A CLOSER LOOK AT ARTIFICIAL PHOTOSYNTHESIS: THE HUMBLE PORPHYRIN

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Abstract

Presently, the world is faced with an urgent dilemma in issues of energy harness and storage, highlighted by the global reliance on fossil fuels to power economies. This has been proven to increase carbon dioxide levels in the earth's atmosphere, which has been shown to cause global warming (Klare, 2015). While natural processes such as photosynthesis successfully reduce carbon dioxide to biomass, the rate at which this happens is mundane relative to the current levels of carbon dioxide being produced. Artificial photosynthesis seeks to mimic natural photosynthesis, potentially maximizing efficiency in individual stages, such as light absorption, energy transfer, proton-coupled electron transfer (PCET), etc. The result can provide both carbon-based fuels and lower atmospheric levels of carbon dioxide (Llansola-Portoles et al., 2017). Several benzimidazole phenol (BIP) porphyrin dyads have been synthesized to study PCET reactions that occur in a photoactive cluster in Photosystem II (Huynh et al., 2017). This report serves as an introductory insight to the development of a specific porphyrin, known as 4OH-PF_{15}, which the Moore lab combines with varieties of BIP molecules to form a BIP porphyrin dyad for the further study of the PCET reactions. After incorporating various techniques including column chromatography and preparative thin layer chromatography (prep TLC), the molecule was successfully prepared and purified. The porphyrin can effectively be used to be combined to various BIPs for the future study of these transfer pathways.

1. Introduction

Artificial photosynthesis is a term that refers to the biomimicry that seeks to convert sunlight, water, and carbon dioxide into a storable fuel. For plants and many photosynthetic bacteria, that fuel is carbohydrates which then is used to carry out other functions for life. But for manufacturing purposes, this mechanism aims to convert photo energy to chemical energy that can be stored, giving way to wide-ranging applications, notably including solar energy utilization by photovoltaic cells, and solar fuels. A rather young discipline, artificial photosynthesis currently focuses on understanding and replicating the steps taken in natural photosynthesis to produce high yield energy sources (Llansola-Portoles et al., 2017).

In nature, photosynthetic processes begin with the capture of solar energy by a pigment protein or cluster of proteins. Here, electromagnetic energy is converted to chemical potential energy, which is of an excited molecular state. This energy is transferred to an organism's reaction center, where most energy-conversion processes occur through a series of redox reactions to harvest a source of energy that the organism can use. Plants, algae, and cyanobacteria host these processes in Photosystem II (PSII) within the thylakoid membranes of their cells (Liu, 2016).

Porphyrins are organic compounds with a macrocycle structure (Figure 1) that consist of compounds known as pyrroles. Similar naturally occurring molecules, such as heme in blood, play a vital role in supporting aerobic life (Barona-Castaño et al., 2016).

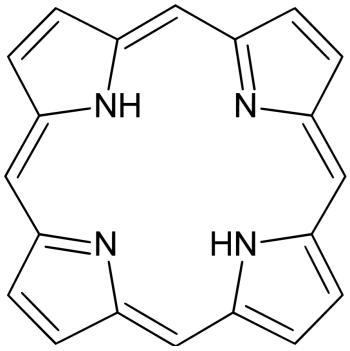

Figure 1: Structural drawing of a general porphyrin compound

Porphyrins have numerous applications beyond being a topic of theoretical study. Photodynamic therapy has emerged as a promising field in combating cancer (Pushpan et al., 2002). Within the field of artificial photosynthesis, porphyrins are vital in their role as primary electron donors and/or acceptors. Pyrroles linked together in this macrostructure are quite stable due to the symmetrical structure, but perhaps more importantly, they provide the ability to bond with specially designed substitutions. The porphyrin in this report, 4OH-PF_{15} (Figure 2), is one composed of a pyrrole macrocycle substituted with pentafluorobenzene groups, a hydroxyl group, and an aldehyde. The aldehyde in this modified porphyrin will enable the binding of the benzimidazole in the final dyad, which will be used to study PCET reactions (Mora et al., 2018; Mora et al., 2019).

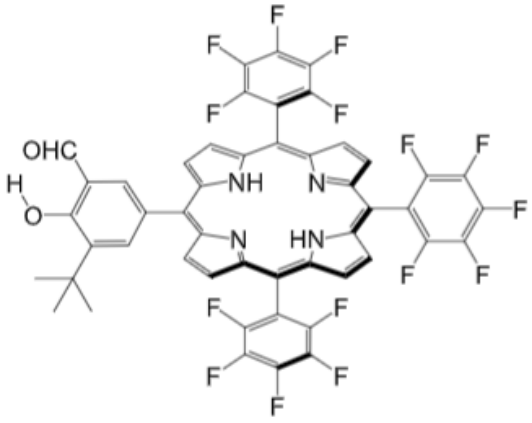

Figure 2: Chemical structure of 4OH-PF_{15}

Significant research has been made in understanding symmetric porphyrins, which are relatively more simple to synthesize in the lab and are produced in higher yields. The porphyrin that will

be discussed in this report is asymmetric in its structure. This is significantly more representative of natural porphyrins that occur in nature, notably of which are found to help form hemoglobin to carry oxygen in blood. Asymmetric porphyrins are also more difficult to synthesize successfully and often synthetic reactions produce lower yields (Vicente & Smith, 2014).

2. Experimental

2.1 Chemicals and Materials

The chemicals used in synthesis were purchased from Aldrich, Acros and Alfa Aesar. Dichloromethane and hexane were distilled before use. Chloroform stabilized with 0.75% ethanol was used. The compounds 5-(pentafluorophenyl)dipyrromethane (F5-DMP) and 5-formyl-3-tert-butyl-2-hydroxy-benzaldehyde were previously synthesized in the lab (Meggiato et al., 2012). The other reactants, 2,3,4,5,6-pentafluorobenzaldehyde, the catalyst boron-trifluoride etherate (BF_3OET_2), and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) had already been purchased. Thin layer chromatography (TLC) was performed with silica-gel-coated glass plates from Merck Millipore. The column chromatography purification was conducted with silica gel 60 (230-400 mesh).

2.2 Synthesis

Procedure for synthesizing 5-(3-*formyl*-4-*hydroxy*-5-*tert*-*butylphenyl*-1-*yl*)-10,15,20-*tris*(2,3,4,5,6-*pentafluorophenyl*)*porphyrin*, *4OH-PF*$_{15}$.

Chloroform was poured into a three-neck round bottom flask (1 L). In order to bubble the system with Argon directly, an exit needle was inserted into the top mouth. This was essential to maintain the flow of Argon. After bubbling for 20 minutes, 330 mg (1.6 mmol) of 5-formyl-3-*tert*-butyl-2-hydroxy-benzaldehyde was added and left to stir for a few minutes. Following this, the 2,3,4,5,6-pentafluorobenzaldehyde (237μL, 1.68 mmol) and 1g (3.2 mmol) of F_5-DMP were combined in a flask with the rest of the reactants under an Argon atmosphere. Immediately covering the round bottom flask system with aluminum foil, we added the catalyst, Boron-trifluoride etherate (BF_3OET_2) (156 μL, 1.25 mmol) to the system and left the reaction to stir at room temperature for 3 hr. The resulting product was oxidized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (1.45g, 6.4 mmol) at 40 ℃ overnight (Mora et al., 2019).

2.3 Workup

The next morning, the reaction was filtered using a silica pad under reduced pressure. The pad was washed using

dichloromethane. The crude reaction was collected in two fractions and the solvent was evaporated.

The two fractions obtained after the filtration underwent another TLC. The primary fraction consisted of most of the porphyrin obtained from the reaction. The secondary flask held trace amounts of porphyrin and a substantial amount of DDQ. Upon the results of the TLC, it was concluded that the secondary fraction could be discarded, as the fraction did not have the desired 4OH-PF_{15}. The primary fraction was then purified using column chromatography.

2.4 Purification

2.4.1 Column chromatography

The silica was packaged with hexane:dichloromethane 90:10 and added to the column. To effectively pack the column, the system remained under an Argon atmosphere. Once enough silica was in the column, the crude reaction was dissolved in dichloromethane, mixed with silica, and then added to the column to an appropriate level, followed with sand. The solid was initially purified on silica gel using a gradient of solvents - hexane:dichloromethane from 9:1 to 1:1 - pouring ~300 mL into the column every round. The gradient of solvents were then used to flush the column to separate out all of the fractions in the crude reaction. The different fractions were collected.

2.4.2 Preparative TLC

The procedure followed with a prep TLC plate, used to separate the impurity within fraction 5 (obtained from the column chromatography) from the 4OH-PF_{15}.

The best mixture of solvents was evaluated using TLC plates. Three solvent combinations were tested with different polarity varieties. A 1:1 hexane:dichloromethane mixture was concluded to be the most ideal for the prep TLC. Only in a 1:1 mixture did the two fractions seem most visibly apart, providing the best chance for an effective separation by prep TLC.

Placed into a hexane:dichloromethane mixture, Fraction 5 was spotted on the prep TLC plate and placed inside the glass compartment and left to run. The plate produced a clear distinction between the impurity and the 4OH-PF_{15}. The porphyrin was recovered by scraping off the silica with a blade. Then, the 4OH-PF_{15} was dissolved in dichloromethane and filtered to separate it from the silica. The solution was evaporated, thus leaving pure porphyrin ready for further analysis.

2.5 Analysis Techniques

2.5.1 Thin Layer Chromatography

The general purpose of a TLC is to evaluate the different compounds in a mixture by separating them. This technique can help to determine the number of compounds in a mixture, the compounds' identity, and their relative purities. Taking no more than 10 minutes, it is a simple way to perform an analysis of products.

The first step is to spot the film. After the sample has been dissolved in a volatile solvent, it is transferred using a micropipette to a glass plate covered with powdered silica gel. The solvent evaporates in a short period of time, leaving only the sample on the plate. Next, the sample is placed into a container with a solvent (sometimes a combination of solvents). The solvent chosen is based on an evaluation of polarity. The polarity of the sample must be considered as well when choosing which solvent to add to the container. The plate is placed inside the container, left to rest until the TLC has developed fully.

Once the solvent in the container has run up the full plate, the plate must be taken out. The number of spots indicates the number of compounds in the mixture. Full purity is when only one spot appears on the plate. This means that a more specialized analysis technique can be used to evaluate the composition of a compound.

2.5.2 ^{1}H Nuclear Magnetic Resonance (NMR)

^{1}H NMR applies nuclear magnetic resonance in relation to Hydrogen atom nuclei within the molecules of a sample. This is meant to help visualize the structure of the molecules (Silverstein et al., 1991).

The sample was dissolved in deuterated chloroform (so that solvent protons do not interfere with the signal of the protons of the sample). The tube with the dissolved sample is placed in the NMR machine and left to run.

^{1}H NMR spectra of these types of porphyrins are characterized by chemical shifts and by proton coupling. Atom nuclei are spinning charged particles which thereby generate a magnetic field. Without outer magnetic fields, these nuclear spins are random and the direction of these spins are random. The ^{1}H NMR applies an external magnetic force, causing the nuclei to align themselves in a unique way with or against the magnetic field. This data aids in the determination of a compound's chemical structure (Soderberg, 2016). The different types of protons are indicated by chemical shifts, where the number of protons of each type is indicated by the integration - or relative area - under the signal.

3. Results

3.1 Discussion

The reaction conducted aimed to form the 4OH-PF_{15} and it was successful. However, the product was not fully oxidized when the reaction was stopped. The reaction was checked with TLC with a hexane:dichloromethane 1:1 mixture.

After filtration in the workup, two fractions were obtained: one with the primary porphyrin, the second with a substantial amount of DDQ without any apparent porphyrin. The second one fraction was discarded.

The column chromatography of the first fraction resulted in new fractions after the purification. Fractions 1-3 seemed to only have PF_{20} (a byproduct of the reaction), which is not the desired porphyrin. Fraction 4 and fraction 5 seemed the most pure, which was the desired outcome. Fractions 6, 7, 8, and 9, had significant impurities and only trace amounts of 4OH-PF_{15}. None of the further fractions seemed to have the desired porphyrin and were discarded. TLCs (2.5.1 Thin Layer Chromatography) were conducted on all of the remaining fractions. As a consequence, fractions 6, 7, 8, and 9 were combined because they were not pure at all and only had trace amounts of 4OH-PF_{15}. Fraction 4 had a couple of impurities, too, but was left separate because it had a substantial amount of porphyrin. Fraction 5 became the candidate for further purification to obtain clean 4OH-PF_{15}. Figure 3 below depicts the purity of the different fractions obtained from the column chromatography.

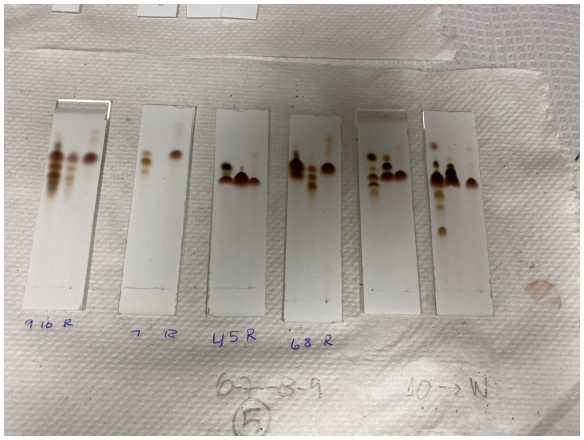

Figure 3: TLC to evaluate fractions obtained from column chromatography.

The prep TLC (2.4.2 Preparative TLC) produced a significant yield of pure 4OH-PF_{15}. However, due to logistical complications arising from the novel coronavirus COVID 19, the rest of the fraction could not be purified. Only about half of fraction 5 was able to be fully purified.

With the fraction that was purified by Prep TLC, the NMR analysis was successful and showed that the porphyrin is very pure (Figures 4-8). We did not see any other product or impurity, confirming that all of the purification processes were successful. In Figure 4, we can see the full NMR spectrum in which there are all of the signals of the protons of the 4OH-PF_{15}. The different protons present distinct

chemical shifts according to the chemical environment of each proton. Also in the spectrum, we can see the integration area for each signal, which is related with the number of the protons that give rise to the signal. The Figures 5-8 are expanded portions of the full NMR spectrum, where we assigned the different signals to the given protons.

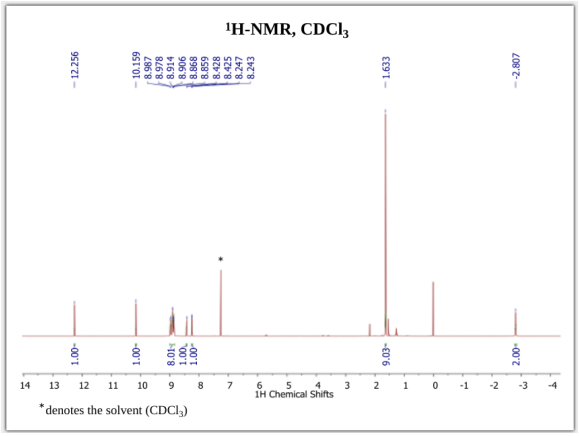

Figure 4: Full NMR spectrum of 4OH-PF_{15}.

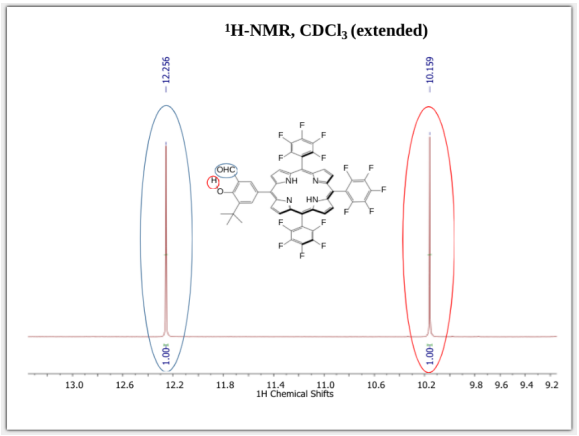

Figure 5: Expanded NMR data depicting the presence of protons of the hydroxide and aldehyde groups.

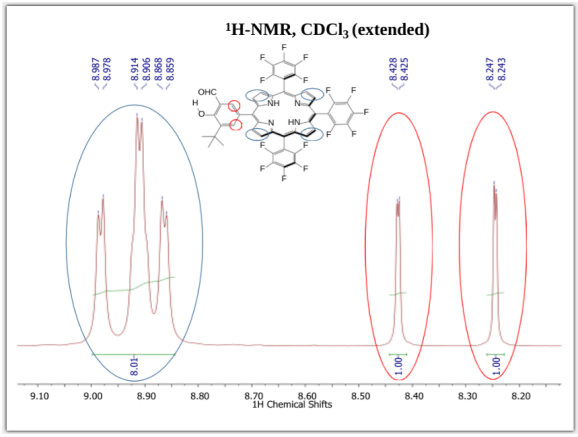

Figure 6: Expanded NMR data depicting the presence of protons directly attached to pyrrole and substituted benzene moiety.

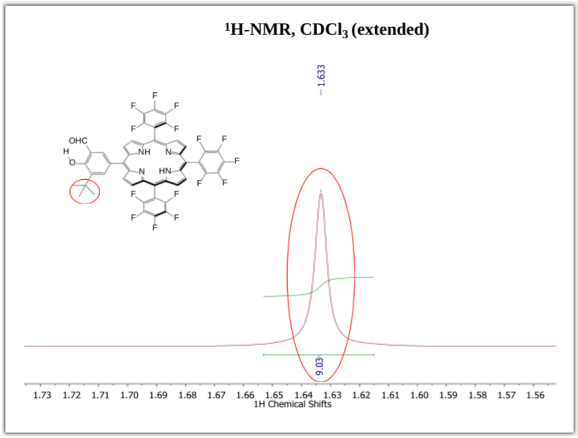

Figure 7: Expanded NMR spectrum depicting the presence of the protons and belonging to the *t*-butyl group attached to the substituted benzene moiety.

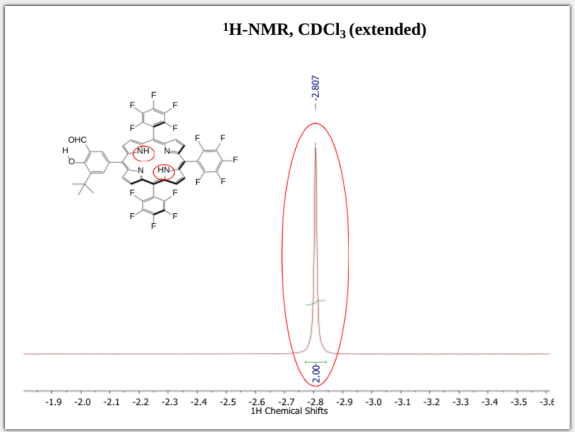

Figure 8: Expanded NMR data representing the presence of the two protons from the amine groups within the pyrrole.

Because Fraction 5 was not fully purified, the yield of the reaction is not known.

4. Conclusion

Overall, the aim to fully synthesize and characterize 4OH-PF_{15} was successful, albeit a few shortcomings with the experimental process. The reaction successfully produced a high yield of porphyrin that could be purified. The column was effective in separating most of the impurity of the crude reaction. Finally, the prep TLC made it possible to more precisely separate the porphyrin from the other impurity, allowing for the opportunity to run the ^{1}H NMR analysis.

Furthermore, with better techniques handling the column and filtration, more of the porphyrin could have been purified and preserved. Unfortunately, a little portion of the 4OH-PF_{15} was lost due to workup error detailed further in the experimental and results.

4.1 Future Directions

If this project were to be continued, interesting avenues of exploration would entail fully completing the characterization of the porphyrin by the team in the Moore lab. According to the NMR spectrum, the porphyrin is pure and possible to be synthesized to create the BIP-porphyrin dyad with the BIP created by the other team members.

Nonetheless, the final product described in this report effectively contributes to the research goals the team was attempting to achieve. The product will be combined with the BIP moiety to establish a stable molecule that will be effective in further modeling the Tyrz-His190 pair of PSII, which is a redox mediator that shuttles electrons from the water oxidation catalyst to P680.

4.2 Acknowledgements

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