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Carbon Monoxide on Demand: Light-Induced CO Release of Flavonols

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CARBON MONOXIDE ON DEMAND: LIGHT-INDUCED CO RELEASE OF

FLAVONOLS

by

Stacey N. Anderson

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ABSTRACT

Carbon Monoxide on Demand: Light-Induced CO Release of Flavonols

by

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Utah State University, 2018

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The research herein outlines the development of flavonol-based compounds that when triggered, release carbon monoxide using visible light. Use of 1) microenvironment effects; 2) modifications to the core structure of 3-hydroxyflavone; and 3), zinc stabilization of the anionic form of flavonols, enabled the development of a family of novel compounds that exhibit a wide array of CO release reactivity

An initial study of zinc 3-hydroxyflavone compounds provided evidence that modulation of the microenvironment surrounding the 3-hydroxyflavonolato moiety influences the quantum yield for light-induced CO release. A hydrophobic microenvironment gives the highest quantum yield for CO release whereas a hydrophilic microenvironment slows light-induced CO release. These properties mimic those found for light-induced CO-release from free flavonols in organic versus aqueous environments.

A new family of extended flavonols containing an additional fused aromatic ring were prepared, characterized and evaluated for CO release reactivity. Members of this family of compounds were tuned to absorb light from 400-600 nm using substituent and heavy atom effects. Notable attributes of this family of compounds include: 1) ease and high yield of synthesis; 2) solubility in aqueous dimethyl sulfoxide (DMSO); 3) thermal stability in aqueous, aerobic media in the absence of light; 4) quantitative CO release reactivity upon exposure to visible light in air; 5) low toxicity, including for the CO release product; and 5) fluorescence prior to CO release, which enables tracking of the compounds within cells. Anaerobic CO release reactivity was also discovered for the derivative that absorbs lowest energy visible light. Overall, this family of compound has many properties that make them desirable for use in biological applications.

Stabilization of the monoanionic form of the extended flavonols via zinc coordination results in compounds that exhibit red-shifted absorption spectra, enhanced quantum yields for quantitative CO release, and most notably, solid state CO release reactivity. Notably, zinc complexes containing two deprotonated extended flavonols exhibit visible-light induced release of two equivalents of CO in the presence of air as either solutions in pyridine or as solids. A compound of this type can be used as an *in-situ* solid-state source for CO in a palladium-catalyzed carbonylation reaction.

(326 pages)

PUBLIC ABSTRACT

Carbon Monoxide on Demand: Light-Induced CO Release of Flavonols

Stacey N. Anderson

Carbon monoxide (CO) is an extremely useful molecule with applications in industrial manufacturing, synthetic procedures as a C1 building block, and as a potential pharmaceutical to produce anti-inflammatory effects and vasodilation. However, the toxicity associated with CO has prevented its full utilization. In order to safely handle CO, compounds and molecules have been developed that act as storage materials for the gas. Ideal storage platforms only release CO upon stimulation via a trigger. Light activation is the most desirable trigger as it can be regulated in terms of the intensity and the wavelength of light used. The majority of light-induced CO-storage platforms that have been reported to date consist of metal carbonyl compounds where CO is bound directly to a metal center. However, disadvantages inherent to this motif, such as potential toxicity associated with the metal and lack of characterization of CO release remnant(s), has pushed the research community to search for alternative CO storage structures.

The research presented in this dissertation outlines our approach toward the development of safe-to-handle, light-induced CO release platforms. We use a flavonol structure similar to those found in fruits and vegetables, such as quercetin, as a light-induced CO release unit. Through changes in the structure of the flavonol and its surrounding environment in chemical compounds, we have found ways to strategically control the light-induced CO release reactivity of the flavonol. Chemical compounds developed in this project are of interest for studying the effects of CO in biological systems and applications in synthetic processes.

ACKNOWLEDGMENTS

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

CHAPTER 1

INTRODUCTION

Carbon Monoxide – Overview

Carbon monoxide (CO) (Figure 1-1) is a colorless, odorless, diatomic gas

with a molecular weight of 28.0 g/mol, making it slightly lighter than the average

molecular weight of air (28.8 g/mol). The boiling point of CO is 82 K and the

melting point is 68 K. The bond dissociation energy of this diatomic molecule is

1072 kJ/mol, making it the strongest chemical bond known.[1]

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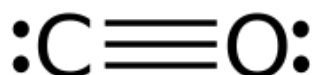
Figure 1-1. Structural representation of carbon monoxide.

The History of Carbon Monoxide

The toxic properties of carbon monoxide have been known for centuries.

Aristotle (384-322 BC) was the first to record that burning coals produced a toxic

gas. It wasn't until 1800 that the Scottish chemist William Cumberland

Cruikshank, collecting gases produced by burning charcoal over red-hot metal

oxides, identified an inflammable gas containing carbon and oxygen, CO.[2]

Exploitation of the poisonous effect of this gas has occurred from antiquity, when

convicts were enclosed in rooms with burning coals to modern times where it

was used in large scale at Nazi extermination camps.[3] While CO is no longer

utilized as a means of execution, CO poisoning is the most common type of fatal

poisoning in many countries.[4] From 1999-2010, there were 5,149 fatalities in the

United States resulting from unintentional CO inhalation.[5] The commonality in

everyday life of processes that produce CO, such as the incomplete combustion

of fossil fuels used to heat homes and power automobiles, has caused CO

poisoning to remain relevant.

It is the chemical properties of CO that make it a potent poison. It is

colorless, odorless, and tasteless, earning it the moniker of "silent killer."

However, its toxicity truly arises from the ability of this gas to irreversibly bind to

metal centers. CO binds to metal centers through σ donation from the highest

occupied molecular orbital (HOMO) on CO (σ*) to an empty d orbital of a metal

and through π back-bonding from a filled d orbital on a metal to the lowest

occupied molecular orbital (LUMO) on CO (π*) (Figure 1-2). For example, this

strong binding of CO to a metal center results in a 230-times stronger affinity for

CO than molecular oxygen (O_2) in hemoglobin, the iron-containing metalloprotein

responsible for oxygen-transport.[7-9] Since 1921, it has been thought that the

irreversibility of the binding between CO and the iron-center in hemoglobin, which

disrupts O_2 delivery to tissues, causes the toxicity of CO. While the binding of CO

to hemoglobin has long been touted as the root of its harmfulness, a subsequent

study reported in 1976 has cast some doubt onto this theory. In this study, dogs

were administered similar concentrations of carbon monoxide through inhalation

or by transfusing the blood with carboxyhemoglobin saturated blood.

π bond from filled metal d orbital
to vacant π* LUMO on CO
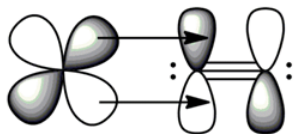
σ bond from HOMO σ* on CO to
vacant d orbital on metal
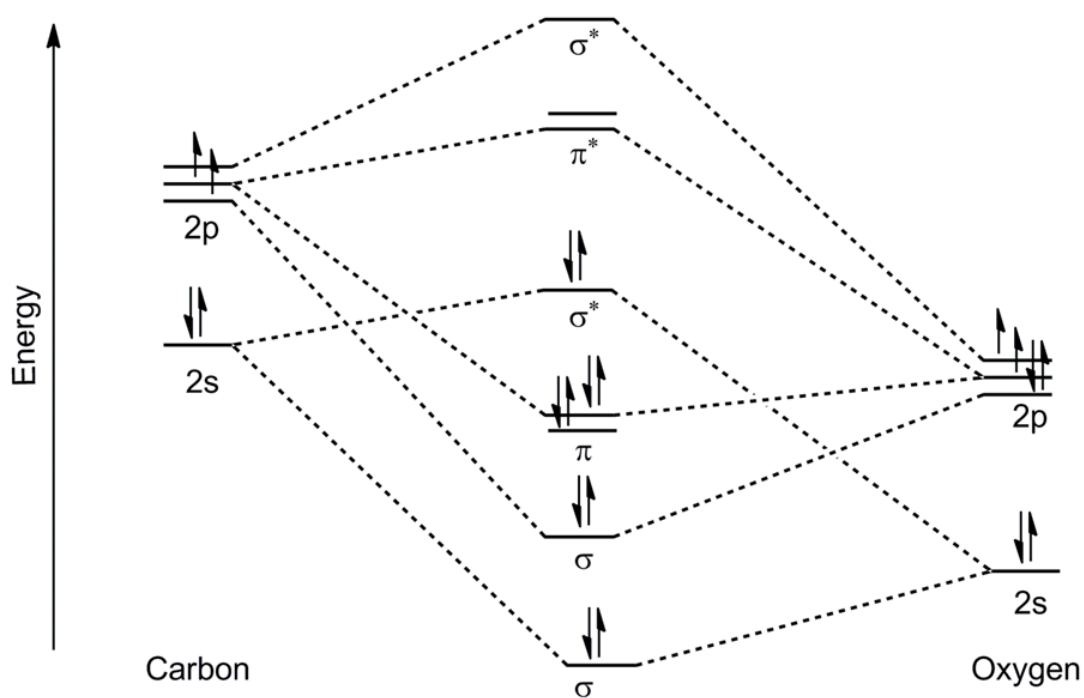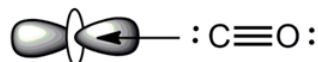
Figure 1-2. (top) Bonding scheme of carbon monoxide to a metal center; (bottom) Molecular orbital diagram of carbon monoxide.

Unfortunately, all of the dogs from the inhalation trials died, while none of the dogs with carboxyhemoglobin saturated blood passed away.[6] These results indicate that the harmfulness of CO is not entrenched in its ability to bind hemoglobin.

Beneficial Biology of Carbon Monoxide

Compared to the long known history of carbon monoxide as a lethal gas, its beneficial effects were only recently discovered in the 20th century. Seminal work by Swedish physician Torgny Sjöstrand demonstrated that not only is CO endogenously produced in humans, but that the oxidative decomposition of heme was the source.[7] Additional work by Coburn and associates demonstrated that the concentration of CO exhaled was greater than the concentration inhaled, particularly under physiologically stressful conditions.[8] These initial findings indicated that CO might serve in a beneficial capacity.

Heme Oxygenase

The oxidation catalyzed by the enzyme heme oxygenase (HO) is the rate-limiting step in the degradation of heme in mammals.[9] HO catalyzes the oxidation of the α-*meso* carbon of the protoporphyrin ring leading to the formation of carbon monoxide, free iron in the +2 oxidation state, and biliverdin, which is rapidly converted to bilirubin by biliverdin reductase (Figure 1-3).[9-10] Originally characterized in 1969, this enzyme has two isoforms.[11] The isoform constitutively expressed in tissues, heme oxygenase-2 (HO-2; ~36 kDa, enzyme classification (EC) 1.14.99.39) functions mainly to regulate necessary levels of free heme.[12] The other isoform, heme oxygenase-1 (HO-1; ~32 kDa, EC 1.14.99.3), is inducible and its regulation and expression is modulated in response to oxidative stress. Notably, an increasing number of reports demonstrate that HO-1 is over
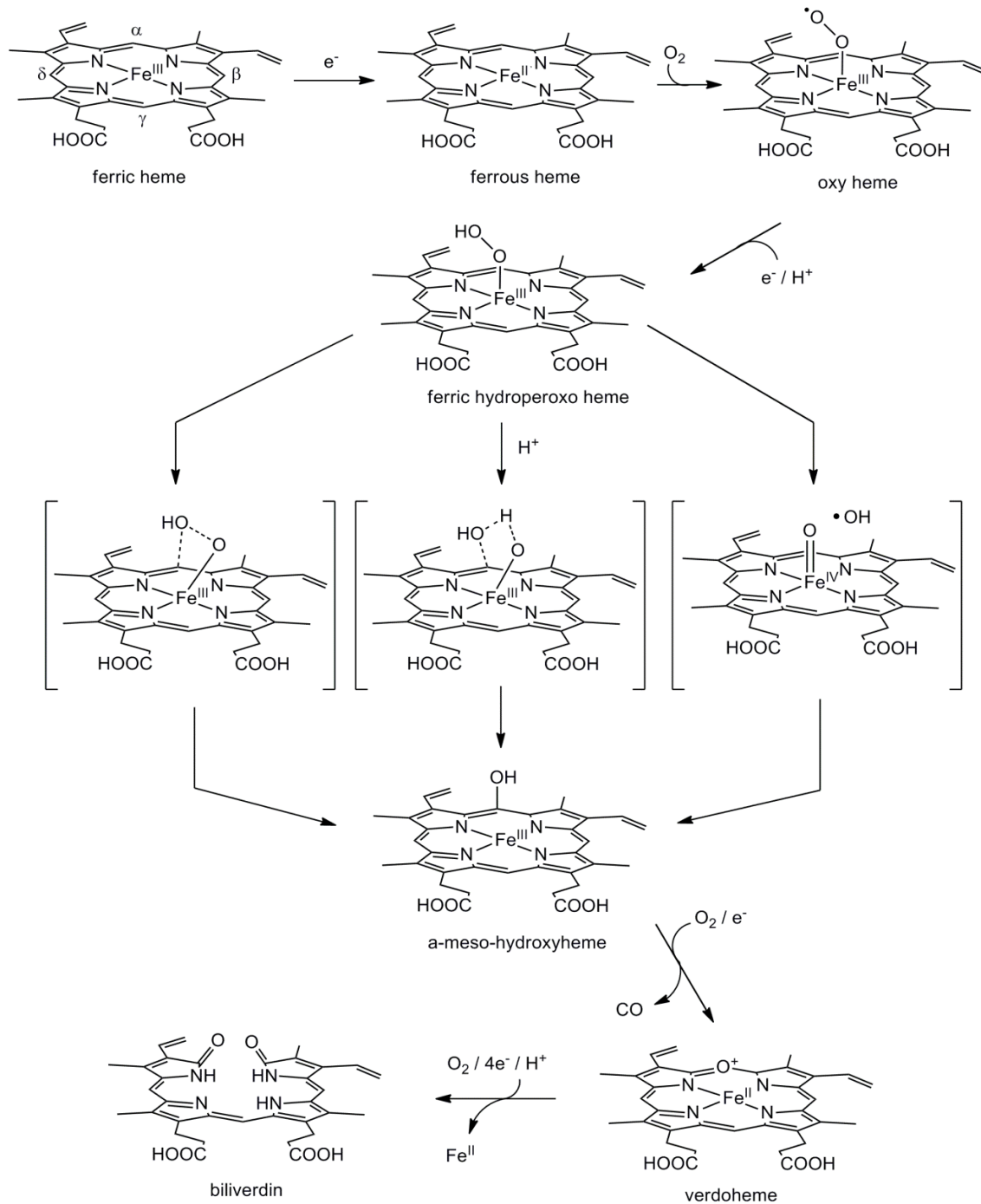
Figure 1-3. Proposed mechanism of COrelease from heme via heme oxygenase. Adapted from Ref 10 with permission of The Royal Chemical Society.

expressed in a variety of diverse disease states such as cardiovascular, neurodegenerative, and immune diseases.[12-13] The over-expression of HO-1 in a number of disease states led to the hypothesis that instead of heme being merely catabolized, that there exists a dynamic equilibrium between destructive and constructive metabolism of heme.[14] In destructive metabolism, the end products of heme degradation are simply eliminated from the organism, whereas in constructive metabolism, CO and bilirubin are purposely produced to modulate vital biological activities.[14] This hypothesis is supported by multiple reports of bilirubin acting as an antioxidant and antinitrosative agent and the ever increasing body of literature demonstrating the efficacy of CO in the treatment of numerous disease states in animal models (Table 1.1).[15]

Cellular Targets of Carbon Monoxide

Carbon monoxide, as a small, gaseous molecule, is freely diffusible across membranes and does not require transporters.[25] Therefore, CO can rapidly facilitate transformations in the cell. Due to the ease with which CO can move across membranes, multiple targets both on or near the cellular surface and within cells have been proposed. The targets proximal to the cellular surface include heme-containing potassium channels, caveolar nitric oxide synthase (NOS; the endothelial isoform of NO synthase), surface NADPH oxidase, and soluble guanylyl cyclase (sGC).[26] Distal cellular targets are thought to include heme-containing transcription factors such as BACH1 and NPAS2 and mitochondria.[26-27] All of the aforementioned targets of CO contain transition

Table 1-1. Preclinical efficacy of inhaled carbon monoxide. Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Drug Discovery* (Ref 16), copyright 2010.

Models of disease	CO gas effect
Bacterial infection in mice	250 ppm improves survival and prevents multiple-organ failure when administered after gram-positive or gram-negative bacterial infection.[16]
Sepsis and endotoxemia in mice, rats, and pigs	50-250 ppm has anti-inflammatory effects; 250 ppm improved lung derangement by endotoxin; 10-250 ppm as a pretreatment or as a post-treatment improves survival, and lung and liver injury in response to lipopolysaccharides.[17]
Pulmonary hypertension in rats and mice	250 ppm for 1 hour per day beginning at peak hypertension reverses right heart size and pulmonary arterial pressure; 50 ppm for 21 days prevents pulmonary hypertension.[18]
Cardiac graft rejection in mice and rats; mouse to rat xenografts	20 ppm prolongs survival; 250-400 ppm for 5-6 days prevents rejection.[19]
Myocardial infarction in mice; cardiac ischemia-reperfusion injury in rats	250-1,000 ppm after injury prevents infarct mass induced by left anterior descending artery ligation.[20]
Hemorrhagic shock in mice	250 ppm after resuscitation prevents organ failure.[21]
Model of acute inflammation in mice; acute lung injury	250 ppm prevents hypoxia-induced lung inflammation and injury.[22]
Aortic allograft rejection in mice and rats	250-400 ppm prevents transplant vascular stenosis.[23]
Lung allograft rejection in mice and rats	250 ppm prevents chronic rejection, ischemia reperfusion injury, and apoptosis.[24]

metals and while it has been postulated that CO binds to many targets, direct

evidence of CO interaction with cellular components that do not contain transition

metals is lacking.[17a, 28] It has been demonstrated that the binding of CO to heme

in proteins alters their conformation and thereby controls activity. In the case of

sGC and NOS, the interaction of CO with the heme moiety leads to an increased

production of cyclic guanosine monophosphate (cGMP) and nitric oxide (NO),

respectively. cGMP and NO are both potent regulators of vascular tone and

neurotransmission.[26] CO can also have an inhibitory effect such as with NADPH

oxidase where it interferes with the production of superoxide and in cytochrome c

oxidase in mitochondria, where it is thought to interfere with electron transport

and oxidative phosphorylation thus increasing the production of reactive oxygen

species (ROS).[29] While there is a significant amount of evidence implicating

potential cellular targets of carbon monoxide, a clear understanding of the

molecular mechanisms by which CO imparts its biological effects is lacking.

Compounds capable of releasing discrete quantities of CO in a directed manner

have the potential to expand the current working knowledge of this potentially

therapeutic molecule.

Carbon Monoxide-Releasing Molecules (CORMS)

  The numerous health benefits observed for administration of low doses of

carbon monoxide, coupled with the dangers of exposure to large amounts of

carbon monoxide, led researchers to develop safe-to-handle storage platforms

for CO. Metal carbonyl compounds (coordination complexes of transition metals

with CO as ligands) have been utilized in synthetic organic chemistry as CO sources, such as in palladium-catalyzed carbonylation reactions.[30] For this reason and the commercial availability of metal carbonyls, the first reported CORMs belong to this class of compounds. CORM-1 ($Mn_2(CO)_{10}$) (Figure 1-4 (left)) and CORM-2 ($[Ru(CO)_3Cl]_2$) (Figure 1-4 (middle)) were the first reported metal carbonyl compounds that exhibited biological effects similar to gaseous CO.[31] Both of these highly lipophilic compounds exhibit CO release upon dissolution in dimethylsulfoxide (DMSO) albeit through different mechanisms. CORM-1 was observed to liberate CO only upon illumination while CO was displaced from CORM-2 through ligand exchange with DMSO and the formation of tricarbonyl and dicarbonyl monomers. Due to the poor solubility of CORM-1 and CORM-2 in aqueous media, for initial biological studies a cell line was chosen that displayed resistance to toxic insult, bovine vascular smooth muscle cells (2% DMSO had no effect on cell viability).[32] While the originally reported IC_{50} values for CORM-1 and CORM-2 were > 100 μM and > 400 μM respectively, in subsequent studies, CORM-2 induced cell death at concentrations as low as 10 nM in HL-1 heart cells.[33] The CO released from CORM-1 and CORM-2 produced a vasodilatory effect (CORM-2; using isolated aortic ring model; CORM-1; using isolated rat heart) and the ability to modulate mean arterial carbonyl compound could deliver CO *in vivo* and evoke biological responses spurred the further development of metal carbonyl CORMs by derivatization of supporting ligands in order to impart desirable characteristics, such as aqueous solubility.

Compounds need to be able to move across lipid bilayers of cell membranes before entering the blood stream and distributing throughout the body. Lipophilic compounds, such as CORM-2, are generally able to diffuse across cell membranes whereas hydrophilic compounds are usually unable to penetrate the lipid bilayer.[34] A method used to overcome the potential toxicity associated with lipophilic CORM-2, was to increase the hydrophilicity of the compound through the introduction of a supporting ligand. A little more than a year after the initial report of CORM-2 was published, the same authors described a tricarbonylchloro(glycinato)ruthenium(II) compound, CORM-3 (Figure 1-4 (right)).[35] The use of deprotonated glycine as a supporting ligand imparted aqueous solubility to the CORM. Introduction of CORM-3 produced no change in

CORM-1

- Soluble in organic solvents
- CO release through photo-dissociation

CORM-2

- Soluble in organic solvents
- CO release through ligand exchange

CORM-3

- Soluble in aqueous buffers
- CO release through ligand exchange
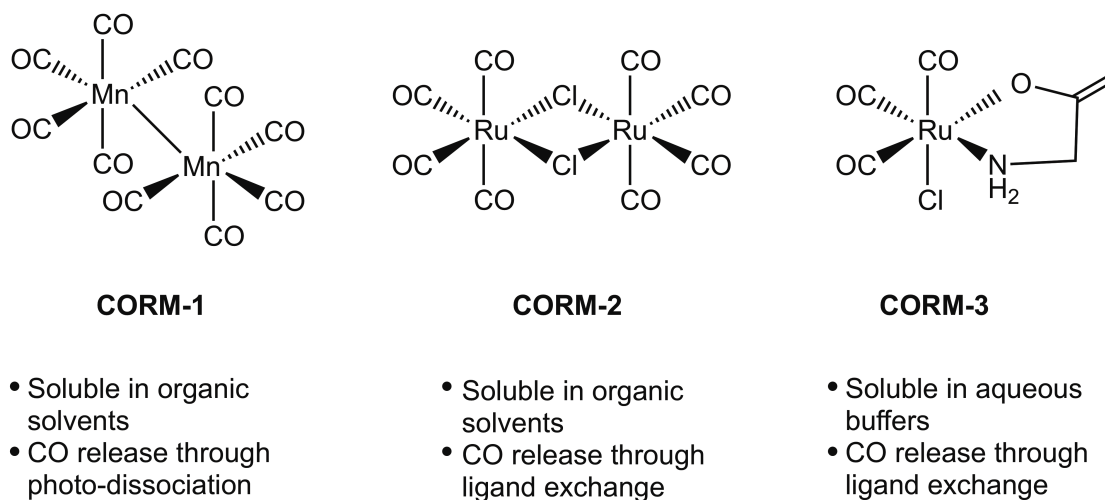
Figure 1-4. Selected properties of CO-releasing molecules CORM-1, CORM-2 and CORM-3.

the cell viability of porcine aortic endothelial cells or primate peripheral blood

mononuclear cells *in vitro* at concentrations up to 500 µM.[36] CORM-3 releases

approximately one equivalent of carbon monoxide per equivalent of compound.

In vitro and *in vivo* experiments using CORM-3 have revealed a host of beneficial

health effects, such as the protection of cardiac cells against oxidative stress,

cardioprotective effects against myocardial ischemia-reperfusion injury, and the

prevention of allograft rejection.[35, 37] These findings propelled CORM-3 into use

to probe the biological effects of CO.[38] The excellent biological properties

coupled with the low toxicity of CORM-3 indicated that this compound had

potential to enter clinical trials; however, complicated aqueous chemistry in both

the synthesis and its CO release mechanism limited its potential (Figure 1-5(a)).

While the synthesis of CORM-3 is straight forward, with $[Ru(CO)_3Cl_2]_2$ (CORM-2)

and glycine combined with sodium ethoxide in methanol under nitrogen, the

purification is problematic.[39] The IR spectrum of each preparation of CORM-3

displayed stretches at 2137, 2072, and 2058 cm^{-1}, consistent for a *fac*-$Ru(CO)_3$

group lacking C_3 symmetry.[39] However, another IR stretch at 1985 cm^{-1} was

observed consistent with a *cis*- $Ru(CO)_2$ impurity.[39] It was initially assumed that

this impurity was a result of CO loss, until it was realized that

$[Ru(CO)_3Cl(glycinato)]$ was reacting with water in the solvent to form

$[Ru(CO)_2(CO_2H)Cl(glycinato)]^-$.[39] This was additionally confirmed through the

observation that CORM-3, upon dissolution in neutral water, lowered the pH to

approximately 3.[40] The original report of CORM-3 indicated that despite the

complex having three carbonyl ligands, only 1 equivalent of CO was released per

equivalent of compound.[35] Subsequent studies demonstrated that in addition to the formation of CO, CORM-3 also released carbon dioxide (CO_2), indicating the presence of other isomer(s) at physiological pH (Figure 1-5(a)).[40] It was subsequently determined that in the pH range of 6-10 one of the carbonyl ligands has been transformed into CO_2 (Figure 1-5(b)).[40]

A

B
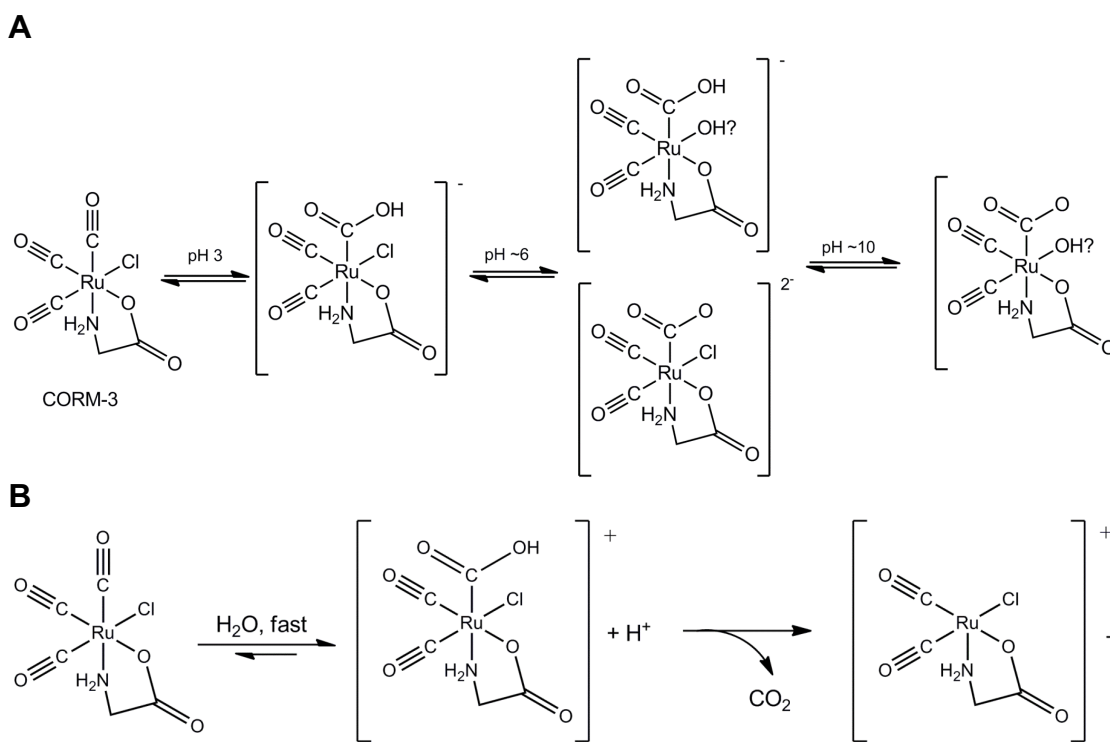
Figure 1-5. Aqueous reactivity of CORM-3: (a) The most probable species formed at equilibrium at different pHs involving CORM-3, and (b) Water-gas shift reaction of CORM-3 in solution to rapidly release CO_2, creating an open site at the ruthenium center which enables coordination to histidine residues. Adapted with permission from Mann, B. E. *Organometallics* **2012**, 31, 5728-5735. Copyright 2012 American Chemical Society.

The formation of a metal coordinated CO_2 and subsequent loss had an unintended side-effect for the biological reactivity of this compound. As CO_2 has a much lower affinity towards metal centers as compared to CO, this ligand was readily lost leaving an open coordination site that was readily filled by binding with protein residues. In biological environments, the protein bound ruthenium carbonyl is the actual CO-releasing molecule.[38e] While CORM-3 remains the compound of choice for elucidating the biological effects of CO, the complicated aqueous chemistry has hindered further development as a pharmaceutical.

Ruthenium complexes, in addition to being utilized as CORMs, have been widely explored as anticancer agents.[41] Due to potential of biological activity of the metal remnants left after CO release occurs from CORM-2 and CORM-3, various other metal centers have been explored. The majority of CORMs reported thus far contain iron or manganese, with a few featuring chromium, rhenium, iridium, tungsten, cobalt, molybdenum, or rhodium.[42] However, none of these compounds have achieved the same level of widespread application as CORM-2 or CORM-3. These subsequent CORMs all display disadvantages that have hampered continued development. Metal carbonyl CORMs have two potential environments that can be tuned for the effective development of a pharmaceutical candidate; the "CORM sphere," defined by the number of carbonyl ligands and the "drug sphere," defined by the supporting ligands which can modulate the pharmacological profiles of the compounds (Figure 1-6).[43] Key parameters of the "CORM sphere" are the number of CO molecules that can be released from the compounds, the kinetics of CO release, and the mechanism
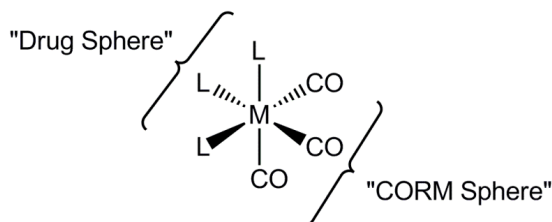
Figure 1-6. The ligand environment surrounding the metal center in a metal-carbonyl CORM is divided into the "drug sphere" and the "CORM sphere". The "drug sphere" is defined by the supporting ligands which can be modulated for pharmacokinetics. The "CORM sphere" is defined by the stoichiometry and kinetics of CO release from the metal center. Adapted by permission from John Wiley and Sons: British Journal of Pharmacology (Ref 42). Copyright 2014.

that leads to CO release. It is important to note that there is no current consensus in the scientific community as to whether a large or small number of carbonyl ligands is desirable and whether the CO release rate should be slow or fast. Rather, it is likely that there is no single answer to these questions and instead a variety of CORMs should be developed to address specific pharmacological applications. However, the majority of CORMs reported have focused almost exclusively on the development of the "CORM sphere" with the "drug sphere" largely neglected. The development of the "drug sphere" is vital to the progression of CORMs beyond simply compounds capable of studying the biological effects of CO to the end goal of real pharmaceuticals. The lack of

progress on the "drug sphere" of CORMs is likely due to other undesirable

characteristics present in metal carbonyl CORMs. These complexes release CO

through ligand exchange (Figure 1-7). Most have been tested for CO release in

aqueous solution using
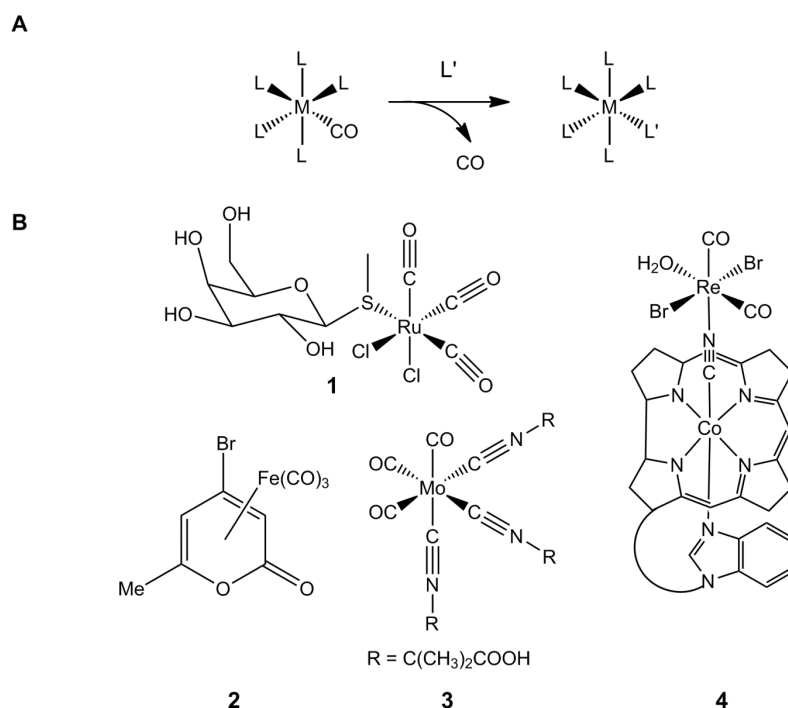
Figure 1-7. Metal carbonyl carbon monoxide-releasing molecules (CORMs).

(a) CO release from metal carbonyl CORMs through ligand exchange, where

L' is either solvent or a biologically relevant coordinating species such as a

histidine residue, and (b) selected examples of metal carbonyl CORMs that

release CO via ligand exchange.

what is known as the myoglobin assay. This assay relies on the different

absorption features of deoxymyoglobin and dicarboxymyoglobin and the reported

CO release from CORMs using this method was reproducible. However, using alternative methods to test for CO release, such as gas chromatography or CO-specific electrodes, it has been determined that CORMs release very little CO in solution and it was the presence of an additional component in the myoglobin assay solution, sodium dithionite, that induced CO release.[44] The small amount of CO release detected for CORMs using gas chromatography or CO-specific electrodes is thought to be due to the reversibility of the ligand exchange reaction liberating CO from the metal center.[45] The reversibility of the CO release reaction is an undesirable characteristic of metal carbonyl compounds and has hindered their further development as CORMs.

While CO release from CORMs in solution via ligand exchange is slow it is not controllable in terms of location and timing. The spontaneity of the CO release calls into question the numerous reports of the biological activity of CO derived from CORMs as the response could have been due to the presence of CO, the CORM, the metal-containing fragment remaining after CO release, or a combination of all of these things. Additionally, for the majority of CORMs reported no information regarding the product(s) remaining after CO release is available. The majority of CORMs contain redox active metal centers. Once CO has been fully liberated, an open coordination site at the metal center is formed. These remnants have the potential to be extremely toxic via the formation of reactive radical species from components common to biological environments, such as O_2. However, as the metal-containing compound(s) formed post CO release are rarely characterized or their toxicity examined, biologically adverse

reactions can only be estimated at this point. The numerous disadvantages associated with metal carbonyl CORMs have hindered their development as pharmaceutical pro-drugs.

Triggered Carbon Monoxide-Releasing Molecules

Since CORMs serve as a storage platform for the biologically active agent CO, the trigger mechanism is a fundamental aspect of the compounds. As noted above, CORMs, such as CORM-3, liberate CO through ligand exchange, which imparts the disadvantage of the lack of temporal control and site-specific delivery of CO. In order to side-step this problem, CORMs have been developed that are stable in solution and only release CO upon stimulation via either an internal (enzyme triggered; ET-CORMs) or external source (magnetic heating or photoexcitation; photoCORM). The CORMs reported to date that are able to release CO when supplied with certain triggers are still almost exclusively metal carbonyl compounds.

Enzyme Triggered Carbon Monoxide-Releasing Molecules (ET-CORMs)

ET-CORMs (Figure 1-8) were developed as a means of slowing down the rate of CO release from metal carbonyl CORMs.[46] The ET-CORMs developed to date utilize supporting ligands susceptible to enzymatic degradation and an $Fe(CO)_3$ "CORM sphere".[46-47] Prior to incubation in the presence of appropriate enzymes (i.e. esterase or phosphatase), ET-CORMs are stable with respect to hydrolysis.
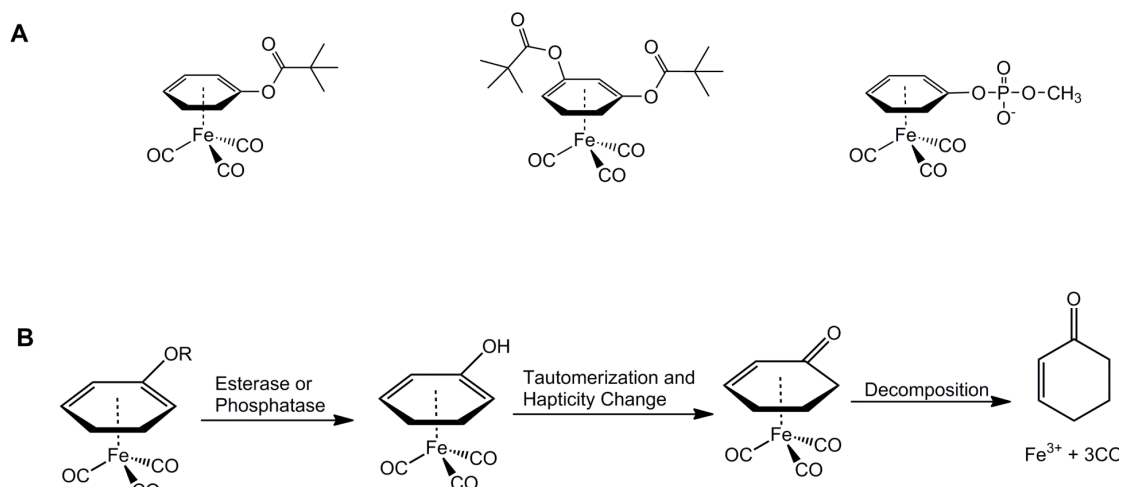
Figure 1-8. Enzyme triggered CO-releasing molecules (ET-CORMs). (A) The ET-CORMs reported to date, and (B) Proposed CO release mechanism from ET-CORMs. Adapted by permission from John Wiley and Sons: British Journal of Pharmacology (Ref 42). Copyright 2014.

The enzymes transform the compounds into highly unstable intermediates that readily decompose and release CO.[48] While it is known that these compounds release CO, the other products of the reaction are less well defined. The proposed reaction mechanism involves release of the transformed supporting ligand as well as Fe^{3+}.[46] The potential release of Fe^{3+} is problematic for the development of these compounds into pharmaceuticals in that while iron is essential to life, the storage and regulation is tightly controlled due to its redox capabilities. Additionally, the classes of enzymes used for these transformations tend to be ubiquitous and therefore targeting to specific biological sites is currently unavailable.

Magnetic Heat Triggered Carbon Monoxide-Releasing Molecules

An alternative and interesting approach to triggering CO release from

CORMs is the use of magnetic heating. Ruthenium carbonyl compounds were

attached to the surface of iron oxide nanoparticles (Figure 1-9).[49] These types of
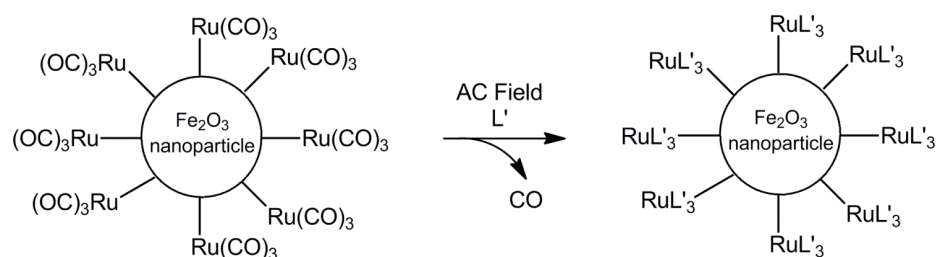
Figure 1-9. Magnetic heat triggered carbon monoxide-releasing molecule. CO

release from ruthenium carbonyl moieties appended to iron oxide

nanoparticles. CO release is triggered using an alternating current magnetic

field (AC field).

compounds seem promising in that magnetic nanoparticles would allow the

compounds to be directed to specific physiological locations by magnetic control.

CO release from the CORM-appended nanoparticle increased two-fold upon

magnetic heating.[49] Unfortunately, due to the type of CORM used in this report

(CORM-3 derivative), magnetic heating of the nanoparticle was not necessary for

CO release to occur and acted more as an aid rather than a trigger.[49]

Photoinduced Carbon Monoxide-Releasing Molecules (photoCORMs)

The most popular type of trigger used to initiate CO release from CORMs is light. The use of light as a trigger is popular because it would allow for site directed CO release. While the photoCORM itself would be systemic, CO release has the potential to be confined simply by limiting illumination to certain areas of the body. The earliest CORMs tested, $Fe(CO)_5$ and $Mn_2(CO)_{10}$, fall into this category, now known as photoCORMs, with CO release occurring only upon illumination. Due to the strength of the CO-metal bonding interaction, these photoCORMs required UV-light to initiate CO liberation. The requirement of UV-light is problematic not only due to the harmful nature of UV-light on living organisms, but also due to its inability to penetrate tissues. The penetration depth of light is wavelength dependent with visible light having greater penetration depth as well as being less harmful than UV-light. However, compounds capable of undergoing photoinduced CO release upon illumination with red light would be the most desirable as wavelengths in the range of 620 - 910 nm have the greatest penetration depth due to the lack of biologically relevant species absorbing in this window (Figure 1-10). Utilizing supporting ligands with specific properties, metal carbonyl photoCORMs have been developed that are capable of undergoing photoinduced CO release at wavelengths ≥ 520 nm.[51] The design strategy for the supporting ligands in these compounds employs a combination of a strong σ-donating ligand like Br⁻, to destabilize occupied orbitals, and a highly conjugated ligand with low-lying π^* molecular orbitals to further destabilize the metal carbonyl bonds through a metal-to-ligand (MLCT) charge transfer.[52] While
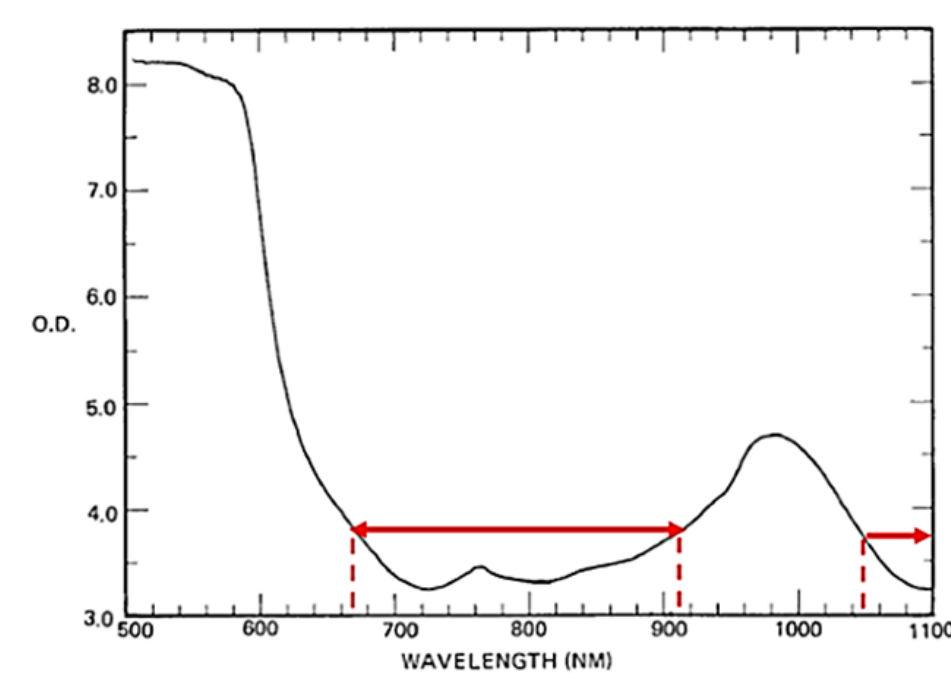
Figure 1-10. The absorption spectrum of a human hand. The best transmission through a hand is between approximately 670 nm and 910 nm and from approximately 1050 nm and higher.[50] Optical density is abbreviated O.D. unpublished data of Karl H. Norris, from The Science of Photobiology, KC Smith, ed., Plenum Press, 1977; p. 400.

these compounds release CO upon illumination with low energy light, as a consequence of destabilizing the metal carbonyl bonds enough to allow for the use of red light, they are not entirely stable in solution and will also release CO through ligand exchange over time (Figure 1-11(b1)).[53]
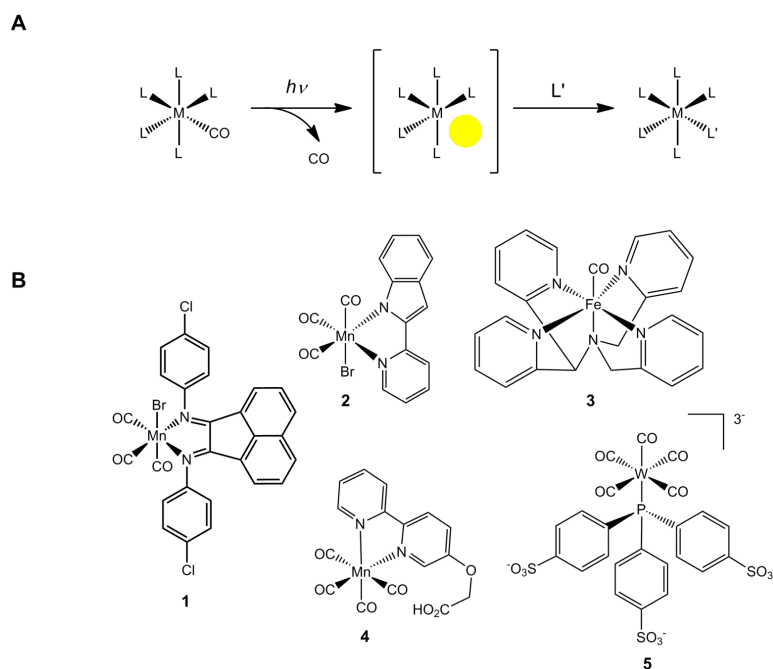
Figure 1-11. Photoinduced carbon monoxide-releasing molecules (photoCORMs). (a) CO release from metal carbonyl photoCORMs via illumination where L' is proposed to be solvent, and (b) selected examples of metal carbonyl photoCORMs.

Upconverting Nanoparticles (UCNP)

A different method to obtaining compounds capable of using low energy red light for CO release is the use of an upconverting nanoparticle (UCNP) together with a photoCORM that requires high-energy visible light (i.e. blue-light). UCNPs are $NaGdF_4$ nanoparticles doped with ytterbium (20%) as the red-light absorber and thulium (0.1%) as the blue light emitter (Figure 1-12).[54] UCNPs
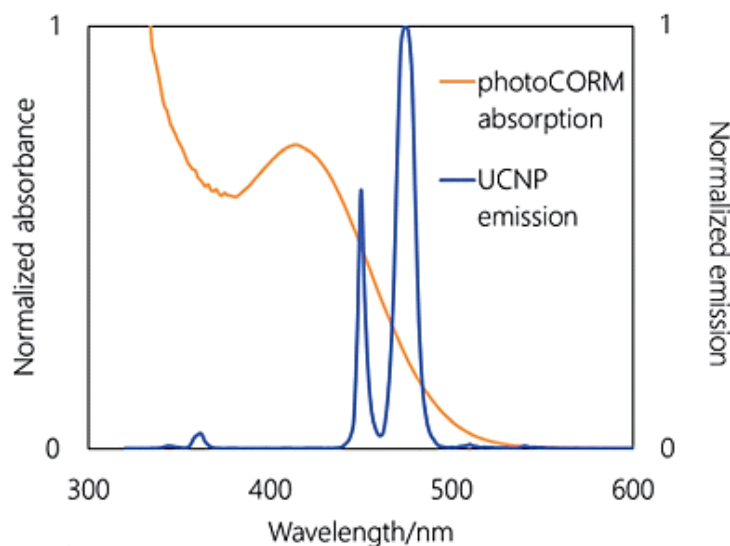
Figure 1-12. Electronic absorption spectrum (yellow) of a Mn(I) carbonyl

photoCORM and emission spectrum (blue) of an upconverting nanoparticle.

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(RSC) on behalf of the European Society for Photobiology, the European

Photochemistry Association and the RSC.

generate high-energy visible light when multiple Yb^{3+} ions absorb low energy red

light or near infrared light and transfer energy to Tm^{3+} thereby populating excited

states that emit higher energy light. The high energy emitted light can then be

absorbed by a photoCORM tethered to the nanoparticle and undergo CO

liberation. UNCPs offer an exciting alternative to achieve CO release from

photoCORMs using low energy light without compromising the stability of the

photoCORM itself, although the biological compatibility of UCNPs has yet to be

determined.

Organic PhotoCORMs

PhotoCORMs offer a distinct advantage over traditional CORMs in that CO release can be controlled with the use of photoexcitation. However, in retaining the metal carbonyl framework as the CORM sphere, photoCORMs retain the same disadvantage as their earlier predecessors in the potentially toxic and often uncharacterized metal containing fragments left after CO release. In reaction to the perceived toxicity of metal carbonyl photoCORMs, CORMs, and/or the metal-containing byproduct(s) of the photoreaction, several purely organic compounds capable of undergoing CO release either through photoreaction or some other mechanism have been recently reported (Figure 1-13).[55] These compounds represent a significant divergence from traditional metal carbonyl CORMs. Wowever, fundamental disadvantages have hampered their significant development as well.

The diketone compound (Figure 1-13(a)) exhibits photoinduced CO-release using visible light (λ_{irr} = 470 nm) with two equivalents of CO liberated per equivalent of compound and the production of a fluorescent photoproduct which could enable tracking of CO liberation in cellular environments.[55b] However, the synthesis of the compound requires multiple steps with an overall yield of 19%. Additionally, the toxicity of the photoCORM and the organic photoproduct has not been evaluated, and importantly, the diketone moiety, necessary for CO release, has been shown to hydrate in aqueous media thereby deactivating the CO-release capability.

Peng, P.; et. al *Org. Biomol. Chem.,* **2013**, 11, 6671-6674.

Antony, L. A. P.; et. al *Org. Lett.,* **2013**, 15, 4552-4555.

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Wang, D. Z.; et. al *Chem. Commun.,* **2014**, 50, 15890-15893.
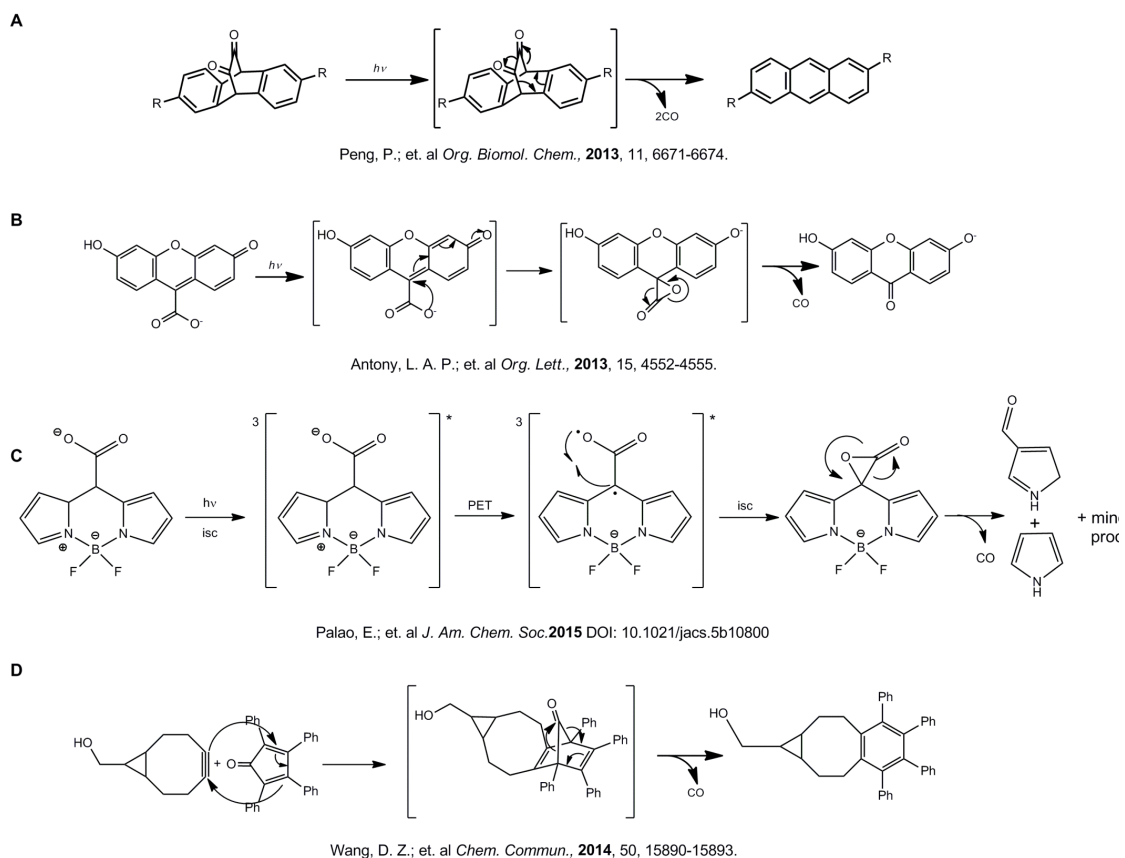
Figure 1-13. Proposed mechanism for CO release from the four reported

organic CORMs. (a) photoinduced CO release from a diketonate moiety, (b)

photoinduced CO release from a carboxylate, (c) photoinduced CO release

from a carboxylate BODIPY compound, and (d) the formation of an unstable

intermediate through a Diels-Alder reaction which decomposes to release CO.

The carboxylate compound (Figure 1-13(b)) undergoes photoreaction to

liberate one mole of CO per mole of molecule and is fluorescent.[55a] While the

toxicity of neither the photoCORM nor the photoproduct has been determined,

the main disadvantage to this compound is the synthesis needed to produce the photoCORM. It is generated using a photoreaction; therefore, isolating the photoCORM before it can undergo further reaction to release CO is problematic and results in an extremely low yield.

A third organic photoCORM has recently been reported that relies on a boron dipyrromethene (BODIPY; Figure 1-13(c)) framework.[56] Derivatives using this framework undergo photoinduced CO-release at wavelengths greater than 500 nm with an extended conjugation derivative capable of CO release upon activation with near-IR light. Additionally, this compound is fluorescent whereas the photoproduct(s) are not allowing for the potential to track CO release in a cellular environment. Minimal toxicity was observed for cells (cell viability did not drop below 50%; neuroblastoma and SH-SY5Y cell lines) at all concentrations tested (up to 100 µM). An almost quantitative amount of CO was produced from the photoreaction of the BODIPY carboxylate under anaerobic conditions (87% yield). However, in aerated solution, the quantity of CO produced decreases dramatically (44% yield). This is thought to be due to the formation of $^1O_2$, which then reacts with the starting material, forming a heretofore unidentified species that is unable to undergo CO release. The efficacy of the lead compound was also tested on hairless mice which displayed increased carboxyhemoglin levels, particularly in the liver and kidneys. Unfortunately, the toxicity of the organic photoproducts was not tested. This was probably due to the fact that a mixture of products is generated, only two of which have been identified as 2-methylpyrrole and 2*H*-pyrrole-4-carbaldehyde.

A fourth compound that doesn't require an external trigger to release CO has also been reported (Figure 1-13(d)). This organic CORM is produced *in situ* via a Diels-Alder reaction and subsequently decomposes to produce CO and an aromatic organic product.[55c] Cytotoxicity assays of the reactants necessary to form the CORM and the CO release product were examined and one of the reactants displayed a relatively high toxicity (IC_{50} 12.5 µM) while the other reactant was minimally toxic (IC_{50} 1 mM). The organic CO release product was non-toxic at all concentrations tested (0.78 - 100 µM). Unfortunately, this CO release system utilizes an *in situ* bimolecular process that could be severely hampered in the complex environments of biological organisms. While organic compounds may represent the future of CO-releasing materials for use as possible therapeutics, those described above have limitations that hamper their continued development.

Alternative Platforms for Carbon Monoxide Release

A notable alternative to metal carbonyl or organic compounds capable of undergoing CO release reactions either triggered or through ligand exchange is CORM-A1 and derivatives thereof (Figure 1-14(a)).[57] CORM-A1 is a water soluble sodium boranocarbonate compound ($Na_2[H_3BCO_2]$) that decomposes to release CO upon interaction with protons. The slow, pH-dependent liberation of CO ($t_{1/2}$ 27.06 min. at pH 7.4, 37 °C) has been considered key for the therapeutic effects observed in a variety of *in vitro* tests and *in vivo* animal disease models.[58] Prior to its use as a CORM, CORM-A1 was utilized as a key component of the
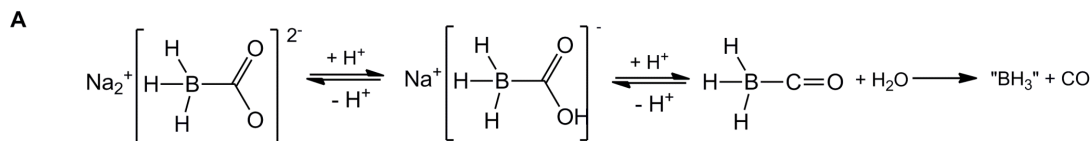
A

B
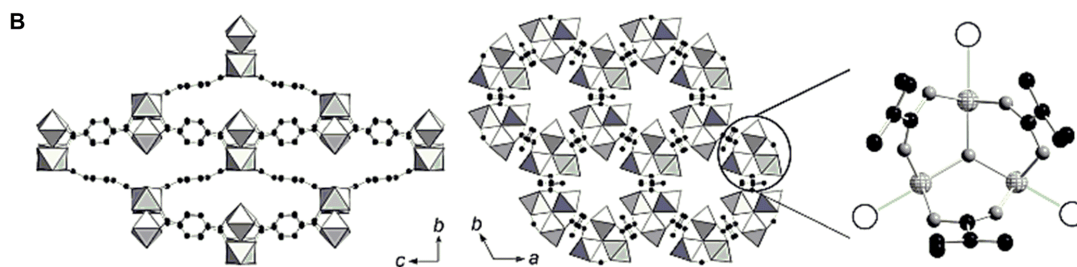
Figure 1-14. Alternative CO release platforms to metal carbonyl complexes and organic compounds. (a) pH-dependent CO release from sodium boranocarbonate (CORM-A1), and (b) Iron(III) terephthalate metal organic framework (MOF) utilized for CO storage; viewed along the *a* axis (left), *c* axis (middle), and one of the μ_3-oxo-bridged timeric unit made of iron octahedrons and terephthalate linkers (right). Empty circles represent terminal ligands, iron atoms are displayed as light gray spheres, oxygen atoms as gray spheres and carbon atoms as black spheres. Reproduced by permission from John Wiley and Sons: Chemistry - A European Journal (Ref. 62). Copyright 2013.

IsoLink kit that *in situ,* reduces $[^{99m}Tc(O_4)]^-$ to $[^{99m}Tc(CO)_3]^+$ for use in radiopharmaceutical diagnostics.[59] Additionally, the first biological studies of this compound were performed in the 1990s well before the beneficial health effects

of CO were recognized.[60] While the CO release from CORM-A1 cannot be controlled in terms of location and timing, the main disadvantage of this compound likely comes from its hydride donor character, with a reducing capability equivalent to that of sodium borohydride ($Na[BH_4]$).[61]

An additional alternative to traditional CORMs has been reported in the form of two metal organic frameworks (MOFs). MOFs are porous coordination polymers constructed from metal nodes and organic linkers. The MOFs reported as CO storage and release platforms are comprised of iron in the +3 oxidation state and dicarboxylate, terephthalate linkers (Figure 1-14b).[62] In order to load CO into the MOF, an activation process is required to remove non-bridging ligands, such as water and chloride, from the iron centers. This process involves heating the MOF above 100 °C in a CO gas stream. CO is released from the platforms via decomposition of the MOF in physiological media. Neither the toxicity of the MOFs nor the decomposition products were reported. However, it is particularly troubling that the decomposition products were not characterized at all. While iron is a biologically required metal ion, its storage is highly controlled as free iron ions are capable of undergoing reactions to produce compounds that are extremely damaging, such as through Fenton reactions. Additionally, the amount of CO loaded and released from the MOFs is unknown, which is extremely problematic for a compound that is therapeutic in low doses and extremely toxic at high concentrations.

The Future of CORMs

Since discovering the potential health benefits of low doses of CO, a considerable number of storage and release platforms for this small gaseous molecule have been developed. However, none of the compounds or materials developed to date represents viable constructs for further development into pharmaceuticals. It is clear that the field of CORMs needs a revolutionary future direction verses a continued investment in the evolutionary development of the previously described CORMs. Nature has the potential to serve as inspiration for a new direction. In higher organisms, the enzyme heme oxygenase endogenously produces CO through the breakdown of hemoglobin. This process can be visually monitored through bruise healing. The utilization of hemoglobin or hemoglobin-like compounds as CORMs could be problematic as it would rely on pre-existing enzymes for CO release and could potentially lead to the destruction of naturally occurring hemoglobin to restore homeostasis. Bacteria and fungi utilize enzymes known as quercetin dioxygenases (QDOs) to produce CO (Figure 1-15). QDOs make use of a class of naturally occurring compounds called flavonols to yield CO. Flavonols are potent antioxidants found in a variety of fruits and vegetables and can serve as inspiration for a revolutionary new class of CORMs.[63]
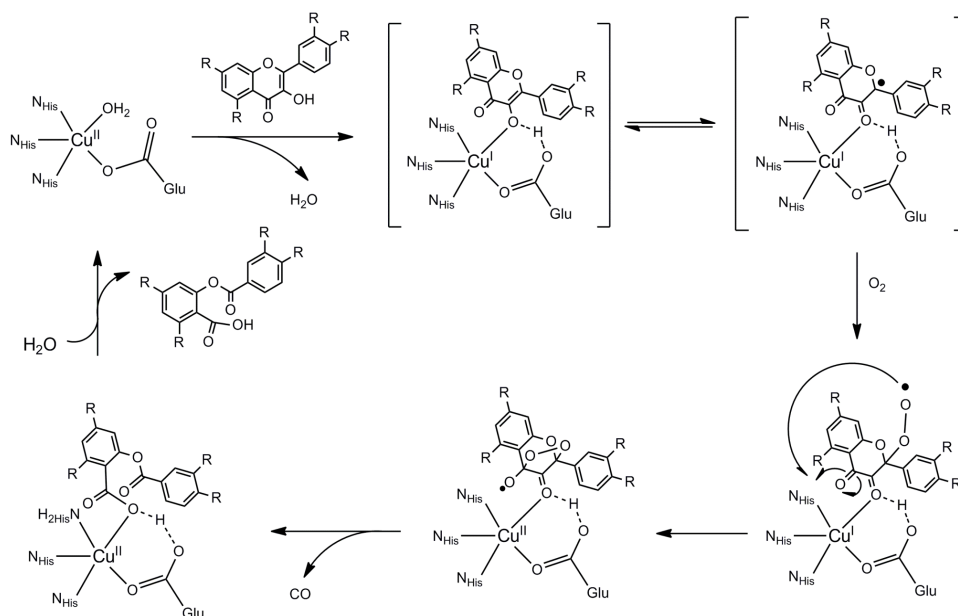
Figure 1-15. Proposed mechanism of CO release from a flavonol in copper

containing quercetin dioxygenase.

Industrial Significance of Carbon Monoxide

Carbon monoxide is used in the production of a wide variety of products

from the purification of metals to the synthesis of chemicals.[65] Well known

compounds manufactured using CO include drugs, such as ibuprofen, and

familiar household products such as vinegar, detergents, and plastics.[66] It is also

routinely used by workers in small-scale drug discovery companies and in

academic laboratories. Additionally, CO is utilized in atmospheric packaging

systems for meats in order to lower microbial load and extend shelf life.[67] The

use of CO gas in industrial processes have become incredibly pervasive to its

low cost and excellent atom economy and is produced (Figure 1-16) by the

megaton (Mt) each year for its multiple applications. The dangers of carbon

monoxide are well documented and can result in fatal poisoning. The symptoms

of CO poisoning - headache, nausea, vomiting, and dizziness - can be difficult to

recognize as they may be accredited to other maladies (e.g. virus, food

Burning of elemental carbon in a restricted supply of oxygen gas

- $C + O_2 \xrightarrow{\text{heat}} CO$
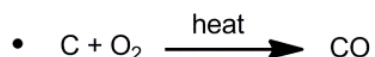
Reduction of carbon dioxide with coke

- $CO_2 + \text{coke} \xrightarrow{> 800\ ^oC} CO$
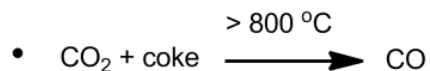
Water-gas shift reaction (preparation of synthesis gas)

- $C \text{ or } CH_4 + H_2O \xrightarrow[> 900\ ^oC]{[Ni]} CO + H_2$

- $CO + H_2O \xrightarrow[> 400\ ^oC]{Fe_xO_y} CO_2 + H_2$
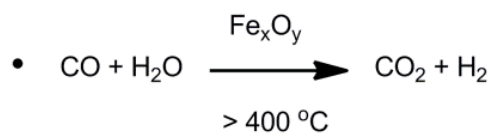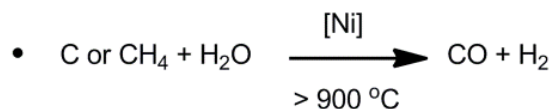
Figure 1-16. Common methods for large-scale industrial production of carbon

monoxide.[64]

poisoning). In large-scale industrial environments, elaborate and expensive gas

handling systems are incorporated to minimize the risk of workers being exposed

to CO. However, smaller scale facilities and academic environments lack the

resources to invest in such an infrastructure and as such the gas is used under

much less rigorous safety conditions. Recognition by the chemical community

that this is a severe hazard has led to the consideration of alternative means of delivering CO into chemical reactions. A general approach has been to develop molecules that will release CO when triggered.[68] To date, the majority of these compounds are used in an *ex situ* fashion, meaning that gaseous CO is produced and then transferred into the reaction chamber.

The most commercially significant CO-release system for use in chemical synthesis reported to date is the "COgen"/"COware" system (Figure 1-17).
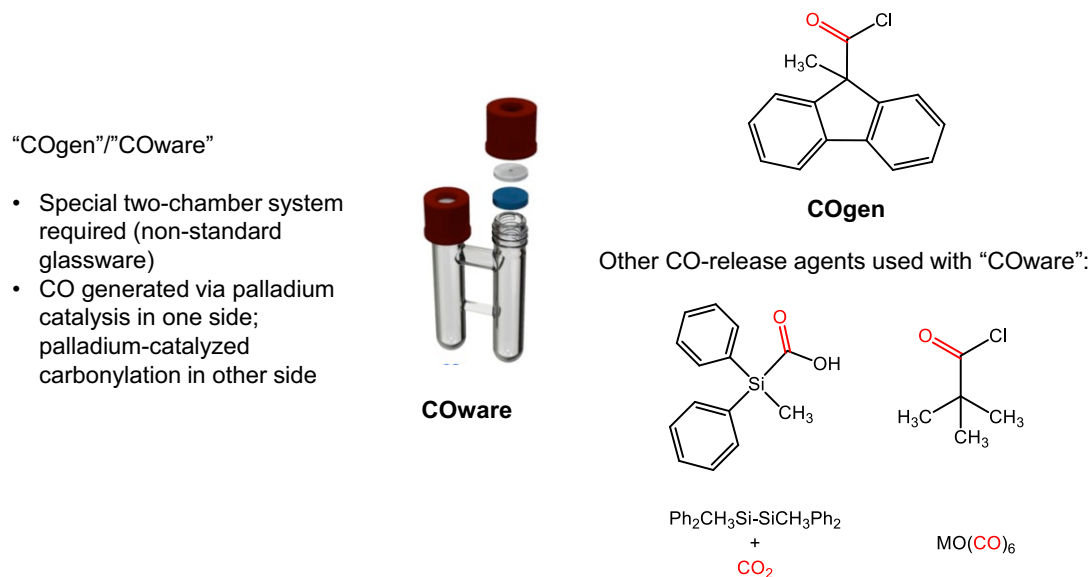
Figure 1-17. "COgen"/"COware". Listed on the right are compounds that are also patented for use as CO-release agents using COware.

This approach involves using chemical compounds to generate CO *ex situ* and deliver the gas via diffusion into the catalytic reaction using a two chambered

system. The utility of this system has been examined on palladium-catalyzed carbonylation reactions. Reactions of this type were first discovered by Richard Heck (2010 Nobel Prize winner) and can be visualized as shown in Figure 1-18.
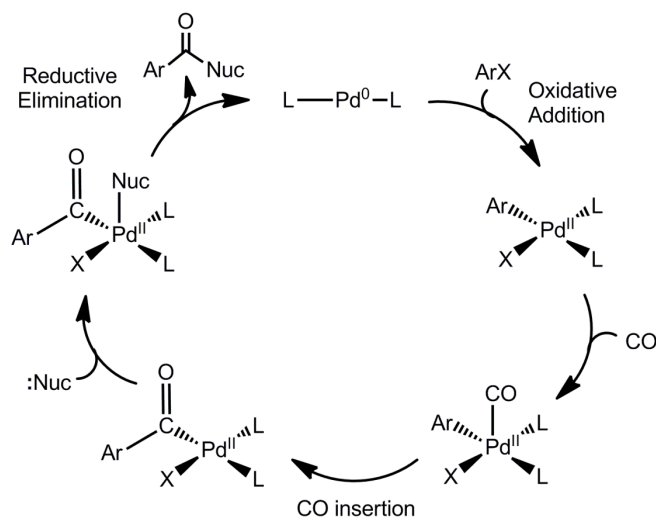
Figure 1-18. Proposed catalytic cycle of a carbonylative Heck reaction where L is a triphenyl phosphine ligand.

Using a palladium catalyst, two molecules are joined via the formation of two new bonds on either side of an inserted C=O unit. The broad generality of this reaction in terms of the compounds that can be coupled make this synthetic approach incredibly powerful. For example, a palladium-catalyzed carbonylation reaction is used in the synthesis of ibuprofen.[66] However, due to size limitations with the required glassware, the primary users of "COgen"/"COware" are likely

small-scale synthetic laboratories (academic and industrial). Notably, this system is able to deliver stoichiometric quantities of CO and has the ability to incorporate carbon isotopes in the CO unit.[68a-ab, 69]

"COgen"/ "COware" has also demonstrated some drawbacks. These include: (1) the requirement of the two-chamber glassware that is not common in most synthetic organic laboratories and must be specially purchased; (2) both sides of the chamber are heated at the same temperature; and (3) the use of expensive palladium catalysts in both chambers as both the release of CO from COgen and the incorporation of CO into the product require palladium catalysts. A few other compounds have been reported to date that can be used as *in situ* CO-release agents (formamides[68ai-ap], $CHCl_3/CsOH$[68aq, 68as, 70], $Mo(CO)_6$[68ar, 68at-ba]), however all require the presence of additional reagents or heat to induce CO release, which can be problematic with regard to reactivity of substrates. For example, $Mo(CO)_6$ acts as a reducing agent toward nitro-substituted substrates and thus limits the substrate scope that can be examined using this CO-release agent *in situ*.[71]

A significant advance for the use of carbon monoxide for the production of value added compounds would be the development of a safe-to-handle, heterogeneous, triggered CO-release material that could be used *in situ* in a broad array of reactions under a variety of reaction conditions. The importance of a material that is safe-to-handle cannot be understated as it could expand the synthetic utility of carbon monoxide to small chemical companies and academic

environments that may not have the capital to invest in the expensive and elaborate infrastructure to use CO safely. The heterogeneous nature would differentiate such a material from CO release agents currently employed and would be especially advantageous as reaction work-up would only involve separation of the CO release material via simple filtration. If such a material could be prepared at low cost and used in standard laboratory glassware, it would be uniquely useful relative to all other reported CO release compounds.

Conclusions

Carbon monoxide is a small gaseous molecule with a long history of toxicity. More recently, the importance of CO in human health as well as in a wide variety of chemical processes has been demonstrated. Due to the ever-increasing significance of this small molecule, the development of safe-to-handle, storage platforms that have properties desirable to the specific application is vital to the continued advancement of CO both as a pharmaceutical and in the production of value-added compounds. In the Berreau group, we are interested in the development and evaluation of novel compounds capable of undergoing photoinduced CO-release based on the framework of naturally occurring flavonols. We hypothesized that flavonols could be developed into a new family of tunable visible light-triggered photoCORMs. In the subsequent chapters of this dissertation, the following topics will be presented: (1) the effect of microenvironment on the photoinduced CO release reactivity of zinc-bound 3-hydroxyflavonolato ligand; (2) the development and biological properties of a new

family of flavonols capable of CO release upon illumination with visible light; (3)

the optimization of photochemical efficiency and solid state photoreactivity of zinc

bound flavonolato compounds utilizing a hydrophobic microenvironment; and (4)

the development of zinc bis-flavonolato compounds for use as heterogeneous *in

situ* CO sources in oxidative palladium catalyzed carbonylation reactions.

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CHAPTER 2

INFLUENCE OF SUPPORTING LIGAND MICROENVIRONMENT ON THE

AQUEOUS STABILITY AND VISIBLE LIGHT INDUCED CO RELEASE

REACTIVITY OF ZINC FLAVONOLATO SPECIES[‡]

Abstract

The visible light induced CO release reactivity of the zinc flavonolato

complex [(6-Ph$_2$TPA)Zn(3-Hfl)]ClO$_4$ (**1**) has been investigated in 1:1 H$_2$O:DMSO.

Additionally, the effect of ligand secondary microenvironment on the aqueous

stability and visible light-induced CO release reactivity of zinc flavonolato species

has been evaluated through the preparation, characterization, and examination

of the photochemistry of compounds supported by chelate ligands with differing

secondary appendages, [(TPA)Zn(3-Hfl)]ClO$_4$ (**3**; TPA = tris-2-

(pyridylmethyl)amine) and [(bnpapa)Zn(3-Hfl)]ClO$_4$ (**4**; bnpapa = *N,N*-bis((6-

neopentylamino-2-pyridyl)methyl)-*N*-((2-pyridyl)methyl)amine)). Compound **3**

undergoes reaction in 1:1 H$_2$O:DMSO resulting in the release of the free neutral

flavonol. Irradiation of acetonitrile solutions of **3** and **4** at 419 nm under aerobic

conditions results in quantitative, photoinduced CO release. However, the

reaction quantum yields under these conditions are lower than that exhibited by

1, with **4** exhibiting an especially low quantum yield. Overall, the results of this study indicate that positioning a zinc flavonolato moiety within a hydrophobic microenvironment is an important design strategy toward further developing such compounds as CO release agents for use in biological systems.

Introduction

The development of carbon monoxide releasing molecules (CORMs) for use in biological systems is of significant current interest.[1-7] This is due to the identification of several beneficial health effects associated with the controlled administration of small amounts of CO. These include anti-inflammatory and antiapoptotic effects, as well as the promotion of vasodilation and protection of tissues against reperfusion injury.[4] To date, the vast majority of CORMs reported are low-valent metal carbonyl compounds. These compounds, while exhibiting a number of favorable properties for biological use, also have limitations, including the fact that many cannot be controlled in terms of the location and timing of the CO release. In this regard, metal-carbonyl based CORMs that exhibit photoinduced CO release reactivity (photoCORMs) using low energy visible light are of particular current interest.[1, 2, 8-13] However, an important unresolved issue for these compounds is the potential for toxicity resulting from the remaining metal/ligand fragment following CO release.

We are interested in developing new types of photoinduced CO-releasing

molecules that are not based on the metal-carbonyl unit. In this regard, it is known that metalloenzymes termed quercetin dioxygenases (QDOs) catalyze the oxidative breakdown of flavonols to give CO and an O-benzoylsalicylic acid (O-bs) derivative as products.[14] In studies of synthetic complexes of relevance to the enzyme/substrate (ES) adducts of QDOs, we discovered stoichiometric, UV-light induced CO-release reactivity for a family of divalent metal complexes ([(6-Ph$_2$TPA)M(3-Hfl)]X, M = Zn(II), Co(II) Cu(II), Ni(II), Mn(II); X = ClO$_4^-$ or OTf$^-$) containing a deprotonated 3-hydroxy-4-flavonolato ligand (3-Hfl) and supported by a tetradentate N_4-donor chelate ligand (6-Ph$_2$TPA, N,N-bis((6-phenyl-2-pyridyl)methyl)-N-((2-pyridyl)methyl)amine).[15,16] For these complexes, CO release occurs via a photoinduced, dioxygenase-type reaction akin to the thermal reaction catalyzed by QDOs. The reaction observed for [(6-Ph$_2$TPA)Zn(3-Hfl)]ClO$_4$ (**1**) in CH$_3$CN under O$_2$ is shown in Scheme 2-1. The quantum yield for this reaction with irradiation at 300 nm (ϕ = 0.09(1)) [16] is an order of magnitude higher than that exhibited by structurally similar Mn(II), Co(II), Ni(II), Cu(II) complexes (ϕ = 0.005-0.008).[16] This is the result of quenching of the excited state in the complexes containing an open-shell d^n metal ion. The photoinduced reaction pathway for O$_2$-dependent CO-release from [(6-Ph$_2$TPA)Zn(3-Hfl)]ClO$_4$ (**1**) likely involves either the coordinated flavonolato ligand acting as a photosensitizer to generate singlet oxygen (^{1}O$_2$), which can then react with ground-state **1**, or an excited state singlet to triplet conversion of the zinc-bound flavonolato anion, which may then undergo reaction with ^{3}O$_2$.
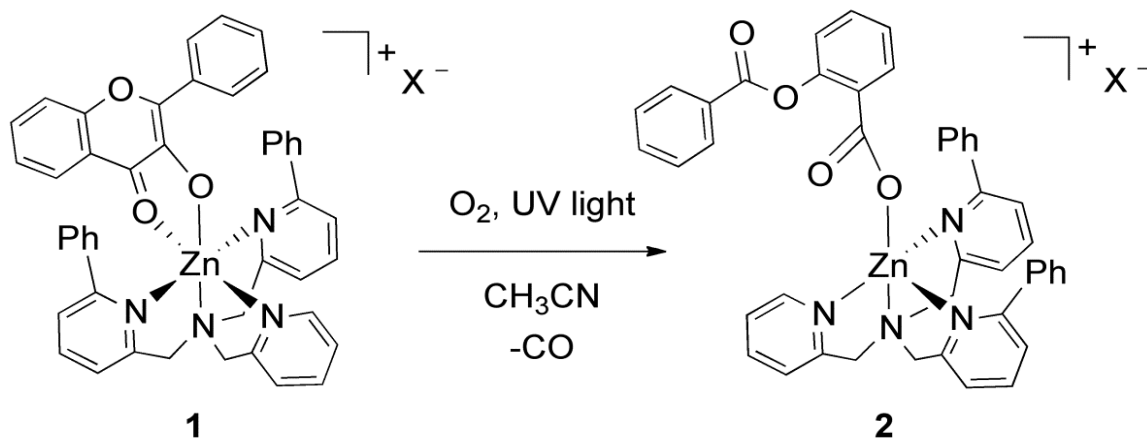
Scheme 2-1. Photoinduced CO release from zinc-bound flavonolato.

The initial studies outlined above have led us to further examine the chemistry of zinc flavonolato species. In the results described herein, we have evaluated the visible light induced CO release reactivity of **1** in 1:1 H_2O:DMSO. Additionally, we have evaluated how the nature of the supporting chelate ligand, particularly with respect to the secondary microenvironment surrounding the zinc flavonolato moiety, influences the aqueous stability and visible light induced CO-release reactivity of this unit. These combined results provide evidence that maintaining a hydrophobic microenvironment surrounding the zincflavonolato moiety is key toward further developing such compounds for use in biological systems.

Experimental

Chemicals and reagents

All chemicals and reagents were obtained from commercial sources and used as received unless otherwise noted. Anaerobic procedures were performed under N_2 in either a VAC Atmospheres or an MBRAUN Unilab glovebox. Solvents for glovebox use were dried according to published methods and distilled under N_2.[17] The preparation of the chelate ligands tris(2-pyridylmethyl)amine (TPA) [18] and *N*,*N*-bis((6-neopentylamino-2-pyridyl)methyl)-*N*-((2-pyridyl)methyl)amine (bnpapa) [19] was accomplished as previously reported. [(6-Ph$_2$TPA)Zn(3-Hfl)]ClO$_4$ (**1**) was prepared as previously described.[20]

Physical methods

^{1}H NMR spectra were collected using a JEOL ECX-300 or Bruker ARX-400 spectrometer. Chemical shifts (in ppm) are referenced to the residual solvent peaks in CD$_2$*H*CN ([1]H: 1.94 (quintet) ppm; [13]C: 1.39 (quintet) ppm). *J* values are given in Hz. IR spectra were recorded on a Shimadzu FTIR-8400 spectrometer as KBr pellets. UV-vis spectra were recorded at ambient temperature using a Hewlett-Packard 8453A diode array spectrophotometer. Emission spectra were collected using a Shimadzu RF-5301PC spectrofluorophotometer using a slit width of 4 nm with the excitation wavelength corresponding to the absorption

maximum of the complex above 400 nm. A Rayonet photoreactor equipped with RPR-4190A lamps was used for all photochemical reactions. Quantum yields were determined using potassium ferrioxalate actinometry as a standard to measure photon flux.[21-23] Carbon monoxide was quantified as previously described.[16] Mass spectral data was collected at the Mass Spectrometry Facility, University of California, Riverside. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA using a PE2400 automatic analyzer.

Caution! Perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts should be prepared and these should be handled with great care.[24]

Photoreactivity of 1 in 1 : 1 H$_2$O : DMSO using visible light

A solution of **1** (0.1 mmol in 10 mL 1:1 H$_2$O:DMSO) was placed in a 100 mL round bottom flask under air. This solution was irradiated at 419 nm until the reaction was determined to be complete as evidenced by loss of the 420 nm absorption band. CO release was determined to be quantitative by GC. Performing the same reaction in 1:1 D$_2$O:d_6-DMSO confirmed the formation of **2** via comparison of the ^{1}H NMR features with those previously reported for this compound.[15]

Preparation of zinc flavonolato complexes

[(TPA)Zn(3-Hfl)]ClO$_4$ (3). Under a N$_2$ atmosphere, a methanol solution (2 mL) of Zn(ClO$_4$)$_2$·6H$_2$O (40 mg, 0.11 mmol) was added to solid TPA (39 mg, 0.13 mmol) and the solution was stirred until the chelate ligand had dissolved. The resulting mixture was combined with a methanol solution (2 mL) containing 3-hydroxyflavone (32 mg, 0.13 mmol) and Me$_4$NOH·5H$_2$O (24 mg, 0.13 mmol). This mixture was stirred for 4 hours at ambient temperature. The solvent was then removed under reduced pressure and the residual solid dissolved in CH$_2$Cl$_2$. The CH$_2$Cl$_2$ solution was filtered through a Celite/glasswool plug and the product was precipitated via the addition of excess hexanes (40 mL). The isolated solid was dried under reduced pressure. Recrystallization via Et$_2$O diffusion into CH$_3$CN yielded yellow crystals suitable for X-ray crystallography (74 mg, 80%). Anal. Calcd. for C$_{33}$H$_{27}$ClN$_4$O$_7$Zn (%): C, 57.24; H, 3.93; N, 8.09. Found: C, 57.55; H, 4.00; N, 7.84. ^{1}H NMR (CD$_3$CN, 300 MHz) δ 8.9 (br, 2H), 8.80-8.45 (br, 4H), 8.03-7.77 (br, 3H) 7.76-7.59 (m, 4H), 7.58-7.20 (m, 8H), 4.79-4.02 (bs, 6H) ppm; UV-vis (CH$_3$CN), nm (ε, M^{-1}cm^{-1}) 415 (19000), 313 (7600); FTIR (KBr, cm^{-1}) 1558 ($\nu_{C=O}$), 1088 (ν_{ClO4}), 619 (ν_{ClO4}); MALDI-MS, *m/z* (relative intensity) C$_{33}$H$_{27}$N$_4$O$_3$Zn: Calcd: 591.1369; Found: 591.1385 ([M-ClO$_4$]$^+$, 100).

[(bnpapa)Zn(3-Hfl)]ClO$_4$ (4). Prepared using a synthetic procedure similar to that employed for the preparation of [(6-Ph$_2$TPA)Zn(3-Hfl)]ClO$_4$.[20] Recrystallization of the crude product via slow Et$_2$O diffusion into CHCl$_3$ yielded yellow crystals suitable for single crystal X-ray crystallography (87 mg, 75%).

Anal. Calcd. for $C_{43}H_{49}ClN_6O_7Zn$(%): C, 59.86; H, 5.72; N, 9.74. Found: C, 60.05;

H, 6.02; N, 9.38. ^{1}H NMR (CD$_3$CN, 300 mHz) δ 8.87 (br, 2H, N-H), 8.73 (d, J =

7.9 Hz, 1H), 8.10 (d, J = 7.9 Hz, 1H), 7.82-7.63 (m, 5H), 7.61-7.52 (m, 2H), 7.50-

7.32 (m, 4H), 7.30-7.18 (m, 2H), 6.49 (d, J = 7.2 Hz, 2H), 6.36 (d, J = 8.6 Hz,

2H), 4.52 (d, J = 14.3 Hz, 2H), 4.31 (s, 2H), 4.23 (d, J = 14.3 Hz, 2H), 2.87 (d, J =

7.2 Hz, 1H), 2.83 (d, J = 7.5 Hz, 1H), 2.62 - 2.48 (m, 2H), 0.63 (s, 18H) ppm; UV-

vis (CH$_3$CN), nm (ε, M^{-1}cm^{-1}) 401 (16500), 321 (18400); FTIR (KBr, cm^{-1}) 3307

(br, ν_{N-H}), 1560 ($\nu_{C=O}$), 1085 (ν_{ClO4}), 620 (ν_{ClO4}); MALDI-MS, m/z (relative

intensity) $C_{43}H_{49}N_6O_3Zn$: Calcd: 761.3152; Found: 761.3120 ([M-ClO$_4$]$^+$, 100).

Photoreactivity of [(L)Zn(3-Hfl)]ClO$_4$ (L = TPA (3) or bnpapa (4))

Identification of [(L)Zn(*O*-bs)]ClO$_4$ products (*O*-bs = *O*-benzoylsalicylato)

A solution of each complex ([(L)Zn(3-Hfl)]ClO$_4$ (L = TPA (**3**) or bnpapa (**4**);

0.1 mmol in 10 mL CH$_3$CN) was placed in a 100 mL round bottom flask under air.

Each solution was irradiated at 419 nm until the reaction was determined to be

complete as evidenced by loss of the ~400-415 nm absorption band. The solvent

was then removed under reduced pressure and the residual solid was

redissolved in a minimal amount of CH$_3$CN (ca. 2 mL). Addition of excess Et$_2$O

(~20 mL) resulted in the deposition of a beige solid that was dried *in vacuo* to

give the reported yields for [(TPA)Zn(*O*-bs)]ClO$_4$ (**5**) and [(bnpapa)Zn(*O*-bs)]ClO$_4$

(**6**). $^{18}O_2$-labeled samples were prepared by transferring aliquots of $^{18}O_2$ into frozen CH_3CN solutions of **3** and **4** in 100 mL solvent transfer flasks, followed by irradiation and work-up as described above.

[(TPA)Zn(O-bs)]ClO₄ (5). Yield: 91%. ^1H NMR (CD_3CN, 300 MHz) δ 8.74 (s, 3H), 8.02 (td, J_1= 7.8, J_2 = 1.5, 4H), 7.73-7.23 (m, 14 H), 4.13 (s, 6H); FTIR (KBr, cm^{-1}) 1761 ($vC=O$); $^{18}O_2$ sample: MALDI-MS, *m/z* (relative intensity); $C_{32}H_{27}N_4{}^{16}O_2{}^{18}O_2Zn$: Calcd. 599.1403. Found: 599.1407 ([M-ClO₄]⁺; 27).

[(bnpapa)Zn(O-bs)]ClO₄ (6). Yield: 88%. ^1H NMR (CD_3CN, 300 MHz) δ 8.57 (s, 1H), 8.19 - 7.00 (m, 14H), 6.65 (s, 2H), 6.47 (d, *J* = 6.4 Hz, 2H), 4.06 (s, 2H), 3.81-3.62 (m, 4H), 3.12-2.87 (m, 6H), 0.85 (s, 18H); FTIR (KBr, cm^{-1}) 1743 ($vC=O$); MALDI, *m/z* (relative intensity); $C_{42}H_{49}N_6{}^{16}O_4Zn$: Calcd: 765.3101. Found: 765.3124 ([M-ClO₄]⁺, 30). $^{18}O_2$ sample: MALDI-MS, *m/z* (relative intensity); $C_{42}H_{49}N_6{}^{16}O_2{}^{18}O_2Zn$: Calcd. 769.3186. Found: 769.3196 ([M-ClO₄]⁺, 58).

Dark control reactions

Solutions of **3** and **4** in CD_3CN (~0.02 M) were prepared in air under minimal red light and placed in NMR tubes. Each NMR tube was then covered with foil and irradiated at 419 nm for a specific amount of time (**3**: 24 h; **4**: 96 h)). For both, evaluation of the solution by ^1H NMR indicated that no reaction had occurred. These results indicate that the CO-release reactions of **3** and **4** are

photoinduced and not thermal processes.

Anaerobic control reactions

Solutions of **3** and **4** in CD_3CN (~0.02 M) were prepared under N_2 in a glove box and placed in NMR tubes. Each NMR tube was then irradiated at 419 nm for a specific amount of time (**3**: 24 h; **4**: 96 h)). For both, evaluation of the solution by ^{1}H NMR indicated that no reaction had occurred. These results indicate that the CO release reactions of **3** and **4** require O_2.

X-ray crystallography

Data collection and refinement

Single crystals of **3·CH₃CN** and **4·CHCl₃** were each mounted on a glass fiber using viscous oil and then transferred to a Nonius Kappa CCD diffractometer for data collection using Mo Kα radiation (λ = 0.71073 Å). Methods of unit cell refinement and determination of final cell constants have been previously reported.[25] The data collected for each compound was corrected for Lorentz, polarization, and absorption effects using DENZO-SMN and

SCALEPAC.[26] Each structure was solved using a combination of direct methods and heavy atoms using SIR 97.

Structure solution

Complexes **3·CH₃CN** and **4·CHCl₃** crystallize in the space groups *P2₁/n* and *P-1*, respectively. All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms were assigned isotropic displacement coefficients ($U(H) = 1.2U(C)$ or $= 1.5U(C_{methyl})$) and their coordinates were allowed to ride using SHELXL97.[27] In **3·CH₃CN** the perchlorate anion exhibits disorder of three of the oxygen atoms. In **4·CHCl₃**, hydrogen bonding interactions are found between the secondary amine NH units of O(1) of the coordinated flavonolato ligand.

Results and discussion

Spectroscopic features and photoinduced CO-release reactivity of 1 in 1:1 H₂O:DMSO

Absorption and emission properties

Compound **1** is not soluble in water, but exhibits good solubility in 1:1 H_2O:DMSO. As shown in Figure 2-1(a), **1** exhibits a ~420 nm absorption band in this solvent mixture which is similar to the spectrum produced in CH_3CN. Evaluation of this solution after 24 h of storage at ambient temperature in the dark revealed no spectral changes. Hence, the compound is stable in an aqueous environment under dark, aerobic conditions.
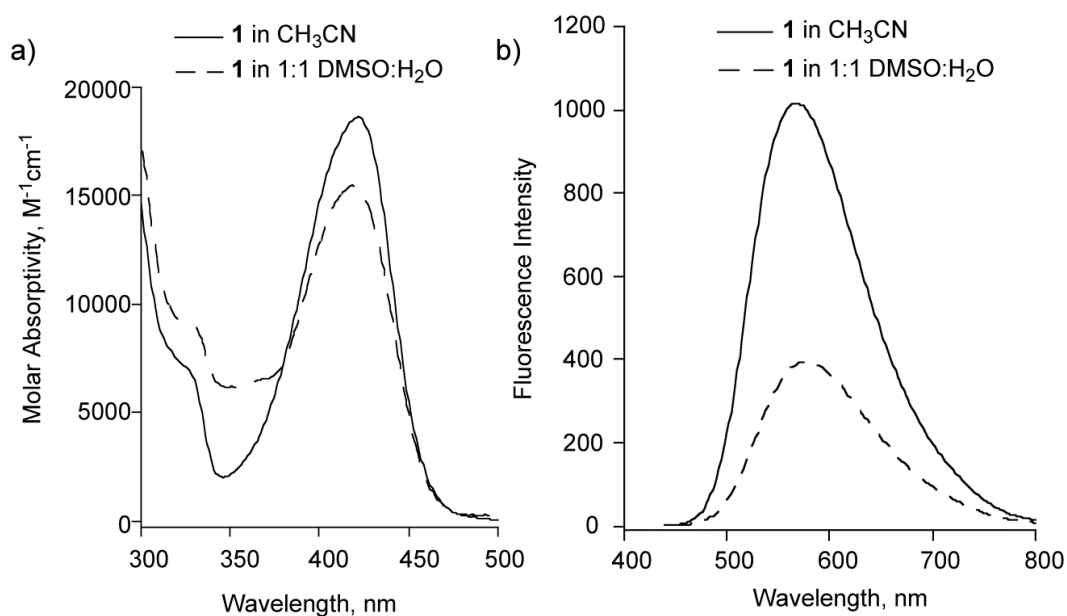
Figure 2-1. (a) Absorption spectra of **1** in CH_3CN and 1:1 DMSO:H_2O. (b) Fluorescence emission spectra of **1** in CH_3CN and 1:1 DMSO:H_2O generated upon excitation at 420 nm.

Photoinduced CO-release reactivity of 1 using visible light

Irradation of **1** in 1:1 H_2O:DMSO using visible light (419 nm) under air results in the release of one equivalent of CO (Table 2-1) and the formation of [(6-Ph$_2$TPA)Zn(*O*-bs)]ClO$_4$ (**2**). The quantum yield for this reaction (ϕ = 0.006) is only two-fold lower than that observed in acetonitrile under similar conditions.

Table 2-1. Reaction quantum yields and CO quantification for the visible light induced aerobic CO release reactions of **1**, **3**, and **4**.

Compound	Solvent	$\phi^{a,b}$	Eq. CO^b
1	CH$_3$CN	0.012(2)	0.99(2)
	1:1 H$_2$O/DMSO	0.006(1)	0.977(6)
3	CH$_3$CN	0.0060(9)	0.99(1)
4	CH$_3$CN	0.000269(8)	1.00(4)

$^a\lambda_{irr}$ = 419 nm; ^{b}Average of three independent determinations.

Synthesis and characterization of 3 and 4

To investigate the influence of the chelate ligand on the aqueous stability and photoinduced CO release reactivity of **1**, analogs were prepared and characterized using tetradentate chelate ligands that either lacked secondary appendages (TPA) or contained hydrogen bond donor moieties (bnpapa).

Synthesis of 3 and 4.

Admixture of the appropriate chelate ligand (TPA or bnpapa) with

Zn(ClO$_4$)$_2$·6H$_2$O, followed by treatment with a solution containing a mixture of 3-

hydroxyflavone (3-HflH) and Me$_4$NOH·5H$_2$O, respectively, produced yellow

reaction mixtures. After workup, each complex was isolated as a crystalline solid

(Scheme 2-2) in 75-80% yield. The zinc flavonolato complexes [(TPA)Zn(3-

Hfl)]ClO$_4$ (**3**) and [(bnpapa)Zn(3-Hfl)]ClO$_4$ (**4**) were characterized by elemental

analysis, X-ray crystallography, absorption and emission spectroscopy, IR, ^{1}H

NMR, and mass spectrometry.
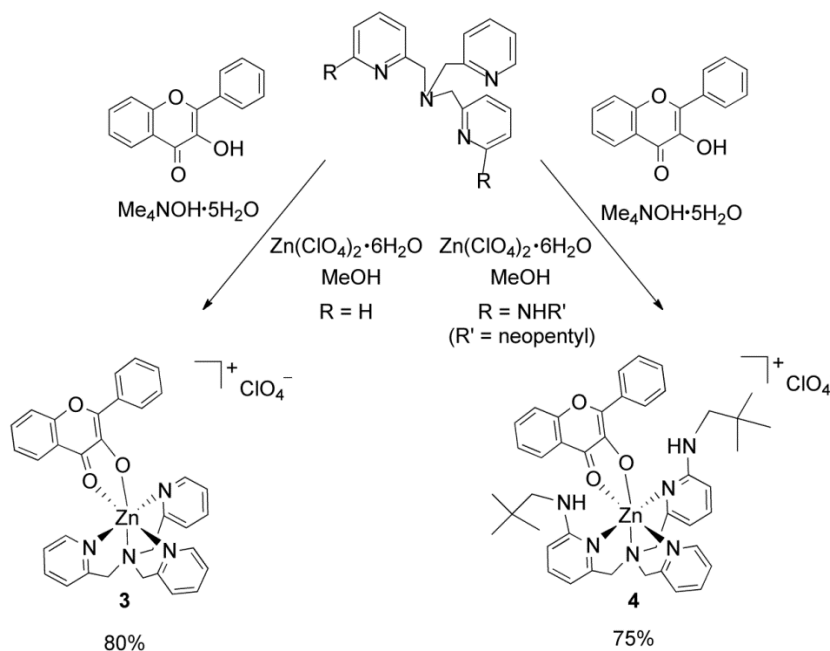
Scheme 2-2. Synthesis of **3** and **4**.

Elemental analysis

The elemental analysis data for bulk samples of **3** and **4** is consistent with the dried powder form of each complex having an analytical formulation that lacks the CH_3CN and $CHCl_3$ solvate molecules, respectively, that are found in the X-ray structures of these complexes (vide infra).

X-ray crystallography

Representations of the cationic portions of **3·CH₃CN** and **4·CHCl₃** are shown in Figure 2-2. Details of the X-ray data collection and refinement are givenin Table A-1. Bond distances and angles within the cationic portions of these structures are given in Table A-2. The Zn(II) centers in the cationic portions of **3·CH₃CN** and **4·CHCl₃** have a coordination number of six and exhibit a distorted psuedoctahedral geometry. This differentiates the solid-state structures of these cations from that found for **1·2CH₂Cl₂**, which exhibits an overall coordination number of five, a distorted square pyramidal geometry ($\tau = 0.35$) [28], and a dissociated phenyl-appended pyridyl donor. All three complexes exhibit bidentate coordination of the flavonolato ligand, with the shorter zinc-oxygen distance in each involving the deprotonated hydroxyl donor (**1·2CH₂Cl₂**: 1.951(2) Å; **3·CH₃CN**: 1.9834(17) Å; **4·CHCl₃**: 2.020(2) Å) and a longer bond to the ketone
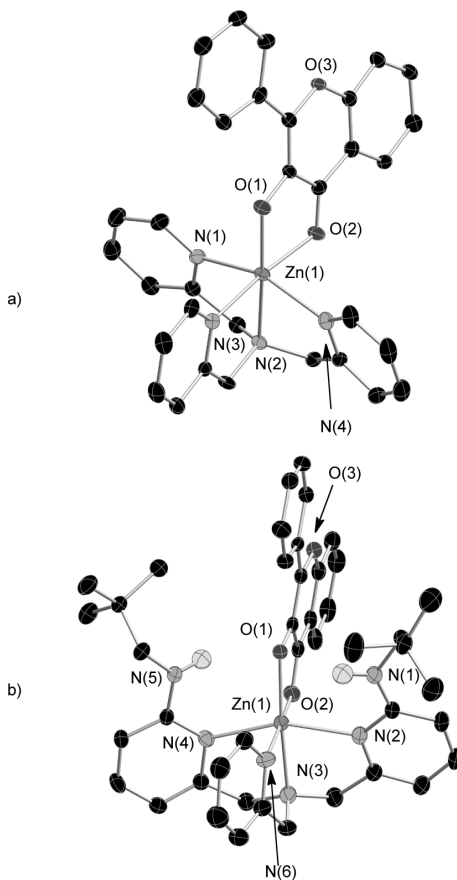
Figure 2-2. Thermal ellipsoid (50%) representations of the cationic portions of (a) **3** and (b) **4**. Hydrogen atoms except those of the N-H units have been omitted for clarity.

oxygen (**1·2CH₂Cl₂**: 2.1175(19) Å; **3·CH₃CN**: 2.1373(17) Å; **4·CHCl₃**: 2.159(2) Å). The Δ_{Zn-O} value is similar in this family of compounds, with all being found in the range of 0.14-0.17 Å. Generally, the observed bond lengths and Δ_{Zn-O} values for the complexes described herein fall within the ranges of other previously reported zinc flavonolato complexes (1.98-2.24 Å; Δ_{Zn-O} = 0.16-0.26 Å).[29-31] Notably, both

Zn-O distances elongate across the supporting chelate ligand series in the order 6-Ph$_2$TPA < TPA < bnpapa. The same trend is found in the average Zn-N$_{py}$ distance, which increases from 2.11 Å in the 6-Ph$_2$TPA-ligated complex to 2.16 and 2.17 Å in the TPA- and bnpapa-ligated complexes, respectively. This latter trend is likely due to the effect of overall coordination number, as well as the presence of the secondary amine donor appendages in the bnpapa-ligated system, which introduces additional steric hindrance.

Examination of the bond lengths within the coordinated flavonolato ligand shows that all three complexes exhibit a similar slight elongation (~0.02 Å) of the ketone carbonyl C-O bond and a slight contraction (~0.02-0.04 Å) of the C-O bond of the hydroxyl donor relative the distances found in the free flavonol (1.232(3) and 1.357(3) Å, respectively).[32] The C=C bond distance within the flavonolato chelate ring is similar in all three complexes (**1·2CH$_2$Cl$_2$**: 1.458(4) Å; **3·CH$_3$CN**: 1.439(3) Å; **4·CHCl$_3$**; 1.460(4) Å) and is elongated relative to that found in the free flavonol (1.363(4) Å).[32] Overall, the bond lengths within the flavonolato ligands of this series of complexes are only minimally affected by the nature of the supporting chelate ligand.

In **4·CHCl$_3$,** hydrogen bonds are found between the two secondary amine NH units of the bnpapa chelate ligand and O(1) of the coordinated flavonolato ligand. The heteroatom distances (2.93 and 3.01 Å) and corresponding N-H…O angles (136.5° and 155.2°) associated with these interactions suggest that these are moderate hydrogen bonds with individual energies of 4-14 kcal/mol.[33]

Spectroscopic characterization

Powdered samples of **3** and **4** were characterized by solid state IR

spectroscopy, and solutions of these complexes were examined using ^{1}H NMR,

mass spectrometry, and absorption and emission spectroscopy.

Infrared spectra

The solid-state FTIR spectra of **1**, **3** and **4** contain a $\nu_{C=O}$ vibration at

~1550-1560 cm^{-1} for the coordinated ketone moiety, as well as vibrations for the

perchlorate counterion. For **4**, a broad vibration at ~3300 cm^{-1} is consistent with

the presence of the secondary amine appendages and their involvement in

hydrogen bonding interactions with the coordinated flavonolato ligand.

^{1}H NMR

Complex **1** exhibits a ^{1}H NMR spectrum with the appropriate number of

signals for C_s symmetry in CD$_3$CN (Figure A-1). This is consistent with

coordination of all of the nitrogen donors of the 6-Ph$_2$TPA ligand in the solution

structure of the complex. Complex **3** exhibits broadened ^{1}H NMR signals

suggestive of fluxional behavior when dissolved in CD$_3$CN (Figure A-2) or CD$_2$Cl$_2$

(Figure A-3(a)) at 25 ^{o}C. The broadened benzylic methylene resonance at ~4.4

ppm suggests that the fluxionality could involve dissociation and reassociation of

pyridyl donors (Figure A-3). This type of behavior has been previously identified in d^{10} Cu(I) complexes of TPA.[34] Cooling of a CD_2Cl_2 solution of **3** from 25 °C to -53.5 °C (Figure A-3(a-h)) produced a sharpening of some of the resonances that was reversible upon returning the sample to room temperature. Further evaluation of the ^1H NMR properties of **3** are underway. Complex **4** exhibits ^1H NMR features (Figure A-4) consistent with the cationic portion having C_s symmetry. ^1H NMR spectra of photoproducts **5** (Figure A-5) and **6** (Figure A-6) were as expected.

Mass spectrometry

Complexes **3** and **4** as well as their photoproducts, **5** and **6**, respectively, were evaluated by MALDI mass spectrometry (Figures A-7 – A-12). Each exhibits an isotopic cluster molecular ion that is consistent with the proposed cationic formulation. $^{18}O_2$ incorporation into **5** and **6** was evaluated by MALDI mass spectrometry. Each exhibits an isotopic cluster molecular ion consistent with the incorporation of both atoms of $^{18}O_2$ into the compounds.

Absorption and emission spectra in CH_3CN and 1:1 H_2O:DMSO

Free 3-hydroxyflavone (3-HflH) exhibits absorption bands at 304 and 343 nm, respectively, when dissolved in methanol.[35,36] The lower energy feature (Band I)

is assigned to a π→π* HOMO to LUMO transition, while the higher energy Band II corresponds to a HOMO-1 to LUMO transition. The absorption spectra of **3** and **4** in CH_3CN are shown in Figure 2-3. The Band I absorption feature for **1**, **3** and **4** is red-shifted relative to the neutral flavonol, with the degree of shift depending
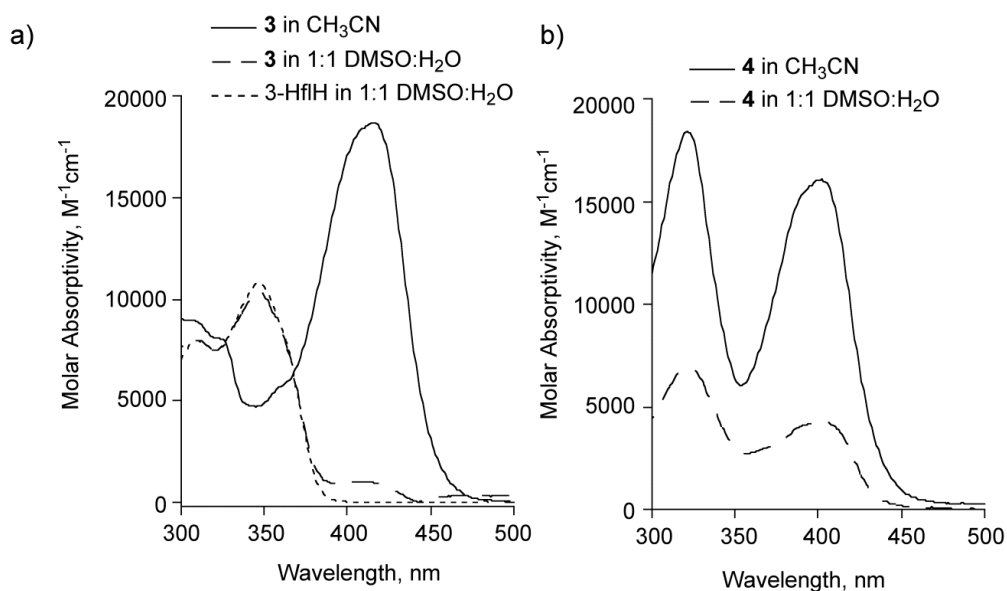
Figure 2-3. (a) Absorption spectra of **3** in CH_3CN and 1:1 $DMSO:H_2O$ and the absorption spectrum of 3-hydroxyflavone in 1:1 $DMSO:H_2O$. (b) Absorption spectra of **4** in CH_3CN and 1:1 $DMSO:H_2O$.

on the nature of the supporting chelate ligand. Specifically, the most red-shifted Band I absorption is found for the 6-Ph_2TPA-ligated complex **1** (λ_{max} = 420 nm (21,000 $M^{-1}cm^{-1}$))[16], followed by those of **3** (λ_{max} = 415 nm (16,500 $M^{-1}cm^{-1}$))

and **4** (λ_{max} = 401 nm (16,500 $M^{-1}cm^{-1}$)). In 1:1 DMSO:H_2O, the TPA-supported

complex **3** exhibits spectral changes consistent with protonation of the

coordinated flavonolato ligand and displacement from the Zn(II) center (Figure 2-

3(a)). A new absorption feature at ~350 nm is consistent with the presence of

free 3-hydroxyflavone (Figure 2-3(a)). The UV-vis absorption spectrum of **4** in 1:1

DMSO:H_2O is similar to that found in CH_3CN (Figure 2-3(b)) albeit with

significantly lower molar absorptivity values. Excitation into the ~400-415 nm

absorption maximum of **3** and **4** dissolved in anaerobic CH_3CN (5 x 10^{-5} M)

produced the emission spectra shown in Figure 2-4. Compound **3** exhibits a

similar Stokes shift (~72 nm) to that found for **1**, but with an overall lower intensity

fluorescent emission. Essentially no fluorescent emission is observed for **4** upon

excitation at its absorption maximum (401 nm). Thus, the fluorescence intensity

decreases in the order of **1** > **3** > **4** in CH_3CN. We propose that this difference

relates to the microenvironment in the 6-Ph_2TPA chelate in **1,** resulting in the

phenyl appendages surrounding the coordinated flavonolato ligand. Specifically,

the presence of the phenyl appendages in the 6-Ph_2TPA chelate in **1** result in the

flavonolato ligand being positioned in a hydrophobic microenvironment. This

motif more limits solvent access to the flavonolato ligand relative to that in **3**

thereby reducing collisional quenching. That being said, in 1:1 H_2O:DMSO, the

emission of **1** decreases in intensity relative to that found in CH_3CN (Figure 2-

1(b)), suggesting that there is some solvent access to the flavonolato moiety. For
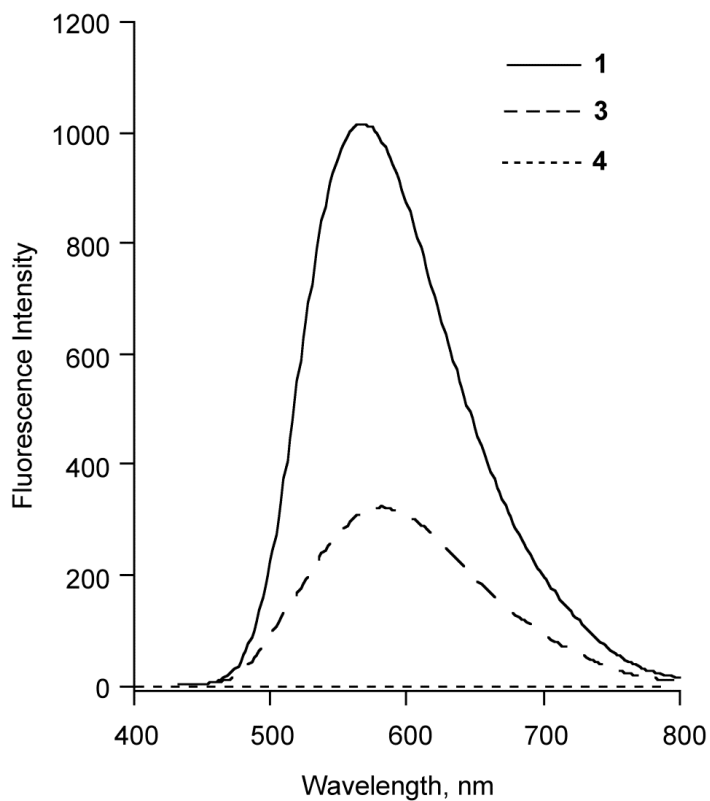
Figure 2-4. Fluorescence emission spectra of **1**, **3**, and **4** in CH_3CN. All spectra were obtained with λ_{ex} at the absorption maximum at ~400-420 nm.

complex **4**, the presence of the rigid intramolecular hydrogen bond donors, which may undergo excited state lengthening or shortening, results in significant fluorescence quenching. [37]

Visible light induced reactivity of 3 and 4

As **3** undergoes reaction in 1:1 H_2O:DMSO to produce free 3-hydroxyflavone, we have only examined the visible light induced CO release reactivity of this compound in aerobic CH_3CN using 419 nm irradiation. Complex **4** was also examined in aerobic CH_3CN and was found to exhibit such low photochemical efficiency (vide infra) that further studies in 1:1 H_2O:DMSO were not performed. Each reaction was irradiated until judged to be complete by the disappearance of the ~400-415 nm absorption band.

Product Identification

GC headspace gas analysis, ^1H NMR, IR, and mass spectrometry were used to characterize the products generated upon photoirradiation of **3** and **4** in CH_3CN at 419 nm. For each complex, the photoreaction resulted in the formation of one equivalent of CO (Table 2-1) as well as a [(L)Zn(*O*-bs)]ClO_4 complex (TPA: **5**; bnpapa: **6**). The latter complexes were identified by their ^1H NMR spectral features (Figures 2-7 and 2-8), molecular ions in MALDI mass spectral experiments (Figures 2-11, 2-12, 2-13 and 2-14), and by their characteristic $\nu_{C=O}$ ester vibration at 1740-1770 cm^{-1} for the *O*-benzoysalicylate ligand. Quantitative ^{18}O incorporation from $^{18}O_2$ for the reactions of **3** and **4** provides conclusive evidence for a dioxygenase-type reaction. Appropriate dark and anaerobic

control reactions demonstrate that the observed reactions for **3** and **4** are O_2 dependent photoinduced processes.

Reaction Quantum Yields

The quantum yields for the visible light induced CO release reactions of **3** and **4** in CH_3CN are shown in Table 2-1. The structure of the supporting chelate ligand has a significant effect on the photochemical efficiency of the CO release reactions, with the quantum yield for **1** being two-fold higher than that of **3**, and both of these values being >20 fold higher than the quantum yield for the reaction of **4**. These results demonstrate that positioning a zinc flavonolato moiety within a rigid hydrogen bond donor environment significantly stabilizes the compound with respect to light induced reactivity.

Conclusions

In the studies outlined herein, we have examined the aqueous, visible light induced CO release reactivity properties of **1**. The results indicate that this complex is stable in an aqueous, aerobic environment in the dark, but will exhibit quantitative, visible light induced CO release to generate a single zinc-containing product. The quantum yield for CO release in 1:1 H_2O:DMSO is only of factor of two lower than that observed in organic solvent.

We have also examined how the supporting chelate ligand influences the aqueous stability and visible light induced CO-release reactivity of zinc flavonolato species. We were particularly interested in examining the influence of the aryl appendages of the 6-Ph$_2$TPA ligand in **1** as it is well known that the photochemical reactivity of organic and inorganic molecules can be modulated via encapsulation within a confined microenvironment such as a cyclodextrin cavity, hydrogen-bonded capsules, or hollow molecular structure [38,39]. Our results demonstrate that the secondary environment introduced by the tetradentate chelate ligand influences the chemistry of zinc flavonolato complexes in multiple ways. First, the presence of the hydrophobic microenvironment in **1** stabilizes the zinc flavonolato moiety with respect to water and differentiates the chemistry of **1** from that of **3** in aqueous DMSO solution. The observation of flavonol displacement from **3** in water is noteworthy not only to this study, but also to investigations being performed in other laboratories wherein simple zinc flavonolato species such as [Zn(3-Hfl)(acetate)] are being evaluated in aqueous media for their antidiabetic and DNA binding properties [40-42]. Second, the lower quantum yields observed for photoinduced CO release from **3** and **4** in CH$_3$CN relative to **1** is likely a consequence of the enhanced accessibility of solvent to the flavonolato moiety and/or secondary hydrogen bonding. These interactions offer additional pathways for non-radiative decay thus leading to the observed excited state quenching and less efficient CO-release processes relative to **1**.

Overall, these investigations revealed that the presence of a hydrophobic microenvironment is important toward providing aqueous stability and maximizing the photochemical efficiency of the CO-release reaction of a zinc flavonolato moiety. Therefore, if zinc flavonolato species are to be advanced for use as photoinduced CO release agents in biological systems, careful consideration will need to be given to the supporting microenvironment within the compound.

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CHAPTER 3

A STRUCTURALLY-TUNABLE 3-HYDROXYFLAVONE MOTIF FOR VISIBLE

LIGHT-INDUCED CO-RELEASING MOLECULES (CORMS)[‡]

Abstract

Molecules that can be used to deliver a controlled amount of CO have the potential to facilitate investigations of the roles of this gaseous molecule in biology and advance therapeutic treatments. This has led to the development of light-induced carbon monoxide-releasing molecules (photoCORMs). A goal in this field is the development of molecules that exhibit a combination of controlled CO release, favorable biological properties (e.g. low toxicity and trackability in cells), and structural tenability to affect CO release. Herein we report a new biologically-inspired organic photoCORM motif that exhibits several features that are desirable in a next generation photoCORM. We show that 3-hydroxyflavone-based compounds are easily synthesized and modified to impart changes in absorption features and quantum yield for CO release, exhibit low toxicity, are trackable in cells, and can exhibit both O_2-dependent and –independent CO release reactivity.

Introduction

Carbon monoxide-releasing molecules (CORMs) are of significant current interest due to the potential of CO as a therapeutic molecule.[1] The vast majority of CORMs developed to date are based on a metal-carbonyl unit as the CO-releasing moiety.[2] Many molecules of this type, including protein-bound derivatives of [RuCl(glycinato)(CO)₃] (CORM-3, Figure 3-1) and analogs,

CORM-3

R = Ph, R' = H
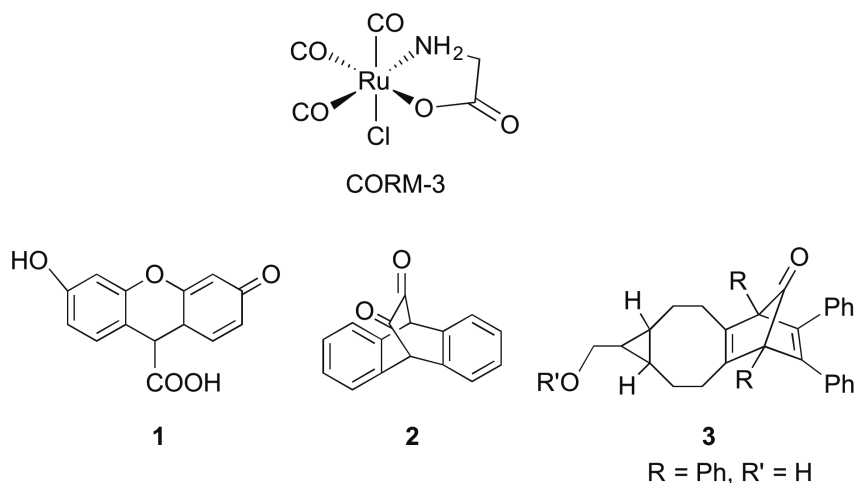
Figure 3-1. Structural motifs of selected previously reported CORMs.

release CO spontaneously through ligand exchange in an aqueous environment.[3] The lack of temporal control for CO release in such systems has led to the use of metal carbonyl complexes that release CO only when triggered.[4,5] Examples of such complexes include photoCORMs, which release CO from a metal carbonyl unit upon illumination with UV or visible light.[4] Recent advances in the field of metal carbonyl photoCORMs demonstrate that CO

release can be tuned to occur upon illumination with low-energy red or NIR light through modification of supporting ligands or through approaches using nanoparticles.[4] However, a concern associated with some metal-carbonyl based photoCORMs are side effects related to the metal-containing photoproducts.[6] A limited number of organic photoCORMs (**1**-**3**, Figure 3-1) have also been recently reported.[7] However, these molecules also have limitations. For example, **1** and **2** are derived from relatively low-yield, multistep synthetic routes that have not been shown to be amenable to structural modification for the tuning of physical properties or biological targeting. The Diels-Alder product **3** can be generated in good yield and subsequently undergoes CO release. However, this compound cannot be isolated and stored.

Desirable features in a next generation organic photoCORM motif include: (1) a high-yield synthesis that enables the preparation of gram quantities of analytically pure compound; (2) solubility in water or aqueous DMSO; (3) thermal stability in aerobic, aqueous environments; (4) controllable, triggered CO release, preferably using light at wavelengths that do not have the potential to impart cellular damage; (5) low toxicity of the photoCORM and its post-CO-release byproducts; (6) ease of structural modification to modulate aqueous solubility, photochemical properties (e.g. light absorption properties), and biocompatibility, and (7) exhibits fluorescence so as to enable tracking of the localization and CO release reactivity of the molecule within cells.[8] In the results reported herein, we describe a new class of biologically-inspired photoCORMs that exhibit all of the desirable features noted above. The parent structure contains a 3-

hydroxyflavone motif, which is found in naturally-occurring molecules that are already known to exhibit several types of biological activity, including antioxidant, anti-inflammatory, and anti-cancer activity, as well as protection against cardiovascular disease.[9] The new flavones reported herein all exhibit visible light-induced CO-release in O_2-containing environments, with one derivative also exhibiting O_2-independent CO release reactivity.

Discussion

Naturally-occurring 3-hydroxyflavone derivatives, such as quercetin (Scheme 3-1(top)), are known to undergo O_2-dependent, *enzyme-catalyzed* degradation to produce CO in bacteria and fungi.[10] In the absence of enzyme, quercetin is known to undergo various types of oxidative reactions, including UV light-induced reactions, which can result in CO release.[11] It is known that unsubstituted 3-hydroxyflavone (3-HflH) will undergo incorporation of both atoms of O_2 and expulsion of CO in the presence of a photosensitizer, or via direct illumination using UV light (Scheme 3.1(middle)).[12] These reactions are proposed to proceed from the normal and tautomeric excited state forms of 3-HlfH, respectively. We have reexamined the photoinduced (λ = 300 nm) reactivity of 3-HflH under O_2 and found that while a nearly quantitative amount of CO is generated (0.95 eq), multiple organic products are detected by GC-MS. Finally, 3-HflH is also known to undergo UV light-induced rearrangement resulting in CO release under anaerobic conditions (Scheme 3-1(bottom)).[13] These combined
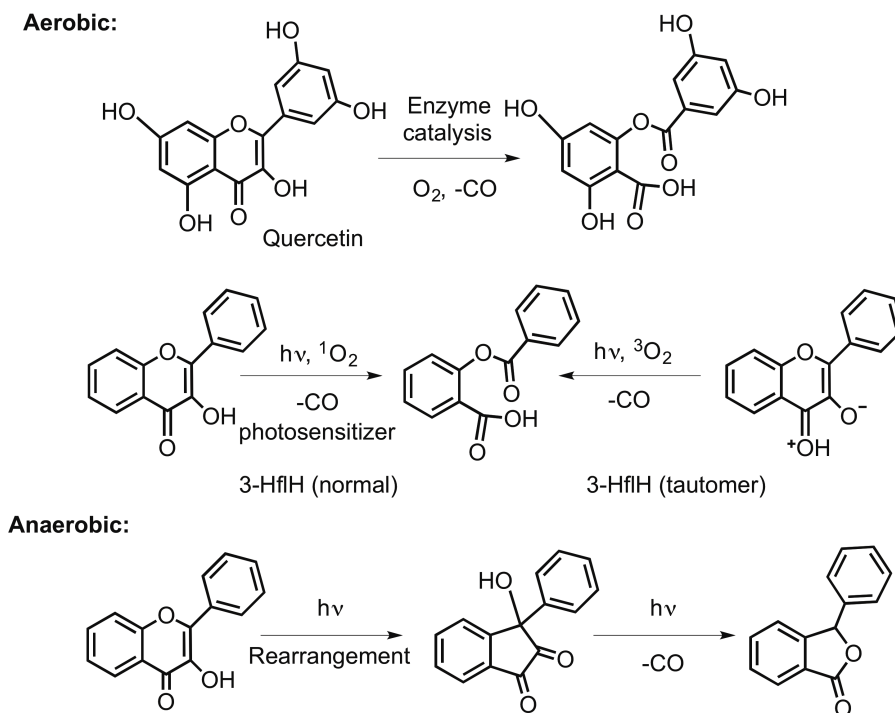
Scheme 3-1. Top) O_2-dependent CO release reactivity of quercetin and 3-HflH.

Bottom) O_2-independent, UV-light induced isomerization reactivity of 3-HflH.

results indicate that 3-hydroxyflavone derivatives have multiple reaction

pathways by which light-induced CO-release reactivity can occur.

We hypothesized that the 3-hydroxyflavone structural motif could be tuned

to undergo visible light-induced CO release. With this strategy in mind, the new

3-hydroxyflavone **4** was designed and prepared using Alger-Flynn-Oyamada

methodology (Figure 3-2(top)).[14] X-ray quality crystals of **4** were obtained via

slow evaporation of a CH$_2$Cl$_2$ solution. Compound **4** was additionally

characterized by elemental analysis, spectroscopic methods (Figures B-1 and B-3 – B-5), and mass spectrometry (Figure B-2). Compound **4** is readily soluble in organic solvents and is also soluble in aqueous DMSO at concentrations suitable for spectroscopic measurements (1:1 H_2O:DMSO) and biological experiments (1% DMSO). Compound **4** crystallizes in the monoclinic space group C2/c.[15] A representation of the molecular structure of **4** is shown in Figure 3-2(bottom).
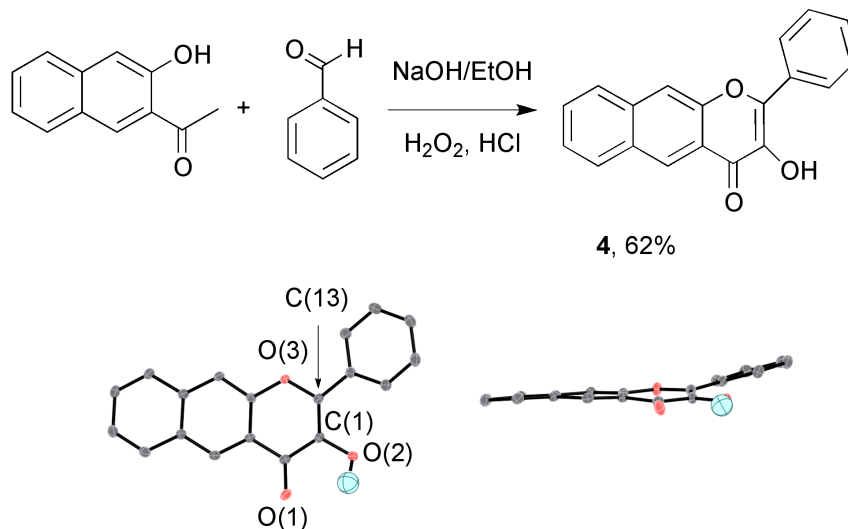
Figure 3-2. Top) Synthetic route for the preparation of **4**. Bottom left) A representation of the molecular structure of **4** as determined by X-ray crystallography. Bottom right) Side-on structural view.

In the solid state, the 3-hydroxy-4-pyrone units from two molecules form centrosymmetric hydrogen bonded dimers with two identical intermolecular O-H..O hydrogen bonds (O…O 2.69 Å; 145.3°).[16] The naphthyl-fused 3-hydroxy-4-pyrone ring structure is nearly planar, and the phenyl appendage twists only

slightly out of this plane. This overall structure favors conjugation of the two electronic systems. Compound **4** has bond lengths and angles very similar to those of 3-hydroxyflavone (3-HflH).[16]

The extended conjugation in **4** produces a red-shift of the absorption features relative to those found for 3-HflH in CH_3CN (Figure B-3).[13] The lowest energy band for **4** is found in the visible region with maximum intensity at 409 nm (ε = 16,600 $M^{-1}cm^{-1}$) in CH_3CN, whereas there is no absorption feature above 400 nm for 3-HflH. In 1:1 H_2O:DMSO, **4** exhibits similar absorption features but with overall lower intensity (Figure B-3). Excitation into any of the absorption features exhibited by **4** in CH_3CN produces a single broad emission feature centered at 582 nm (Figure B-4). Based on literature precedent for 3-HflH, the large Stokes shift ($\geq$ 177 nm) suggests the formation of an excited state tautomeric form wherein intramolecular proton transfer has occurred to give a zwitterionic species. [17] When dissolved in 1:1 H_2O:DMSO and excited in the lowest energy absorption band, **4** exhibits two emission bands at 475 and 582 nm, respectively (Figure B-5). The former is of relatively low intensity and likely represents emission from an excited state normal form of the molecule, whereas the latter matches the emission feature produced in organic solvent.[17]

Solutions of **4** in acetonitrile, 1:1 H_2O:DMSO, or cell culture media (RPMI-1640; pH = 7.4) are stable in the presence of ambient O_2 for >2 weeks when protected from light. Exposure of an aerobic CH_3CN solution of **4** to visible light (419 nm) results in quantitative CO release (0.96(2) eq) as determined by GC

headspace analysis and the formation of 3-(benzoyloxy)-2-naphthoic acid (**5**, Scheme 3-2; Figures B-6 – B9). This organic product is pale yellow in color and
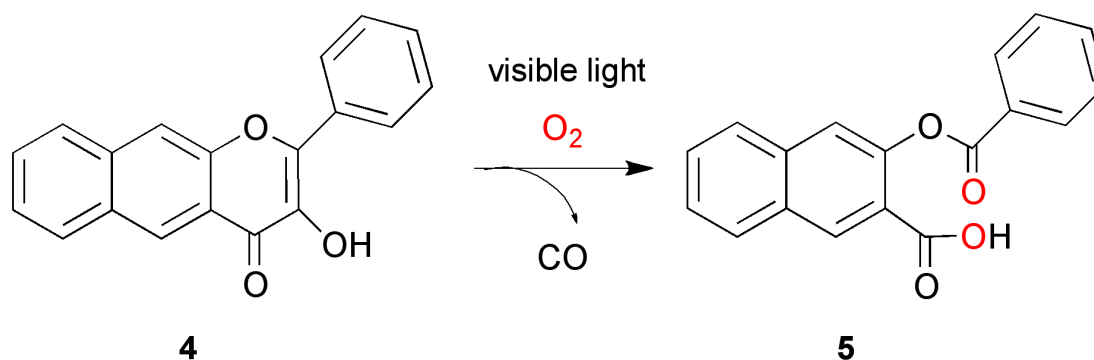
Scheme 3-2. Photoinduced CO release reactivity of **4**.

does not exhibit any emission features in the visible region (Figure B-10). The quantum yield for the CO-release reaction of **4** is 0.007(3). The same reaction occurs in methanol (Figure B-8) and 1:1 H_2O:DMSO as determined by ^1H NMR and GC head space gas analysis. Control reactions indicate that both O_2 and visible light are needed for the CO release reaction of **4**. An $^{18}O_2$ labeling experiment demonstrates that both oxygen atoms from O_2 are incorporated into the organic photoproduct. Compound **4** exhibits several features that suggest that it could be a useful CO release agent in biological systems. First, it exhibits minimal toxicity, as determined by MTT cell viability assays using A549 cells (IC_{50} = 41.5 µM; Figure B-11), and the organic product remaining following CO release is non-toxic. Importantly, the fluorescent nature of **4** makes it trackable in cells

prior to CO release. The cellular uptake properties of **4** were evaluated in A549 cells, which were exposed to Hoechst stain for 10 minutes (to enable visualization of nuclei), followed by incubation with **4** for 1 hour in the dark. Fluorescence microscopy images (Figure 3-3; Figure B-12) of the cells were collected after 30 seconds, 3 minutes, and 10 minutes of visible light exposure.[18]

The observed green emission at the first two time points provides evidence that **4** is taken up by almost all cells. The compound is not associated with the plasma membrane, but is distributed throughout the cytoplasm and appears to concentrate around the nucleus. Importantly, continued exposure of the cells to visible light results in a decrease in the observed green fluorescence of the compound after three minutes, with complete loss after ten minutes. This observation provides strong evidence for the photoinduced cleavage of the 3-hydroxy-4-pyrone ring and CO release reactivity within the cells as the photoproduct does not display any emission. It should be noted that use of the intracellular CO probe COP-1 is not feasible in this system because the emission of **4**, which disappears upon CO release, overlaps with the emission feature of CO-incorporated COP-1. [19]

A key feature of the structural motif of **4** that distinguishes it from all previously described organic photoCORMs is the ease with which structural modifications can be introduced to tune its physical properties. For example, a dialkylamino substituent can be incorporated on the phenyl ring or the carbonyl oxygen can be substituted with sulphur to red-shift absorption features toward

the therapeutic window. Dialkylamino-substituted flavonols have been previously

used as
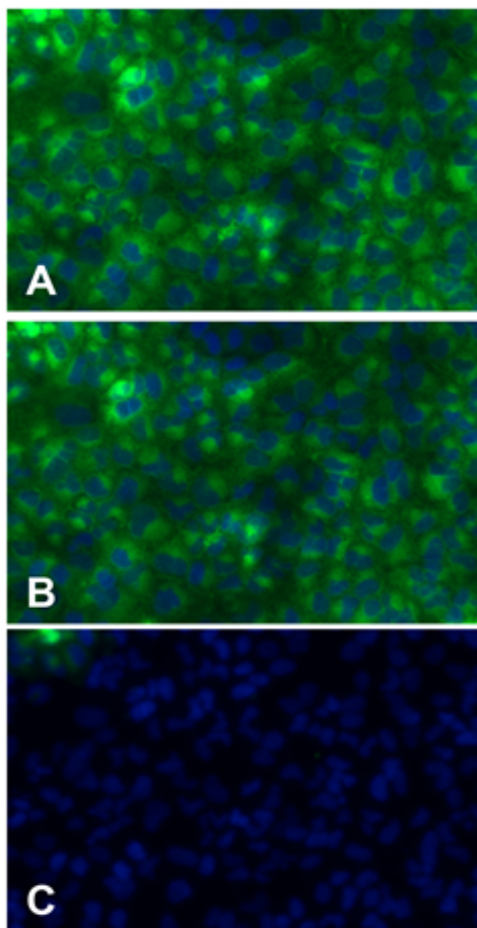
Figure 3-3. Fluorescence microscopy images of human lung cancer (A549) cells

treated with **4** for 1 hr, then exposed to visible light (X-Cite 120 LED light source

(Lumen Dynamics) with a 120-watt lamp used at 18% power (~4 x 10^{16}

photons/sec) and 38HE filter) for a) 30 sec, b) 3 min, and c) 10 min.[18] Pictures

represent overlay images for fluorescence detection of **4** (green) and the nuclear

Hoechst stain (blue). Loss of fluorescence with increasing length of exposure to

visible light is consistent with photoinduced CO release from **4**. See Figure B-12

for separate images of each detection channel and the complete field of view observed.

environment-sensitive probes in biological systems.[20] However, neutral flavonols of this type have *not* been previously shown to exhibit photoinduced CO-releasing reactivity. Flavothiones, have been reported to undergo O_2-dependent photodegradation to give non-toxic byproducts, but these reactions have *not* been fully explored in terms of product identification.[21] Molecules **6**-**8** (Figure 3-4) were easily prepared using standard synthetic methods and were isolated in analytically pure forms via precipitation. Each compound was characterized by elemental analysis, UV-vis, fluorescence, IR, and mass spectrometry (Figure B-13 – B-21). These molecules exhibit red-shifted absorption features and higher molar absorptivity values than were observed for **4** (Figure 3-4). Quantitative CO release occurs when aerobic CH_3CN solutions of **6**-**8** are exposed to visible light (**6,7**: 419 nm; **8**: >546 nm) (Table 3-1). For **6** and **7**, O_2-incorporated organic products akin to that found in the reaction of **4** were identified by ^{1}H NMR, IR, and mass spectral analysis (Figures B-22 – B-26). The quantum yield associated with the reaction of **7** is significantly enhanced relative to that found for **4**. The reaction involving **8** is noteworthy in that, while a full equivalent of CO is released under aerobic conditions, significant light-induced CO release reactivity (0.32(7) eq) also occurs under anaerobic conditions. Both the aerobic and anaerobic pathways for CO release from **8** result in the production of a mixture of products, including some that appear to result from photoisomerization reactivity (Figures

B-27 – B28). Photoinduced CO-release also occurs when **6-8** are dissolved in
other solvents, including DMSO and 1:1 H_2O:DMSO.
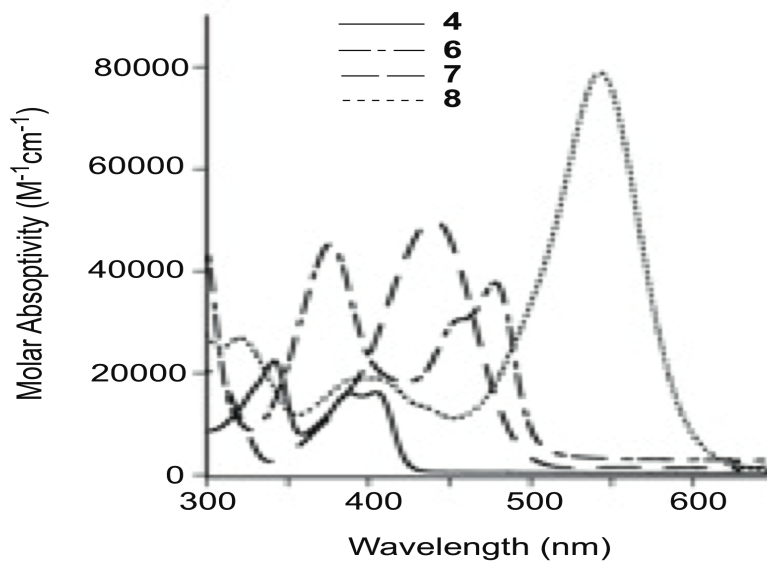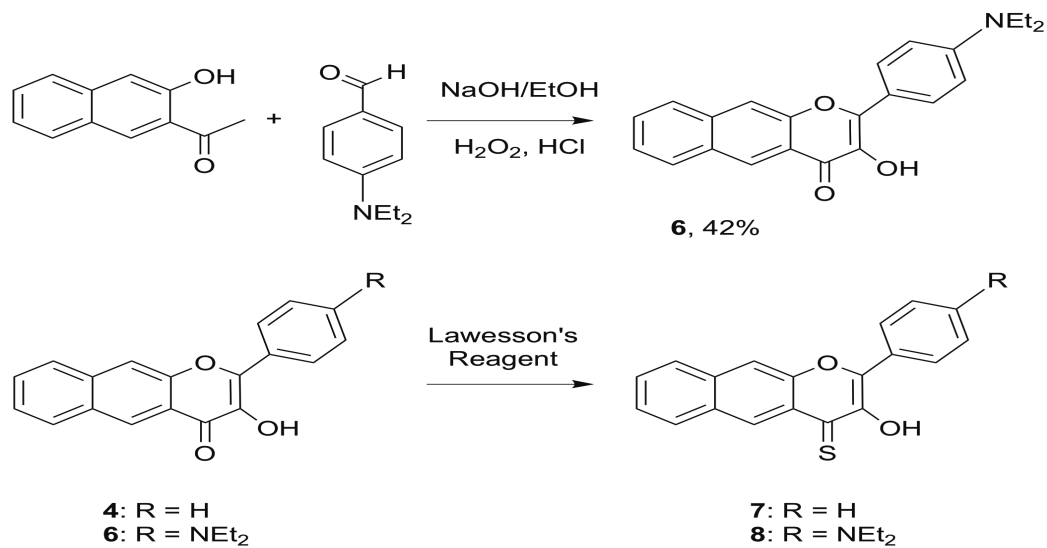
Figure 3-4. (top) Synthetic procedures for **6-8**. (bottom) Absorption spectra of **4**
and **6-8** in CH_3CN.

Table 3-1. CO quantification and quantum yields for the reactions of **4** and **6-8** with O_2 upon illumination with visible light in CH_3CN.

Compound	eq. CO[c]	Φ[c]
4[a]	0.96(2)	0.007(3)
6[a]	0.99(1)	0.006(1)
7[a]	1.00(1)	0.426(3)
8[b]	1.00(1)	[d]
8[b]	0.32(7)[e]	[d]

[a] Measured using 419 nm light. [b] Measured using >546 nm light. [c]Reported values are the average of three independent trials. [d]Not reported due to mixture of products. [e]Anaerobic conditions.

Conclusion

The use of a 3-hydroxyflavone-based structural motif offers many advantages in terms of photoCORM design. Compounds of this type can be prepared and isolated in analytically pure form using simple organic chemistry. Compounds **4** and **6-8** are soluble in organic solvents as well as aqueous DMSO. Solutions of these compounds are stable with respect to O_2 for weeks when protected from light. CO release is triggered under aerobic conditions by the introduction of visible light, the wavelength of which can be tuned through structural modification of the molecule. A representative example of this family of compounds (**4**) exhibits minimal toxicity and its organic byproduct following CO release is non-toxic. The fluorescent nature of **4** and analogs makes these molecules trackable in cells up to the point of CO-release. The observed

photoinduced reactivity of **6-8** demonstrates that structural modifications can be made without loss of the CO-release reactivity, and that both aerobic and anaerobic CO-release reaction pathways can be accessed. Overall, this family of compounds thus meets key criteria set forth for next generation photoCORMs. Further evaluation of the applications of these novel molecules and analogs for CO-release in biological systems are in progress.

Experimental Section

Chemicals and Reagents

All chemicals and reagents were obtained from commercial sources and used as received unless otherwise noted. Anaerobic procedures were performed under N_2 in a VAC Atmosphere glovebox. Solvents for glovebox use were dried according to published methods and distilled under N_2.[22]

Physical Methods

^{1}H and ^{13}C{^{1}H} NMR spectra (in ppm) were collected using JEOL ECX-300 MHz spectrometer and are referenced to the residual solvent peak in $CDCl_3$ (^{1}H: 7.26 (singlet) ppm; ^{13}C 77.16 (singlet) ppm). *J* values are given in Hz. IR spectra were collected using a Shimadzu FTIR-8400 spectrometer. UV-vis spectra were recorded at ambient temperature using a Hewlett-Packard 8453A

diode array spectrophotometer. Fluorescence emission spectra were collected

using a Shimadzu RF-530XPC spectrometer in the range of 400-800 nm, with

the excitation wavelength corresponding to the absorption maxima of the

molecules. The excitation and emission slit widths were set at 1.5 nm for all

molecules. Mass spectral data was collected at the Mass Spectrometry Facility,

University of California, Riverside. ESI/APCI mass spectra were recorded on an

Agilent LCTOF (2006) with a Windows XP based operating system. Elemental

analyses were performed by Atlantic Microlab, Inc., Norcross, GA, using a

PE2400 automatic analyzer. A Rayonett photoreactor equipped with either RPR-

4190A or white light lamps with 546 nm cutoff filters were used for all

photochemical reactions. Quantum yields were determined using potassium

ferrioxalate or potassium reinekate as standards to measure photon flux.[23]

Carbon monoxide was quantified as previously described.[24]

1-(3-hydroxynaphthalen-2-yl)ethenone. 2-hydroxynaphthoic acid

(1.8818 g, 10 mmol) was dissolved in dry, freshly distilled THF (40 mL). This

solution was purged with N_2 and subsequently cooled to 0 °C using an ice bath.

Methyllithium (30 mmol, 18.7 mL, 1.6 M in hexanes) was then slowly added in

aliquots via air-tight syringe. The reaction was allowed to stir at 0 °C for 3 hours

and then quenched with 0.5 M HCl dropwise until any frothing ceased. The THF

was then removed under reduced pressure and 0.5 M HCl (60 mL) was added to

the residue. The acidic aqueous solution was then extracted using

dichloromethane (50 mL x 3). The combined organic fractions were dried over

sodium sulfate, filtered, and the solvent was then removed under reduced

pressure yielding the product as a bright yellow solid (1.84 g, 99%). ^{1}H NMR

(CDCl$_3$, 300 MHz) δ 11.54 (s, 1H), 8.35 (s, 1H), 7.79 (d, J = 6.0 Hz, 1H), 7.65 (d,

J = 6.0 Hz, 1H), 7.50 (t, J = 5.1 Hz, 1H), 7.32 (t, J = 5.1 Hz, 1H), 7.24 (s, 1H),

2.78 (s, 3H) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz) 204.9, 157.3, 138.4, 133.7, 129.8,

129.5, 127.0, 126.4, 124.2, 121.5, 112.4, 27.1 ppm (12 signals expected and

observed). Melting point 111-113 °C. The ^{13}C NMR and melting point data are

consistent with reported literature values.[25]

3-Hydroxy-2-phenyl-benzo[g]chromen-4-one (**4**). Sodium hydroxide (21

mL. 5M, 104 mmol) was added to a suspension of 1-(3-hydroxynaphthalen-2-

yl)ethanone (3.90 g, 21 mmol) in ethanol (60 mL) and allowed to stir for 30

minutes at room temperature. Benzaldehyde (2.14 mL, 21 mmol) was added to

the reaction and the resulting mixture was stirred for 5 hours resulting in the

formation of a dark red solution. The reaction was then cooled to 0 °C in an ice

bath, hydrogen peroxide (7.6 mL, 30%) was added drop-wise, and the resulting

mixture was stirred overnight while warming to room temperature. Acidification of

the solution to pH = 6.5 with 0.5 M HCl resulted in the formation of a bright yellow

precipitate which was isolated by filtration and washed with ethanol (3.74 g,

62%). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.88 (s, 1H), 8.35 (d, J = 5.4 Hz, 2H), 8.08 (t,

J = 3.6 Hz, 2H), 7.95 (d, J = 6.3 Hz, 1H), 7.67 – 7.45 (m, 5H), 6.93 (s, 1H) ppm.

^{13}C NMR (CDCl$_3$, 100 MHz) 174.6, 151.7, 145.8, 137.3, 136.1, 131.4, 130.5,

130.0, 129.6, 129.0, 128.8, 128.2, 127.4, 126.7, 126.0, 120.1, 114.4 ppm (17

signals expected and observed). FTIR (KBr, cm^{-1}) 3290 (v_{O-H}), 1596 ($v_{C=O}$). UV-vis (CH$_3$CN, nm) (ε, M^{-1}cm^{-1}) 409 (16,600), 392 (15,900), 345 (22,800). Melting point 208-210 °C. Anal. Calc. C$_{19}$H$_{12}$O$_3$: C, 79.16; H, 4.20. Found: C, 78.96; H, 4.23. ESI/APCI-MS (relative intensity) calcd. for C$_{19}$H$_{13}$O$_3$ [MH]$^+$: 289.0859; found: 289.0866 (100%).

Photoreactivity of 4 in the presence of O$_2$. Production of 3-(benzoyloxy)-2-naphthoic acid (5). A solution of **4** (~0.05 mmol) in 5.0 mL CH$_3$CN was placed in a 50 mL round bottom flask under air. The solution was then placed in a Rayonette photoreactor equipped with 419 nm lamps and was irradiated until the reaction was determined complete as evidenced by the disappearance of the lowest energy absorption band. The solvent was then removed under reduced pressure yielding an off-white solid (100%). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.73 (s, 1H), 8.25 (d, *J* = 7.5, 2H), 7.98 (d, *J* = 7.8, 1H), 7.86 (d, *J* = 7.8, 1H), 7.72 - 7.46 (m, 6H) ppm. FT-IR (KBr, cm^{-1}) 1743 ($v_{C=O}$). ESI/APCI-MS (relative intensity) calcd. for C$_{18}$H$_{13}$O$_4$ [MH]$^+$: 293.0808; found: 293.0811 (83%).

^{18}O-labeling studies. Aliquots of ^{18}O$_2$ were transferred into a frozen CH$_3$CN solution of **4** in a 100 mL solvent transfer flask followed by irradiation at 419 nm and removal of the solvent under reduced pressure. ESI/APCI-MS (relative intensity) calcd. for C$_{18}$H$_{13}$O$_2$^{18}O$_2$ [MH]$^+$: 297.0892; found: 297.0899 (18%).

Dark Control Reaction. A solution of **4** in CD_3CN (~3 mM) was prepared in air under minimal red light and placed in an NMR tube. The NMR tube was then covered with foil, placed in a photo reactor, and irradiated using 419 nm lamps for 24 hours. Evaluation of the solution by ^1H NMR indicated that no reaction had occurred.

Anaerobic Control Reaction. A solution of **4** in CD_3CN (~3 mM) was prepared under N_2 in a glove box and placed in an NMR tube. The NMR tube was then placed in a photo reactor and irradiated using 419 nm lamps for 24 hours. Evaluation of the solution by ^1H NMR indicated that no reaction had occurred.

Cell Culture and Viability Assays. Jurkat (JM) cells were maintained as previously described by Shorey *et al.*[26] A549 human adenocarcinoma cells (ATCC, Manassas, VA) cells were grown in DMEM/Ham's F-12 1:1 mixture medium supplemented with 10% charcoal-stripped, heat-inactivated fetal bovine serum (FBS; Caisson Laboratories, Logan, UT) in a humidified incubator at 37°C with 5% CO_2. Cytotoxicity was determined by the colorimetric MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoluim bromide] assay as previously described.[26] Briefly, 2.1 x 10^3 cells/ml for A549 cells or 1 x 10^5 cells/ml for Jurkat cells. Test compounds were prepared in DMSO, then added to culture media for a maximum final DMSO concentration not greater than 0.1% (v/v). After addition of test compounds at concentrations ranging from 80 nM to 100 µM, cells were incubated for 24 h in the dark, then treated with MTT to assess cell viability as

outlined previously.[26] All experiments were independently replicated three times, and each experimental treatment was performed in triplicate.

Fluorescence microscopy. A549 cells were maintained in culture as described above, then seeded into Millicell E-Z-Slide culture chambers (EMD Millipore, Billerica, MA) at an initial density of 7.5×10^4 cells/cm^3 and allowed to adhere to the chamber slides for 24 hr. The cells were then treated for 1 hr with the nuclear dye Hoechst 33342 (0.5% v/v) for 10 min, followed by three washes with plain culture media to remove residual dye. All of incubation and wash steps described below were performed under minimal red light. A 100 mM stock solution of compound **4** was prepared in DMSO and then diluted to a working concentration of 2 mM in culture media (DMEM/Ham's F-12). The compound **4** working stock was diluted again to a final concentration of 50 µM in the culture chamber media, and the cells were incubated for 1 hr. The culture chamber was then gently washed thrice with plain culture media. Cells were fixed with a 1:10 (v/v) solution of formaldehyde fixative (Immunochemistry Technologies, Bloomington, MN) and culture media for 5 min, then washed twice with plain culture media. Finally, cells were imaged using a Zeiss Axio Observer inverted microscope (Carl Zeiss Microscopy, Thornwood, NY) equipped with fluorescence detection. Images were acquired using a 10x objective with excitation λ of 450-490 nm (BP 470/40 filter) and emission λ of 500-550 nm (BP 525/50 filter) for detection of compound **4** in A549 cells following 30 sec, 3 min or 10 min exposure to visible light. Also, for localization of the Hoechst dye, a single

fluorescence image was acquired at excitation λ at 365 nm; emission λ of 420-470nm (BP 445/50 filter). Acquired images were universally adjusted to enhance contrast levels (same settings for all acquired images for each detection channel) using Adobe Photoshop CS6 (Adobe, San Jose, CA).

2-(4-(diethylamino)phenyl)-3-hydroxy-4H-benzo[g]chromen-4-one (**6**). Sodium hydroxide (4.0 mL, 5M, 20 mmol) was added to a suspension of 1-(3-hydroxynaphthalen-2-yl)ethanone (0.931 g, 5 mmol) in ethanol (14 mL) and the resulting mixture was allowed to stir for 30 minutes at room temperature. 4-diethylaminobenzaldehyde (0.886 g, 5 mmol) was added to the mixture and the solution stirred for 24 hours. The reaction mixture was then cooled to 0 °C in an ice bath, hydrogen peroxide (3 mL, 30%) added drop-wise, and the solution was stirred overnight. Acidification of the solution to pH = 6.5 with 0.5 M HCl caused the formation of an orange precipitate. The solid was filtered from solution and washed with ethanol   (0.754 g, 42% yield). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.83 (s, 1H), 8.27 (d, J = 9.27 Hz, 2H), 8.06 (d, J = 8.22 Hz, 1H), 8.02 (s, 1H), 7.93(d, J = 8.22 Hz, 1H), 7.60 (t, J = 7.89 Hz, 1H), 7.51 (t, J = 7.89 Hz, 1H), 6.9 – 6.76 (m, 3H), 3.48 (q, J = 7.2 Hz, 4H), 1.26 (t, J = 7.2 Hz, 6H) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz) 173.2, 151.7, 149.4, 147.9, 135.8, 135.7, 130.1, 129.6, 128.6, 127.4, 126.2, 125.8, 120.6, 117.6, 114.1, 111.2, 44.7, 12.8 ppm (19 signals expected and observed). FT-IR (KBr, cm^{-1}) 3270 (v_{O-H}), 1588 ($v_{C=O}$). UV-vis (CH$_3$CN), nm (ε, M^{-1}cm^{-1}) 442 (51,000), 301 (22,100). Melting point 191-192 °C. Anal. Calc. C$_{23}$H$_{21}$NO$_3$: C, 76.86; H, 5.89; N, 3.90. Found: C, 76.77; H, 5.80; N, 3.96.

ESI/APCI-MS (relative intensity) calcd. for $C_{23}H_{22}NO_3$ [MH]$^+$: 360.1594; found: 360.1594 (100%).

Synthesis of 7 and 8. Lawsson's Reagent (1.01 g, 2.5 mmol) was added to a solution of **4** or **6** in toluene (120 mL), thoroughly purged with N_2, and refluxed for 4 hours producing a dark red solution. The solution was cooled to room temperature, filtered, and the solvent removed under reduced. The residual solid was washed with methanol and hexanes yielding **7** (61% yield) or **8** (60% yield) as a dark orange and dark purple solid respectively.

3-Hydroxy-2-phenyl-4H-benzo[g]chromene-4-thione (7). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 9.15 (s, 1H), 8.61 (bs, 1H), 8.49 (d, J = 8.1 Hz, 2H), 8.17-8.07 (m, 2H), 7.94 (d, J = 8.4 Hz, 1H), 7.70-7.45 (m, 5H) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz) δ 189.7, 147.5, 145.4, 142.1, 135.8, 131.4, 131.2, 129.8, 129.6, 129.3, 129.1, 129.0, 127.3, 126.7, 126.4, 114.8 ppm (17 signals expected, 16 observed). UV-vis (CH$_3$CN), nm (ε, M^{-1}cm^{-1}) 478 (36,700), 456 (29,600), 376 (40,300). Melting Point 151-152 °C. Anal. Calc. $C_{19}H_{12}O_2S \cdot 0.1H_2O$: C, 74.54; H, 4.02. Found: C, 74.42; H, 4.09. The presence of 0.1 eq H_2O was confirmed by integration of the peak at 1.56 ppm in the ^{1}H NMR spectrum. ESI/APCI-MS (relative intensity) calcd. for $C_{19}H_{13}O_2S$ [MH]$^+$: 305.0631; found: 305.0638 (100%).

2-(4-(diethylamino)phenyl)-3-hydroxy-4H-benzo[g]chromene-4-thione (4). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 9.10 (s, 1H), 8.75 (bs, 1H), 8.46 (d, J = 9.0 Hz,

2H), 8.08 (d, J = 8.1 Hz, 1H), 8.03 (s, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.57 (t, J =

7.5 Hz, 1H), 7.49 (t, J = 7.5 Hz, 1H), 6.82 (d, J = 9.0, 2H), 3.49 (q, J = 7.2 Hz,

4H), 1.27 (t, J = 7.2 Hz, 6H) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz) 182.6, 150.3,

147.4, 145.0, 134.9, 131.8, 131.0, 129.6, 129.0, 128.3, 127.2, 126.4, 125.9,

116.8, 114.0, 111.5, 44.9, 12.8 ppm (19 signals expected, 18 observed). UV-vis

(CH$_3$CN), nm (ε, M^{-1}cm^{-1}) 544 (85,800), 400 (19,500), 320 (22,800). Melting

point: 176-177 °C. Anal. Calc. C$_{23}$H$_{21}$NO$_2$S: C, 73.57; H, 5.64; N, 3.73. Found:

C, 73.30; H, 5.79; N, 3.70. ESI/APCI-MS (relative intensity) calcd. for

C$_{23}$H$_{22}$NO$_2$S [MH]$^+$: 376.1366; found: 376.1364 (100%).

Photoreactivity of 6-8. Identification of 9-11. A solution of each

molecule **6, 7** and **8** (~0.05 mmol) in 5.0 mL CH$_3$CN was placed in a 50 mL round

bottom flask under air. The solution was then placed in a Rayonette photoreactor

equipped with lamps of appropriate wavelength (**6,7**: 419 nm lamps; **8**: white light

lamps with 546 nm cutoff filters) and irradiated until the reaction was determined

complete as evidenced by the loss of the lowest energy absorption band (409 -

544 nm). The solvent was then removed under reduced pressure yielding

molecules **9-11**.

3-((4-(diethylamino)benzoyl)oxy)-2-naphthoic acid (**9**). ^{1}H NMR

(CDCl$_3$, 300 MHz) 8.69 (s, 1H), 8.07 (d, J = 9.0 Hz, 2H), 7.96 (d, J = 8.4 Hz, 1H);

7.84 (d, J = 8.1 Hz, 1H), 7.68 (s, 1H), 7.61 (t, J = 8.4 Hz, 1H), 7.53 (t, J = 8.1 Hz,

1H), 6.70 (d, J = 9.0, 1H), 6.61 (d, J = 9.0 Hz, 1H), 3.27 (q, J = 6.9 Hz, 4H), 1.31

(t, J = 6.9 Hz, 6H) ppm. FT-IR (KBr, cm^{-1}) 1716 ($v_{C=O}$). ESI/APCI-MS (relative

intensity) calcd. for $C_{22}H_{22}NO_4$ [MH]$^+$: 364.1543; found: 364.1544 (100%).

3-(benzoyloxy)naphthalene-2-carbothioic-O-acid (**10**). ^{1}H NMR (CDCl$_3$,

300 MHz) 8.67 (s, 1H), 8.24 (d, J = 7.8 Hz, 2H), 7.99 (d, J = 8.7 Hz, 1H), 7.85 (d,

J = 7.8 Hz, 1H), 7.76 (s, 1H), 7.72 - 7.39 (m, 5H) ppm. FT-IR (KBr, cm^{-1}) 1704

($v_{C=O}$). ESI/APCI-MS (relative intensity) calcd. for $C_{36}H_{22}O_6S_2Na$ [2M+Na]$^+$:

637.0750; found: 637.0757 (37%).

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15. CCDC 1025106 contains the supplementary crystallographic data for this paper. These data may be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

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18. Light intensity for fluorescent microscope source with 38HE filter: 3.67 x 10^{16} photons/sec. This is similar to the photon flux (1.58 x 10^{17} photos/sec) delivered by the Rayonet photoreactor used in the CO-release studies of **4**. The absorption spectrum of the fluorescence microscope source under the conditions described above overlaps with the low energy portion of the visible absorption band of **4**. Notably, a solution of **4** in 1:1 DMSO:H_2O was found to undergo visible light-induced CO-release reactivity upon illumination with the fluorescence microscope source under identical conditions.

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CHAPTER 4

SOLUTION OR SOLID - IT DOESN'T MATTER: DIOXYGENASE-TYPE VISIBLE

LIGHT-INDUCED CO RELEASE CHEMISTRY OF ZINC FLAVONOLATO

COMPLEXES

Abstract

A family of zinc flavonolato complexes, $[(6\text{-}Ph_2TPA)Zn(\text{flavonolato})]X$, of

four extended flavonols has been prepared, characterized, and evaluated for

visible light-induced CO release reactivity. Zinc coordination of each flavonolato

anion results in a red-shift of the lowest energy absorption feature and in some

cases enhanced molar absoptivity. The zinc-coordinated flavonolato ligands

undergo visible light-induced CO release with enhanced reaction quantum yields

relative to the neutral flavonols. Most notable is the disovery that zinc flavonolato

derivatives undergo similar visible light-induced CO release reactivity in solution

and in the solid state. This discovery suggests the possible application of solid

zinc flavonolato species in applications requiring controlled, light-triggered CO

release in biological and synthetic applications.

Introduction

Delivery of controlled amounts of carbon monoxide (CO) is of significant

current interest both in biology and synthetic chemistry. In humans, CO is

produced endogenously via the enzyme-catalyzed breakdown of heme.[1] Studies

of the effects of CO have demonstrated its potential to produce a variety of beneficial health effects including anti-inflammatory, anti-apoptotic, vasodilation, and anti-bacterial effects.[2,3] To explore the biology of CO and its possible uses as a therapeutic, CO-releasing molecules (CORMs) have been developed for the delivery of controlled amounts of CO.[4] To date, the majority of CORMs investigated have been metal carbonyl compounds (MCCs), with the most extensively studied being [Ru(CO)$_3$(glycinate)Cl] (CORM-3).[5] This compound coordinates to histidine residues of proteins and spontaneously releases CO via ligand exchange.[6-8] Other MCC CORMs release CO via enzyme-induced reactivity[9,10], magnetic heating[11], or via visible light-induced reactivity (photoCORMs).[12-14] CORMs that can be controlled in terms of their CO release reactivity are especially attractive as they offer the opportunity for localized release of CO at specific sites for biological studies or therapeutic purposes.

To address issues of solubility and concerns regarding the use of redox-active and heavy metals in MCCs, efforts to encapsulate these compounds in micelles, dendrimers, and solid supports have been reported.[11,15-19] Efforts to develop metal-free organic photoCORMs have also advanced.[20-23] The development of organic photoCORMs holds significant promise as standard approaches employed in medicinal chemistry for tuning and targeting could be possible.[24-26]

CO-releasing molecules are also of significant current interest for applications in synthetic organic chemistry. For example, because of the health hazards of handling CO gas, efforts in recent years have focused on the

development of molecules that release CO on demand for synthetic applications, such as palladium-catalyzed carbonylation reactions.[27-32] The majority of the CO-release compounds currently being used in synthetic organic applications either are used in an *ex situ* fashion or are used *in situ* but require the introduction of additional reagents, microwave irradiation, or heating to induce CO release.

An area that has seen little development is that of solid-state coordination compounds capable of visible light-induced CO release. Such compounds offer the possibility of CO release materials with no soluble byproducts, which could have tremendous advantages for both biological and synthetic applications. Mascharak recently reported two visible light induced MCC photoCORMs that are reported to undergo solid-state CO release.[33] However, the quantity of CO released and the other products of this reactivity have not been reported.

Our laboratory is developing CO release compounds based on the 3-hydroxy-4-flavone moiety found in naturally occurring flavonols. We recently reported a new family of flavonol derivatives (**1-4**, Figure 4-1) that undergo visible light-induced quantitative CO release when dissolved in organic solvents or organic/aqueous mixtures in the presence of O_2.[34] As shown in Table 4-1, this class of compounds can be structurally tuned to undergo quantitative CO release under aerobic or anaerobic conditions. These flavonol derivatives are fluorescent and therefore are trackable in cells. Compound **1** has been demonstrated to penetrate cells and then undergo CO release upon exposure to visible light. The structural framework in **1-4** also offers the opportunity for chelation to a metal
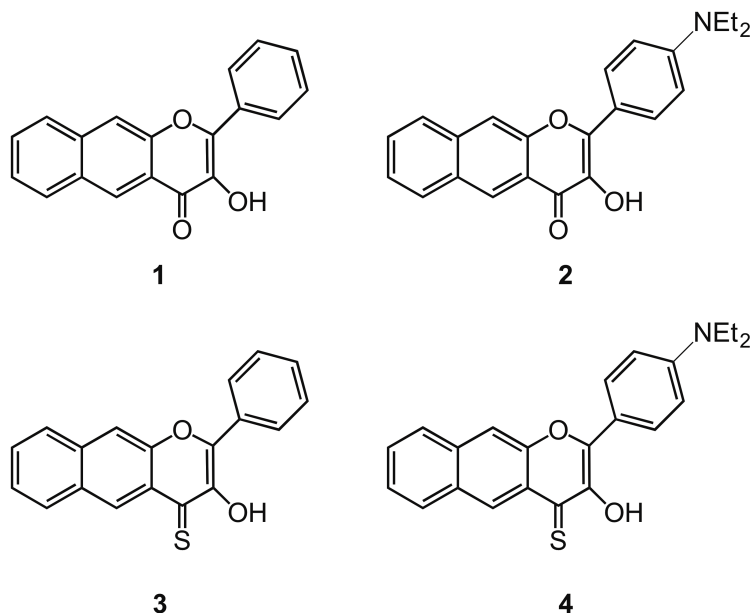
Fig 4-1 Structures of flavonol-based CO releasing molecules.

Table 4-1 Properties of **1-4**.

Compound	λ_{max} (nm)[a] ε (M^{-1}cm^{-1})	Aerobic CO- release[b]	Φ^c	Anaerobic CO-release
1	409 (16,600)	Yes	0.007(3)	No
2	442 (51,000)	Yes	0.006(1)	No
3	478 (36,700)	Yes	0.426(3)	No
4	544 (85,500)	Yes	*d*	Yes

[a]Most red-shifted absorption feature. [b]Measured in acetonitrile in the presence of air. [c]Average of three independent trials; values in parenthesis represent standard error. [d]Quantum yield for CO release not reported due to concurrent aerobic and anaerobic CO release reactions.

center as a means of tuning the reactivity of the CO-releasing moiety. In the studies reported herein, we have used a structural template from our laboratory that has been employed in studies of other metal flavonolato compounds[35-37] to evaluate how zinc stabilization of **1-4** affects their CO-release reactivity. Key findings include that zinc coordination: (1) red shifts the absorption spectral features of the compounds, with some being in the therapeutic window (>650 nm), (2) significantly enhances the quantum yield for visible-light induced CO release, and (3) enables solid-state CO release activity that is identical to the reactivity seen in solution. The discovery of light-induced solid-state release reactivity is particularly significant as it suggests that solid-state CO-release materials based on a zinc flavonolato motif can be developed. Overall, our results suggest that the structural motif in **1-4** is a highly versatile CO release unit that can be developed for various CO release applications.

Experimental Section

General

Chemicals and reagents. All chemicals and reagents were obtained from commercial sources and used as received unless otherwise noted. The synthesis of the extended flavonols **1-4** proceeded as previously reported.[34] The 6-Ph$_2$TPA (*N,N*-bis(((6-phenyl-2-pyridyl)methyl)-*N*-((2-pyridyl)methyl)amine*)* ligand was prepared and purified as previously described.[35]

Physical Methods. Anaerobic procedures were performed in a Vacuum Atmospheres glovebox under N_2. Solvents for glovebox use were dried according to published methods and distilled under N_2.[36] UV-vis spectra were recorded at room temperature using a Hewlett-Packard 8453A diode array spectrophotometer. Fluorescence emission spectra were collected using a Shimadzu RF-530XPC spectrometer in the range of 400-800 nm with the excitation wavelength corresponding to the most red-shifted absorption maximum of the compound. Infrared spectra were recorded using a Shimadzu FTIR-8400 as KBr pellets. ^1H NMR spectra (in ppm) are referenced to the residual solvent peaks in acetonitrile-d_3 (^1H: 1.94 (quintet) ppm). *J* values are given in Hz. Mass spectral data was collected at the Mass Spectrometry Facility, University of California, Riverside. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, or by Robertson Microlit Laboratories, Ledgewood, NJ. Photochemical experiments were performed using a Luzchem photoreactor equipped with either RPR-4190A, RPR-5750A, or Sylvania cool white lamps. Carbon monoxide quantification was performed by gas chromatography as previously described.[37] Quantum yields were determined by ferrioxalate or potassium reineckate actinometry using an integrative analysis method.[38-40] The output measured during the quantum yield determination for **5** was 1.46091 x 10^{17} photons/sec when illuminated with RPR-4190A lamps and for **6**, **7**, and **8** was 6.87824 x 10^{15} photons/sec when illuminated with white light lamps equipped with 546 nm cut off filters.

Caution! Perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts of material should be prepared, and these should be handled with great care.[42]

Synthesis of 5-8. In a N_2-filled glovebox, a methanolic solution (2 mL) of $Zn(ClO_4)_2 \cdot 6H_2O$ (0.0500 g, 0.134 mmol) was added to solid 6-Ph_2TPA (0.0594 g, 0.134 mmol) and the resulting mixture was stirred until all of the chelate ligand dissolved. The solution was then added to a methanolic solution (2 mL) containing **1**, **2**, **3**, or **4** (0.134 mmol) and $Me_4NOH \cdot 5H_2O$ (0.0243 g, 0.134 mmol). The mixture was allowed to stir for 4 hours at ambient temperature. The solvent was then removed under reduced pressure and the residual solid was dissolved in CH_2Cl_2. The solution was then filtered through a Celite/glasswool plug and the final product was precipitated by the addition of excess hexanes. Each solid product was dried under reduced pressure.

[(6-PhTPA)Zn(1⁻)]ClO₄ (5). Orange solid (89%). ^{1}H NMR (CD₃CN, 300 MHz) δ 8.62 (*d*, *J* = 5.1, 1H), 8.27 (*t*, *J* = 1.5, 1H), 8.25 (*t*, *J* = 1.5, 1H), 8.21 (d, J = 21.6, 2H), 8.03 (m, 2H), 7.96 (*t*, *J* = 7.5, 1H), 7.78 (*t*, *J* = 7.8, 2H), 7.66 - 7.37 (m, 9H), 7.33 (d, *J* = 7.8, 2H), 7.16 - 6.88 (m, 10H), 4.92 (d, *J* = 14.7, 2H), 4.57 (d, *J* = 14.7, 2H), 4.43 (s, 2H) ppm. FTIR (KBr, cm⁻¹) 1094 (v_{ClO4}), 622 (v_{ClO4}). UV-vis (CH₃CN) nm (ε, M⁻¹cm⁻¹) 480 (6300), 384 (1700). ESI/APCI-MS, *m/z* (relative intensity) 793.2152, calc. 793.2157 ([M-ClO₄]⁺, 34%). Anal Calc. $C_{49}H_{37}N_4O_7ClZn \cdot 0.7CH_2Cl_2$: C, 62.56; H, 4.06; N, 5.87. Found: C, 62.62; H, 4.26; N, 6.16. The presence and quantity of dichloromethane in the solid sample was confirmed through integration of the CH_2Cl_2 resonance in the ^{1}H NMR spectrum.

[(6-Ph₂TPA)Zn(2⁻)]ClO₄ (6).Orange-red solid (93%). ¹H NMR (CD₃CN,

300 MHz) δ 8.50 (d, *J* = 4.8 Hz, 1H), 8.31 (d, *J* = 9.3 Hz, 2H), 8.22 (s, 1H), 8.09

(s, 1H), 8.05 - 7.93 (m, 2H), 7.85 (t, *J* = 7.8 Hz, 1H), 7.78 (t, *J* = 7.5 Hz, 2H), 7.64

- 7.45 (m, 4H), 7.44 - 7.34 (m, 3H), 7.31 - 7.21 (m, 5H), 7.11 (t, *J* = 7.5 Hz, 2H),

7.01 (t *J* = 7.5 Hz, 4H), 6.80 (d, *J* = 9.0 Hz, 2H), 4.93 (d, *J* = 14.4 Hz, 2H), 4.54

(d, *J* = 14.4 Hz, 2H), 4.37 (s, 2H), 3.51 (q, *J* = 7.2 Hz, 4H), 1.24 (t, *J* = 7.2 Hz,

6H) ppm. FT-IR (KBr, cm⁻¹) 1088 (v_{ClO4}), 618 (v_{ClO4}). UV-vis (CH₃CN) nm (ε, M⁻¹cm⁻¹) 524 (80,000). ESI/APCI-MS, *m/z* (relative intensity) 864.2887, calc.

864.2882 ([M-ClO₄]⁺, 7%). Anal Calc. $C_{53}H_{46}N_5O_7ClZn \cdot H_2O$: C, 64.70; H, 4.92; N,

7.12. Found: C, 64.84; H, 4.63; N, 6.52. The presence and quantity of water in

the elemental analysis sample was confirmed through integration of the H₂O

resonance in the ¹H NMR spectrum.

[(6-Ph₂TPA)Zn(3⁻)]ClO₄ (7).Dark red solid (93%). ¹H NMR (CD₃CN, 300

MHz) δ 8.80 (s, 1H), 8.47 - 8.39 (m, 2H), 8.38 - 8.30 (m, 2H), 8.13 (d, *J* = 9.0 Hz,

1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.85 (t *J* = 7.8 Hz, 1H), 7.79 (t, *J* = 7.8 Hz, 2H),

7.64 (t, *J* = 6.6 Hz, 1H), 7.60 - 7.52 (m, 4H), 7.43 (t, *J* = 6.6 Hz, 5H), 7.28 (d, *J* =

6.9 Hz, 5H), 7.08 (t, *J* = 7.2 Hz, 2H), 6.99 (t, *J* = 7.2 Hz, 4H), 4.82 (bd, *J* = 14.4

Hz, 2H), 4.49 (bd, *J* = 14.4 Hz, 2H), 4.41 (s, 2H) ppm. FT-IR (KBr, cm⁻¹) 1086

(v_{ClO4}), 619 (v_{ClO4}). UV-vis (CH₃CN) nm (ε, M⁻¹cm⁻¹) 550 (9,300), 396 (7,200).

ESI/APCI-MS, *m/z* (relative intensity) 809.1930, calc. 809.1923 ([M-ClO₄]⁺, 25%).

Anal Calc. $C_{49}H_{37}N_4O_6ClSZn \cdot 0.5H_2O \cdot 1CH_2Cl_2$: C, 59.77; H, 4.01; N, 5.58.

Found: C, 59.29; H, 3.51; N, 5.57. The presence and quantity of water and

dichloromethane in the elemental analysis sample was confirmed through integration of the respective resonances in the ^{1}H NMR spectrum.

[(6-Ph$_2$TPA)Zn(4$^-$)]ClO$_4$ (8).Dark blue solid (91%). ^{1}H NMR (CD$_3$CN, 300 MHz) δ 8.69 (s, 1H), 8.58 (d, J = 9.3 Hz, 2H), 8.43 (d, J = 5.4 Hz, 1H), 8.21 (s, 1H), 8.10 (d, J = 8.1 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.89 - 7.77 (m, 3H), 7.64 - 7.42 (m, 10H), 7.38 (t, J = 7.2 Hz, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.24 - 7.17 (m, 2H), 7.16 - 7.07 (m, 4H), 6.86 (d, J = 9.3 Hz, 2H), 4.83 (d, J = 13.8 Hz, 2H), 4.49 (d, J = 13.8 Hz, 2H), 4.41 (s, 2H), 3.54 (q, J = 7.2 Hz, 4H), 1.23 (t, J = 7.2 Hz, 6H) ppm. FT-IR (KBr, cm^{-1}) 1084 (v_{ClO4}), 622 (v_{ClO4}). UV-vis (CH$_3$CN) nm (ε, M^{-1}cm^{-1}) 600 (111,700). ESI/APCI-MS, m/z (relative intensity) 880.2659, calc. 880.2658 ([M-ClO$_4$]$^+$, 15%). Anal Calc. C$_{53}$H$_{46}$N$_5$O$_6$ClSZn·H$_2$O: C, 63.67; H, 4.84; N, 7.00. Found: C, 63.37; H, 4.57; N, 6.75. The presence and quantity of water in the elemental analysis sample was confirmed through integration of the H$_2$O resonance in the ^{1}H NMR spectrum.

Photoinduced Reactivity of 5-8 in CH$_3$CN. Illumination of aerobic CH$_3$CN solutions of **5-8** using visible light (**5**: 419 nm; **6**, **7**, **8**: >546 nm) results in the release of one equivalent of CO (Table 4-2) and the formation of the carboxylate derivatives [(6-Ph$_2$TPA)Zn(carboxylate)]ClO$_4$ (**9-12**) which were characterized by ^{1}H NMR, IR, and ESI/MS.

[(6-Ph$_2$TPA)Zn(((3-benzoyl)oxy)-2-naphthanoate)]ClO$_4$. (9). ^{1}H NMR (CD$_3$CN, 300 MHz) δ 8.22 (d, J = 4.5 Hz, 1H), 8.14-7.99 (m, 3H), 7.93-7.78 (m, 4H), 7.66 (d, J = 7.5 Hz, 2H), 7.64-7.51 (m, 6H), 7.50 - 7.37 (m, 5H), 7.34-7.23 (m, 2H), 7.20-7.05 (m, 9H), 4.58-4.26 (m, 6H) ppm. FT-IR (KBr, cm^{-1}) 1724

($\nu_{C=O}$). ESI/APCI-MS, *m/z* (relative intensity), 797.2113, calc. 797.2201 ([M-

ClO$_4$]$^+$, 35%).

Table 4-2 Properties of **5-8**.

Compound	λ_{max} (nm)^a ε (M^{-1}cm^{-1})	Solution CO-release (eq CO)^b	Φ^c	Solid-state CO release (eq CO)^f
5	480 (6300)	0.94(4)	0.651(2)^d	0.96(3)
6	524 (80,000)	0.98(1)	0.583(4)^e	0.93(2)
7	550 (9300)	0.92(3)	0.951(4)^e	0.97(1)
8	600 (111,700)	0.97(2)	0.947(7)^e	0.94(1)

[(6-Ph₂TPA)Zn(3-((4-(diethylamino)benzoyl)oxy)-2-naphthoate)]ClO₄

(**10**). ^{1}H NMR (CD$_3$CN, 300 MHz) δ 8.24 (d, *J* = 5.4 Hz, 1H), 8.15-7.97 (m, 3H),

7.88-7.77 (m, 2H), 7.68 (t, *J* = 9.0 Hz, 4H), 7.61-7.50 (m, 4H), 7.49-7.39 (m, 3H),

7.34-7.24 (m, 2H), 7.21 - 6.84 (m, 9H), 6.60 (d, *J* = 9.0 Hz, 2H), 4.65-4.27 (m,

6H), 3.41 (q, *J* = 7.2 Hz, 4H), 1.15 (t, *J* = 7.2 Hz, 6H) ppm. FT-IR (KBr, cm^{-1})

1722 ($\nu_{C=O}$). ESI/APCI-MS, *m/z* (relative intensity), 868.2870, calc. 868.2836 ([M-

ClO$_4$]$^+$, 3%).

[(6-Ph₂TPA)Zn(3-((benzoyloxy)naphthalene-2-carbothiolate)]ClO₄ (**11**).

^{1}H NMR (CD$_3$CN, 300 MHz) 8.46 (d, *J* = 5.4 Hz, 1H), 8.15-7.84 (m, 6H), 7.83-

7.74 (m, 3H), 7.70 (t, *J* = 7.5 Hz, 1H), 7.65-7.32 (m, 13H), 7.27 (t, *J* = 7.8 Hz,

4H), 7.09 (d, J = 7.8 Hz, 3H), 4.60-4.16 (m, 6H) ppm. FT-IR (KBr, cm^{-1}) 1742

($\nu_{C=O}$). ESI/APCI-MS, m/z (relative intensity), 813.1889, calc. 813.1872 ([M-

ClO$_4$]$^+$, 100%).

[(6-Ph$_2$TPA)Zn(3-((4-diethylamino)benoyl)oxy)naphthalene-2-

carbothiolate)]ClO$_4$ (12). ^{1}H NMR (CD$_3$CN, 300 MHz) 8.46 (d, J = 5.1 Hz, 1H),

8.00 - 7.88 (m, 4H), 7.85 - 7.75 (m, 2H), 7.65 (d, J = 9.3 Hz, 2H), 7.61 - 7.52 (m,

4H), 7.48 - 7.35 (m, 7H), 7.25 (t, J = 7.8 Hz, 4H), 7.10 (d, J = 7.5 Hz, 4H), 6.68

(d, J = 9.3 Hz, 2H), 4.62 - 4.28 (m, 6H), 3.51 (q, J = 6.9 Hz, 4H), 1.25 (t, J = 6.9

Hz, 6H) ppm. FT-IR (KBr, cm^{-1}) 1739 ($\nu_{C=O}$). ESI/APCI-MS, m/z (relative

intensity), 884.2641, calc. 884.2607 ([M-ClO$_4$]$^+$, 50%).

Control reactions. Solutions of **5-8** (~10 mM) in d_3-acetontirile were

prepared in air under minimal red light and placed in NMR tubes. The tubes were

then covered with foil and illuminated with white light for 24 hours. Evaluation of

each solution by ^{1}H NMR indicated that no reaction had occurred.

Anaerobic control reactions. Solutions of **5-8** (~10 mM) in d_3-acetontrile

were prepared in a Vacuum Atmospheres glovebox under N$_2$ and placed in NMR

tubes. The caps of the NMR tubes were wrapped securely with Parafilm and the

tubes were illuminated with white light lamps for 24 hours. Evaluation of the

samples by ^{1}H NMR indicated that no reaction had occurred.

CO release quantification of 5-8 in acetonitrile. 50 mL round bottom

flasks were loaded with **5-8** (ca. 10 mg) dissolved in acetonitrile (5.0 mL). The

flask was then sealed with a septum, purged with O$_2$ for 45 seconds, and

illuminated with white light for 24 hours resulting in bleaching of each solution to colorless. A sample (10 mL) of the headspace gas was then analyzed by gas chromatography and the area of the peak associated with CO applied to a calibration curve created specifically for reactions done in acetonitrile. The solvent was then removed under reduced pressure and the residual solid analyzed by ^{1}H NMR for completeness of reaction.

Photoinduced Reactivity of 5-8 as Solids. Compounds **5-8** (ca. 10 mg) were loaded into 50 mL round bottom flasks under O_2. Exposure of solid **5-8** to white light for 48 hours results in clean conversion to **9-12** as determined by ^{1}H NMR of the final product. Additionally, the color of the solid compounds changed to beige.

Results

The development of CO-releasing structural motifs that can be easily prepared and tuned via multiple strategies (e.g. organic synthesis and/or metal coordination) offers advantages toward the development of compounds that can be applied in biology and synthesis. Our approach focuses on the use of extended 3-hydroxy-4-pyrone based frameworks which are easily structurally modified to modulate spectroscopic and CO release properties. The presence of the 3-hydroxy-4-pyrone unit also offers the possibility of metal coordination. Compounds **5-8** were prepared via the approach shown in Scheme 4-1. Each was obtained in high yield as an analytically pure solid following precipitation from hexanes. Although X-ray quality crystals could not be obtained for a
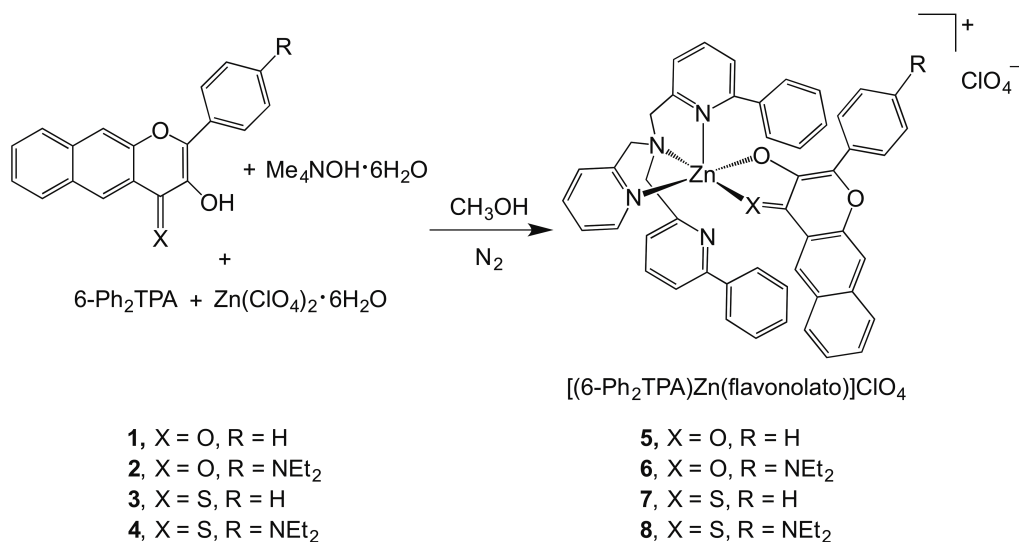
Scheme 4-1 Synthesis of **5-8**.

1, X = O, R = H	**5**, X = O, R = H
2, X = O, R = NEt₂	**6**, X = O, R = NEt₂
3, X = S, R = H	**7**, X = S, R = H
4, X = S, R = NEt₂	**8**, X = S, R = NEt₂

member of this family of compounds, the ^{1}H NMR spectral features of **5-8** (Figures C-1, C-6, C-11, and C-16) suggest a mononuclear pseudo-octahedral Zn(II) center with the flavonolato ligand positioned between the phenyl-appended pyridyl donors of the 6-Ph₂TPA ligand. Evidence for this type of structure is the ^{1}H NMR features of the benzylic hydrogens which are consistent with C_s (mirror plane) symmetry in the cation. When dissolved in CH_3CN, each compound exhibits a molecular ion consistent with the proposed mononuclear formulation (Figures C-5, C-10, C-15, and C-20). The outer sphere ClO_4^- is evidenced in the solid state IR spectra of the compounds (Figures C-2, C-7, C-12, and C-17).

Each compound **5-8** exhibits a ~60-90 nm red-shift for the lowest energy absorption feature relative to neutral **1-4** (Tables 4-1 and 4-2; Figures C-3, C-8, C-13, C-18). While **5** and **7** exhibit lower molar absorptivity values for the lowest

energy absorption band than the neutral flavonols, those of the NEt$_2$-substituted

6 and **8** are enhanced relative to the neutral flavonols. In terms of previously

reported photoCORMs, the molar absorptivity values of **6-8** at >500 nm exceed

those exhibited by molecular metal carbonyl photoCORMs that absorb above

500 nm.[12] *Most red-shifted absorption feature.*[b] Compound dissolved in

acetonitrile in the presence of O$_2$. CO quantification performed by GC after

illumination with 419 nm lamps (**5**) or white light lamps equipped with 546 nm cut

off filters (**6**, **7, 8**) for 24 h. Average CO release of three independent samples;

values in parentheses represent standard error. [c]Average of three independent

trials; values in parenthesis represent standard error. [d]Φ obtained for solution

(CH$_3$CN) CO release via illumination using 419 nm lamps and potassium

ferrioxalate actinometer. [e]Φ obtained for solution (CH$_3$CN) CO release via

illumination with white light lamps equipped with 546 nm cut off filters and

potassium reineckate actinometer. [f]Powdered compound in the presence of O$_2$.

CO quantification performed by GC after illumination with 419 nm light (**5**) or

white light lamps equipped with 546 nm cut off filters (**6**, **7, 8**) for 48 h. Average

CO release of two independent samples; values in parentheses represent

standard error.

Compounds **6** and **8** have molar absorptivity values at >500 nm that

exceed all organic photoCORMs[20-23], including recently reported BODIPY

derivatives.[20] Illumination into the lowest energy absorption band of **5-8** produces

a fluorescent emission with a Stokes shift of 30-72 nm (Figures C-4, C-9, S14,

and C-19). The sulfur-containing **7** and **8** exhibit smaller Stokes shifts relative to the oxygen analogs.

Photoinduced O$_2$ reactivity of 5-8 in CH$_3$CN

Illumination of CH$_3$CN solutions of **5-8** with visible light results in the release of one equivalent of CO and the clean formation of a single zinc carboxylate compound (**9-12**; Scheme 4-2) as determined by ^{1}H NMR, IR, and ESI-MS. The ^{1}H NMR spectra of **9-12** again exhibit features for the benzylic hydrogens consistent with C_s symmetry (Figures C-21, C-25, C-28, C-31). Infrared and ESI/APCI mass spectral data also supports to proposed formulation

5, X = O, R = H
6, X = O, R = NEt$_2$
7, X = S, R = H
8, X = S, R = NEt$_2$

9, X = O, R = H
10, X = O, R = NEt$_2$
11, X = S, R = H
12, X = S, R = NEt$_2$
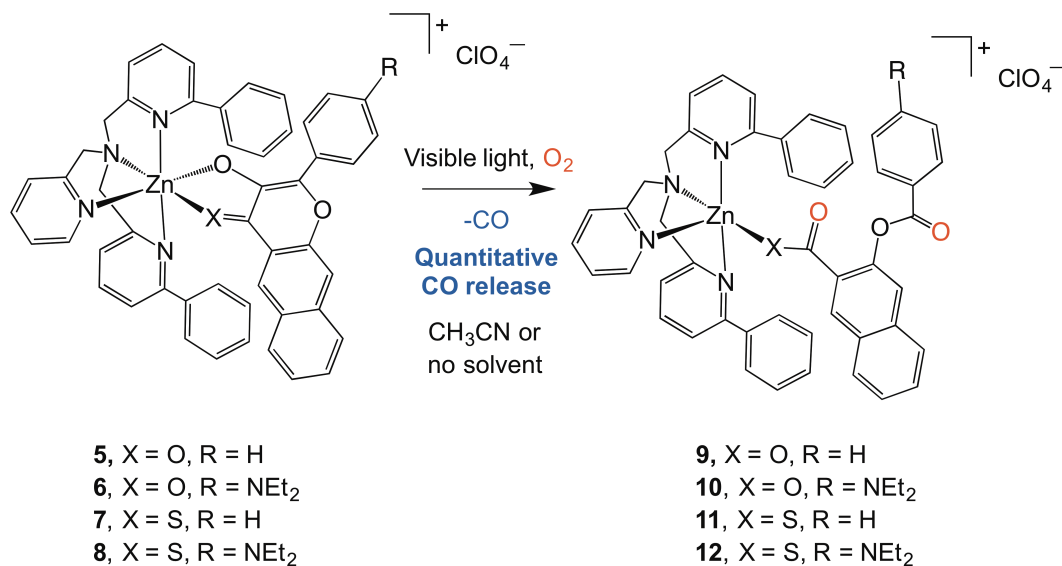
Scheme 4-2 Visible light induced CO release reactivity of **5-8** in CH$_3$CN or the solid state.

of mononuclear carboxylate compounds that have resulted from dioxygenase-type reactivity and CO-release (IR: Figures C-22, C-26, C-29, C-32; ESI/APCI: Figures C-23, C-27, C-30, C-33). The incorporation of two oxygen atoms from O_2 into the product is also evidenced by the reaction of **5** in the presence of $^{18}O_2$, which yields **9** containing two labeled oxygen atoms (ESI/APCI MS Figure C-24). The reaction quantum yields for CO-release from **9-12** (Table 4-2) in CH_3CN significantly exceed those exhibited by the neutral flavonols **5-7**, metal carbonyl photoCORMs[12], and all reported neutral organic photoCORMs[20-23].

Solid-state photoinduced CO release reactivity of 5-8

Light induced CO release compounds offer the possibility of control over the temporal and spatial release of CO. To date, compounds developed for biological and *in situ* synthetic applications have focused on soluble species. However, insoluble solid-state materials or compounds that release CO upon introduction of visible light could also be very useful in various applications including in implantable systems and for CO release within reaction mixtures. Visible and near infra-red light-driven NO-releasing solid-state materials (photoNORMs) have been previously developed for biological applications.[43-51] A current limitation in terms of photoCORM development is the almost complete lack of availability of compounds that exhibit solid state CO release reactivity upon illumination with visible light. Recently, Mascharak, et al. reported two Mn(I) tricarbonyl compounds that will release CO upon illumination of the crystals with

microscope light. However, these compounds were not characterized in terms of the amount of CO released or the products remaining following CO release.[33]

To our knowledge, solid-state light-induced CO release has not been previously reported for flavonol or metal flavonolato complexes.[52] Notably, we have discovered that exposure of powders of **5-8** to visible light in the presence of O_2 for 48 h results in quantitative CO release (Table 4-2). Analysis of the remaining beige powders following light illumination via ^{1}H NMR in CD_3CN indicates the clean formation of **9-12**. Thus, the same reactivity is observed for **5-8** both in solution and in the solid state. It is essential to note that the neutral flavonols **1-4** are unreactive as solids upon extended illumination (one week) with visible light. Both the neutral flavonols (**1-4**) and the zinc flavonolato derivatives (**5-8)** are also unreactive as solids under anaerobic conditions.

Conclusions

The dioxygenase-type CO release chemistry of metal complexes of 3-hydroxy-4-pyrone derivatives (flavonols) has received considerable attention due to its relevance to quercetin dioxygenase enzymes.[53-60] Our discovery of light-induced CO release chemistry for divalent metal flavonolato compounds[37,38] has spurred us to further evaluate the light-induced CO release reactivity of flavonol and flavonolato species for applications as photoCORMs. Construction of the extended 3-hydroxy-4-pyrone derivatives **1-4** led to the discovery that unlike neutral 3-hydroxyflavone[52], which undergoes multiple types of reactions upon illumination with UV light, the neutral extended flavonols undergo clean

dioxygenase-type visible-light induced CO release in the presence of O_2.[34] In this contribution we have evaluated how stabilizing the flavonolato anion of **1-4** via zinc coordination affects the CO release chemistry. Importantly, we have discovered that zinc coordination results in a red-shift of the absorption features, with **8** exhibiting a large absorption feature that extends into the therapeutic window (>650 nm). The reaction quantum yields for CO release are enhanced in the flavonolato derivatives relative to the neutral flavonols, in some cases by two orders of magnitude. Perhaps most importantly, the flavonolato derivatives exhibit CO release both in solution and in the solid state. Overall, these results demonstrate that 3-hydroxy-4-pyrone (flavonol) derivatives are tunable using both organic and inorganic approaches for the development of light-driven CO-releasing molecules. The ease with which structural modifications can be made positions this family of CO release molecules to contribute to various goals in understanding the biological effects of CO. Specifically, the tunability of the quantum yield in flavanol/flavonolato derivatives suggests that such compounds could be used to deliver various fluxes of CO under site and temporal control. The discovery of light-induced solid-state release reactivity in **9-12** also opens up the opportunity for the development of metal flavonolato-based solid-state CO-releasing materials that can be triggered using visible light. Such materials could be useful for antibacterial applications[61] and well as CO release in synthesis (e.g. carbonylation chemistry). We note that replacement of the 6-Ph$_2$TPA ligand with other supporting ligands dramatically affects the organic and aqueous solubility of the zinc flavonolato compounds while light-induced CO release reactivity is

retained. Studies of applications of various compounds of this class are currently in progress in our laboratory.

Acknowledgments

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CHAPTER 5

SOLUTION OR SOLID - IT DOESN'T MATTER: VISIBLE-LIGHT INDUCED CO

RELEASE REACTIVITY OF ZINC FLAVONOLATO COMPLEXES

Abstract

Zinc flavonolato complexes, $Zn(flavonolato)_2$, of four extended flavonols
have been prepared, characterized, and evaluated for visible light-induced CO
release reactivity. Zinc coordination of each flavonolato anion results in a red-
shift of the lowest energy absorption feature and in some cases enhanced molar
absorptivity relative to the free flavonol. The zinc-coordinated flavonolato ligands
undergo visible light-induced CO release producing 2 equivalents of CO per
equivalent of compound. Most notable is the discovery that zinc flavonolato
derivatives undergo similar visible light-induced CO release reactivity in solution
and in the solid state. A solid film of a $Zn(flavonolato)_2$ derivative was evaluated
as an *in situ* CO release agent for aerobic oxidative palladium-catalyzed
alkoxycarbonylation to produce esters in ethanol. The CO release product was
found to undergo ester alcolysis under the conditions of the carbonylation
reaction.

Introduction

Delivery of controlled amounts of carbon monoxide (CO) is of
significant current interest both in biology and synthetic chemistry. In

humans, CO is produced endogenously via the enzyme-catalyzed breakdown of heme.[1] Studies of the effects of CO have demonstrated its potential to produce a variety of beneficial health outcomes including anti-inflammatory, anti-apoptotic, vasodilation, and anti-bacterial effects.[2-3] To explore the biology of CO and its possible uses as a therapeutic, CO-releasing molecules (CORMs) have been developed for the delivery of controlled amounts of CO.[4] To date, the majority of CORMs investigated have been metal carbonyl compounds (MCCs), with the most extensively studied being $[Ru(CO)_3(glycinate)Cl]$ (CORM-3).[5] This compound coordinated to histidine residues of proteins and spontaneously releases CO via ligand exchange.[6-8] Other MCC CORMs release CO via enzyme-induced reactivity[9,10], magnetic heating[11], or via visible light-induced reactivity (photoCORMs).[12-14] CORMs that can be easily controlled in terms of their CO release reactivity are especially attractive as they offer the opportunity for localized release of CO at specific sites for biological studies or therapeutic purposes.

To address issues of solubility and concerns regarding the use of redox-active and heavy metals in MCCs, efforts to encapsulate these compounds in micelles, dendrimers, and solid supports have been reported.[11, 15-19] Efforts to develop metal-free organic photoCORMs have also advanced.[20-23] The development of organic photoCORMs holds significant promise as standard approaches employed in medical chemistry for tuning and targeting could be possible.[24-26]

CO-releasing molecules are also of significant current interest for applications in synthetic organic chemistry. Due to the health hazards of handling CO gas, efforts in recent years have focused on the development of molecules that release CO on demand for synthetic applications, such as palladium-catalyzed carbonylation reactions.[27-32] The majority of the CO release compounds currently being used in synthetic organic application wither are used in an *ex situ* fashion or are used *in situ* but require the introduction of additional reagents, microwave irradiation, or heating to induce CO release. *Ex situ* approaches require either the use of specialized two-chamber glassware to separate the CO-generation process from the substrate reaction chamber[27-31-,] or manual transfer of the gas (e.g. via a balloon).[32] *In situ* sources for CO have also been previously reported. These include metal carbonyls (e.g. $Mo(CO)_6$), which can serve as *in situ* CO release agents for palladium-catalyzed carbonylations, albeit not at a stoichiometric level with respect to CO.[33-43] When metal carbonyls are used, additional reagents, high temperatures and/or microwave assistance are generally needed for CO release. It is also important to note that low-valent metal carbonyls typically cannot be used *in situ* for substrates containing functional groups that can be reduced (e.g. $-NO_2$ derivatives) due to the reducing nature of the low-valent metal complex.[34-40, 44] Two chamber systems can be used to address this issue.[45] Formamides[46] (including *N*-formylsaccharin[47,48]), formats, and formic acid[49,50] derivatives, as well as $CHCl_3$[51], and diethylpyrocarbonate[52,53] have

been recently investigated as *in situ* CO release agents at near stoichiometric levels. However, all of these CO release compounds require either significant heat (formamides and formic acids, diethylpyrocarbonate), or the presence of additional reagents (e.g. *N*-formylsaccharin (mild base or KF); formats (mild base); formic acid (strongly acidic conditions); $CHCl_3$ (metal hydroxide) to induce CO release.

An area that has seen little development is that of solid compounds capable of visible light-induced CO release. Such compounds could offer the possibility of CO release materials that could have tremendous advantages for both biological and synthetic applications. Mascharak recently reported two visible light-induced MCC photoCORMs that undergo solid-state CO release.[54] However, the quantity of CO released and the byproducts of this reactivity have not been reported.

Our laboratory is developing visible light-induced CO release compounds based on the 3-hydroxy-4-flavone moiety found in naturally occurring flavonols. Prior to our work, metal flavonolato species were well known to undergo dioxygenase-type degradation to release CO via either enzyme catalysis[55] or thermal reactivity[56] involving O_2 activation. However, reports of light-driven CO release reactivity involving O_2 activation with either free or metal-coordinated flavonols were scarce.[57] We recently reported a new family of neutral flavonol derivatives (**1-4**, Figure 5-1) that
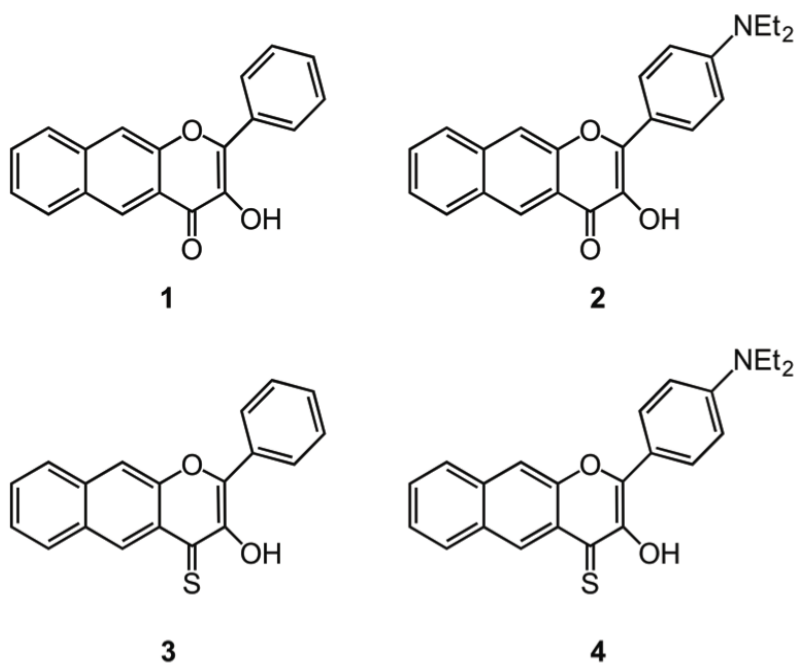
Fig 5-1 Structure of flavonol-based CO releasing molecules

undergo dioxygenase-type visible light-induced quantitative CO release

when dissolved in organic solvents or organic/aqueous mixtures.[58] As

shown in Table 5-1, this class of compounds can be tuned to undergo

quantitative CO release under aerobic or anaerobic conditions. These

flavonol derivatives are fluorescent and therefore trackable in cells.

Compound **1** has been demonstrated to penetrate cells and then undergo

quantitative CO release upon exposure to visible light. The structural

framework in **1-4** also offers the opportunity for chelation to a metal center

as a means of tuning the reactivity of the CO-releasing moiety. In the

studies reported herein, we have identified key findings related to zinc

Table 5-1 Properties of **1**-**4**.

Compound	λ_{max} (nm)[a] ε (M^{-1}cm^{-1})	Aerobic CO Release	Φ^c	Anaerobic CO Release
1	409 16,600	Yes	0.007(3)	No
2	442 51,000	Yes	0.006(1)	No
3	478 36,700	Yes	0.43(3)	No
4	544 85,500	Yes	*d*	Yes

[a]Most red-shifted absorption feature. [b]Measured in acetonitrile in the presence of air. [c] Average of three independent trials; values in parenthesis represent standard error. [d]Quantum yield for CO release not reported due to concurrent aerobic and anaerobic CO release reactions.

coordination. The absorption spectral features of the compounds red shift with some being in the therapeutic window (>650 nm) and solid-state CO release activity can occur that is identical to the reactivity seen in solution. The discovery of light-induced solid-state CO release reactivity is particularly significant as it suggests that CO release materials based on a zinc flavonolato motif could be developed. Building on this discovery, we sought to design zinc flavonolato derivatives that are insoluble in organic solvents, which could facilitate their use as *in situ* light-driven CO release agents in synthetic organic processes. Our experience with simple Zn(II) bis-flavonolato compounds (Zn(flavonolato)$_2$) suggested that such compounds could exhibit the desired minimal solubility.[59] Therefore

derivatives of this type using **1-4** were synthesized, characterized, and evaluated as solid-state CO release agents. As outlined herein, we have found that a member of this family can be used in aerobic oxidative palladium-catalyzed carbonylation processes that employ O_2 as the sole oxidant and are performed under very mild conditions.[62] Overall, our results suggest that the structural motif in **1-4** is a highly versatile CO release unit that can be developed for various CO release applications.

Experimental Section

General

Chemicals and Reagents

All chemicals and reagents were obtained from commercial sources and used as received unless otherwise noted. The synthesis of the extended flavonols **1-4** proceeded as previously reported.[58]

Physical Methods

Anaerobic procedures were performed in a Vacuum Atmospheres glovebox under N_2. Solvents for glovebox use were dried according to published methods and distilled under N_2.[64] UV-vis spectra were recorded at room temperature using a Hewlett-Packard 8453A diode array spectrophotometer. Fluorescence emission spectra were collected using a

Shimadzu RF-530XPC spectrometer in the range of 400-800 nm with the excitation wavelength corresponding to the most red-shifted absorption maximum of the compound. Infrared spectra were recorded using a Shimadzu FTIR-8400 as KBr pellets. ^{1}H NMR spectra (in ppm) are referenced to the residual solvent peaks in pyridine-d_5 (^{1}H: 7.22 (singlet) ppm). *J* values are given in Hz. Mass spectral data was collected at the Mass Spectrometry Facility, University of California, Riverside. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, or by Robertson Microlit Laboratories, Ledgewood, NJ. Photochemical experiments were performed using a Luzchem photoreactor equipped with RPR-4190A, RPR5750A, or Sylvania cool white lamps. Carbon monoxide quantification were performed by gas chromatography as previously described.[61]

General Synthetic Method for Zinc Bis(flavonolato) Compounds. A 500 mL round bottom flask, protected from light with aluminium foil, was charged with the flavonol (21 mmol), Me$_4$NOH·5H$_2$O (21 mmol), and MeOH (50 mL) and the resulting mixture was stirred at room temperature for 2 hours. Zinc triflate (10.3 mmol) was then transferred to the flask using MeOH (15 mL). The mixture was stirred overnight after which time the product was collected by filtration, washed with water and dried *in vacuo*.

Zn(1⁻)$_2$·4H$_2$O (5). 88% yield. Orange solid. ^{1}H NMR (pyridine-d_5, 300 MHz) 9.35 (d, *J* = 7.8 Hz, 4H), 8.86 (s, 2H), 8.07 (s, 2H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.80-7.77 (m, 6H), 7.54-7.39 (m, 4H), 7.27 (t, *J* = 7.2 Hz, 2H) ppm.

UV-vis (pyridine), nm (ε, M^{-1}cm^{-1}) 483 (23,300), 375 (7,600), 306 (25,700).

LIFDI-MS, *m/z* (relative intensity) 638.0710, calc. 638.0702 ([M]$^+$, 100%).

Anal. Calc. $C_{38}H_{22}O_6Zn \cdot 4H_2O$: C, 64.10; H, 4.25. Found: C, 63.48; H, 4.08.

The presence and quantity of water present in the solid sample was

confirmed through integration of the water peak in the ^{1}H NMR spectrum.

Zn(2$^-$)$_2$·2H$_2$O (6). 86% yield. Red Solid. ^{1}H NMR (pyridine-d_5, 300

MHz) 9.37 (d, *J* = 8.7 Hz, 4H), 9.03 (s, 2H), 8.10 (s, 2H), 7.91 (d, *J* = 8.1

Hz, 2H), 7.88 (d, *J* = 8.7 Hz, 2H), 7.46 (t, *J* = 8.7 Hz, 2H), 7.34 (t, *J* = 8.1

Hz, 2H), 6.94 (d, *J* = 8.7 Hz, 4H), 3.30 (q, *J* = 7.2 Hz, 8H), 1.08 (t, *J* = 7.2

Hz, 12H) ppm. UV-vis (pyridine), nm (ε, M^{-1}cm^{-1}) 525 (92,500), 402

(17,600), 305 (47,800). LIFDI-MS, *m/z* (relative intensity) 780.2163, calc.

780.2172 ([M$^+$], 100%). Anal. Calc. $C_{46}H_{40}N_2O_6Zn \cdot 2H_2O$: C, 67.52; H, 5.42;

N, 3.42. Found: C, 67.30; H, 5.29; N, 3.50. The presence and quantity of

water present in the solid sample was confirmed through integration of the

water peak in the ^{1}H NMR spectrum.

Zn(3$^-$)$_2$·2H$_2$O (7). 92% yield. Purple solid. ^{1}H NMR (pyridine-d_5, 300

MHz) 9.48 (d, *J* = 7.5 Hz, 4H), 9.45 (s, 2H), 8.26 (s, 2H), 8.10 (d, *J* = 8.7

Hz, 2H), 8.01 (d, *J* = 8.4 Hz, 2H), 7.70 (t, *J* = 7.5 Hz, 4H), 7.56-7.43 (m, 6H)

ppm. UV-vis (pyridine), nm (ε, M^{-1}cm^{-1}) 564 (31,700), 405 (27,500), 306

(22,800). LIFDI-MS, *m/z* (relative intensity) 670.0259, calc. 670.0246 ([M$^+$],

72%). Anal. Calc. $C_{38}H_{22}O_4S_2Zn \cdot 2H_2O$: C, 64.45; H, 3.70. Found: C, 64.21;

H, 3.73. The presence and quantity of water present in the solid sample

was confirmed through integration of the water peak in the ^{1}H NMR

spectrum.

Zn(4⁻)$_2$·2H$_2$O (8). 91% yield. Blue solid. ^{1}H NMR (pyridine-d_5, 300

MHz)   9.55 (d, J = 9.3 Hz, 4H), 9.44 (s, 2H), 8.16 (s, 2H), 8.05 (d, J = 8.1

Hz, 2H), 7.95 (d, J = 8.7 Hz, 2H), 7.55-7.37 (m, 4H), 6.91 (d, J = 6.9 Hz,

4H), 3.33 (q, J = 6.9 Hz, 8H), 1.09 (t, J = 6.9 Hz, 12H) ppm. UV-vis

(pyridine), nm (, M^{-1}cm^{-1}) 609 (143,300), 303 (52,200). LIFDI-MS, m/z

(relative intensity) 812.1711, calc. 812.1715 ([M$^+$], 100%). Anal. Calc.

C$_{46}$H$_{40}$N$_2$O$_4$S$_2$Zn·2H$_2$O: C, 64.97; H, 5.22; N, 3.29. Found: C, 64.75; H,

5.08; N, 3.27. The presence and quantity of water present in the solid

sample was confirmed through integration of the water peak in the ^{1}H NMR

spectrum.

Photoinduced Reactivity of 5-8 in Pyridine. Solutions of **5-8**

(~0.01 mmol) in pyridine-d_5 (~0.7 mL) were placed in NMR tubes under air.

Each tube was then placed in a Luzchem photoreactor equipped with 419

nm or white light lamps and illuminated until the reaction was determined to

be complete by ^{1}H NMR. The solvent was then removed under reduced

pressure and an FT-IR spectrum was obtained.

Zinc bis(3-benzoyloxy)-2-naphthoate) (9). Beige solid. ^{1}H NMR

(pyridine-d_5, 300 MHz)   9.14 (s, 2H), 8.48 (d, J = 6.9 Hz, 4H), ^{1}H NMR

(pyridine-d_5, 300 MHz) 7.94-7.82 (m, 6H), 7.56-7.37 (m, 10H) ppm. FT-IR

(KBr, cm^{-1}) 1728 ($\nu_{C=O}$).

Zinc bis(3-((4-(diethylamino)benzoyl)oxy)-2-naphthoate) (10).

Beige solid. ^{1}H NMR (pyridine-d_5, 300 MHz) 9.11 (s, 2H), 8.47 (d, J = 7.2

Hz, 4H), 8.00-7.75 (m, 6H), 7.51-7.26 (m, 4H), 6.71 (d, J = 7.2 Hz, 4H),

3.19 (q, J = 6.9 Hz, 8H), 0.99 (t, J = 6.9 Hz, 12H) ppm. FT-IR (KBr, cm^{-1})

1714 ($\nu_{C=O}$)

Zinc bis(3-(benzoyloxy)naphthalene-2-carbothiolate (11). Beige

solid. ^{1}H NMR (pyridine-d_5, MHz) 9.14 (s, 2H), 8.36 (d, J = 8.6 Hz, 4H),

7.97 (d, J = 8.1 Hz, 2H), 7.84 (d, J = 8.1 Hz, 2H), 7.79 (s, 2H), 7.55-7.35

(m, 10H) ppm. FT-IR (KBr, cm^{-1}) 1739 ($\nu_{C=O}$).

Zinc bis(3-(4-(diethylamino)benzoyl)oxy)naphthalene-2-

carbothiolate (12). Beige solid. ^{1}H NMR (pyridine-d_5, 300 MHz) 9.06 (s,

2H), 8.36 (d, J = 7.5 Hz, 4H), 8.00-7.65 (m, 6H), 7.57-7.29 (m, 4H), 6.73 (d,

J = 7.5 Hz, 4H), 3.26 (q, J = 6.9 Hz, 8H), 1.04 (t, J = 6.9 Hz, 12H) ppm. FT-

IR (KBr, cm^{-1}) 1713 ($\nu_{C=O}$).

Dark control reactions of 5-8. Solutions of **5-8** (~2 mM) in pyridine-

d_5 were prepared in air under minimal red light and each was placed in a

NMR tube. Each NMR tube was then covered in foil, placed in a

photoreactor, and illuminated using 419 nm (**5** and **6**) or white light lamps

(**5-8**) for 24 hours. Evaluation of each solution by ^{1}H NMR indicated that no

reaction had occurred.

Anaerobic control reaction. Solutions of **5-8** in pyridine-d_5 were

prepared, each was placed in a NMR tube, and N$_2$ was bubbled through

each solution for ~5 minutes. Each NMR tube was then placed in a photoreactor and illuminated with 419 nm (**5** and **6**) or white light lamps (**5-8**) for 24 hours. Evaluation of each solution by ^{1}H NMR indicated that no reaction had occurred.

CO release quantification of 5-8 in solution. 50 mL round bottom flasks were loaded with **5-8** (ca. 10 mg) dissolved in pyridine (5 mL). Each flask was then sealed with a septum, purged with O_2 for 45 seconds, and illuminated with white light for 24 hours, which resulted in the bleaching of each solution to colorless. A sample (10 mL) of headspace gas was then analyzed by gas chromatography and the area of the peak associated with CO applied to a calibration curve created specifically for reactions performed in pyridine. The solvent was then removed under reduced pressure and the residual solid was analyzed by ^{1}H NMR for completeness of reaction.

Photoreactivity fo 5-8 as powdered solids. Compounds **5-8** were placed in 50 mL round bottom flasks as powdered solids. Each flask was then sealed with a septum, gently purged with O_2 for 1 minute, and placed in a photoreactor with 419 nm (**5** and **6**) or white light lamps (**5-8**) for 72 hours. The headspace gas of the flask was then analyzed by gas chromatography for the production of CO. Compounds **5** and **6** as powdered solids produced 2 equivalents of CO per equivalent of compound when illuminated 419 nm or white light lamps. Compounds **7** and **8** did not produce CO upon illumination with white light lamps. The residual solid in

the flask was then dissolved in pyridine-d_5 and the solution evaluated by ^{1}H NMR, indicating **5** and **6** had fully converted to the photoproducts **9** and **10**, while no reaction occurred for **7** and **8**.

Photoreactivity of 5 as a film deposit. Compound **5** (52.6 mol, 33.7 mg) was deposited in a round bottom flask and dissolved in pyridine (~ 10 mL). The solvent was then removed via rotary evaporator using the highest rpm setting to deposit the compound on the vessel walls. The flask was sealed with a septum and placed in a photoreactor with 419 nm or white light lamps and illuminated for 48 hours. Reactions were determined to be complete by ^{1}H NMR.

CO quantification for solid samples 5 and 6. A GC calibration curve for the quantification of CO was created using sodium bicarbonate as an inert solid in a 50 mL round bottom flask. Each flask was sealed with a septum, evacuated, and varying volumes of O_2 and CO were injected. For each mixture, GC analysis was performed using the number of moles of CO calculated to be in the flask based on volume.

Aerobic oxidative palladium-catalyzed carbonylation reactions. The general reaction conditions were as follows. Compound **5** was deposited as a film in a 50 mL round bottom flask as described above. The flask was then loaded with phenyl boronic ester (52.6 mol, 10 mg), $PdCl_2(PPh_3)_2$ (2.63 mol, 1.8 mg), NEt$_3$ (105.2 mol, 15 L) and ethanol (5 mL, 190 proof). The flask was then sealed with a septum and placed in an oil bath pre-heated to 40 °C. Each reaction mixture was illuminated with

two CFL blue bulbs set at an average distance of 5 cm from the flask for 24 hours. The flask was removed from the oil bath and allowed to cool to room temperature. The reaction mixture was then passed through a short silica plug (~600 mg) 1 L of the filtrate was then analyzed by GC-MS. The remaining filtrate from the reactions was then brought to dryness under reduced pressure and analyzed by ^{1}H NMR.

GC-MS parameters. GC-MS spectral data was collected on a Shimadzu GCMS-QP5000 equipped with an EC-5 column that is 5% phenyl:95% methylpolysiloxane with a fused silica coating. The column is 30 m in length with a 0.25 m coating and 0.25 mm diameter. The injection temperature was 185 °C with an interface temperature of 250 °C. The column initial temperature was 70 °C with a final temperature of 250 °C and a ramp rate of 12 °C/minute. The column inlet pressure was 43.5 kPa with a column flow of 0.8 mL/min and a linear velocity of 32.8 cm/sec for a total flow of 35.4 mL/min. Analysis of product mixtures was performed on 1 L of EtOH solution.

Evaluation of CO release reactivity of 5 (film deposit) in the presence of ethanol. A film deposit of **5** in a 50 mL round bottom flask was generated as outlined above. To this flask was added ethanol (5 mL). The flask was then sealed with a septum and placed in an oil bath pre-heated to 40 °C. The flask was illuminated with two CFL blue bulbs set at an average distance of 5 cm from the flask for 24 hours. The flask was then removed from the oil bath and allowed to cool to room temperature. The

ethanol solution was then passed through a short silica plug (~600 mg). The filtrate was then analyzed by GC-MS, which revealed the formation of ethyl benzoate. The remaining filtrate was then brought to dryness under reduced pressure and analyzed by ^{1}H NMR.

Results

The development of CO-releasing structural motifs that can be easily prepared and tuned via multiple strategies (e.g. organic synthesis and/or metal coordination) offers advantages toward the generation of compounds that can be easily applied in biology and synthesis. Our approach focuses on the use of extended 3-hydroxy-4-pyrone based frameworks, which are easily structurally modified to modulate spectroscopic and CO release properties. The presence of the 3-hydroxy-4-pyrone unit also offers the possibility of metal coordination.

Mixing **1-4** with Zn(OTf)$_2$ in MeOH in the presence of base yielded precipitates of the analytical formulation [Zn(flavonolato)$_2$·nH$_2$O (**5**, n=4; **6-8**, n=2; Scheme 5-1). All exhibit poor solubility in common organic solvents except pyridine. Each compound was characterized as a solid using elemental analysis and FT-IR. ^{1}H NMR, LIFDI-MS, UV-vis, and fluorescence spectra (Figures D-1-D-16) were obtained in pyridine. The ^{1}H NMR features are consistent with equivalent flavonolato ligands,
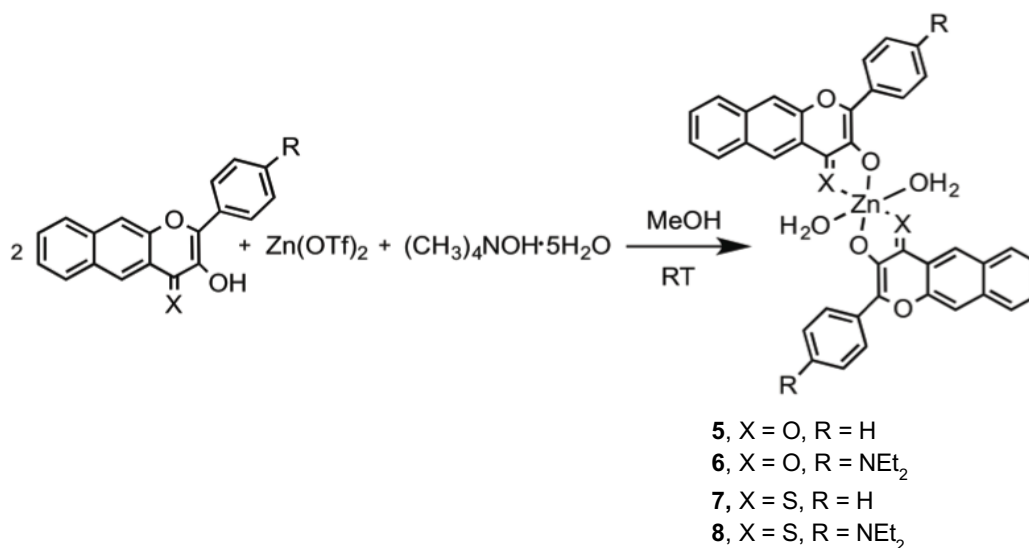
Scheme 5-1 Synthesis of **5-8**.

suggesting possible *trans* water ligands (Scheme 5-1) in a pseudo-octahedral structure. As shown in Figure 5-2, the absorption features of **5-8** in pyridine (Table 5-2) span the visible region from ~400-650 nm. The NEt_2-containing derivatives **6** and **8** have high extinction coefficients, consistent with the presence of two flavonolato ligands.

Photoinduced O_2 reactivity of 5-8

When dissolved in pyridine and illuminated with white (**5-8**) or blue light (**5** and **6**), the Zn(II) bis(flavonolato) compounds exhibit dioxygenase-type photoinduced release of two equivalents of CO (Table 5-2). The zinc containing products (**9-12**, Scheme 5-2) are bis-carboxylate derivatives based on ^{1}H NMR and IR spectral features (Figures D-17-D-24). Thus,
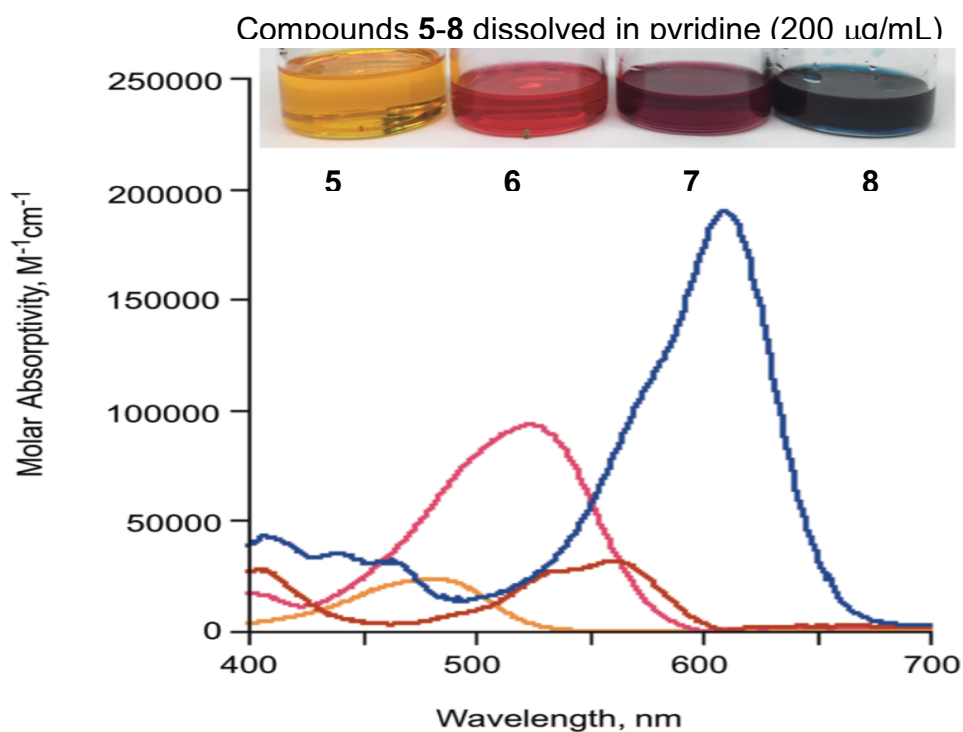
Fig 5-2 Absorption spectral features of **5**-**8**.

Table 5-2 Absorption and CO release properties of **5-8**

Compound	λ_{max} (nm)[a,b] ϵ (M^{-1}cm^{-1})	Solution CO-release (eq. CO)[b-d]	Solid-state CO-release (eq. CO)[e]
5	483 (23,300)	1.86(2)	1.77(4)
6	525 (92,500)	1.91(4)	1.63(2)
7	506 (31,700)	1.89(3)	No
8	609 (193,300)	1.95(4)	No

[a]Most re-shifted absorption feature. [b]Compound dissolved in pyridine in the presence of O2. [c]Determined using GC; average of three independent measurements. [d]Illuminated with white compact fluorescent (CFL) bulbs for 48h. Compounds **5** and **6** will also undergo CO release using blue CFL bulbs in 48h. [e]Illuminated with white CFL bulbs for 4 days; average of three independent measurements.

5, X = O, R = H
6, X = O, R = NEt$_2$
7, X = S, R = H
8, X = S, R = NEt$_2$

9, X = O, R = H
10, X = O, R = NEt$_2$
11, X = S, R = H
12, X = S, R = NEt$_2$
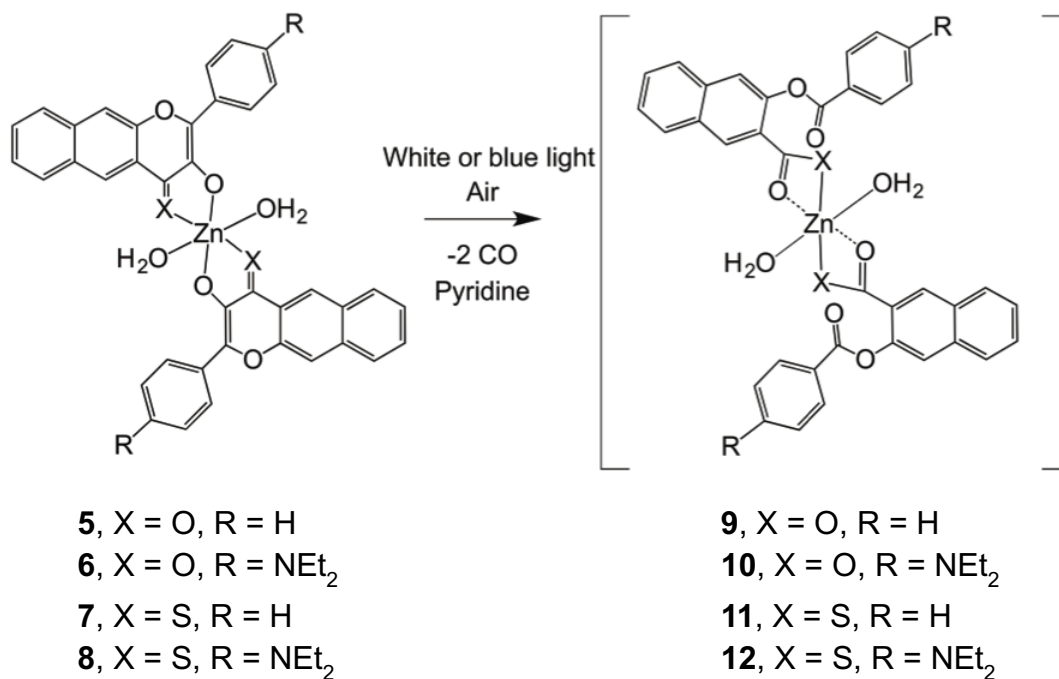
Scheme 5-2 Visible light induced CO release reactivity of **5**-**8** in pyridine.

both flavonolato ligands in **5**-**8** undergo light-induced CO release when dissolved in pyridine. We note than Tran and Cohen previously reported light-induced reactivity for a zinc bis(flavonothianoato) complex in CHCl$_3$ but did not report the nature of the products.[63]

Solid-state photoinduced CO-release reactivity of 5 and 6

Light induced CO release compounds offer the possibility of control over the temporal and spatial release of CO. To date, compounds developed for biological and *in situ* synthetic applications have focused on soluble species. However, solid-state materials or compounds that release

CO upon introduction of visible light could also be very useful in various applications, including in implantable systems and for CO release within reaction mixtures. Visible and near infra-red light-driven NO-releasing solid-state materials (photoNORMs) have been previously developed for biological applications.[64-72] A current limitation in terms of development of photoCORM materials is the almost complete lack of availability of compounds that exhibit solid state CO release reactivity upon illumination compounds that will release CO upon illumination of the crystals with microscope light. However, these compounds were not characterized in terms of the amount of CO released or the products remaining following CO release.[54]

To our knowledge, solid state light-induced CO release has not been previously reported for flavonol or metal flavonolato complexes. Notably, we have discovered that exposure of powders of **5** and **6** to visible light in the presence of O_2 for 48 h results in quantitative CO release (Table 5-2). Analysis of the remaining beige powders following light illumination via ^{1}H NMR in pyridine-d_5 indicated the clean formation of **9** and **10**. Thus the same reactivity of observed for **5** and **6** both in solution and in the solid state. The solid thiocarbonyl derivatives did not exhibit CO release even after extensive periods of illumination (1 week). These results demonstrate that there are factors that influence solid-state CO release within this family of compounds that remain to be determined. It is important to note that the neutral flavonols **1-4** are unreactive as solids upon extended illumination (1

week) with visible light. The zinc bis(flavonolato) complexes (**5** and **6**) are unreactive as solids under anaerobic conditions.

Initial evaluation of 5 as a CO source for oxidative palladium catalyzed carbonylation in ethanol

Building on the discovery of solid-state CO release reactivity for **5**, we performed initial studies to examine the possibility of using this compound as an *in situ* heterogeneous visible light-induced CO release agent for oxidative palladium-catalyzed alkoxycarbonylation processes such as the reactions outlined in Scheme 5-3. Liu, et al. have previously reported that such reactions can be performed using air:CO (ratio 3:1-5:1) to give carbonylated products in >70% yield using various alcohols as the solvent.[62] To our knowledge, CO-releasing molecules have not been previously explored as alternatives to CO gas under this type of low temperature, aerobic conditions. Our initial screening reactions focused on the use of **5** at near stoichiometric level in terms of available CO in reactions with boronic esters of varying electronic substituents (Scheme 3b). For each reaction, a 50 mL round bottom flask was coated with 1 equivalent of **5** (up to 2 eq CO) as a film via rotary evaporation of a pyridine solution of the complex. The boronic ester, NEt_3, and $PdCl_2(PPh_3)_2$ were then added along with ethanol (5 mL). Each reaction mixture was heated under air at 40 °C using an oil bath and was illuminated with two blue CFL
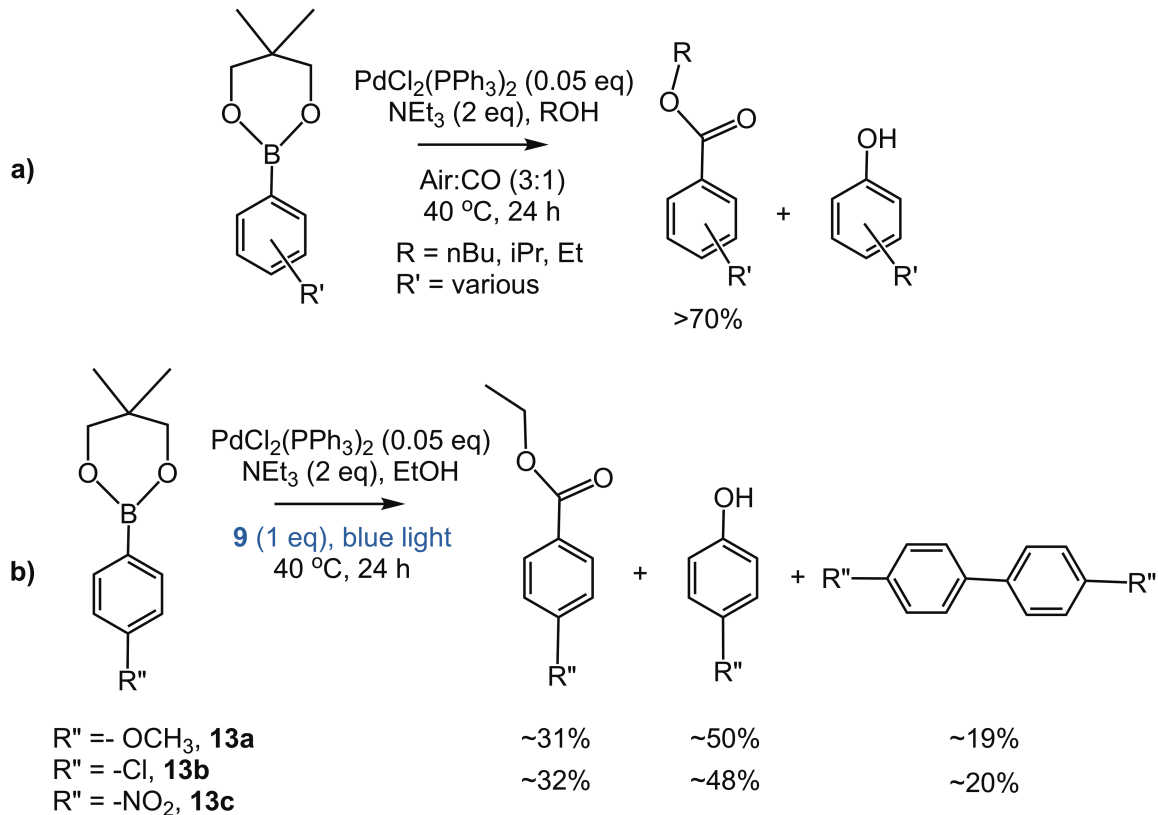
Scheme 5-3 Aerobic palladium catalyzed alkoxycarbonylation reactions.

The relative yields of the products generated for R" = -NO$_2$ in b) could not

be determined due to peak overlap in the GC-MS trace.

bulbs to initiate CO release. After 24 hours of heating and illumination, the

products of the reactions were evaluated using GC-MS and ^{1}H NMR. In the

reactions of **13a** and **13b**, the desired carbonylation ester products are

generated, along with phenol and biphenyl products (Scheme 5-3, Figures

D-25 and D-26). Generally similar results were obtained for the nitro

derivative **13c** albeit no biphenyl product was identified (Figure D-27). The

production of significant amounts of phenyl and biphenyl byproducts

indicates that the CO flux in the reaction mixture needs to be further

optimized to limit the formation of these oxidation byproducts. However,

this was not pursued in these reactions due to the additional identification

of the formation of ethyl benzoate in each reaction mixture. This ester is

generated via the breakdown of the flavonol-based CO-release product by

ester alcoholysis (Scheme 5-4). Compound **5** releases ~1 eq of CO after
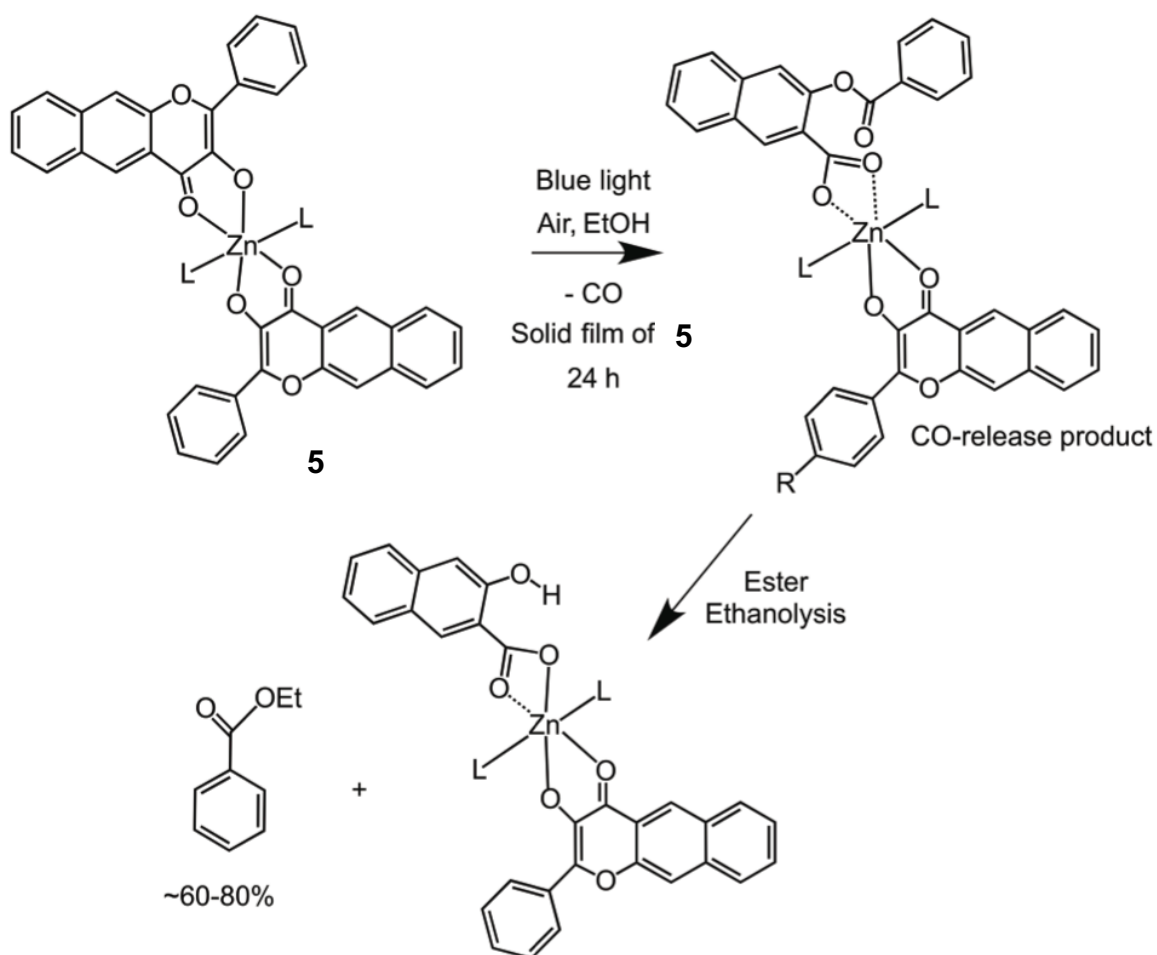
Scheme 5-4 Proposed ester alcoholysis reactivity of CO release product.

24 hours of illumination of the deposited film (Figure D-28). Quantification (via GC-MS) of the ethyl benzoate generated in reaction mixtures shown in Scheme 5-4 after 24 hours indicated that ~60-80% of the expected CO-release product (1 eq) has undergone ethanolysis to produce ethyl benzoate. Overall, these results indicate that while the starting heterogeneous zinc bis(flavonolato) complex is a viable source of CO for carbonylation reactivity, the CO-release product is reactive with alcohols. Minimizing this background alcoholysis reactivity of the CO release agent so as to obtain cleaner carbonylation reaction product mixtures is the subject of ongoing studies. We believe that such studies are justified by the combination of the mild reaction conditions and safe CO-handling present in these reactions.

Conclusions

The dioxygenase-type CO-release chemistry of metal complexes of 3-hydroxy-4-pyrone derivatives (flavonols) has received considerable attention due to its relevance to quercetin dioxygenase enzymes.[56,73-79] Our discovery of light-induced CO-release chemistry for divalent metal flavonolato compounds[60,61,67] has spurred us to further evaluate the light-induced CO release reactivity of flavonols and flavonolato species for applications as photoCORMs. Construction of the extended 3-hydroxy-4-pyrone derivatives **1**-**4** led to the discovery that unlike neutral 3-hydroxyflavone[57], which undergoes multiple types of reactions upon

illumination with UV light, the neutral extended flavonols undergo clean

dioxygenase-type visible-light induced CO release in the presence of O_2.[58]

In this contribution we have evaluated how stabilizing the flavonolato anion

of **1**-**4** via zinc coordination affects the CO release chemistry. Importantly,

we have discovered that zinc coordination results in a red-shift of the

absorption features, with **8** exhibiting a large absorption feature that

extends into the therapeutic window (>650 nm). Perhaps most importantly,

the flavonolato derivatives exhibit CO release both in solution and in the

solid state. Overall, these results demonstrate that 3-hydroxy-4-pyrone

(flavonol) derivatives are tunable using both organic and inorganic

approaches for the development of light-driven CO-releasing molecules.

The ease with which structural modifications can be made positions this

family of CO-releasing molecules to contribute to various current goals in

the biology and chemistry of carbon monoxide. The discovery of light-

induced solid-state CO release reactivity in **5** and **6** also opens up the

opportunity for the development of metal flavonolato-based solid-state CO-

releasing materials that can be triggered using visible light. As

demonstrated herein, solid zinc bis(flavonolato) compounds can be used

as *in situ* CO-release compounds in oxidative palladium-catalyzed

carbonylation reactions albeit with some degradation of the CO release

product. Further studies of the applications of these solids in carbonylation

processes are currently in progress.

Acknowledgements

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CHAPTER 6

SUMMARY

Supplied in large enough doses, carbon monoxide is toxic. This has been known since antiquity. It is only relatively recently that carbon monoxide has been recognized as an incredibly useful compound with roles in chemical reactions and human health. However, the risk associated with using gaseous CO has precluded its wide spread application. In order to overcome this difficulty, the research community has been developing compounds that are essentially storage devises for carbon monoxide that are safe-to-handle and eliminate the safety risk associated with the gas. Great strides have been made in this area, with numerous compounds earning the moniker of CO-releasing molecules (CORMs) having been reported. These compounds have demonstrated the feasibility of storing and releasing CO from a compound. Unfortunately, the CORMs reported thus far all exhibit significant drawbacks that have hindered the further development of this field. In the Berreau Lab, we are interested in the development of novel extended flavonols that act as visible light-induced CORMs. These compounds, based on a naturally occurring framework, display numerous characteristics desirable in a CORM. Couple these characteristics with the ease of synthesis and their unprecedented ability to be modified (Figure 6-1) and we have identified a class of compounds that has the potential to revolutionize the field of CORMs. The research described in this dissertation
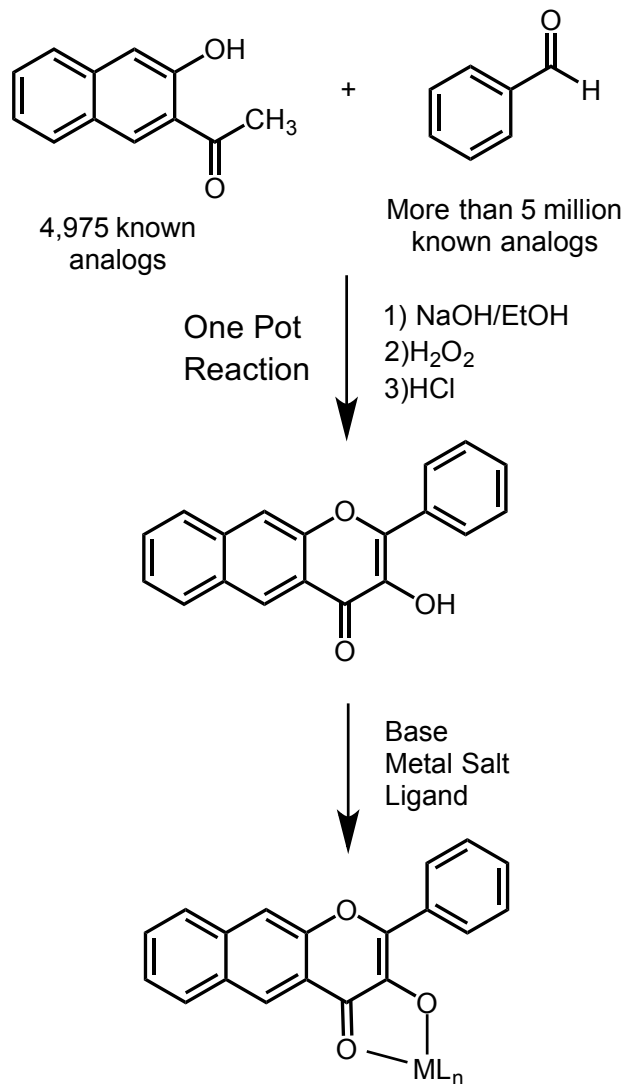
Figure 6-1. The ease of synthesis and potential for analogs of the extended flavonol class of compounds.

outlines the means by which modifications to the core structure of flavonols affect the visible light-induced CO-releasing properties of this class of compounds.

The use of visible light to activate CO-releasing molecules was an important milestone. UV-light is higher in energy than visible light and therefore

light-driven CO release from CORMs is typically easier when performed with UV-light. However, UV-light is also extremely damaging and lacks penetration depth into tissue. Therefore, researchers sought to develop CORMs capable of triggered CO release using less damaging visible light that also has better tissue penetration. A significant number of compounds have been reported that have this capability. However, none of these reported compounds can be tuned in terms of the wavelength of visible light utilized for the CO release reaction. Through simple modifications to the core flavonol structure, we have developed CORMs that have absorption features encompassing almost the entire visible spectrum (Figure 6-2). Additionally, neutral extended flavonols display numerous
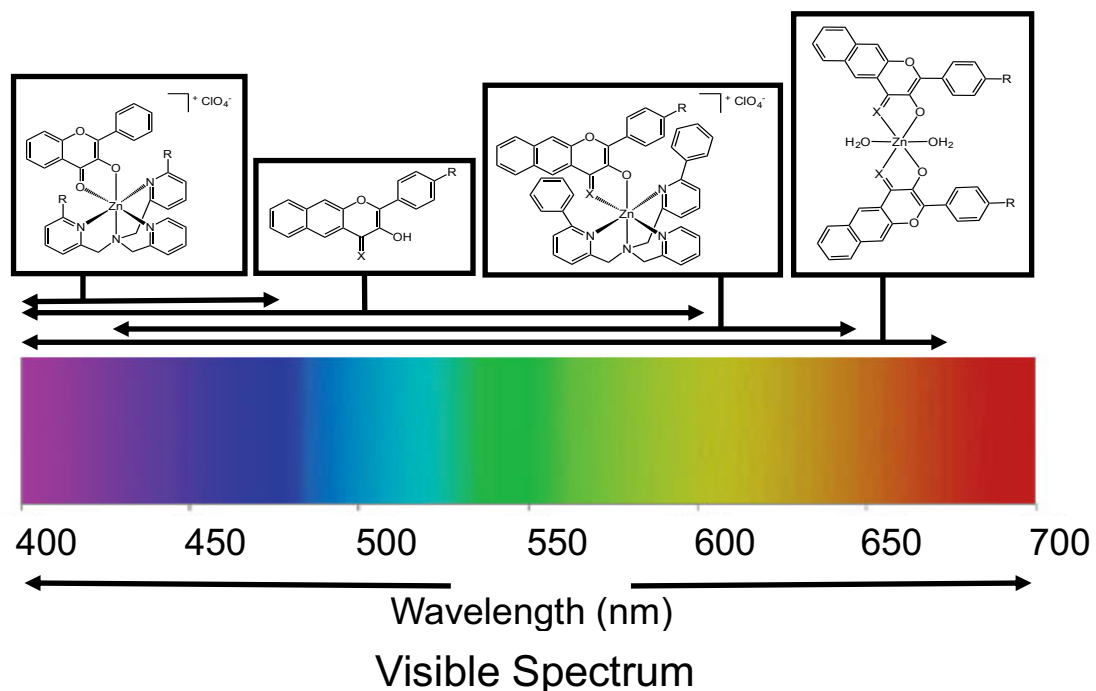
Figure 6-2. Ranges of the lowest energy absorption features for the flavonol compounds described herein.

features desirable in a CO-releasing molecule. For example, the parent

compound of the neutral extended flavonols displays low toxicity and,

importantly, the photoremnant is non-toxic and the fluorescence properties of

these compounds enable tracking of the localization and the CO-release

reactivity within cells. The ease with which these compounds can be modified to

absorb across the visible spectrum means that they have significant potential for

future commercial development.

The role of carbon monoxide has been implicated in the regulation of a

wide range of physiological processes. It therefore seems logical that different

biological processes would require CO at different rates. For light-triggered

CORMs, the efficiency of the CO release photoreaction is measured in terms of a

quantum yield, with compounds typically falling somewhere between 0 and 1. A

compound with a quantum yield of 1 displays 100% photochemical efficiency. We

have demonstrated that flavonols can be modified to achieve many different

quantum yields (Figure 6-3). Not only have we developed photoCORMs with the

highest quantum yields reported to date, we have compounds that display a

range of different quantum yields. I believe that the principles used to modulate

the quantum yields of these compounds will have a significant role in their future

use and development.

The usefulness of carbon monoxide goes beyond its potential as a

therapeutic. It is routinely used in numerous synthetic processes as a carbonyl

source, such as in the synthesis of ibuprofen and acetic acid. However, the
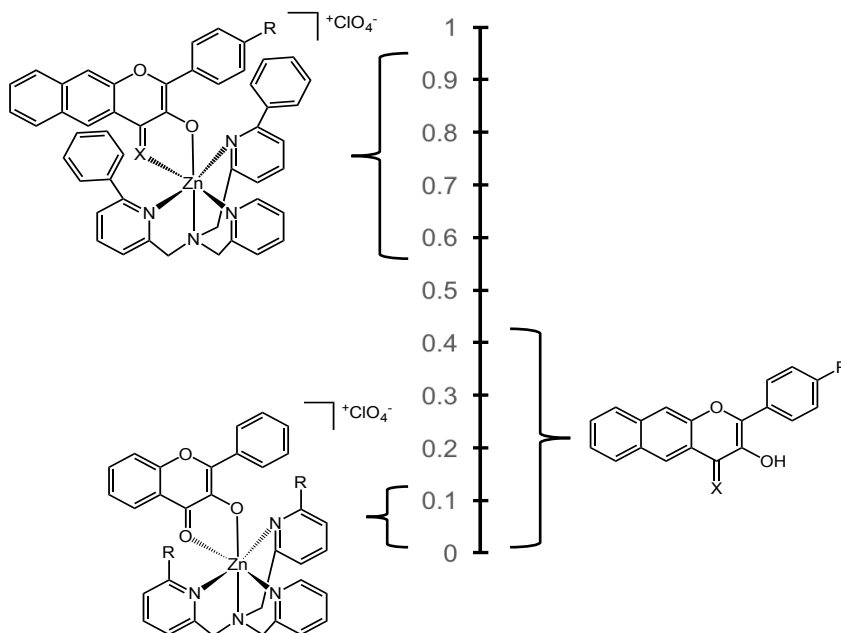
Figure 6-3. Ranges of quantum yields for the differing flavonol compounds.

toxicity of the gas required a significant investment in infrastructure in order to ensure the safety of workers. Small-scale chemical companies and academic institutions lack the capital to ensure its safe usage. In coordinating the extended flavonols to zinc, we discovered that the compounds would undergo photoinduced CO-release as solids. The discovery of this type of reactivity has the potential to expand the use of photoCORMs into the realm of CO sources for synthetic reactions. It was with this in mind that the zinc(bis(flavonolato)) compounds were developed. Zn(bis(flavonolato)) compounds are notoriously insoluble in almost all solvents and the compounds that we developed are no different, the exception being pyridine. These compounds were used as a heterogeneous *in situ* CO source for oxidative palladium-catalyzed carbonylation

reactions. The discovery of solid-state photoinduced CO-release in these compounds has the potential to develop a new use for photoCORMs.

The future of this work consists of two distinct pathways: (1) the further creation of analogs to enhance the biological properties of the compounds (i.e. targeting); and (2) the development of different applications for the solid state CO-releasing compounds. The results of these future studies will only serve to enhance the already tremendous potential of this class of compounds.

APPENDICES

APPENDIX A

CHAPTER 2 SUPPLEMENTARY MATERIAL

Table A-1. Summary of X-ray data collection and refinement.

	3·CH$_3$CN	4·CHCl$_3$
empirical formula	C$_{35}$H$_{30}$ClN$_5$O$_7$Zn	C$_{44}$H$_{50}$Cl$_4$N$_6$O$_7$Zn
formula weight	733.46	982.07
crystal system	Monoclinic	Triclinic
space group	*P2$_1$/n*	*P-1*
a (Å)	10.7484(2)	11.8486(3)
b (Å)	17.7387(3)	12.4945(4)
x (Å)	17.7291(2)	16.2785(5)
α (deg)	90	88.8622(12)
β (deg)	105.7229(10)	75.3891(15)
γ (deg)	90	81.2326(16)
V (Å^3)	3253.80(9)	2304.29(12)
Z	4	2
density (calcd), Mg m^{-3}	1.497	1.415
temp (K)	150(1)	150(1)
crystal size (mm^3)	0.38 x 0.23 x 0.08	0.35 x 0.28 x 0.10
diffractometer	Nonius Kappa CCD	Nonius Kappa CCD
Abs. coeff. (mm^{-1})	0.896	0.821
2θ max (deg)	54.96	50.68
Reflections collected	14263	15983
indep. reflections	7433	8410
variable parameters	443	588
*R*1 / w*R*2^b	0.0405/0.0962	0.0490/0.1235
goodness-of-fit (*F^2*)	1.037	1.027
largest diff. (e Å^{-3})	0.919; -0.530	1.106; -0.664
CCDC number	1006160	1006159

^{a}Radiation used: Mo Kα (λ = 0.71073 Å). ^{b}*R*1 = $\sum | |F_o| - |F_c| | / \sum |F_o|$;

w*R*2 = $[\sum[w(F_o^2-F_c^2)^2]/[\sum(F_o^2)^2]]^{1/2}$ where w = 1/[$^2(F_o^2)$ + (aP)^2 + bP].

Table A-2. Selected Bond Distances (Å) and Angles (deg) for **3·CH₃CN**

and **4·CHCl₃**[a]

3·CH₃CN			
Zn(1)-O(1)	1.9834(17)	O(1)-Zn(1)-O(2)	81.57(7)
Zn(1)-O(2)	2.1373(17)	O(1)-Zn(1)-N(3)	103.21(7)
Zn(1)-N(1)	2.186(2)	N(3)-Zn(1)-O(2)	174.39(7)
Zn(1)-N(2)	2.2116(18)	O(1)-Zn(1)-N(4)	108.68(7)
Zn(1)-N(3)	2.1179(19)	N(3)-Zn(1)-N(4)	87.76(7)
Zn(1)-N(4)	2.171(2)	O(2)-Zn(1)-N(4)	87.90(7)
O(1)-C(19)	1.334(3)	O(2)-Zn(1)-N(2)	94.34(6)
O(2)-C(20)	1.249(3)	N(4)-Zn(1)-N(2)	77.13(7)
C(19)-C(20)	1.439(3)	N(1)-Zn(1)-N(2)	76.83(7)
O(1)-Zn(1)-	172.64(7)	O(1)-Zn(1)-N(1)	97.19(7)
N(3)-Zn(1)-	81.23(7)	N(3)-Zn(1)-N(1)	89.31(7)
N(4)-Zn(1)-	153.95(8)	O(2)-Zn(1)-N(1)	93.04(7)
4·CHCl₃			
Zn(1)-O(1)	2.020(2)	O(1)-Zn(1)-N(6)	106.46(9)
Zn(1)-O(2)	2.159(2)	O(1)-Zn(1)-N(3)	171.00(10)
Zn(1)-N(2)	2.200(3)	N(6)-Zn(1)-N(3)	82.28(11)
Zn(1)-N(3)	2.144(3)	O(1)-Zn(1)-O(2)	80.24(8)
Zn(1)-N(4)	2.181(3)	N(6)-Zn(1)-O(2)	172.05(9)
Zn(1)-N(6)	2.124(3)	N(3)-Zn(1)-O(2)	91.21(10)
O(1)-C(29)	1.320(4)	N(3)-Zn(1)-N(4)	80.08(10)
O(2)-C(30)	1.254(4)	O(2)-Zn(1)-N(4)	91.51(9)
C(29)-C(30)	1.460(4)	O(1)-Zn(1)-N(2)	99.41(9)
N(6)-Zn(1)-	95.04(11)	O(1)-Zn(1)-N(4)	102.80(9)
N(3)-Zn(1)-	77.30(10)	N(6)-Zn(1)-N(4)	82.91(11)
N(4)-Zn(1)-	157.35(10)	O(2)-Zn(1)-N(4)	91.51(9)

[a]Estimated standard deviations in the last significant figure are given

in parentheses.
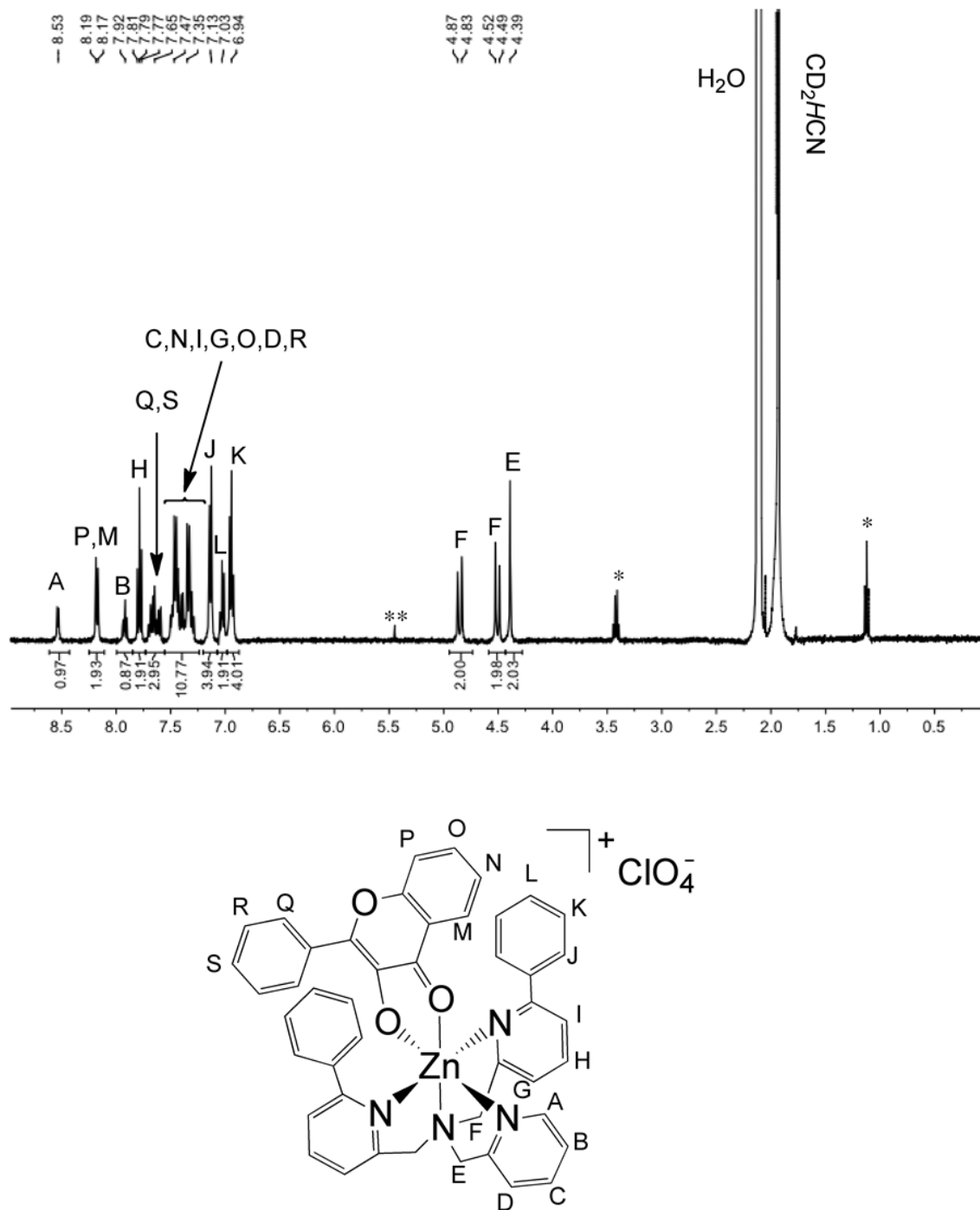
Figure A-1. ^{1}H NMR spectrum of [(6-Ph$_2$TPA)Zn(3-Hfl)]ClO$_4$ (**1**) in CD$_3$CN at 25 °C. * denotes residual Et$_2$O; ** denotes residual CH$_2$Cl$_2$ in the sample.
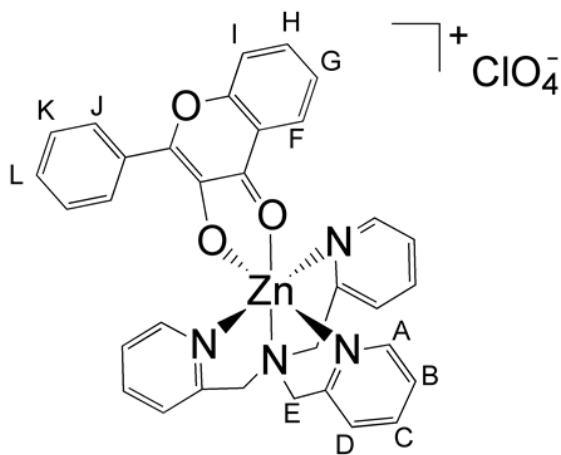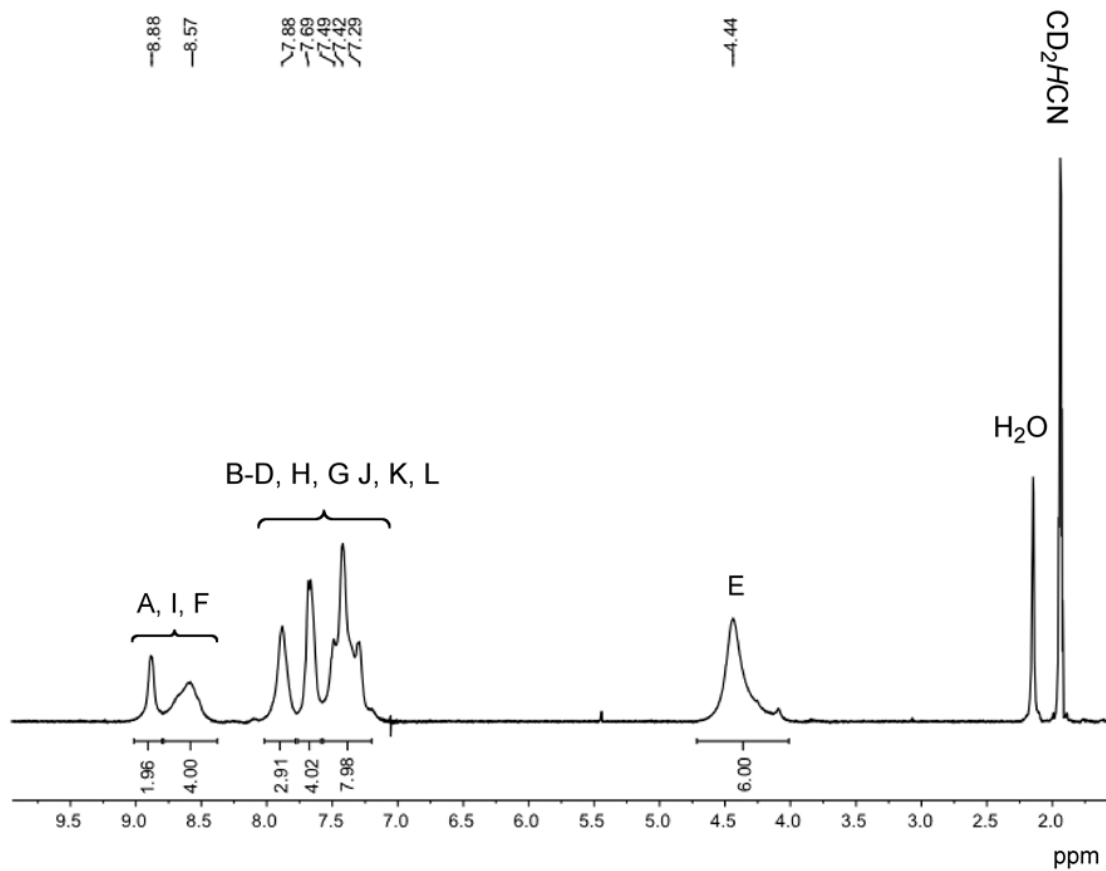
Figure A-2. ^{1}H NMR spectrum of [(TPA)Zn(3-Hfl)]ClO$_4$ (**3**) in CD$_3$CN at 25 °C.

Note: The grouping of resonances at ~8.5-9.0 ppm integrates to 6 H's. This

indicates that one of the H's listed for the other aromatic group is located here.

However, we are unable to assign which hydrogen this may be.
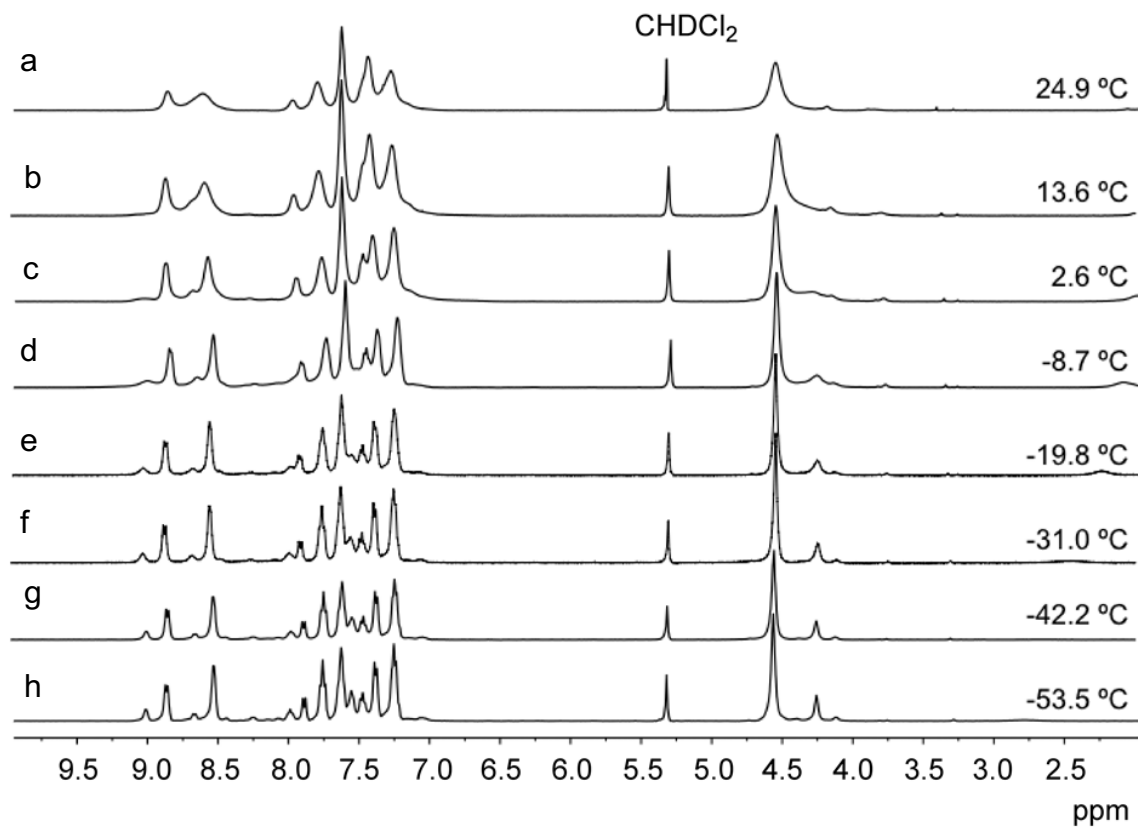
Figure A-3. ^{1}H NMR spectra of [(TPA)Zn(3-Hfl)]ClO$_4$ (**3**) at various temperatures in CD$_2$Cl$_2$.
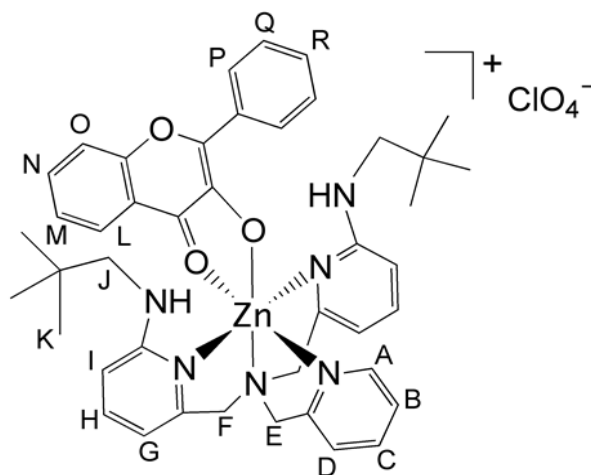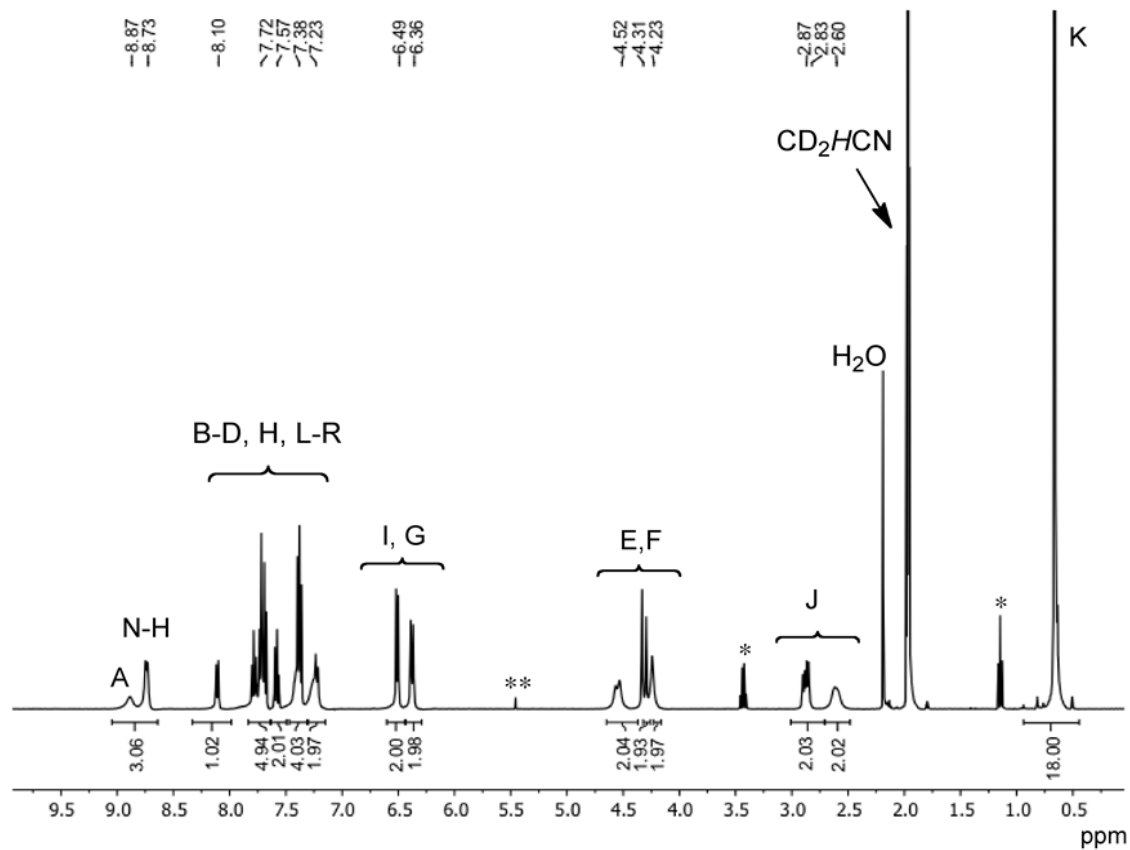
Figure A-4. ^{1}H NMR spectrum of [(bnpapa)Zn(3-Hfl)]ClO$_4$ (**4**) in CD$_3$CN at 25 °C.

* denotes residual Et$_2$O; ** denotes residual CH$_2$Cl$_2$ in the sample.
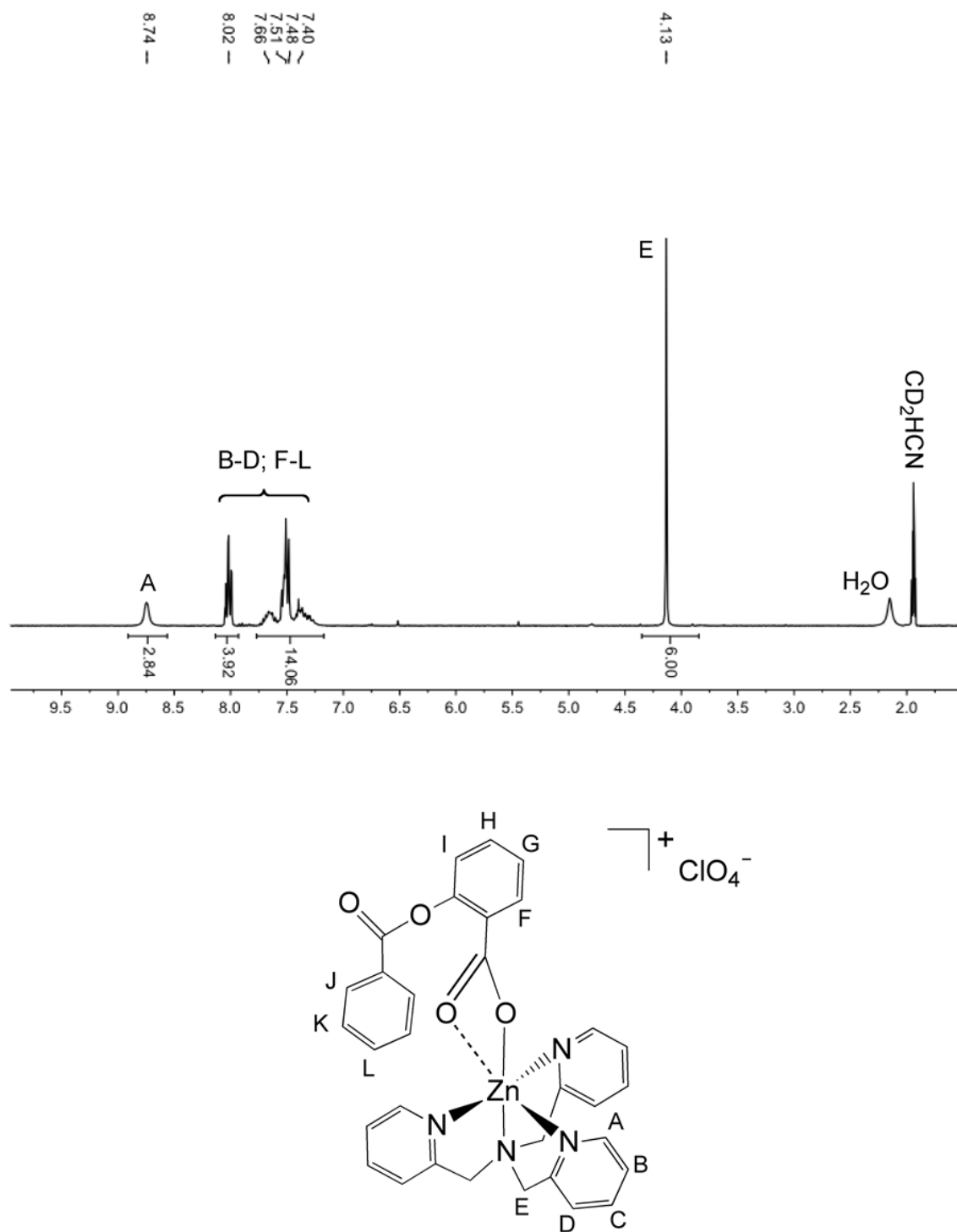
Figure A-5. ^{1}H NMR spectrum of [(TPA)Zn(O-bs)]ClO$_4$ (**5**) in CD$_3$CN at 25 ^{o}C.
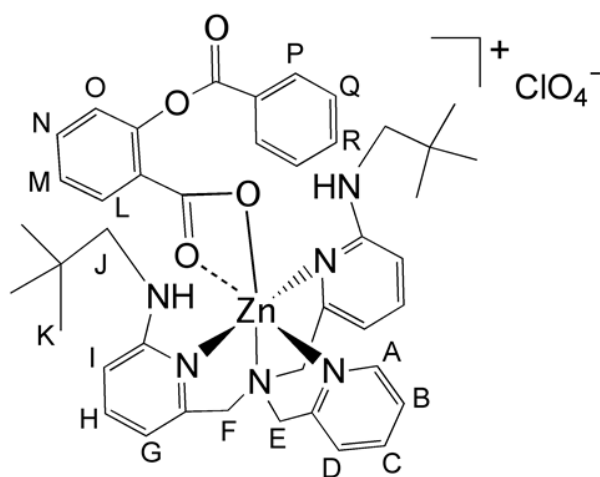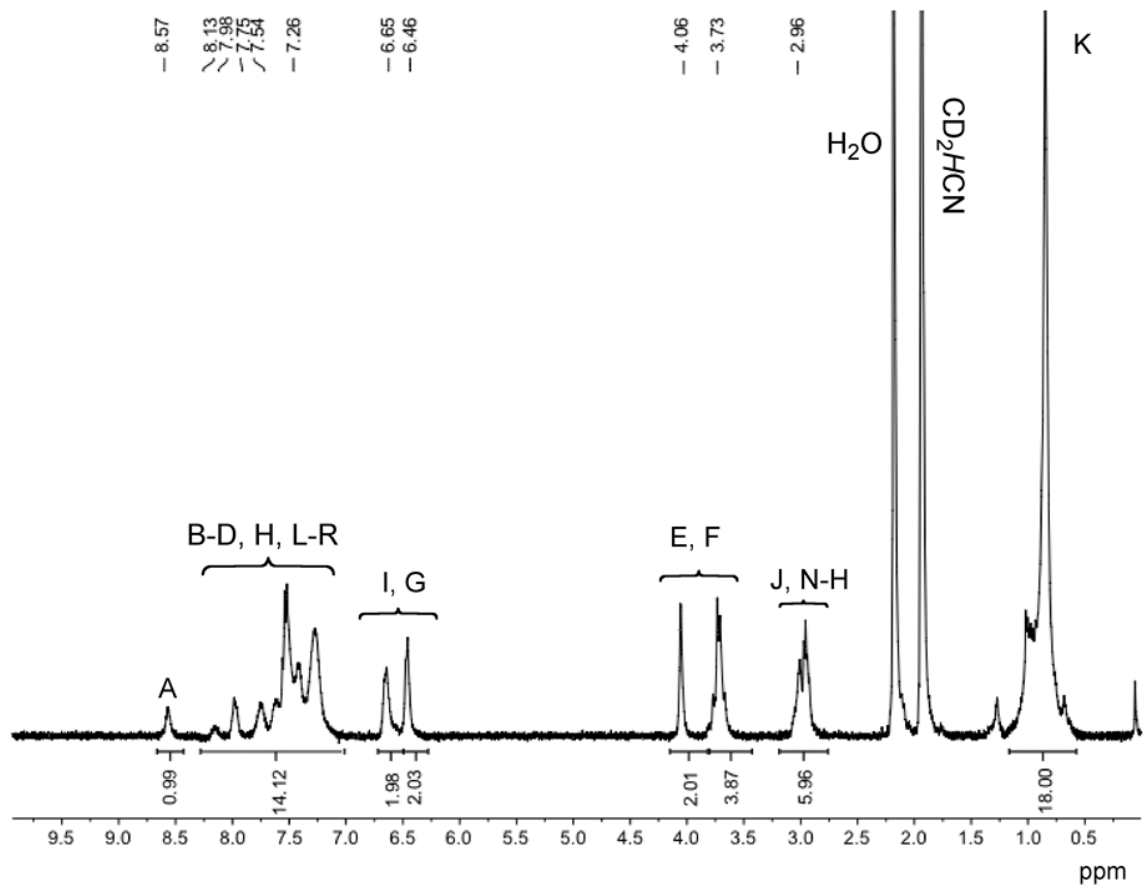
Figure A-6. ^{1}H NMR spectrum of [(bnpapa)Zn(O-bs)]ClO$_4$ (**6**) in CD$_3$CN at 25 ^{o}C.

[M-ClO₄]⁺

Berreau (Utah St.) #492 ACN/DIT

[M-ClO₄]⁺

Ref. #1

Ref. #2

Berreau (Utah St.) #492 ACN/DIT/MX 1A
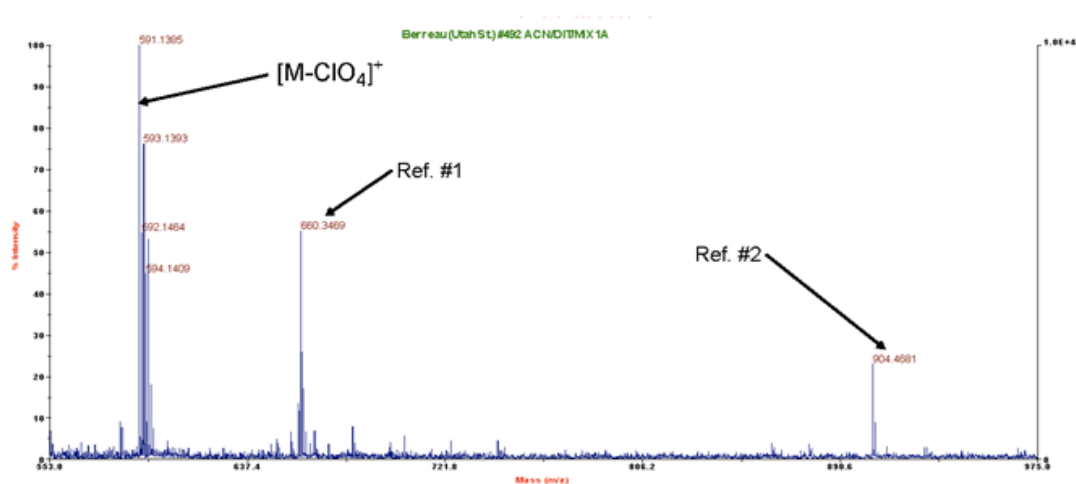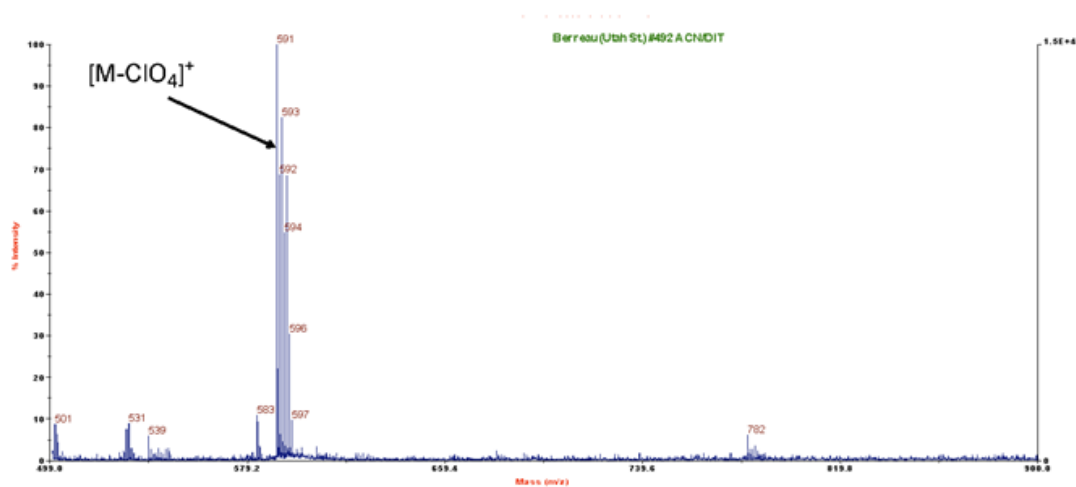
Measured Mass		591.1385

Element	Low Limit	High Limit
C	28	38
H	15	35
N	0	5
O	0	5
Zn	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C35 H29 N O4 Zn	591.1383	0.2	0.4	22
C36 H25 N5 Zn	591.1396	-1.1	-1.8	27
C33 H27 N4 O3 Zn	591.1369	1.6	2.7	22.5
C38 H27 N2 O Zn	591.1409	-2.4	-4.1	26.5

Figure A-7. MALDI mass spectrum of [(TPA)Zn(3-Hfl)]ClO₄ (**3**).
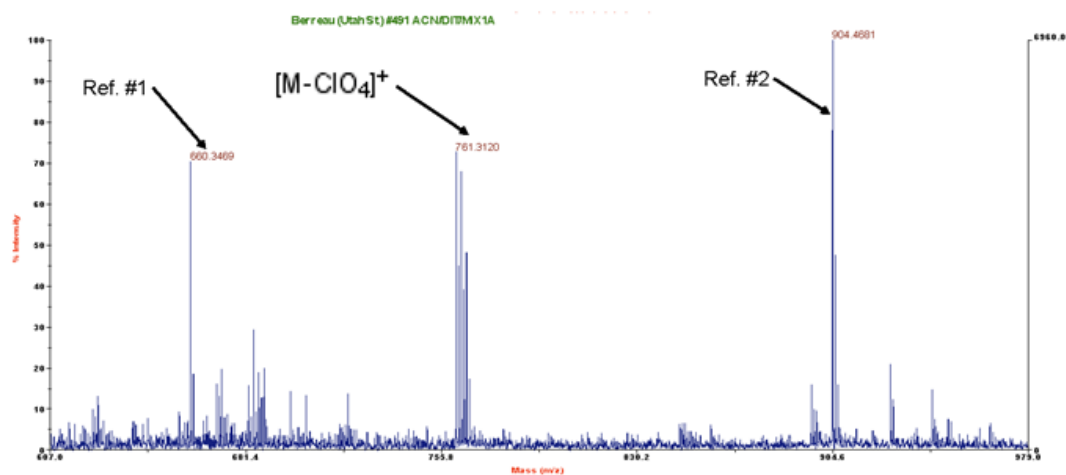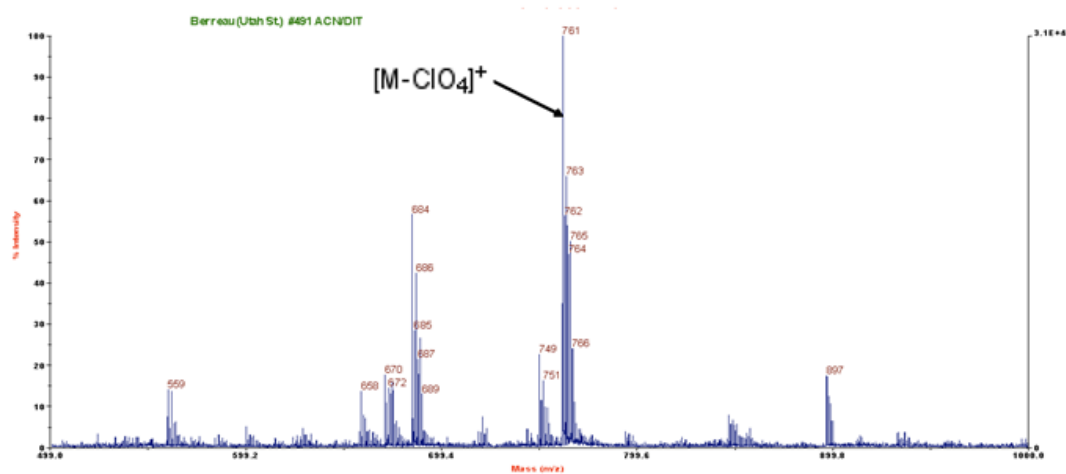
Measured Mass: 761.312

Element	Low Limit	High Limit
C	38	48
H	40	60
N	4	8
O	0	5
Zn	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C38 H49 N8 O5 Zn	761.3112	0.8	1.1	18.5
C43 H49 N6 O3 Zn	761.3152	-3.2	-4.2	22.5

Figure A-8. MALDI mass spectrum of [(bnpapa)Zn(3-Hfl)]ClO$_4$ (**4**).
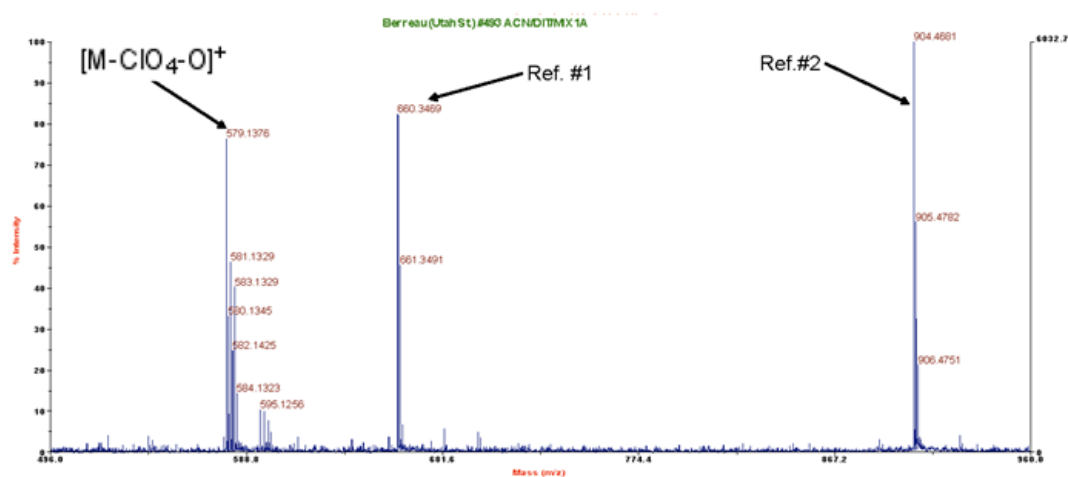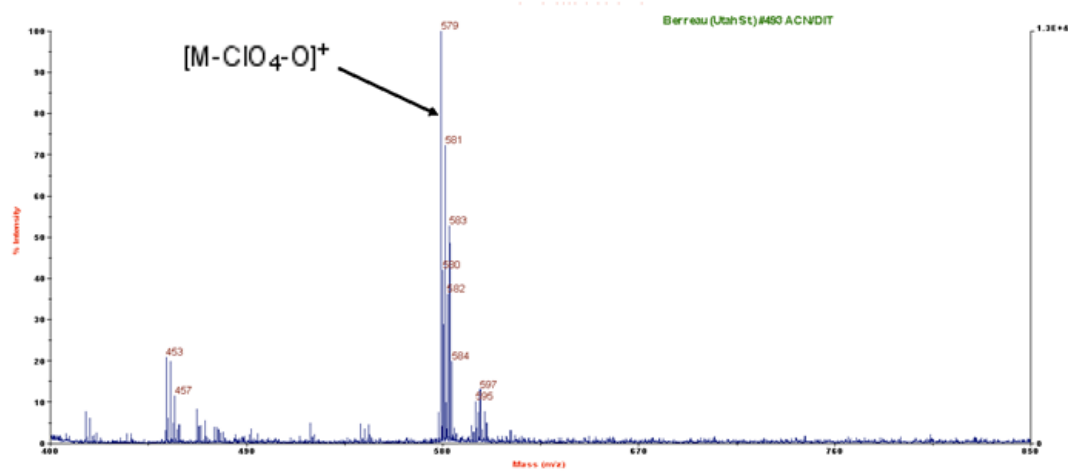
Measured Mass	579.1376			

Element	Low Limit	High Limit		
C	27	37		
H	20	40		
N	0	5		
O	0	5		
Zn	0	1		

Formula	Calculated Mass	mDaError	ppmError	RDB
C34 H29 N O4 Zn	579.1383	-0.7	-1.1	21
C32 H27 N4 O3 Zn	579.1369	0.7	1.2	21.5
C35 H25 N5 Zn	579.1396	-2.0	-3.4	26

Figure A-9. MALDI mass spectrum of [(TPA)Zn(O-bs)]ClO$_4$ (**5**).
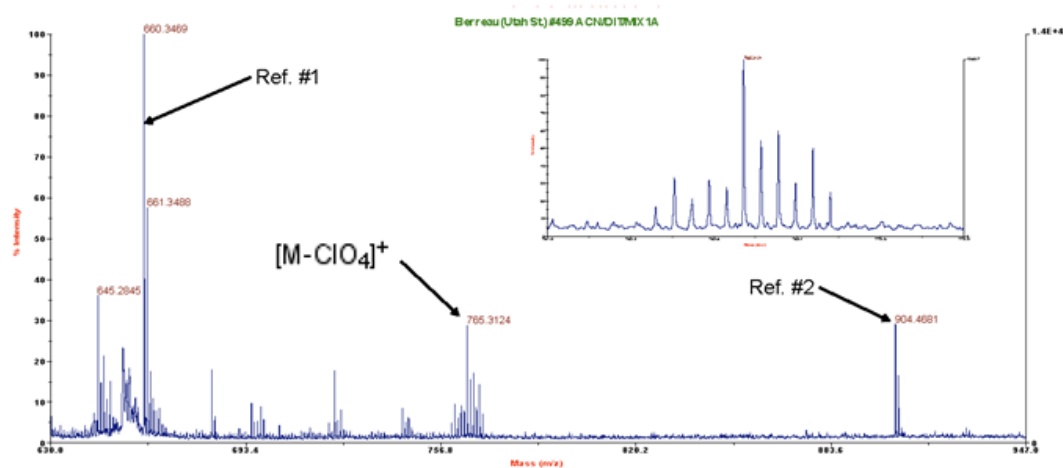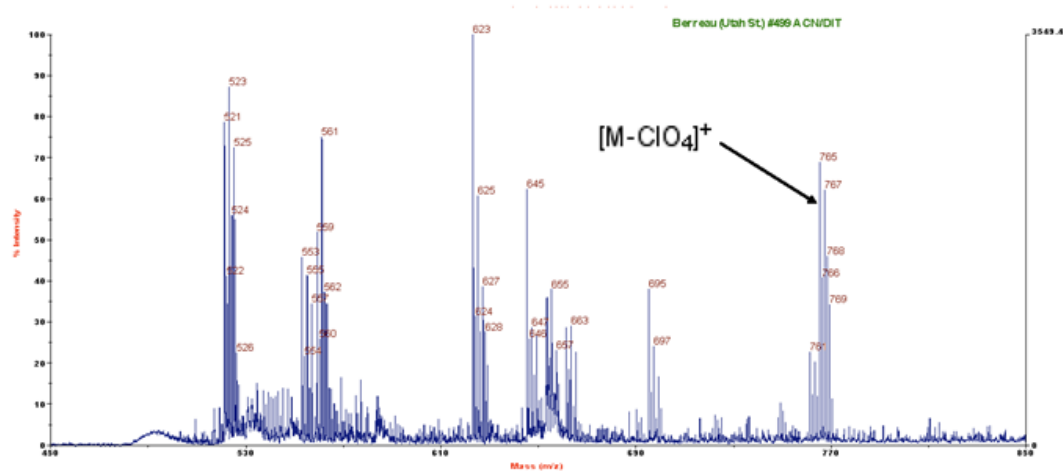
Measured Mass		765.3124		
Element	Low Limit	High Limit		
C	37	47		
H	40	50		
N	4	8		
O	0	5		
Zn	0	1		
Formula	Calculated Mass	mDaError	ppmError	RDB
C45 H47 N7 O Zn	765.3128	-0.4	-0.5	26
C47 H49 N4 O2 Zn	765.3141	-1.7	-2.3	25.5
C42 H49 N6 O4 Zn	765.3101	2.3	3.0	21.5

Figure A-10. MALDI mass spectrum of [(bnpapa)Zn(*O*-bs)]ClO₄ (**6**).
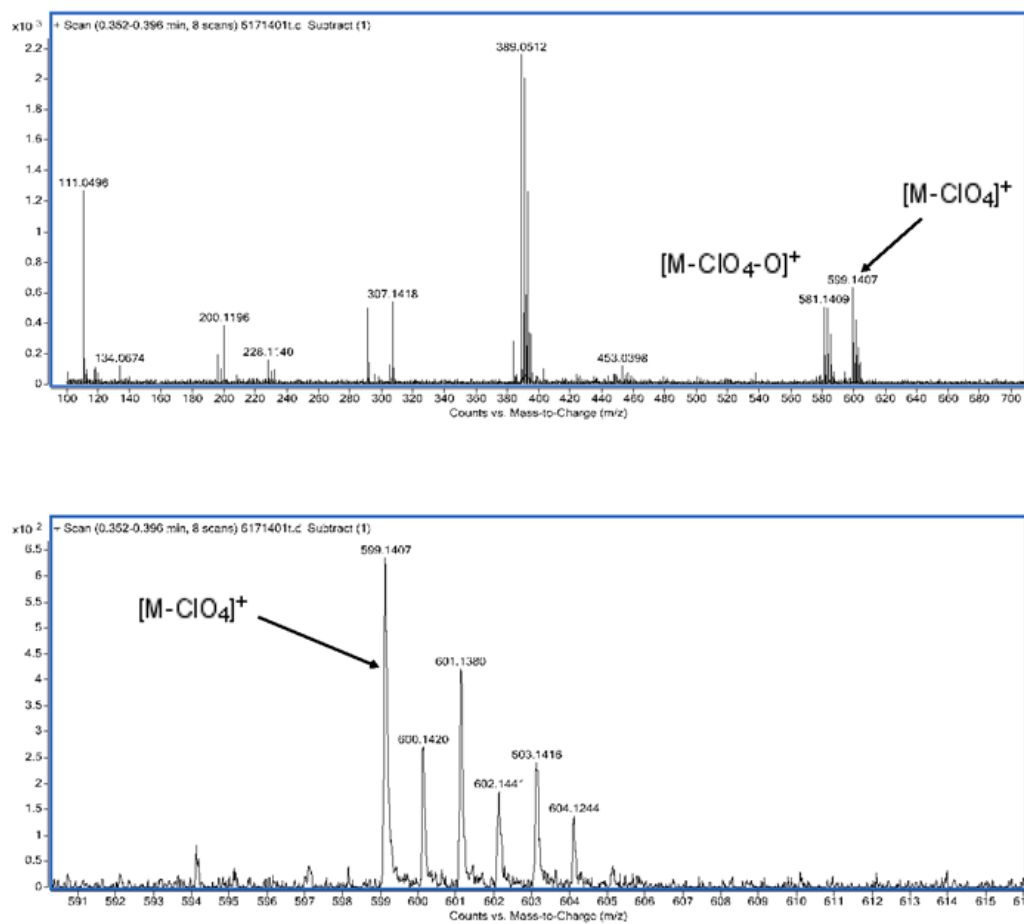
Measured Mass   599.1407

Element	Low Limit	High Limit
C	27	37
H	20	40
N	2	6
O	0	2
18O	0	2
Zn	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C32 H27 N4 O2 18O2 Zn	599.1403	0.4	0.6	21.5
C34 H25 N6 18O Zn	599.1418	-1.1	-1.9	25.5
C35 H27 N4 O2 Zn	599.1420	-1.3	-2.2	24.5
C36 H27 N3 O 18O Zn	599.1432	-2.5	-4.1	25

Figure A-11. MALDI mass spectrum of $[(TPA)Zn(O\text{-bs-}^{18}O_2)]ClO_4$ (**5-$^{18}O_2$**).
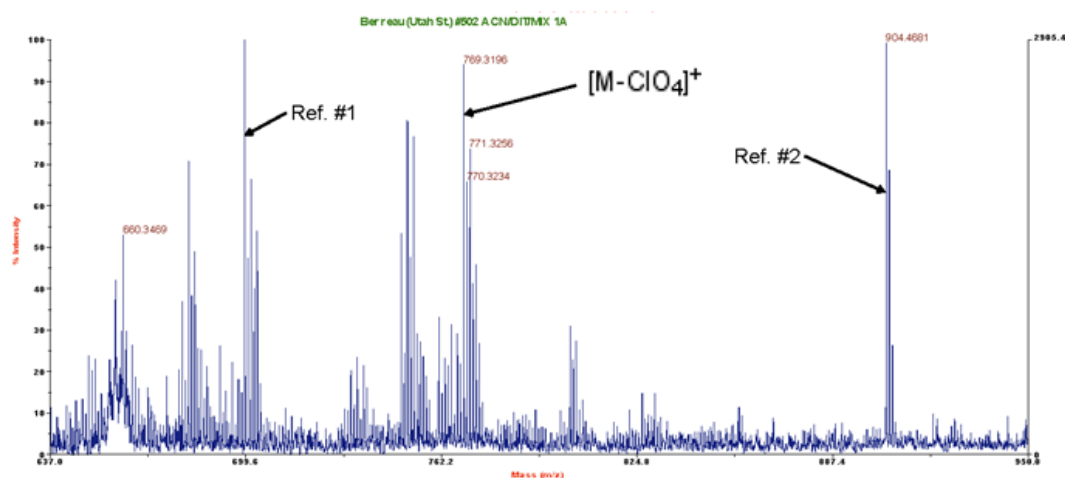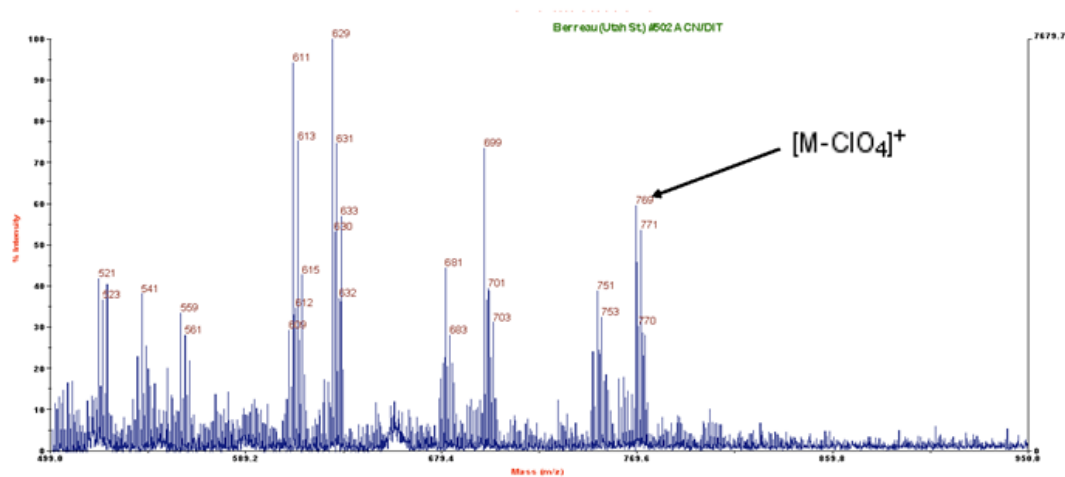
Measured Mass		769.3196			
Element	Low Limit	High Limit			
C	37	47			
H	40	60			
N	5	7			
O	0	3			
18O	0	3			
Zn	0	1			
Formula	Calculated Mass	mDaError		ppmError	RDB
C43 H49 N5 O 18O3 Zn	769.3198	-0.2		-0.2	22
C45 H49 N6 O2 Zn	769.3203	-0.7		-0.9	24.5
C42 H49 N6 O2 18O2 Zn	769.3186	1.0		1.3	21.5
C46 H49 N5 O 18O Zn	769.3215	-1.9		-2.4	25
C41 H49 N7 O3 18O Zn	769.3174	2.2		2.8	21
C38 H49 N7 O3 18O3 Zn	769.3158	3.8		5.0	18

Figure A-12. MALDI mass spectrum of [(bnpapa)Zn(*O*-bs-$^{18}O_2$)]ClO$_4$ (**6-$^{18}O_2$**)

APPENDIX B

CHAPTER 3 SUPPLEMENTARY MATERIAL
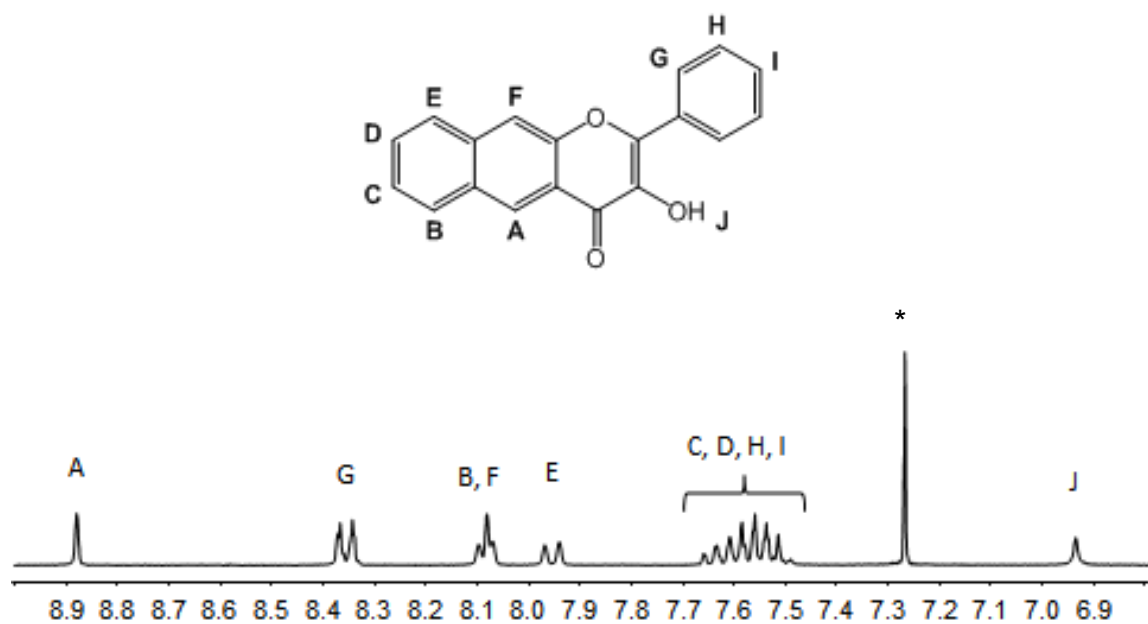
Figure B-1. ^{1}H NMR spectrum of **4** in CDCl$_3$. The * indicates the residual signal of the solvent (C*H*Cl$_3$).
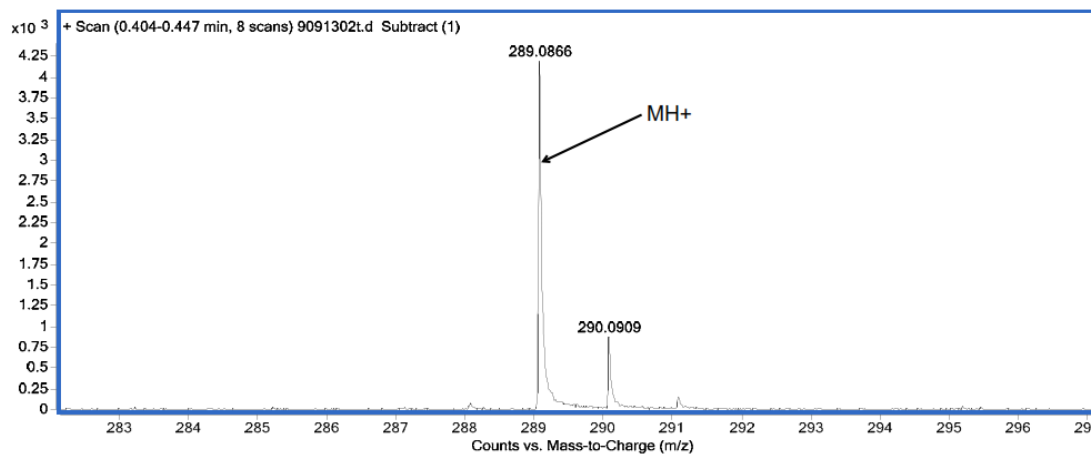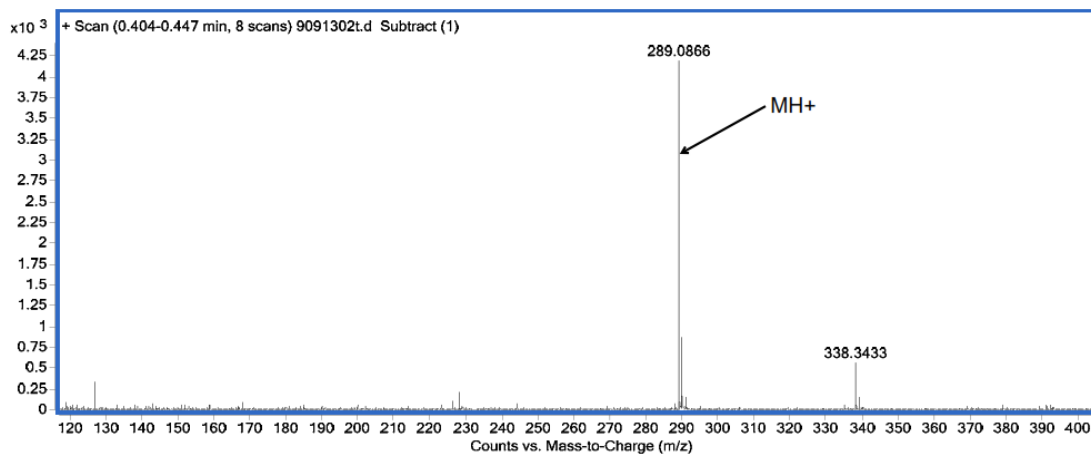
Figure B-2. ESI/APCI-MS of **4**.
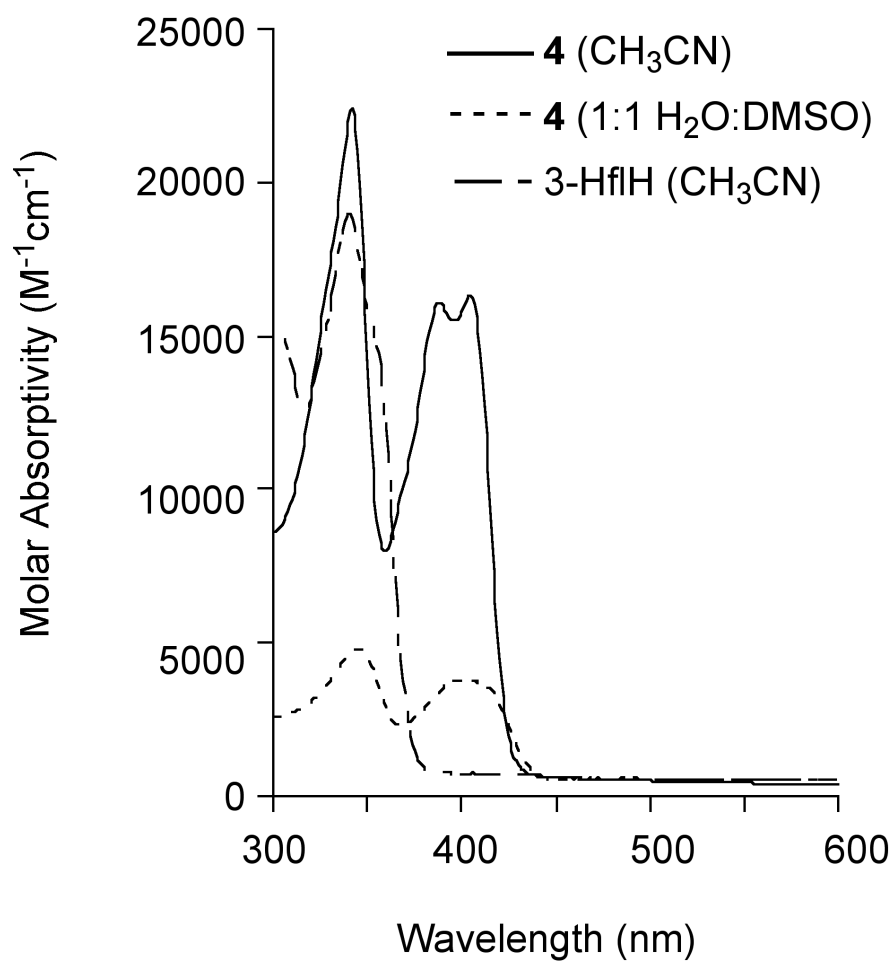
Figure B-3. Absorption spectra of **4** in dry CH_3CN and 1:1 H_2O:DMSO, and 3-hydroxyflavone in CH_3CN.
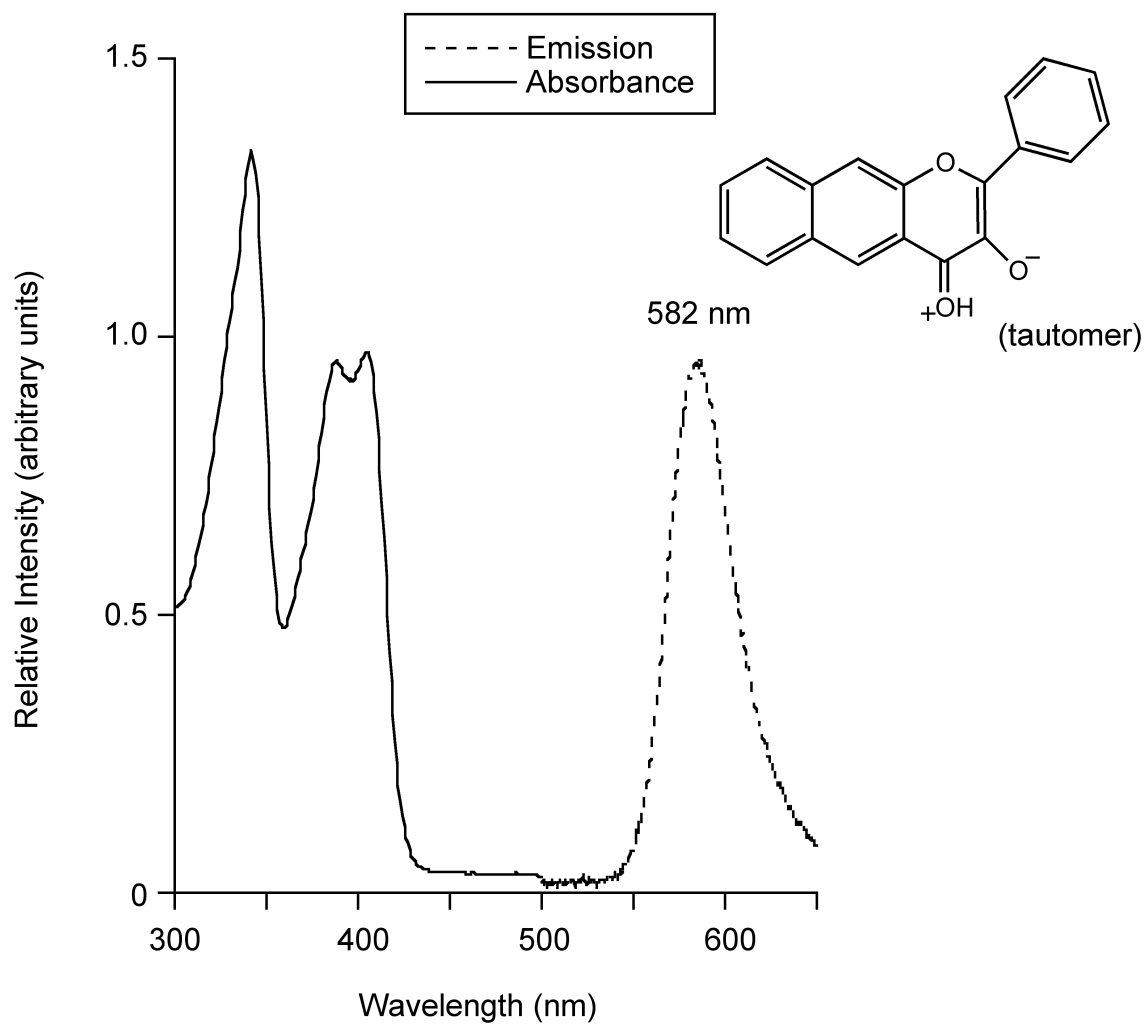
Figure B-4. Absorption and emission spectra of **4** in dry CH₃CN.
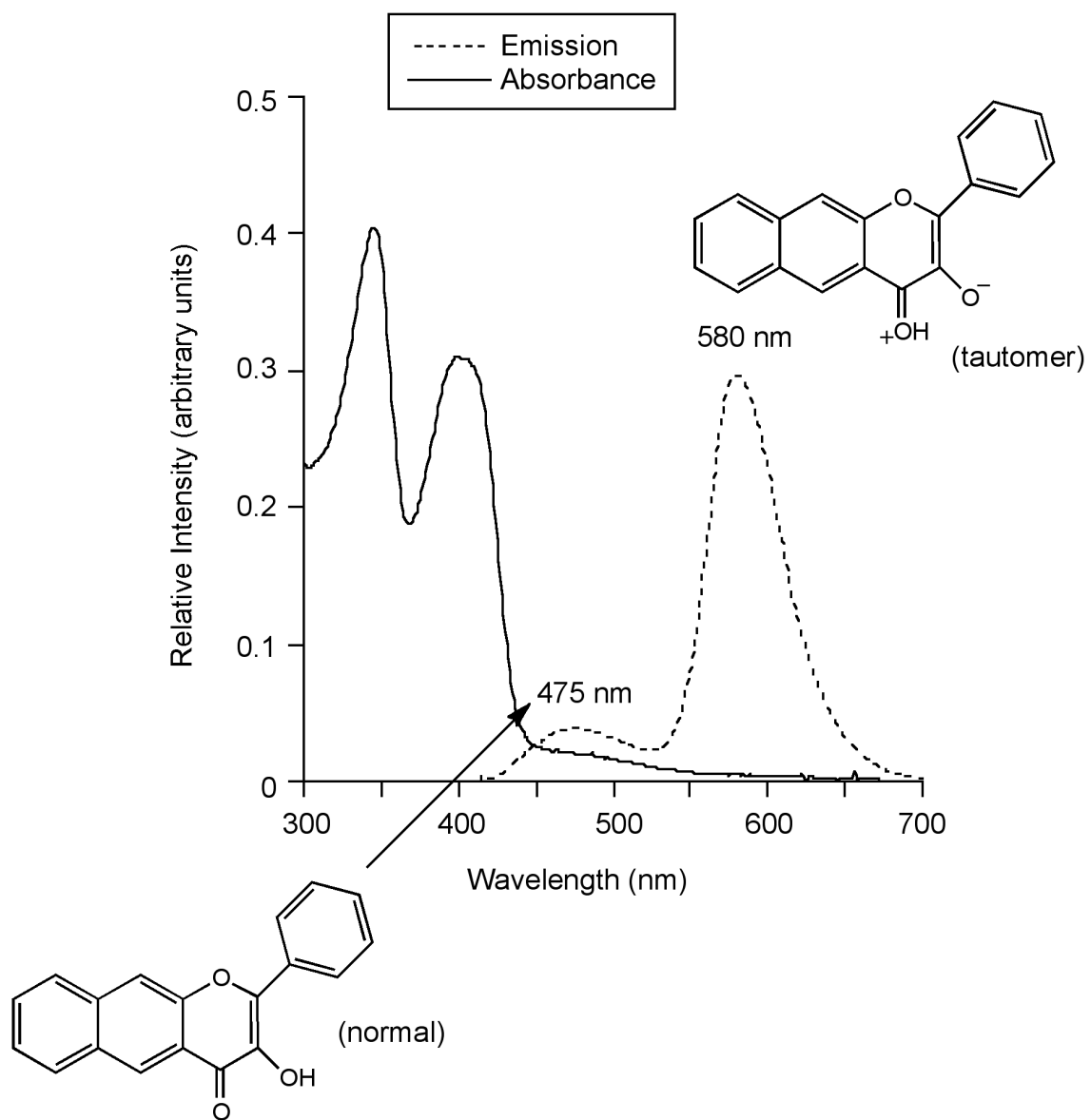
Figure B-5. Absorption and emission spectra of **4** in 1:1 H$_2$O:DMSO.
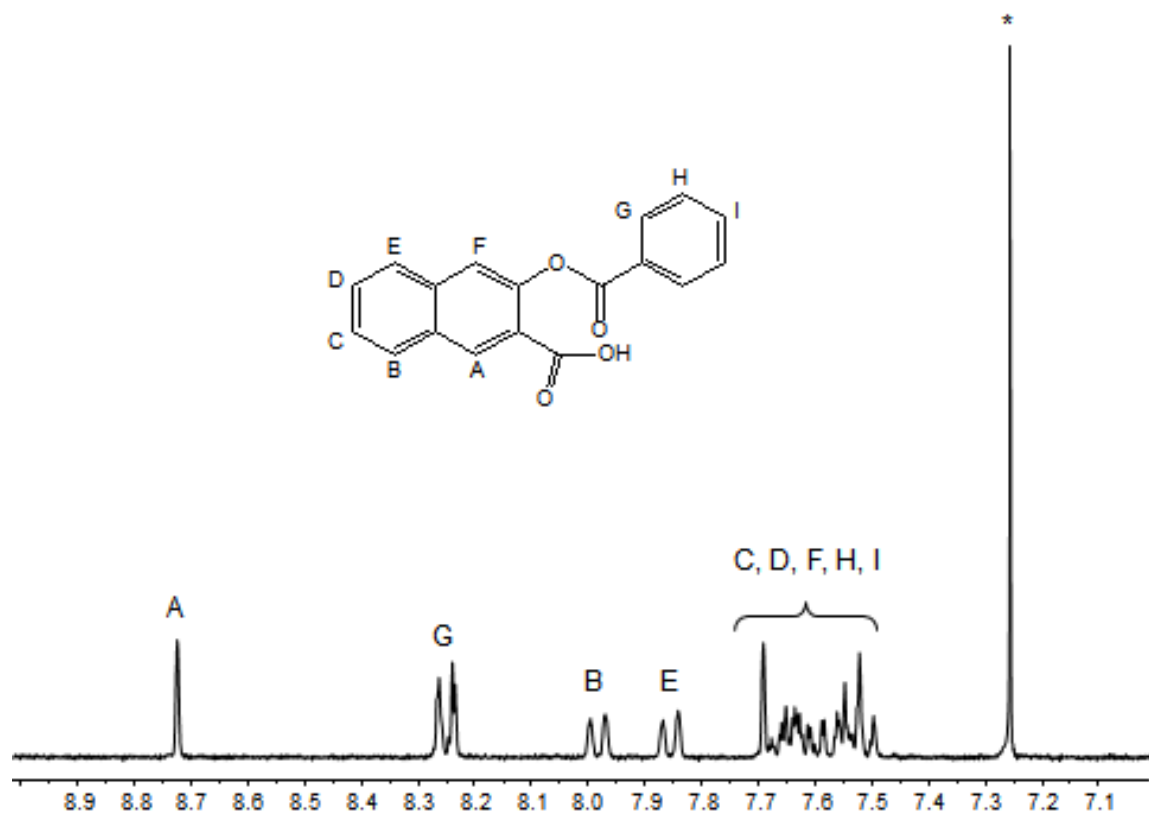
Figure B-6. ^{1}H NMR spectrum of **5** in CDCl$_3$. The * indicates the residual signal of the solvent (C*H*Cl$_3$).
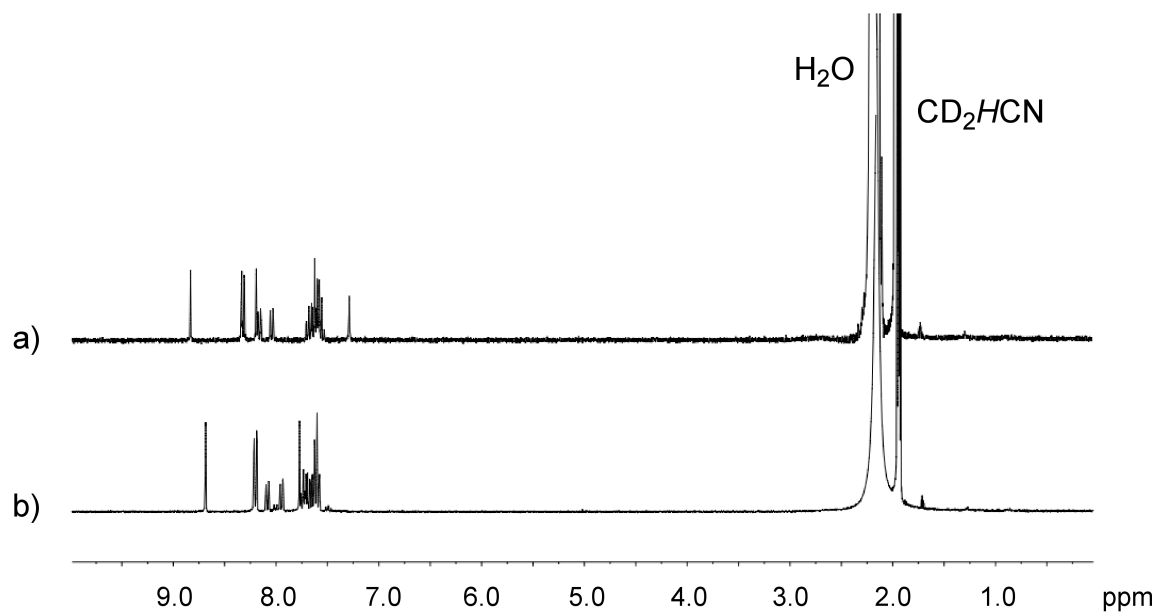
Figure B-7. a) ^{1}H NMR spectrum of **4** in CD$_3$CN at ambient temperature. b) ^{1}H NMR spectrum obtained after irradiation of the sample at 419 nm for 24 h. The results indicate clean conversion to **5**.
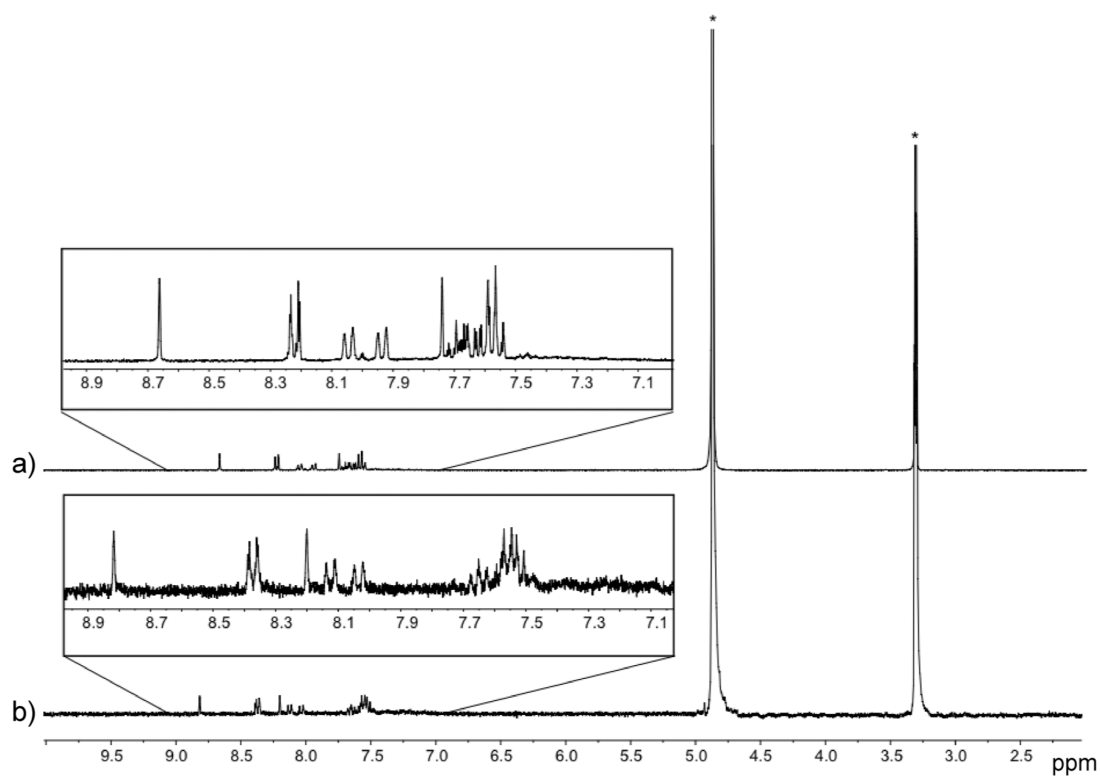
Figure B-8. a) ^{1}H NMR spectrum of **4** in CD$_3$OD at ambient temperature. b) ^{1}H NMR spectrum obtained after irradiation of the sample at 419 nm for 24 h. The results indicate conversion to **5**. The * indicates the residual signals of the solvent (CHD$_2$OH).

Figure B-9. ESI/APCI-MS of **5**.

Figure B-10. Emission spectra of **4** and **5** in CH_3CN under N_2 generated upon excitation into the lowest energy absorption maximum (**4**: 409 nm; **5**: 323 nm).
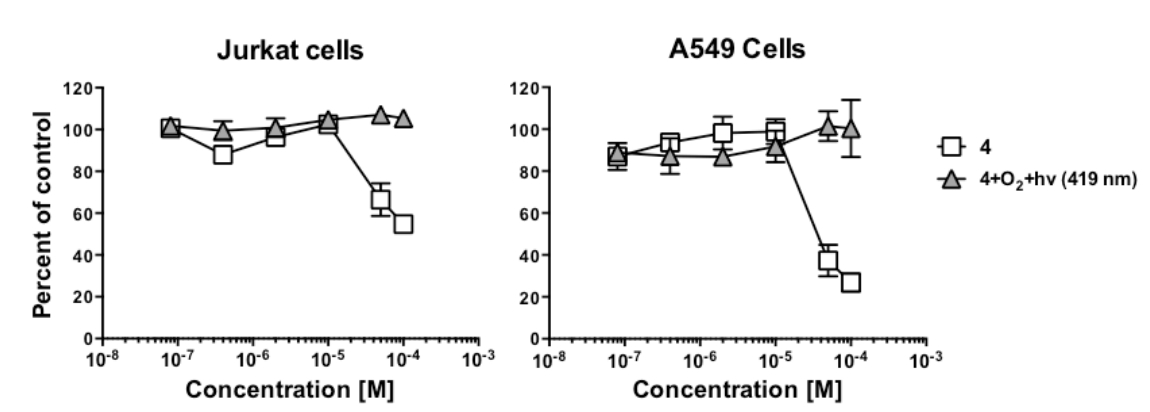
Figure B-11. Plots of human leukemia (Jurkat) and non-small cell lung

carcinoma (A549) cell viability versus complex concentration for **4** and its

photoinduced reaction product (**5**). *IC*$_{50}$ values were determined using a four-

parameter nonlinear regression for assays wherein at least a 50% reduction in

cell viability was observed, in this case only for A549 cells treated with compound

4 (IC$_{50}$ = 41.5 µM). Values shown represent the average ± SEM of three

independently replicated experiments.
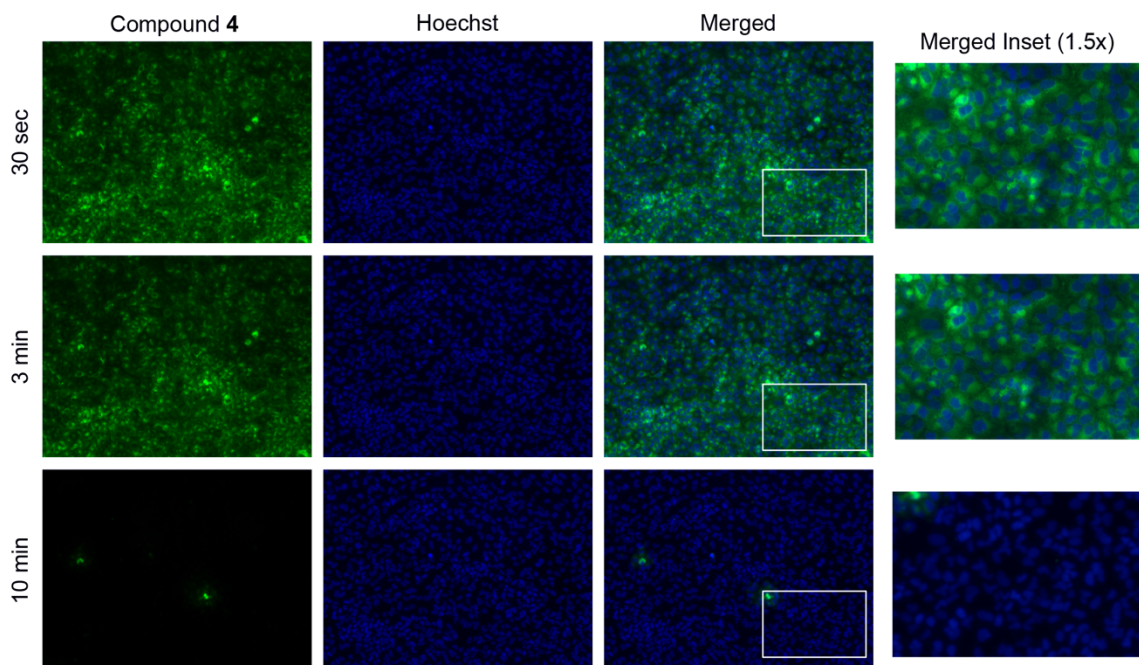
Figure B-12. Fluorescence microscopy of human lung cancer (A549) cells

treated with compound **4** and then exposed to visible light. Detection of

compound **4** (shown above as green) was performed using Zeiss filter set 38:

excitation λ of 450-490 nm (BP 470/40 filter) and emission λ of 500-550 nm (BP

525/50 filter). Following a 1 hr incubation with compound **4**, cells were excited

with visible light for 30 sec, 3 min and 10 min. For localization of the Hoechst

dye, a single fluorescence image was acquired at excitation λ at 365 nm;

emission λ of 420-470nm (BP 445/50 filter), shown in blue above (repeated down

rows for visual comparison). Overlay images indicated that compound **4** was cell

permeable, was present in most cells in the field of view, and was localized

primarily to the cytoplasm. The apparent loss of fluorescence with increasing

length of exposure to visible light suggested photoinduced CO release.
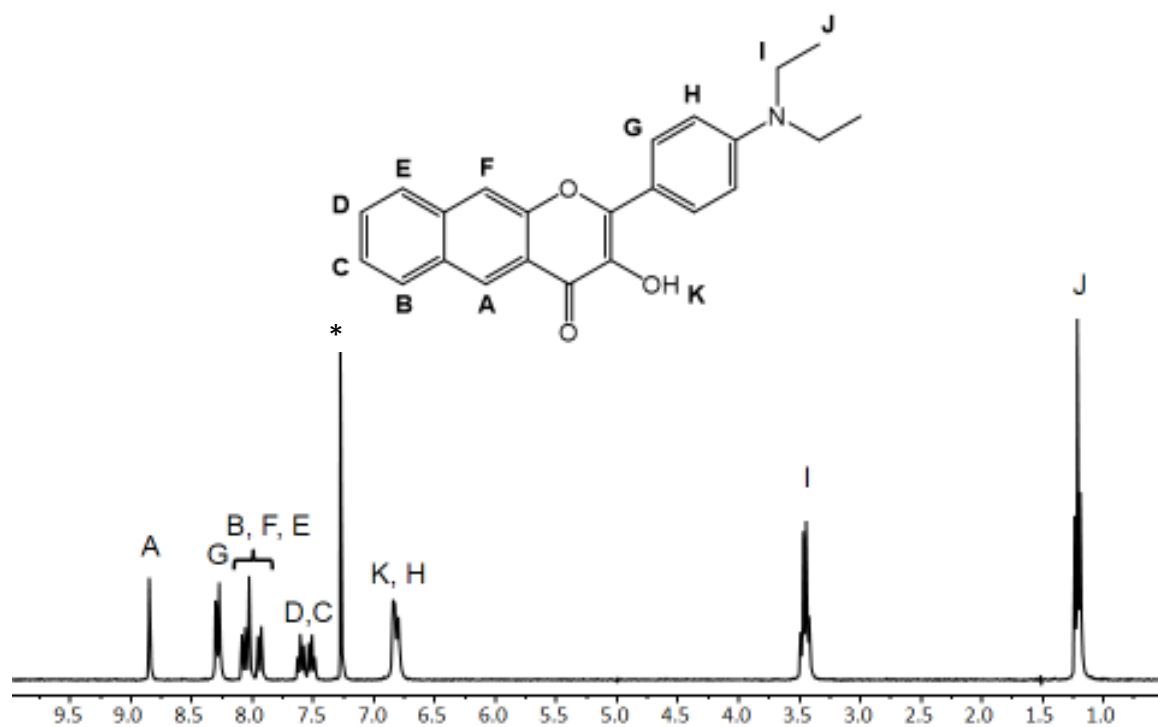
Figure B-13. ^{1}H NMR spectrum of **6** in CDCl$_3$. The * indicates the residual signal of the solvent (CHCl$_3$).

Figure B-14. ESI/APCI-MS of 6.
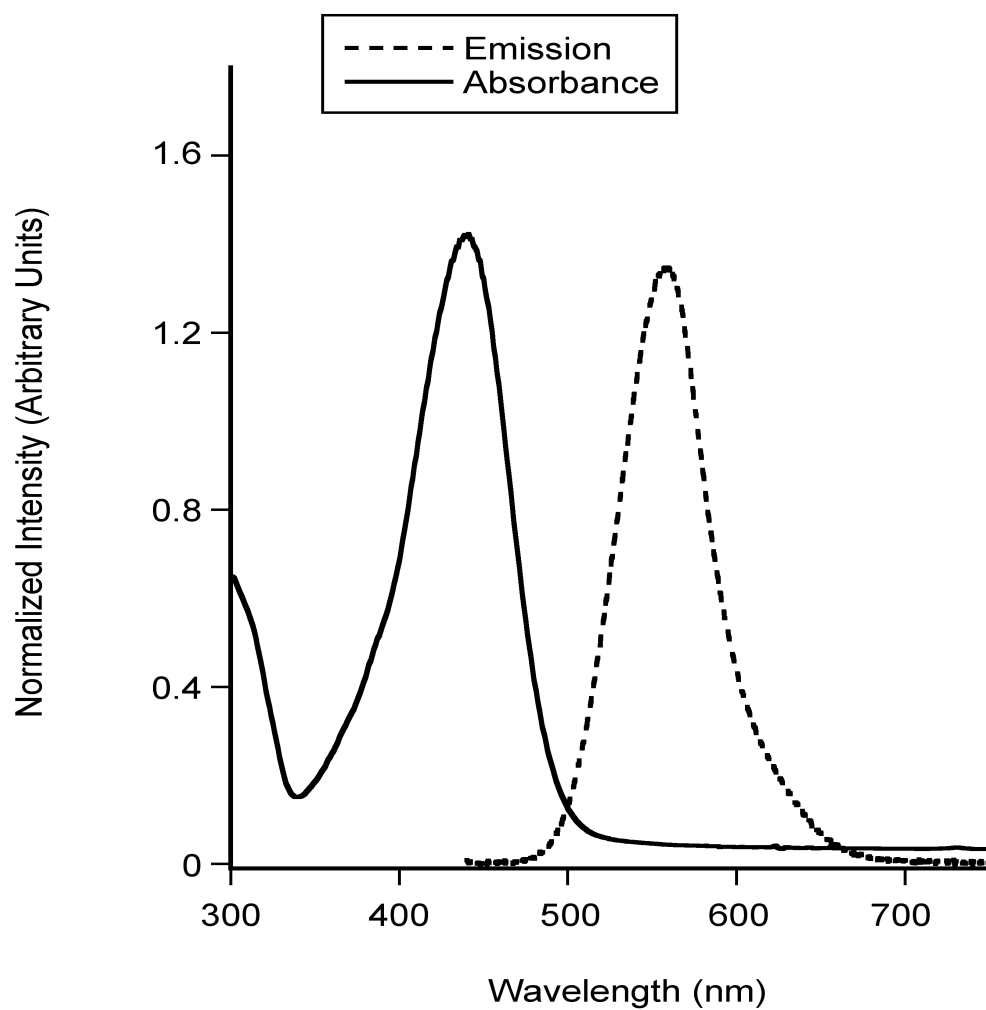
Figure B-15. Absorption and emission spectra of **6** in dry CH₃CN.
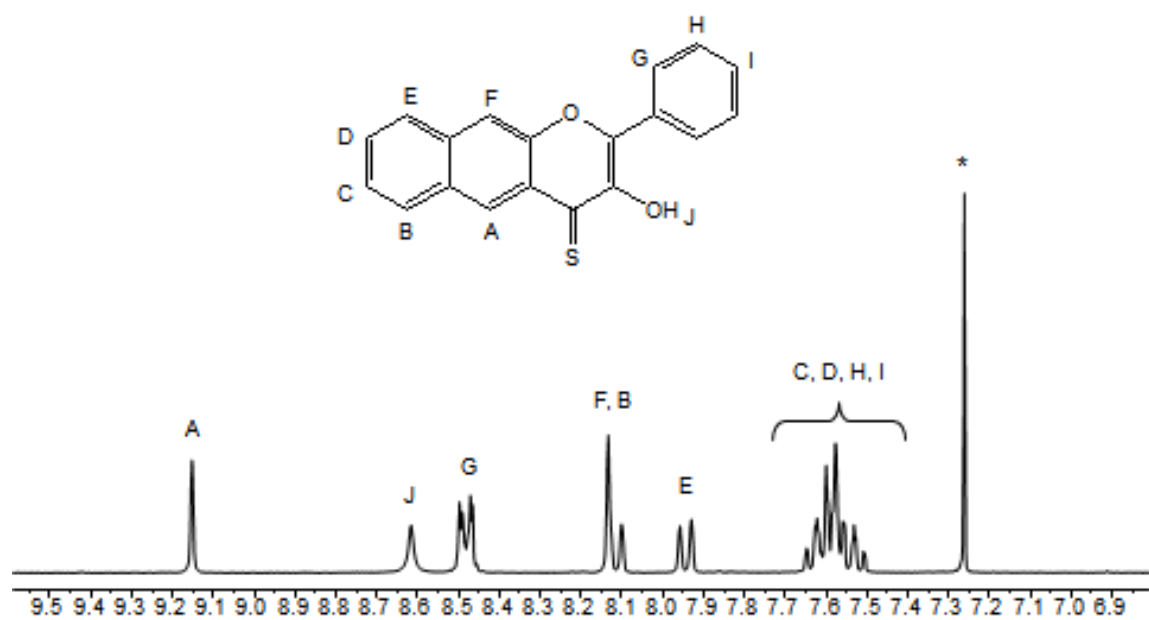
Figure B-16. ^{1}H NMR spectrum of **7** in CDCl$_3$. The * indicates the residual signal of the solvent (C*H*Cl$_3$).
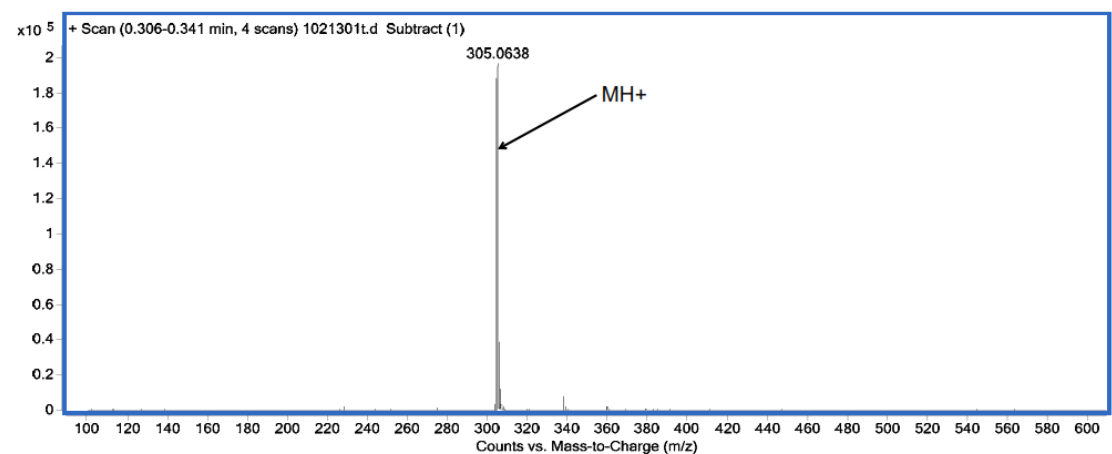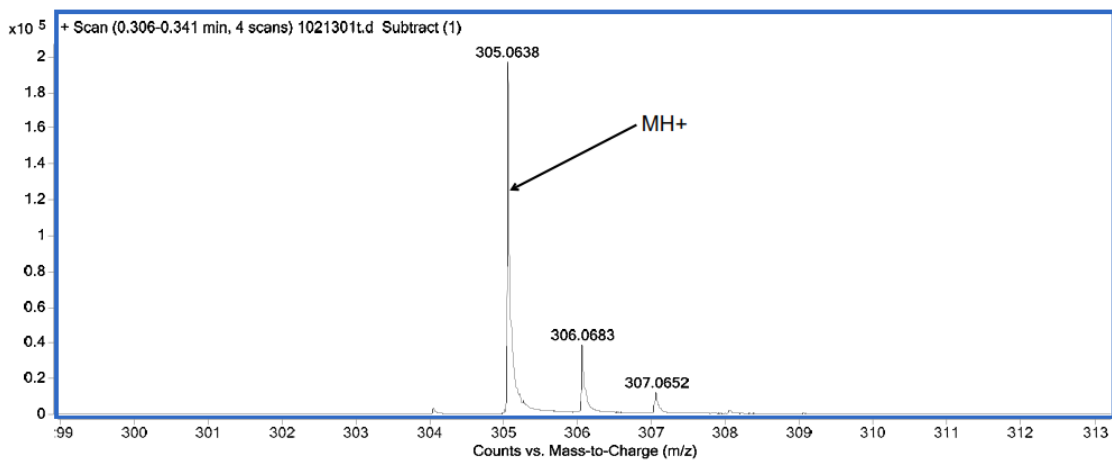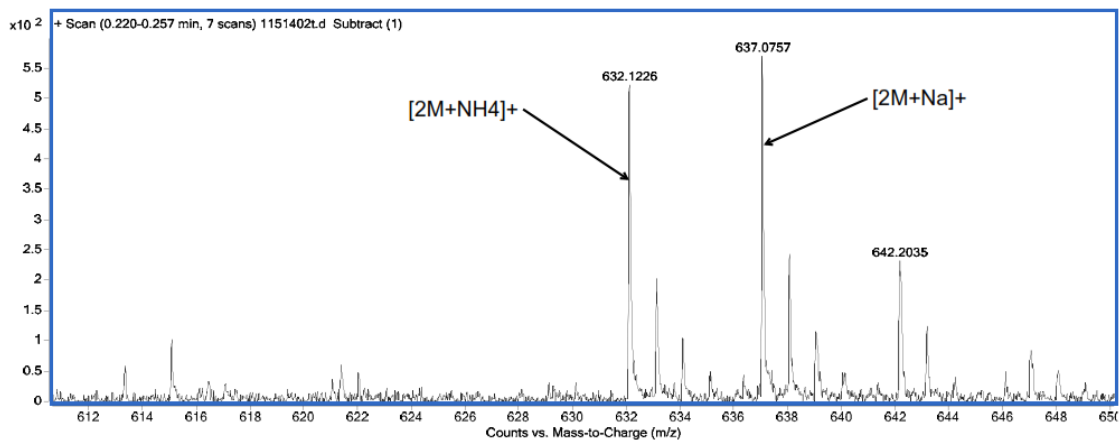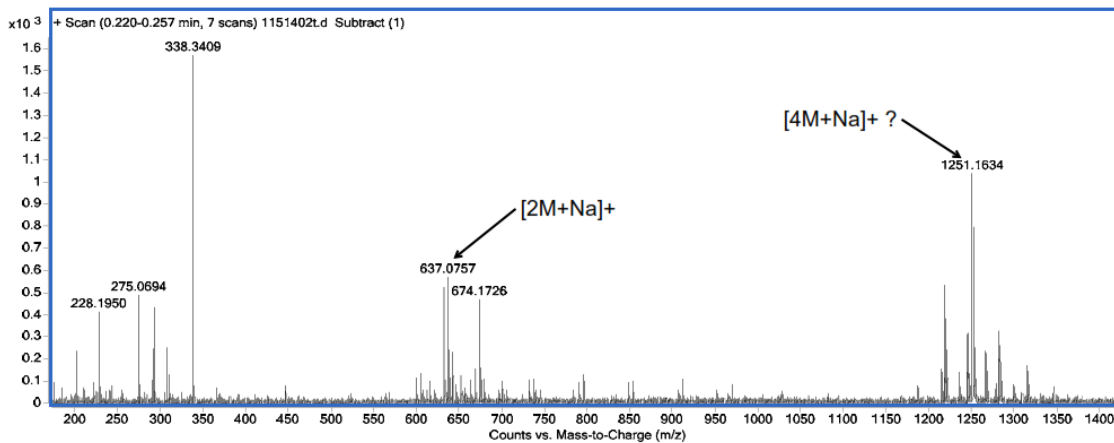
Measured Mass   305.0638

Element	Low Limit	High Limit
C	17	25
H	5	25
O	0	3
S	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C19 H13 O2 S	305.0631	0.7	2.4	13.5

Figure B-17. ESI/APCI-MS of **7**.
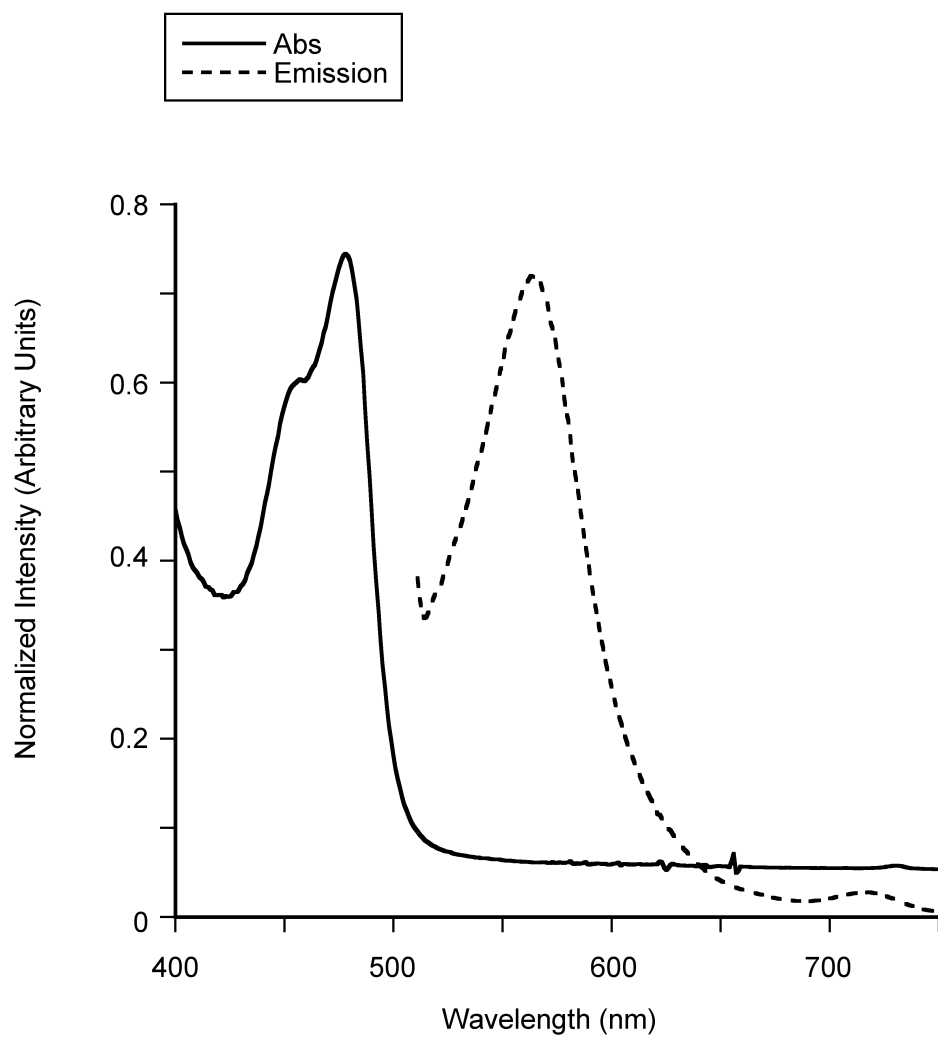
Figure B-18. Absorption and emission spectra of **7** in dry CH₃CN.
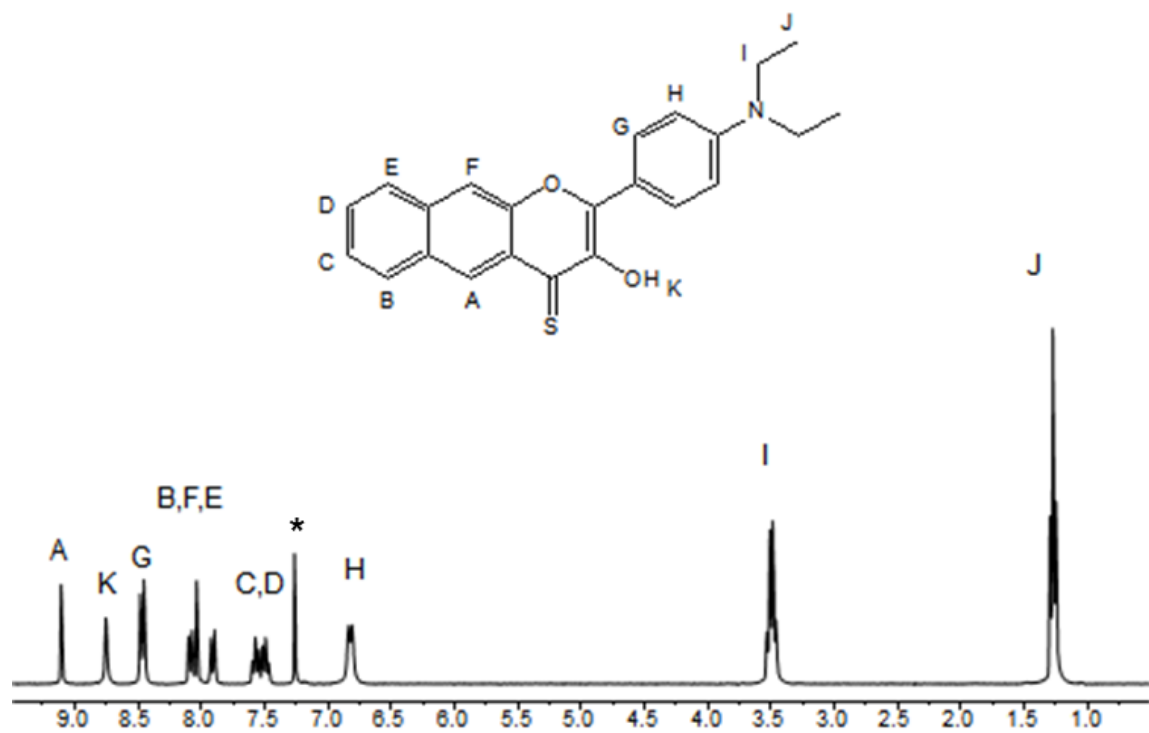
Figure B-19. ^{1}H NMR spectrum of **8** in CDCl₃. The * indicates the residual signal of the solvent (C*H*Cl₃).
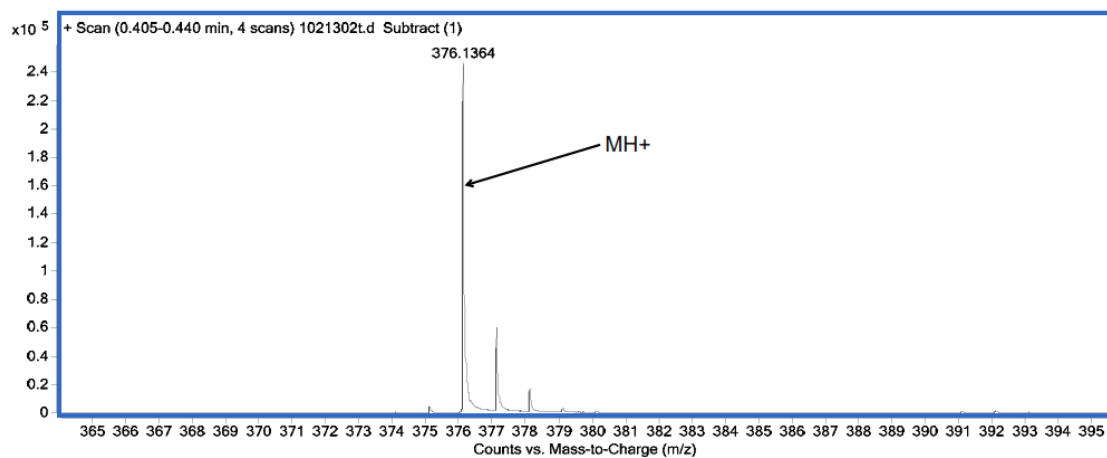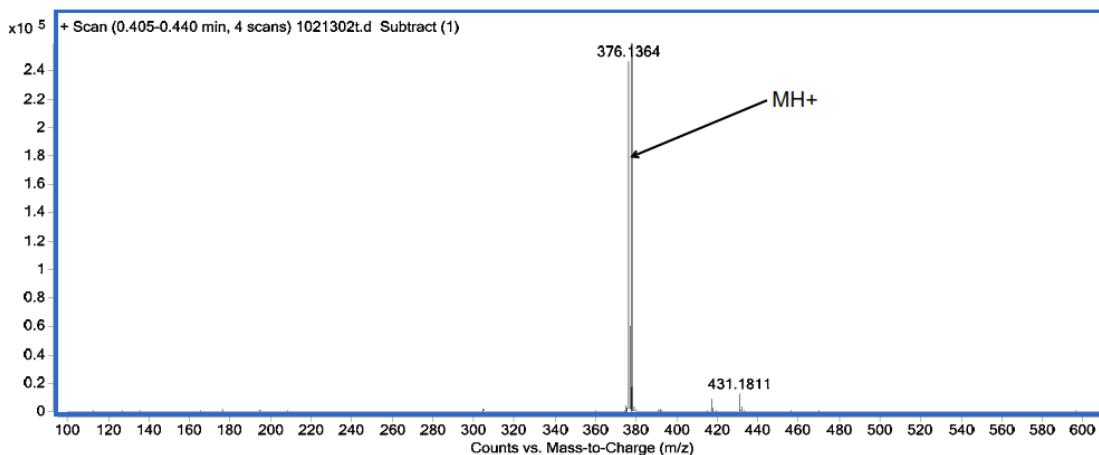
Measured Mass   376.1364

Element	Low Limit	High Limit
C	17	25
H	5	25
O	0	3
S	0	1
N	0	2

Formula	Calculated Mass	mDaError	ppmError	RDB
C23 H22 N O2 S	376.1366	-0.2	-0.5	13.5

Figure B-20. ESI/APCI-MS of **8**.
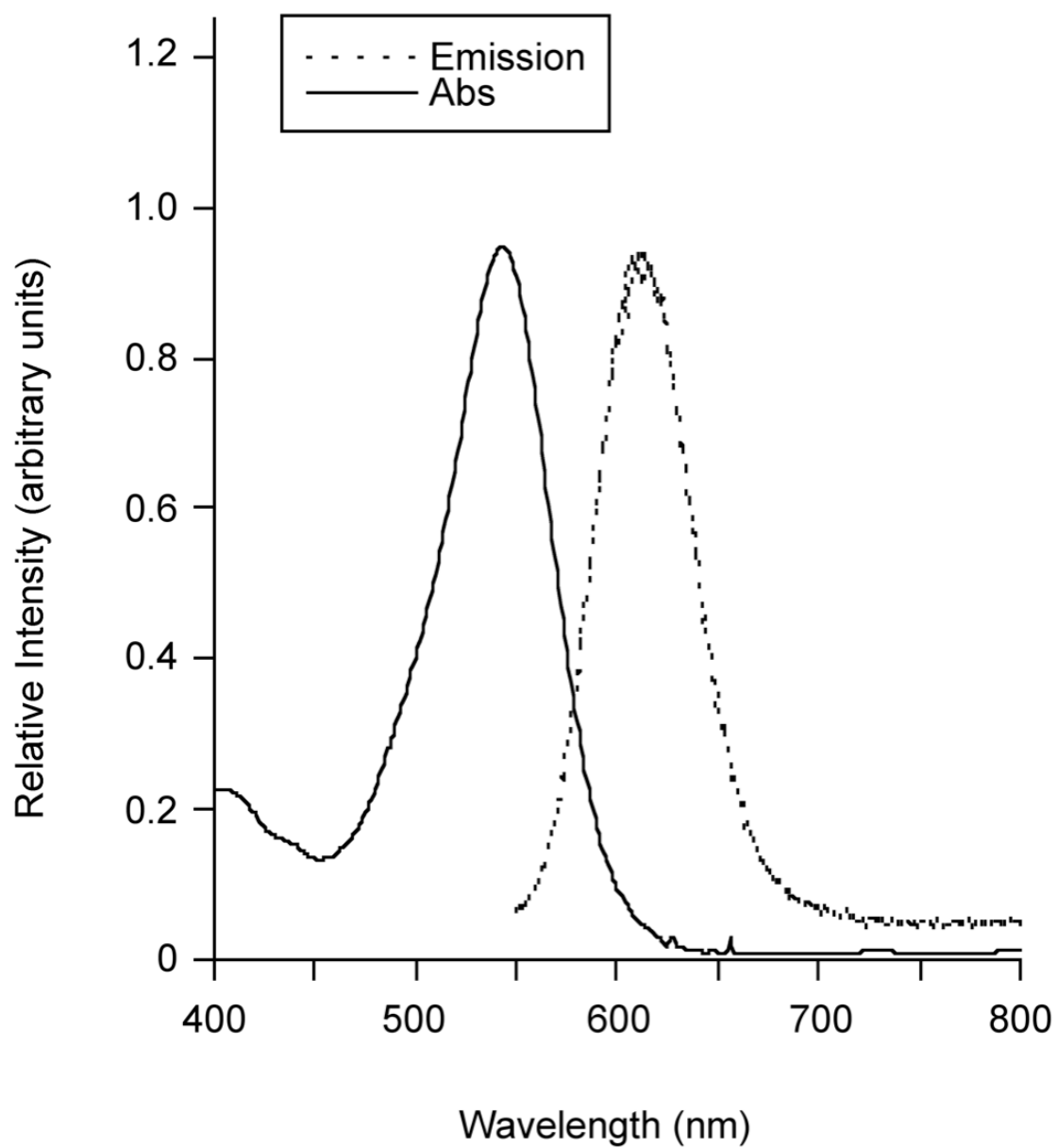
Figure B-21. Absorption and emission spectra of **8** in dry CH3CN.
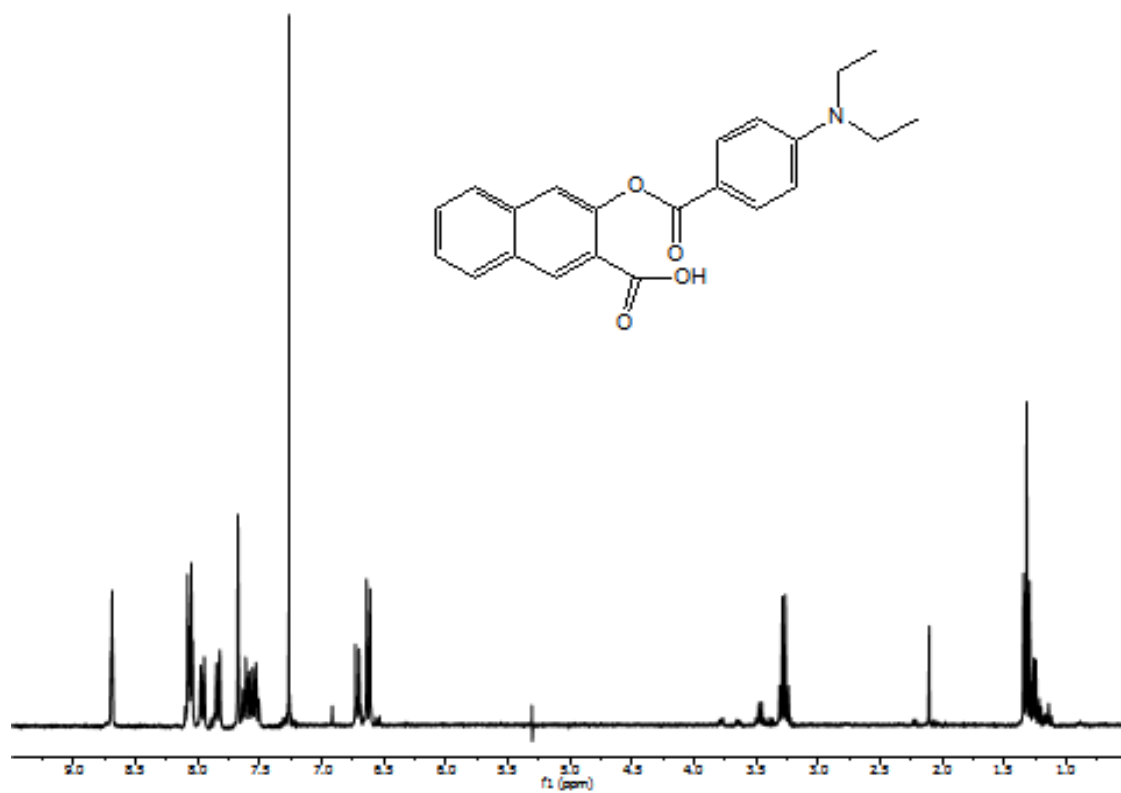
Figure B-22. ^{1}H NMR spectrum of **9** in CDCl$_3$. The * indicates the residual signal of the solvent (C*H*Cl$_3$).
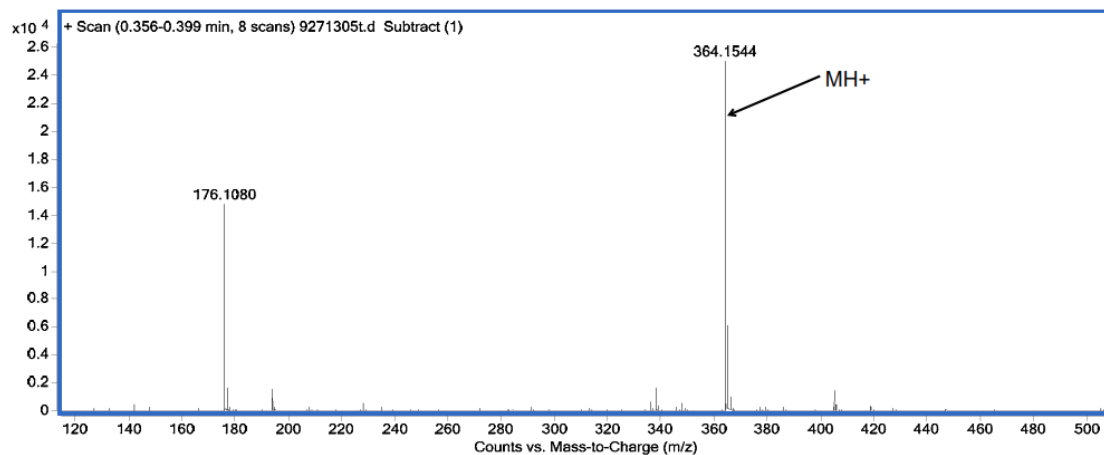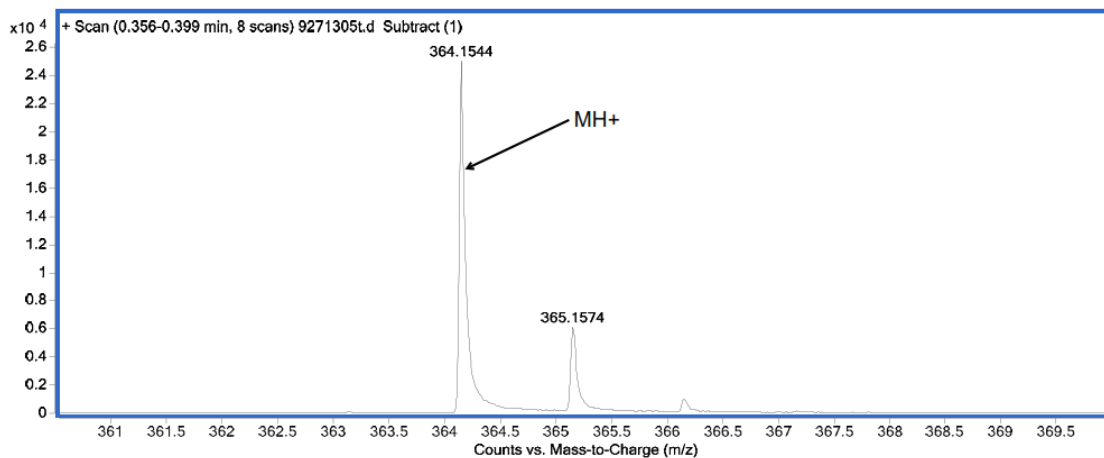
Measured Mass   364.1544

Element	Low Limit	High Limit
C	17	27
H	15	35
N	0	2
O	2	5

Formula	Calculated Mass	mDaError	ppmError	RDB
C22 H22 N O4	364.1543	0.1	0.2	12.5

Figure B-23. ESI/APCI-MS of **9**.
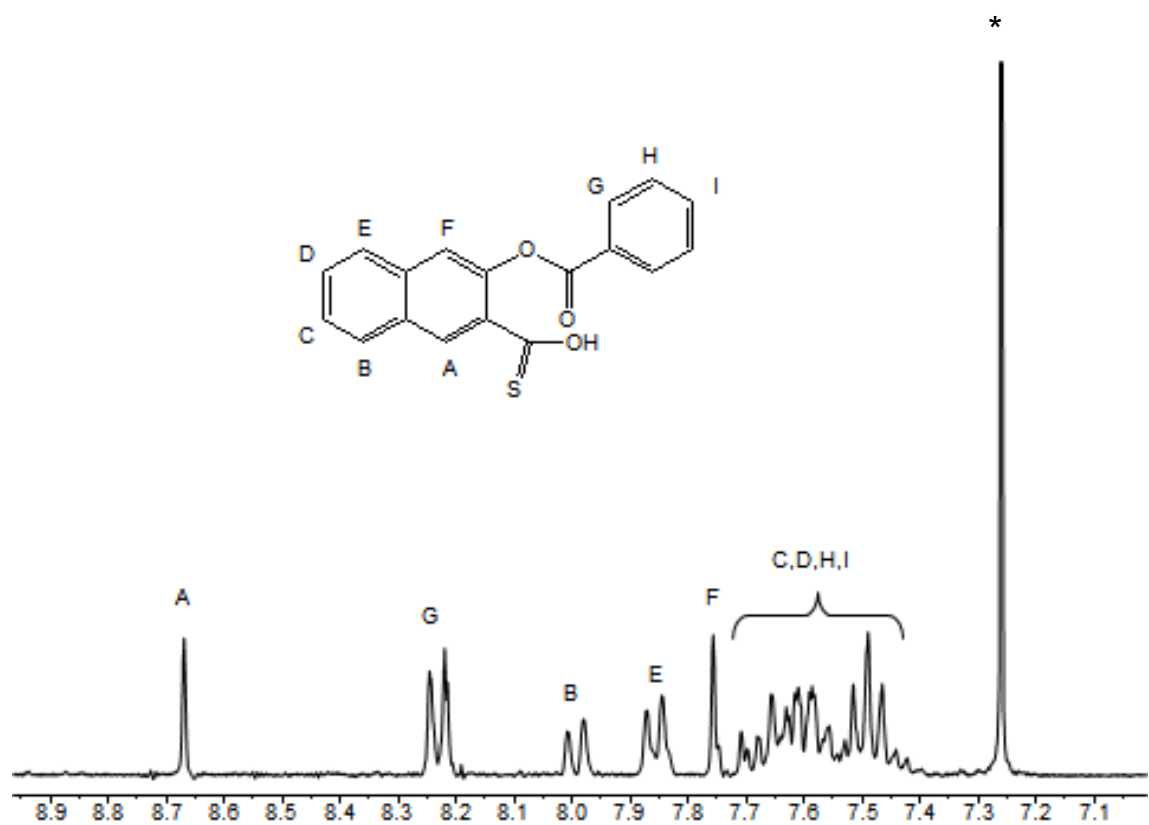
Figure B-24. ^{1}H NMR spectrum of **10** in CDCl$_3$. The * indicates the residual signal of the solvent (C*H*Cl$_3$).

Measured Mass	637.0757				

Element	Low Limit	High Limit
C	30	40
H	15	35
O	4	8
S	0	2
Na	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C36 H22 O6 Na S2	637.0750	0.7	1.1	25.5
C38 H21 O6 S2	637.0774	-1.7	-2.7	28.5

Figure B-25. ESI/APCI-MS of **10**. The results provide evidence for the oxidized disulfide form of **10** (see drawing below).
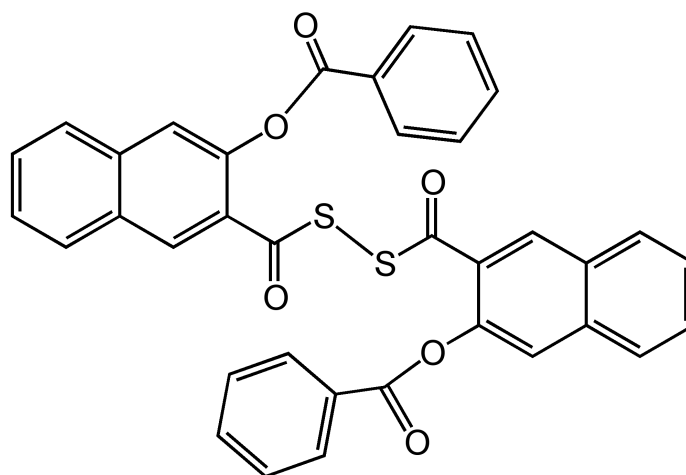
Figure B-26. Proposed structure of oxidized disulfide form of **10.**
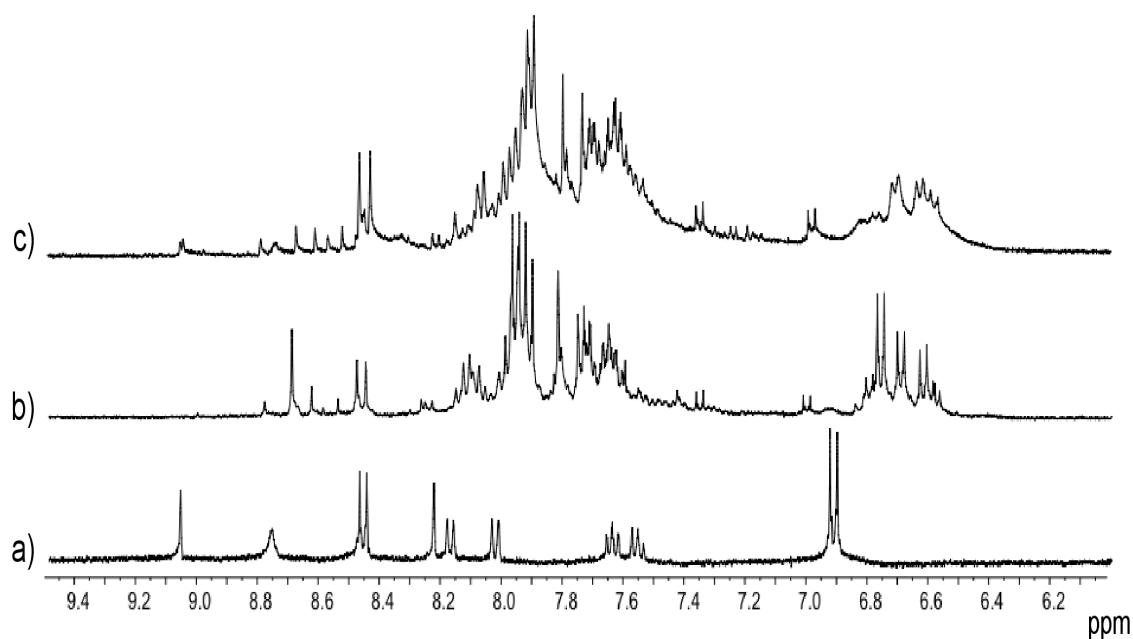
Figure B-27. ^{1}H NMR spectra of **8** demonstrating anaerobic

photoreactivity. a) Aromatic region of the ^{1}H NMR spectrum of **8** in CD$_3$CN.

b) ^{1}H NMR spectrum after 24 h of irradiation (λ_{max} > 546 nm) of **8** in

CD$_3$CN under anaerobic conditions. c) ^{1}H NMR spectrum after 24 h of

irradiation (λ_{max} > 546 nm) of **8** in CD$_3$CN under aerobic conditions.
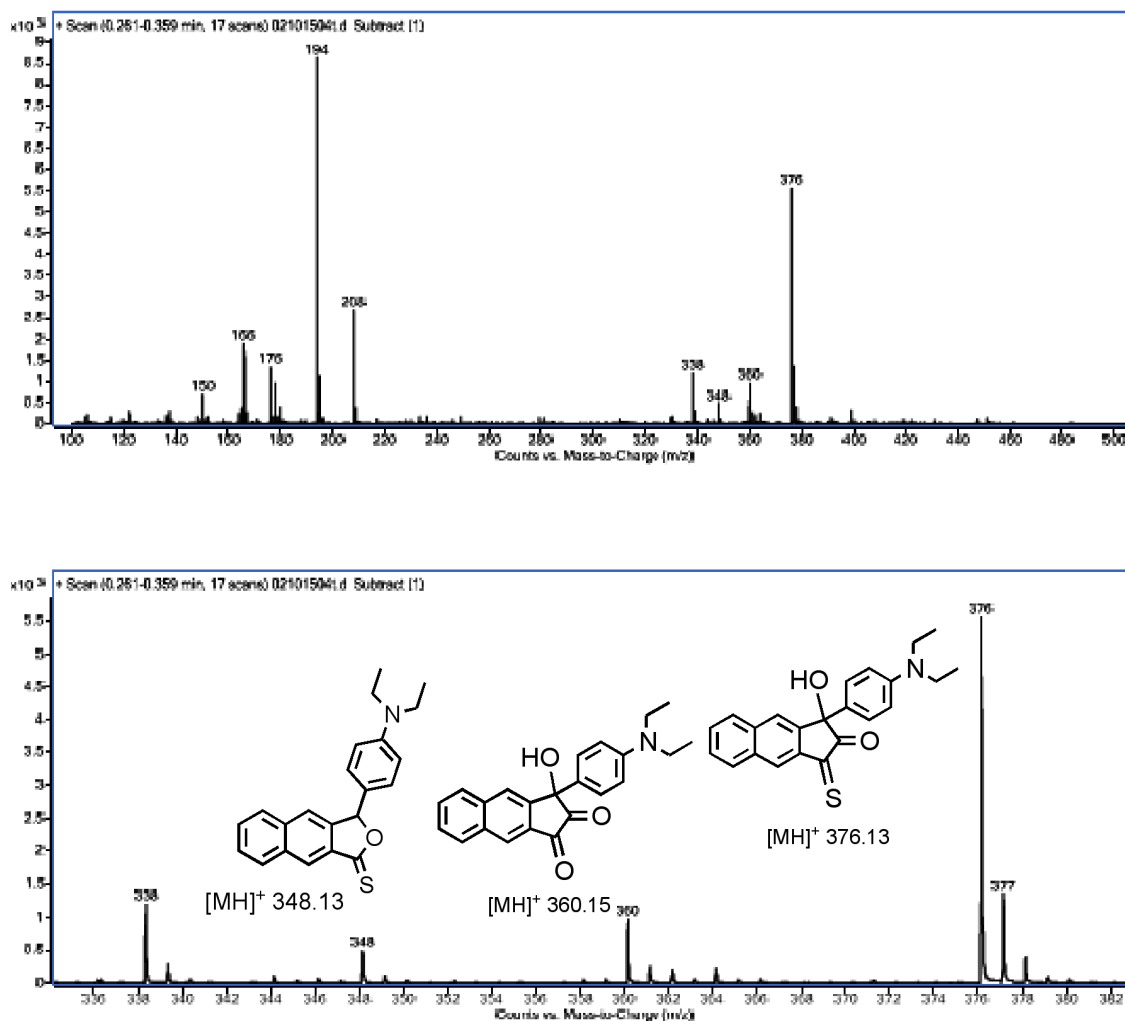
Figure B-28. ESI/APCI-MS of the reaction mixture produced upon irradiation of **8** in CH$_3$CN (λ_{max} > 546 nm) under anaerobic conditions. The observed ions suggest an isomerization pathway leading to CO release. Under aerobic conditions, the *m/z* 376 is observed, along with an insoluble precipitate.

APPENDIX C

CHAPTER 4 SUPPLEMENTARY MATERIAL
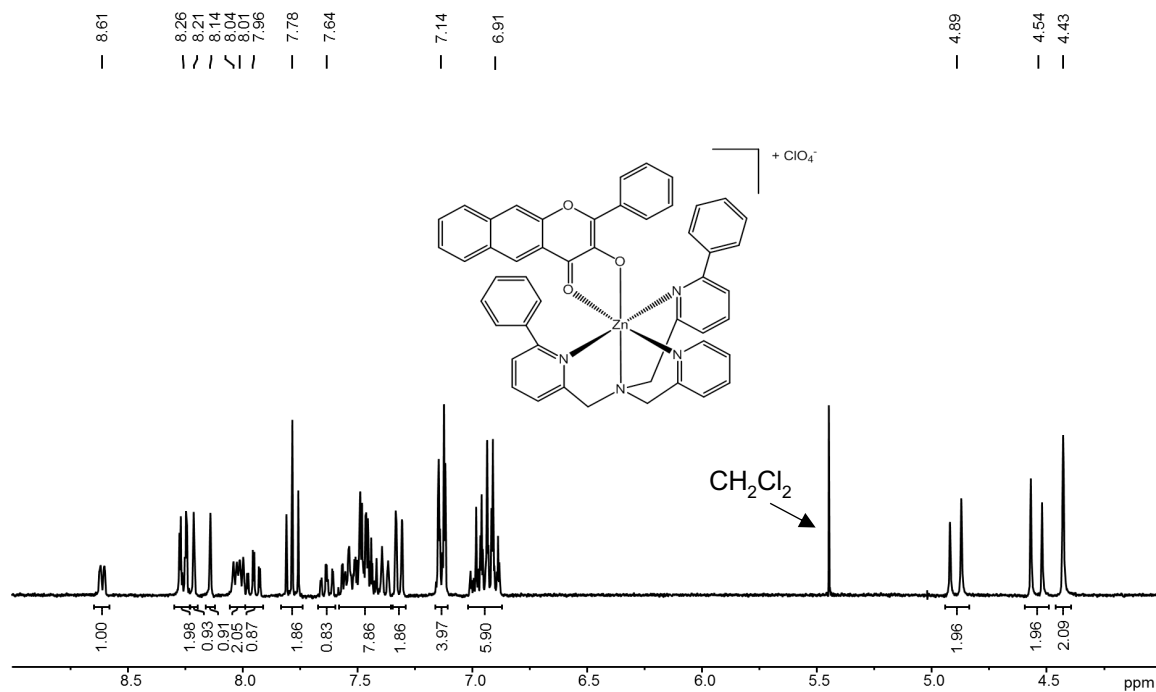
Figure C-1. ^{1}H NMR of **5** in CD$_3$CN.
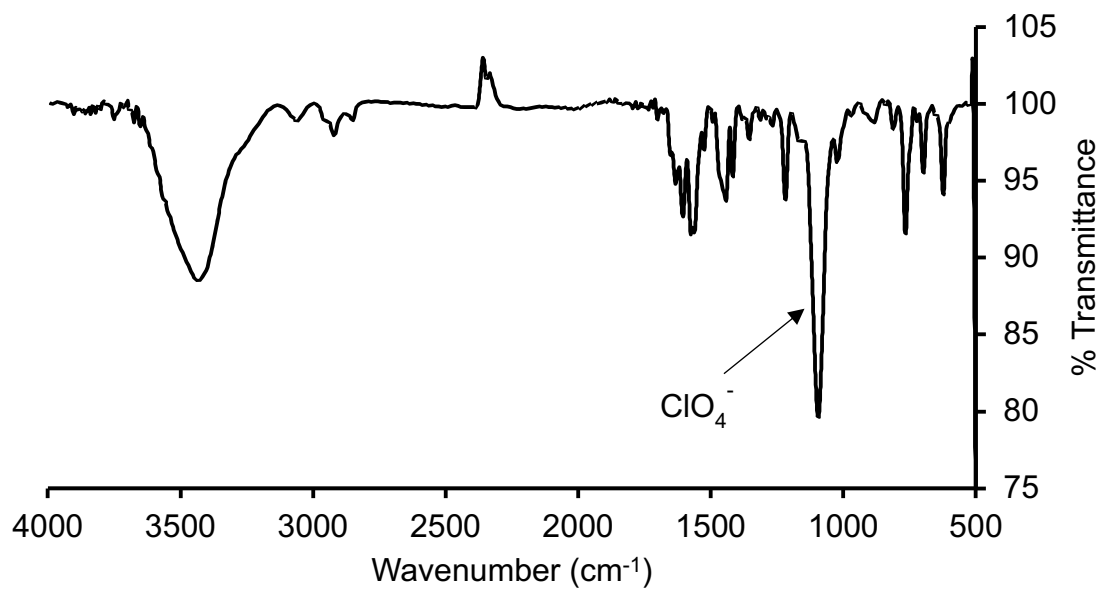
Figure C-2. FT-IR of **5** in KBr.
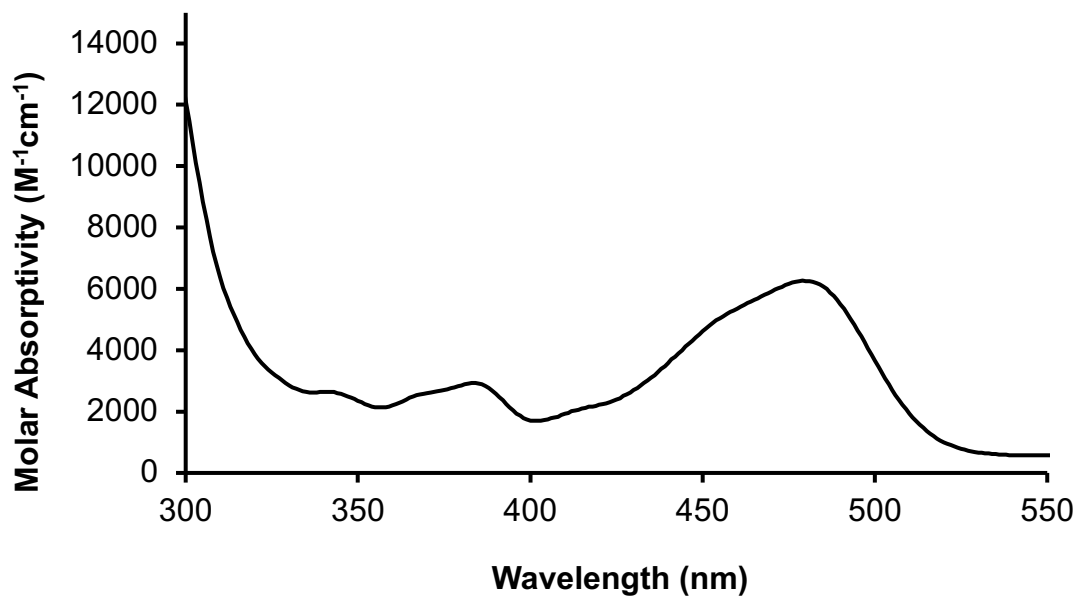
Figure C-5. ESI/APCI MS of **5**.
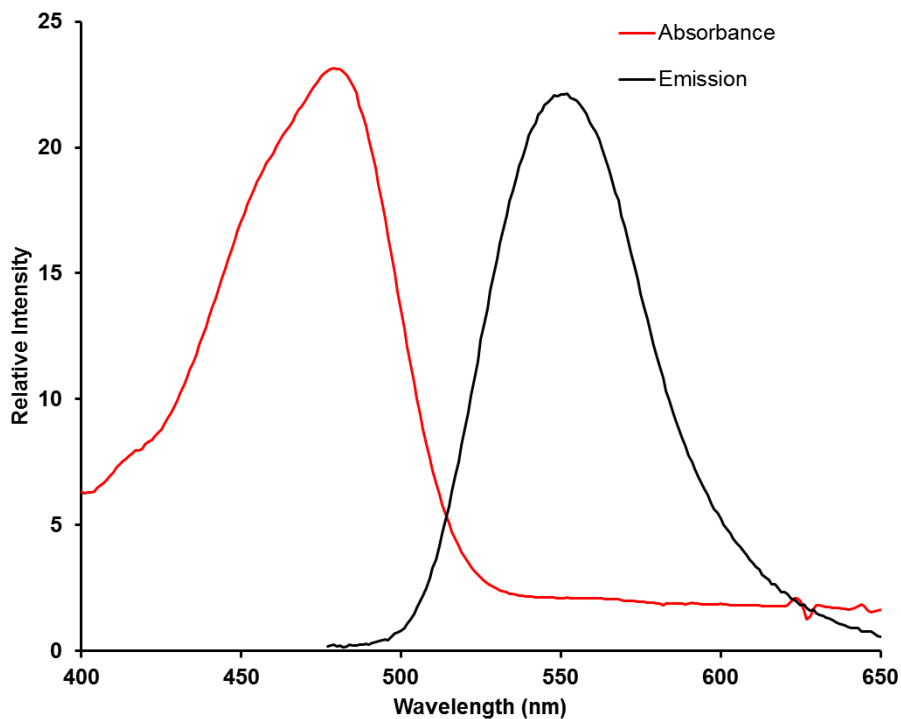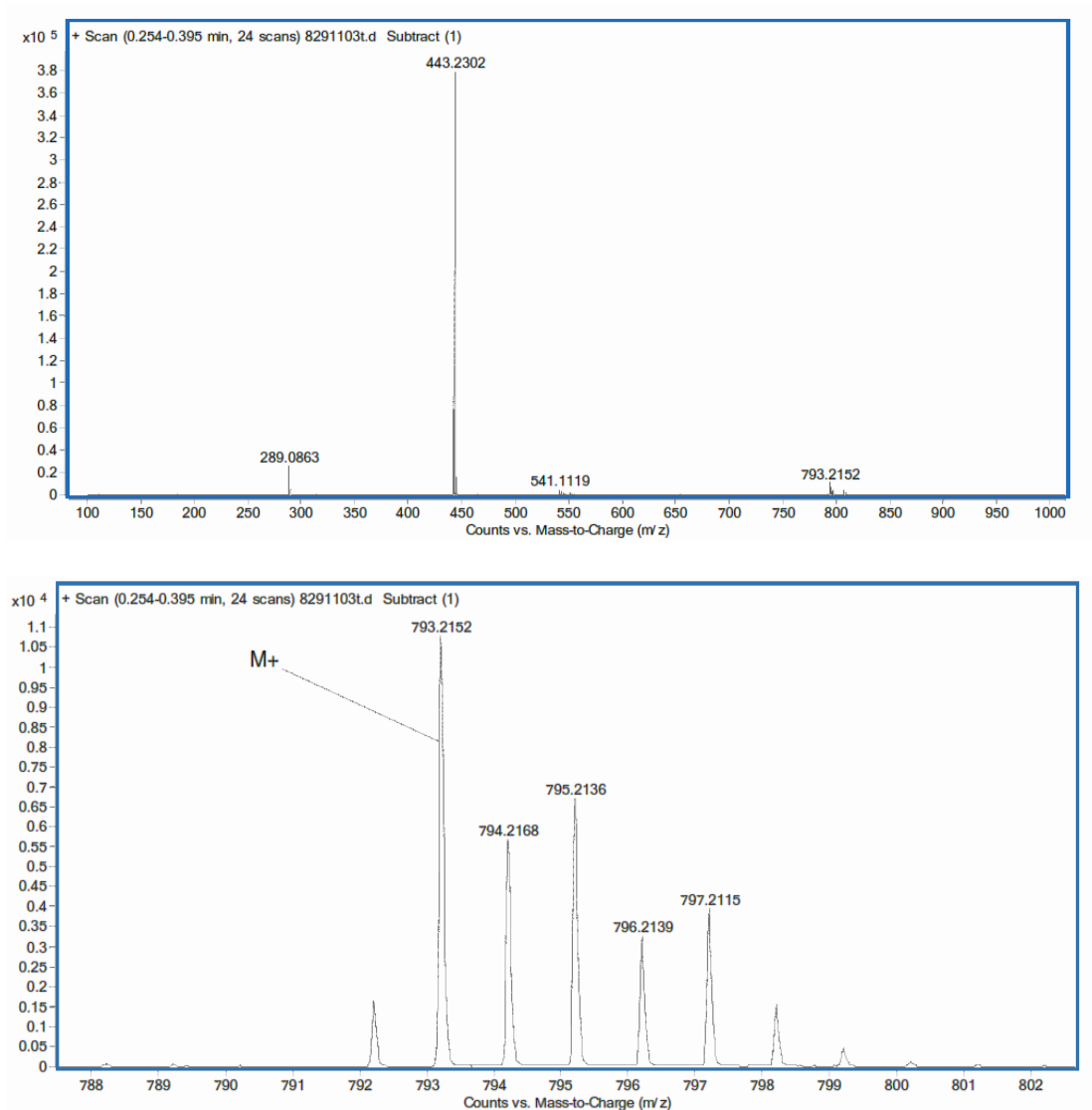
Measured Mass   793.2152

Element	Low Limit	High Limit
C	44	54
H	30	50
N	2	6
O	0	5
Zn	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C49 H37 N4 O3 Zn	793.2152	0.0	0.0	33.5
C52 H35 N5 Zn	793.2178	-2.6	-3.3	38
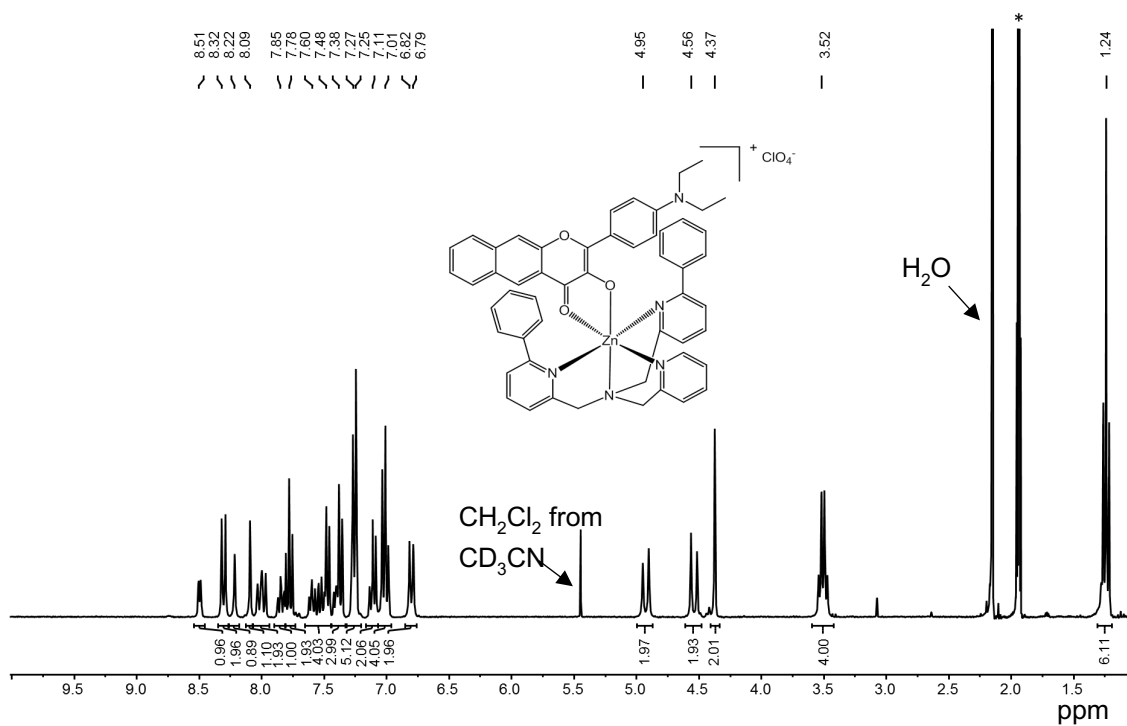
Figure C-6. ^{1}H NMR spectrum of **6** in CD$_3$CN. (* denotes residual solvent)
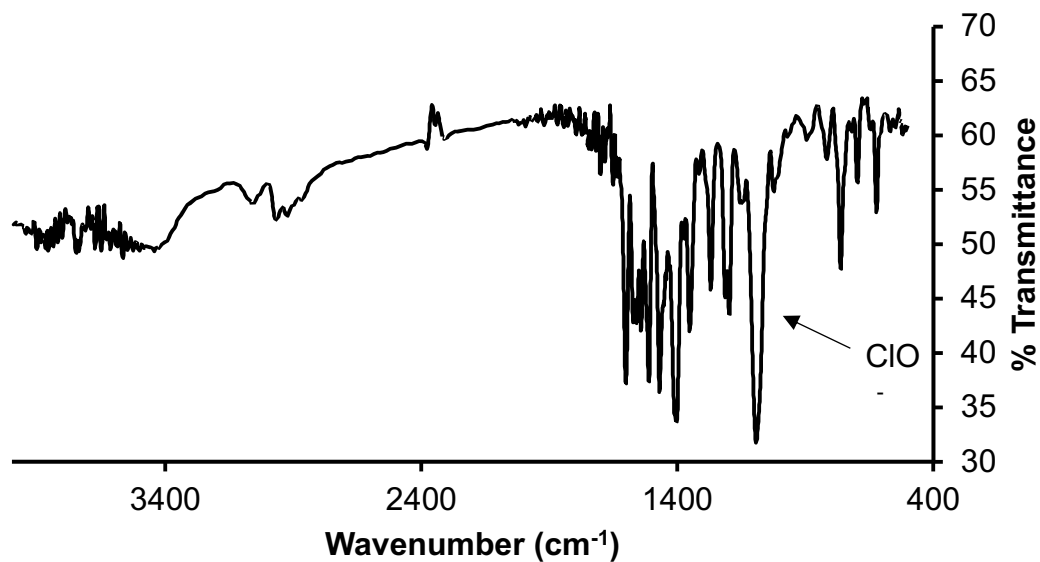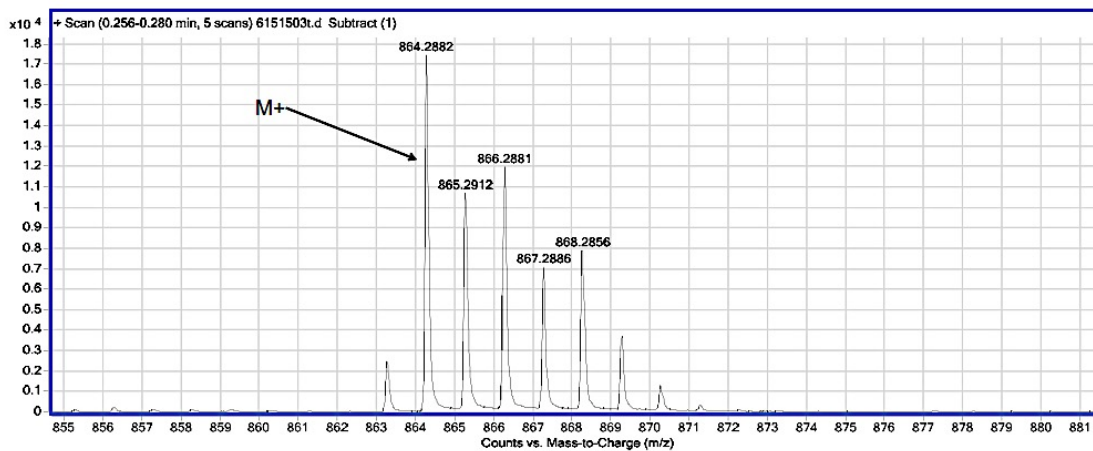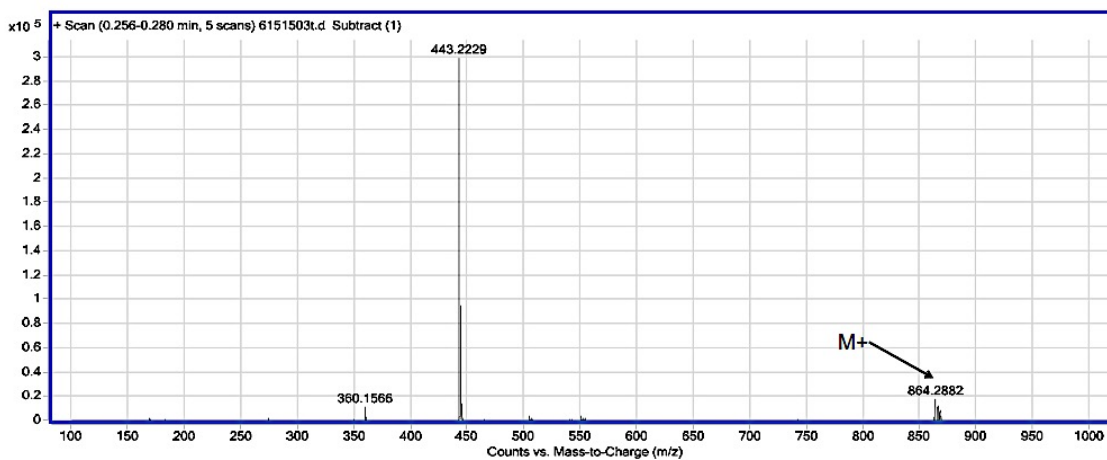

Figure C-7. FT-IR of **6** in KBr.
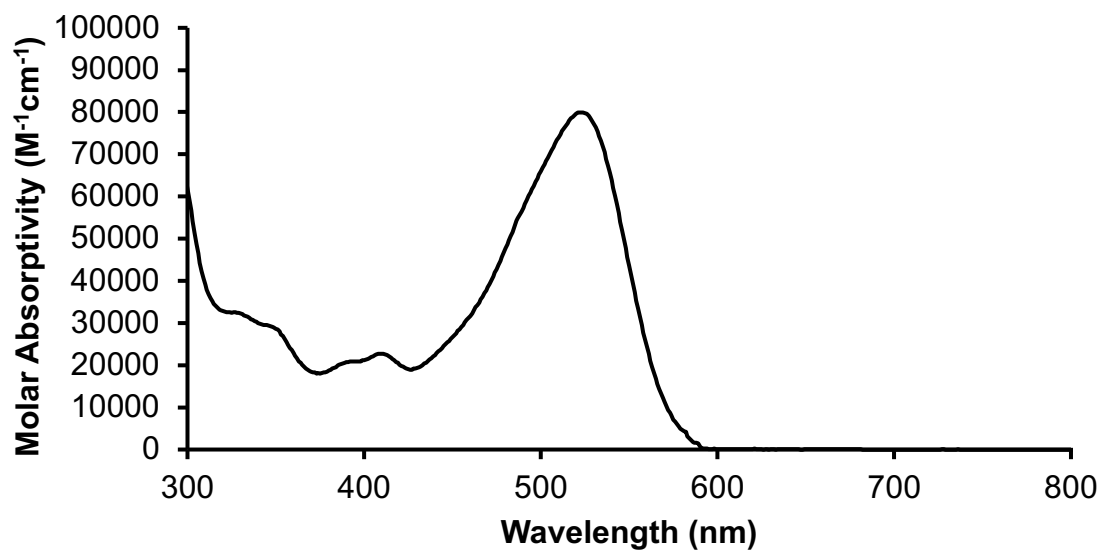
Figure C-8. Absorption spectrum of **6** in acetonitrile.
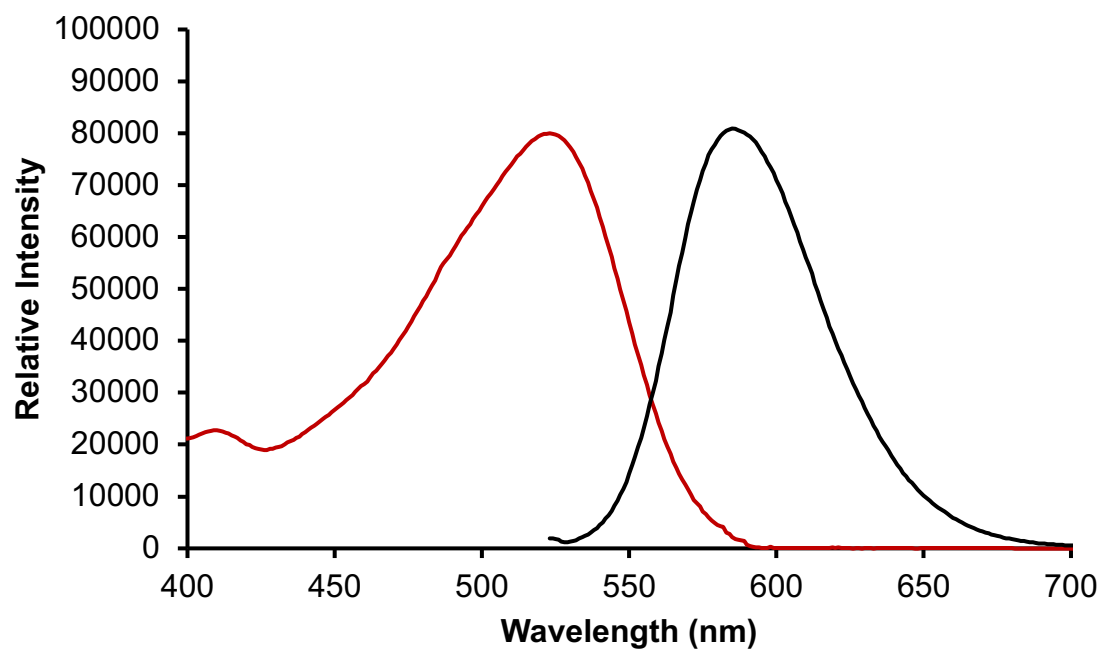
Figure C-9. Overlay of the lowest energy absorption feature of **6** with the

emission spectrum in acetonitrile.

Measured Mass		864.2882		

Element	Low Limit	High Limit		
C	48	58		
H	35	55		
N	3	7		
O	2	6		
Zn	0	1		

Formula	Calculated Mass	mDaError	ppmError	RDB
C53 H46 N5 O3 Zn	864.2887	-0.5	-0.5	33.5
C50 H48 N4 O6 Zn	864.2860	2.2	2.6	29
C48 H46 N7 O5 Zn	864.2846	3.6	4.1	29.5
C58 H36 N6 O3	864.2843	3.9	4.5	44

Figure C-10. ESI/APCI MS of **6**.
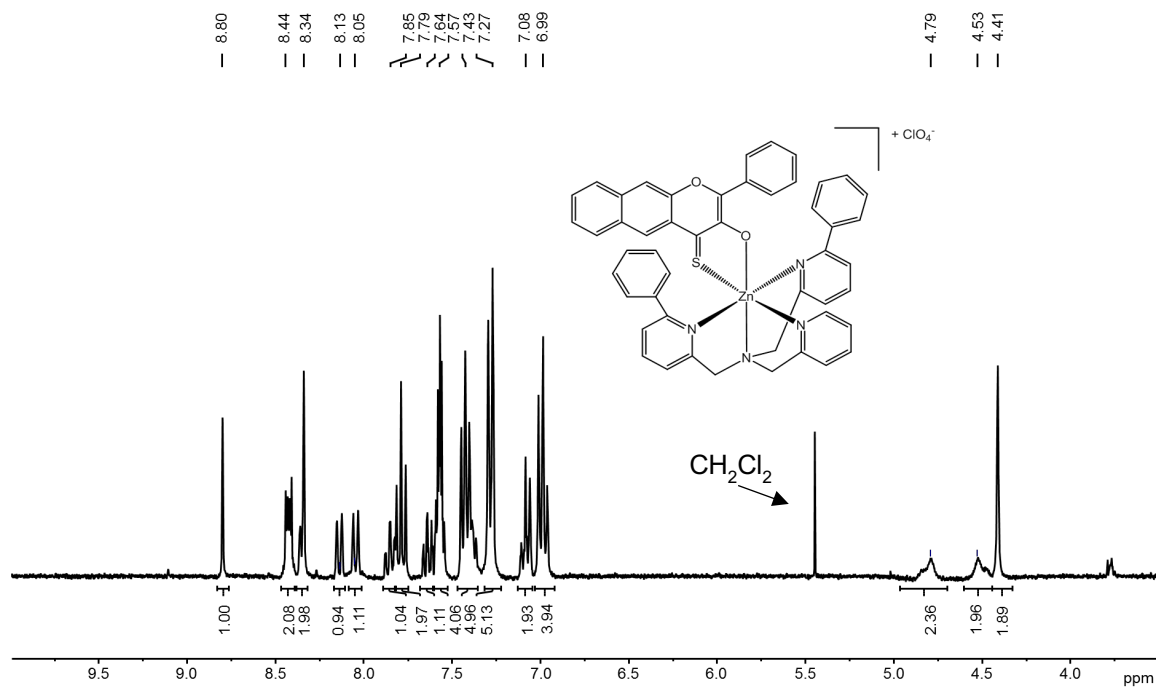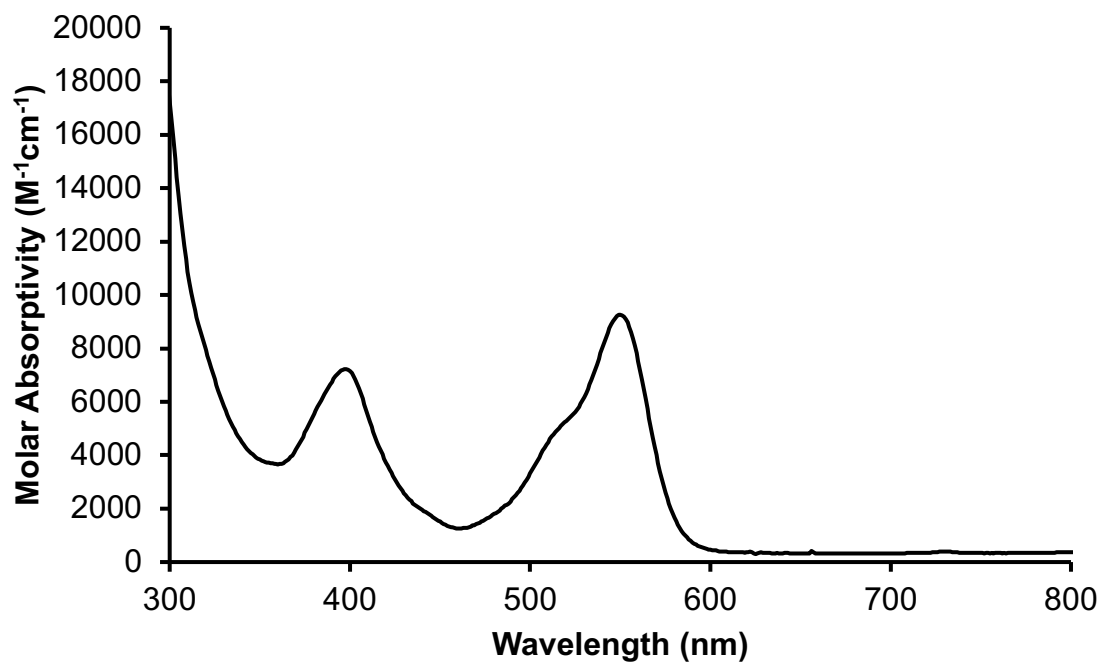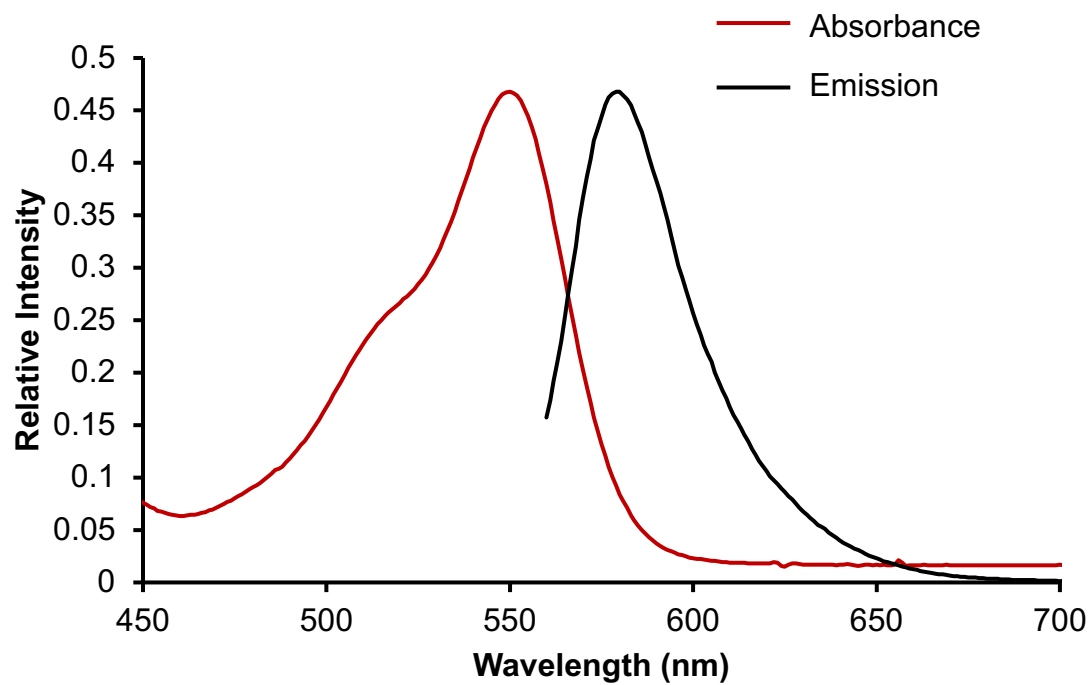
Figure C-11. ^{1}H NMR of **7** in CD$_3$CN.
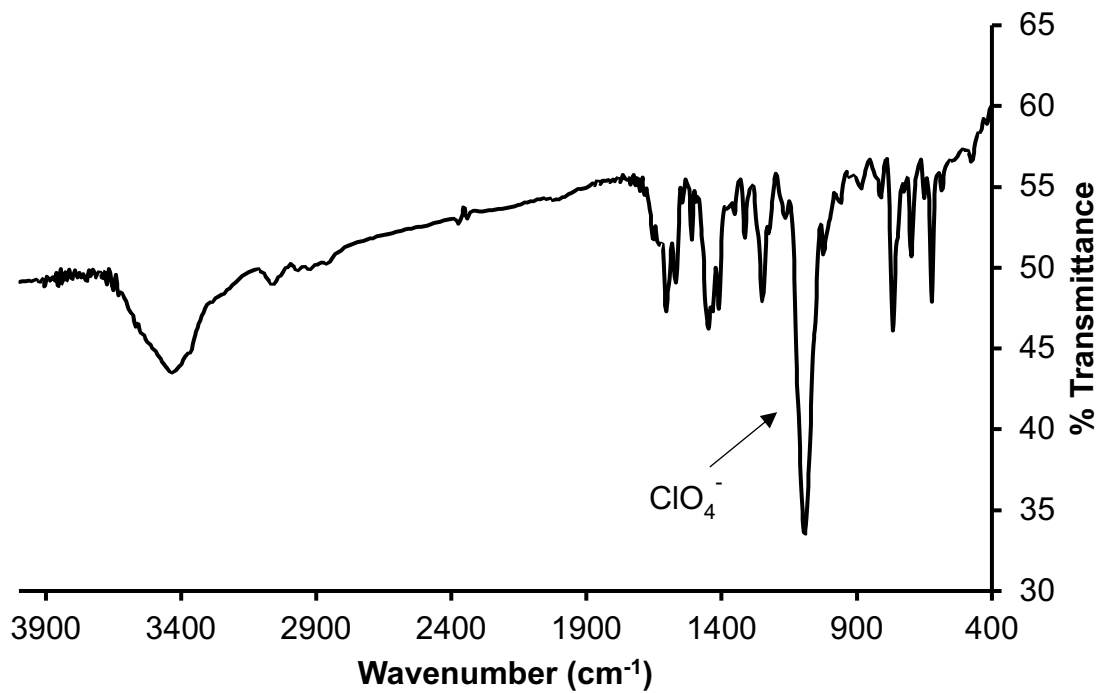
Figure C-12. FT-IR of **7** in KBr.

Figure C-13. Absorption spectrum of **7** in acetonitrile

Figure C-14. Overlay of the lowest energy absorption feature of **7** with the

emission spectrum in acetonitrile.

Measured Mass 809.193

Element	Low Limit	High Limit
C	44	54
H	30	50
N	0	5
O	0	3
S	0	1
Zn	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C51 H39 N O3 S Zn	809.1937	-0.7	-0.8	33
C49 H37 N4 O2 S Zn	809.1923	0.7	0.8	33.5
C54 H35 N O3 Zn	809.1903	2.7	3.3	38
C54 H37 N2 S Zn	809.1963	-3.3	-4.1	37.5

Figure C-15. ESI/APCI MS of **7**.
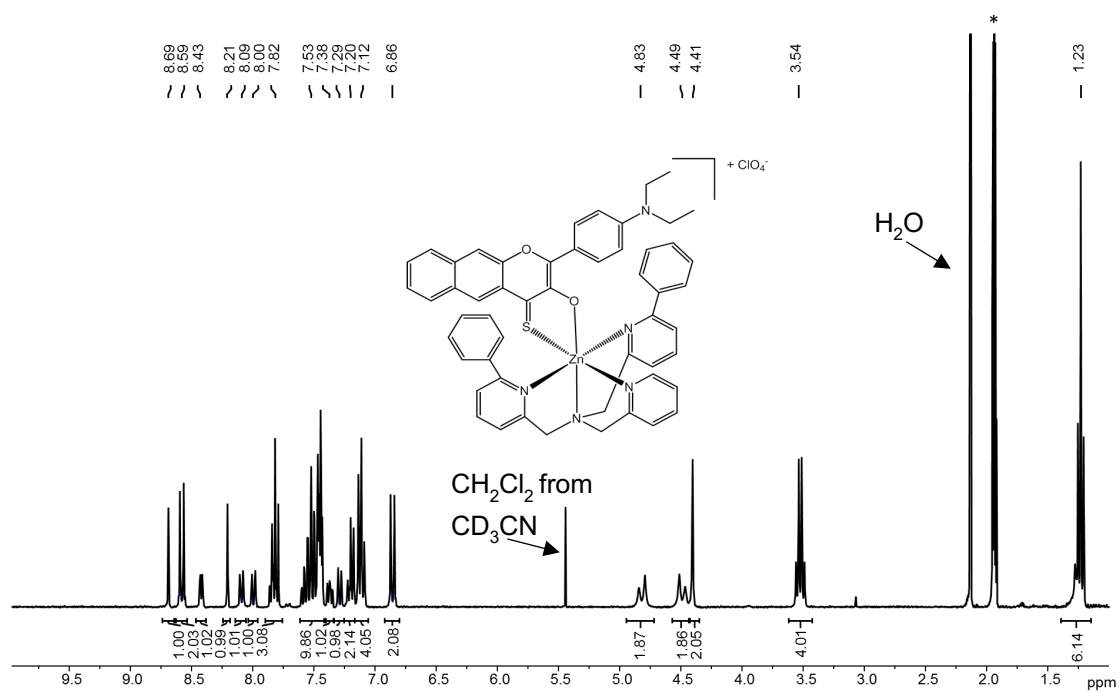
Figure C-16. ^{1}H NMR of **8** in CD$_3$CN. (* denotes residual solvent)
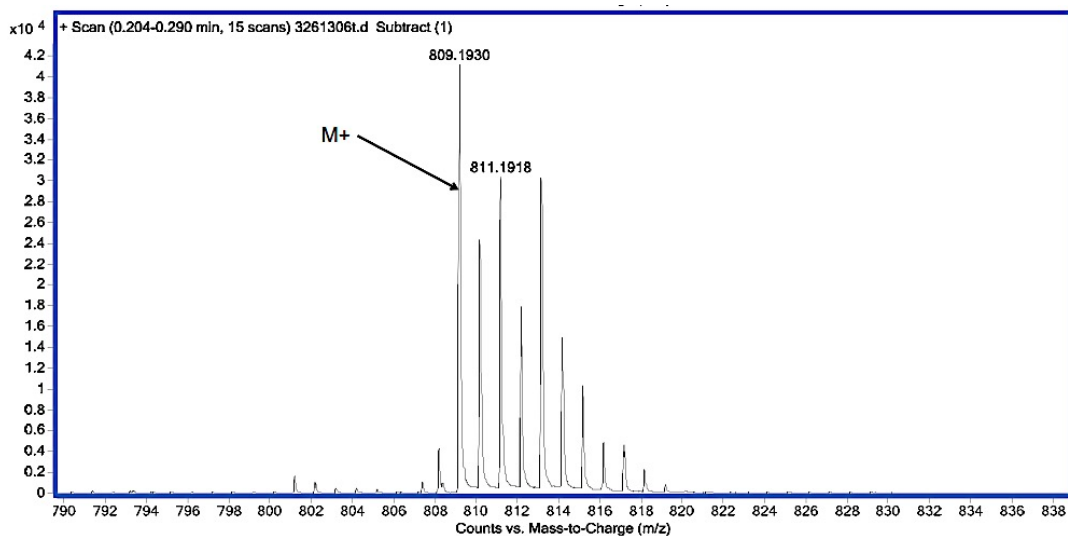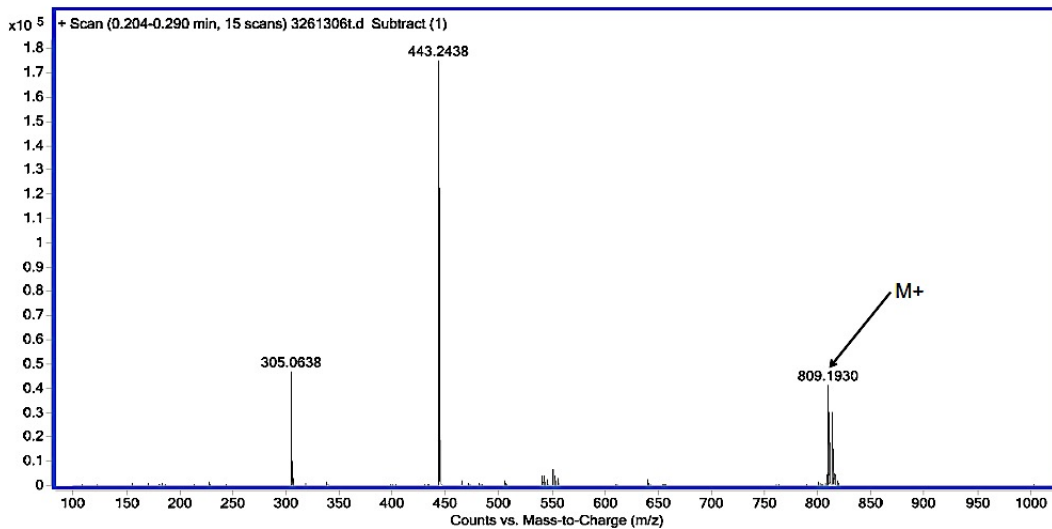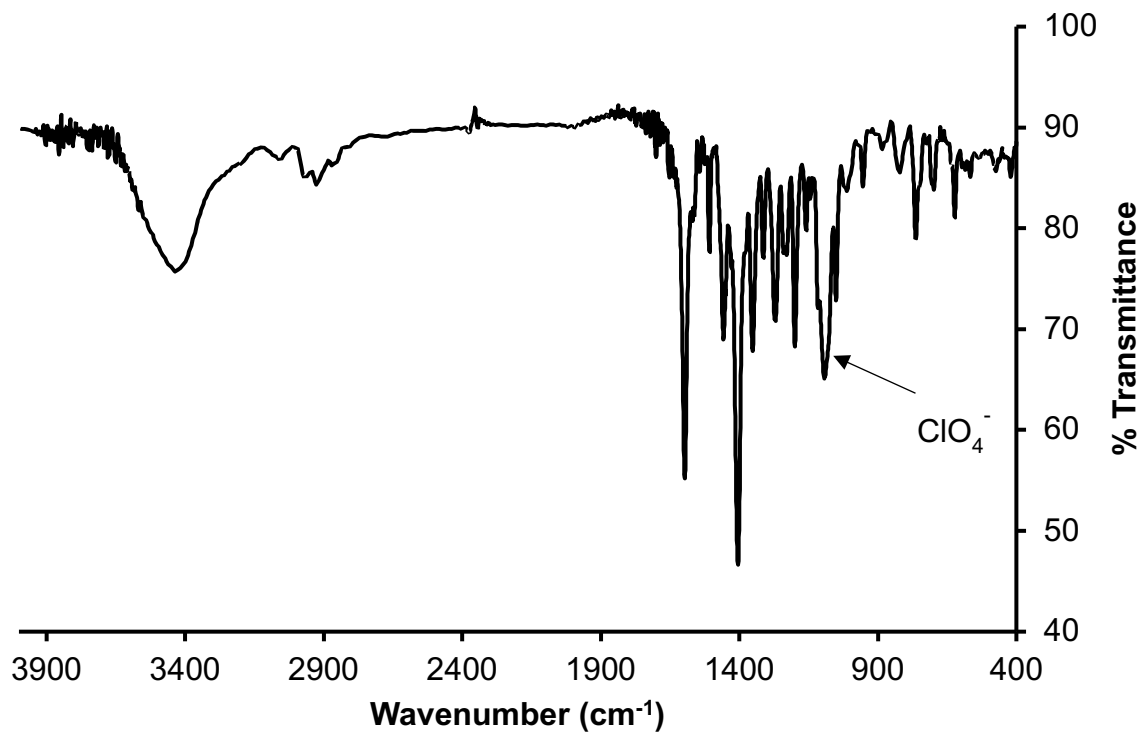

Figure C-17. FT-IR of **8** in KBr.
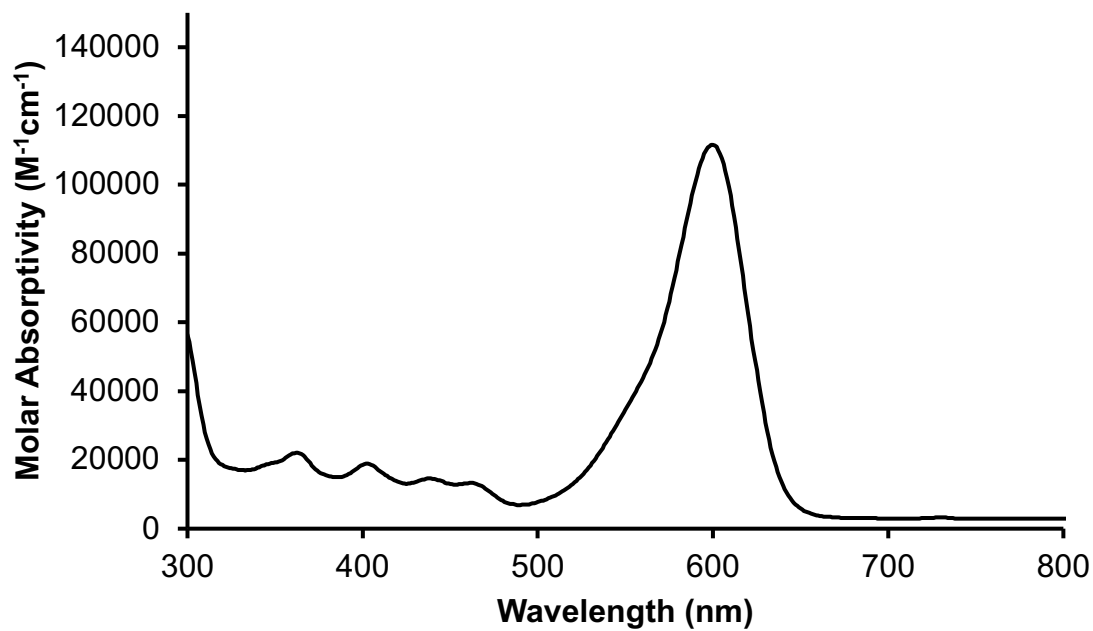
Figure C-18. Absorption spectrum of **8** in acetonitrile.
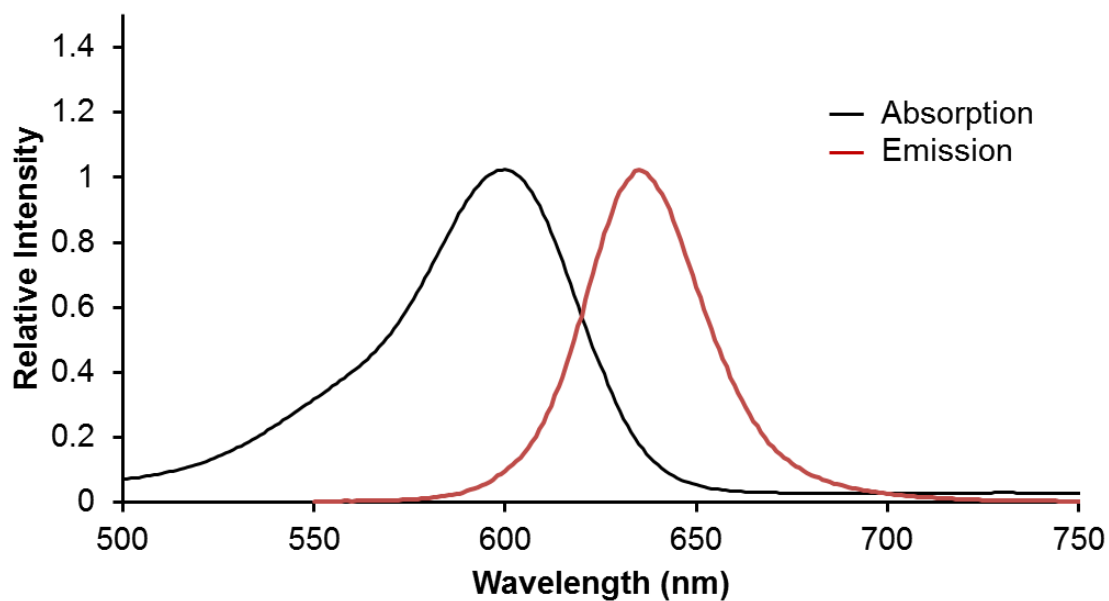
Figure C-19. Overlay of the lowest energy absorption feature of **8** with the

emission spectrum in acetonitrile
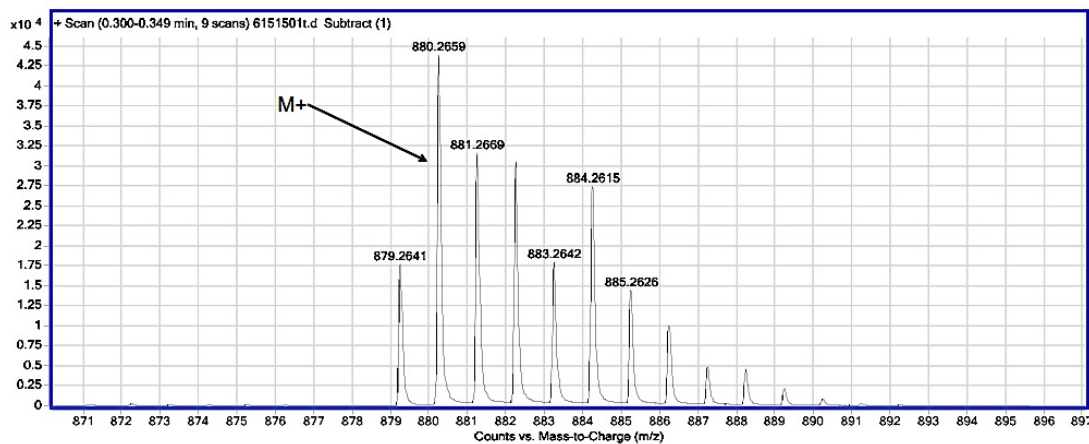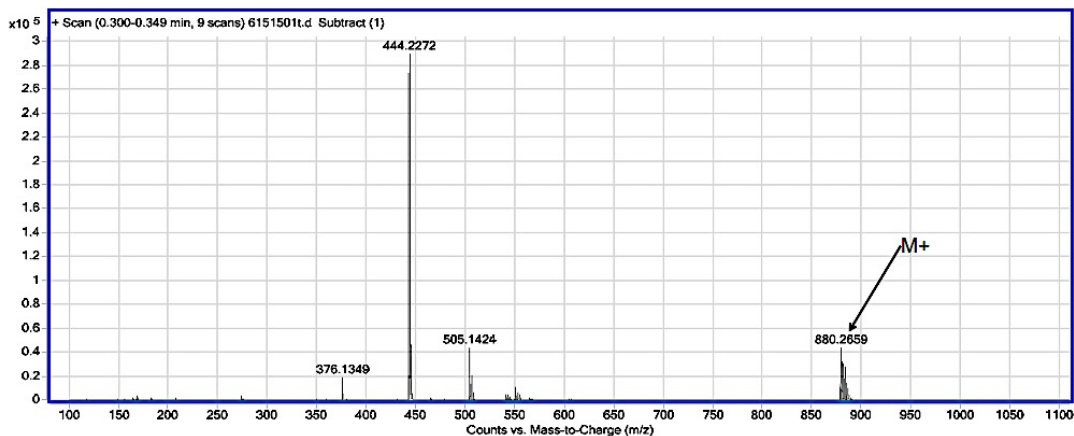
Measured Mass		880.2659		

Element	Low Limit	High Limit		
C	48	58		
H	35	55		
N	3	7		
O	0	3		
S	0	1		
Zn	0	1		

Formula	Calculated Mass	mDaError	ppmError	RDB
C53 H46 N5 O2 S Zn	880.2658	0.1	0.1	33.5
C56 H42 N5 O2 Zn	880.2624	3.5	3.9	38.5
C58 H46 N3 S Zn	880.2698	-3.9	-4.5	37.5

Figure C-20. ESI/APCI MS of **8**.
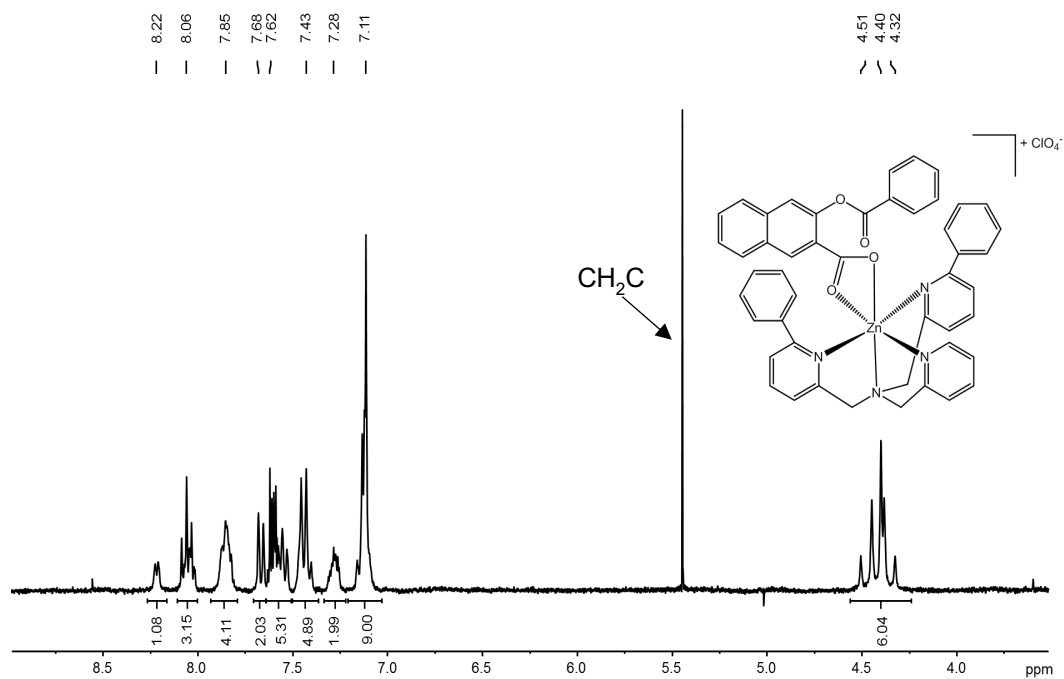
Figure C-21. ^{1}H NMR of **9** in CD$_3$CN.
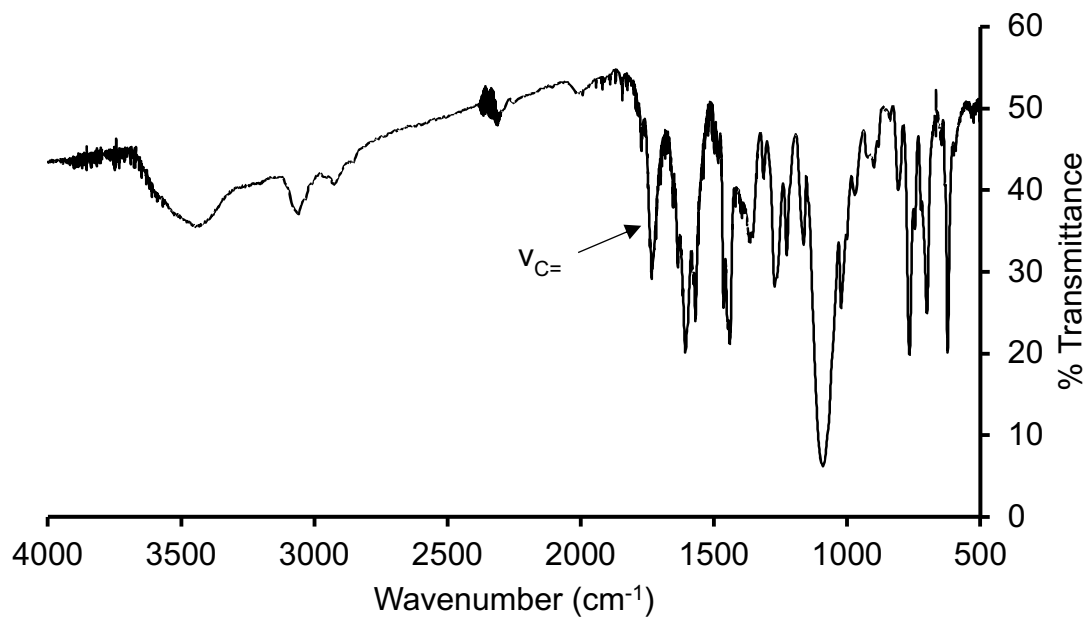
Figure C-22. FT-IR of **9** in KBr.

Figure C-23. ESI/APCI MS of **9**.
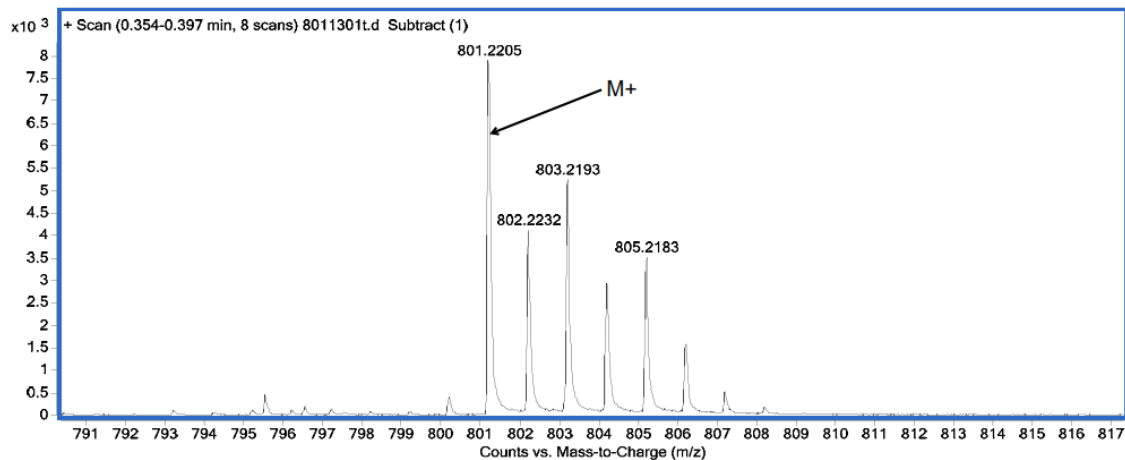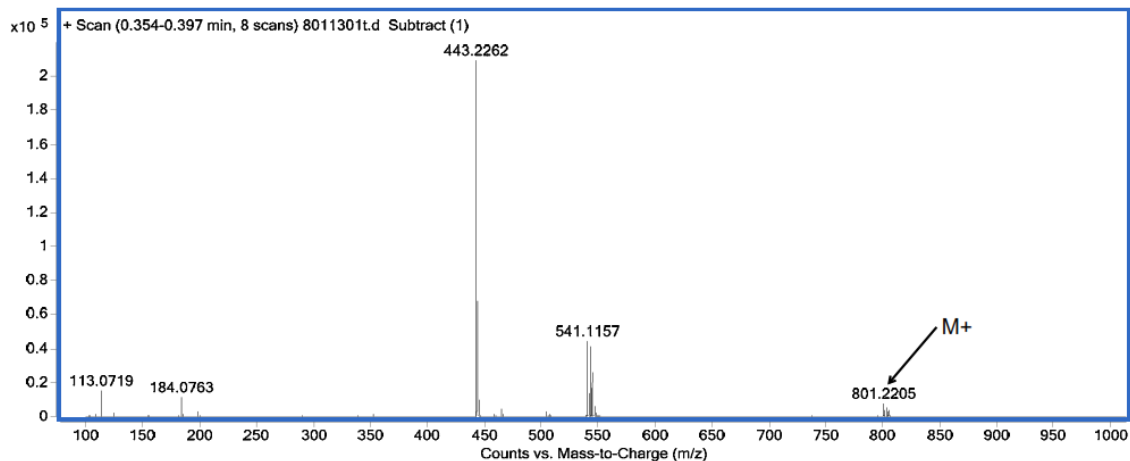
Measured Mass		801.2205		

Element	Low Limit	High Limit		
C	43	53		
H	30	50		
N	0	5		
O	0	2		
18O	0	2		
Zn	0	1		

Formula	Calculated Mass	mDaError	ppmError	RDB
C51 H37 N4 O2 Zn	801.2202	0.3	0.3	35.5
C52 H37 N3 O 18O Zn	801.2214	-0.9	-1.1	36
C48 H37 N4 O2 18O2 Zn	801.2186	1.9	2.4	32.5
C53 H37 N2 18O2 Zn	801.2226	-2.1	-2.6	36.5

Figure C-24. ESI/APCI MS of **9** demonstrating $^{18}O_2$ incorporation.
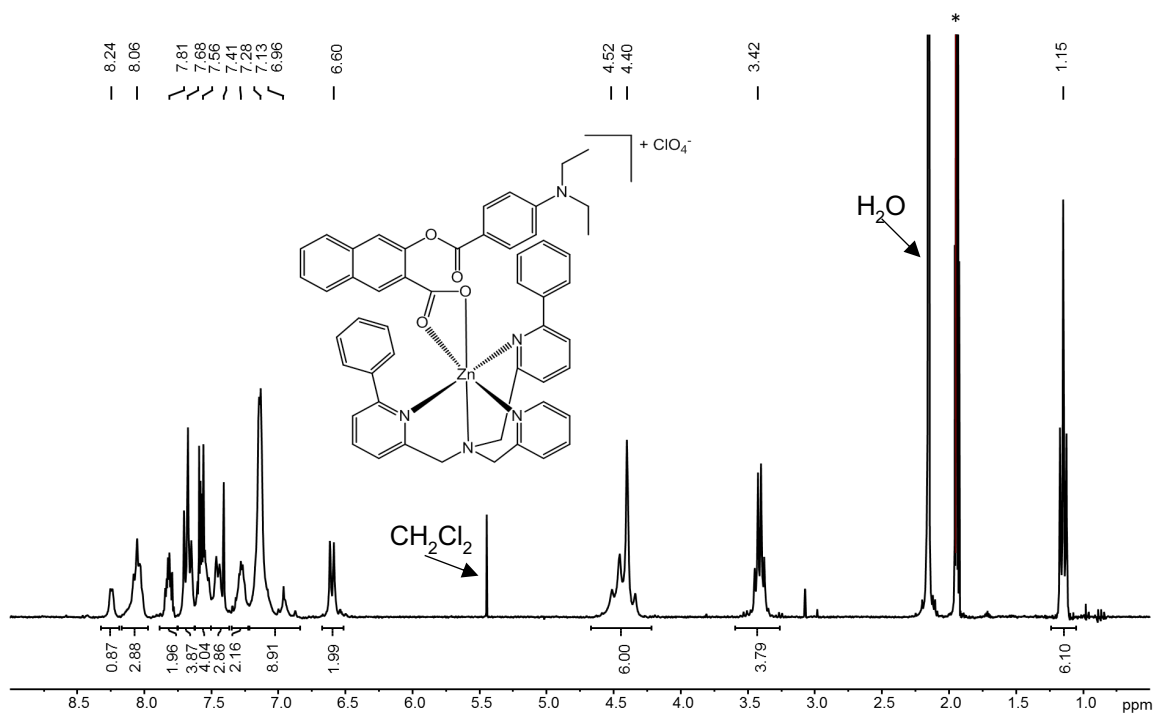
Figure C-25. ^{1}H NMR of **10** in CD$_3$CN. (* denotes residual solvent)
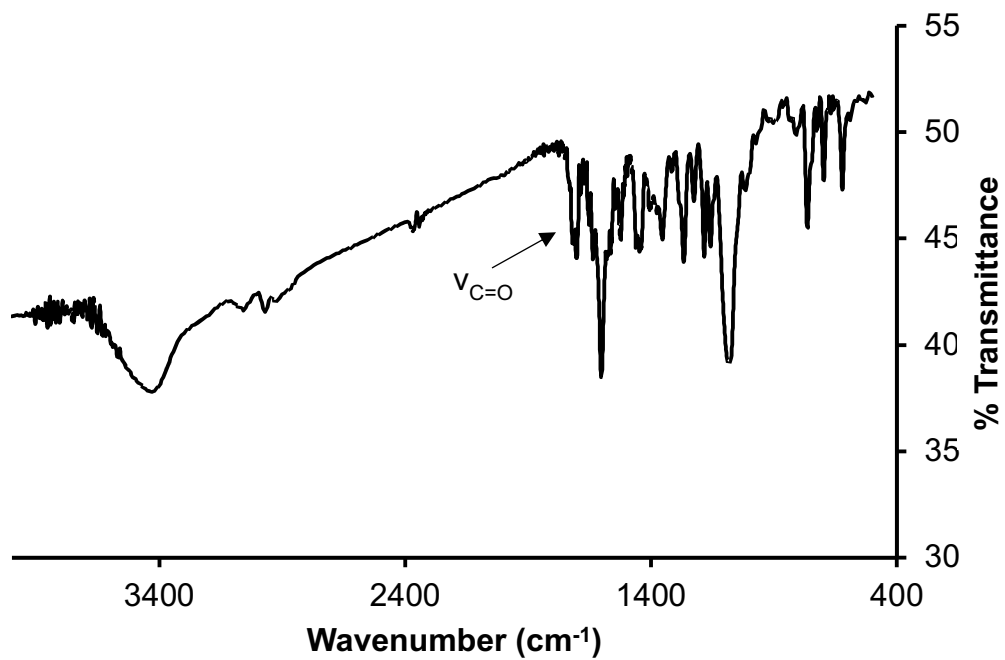
Figure C-26. FT-IR of **10** in KBr.
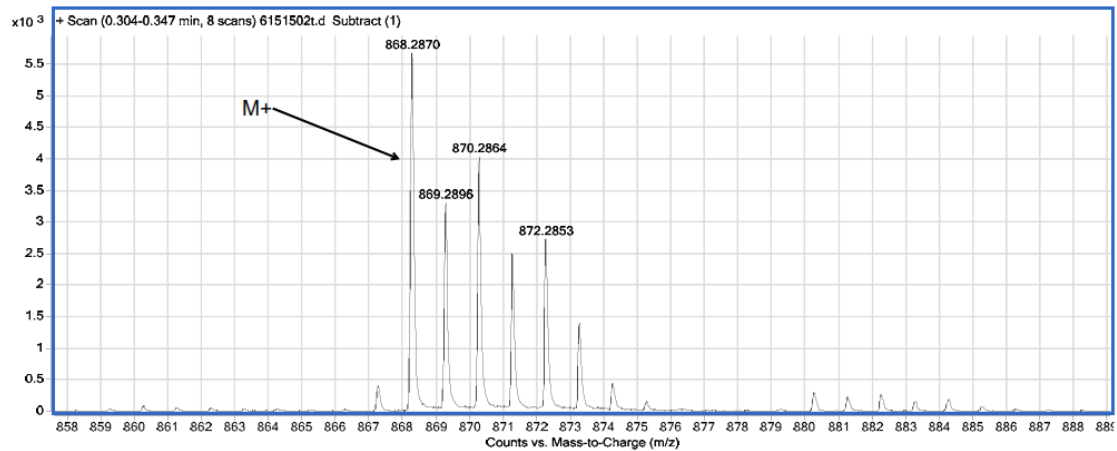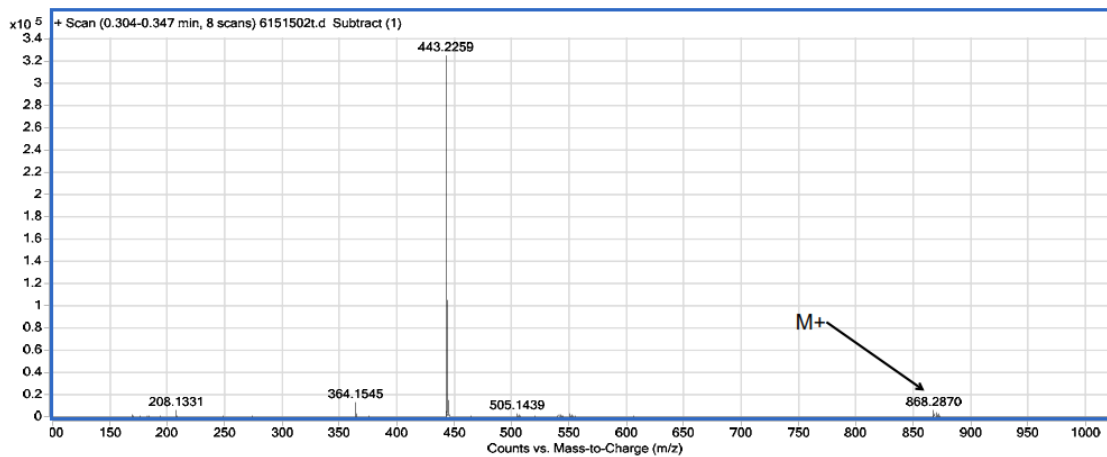
Measured Mass   868.287

Element	Low Limit	High Limit
C	48	58
H	35	55
N	3	7
O	2	6
Zn	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C57 H46 N3 O2 Zn	868.2876	-0.6	-0.7	36.5
C53 H38 N7 O6	868.2878	-0.8	-0.9	38.5
C52 H46 N5 O4 Zn	868.2836	3.4	3.9	32.5

Figure C-27. ESI/APCI MS of **10**.
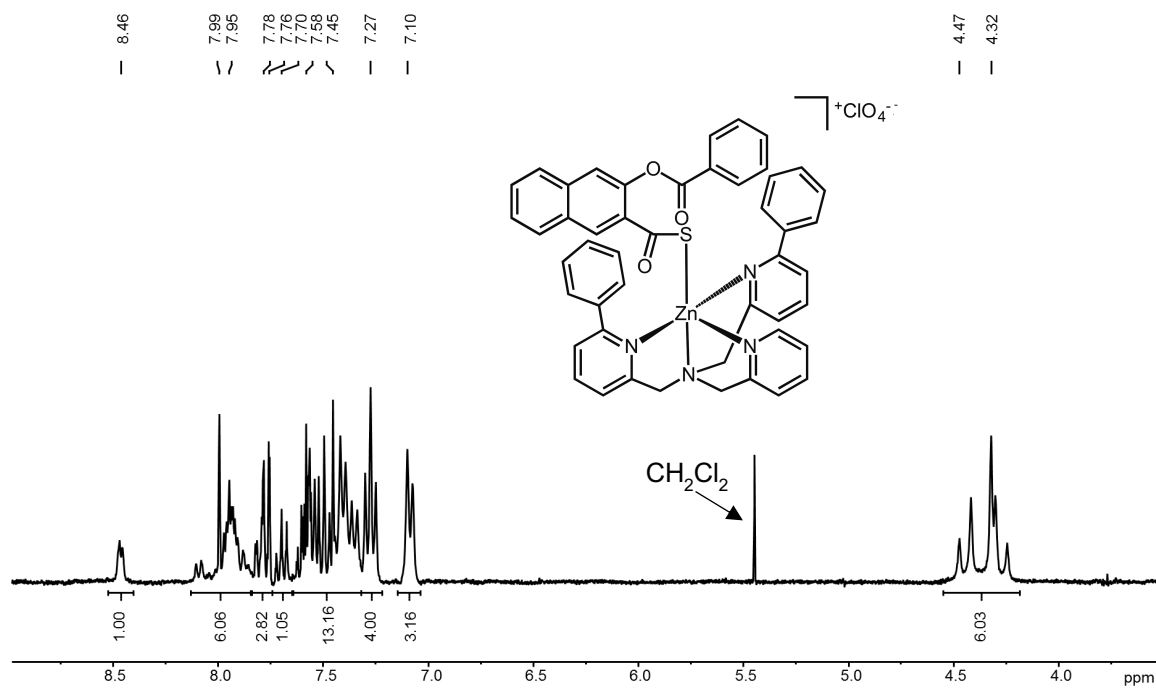
Figure C-28. ^{1}H NMR of **11** in CD$_3$CN.
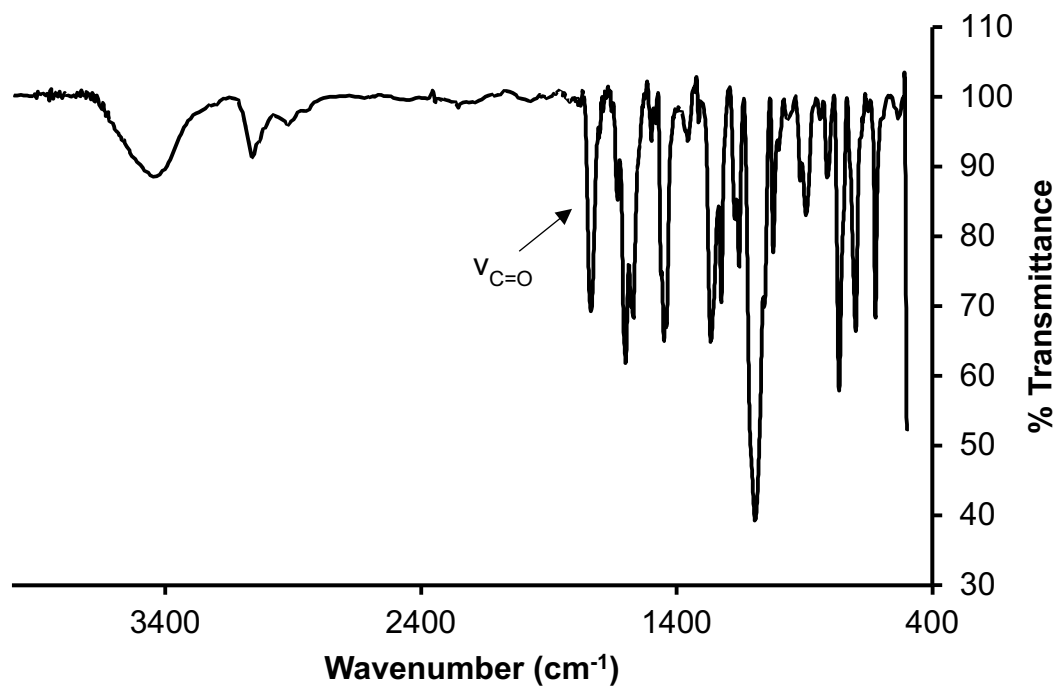
Figure C-29. FT-IR of **11** in KBr.

Figure C-30. ESI/APCI MS of 11.
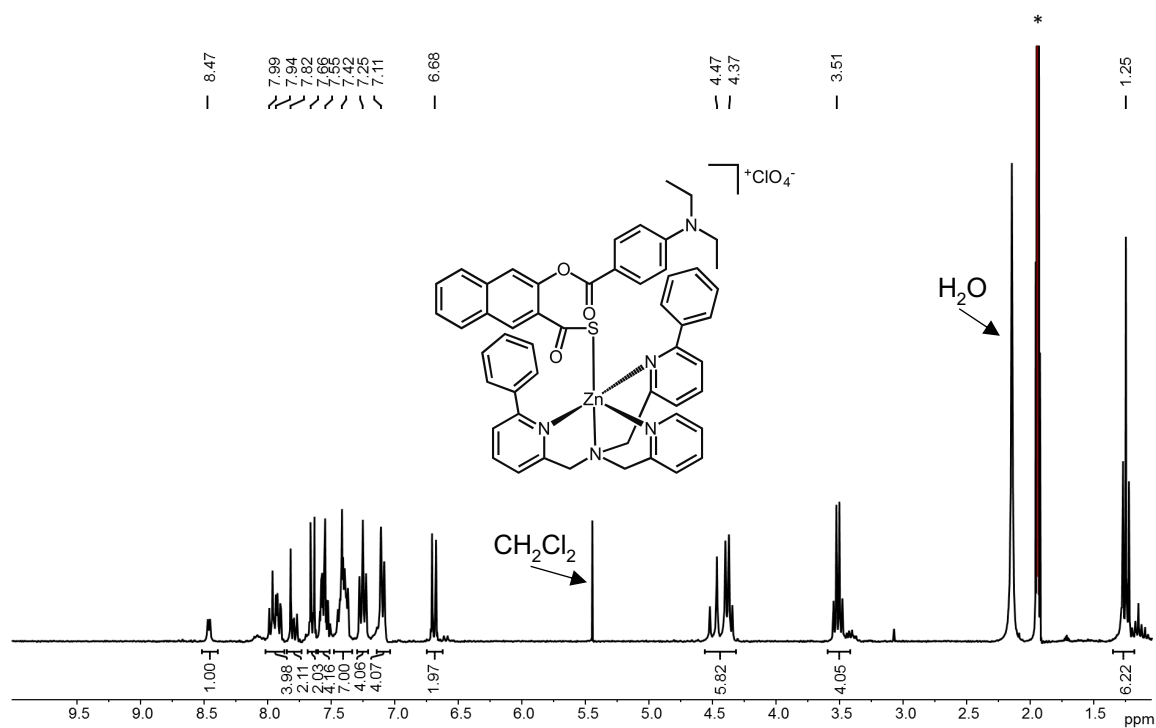
Figure C-31. ^{1}H NMR of **12** in CD$_3$CN. (* denotes residual solvent)
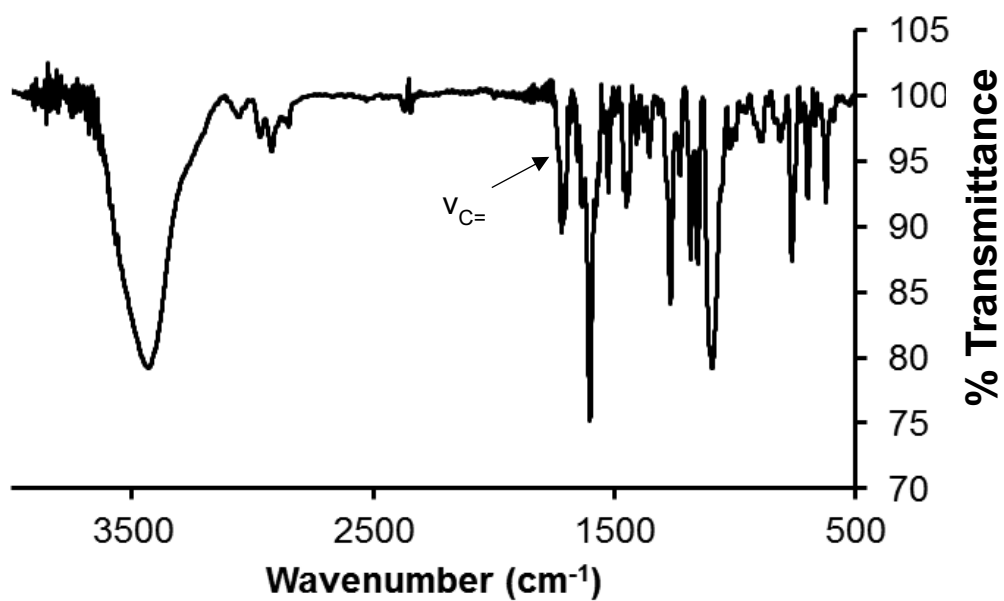
Figure C-32. FT-IR of **12** in KBr.
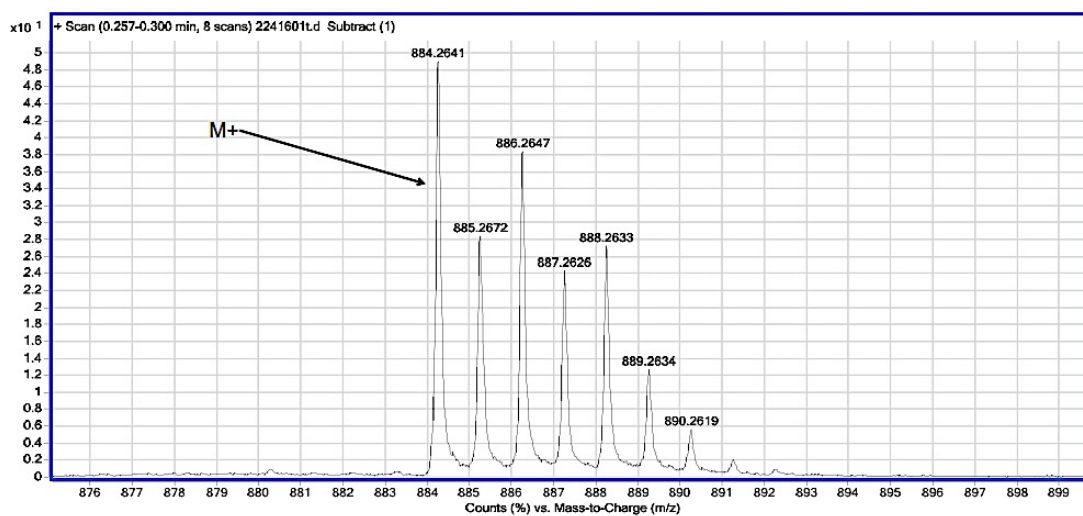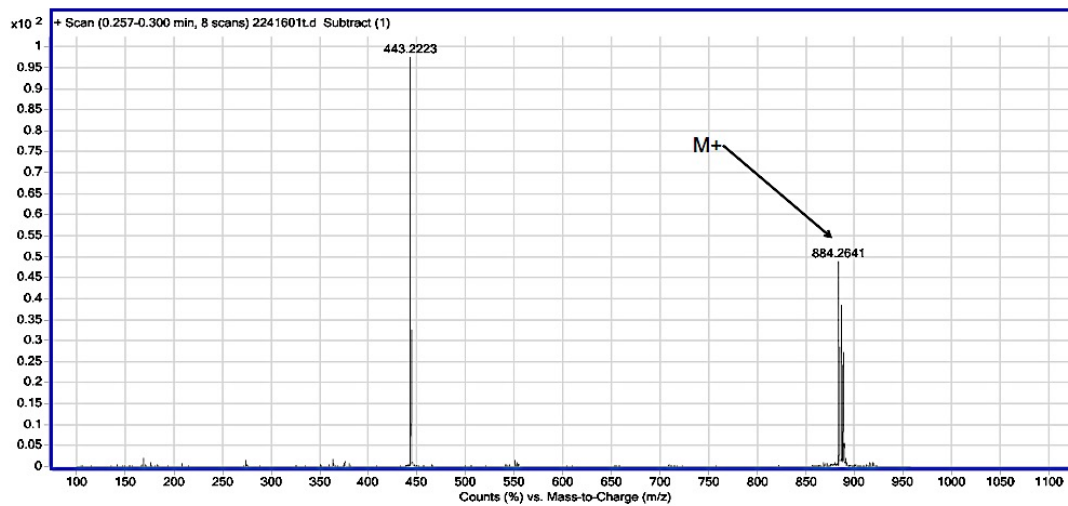
Measured Mass   884.2641

Element	Low Limit	High Limit
C	47	57
H	35	55
N	3	7
O	0	5
S	0	1
Zn	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C57 H46 N3 O S Zn	884.2648	-0.7	-0.7	36.5
C55 H44 N6 S Zn	884.2634	0.7	0.8	37
C53 H38 N7 O5 S	884.2650	-0.9	-1.0	38.5
C51 H44 N6 O5 Zn	884.2659	-1.8	-2.1	33
C52 H46 N5 O3 S Zn	884.2607	3.4	3.8	32.5

Figure C-33. ESI/APCI MS of **12**.

APPENDIX D

CHAPTER 5 SUPPLEMENTARY MATERIAL
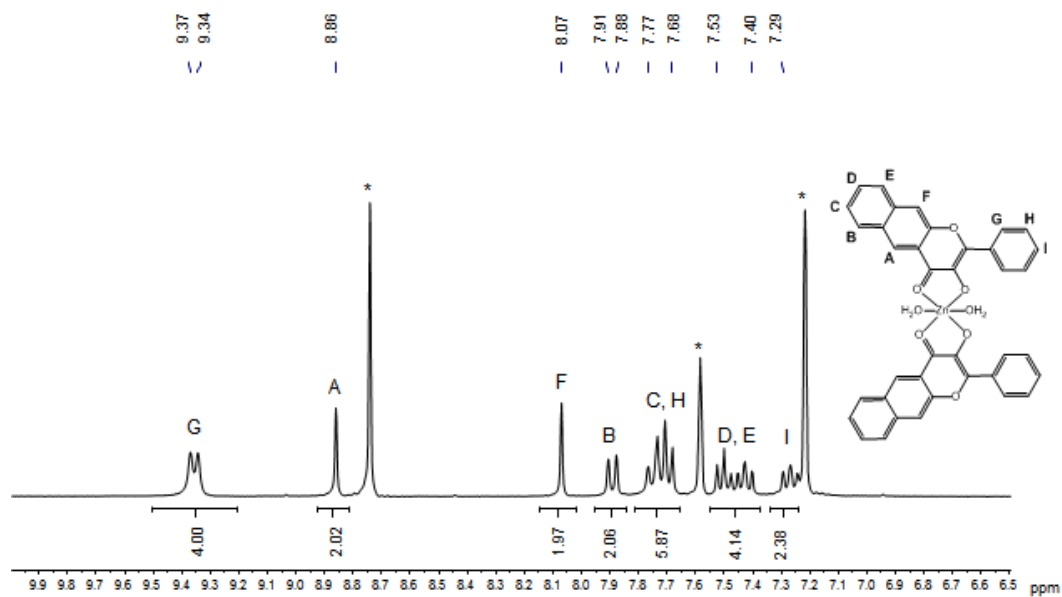
Figure D-1. ^{1}H NMR of **5** in pyridine-d_5 (* indicates solvent peaks).
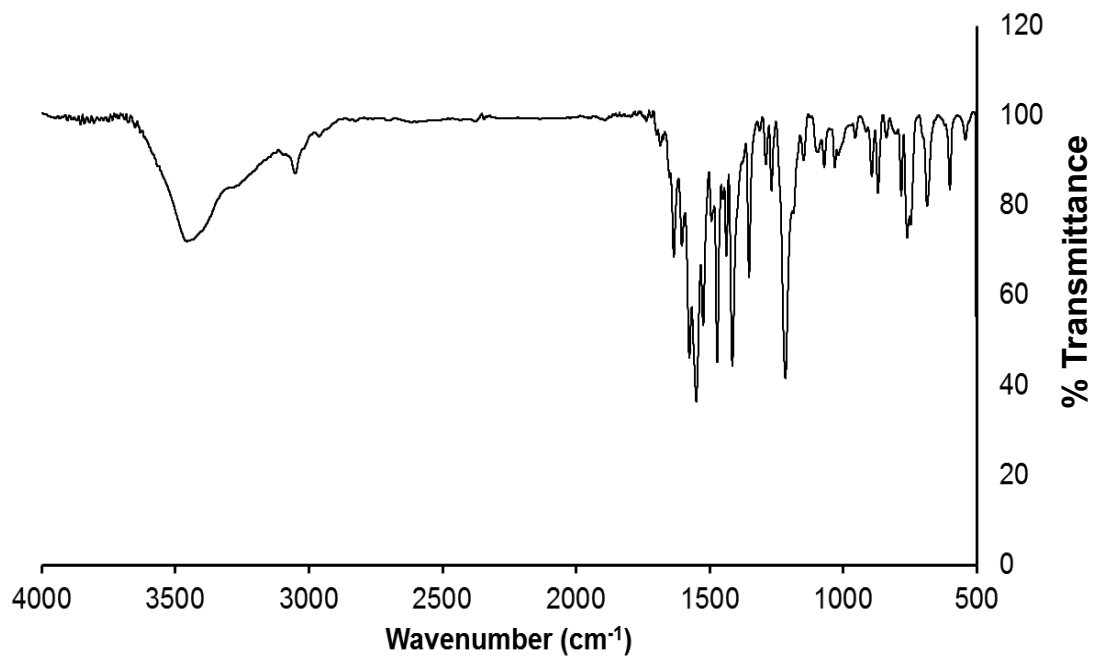
Figure D-2. FTIR spectrum of **5**.
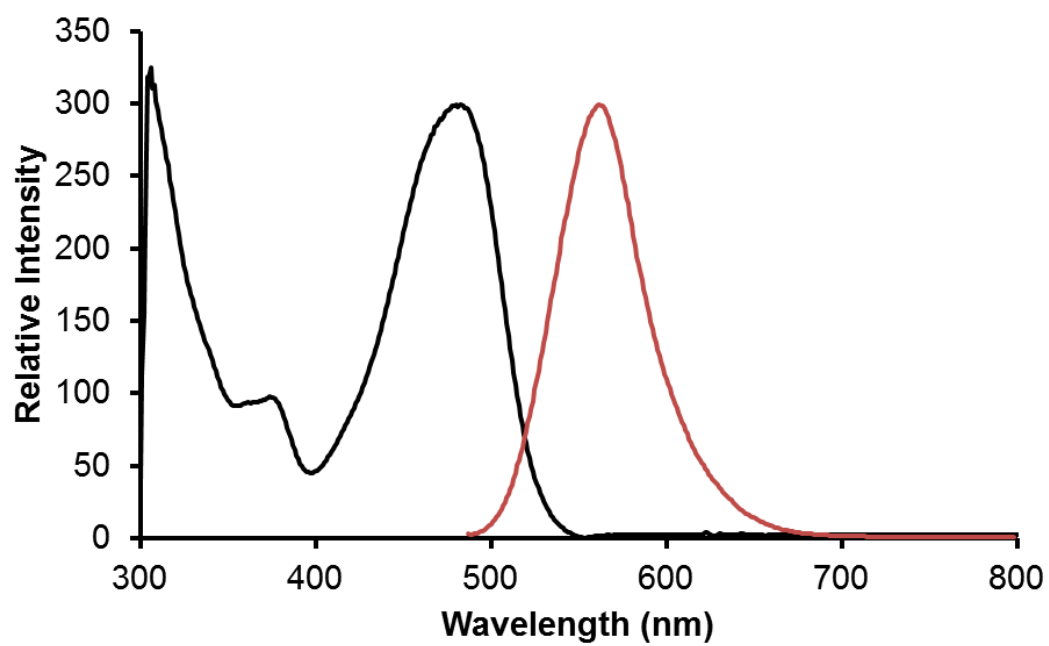
Figure D-3. Absorption (black) and emission (red) spectra of **5**.
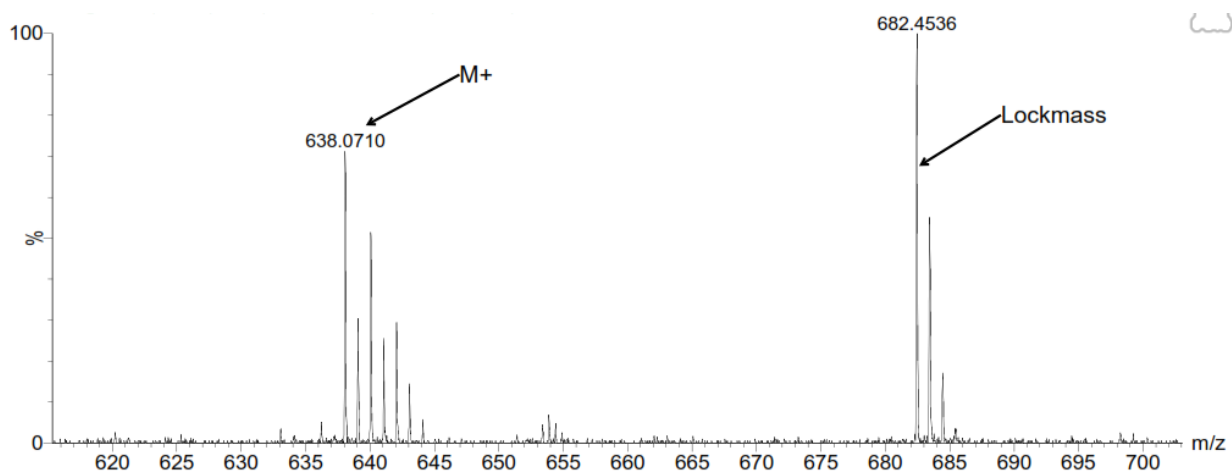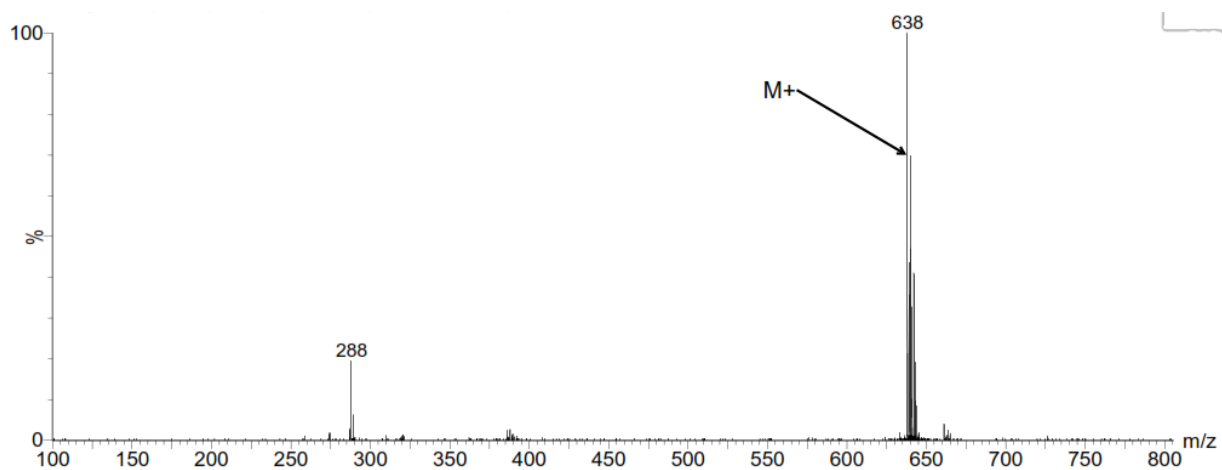
Figure D-4. LIFDI-MS of **5**.
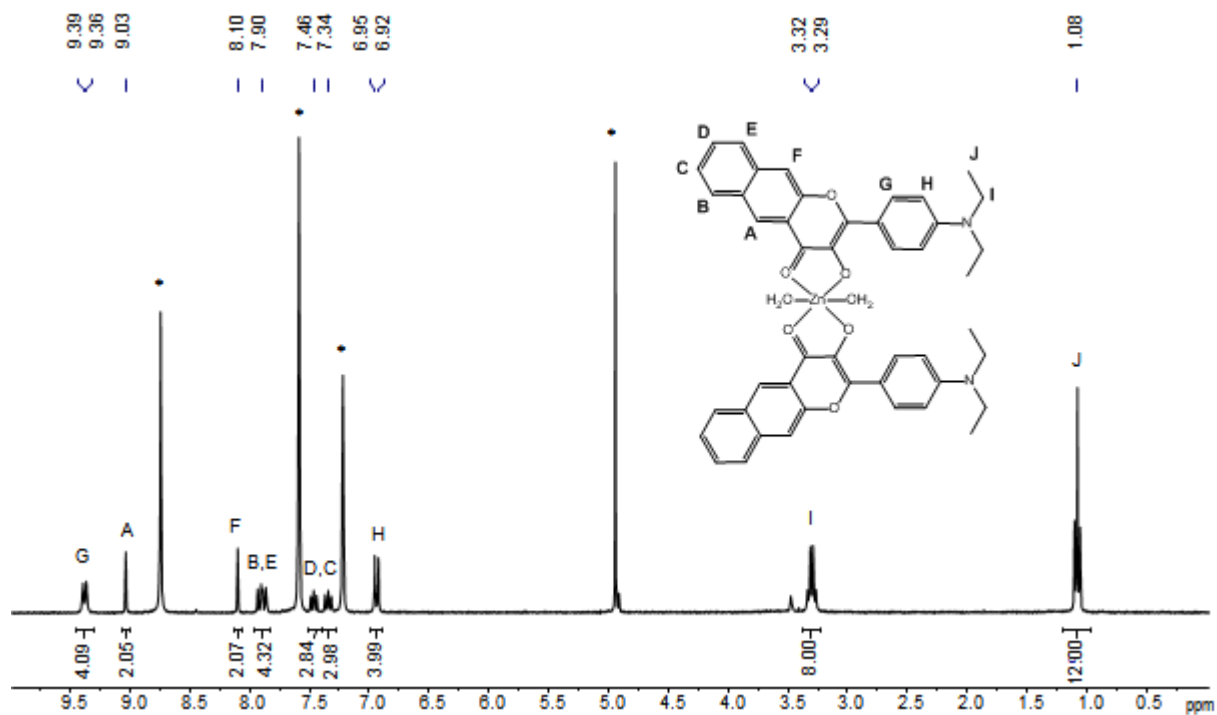
Figure D-5. ^{1}H NMR of **6** in pyridine-d_5 (* denotes residual solvent peaks).
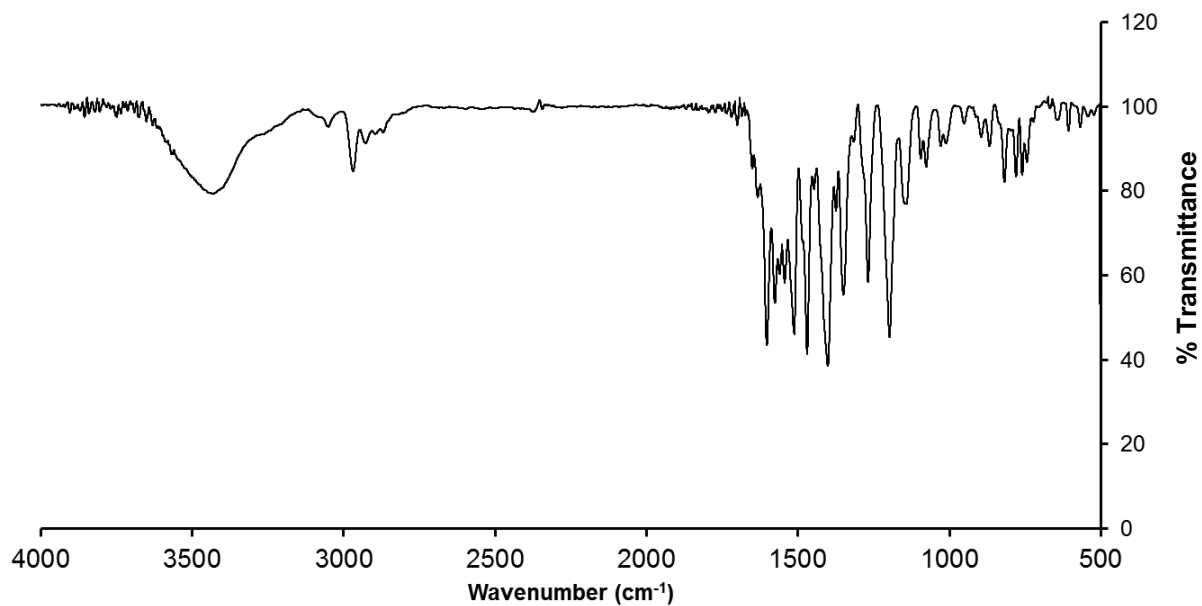
Figure D-6. FTIR spectrum of **6**.
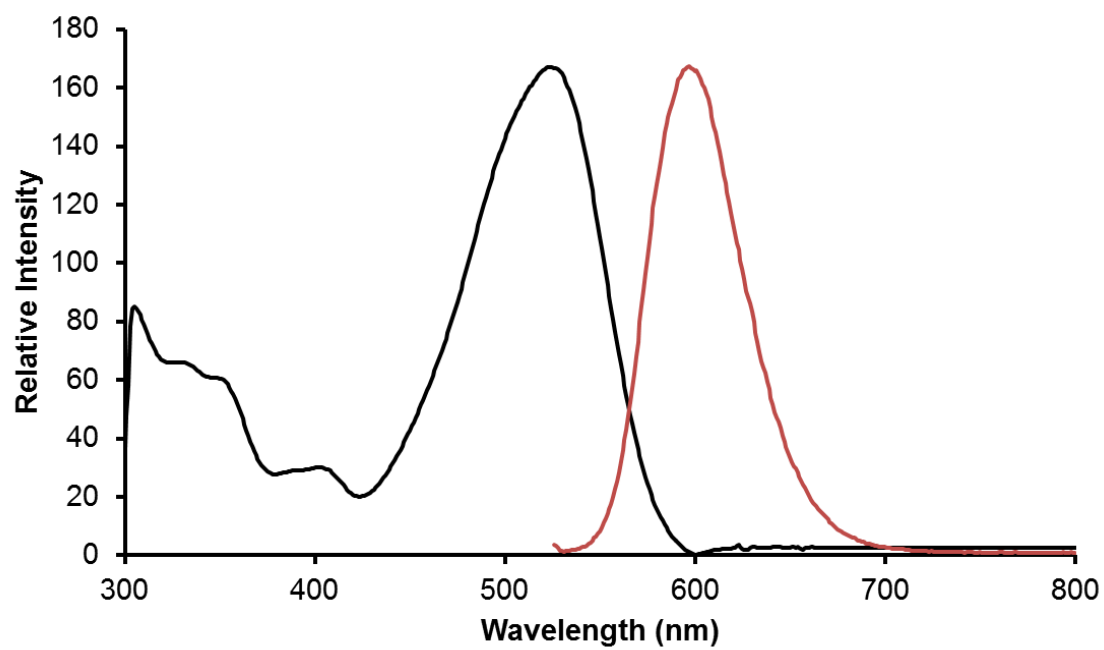
Figure D-7. Absorption (black) and emission (red) spectra of **6**.
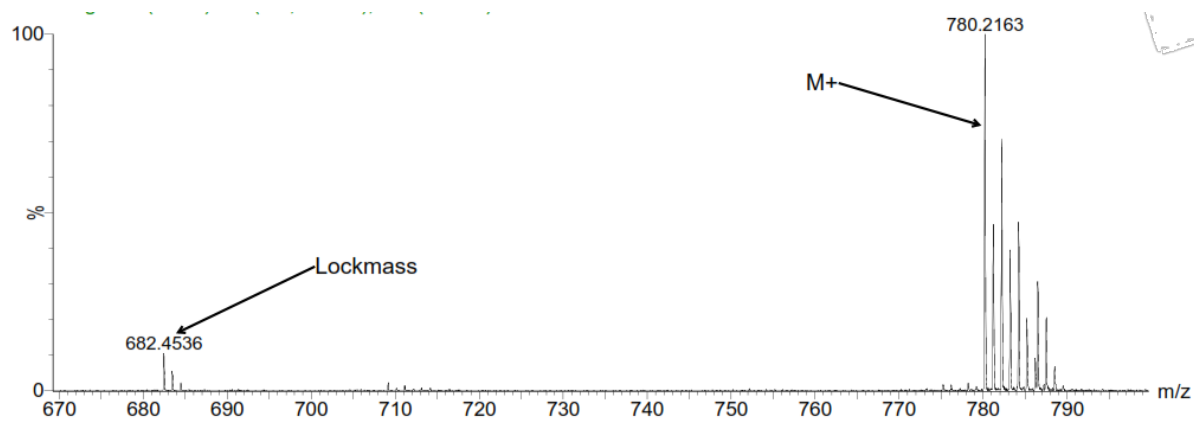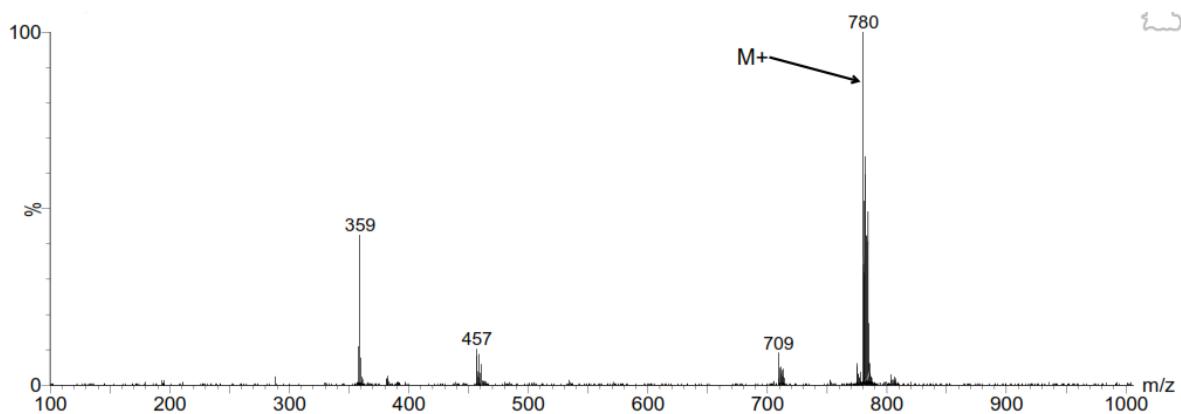
Measured Mass		780.2163			

Element	Low Limit	High Limit
C	41	51
H	30	50
N	0	2
O	4	8
Zn	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C46 H40 N2 O6 Zn	780.2172	-0.9	-1.2	28

Figure D-8. LIFDI-MS of **6**.
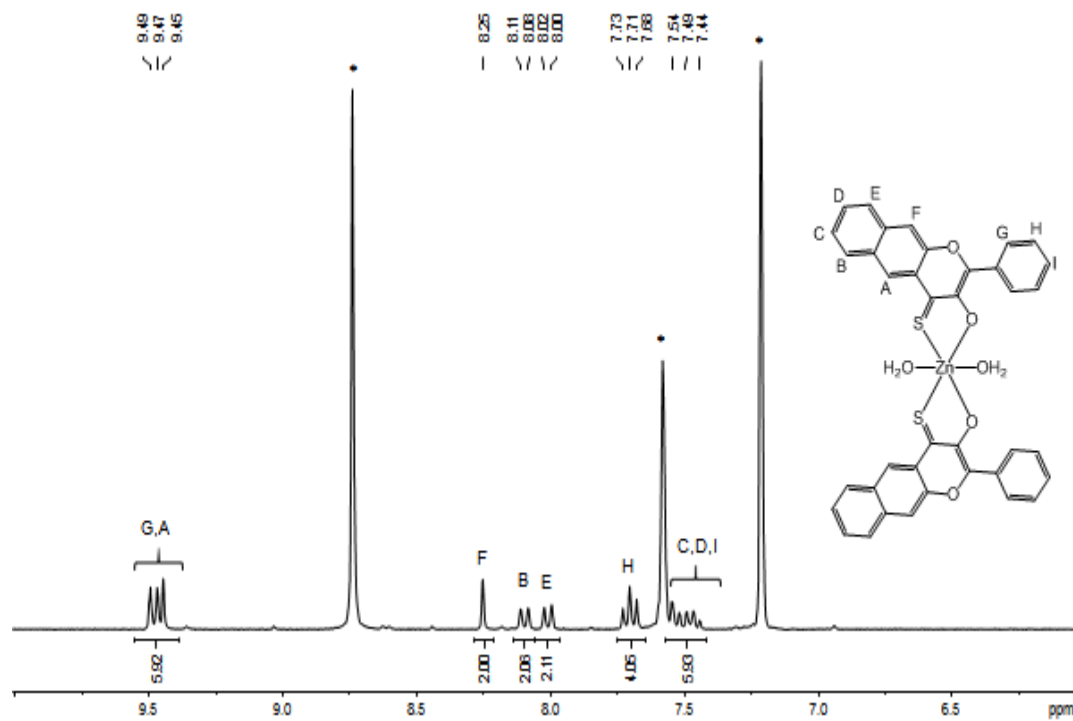
Figure D-9. ^{1}H NMR of **7** in pyridine-d_5 (* denotes residual solvent peaks).
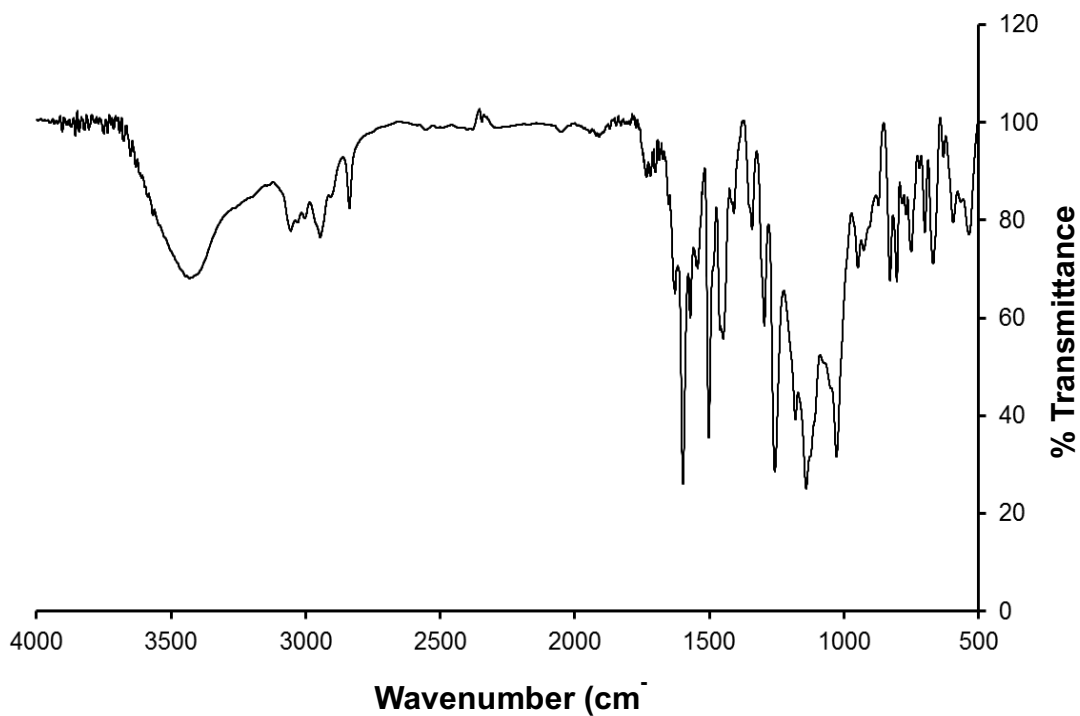
Figure D-10. FTIR spectrum of **7**.
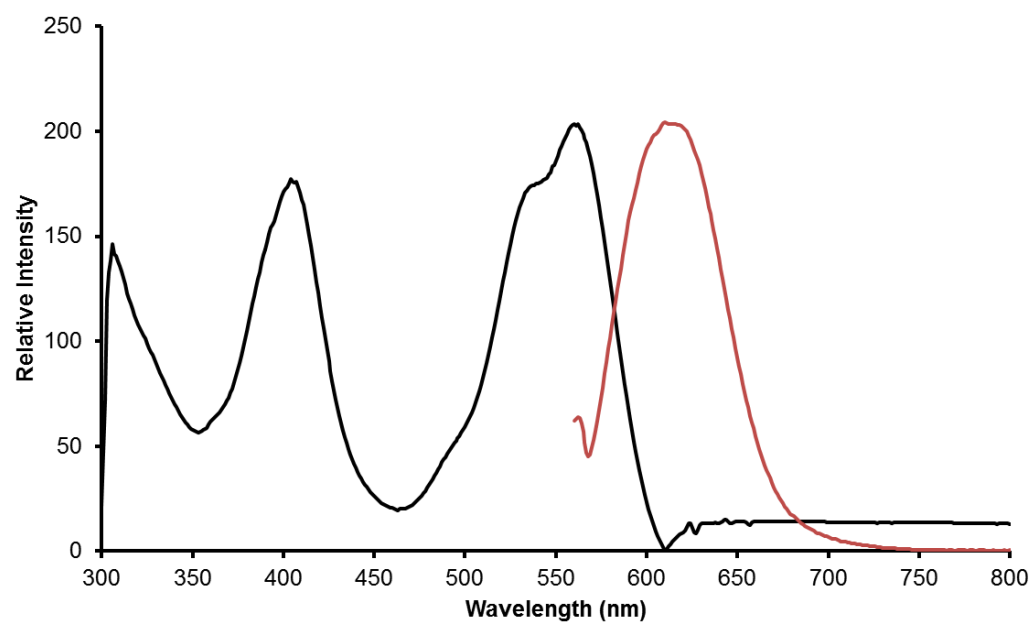
Figure D-11. Absorption (black) and emission (red) spectra of **7**.
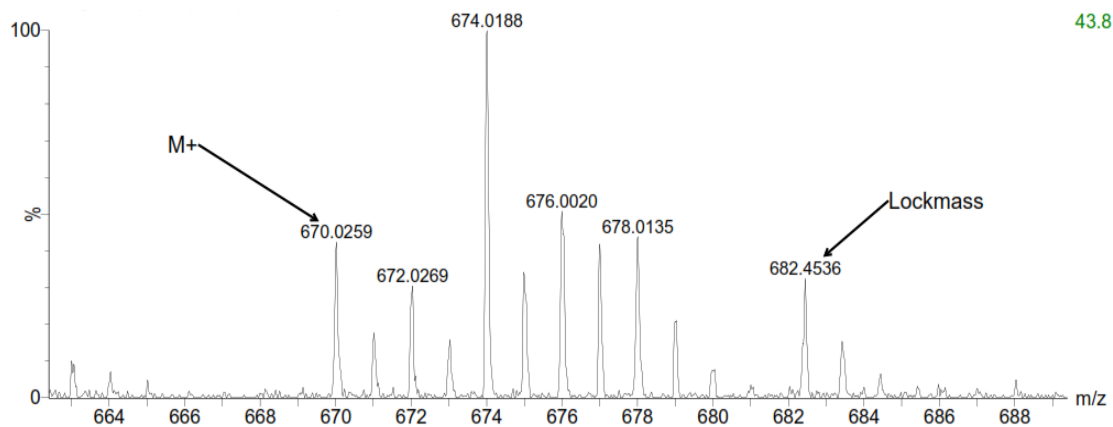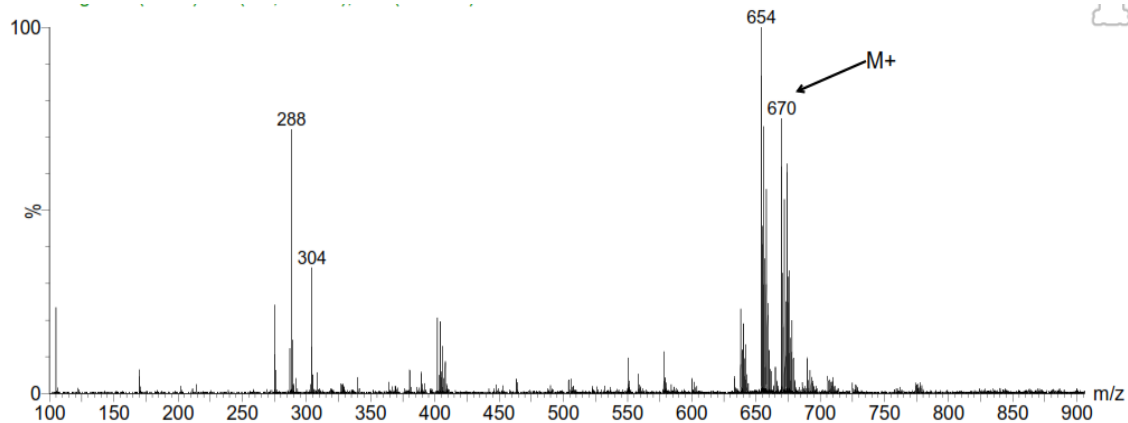
Measured Mass		670.0259			

Element	Low Limit	High Limit
C	33	43
H	15	35
S	0	2
O	2	6
Zn	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C38 H22 O4 S2 Zn	670.0246	1.4	2.0	28

Figure D-12. LIFDI-MS of **7**.
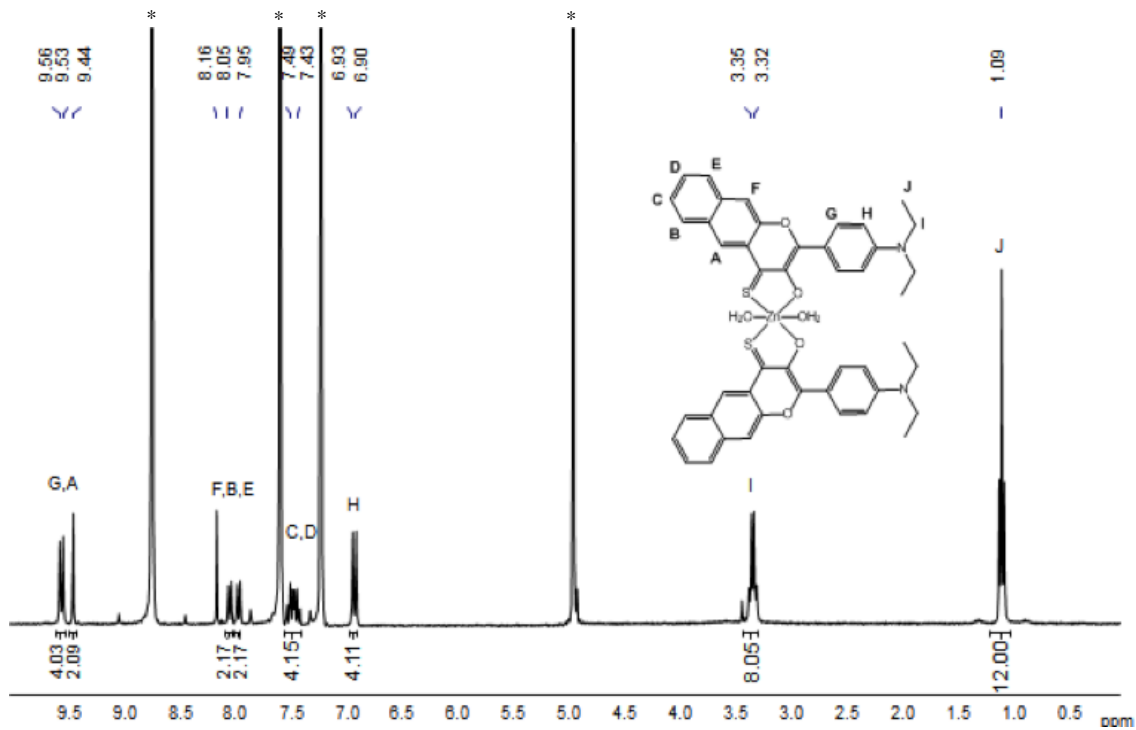
Figure D-13. ^{1}H NMR of **8** in pyridine-d_5 (* denotes residual solvent peaks).
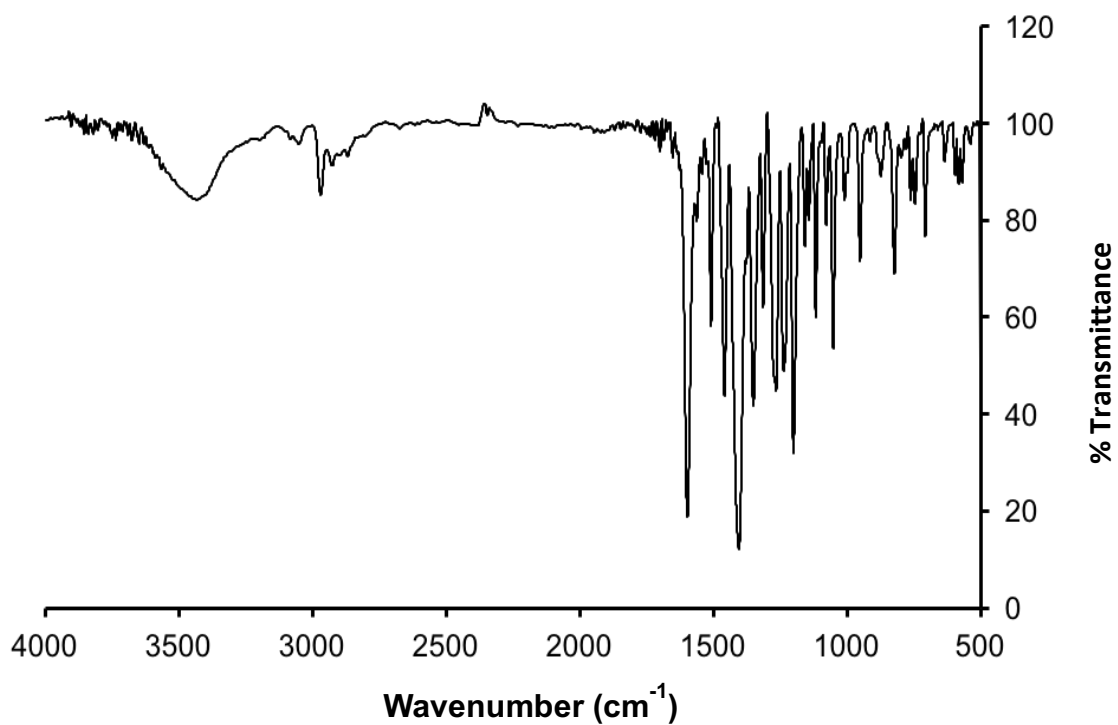
Figure D-14. FTIR spectrum of **8**.
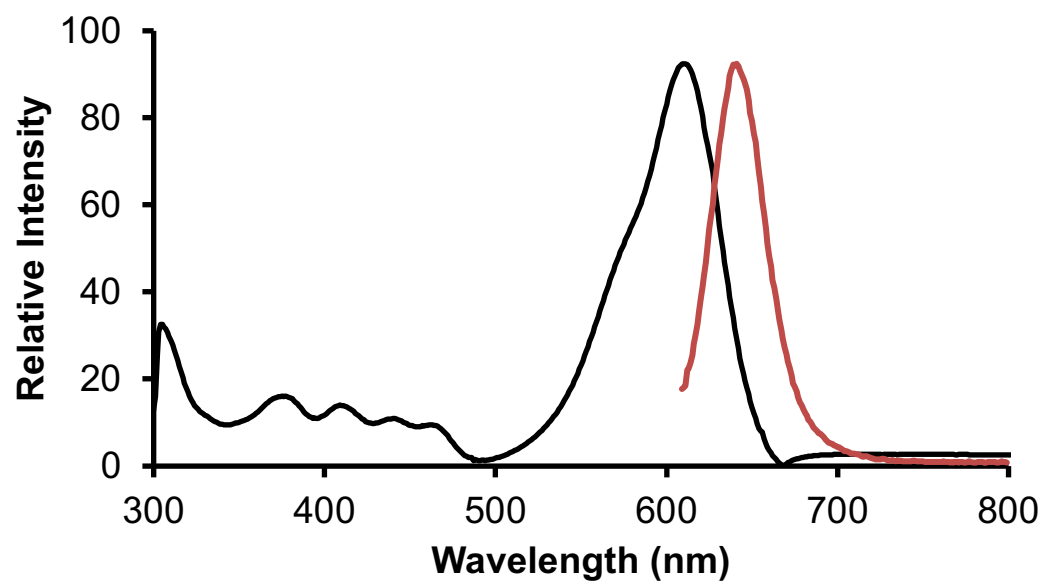
Figure D-15. Absorption (black) and emission (red) spectra of **8**.
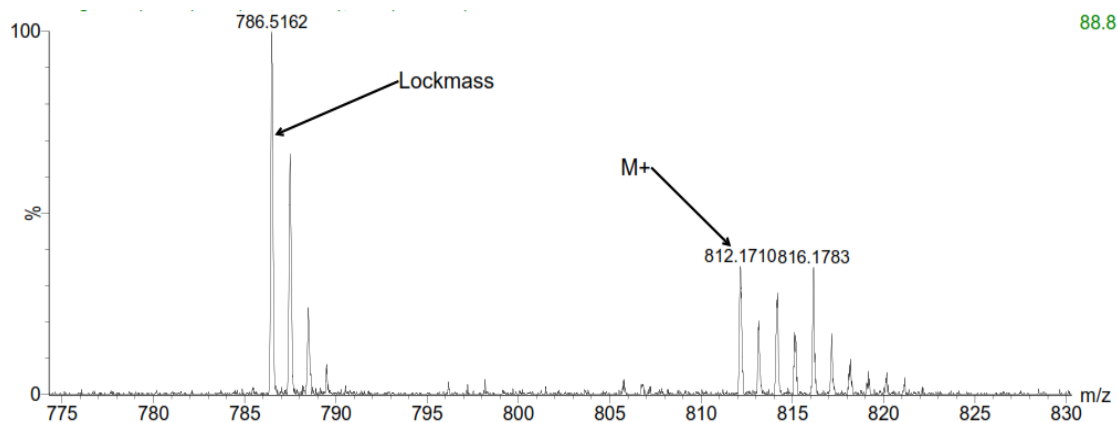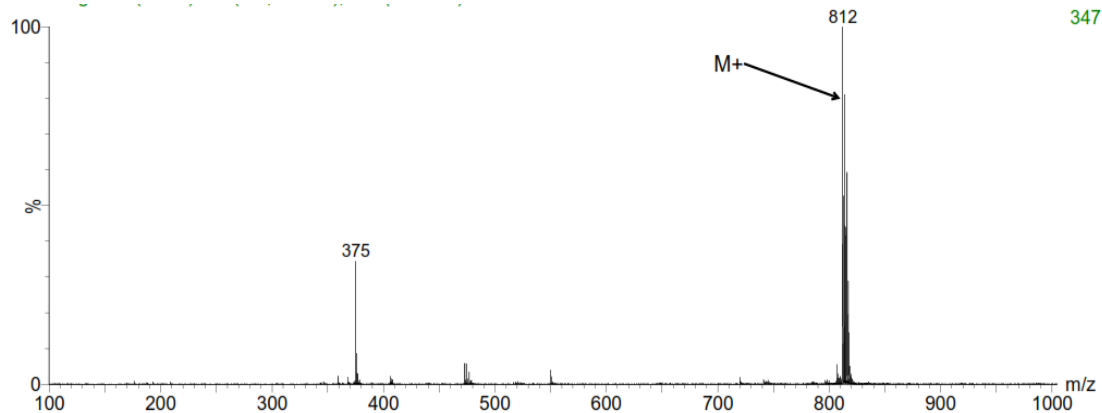
Measured Mass		812.171

Element	Low Limit	High Limit
C	41	51
H	30	50
N	0	2
O	2	6
S	0	2
Zn	0	1

Formula	Calculated Mass	mDaError	ppmError	RDB
C46 H40 N2 O4 S2 Zn	812.1715	-0.5	-0.7	28
C49 H36 N2 O4 S Zn	812.1682	2.8	3.5	33

Figure D-16. LIFDI-MS of **8**.
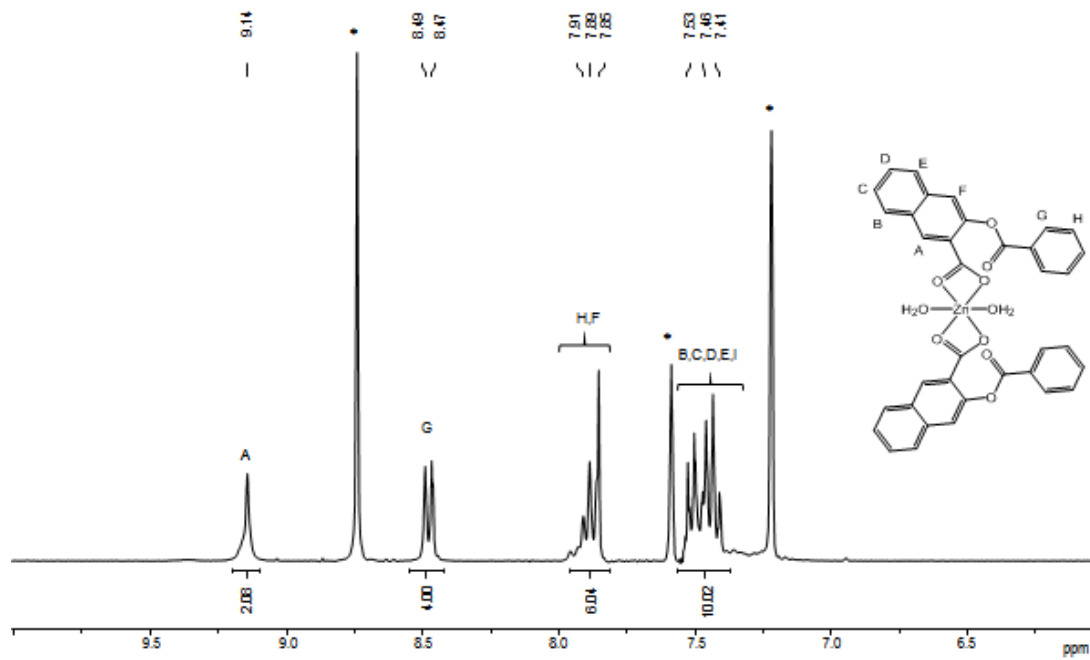
Figure D-17. ^{1}H NMR spectrum of **9** in pyridine-d_5. (* denotes residual solvent peaks)
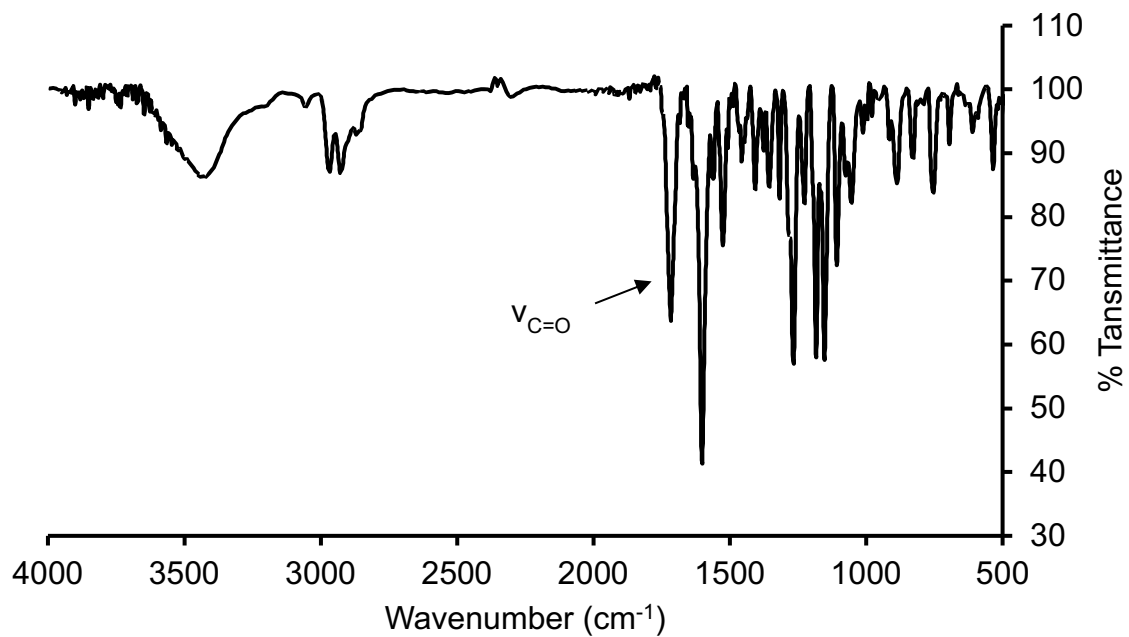
Figure D-18. FT-IR spectrum of **9**.
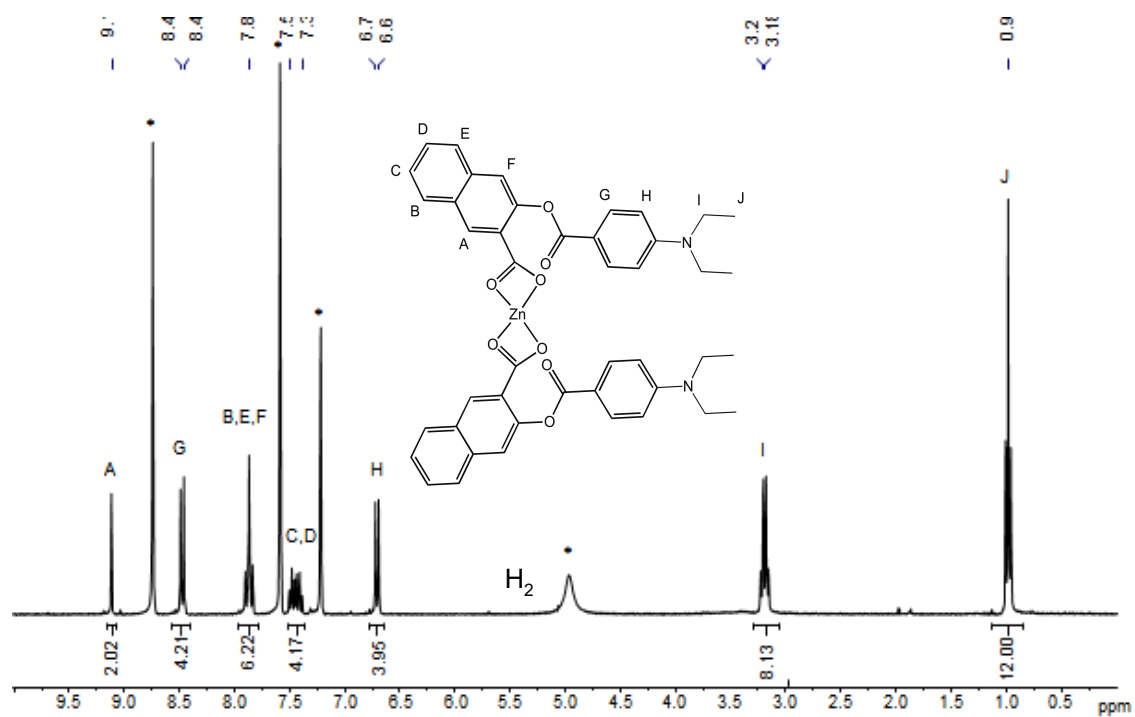
Figure D-19. ^{1}H NMR spectrum of **10** in pyridine-d_5 (* denotes residual solvent peaks).
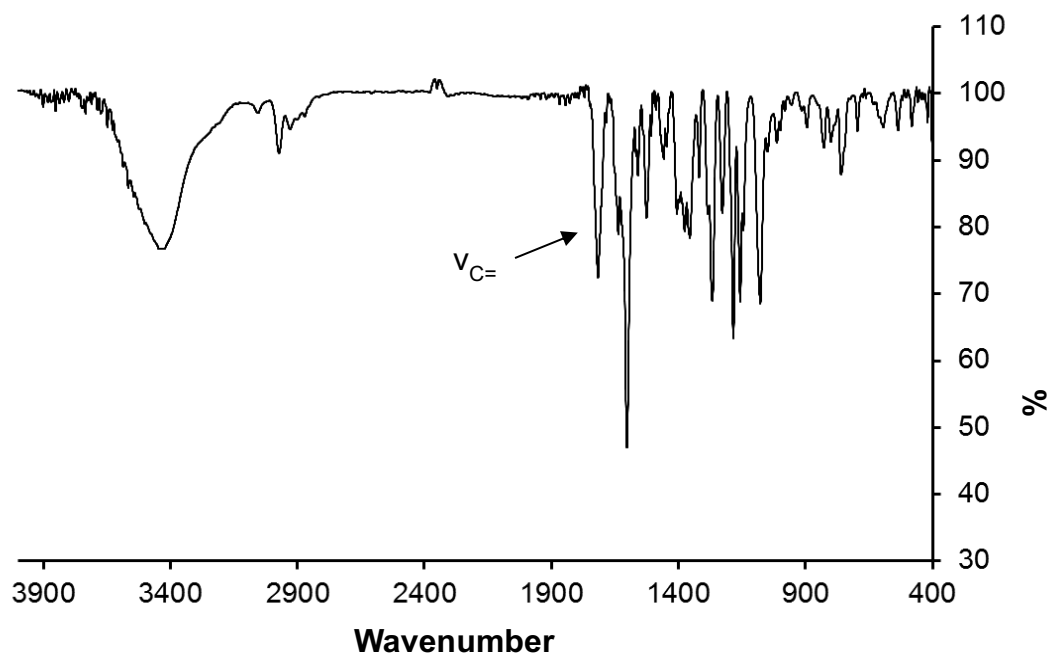
Figure D-20. FT-IR spectrum of **10**.
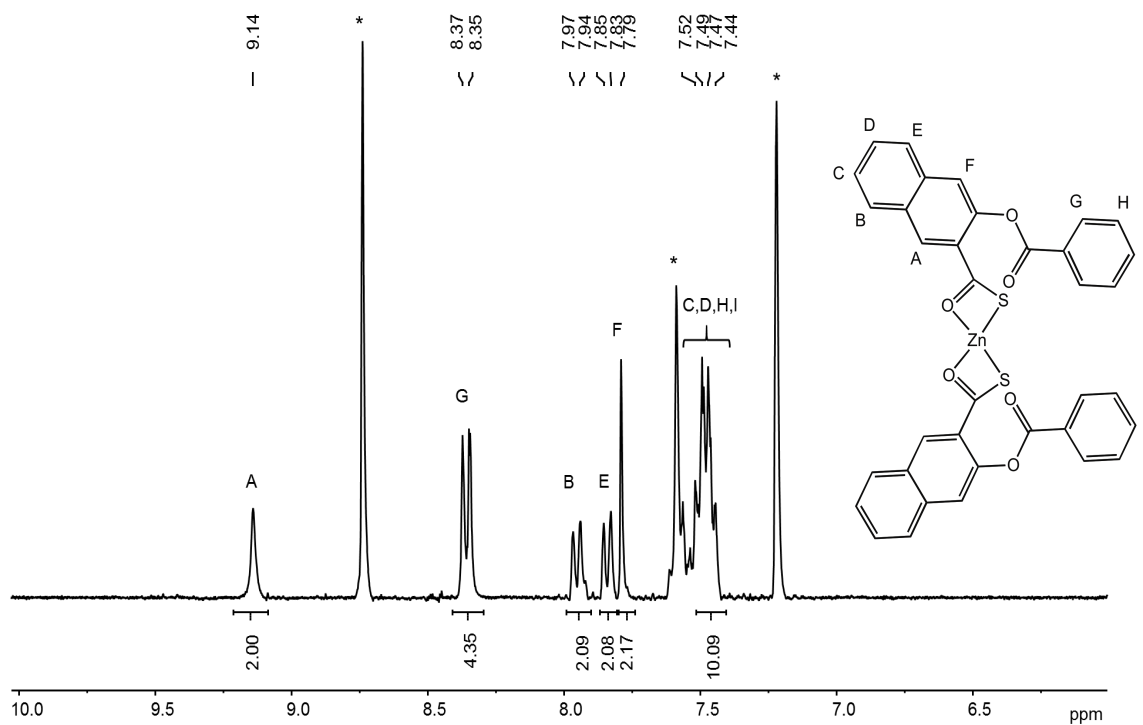
Figure D-21. ^{1}H NMR spectrum of **11** in pyridine-d_5 (* denotes residual solvent).
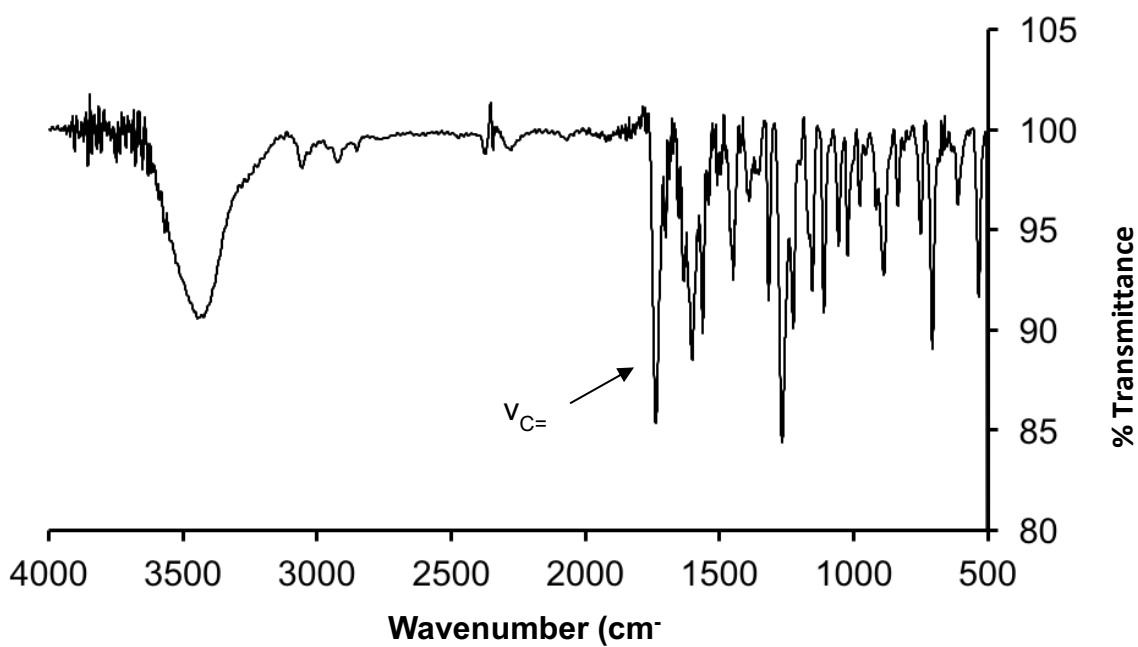
Figure D-22. FT-IR spectrum of **11**.

Figure D-23. ^{1}H NMR spectrum of **12** in pyridine-d_5. (* denotes residual solvent)
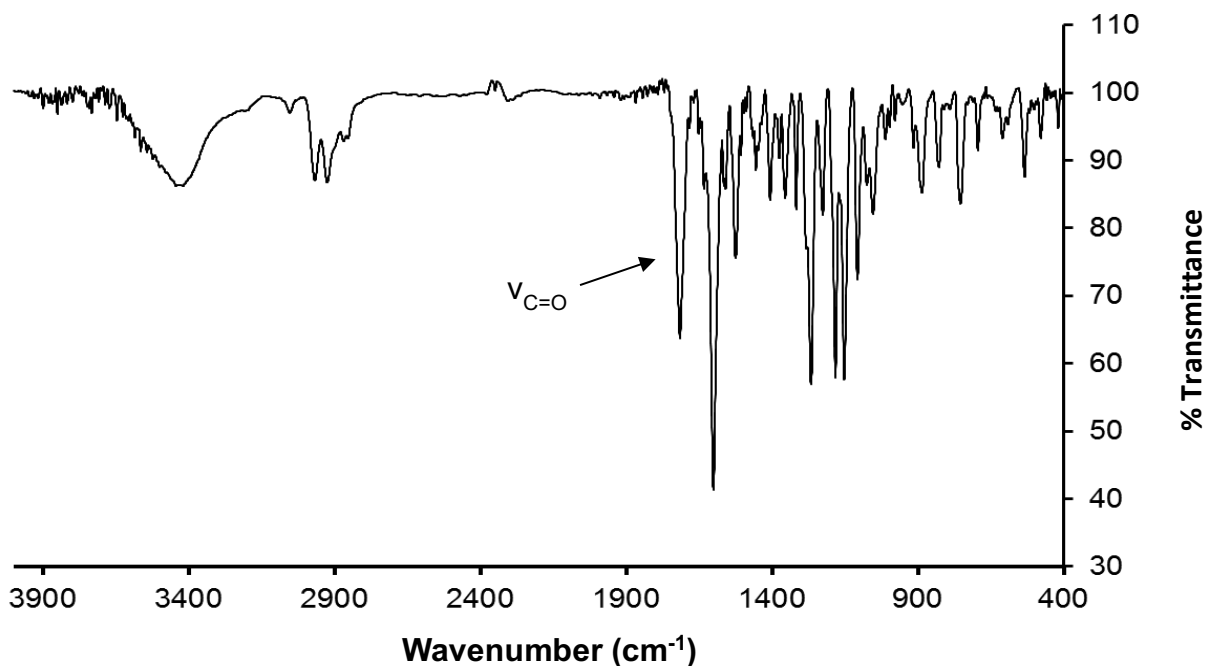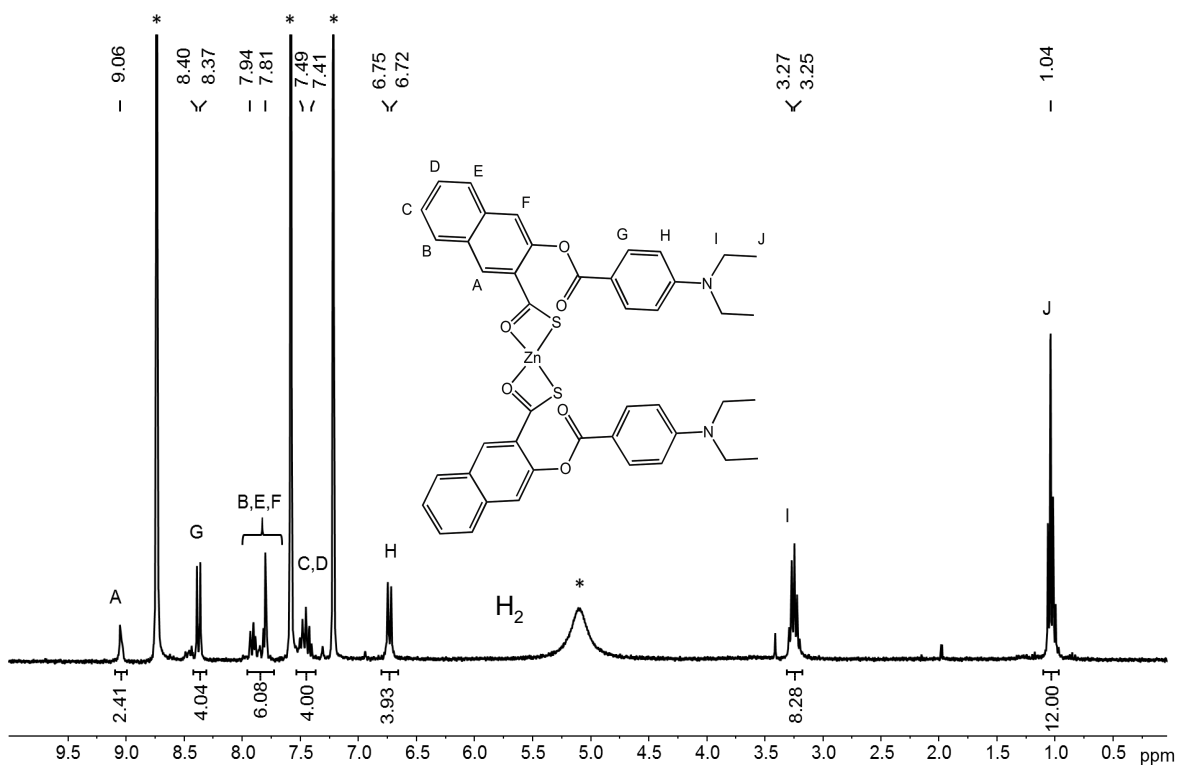
Figure D-24. FT-IR spectrum of **12**.
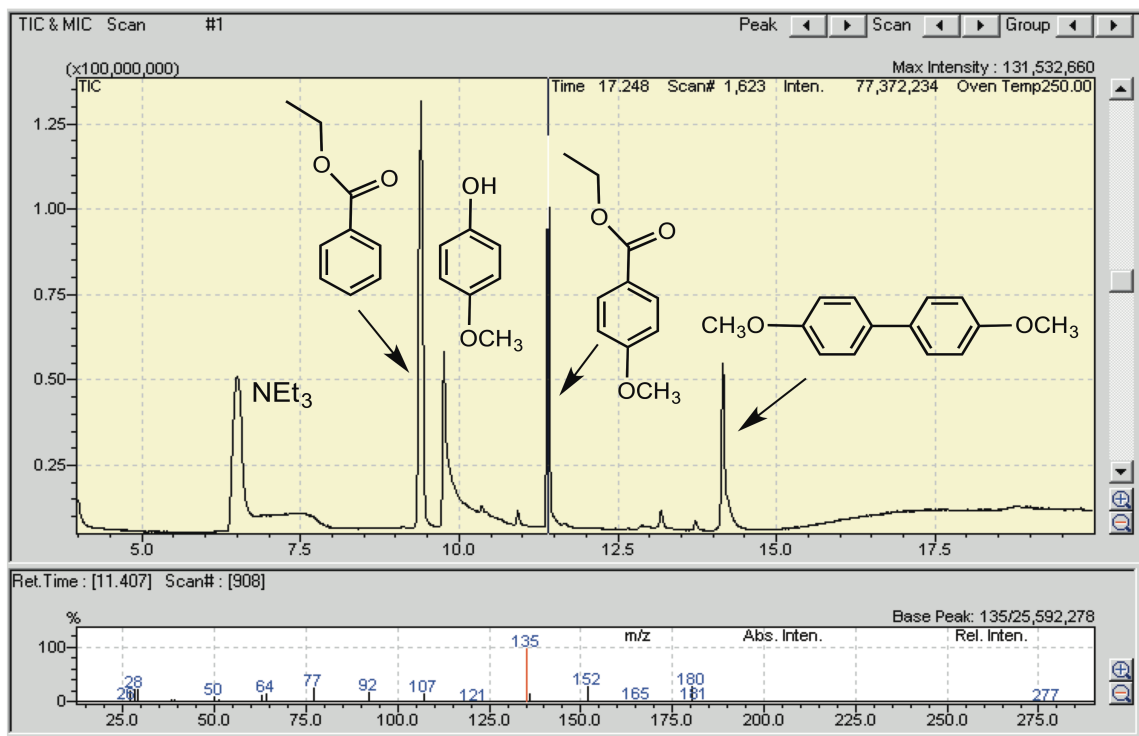
Figure D-25. GC-MS results for the carbonylation reaction of **13a** using a film of

5 as the light-induced CO source.
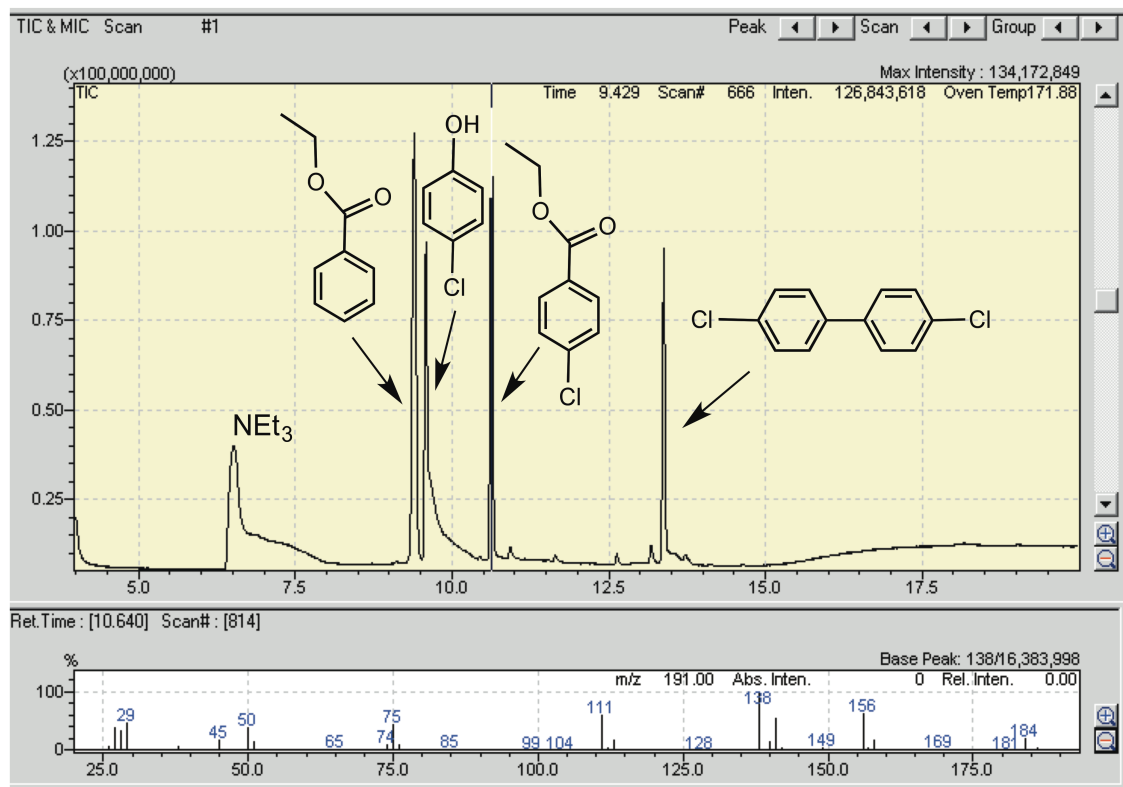
Figure D-26. GC-MS results for the carbonylation reaction of **13b** using a film of

5 as the light-induced CO source.
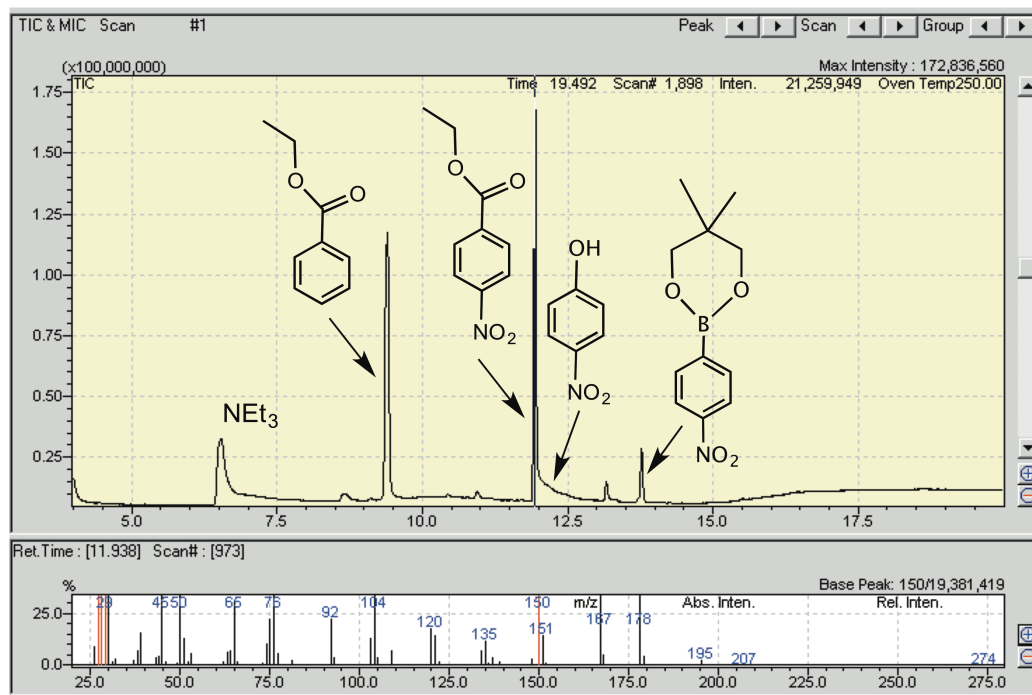
Figure D-27. GC-MS results for the carbonylation reaction of **13c** using a film of **5** as the light-induced CO source.
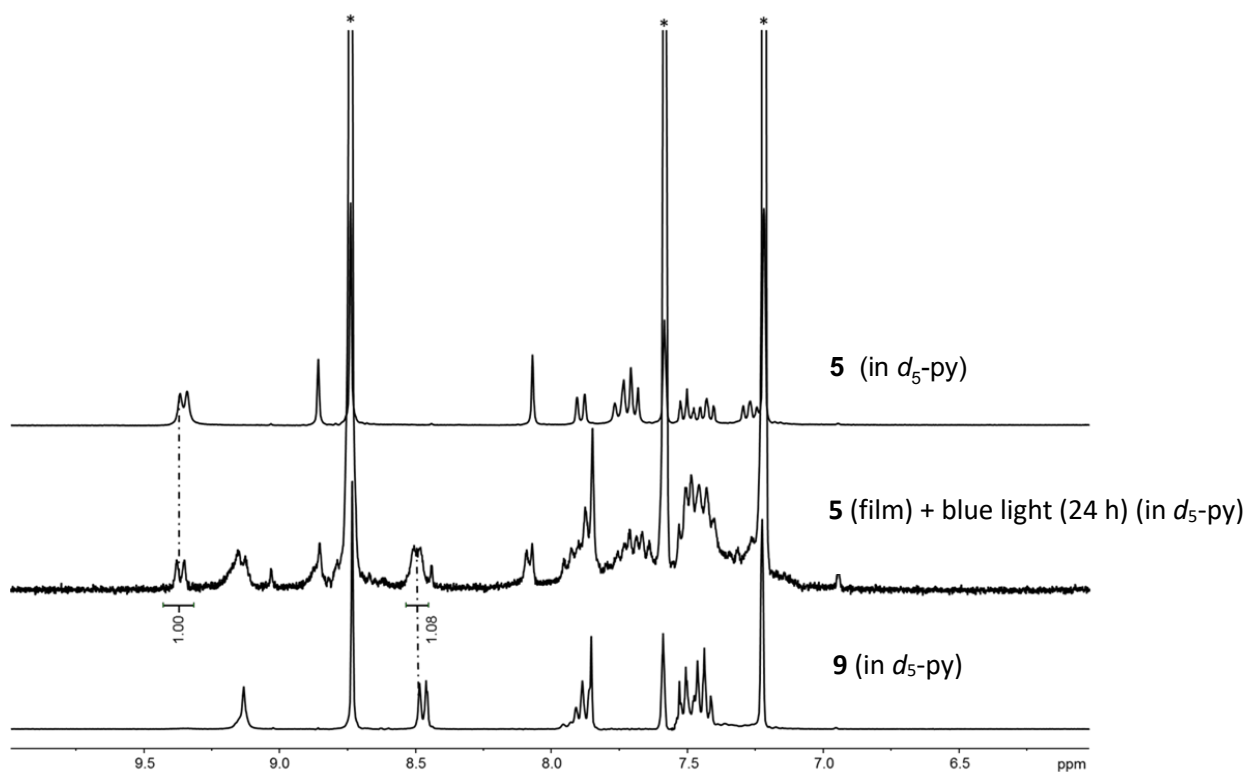
Figure D-28. ^{1}H NMR spectrum of **5** in pyridine-d_5 (top). ^{1}H NMR spectrum of remaining solid following illumination of **5** (film) with two blue CFL bulbs for 24 hours (middle). ^{1}H NMR spectrum of **9** in pyridine-d_5 (bottom) (* denotes residual solvent).

APPENDIX E

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Dear Hector Esquer,

I am in the process of preparing my dissertation in Chemistry and Biochemistry Department at Utah State University.

I am requesting your permission to include the following manuscripts in their entirety as a chapter in my dissertation:

1. Anderson, S. N.; Richards, J. M.; Esquer, H. J.; Benninghoff, A. D.; Arif, A. M.; Berreau, L. M. "A structurally-tunable 3-hydroxyflavone motif for visible-light induced carbon monoxide-releasing molecules (CORMs)," *Chemistry Open* **2015**, 4, 590-594.

I will acknowledge your contribution to this part of my dissertation by the inclusion of a footnote on the title page for that chapter. Additionally, a copy of this letter will become an Appendix to the dissertation. Please advise me of any changes you require.

Please, indicate your approval of this request by signing the endorsement below. If you have any questions, please call me at the number above.

If possible, please provide your reply immediately. Thank you very much for your consideration, Stacey Anderson.

I hereby give permission to Stacey Anderson to reprint the manuscripts listed above in her dissertation.

Signed_____ Date 03/08/2016

CURRICULUM VITAE

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Education

August 2010-
May 2016

Utah State University

Department of Chemistry & Biochemistry; Logan, UT

PhD program in Inorganic Chemistry

Advisor: Lisa M. Berreau

Dissertation Title: "CO Release on Demand: Light-Induced

CO Release of Flavonols."

August 2005-
May 2010

Columbia College

BA Chemistry, minor concentration in Biology

Advisor: Alan James

Publications

1. <u>S. N. Anderson</u>, M. T. Larson, and L. M. Berreau, "Solution or solid – it doesn't matter: visible light-induced CO release reactivity of zinc flavonolato complexes," *Dalton Trans.* **2016**, 45, 14570-14580.

2. <u>S. N. Anderson</u>, J. M. Richards, H. J. Esquer, A. D. Benninghoff, A. M. Arif, and L. M. Berreau, "A structurally-tunable organic motif for carbon monoxide-releasing molecules (CORMs)," *ChemistryOpen*, **2015**, 4, 590-594.

3. <u>S. N. Anderson</u>, M. Noble, K. Grubel, B. Marshall, A. M. Arif, and L. M. Berreau, "Influence of supporting ligand microenvironment on the aqueous stability and visible light-induced CO-release reactivity of zinc flavonolato species," *J. Coord. Chem.* **2014**, 67, 4061-4075.

4. K. Grubel, A. M. Marts, S. M. Greer, D. L. Tierney, C. J. Allpress, <u>S. N. Anderson</u>, B. J. Laughlin, R. C. Smith, A. M. Arif, and L. M. Berreau, "Photoinitiated dioxygenase-type reactivity of open-shell 3d divalent metal flavonolato complexes," *Eur. J. Inorg. Chem.* **2012**, 4750-4757.

5. K. Grubel, S. L. Saraf, <u>S. N. Anderson</u>, B. J. Laughlin, R. C. Smith, A. M. Arif, and L. M. Berreau, "Synthesis, characterization, and photoinduced CO-release reactivity of a Pb(II) flavonolate complex," *Inorg. Chim. Acta* **2012**, 407, 91-97.

Patent Disclosure

- L. M. Berreau and <u>S. N. Anderson</u>, "Carbon monoxide releasing molecules and associated methods," Patent US 9,840,488; Dec 12,2017

Presentations

- <u>S. N. Anderson</u>, R. Zhang, J. M. Richards, M. Noble, A. M. Arif, and L. M. Berreau, "A new family of flavonols: tunable photoinduced carbon monoxide releasing agents," Present at the 248th American Chemical Society National Meeting and Symposium, San Francisco, CA, August 10-14, 2014. (Oral presentation)

- <u>S. N. Anderson</u> and L. M. Berreau, "New family of photoCORMs: photoinduced CO release in zinc 3-hydroxy-4-pyrone complexes," 245th American Chemical Society National Conference, New Orleans, LA, April 7-11, 2013. (Poster presentation)

- <u>S. N. Anderson</u> and L. M. Berreau, "New family of photoCORMs: photoinduced CO release in zinc 3-hydroxy-4-pyrone complexes," Utah State University Graduate Research Symposium, Logan, UT, April 12, 2013.

Awards and Honors

- Seely-Hinkley Scholar (2014-2015)

- Department of Chemistry and Biochemistry Teaching Award (2014)

- Joseph Rueul Harris Scholarship (2014)

Professional Memberships and Other Activities

- American Chemical Society (2010-present)

- USU Student Safety Committee, Founder and Chair

Teaching Experience

August 2010 – May 2015	1210 General Chemistry I Recitation
	1215 General Chemistry I Laboratory
	1220 General Chemistry II Recitation
	2325 Organic Chemistry II Laboratory
	3005 Quantitative Analysis Laboratory
	3520 Inorganic Chemistry Laboratory

Previous Work Experience

June 2005 – July 2010	Inpatient Pharmacy Technician, Columbia Regional Medical Hospital, Columbia, MO. Manager: Steven Lee
July 1999 – June 2005	Inpatient Pharmacy Technician, University of Missouri Hospital, Columbia, MO. Manager: Steven Calloway