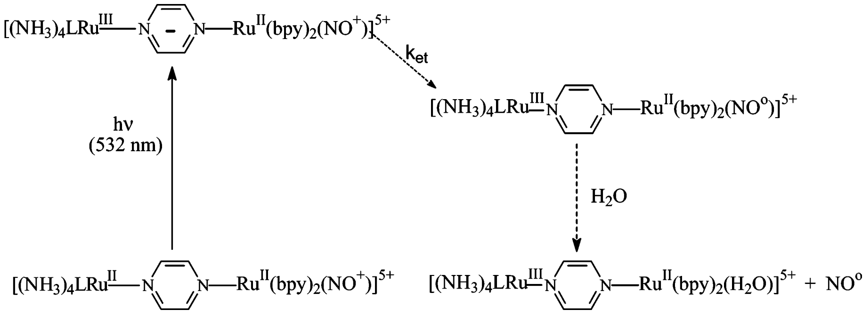


Figure 4. UV−visible spectra of the $[\text{Ru}^{\text{II}}(\text{py})(\text{NH}_3)_4(\text{pz})\text{Ru}^{\text{II}}(\text{bpy})_2(\text{NO})]^{5+}$ complex in acetate buffer solution (pH = 4.5) during flash-photolysis at $\lambda_{\text{irr}} = 532$ nm. Inset: chronoamperograms of NO release as measured by the NOmeter.

Scheme 2



Scheme 3



$[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ decreases upon light irradiation at 532 nm, and an isosbestic point can be seen at 490 nm, as observed in the case of the $[Ru^{II}(py)(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ complex (Figure 4). This is consistent with the formation of $[Ru^{III}L(NH_3)_4(pz)Ru^{II}(bpy)_2(H_2O)]^{5+}$ once the nitric oxide release was observed by NO measurement. The most probable mechanism for NO release from $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ upon light irradiation at 532 nm comes from a photoinduced electron transfer. The nitrosyl binuclear complex can be photoexcited at 532 nm, generating the mixed-valent $[Ru^{III}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$complex. Considering that the excited electron in the MLCT transition of $[Ru(NH_3)_5(py\text{-}X)]^{2+}$ complex (py-X is pyridine derivative ligands) is mainly localized on the pyridine ligand,[42] we could attribute the formation of the intermediate $[Ru^{III}L(NH_3)_4(pz^{\bullet -})Ru^{II}(bpy)_2(NO)]^{5+}$ species under light irradiation at 532 nm. Tentatively, we can infer that an electron-transfer reaction occurs due to the high driving force from pz^- to NO^+ in the $\{Ru^{III}(pz^-)Ru(NO^+)\}^*$ fragment. Similar photolysis with *trans*-$[RuL(NH_3)_4pz]^{2+}$ in pH 1.0 and 7.4 was performed, but we found no existent reaction by UV−vis spectrum analysis, since the intensity of the MLCT band remained virtually unchanged. Therefore, we can concluded that NO was dissociated after a photoinduced electron-transfer process, as shown in Scheme 3. However, the univocal understanding of the electron-transfer mechanism of the excited state requires a detailed photophysical study with time-resolved transient absorption spectroscopy but this is beyond the scope of this work.

5. Conclusions

The strategy to obtain nitric oxide release from $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ by light irradiation in the visible region has significant implications for the use of these complexes in PDT. The ability to control the energy of the MLCT band by changing the ligand, L, in binuclear complexes was achieved by photoinduced electron transfer in this present work. Apparently, the amount of NO is dependent on the electron-transfer constant (k_{et}), once the NO quantum yield for the light irradiation at 532 nm of $[Ru^{II}L(NH_3)_4(pz)Ru^{II}(bpy)_2(NO)]^{5+}$ complex in aqueous solution is lower that the complex irradiated at 355 nm.

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