Synthesis and Characterization of some Photo-CORM and Photosensitizers

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0. Populärvetenskaplig Sammanfattning

Det är väl känt att kolmonoxid (CO) är en giftig och hälsofarlig gas som är luktlös, färglös och smaklös vilket gör den svår att detektera utan analytisk utrustning. Men under senaste åren så har forskning bevisat att i små doser så kan kolmonoxid användas som en terapeutisk substans för att lindra malaria, cancer, lung-, hjärt- och kärlsjukdomar men även reducera mängden inflammatoriska substanser i kroppen. Hur får man då in kolmonoxiden i kroppen? Det mest självklara valet är att andas in gasen i små mängder men där uppstår ett problem, då man inte kan kontrollera vart i kroppen kolmonoxiden kommer att distribueras. Istället så kan man då använda så kallade CORMs (Carbon monoxide releasing molecules) för att kunna kontrollera mängden, och distributionen av kolmonoxid i patientens kropp. Dessa CORMs består av ett metall-center (i detta fall rhenium) med kolmonoxid bundet direkt till metallen med diverse ligander bundna till metallen för att ändra hur CORM:en beter sig.

I detta arbete så syntetiserades ett par photo-CORMs, alltså en molekyl som släpper ifrån sig kolmonoxid genom ljusstrålning, för att testa dess effektivitet i att släppa kolmonoxid i lösning. Det gjordes med UV-strålning då CORM:en endast reagerar med UV-strålning. Experimenten var lyckade och kolmonoxid släpptes i lösning och kunde detekteras med hjälp av IR, UV-vis spektroskopi och elektrokemi.

Det är inte alltid gynnsamt att använda UV-strålning då det är farligt för celler, och har en dålig penetrationsförmåga. Det är mycket mer gynnande att använda IR-strålning, men det går inte att ändra beteendet på en CORM så drastiskt så då syntetiserades några PS (Photosensitizers) för att lösa det problemet. En photosensitizer är en molekyl som tar emot elektromagnetisk strålning som exciterar sina elektroner, och överför sedan den exciterade energin till en annan molekyl genom kollision, bland annat. Syntetiseringen av ett par rutenium, järn och koppar komplex var lyckade, men hann aldrig testas i lösning med CORMs, så effektiviteten är ännu okänd.

1. Abstract

It is well known that Carbon monoxide is a toxic and hazardous gas, but during recent years it has been proven that using a controlled concentration, carbon monoxide can in fact be used as a therapeutic agent, and can contribute to decrease malaria, treat cardiovascular and lung diseases and potentially even treat cancer, among other things. The main problem in using carbon monoxide directly is the fact that it is not trivial to control the volume of gas inhaled, compromising its use as a therapeutic agent. Much effort has been made to synthesize molecules that can release carbon monoxide, so-called CORMs. The release of the carbon monoxide present in these molecules can occur in different ways: a) exchanging the carbon monoxide with solvent (e.g. water). Although this is an easy and controlled method to release a known quantity of carbon monoxide, it is not possible to guarantee the time and tissue the CO is released. b) triggering the release of carbon monoxide, for example by light exposure (Photo-CORMs). This can be an efficient method, since metal carbonyls are well known as photosensitive systems.

Two Photo-CORMs complexes, Re(aaz)(CO)3]Cl (aaz = 6-amino-6-methylperhydro-1,4-diazepine) and [Re(tacn)(CO)3]Cl (tacn = 1,4,7-triazacyclononane), were synthesized, fully characterized and tested for their ability to release carbon monoxide when exposed to light radiation. By determining the change in oxidation potential through square wave electrochemistry, as well as UV- and IR-spectra, there was enough proof to conclude that the complexes release at least 2 equivalents of carbon monoxide per Photo-CORM when radiated by UV light.

Four new complexes, containing photosensitizers were also synthesized and characterized [Ru(Me2bpy)2Cl2] (bpy = 2,2'-bipyridine) , [Ru(phen)2Cl2] (phen = 1,10-phenanthroline), [Cu(Me2-bpy)2]BF4 (4,4'-dimethyl-2,2'-bipyridine) and [Fe(η5-C5H4CHO)(η6-C6H6)]PF6 ((η5-C5H4CHO)(η6-C6H6) = η6-benzene,η5-cyclopentadienyl). All the four complexes absorb IR radiation and excite the Photo-CORM through triplet energy transfer. The IR spectroscopy showed that all photosensitizers were formed, since there were displacements of the bands and formation of new bands.

2. Introduction

1. Carbon Monoxide

It is well known that carbon monoxide (CO) is a highly toxic and deadly in high concentrations.[1] What makes CO deadly is that it has a high affinity to binding to hemoglobin competitively against oxygen (200 times higher affinity than oxygen), resulting in cellular asphyxiation. CO is a colorless, tasteless and odorless gas which makes it hard to detect by natural means resulting in unintentional poisoning.[2] CO is responsible for an average of 439 deaths annually in the U.S. caused by unintentional poisoning.[3] Although in recent years it has been found that CO can be used to treat lung diseases, malaria, sepsis, cardiovascular diseases, increase anti-inflammatory species, decrease pro-inflammatory species, and even potentially treat cancer.[1,4,5]

CO inhibits carboxyhemoglobin (COHb) and hemoglobin which prevents the red blood cells from using oxygen resulting in chemical asphyxiation. As the amount of inhibited COHb increases, the active sites for oxygen to bind in increases its affinity in the presence of CO, which results in the hemoglobin not being able to release any oxygen into the cell. CO competitively inhibits hemoglobins tetramer receptor site whereas CO has a much higher affinity than oxygen for the receptor site at hemoglobin which makes it impossible for oxygen to bind in to the active site.[4,6]

CO has shown to have therapeutic effects in both human and rodent cells. CO selectively inhibits the expression of pro-inflammatory cytokines and increases the production of anti-inflammatory cytokines both in vivo and in vitro. Which contributes to reduce sepsis, cardiovascular diseases and malaria.[7]

Heme oxygenase-1 (HO-1) has a protective effect against cerebral malaria (CM) because it catabolizes free heme groups, which are cytotoxic in the host circulation system. When HO-1 catabolizes hemoglobin; iron, CO and biliverdin is formed, which is the one of the biological ways to produce CO in the metabolism. It has been discovered that CO can, by inhibiting the free heme ring, disrupt the malaria parasite from polymerizing the heme-rings, rendering the parasite harmless, thus depressing the parasite. Research has shown that releasing CO molecules into CM infected mice reduces the infection completely.[8,9]

CO is inexpensive, readily available and can be distributed by different means, such as by inhalation of CO-gas, or through Carbon monoxide releasing molecules (CORMs). Metallic CORMs have very versatile characteristics, such as biocompatibility, low toxicity, water solubility, and are stable compounds. Different metals can be used as well as different ligands to alter the chemical properties of the CORM to easier deliver the CO to target destination in the host's body.[1,10]

2. CORMs

Inhalation of CO-gas is very unspecific and limited since the distribution in the body cannot be controlled and can be dangerous if the CO reaches the bloodstream.[5] By using CORMs the distribution becomes specific since the release of the CO is easily controlled by an external factor, such as an enzyme, organic/inorganic species, or light irradiation. This controls delivery location, dosage and timing for release of CO. A CORM releasing its CO through light irradiation is called a photo-CORM. It has been shown that it requires high energy excitation to dissociate CO from the CORM metallic center, since the Metal-CO binding has a strong affinity towards each other. If the excitation energy is low the only dissociation that is possible is the Metal-Metal bond, if it is present.[11,12] The CORMs are very stable in low oxidation states because of a combination σ-bonding and π-back-bonding between the metal

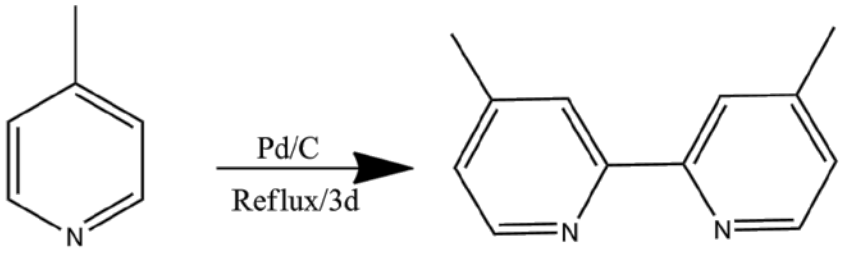
and the CO. The π-back-bonding is contributed by an empty low energy π*-antibonding orbital from CO interacting with a filled d-orbital. This relieves the metal of electron density added during σ-bond formation because the CO is electron withdrawing. Using light irradiation causes the d orbital electron density to decrease, as the HOMO (d_{π}-orbital) excites electrons to the LUMO ($d_{\pi*}$-orbital), thus weakening the π back-bonding affinity, which provides a way to dissociate CO from the metal center of the CORM.[11] Dissociating the CO from the metal center by light irradiation is caused by an excitation from the $d_{\pi*}$ to the $CO_{\sigma*}$ orbital which is from a nonbonding orbital to an antibonding orbital destabilizing the bond and thus breaking the sigma bond.[13]

High energy excitation is within the UV-range which is an issue for distributing drugs into a host since UV-light is toxic towards cells and tissue as well as a weak ability to penetrate skin as it can only penetrate 1 mm, which is an issue to activate photo-CORMs in the bloodstream or in organs.[12]

3. Photosensitizers (PS)

PS are compounds that absorbs electromagnetic radiation to elevate into an excited state to then transfer the excited energy unto a substrate by intermolecular collision, or intramolecular conformation change at the PS (such as oxidizing the PS) where the substrate normally cannot be activated. PS can be considered catalysts if they are not consumed during the reaction. A PS is useless on its own and needs a substrate to function properly.[14] When light is radiated on the PS the species excites into an excited state (singlet, or triplet state) which has a short lifetime of 10^{-10}-10^{-9} s. Through radiative (fluorescence) or non-radiative processes (internal conversion or intersystem crossing) the species deactivates into an excited triplet state which have a much longer lifetime of about 10^{-6}-10^{-3} s which is beneficial to increase the odds of conversion with the substrate.[15,16]

4. Experimental



4-picoline **4,4'-dimethyl-2,2'-bipyridine**

Figure 1: Reaction scheme for synthesis of 4,4'-dimethyl-2,2'-bipyridine from 4-picoline and Pd/C.

4,4'-dimethyl-2,2'-bipyridine[17]: 4-picoline and Pd/C (50:1 ; m/m) was added to a round bottom flask and refluxed at 140-150 ºC with stirring for 3 days. After the reflux the resulting solution was filtered hot to separate the catalyst from the product and 4-picoline. The filtrate was then distilled, to separate the 4-picoline from the product, and collected as white/yellowish crystals. The crystals were recrystallized over ethyl acetate and the solvent evaporated slowly under standard conditions (22 ºC, 1 atm). The white powder was then collected and analyzed with a yield of 10 % or 0.5 % (1H NMR (400 MHz, CDCl3): δ 8.54

(d, J = 4 Hz, 2H), 8.22 (s, 2H), 7.14 (d, J = 4 Hz, 2H), 2.44 (s, 6H), 13C (400 MHz, CDCl3): δ 156.03, 148.91, 148.12, 124.63, 122.00, 94.90, 21.18).

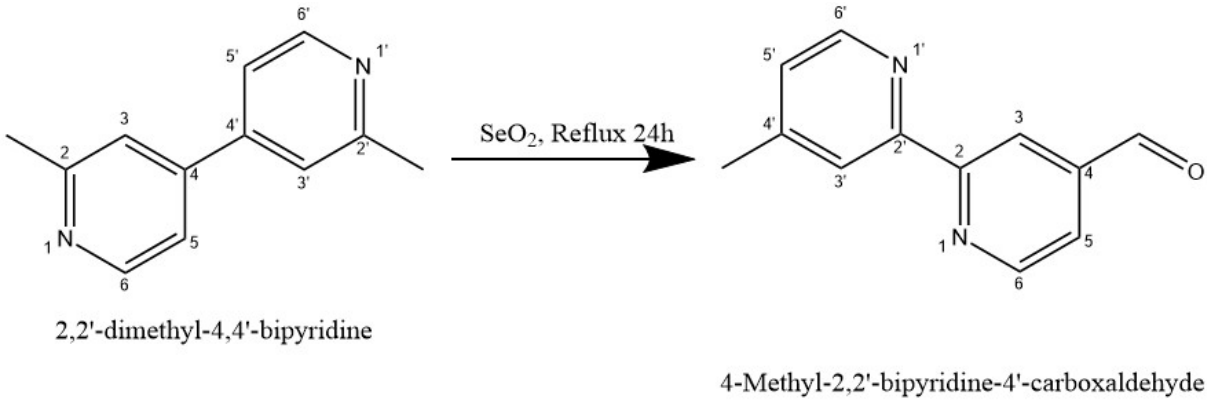


***Figure 2: Reaction scheme for synthesis of* 4-Methyl-2,2'-bipyridine-4'-carboxaldehyde from 2,2'-dimethyl-4,4'-bipyridine and SeO_2.**

4-Methyl-2,2-bipyridine-4-carboxaldehyde[18]: SeO_2 (1.44 g, 13 mmol) was dissolved in 1-4-dioxane (5 mL containing 4% H_2O) 2,2'-dimethyl-4,4'-bipyridine (1.84 g, 10 mmol) to 40 mL of 1-4-dioxane. The solution was heated and refluxed for 24 h and then the warm solution suction filtered through celite and washed with ethanol. The washed solution was mixed with the filtrate and then rotary evaporated to remove all solvent. The resulting beige/brown residue was suspended in saturated $NaHCO_3$ (50 mL) and then liquid-liquid extracted using DCM (5 x 50 mL). The combined organic phase was dried over $MgSO_4$ and the solvent (DCM) was removed through rotary evaporation. The yellowish solid was suspended in $Na_2S_2O_5$ (100 mL, 0.3 mol*L^{-1}) and stirred for 30 minutes and then suction filtered. This was repeated a second time for the solid using half the amount of $Na_2S_2O_5$ (50 mL, 0.3 mol*L^{-1}). The residue was washed with ethyl acetate (50 mL) and $NaHCO_3$ (18 g) was dissolved in the filtrate and then extracted with DCM (30 x 40 mL). The organic phase was dried over $MgSO_4$ and removed by rotary evaporation to yield the 4-Methyl-2,2-bipyridine-4-carboxaldehyde as a white solid with a yield of 26 % (0,663 g, 2.67 mmol).

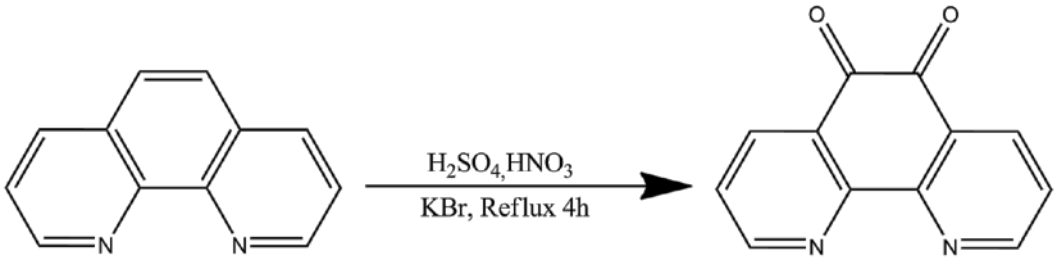


Figure 3: Reaction scheme for synthesis of 1,10-Phenanthroline-5,6-dione from 1,2-Phenantroline, H_2SO_4, HNO_3 and KBr.

1,10-Phenanthroline-5,6-dione[19]: 1,10-Phenanthroline (4.0 g, 22 mmol) and KBr (4.0 g, 33 mmol) were dissolved in an ice cold mixture of H_2SO_4 (6% in water) (40 mL) and concentrated HNO_3 (20 mL) dropwise. The solution was refluxed for 4 hours. The resulting hot yellow solution was poured over ice cold water (600 mL) and neutralized gently with solid NaOH until the pH reached a value of 6-7. The neutral solution was extracted with chloroform (50 mL x 5) and was dried over Na_2SO_4 to then be rotary evaporated and recrystallized from absolute ethanol. The 1,10-phenanthroline-5,6-dione (4.1 g, 19.8 mmol) was collected with a yield of 90 % (1H NMR (400 MHz, CDCl3): δ 9.14 (d, J = 4 Hz, 2H) 8.52 (d, J = 8 Hz, 2H), 7.61 (q, J = 7.6, 4.8 Hz, 2H, 13C (400 MHz, CDCl3): δ 178.67, 156.43, 152.91, 137.33, 128.07, 125.62).

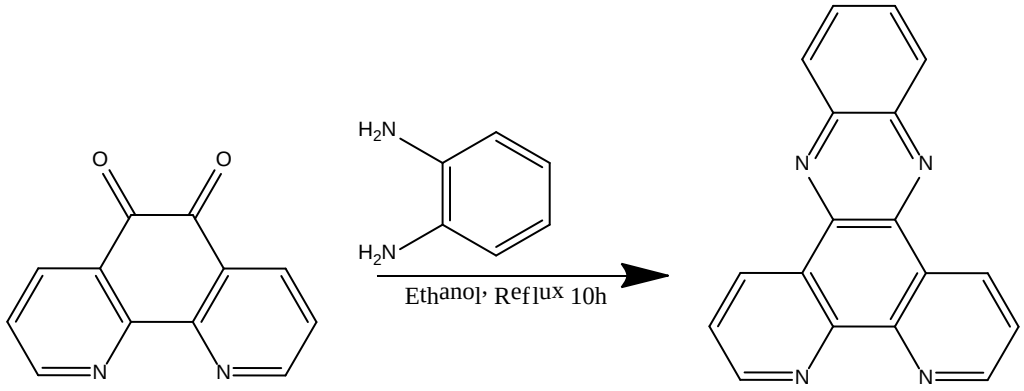


Figure 4: Reaction scheme for synthesis of Dipyridophenazine from* 1,10-Phenanthroline-5,6-dione*, o-phenylenediamine and ethanol.

Dipyridophenazine[20]: 1,10-phenanthroline-5,6-dione (0.40 g, 1.9 mmol) were dissolved in ethanol (20 mL) and o-phenylenediamine (0.24 g, 2.2 mmol), 4-methylbenezenesulfonic acid (8.75 mg, 0.05 mmol) were added respectively. The solution was refluxed for 10 hours. The resulting solution was filtered and recrystallized from ethanol to give the Dipyridophenazine (0.43 g, 1.52 mmol) with a yield of 80 %

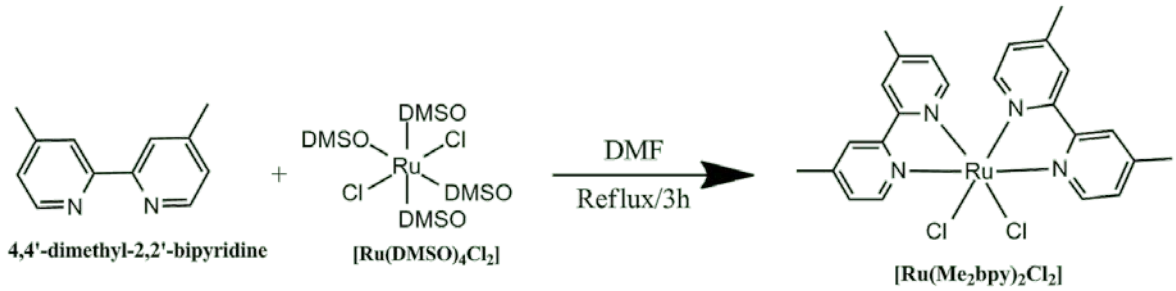


Figure 5: Reaction scheme for synthesis of [Ru(Me$_2$bpy)$_2$Cl$_2$] from 4,4'-dimethyl-2,2'-bipyridine, [Ru(DMSO)$_4$Cl$_2$] and DMF

[Ru(Me$_2$bpy)$_2$Cl$_2$] (a)[21]: One equivalent of [Ru(DMSO)$_4$Cl$_2$] was dissolved in DMF with two equivalents of 4,4'-dimethyl-2,2'-bipyridine and an excess of LiCl, and refluxed under controlled atmosphere at 110-130 ^{o}C for 3 hours, a colour change from yellow to red/purple was observed during reflux. After the reflux the resulting solution was washed with absolute ethanol and cooled down overnight to form precipitate. The cooled down solution was filtered to remove any bi-products. [Ru(Me$_2$bpy)$_2$Cl$_2$] was collected with a 60.6-64.8 % yield.

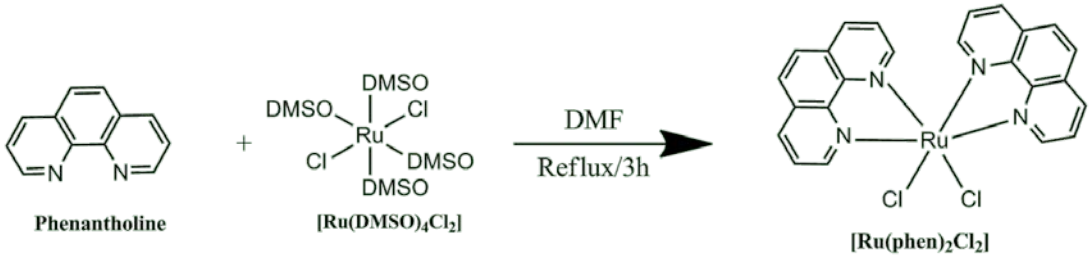


Figure 6: Reaction scheme for synthesis of [Ru(phen)$_2$Cl$_2$] from Phenanthroline, [Ru(DMSO)$_4$Cl$_2$] and DMF

[Ru(phen)$_2$Cl$_2$] (b)[21]: The [Ru(phen)$_2$Cl$_2$] was synthesized using the same method as for [Ru(Me$_2$bpy)$_2$Cl$_2$] only using phenanthroline as ligand with a similar yield between 61.1-65.0 % yield.

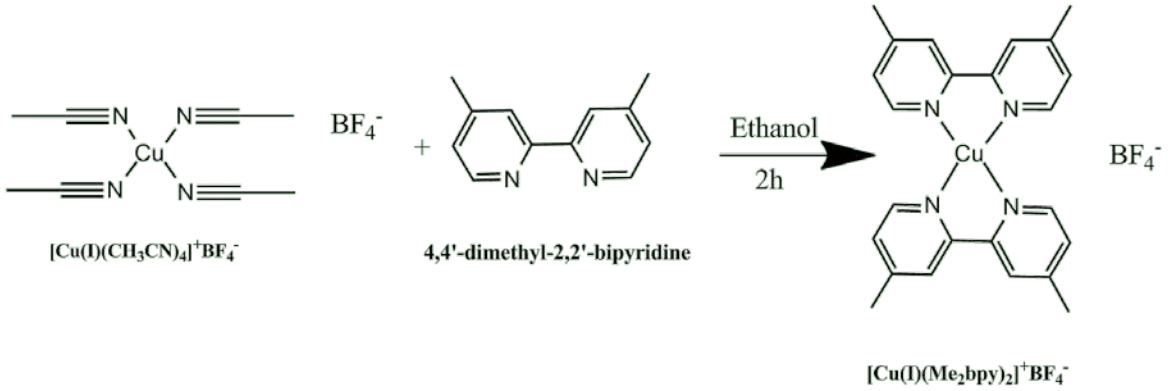


Figure 7: Reaction scheme for synthesis of $[Cu(I)(Me_2\text{-}bpy)_2]BF_4$ from $[Cu(CH_3CN)_4]BF_4$, 4,4'-dimethyl-2,2'-bipyridine and Ethanol

$[Cu(I)(Me_2\text{-}bpy)_2]BF_4$[22]: one equivalent of $[Cu(CH_3CN)_4]BF_4$ was dissolved in absolute ethanol where after two equivalents of 4,4'-dimethyl-2,2'-bipyridine was added to the solution slowly under stirring for 2h. Afterwards the solution was suction filtered, the precipitate was dried in the oven (65 ^oC) and collected once dry meanwhile the filtrate was rotor evaporated. The resulting brown solid was then washed with DCM and left in the freezer overnight to form precipitate. $[Cu(Me_2\text{-}bpy)_2]BF_4$ was collected as a brown oil with a yield of 16.2 % and further analyzed with ATR.

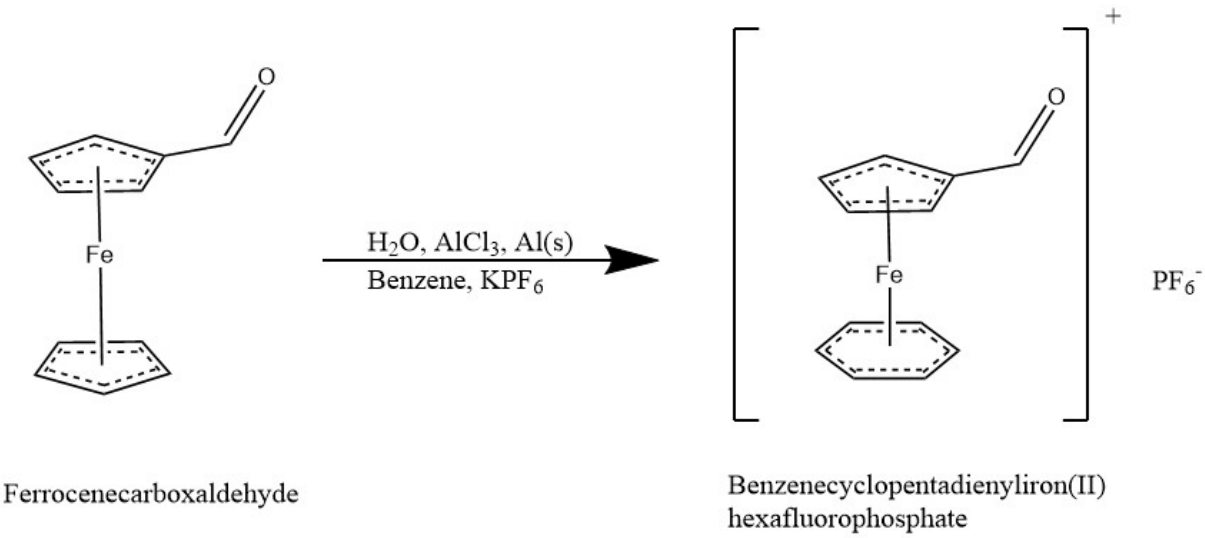


Figure 8: Reaction scheme for synthesis of $[Fe(\eta\text{-}C_5H_4CHO)(\eta\text{-}C_6H_6)]PF_6$ from Ferrocenecarboxaldehyde, H_2O, $AlCl_3$, Al(s), benzene and KPF_6

$[Fe(\eta\text{-}C_5H_4CHO)(\eta\text{-}C_6H_6)]PF_6$[23]: Ferrocenecarboxaldehyde (2.0 g, 9.34 mmol) was dissolved in benzene (10 mL) and Aluminum powder (0.2 g), $AlCl_3$ (4.0 g, 30.0 mmol) and water (0.2 mL) was added respectively under stirring solution. The solution was gently refluxed for 45 minutes, cooled down and ice-cold water (25 mL) was added to the resulting solution. The aqueous phase was extracted and diluted up to 50 mL using water. KPF_6 (2.5 g) was dissolved to the solution and stirred for 10 minutes. Thereafter the solution was suction filtered and washed with water, ethanol and diethyl ether respectively and the green precipitate was dried overnight. The precipitate was then dissolved in acetonitrile (10 mL) and filtered to then have tetrachloroethane (3 mL) added and be rotary evaporated until 3 mL of liquid remains. The solid was filtered and washed with dichloromethane and the $[Fe(\eta\text{-}C_5H_4CHO)(\eta\text{-}C_6H_6)]PF_6$ was collected as a brown solid with a yield of 25.7 % (0.54 g, 2.40 mmol)

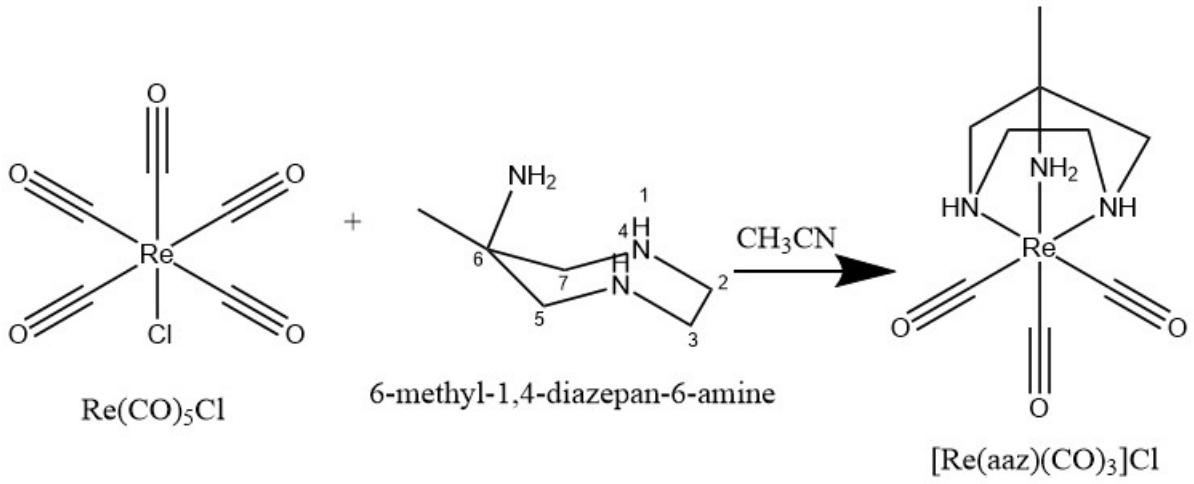


Figure 9: Reaction scheme for synthesis of [Re(AAZ)(CO)$_3$]Cl from [Re(CO)$_5$]Cl, aaz and acetonitrile.

[Re(aaz)(CO)$_3$]Cl[24]: aaz (0.094g, 0.3 mmol) was dissolved in acetonitrile (10 mL) to then be dried over liquid-N_2 and heated up to room temperature two times. [Re(CO)$_5$]Cl (0.094 g, 0.3 mmol) was added to the solution and the round bottom flask is dried a third time and was then covered in aluminum foil. The solution was then heated to 80 ºC and stirred for 12 hours without any light exposure. During the stirring a yellow precipitation could be observed. The precipitate was filtered and washed with DCM and the [Re(aaz)(CO)$_3$]Cl (0.091 g, 0.21 mmol) was collected with a yield of 70 %

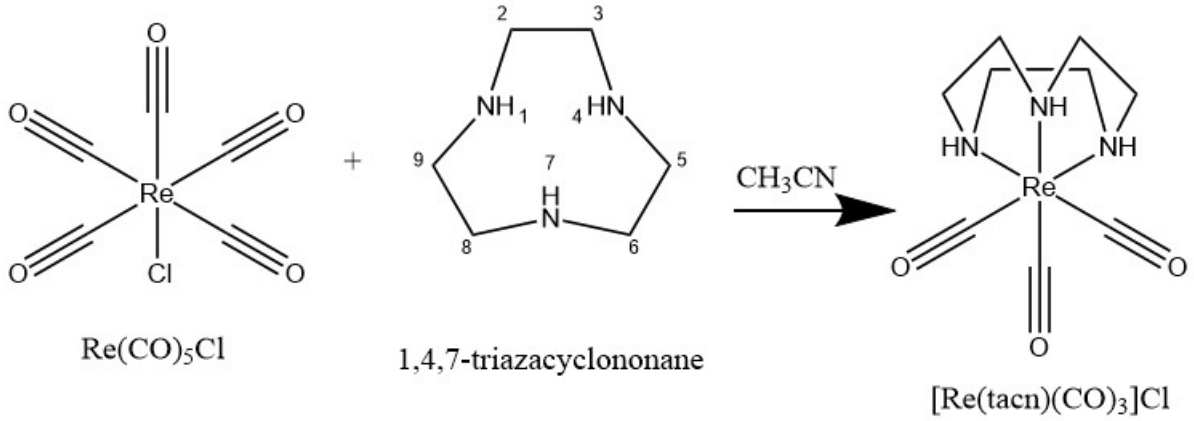


Figure 10: Reaction scheme for synthesis of [Re(tacn)(CO)$_3$]Cl from Re(CO)$_5$Cl, 1,4,7-triazacyclononane and acetonitrile

[Re(tacn)(CO)$_3$]Cl[24]: The [Re(tacn)(CO)$_3$]Cl was synthesized using the same method described as [Re(aaz)(CO)$_3$]Cl with a yield of 74%

5. Results and Discussion

1. Ligands

The 4,4'-dimethyl-2,2'-bipyridine synthesis literature shows low yields and long reaction times, which meant the synthesis had to be repeated several times to get enough ligands for the complexes. The literature implied the product would be separated from the reactant using rotary evaporation, but we chose to distill the solution as the distillation setup was more readily available and more controlled than the evaporation. Since the yield was so low, we wanted to extract as much of the product as possible. Carrying out the reaction by reflux for

three days using roughly 150 mL of 4-methylpyridine and 1/50 the mass of Pd/C only gave trace amounts of the product, so we decided to reduce the amount of reactant to 70 mL and increase the reflux to 5 days which greatly increased the yield to roughly 10%. The low yields were suspected to be caused by the reflux being too gentle, so we tried a more aggressive reflux which resulted in higher yields. Some of the reactions were suspected to be contaminated by water since the solution did not boil at the right temperatures under vacuum, and when it boiled, it was highly aggressive. The contamination came from the condenser, as it was an open system and the conditions had high relative humidity. No bi-products were taken into account since the addition of the C-C bond is exclusively meta-directing to the methyl groups, and para/ortho directing to the nitrogen. Purifying the product through distillation removed any unwanted species Both the carbon and hydrogen NMR spectra were in agreement with the structure which proves successful synthesis and the purity of the crystals.

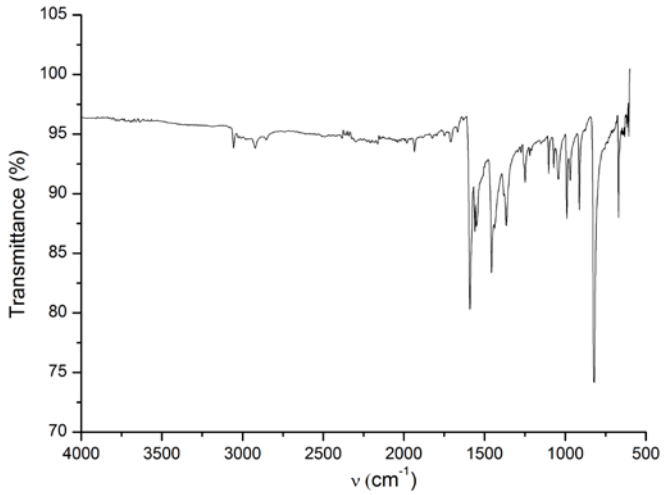


Figure 11: ATR-spectrum of 4,4'-dimethyl-2,2'-bipyridine

The 4-Methyl-2,2-bipyridine-4-carboxaldehyde were synthesized with a yield of 26% without any trouble since the synthesis is well established. The synthesis was altered in the first reflux to fit the amount of reactant we used (10 mmol 4,4'-dimethyl-2,2'-bipyridine) to not waste any excess of 4,4'-dimethyl-2,2'-bipyridine. The rest of the reaction used the same amount as the referenced literature. Although the liquid-liquid extraction was not so successful as the referenced reaction, as they suggested 20 extractions. Though after 20 extractions there was still product in the organic phase, which was checked with TLC, so we did five further extractions where in there was still a small spot on the TLC plate, so the yield could perhaps have been improved. Comparing the IR spectra between the reactant and the product shows three peaks at 2914, 2850 and 1700 cm^{-1} at the product spectrum which are not present at the reactant spectrum. This suggests that the 2914 and 2850 cm^{-1} are the C-H stretches for the aldehyde and the 1700 cm^{-1} is the stretch for C=O for the aldehyde.

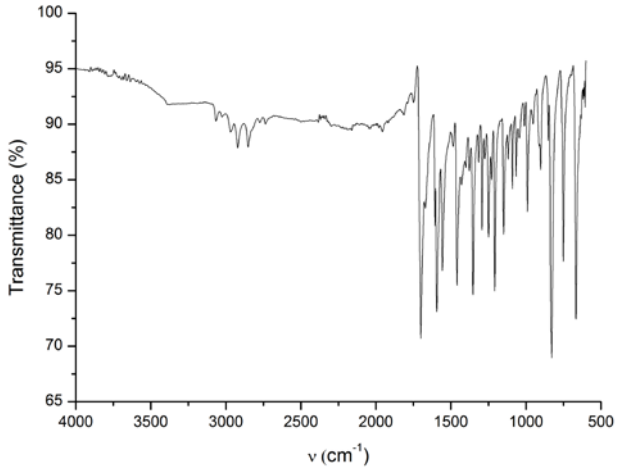


Figure 12: ATR-spectrum of carboxaldehyde bipyridine

The 1,10-Phenanthroline-5,6-dione were synthesized with a yield of 90 %. The steps in the synthesis were followed according to the literature but with different amounts of reactants. The refluxed solution was neutralized to make sure that the intermediate is protonated and not in its anion state, so the species will exclusively end up in the organic phase for the extraction. ^{1}H as well as ^{13}C NMR analyses concluded that the synthesis was successful since the NMR data were in agreement with the structure.

Dipyridophenazine (dppz) was synthesized with a yield of 80 % and the reaction was followed according to the literature. It is important to add the reactants in the right order as bi-reactions may occur if they are added in the wrong order. There was no NMR data for the dppz ligand since we had trouble dissolving the product in $CDCl_3$ so the product was analyzed by IR instead. The IR data shows a strong band at 1339 cm^{-1} that implies there is aromatic N-C stretches. There is also a weak band at 3065 cm^{-1} which corresponds to C-H aromatic stretch and a weak band at 1630 cm^{-1} which corresponds to C=C conjugated stretch. This does not prove the exact structure of the compound but there is certain evidence that all the bonds that are in the IR spectrum are in the product.

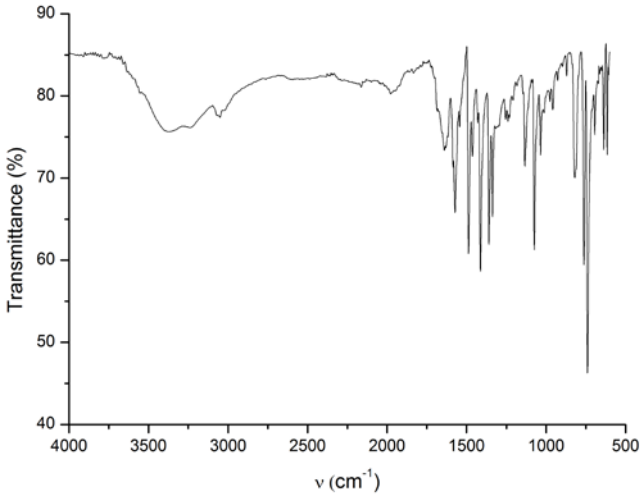


Figure 13: ATR-Spectrum of dppz

2. Photosensitizers

For the $[Ru(Me_2bpy)_2Cl_2]$ and $[Ru(phen)_2Cl_2]$ synthesis an excess of LiCl was used for the purpose of having chloride ions present in solution incase a bound chloride dissociates from the complex during the reflux, making sure that no solvent nor bi-products binds competitively to the complex. It is important to have chloride bound to the complex for further reactions such as binding another Me_2bpy to the complex or creating a Ru-Ru bridge as the chloride acts as good leaving group for further synthesis. Having a controlled atmosphere, in this case a nitrogen atmosphere is important to keep any water and oxygen out of the reaction vessel in case any bi-products would form. The reactant complex is a trans isomer since the steric hindrance and the electron withdrawing effects of the chloride ions are dominant for the isomerism. There was no characterization done for the product to see if the isomerism was changed. It is possible for the product to react with another bipyridyl ligand in both trans and cis states as the chlorides dissociate before the ligand binds to the metal since it is not favored to put electrons into a higher d orbital for the metal, it is more energy efficient to first dissociate the chloride into at 16 electron complex and then attach the bipyridyl. The equipment used for the ATR spectra, did not allow measurements below 500 cm^{-1} so there was no way of actually report the Ru-N stretch although it can be proven that the bond exists by looking at the displaced N=C bonds for the ligands, and that band should be at 1342-1266 cm^{-1} but shows up at 1416 cm^{-1} for our spectrum which proves there is a metal center present.

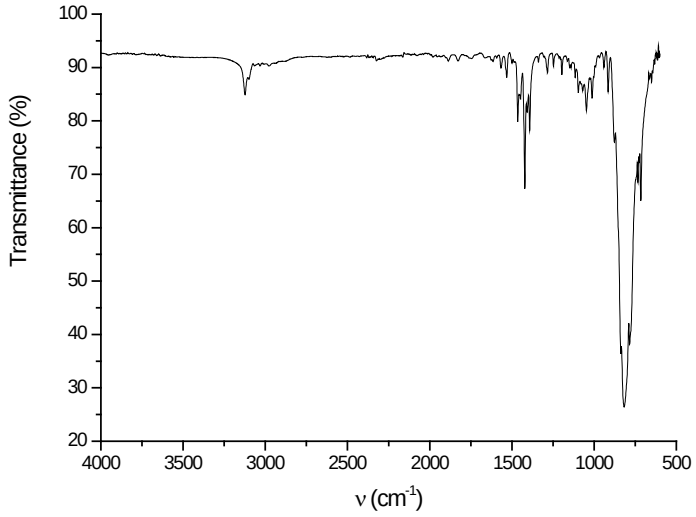


Figure 14: ATR-spectrum of $Ru(Me_2Bpy)_2Cl_2$

$[Cu(Me_2\text{-}bpy)_2]BF_4$ was synthesized according to the literature but the yield (16.2%) was surprisingly low as the literature was considerably higher. There is no known reason for why the yield was so low, as the Me_2-bpy and $Cu(CH_3CN)_4$ was pure. The reason is ultimately human error as there was little to no equipment used that could cause the low yield. The same can be said about the IR-spectrum from the $[Ru(Me_2bpy)_2Cl_2]$ as for the $[Cu(Me_2\text{-}bpy)_2]BF_4$ IR-spectrum as the N=C stretch is reported at 1420 cm^{-1} which again proves that a metal center is present.

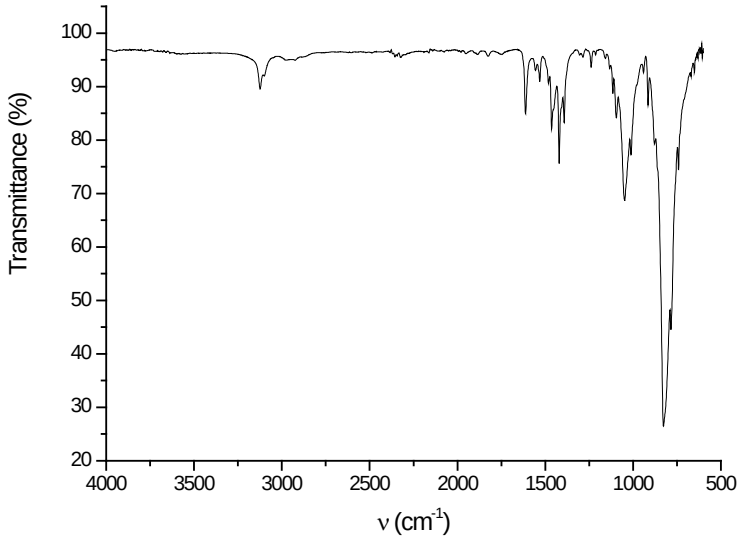


Figure 15: ATR-spectrum of $Cu(Me_2Bpy)_2$.

$[Fe(\eta\text{-}C_5H_4CHO)(\eta\text{-}C_6H_6)]PF_6$ was synthesized according to the literature and analyzed with ATR IR. The sample was not completely pure as seen by the ATR-spectrum in the 1200-800 cm^{-1} range which only has one broad peak. Using ferrocene carboxaldehyde as reference one difference is the absence of the peak at 3050 cm^{-1} which corresponds to the C-H stretch for the aromatic ring, which should still be visible as benzene exchanged from the cyclopentadiene should have a stronger peak. There is no visible peak at 1750-1700 cm^{-1} for neither the reactant nor the product, which is the C=O aldehyde stretch which makes it impossible to know if the synthesis was successful or not as the aldehyde stretch would prove if only the cyclopentadiene would be substituted or if both the carboxy cyclopentadiene and the cyclopentadiene would be substituted.

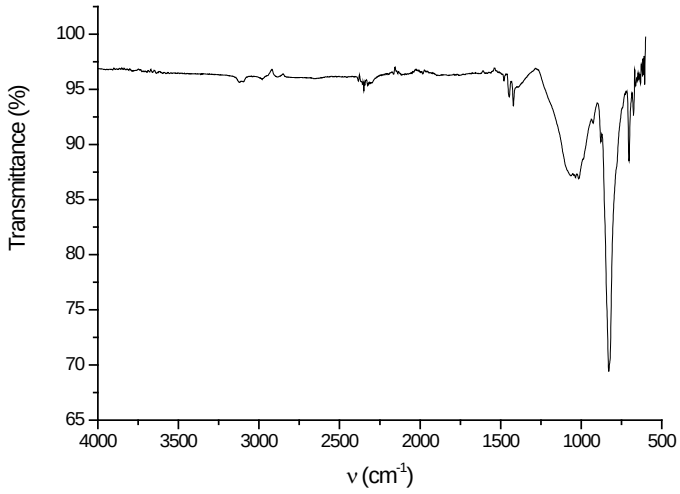


Figure 16: ATR-spectrum of $[Fe(\eta\text{-}C_5H_4CHO)(\eta\text{-}C_6H_6)]PF_6$.

3. Photo-CORM

The synthesis for [Re(aaz)$(CO)_3$]Cl and [Re(tacn)$(CO)_3$]Cl are very light-sensitive as the product and the reactant can photo decompose so it was important to keep light away from the reaction vessel using aluminum foil and reduced lightning in the lab. The solution was cooled down using liquid nitrogen to exclude any oxygen to the reaction vessel which is important so no bi-products can be created. The oxygen is excluded since the freezing point for oxygen is below the temperature of the liquid nitrogen, so when the solvent is freezing, the gaseous oxygen is pushed out of the solution into the atmosphere. the cooling is done three times to ensure complete absence from oxygen.

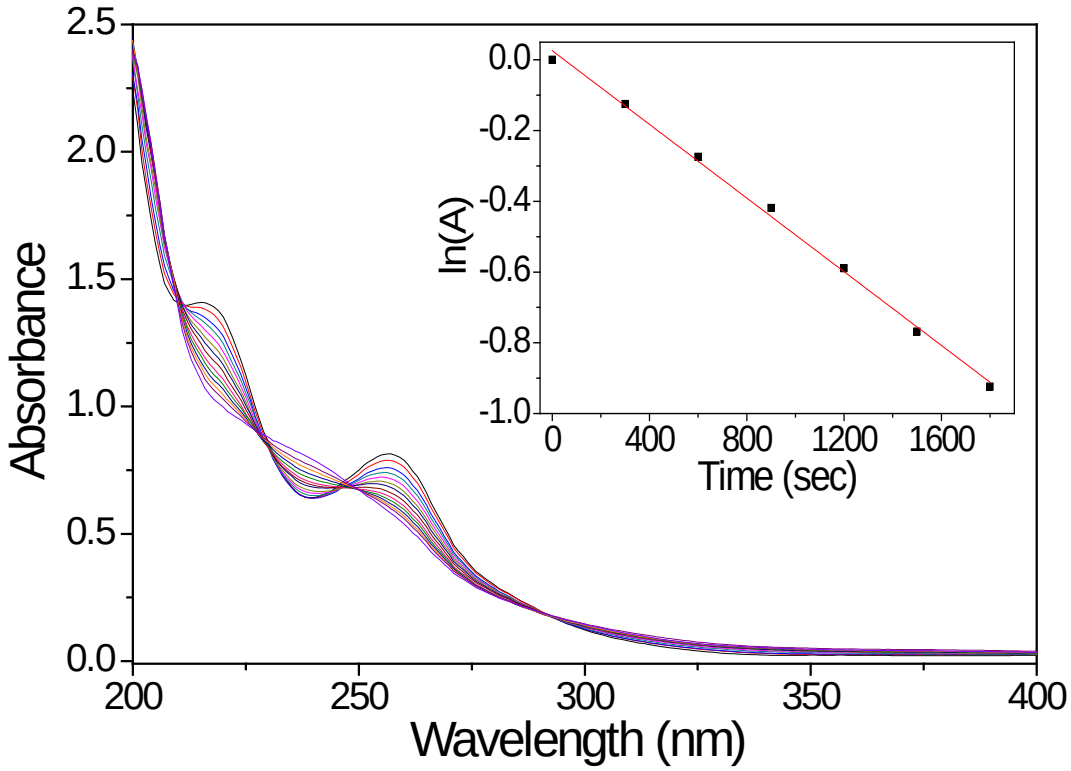


Figure 17: UV-VIS spectrum and rate of reaction for [Re(TACN)$(CO)_2$]Cl (1 mmol*L^{-1} in water) after light exposure from 400 to 200 nm, showing the initial rate constant at 255 nm, where the intervals between each measurement is 5 minutes and the blanc is water.

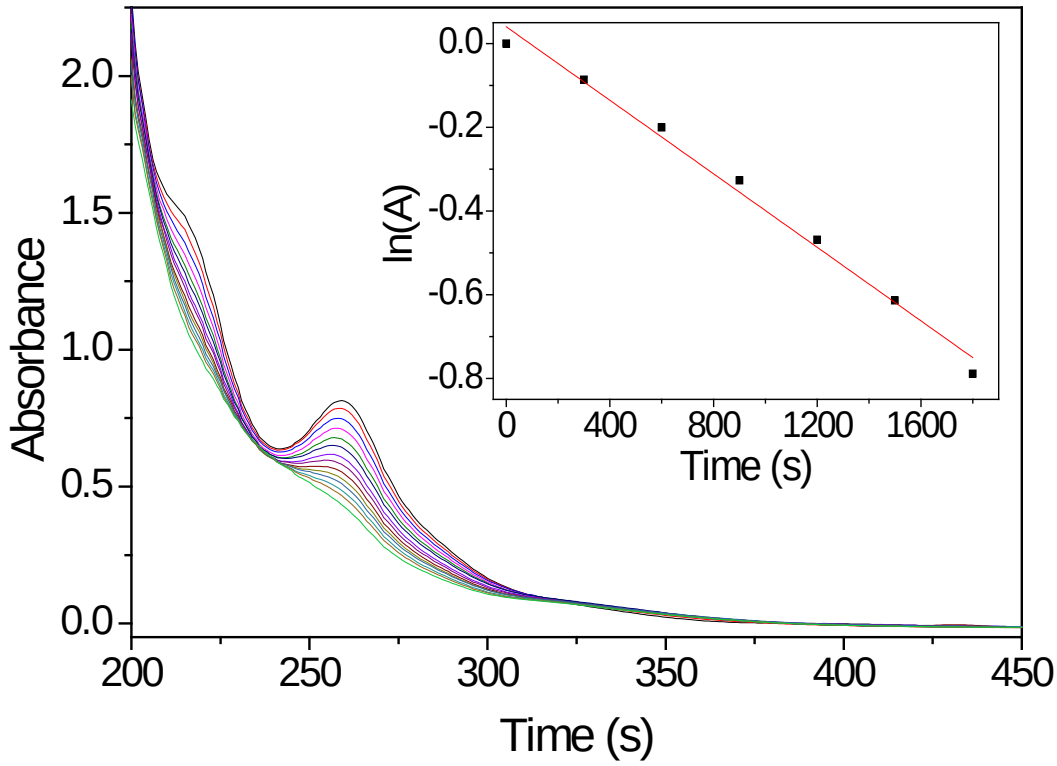


Figure 18: UV-VIS spectrum and rate of reaction for [Re(AAZ)(CO)$_2$]Cl (1 mmol*L^{-1}) after light exposure from 450 to 200 nm, showing the initial rate constant at 259 nm, where the intervals between each measurement is 5 minutes and the blanc is water.

As seen in Figure 17 and Figure 18 the UV-VIS experiments for both the aaz and the tacn complexes shows the same results. The peak at 255 and 259 nm is decreasing as the light is exposed to the complex. The irradiated light is at 255 nm for both complexes but the peak for the aaz complex is still decreasing, so the experiments was irradiated at the same wavelength. The peak that is decreasing is the result of the complexes releasing CO, thus increasing the electron density at the metal center, making it less readily available for absorption. As the peak in the 255-259 nm area is decreasing, a peak is starting to form in the 220 nm area for the aaz complex and 235 nm for the tacn complex as that is the intermediate product where the complex has bonded a solvent molecule to stabilize itself. Although there is an obvious difference for the tacn and the aaz complex as the tacn complex has a peak decrease in the 220 nm when the aaz has an increase at that range. This is suspected to be because the tacn complex forms 2 different intermediates as aaz does not.

Reaction rate (k)	s^{-1} (10^{-4})
[Re(tacn)CO_3]Cl	5.21
[Re(aaz)CO_3]Cl	4.39

Table 1: Showing the initial reaction rates taken from figure 17 and 18 of the Rhenium complexes at 255 nm and 259 nm respectively where the activity is at a maximum.

As seen by Table 1, which is the calculated rate constant for each complex the tacn has a slightly higher reaction rate than aaz does. It is important to note that the rate constant graph in Figure 7 and 8 is not linear but is treated as pseudo-first order reaction as the only wanted interaction is the one the complex makes with the irradiated light.

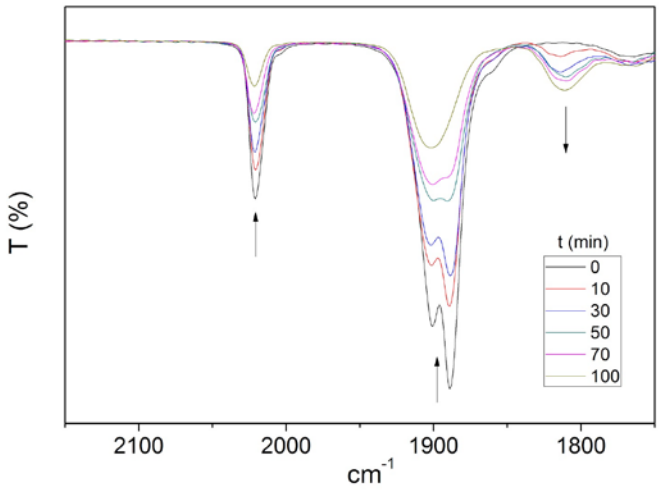


Figure 19: IR-spectrum for [Re(TACN)(CO)$_2$]Cl after light exposure at 255 nm where the intervals between each measurement is shown to the right in the figure.

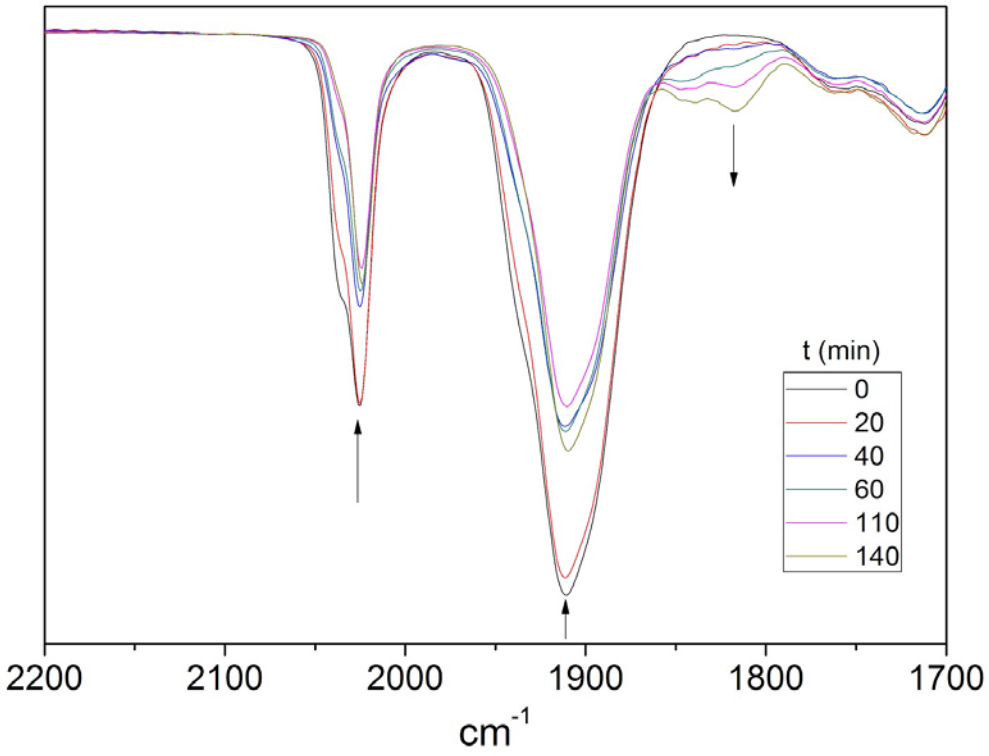


Figure 20: IR-spectrum for [Re(AAZ)(CO)$_2$]Cl after light exposure at 259 nm where the intervals between each measurement is shown to the right in the figure.

The same can be said for the IR measurements as for the UV-VIS. As the CO releases from the complex, the intensity of the peak is decreasing, as well as an increase in a new broader peak for the intermediate compound that is formed. But the difference between the UV-VIS

and the IR is that both the complexes reacts the same way in the IR and differently in the UV-VIS since the IR has 2 decreasing peaks and 1 increasing peak.

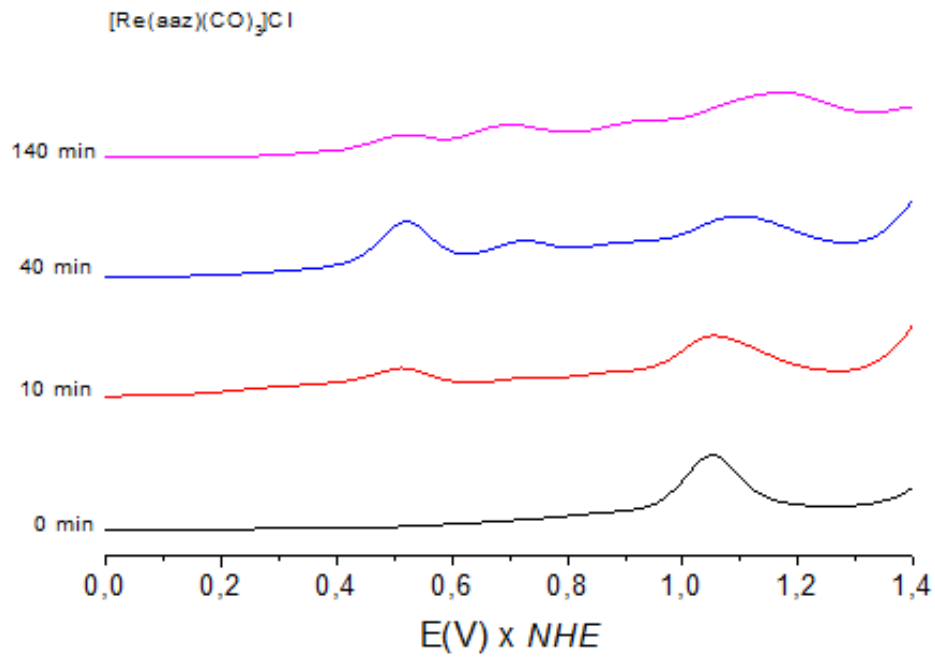


Figure 21: Square wave voltammetry graph of [Re(aaz)(CO)$_3$Cl dissolved in water after t minutes of light exposure at 255 nm with 200 mV/s intervals.

The same experiment was done using square wave voltammetry to see the oxidation capacity of the complexes and how it changes when the CO is released. At t = 0 there is only one peak, at roughly 1.05 V but after 10 minutes of exposure a peak is formed at 0.5 V, this proves that the CO is released, and an intermediate is created as a result. After 40 minutes the peak at 0.5 V is at its highest but a peak at 0.7 V is starting to show up, as well as the unreacted complex is displacing towards higher potentials. This suggests another intermediate is formed and could be the results of the complex releasing another CO which enables new intermediated such as M=M bond intermediates.

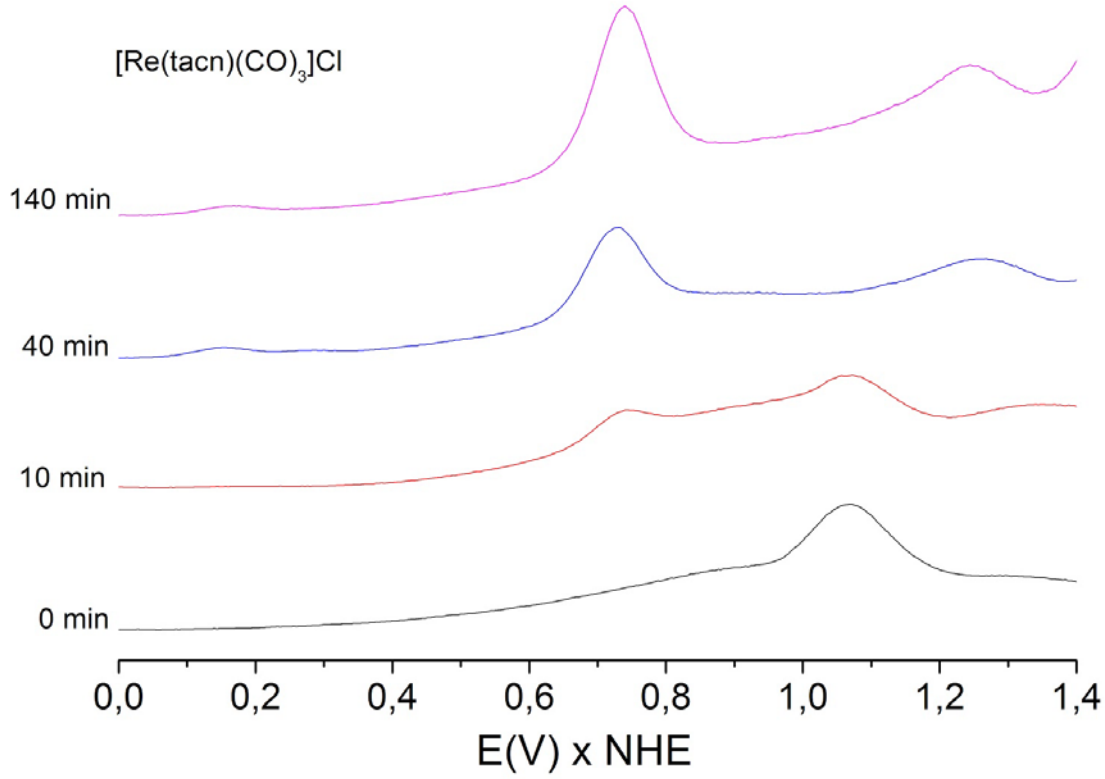


Figure 22: Square wave voltammetry diagram of [Re(tacn)(CO)$_3$Cl dissolved in water after t minutes of light exposure at 255 nm with 200 mV/s intervals.

The same can be seen for the tacn complex although the peak formed from the first intermediate is much stronger than for the aaz complex and is at a higher potential but decreases rapidly as the first intermediate peak is quite strong. The displacement of the unreacted complex is bigger than for the aaz complex but is suspected to be the second CO release intermediate, but it is unsure as the first intermediate does not decrease as it does the aaz complex. This could mean that the second intermediate is not dependent on the first intermediate for the tacn complex although it is not proven.

4. Conclusion

A series of ligands and complexes were synthesized with varied yields ranging from trace amounts, up to 90 % yield. The Bipyridine syntheses was optimized, since the yields were so low, to yield the highest amount of product as there was a high demand for the ligand. The rest of the ligands were easily synthesized with a high yield which meant that the reaction did not have to be repeated. The purity of the ligands was proved by IR and NMR spectroscopy. The complex syntheses were yielded high amounts of product and the Re complexes were characterized to test their effectivity in releasing CO molecules under light irradiation with successful results and the rest of the complexes were characterized to prove their purity.

5. References

1.Malamati Kourti, Wen G. Jiang, and Jun Cai, “Aspects of Carbon Monoxide in Form of CO-Releasing Molecules Used in Cancer Treatment: More Light on the Way,” Oxidative

Medicine and Cellular Longevity, vol. 2017, Article ID 9326454, 12 pages, 2017. https://doi.org/10.1155/2017/9326454
2.Mark Goldstein. Carbon Monoxide Poisoning, Journal of Emergency Nursing, Volume 34, Issue 6, 2008, Pages 538-542.
3. Neil B. Hampson, Cost of accidental carbon monoxide poisoning: A preventable expense, Preventive Medicine Reports, Volume 3, 2016, Pages 21-24, ISSN 2211-3355, https://doi.org/10.1016/j.pmedr.2015.11.010.
4. Motellini, R, Otterbein L. E. The therapeutic potential of carbon monoxide *Nature Reviews Drug Discovery* volume 9, pages 728–743 (2010) https://doi.org/10.1038/nrd3228
5. Gullotta, F. , Masi, A. d. and Ascenzi, P. (2012), Carbon monoxide: An unusual drug. IUBMB Life, 64: 378-386. doi:10.1002/iub.1015
6Blumenthal I. Carbon monoxide poisoning. *J R Soc Med*. 2001;94(6):270-2.
7. Stefan W Ryter, Augustine MK Choi, Therapeutic applications of carbon monoxide in lung disease, Current Opinion in Pharmacology, Volume 6, Issue 3, 2006, Pages 257-262, ISSN 1471-4892, https://doi.org/10.1016/j.coph.2006.03.002
8. Viktória Jeney, Susana Ramos, Marie-Louise Bergman, Ingo Bechmann, Jasmin Tischer, Ana Ferreira, Virginia Oliveira-Marques, Chris J. Janse, Sofia Rebelo, Silvia Cardoso, Miguel P. Soares, Control of Disease Tolerance to Malaria by Nitric Oxide and Carbon Monoxide, Cell Reports, Volume 8, Issue 1, 2014, Pages 126-136, ISSN 2211-1247, https://doi.org/10.1016/j.celrep.2014.05.054
9. Ana Pamplona, Ana Ferreira, József Balla, Viktória Jeney, György Balla, Sabrina Epiphanio, Ângelo Chora, Cristina D Rodrigues, Isabel Pombo Gregoire, Margarida Cunha-Rodrigues, Silvia Portugal, Miguel P Soares, Maria M Mota. Heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria. Nature Medicine volume 13, pages 703–710 (2007), https://doi.org/10.1038/nm1586
10. Pickens, R. N. Inorg. Chem. 2018, 57: 11616-11625.
11. Kubeil, M. Inorg. Chem. 2017 56(10): 5941-5952.
12. Askes, S. H. C. JACS 2017 139(43): 15292-15295.
13. Rosa, A., Ricciardi, G., Baerends, E. J., & Stufkens, D. J. Inorg. Chem 1996, 35, 2886-2897.
14. Gilbert Laustriat,Molecular mechanisms of photosensitization,Biochimie,Volume 68, Issue 6,1986,Pages 771-778.
15. Quintero, Bartolome & Miranda, Miguel. (2000). Mechanisms of photosensitization induced by drugs: A general survey. Ars Pharmaceutica. 41. 27-46.
16. Béatrice M. Aveline,Chapter 2 Primary processes in photosensitization mechanisms,Editor(s): Piergiacomo Calzavara-Pinton, Rolf-Markus Szeimies, Bernhard Ortel,Comprehensive Series in Photosciences, Elsevier, Volume 2, 2001, Pages 17-37.
17. Eur. J. Org. Chem. 2004, 235-254
18. Busche, C. , Comba, P. , Mayboroda, A. and Wadepohl, H. (2 Eur. J. Inorg. Chem., 2010: 1295-1302.
19. Akbarzadeh-Torbati N, Sarani Y. Synthesis and Characterization of Cd Complexes Containing Phenanthroline Derivatives. Biomed Pharmacol J 2016;9(2).
20. Yue Wang, Renfeng Song, Ke Guo, Qingtao Meng, Run Zhang, Xiangfeng Konga and Zhiqiang Zhang. Dalton Trans., 2016,45, 17616-17623
21. McCusker C. E, McCusker J. K. Inorg. Chem. 2011, 50, 1656–1669.
22. Pallenberg A. J, Koenig K. S, Barnhartlb D. M. Inorg. Chem. 1995, 34, 2833-2840.
23. Didier Astruc, Organo-iron complexes of aromatic compounds. Applications in synthesis, Tetrahedron, Volume 39, Issue 24 1983, Pages 4027-4095.

24. Gonzalez, M. A.; Yim, M. A.; Cheng, S.; Moyes, A.; Hobbs, A. J.; Mascharak, P. K. Manganese Carbonyls Bearing Tripodal Polypyridine Ligands as Photoactive Carbon Monoxide-Releasing Molecules. Inorganic Chemistry, v. 51, p. 601-608, 2012.

6. Appendix

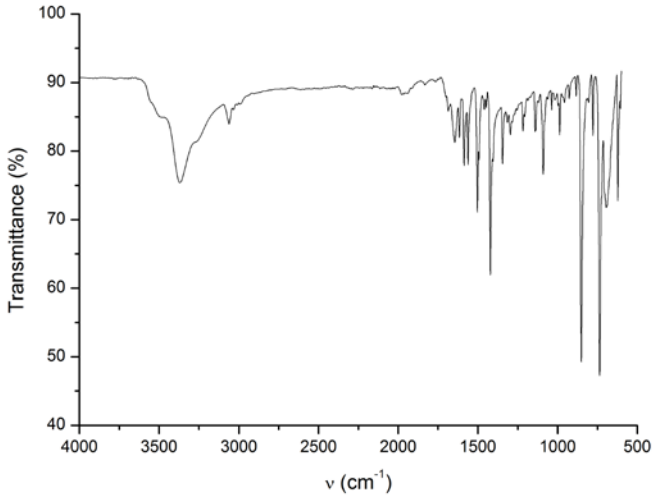


Figure 23: ATR-spectrum of Phenanthroline

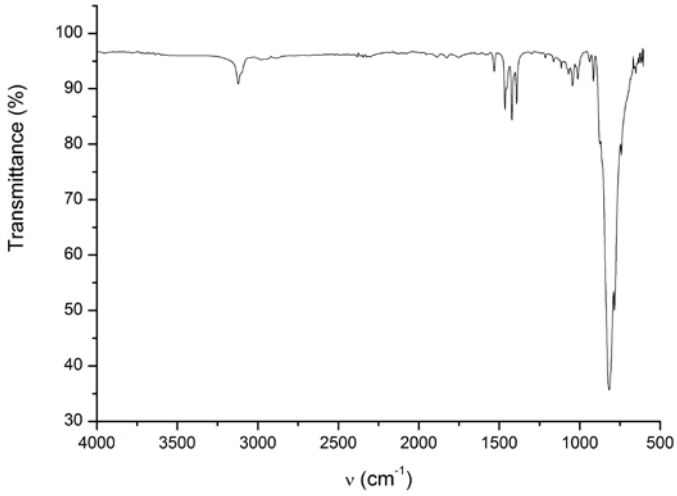


Figure 24: ATR-Spectrum of Ferrocene

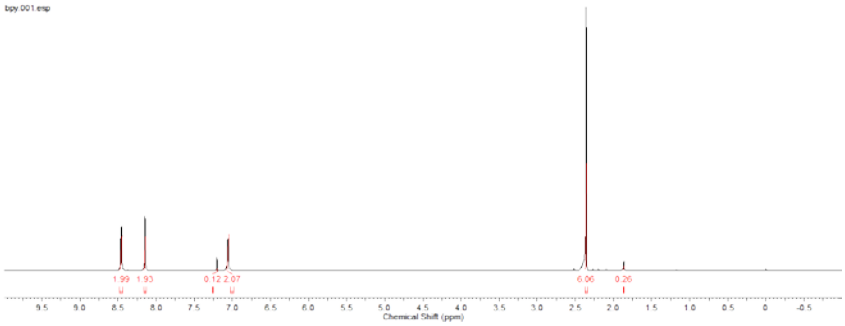


Figure 25: 1H NMR-spectrum of 4,4'-dimethyl-2,2'-bipyridine. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (d, J = 4 Hz, 2H), 8.22 (s, 2H), 7.14 (d, J = 4 Hz, 2H), 2.44 (s, 6H)

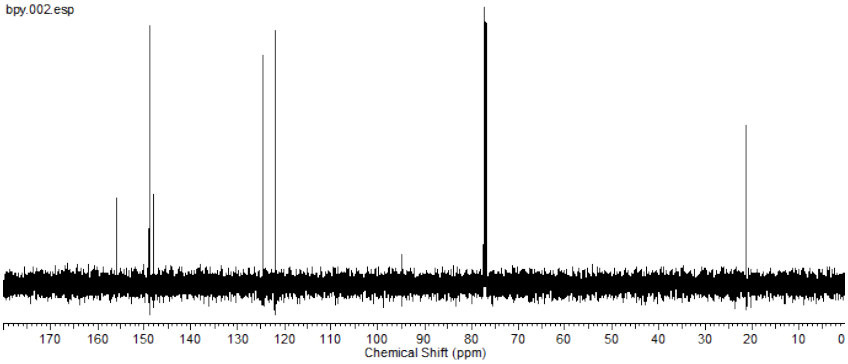


Figure 26: 13C NMR-spectrum of 4,4'-dimethyl-2,2'-bipyridine ^{13}C (400 MHz, $CDCl_3$): δ 156.03, 148.91, 148.12, 124.63, 122.00, 94.90, 21.18

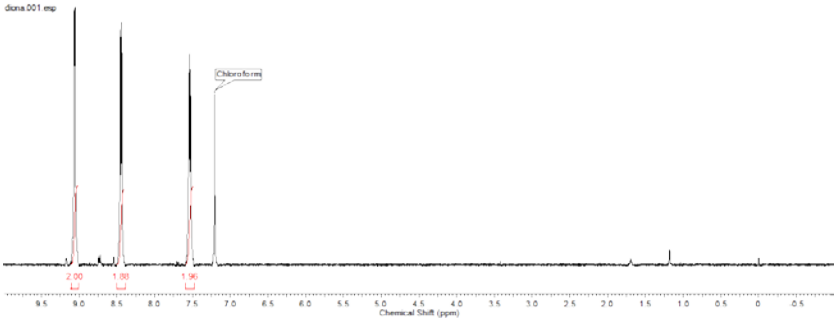


Figure 27: 1H NMR-spectrum of diona. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.14 (d, J = 4 Hz, 2H) 8.52 (d, J = 8 Hz, 2H), 7.61 (q, J = 7.6, 4.8 Hz, 2H)

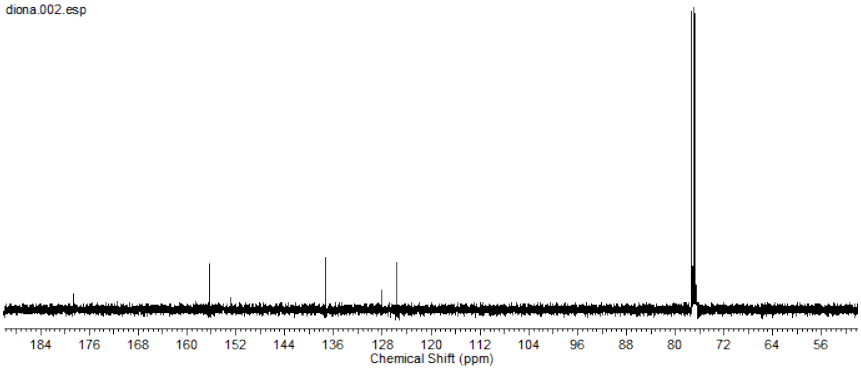


Figure 28: 13C NMR-spectrum of diona, ^{13}C (400 MHz, $CDCl_3$): δ 178.67, 156.43, 152.91, 137.33, 128.07, 125.62.

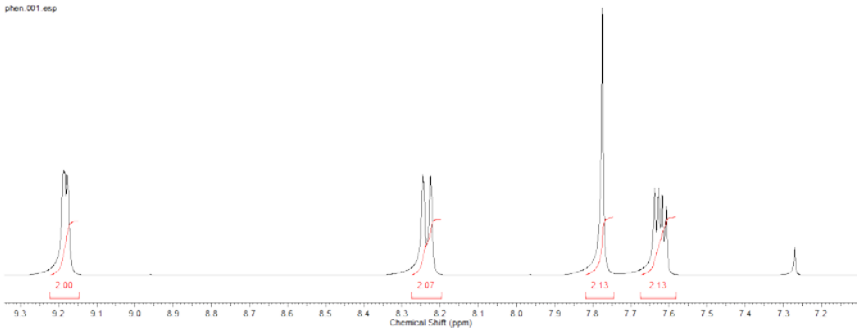


Figure 29: 1H NMR-spectrum of phenanthroline, ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.19 (m, 2H), 8.24 (d, J = 4 Hz, 2H), 7.78 (s, 2H), 7.63 (dd, J = 7.9, 4.4 Hz, 2H)

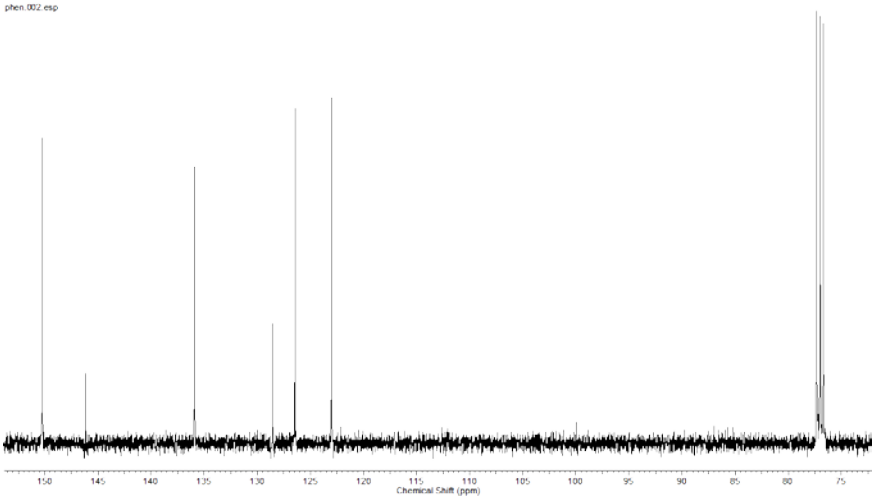


Figure 30: 13C NMR-spectrum of phenanthroline ^{13}C (400 MHz, $CDCl_3$): δ 150.29, 146.22, 135.96, 128.61, 126.50, 123.05.