Reagents for Phototherapy: Design of Transition Metal Diazo Compounds

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Abstract

In this paper is discussed the synthesis of compounds that will absorb low energy light, 600-900 nm, where skin is transparent. In order to synthesize these compounds, first a ligand was designed that had the properties to allow for absorption in the low energy region and also had promise for generating radical intermediates for DNA degradation.

These ligands will then be further functionalized with a diazo unit that along with the ligand-to-metal charge transition, will lead to generation of radical species of interest.

Introduction

Preparation of complexes with ligands that absorb in the low energy region of the spectrum has been shown to lead to ligand-to-metal charge transfer. Upon charge transfer, radical intermediates could be generated capable of DNA degradation that could be used in the development of radical-based theraputics.[1,2] Radicals are extremely important in many fields of chemistry and can be used as reagents for synthetic chemistry and even used in DNA cleavage.

Photodynamic therapy (PDT) is one type of treatment that utilizes radical oxygen to kill tumor cells in cancer patients. The problem with this type of treatment of cancer tumors is that not all tumors occur in oxygen rich environments. In order to solve these issues radicals, like carbenes, can be used to attack and kill tumors in oxygen deficient, or hypoxic, environments. One way to do this is to release N_2 from a diazo unit resulting in a reactive carbene.[2,3] Radicals and diazo units are notoriously unstable to reduction-oxidation (redox) processes, so one way to stabilize these processes would be to couple them with metal ions. Metal ions can adopt many different oxidation states and can stabilize unpaired electrons. Therefore, to better utilize the usefulness of radical species, our group has been coupling metal ions with radical generating species. There have been many different studies on these coupled complexes, such as the activation of 3-hydroxy-1,2,3-benzotriazine-4(3H)-one by Fe(III), by our group and they have been used to modify DNA.[4] While the tris[(3-hydroxy-1,2,3-benzotriazine-4(3H)-one]iron(III) did show to produce localized ligand radical intermediates which were capable of cleaving DNA, the

issue with these complexes is that they did not absorb strongly at long wavelengths where PDT functions.[1,5]

It is, therefore, important that the ligand system chosen be able to absorb low energy light and be able to exhibit a low energy ligand-to-metal charge transfer when coordinated to a metal. Using this electronic transition, the metal should become oxidized as a function of time and accordinly activate the ligand and release the diazo, N_2, unit. Choice of appropriate metals that will have the best qualities to allow for the design to be effective is extremely important to making a useful system.

Our group has had much experience with V(V)-catechol charge transfer chemistry, which have charge transfer transitions in the wavelength ranges of 600-900nm. In addition to our experience with these compounds, other groups have also observed that vanadium complexes of nitrogen and oxygen ligands have low-energy ligand-to-metal charge transfer transitions, which are directly associated with non-innocent ligands such as catecholate.[6] Our idea is to couple catechol-derived ligands with metals such as vanadium (V), as stated above, possess the long wavelength/low energy ligand-to-metal charge transfer transition character, as well as Molybdenum (VI) and Fe (III) which are also thought to possess these characteristic electronic properties when coupled with catechols. These metals are also attractive because both Molybdenum (VI) and Vanadium (V) are d^0 and Fe (III) is high spin d^5. Having these electronic configurations makes them diamagnetic which allows for use of Nuclear Magnetic Resonance (NMR) studies.

Results and Discussion

Two ligands were synthesized,(Figure 1) one of which is a new compound (**3b**), by using 1,2-benzenediol (catechol,1) as a starting material. To a mixture of catechol and aluminum chloride in chlorobenzene, an equimolar amount of benzoyl chloride (**2**) was added dropwise, while being maintained at 5-7°C. The reaction proceeds by the well understood Friedel-Crafts acylation mechanism as shown in Scheme 1.

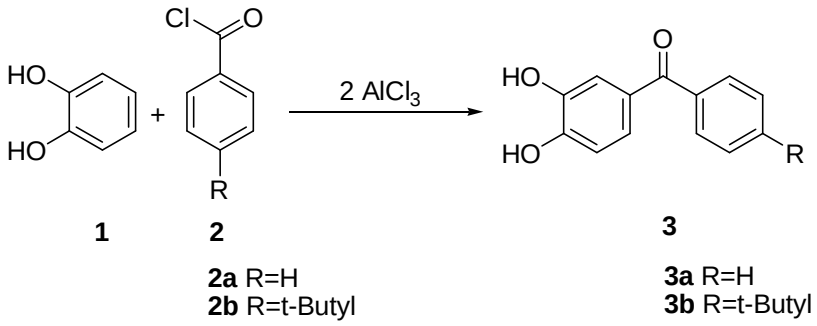
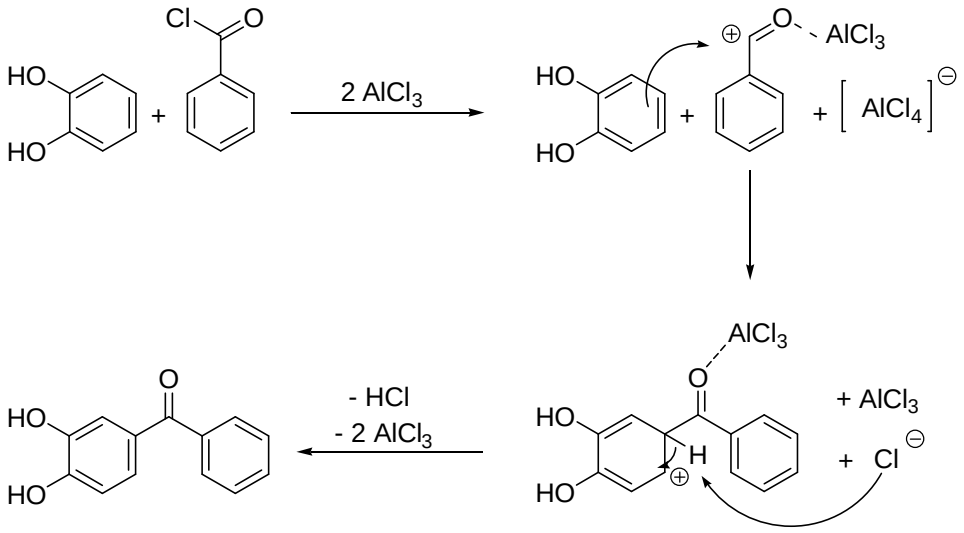


Figure 1. General reaction for making desired ligands

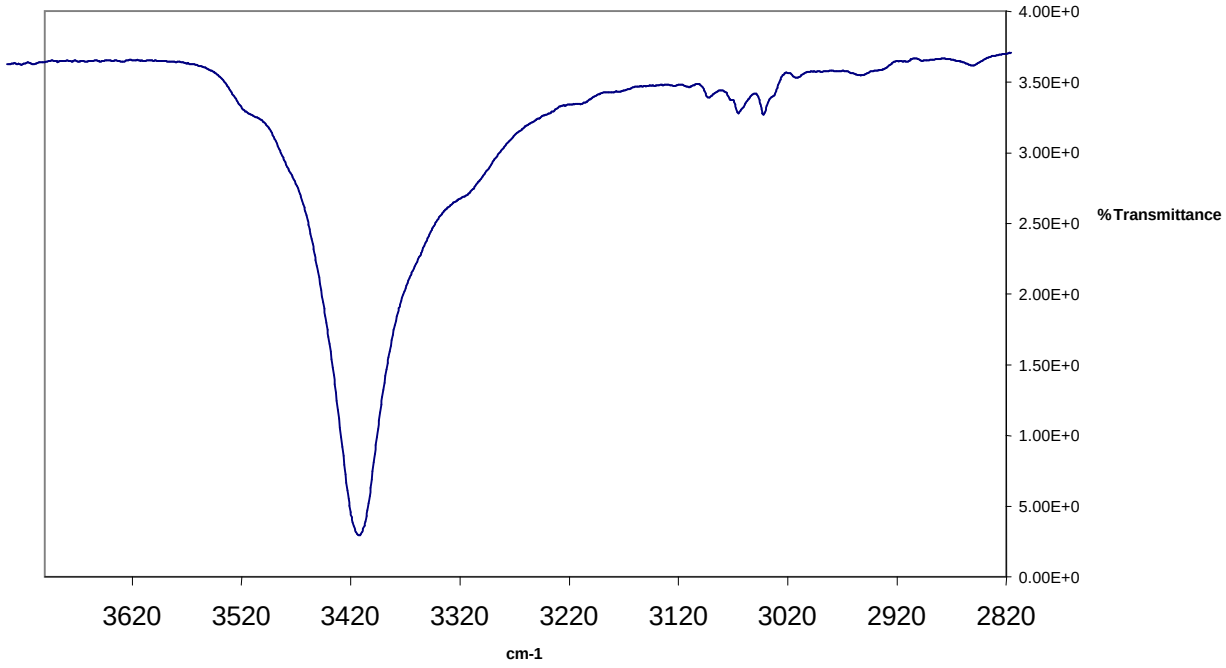


Scheme 1. Synthetic mechanism of Friedel-Crafts acylation for 3-4-dihydroxybenzophenone.

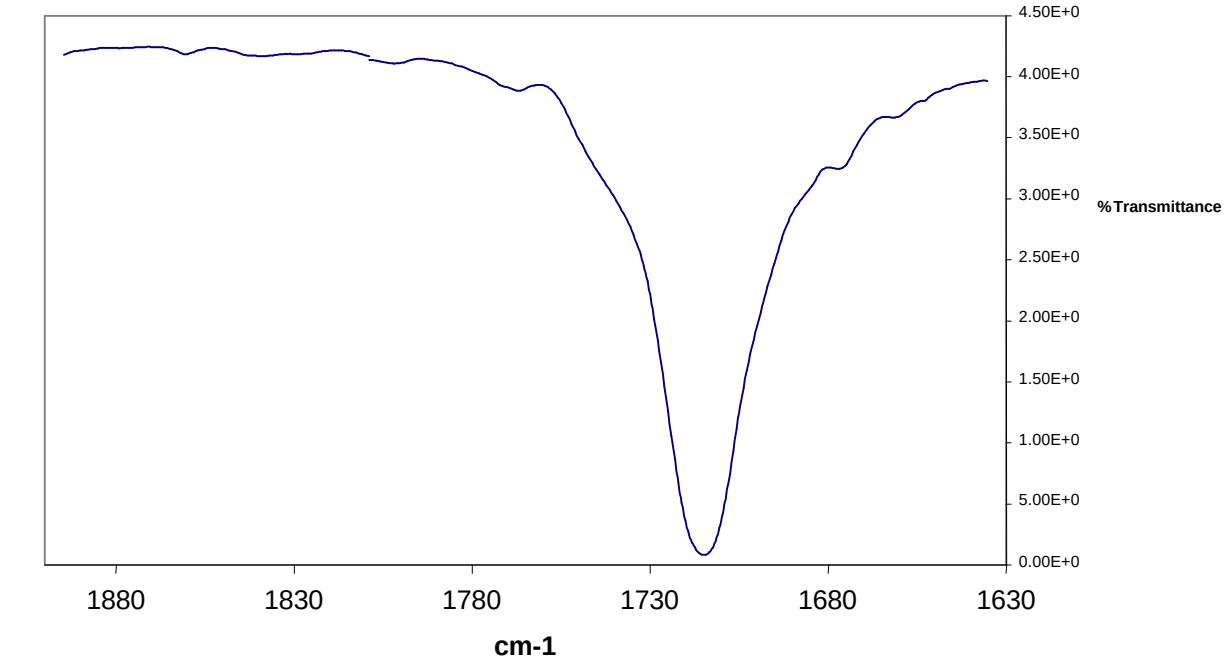
The first step of the Friedel-Crafts acylation is the formation of an oxonium complex or an ion pair. Upone formation of this ion pair electrophillic substitution of the catechol occurs while the aluminum chloride is still attached to the oxygen atom of the oxonium fragment. In the next step, a chloride ion abstracts on hydrogen atom and leaves the system as hydrochloric acid gas.[10] This results in regeneration of the aluminum chloride catalyst and generation of the desired 3-4-dihydroxybenzophenone (**3a**). This reaction is also used to generate 3-4-dihydroxybenzo-4-tertbutylphen-1-one (**3b**) if 4-tert-butyl benzoyl chloride (**2b**) was used as starting material. These compounds were characterized by NMR, mass spectrometry, and IR. Along with these methods, thin layer chromatography (TLC) with solvent mixture dichloromethane/hexanes (2:1) showed a solubility difference between the t-butyl (**3b**) and **3a**. **3b** traveled much farther than **3a** on the TLC which shows what was expected that t-butyl makes these compounds more soluble. This might aid in making sure that solubility won't be a problem in the future.

IR coupled with mass analysis has been the biggest aid in making sure the compounds synthesized are actually the desired compounds. Seen in **Figure 2** (four selections) are the IR areas of interest for these compounds, the carbonyl, C=O, stretch around 1700 cm^{-1} which indicates that indeed the acylation was successful because the product was a powder and the C=O came from a liquid reactant so the only possibility to see the C=O would be if it was indeed in the compound, and the other area of interest in the compounds are the sharp OH stretches around 3400 cm^{-1} from the catechol-like portion of the ligand and for **3b** C-H stretches around 2600-2900 cm^{-1}, relating to the t-butyl group substituted on the phenyl ring.

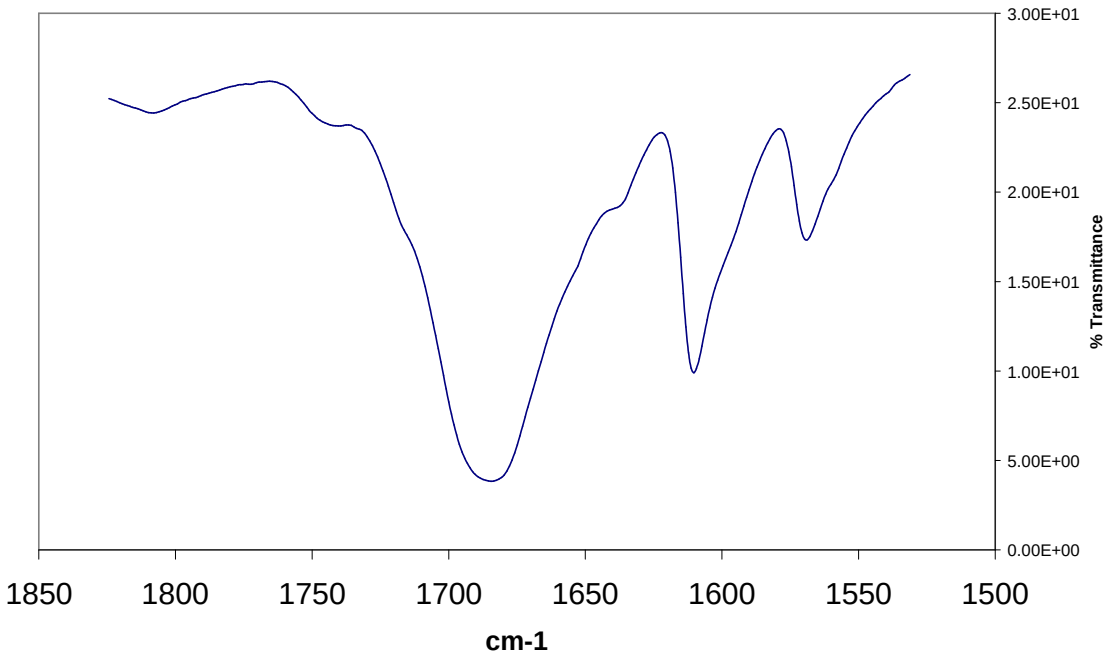
OH stretch of 3-4-dihydroxybenzophenone ligand



C=O stretch 3-4-dihydroxybenzophenone ligand



C=O stretch for t-butyl ligand



C-H Stretch of t-butyl ligand

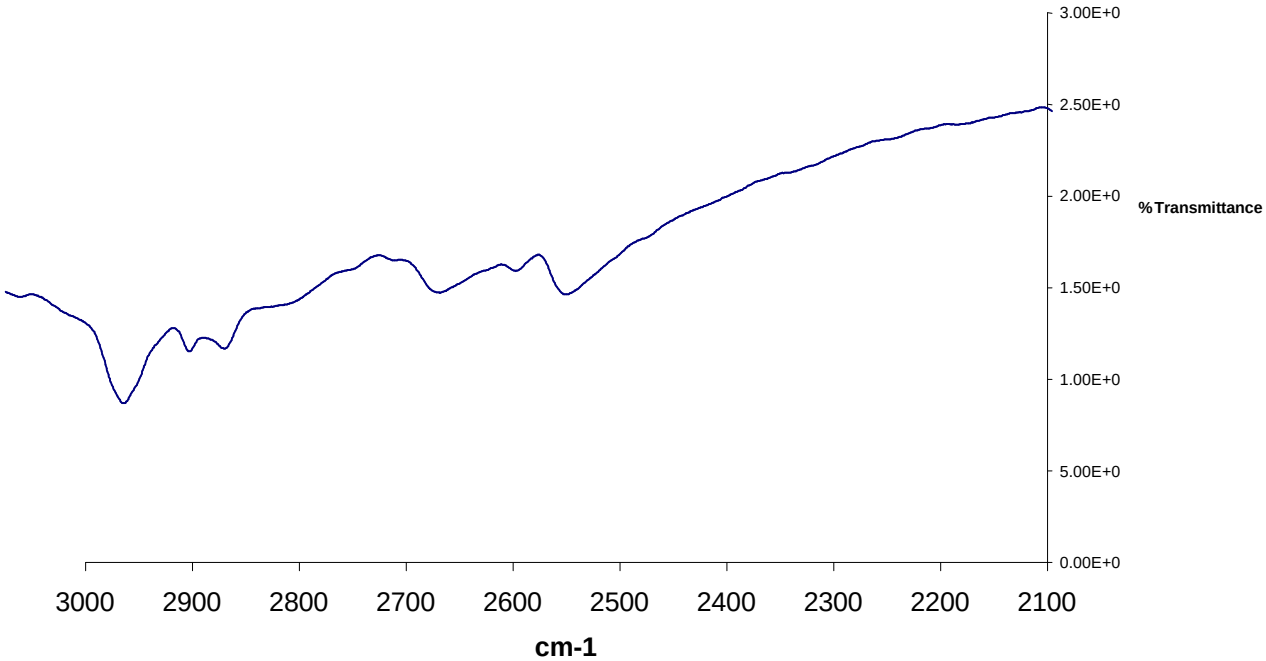
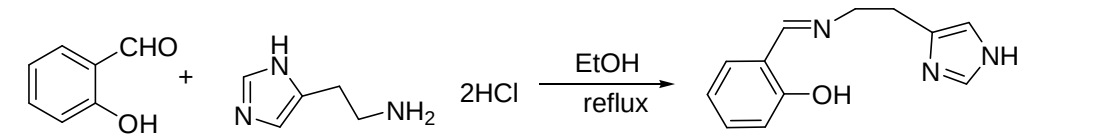


Figure 2. IR spectra for compounds **3a** and **3b**. Ranges were selected to show the most significant peaks.

Once the desired catechol derivatives (**3a and 3b**) were synthesized, the next step of the project involved complexation of these ligands to vanadium cations. The first attempt was to react **3a** with bisacetylacetonato oxo-vanadium(IV) (bisacac) ($VO(acac)_2$). The major reason for this experiment was that not only is a mono-substituted metal complex possible, but also eventually a di- and tri- substituted metal complex. Unfortunately, this reaction turned out to not be a viable path because in order to displace the acac ligand there must be an ancillary ligand in the place where the second acac is located in $VO(acac)_2$.

A very common ancillary ligand for $VO(acac)_2$ is 4-(2-(Salicylideneamino)ethyl)imidazole, HSALIMH[8], **4**. The straight forward synthesis of HSALIM can be seen below in **Scheme 2**.



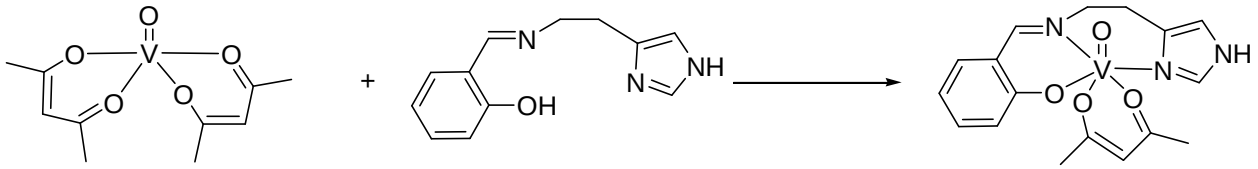
Salicylaldehyde Histamine dihydrochloride 4

4- 2- (Salicylideneamino)ethyl)imidazole

Scheme 2. Synthesis of HSALIMH.

This reaction is a Schiff base condensation reaction of salicylaldehyde with histamine dihydrochloride to form 4-2-(salicylideneamino)ethyl)imidazole.[8] 4-2-(salicylideneamino)ethyl)imidazole is a yellow oil and was isolated before coordination to

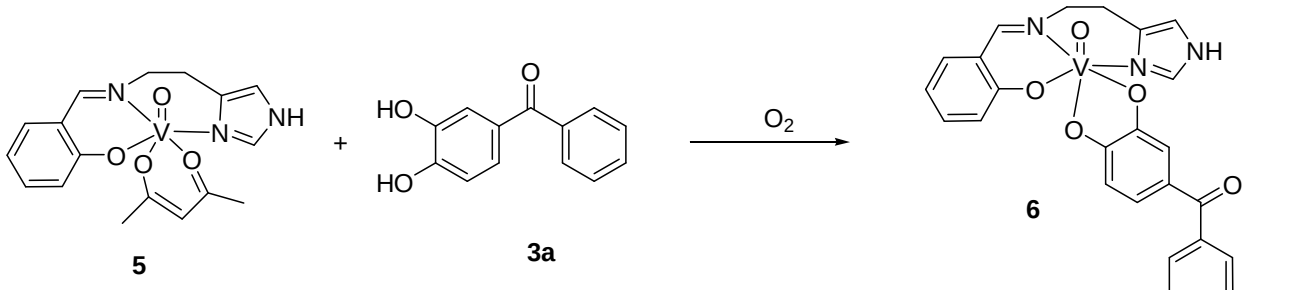
$VO(acac)_2$ for purpose of characterization. HSALIMH was then reacted with $VO(acac)_2$ to produce (Acetylacetonato)[4-(2-(salicylideneamino)ethyl)imidazolyl]oxo-vanadium(IV)·CH_3OH (**Scheme 3**).



$VO(acac)_2$ — **4** ancillary ligand — **5** (Acetylacetonato)[4-(2-(salicylideneamino)ethyl)imidazolyl] oxo-vanadium(IV)

Scheme 3. Synthesis of VO(SALIMH)ACAC.

The next objective for this project was to complex the catechol derivative ligands (**3a** and **3b**) to complex **5**. Synthesis of (3-4-dihydroxybenzophenone)[4-(2-(salicylideneamino)ethyl)imidazolyl]oxo-vanadium(V)[9] (6) is show in **Scheme 4**.

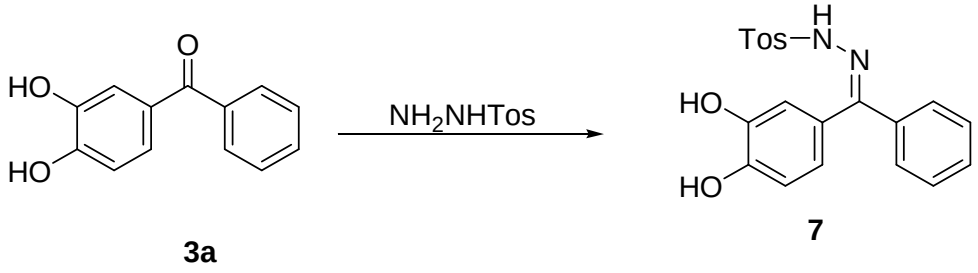


5 $VO(acac)_2$ — **3a** — **6** (3-4-dihydroxybenzophenone)[4-(2-(salicylideneamino)ethyl)imidazolyl]oxo-vanadium(V)

Scheme 4. Synthesis of (3-4-dihydroxybenzophenone)[4(2-(salicylideneamino)ethyl)imidazolyl]oxo-vanadium(V).

Compound **5** was dissolved in dry acetonitrile and to that solution **3a** was added. After addition of **3a** to the solution, the reaction was opened to air and the reaction turned from a red color to a blueish-purple color. This color is indicative of oxidation of vanadium from IV to V, and this reaction is left open to air for 12 hours. After about a week this deep purple compound turned a brownish color, so it is assumed to decompose if left open to air for over a week. In the next attempt to synthesize this complex, the product will be stored in an air tight vial.

The next reaction attempted was to convert the catechol derivative ligands to the hydrazone with tosylhydrazine the reaction can be seen in **Scheme 5**.



3a **7**

Scheme 5. Synthesis of 3-4-dihydroxybenzotosylhydrazone.

Compound **3a** was dissolved in dichloromethane and to this p-tosylhydrazine was added while the temperature was maintained in an ice bath. No reaction was observed and the ice bath was removed. The reaction was allowed to come to room temperature and maintained at room temperature for two hours with no reaction observed. The reaction was monitored by thin layer chromatography (TLC) and the spots observed for the reaction mixture were no different than starting materials. The reaction was attempted since tosylhydrazine is known to convert diketones to diazo-ketones.[10] This system has only one ketone, therefore this might have a significant effect on the outcome because two

ketones might aid in stabilization via hydrogen bonding with the tosylhydrazine. Another factor might be that the activation energy barrier might be higher for this reaction and therefore more energy might be needed to convert the ketone to the hydrazone. In this instance, the solvent might be changed to chlorobenzene and then the reaction might be heated to about 40°C and left to react overnight this might overcome any possible barriers to the hydrazone product. Also the equivalents of p-tosylhydrazine could be increased from 1 equivalent to 2 equivalents to aid in conversion to the hydrazone product.

Experimental

Materials and General Procedures

Chemicals used were obtained from Aldrich and Alfa Aesar and were of the highest purity available. Ligands were purified by recrystallization with benzene. Proton (^{1}H) NMR was recorded on a VXR 400 spectrometer using the residual proton resonance of the solvent as an internal reference. Infrared spectra (KBr) were recorded on a Nicolet 510P FT IR spectrophotometer. Mass Spectra were obtained using either a MAT 95XP, which is an electron impact/chemical ionization high resolution and high mass accuracy mass spectrometer, or by using this instrument with an Electrospray ionization source.

Synthesis of Compounds

3-4-dihydroxybenzophenone (3a): Synthesized by following literature procedure except benzoyl chloride was used and the solvent used was chlorobenzene.[7] ^{1}H NMR ($CDCl_3$): δ

6.99-8.24 ppm corresponding to 8 aromatic protons and δ 5.43 ppm corresponding to 2 protons for OH groups. EI/CI *m/z* = 214.0647 (M^+), *m/z* = 215.0725 (MH^+). IR: 3420 cm^{-1} stretch corresponding to the OH groups, and 1720 cm^{-1} stretch corresponding to the C=O functionality.

3-4-dihydroxybenzo-4-tertbutylphen-1-one (3b): Synthesized by adapting literature procedure by using 4-tertbutyl benzoyl chloride and using chlorobenzene as the solvent.[7] EI/CI *m/z* = 271.133 (MH^+). IR: 2560-2970 cm^{-1} stretches corresponding to C-H stretches of the t-butyl functional group and 1690 cm^{-1} stretch corresponding to the C=O functionality.

4-(2-(Salicylideneamino)ethyl)imidazole, HSALIMH (4): Synthesized by following literature procedure.[8] Isolated as a yellow oil and spectroscopic data matched literature values. EI/CI *m/z* = 215.3 (M^+).

(Acetylacetonato)[4-(2-(salicylideneamino)ethyl)imidazolyl]oxo-vanadium(IV)·CH_3OH (5): Synthesized by following literature procedure.[8] Isolated as a red powder and spectroscopic data matched literature values. EI/CI *m/z* = 403.2 (M^+Na^+).

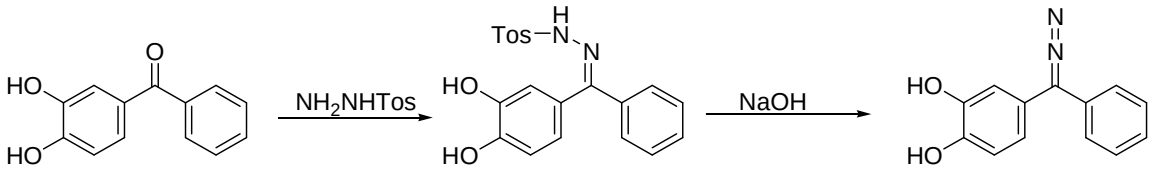
(3-4-dihydroxybenzophenone)[4-(2-(salicylideneamino)ethyl)imidazolyl]oxo-vanadium(V) (6): Isolated as a deep blueish- puple powder, synthesized by adapting literature procedure by using 3-4-dihydroxybenzophenone.[9] EI/CI *m/z* = 515.0 (M^+Na^+).

3-4-dihydroxybenzotosylhydrazone (7): Synthesized using a procedure frequently used in the Zaleski lab by taking 20 mg (1 equivalent) of **3** in 20 mL of dichloromethane and to this 17 mg (1 equivalent) of p-tosylhydrasine was added. This reaction was maintained in an ice bath for one hour and after no reaction was observed the ice bath was removed and let warm to room temperature. The reaction was left to react at room temperature for 2 hours and still no reaction was observed.

Future Work

Along with both of the 3 ligands, another ligand with a methyl will be synthesized using toluyl chloride. This would be desirable considering that the metyl group will also affect the solubility of these ligands, but not as much as the t-butyl group, so it would be a nice range for analyzing solubility. Once this other ligand is synthesized then both the t-butyl substituted and the methyl substituted will be complexed to vanadium.

As suggested in the results and discussion section of this paper, the next reaction to take place will be the hydrazine reaction to convert the ketone of **3a** and **3b** into the tosylhydrazone and then converting the tosylhydrazone into the diazo unit by a base-catalyzed step. The reaction scheme can be seen below in **Scheme 6**.



Scheme 6. Synthesis of the diazo compound from ligand **3**.

Following the diazidization of these ligands, they will be complexed to vanadium and the charge transfer characterization of these complexes will then be persued. In addition to complexation to the metal vanadium, two other metals will also be evaluated, Fe(III) and Mo(VI). These metals are of interest because they are either half filled or empty and d^5 and d^0 complexes are diamagnetic which allow for characterization with NMR techniques. Of additional interest to this project in the future would be to make complexes with not only one, but two or three of the desired ligands. There is plenty yet to be accomplished in this project with lots of promising possibilities.

Conclusions

At the writing of this paper, two catechol derivative ligands have been synthesized that have carbonyl functional groups. Carbonyl functional groups are good in order for conversion to the diazo, which can lead to the generation of radicals. These ligands also absorb in the low energy light region which makes them excellent candidates for phototherapy agents. The precursor catechol derivative ligand, **3a**, synthesized in this paper has been attached to vanadium in order to better understand the properties of these complexes prior to complexation with the more reactive diazo ligand.

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I would like to thank Dr. Jeff Zaleski for all of his guidance with my project. In addition, I would like to especially acknowledge Tillmann Koepke for all of his support and advisement during this project especially in the synthetic area of this project. Thanks to the Zaleski group as well.

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