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Organometallic Rhenium Dyes for Nitric Oxide Detection and Imaging

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

ORGANOMETALLIC RHENIUM DYES FOR NITRIC OXIDE DETECTION AND
IMAGING

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Lissette Irene Lozano-Lewis

2015

To: Dean Michael R. Heithaus
College of Arts and Sciences

This dissertation, written by Lissette Irene Lozano-Lewis, and entitled Organometallic Rhenium Dyes for Nitric Oxide Detection and Imaging, having been approved in respect to style and intellectual content, is referred to you for judgement.

We have read this dissertation and recommended that it be approved.

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DEDICATION

I dedicate this dissertation to my parents Jaime & Irene for their immense love and for always being there with me, to my husband Joseph for having the patience and understanding of my vocation, and to my grandma Julia for guiding my steps from heaven.

ACKNOWLEDGMENTS

I wish to thank Dr. Kavallieratos, my advisor, for his guidance throughout this graduate school experience, and all the members of my committee for their support and patience. Their gentle, but firm direction has been most appreciated. Dr. Miksovskva was very helpful in instructing me in all the photophysical background and also allowing me access to her laboratory to perform some key fluorescence lifetime experiments. Dr. Nikolaos Tsoukias and Dr. Kumpal Madrasi collaborated with us in the cell fluorescence microscopy studies. I would like to thank Dr. Kevin O'Shea and his research group for access to the photoreactor apparatus for photochemical reactions. I also would like to thank Dr. Daniel Rabinovich, my former mentor at UNC-Charlotte for his continuing mentorship, friendship, and research collaboration on Chapter 4 of this work (Re- α -diimine complexes). Finally, I would like to thank all of Dr. Kavallieratos's group members, past and present, for the patience and good humor, as they made my lab time really enjoyable.

I have found my coursework throughout the graduate curriculum and institutional programs to be stimulating and thoughtful, providing me with the tools to explore and solve both past and present challenges.

ABSTRACT OF THE DISSERTATION
ORGANOMETALLIC RHENIUM DYES FOR NITRIC OXIDE DETECTION AND
IMAGING

by

Lissette Irene Lozano-Lewis

Florida International University, 2015

Miami, Florida

Professor Konstantinos Kavallieratos, Major Professor

The importance of sensing Nitric Oxide (NO) in physiology and medicine has led us to explore the reactivity of NO with organometallic Re dyes. Rhenium complexes were synthesized with the ability to react with NO and sense it under physiological conditions. Fluorescent 1,10-phenanthroline complexes (phen)Re(PPh₃)(CO)₂OSO₂CF₃ (1) and (phen)Re(CH₃CN)(CO)₂OSO₂CF₃ (3) can sense NO in the range of 10 - 150 μM showing a decrease in fluorescence response at 514 nm and 532 nm respectively, upon NO-donor addition ($\lambda_{exc} = 360$ nm). (phen)Re(THF)(CO)₂OSO₂CF₃ (2) showed a ratiometric response with a decrease in fluorescence intensity at 585 nm and an increase at 445 nm. These complexes were characterized by FT-IR, ¹H-NMR, ¹³C-NMR, and ¹⁹F-NMR. The quantum yields (Φ) and lifetimes (τ) were also determined. On the basis of ¹⁹F-NMR and ¹H-NMR spectroscopic evidence, the proposed mechanism of action involves dissociation of CF₃SO₃⁻ (abbreviated as OTf⁻) together with PPh₃, (for 1), THF (for 2), or CH₃CN (for 3). Fluorescence microscopy showed that 1 permeates through the cell membrane of rat aortic endothelial cells (RAOEC) and 2 gives increase in cell fluorescence response at 450 nm when exogenous NO is added to RAOEC. The

Re complexes of dppz (dppz = 2,2'-dipyrido[3,2-a:2,3'-c]phenazine) (dppz)Re(THF)(CO)₂OSO₂CF₃ (A2) and (dppz)Re(CH₃CN)(CO)₂OSO₂CF₃ (A3) were synthesized using the same methods as for complexes 1 and 3. Even though the dppz complexes exhibited similar behavior than the phenanthroline analogs, the signal changes were small and the results were inconclusive. The α -Diimine Re complexes of Xy-DAD (XyDAD = 1,4-bis-[2,6-dimethylphenyl]-1,4-diazo-1,3-butadiene) (Xy-DAD)Re(THF)(CO)₂OSO₂CF₃ (8) and (Xy-DAD)Re(CH₃CN)(CO)₂OSO₂CF₃ (9) showed reactivity with NO and CN⁻ with a response in the visible range of the absorption spectrum. These diazadiene (DAD) complexes showed decreased reactivity with NO and CN⁻ in the presence of oxidants. The *i*-PrDAD complexes (*i*-PrDAD = 1,4-bis-[2,6-diisopropylphenyl]-1,4-diazo-1,3-butadiene) (*i*-Pr-DAD)Re(THF)(CO)₂OTf (10) and (*i*-Pr-DAD)Re(CH₃CN)(CO)₂OTf (11) exhibited similar behavior with the Xy-DAD analogs, but the spectral changes were much smaller and the results were inconclusive. The ESI-MS Mass Spectrometry studies of the phenanthroline complexes 1, and 2, after reaction with NO-donor showed that 1 and 2 react with NO to give [Re(CO)₂(NO)₂S₂]⁺ species, while (phen)Re(CO)₃Cl and (phen)Re(CO)₃OSO₂CF₃ do not show any metal-NO containing products, which is consistent with the fluorescence studies.

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ACRONYMS AND ABBREVIATIONS

CN ⁻	Cyanide
CO	Carbon monoxide
DAD	1,4-diazo-1,3-diene
dppz	2, 2'-dipyrido[2,3 -a:2',3' -c]phenazine
EDRF	Endothelial Derived Relaxation Factor
ESI-MS	Electrospray Ionization Mass Spectrometry
FT-IR	Fourier Transform Infrared Spectroscopy
<i>i</i> -Pr	Isopropyl
λ_{em}	Emission wavelength
λ_{exc}	Excitation wavelength
M	Molarity (mol/L)
MeCN	Acetonitrile
³ MLCT	Triplet – Metal to ligand charge transfer
μ M	micromolar
mM	millimolar
NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide
OSO ₂ CF ₃ ⁻	CF ₃ SO ₃ ⁻ (trifate)
PBS	Phosphate Buffer Saline
phen	1,10-phenanthroline

PPh ₃	Triphenyl Phosphine
RAOEC	Rat Aortic Endothelial Cells
THF	Tetrahydrofuran
UV-Vis	Ultra Violet - Visible
Xy	Xylene

1. INTRODUCTION

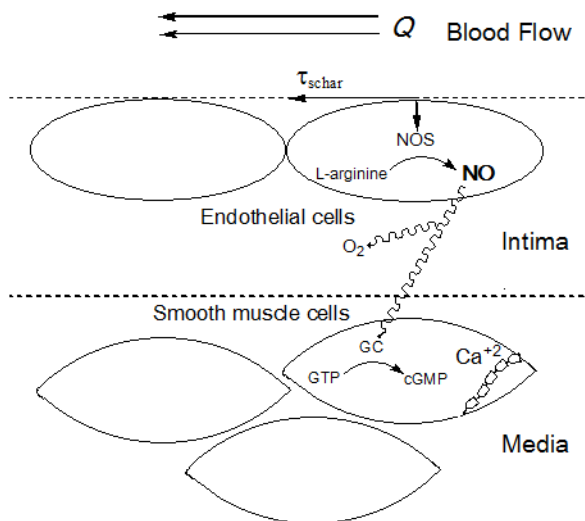
Nitric oxide, or oxidonitrogen (NO),¹ is a free radical produced as byproduct of nitrogenized fossil fuels and electrical discharges. Nitric Oxide is catalogued as an oxidizer and will both support and propagate combustion. Because of the presence of oxygen, NO is converted into NO₂ rapidly in air. Nitric Oxide is converted to HNO₂, nitrous acid, in aqueous solutions in the presence of oxygen.

1.1. BIOLOGICAL ROLES OF NITRIC OXIDE

The importance of NO in biology and physiology was highlighted in 1998 when Drs. Furchgott, Murad and Ignarro won the Nobel Prize in medicine and physiology for their research in NO physiology. Currently, there are over 5,000 publications per year that deal with the role of NO in biomedicine.

In biological systems, NO is involved in signaling and several physiological and pathological processes. Nitric Oxide is short-lived in the blood and is known as an endothelial derived relaxing factor (EDRF) because of its vasodilator properties.³ Nitric Oxide is produced in cells by the combined role of three isoforms of Nitric Oxide Synthase (NOS) enzymes: i) endothelial (eNOS or NOS-3), ii) neuronal (nNOS or NOS-1) and iii) inducible (iNOS or NOS-2), the last of which is calcium-dependent. The process involves arginine oxidation followed by the reduction of molecular oxygen.⁴

Nitric Oxide is involved in important functions in the smooth muscle, such as muscle relaxation, vasodilatation, and regulation of blood flow.⁵ Nitric Oxide is also involved in neuronal activities acts as neurotransmitter.⁶ It is important in the immune system's attack against pathogens and neoplasia.⁷ Homeostasis of NO in biological systems is a key component of the cellular health. Nowadays, there is increasing research in exogenous NO sources.⁸ There is a vast selection of novel compounds that release NO (or NO-like substrates) to specific targets under special triggers (like nitrites under hypoxic conditions).⁹ Nitric oxide promotes the generation of the messenger cyclic 3',5'-guanosine monophosphate (cGMP), activating the nucleotide-dependent protein kinase G (PKG or cGKI). Protein kinase G is an enzyme involved in the phosphorylation of several calcium regulators.¹⁰ Actin-myosin dynamic alterations derive from varying calcium concentration-sensitization and hyperpolarizing potassium channels, which leads to smooth muscle relaxation (Scheme 1.1).¹⁰ Nitric Oxide functions as an immune system booster, killing invasive bacteria by oxidative damage.¹¹ However, in extreme cases, these increments in intracellular NO concentration lead to profuse vasodilation, causing the hypotension characteristics in sepsis cases or reaching cytotoxic levels leading to an apoptotic cascade.¹²



Scheme 1.1 Stress-induced EDRF-NO mechanism¹⁰

Because of its relatively low polarity, NO is less soluble in oxygen-free water (1.9 mM) than in organic solvents, such as methanol (14 mM), toluene (11 mM), and hexane (18 mM).¹³ Nitric Oxide is sufficiently lipophilic to permeate through cell membranes. It is oxidized to NO_2^- in oxygen-containing aqueous solutions with a rate constant ranging from $3 \times 10^6 \text{ M}^2 \text{ s}^{-1}$ to $12 \times 10^6 \text{ M}^2 \text{ s}^{-1}$.¹⁴ The NO oxidation reaction is 300 times faster in aqueous conditions than in hydrophobic conditions.¹⁵ Micromolar concentrations of NO can trigger formation of reactive nitrogen species, which produces cell damage, carcinogenesis, and neurodegenerative disorders.¹⁶

1.2. NITRIC OXIDE QUANTIFICATION

The quantification of NO^{17} in tissues remains a challenge because of the absence of a detection method that can combine significant spatial and temporal resolution with high NO specificity.¹⁸ The challenges lie in NO's short lifetime ($t_{1/2} = \text{ca. } 5 \text{ s}$) and the fact that it is found in low concentrations in living tissues.

Detection methods for NO include chemiluminescence, electrochemistry, and spin-trapping followed by EPR (Electron Paramagnetic Resonance). Chemiluminescence is produced when NO is exposed to ozone;¹⁹ the limitation of this technique is the difficulty of adding the analytes to the detector, making it unsuitable for monitoring NO in biological systems. Another method uses luminol,²⁰ which has the same response as peroxides even though it is less cytotoxic than using hydrogen peroxide. Electrochemical microsensors can provide real-time detection;²¹ but spatial information is limited to the electrode tip, making it unsuitable for NO-related biochemistry studies. EPR has been proposed for studies *in vitro* and *in vivo*.²² However, as a result of its high reactivity and low concentration, NO cannot be reliably quantified by EPR techniques. In order to improve the EPR technique, spin-trapping reagents can be used to form stable radicals, giving enough time for detection; There are different types of NO spin trapping reagents: nitronone (DMPO) and nitroso (MNP, DNBNS), which are used in cell cultures;²³ nitrosylhemoprotein, ferrous-Hb;²⁴ nitronyl nitroxides;²⁵ Dithiocarbamates/Iron-dithiocarbamate (DTC/Fe-DTC), which is the only type of spin trapping reagent used *in vivo*;^{26a} Dithiocarbamates have antioxidant properties and react with NO almost at the diffusion-controlled rate.^{27b}

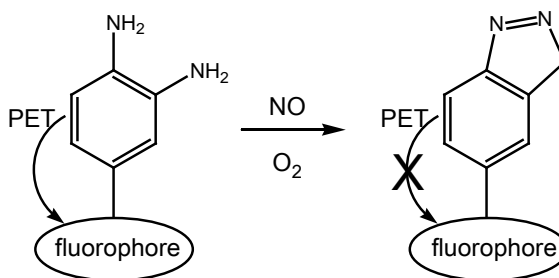
1.3. FLUORESCENT DYES FOR NO SENSING

Fluorescence imaging is a form of epifluorescence or confocal microscopy in which using objective lens focuses the excitation ray going through a pinhole, limiting the section of the sample to analyze.²⁷ Fluorescent dyes have been used for quantification and imaging of NO at inter- and intracellular concentrations.²⁸ The advantage of such dyes is that they can be irradiated at specific intensities and emissive dye-substrate

complexes formed upon reaction with NO can be observed. Some fluorescent dyes have high selectivity and can quantify NO at sub-micromolar concentrations. These dyes can be separated into six different groups: i) Dyes based on NO-Induced diamine cyclization process; ii) dyes based on NO-Induced diazo-ring-generation process; iii) based on NO-Induced spirolactam-ring-opening; iv) dyes based on NO-Triggered selective ligands dissociation of Metal complex; v) dyes based on NO-Triggered reduction of copper(II) and release of fluorescent ligand; vi) dyes based on NO-Induced spirolactam ring-opening, reduction of Cu^{+2} , and nitrosylation of fluorescent ligand.

1.3.1. Dyes based on NO-induced diamine cyclization process:

NO sensing by these dyes is based on formation of a highly fluorescent triazole in the presence of N_2O_3 ²⁹ (oxidized product of NO in presence of oxygen). The change in response can be attributed to the less electron donating group triazole, disturbing the PET process (Photo-Electron Transfer) recovering the fluorescence intensity. The core of this type of fluorescent dye is shown in Scheme 2:



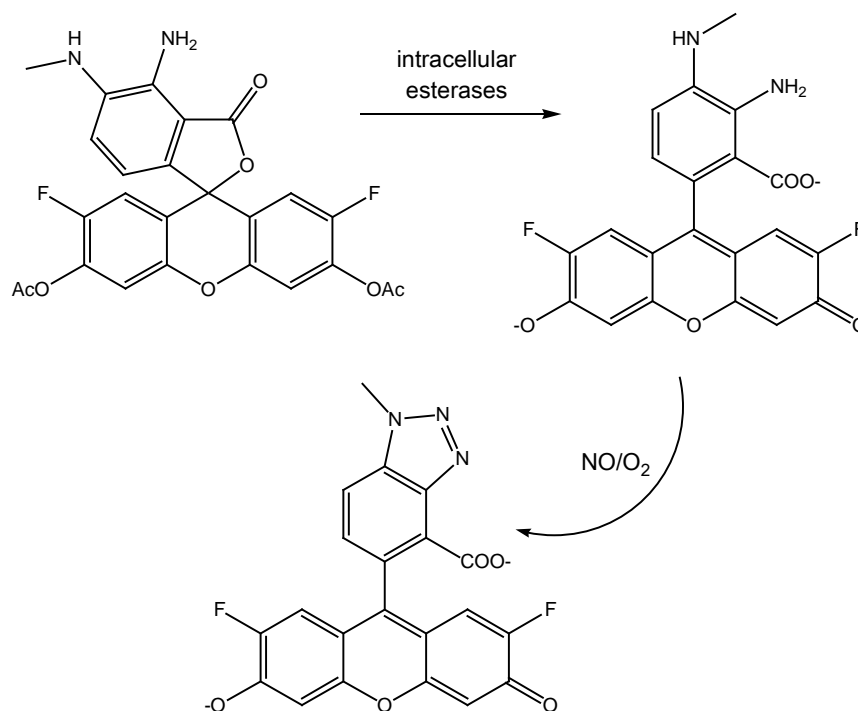
Scheme 1.2 Mechanism of NO fluorescent sensors based on NO-induced diamine cyclization process^{17b}

These sensors contain different types of fluorophores: i) Fluorescein,³⁰ *e.g.*, DAF and DAF-FM; ii) BODIPY³¹ (boron dipyrromethene complexes that have stability in large ranges of pH); iii) Acridine;³² iv) Anthraquinone;³³ v) Tricarbocyanine,³⁴ (highly

soluble in water near infrared luminescent probes, designed for human tissue NO imaging); vi) Phosphorescent heavy-metal-complexes as lumimophores,³⁵ (e.g., ruthenium-bipy core). Using this mechanism ratiometric sensors can also be developed, in which the ratiometric sensing is realized through the fluorescence resonance energy transfer (FRET) process;^{30d} The carbon dots serve as both the energy donor and the anchoring site for the NO probe moiety, which turns into the energy acceptor in the presence of NO.

1.3.1.1. Diaminofluorescein (DAF)

Since 1995, the Nagano group has been developing fluorometric methods to detect NO using diaminonaphthalene (DAN). In the presence of oxygen DAN yields the fluorescent naphthalene triazole (NAT) under neutral conditions.³⁶ Fluorescence intensity increases incrementally with NO concentration. However, DAN cannot be used for quantification in biological systems because it leaks out through the cell membrane. Using the same methodology, Diaminofluorescein (DAF) was shown to react with NO/O₂, producing DAF-T (triazole) with a small change in absorbance and a large increase in fluorescence (180-fold). DAF is suitable for cell studies; its excitation wavelength is in the visible range, not making auto-fluorescence possible.³⁷ Different series of diaminofluorescein dyes have been synthesized in order to modify the pK_a, increase quantum yield, increase stability in the presence of pH changes, increase cell membrane permeability, decrease autofluorescence, decrease photobleaching, and increase cell trappability of the DAF-T complex.

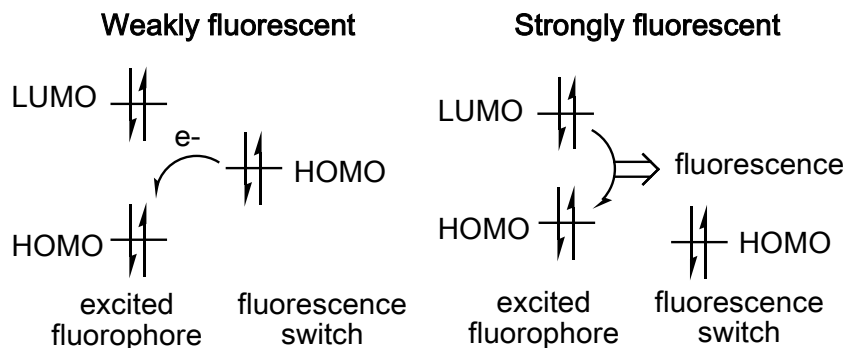


Scheme 1.3 DAF-FM mechanism of action toward NO/O₂ in the absence of extracellular leaking.³⁸

Fluorinated DAFs were shown to be more resistant to photobleaching; DAF-FM is the most sensitive and stable within the DAF series,³⁸ with an excitation wavelength of 488 nm and emission wavelength of 515 nm. It is the most widely used fluorescent probe for NO imaging. Even though the mechanism remains unclear, the fluorescence enhancement is ascribed to the interruption of photoinduced electron transfer (PET) between the benzoic acid and the xanthene moiety.³⁹ Kinetic studies of NO reactivity with DAF-FM, by Tsoukias et al. have shown that the dye response depends on N₂O₃ concentration.^{39d}

Photoinduced electron transfer is a widely accepted mechanism for fluorescence quenching. The fluorescein structure has two parts: the benzoic acid moiety as the PET donor and the xanthene ring as the fluorophore. The electron transfer from the PET donor

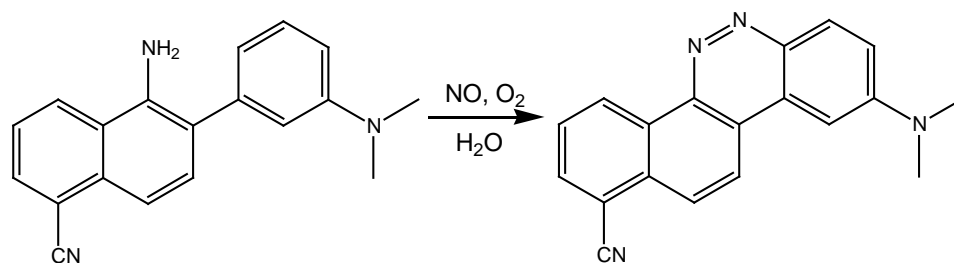
decreases the fluorescence intensity of xanthene. The small variations between excitation and emission wavelengths of DAF and DAF-T can be attributed to the dihedral angle between the benzoic acid moiety and the xanthene ring being almost 90° , as shown in the X-ray structures of analogous compounds.^{39c}



Scheme 1.4 Frontier orbital energy diagram: Illustration of the thermodynamic situation of the fluorescence OFF/ON switch including the PET process³⁹

1.3.2. Dyes based on NO-induced diazo-ring-generation

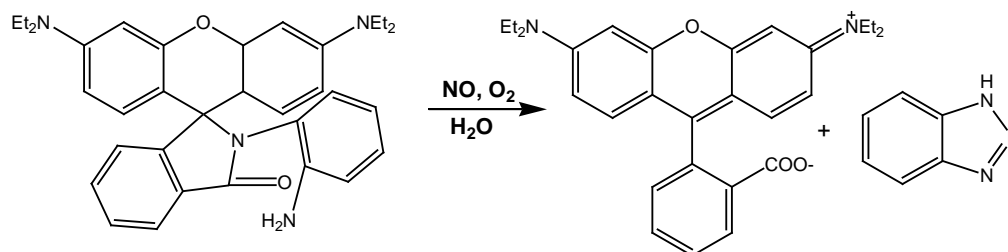
These fluorophores react with NO, forming a rigid π -conjugation system that enhances the fluorescence intensity up to three orders of magnitude,⁴⁰ as shown in Scheme 1.5. These dyes have low cytotoxicity and cross the cell membranes, but not the nuclear membrane. Anslyn and coworkers have developed a dye which in the presence of NO/N₂O₃ generates a diazo-ring fluorophore. The dye reacts with NO forming an N-nitrosoaniline intermediate, which then undergoes electrophilic substitution to form the diazo- product. The probe has preference for NO/N₂O₃ over hydroxyl radical, nitrite, nitrate and peroxyxynitrite. The product generated is stable in a wide range of pH and the fluorescence intensity does not increase over time.



Scheme 1.5 Mechanism of NO fluorescent sensors based on NO-induced diazo-ring generation process.⁴⁰

1.3.3. Dyes based on NO-induced spirolactam-ring-opening process:

These sensors use rhodamine as fluorophore and a reactive lactam group.⁴¹ After reacting with NO, the lactam ring opens and fluorescence intensity increases because of the extension in the conjugation of the rhodamine fluorophore. The dual fluorophore system is used as the Förster resonance energy transfer (FRET) dyad. There is a change in the conjugated system that allows the turn on/off of both dyes. The system is selective toward NO over other competitive radicals and ions. Although this dye is efficient, its synthesis and purification is complex.

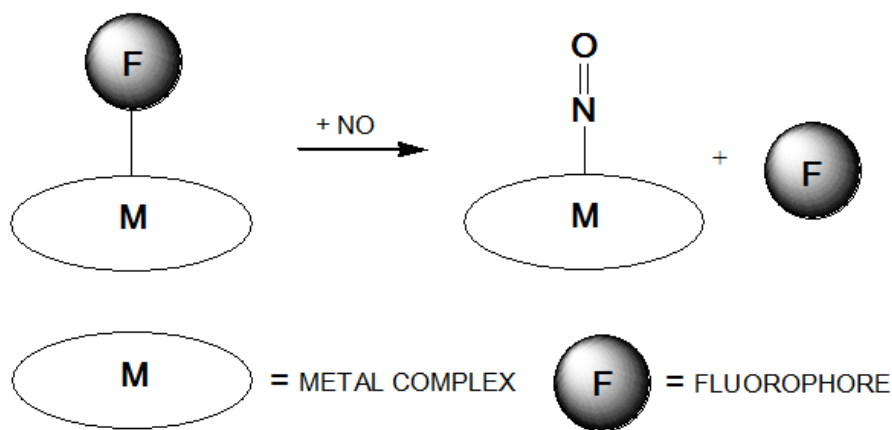


Scheme 1.6 Mechanism of NO fluorescent sensors based on NO-induced spirolactam-ring opening process

1.3.4. Dyes based on NO-triggered ligand dissociation from a metal complex

Metal to ligand charge transfer (MLCT) between the partially filled d-orbitals of a metal and the excited states of the fluorophore can cause fluorescence quenching.

Formation of a Metal-NO complex displaces the fluorophore and turns on the fluorescence emission (Scheme 1.7). Using this mechanism of action, Co(II),⁴² Rh(II),⁴³ Ru(II),⁴⁴ Fe(II),⁴⁵ and Cu(II) complexes have been designed for NO detection. Most of these complexes react directly with NO. However, they are insoluble or unstable in aqueous solutions because water displaces fluorophores from the coordination sphere or hydrolyses the fluorophores.

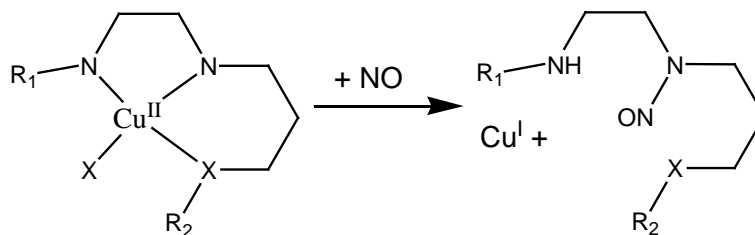


Scheme 1.7 Mechanism of NO fluorescent sensors based on NO-triggered dissociation of a fluorescence ligand from a metal complex

1.3.5. Dyes based on NO-triggered reduction of Cu^{+2} and release of fluorescent ligand:

Most metal nitrosyl complexes described in the previous section will slowly hydrolyze if their solutions are exposed to air for several days, as water can outcompete the fluorophore for coordination to the metal center. The metal nitrosyl hydrolysis side reaction is why a new type of dye was introduced using Cu(II) complexes for NO detection. The new strategy involves the formation of a Cu(I) species via NO-triggered reduction of paramagnetic Cu(II) that was associated with NO coordination to a Cu center and the release of the fluorescent nitrosylated ligand, corresponding to

fluorescence enhancement. Lippard's group explored this idea by preparing a new probe that include a modified Cyclam ligand in the metal coordination sphere.⁴⁶ Initially this probe was prepared to sense Hg(II).



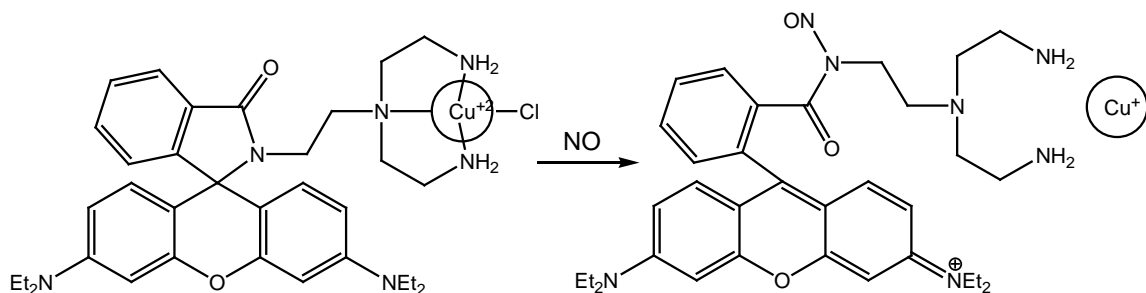
Scheme 1.8 Mechanism of NO fluorescent sensors based on NO-triggered reduction of Cu(II) and release of fluorescent ligand

Lippard *et al.* have developed a series of copper-fluorescein complexes that can detect NO under neutral aqueous conditions, in the absence of oxygen.⁴⁷ The addition of hydrolysable esters or conjugation to dextran are used to add cell-trappability.^{48,49} The major improvement compared to DAF is that this dye responds to NO and not to an oxidized product of NO. Cu-FL is selective for NO over hypochlorite, superoxide, peroxide, nitrite, nitrate, peroxynitrite and HNO. The quantum yield of Cu-FL ($\Phi = 0.077$) is substantially lower than for fluorescein ($\Phi = 0.95$). After reaction with NO and elimination of Cu(I), the quantum yield increases again in the nitrosylated fluorescein ($\Phi = 0.58$). Thus the mechanism is called turn-off/turn-on.

1.3.6. Dyes based on NO-induced spirolactam ring-opening, reduction of Cu⁺², and nitrosylation of fluorescent ligand

Recently, there have been more examples of sensors that use the combination of two or more described mechanisms. In a recent example, by Duan and coworkers; the

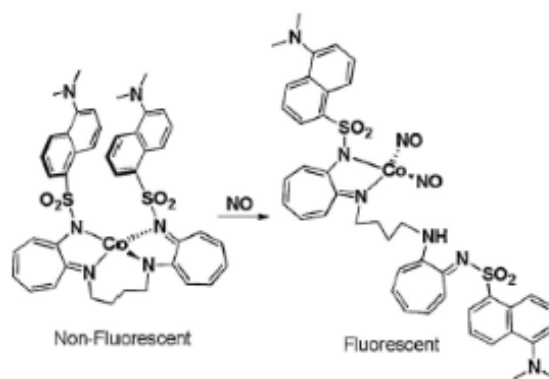
dye mode of action combines spirolactam ring opening and reduction of Cu(II).⁵⁰ The mechanism is similar to the one described in the previous section 1.3.5.



Scheme 1.9 Dyes based on NO-induced spirolactam ring-opening, reduction of Cu⁺², and nitrosylation of fluorescent ligand⁵⁰

1.3.7. Dyes based on NO coordination to a transition metal and detachment of a fluorophore

When a fluorophore is coordinated to a transition metal, its intensity is suppressed because of the interaction with the partially filled d-orbitals. Under the presence of NO, NO displaces the fluorophore from the metal center, forming stable nitrosyl complexes and the fluorophore regains its initial fluorescence intensity.¹³² For example, there is the dansylated animotroponimate Co(II) complex prepared by Lippard¹³³ in which a 4-fold increase in fluorescence intensity is observed in the presence of NO. The major challenge for these selective dyes is solubility in aqueous physiological media in order for them to be suitable for fluorescence cell microscopy. Many transition metal complexes do not pass this challenge.



Scheme 1.10 Lippard's Co(II) complex used as NO sensor. (Figure reproduced from reference 133)

1.4. USE OF METALS IN BIOLOGICAL SYSTEMS

Metals are used in biological systems to perform several roles, such as active sites in enzymes, regulators, electrolytes, messengers, transport agents, and transmitters.⁵¹ They also provide accessible scaffolds important for structural stability.⁵² Table 1.1 provides a summary of these roles.

Table 1.1 Functional roles of inorganic elements applied to biology.

Function	Inorganic Element	Representative Examples
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Structure	Ru, Rh, Pt	Kinetically inert complexes serve as structural scaffolds that interact in novel ways with biomolecules
Signaling	Ca, NO	Compounds that release Ca, NO, or other signaling molecules
Acid-base catalysis	Co, Zr, Pt, Ln	Complexes that induce hydrolytic cleavage of biomolecules
Luminescence	Tb, Eu, other Ln	Subcellular imaging agents
Magnetic resonance	Gd, Mn	Metal-responsive MRI imaging
Radioactive	^{99m} Tc, ⁶⁴ Cu, others	SPECT, PET imaging
X-ray	Os, Ln	Heavy atom derivatives for biomolecular X-ray structure determination
Alteration of metal bioavailability	Fe, Cu, Zn, Ca, other	Chelating agents that alter normal metal homeostasis
Bonding	Zn, Ni, other	Metal-ligands bond formation for analyte sensing, metalloprotein inhibition, or protein labeling
Ligand exchange	Gd, Co, others	Change in coordination sphere to increase MRI signal or release bioactive molecule
Electron transfer	Rh, Ru	Photoinduced electron transfer to produce potent photooxidants
Redox	Fe, Cu, others	Oxidative degradation of proteins, DNA; chelation to manipulate redox activity

The technicium isotope ^{99m}Tc has been used for tumor imaging.⁵³ By using a combination of photophysical and radiolabeling, cancer therapy and the treatment response can be monitored.⁵⁴ These two elements have similar electronic properties. Technicium and rhenium form analogous complexes with similar physical and chemical

properties, these complexes are expected to have the same pharmacokinetic behavior and are, therefore, considered a “matched pair” for diagnosis and therapy. ^{99m}Tc biocomplexes can be used as an alternative to PET radioligands such as ^{11}C ,⁵⁵⁻⁵⁶ ^{18}F ,⁵⁷ and ^{124}I .⁵⁸

1.4.1. Metal complexes with fluorescent properties

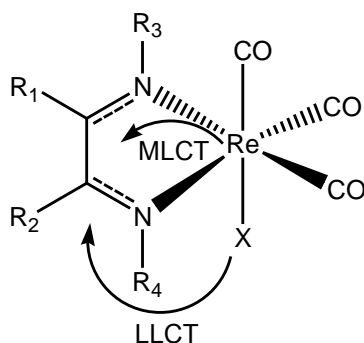
Fluorescence microscopy is a resourceful, high-resolution technique that can be used for imaging and the study of luminescent molecular probes. Metal complexes that exhibit MLCT usually possess long-lived excited states in the order of hundreds of nanoseconds to milliseconds. The long-lived excited state allows the use of time-resolved luminescence microscopy (TRLM) in order to eliminate autofluorescence.⁵⁹ Metal complexes can have long Stokes shifts, low cytotoxicity, and high cellular uptake. Targeted intracellular localization can be achieved by carefully choosing the fluorophores.⁶⁰ Several transition metals and lanthanides have been used as luminophores for *in vivo* imaging, including Ir(III),⁶¹ Pt(II),⁶² Eu(III),⁶³ and Tb(III).⁶⁴

1.4.2. Metal complexes for NO Release

There are metal complexes that release NO after irradiation.⁶⁵ The Fe-S-NO clusters, such as Roussin's salt,⁶⁶ and simple Ru complexes, like $[\text{Ru}(\text{NO})\text{Cl}_5]^{2-}$, can be used for this purpose.⁶⁷ The PaPy₃H (*N,N*-bis(2-pyridylmethyl)amine-*N*-ethyl-2-pyridine-2-carboxamide) group was introduced by Mascharak and coworkers as a pentacoordinated ligand that can provide four equatorial nitrogen sites in an octahedral metal complex with an apical NO.⁶⁸ The Ru(II)-PaPy₃H complex can release NO and it is soluble and stable in aqueous conditions.⁶⁹ The incorporation of fluorescent dyes into NO releasing compounds provides a path for tracking and monitoring targeted cellular

NO uptake.⁷⁰ The promotion of an electron from the metal molecular orbital to a π^* (NO) antibonding orbital is required for the metal nitrosyl photoactivity.⁷¹ The NO-releasing complexes, which can be excited in the near infrared (NIR) region, are needed because of mammalian tissue penetration restriction.⁷²

Rhenium tricarbonyl complexes have been used for imaging applications because of their luminescent properties provided by the $^3\text{MLCT}$: large Stokes shifts, fluorescent lifetimes in the hundreds of nanoseconds range, and good quantum yields.⁷³ Lately, it has been discovered that these qualities also can be used in biological probes.⁷⁴ In $(\text{L})\text{Re}(\text{CO})_3\text{X}$ type complexes (L = bidentate nitrogen ligand with extended conjugated system), the UV-Vis spectrum shows three transitions (Scheme 1.11): (i) a high energy band $\pi \rightarrow \pi^*$, (ii) a lower MLCT energy band $\text{Re} \rightarrow \pi^*(\text{L})$, and (iii) a $\text{X} \rightarrow \text{L}$, LLCT transition.⁷⁵ These tricarbonyl complexes exhibit solvatochromism⁷⁶ and are greatly affected by substitutions in the polypyridine ligand. These complexes possess a C_s geometry with three active vibrational modes [$\text{A}'(1) + \text{A}'(2) + \text{A}''$] corresponding to the three CO in facial configuration.⁷⁷



Scheme 1.11 UV-Visible transitions observed for $(\text{L})\text{Re}(\text{CO})_3\text{X}$

1.5. FAMILIES OF NO-DONORS

Nitric Oxide has a solubility in water of 2 - 3 mM and reacts rapidly with several radicals and water.⁷⁸ Some advantages of using NO-donors are: Ease of use vs. NO gas, targeted delivery, and production modulation in order to avoid side reactions.⁷⁹ For almost 150 years, glyceryl trinitrate (GTN) has been used as an NO-donor for medical purposes.⁸⁰ There are seventeen different classes of NO-donors, which vary from enzymatic generators to redox generators. These NO-donors have different biological applications.⁸¹

1.5.1. Organic nitrates (RONO₂)

Organic nitrates are alcohol nitric acid esters. Organic nitrates are the oldest class, medically applied. They are synthesized directly from nitric acid and alcohol or from alkyl halide and AgNO₃.⁸² They have low solubility in water and they suffer from hydrolysis and β-H elimination in alkaline conditions.⁸³ The biggest shortcoming is “nitrate tolerance”; even though the mechanism is not clear, it may result in sulfhydryl depletion.⁸⁴

1.5.2. Organic nitrites.

These are esters of alcohols and nitrous acid. They have been widely used as vasodilators. They can be synthesized by esterification of alcohols with nitrous acid,⁸⁵ reaction with other nitrosilating agents, or by transesterification.⁸⁶ Organic nitrites have lower boiling points than the corresponding alcohols, and react rapidly in the presence of nucleophiles, transferring the nitrosyl group.⁸⁷

1.5.3. Metal-NO complexes

This group has been discussed before (1.3.4-1.3.7). Sodium nitroprusside (SNP), dinitrosyl iron thiol complexes (DNICs), iron-sulfur clusters nitrosyl, ruthenium nitrosyls, and iron-porphyrin nitrosyls are members of the Metal-NO family.

1.5.4. N-Nitrosamines

N-nitrosamines have been used sparingly as solvents or intermediates in the synthesis of hydrazines and diazoalkanes.⁸⁸ They are considered carcinogenic because they can alkylate biomolecules.⁸⁹

1.5.5. N-hydroxy-N-Nitrosamines.

N-hydroxy-N-Nitrosamines decompose to generate NO under physiological conditions. Therefore, they can be used as anti-hypertensive agents and inhibitors of platelet aggregation.⁹⁰ They are prepared by direct nitrosation of N-hydroxyamines.⁹¹ Cupferron is used in extraction, separation, and colorimetric detection of metals.⁹² It releases NO under enzymatic,⁹³ electrochemical,⁹⁴ and oxidative conditions.⁹⁵

1.5.6. N-Nitrosimines.

Heterocyclic compounds produced from the correspondent imines. There are seven subtypes according to the substituents.⁹⁶ They release either NO or N₂O from the decomposition of HNO. In the presence of thiols, the production of N₂O is favored over NO.

1.5.7. Nitrosothiols (RSNOs).

Nitrosothiols were first synthesized in 1909.⁹⁷ There are few RSNOs that are stable. They are proposed as storing, transferring and delivering vehicles for NO. They are synthesized from thiols and NO derivatives.⁹⁸ The reaction rates with NO are influenced by steric effects; bulky substrates react slower than less hindered ones.⁹⁹

1.5.8. C-Nitroso compounds

Tertiary C-nitroso compounds with germinal electron withdrawing groups can be used as NO-donors. These compounds are prepared from their corresponding hydroxylamines.¹⁰⁰ They release NO under thermolysis and photolysis.¹⁰¹ In biological systems, they can produce *in vitro* vasodilation and decrease the presence of oxyhemoglobin.¹⁰²

1.5.9. Diazetidine dioxides

These four-member ring heterocycles were first introduced in 1971.¹⁰³ They decompose in physiological aqueous solutions.¹⁰⁴ Diazetidine dioxides were used to decrease the systolic high blood pressure in rats.¹⁰⁵

1.5.10. Furoxans and benzofuroxans.

These five member heterocycles may lead to positive and negative biological outcomes.¹⁰⁶ These compounds are thermally stable; they are reactive in presence of nucleophiles and bases. They release NO in presence of thiols¹⁰⁷ and undergo ring opening reactions with biological thiols, such as cysteine or glutathione.¹⁰⁸

1.5.11. Oxatriazole-5-imines

These mesoionic substituted five-member heterocycles were first reported in 1965.¹⁰⁹ They have been used as blood pressure depressants.¹¹⁰ They were initially synthesized from monohydrazines through substituted cyanohydrazines.¹¹¹

1.5.12. Sidnonimines

Sidnonimines is an important class of NO-donors¹¹² introduced in 1957.¹¹³ These reagents produce stoichiometric amounts of superoxide anions; NO and superoxide react with each other, producing peroxynitrite. Their metabolites are potent vasorelaxants.¹¹⁴

1.5.13. Oximes

The alkyl- and aryl- oximes can be converted to NO under oxidative conditions.¹¹⁵ These compounds are vasoactive, with similar activity to NO. They are synthesized by addition of a nitro and a hydroxyimino group into a diene by a nitro-nitrosation step.¹¹⁶ Oximes decompose into NO and the corresponding ketone, under physiological conditions.¹¹⁷

1.5.14. Hydroxylamines:

Hydroxylamines are hygroscopic free-base products of mammalian cells.¹¹⁸ In alkaline solutions, they disproportionate into ammonia, nitrogen and nitrous oxide. Hydroxylamines can produce NO under enzymatic conditions.¹¹⁹

1.5.15. N-Hydroxyguanidines

N-Hydroxyguanidines are products of guanidine and hydroxyurea that exhibit cytotoxicity against leukemic cells and antitumor activity *in vivo*.¹²⁰ In contrast to other NO-donors, hydroxyguanidines do not produce NO spontaneously: They need NOS¹²¹ or P450¹²² enzymes to produce NO.

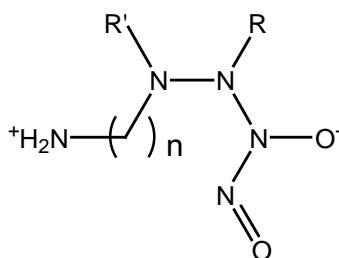
1.5.16. Hydroxyurea

Hydroxyurea is a well-known antitumor agent.¹²³ It shows some activity against some cancers,¹²⁴ skin diseases,¹²⁵ and inhibits HIV virus replication.¹²⁶ In 1998, the first *in vitro* reaction of oxyhemoglobin with hydroxyurea was reported. It involved the

specific transfer of NO from the NHOH group of hydroxyurea in biomolecules taken from sickle cell anemia patients.¹²⁷

1.5.17. Zwitterionic polyamine/NO adducts (NONOates)

Bubbling NO to amines produces $\text{RN}[\text{N}(\text{O})\text{NO}]^-(\text{CH}_2)_x\text{NH}_2^+$.¹²⁸ The half-lives at physiological conditions may vary from 1.8 min to 56 h, depending on the amine substituents.¹²⁹ These adducts are affected by slow decomposition even in the solid state, and thus are stored at low temperatures (-78 °C).¹³⁰ NONOates may release two full equivalents of NO in solution¹³¹ and some have extended shelf life.



Scheme 1.12 Structure of a generic zwitterionic NONOate.

Table 1.2 Spectroscopic properties and NO-release half-lives for different NONOate type NO-donors. Measured in 0.01 M NaOH. Determined at 22 °C and pH 7.4 in 0.1 M phosphate buffer.

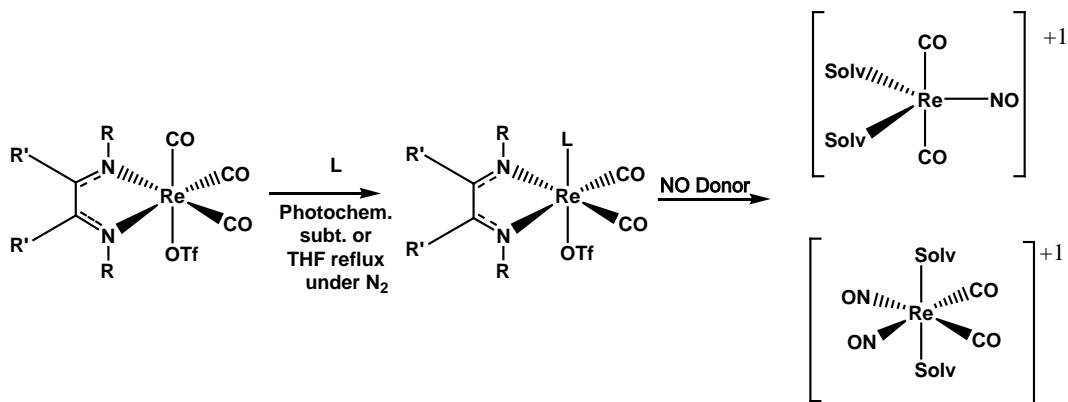
$\text{RN}[\text{N}(\text{O})\text{NO}]^-(\text{CH}_2)_x\text{NH}_2^+\text{R}'$					
x	R	R'	UV λ_{max} (nm)	$\epsilon \times 10^{-3}$ ($\text{M}^{-1}\text{cm}^{-1}$)	$t_{1/2}$ (min)
2	Me	Me	250	7.31	36.1

3	Me	Me	250	7.68	10.1
4	Me	Me	250	8.59	1.3
6	Me	Me	250	7.25	3.7
2	Me	H	250	7.8	40
2	Et	H	250	7.96	333
2	Et	Et	250	7.61	327
3	Me	H	250	7.77	13.7
3	Et	Et	250	8.55	71.8
3	Pr	H	250	8.05	76.6
3	i-Pr	H	250	7.44	93
3	i-Pr	i-Pr	250	7.89	88.5
3	cyclohex	H	250	9.13	115
2	(CH ₂) ₂ NH ₂	H	252	7.64	3400
3	(CH ₂) ₃ NH ₂	H	252	7.83	284
3	(CH ₂) ₄ NH ₂	H	250	9.42	165

1.6. PROJECT RATIONALE

The objective of this project is the synthesis, characterization and NO sensing and imaging study of Re-based ratiometric fluorescent dyes. The unique mechanism we envisioned for NO sensing depends on change in fluorescence properties that occurs upon NO coordination. In order to obtain a ratiometric fluorescent probe that can detect changes in NO concentration with high sensitivity and selectivity, an entirely new approach on NO sensing is employed that combines the following elements: 1) NO binding to a metal center, such as Re(I); 2) a bidentate or tridentate phenanthroline (phen), dipyrrophenazine (dppz), or α -diimine framework attached on the metal, which provides favorable photophysical characteristics, as well potential for modification; and 3) two weakly-coordinated ligands: i) The X-type CF₃SO₃⁻ and ii) An L-type ligand, such as

THF, CH₃CN, or PPh₃. The sensing mechanism may involve the formation of nitrosyl complexes with concurrent alteration of photophysical properties of an organometallic complex upon reaction with NO.



Scheme 1.13 Proposed mechanism for the ratiometric response for Re-based NO sensors.

The mechanism of NO sensing is based on the optical and photophysical properties of rhenium, and the affinity that rhenium has for Nitric Oxide (NO) to form nitrosyl complexes. These complexes sense NO in aqueous media, at 37 °C and pH 7 and are expected to be able to permeate the cell membrane and get trapped in the cytosolic media. The strong ³MLCT is a result of the presence of carbonyl groups. Dissociation of labile ligands (L and CF₃SO₃⁻) and NO attachment leads to a different fluorescence maximum, promoting ratiometric response. Aromatic ligands (polypyridines, phenanthrolines) can give this probe membrane cell permeability, providing the complex is neutral. Change in polarity or overall charge of the complex upon NO reaction could potentially trap the product inside the cell.

Previous attempts of rhenium phenanthroline complexes^{35b} as NO sensors have used diaminopyridine, which transforms into a triazole in a mechanism similar to DAF family of dyes (1.3.1). In those dyes there was no coordination of NO to Re.

Rhenium Tricarbonyl $[Re(CO)_3(N-N)(X)]^+$ systems: This type of kinetically inert Re(I) d^6 18-electron complexes displays a 3MLCT phosphorescent emission after near visible ultra-violet and visible light excitation. The function of these carbonyl ligands is to avoid the deactivation of Re(I) metal center. The 3MLCT transition is characteristic of d^6 -Rhenium(I)-carbonyl complexes. There are also non-carbonyl rhenium complexes that able to display this transition. However, their ligand field splitting is very small; for example, $Re(bipy)_3$ is non-luminescent. Several other ligands like polypyridines, bis-quinolines and pyridazines have been studied as probes for cell imaging.¹³⁴

Rhenium imaging probes have shown reliable responses. Changing fluorophores selectively may target different subcellular structures. Rhenium(I) reacts with specific biochemical agents localized in the cell, such as sulfur-containing aminoacids in the mitochondria, or with macromolecules by non-covalent interactions. None of these precursors uses NO-triggered selective ligand dissociation from a metal complex, as a mechanism to provide NO sensing with a ratiometric response. The proposed probe is expected to selectively sense NO even under anaerobic conditions, because it does not need O_2 for activation. Several current NO dyes respond to NO indirectly with the real substrates being N_2O_3 or NO_2 radical formed by reaction of NO with oxygen. The Re-NO dyes we propose are soluble in aqueous media, (pH between 7.2 and 7.4), by using only a minimum amount of organic solvent. In order for the Re-NO dyes to meet requirements for widespread applicability, they need to be stable under physiological conditions, permeable through the cell membranes and modifiable to be trapped inside the cell.

1.7. REFERENCES

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2. (phen)Re(PPh₃)(CO)₂OSO₂CF₃ AS AN ORGANOMETALLIC FLUORESCENT NO SENSOR.

2.1. ABSTRACT

The compound (phen)Re(PPh₃)(CO)₂OSO₂CF₃ (**1**), which can be obtained photochemically or thermally from (phen)Re(CO)₃OSO₂CF₃ shows ratiometric fluorescence sensing response upon NO addition, which was added in the form of NO-donor Z-1-N-methyl-N-[6-(N-methylammoniohexyl)amino]diazene-1,2-diolate (MAHMA-NONOate). Addition of 10-150 μM of NO-donor into a 25 μM solution of (phen)Re(PPh₃)(CO)₂OSO₂CF₃ in 25% v/v acetone/phosphate saline (PBS) buffer gave fluorescence quenching at 518 nm, and fluorescence enhancement at 426 nm upon excitation at 360 nm. The FT-IR spectra after reaction with NO showed bands at 1720 cm⁻¹ and 1631cm⁻¹ that can be ascribed to Re-NO complex formation. The ¹H-NMR and ¹⁹F-NMR spectra after reaction with NO showed characteristic resonances for non-coordinated triflate and PPh₃, suggesting that the organometallic product formed upon reaction with NO releases both these ligands. Fluorescence microscopy studies on Rat Aortic Endothelial Cells in 2% v/v acetone/phosphate saline (PBS) buffer showed distinct incorporation of the dye into cells. Compound **1** is an isolable complex, which is stable at room temperature and therefore can be used as an NO sensing organometallic dye, as prepared, without further treatment.

2.2. INTRODUCTION

Nitric Oxide sensing has been a topic of intense research interest since the early 90's (references 1-16 in Chapter 1). Diaminofluoresceins (DAF) are the most commonly used dyes for fluorescent detection, because of their high sensitivity and good cell

absorptivity.¹ However DAF-like sensors have a complex NO sensing mechanism, that relies on activation of NO with dioxygen.² Another strategy for selective sensing of NO is by using metal-containing dyes, such as fluorescein containing-Cu(II) and Fe(III) centers introduced by Lippard *et al.*³ Surprisingly, third-row transition metal organometallics, have found very limited application to NO sensing, despite their well-studied photophysical properties and ability to bind NO. Several transition metal nitrosyl complexes have been reported,⁴ including some Re(I) complexes.⁵ There are only a few reported examples of mixed-ligand phosphine/nitrosyl rhenium complexes.⁶ These complexes are typically synthesized from rhenium nitrosyls, but not vice-versa.⁷

Several Re(I) complexes have photoluminescent properties⁸ and exhibit photoactivity in solution because of metal-to-ligand charge transfer (MLCT).⁹ Control of Re(I) photochemical, photophysical, and electrochemical properties has generated interest because of the use of these complexes as electro- and photocatalysts.¹⁰ Typically, Re-phosphine complexes have higher quantum yields than [Ru(bipy)₃]²⁺.¹¹ Excited state properties of diimine rhenium(I) carbonyl complexes occur in the lowest triplet excited state, because of non-radiative processes that occur from the highest vibrational energy levels.¹² The nature of the π -acceptor ligand (diimine or bridging ligand) determines the photophysical/photochemical properties. Mononuclear diimine rhenium(I) carbonyl complexes have quantum yields varying from 0.001 to 0.1 and long emission lifetimes (10 ns to 1 μ s) as result of the lowest energy MLCT.¹³ There are two general methods for tuning the metal complex photophysical properties: (a) using a ligand with a different field strength to adjust the energy of the bonding orbitals in the metal center; (b) adding different substituents to the diimine ligand to alter the π^* orbital energy level, producing

a change in the absorption/emission wavelength, within the desired window range.¹³ Recently, a Re(I)-phenanthroline-tricarbonyl-pyridine complex showed fluorescence enhancement upon NO addition. The fluorescence change is a result of triazole formation, occurring after oxidative reaction with activated NO (in the form of N₂O₃) in a similar fashion as DAF-FM.¹⁴ This compound was not found to be responsive in cells upon exogenous NO-donor addition. Our research group has recently proposed a new method for NO sensing and detection based on the favorable photophysical and NO complexation properties of metal polypyridine complexes and analogs.¹⁵

The triphenylphosphine organometallic complex (phen)Re(PPh₃)(CO)₂OSO₂CF₃ (**1**) introduced in this Chapter showed fluorescence responses, that can be ascribed directly to NO complexation by Re, as indicated by NMR and IR spectroscopic studies. **1** also has the additional advantages that it can be prepared in an isolable form, and is stable at room temperature. Preliminary fluorescence microscopy studies in cells have shown good absorptivity, thus indicating its promise as a fluorescent probe for nitric oxide imaging.

2.3. EXPERIMENTAL SECTION

2.3.1. Materials and Methods

(phen)Re(CO)₃Cl and (phen)Re(CO)₃OSO₂CF₃ were prepared as previously reported and were found spectroscopically identical to the reported compounds.^{16,17} All other materials (purchased from Aldrich Chemical Co., ACROS Organics, Cayman Chemicals, or Alfa Aesar) were standard reagent grade and were used without further purification, unless otherwise noted. Tetrahydrofuran (THF) used for the preparation of **1**, was anhydrous (purchased as an Aldrich Sure-Seal™ product), or distilled and dried

before use. PBS buffers A (0.002 M in total phosphate, pH = 7.2, [NaCl] = 0.04 M) and B (0.002 M in total phosphate, pH = 7.2, [NaCl] = 0.02 M) were prepared from K₂HPO₄, KH₂PO₄, and NaCl (all from Fisher Scientific). Water used for preparation of the buffers and experiments with Nitric oxide was filtered water (18 MΩ·cm⁻¹) produced from a Millipore® nanopure diamond lab water system (Barnstead Thermolyne Corporation, Dubuque, IA). Deuterated solvents were purchased from Cambridge Isotope Laboratories Ltd. Rat Aortic Endothelial Cells were acquired from Cell Biologics. Fetal Bovine Serum (FBS) and DMSO were bought from ATCC Inc. Penicillin-Streptomycin and endothelial cell growth supplement from bovine neural tissue were purchased from Sigma-Aldrich. DMEM/F-12 medium, PBS (for fluorescence microscopy experiments) and Trypsin (0.05% EDTA) were purchased from GIBCO/Invitrogen Inc. Cell culture flasks and dishes were purchased from Fisher Scientific. Photochemical reactions were carried out using a Rayonet photochemical reactor (Southern New England Ultraviolet, model RPR-100), equipped with a cooling fan and 10 phosphor-coated low-pressure mercury lamps ($\lambda_{\text{max}} = 300 \text{ nm}$, light intensity = $5.2 \pm 0.1 \times 10^6 \text{ photon/sec/cm}^3$). Fluorescence Spectra were recorded on a Jobin Yvon Horiba Fluoromax-3 fluorimeter. Fluorescence Microscopy was performed using an Olympus IX-81 Fluorescence Microscope and analyzed with IPLab v 4.04 Scientific Imaging Processing Software. Frequency-domain fluorescence lifetime measurements were performed using a ChronosFD spectrofluorometer (ISS, Champaign, IL). Samples were excited with a 370 nm modulated diode, and emission was collected using 400 nm long-pass filters (Andover, Salem, NH). The ¹H and the ¹³C-NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer and were referenced, using the residual solvent resonances. All

chemical shifts, δ , are reported in ppm. The FT-IR spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR Spectrometer.

2.3.2. Synthesis of (phen)Re(PPh₃)(CO)₂OSO₂CF₃:

Method 1 - Thermal Substitution: 20.0 mg of (phen)Re(CO)₃OSO₂CF₃ (33.3 μ mol) and 10 mL of THF (final concentration: 3.33 mM) were placed in a 50 mL round bottom flask, under N₂ flow. The solution was refluxed for 48 h. The color changed during reflux from yellow to amber. The volatiles were evaporated to dryness and 1.0 mL of dichloromethane was added. The product was precipitated at room temperature by adding 4.0 mL of diethyl ether. The bright yellow powder product was filtered and was dried *in vacuo* at 40 °C. 20.5 mg, (74%). ¹H-NMR (CDCl₃): 6.96 (t, 3H, ³J_{H-H} = 7.0 Hz), 7.39 (dd, 6H, ³J_{H-H} = 7.6 Hz, ³J_{H-H} = 2.0 Hz), 7.62 (dd, 6H, ³J_{H-H} = 12.5 Hz, ³J_{H-H} = 7.2 Hz), 7.95 (d, 2H, ³J_{H-H} = 8.2 Hz, ³J_{H-H} = 5.1 Hz), 8.36 (s, 2H), 8.99 (d, 2H, ³J_{H-H} = 8.28 Hz), 9.14 (d, 2H, ³J_{H-H} = 4.7 Hz). ¹³C-¹H-NMR (CDCl₃): 128.92 (*p*-CH, s, 3C), 129.15 (*m*-CH, d, 6C, ³J_{C-P} = 37.8 Hz), 131.23 (CH, s, 2C), 132.38 (*o*-CH, d, 6C, ¹J_{C-P} = 40.5 Hz), 137.14 (CH, s, 2C), 140.04 (CH, s, 2C), 153.32 (CH, s, 2C). ¹⁹F-¹H-NMR (CDCl₃): -78.3 ppm FT-IR (cm⁻¹): 2029 (ν_{CO}, s), 1926 (ν_{CO}, s), 1906 (ν_{CO}, s), 1433, 1266, 1152, 1031, 854, 725, 638. Anal. Calc. C₃₃H₂₃F₃N₂O₆PreS: C 46.64%, H 2.72%, N 3.29%. Found: C 46.28%, H 2.32%, N 3.20%. UV-Vis (in ethanol): λ_{max} = 360 nm (ε = 4500 mol⁻¹cm⁻¹), fluorescence emission in acetone (λ_{exc} = 360nm): 585nm Φ₁=0.027 (at 370 nm, in 1:3 acetone/PBS buffer, using quinine sulfate in sulfuric acid 0.1 M as reference Φ_R=0.546).

Lifetime method 1: Frequency dependent, useful for fast lifetimes: τ₁ = 21.3 ± 0.3 ns, f₁ = 0.0626 ± 0.0008, α₁ = 0.00294; τ₂ = 389 ± 5 ns, f₂ = 0.601 ± 0.007, α₂ = 0.00155.

Lifetime method 2: Time-dependent, useful for slow lifetimes: $\tau_1 = 35.4 \pm 0.05$ ns, $f_1 = 0.869 \pm 0.047$; $\tau_2 = 225 \pm 0.06$ ns, $f_2 = 0.094 \pm 0.0003$; $\tau_3 = 1995 \pm 15$ ns, $f_3 = 0.006 \pm 0.00003$; with fluorescence still observable after 1 μ s. Differences between the two methods can be attributable to in the second method the values are result of an algorithmic fitting instead of real measurement.

Method 2 - Photochemical Substitution: 1.0 mg of (phen)Re(CO)₃OSO₂CF₃ (1.67 μ mol), 3.0 mg of triphenylphosphine and 1.00 mL of THF (final concentration 1.67 mM) were placed in a NMR tube. The solution was irradiated with a photochemical reactor ($\lambda_{\max} = 300$ nm, light intensity = $5.2 \pm 0.1 \times 10^6$ photon/sec/cm³). After irradiation the color of the solution changed from yellow to amber. This solution was used without further treatment. FT-IR (cm⁻¹): 2029 (ν_{CO} , vs), 1921 (ν_{CO} , vs), 1905 (ν_{CO} , vs), 1722 ($\nu_{\text{CO-O}}$, vs), 1633 ($\nu_{\text{CO-O}}$, vs), 1437, 1279, 1261, 1180, 1118, 1030, 749, 693, 637.

2.3.3. Fluorescence experiments with NO-donor

In a typical experiment, 1.000 mL of a solution of (phen)Re(THF)(CO)₂OSO₂CF₃ or (phen)Re(PPh₃)(CO)₂OSO₂CF₃ complex (100 μ M) was prepared by dilution with acetone of a 1.67 mM THF stock solution. Aqueous PBS buffer A (pH=7.2, [NaCl] = 0.04 M, 2.75 mL to 3.00 mL) was added. Then 0-250 μ L of 4 mM stock solution of MAHMA-NONOate NO-donor, in 0.04 M NaOH were added, in order to obtain 75% v/v aqueous/organic solution and a final NO-donor concentration in the 0-200 μ M range, while the concentration of the Re complex remains constant at 25 μ M. No more than 10 min. elapsed from preparation of the organic phase to the addition of NO-donor. The solution was mixed thoroughly in a vortex and sonicated for 90 min. at

room temperature. The fluorescence of all solutions was measured at $\lambda_{\text{exc}} = 360$ nm and recorded from 400 nm to 700 nm. For a control experiment a NaOH stock solution was used instead of an NO-donor/NaOH stock, in order to account for any possible variations in the fluorescence due to minor pH changes. The result of the control titrations gave identical spectra for all titration points in the 1-150 μL range. These experiments were also performed in identical conditions for control compounds (phen)Re(CO)₃Cl and (phen)Re(CO)₃OTf and no significant changes were observed for their fluorescence emissions upon NO addition.

2.3.4. Fluorescence titrations with gradual addition of NO-donor under anaerobic and aerobic conditions.

In a typical experiment, 0.750 mL of a (phen)Re(THF)(CO)₂OSO₂CF₃ or (phen)Re(PPh₃)(CO)₂OSO₂CF₃ solution (100 μM) was prepared from dilution of 20 μL of a 1.67 mM THF stock solution with acetone. A quantity of 2.25 mL of aqueous PBS buffer A (pH=7.2, [NaCl] = 0.04 M) was added. The fluorescence emission was monitored using $\lambda_{\text{exc}} = 360$ nm. Then 3 mM stock solution of MAHMA-NONOate NO-donor in 0.04 M NaOH was added progressively 300 μL in 25 μL portions. After each addition, the solution was left react for 10 minutes (approximately 3 MAHMA NONOate half-life), at room temperature before measuring fluorescence using the same parameters as above.

2.3.5. Fluorescence titrations with low concentrations of NO-donor.

In a typical experiment, 0.750 mL of a (phen)Re(THF)(CO)₂OSO₂CF₃ or (phen)Re(PPh₃)(CO)₂OSO₂CF₃ solution (100 μM) was prepared from dilution of 20 μL of a 1.67 mM THF stock solution with acetone. A quantity of 2.25 mL of aqueous PBS

buffer B (pH=7.2, [NaCl] = 0.02 M) was added. The solution was bubbled with nitrogen for 30 minutes. The fluorescence emission was monitored using $\lambda_{\text{exc}} = 360$ nm. Then 3 mM stock solution of MAHMA-NONOate NO-donor in 0.04 M NaOH was added progressively 150 μL in 1 - 25 μL portions. After each addition, the solution was left react for 10 minutes (approximately 3 MAHMA NONOate half-life), at room temperature before measuring fluorescence using the same parameters as above.

2.3.6. ^1H -NMR and ^{19}F -NMR study of reactivity of Re complexes with NO:

(phen)Re(PPh₃)(CO)₂OSO₂CF₃ (1 mg) was added to 0.05 mL of THF in four separate 10 mL vials. Acetone (1.6 mL) was added to each vial. Then 2.4 mL of PBS buffer A (pH=7.2, [NaCl] = 0.04 M) and 0.2 mL of stock NO-donor solution (5 mM in 0.04 M NaOH) were added sequentially. The solutions were vortexed for 30 sec. and then sonicated for 3h at room temperature. Dichloromethane was added (3 x 10 mL), and the organic layer was dried with anhydrous sodium sulfate. The solvent was evaporated and the non-volatiles were taken up in chloroform-D and the NMR spectra were collected. In order to remove uncomplexed phenanthroline products and collect only the NMR spectra of complexes, the organic phases were further diluted with dichloromethane and washed with 1 M hydrochloric acid, followed by 1 M sodium bicarbonate, rinsed with deionized water, and dried with anhydrous sodium sulfate. The volatiles were evaporated, the solid residues were redissolved in chloroform-D, and the ^1H -NMR and ^{19}F -NMR spectra were recorded. These experiments were also performed under identical conditions for control compounds (phen)Re(CO)₃Cl and (phen)Re(CO)₃OTf. After HCl/NaHCO₃ washing no changes were observed in the ^1H -

NMR spectrum of (phen)Re(CO)₃Cl, while for (phen)Re(CO)₃OTf, resonances identical to (phen)Re(CO)₃Cl were observed.

2.3.7. Fluorescence microscopy studies of (phen)Re(PPh₃)(CO)₂OSO₂CF₃ in Rat Aortic Endothelial Cells:

Cell preparation: Rat Aortic Endothelial Cells were grown in Duplecco's Modified Eagle Medium: Nutrient Mixture F-12 medium (DMEM/F-12) supplemented with 10% Fetal Bovine Serum (FBS), 1% penicillin-streptomycin, and endothelial cell growth supplement in a humidified tissue culture incubator at 37°C equilibrated with 95/5 v/v% Air/CO₂ mixture. Cells were cultured in 35 × 10 mm dishes seeded with 2 × 10⁵ cells and used at 60-80% confluency.

Solution Preparation: A 500 μM solution of Re(phen)(THF)(CO)₂OSO₃CF₃ in THF/acetone (Solution B) was prepared from dilution of 62.5 μL of a stock solution of Re(phen)(THF)(CO)₂OSO₃CF₃ in THF (1.6 × 10⁻³ M - Solution A) with 138 μL of acetone. This solution was added to 800 μL of commercial 10 mM PBS buffer solution (pH=7.2), resulting in a final 100 μM solution of **1** in 80/14/6 v/v aqueous buffer/acetone/THF. This new solution was further diluted 1/10 v/v with PBS Buffer before adding it to the cells, resulting in a final solution of 10 μM of **1** in 98/1.4/0.6 v/v aqueous PBS buffer/acetone/THF (Solution C). Various quantities of 6 mM NO-donor solution (MAHMA-NONOate) in 0.01 M NaOH were diluted with 0.01 M NaOH to give 0, 100, 500 & 1000 μM solutions of NO-donor (Solutions D-G).

Loading of solution C (10 μM of Re complex 1): The old media were poured out from the plates; then the plates were rinsed twice with 1 mL of 10 mM PBS (pH = 7.2). Then 100

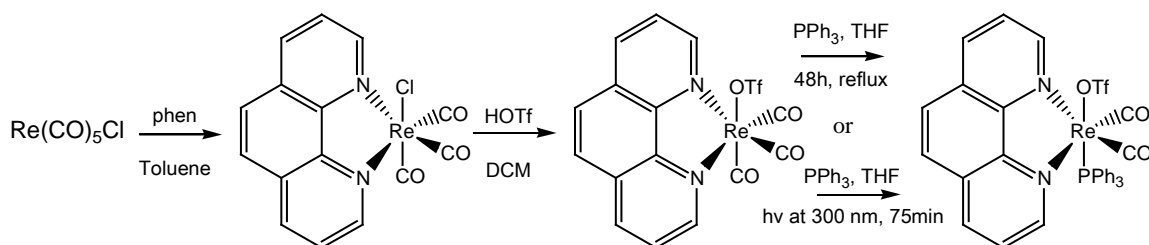
μL of DMEM/FBS/Penicillin Media, 800 μL of PBS 10 mM, and 100 μL of Re complex stock (Solution C) were added with this specific order. The plates were incubated for 75 min. at 37 °C equilibrated with 95/5 v/v% Air/CO₂ mixture.

Fluorescence Microscopy Measurements: Each plate was placed in the fluorescence microscope and measured under a filter an excitation wavelength of 350 nm (330-380 nm window) and an emission window of 440-460 nm. The images were collected with a total exposure time of 2668 ms and 20X zoom.

2.4. RESULTS AND DISCUSSION

2.4.1. Synthesis of (phen)Re(PPh₃)(CO)₂OSO₂CF₃ (**1**)

Complex **1** was synthesized by photochemical or thermal substitution of CO from (phen)Re(CO)₃SO₃CF₃. Only the thermal preparation was performed on a larger scale and led to an isolable quantity of the product. However the product was found to have identical UV-Vis and NMR spectra, regardless of the preparation method. The isolated product was characterized by ¹H-NMR, ¹³C-NMR, and FT-IR, and its purity was confirmed by elemental analysis.



Scheme 2.1 Synthesis of (phen)Re(PPh₃)(CO)₂OSO₂CF₃ (**1**)

2.4.2. Fluorescence studies with addition of NO-donor

Complex **1** showed significant fluorescence response to NO addition. The fluorescence spectrum of **1** in acetone/buffer 25/75 v/v shows one emission at 514 nm

($\Phi = 0.03$). Figure 2.1 shows the effects on the fluorescence spectrum of **1** upon NO gradual addition, in the form of NO-donor MAHMA-NONOate (0 - 150 μM) dissolved in 0.04 M NaOH. The emission at 514 nm decreases rapidly upon NO addition and its intensity shows linear dependence with NO-donor concentration, thus allowing NO quantification in the 0-150 μM range (Figure 2.2).

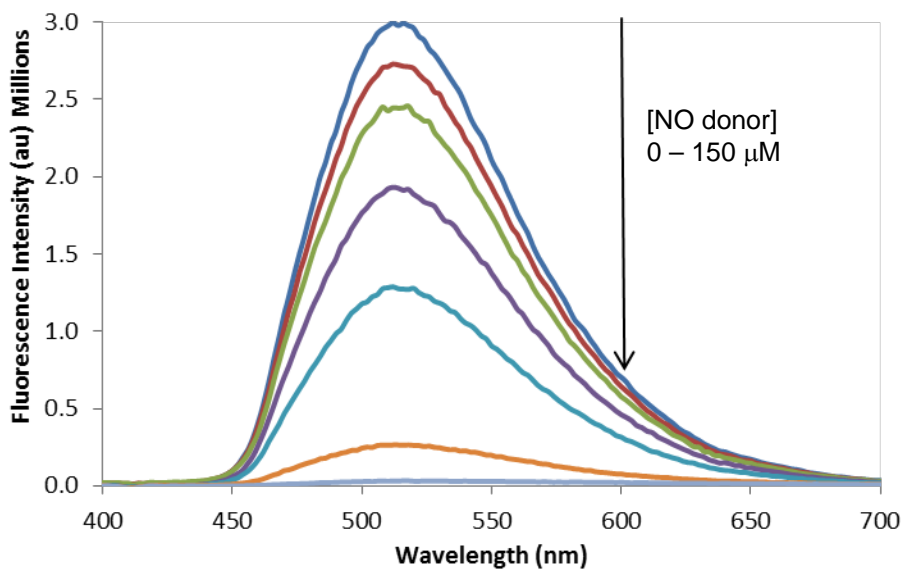


Figure 2.1 Fluorescence Response upon NO-donor addition (0-150 μM) to 20 μM of Re complex **1** in acetone/PBS aqueous buffer 25/75 v/v, ($\lambda_{\text{exc}} = 360 \text{ nm}$)

A control experiment in the absence of NO-donor, (addition of 0.04 M NaOH) shows no observable changes in fluorescence intensity, proving that the sensor responds to NO-donor addition and not any other substance present in the matrix of the added solution (Figure 2.2). It should also be noted that by using a 0.002 M PBS buffer, in 0.04 M NaCl, and a NaOH 0.04 M solution for the N donor no precipitation is observed under the concentration range of these experiments. These experiments were also performed in identical conditions for control compounds (phen)Re(CO)₃Cl and (phen)Re(CO)₃OTf and no significant changes were observed at their fluorescence

emissions at 565 nm, upon NO addition, indicating that the presence of a labile L ligand is necessary for reaction with NO.

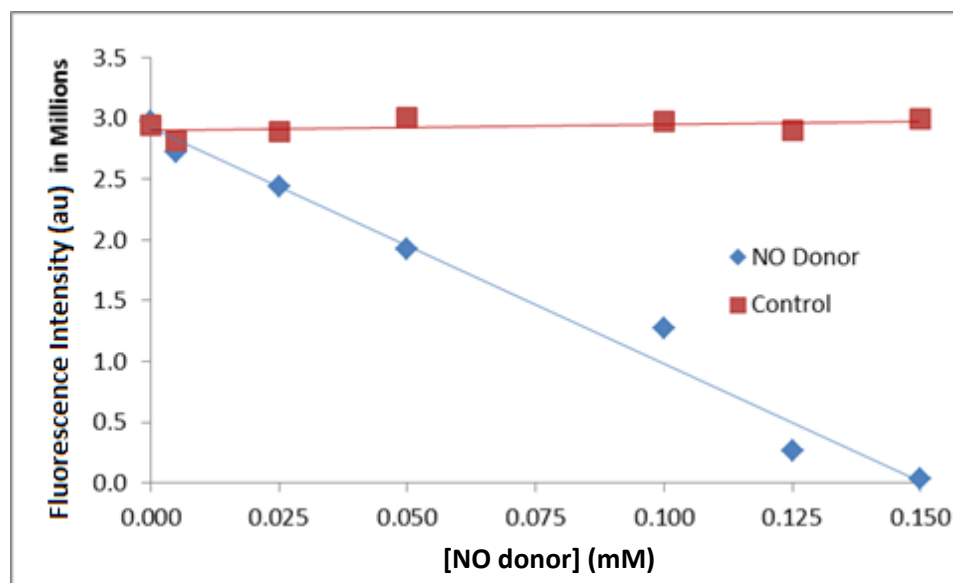


Figure 2.2 Observed Fluorescence intensities of **1** (20 μ M) at $\lambda_{\max} = 514$ nm upon NO-donor addition vs addition of control solution (NaOH 0.04 M). $\lambda_{\text{exc}}=360$ nm.

Complex **1** prepared photochemically on a 1 mg scale (1.67 mM in THF) showed also significant fluorescence response to NO addition both at 560 nm (decrease) and at 455 nm (increase). The fluorescence spectrum of **1** in THF/acetone/buffer 2/23/75 shows one emission at 562 nm. The shift in emission compared to the isolated complex could be ascribed both to the difference in the solvent system, and the presence of unreacted (phen)Re(CO)₃SO₃CF₃, or (phen)Re(CO)₂(THF)SO₃CF₃ which emit at 562 nm and 535 nm respectively (see Chapter 3). Figure 2.3 shows the effects on the fluorescence spectrum of **1** upon NO gradual addition, in the form of NO-donor MAHMA-NONOate (0-150 μ M) dissolved in 0.04 M NaOH. The emission at 560 nm decreases rapidly upon NO addition (with a gradual low-energy shift), while the increase

at 452 nm is more pronounced a higher NO-donor concentrations leading to a pseudo-ratiometric response, and an “isosbestic area” at 485 nm.

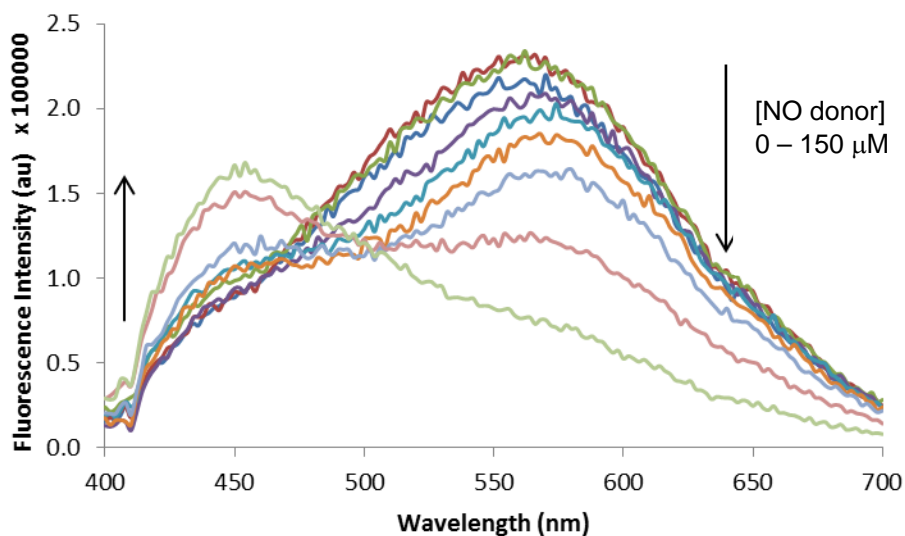


Figure 2.3 Fluorescence Response upon NO-donor addition (0 - 150 μM) to 25 μM of Re complex **1** prepared photochemically, used as THF solution, in acetone/PBS aqueous buffer 25/75 v/v ($\lambda_{\text{exc}} = 360 \text{ nm}$)

The plot of intensity ratio for the two signals at 452 nm and 562 nm vs. NO-donor concentration, (Figure 2.4) allows NO quantification using the ratio of the two responses. The rapid increase on the ratiometric response for higher NO concentrations are result of to the different range of NO in which the intensities at 565 nm and 454 nm are most sensitive. Specifically the 562 nm intensity decrease shows high sensitivity for low NO concentration, while the increase at 452 nm becomes more notable for high concentrations.

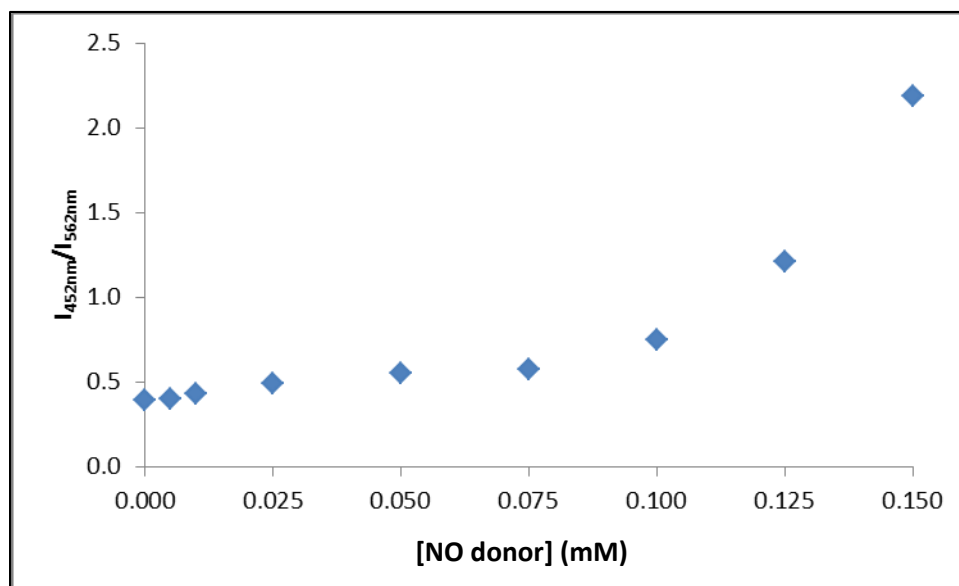


Figure 2.4 Fluorescence intensity ratio of 454 nm/562 nm for **1** (25 μM) with NO-donor addition (0 - 150 μM)

2.4.3. Fluorescence titration with gradual addition of NO-donor under anaerobic and aerobic conditions.

Complex **1** showed stability under aqueous media, 37 $^{\circ}\text{C}$ and pH 7. Figures 2.5 and 2.6 show the effects on the fluorescence spectrum of **1** upon titrations with NO, in the form of NO-donor MAHMA-NONOate (0-150 μM) dissolved in 0.04 M NaOH, added in 25 μL portions after 10 minutes intervals, under anaerobic (Figure 2.5) or aerobic (Figure 2.6) conditions. The emission at 514 nm decreases rapidly upon NO addition and its intensity shows linear dependence with NO-donor concentration. The experiment was performed under nitrogen atmosphere in order to avoid NO oxidation to N_2O_3 .

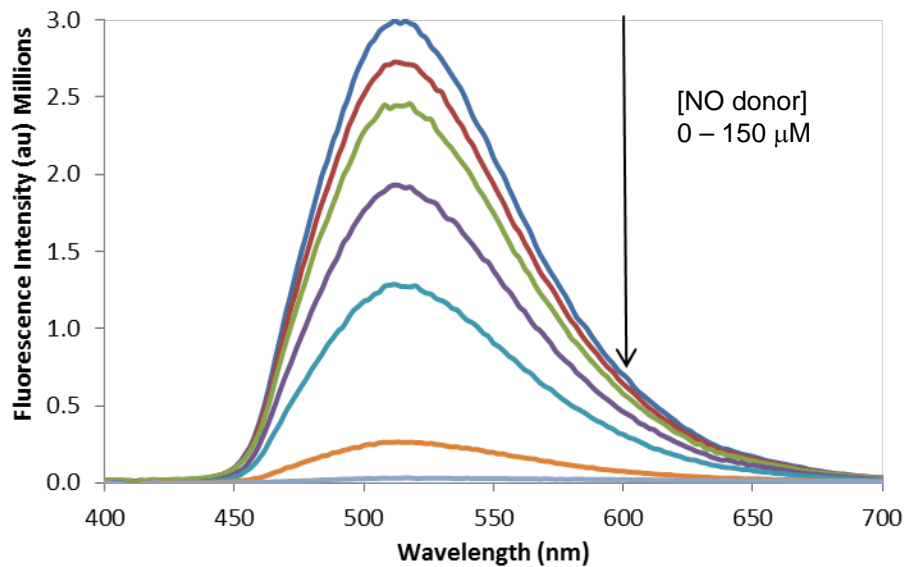


Figure 2.5 Fluorescence Spectra of 10 μM **1** with NO, acetone/buffer 25:75, $\lambda_{\text{exc}} = 360 \text{ nm}$, under nitrogen

Quenching of the 514 nm emission, was observed under both anaerobic and aerobic conditions. However, under anaerobic conditions (Fig. 2.5) the effect is more pronounced, indicating that oxygen is not needed in order for the sensing of NO to take place. Such observation is consistent with a mechanism that does not involve O_2 -activated intermediates such as NO_2 and N_2O_3 , and perhaps implies that sensing is a direct result of a reaction of NO with the Re(I) dye.

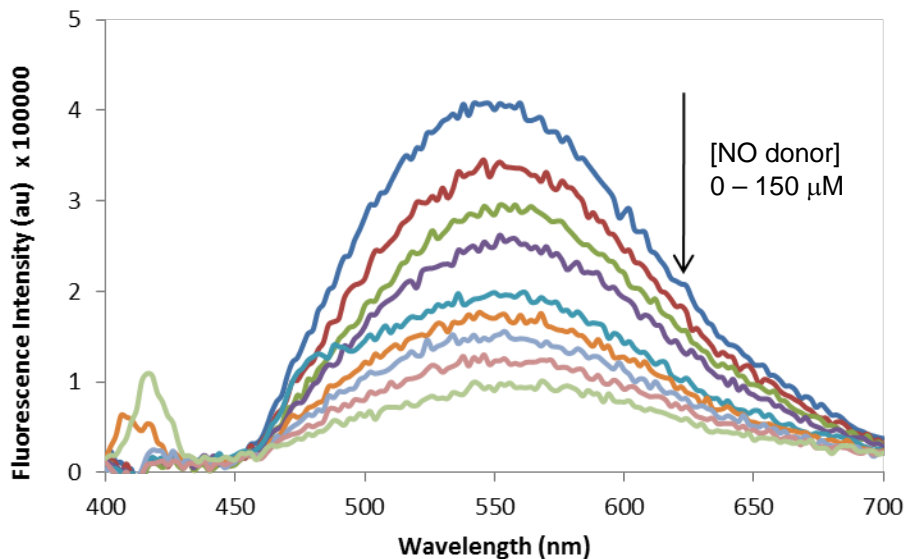


Figure 2.6 Fluorescence Spectra of 10 μM **1** with NO-donor, acetone/buffer 25:75, $\lambda_{\text{exc}} = 360 \text{ nm}$

In the comparison plot (Figure 2.7) of I_{514} from titration spectra under nitrogen (Figure 2.5) and under air (Figure 2.6) the consistency of response in aerobic and anaerobic conditions is readily observable. The higher sensitivity for low NO concentrations under anaerobic conditions could be attributed to the rapid reaction of NO with O_2 forming non-reactive oxidized products, such as NO_2 and N_2O_3 .

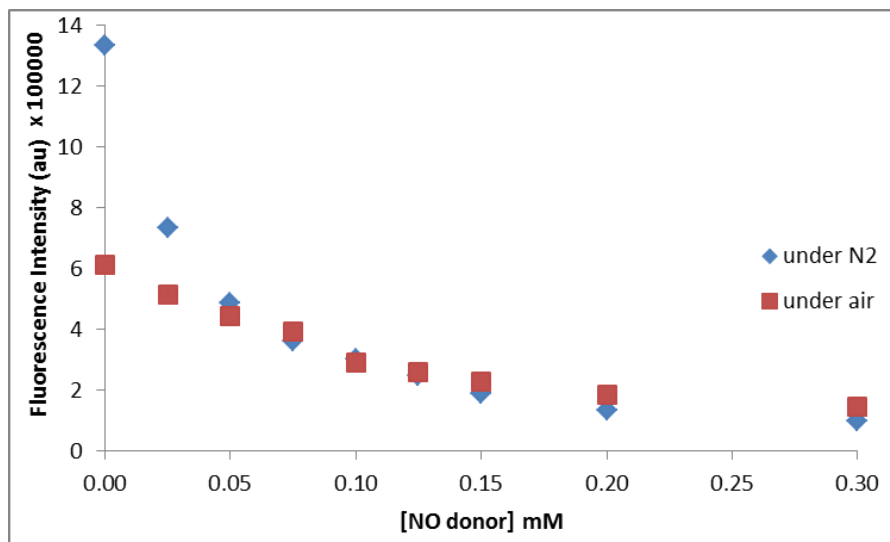


Figure 2.7 Fluorescence Titrations of **1** (10 μM) with NO-donor (0-300 μM), under N_2 vs. under air. $I_{514\text{nm}}$ vs. NO-donor concentration.

The same comparison for titration of **1** with NO under anaerobic *vs.* aerobic conditions was carried out also after photochemical preparation of the complex. Under such conditions the ratiometricity in anaerobic *vs.* aerobic conditions can be compared. Complex **1** prepared photochemically on a 1 mg scale (1.67 mM in THF) showed ratiometric response to NO addition at 560 nm (decrease) and at 455 nm (increase). The fluorescence spectrum of **1** in THF/acetone/buffer 2/23/75 shows one emission at 562 nm. The shift in emission compared to the isolated complex could be ascribed both to the difference in the solvent system, and the presence of unreacted (phen)Re(CO)₃SO₃CF₃, or (phen)Re(CO)₂(THF)SO₃CF₃ which emit at 560 nm and 585 nm respectively (see Chapter 3). Figure 2.8 shows the comparison of fluorescence intensity ratio (I_{454}/I_{562}) upon titration of **1** with NO, in the form of NO-donor MAHMA-NONOate (0-300 μ M) dissolved in 0.04 M NaOH in 25 μ L portions. The two plots in the graph compare the intensity ratios observed under nitrogen and under air. The ratiometric response under N₂ appears more consistent and linear, suggesting that the Re-dye reacts directly with NO and not with oxidized products, such as NO₂ or N₂O₃.

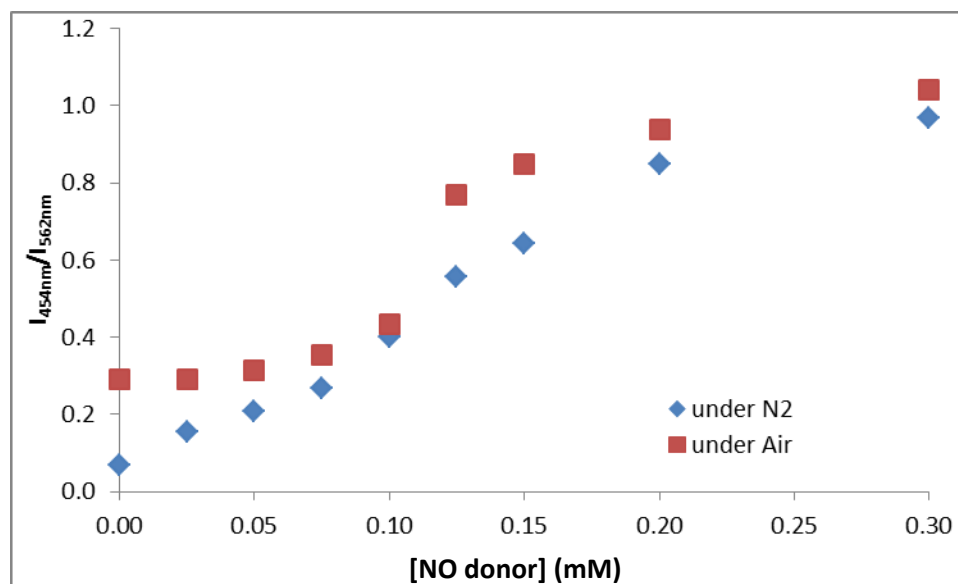


Figure 2.8 Graph of photochemically substituted (phen)Re(PPh₃)(CO)₂OSO₂CF₃ in THF 10 μM with NO, acetone/buffer 25:75, λ_{exc}=360 nm, comparison nitrogen vs air

2.4.4. Fluorescence titration with low concentrations of NO-donor.

Figure 2.9 shows the fluorescence emission of **1** upon titration with NO, in the form of NO-donor MAHMA-NONOate (0 - 150 μM) dissolved in 0.04 M NaOH, added in 1 - 25 μL portions after 10 min. intervals, under anaerobic conditions. The 514 nm decreases rapidly upon NO addition and emission at 426 nm increases correspondingly with NO-donor concentration. The NaCl concentration was lower than in the titration experiment described in 2.4.3 and this was found to reduce the intensity of the 514 nm emission, but not affecting the intensity of the emerging emission at 426 nm. The experiment was performed under nitrogen atmosphere in order to avoid NO oxidation to N₂O₃. The observed response under anaerobic conditions implies that sensing is a direct result of a reaction of NO with the Re(I) dye.

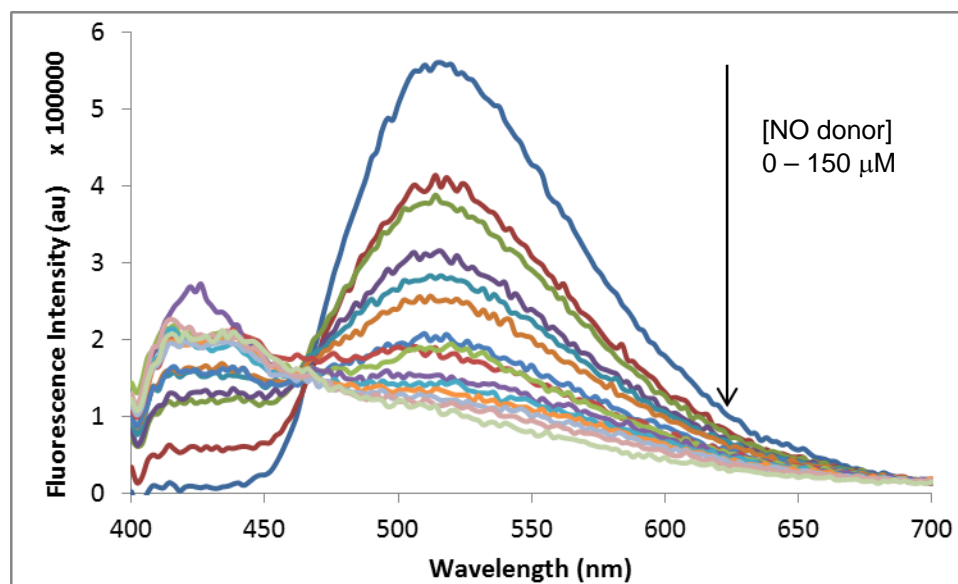


Figure 2.9 Fluorescence Spectra of 25 μM **1** (thermally prepared) with NO, acetone/buffer 25:75, $\lambda_{\text{exc}} = 360$ nm, under nitrogen.

Figure 2.10 exhibits complex **1** prepared photochemically on a 1 mg scale (1.67 mM in THF) showed ratiometric response to NO addition at 516 nm (decrease) and at 436 nm (increase).

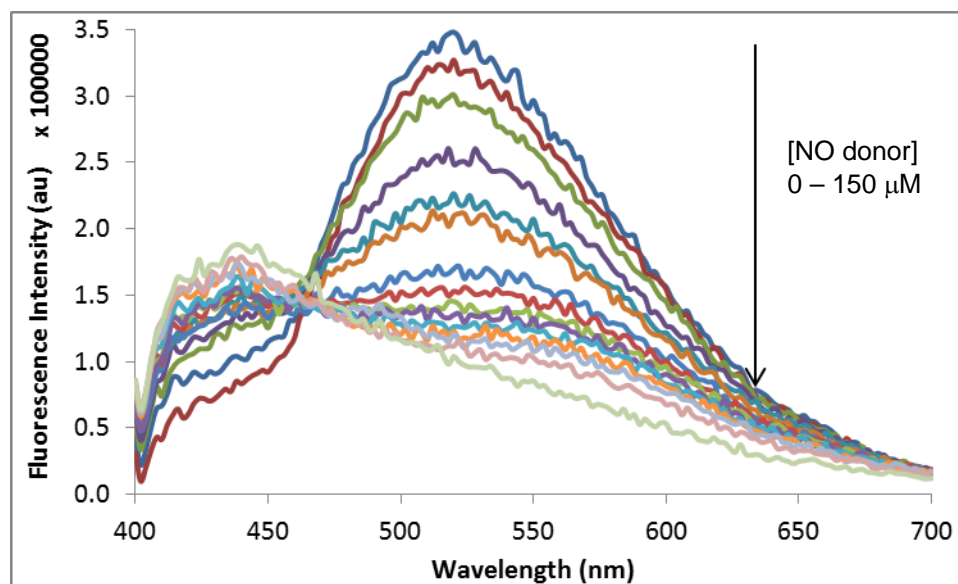


Figure 2.10 Fluorescence Spectra of 25 μM **1** (photochemically prepared) with NO, acetone/buffer 25:75, $\lambda_{\text{exc}} = 360$ nm, under nitrogen.

Figure 2.11 shows the change in fluorescence intensity with increasing NO-donor concentration. As exhibited in Figure 2.7, the intensity of the isolated (thermally produced) complex **1** is double than the photochemically produced mixture.

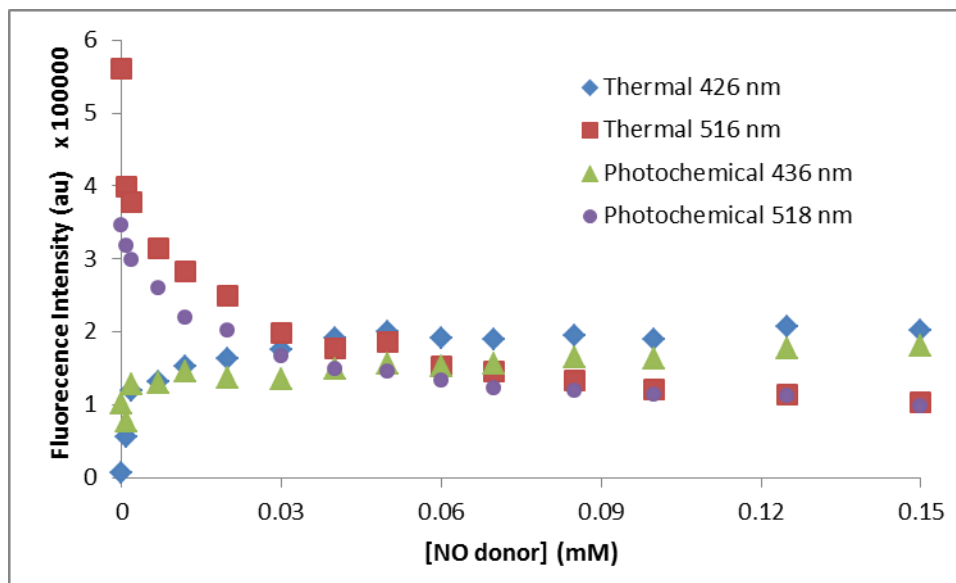


Figure 2.11 Observed fluorescence intensities of 25 μM **1** (thermally prepared) with gradual addition of NO-donor, acetone/buffer 25:75, $\lambda_{\text{exc}} = 360 \text{ nm}$

Figure 2.12 exhibits the comparison of fluorescence intensity ratios when the NO-donor concentration increases. The chosen signals were 426 nm and 515 nm for complex **1** prepared thermally; and 436 nm and 518 nm for complex **1** prepared photochemically. An important observation after analyzing the ratio of **1** prepared on either technique (photochemically or thermally) is the presence of two phenomena, higher response at concentrations lower than 5 μM of NO-donor than at high ones.

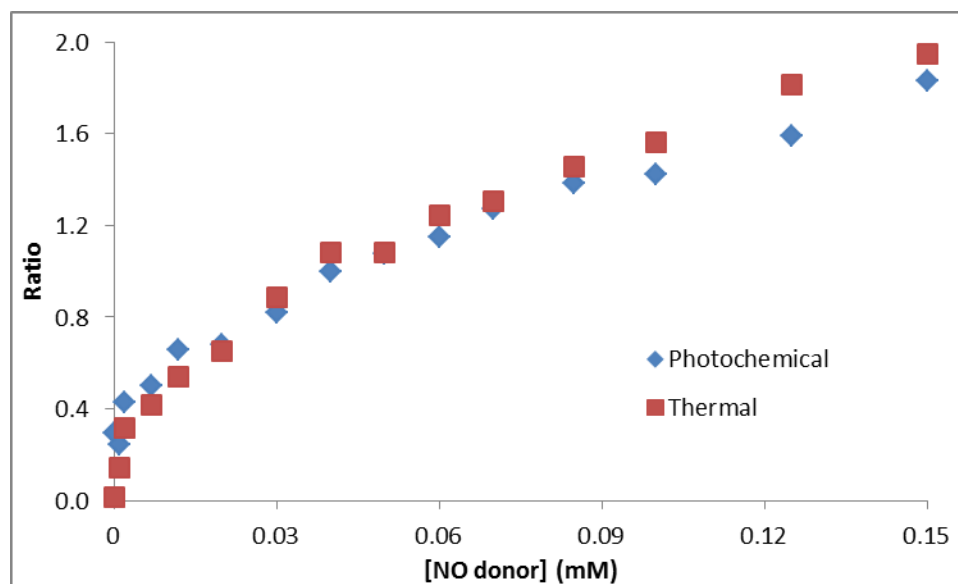


Figure 2.12 Observed fluorescence ratio of 25 μM **1** (photochemically and thermally prepared) with gradual addition of NO-donor, acetone/buffer 25:75, $\lambda_{\text{exc}} = 360 \text{ nm}$

2.4.5. FT-IR spectroscopy:

The FT-IR spectra were collected before (Fig. 2.9) and after the addition of NO-donor to $(\text{phen})\text{Re}(\text{PPh}_3)(\text{CO})_2\text{OSO}_2\text{CF}_3$. After adding NO, the solution was let evaporate for 12 hours, for removing acetone, the solids settled down in the tube. The remaining solution was decanted to remove basic impurities, such as MAHMA-NONOate reaction products and uncoordinated phen and analogs, and the spectrum was collected (Fig. 3.10). In the spectrum of the product after NO addition bands were observed at 1739 cm^{-1} , 1660 cm^{-1} and 1633 cm^{-1} , which are not present in the starting material and can be assigned to metal-nitrosyl complexes. These typically show stretching bands in the $1600 \text{ cm}^{-1} - 1750 \text{ cm}^{-1}$ range.¹⁸ Nitroso compounds (organic NO) typically appear below 1600 cm^{-1} . Even though NO stretching bands are typically strong they appear rather weak in our spectra, thus indicating that the Nitrosyl complex may not be the major product of the reaction of NO with the Re complex, nevertheless the

presence of these bands, which do not appear when there is no reaction with NO, are indicative of a Re-NO complex. The typical triflate signals between 1122 cm^{-1} and 1267 cm^{-1} are missing in figure 2.10.

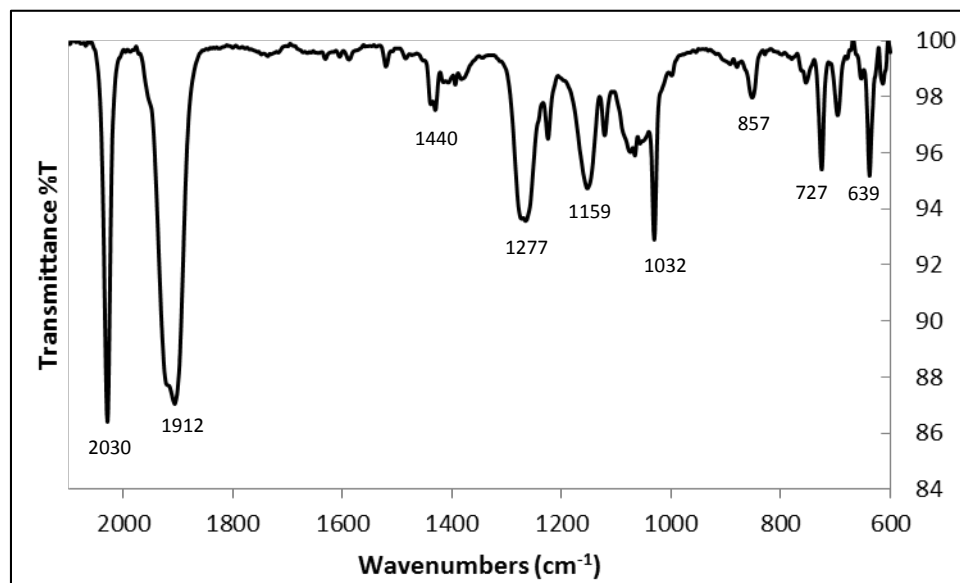


Figure 2.13 FT-IR spectrum of $(\text{phen})\text{Re}(\text{CO})_2(\text{PPh}_3)\text{OSO}_2\text{CF}_3$ (**1**)

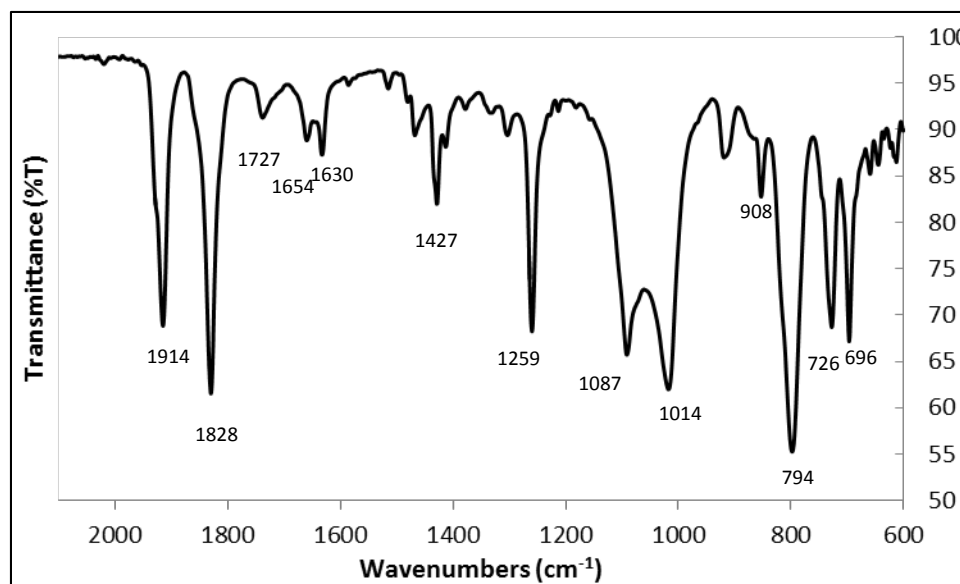


Figure 2.14 FT-IR spectrum of **2** after reaction with NO-donor.

2.4.6. ^1H -NMR and ^{19}F -Study of Re complex reactivity:

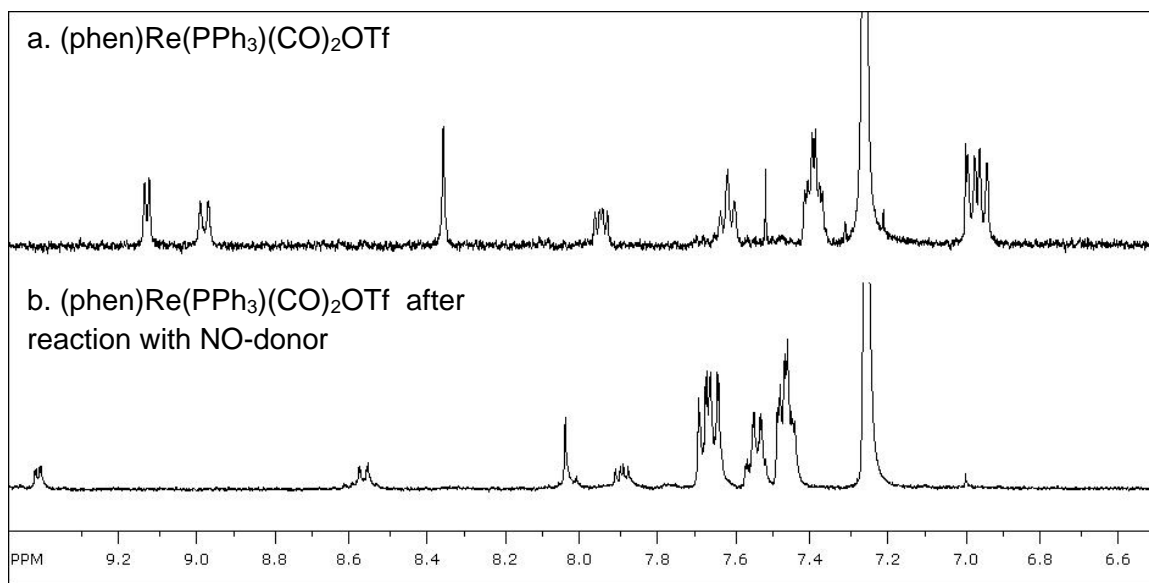


Figure 2.15 Aromatic region of NMR of **2** before (a) and after (b) reaction with NO-donor

Figure 2.15 shows the spectrum of (phen)Re(PPh₃)(CO)₂OSO₂CF₃ before (Fig. 2.15a) and after (Fig. 2.15b) reaction with NO-donor. Spectrum 2.15b clearly shows the resonances of free triphenylphosphine. Furthermore, experiments performed in identical conditions for control compounds (phen)Re(CO)₃Cl and (phen)Re(CO)₃OTf showed no changes after HCl/NaHCO₃ washing in the spectrum of (phen)Re(CO)₃Cl, while for (phen)Re(CO)₃OTf, resonances identical to (phen)Re(CO)₃Cl were observed, presumably because of substitution of triflate with Cl⁻. These experiments underscore the need for a labile L-type ligand, and suggest that triphenylphosphine dissociates for NO can react with the metal center. ^{19}F -NMR after NO-donor reaction showed no coordinated triflate signals at -78.4 ppm, before or after acid wash. Thus, it can be concluded that CF₃SO₃⁻ got dissociated after reaction with NO, suggesting that NO

displaces both triflate and PPh_3 in order to coordinate to Re(I) . Such mechanism is consistent with the rationale of using Re complexes containing labile L and X ligands for NO sensing.

2.4.7. Cell Studies & Fluorescence Microscopy:

The solution results and the observed fluorescence responses upon NO addition to **1**, suggested that these responses could also be observed in cells under aqueous media, 37 C and neutral pH. A first step towards applicability of this dye in living cells is to test its absorptivity and emission under physiological conditions. **1** was used as an initial model of absorptivity into Rat Aortic Endothelial Cells (RAOEC), since it could be readily prepared in a solid form and in dilute solutions.

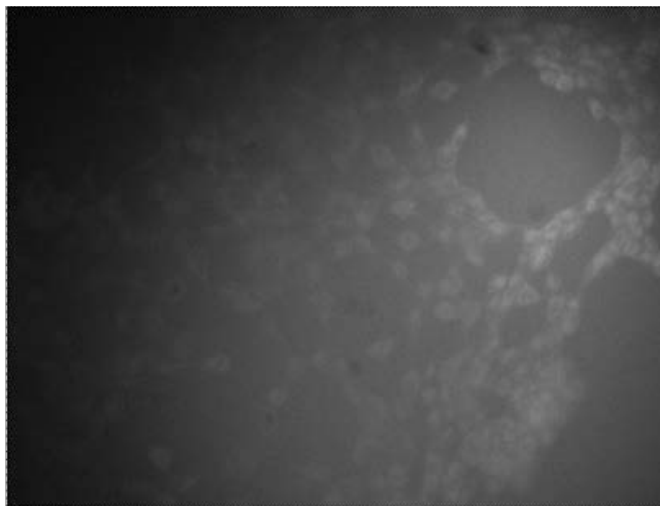


Figure 2.16 Fluorescence response of **2** ($10\ \mu\text{M}$) in RAOEC at $\lambda_{\text{em}} = 450\ \text{nm}$ ($\lambda_{\text{exc}} = 350\ \text{nm}$) showing the absorption of **2** into living cells.

Figure 2.16 shows that $(\text{phen})\text{Re}(\text{PPh}_3)(\text{CO})_2\text{OSO}_2\text{CF}_3$ demonstrated good absorptivity into cells via diffusion, in the same fashion that other fluorescent dyes.¹⁸

2.5. CONCLUSION

The complex (phen)Re(PPh₃)(CO)₂OSO₂CF₃ has been synthesized and characterized. This complex can react with NO under physiological conditions, producing a quenching in fluorescence that can be monitored. This complex has permeability through the cell membrane by diffusion and it can be used as fluorescent probe for NO oxide imaging. This is the first example of a rhenium phosphine complex used as an NO sensor.

2.6. REFERENCES

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3. (phen)Re(THF)(CO)₂OSO₂CF₃ AS A FLUORESCENT ORGANOMETALLIC COMPLEX NO SENSOR.

3.1. ABSTRACT

The complex (phen)Re(THF)(CO)₂OSO₂CF₃ (**2**) that can be obtained photochemically or thermally shows ratiometric response upon NO addition, which was added using the Z-1-N-methyl-N-[6-(N-methylammoniohexyl)amino]diazene-1,2-diolate (MAHMA-NONOate) as an NO-donor. Addition of 5-150 μM of NO-donor into a 20 μM solution of (phen)Re(THF)(CO)₂OSO₂CF₃ obtained thermally or photochemically from (phen)Re(CO)₃OSO₂CF₃ in 25% v/v acetone/phosphate saline(PBS) buffer gave fluorescence quenching at 582nm and a concurrent increase in emission at 450nm, upon excitation at 360nm. The FT-IR spectroscopy spectrum, upon addition of NO-donor, showed bands at 1720 cm⁻¹ and 1631cm⁻¹ that can be ascribed to nitrosyl complexes formed upon reaction with NO. Nuclear Magnetic Resonance (NMR) studies have shown that the NO fluorescence responses can be ascribed to the reactivity of the NO with the organometallic complex. Fluorescence microscopy studies on Rat Aortic Endothelial Cells in 2% v/v acetone/phosphate saline (PBS) buffer using 1 μM of **2**, and 1-10 μM of NO-donor gave notable increases in fluorescence emission at 450 nm ($\lambda_{exc} = 350\text{nm}$) and distinct incorporation of the dye into the cell. The system demonstrates the potential of a new approach for the design of fluorescent NO sensors based on the reactivity and fluorescence changes upon NO coordination to third-row transition-metal organometallic fluorescent complexes.

3.2. INTRODUCTION

Nitric oxide has several functions of particular importance for human health.^{1,2} It is now recognized as a key signaling molecule in the cardiovascular system because it regulates blood pressure via vasodilation.³ Despite numerous significant scientific contributions recently on NO physiology and biomedical functions,⁴ fundamental questions about basic physiological functions of NO remain unclear.^{5,6} Over the last decade, there have been several efforts to model NO-dependent signaling in the cardiovascular system^{7,8,9,10} and controversies regarding the role of NO in the vasculature have been discussed.¹¹ Thus, effective quantification and detection of NO in biological tissues is of paramount importance in understanding its role and physiology.

Quantification and imaging of NO in tissues remains a challenge because of the absence of a detection method that can combine significant spatial and temporal resolution with high NO selectivity¹². Challenges lie in NO's short lifetime ($t_{1/2} = ca. 5$ s) and the fact that it is found in low concentrations in living tissues.¹³

The NO-sensitive fluorescent dyes, such as diaminofluorescein, DAF-FM¹⁴ and Copper-Fluorescein Cu-FL,¹⁵ can provide practical methods for quantification and imaging of NO *in vitro* and *in vivo*. Some recent studies¹⁶ have reported fluorescent probes using different chemical concepts, including what is claimed to be the first ratiometric fluorescent probe for NO.^{16c} In a recent report a combined rhodamine-coumarin fluorophore showed ratiometric sensing for NO, with an fluorescence emission increase at 583 nm and a decrease at 473 nm even in cells.

2,2'-bipy and phenanthroline complexes of Re(I) and Ru(II) have been used as fluorescent platforms, and in photoluminescence applications.¹⁷ Their favorable

photochemical properties, stability of NO complexes,¹⁸ and flexibility in accommodating conjugated ligand systems offer potent scaffolds for the design of novel NO-selective dyes. Recently Choi and Lo¹⁹ have incorporated a diaminopyridine into a Re(phen) system to achieve sensing for NO, via the formation of a triazole derivative in the same fashion as it has been proposed for the DAF fluorophore reactivity with NO (via initial N₂O₃ formation). They observed an increase of fluorescence emission at 560 nm, yet this Re sensor was not found to be responsive at HeLa cells upon NOC-7 NO-donor addition. Mascharak and Fry synthesized and tuned ruthenium nitrosyls complexes for targeted NO delivery to cells to induce apoptosis.²⁰

As part of our efforts to explore new mechanisms of NO sensing using fluorescent organometallics, we are now demonstrating a concept of NO sensing based on NO coordination to a Re(I) center, resulting in a ratiometric response, upon NO addition in the form of the NO-donor MAHMA-NONOate. The ratiometric response is dependent on NO concentration, and is observed at pH 7.2 in solution (25% v/v acetone/PBS buffer) and also in Rat Aortic Endothelial Cells (2% v/v acetone/PBS), by fluorescence microscopy. The FT-IR and NMR spectra were consistent with the formation of nitrosyl complexes upon reaction of the Re(I)-THF-complex that may be responsible for the observed changes in fluorescence. These new complexes provide a novel concept for Nitric Oxide detection in biological tissues that is based on a radically different concept that takes advantage of the photophysical properties and synthetic versatility and reactivity of the Re(phen)system to give a ratiometric organometallic NO sensor. To our knowledge this is the first example of a ratiometric NO sensor that uses coordination of NO to a metal center, without a redox reaction at the metal center, and taking advantage

of the photophysical properties of phenanthroline complexes of third row transition metal organometallics.

In prior work (Chapter 2), it was shown that (phen)Re(PPh₃)(CO)₂OSO₂CF₃, prepared thermally or photochemically from (phen)Re(CO)₃OSO₂CF₃ has the ability to react with NO under physiological conditions, producing a fluorescence change at 514 nm and it has cell membrane permeability.

3.3. EXPERIMENTAL SECTION

3.3.1. Material and Methods

(phen)Re(CO)₃Cl and (phen)Re(CO)₃OSO₂CF₃ were prepared as previously reported and were found spectroscopically identical to the reported compounds.^{21,22} All other materials (purchased from Aldrich Chemical Co., ACROS organics, Cayman Chemicals, or Alfa Aesar) were standard reagent grade and were used without further purification, unless otherwise mentioned. Tetrahydrofuran (THF) used for the preparation of **2**, was anhydrous (purchased as an Aldrich Sure-Seal™ product), or distilled and dried before use. PBS buffer (0.002 M in total phosphate, pH = 7.2, [NaCl] = 0.04 M) was prepared from K₂HPO₄, KH₂PO₄, and NaCl (all from Fisher Scientific). Water used for preparation of the buffers and experiments with Nitric oxide was filtered water (18 MΩ·cm⁻¹) produced from a Millipore® nanopure diamond lab water system (Barnstead Thermolyne Corporation, Dubuque, IA). Deuterated solvents were purchased from Cambridge Isotope Laboratories Ltd. Rat Aortic Endothelial Cells acquired from Cell Biologics were used for the cell based experiments. Fetal Bovine Serum (FBS) and DMSO were bought from ATCC. Penicillin-Streptomycin and endothelial cell growth supplement from bovine neural tissue were bought from Sigma

Aldrich. DMEM/F-12 medium, Phosphate Buffer Saline (PBS) and Trypsin (0.05% EDTA) were bought from Invitrogen Inc. Cell culture flasks and dishes were purchased from Fisher Scientific. Photochemical reactions were carried out using a Rayonet photochemical reactor (Southern New England Ultraviolet, model RPR-100), equipped with a cooling fan and 10 phosphor coated low-pressure mercury lamps ($\lambda_{\max} = 300$ nm, light intensity = $5.2 \pm 0.1 \times 10^6$ photon/sec/cm³). Fluorescence Spectra were recorded on a Jobin Yvon Horiba Fluoromax 3 spectrometer. Fluorescence Microscopy was performed using an Olympus IX 81 Fluorescence Microscope and analyzed with IPLab v 4.04 Scientific Imaging Processing Software. Frequency-domain fluorescence lifetime measurements were performed using a ChronosFD spectrofluorometer (ISS, Champaign, IL). Samples were excited with a 370 nm modulated diode, and emission was collected using 400 nm long-pass filters (Andover, Salem, NH). The ¹H and ¹³C NMR spectra were recorded on a 400 Bruker NMR spectrometer and were referenced, using the residual solvent resonances. All chemical shifts, δ , are reported in ppm. The FT-IR spectra were recorded with a Perkin Elmer Spectrum 100 FT-IR Spectrometer.

3.3.2. Conversion of (phen)Re(CO)₃OSO₂CF₃ to (phen)Re(CO)₂(THF)OSO₂CF₃

3.3.2.1. Method 1 - Thermal Substitution:

(phen)Re(CO)₃OSO₂CF₃ (1 mg, 1.67 μ mol) and 1.00 mL of anhydrous tetrahydrofurane (final concentration 1.67 mM) were placed in a 10 mL round bottom flask, under N₂ flow. The solution was refluxed for 48 h. After refluxing, the color of the solution changed from yellow to amber. This solution was used without further treatment.

3.3.2.2.Method 2 - Photochemical Substitution:

(phen)Re(CO)₃OSO₂CF₃ (1 mg, 1.67 μmol) and 1.00 mL of anhydrous tetrahydrofuran (final concentration 1.67 mM) were placed in a NMR tube. The solution was irradiated with a Rayonet photochemical reactor (Southern New England Ultraviolet, model RPR-100), equipped with a cooling fan and 10 phosphor coated low-pressure mercury lamps ($\lambda_{\max} = 300$ nm, light intensity = $5.2 \pm 0.1 \times 10^6$ photon/sec/cm³). After irradiation the color of the solution changed from yellow to amber. This solution was used without further treatment. When this procedure was performed in d₈-THF, a set of signals different from the starting material were observed. ¹H-NMR (THF-d₈): 7.98 (d, 2H, ³J_{H-H} = 8.2 Hz, ³J_{H-H} = 5.1 Hz), 8.36 (s, 2H), 8.99 (d, 2H, ³J_{H-H} = 8.28 Hz, ⁴J_{H-H} = 1.4 Hz), 9.14 (d, 2H, ³J_{H-H} = 4.7 Hz, ⁴J_{H-H} = 1.4 Hz). ¹⁹F-¹H-NMR (CDCl₃): -78.1 ppm. FT-IR (cm⁻¹): 2033 (ν_{CO}, vs), 1910 (ν_{CO}, vs), 1430, 1290, 1246, 1170, 1031, 848, 725, 637. UV-Vis (in ethanol): $\lambda_{\max} = 360$ nm - br ($\epsilon = 4500$ mol⁻¹cm⁻¹), Fluorescence emission in acetone ($\lambda_{\text{exc}} = 360$ nm): 585nm $\Phi_1=0.002$ (at 370 nm, in 1:3 acetone:PBS, using quinine sulfate in sulfuric acid 0.1 M as reference $\Phi_R=0.546$). Lifetime: $\tau_1 = 2.26 \pm 0.6$ ns, $f_1 = 0.114 \pm 0.005$, $\alpha_1 = 0.0504$; $\tau_2 = 41.3 \pm 1$ ns, $f_2 = 0.457 \pm 0.01$, $\alpha_2 = 0.0111$; $\tau_3 = 144 \pm 6$ ns, $f_3 = 0.436 \pm 0.01$, $\alpha_3 = 0.00302$. The presence of 3 lifetimes symbolizes three different processes that happen at the same time. There is an ultrafast reaction, it may be a rapid exchange solvent substitution (substitution of THF by water), leading to inactivation.

3.3.3. Fluorescence studies with addition of NO-donor

In a typical experiment a THF solution of Re complex prepared as per 2.3.2.1 or 2.3.2.2. (24 μL , 1.67 mM) is added to 970 μL of acetone to result in 1.000 mL of a 100 μM solution in 97:3 v/v acetone:THF. A quantity of 2.75 mL to 3.00 mL of aqueous PBS buffer (pH=7.2) was added. Then 0 to 150 μL of 4 mM stock solution of MAHMA-NONOate NO-donor, in 0.04 M NaOH were added, in order to obtain 75% v/v aqueous/organic solution and a final NO-donor concentration from 0 to 150 μM range, while to concentration of the Re complex remains constant at 20 μM . No more than 10 minutes elapsed from preparation of the organic phase to the addition of NO-donor. The solution was mixed thoroughly in a vortex and sonicated for 90 min at room temperature. The fluorescence of all solutions was measured at $\lambda_{\text{exc}} = 360$ nm and recorded from 400 nm to 700 nm. For a control experiment a NaOH stock solution was used instead of an NO-donor/NaOH stock, in order to account for any possible variations in the fluorescence due to pH changes. The result of the control titrations gave identical spectra for all titration points for the 1-150 μL range.

3.3.4. ^1H -NMR and ^{19}F -NMR study of reactivity of Re complexes with NO:

(phen)Re(THF)(CO)₂OSO₂CF₃ (1 mg), (phen)Re(PPh₃)(CO)₂OSO₂CF₃ (1 mg),²³ (phen)Re(CO)₃Cl (1 mg), or (phen)Re(CO)₃OSO₂CF₃ (1 mg) were added to 0.05 mL of THF in four separate 10 mL vials. 1.6 mL of acetone was added. Then 2.4 mL of PBS aqueous buffer (pH = 7.2) and 0.2 mL of stock NO-donor solution 5 mM in 0.04 M NaOH were added sequentially. The mixtures were vortexed for 30 sec and then sonicated for 3h at room temperature. Dichloromethane was added (3 x 10 mL), and the

organic layer was dried with anhydrous sodium sulfate. The solvent was evaporated and the non-volatiles were taken up in chloroform and the NMR spectra were collected. In order to remove uncomplexed phenanthroline products and collect only the NMR of complexes the organic phases were further diluted with dichloromethane and washed with 1 M hydrochloric acid, followed by 1 M sodium bicarbonate, rinsed with deionized water, and dried with anhydrous sodium sulfate. The volatiles were evaporated, the solid residues were redissolved in chloroform-d, and the NMR spectra were collected. These experiments were also performed under identical conditions for control compounds (phen)Re(CO)₃Cl and (phen)Re(CO)₃OTf. After HCl/NaHCO₃ washing no changes were observed in the ¹H-NMR spectrum of (phen)Re(CO)₃Cl, while for (phen)Re(CO)₃OTf, resonances identical to (phen)Re(CO)₃Cl were observed.

3.3.5. Fluorescence microscopy studies of addition of NO-donor to (phen)Re(THF)(CO)₂OSO₂CF₃ in Rat Aortic Endothelial Cells:

Cell preparation: Rat Aortic Endothelial Cells were grown in Duplecco's Modified Eagle Medium: Nutrient Mixture F-12 medium (DMEM/F-12) supplemented with 10% Fetal Bovine Serum (FBS), 1% penicillin-streptomycin, and endothelial cell growth supplement in a humidified tissue culture incubator at 37°C equilibrated with 95/5 v/v% Air/CO₂ mixture. Cells were cultured in 35 × 10 mm dishes seeded with 2 × 10⁵ cells and used at 60-80% confluency.

Solution Preparation: A 500 μM solution of Re(phen)(THF)(CO)₂OSO₃CF₃ in THF/acetone (Solution B) was prepared from 62.5 μL of a stock solution of Re(phen)(THF)(CO)₂OSO₃CF₃ in THF (1.6 × 10⁻³ M - Solution A) and 138 μL of acetone. This solution was added to 800 μL of 10 mM PBS buffer solution (pH=7.2),

resulting in a final 100 μM solution of **1** in 80/14/6 v/v aqueous buffer/acetone/THF. This new solution was further diluted 1/10 v/v with PBS Buffer before adding it to the cells, resulting in a final solution of 10 μM of **1** in 98/1.4/0.6 v/v aqueous PBS buffer/acetone/THF (Solution C). Various quantities of 6 mM NO-donor solution (MAHMA-NONOate) in 0.01M NaOH were diluted with 0.01M NaOH to give 0, 100, 500 & 1000 μM solutions of NO-donor (Solutions D-G).

Loading of solution C (10 μM of Re complex **1**): The old media was poured out from the plates; then the plates were rinsed twice with 1 mL of 10 mM PBS (pH = 7.2). Then 100 μL of DMEM/FBS/Penicillin Media, 800 μL of PBS 10 mM, and 100 μL of Re complex stock (Solution C) were added with this specific order. The plates were incubated for 75 min. at 37 °C equilibrated with 95/5 v/v% Air/CO₂ mixture.

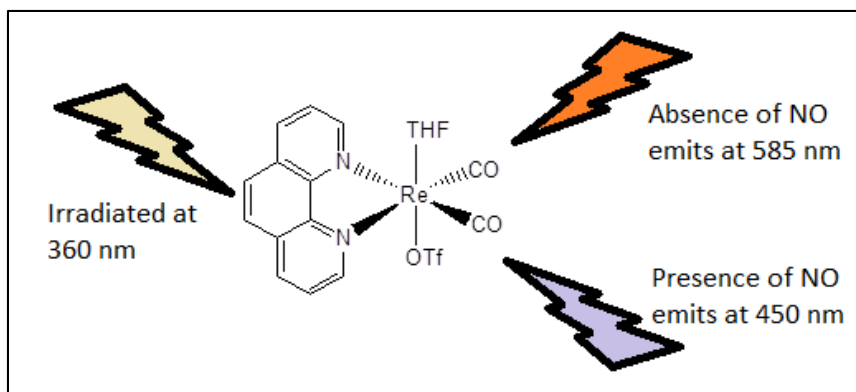
Loading of NO-donor: First the reactive media was poured out from the plate; then the plates were rinsed twice with 1 mL of 10 mM PBS (pH = 7.2). Then 100 μL of DMEM/FBS/Penicillin Media, 890 μL of PBS 10 mM, and 10 μL of NO-donor solution in NaOH from 0-1000 μM range (Solutions D-G) were added to give final NO-donor concentrations of 0 μM , 1 μM , 5 μM , and 10 μM . The plates were incubated for 15 min; the reactive media was poured out from the plate, and 1 mL of fresh PBS 10 mM was added to each plate.

Fluorescence Microscopy Measurements: Each plate was placed in the fluorescence microscope and measured under a filter an excitation wavelength of 350 nm (330-380 nm window) and an emission window of 440-460 nm. The images were collected with a total exposition time of 2668 ms and 20X zoom.

3.4. RESULTS AND DISCUSSION

3.4.1. Design Considerations

To achieve our goal of obtaining a ratiometric fluorescent probe that can detect changes in NO concentration with high sensitivity and selectivity, we are using an entirely new approach for NO sensing that combines the following elements: 1) NO binding to a Re(I) metal center, 2) a phenanthroline, attached on the metal, which provides favorable photophysical characteristics,¹⁷ as well potential for modification, for fine-tuning the emission wavelength, and 3) a labile ligand (THF or OSO_2CF_3) which is expected to be displaced by NO altering the fluorescence emission upon NO addition. NO coordination to the Re center is expected to shift the MLCT band to higher frequency, since NO is a π -acceptor, unlike THF that is a donor.



Scheme 3.1 Concept diagram for phenanthroline-rhenium NO sensors

3.4.2. Fluorescence Studies:

Solutions of $(\text{phen})\text{Re}(\text{THF})(\text{CO})_2\text{OSO}_3\text{CF}_3$ prepared photochemically or thermally were let to react with NO-donor in PBS buffer, without prior isolation of these species. Absorption Spectra of $(\text{phen})\text{Re}(\text{THF})(\text{CO})_2\text{OSO}_2\text{CF}_3$ in methanol and

fluorescence spectra of solutions of (phen)Re(THF)(CO)₂OSO₂CF₃ in acetone show characteristic differences from the spectra of the triflate complex with a single intense fluorescence emission at 585 nm. However in the presence of water, such as in PBS buffer before addition of NO-donor, there is also a weak emission at 435 nm, which could be ascribed to decomposition species. An NMR study of the conversion of the triflate complex to **1** showed clearly the resonances of a new complex consistent with the formulation presented and the THF and triflate in a *trans*- configuration. For the isolated triphenylphosphine analog **2**, a single emission at 514 nm is observed.

For complex **1** prepared photochemically as soon as NO-donor was added to the solution, (10 μM to 250 μM concentration range) the emission intensity at 585 nm decreased while the emission intensity at 435 nm increased and its maximum was shifted to 445 nm (Figures 3.1 and 3.2). However, it appears to be an anomaly for the 10 μM and 20 μM points, (Figure 3.2), since the trend is opposite for small additions of NO-donor/NaOH solution. This could be ascribed to the fact that the zero point does not have any NaOH. Similar results were observed when the complex was prepared thermally (Figure 3). The emission at 585 nm appears to be quenching before the increase of the emission at 445 nm becomes prominent. This may be indicative of a two-step process, with a first step that may involve the partial dissociation of the THF ligand followed by radiation-less radical reactions, and a second step forming a species with a fluorescence emission at 445 nm. As a control, experiments with (phen)Re(CO)₃Cl and (phen)Re(CO)₃OSO₂CF₃ under identical conditions showed no changes in the fluorescence spectra.

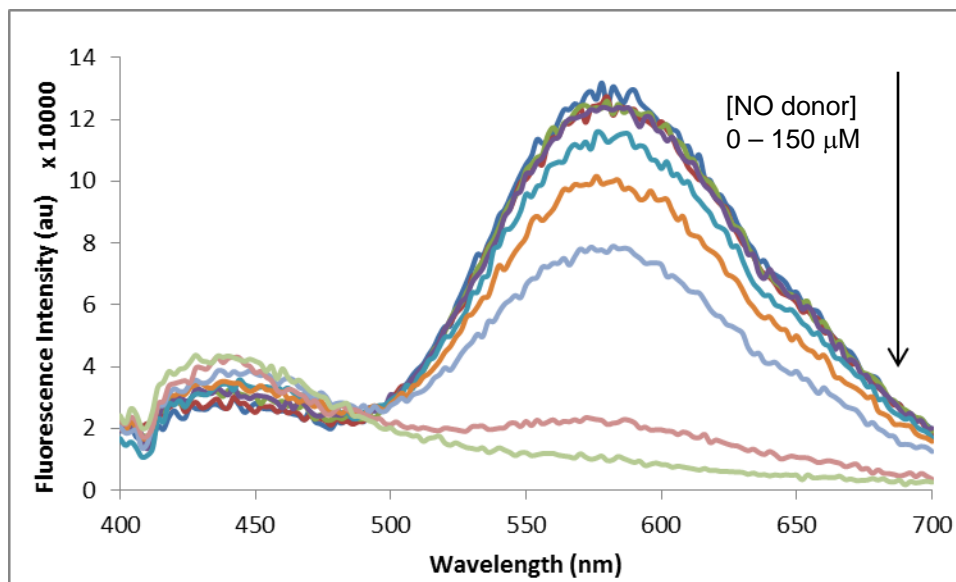


Figure 3.1 Fluorescence Response upon NO-donor addition (0-150 μM) to 20 μM of Re complex **2** (generated by photochemical substitution) in acetone/PBS aqueous buffer 25/75 v/v, ($\lambda_{\text{exc}} = 360 \text{ nm}$)

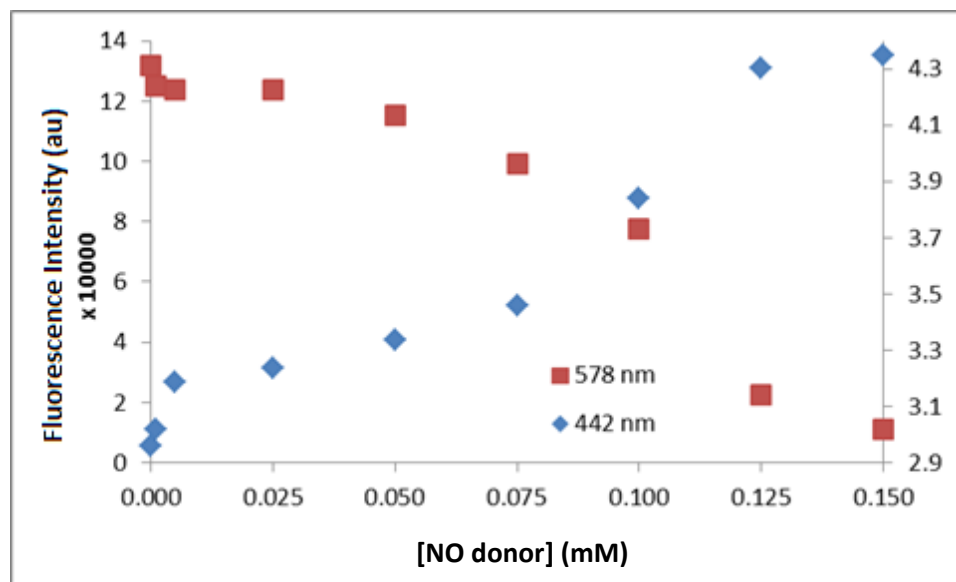


Figure 3.2 Observed Fluorescence intensities $\lambda_{\text{max}} = 450 \text{ nm}$ and $\lambda_{\text{max}} = 582 \text{ nm}$ upon NO-donor addition $[\mathbf{2}] = 40 \mu\text{M}$ ($\lambda_{\text{exc}} = 360 \text{ nm}$).

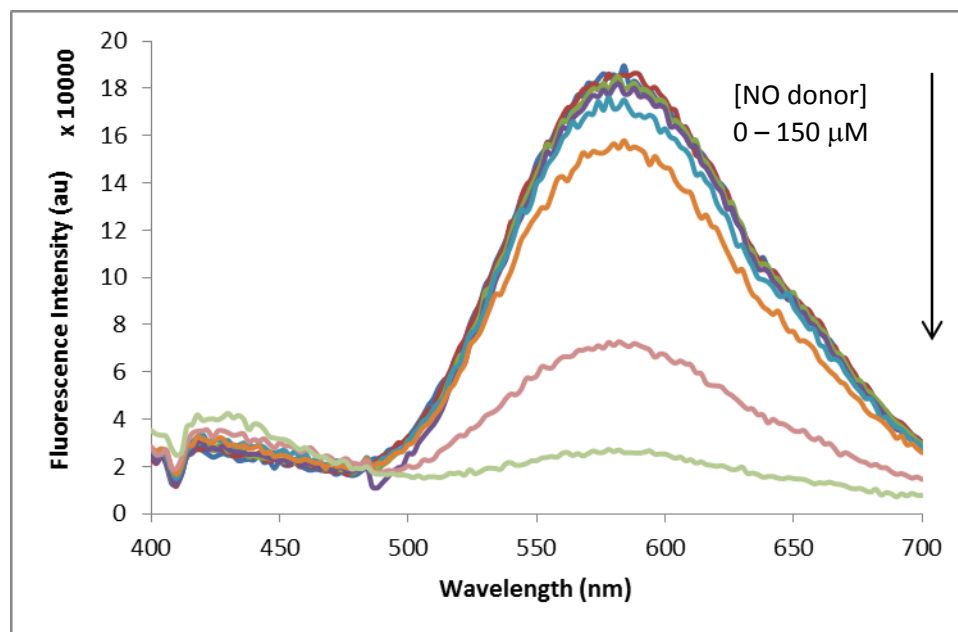


Figure 3.3 Fluorescence Response upon NO-donor addition (0-150 μM) to 20 μM of Re complex **2** (generated by thermal substitution) in acetone/PBS aqueous buffer 25/75 v/v, ($\lambda_{\text{exc}}=360$ nm)

Having similar results but not identical suggest that the degree of conversion may vary between the two methods but the identity of the final product is the same. A blank experiment was performed using only gradual increasing amounts of sodium hydroxide solution in absence of MAHMA NONOate; in the outcome there was no reaction at all, this confirm that it is the reaction with NO-donor the responsible of the change in fluorescence.

Fluorescence titration with gradual addition of NO-donor under anaerobic and aerobic

conditions: For fluorescence titration measurements, a stock solution of (phen)Re(CO)₃OSO₂CF₃ in THF with a mass concentration of 1 mg/mL was freshly prepared and irradiated at 300 nm in a 10 lamp photoreactor for 75 minutes, obtaining a solution of the complex. Then, 67 μL of this stock solution was diluted in 683 μL of acetone and 2250 μL of phosphate buffer 0.002 M pH 7.2 with sodium chloride 0.04 M

(resulting in a total volume of 3 mL and a final mass concentration of 25 μM) in a quartz glass cuvette. The fluorescence emission was monitored using λ_{exc} of 360 nm. The bandpass for excitation and emission was 2 nm. A stock solution of MAHMA NONOate in NaOH 0.04M with a mass concentration of 3 mmol/mL was freshly prepared; this stock solution was dispensed stepwise in portions of 25 μL from a 50 μL syringe into the fluorescence cuvette. After each step, the solution was left react for 10 minutes (approximately, 3 MAHMA NONOate half-life) of at room temperature before measuring fluorescence using the same parameters as above.

In another cuvette, it was prepared other 3000 μL of (phen)Re(THF)(CO)₂OSO₂CF₃ in the same THF/acetone/buffer (see above) was bubbled with nitrogen for 30 minutes. The fluorescence titration was performed under the same conditions than above.

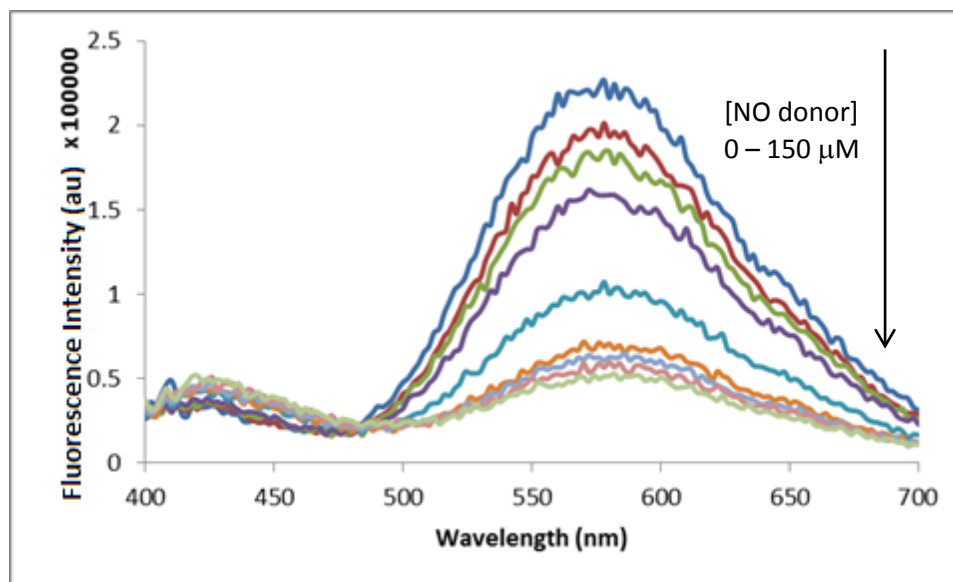


Figure 3.4 Fluorescence Spectra of 2 25 μM with NO, acetone/buffer 25:75, λ_{exc} =360 nm, under nitrogen

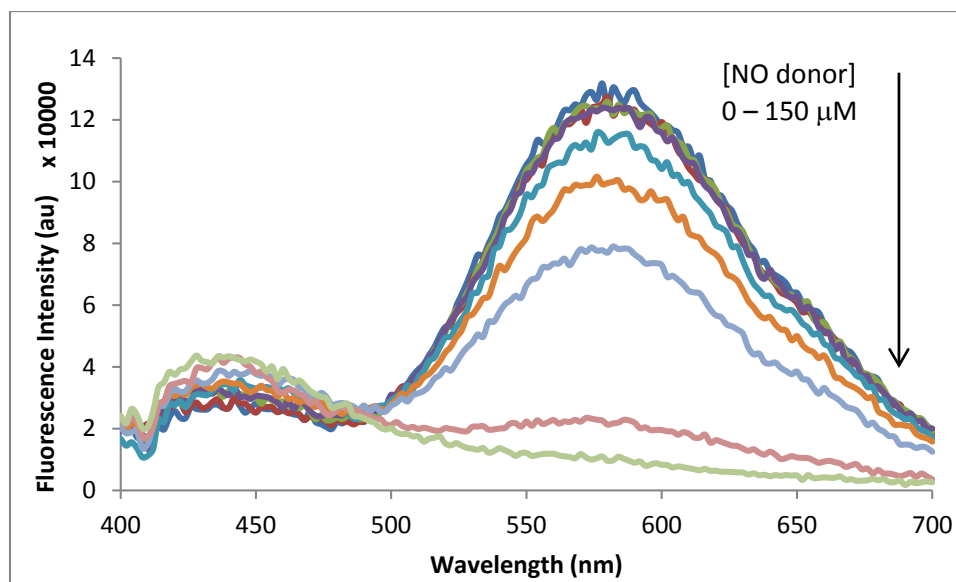


Figure 3.5 Fluorescence Spectra of **2** 25 μ M with NO, acetone/buffer 25:75, λ_{exc} =360 nm

The results were as expected, quenching signal at 585 nm versus a slight increment in the signal around 430nm. After performing the titration under air or under nitrogen exhibiting similar behaviors, it can be concluded that it is no need to an oxidant environment in order the reaction to occur; like in the triazol formation based dyes. On the same tenor, it can be concluded that NO is the real substrate.

The intensity of the titration performed under air is 40% lower than under nitrogen, and the response towards NO addition is lower in the same extend, that can be explained by oxidation of the real substrate NO to NO₂, and this second one cannot be used as a substrate for this Re complex.

One noticeable result is that in both cases the increment in the 430 nm signal is the same in both cases. It can be concluded that this signal depends only on the product after addition of NO; and no on the side reaction of the starting material.

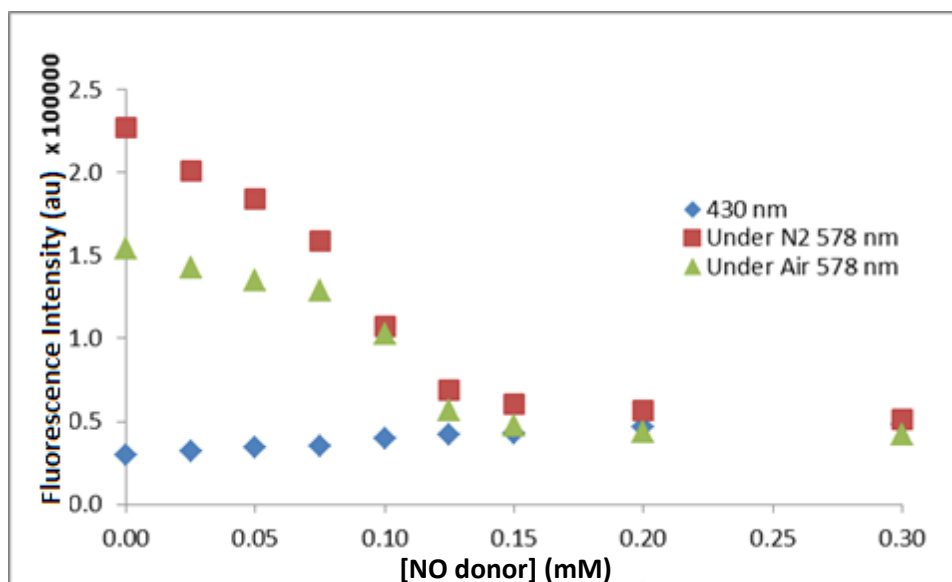


Figure 3.6 Fluorescence Spectra of (phen)Re(THF)(CO)₂OSO₂CF₃/THF 30 μ M with NO, acetone/buffer 25:75, $\lambda_{exc} = 360$ nm, comparison nitrogen vs air

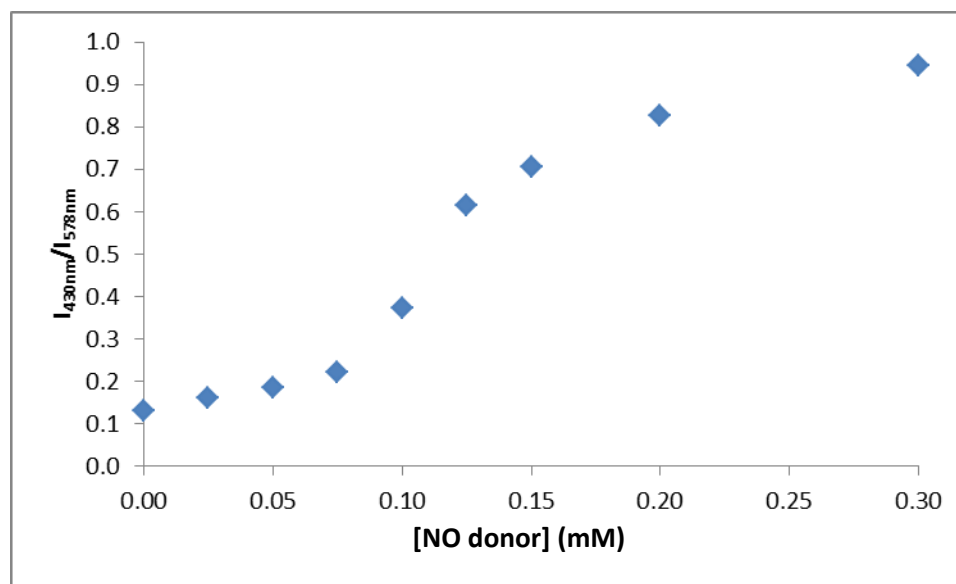


Figure 3.7 Fluorescence intensity ratio of 430 nm/578 nm for **2**(25 μ M) with NO-donor addition (0 - 150 μ M)

There was a linear response at the lower range; until 3 times (phen)Re(THF)(CO)₂OSO₂CF₃ complex concentration was reached; saturation was not reached.

3.4.3. FT-IR spectroscopy:

The FT-IR spectra were collected before (Fig. 3.8) and after the addition of NO-donor to **1**. After adding NO, the solutions were treated by an acid/base wash (1 M HCl / 1 M NaHCO₃ in order to remove basic impurities, such as MAHMA-NONOate reaction products and uncoordinated phen and analogs, and the spectrum was collected (Fig. 3.9). In the spectrum of the product after NO addition bands were observed at 1720 cm⁻¹ and 1631 cm⁻¹, which are not present in the starting material and can be assigned to metal-nitrosyl complexes. These typically show stretching bands in the 1600 cm⁻¹ - 1750 cm⁻¹ range.^{18c,18d} Nitroso compounds (organic NO) typically appear below 1600 cm⁻¹. Even though NO stretching bands are typically strong they appear rather weak in our spectra, thus indicating that the Nitrosyl complex may not be the major product of the reaction of NO with the Re complex, nevertheless the presence of these bands, which do not appear when there is not reaction with NO, are indicative of a Re-NO complex.

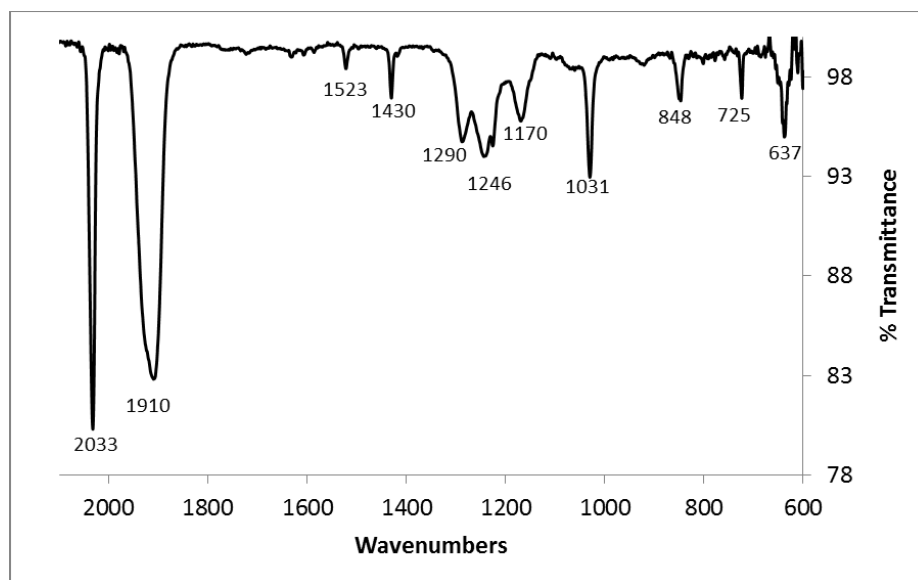


Figure 3.8 FT-IR spectrum of **2**

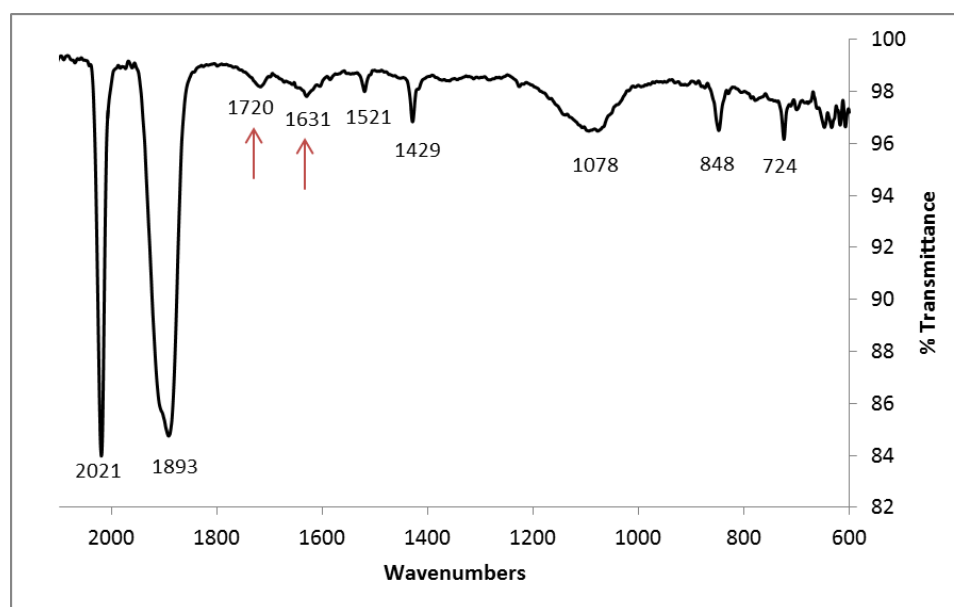


Figure 3.9 FT-IR spectrum of **2** after reaction with NO-donor and wash with 1 M HCl/1 M NaHCO₃.

3.4.4. ^1H -NMR and ^{19}F -NMR Study of Re complex reactivity:

Formation of **1** from $(\text{phen})\text{Re}(\text{CO})_3(\text{OSO}_2\text{CF}_3)$ in d_8 -THF:

Photosubstitution of $(\text{phen})\text{Re}(\text{CO})_3\text{OSO}_2\text{CF}_3$ in d_8 -THF allowed monitoring of conversion into complex **1** by NMR spectroscopy (Fig. 2.10). Two different species (A and B) can be identified in the spectrum with integration of ratios of 80/20 after 30 min, while a third species (C) appears after 90 min with an integration ratio of 7 to 62(for A) to 32 (For B). These three species are presumed to be the starting material (A), and the *trans*-THF/triflate *cis*-CO complex (B), and the *cis*-THF/triflate *cis*-CO complex (C). The assignments are based on the higher observed symmetry of the spectral pattern for B.

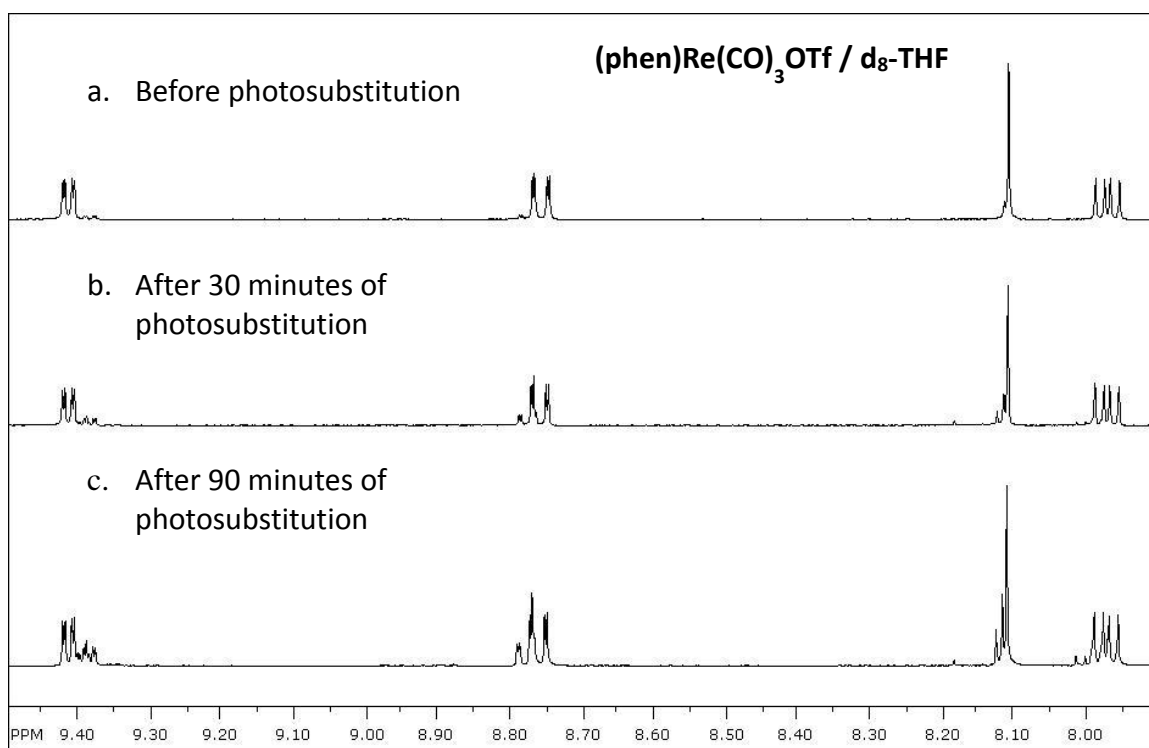


Figure 3.10 Aromatic region of ^1H -NMR spectra (d_8 -THF) for **2** (top), **2** after 30 min of UV irradiation at 300 nm (middle), and after 90 min of UV irradiation (bottom).

Even before the reaction mixture was irradiated there was a small conversion to the *trans*-THF-triflate complex (Fig. 3.10a). The isolated and spectroscopically

characterized phosphine analog **1**, (Chapter 2), which is in the *trans* PPh₃-triflate configuration was found to have analogous spectroscopic symmetric splitting pattern with the major product of the reaction in d₈-THF assigned as *trans*- (Species B).

NMR study of reactivity of rhenium complexes with NO: The ¹H-NMR spectra of (phen)Re(CO)₃Cl and phenanthroline do not show spectral changes under irradiation conditions with THF, and furthermore no spectroscopic changes are observed even after the addition of NO-donor to the irradiated solutions. The triflate complex however showed some spectral changes upon reaction with 20-fold excess of NO donor (or 40-fold excess of NO as each MAHMA-NONOate gives 2 NO molecules which can be compared with the NMR spectra of complexes **1** and **2** under the same conditions(Figure 3.11a).

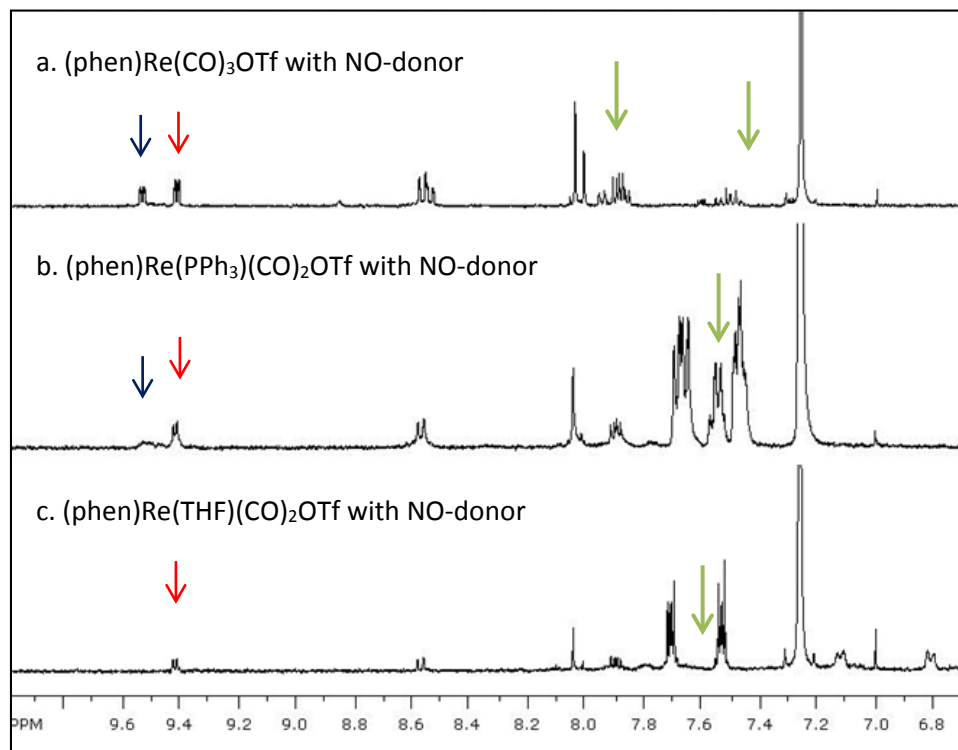


Figure 3.11 Aromatic region of ¹H-NMR spectra after reaction with NO-donor (d₈-THF) of (a) (phen)Re(CO)₃OSO₂CF₃, (b): **1** (c): **2**.

The spectrum of (phen)Re(CO)₃OSO₂CF₃ upon reaction with NO shows two sets of phenanthroline signals (4 signals), and the appearance of a new set of signals at δ 7.4 and 7.8 ppm (Figure 3.11a). For the phosphine complex the integration between the two sets of signals (products vs. starting material) shows higher ratio of products vs. starting material, with an additional set of signals overlapping with the triphenylphosphine signal (Fig. 3.11b). The integration ratios and chemical shift changes indicate triphenylphosphine dissociation. Phenanthroline is also dissociated upon NO reaction and phenanthroline-containing byproducts are formed. For the THF complex **2**, the signals from the starting material are completely consumed; signals at δ = 7.4 and 7.8 ppm are the most intense and those signals do not exhibit phenanthroline splitting pattern. Integration of THF resonances in the 1.8 - 2.8 ppm region also indicate THF dissociation from Re upon reaction with NO.

Acid/Base wash studies: Phenanthrolines and substituted phenanthrolines, which are not bound to the rhenium center, are basic and can be removed (together with the amines formed as byproducts from reaction of MAHMA NONOate) by acid wash with 1.0 M HCl, (Fig. 3.12).²³ After **2** reacts with NO-donor (5-fold excess) it displays a complicated spectrum, (Fig. 3.12a) caused by the presence of six different species, of which only two appear to contain Re. After treatment with 1M HCl, and then 1M NaHCO₃, the basic organic species are removed, and only the Re-containing species remain in the spectrum.

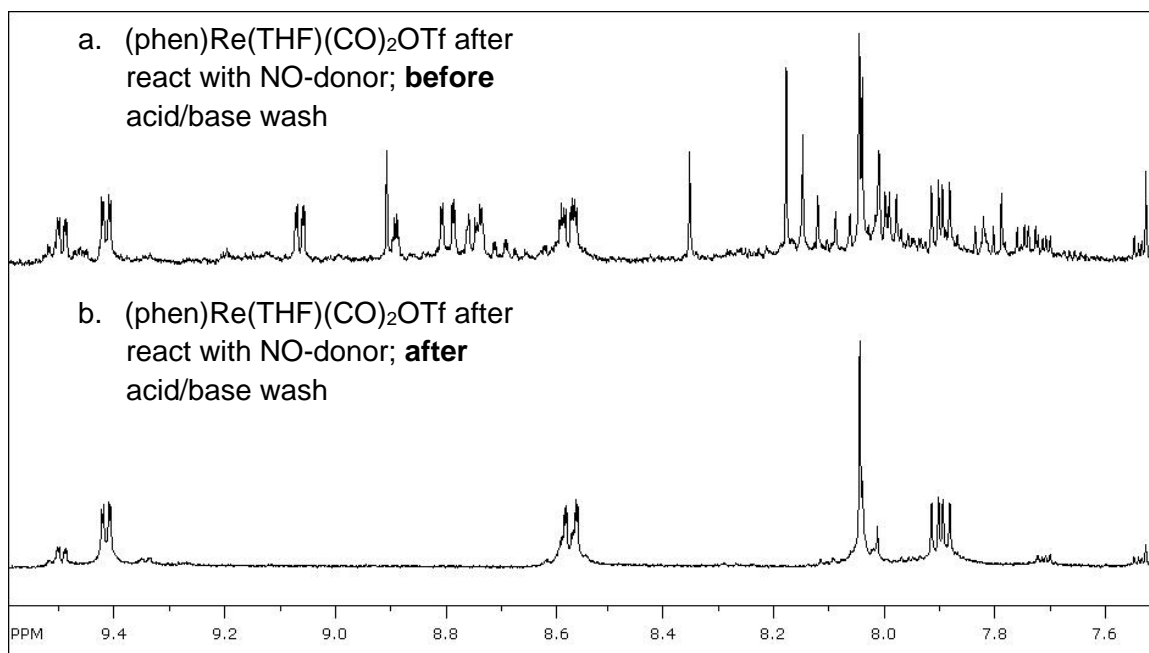


Figure 3.12 NMR spectra (aromatic region) for the reaction of **2** with NO-donor before (a) and after (b) wash with 1M HCl / 1M NaHCO₃.

One of the species present is (phen)Re(CO)₃Cl, possibly formed after reaction of the coordinatively unsaturated Re(I) center resulting after dissociation of THF and OSO₂CF₃⁻ ligands. The chemical shifts of the second species are completely different from complex **2** and are consistent with a species that has no longer contains a coordinated CF₃SO₃⁻ ligand and in which the THF has been replaced by a π -acid such as NO. In summary, with the exception of (phen)Re(CO)₃Cl, all the NMR spectra show a pattern of a) signals corresponding to non-coordinated to Re ortho-di-substituted phenanthroline which practically disappear after treatment with 1M HCl / 1M NaHCO₃ b) signals corresponding to possibly a Re nitrosyl complex (or complexes) and c) signals corresponding to (phen)Re(CO)₃Cl presumably formed from unreacted (phen)Re(CO)₃OSO₂CF₃. The intensity patterns for those peaks indicate a decrease in reactivity moving from **2**, to **1**, to (phen)Re(CO)₃OSO₂CF₃.

These experiments underscore the need for a labile L-type ligand, and suggest that triphenylphosphine dissociates in order for NO to react with the metal center. ^{19}F -NMR after NO-donor reaction showed no coordinated triflate signals at -78.4 ppm, before or after acid wash. Thus, it can be concluded that CF_3SO_3^- got dissociated after reaction with NO, suggesting that NO displaces both triflate and PPh_3 in order to coordinate to Re(I) .

3.4.5. Cell Studies & Fluorescence Microscopy:

Fluorescent dyes should be able to detect NO in biological systems, such as cells and endothelial tissues, in order to be practical for NO imaging and study of mechanistic aspects of its physiology.

Reactivity of 2 in RAOEC: Fluorescent dyes should be able to give a visible fluorescence response to different intracellular NO concentrations. Fig 3.13 shows that increasing the concentration of NO leads to increases in fluorescence intensity. Beyond 10 μM NO, the RAOEC cells showed signs of shrinking, indicative of cell death.

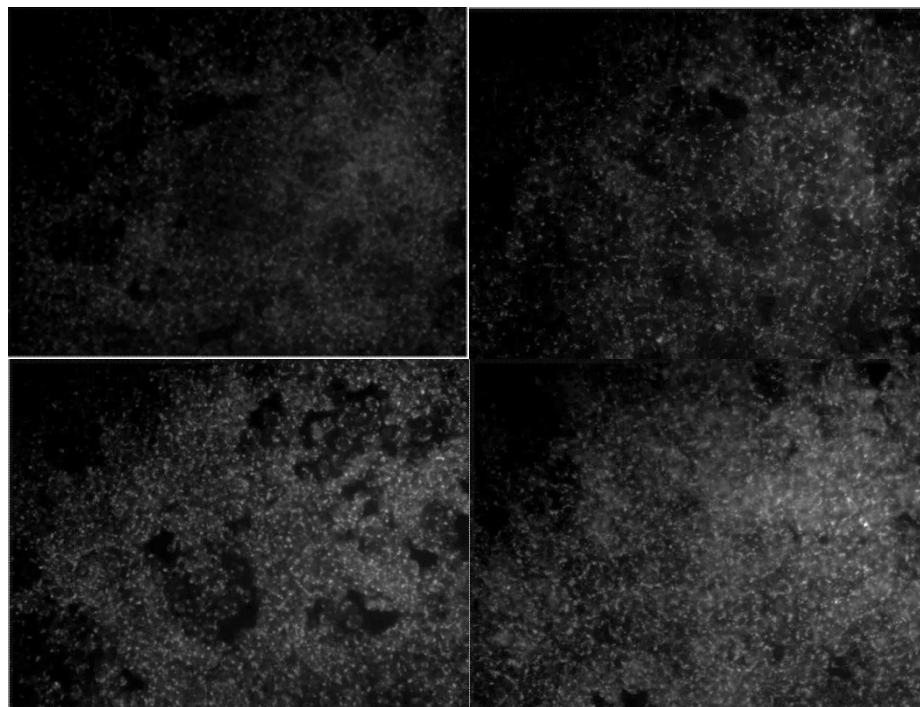
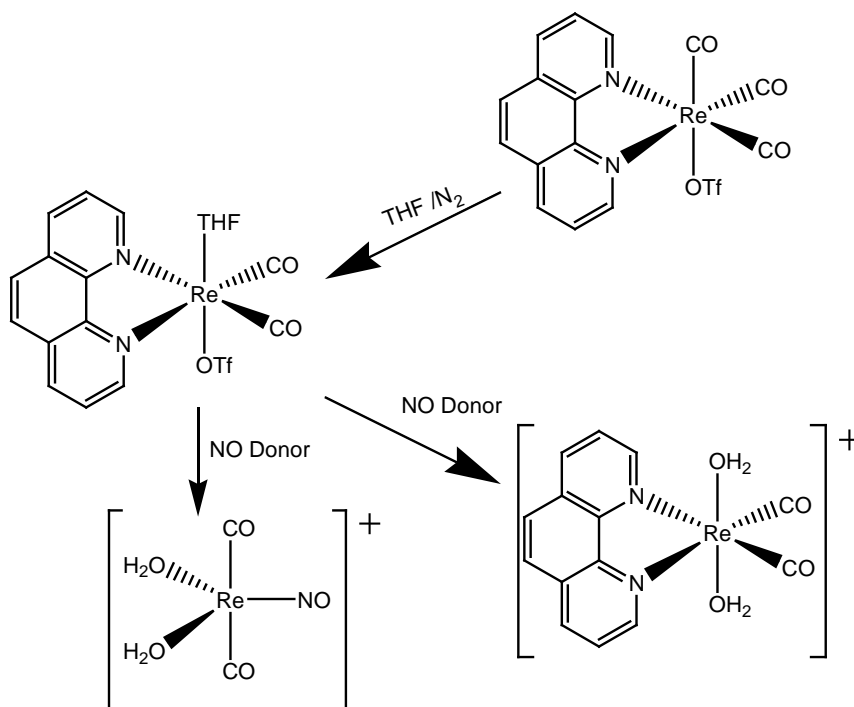


Figure 3.13 Picture sequence demonstrating fluorescence response in RAOEC at $\lambda_{em} = 450$ nm, Dye was generated by photochemical substitution (50 min). (a) with no NO-donor added (b) with 1 μ M NO-donor (c) with 5 μ M NO-donor and (d) with 10 μ M NO-donor. $\lambda_{exc} = 350$ nm.

The reaction of rhenium complexes with NO-donor is complex and produces several species, some of them fluorescent. Both the NMR and fluorescence studies are consistent with an initial dissociation of the labile THF or PPh₃ ligand, followed by reaction with NO either i) on the coordinated phenanthroline with subsequent dissociation from the metal center, as it has been observed before by Hill²⁵, Mann²⁶ and Halper²⁷ or ii) on the Re center forming a nitrosyl complex. The presence of CFSO₃⁻ in the Re coordination sphere is important for reaction with NO. Scheme 2 summarizes possible mechanistic pathways:



Scheme 3.2 Proposed mechanistic pathways for NO reactivity with $(\text{phen})\text{Re}(\text{CO})_2(\text{THF})\text{OSO}_2\text{CF}_3$ (**2**)

3.5. CONCLUSIONS

The organometallic Re(I) complex was shown to react with NO at room temperature, producing fluorescence responses. The presence of labile ligands (THF, triflate, PPh_3) is critical for reactivity with NO. Comparison studies show that rhenium complexes are prone to partial deactivation by side-reactions. These probes are able to permeate the cell membrane by diffusion and also exhibit fluorescence responses upon NO-donor addition inside the cell.

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23. 20.0 mg of (phen)Re(CO)₃OSO₂CF₃ (33.3 μmol) and 10 mL of anhydrous THF (final concentration of 3.33 mM) were placed in a 50 mL round bottom flask, under N₂ flow. The solution was refluxed for 48 h. The color changed during reflux from yellow to amber. The volatiles were evaporated to dryness and 1.0 mL of dichloromethane was added. The product was precipitated at room temperature by adding 4.0 mL of diethyl ether. The bright yellow powder product was filtered and was dried in vacuo at 40 °C.
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4. STUDY OF THE FLUORESCENT ORGANOMETALLIC COMPLEX (phen)Re(CH₃CN)(CO)₂OSO₂CF₃ AS A NO SENSOR.

4.1 ABSTRACT

The complex (phen)Re(CH₃CN)(CO)₂OSO₂CF₃ (**3**), which can be obtained thermally from (phen)Re(CO)₃OSO₂CF₃ shows fluorescence quenching response upon NO addition, which was added in the form of NO-donor Z-1-N-methyl-N-[6-(N-methylammoniohexyl)amino]diazene-1-ium-1,2-diolate (MAHMA-NONOate). Addition of 10-150 μM of NO-donor into a 25 μM solution of (phen)Re(CH₃CN)(CO)₂OSO₂CF₃ in 25% v/v acetone/phosphate saline (PBS) buffer gave fluorescence quenching at 532 nm, upon excitation at 360 nm. The ¹H-NMR and ¹⁹F-NMR spectra after reaction with NO showed characteristic resonances for non-coordinated triflate and CH₃CN, suggesting that the organometallic product formed upon NO releases both these ligands upon reaction with NO. The present study expands our newly demonstrated approach for the design of fluorescent NO sensors based on NO coordination to Re;¹ **3** is an isolable complex, which is stable at room temperature and therefore can be used as an NO sensing organometallic dye, as prepared, without further treatment.

4.2 INTRODUCTION

Acetonitrile transition metal complexes have been used as intermediaries in inorganic and organometallic synthesis.² These complexes are stable at room temperature and react only under photochemical conditions or at high temperatures.³ Rhenium acetonitrile complexes are typically synthesized by reflux in acetonitrile⁴ or using trimethylamine N-oxide dehydrate.⁵

Rhenium tricarbonyl complexes have interesting photophysical properties, and they are used as luminophores.⁶ Rhenium imaging probes have shown reliable responses. Changing fluorophores selectively may target different subcellular structures. Rhenium(I) reacts with specific biochemical agents localized in the cell, such as sulfur-containing aminoacids in the mitochondria.⁷

In prior work (Chapters 2 and 3), it was shown that (phen)Re(THF)(CO)₂OSO₂CF₃, and (phen)Re(PPh₃)(CO)₂OSO₂CF₃ prepared *in situ* thermally or photochemically from (phen)Re(CO)₃OSO₂CF₃ have the ability to react with NO under physiological conditions, producing a fluorescence change. The new acetonitrile analog **3** introduced in this Chapter shows analogous fluorescence responses, that can be ascribed directly to NO complexation by Re, as indicated by NMR and IR spectroscopic studies. **3** also has the additional advantages that it can be prepared in an isolable form, and is stable at room temperature.

4.3 EXPERIMENTAL SECTION

4.3.1. Materials and Methods

The complexes (phen)Re(CO)₃Cl and (phen)Re(CO)₃OSO₂CF₃ were prepared as previously reported and were found spectroscopically identical to the reported compounds.^{8,9} All other materials (purchased from Aldrich Chemical Co., ACROS organics, Cayman Chemicals, or Alfa Aesar) were standard reagent grade and were used without further purification, unless otherwise noted. PBS buffer (0.002 M in total phosphate, pH = 7.2, [NaCl] = 0.04 M) was prepared from K₂HPO₄, KH₂PO₄, and NaCl (all from Fisher Scientific). Water used for preparation of the buffers and experiments with Nitric oxide was filtered water (18 MΩ·cm⁻¹) produced from a Millipore® nanopure

diamond lab water system (Barnstead Thermolyne Corporation, Dubuque, IA). Deuterated solvents were purchased from Cambridge Isotope Laboratories Ltd. ^1H and ^{13}C NMR spectra were recorded on a 400 Bruker NMR spectrometer and were referenced, using the residual solvent resonances. All chemical shifts, δ , are reported in ppm. FT-IR spectra were recorded with a Perkin Elmer Spectrum 100 FT-IR Spectrometer. Frequency-domain fluorescence lifetime measurements were performed using a ChronosFD spectrofluorometer (ISS, Champaign, IL). Samples were excited with a 370 nm modulated diode, and emission was collected using 400 nm long-pass filters (Andover, Salem, NH).

4.3.2. Synthesis of (phen)Re(CH₃CN)(CO)₂OSO₂CF₃:

20.0 mg of (phen)Re(CO)₃OSO₂CF₃ (33.3 μmol) and 20 mL of acetonitrile (final concentration: 16.5 mM) were placed in a 50 mL round bottom flask under N₂ flow. The solution was refluxed for 48 hours. The color changed during reflux from yellow to amber. The volatiles were reduced to 1.0 mL. The product was precipitated at room temperature by adding 4.0 mL of diethyl ether. The bright yellow powder product was filtered and was dried *in vacuo* at 40 °C. 18.4 mg, (84%). ^1H -NMR (CDCl₃): 2.18 (3H, s), 8.04 (d, 2H, $^3\text{J}_{\text{H-H}} = 8.3$ Hz, $^3\text{J}_{\text{H-H}} = 5.1$ Hz), 8.20 (s, 2H), 8.79 (d, 2H, $^3\text{J}_{\text{H-H}} = 8.3$ Hz, $^3\text{J}_{\text{H-H}} = 1.3$ Hz), 9.37 (d, 2H, $^3\text{J}_{\text{H-H}} = 5.1$ Hz, $^3\text{J}_{\text{H-H}} = 1.2$ Hz). ^{13}C - $\{^1\text{H}\}$ -NMR ((CD₃)₂CO): 3.17 (CH₃CN, s, 1C), 127.93 (CH, s, 2C), 129.11 (CH, s, 2C), 132.27 (CH, s, 2C), 147.84 (CN, s, 1C), 155.74 (CH, s, 2C). ^{19}F - $\{^1\text{H}\}$ -NMR (CDCl₃): -78.2 ppm. FT-IR (cm⁻¹): 2295 (ν_{CN} , w), 2033 (ν_{CO} , s), 1927 (ν_{CO} , s), 1904 (ν_{CO} , s), 1631, 1521, 1431, 1417, 1255, 1222, 1156, 1030, 877, 851, 724, 637. UV-Vis (in ethanol): $\lambda_{\text{max}} = 360$ nm - br ($\epsilon = 1840$ mol⁻¹cm⁻¹), Fluorescence emission in acetone ($\lambda_{\text{exc}} = 360\text{nm}$): 532 nm

$\Phi_1=0.006$ (at 370 nm, in 1:3 acetone/PBS buffer, using quinine sulfate in sulfuric acid 0.1 M as reference $\Phi_R=0.546$). Lifetime: $\tau_1 = 15.1 \pm 0.4$ ns, $f_1 = 0.257 \pm 0.004$, $\alpha_1 = 0.017$; $\tau_2 = 141 \pm 3$ ns, $f_2 = 0.424 \pm 0.003$, $\alpha_2 = 0.00301$. There are two major processes occurring. The adding contributions only add up 70%. There are minor reactions not fully described.

4.3.3. Fluorescence experiments with NO-donor

In a typical experiment, a 100 μM solution of $(\text{phen})\text{Re}(\text{CH}_3\text{CN})(\text{CO})_2\text{OSO}_2\text{CF}_3$ complex was prepared from 1.67 mM stock solution of $(\text{phen})\text{Re}(\text{CH}_3\text{CN})(\text{CO})_2\text{OSO}_2\text{CF}_3$ in THF and acetone was added up to 1.000 mL of total solution of Re complex. 2.75 mL to 3.00 mL of aqueous PBS buffer (pH = 7.2) was added. Then 0 to 250 μL of 4 mM stock solution of MAHMA-NONOate NO-donor, in 0.04 M NaOH, were added in order to obtain 75% v/v aqueous/organic solution and a final NO-donor concentration from 0 to 200 μM range, while the concentration of Re complex remained constant at 25 μM . No more than 10 minutes elapsed from preparation of the organic phase to the addition of the NO-donor. The solution was mixed thoroughly in a vortex and sonicated for 90 minutes at room temperature. The fluorescence of all solutions was measured with a $\lambda_{\text{exc}} = 360$ nm and the emission was recorded from 400 nm to 700 nm. For a control experiment, a NaOH solution was used instead of an NO-donor/NaOH stock solution in order to account for any possible variations in the fluorescence due to minor pH changes. The result of the control titrations gave identical spectra for all titration points for the 1-150 μL range.

4.3.4. Fluorescence titration with gradual addition of NO-donor under anaerobic and aerobic conditions.

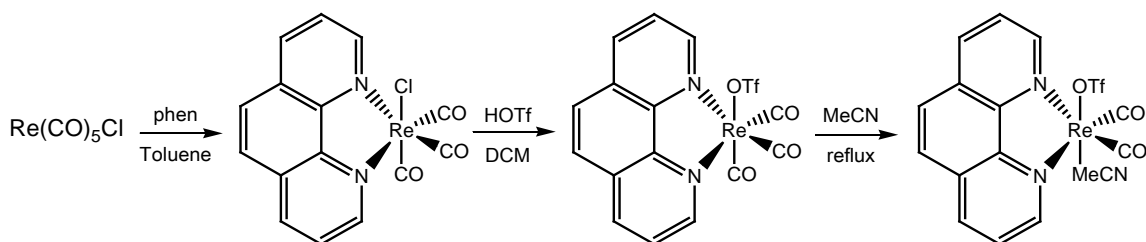
In a typical experiment, a 200 μM of solution of (phen)Re(CH₃CN)(CO)₂OSO₂CF₃ complex was prepared from 1.67 mM stock solution in acetone up to 0.750 mL of total solution of Re complex. 2.25 mL of aqueous PBS buffer (pH=7.2) was added. The fluorescence emission was monitored using $\lambda_{\text{exc}} = 360$ nm. Then, 3 mM stock solution of MAHMA-NONOate NO-donor in 0.04 M NaOH was added progressively in 25 μL portions up to the total of 300 μL . After each addition, the solution was left to react for 10 minutes (approximately 3 MAHMA NONOate half-lives) at room temperature before measuring fluorescence using the same parameters as above mentioned.

In another cuvette, another 3000 μL of (phen)Re(CH₃CN)(CO)₂OSO₂CF₃ was prepared in the same acetone/buffer system (see above); this solution was bubbled with nitrogen for 30 minutes. The fluorescence titration was performed under the same conditions mentioned above.

4.4. RESULTS

4.4.1. Synthesis of (phen)Re(CH₃CN)(CO)₂OSO₂CF₃ (**3**)

Complex **3** was synthesized by thermal substitution of CO from (phen)Re(CO)₃SO₃CF₃. The isolated product was characterized by ¹H and ¹³C-NMR and FT-IR.



Scheme 4.1 Synthesis of (phen)Re(CH₃CN)(CO)₂OSO₂CF₃ (**3**)

4.4.2. Fluorescence studies with addition of NO-donor

Complex **3** showed significant fluorescence response to NO addition. The fluorescence spectrum of **3** in acetone/buffer 25/75 v/v shows one emission at 565 nm ($\Phi = 0.006$). Figure 4.1 shows the effects on the fluorescence spectrum of **3** upon NO gradual addition, in the form of NO-donor MAHMA-NONOate (0-150 μ M) dissolved in 0.04 M NaOH. The emission at 562 nm decreases rapidly upon NO addition and its intensity shows linear dependence with NO-donor concentration, thus allowing NO quantification in the 0-150 μ M range (Figure 4.1).

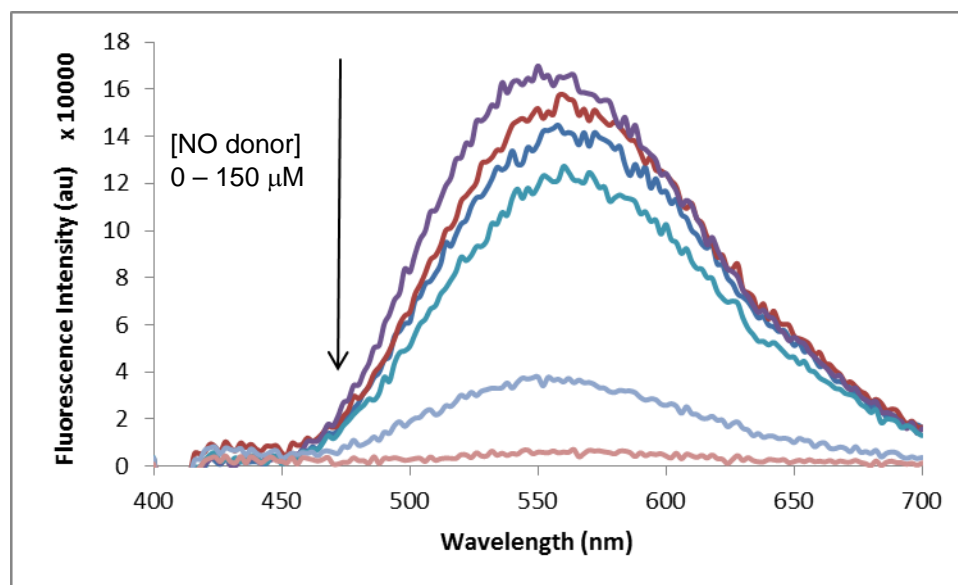


Figure 4.1 Fluorescence Response upon NO-donor addition (0-150 μ M) to 20 μ M of Re complex **3** in acetone/PBS aqueous buffer 25/75 v/v, ($\lambda_{exc} = 360$ nm)

4.4.3. Fluorescence titration with gradual addition of NO-donor under anaerobic and aerobic conditions.

Complex **3** showed significant stability under physiological conditions. Figure 4.2 shows the effects on the fluorescence spectrum of **3** upon NO consecutive addition, in the form of NO-donor MAHMA-NONOate (0 – 300 μM) dissolved in 0.04 M NaOH, in 25 μL portions after 10 minutes intervals. The emission at 532 nm decreases rapidly upon NO addition and its intensity shows linear dependence with NO-donor concentration, this experiment was performed under nitrogen to avoid NO oxidation to N_2O_3 , in a titration fashion.

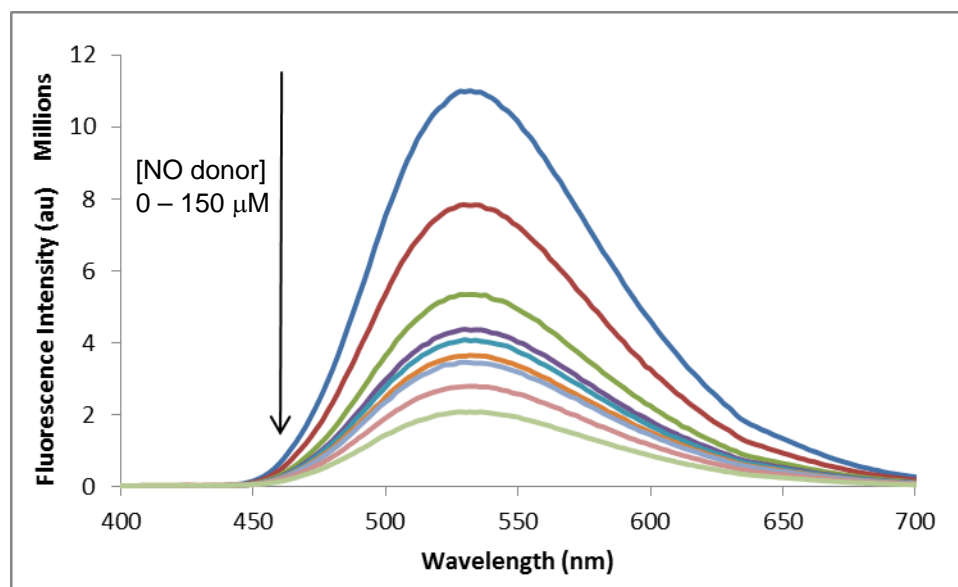


Figure 4.2. Fluorescence Spectra of 50 μM **3** with NO, acetone/buffer 25:75, $\lambda_{\text{exc}} = 360 \text{ nm}$, under nitrogen

Figure 4.3 shows the effects on the fluorescence spectrum of **1** upon NO consecutive addition, in the form of NO-donor MAHMA-NONOate (0 - 300 μM) dissolved in 0.04 M NaOH, in 25 μL portions after 10 minutes intervals. The emission at 532 nm decreases rapidly upon NO addition and its intensity shows linear dependence

with NO-donor concentration, this experiment was performed under air, in a titration fashion; some NO got consumed by oxidation. The intensity of the titration performed under air is 30% lower than under nitrogen, as exhibit in Figure 4.3; and the response towards NO addition is lower in the same extend, that can be explained by oxidation of the real substrate NO to NO₂, and this second one cannot be used as a substrate for this Re complex.

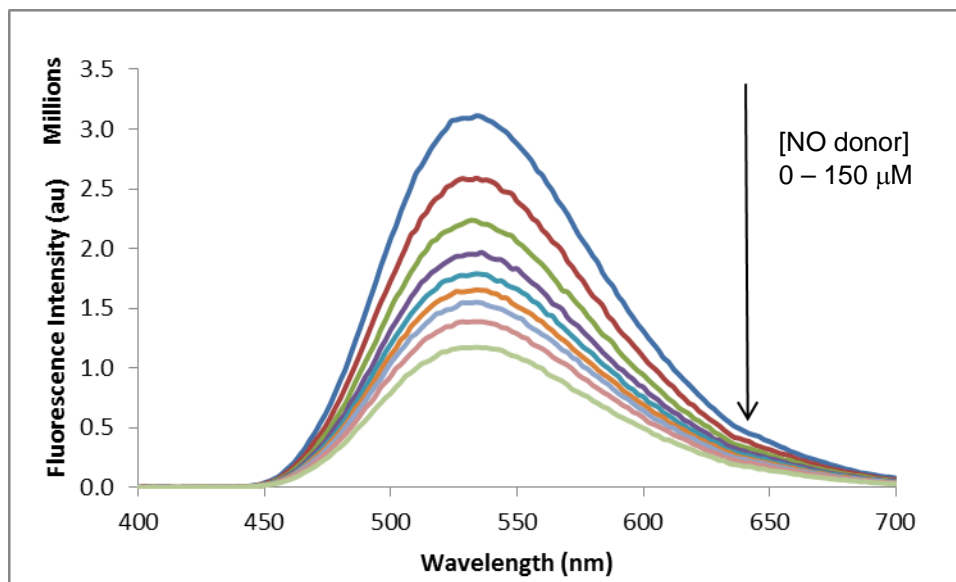


Figure 4.3. Fluorescence Spectra of 50 μM **6** with NO, acetone/buffer 25:75, $\lambda_{\text{exc}} = 360 \text{ nm}$

The fluorescence titration comparison plot (Figure 4.4) of I_{532} vs [NO-donor] under nitrogen (Figure 4.2) and under air (Figure 4.3) shows the similarity of responses in the two experiments, and also higher sensitivity in lower concentrations of NO under anaerobic conditions. Thus, it can be concluded that there is no need for an oxidant in order for the reaction to occur. This result is indicative of direct interaction of the dye with NO and not with NO-derived oxidized products.

^{19}F -NMR after NO-donor reaction showed no signals. It can be concluded that CF_3SO_3^- got dissociated after reaction with NO; this proves one part of the mechanism.

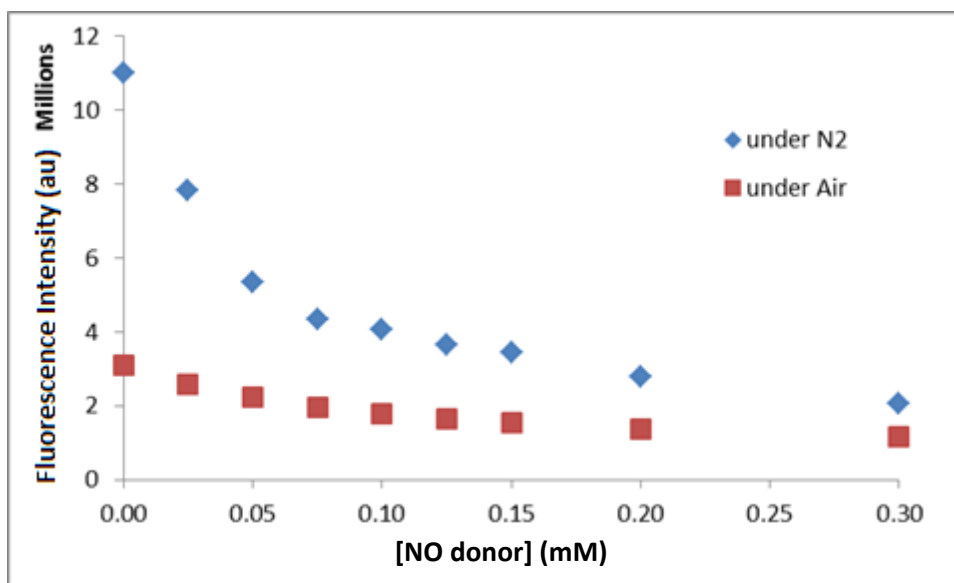


Figure 4.4 Fluorescence Spectra of **3** $50\mu\text{M}$ with NO, comparison nitrogen vs air at 532 nm.

4.4 CONCLUSION

The new complex $(\text{phen})\text{Re}(\text{CH}_3\text{CN})(\text{CO})_2\text{OSO}_2\text{CF}_3$ has been synthesized and characterized. This complex can react with NO under physiological conditions, producing a quenching in fluorescence that can be monitored.

4.5 REFERENCES

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5. α -DIIMINE RHENIUM COMPLEXES, AS COLORIMETRIC SENSORS FOR NO and CN⁻.

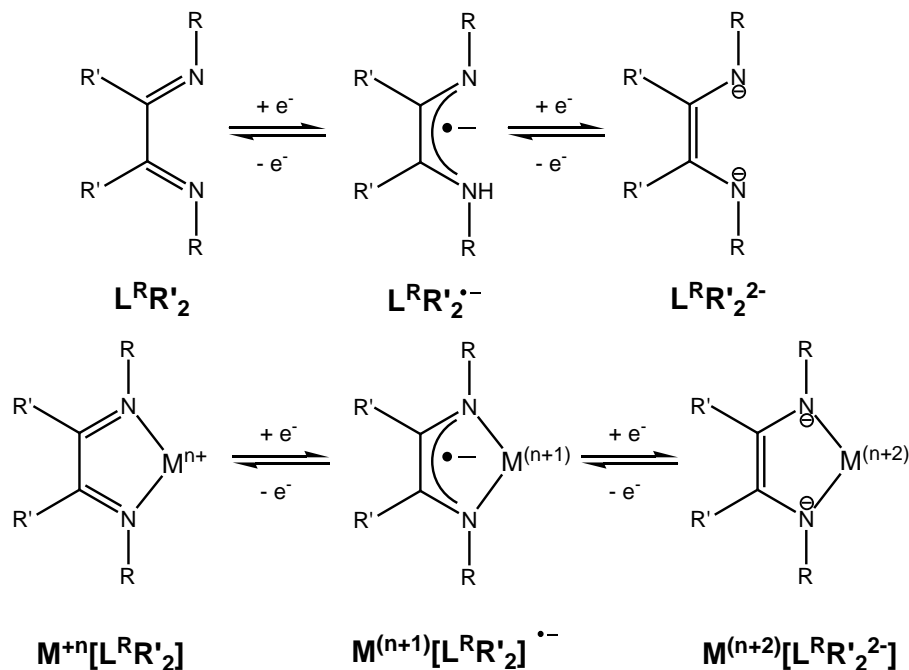
5.1. ABSTRACT

The complexes (Xy-DAD)Re(THF)(CO)₂OTf (**8**), (Xy-DAD)Re(CH₃CN)(CO)₂OTf (**9**), (iPr-DAD)Re(THF)(CO)₂OTf (**10**), and (iPr-DAD)Re(CH₃CN)(CO)₂OTf (**11**) obtained photochemically from (Xy-DAD)Re(CO)₃OTf (**5**) and (iPr-DAD)Re(CO)₃OTf (**7**) respectively show colorimetric response upon NO and CN⁻ addition, which was added in the form of NO-donor Z-1-N-methyl-N-[6-(N-methylammoniohexyl)amino]diazene-1,2-diolate (MAHMA-NONOate) and in the form of NaCN. Addition of 10-100 μ M of NO-donor into a 50 μ M solution of **8**, **9**, **10**, and **11** in 50% v/v acetone/phosphate saline (PBS) buffer gave hypsochromic shift from 478 nm to 408 nm upon NO addition in the case of complex **7**, decreased the signal at 500 nm upon NO addition in the case of complex **8**, increased the signal at 470 nm upon CN⁻ addition in the case of complex **9**, and hypsochromic shift from 500 nm to 348 nm upon CN⁻ addition in the case of complex **10**. The ¹H-NMR and ¹⁹F-NMR spectra after reaction with CN⁻ and NO showed characteristic resonances for non-coordinated triflate, THF, and CH₃CN, suggesting that the organometallic product formed upon NO and CN⁻ release both these ligands upon reaction with the respective substrate. This project tries to explore the ability of rhenium complexes to bind to NO;¹ and this change in the coordination sphere may produce an optical change that may be quantifiable.

5.2. INTRODUCTION

Nitric oxide has several functions of particular importance for human health.^{2,3} It is now recognized as a key signaling molecule in the cardiovascular system and is also called endothelial derived relaxation factor (EDRF),⁴ as NO regulates blood pressure by relaxing blood capillaries, veins and arteries. Several transition metal nitrosyl complexes have been reported,⁵ including some Re(I) complexes.⁶ Therefore, late transition metal chemistry should be explored for NO radical binding.

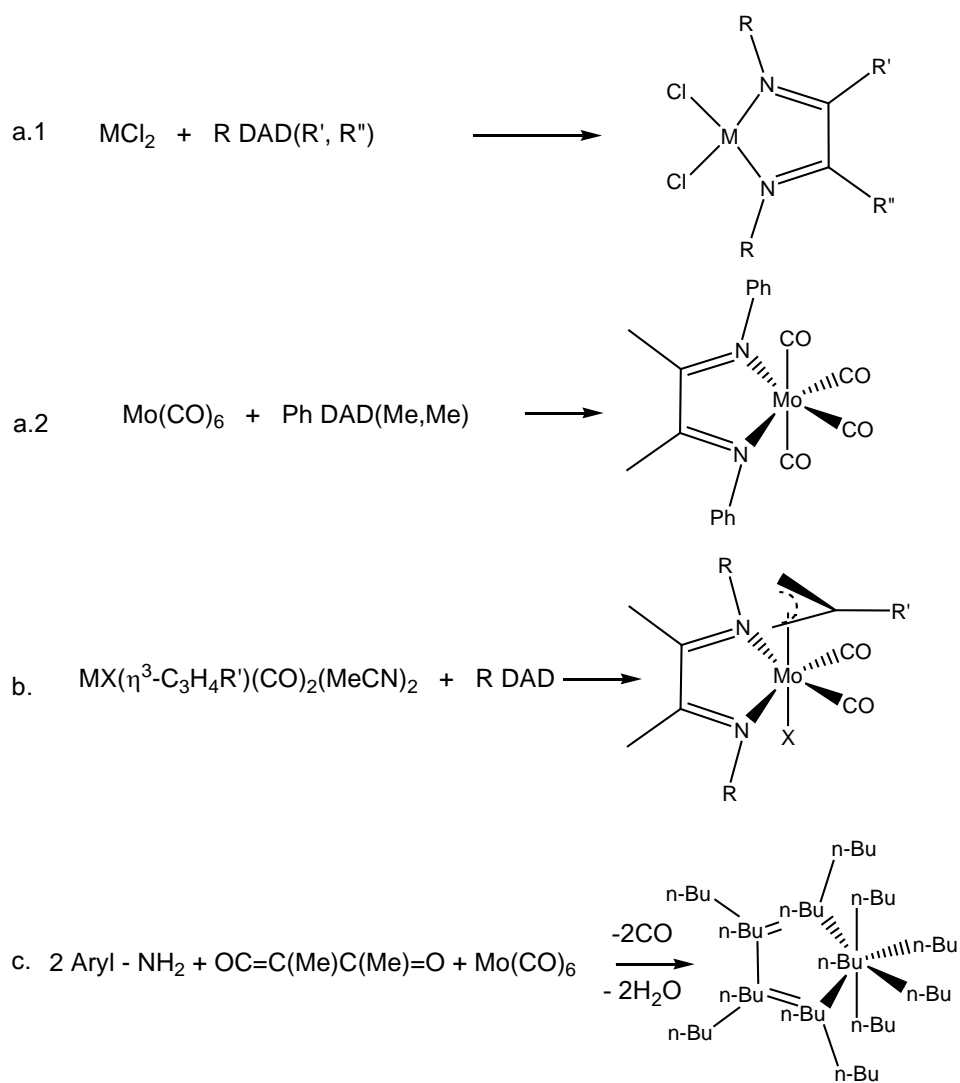
The 1,4-Diazabutadienes (DAD or DAB) are ligands where intramolecular electron transfer can be studied. These intramolecular electron transfers lead to valence tautomerization (VT). 1,4-diazabutadienes have two redox centers, which create the possibility of a bistable electronic state.⁷ Coordinating transition metals to non-innocent redox ligands may generate bistable electronic complexes, as shown in Scheme 5.1.



Scheme 5.1. Valence tautomerization (VT) produced by bistable electron state DAD.

Another possibility of “distortion” is “back-bonding” from the metal d-orbitals to the empty LUMO π^* of the diamine ligand.⁸ There are complexes with osmium, ruthenium and rhodium;⁷ but rhenium chemistry with DAD has been barely explored.

The synthesis of metal-DAD complexes can be summarized as substitution from metal halides or metal carbonyls with the DAD ligand, or by chelating ligand substitution, or by Schiff-base condensation.⁹



Scheme 5.2 Summary of synthesis of metal-DAD complexes: a. from metal halide or carbonyl; b. from chelating ligand substitution; c. from Schiff-base condensation.

In prior work (Chapter 2- 4), it was shown that (phen)Re(PPh₃)(CO)₂OSO₂CF₃, (phen)Re(THF)(CO)₂OSO₂CF₃, and (phen)Re(CH₃CN)(CO)₂OSO₂CF₃ prepared *in situ* thermally or photochemically from (phen)Re(CO)₃OSO₂CF₃, have the ability to react with NO under physiological conditions, producing a fluorescence response. These new DAD-Re-THF and DAD-Re-CH₃CN analogs **8**, **9**, **10** and **11**, which are introduced in this Chapter, show colorimetric responses to NO and CN⁻ addition that can be ascribed to NO or CN⁻ complexation by the Re center.

5.3. EXPERIMENTAL PROCEDURES

5.3.1. Materials and Methods

All materials (purchased from Aldrich Chemical Co., ACROS organics, Cayman Chemicals, or Alfa Aesar) were standard reagent grade and were used without further purification, unless otherwise mentioned. Toluene, used for the preparation of **3** and **5**, was distilled and dried before use. PBS buffer (0.002 M in total phosphate, pH = 7.2, [NaCl] = 0.04 M) was prepared from K₂HPO₄, KH₂PO₄, and NaCl (all from Fisher Scientific). Water, used for preparation of the buffers and experiments with Nitric oxide, was filtered water (18 MΩ·cm⁻¹) produced from a Millipore® nanopure diamond lab water system (Barnstead Thermolyne Corporation, Dubuque, IA). Deuterated solvents were purchased from Cambridge Isotope Laboratories Ltd. Photochemical reactions were carried out using a Rayonet photochemical reactor (Southern New England Ultraviolet, model RPR-100), equipped with a cooling fan and 10 phosphor coated low-pressure mercury lamps ($\lambda_{\text{max}} = 300 \text{ nm}$, light intensity = $5.2 \pm 0.1 \times 10^6 \text{ photon/sec/cm}^3$). Fluorescence Spectra were recorded on a Jobin Yvon Horiba Fluoromax 3

spectrometer. ^1H and ^{13}C NMR spectra were recorded on a 400 Bruker NMR spectrometer and were referenced, using the residual solvent resonances. All chemical shifts, δ , are reported in ppm. FT-IR spectra were recorded with a Perkin Elmer Spectrum 100 FT-IR Spectrometer. UV-Visible spectra were recorded with a Cary 100 Spectrophotometer.

5.3.2. Synthesis of (Xy-DAD)Re(CO)₃Cl (4):

100.0 mg of Re(CO)₅Cl (276 μmol) and 73.0 mg of Xy-DAD (276 μmol) were suspended in 25 mL of distilled anhydrous Toluene (final concentration of 11.0 mM), under N₂ flow. The suspension was refluxed for 2 hours. The volatiles were evaporated to half volume. The solution changed from yellow to dark purple. The bright purple powder product was filtered and was dried *in vacuo* at 40 °C. 117.3 mg, (70%). $^1\text{H-NMR}$ (CDCl₃): 2.29 (s, 6H), 2.59 (s, 6H), 7.20 (m, 6H, $^3J_{\text{H-H}} = 4.5$ Hz), 8.75 (d, 2H, $^3J_{\text{H-H}} = 6.7$ Hz). $^{19}\text{F}\{-^1\text{H}\}$ -NMR (CDCl₃): -78.3 ppm. $^{13}\text{C-NMR}$ (CDCl₃): 18.8 (s, 2C); 20.1 (s, 2C); 128.1 (s, 2C); 128.3 (s, 2C); 128.8 (s, 2C); 129.6 (s, 2C); 150.3 (s, 1C); 166.6 (s, 1C). FT-IR (cm⁻¹): 2021 (v_{CO}, vs), 1931 (v_{CO}, vs), 1885 (v_{CO}, vs), 1637, 1499, 1468, 1382, 1265, 1185, 1098, 1029, 771. UV-Vis (in ethanol): $\lambda_{\text{max}} = 440$ nm - br ($\epsilon = 8075$ mol⁻¹cm⁻¹). Anal. Calc. for C₂₁H₂₀ClN₂O₃Re: C 44.24%, H 3.37%, N 4.91%. Found: C 43.96%, H 3.37%, N 4.89%.

5.3.3. Synthesis of (Xy-DAD)Re(CO)₃OSO₂CF₃ (5):

50.0 mg of (Xy-DAD)Re(CO)₃Cl (3) (87.7 μmol) and 20 mL of dichloromethane (4.3 μM of concentration) was placed in a 50 mL round bottom flask under N₂ flow. 0.15 mL of triflic acid was added dropwise. The solution was stirred for 2 hours at room temperature. The volatiles were evaporated to 1.0 mL. The product was precipitated at

room temperature by adding 4.0 mL of diethyl ether. The bright purple powder product was filtered and was dried *in vacuo* at 40 °C. 59.5 mg, (98%). ¹H-NMR (CDCl₃): 2.36 (s, 6H), 2.39 (s, 6H), 7.23 (m, 6H, ³J_{H-H} = 4.5 Hz), 8.77 (s, 2H). ¹⁹F-¹H-NMR (CDCl₃): -78.2 ppm. ¹³C-NMR (CDCl₃): 18.6 (s, 2C); 18.9 (s, 2C); 128.1 (s, 2C); 128.7 (s, 2C); 129.2 (s, 2C); 129.7 (s, 2C); 168.9.3 (s, 1C); 171.6 (s, 1C). FT-IR (cm⁻¹): 2038 (ν_{CO}, vs), 1951 (ν_{CO}, vs), 1909 (ν_{CO}, vs), 1313, 1233, 1204, 1190, 1005, 773, 624. Anal. Calc. for C₂₁H₂₀F₃N₂O₆ReS: C 38.59%, H 3.09%, N 4.09%. Found: C 38.25%, H 2.82%, N 3.99%.

5.3.4. Synthesis of (*i*-Pr-DAD)Re(CO)₃Cl (6):

100.0 mg of Re(CO)₅Cl (276 μmol) and 105.0 mg of *i*Pr-DAD (276 μmol) were suspended in 25 mL of distilled, anhydrous Toluene (final concentration of 11.0 mM), under N₂ flow. The suspension was refluxed for 2 h. The volatiles were evaporated to half volume. The solution changed from yellow to dark purple. The bright purple powder product was filtered and was dried *in vacuo* at 40 °C. 123.0 mg, (55%). ¹H-NMR (CDCl₃): 1.16 (dd, 12H, ³J_{H-H} = 14.5 Hz, ³J_{H-H} = 6.8 Hz), 1.36 (d, 12H, ³J_{H-H} = 6.8 Hz), 2.80 (m, 2H, ³J_{H-H} = 6.7 Hz), 3.92 (m, 2H, ³J_{H-H} = 6.7 Hz), 7.72 (m, 6H, ³J_{H-H} = 4.5 Hz), 8.70 (s, 2H). ¹⁹F-¹H-NMR (CDCl₃): -78.3 ppm. ¹³C-NMR (CDCl₃): 22.8 (s, 2C); 22.9 (s, 2C); 26.2 (s, 2C); 26.8 (s, 2C); 28.0 (s, 2C); 28.2 (s, 2C); 123.8 (s, 2C); 124.9 (s, 2C); 128.8 (s, 2C); 139.2 (s, 2C); 147.8 (s, 1C); 166.7 (s, 1C). FT-IR (cm⁻¹): 2025 (ν_{CO}, vs), 1934 (ν_{CO}, vs), 1916 (ν_{CO}, vs), 1501, 1464, 1441, 1362, 1355, 1321, 1170, 1102, 796, 752, 638. Anal. Calc. for C₂₁H₂₀ClN₂O₃Re: C 51.05%, H 5.31%, N 4.10%. Found: C 50.86%, H 5.15%, N 4.10%.

5.3.5. Synthesis of (*i*-Pr-DAD)Re(CO)₃OSO₂CF₃ (7):

50.0 mg of (*i*-Pr-DAD)Re(CO)₃Cl (**5**) (87.7 μmol) and 20 mL of dichloromethane (4.3 mM of concentration) was placed in a 50 mL round bottom flask under N₂ flow. 0.15 mL of triflic acid was added dropwise. The solution was stirred for 2 hours at room temperature. The volatiles were evaporated to 1.0 mL. The product was precipitated at room temperature by adding 4.0 mL of diethyl ether. The bright purple powder product was filtered and was dried *in vacuo* at 40 °C. 59.5 mg, (98%). ¹H-NMR (CDCl₃): 1.15 (dd, 12H, ³J_{H-H} = 67.4 Hz, ³J_{H-H} = 6.7 Hz), 1.38 (d, 12H, ³J_{H-H} = 18.3 Hz, ³J_{H-H} = 6.7 Hz), 2.77 (m, 2H, ³J_{H-H} = 6.7 Hz), 3.16 (m, 2H, ³J_{H-H} = 6.7 Hz), 7.29 (m, 6H, ³J_{H-H} = 3.5 Hz), 7.37 (m, 6H, ³J_{H-H} = 3.5 Hz), 8.75 (s, 2H). ¹⁹F-¹H-NMR (CDCl₃): -78.2 ppm. ¹³C-NMR (CDCl₃): 22.2 (s, 2C); 23.2 (s, 2C); 26.3 (s, 2C); 26.9 (s, 2C); 28.3 (s, 2C); 28.4 (s, 2C); 124.3 (s, 2C); 124.9 (s, 2C); 129.4 (s, 2C); 139.2 (s, 2C); 141.0 (s, 2C); 146.9 (s, 1C); 169.0 (s, 1C). FT-IR (cm⁻¹): 2038 (ν_{CO}, vs), 1953 (ν_{CO}, vs), 1922 (ν_{CO}, vs), 1510, 1325, 1316, 1235, 1202, 1192, 1001, 796, 750, 633.

5.3.6. Photochemical substitution reactions:

All the solutions were irradiated with a Rayonet photochemical reactor (Southern New England Ultraviolet, model RPR-100), equipped with a cooling fan and 10 phosphor coated low-pressure mercury lamps ($\lambda_{\max} = 300$ nm, light intensity = $5.2 \pm 0.1 \times 10^6$ photon/sec/cm³). After 75 minutes of irradiation, the colors of the solutions changed to darker colors. Unfortunately, all of the organometallic complexes could not be isolated and it had to be prepared *in situ*.

5.3.6.1. Synthesis of (Xy-DAD)Re(THF)(CO)₂OSO₂CF₃ (**8**):

(Xy-DAD)Re(CO)₃OSO₂CF₃ (**4**) (1 mg, 1.46 μmol) 1.00 mL of anhydrous THF (final concentration 1.46 mM) were placed in a NMR tube. The solution was irradiated

for 75 minutes at room temperature. This solution was used without further treatment. $^1\text{H-NMR}$ (CDCl_3): 2.24 (s, 6H), 2.36 (s, 6H), 7.10 (m, 6H, $^3J_{\text{H-H}} = 3.4$ Hz), 7.12 (m, 6H), 9.04 (s, 2H). $^{19}\text{F-}\{^1\text{H}\}\text{-NMR}$ (CDCl_3): -78.1 ppm. FT-IR (cm^{-1}): 2036 (ν_{CO} , vs), 1945 (ν_{CO} , vs), 1918 (ν_{CO} , vs), 1720 ($\nu_{\text{CO-O}}$, vs), 1461, 1237, 1177, 1143, 1091, 1030, 994, 921, 775, 638, 614.

5.3.6.2. Synthesis of (Xy-DAD)Re(CH₃CN)(CO)₂OSO₂CF₃ (9):

(Xy-DAD)Re(CO)₃OSO₂CF₃ (**4**) (1 mg, 1.46 μmol) 1.00 mL of acetonitrile (final concentration 1.46 mM) were placed in a NMR tube. The solution was irradiated for 75 minutes at room temperature. This solution was used without further treatment. $^1\text{H-NMR}$ (CDCl_3): 2.32 (s, 6H), 2.41 (s, 6H), 7.26 (m, 6H), 7.26 (m, 6H), 8.86 (s, 2H). $^{19}\text{F-}\{^1\text{H}\}\text{-NMR}$ (CDCl_3): -78.2 ppm. FT-IR (cm^{-1}): 2307 (ν_{CN} , w), 2040 (ν_{CO} , vs), 1939 (ν_{CO} , vs-br), 1470, 1385, 1327, 1262, 1225, 1200, 1160, 1098, 1030, 775, 701, 638.

5.3.6.3. Synthesis of (*i*-Pr-DAD)Re(CH₃CN)(CO)₂OSO₂CF₃ (10):

(*i*-Pr-DAD)Re(CO)₃OSO₂CF₃ (**6**) (1 mg, 1.26 μmol) 1.00 mL of anhydrous tetrahydrofuran (final concentration 1.46 mM) were placed in a NMR tube. The solution was irradiated for 75 minutes at room temperature. This solution was used without further treatment. $^1\text{H-NMR}$ (CDCl_3): 0.96 (dd, 12H, $^3J_{\text{H-H}} = 6.6$ Hz), 1.15 (dd, 12H, $^3J_{\text{H-H}} = 6.5$ Hz), 1.27 (dd, 12H, $^3J_{\text{H-H}} = 6.4$ Hz), 1.28 (d, 12H, $^3J_{\text{H-H}} = 4.5$ Hz), 1.33 (m, 2H, $^3J_{\text{H-H}} = 5.4$ Hz), 3.13 (m, 2H, $^3J_{\text{H-H}} = 6.6$ Hz), 7.27 (m, 3H), 7.68 (m, 3H), 9.05 (s, 2H). $^{19}\text{F-}\{^1\text{H}\}\text{-NMR}$ (CDCl_3): -78.1 ppm. FT-IR (cm^{-1}): 2037 (ν_{CO} , vs), 1947 (ν_{CO} , vs), 1922 (ν_{CO} , vs), 1722 ($\nu_{\text{CO-O}}$, vs), 1460, 1335, 1238, 1174, 1143, 1091, 1032, 989, 919, 802, 756, 637, 614.

5.3.6.4. Synthesis of (*i*-Pr-DAD)Re(CH₃CN)(CO)₂OSO₂CF₃ (11):

(*i*-Pr-DAD)Re(CO)₃OSO₂CF₃ (**6**) (1 mg, 1.26 μmol) 1.00 mL of anhydrous tetrahydrofuran (final concentration 1.46 mM) were placed in a NMR tube. The solution was irradiated for 75 minutes at room temperature. This solution was used without further treatment. ¹H-NMR (CDCl₃): 1.26 (dd, 12H, ³J_{H-H} = 7.0 Hz, ³J_{H-H} = 7.0 Hz), 1.41 (d, 12H, ³J_{H-H} = 9.8 Hz, ³J_{H-H} = 6.9 Hz), 2.81 (m, 2H, ³J_{H-H} = 6.8 Hz), 3.07 (m, 2H, ³J_{H-H} = 6.8 Hz), 7.45 (m, 4H), 7.48 (m, 2H), 8.99 (s, 2H). ¹⁹F-¹H-NMR (CDCl₃): -78.2 ppm FT-IR (cm⁻¹): 2298 (ν_{CN}, w), 2041 (ν_{CO}, vs), 1942 (ν_{CO}, vs), 1722, 1633, 1437, 1279, 1261, 1180, 1118, 1030, 749, 693, 637.

5.3.7. Reactivity of α-diimine complexes with NO-donor:

The amount of Re-DAD (stock solution in THF or CH₃CN) complex solution needed for the final concentration was calculated assuming 4 mL of final 50 μM solution. Up to 2.0 mL of acetone was added in order to obtain a solution 50% v/v of total organic solvent. Up to 2.0 mL of aqueous buffer was added in order to obtain 50% v/v aqueous solution total between Phosphate Buffer Saline pH=7.4; and 4 mM NO-donor stock solution was prepared in 0.04M NaOH; specific amounts were added to achieve a final NO-donor concentration going from 1 to 200 μM range. No more than 10 minutes elapsed from the dilution of the THF/CH₃CN stock rhenium complex solution to the final addition of NO-donor. The solution was mixed thoroughly in a vortex and sonicated for 90 minutes at room temperature. At higher concentrations of NO-donor, the solutions became clear. The UV-Visible spectra of this set of solutions were recorded from 300 nm to 650 nm.

5.3.8. Reactivity of α-diimine complexes with NaCN:

The amount of Re-DAD (stock solution in THF or CH₃CN) complex solution needed for the final concentration was calculated assuming 4 mL of final 50 μM solution. Up to 2.0 mL of acetone was added in order to obtain a solution 50% v/v of total organic solvent. Up to 2.0 mL of aqueous buffer was added in order to obtain 50% v/v aqueous solution total between Phosphate Buffer Saline pH=7.4; and 4 mM NaCN stock solution was prepared in DI water; specific amounts were added to achieve a final NaCN concentration going from 1 to 100 μM range. No more than 10 minutes elapsed from the dilution of the THF/CH₃CN stock rhenium complex solution to the final addition of NO-donor. The solution was mixed thoroughly in a vortex and sonicated for 90 minutes at room temperature. At higher concentrations of NO-donor, the solutions became yellow. The UV-Visible spectra of this set of solutions were recorded from 300 nm to 650 nm.

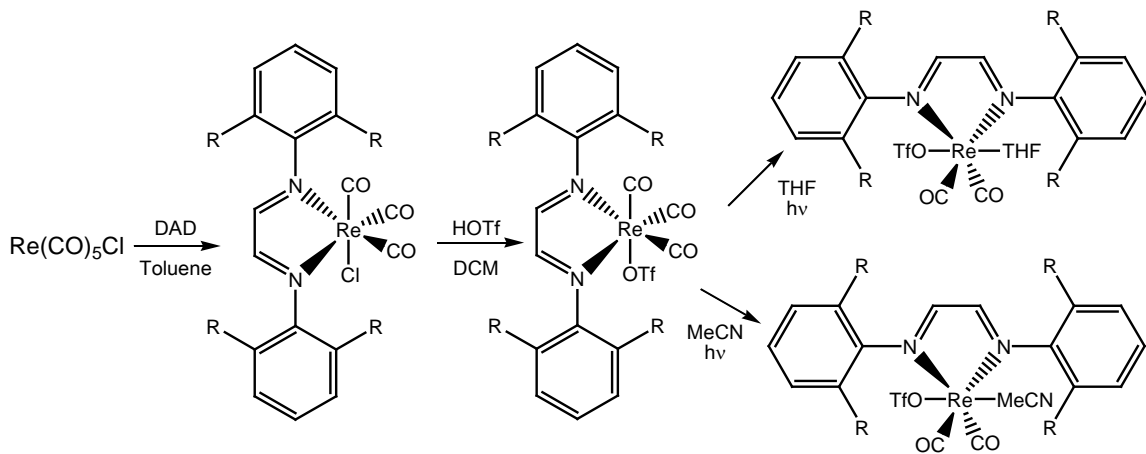
5.3.9. Comparison studies with other ions:

The solutions of 4 mM NaNO₂, NaNO₃, NaClO₄, NaOCl, H₂O₂, and NaCN were prepared in ultrapure water. ¹O₂ was prepared in situ, mixing same the amounts of NaOCl and H₂O₂. Stock solutions of (Xy-DAD) rhenium complexes were diluted in acetone for preparing 200 μM solutions. 2.8 mL of 0.002M PBS pH 7.2 was added to 1 mL of the previous prepared Re solutions. 100 μL of PBS was added to the first tube, this tube will be the standard (std). To the rest there were added 100 μL of each of the competitive ions and/or species (NO₂⁻, NO₃⁻, ClO₄⁻, ClO⁻, H₂O₂, CN⁻, and ¹O₂). After the solutions were allowed to react for 15 min, a UV-Visible spectrum was measured. 100 μL of NO-donor were added to each tube and all the solutions were allowed to react for 1 h, a second UV spectrum was recorded.

5.4. RESULTS

5.4.1. Synthesis of (R-DAD)Re(L)(CO)₂OSO₂CF₃ (R = Me or *i*-Pr; L = THF or CH₃CN)

Complexes **8**, **9**, **10**, and **11** were synthesized *in situ* by photochemical substitution of CO from (R-DAD)Re(CO)₃SO₃CF₃. The products were characterized by ¹H and ¹³C-NMR and FT-IR.



Scheme 5.3 Synthetic path for DAD-rhenium complexes

5.4.2. Reactivity of α -diimine complexes with NO-donor:

In the case of Figure 5.1, which shows the spectrum UV – Visible of the interaction of (Xy-DAD)Re(THF)(CO)₂OSO₂CF₃ (**8**) with NO-donor, in the form of MAHMA NONOate from 0 – 200 μM . It can be seen that the signal decreases at 478 nm and shifts hypsochromically, meanwhile the absorbance at 408 nm increases with an isobestic point around 451 nm. This color change can be observed by the naked eye.

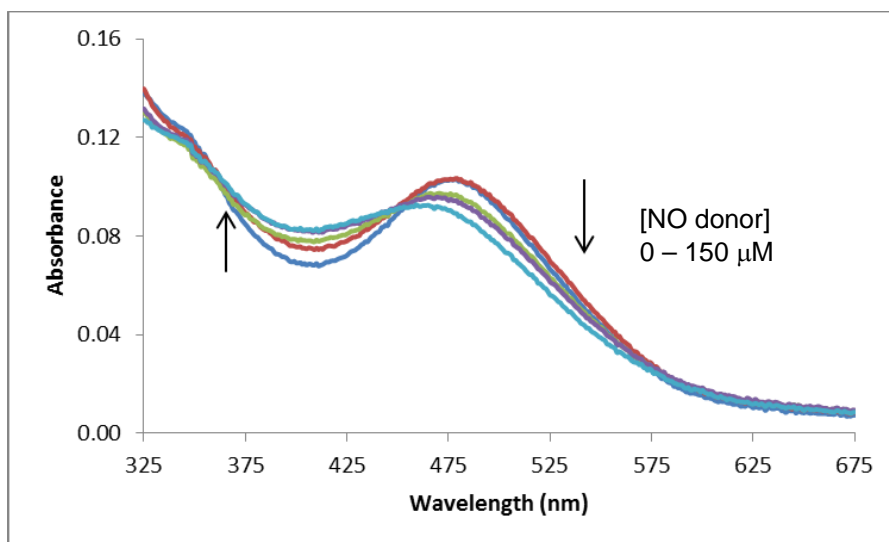


Figure 5.1 Interaction of $(Xy-DAD)Re(CO)_2(THF)OSO_2CF_3$ with NO-donor, acetone/PBS (1:1)

Complex **8** prepared photochemically on a 1 mg scale (1.67 mM in THF) showed also significant colorimetric response to NO addition both at 478 nm (decrease) and at 408 nm (increase). The UV-Vis spectrum of **8** in THF/acetone/buffer 2/48/50 shows one absorption signal at 478 nm. Figure 5.2 shows the plot of the local maxima and minima on the UV-Visible spectrum of **8** upon NO gradual addition, in the form of NO-donor MAHMA-NONOate (0-200 μ M) dissolved in 0.04 M NaOH. The graph also shows the ratio of the absorbance at 408 nm vs the absorbance at 478 nm. This plot shows a linear dependency at lower NO-donor concentrations.

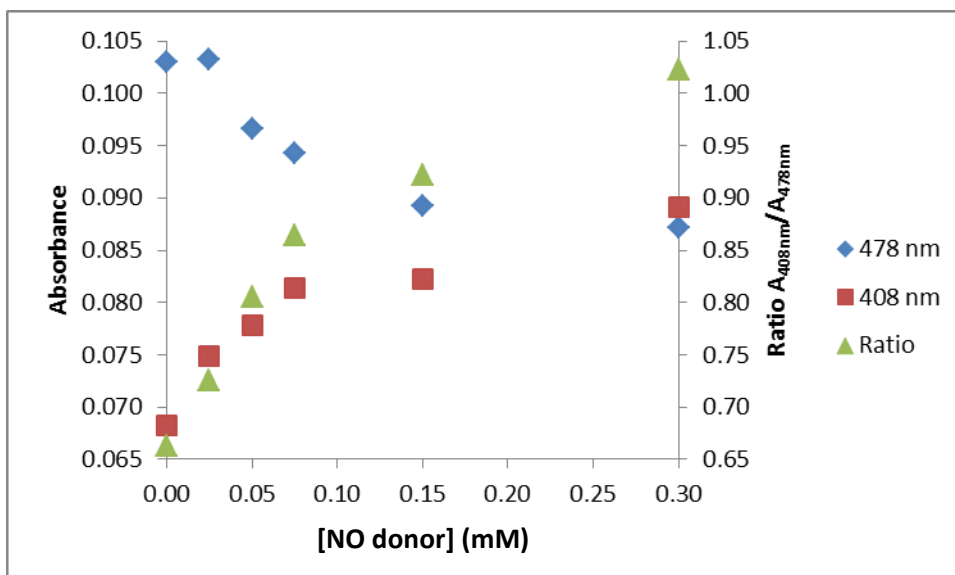


Figure 5.2 Relationship and ratio of the reaction of (Xy-DAD)Re(CO)₂(THF)OSO₂CF₃ with NO-donor, acetone/PBS (1:1)

In the case of Figure 5.3, which shows the spectrum UV – Visible spectrum of **8** in acetonitrile/acetone/buffer 2/48/50 of the interaction of (Xy-DAD)Re(CH₃CN)(CO)₂OSO₂CF₃ (**9**) with NO-donor, in the form of MAHMA NONOate in 0.04 M NaOH, from 0 – 200 μM . It can be seen that the signal decreases at 496 nm and shifts hypsochromically. In the case of figure 5.3 Interaction of **9** with NO-donor, it is shown that the signal at 496 nm decreases consistently when the NO-donor concentration increases. Like the previous case, this color change can be observed by the naked eye.

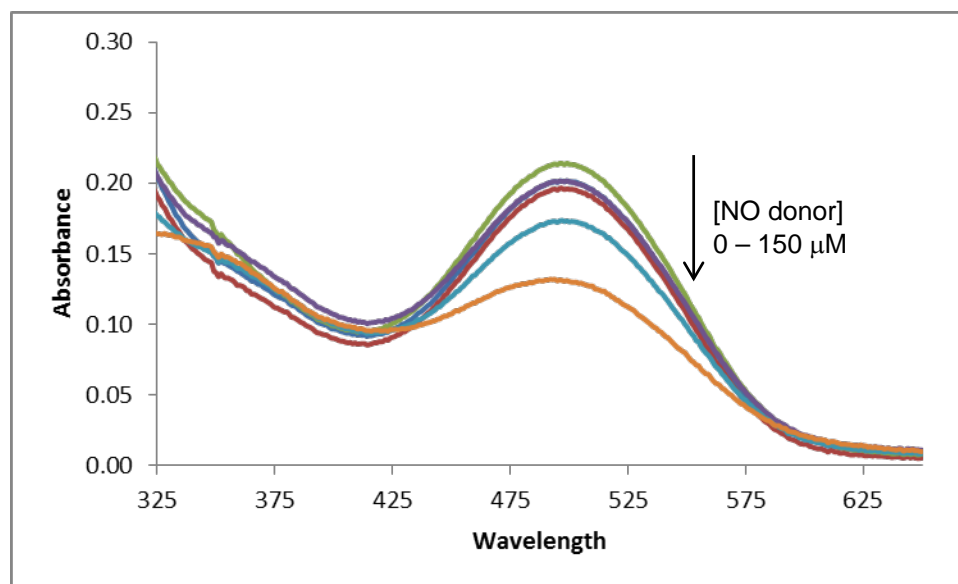


Figure 5.3 Interaction of $(\text{Xy-DAD})\text{Re}(\text{CO})_2(\text{CH}_3\text{CN})\text{OSO}_2\text{CF}_3$ with NO-donor, Acetone/PBS (1:1)

The results of the interaction of NO-donor with $(i\text{-Pr-DAD})\text{Re}(\text{CO})_2(\text{CH}_3\text{CN})\text{OSO}_2\text{CF}_3$ and $(i\text{-Pr-DAD})\text{Re}(\text{CO})_2(\text{THF})\text{OSO}_2\text{CF}_3$ were not clear because of the lower solubility of those complexes in mixed aqueous media. Even though the tendency was still observed, there was precipitation, which produced scattering and gave erratic absorbances.

5.4.3. Reactivity of α -diimine complexes with NaCN:

In the case of Figure 5.4, which shows the spectrum UV – Visible spectrum of **8** in THF/acetone/buffer 2/48/50 of the interaction of $(\text{Xy-DAD})\text{Re}(\text{THF})(\text{CO})_2\text{OSO}_2\text{CF}_3$ (**9**) with NaCN, from 0 – 100 μM . It can be seen that the signal decreases at 480 nm and shifts hypsochromically. In the case of figure 5.4 Interaction of **8** with NaCN, it is shown that the signal at 480 nm increases consistently when the NO-donor concentration increases. Like the previous case, this color change can be observed by the naked eye. There is a little decrease of the signal at 344 nm.

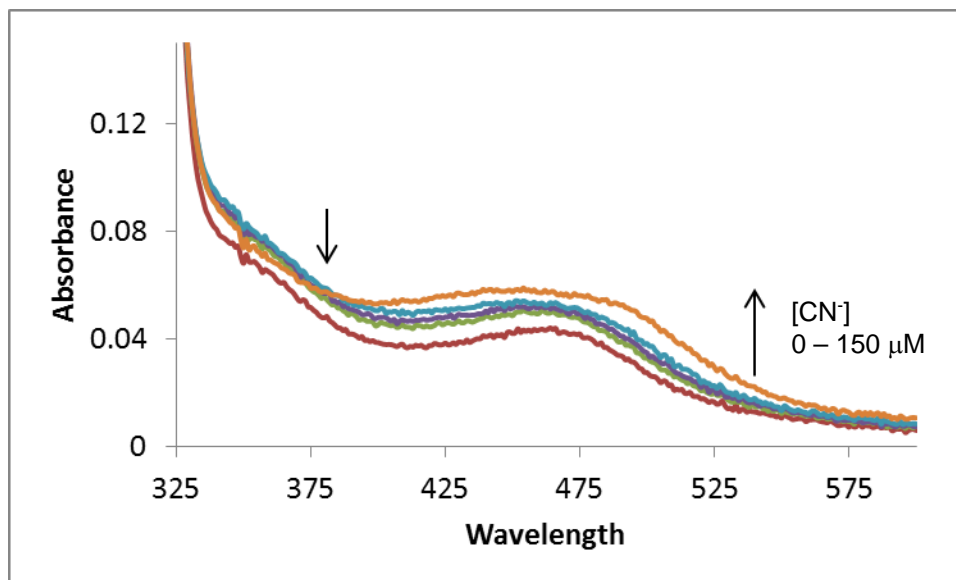


Figure 5.4 Interaction of $(Xy-DAD)Re(CO)_2(THF)OSO_2CF_3$ with NaCN, acetone/PBS (1:1)

In the case of Figure 5.5, which shows the spectrum UV – Visible of the interaction of $(Xy-DAD)Re(CH_3CN)(CO)_2OSO_2CF_3$ (**9**) with NaCN from 0 – 100 μM . It can be seen that the signal decreases at 500 nm and shifts hypsochromically, meanwhile the absorbance at 348 nm increases with an isosbestic point around 361 nm. This color change can be observed by the naked eye.

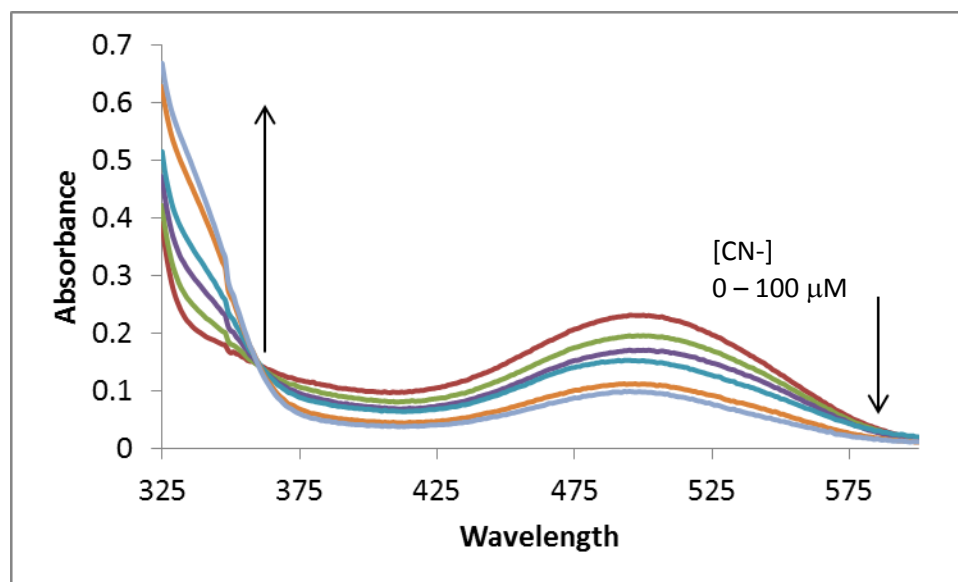


Figure 5.5 Interaction of $(Xy-DAD)Re(CO)_2(CH_3CN)OSO_2CF_3$ with NaCN, acetone/PBS (1:1)

The graph also shows the ratio of the absorbance at 500 nm vs the absorbance at 348 nm. This plot shows a linear dependency at lower NO-donor concentrations. Figure 5.6 shows the plot of the local maxima and minima on the UV-Visible spectrum of **9** upon CN^- gradual addition, in the form of NaCN (0-100 μM). The graph also shows the ratio of the absorbance at 348 nm vs the absorbance at 500 nm. This plot shows a linear dependency at lower NO-donor concentrations.

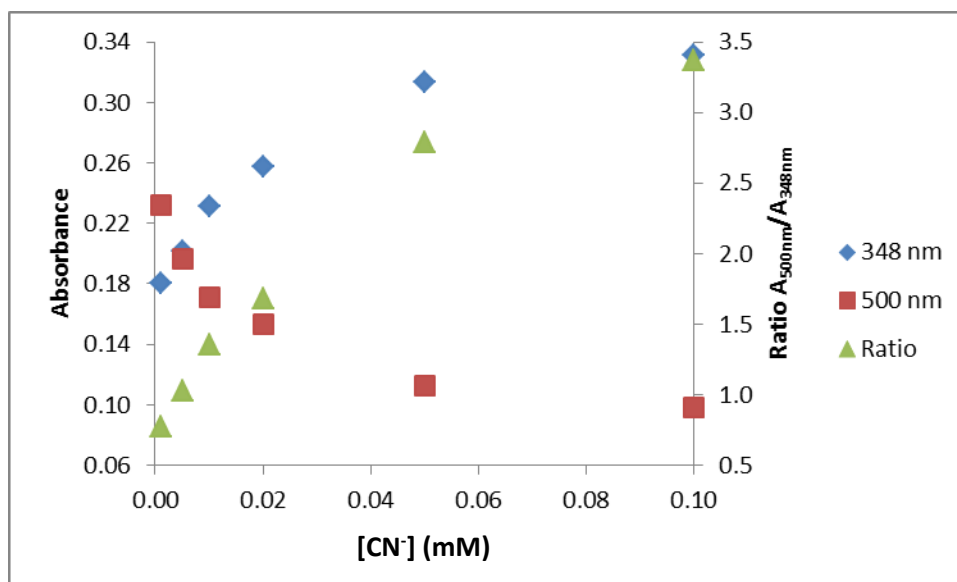


Figure 5.6 Relationship and ratio of the reaction of (Xy-DAD)Re(CO)₂(CH₃CN)OSO₂CF₃ with NaCN, acetone/PBS (1:1)

The results for the interaction of NaCN with (*i*-Pr-DAD)Re(CO)₂(CH₃CN)OSO₂CF₃ and (*i*-Pr-DAD)Re(CO)₂(THF)OSO₂CF₃ were not clear because of the lower solubility of those complexes in mixed aqueous media. Even though the tendency was still observed, there was precipitation, which produced scattering and gave erratic absorbances.

5.4.4. Comparison studies with other ions:

A study was conducted in order to investigate the behavior of these dyes in the presence of competitive anions and/or oxidant species. Re(I) centers and DAD ligands are known by their redox properties. The selected anions and oxidant species were NO₃⁻, NO₂⁻, ClO₄⁻, ClO⁻, H₂O₂, CN⁻ and ¹O₂ (this one was prepared in situ by reacting H₂O₂ with ClO⁻). Also, it was investigated whether the dyes still have activity towards NO after the reaction with these species.

Figure 5.7 shows the UV – Vis maxima of $(Xy-DAD)Re(THF)(CO)_2OSO_2CF_3$ (**8**) at 408 nm, in the presence of strong oxidants the color (qualitative) and the absorbance at 500 nm (quantitative) decreases to almost zero. In the presence of weak oxidants the color was diluted, in the presence of CN^- the solution turned yellow and the spectrum change considerably with a maximum of 348 nm.

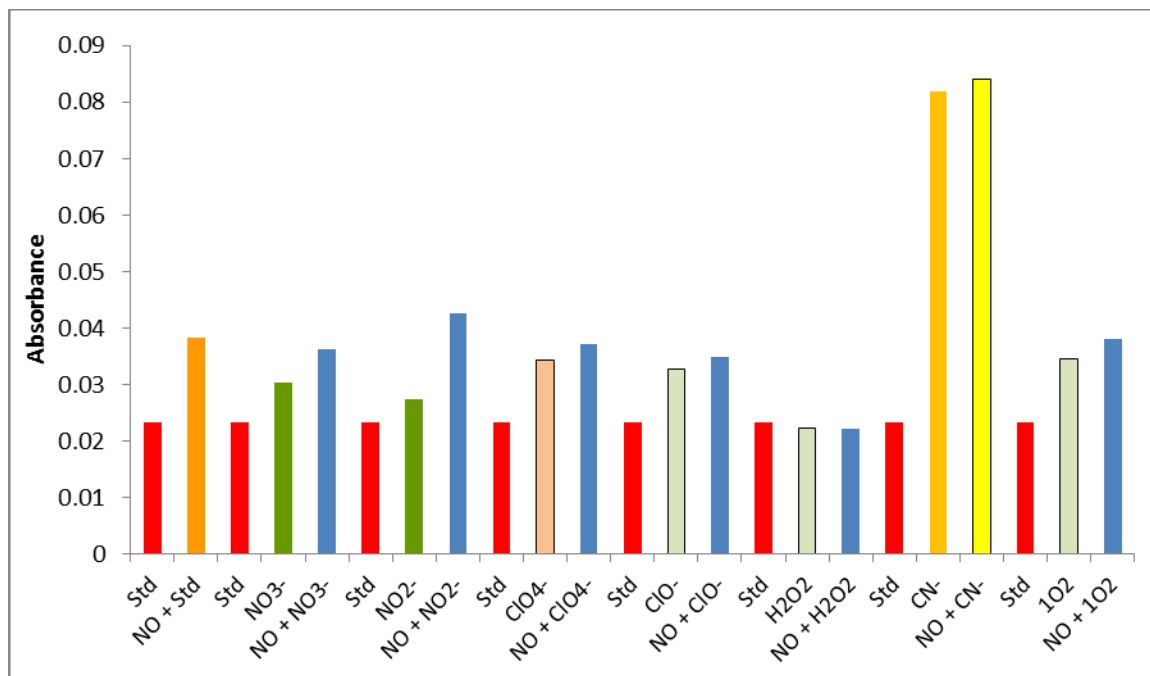


Figure 5.7 Comparison Studies among $(Xy-DAD)Re(THF)(CO)_2OSO_2CF_3$ with NO-donor and Different Ions at 500 nm

Figure 5.8 shows the UV – Vis maxima of $(Xy-DAD)Re(CH_3CN)(CO)_2OSO_2CF_3$ (**9**) at 500 nm, in the presence of strong oxidants the color (qualitative) and the absorbance at 500 nm (quantitative) decreases to almost zero. In the presence of weak oxidants the color was diluted, in the presence of CN^- the solution turned yellow and the spectrum change considerably with a maximum of 348 nm. In the case of CN^- , there is not difference between the **8** and **9** spectra. There is possibly substitution of THF or CH_3CN for CN^- .

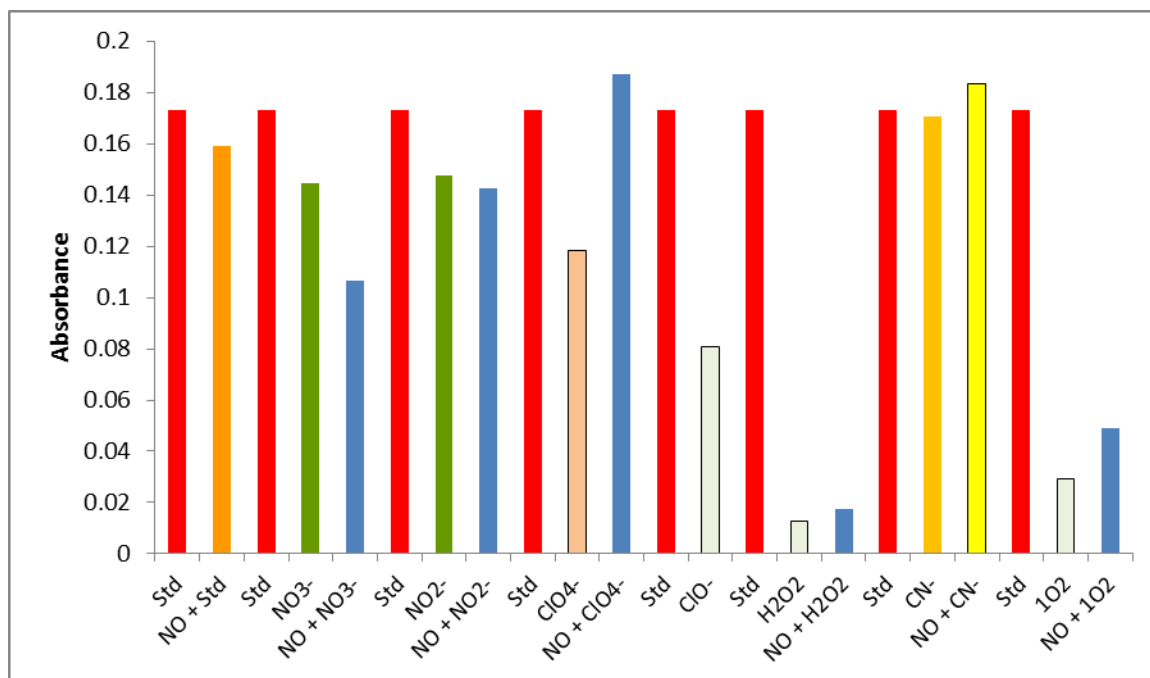


Figure 5.8 Comparison studies among (Xy-DAD)Re(CH₃CN)(CO)₂OSO₂CF₃ with NO-donor and Different Ions at 500 nm

The results obtained by (Xy-DAD)Re(CH₃CN)(CO)₂OSO₂CF₃ and (Xy-DAD)Re(THF)(CO)₂OSO₂CF₃ in the presence of competitive ions can be summarized as follows: a) in the presence of NO₂⁻, for both dyes, there were minor changes, and activity toward NO was not affected; b) in the presence of weak oxidants NO₃⁻ and ClO₄⁻, the results were not conclusive; activity toward NO was partially lost because of the partial oxidation of Re(I) center; c) in the case of CN⁻, there was ligand substitution, and the reaction exhibited a change of color from red to yellow - CN⁻ and NO are iso-electronics/iso-energetic and they compete for the same soft metal center; d) in the case of the strong oxidants ClO⁻, H₂O₂, and ¹O₂, the color was lost completely, in addition to the activity toward NO, d⁶ Re(I) possesses a ³MLCT transition, which is responsible for generating the optical response (as describe in section 1.4.3.) toward NO. Oxidation, partial or total, of the metal center from Re(I) to Re(III) deactivates the

organometallic dye because d^4 Re(III) does not possess 3 MLCT transition, which is required for the optical response.

5.5. CONCLUSIONS

Organometallic d^6 Re(I) complexes, with the DAD ligands, which can be used as NO and cyanide sensors. Re-DAD complexes are less soluble in mixed aqueous media than the phenanthroline analogs; they need twice the amount of acetone in the solvent mixture. Xy-DAD complexes are more soluble than *i*-Pr-DAD ones. In the presence of oxidants, the Re complexes are deactivated by oxidation presumably because non- d^6 Re centers do not possess the 3 MLCT transition state needed for the optical response.

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6. CONCLUSION

As conclusion of this study, $(LN_2)Re(L)(CO)_2OTf$ complexes ($LN_2 = phen$ or DAD ; $L = THF, CH_3CN,$ and PPh_3) can be used as NO fluorescence sensors. The response is significantly stronger when the reaction with NO-donor is performed under anaerobic conditions indicating that NO is the real substrate instead of NO oxidation products. Controlling the NaCl concentration in the buffer allows converting these dyes into ratiometric response dyes. The spectroscopy data elucidate a mechanism involving the detachment of two labile ligands. The complex $(phen)Re(PPh_3)(CO)_2OTf$ (**1**) has the most value as NO sensing fluorescent dye, because of it has the highest quantum yield of the Re-family group, and it is easy to manipulate because of it is isolable and it can be stored in powder form. This project can be extended in the future to other fluorescent ligands and Re complexes with higher quantum yields that can sense NO under physiological conditions.

APPENDIX 1. STUDY OF THE FLUORESCENT ORGANOMETALLIC COMPLEX (dppz)Re(L)(CO)₂OSO₂CF₃ AS A NO SENSOR (L=THF, CH₃CN, and PPh₃).

A1.1. ABSTRACT

Dipyrido[3,2-a:2',3'-c]phenazine (dppz) is used as fluorophore for DNA intercalator and fluorescent dye. (dppz)Re(THF)(CO)₂OSO₂CF₃ (**A2**), (dppz)Re(PPh₃)(CO)₂OSO₂CF₃ (**A3**), and (dppz)Re(CH₃CN)(CO)₂OSO₂CF₃ (**A4**), which can be obtained photochemically from (dppz)Re(CO)₃OSO₂CF₃ (**A1**) shows fluorescence quenching response upon NO addition, which was added in the form of NO-donor Z-1-N-methyl-N-[6-(N-methylammoniohexyl)amino]diazene-1,1,2-diolate (MAHMA-NONOate). Addition of 10-150 μM of NO-donor into a 25 μM solution of (dppz)Re(CH₃CN)(CO)₂OSO₂CF₃ or (dppz)Re(THF)(CO)₂OSO₂CF₃ in 25% v/v acetone/phosphate saline (PBS) buffer gave fluorescence quenching at 458 nm and 586nm, upon excitation at 360 nm and 380 nm. The ¹H-NMR and ¹⁹F-NMR spectra after reaction with NO showed characteristic resonances for non-coordinated triflate and the L ligand (L = THF, PPh₃ or CH₃CN), suggesting that the organometallic product formed upon NO releases both these ligands upon reaction with NO. This study expands our newly demonstrated approach for the design of fluorescent NO sensors based on NO coordination to Re;¹ **A1** is an isolable complex, which is stable at room temperature and therefore can be used as starting material for NO sensing organometallic dyes.

A1.2. INTRODUCTION

Fluorescent materials are attracting considerable interest due to their potential sensory, biochemical, medical, and photoelectronic applications, in addition to other

possible uses.² One type of fluorescent material that have been attracting attention are Dipyrido[3,2-a:2',3'-c]phenazine Ru(II) complexes. They have been used as “light switches” when they bind DNA.³ Through NMR, it was proved that Ru(phen)₂(dppz)⁺² intercalated double stranded DNA employing the dppz moiety. Ru(dppz) complexes were also used as electrochemical indicators for DNA double-helix’s detection.⁴

In many metal polypyridyl complexes, the most important state is the metal-to-ligand charge-transfer (MLCT) state, which is important to defining photophysical properties.⁵ The Barton *et al.* study proved that dppz become emissive when the nitrogens are shielded from water; otherwise, these complexes are non-emissive.⁶

Many photophysical and theoretical studies have suggested that Re(I)–and Ru(II)–dppz complexes exhibit two low-energy triplet metal to ligand charge-transfer (MLCT) states and one triplet ligand-centered state.⁷ The ³MLCT (phen) state is emissive because of environment stabilization, while the ³MLCT (phz) state is a dark state.⁸

dppz complexes are weakly emissive in CH₂Cl₂. For most complexes, their emission is short, decaying in less than 10 ns after the laser pulse.⁹ It was found that [Re(CO)₃(dppz)Cl] complexes possess MLCT states based on the phen portion of the dppz ligands, which does not occur in [Re(CO)₃(dppz)(py)]PF₆, which is L⁺¹, in which the phenazine is responsible of the transition. Many of the molecular orbitals in these compounds show extensive delocalization across the dppz ligand, in particular for these cationic species, which have L⁺¹ extended over the whole dppz system (Figure A1.1) destabilizing the orbital.

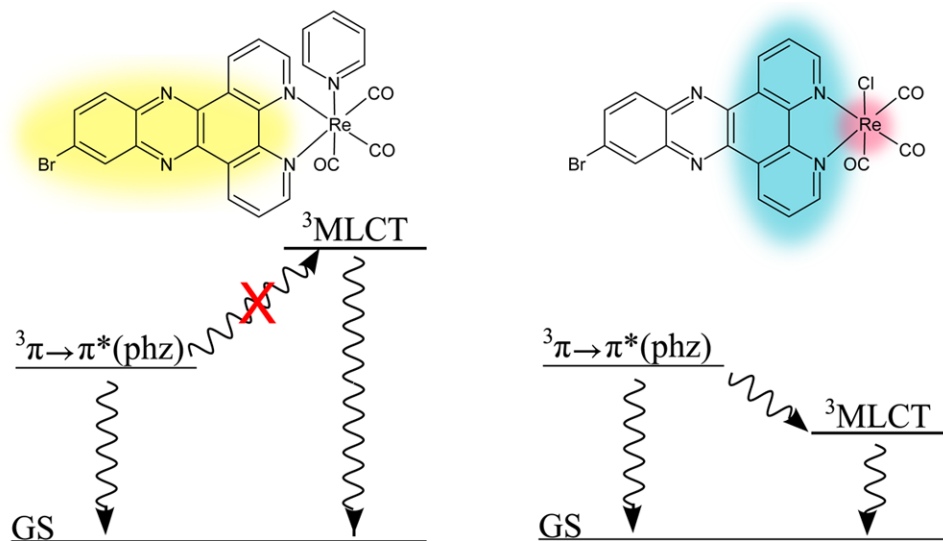


Figure A1.1 Energy diagram for (a) [(dppz)Re(CO)₃(py)]PF₆ and (b) (dppz)Re(CO)₃Cl

A1.3. EXPERIMENTAL SECTION

A1.3.1. Materials and Methods

(dppz)Re(CO)₃Cl¹⁰ was prepared with a modification of the previously reported procedure for (phen)Re(CO)₃Cl.¹¹ All other materials (purchased from Aldrich Chemical Co., ACROS organics, Cayman Chemicals, or Alfa Aesar) were standard reagent grade and were used without further purification, unless otherwise noted. PBS buffer (0.002 M in total phosphate, pH = 7.2, [NaCl] = 0.04 M) was prepared from K₂HPO₄, KH₂PO₄, and NaCl (all from Fisher Scientific). Filtered water (18 MΩ·cm⁻¹) produced from a Millipore® nanopure diamond lab water system (Barnstead Thermolyne Corporation, Dubuque, IA) was used for preparation of the buffers and experiments with NO. Deuterated solvents were purchased from Cambridge Isotope Laboratories Ltd. ¹H and ¹³C NMR spectra were recorded on a 400 Bruker NMR spectrometer and were referenced, using the residual solvent resonances. All chemical shifts, δ, are reported in

ppm. FT-IR spectra were recorded with a Perkin Elmer Spectrum 100 FT-IR Spectrometer.

A1.3.2. Synthesis of (dppz)Re(CO)₃OSO₂CF₃:

The complex (dppz)Re(CO)₃OSO₂CF₃ was prepared by a modification of the previously reported procedure for (phen)Re(CO)₃OSO₂CF₃.¹² 150.0 mg of (dppz)Re(CO)₃Cl (255 μmol) and 10 mL of dichloromethane (final concentration: 25.5 mM) were placed in a 50 mL round bottom flask under N₂ flow. 250 μL of HOSO₂CF₃ were added dropwise in a 30 minute period, after the solution was left stirring for 2 h. The color changed during reflux from orange to red. 250 μL of ethanol were added and the volatiles were evaporated to 1.0 mL. The product was precipitated at room temperature by adding 4.0 mL of diethyl ether. The bright orange powder product was filtered and was dried *in vacuo* at 40 °C. 168.1 mg, (93%). ¹H-NMR (CDCl₃): 8.09 (dd, 2H, ³J_{H-H} = 6.6 Hz, ³J_{H-H} = 3.4 Hz), 8.10 (dd, 2H, ³J_{H-H} = 8.2 Hz, ³J_{H-H} = 5.2 Hz), 8.48 (dd, 2H, ³J_{H-H} = 6.6 Hz, ³J_{H-H} = 3.4 Hz), 9.50 (dd, 2H, ³J_{H-H} = 5.2 Hz, ³J_{H-H} = 1.4 Hz), 9.96 (dd, 2H, ³J_{H-H} = 8.2 Hz, ³J_{H-H} = 1.4 Hz). ¹⁹F-¹H-NMR (CDCl₃): -78.1 ppm. FT-IR (cm⁻¹): 2015 (ν_{CO}, s), 1875 (ν_{CO}, s), 1629, 1604, 1584, 1520, 1427, 1417, 1350, 1321, 1277, 1255, 1223, 1145, 1109, 1058, 1030, 908, 848, 808, 780, 723, 638. UV-Vis (in ethanol): λ_{max} = 360 nm - br (ε = 1840 mol⁻¹cm⁻¹), 380 nm - (ε = 1840 mol⁻¹cm⁻¹).

A1.3.3. Photochemical substitution:

All the solutions were irradiated with a Rayonet photochemical reactor (Southern New England Ultraviolet, model RPR-100), equipped with a cooling fan and 10 phosphor coated low-pressure mercury lamps (λ_{max} = 300 nm, light intensity = 5.2 ± 0.1 × 10⁶

photon/sec/cm³). After 75 min. of irradiation, the colors of the solutions changed to darker colors.

A1.3.3.1. Synthesis of (dppz)Re(THF)(CO)₂OSO₂CF₃ (A2):

(dppz)Re(CO)₃OSO₂CF₃ (A1) (1 mg, 1.46 μmol) 1.00 mL of anhydrous THF (final concentration 1.46 mM) were placed in a NMR tube. The solution was irradiated for 75 min. at room temperature. This solution was used without further treatment. ¹H-NMR (CDCl₃): 7.98 (dd, 2H, ³J_{H-H} = 6.6 Hz, ³J_{H-H} = 2.8 Hz), 8.12 (dd, 2H, ³J_{H-H} = 8.2 Hz, ³J_{H-H} = 5.2 Hz), 8.48 (dd, 2H, ³J_{H-H} = 6.6 Hz, ³J_{H-H} = 3.4 Hz), 9.44 (dd, 2H, ³J_{H-H} = 5.2 Hz, ³J_{H-H} = 1.4 Hz), 9.87 (dd, 2H, ³J_{H-H} = 8.2 Hz, ³J_{H-H} = 1.4 Hz). ¹⁹F-¹H-NMR (CDCl₃): -78.4 ppm. FT-IR (cm⁻¹): 2032 (ν_{CO}, s), 1915 (ν_{CO}, s), 1640, 1460, 1442, 1423, 1344, 1320, 1282, 1241, 1177, 1115, 1064, 1030, 908, 848, 808, 780, 723, 638.

A1.3.3.2. Synthesis of (dppz)Re(CH₃CN)(CO)₂OSO₂CF₃ (A3):

(dppz)Re(CO)₃OSO₂CF₃ (A1) (1 mg, 1.46 μmol) 1.00 mL of acetonitrile (final concentration 1.46 mM) were placed in a NMR tube. The solution was irradiated for 75 minutes at room temperature. This solution was used without further treatment. ¹H-NMR (CDCl₃): 2.24 (s, 3H), 8.04 (d, 2H, ³J_{H-H} = 6.6 Hz, ³J_{H-H} = 3.4 Hz), 8.13 (dd, 2H, ³J_{H-H} = 8.2 Hz, ³J_{H-H} = 5.2 Hz), 8.46 (dd, 2H, ³J_{H-H} = 6.6 Hz, ³J_{H-H} = 3.4 Hz), 9.38 (d, 2H, ³J_{H-H} = 4.4 Hz), 10.01 (dd, 2H, ³J_{H-H} = 7.3 Hz). ¹⁹F-¹H-NMR (CDCl₃): -78.3 ppm. FT-IR (cm⁻¹): 2322 (ν_{CN}, w), 2042 (ν_{CO}, vs), 1953 (ν_{CO}, vs), 1941 (ν_{CO}, vs), 1924 (ν_{CO}, vs), 1642, 1496, 1422, 1358, 1283, 1252, 1226, 1163, 1031, 820, 775, 731, 639.

A1.3.3.3. Synthesis of (dppz)Re(PPh₃)(CO)₂OSO₂CF₃ (A4):

(dppz)Re(CO)₃OSO₂CF₃ (A1) (1 mg, 1.46 μmol) 1.00 mL of acetonitrile (final concentration 1.46 mM) were placed in a NMR tube. The solution was irradiated for 75

minutes at room temperature. This solution was used without further treatment. ^1H -NMR (CDCl_3): 6.96 (t, 3H, $^3J_{\text{H-H}} = 7.0$ Hz), 7.39 (dd, 6H, $^3J_{\text{H-H}} = 7.6$ Hz, $^3J_{\text{H-H}} = 2.0$ Hz), 7.62 (dd, 6H, $^3J_{\text{H-H}} = 12.5$ Hz, $^3J_{\text{H-H}} = 7.2$ Hz), 8.06 (d, 2H, $^3J_{\text{H-H}} = 6.6$ Hz, $^3J_{\text{H-H}} = 3.4$ Hz), 8.09 (dd, 2H, $^3J_{\text{H-H}} = 8.3$ Hz, $^3J_{\text{H-H}} = 5.2$ Hz), 8.49 (dd, 2H, $^3J_{\text{H-H}} = 6.6$ Hz, $^3J_{\text{H-H}} = 3.4$ Hz), 9.56 (dd, 2H, $^3J_{\text{H-H}} = 5.2$ Hz, $^3J_{\text{H-H}} = 1.8$ Hz), 9.93 (dd, 2H, $^3J_{\text{H-H}} = 8.3$ Hz, $^3J_{\text{H-H}} = 1.8$ Hz). ^{19}F - $\{^1\text{H}\}$ -NMR (CDCl_3): -78.4 ppm FT-IR (cm^{-1}): 2032 (ν_{CO} , s), 1932 (ν_{CO} , s), 1914 (ν_{CO} , s), 1648, 1460, 1439, 1366, 1260, 1178, 1120, 1052 (s-br), 897, 803, 780, 723, 696.

A1.3.4. Fluorescence experiments with NO-donor

In a typical experiment, a 100 μM solution of $(\text{dppz})\text{Re}(\text{THF})(\text{CO})_2\text{OSO}_2\text{CF}_3$ complex was prepared from 1.67 mM stock solution of $(\text{dppz})\text{Re}(\text{THF})(\text{CO})_2\text{OSO}_2\text{CF}_3$ in THF and acetone was added up to 1.000 mL of total solution of Re complex. 2.75 mL to 3.00 mL of aqueous PBS buffer (pH = 7.2) was added. Then 0 to 250 μL of 4 mM stock solution of MAHMA-NONOate NO-donor, in 0.04 M NaOH, were added in order to obtain 75% v/v aqueous/organic solution and a final NO-donor concentration from 0 to 200 μM range, while the concentration of Re complex remained constant at 25 μM . No more than 10 minutes elapsed from preparation of the organic phase to the addition of the NO-donor. The solution was mixed thoroughly in a vortex and sonicated for 90 minutes at room temperature. The fluorescence of all solutions was measured with a $\lambda_{\text{exc}} = 360$ nm and 380 nm. The emission spectrum was recorded from 400 nm to 700 nm.

A1.3.5. UV-Visible spectroscopy experiments with NO-donor

The $(\text{dppz})\text{Re}(\text{THF})(\text{CO})_2\text{OSO}_2\text{CF}_3$ (**A2**), $(\text{dppz})\text{Re}(\text{THF})(\text{CO})_2\text{OSO}_2\text{CF}_3$ (**A3**), and $(\text{dppz})\text{Re}(\text{THF})(\text{CO})_2\text{OSO}_2\text{CF}_3$ (**A4**) samples were prepared in the same way that in A1.3.4. The UV-Visible spectra of all solutions were recorded from 300 nm to 600 nm.

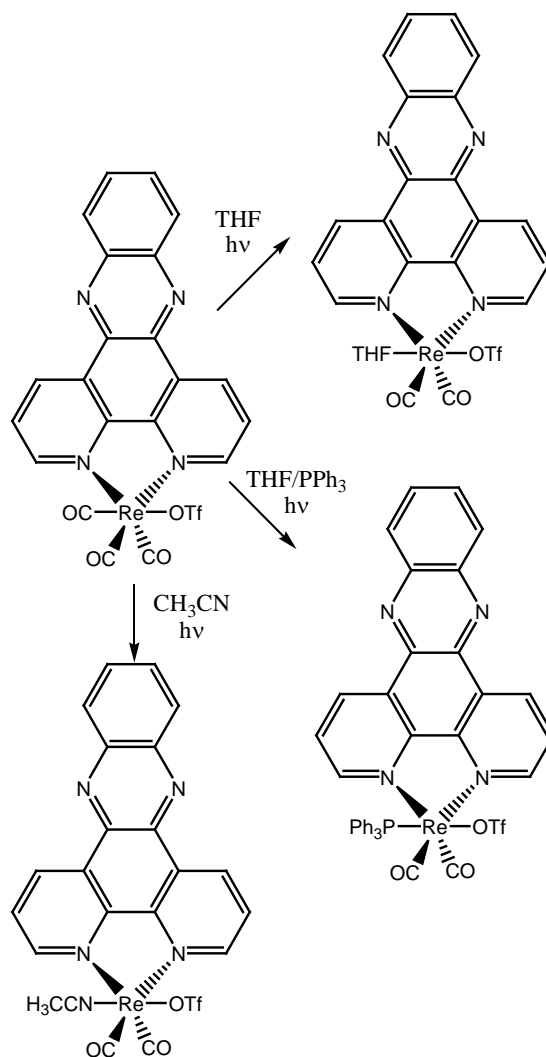
A1.3.6. ^1H -NMR and ^{19}F -NMR Studies of dppz complexes

The performed procedure was similar to sections 2.3.5 and 3.3.4; the stock solutions of **A2**, **A3** and **A4** after reaction with NO-donor were extracted with dichloromethane. The organic fractions were dried with Na_2SO_4 and the volatiles were evaporated. The residue was redissolved with chloroform-d and NMR was performed.

A1.4. RESULTS AND DISCUSSION

A1.4.1. Synthesis of $(\text{dppz})\text{Re}(\text{L})(\text{CO})_2\text{OSO}_2\text{CF}_3$

Complexes **A2**, **A3** and **A4** were synthesized by photochemical substitution of CO from $(\text{dppz})\text{Re}(\text{CO})_3\text{SO}_3\text{CF}_3$ (**A1**). The products were characterized by ^1H and FT-IR.



Scheme A1.1 Synthesis of $(\text{dppz})\text{Re}(\text{L})(\text{CO})_2\text{OSO}_2\text{CF}_3$

A1.4.2. Fluorescence studies with addition of NO-donor

The PPh_3 and CH_3CN complexes **A2** and **A3** show no fluorescence spectra. The $\text{Re}(\text{dppz})(\text{CO})_2(\text{THF})\text{OTf}$ complex **A2** showed small fluorescence response to NO addition; UV-Vis spectrum exhibited two signals at 360 nm and at 380 nm. Figure A1.1 shows the fluorescence spectrum of **A2** at $\lambda_{\text{exc}} = 360$ nm, upon NO gradual addition, in the form of NO-donor MAHMA-NONOate (0 - 150 μM) dissolved in 0.04 M NaOH. The emissions at 570 nm and 424 nm decrease slightly upon NO addition and the intensity

changes show linearity with NO-donor concentration (Figure A1.2).

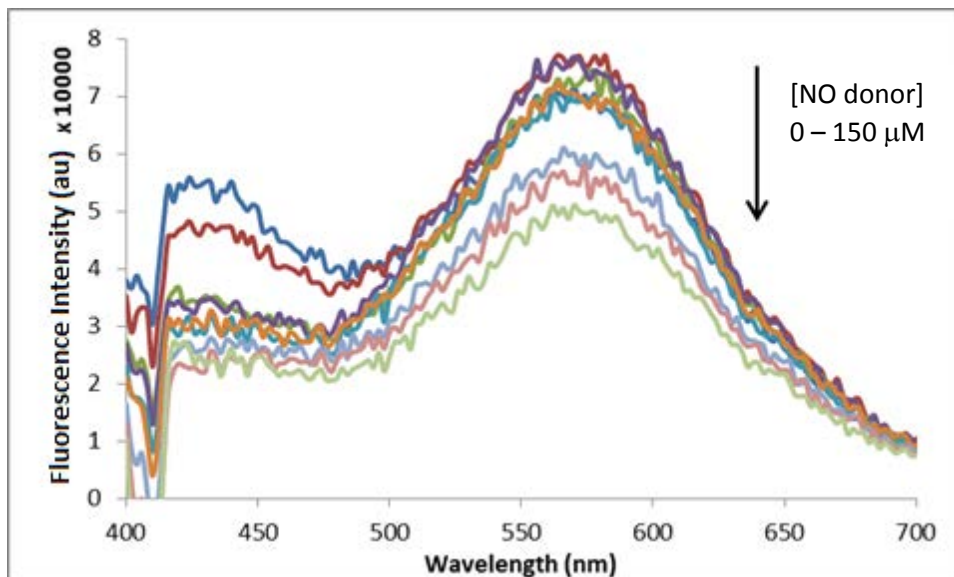


Figure A1.2 Fluorescence Response upon NO-donor addition (0-150 μM) to 25 μM of Re complex **A1** in acetone/PBS aqueous buffer 25/75 v/v, ($\lambda_{\text{exc}} = 360 \text{ nm}$)

Figure A1.3 shows the fluorescence spectrum of **A2** at $\lambda_{\text{exc}} = 380 \text{ nm}$ upon NO gradual addition, in the form of NO-donor MAHMA-NONOate (0-150 μM) dissolved in 0.04 M NaOH. The emission responses were similar to those observed for $\lambda_{\text{exc}} = 360 \text{ nm}$.

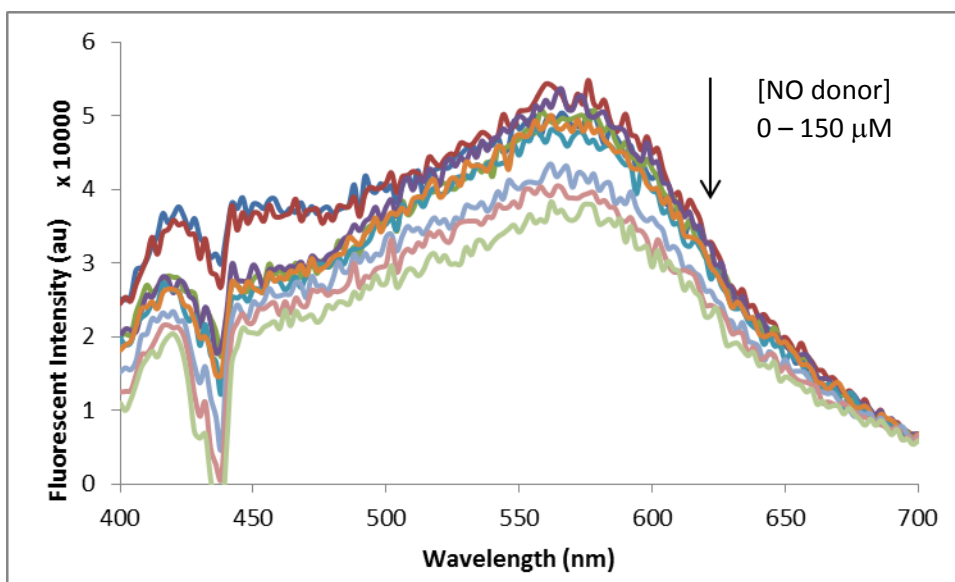


Figure A1.3 Fluorescence Response upon NO-donor addition (0-150 μM) to 25 μM of Re complex **A1** in acetone/PBS aqueous buffer 25/75 v/v, ($\lambda_{\text{exc}} = 380 \text{ nm}$)

A1.4.3. UV-Visible spectroscopy studies with addition of NO-donor

The THF, PPh_3 and CH_3CN complexes (**A2**, **A3** and **A4**, respectively) showed significant response upon NO addition under physiological conditions in UV-Vis spectra. Figure A1.4 shows the effects upon NO addition, in the form of NO-donor MAHMA-NONOate (0 – 150 μM) dissolved in 0.04 M NaOH to the CH_3CN complex **A4**. The absorption at 305 nm, 360 nm, 380 nm increased steadily upon NO addition and its intensity showed linear dependence with NO-donor concentration.

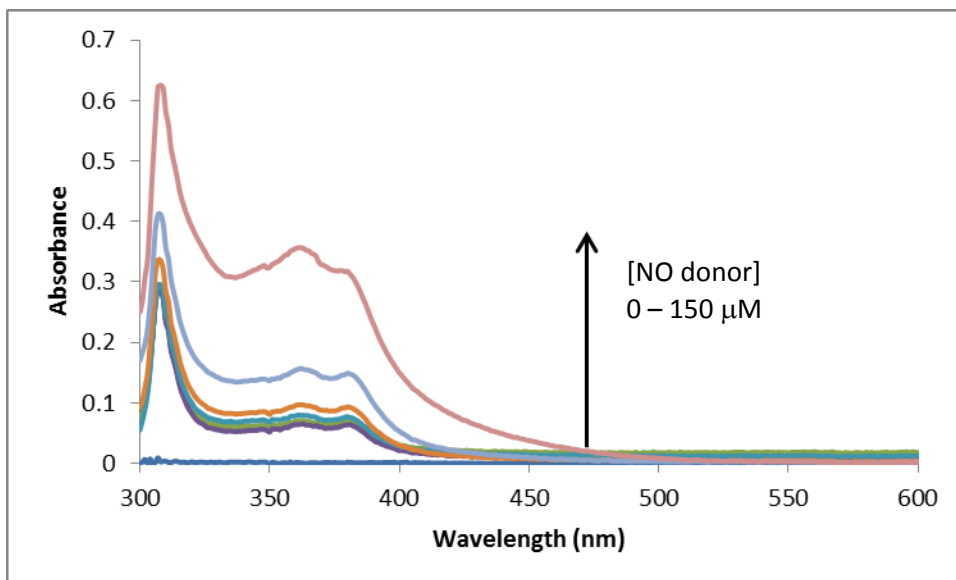


Figure A1.4. UV-Vis Spectra of **A4** (25 μM) after addition of different amounts of NO donor, in acetone/buffer 25:75.

Figure A1.5 shows the UV-Vis response of **A4** with NO concentration. At low concentrations the change is not noticeable. After addition of 100 μM NO, the change in response became more noticeable.

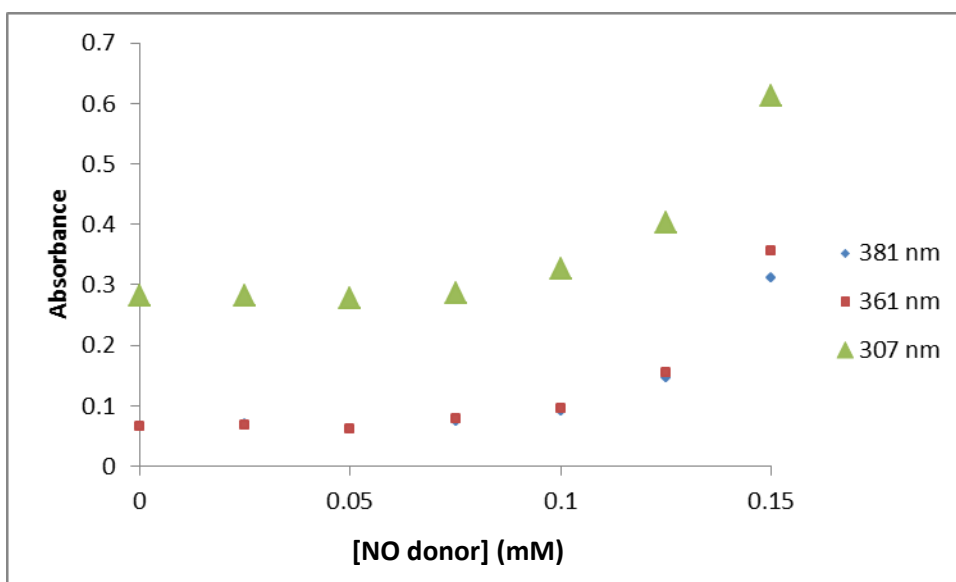


Figure A1.5 UV-Visible absorbance of **A4** 25 μM vs NO concentration, in acetone/buffer 25:75,

Figures A1.5 and A1.6 show the UV-Vis spectral responses upon NO addition to THF and PPh₃ dppz complexes **A2** and **A3**. After 75 μmol NO addition, there is a change in the slope of the linear response.

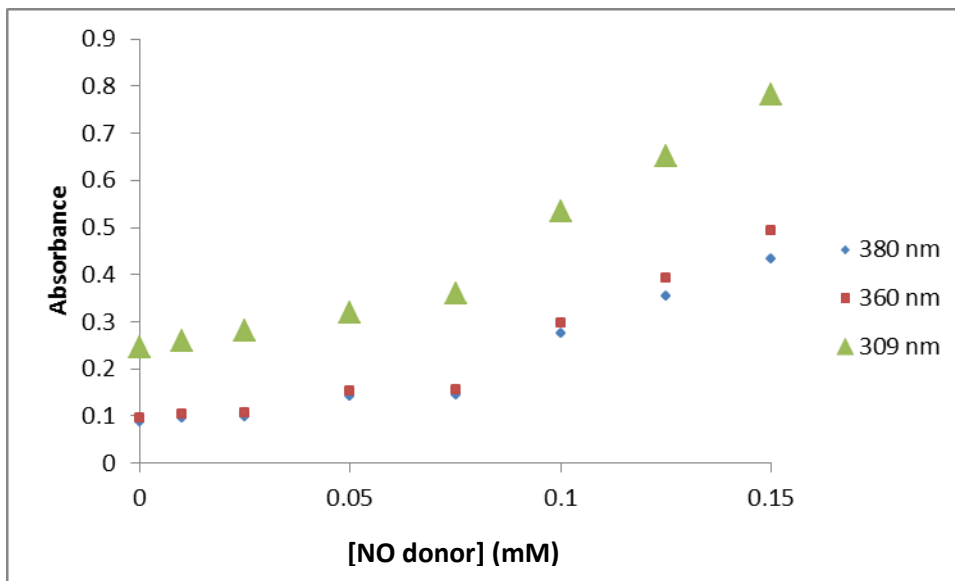


Figure A1.6 UV-Visible absorbance of **A2** 25 μM **6** vs NO concentration, in acetone/buffer 25:75

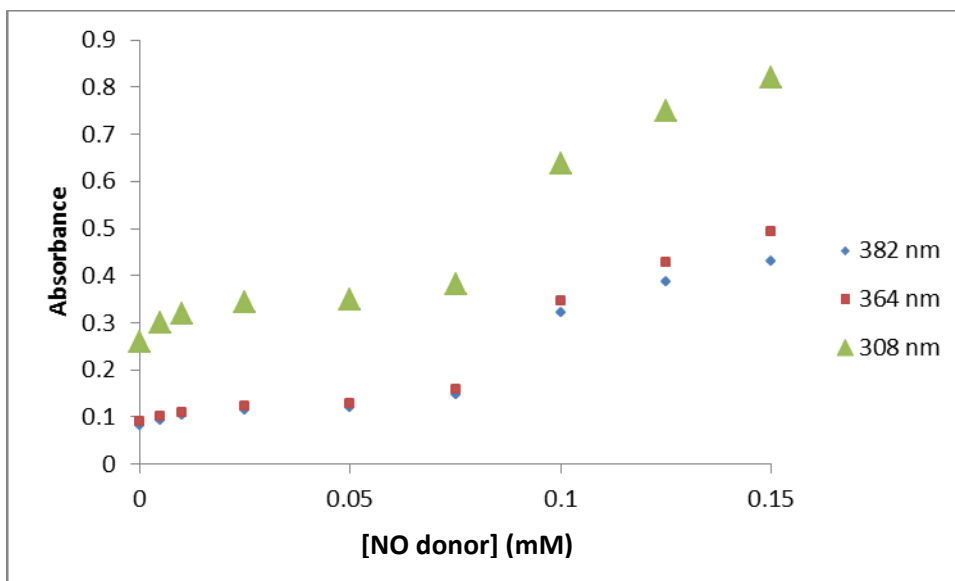


Figure A1.7 UV-Visible absorbance of **A3** 25 μM **6** vs NO concentration, in acetone/buffer 25:75

A1.4.4. ¹H-NMR and ¹⁹F-NMR studies on dppz complexes

Similar to the phenanthroline analogs, ¹H-NMR showed, for all three NO-active dppz complexes (dppz)Re(L)(CO)₂OTf (THF for **A2**, PPh₃ for **A3**, and CH₃CN **A4**), that after reaction with NO-donor, the chemical shift of “free – unbound” L – ligand plus a generic “dppz-Re” signals, which are similar for the three dppz-Re complexes. ¹⁹F-NMR showed that after reaction with NO-donor there is not CF₃SO₃⁻ signal (or other F – containing species). These results help to prove that triflate group (CF₃SO₃⁻) gets detached from the metal center, and it was discarded in the aqueous layer.

All dppz complexes exhibited only small, yet linear changes in response upon NO addition. Both the initial fluorescence intensities, and the responses of dppz complexes upon NO addition were notably lower than for the phen complexes. Partial protonation of the basic N on the dppz framework can explain the lower initial fluorescence intensities in all complexes, because of the dppz ³MLCT is dark; opposite to ³MLCT of phen with is emissive due to environment stabilization. The lower response to NO addition is due to dppz complexes are slightly inert and Re-dppz are less prone to ligand substitution.

A1.5. CONCLUSION

The (dppz)Re(L)(CO)₂OSO₂CF₃ complexes have been synthesized and characterized. These complexes react with NO under physiological conditions, producing a UV-Vis response. The THF complex **A2** showed also small quenching in fluorescence, with linear response to NO addition. NMR Studies of all three NO-active complexes proved that after reaction with NO triflate and L ligand get disassociated from the metal center. The similarities between phen, DAD, and dppz complexes, toward

reaction with NO, help to elucidate the possible mechanisms that undergo these Re(I) complexes.

A1.6. REFERENCES

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6.6 Hz, $^3J_{\text{H-H}} = 3.4$ Hz), 8.46 (dd, 2H, $^3J_{\text{H-H}} = 6.6$ Hz, $^3J_{\text{H-H}} = 3.5$ Hz), 9.47 (dd, 2H, $^3J_{\text{H-H}} = 5.2$ Hz, $^3J_{\text{H-H}} = 1.5$ Hz), 9.87 (dd, 2H, $^3J_{\text{H-H}} = 8.3$ Hz, $^3J_{\text{H-H}} = 1.5$ Hz). UV-Vis (in ethanol): $\lambda_{\text{max}} = 360$ nm - br ($\epsilon = 1840$ mol $^{-1}$ cm $^{-1}$), 380 nm - ($\epsilon = 1840$ mol $^{-1}$ cm $^{-1}$).

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APPENDIX 2. ESI-MS STUDY OF REACTIVITY OF Re-PHENANTHROLINE COMPLEXES WITH NO

A2.1. ABSTRACT

The ESI-MS spectra of the phenanthroline complexes $\text{Re}(\text{phen})(\text{CO})_3\text{LX}$ ($\text{L}=\text{PPh}_3$, CO , THF , $\text{X}=\text{Cl}^-$, OTf^-) were collected, after reaction with NO donor. Spectra of $\text{Re}(\text{phen})(\text{CO})_2\text{PPh}_3\text{OTf}$ (**1**) and $\text{Re}(\text{phen})(\text{CO})_2(\text{THF})\text{OTf}$ (**2**) after reaction with NO showed presence of a peak at $m/z = 339.4$ (for **1**) and $m/z = 391.2$ (for **2**) corresponding to $[\text{Re}(\text{CO})_2(\text{NO})_2(\text{H}_2\text{O})_2]^+$ (for **1**) and $[\text{Re}(\text{CO})_2(\text{NO})(\text{acetone})_2]^+$ (for **2**). The ESI-MS spectra of control reactions of $\text{Re}(\text{phen})(\text{CO})_3\text{Cl}$ and $\text{Re}(\text{phen})(\text{CO})_3\text{OTf}$ with NO, show no peaks of any NO-metal containing species, as expected, since these species do not react with NO (See Chapter 2). Instead those spectra showed a peak at $m/z = 492.2$, which corresponds to the species $[\text{Re}(\text{phen})(\text{CO})(\text{H}_2\text{O})_2(\text{acetone})]^+$. This peak was also observed along with other decomposition products for the spectra of **1** and **2** after NO reaction, yet in lower intensity than the peaks of the NO-containing products.

A2.2. INTRODUCTION

Due to its versatility, electrospray ionization mass spectrometry (ESI-MS) offers the development for identification of several organic and inorganic compounds.¹ ESI-MS can detect and characterize single species in complex matrixes, which are not involved in equilibria or solvation processes. This is very important for the characterization of metal-ligand systems. ESI-MS may indicate the stoichiometry of new complexes.^{2,3,4}

The ESI-MS technique uses soft ionization, this characteristic decreases excessive fragmentation and parent compounds in solution can be detected with this technique. Charge and nature of the analyte is determinant for choosing the mode in which can be

detected more efficiently. This technique offers two modes positive and negative, giving different patterns from the same molecule.^{5a} Important structural information can be recovered directly from the m/z ; in the case of structural conflicts, MS-MS spectra or isotopic pattern can be used for full identification.^{5b} ESI-MS has been used to characterize cationic Re(I)-phenanthroline complexes.⁶

A2.3. SAMPLE PREPARATION

(phen)Re(PPh₃)(CO)₂OSO₂CF₃ (**1**), (phen)Re(THF)(CO)₂OSO₂CF₃ (**2**), (phen)Re(CO)₃Cl, or (phen)Re(CO)₃OSO₂CF₃ (1 mg of each one) were added to 0.05 mL of THF in four separate 10 mL vials. 1.6 mL of acetone was added. Then 2.4 mL of PBS aqueous buffer (pH = 7.2) and 0.2 mL of stock NO-donor solution 5 mM in 0.04 M NaOH were added sequentially. The mixtures were vortexed for 30 sec and then sonicated for 3h at room temperature. Dichloromethane was added (3 x 10 mL), and the organic layer was dried with anhydrous sodium sulfate. In order to remove uncomplexed phenanthroline products, the organic phases were washed with 1 M hydrochloric acid, followed by 1 M sodium bicarbonate, rinsed with deionized water, and dried with anhydrous sodium sulfate. The volatiles were evaporated; the solid residues were redissolved in acetonitrile. The samples were measured using ESI in positive mode.

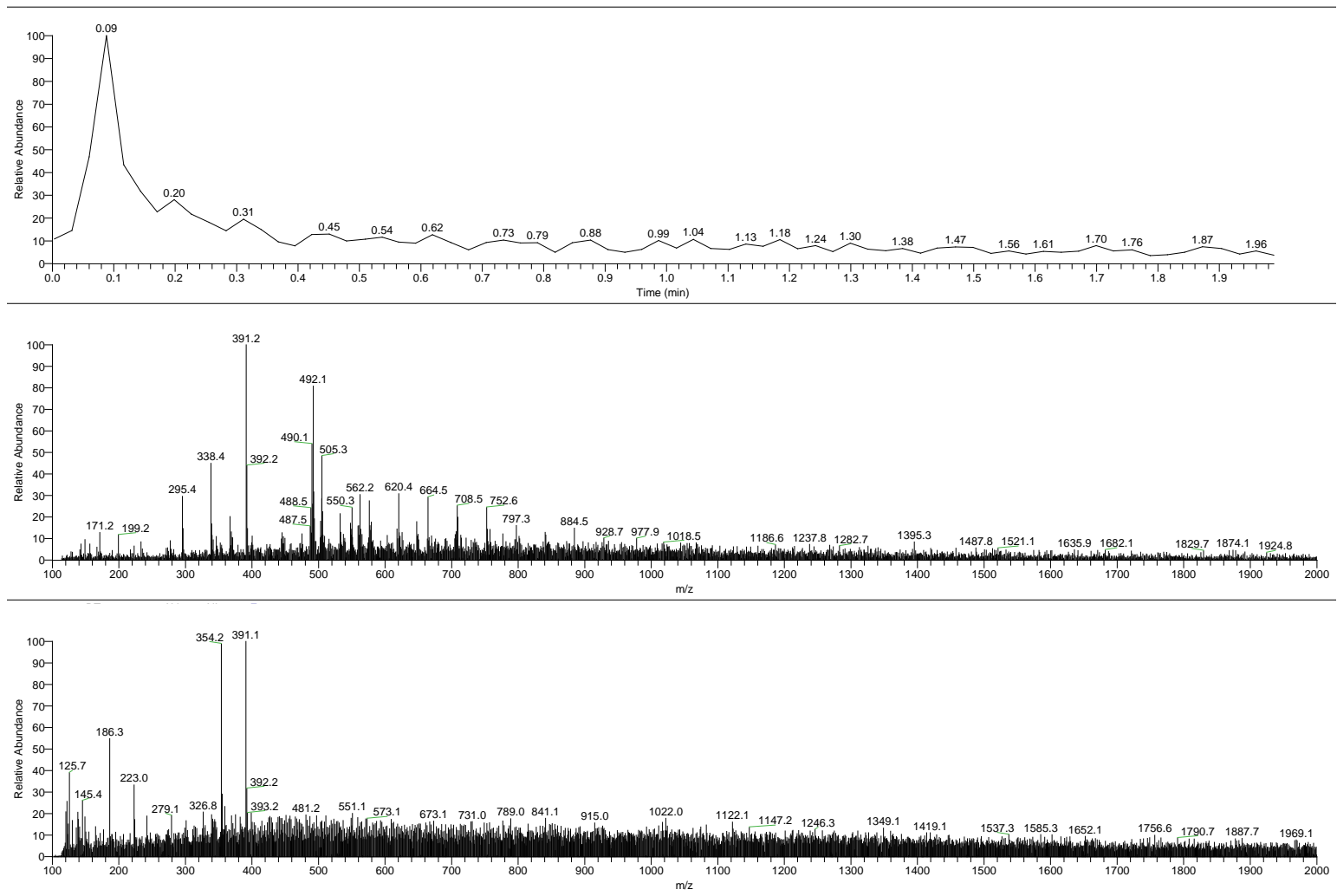


Figure A2.1 ESI-MS positive mode spectrum of (phen)Re(CO)₂(THF)OTf

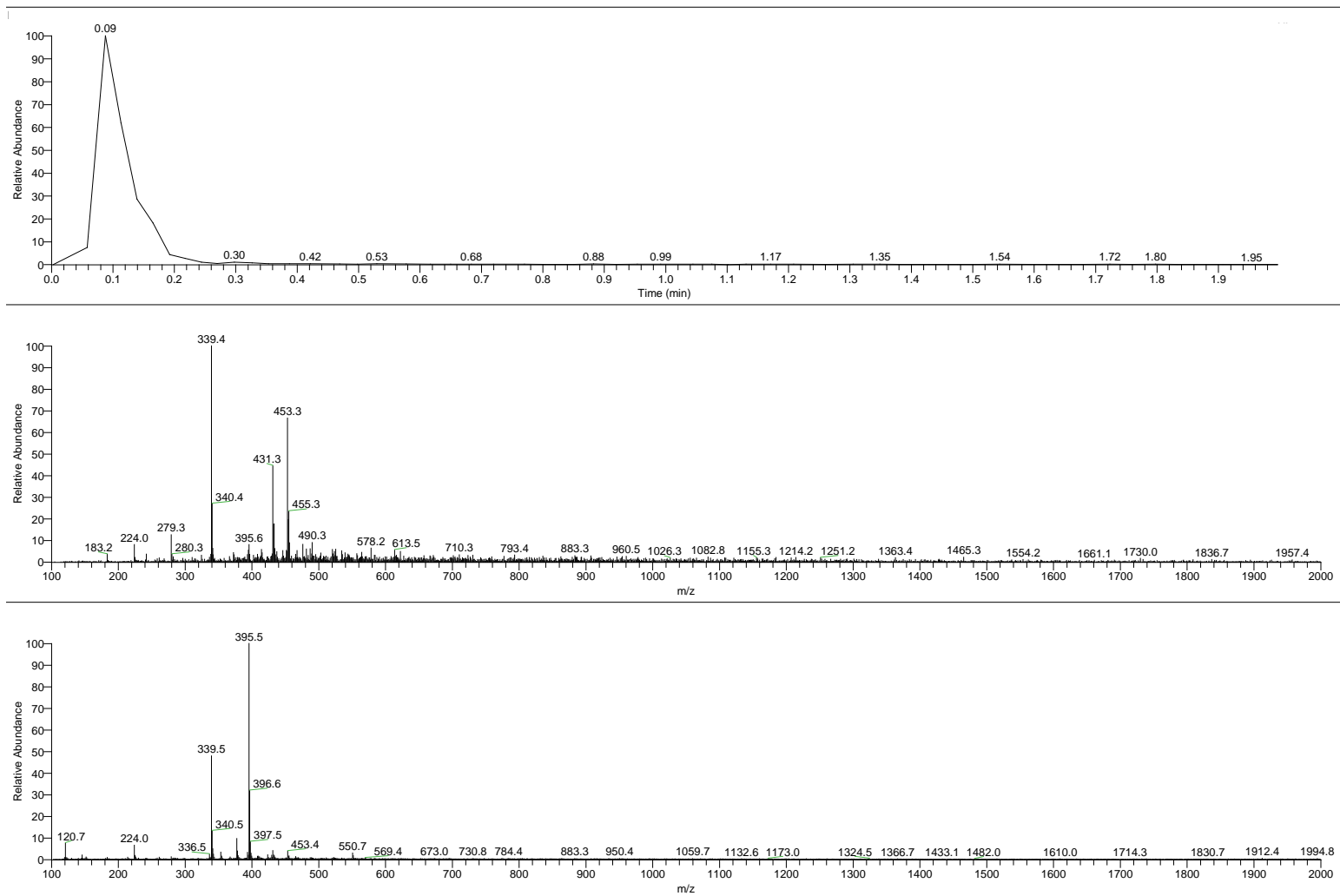


Figure A2.2 ESI-MS positive mode spectrum of (phen)Re(CO)₂(PPh₃)OTf

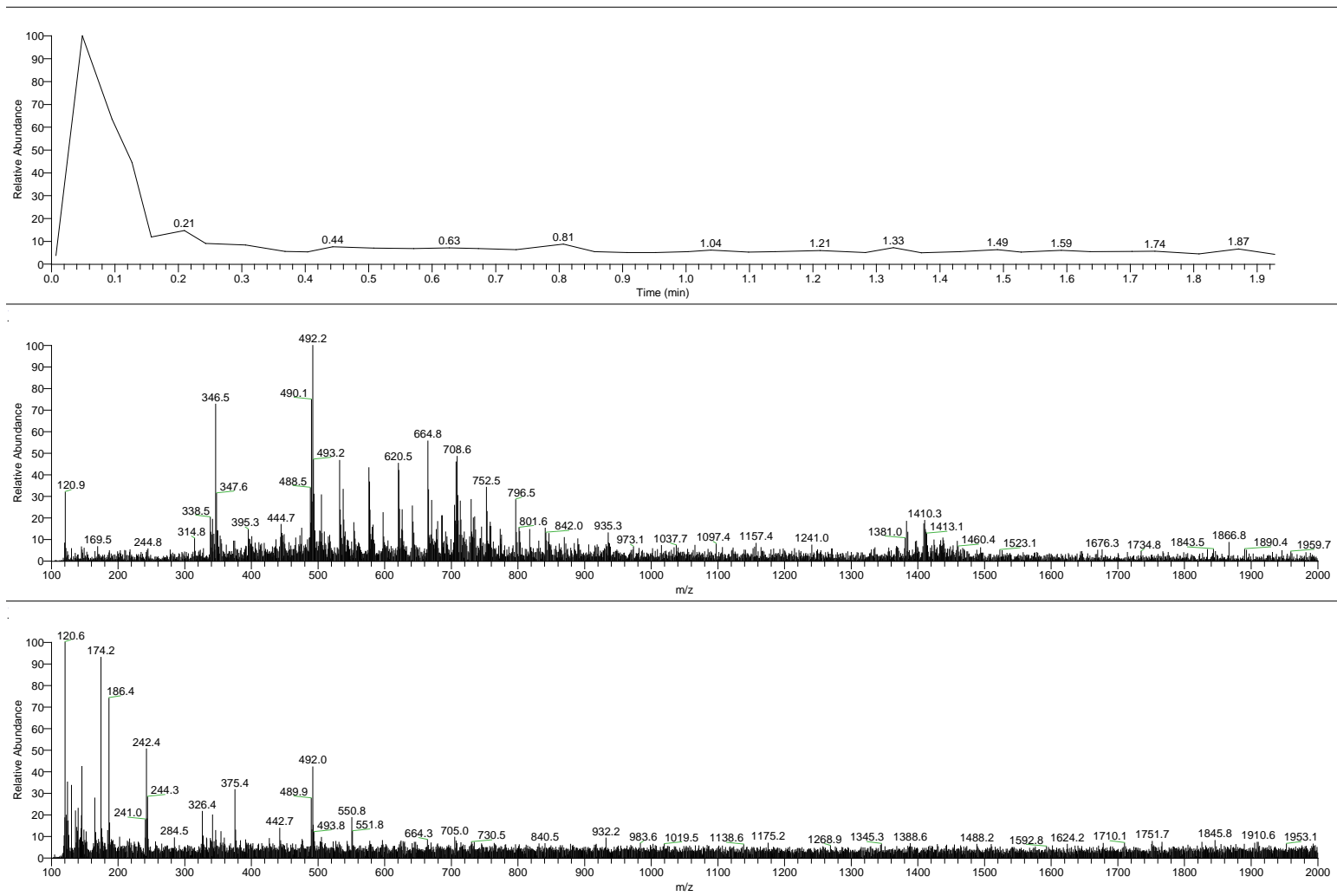


Figure A2.3 ESI-MS positive mode spectrum of (phen)Re(CO)₃Cl

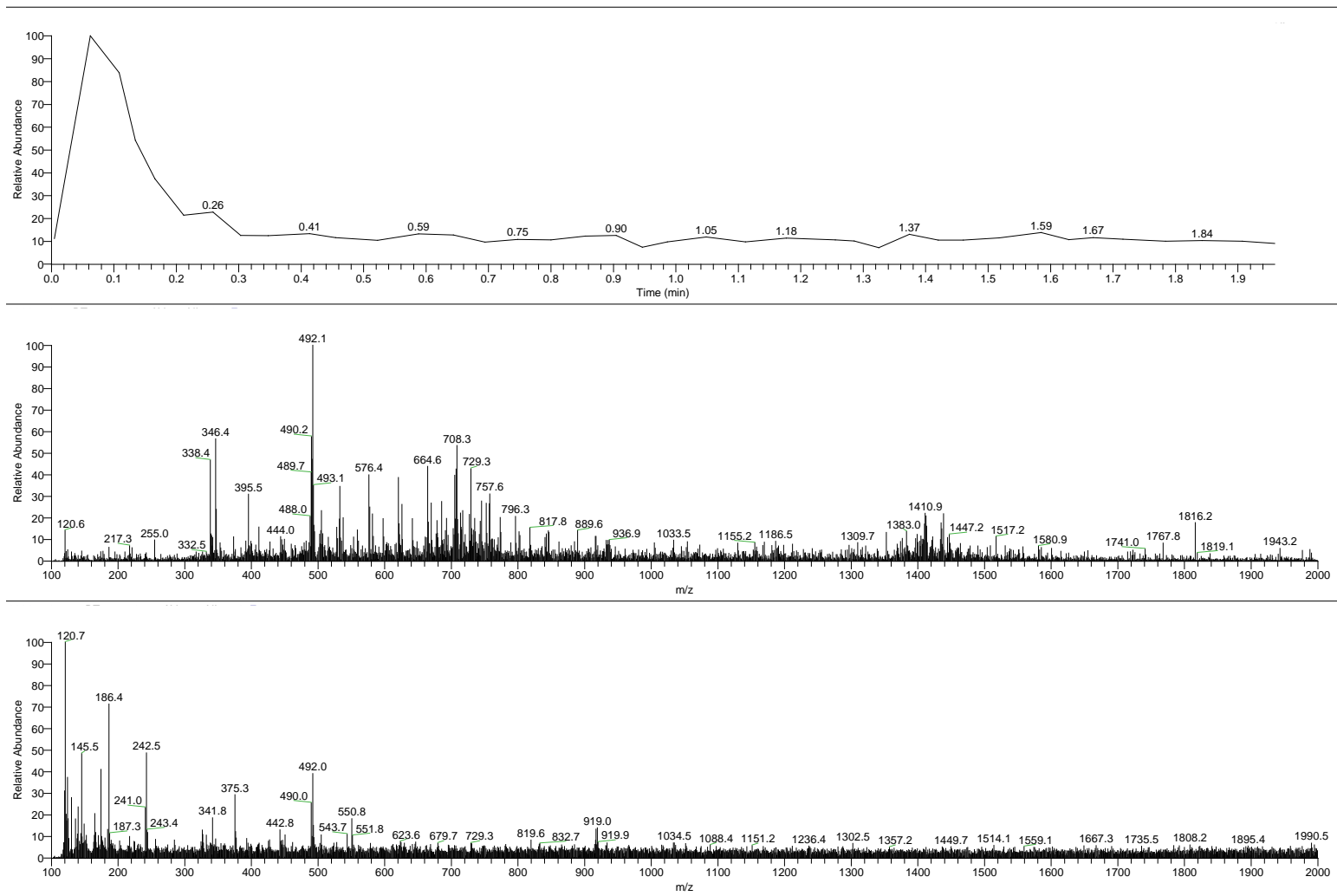
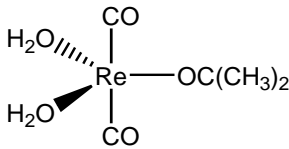
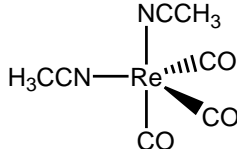
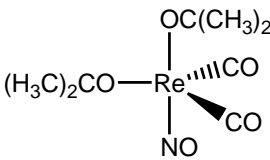
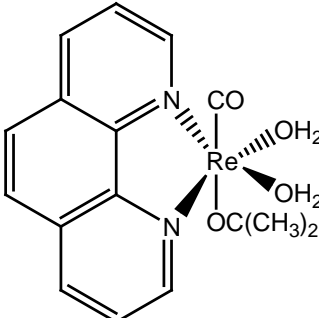


Figure A2.4 ESI-MS positive mode spectrum of (phen)Re(CO)₃OTf

A2.4. RESULTS AND DISCUSSION

Different structures can be assigned according to the given m/z ratio. The spectra obtained for (phen)Re(THF)(CO)₂OTf (figure A2.1) exhibited a major m/z peak at 391.2 corresponding to Re(NO)(CO)₂(acetone)₂, in addition to several solvation products exhibited in Table A2.2.

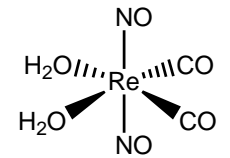
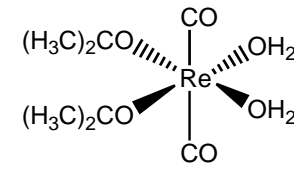
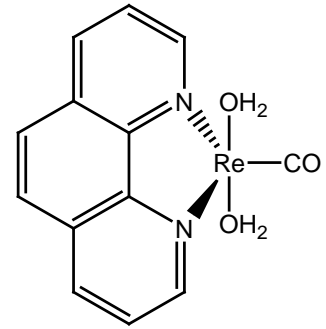
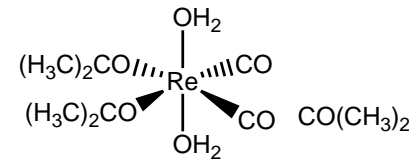
Table A2.1 List of most important ESI-MS positive mode signals of (phen)Re(THF)(CO)₂OTf upon reaction with NO-donor.

m/z ratio	Relative abundance	Possible Structure
186.3	55	Re
338.4	50	
354.2	95	
391.2	100 - 100	
492.1	80	

The spectra obtained for (phen)Re(PPh₃)(CO)₂OTf (figure A2.2) exhibited a major m/z peak at 339.4 corresponding to Re(NO)₂(CO)₂(H₂O)₂, in addition to several solvation products exhibited in Table A2.3

Table A2.2

List of most important ESI-MS positive mode signals of (phen)Re(PPh₃)(CO)₂OTf upon reaction with NO-donor.

m/z ratio	Relative abundance	Possible Structure
339.4	100 - 50	 <p>Chemical structure showing a central Rhenium (Re) atom coordinated to two NO ligands (top and bottom), two CO ligands (right and bottom-right), and two H₂O ligands (left and bottom-left).</p>
395.5	100	 <p>Chemical structure showing a central Rhenium (Re) atom coordinated to two CO ligands (top and bottom), two OH₂ ligands (right and bottom-right), and two (H₃C)₂CO ligands (left and bottom-left).</p>
431.3	45	 <p>Chemical structure showing a central Rhenium (Re) atom coordinated to a phenanthroline ligand (left and top), two OH₂ ligands (right and bottom-right), and one CO ligand (bottom).</p>
453.3	60	 <p>Chemical structure showing a central Rhenium (Re) atom coordinated to two (H₃C)₂CO ligands (left and bottom-left), two OH₂ ligands (top and bottom-right), and two CO ligands (right and bottom-right).</p>

(phen)Re(CO)₃Cl and (phen)Re(CO)₃OTf were used as controls. The mass spectra of these complexes showed no Re –NO containing products (Tables A2.3 and A2.4).

Table A2.3 List of most important ESI-MS positive mode signals of (phen)Re(CO)₃Cl upon reaction with NO-donor.

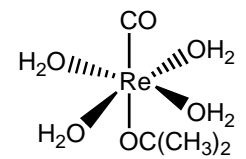
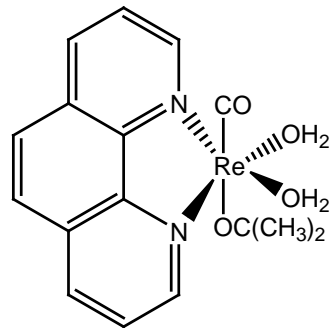
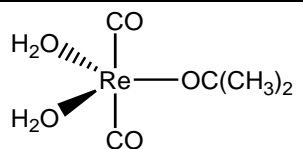
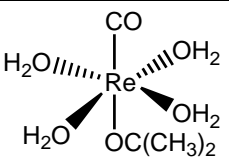
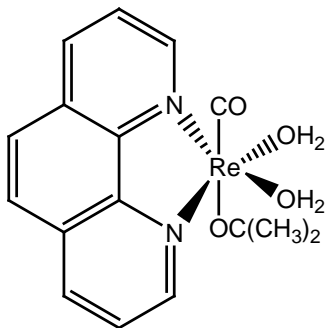
m/z ratio	Relative abundance	Possible Structure
186.4	72	Re
242.4	50	Re(OH) ₃
346.5	70	
492.2	100 - 45	

Table A2.4 List of most prominent ESI-MS positive mode signals of (phen)Re(CO)₃OTf upon reaction with NO-donor.

m/z ratio	Relative abundance	Possible Structure
186.4	70	Re
242.4	50	Re(OH) ₃
338.4	48	
346.4	56	
492.1	100 - 40	

The mechanism of action of these phen-Re(I) fluorescent dyes is complex: Two different paths can be identified by spectroscopic techniques: a. Reaction with NO, by elimination of two ligands; b. Deactivation of the initial complex by solvation (substitution with acetone, acetonitrile or water).

A2.5. CONCLUSION

ESI-MS m/z ratios can be useful to predict possible products after the reaction with NO-donor. NO-active complexes (phen)Re(PPh₃)(CO)₂OTf (**1**) and (phen)Re(THF)(CO)₂OTf (**2**) exhibited m/z ratio signals that can be ascribed as NO-Re containing species. Control complexes (phen)Re(CO)₃OTf and (phen)Re(CO)₃Cl only exhibited solvation byproduct, without NO in them. Other important fact is that all these formed complexes have net charge of +1; charged complexes cannot pass through the phospholipidic bilayer cell membrane.

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