

# **A designed ruthenium nitrosyl and its potential therapeutic application as an NO donor**

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## **Abstract**

A ruthenium nitrosyl compound  $[(\text{Me}_2\text{bpb})\text{Ru}(\text{NO})(4\text{-vypy})]^+$  (**2**) was synthesized and characterized using spectroscopic methods. The synonymous compound,  $[(\text{Me}_2\text{bpb})\text{Ru}(\text{NO})(\text{py})]^+$  (**1**) has previously been characterized using both spectroscopy and x-ray diffraction. These ruthenium nitrosyls have been shown to release nitric oxide (NO) when exposed to UV light.<sup>1</sup> After characterization of **2**, it was inserted into a hydrogel and the rate of release of NO (kinetics), as well as the compound stability were studied. The kinetics of **2** in the gel was compared to the kinetics **2** in a water solution. An additional comparison of kinetics was done using another previously synthesized ruthenium nitrosyl (PapyRu(NO)(BF<sub>4</sub>)<sub>2</sub>). The rate of leeching of the compound itself was compared with the rate of leeching of **1**, which was also inserted into a gel. Although these two compounds are similar in structure and reactivity, the 4-vinyl pyridine group of **2** covalently attaches to the gel matrix, making it a more stable compound. The initial results and the implications of **2** are outlined in this communication.

## **Introduction**

Endogenously occurring nitric oxide (NO) has a multitude of regulatory roles in the human body, such as blood pressure control, and immune and inflammatory responses. In addition, NO is one of the only known gas molecules that acts as a neurotransmitter.<sup>2</sup> The physiological importance of NO can be appreciated by considering some of the pathological conditions that can occur due to its deficiency. Likewise, the established NO therapies that are currently being used via either an NO donor or inhalation of NO gas, are revealing of its importance. Some of the conditions that are currently being successfully treated include, various forms of cardiovascular disease and infant respiratory problems.<sup>3</sup> Many experimental trials using NO are also undergoing scientific and experimental observation. Of particular interest for the designed ruthenium nitrosyl (**2**) discussed in this communication is induced cellular apoptosis, caused by high concentrations of NO. The most apparent use of this treatment would be to target malignant tumor cells.

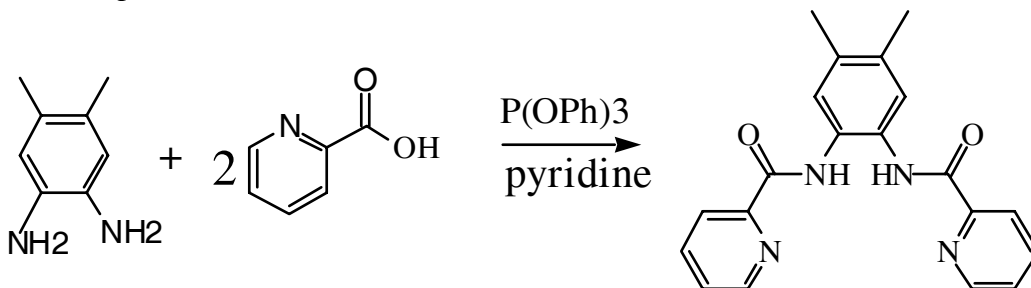
NO induced cellular apoptosis occurs as a result of its biological toxicity at higher concentrations. Although this effect has been documented<sup>4</sup>, there are several obstacles to making an effective NO donor that can be used for the treatment of malignant tumors. If a treatment using NO was going to target only those cells, it would need to 1.) Have a controlled way to activate the release of NO, 2.) Release high enough concentrations of

NO so as to induce apoptosis, 3.) Not have side effects as a result of toxic exposure to the NO donor itself, and 4.) Have a precise release of NO, so that only the cells targeted were exposed to the apoptotic agent (NO).

Obtaining solutions to these issues was the primary interest in the design of **2**. Each of the obstacles outlined were considered and/or addressed in the design of **2** as described by the following solutions 1.) The ruthenium-NO bond is very stable. However, when a ligand with multiple aromatic groups is attached to ruthenium, the Ru-NO bond becomes photosensitive and will break when exposed to low level UV light. This allows for the controlled release of NO. 2.) Initial studies analyzing the concentration of NO released by **2** have been done (see figure 4). However, a future study using cells will be required to determine whether the rate of NO release is sufficient for cell apoptosis. 3.) In-order to address issues of the compound's toxicity, **2** was covalently attached to a polymer gel via a radical reaction between the gel matrix and the vinyl group of the compound. This attachment prevents the compound from leeching out of the polymer gel. 4.) Lastly, the design of the compound-gel matrix will allow the gel to be attached to a fiber-optic device illuminated by a UV light pen. It is foreseeable that this device will allow for the application of tissue specific NO exposure.

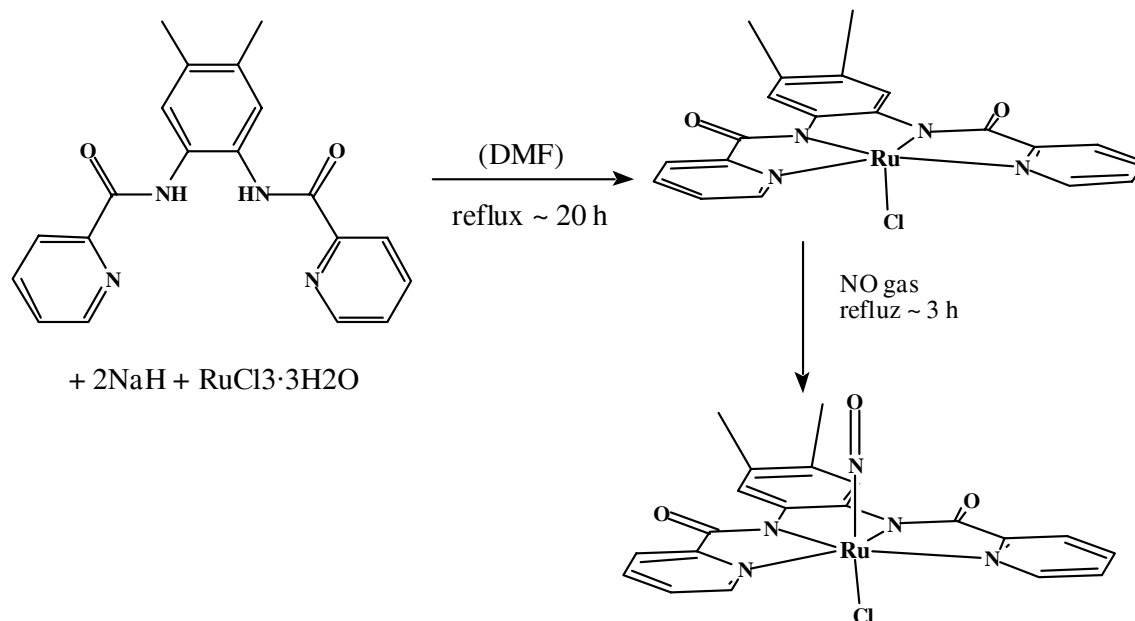
## Experimental Section

Synthesis of the ligand [1,2-bis(pyridine-2-carbozamido)-4,5-dimethylbenzene (H<sub>2</sub>Me<sub>2</sub>bpb)]:



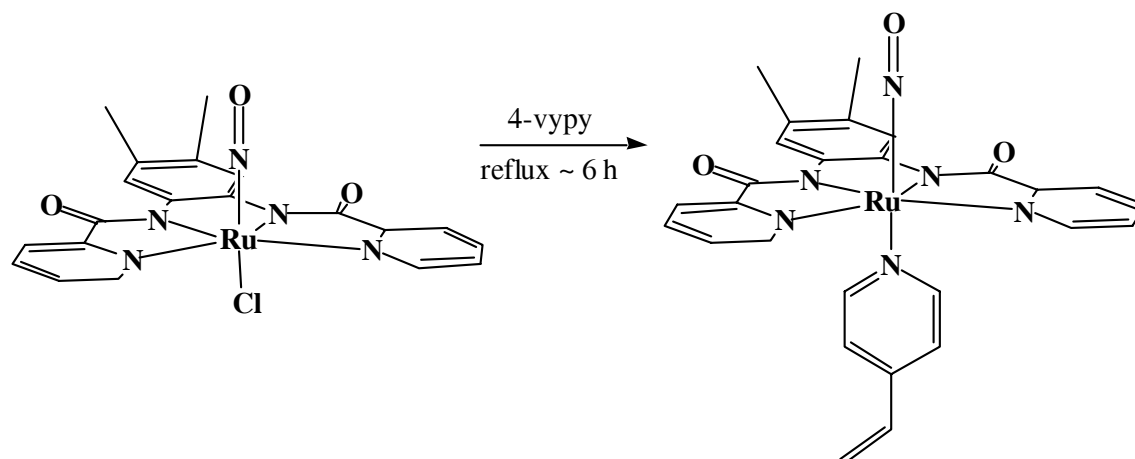
A solution of 7.4 g of 4,5-dimethyl-1,2-phenylenediamine and 13.3 g of picolinic acid in 40 mL of pyridine and 33.5 g of triphenyl phosphite was allowed to reflux for 5 h. Initially, the solution had a transparent brown appearance but it turned a dark red before refluxing. The compound was then concentrated to half its volume using short path distillation and stored at 0° C for one day, allowing the ligand to precipitate out of the solution. The reaction mixture was filtered and the crystallized compound was washed with 30 mL of Et<sub>2</sub>O and dried under vacuum.

Synthesis of [(Me<sub>2</sub>bpb)Ru(NO)(Cl)]·toluene:



A solution of 1.0 g (2.89 mmol) of the ligand, H<sub>2</sub>Me<sub>2</sub>bpb in 30 mL of DMF was set up for reflux in a 100 mL Schlenk flask and a slurry of 0.14 g (5.83 mmol) of NaH in 5 mL of DMF was added. The color of the solution went from dark yellow to clear yellow on completion of the evolution of H<sub>2</sub>. One equivalent, 0.75 g (2.87 mmol) of Ru<sub>3</sub>·3H<sub>2</sub>O was then added, causing the solution to turn dark brown immediately. The solution was then allowed to reflux for approximately 20 h under dinitrogen, resulting in a dark green solution and a gray white precipitate (NaCl). The solution was then cooled and filtered through a sintered glass crucible and the filtrate was again heated to reflux and bubbled with NO gas for 3 h. Using short path distillation, the reaction mixture was concentrated down to 10 mL and a solvent re-crystallization was set up by adding 10 mL of Et<sub>2</sub>O to the reaction mixture. The solution was left under gradual cooling until the compound reached -20°C. After 2-5 days, the crystallized product was formed. It was washed with Et<sub>2</sub>O, dried under a vacuum, and analyzed using IR and NMR.

Synthesis of [(Me<sub>2</sub>bpb)Ru(NO)(4-vypy)]<sup>+</sup> (2):



A solution of 0.05 g (0.257 mmol) of  $\text{AgBF}_4$  in 5 mL of MeCN was added to a stirred solution of 0.1 g of **1** (0.196 mmol) in 25 mL of MeCN, and the reaction mixture was allowed to reflux for 20 h. One equivalent (0.2156 mmol) of 4-vinyl pyridine was then added to the mixture and reflux was continued for an additional 6 h, resulting in **2**.

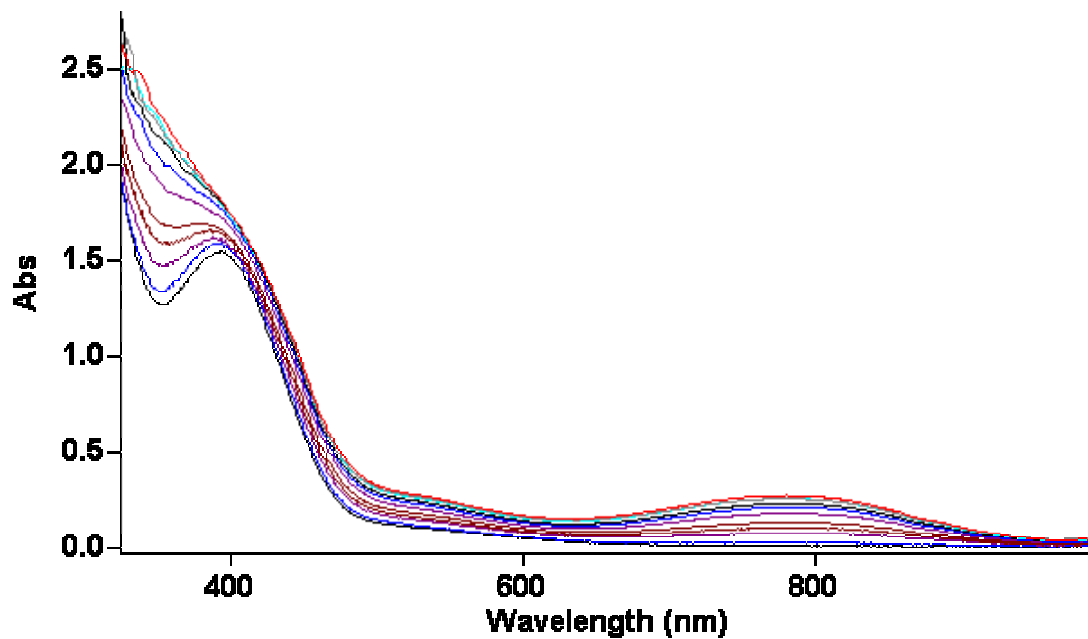
## Results

NMR and IR were the primary means of characterization of **2**. Re-crystallization of **2** is also currently underway for the purpose structure determination using x-ray diffraction. The NMR of **2** was taken from the product after re-crystallization in acetonitrile and ether. Identification of peaks, including solvent peaks ( $\text{CD}_3\text{CN}$ ) were identified and accurately correlated to their expected shifts.

QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

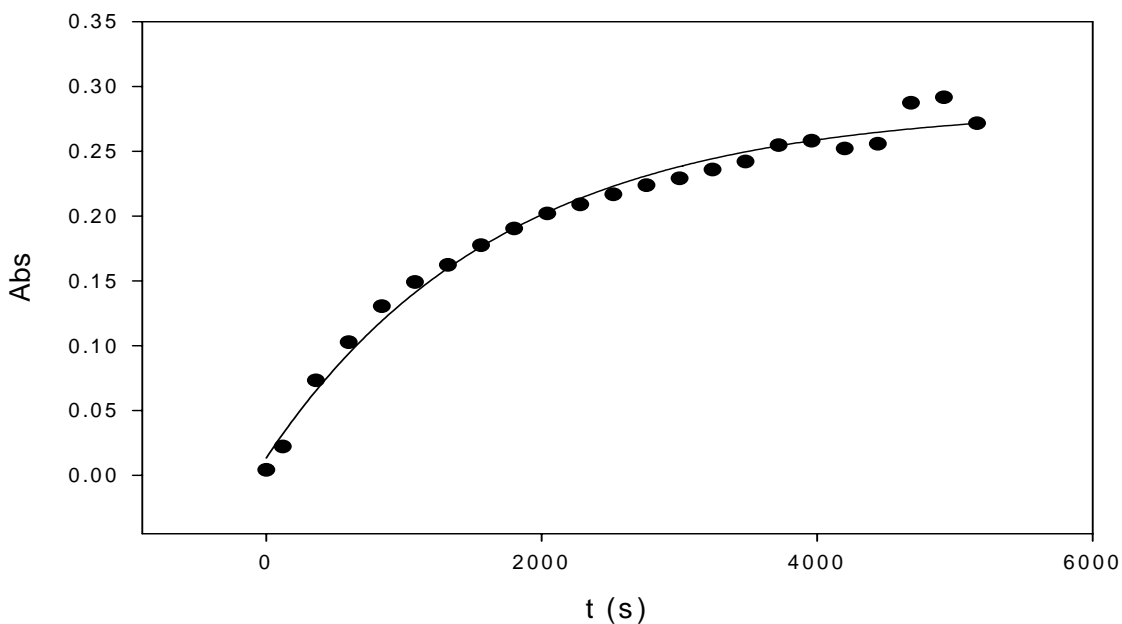
### Figure 1. NMR of **2** in acetonitrile.

Using electronic absorption spectroscopy, further observations were made via a study of the kinetics of **2**. The absorbance revealed that **2** undergoes a shift in the visible wavelength spectrum. Initially, a peak at 400 nm was observed. After several 1-4 minute intervals over a period of ~ 2 h, a new peak was formed in the 650-700 nm range. This indicates that **2** undergoes a chemical change on exposure to UV light. Most likely, this is due to the release of NO from the compound-gel matrix.



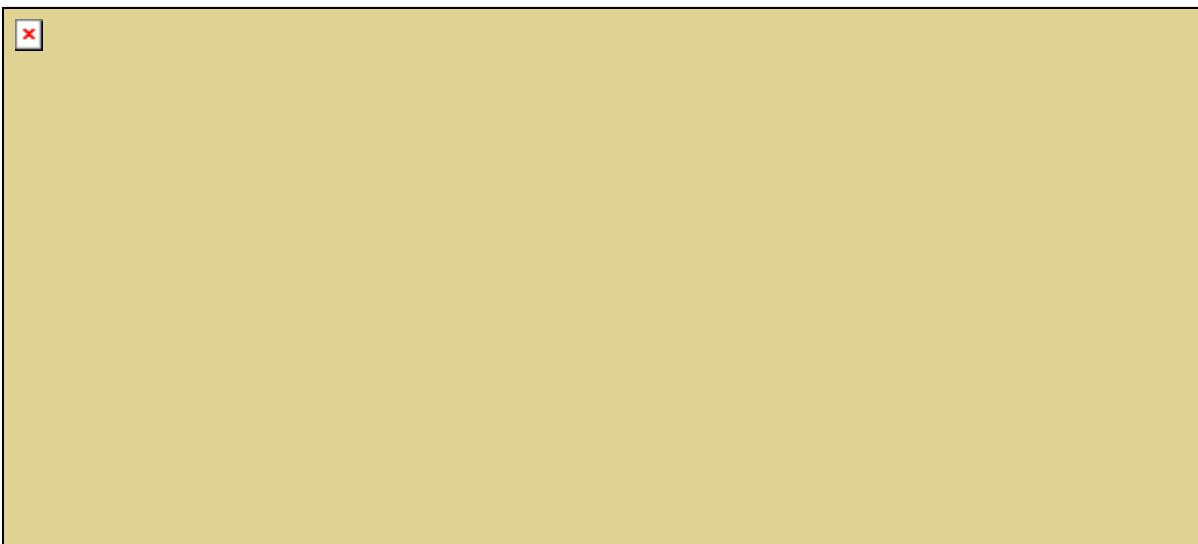
**Figure 2.** Absorbance graph of **2** through several intervals of UV light exposure.

The data collected from the electronic absorption spectroscopy was put into a 3-parameter, exponential rise to max equation and the rate constant was identified in order to determine the rate of NO release. The rate constant of **2** in the gel was compared with **2** in a water solution and was found to be ~ 9 times slower, which was expected. Further research and study will be needed in order to determine what concentrations of NO are necessary for inducing apoptosis.



**Figure 3.** Kinetics for **2** using a 3-parameter, exponential rise to max equation.

An NO electrode was used to determine the release of NO (nM). This initial study did not take the concentration of **2** into account, so it is not yet suitable for in-depth analysis. The NO electrode measured the concentration of NO that was released from the gel when exposed to 15 to 40 second intervals of UV light. One-minute intervals were taken in-between UV exposures, in order to allow the baseline to equilibrate. The NO amperogram verified that the compound is releasing NO during UV light exposure.



**Figure 4.** NO amperogram showing release of NO in nM.

## Conclusion

Although synthesis of **2** was successful, there were challenges in isolating a pure compound. This prevented us from attaining x-ray quality crystals. During the course of synthesis and re-crystallization, many adjustments were made to enhance the procedure in order to attain a pure product. However, the synthesis may still require some

alterations before a pure compound can be consistently isolated. This communication contained only light analysis because the experiments have not been repeated enough times for proper statistical analysis.

After properly analyzing **2**, we hope to design a fiber-optic device with the attached gel matrix. If **2** functions in the way that these early experiments indicate, a second phase of experimentation will be initiated by monitoring the effects of **2** on live cell cultures.

## References

- (1) Patra, A. K.; Mascharak, P. K. *Inorg. Chem.* 2004. 43, 4487-4495.
- (2) Patra, A. K.; Mascharak, P. K. *Angew. Chem. Int. Ed.* 2002. 41, No. 14
- (3) Nitric oxide donors; Wang, P. G., Cai, T.N., Taniguchi, N., Eds.; Wiley-VCH: Weinheim, Germany, 2005.
- (4) Patra, A. K.; Mascharak, P. K. *Angew. Chem. Int. Ed.* 2002. 41, No. 14