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Margarita Romero Ávila, Andrés Felipe León-Rojas, Pascal G. Lacroix, Isabelle Malfant, Norberto Farfán, et al.. Two-Photon-Triggered NO Release via a Ruthenium–Nitrosyl Complex with a Star-Shaped Architecture. *Journal of Physical Chemistry Letters*, 2020, 11 (16), pp.6487-6491. 10.1021/acs.jpcllett.0c01953 . hal-02948930

HAL Id: hal-02948930

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Two-Photon Triggered NO-Release Via a Ruthenium-Nitrosyl Complex With a Star-Shaped Architecture

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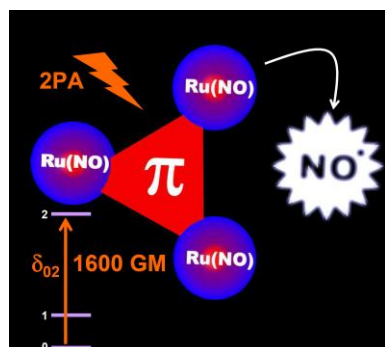
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ABSTRACT. We report herein a molecular engineering strategy based on the design of a multipolar ruthenium-nitrosyl (Ru-NO) complex with a three-branched architecture. The three ruthenium-nitrosyl units are introduced at the periphery of a highly π -delocalized truxene core bearing three terpyridine ligands. The two-photon absorption capabilities of the complex were investigated by the Z-scan technics. The strong electronic coupling among the individual arms gives rise to a very strong two-absorption response ($\delta_{800\text{nm}} \sim 1600 \text{ GM}$), which corresponds to a 16 times enhancement of the capability of a single arm reference, promoting thereby an efficient light-driven NO release process in aqueous medium.

TOC GRAPHICS

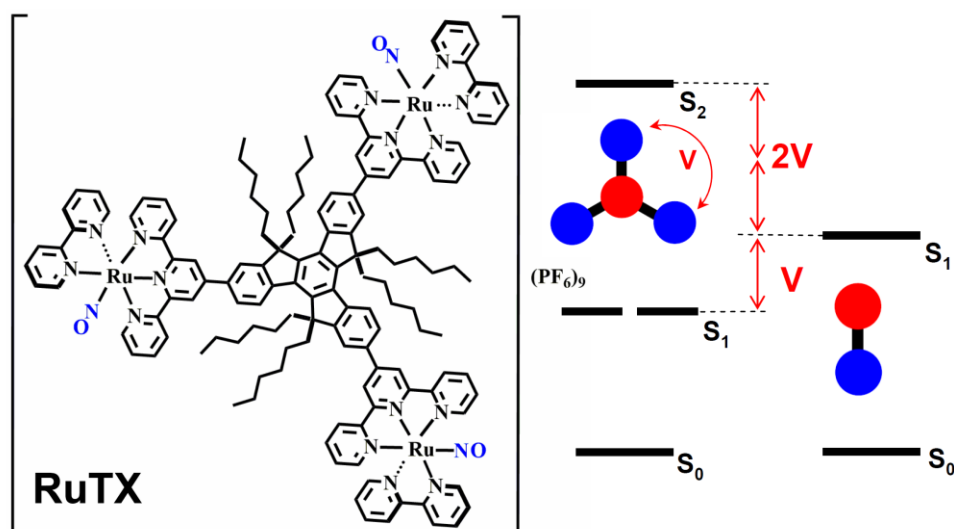


KEYWORDS Coordination Chemistry, Photo-activated Chemotherapy, Two-Photon Absorption, Z-scan, Nitric Oxide, NO delivery.

With the advent of photo-activated chemotherapy, molecular engineering of light-activated nitric oxide (NO) donors¹ has gained an increasing attention in regard to promising applications² devoted to the spatio-temporal control of NO delivery in physiological environments. Indeed, regulating the concentration of this signaling molecule is of primary importance due to specific cellular responses to a local change of NO concentration. Various appealing biological effects of NO have been demonstrated. For instance, the successful action of NO against antibiotics resistance in biofilms in combination with methicillin has recently been reported.³ Additionally, the use of NO as anti-cancer agents⁴ has shown that the increase of NO concentration up to the mM range promoted both apoptosis and cells destruction.^{5,6} In this precise case, it is clear that targeting a malignant cell among a large population of healthy ones requires an efficient discriminating method to spatially localize and exterminate the growing tumor at sub- μ m scale. Two-photon activation constitutes one of these appealing techniques.^{7,8} By tightly focusing a f_s -pulse laser into the biomaterials, the resulting two-photon absorption (2PA) effect intrinsically confines the photoinduced process to a focal volume with feature size that perfectly fulfils such a spatial discrimination requirement. In addition, the near infrared wavelength light used for two-photon excitation allows a deep penetration into living tissues. In this context, various molecular design strategies⁹⁻¹⁴ have been proposed to develop two-photon active chromophores incorporating a light-triggered NO releasable functionality.

Since the pioneer work of Weckler *et al.*¹⁰ who demonstrated the feasibility of two-photon exciting a metal-nitrosyl complex associating an iron/sulfide/nitrosyl cluster and a moderate two-photon active fluorescein dye, various molecular design approaches have proposed alternative solutions which integrate more closely the metal-nitrosyl moiety into the 2PA conjugated subunit.^{11-13,15} By taking advantage of the strong electron withdrawing ability of the metal cation

and its judicious positioning into a multibranched structure, very strong 2PA responses can be accounted. In line with this strategy, we present herein a multipolar ruthenium-nitrosyl (Ru-NO) complex (**Scheme 1**) which has been designed based on a three-branched truxene ligand (TX). This planar conjugated subunit¹⁶ was three-fold substituted with 2, 2': 6', 2''-terpyridine anchoring groups positioned at the external rim of the truxene core. Each Ru-NO center is then coordinated by the three terpyridine nitrogens and also by an ancillary 2,2' bipyridine ligand. Although most of the investigations were carried out in organic environment, it is noteworthy that such a single Ru-NO complex (i.e. [Ru(bpy)(tpy)-NO]³⁺) has previously demonstrated its NO release ability upon light stimulation in aqueous medium.^{15, 17, 18} TX was synthesised by following modified procedures found in the literature, by coupling between a tris-boronated truxene and three iodoterpyridine. The nitrosyl ruthenium (**RuTX**) complex was prepared in four steps following reported methodologies. Details on the synthesis and complete characterisations of the complex are provided as supplementary materials.



Scheme 1. (Left) Molecular structure of the Ru-NO complex (**RuTX**). (Right) Schematic representation of the effects of interbranch coupling on the electronic levels for a C₃ three-branched symmetry.

RuTX displays a star-shaped octupole retaining a C_3 symmetry. In this multibranched architecture, the electronic coupling among the individual arms^{19, 20} should lead to a synergy effect allowing very strong two-photon absorption responses with a concomitant light-triggered NO release ability.

Figure 1 shows the one- (1PA) and two-photon absorption (2PA) spectra of the Ru-nitrosyl complex along with those of its truxene-based ligand (TX). The corresponding spectroscopic data are reported in **Table S1**.

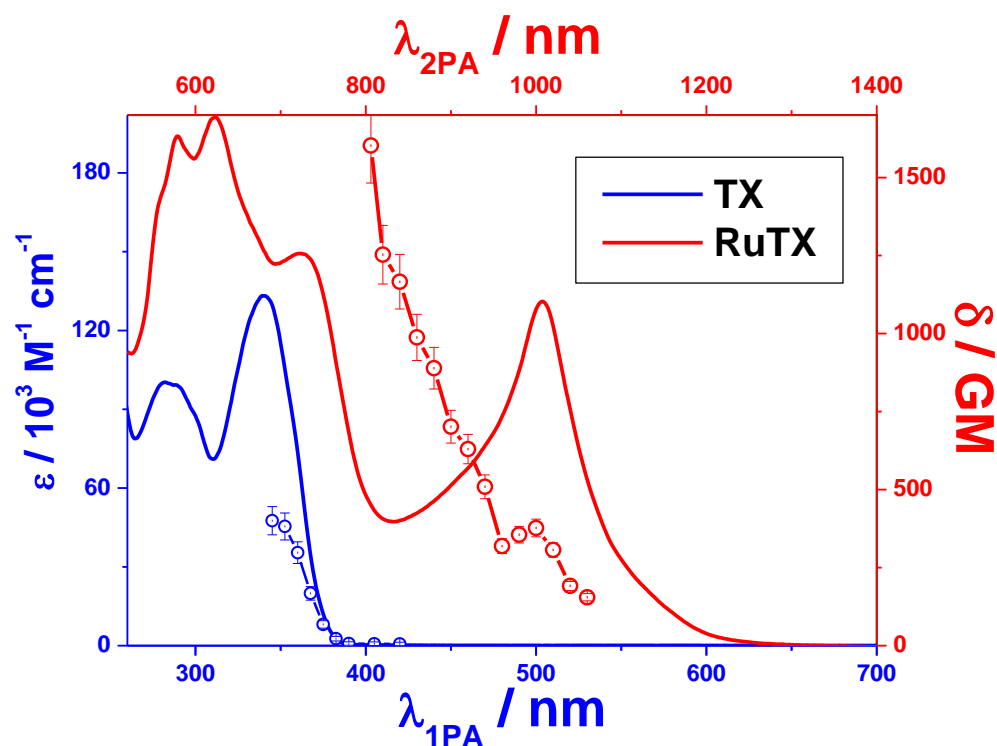


Figure 1. One- and two-photon absorption spectra of TX in dichloromethane (DCM) and **RuTX** in acetonitrile (ACN).

In the 260-400 nm range, the absorption spectrum of **TX** displays two distinctive bands: A structured band located at 282 nm and a very intense one ($\epsilon_{\text{MAX}} \sim 133\,000 \text{ M}^{-1} \text{ cm}^{-1}$) with $\lambda_{\text{MAX}} = 340 \text{ nm}$. The lower energy band should presumably correspond to strongly allowed $\pi-\pi^*$

transitions implying charge delocalization from the truxene core¹⁶ (donor) to the external terpyridine subunits (acceptors). Interestingly, on going from **TX** to **RuTX**, the introduction of strong electron withdrawing Ru-nitrosyl moieties clearly induces a very strong bathochromic shift of the longest wavelength band by about 9585 cm⁻¹ (~ 1.19 eV) which maintains a very strong absorption ($\epsilon_{\text{MAX}} \sim 132\,000\text{ M}^{-1}\text{ cm}^{-1}$).

In parallel, the high-energy side of the absorption spectrum clearly displays two well-separated bands: a vibronically structured one at 311 nm which is typically observed upon metal-ligand bonding with terpyridine groups^{11, 12} and a structureless band which is clearly emerging at 365 nm. This latter band together with the lowest energy one are of particular interest since their occurrence directly results from the interplay and extent of electronic coupling between the three arms of the metal-complex. As illustrated in **Scheme 1**, **RuTX** can be regarded as an assembly of three-dipole like branches with identical S₀-S₁ energy if the interbranching coupling is negligible. However, if this latter interaction is strong (*V* in **Scheme 1**) the Frenkel^{20, 21} exciton model predicts a splitting of the degenerate S₁ levels giving rise to a doubly degenerate S₁ state stabilized by an energy of $-V$ and a third non-degenerated S₂ state which becomes destabilized by an energy of $2V$. According to this exciton coupling scheme, an estimated value of *V* can be obtained by measuring the S₁-S₂ energy gap on the absorption spectrum of **RuTX** (i.e. $\Delta E_{12} = 3V$). In this precise case, one should stress that for a pure C₃-symmetry, the S₀-S₁ transition is one-photon allowed whereas the S₀-S₂ one is theoretically one-photon forbidden. However, the latter transition may become partially allowed upon relaxation to conformations with a lower symmetry.^{22, 23} Therefore, the inter-arm interaction energy can be estimated to a value of *ca.* 0.31 eV for **RuTX**. Such a constant is comparable to that observed for crystal violet¹⁹ (*V* ~ 0.37 eV) and denotes a strong interbranch cross-talk which can be oriented to enhance the 2PA response.

Indeed, the case of the three-branched octupoles is very interesting since Beljonne *et al.*¹⁹ have previously predicted that the 2PA cross-section (δ) into the S_2 excited state can be drastically amplified upon a judicious modulation of V with respect to the S_1 state energy. In addition, such a rationalization has been experimentally confirmed for crystal violet, which exhibits a very high 2PA response into its S_2 state with a δ_{MAX} of about 2000 GM. If we consider now our Ru-nitrosyl complex, one can expect a comparable 2PA amplification. The 2PA spectra of **TX** and **RuTX** are shown in **Figure 1**. According to our spectral resolution, both chromophores display a first resonance located respectively at 690 nm and 1000 nm. These 2PA bands reasonably match their corresponding 1PA ones and display equivalent intensities with δ_{MAX} in the 370-400 GM range. But the most striking effect can be observed for **RuTX** which clearly undergoes an impressive 2PA enhancement in its high energy region (< 880 nm) with δ of about 1600 GM at 800 nm. This very large 2PA cross-section can be readily attributed to the presence of the S_0 - S_2 transition whose nonlinear absorption response nicely confirms the exciton model.¹⁹ Note that this 2PA enhancement can be even higher for $\lambda_{exc} < 800$ nm but it cannot be quantified by our open-aperture z-scan method (see ESI) due to strong interferences of excited states absorption below 790 nm. Therefore, it clearly appears that the photoinduced charge transfer process occurring from the truxene core to the peripheral Ru-nitrosyl subunits boosts the 2PA ability of **RuTX** but this primary process also constitutes a driving force promoting locally the Ru-NO bond dissociation. In order to exemplify this photodissociation reaction, the 1PA photolysis of **RuTX** has been first performed upon irradiation at 532 nm in acetonitrile. **Figure 2A** typically depicts the evolution of the absorption spectrum of **RuTX** during its visible irradiation. The longest wavelength absorption band undergoes a very slight blue shift associated to a subtle decrease of its intensity. In the 270-380 nm range, the photoinduced spectral changes are more

significant. For instance, the structured band located at 290 nm which corresponds to terpyridine-centred transitions increases by a factor 1.25. The presence of several isosbestic points also confirms the occurrence of an equilibrium reaction. To assess the formation of nitric oxides (NO) during the photolysis of **RuTX**, an ESR experiment was performed during this photoreaction in the presence of iron (II) *N*-methyl-D-glucamine dithiocarbamate (Fe-MGD) used as a spin trap which generates a Fe-MGD-NO complex with a characteristic ESR signal.²⁴ As shown in **Figure 2B**, the ESR spectrum of the mixture solution of **RuTX**/Fe-MGD after 20 min photolysis shows a triplet signal with a hyperfine splitting constant value of $a_N = 1.2 \times 10^{-3} \text{ cm}^{-1}$ and a *g*-factor of $g = 2.040$. This ESR signal is consistent with the formation of $[(\text{MGD})_2\text{Fe}^{2+}\text{-NO}]$ complex.

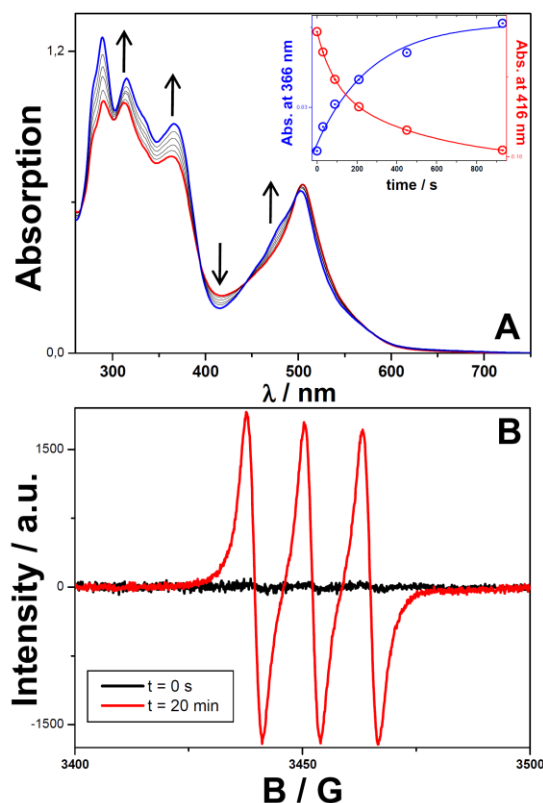


Figure 2. A. Evolution of the absorption spectrum of **RuTX** in solution of ACN upon irradiation at 532 nm. (Inset : Time-dependent absorption changes at 366 nm and 416 nm). B. Triplet ESR signals of **RuTX** in presence of Fe-MGD before and after visible irradiation.

It should be also underlined that if the same solution is kept initially in the dark, the presence of NO is not detected which confirms the photo-triggered character for the generation of NO. We next investigated this photorelease process upon two-photon excitation. For this purpose, two isomolar aqueous solutions of **RuTX** (c : 150 μ M) mixed with Griess agent were irradiated respectively at 800 nm and 1000 nm using a *fs*-pulse laser whose average power was adjusted to the same value for both λ_{exc} . Note that these two distinctive irradiation wavelengths were chosen since at these λ_{exc} the corresponding 2PA cross-sections of **RuTX** are significantly different with a δ -ratio of about 4.2 between 800 nm and 1000 nm. The progress of the photoreaction was monitored by recording the absorption spectrum of the solutions each 20 min irradiation (see Figure S3). The two-photon induced generation of NO is then revealed by its fast and quantitative reaction with the Griess agent yielding an ‘azo’ dye by-product which displays a large absorption band in the 450-600 nm range.^{11, 12}

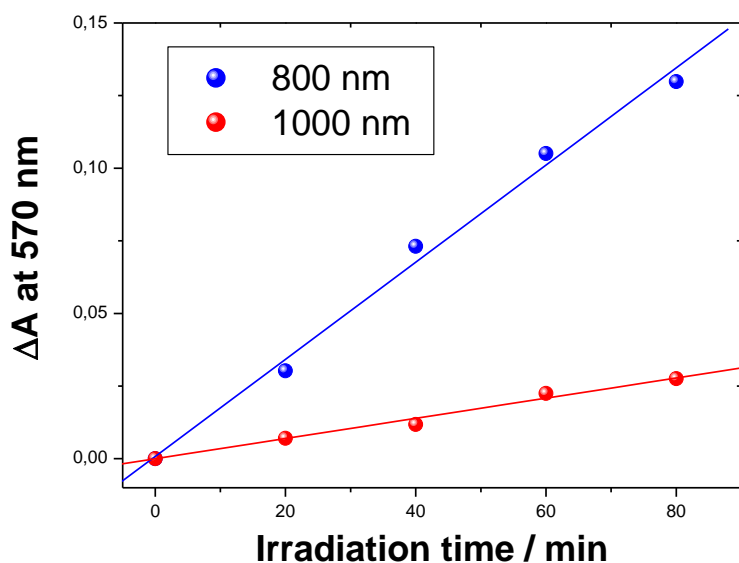


Figure 3. Kinetics of NO release upon two-photon excitation of **RuTX** at 800 nm and 1000 nm in presence of Griess reagent in aqueous medium (H₂O / ACN: 1v: 1v). The average incident power was maintained at 1.2W for both λ_{exc} .

Figure 3 typically shows the time-dependent increase of the absorbance at 570 nm for each **RuTX** solution distinctively irradiated at 800 nm and 1000 nm. As shown in the **Figure 3**, the dynamic for the two-photon release of NO is clearly much faster for the irradiation at 800 nm than that observed for irradiation at 1000 nm. It also appears that the generation of NO increases linearly with the NIR irradiation time suggesting a zero-order kinetics process. This time-dependent effect was previously observed for other two-photon activable Ru-nitrosyl complexes¹¹ and, according to our experimental methodology, the slopes derived from these linear correlations should be directly connected to the two-photon uncaging cross-sections (δ_u). We have therefore estimated the δ_u -ratio between 800 nm and 1000 nm to a value of ca. 4.9. This ratio which nicely parallels that measured for the 2PA cross-sections (i.e. 4.2) suggests that the quantum yield for NO generation is globally invariant upon two-photon excitation in the 800-1000 nm range. It also confirms that the dynamic for two-photon release of NO can be significantly amplified when achieving the resonance into the highly two-photon activable S_2 excited state of **RuTX** as predicted by the exciton model.

In summary, we have developed a new ruthenium-nitrosyl complex whose three-branched architecture allows a strong electronic coupling between each peripheral metal-center. Such an efficient interarm cross-talk in conjunction with the symmetry of the octupole clearly guarantee a synergy effect combining very strong 2PA cross-sections by three orders of magnitude in the NIR region with a light-driven release of nitric oxides in aqueous medium.

ACKNOWLEDGMENT. The work has been performed within the framework of the French-Mexican International Laboratory (LIA-LCMMC). Authors acknowledge support from PAPIIT (IN222819), PAIP and CONACYT and USAII for NMR spectra.

ASSOCIATED CONTENT

Supporting Information. The supporting information is available free of charge at...

1. Figures and Table: optical data (Table S1), optical spectra (Figures S1-S2). 2. Experimental procedures. 3. Synthesis and characterization of **RuTX**.

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