4-20-2023

BACTERIAL-MEDIATED PHOTOCATALYTIC ORGANIC OXIDATION

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by

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A Thesis

Submitted to the
Department of Chemistry and Biochemistry
College of Science and Mathematics
In partial fulfillment of the requirement
For the degree of
Master of Science in Pharmaceutical Sciences
April 3, 2023

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Acknowledgments

I wish to express my sincere gratitude and appreciation to Professor Lark Perez for his invaluable advice and guidance throughout my research. His mentorship enabled me to invest my time in acquiring new skills and knowledge in organic chemistry while maintaining my focus on researching more environmentally-friendly approaches. I am excited to apply this knowledge and take an environmental stance in my future professional endeavors.

I would also like to express my gratitude to my girlfriend, Amanda, whose support and encouragement helped me complete my journey through the master's program. Without her motivation, I would not have been able to achieve this milestone and find balance outside of the program.

Furthermore, I extend my gratitude to my family for their support and advice throughout my academic journey. Their support has been instrumental in enabling me to begin my undergraduate studies at Rowan University and return to complete the master's program.

Once again, thank you to all who have supported me during this journey.

Abstract

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BACTERIAL-MEDIATED PHOTOCATALYTIC OXIDATION
2019-2021
Lark Perez, Ph.D.
Master of Science in Pharmaceutical Sciences

Bacteria, though well-known and widespread in scientific application, have plenty more opportunity to grow in the field of organic synthesis. The primary objective of this project was to apply the use of bioluminescent bacteria in an organic photoredox reaction intending to use the bacteria as a renewable source of light. This sustainable method, as opposed to high wattage bulbs, paves a green pathway for organic photocatalytic reactions. Using bioluminescent E. coli, the focus was on performing organic oxidation reactions with a recyclable photocatalyst. When using bacteria in conjunction with chemicals like nitromethane, it was an obstacle to keep the E. coli alive. By employing toxicology studies, we could determine minimum inhibitory concentration (MIC) values for each chemical that were to be tested. Setting up control experiments and reproducibility studies was necessary in order to confirm the possibility of such reactions. With these constraints and variables under control, we were able to perform our photoredox reactions successfully using bioluminescent E. coli as our light source and a graphitic carbon nitride polymer as our photocatalyst. The reactions targeted in this study gave way to a high yield product, confirming that bioluminescent bacteria can be used as a light source in solution with organic solvents.

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Chapter I

Introduction

Organic synthesis can be a significant generator of waste and inefficiency in the world of chemistry. Organic waste is also often an environmental hazard; having large amounts of solvents or metal catalysts released into our environment with no forethought can create catastrophes. Our industry could help to solve these issues by prioritizing environmentally friendly forms of chemistry. Green, sustainable chemistry would help to reduce or eliminate hazardous waste when introduced in any or all stages of a product's life cycle. From the early stages of design and manufacturing to the late stages of its disposal, a product undergoing a greener chemical process improves efficiency and removes the "need" for hazardous waste in our environment (*1*,*2*,*3*).

With green chemistry as the basis of this study, we formed the concept of running photocatalytic oxidation reactions. Using a green catalyst and renewable light source, could a successful reaction be run? It is typical for reactions of this nature to require high amounts of energy and generous light sources to produce enough light for a reaction to occur. Additionally, these reactions usually use a rare metal catalyst or other metal catalyst to produce the desired product (*3*,*5*). Removing the use of these high energy light bulbs and hazardous metals from catalysts can help to promote more environmentally friendly alternatives. This project focused on a green form of organic photocatalytic oxidation by, more specifically, completing both of the following: replacing the light source with a more renewable resource and switching from a metal catalyst to a polymer that is recyclable and easy to produce (*3*,*4*).

Over the years, photocatalysis has grown as a subject of interest in the matter of green chemistry. Simple oxidation and alkylation reactions can be done using photocatalysis, but there are remaining inefficiencies with these reactions (*1*,*6*). Introducing both a new form of light production along with a new catalyst was our primary goal in this study; as such, we wanted to address the first major inefficiency of the wasteful light source, replacing the light bulbs used for photocatalytic reactions with a bioluminescent bacterium. In this study, a strain of E. coli was selected that would produce light when given enough oxygen and food. If this bacterium is successful in light production, then there is a new approach to reducing waste and confronting the aforementioned inefficiencies. We have changed how this reaction works in a novel way with only one step. The synthesis route described above can be seen in figure 2 and in figure 3 with a more in depth look at the potential mechanism of action..

By using bacteria as our light source, the need for energy input in the form of a light bulb has been removed. Bacteria only require a few particular conditions to stay alive and produce light: a warm and moist environment, "food" with enough nutrients to sustain them, and oxygen. Bacterial cultures can be grown and scaled with ease due to their minimal environmental requirements. To encourage production, bacteria are grown in an aqueous solution of water and lysogeny broth (LB), which is a nutrient-dense growth medium abundant in peptides, vitamins, and trace elements; this provides an ideal environment for bacteria to grow. An initial amount of one mL of bacterial stock can produce up to one hundred mL of bacteria in roughly six hours. This rapid growth requires very little input to reach maximum population. Bacteria also require a fully

aqueous environment, meaning less organic solvents are required. This can be an obstacle for solubility of substrates, as some can be partially or insoluble in water.

The final essential contribution for successful bacteria growth is oxygen. Atmospheric oxygen is sufficient enough in keeping the bacteria alive. This removes yet another issue from the development of this study, in that the reaction can be conducted in a bacterial incubator without oxygen tanks nearby, addressing a potential safety hazard.

The removal of the light bulb resolves another inefficiency with this method, because light typically has a difficult time passing through glassware and penetrating solutions. With the introduction of bacteria, the properties and tendencies of light sources defined by Beer-Lambert or Beer's Law ((A=εℓc), where A represents the absorbance of light by a solution) become reduced. The light is produced in the solution by the bacteria, which has already been integrated with the catalyst and substrates. Therefore, there is no need for the light to move through any glassware reducing absorption by the catalyst. We hypothesized that this would reduce inefficiencies with light production. See Figure 1 below for a reaction vessel using bacteria.

Figure 1

Photon Production via Bioluminescent E. coli in Erlenmeyer Flasks

Figure 2

Predicted Mechanism of Action for the Photocatalytic Oxidation of 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline (1)

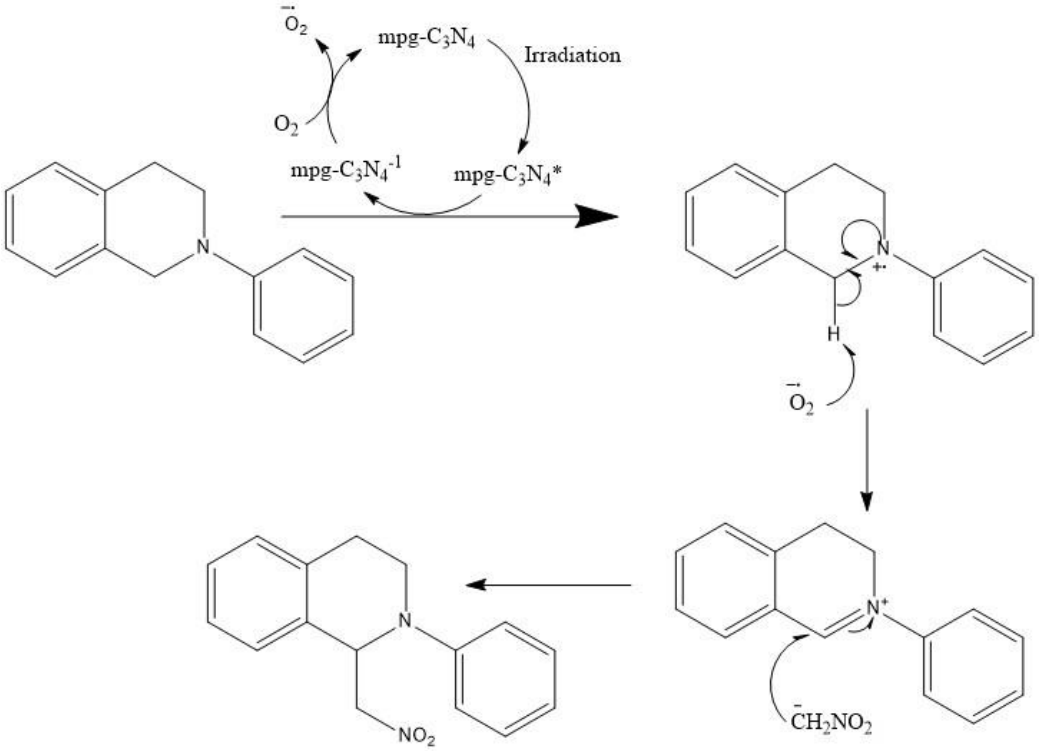
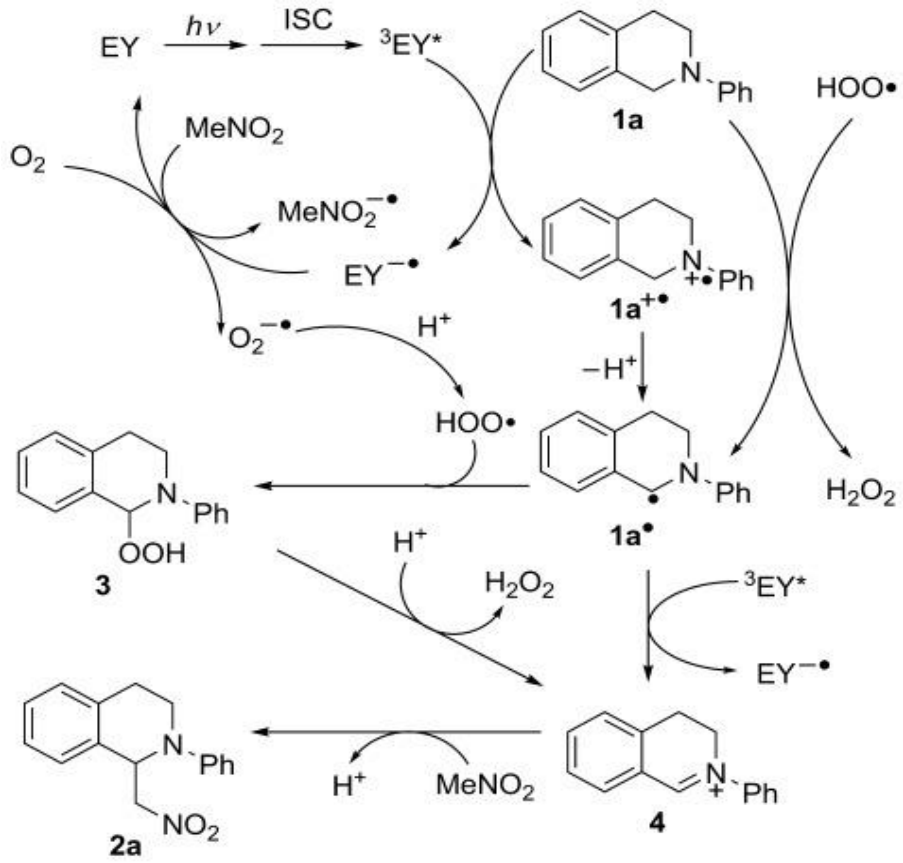


Figure 3

Expanded Mechanism of Photoredox Experiment



Note. The mechanism of the photoredox experiment can undergo different mechanism routes. This scheme details the underlying changes that may happen for the final desired product of 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline.

The next step of this process required us to tackle switching from a precious metal catalyst to a catalyst that would be both recyclable and less expensive to produce (*2*,*3*). Focusing on the catalyst mpg- C_3N_4 (microporous graphitic carbon nitride) as our replacement of expensive and less efficient metal catalysts, we can reduce costs and efficiency issues. Some metal photocatalysts can only absorb certain light spectrums, such as ultraviolet (UV), and when they are able to absorb more wavelengths they become quite expensive (*4*,*5*). The mpg- C_3N_4 can absorb a medium range of wavelengths and is very inexpensive and simple to produce. We moved to a microporous version of g- C_3N_4 to increase the surface area of the catalyst to create the possibility for more photocatalytic interactions. This can help to acknowledge and resolve the issue of a decreased absorbance range (*5*,*6*). By making these changes to the established photocatalytic reactions, both environmental hazards and solvent waste are able to be reduced, and cost can be conserved when scaling up.

Chapter II

Materials and Methods

*All chemicals and materials were purchased from commercial sources unless otherwise stated.

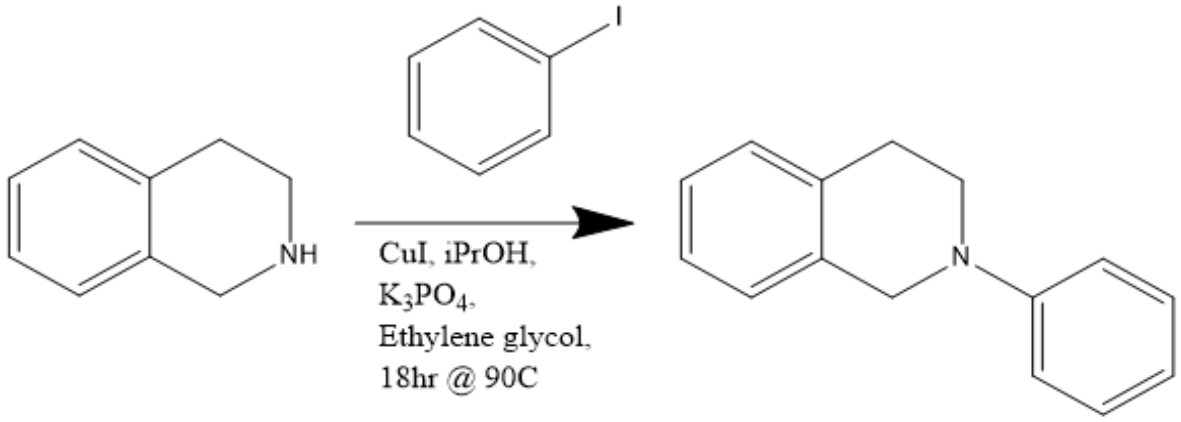
Synthesis of 2-phenyl-1,2,3,4-tetrahydroisoquinoline

In a 20 mL scintillation vial copper iodide (34mg, 0.179mmol, 0.1equivalents) and potassium phosphate (759.9mg, 3.58mmol, 2.0 equivalents) were dissolved in Iso-propyl alcohol (1.79mL, 1.0 M). At this time the reagents showed incomplete solubility. The solution was mixed for ten minutes using a magnetic stir bar. After being thoroughly mixed, 1,2,3,4-tetrahydroisoquinoline (340µL, 2.68mmol, 1.5 equivalents), ethylene glycol (200µL, 3.58mmol, 2.0 equivalents), and iodobenzene (200µL, 1.79mmol, 1.0 equivalents) were all added to the solution. Once added, the solution was set to stir for eighteen hours at ninety degrees Celsius. This process was completed using a thermoregulated hot plate. The following day, a separatory funnel extraction, also known as a workup, was performed on the product. This workup was completed using dichloromethane (DCM) and water. Twenty mL of water was added to the vial and transferred to a separatory funnel; this step was done once more, with any leftover solids broken up with a metal scoopula. Next, the vial was filled with DCM to dissolve any excess product and was then transferred over to the funnel. The product was extracted three times with DCM and then dried in a flask with sodium sulfate to remove any excess water. Purification was then performed using a Biotage flash chromatography instrument along with a 25g sized column filled with silica. Thin layer chromatography (TLC) was

used to help identify each material and the product throughout this synthesis. The column was run with a slow gradient of hexanes and ethyl acetate. The product eluted off the column between 15% and 20% ethyl acetate to provide 2-phenyl-1,2,3,4-tetrahydroisoquinoline (TIQ) (38.8mg, 0.185mmol, 10.4% yield). Below the reaction scheme for 2-phenyl-1,2,3,4-tetrahydroisoquinoline and a detailed scheme showing the Ullman mechanism for the reaction. Previous synthesis of this compound can be found in the supporting information of reference 1. (Figure A6, Figure A7) (*1,2,6*)

Figure 4

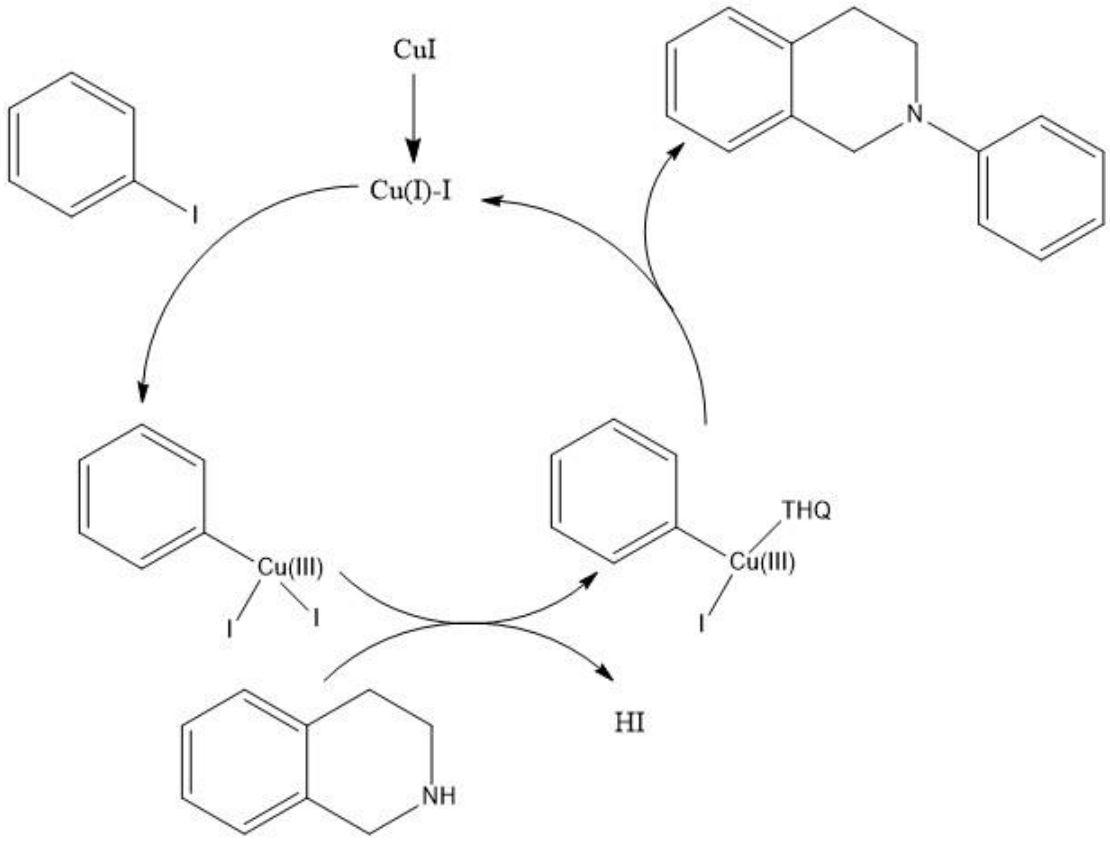
Synthesis of 2-phenyl-1,2,3,4-tetrahydroisoquinoline



Note. A reaction shortly describing the materials and methods used to synthesize 2-phenyl-1,2,3,4-tetrahydroisoquinoline.

Figure 5

Ullman Mechanism of TIQ



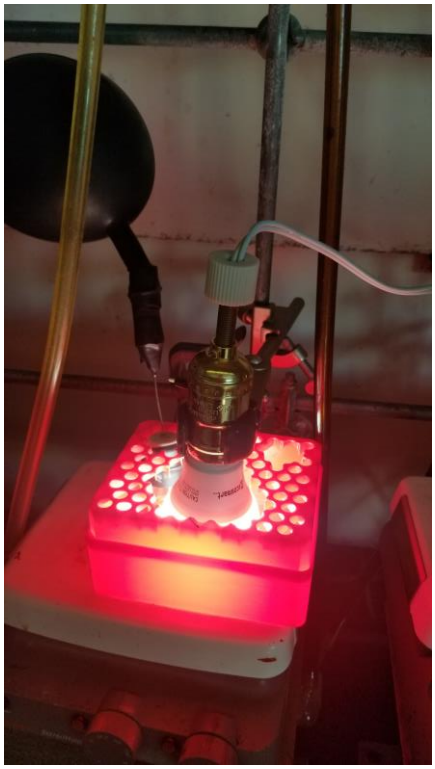
Note. Ullman mechanism describing the underlying mechanism of copper coupling, transmetallation, and reductive elimination providing the final product 2-phenyl-1,2,3,4-tetrahydroisoquinoline.

Synthesis of 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline

This reaction was run in a makeshift chamber to introduce light and oxygen in a safe manner with no extraneous light or oxygen being able to be of use (Figure 6). In a scintillation vial containing 2-phenyl-1,2,3,4-tetrahydroisoquinoline (26mg, 0.125mmol, 1.0 equivalents), nitromethane (0.5mL, 2.72mmol, 0.5 equivalents) was added. Once the substrate was dissolved, mpg- C_3N_4 (15mg, 0.163mmol, 1.3 equivalents) was added to the vial, which was then capped with a rubber septum. The septum was punctured with a needle to introduce the oxygen, held within a balloon attached to a needle. The reaction was run with a 60w frosted light bulb for 24 hours. On the second day, the contents of the vial were put on a Rotovap and stayed until dry. The contents of the vial was moved to a vacuum pump to remove any excess solvents. Once dry, a crude H-NMR in $CDCL_3$(Chloroform)(SI. N1) was taken and the product was purified by Biotage flash chromatography. TLC was employed to identify the product along with H-NMR. The final product eluted between 0% and 10% ethyl acetate to provide 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline (18.8mg, 0.07mmol, 72% yield). Figure 7 below describes the general method to synthesize 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline. Previous synthesis of this compound can be found in the supporting information of reference 2. (Figure A2, Figure A3) (*1*,*2*,*6*)

Figure 6

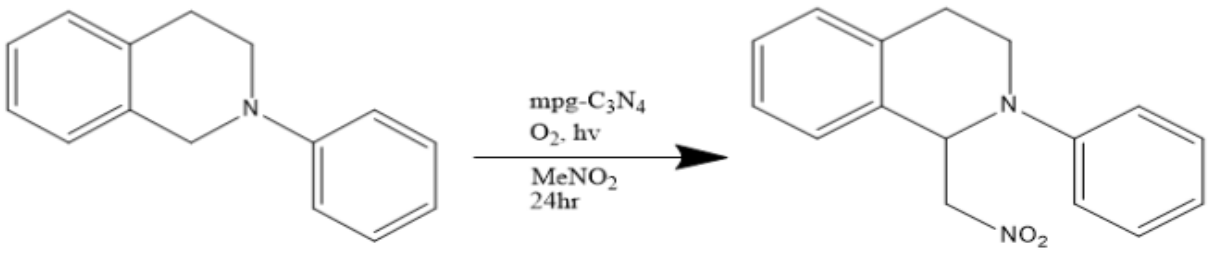
Controlled Reaction Chamber



Note. Reaction chamber used to synthesize photocatalytic oxidation reactions

Figure 7

Synthesis of 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline



Note. Scheme of either the bacterial or light bulb-mediated photoredox reaction of 2-phenyl-1,2,3,4-tetrahydroisoquinoline to 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline.

Synthesis of dimethyl 2-(2-phenyl-1,2,3,4-tetrahydroisoquinolin-1-yl)malonate

This synthesis followed the same procedure as above with changes to the solvent and substrates. In a scintillation vial, 2-phenyl-1,2,3,4-tetrahydroisoquinoline (26mg, 0.125mmol, 1.0 eqiuvalents),dimethyl malonate (71uL, 0.625mmol, 5.0 equivalents), mpg-C_3N_4 (15mg, 0.163mmol, 1.3 equivalents) and Dri-Solv acetonitrile (0.5mL, 0.5 equivalents) were set to mix overnight under light irradiation. Purified and crude products were determined by TLC and H-NMR. Previous synthesis of this compound can be found in the supporting information of reference 2. (Figure A15) (*1,2,6*)

Initial Toxicity Assay of DH5-alpha E. coli

The bacteria was grown overnight at 37 degrees Celsius. 200uL of bacterial solution was added to 20mL of LB media solution. The assay was plated on a 96 well plate beginning with the addition of 200uL of bacterial solution to the wells of rows 1-11. LB media with no bacteria was added to column 12 to act as a negative control while columns 10 and 11 acted as positive controls. An extra 50uL of bacterial solution was added to the first row of wells. Addition of chemicals being studied for toxicity is as follows: 50uL of nitromethane was added to the first row of wells 1-3. 50uL of TIQ was added to the first row of wells 4-6. 50uL of dimethyl malonate was added to the first row of wells 7-9. Then a 3-fold dilution was performed down the plate leaving the final row with the least concentrated solutions. This plate was run on a plate reader for 24 hours and OD600 and Lux was recorded every 15 minutes for those 24 hours. (Figures 13-19):

Mpg-C3N4 Polymer Synthesis

In a scintillation vial, cyanamide (1g, 23.78mmol, 1.0 equivalents) was mixed with an SiO2 water solution (40 wt. % suspension) (1.25mL, 8.32mmol, 0.35 equivalents). This was set to stir at 60 degrees Celsius on a thermoregulated hotplate for 18 hours. The product was then removed from the vial and placed into a crucible. The crucible was placed inside a quartz tube, which was then placed in a furnace. The furnace was set to increase from room temperature to 549 degrees Celsius over a period of 4 hours, after which the furnace maintained a temperature of 549 degrees Celsius for another 4 hours. During the time in the furnace, nitrogen gas was passed through the tube to remove any dangerous by-product gasses while the furnace was left until the next day to cool. The crucible was removed from the furnace and tube. The golden-brown product was removed from the crucible, placed in a 4M NH_4HF_2 acid bath, and left to stir overnight. This process was completed to remove the silica mold that creates the microporous graphitic carbon nitride. The next day, the solution was centrifuged and the acid bath was decanted into the waste. The polymer was then centrifuged twice at 2000 rpm for 10 minutes with deionized water and once more finally with ethanol. The final product was dried overnight in a vacuum and the polymer was moved to long-term storage. (Figure A16, Figure A17) (*3*)

Figure 8

Furnace for Polymer Synthesis

Note. The furnace with quartz tube set inside used to minimize dangerous gaseous buildup during polymer synthesis.

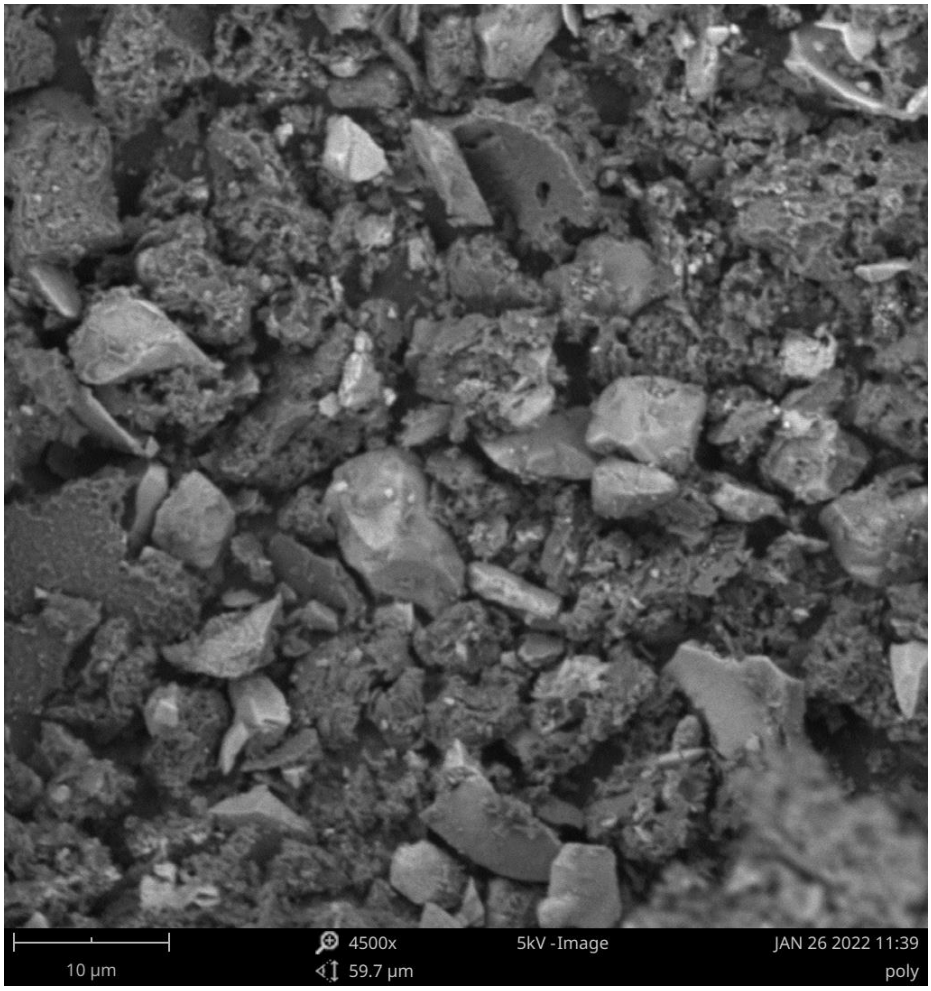
Figure 9

Crucible for Polymer Synthesis

Note. This crucible was used to place precursors of mpg- C_3N_4 into the furnace.

Figure 10

Micro Image of Catalyst



Note. Scanning electron microscope image of mesoporous graphitic carbon nitride.

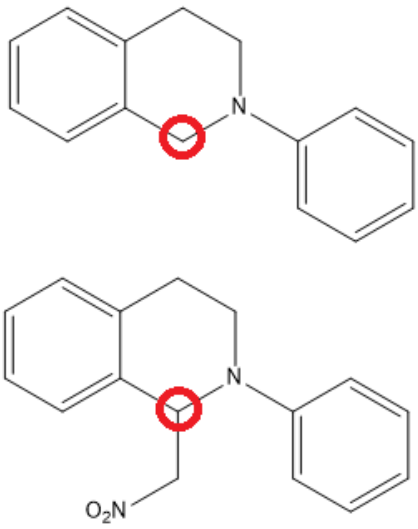
(Thank you to Matt Jackson, AMT, for helping provide the SEM data)

Bacterial Photocatalytic Oxidation

Bacteria was grown overnight at 37 degrees Celsius in a culture tube. The following day, two 250mL Erlenmeyer flasks were filled with 100mL of LB media. To these flasks 1mL of bacterial culture was added to each. A small sample of bacterial culture was taken from each flask every hour to collect growth and light production data using a well plate and a plate reader. After 4 hours had elapsed, 100uL of a 0.5mM IPTG solution was added to each flask. The bacteria was left to grow for another half an hour before TIQ (57mg, 0.27mmol, 1.0 equivalents), nitromethane (214µL, 2.7mmol, 1.0 equivalents), and catalyst (520mg, 5.65mmol, 20.9 equivalents) were added to only one flask. Once the chemicals were added to both flasks, they were covered with aluminum foil to remove any extraneous light from intruding. Readings of lux and OD600 were taken for two more hours before leaving the reaction to run for a subsequent 17 hours. The workup for this reaction was done via vacuum filtration. The media was filtered with a filter paper and filter aid on a Büchner funnel. Then, diethyl ether was run through the filter to dissolve any surplus product that did not make it through the filter. The mixture of ether and water was put into a separatory funnel and extraction was done three times using ether. The layers were combined then put on the Rotovap until dry. A crude H-NMR was taken of the product (SI. N4). Purification was done using a Biotage but the final mass was too small to recover. The conversion was calculated using ppm shifts between product and substrate peaks (Figure 11, Figure 12, Figure 14, Figure 15).

Figure 11

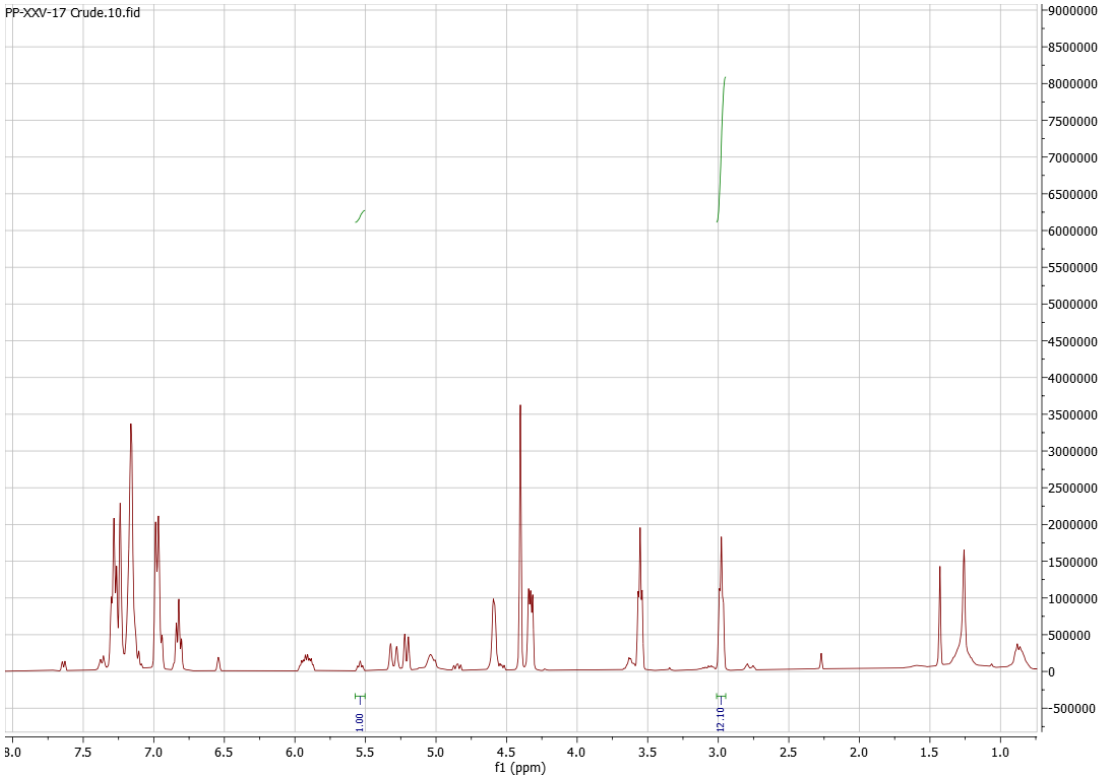
Oxidation of TIQ



Note. The structures of 2-phenyl-1,2,3,4-tetrahydroisoquinoline and 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline with red circles depicting the proton used to calculate percent conversion from starting material to product.

Figure 12

Crude Proton NMR of Bacterial Oxidation Reaction



Note. Using the triplet peaks selected to calculate an estimated conversion from substrate to product. The overall conversion for this experimental run was 4%. Confirming bacteria can produce enough light for a photoredox reaction to occur.

Recycling Bacterial Photocatalysis Oxidation

This reaction was performed in the same manner as described above with changes to the workup. Once the reaction and workup was completed, there was no purification performed. Instead, a crude H-NMR was taken after the workup and the crude product (a mixture of TIQ and the nitromethyl-TIQ) was then reused as the substrate of the above described reaction. This recycling reaction was run 5 times to achieve a 43.5% yield which was sufficient for purification (*3*).

Chapter III

Results and Discussion

The synthesis for 2-phenyl-1,2,3,4-tetrahydroisoquinoline was prepared as stated in the above method. After several trials, a yield weight of 158mg was eventually achieved. This was a yield of 80%, similar to values established in similar experiments documented. This yield was not demonstrated at first due to issues with the workup and chemicals used. One reason for this is that some of the chemicals used were expired and therefore potentially impure. By ordering newer chemicals, we were able to remove that source of error. Initially, the workup was completed using diethyl ether and no separatory funnel. The solids also contained too much of the final product, so those had to be broken up during extraction. Once the extraction and purification was complete, the final product came to be a dark brown, thick oil. We were able to confirm this via H-NMR comparison to the literature values. This called into question the method for measuring out the TIQ for photocatalysis reactions that we intended to run. In order to address this issue, the TIQ was dissolved in a small amount of ethyl acetate and the required amount for each run was removed and placed in another vial. The vial was then dried on vacuum. The first photocatalysis reaction run was very successful, with a conversion of 72%, confirmed via Biotage purification. This supported that the photocatalysis reaction was indeed possible and, more importantly, that the catalyst was successful in its intended use. This also allowed us to continue and plan for future runs of our bacterial-mediated oxidation reactions.

Mpg- C_3N_4 is not a common catalyst for photocatalysis work. Over the last few years, however, graphitic carbon nitride has become a more commonly used catalyst (*2,3,5,6*). Our catalyst was successfully made from the aforementioned procedure but, due to issues with the furnace, there were inconsistencies with the carbonization of the final product. This required a careful removal of any non-carbonized C3N4 before moving forward with the procedure. The high conversion rate of the TIQ can be attributed in part to the microporous nature of the catalyst. By increasing the surface area of the catalyst, there is a potential increase for photocatalytic interactions. This helps to offset any issues with the absorbance range of the catalyst. Another issue that arose was the efficacy of the catalyst; it was determined that the catalyst could only be used a few times before its effectiveness decreased. By washing the catalyst in ethanol and deionized water after a few reactions, the catalyst was capable of completing more reactions, reducing the need to produce new catalyst. This discovery helped to reduce waste in the form of hazardous waste and energy waste from producing more catalyst. (*3*)

There was another reaction identified as a possible secondary photocatalytic oxidation reaction. This was achieved by using dimethyl malonate as the nucleophile and acetonitrile as the solvent instead of nitromethane (*1,2,6*). The photocatalysis reaction was performed to determine a control conversion rate, as discussed previously for nitromethane, which gave a strong yield of 74% conversion. While this indicated that the synthesis could be a second possible course for use in the bacterial model, it was eventually halted due to the volatility of acetonitrile killing the bacteria. Other substrates and solvents were identified as possible synthesis routes, but the chemicals were either too toxic for the bacteria or the reaction did not have a yield once the reaction was run.

Due to these reasons, we were forced to abandon these other bacterial-mediated synthesis approaches.

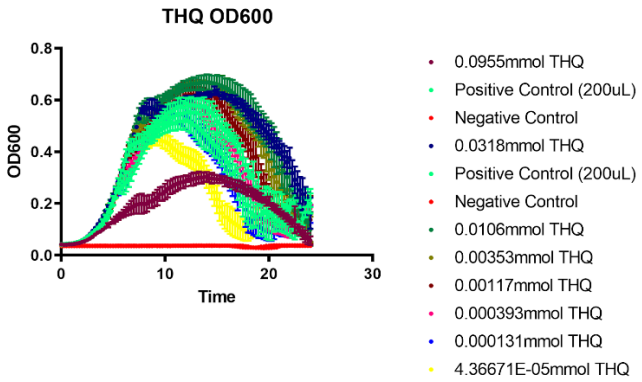
The 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline synthesis via photocatalytic oxidation gave very promising results. The catalysis via light bulb had a 72% yield, creating a large leap for the research by confirming that we could successfully convert large amounts of our TIQ molecule while using our improved mpg- C_3N_4. The photocatalysis was executed multiple times to verify that the reaction could be efficient as well as consistent. This consistency affirmed the reaction as a control for when the bacterial-mediated reaction would be attempted.

The main focus of our research was a successful bacteria-mediated photocatalytic oxidation reaction. By introducing the bacteria as the light source, the catalyst could be activated more frequently due light being produced in the homogenous solution of media and catalyst. Since chemicals would also be flowing freely in this solution, toxicity was incredibly important to monitor during the studies. Figures 1 and 2 below help to illustrate how concentrations and chemical amounts were determined before any bacterial series were run. MIC assays or minimum inhibition concentration assays were used to determine the best choice of chemicals to be incorporated into the bacterial media. These assays were necessary to select which synthesis routes could be feasible to run. The MIC data generated also helped to determine which concentrations inhibited bacterial growth (OD600) and light production (RLU) (Table 1). The concentrations chosen for the future bacterial-mediated reactions were compromised between maximum light production (RLU) and highest tolerable concentration. This tolerable concentration was important in

order to design experiments that can give enough yield for qualitative and quantitative measurements.

Figure 13

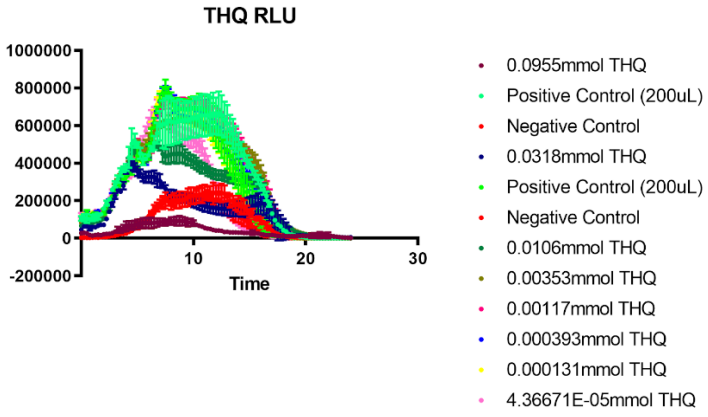
TIQ Toxicity Assay of OD600



Note. Growth curves of E. coli, measuring OD600 (optical density), with varying 2-phenyl-1,2,3,4-tetrahydroisoquinoline concentrations.

Figure 14

TIQ Toxicity Assay of RLU

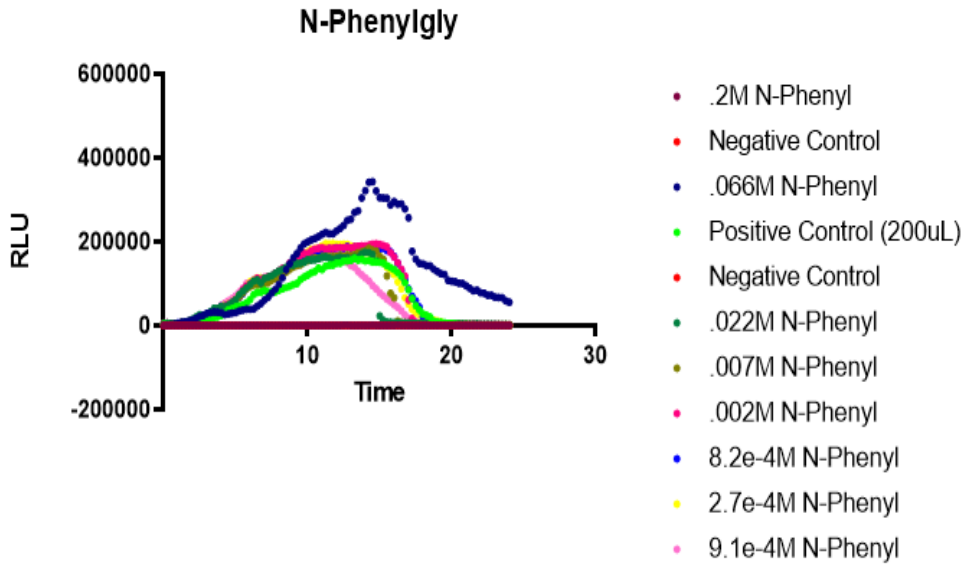


Note. RLU's or relative light units help determine the toxicity of varying amounts of 2-phenyl-1,2,3,4-tetrahydroisoquinoline concentrations.

In addition to evaluation of our TIQ substrate for bacterial toxicity I also determined the usable concentrations of a range of additional reagents and the catalyst including n-phenyl glycine, Cyclohexene, Nitromethane, and mesoporous graphitic carbon nitride. The selected primary bioassay data is provided in Graphs 1 through 6, and the full data set is tabulated in Table 1 below.

Figure 15

MIC Assay of N-phenylglycine



Note. This compares RLU output over time in hours.

Figure 16

MIC Assay of Cyclohexene

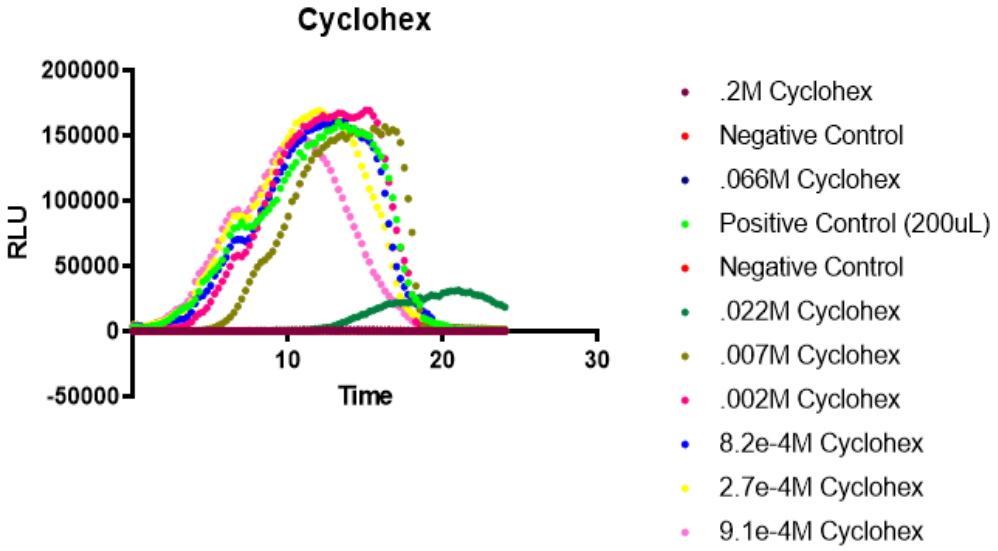


Figure 17

MIC Assay of Nitromethane

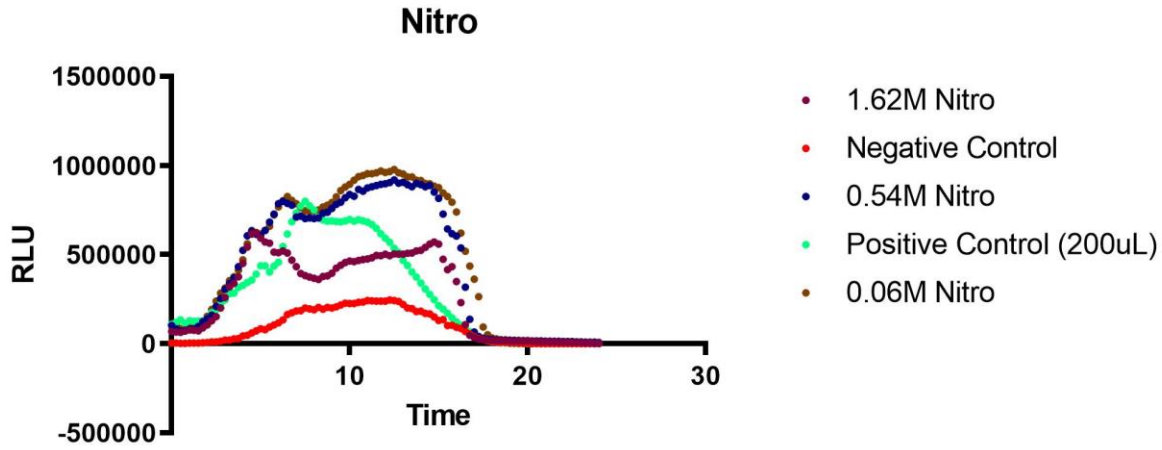


Figure 18
MIC Assay of MPG-C_3N_4

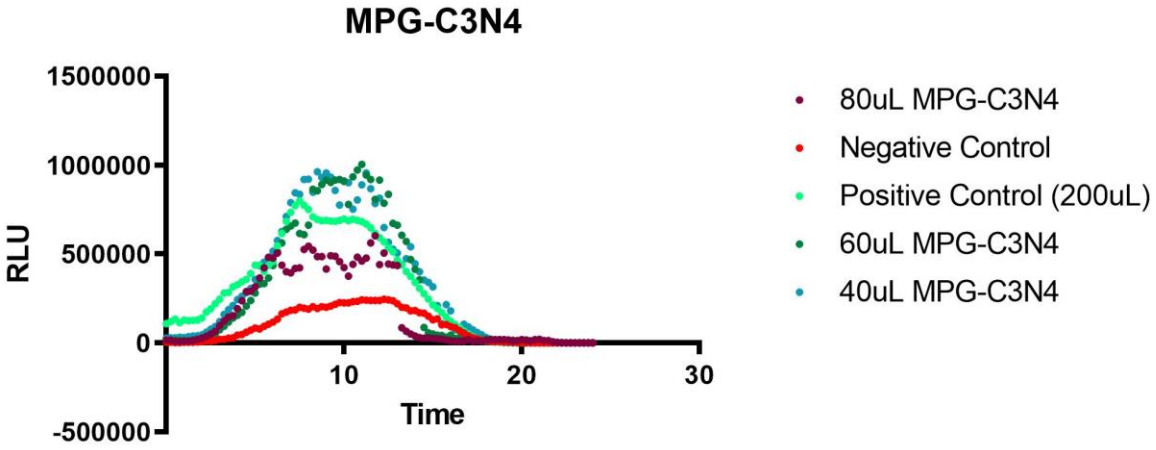


Table 1
Extracted Data from MIC Assays

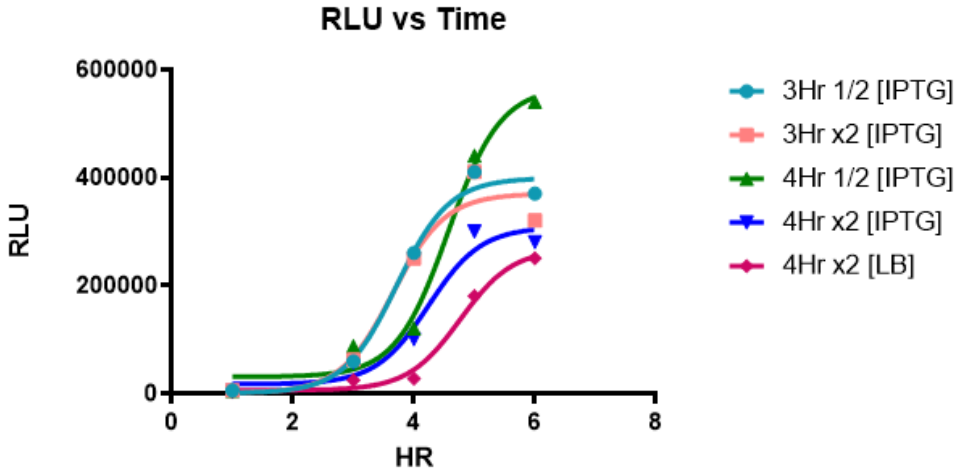
Substrate	Tolerable Concentration (mg/mL)	Max RLU	Tolerable (Comparative)
Diazo	0.03125	1,404,110	x
Furan	0.0781	1,033,490	x
MesIR	0.015	163,235	
(iPr)3PhIR	0.015	1,282,080	x
Me-THQ	1.08	265,005	
Benzly Alcohol	3.13	234,811	
MeOH (S)	0.224	177,448	
Di-Phenyl	0.336	194,898	
DMSO (S)	0.021	199,246	
Isochroman	0.992	177,332	
N-Phenylgly	9.96	343,257	x
Cyclohexene	0.164	169,323	
MPG- C_3N_4 (C)	15	1,004,300	x
Nitromethane	3.66	976,797	x
THQ	0.737	797,196	x

Note. The data from the MIC assay experiments were used to determine which compounds to move forward with.

Initially, these bacterial runs started out with very low conversion rates. The conversion rates averaged within a range of 6% to 10%. At the onset, the chemicals were added into the media once the bacteria were thought to have reached maximal growth. This was determined to be too late in the process and instead the chemicals were added during bacteria growth in order to obtain maximum light absorbance for the catalyst. By changing the time at which the reactants and catalyst were added, along with the addition of IPTG, this helped to achieve increased and consistent conversion rates. IPTG (Isopropyl β- d-1-thiogalactopyranoside), which triggers the lac operon to induce protein expression, pushes the E. coli to produce more light which in turn means more conversion. By changing the interval of time between this addition and how much was added, we can control the conversion rate of the TIQ. Initially, the procedure called for a one hour gap between addition of the IPTG and chemicals. After testing both gap times, it was determined that a 30 minute gap provided more consistent results. Along with these results, studies were conducted to determine if changing the concentrations of IPTG or LB media would produce higher or lower yields of the final product (Figure 19).

Figure 19

Variable Optimizations



Note. The variables changed for each line are concentration of IPTG added, when IPTG was added, and increased concentration of LB media.

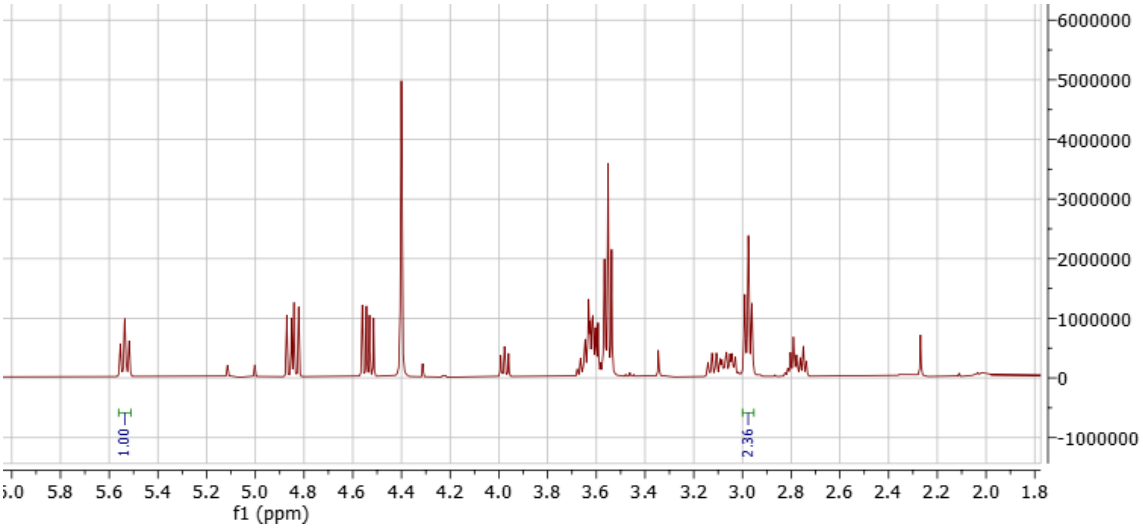
Many iterations of these bacterial runs were completed in order to gain insight on timing and other variables that could be altered to produce a more desirable outcome. Tandem runs were completed following the same procedure previously mentioned for the bacterial oxidation reaction. Instead of adding the substrates and catalyst, the bacteria were left to grow unhindered, with individual variables changed for each of the five runs done in tandem. The results of these runs helped to determine the final variables that were consistently used for the most successful bacterial-mediated reactions. These procedures provided the framework for a more efficient use of the bacterial-mediated experiments.

Recycling bacterial-mediated oxidation allows for the innovative concept of a bacterial-run organic synthesis reaction to reach its full potential. Due to issues with inconsistency and low conversion rates, we decided the most efficient way to highlight the bacterial-driven reaction was through a recycling style reaction. This concept can be compared to bioreactors, which are designed in a similar style. By keeping a continuous flow of new LB media, the bacteria can keep growing and will not run out of food, which incidentally stunts their growth and reduces reaction rates. Due to the expensive nature of bioreactors and maintenance, we decided to set our reaction up in the previously mentioned way. Removing purification from the process allowed us to keep whatever starting material was not converted. This non-converted starting material was then used as our substrate again in a second reaction. By continuing this, we were able to have larger purification fractions of the final product. This eventually led to the highest single conversion of 25%. A 25% conversion in a single run shows the possibility that this reaction can be run only four times to reach 100% conversion. H-NMR data and Table 2

shown below help to describe how the peak ratios were used to confirm conversion rates with no purified product.

Figure 20

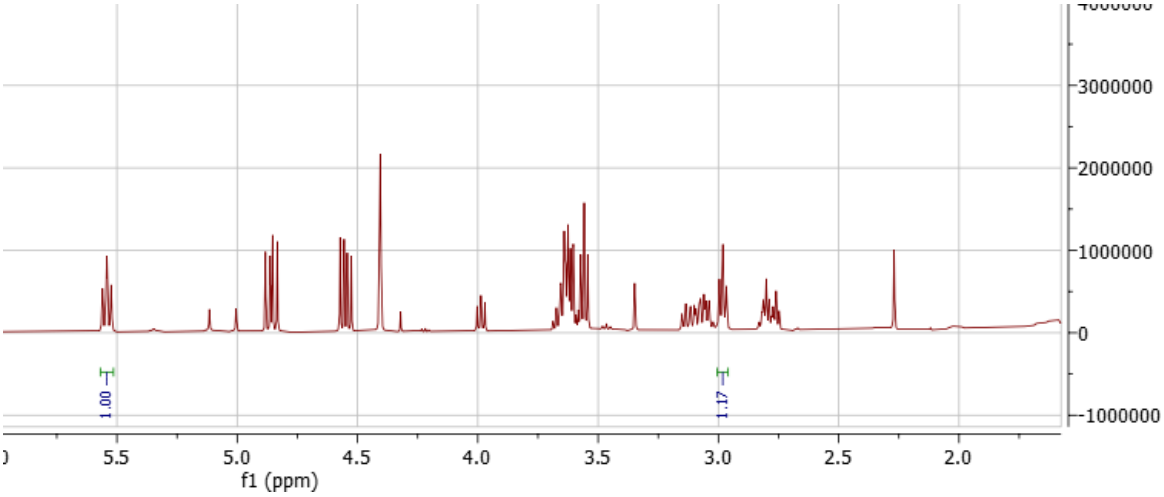
Third Recycling Run



Note. The H-NMR data of a 3rd run recycling data illustrates the comparison ratios of two specific peaks. The left peak shows a triplet of one proton and is linked to the oxidized TIQ molecule. The right peak is a triplet peak of two protons and is linked to the non-oxidated form of TIQ. The ratio is 1:2.36 but because the right peak is that of two protons the ratio must be adjusted which makes the ratio a 1:4.72 or a conversion of 21.1% but this does not show the rate between each run, only total conversion from previous runs.

Figure 21

Fourth Recycling Run



Note. The H-NMR data of the 4th run from the same series of runs as shown from the image above. This image illustrates the specific conversion between 2 runs, runs 3 and 4. The ratio is 1:2.34 a conversion of 42.7%. The corrected conversion between just these two runs is 21.6%.

Table 2

Conversion of Each Bacterial Run

Run #	Weight (mg)	% Converted	% Converted Per Run
1	53	8	x
2	50.4	10	2
3	47.5	21	11.1
4	40	41.6	21.6
5	31.6	43	0.4

Note. The cumulative change in percent conversion over each run for one recycling bacterial photocatalysis oxidation experiment. The "% Converted Per Run" was calculated via H-NMR data in order to rule out inaccurate weight due to loss of sample during transfers.

With consistent results between 15% and 25% conversion, this proved recycling to be a much stronger mechanism to continue using. Issues with this process did arise when a final weight was taken; the original substrate weight of 53mg, resulted in a reduction after each run. After three runs the overall weight of substrate and product was reduced from 53mg to 37mg. This drastic reduction in weight led us to rethink the scheme to address the initial issues with it. It seemed that the weight lost occurred during the workup phase. The original workup phase used a filtration system, rather than a separatory funnel system. This style of workup was not harsh enough to remove all substrate and product from the water layer or any stuck in the filter aid in the funnel. By switching to a separatory funnel style workup after the filtration, we were able to solve the massive loss in substrate. This was confirmed with a loss of only 3mg of substrate/product between 3 recycling runs. While the weight problem was solved, a lack in conversion was found to be due to the use of over-recycled catalyst. Washing the catalyst with an ethanol and water solution followed by centrifugation cleaned the catalyst of excess bacterial or chemical waste. The freshly cleaned and recycled catalyst led to increased rates of conversion when compared against the un-cleaned version. By using this recycled catalyst, the reactions could be run with less time needed between and

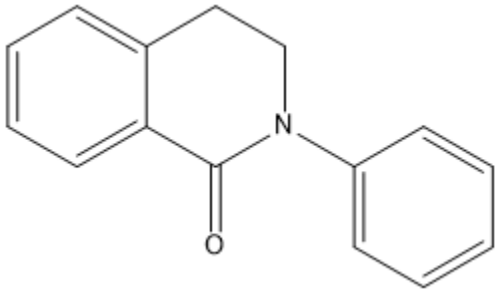
required less catalyst to be made. This mechanism is still an area of interest in our research.

With the new workup method above, a new oxidation product was found. This new oxidation product may have been favored over the desired product. This new oxidation product was seen in some of the first experiments run. In the NMR's (SI. N5, N13) from our earlier experiments it can clearly be seen that the telltale triplets of the TIQ molecule had been shifted up in PPM and the singlet that marks the lone proton bound to the nitrogen of the TIQ molecule, cannot be seen in the HNMR. This shift in PPM and the removal of the singlet marks the oxidation of that single hydrogen leading to a double bonded oxygen molecule which can be seen below in figure 22. (*1*)

While in some of the earlier reactions this secondary oxidation was seemingly un-favored over our preferred Henry reaction, with changes to the workup procedure we found a higher abundance of this 2-phenyl-3,4-dihydroisoquinolin-1(2H)-one molecule over the 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline. We were able to isolate this new oxidation product with a 55% yield in just one reaction. While this compound was not originally planned as a final product, it could possibly be the reason for low yields of the Nitro-TIQ molecule; it became good news for our future experiments. With the knowledge that light producing bacteria can provide the energy source for an oxidative organic reaction and in so producing a greater than 50% yield in only one overnight reaction, our research can keep moving forward with new goals.

Figure 22

Structure of Mystery Oxidation



Note. Structure of 2-phenyl-3,4-dihydroisoquinolin-1(2H)-one.

Chapter IV

Conclusion

We have demonstrated that bacteria can be a suitable replacement as a light source in these photocatalytic reactions. With the new information regarding the single alpha oxidation product, research can target honing procedural settings to focus solely on producing the alpha oxidized product. With a yield of over 50%, there is concrete proof that bioluminescent bacteria can produce enough light for photocatalytic oxidative synthesis. Coupled with a highly recyclable and inexpensive catalyst, this process is likely to drive down cost and increase efficiency, while also being a more environmentally friendly and sustainable reaction. Bacteria can survive and produce the necessary circumstances for these organic photocatalytic oxidation reactions to occur. The introduction of bacteria to these reactions can create alternative solutions with a green form of organic synthesis. The scope of these reactions can have widespread and varying applications.

There are several future studies planned for these types of reactions. During the period where the research labs were closed due to covid-19, there was a strong focus on literary research and planned approaches to introduce new schemes to help address some of the issues discovered during the beginning of this research. Both the bacteria and reaction require an input of oxygen. By using only atmospheric oxygen, we limited the amount that could be introduced to the reaction. By finding a way to implement an O2 source there could be potential increases in reaction rates. An investment into a bioreactor could lead to more consistent studies done on the recycling style reactions.

This can also help limit variability issues in future studies. Other substrates became an area of great interest. While simple oxidation reactions made it easy to prove our hypothesis, we also wanted to focus on other substrates that were more interesting or more easily purchased, saving time and effort. Multiple substrates had been tested and only a few seemed to have promising results come back from the toxicity studies. These substrates seemed to have worse conversion rates than our initial substrate TIQ. Some alternative reactions have also become great areas of interest such as oxidative decarboxylation of alpha-amino acids to make alpha amino alkyl radicals which react with alkenes. Through the literary research, we focused on changing the catalyst either to introduce a new type of environmentally friendly catalyst that could absorb larger wavelength gaps or had a reduced band gap. Potentially, these other catalysts could increase reaction rates. In the same vein of catalyst experimentation, research showed that the mpg-C_3N_4 could be doped with other chemicals to lower band gaps or even increase specific surface area. C_3N_4 nanosheets or nanoparticles are also an area of interest for future studies. With the introduction of a new more favored oxidative reaction (2-phenyl-3,4-dihydroisoquinolin-1(2H)-one) future studies will focus on establishing the correct parameters to make resulting yields higher and more reproducible. This style of photocatalytic reaction can change the typical way organic chemistry is conducted for the better, whether that be through degradation of pollutants, greener forms of organic synthesis, or a decrease in hazardous waste.

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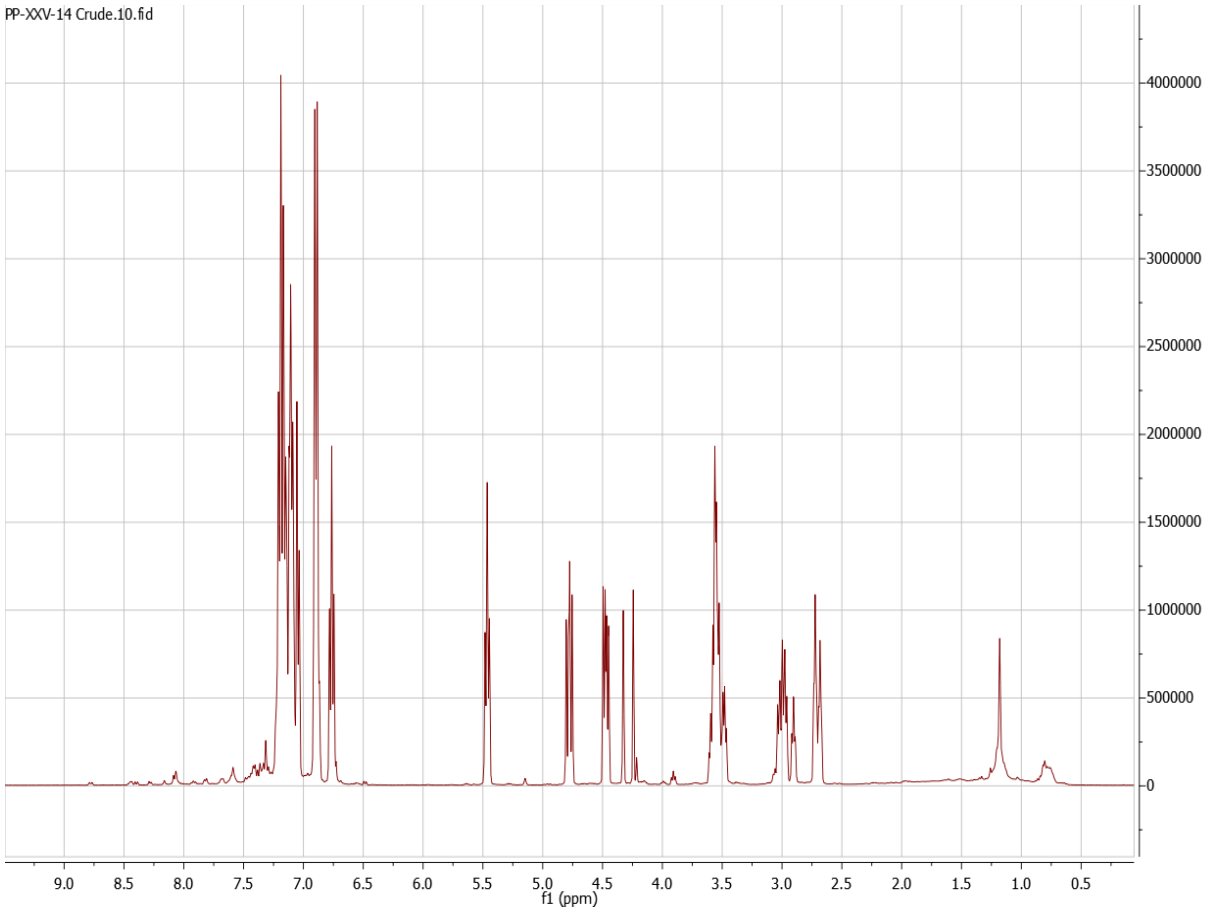
Appendix

Supplementary Data

Figure A1

Proton NMR of 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline



Note. NMR of crude product.

Figure A2

Proton NMR of 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline

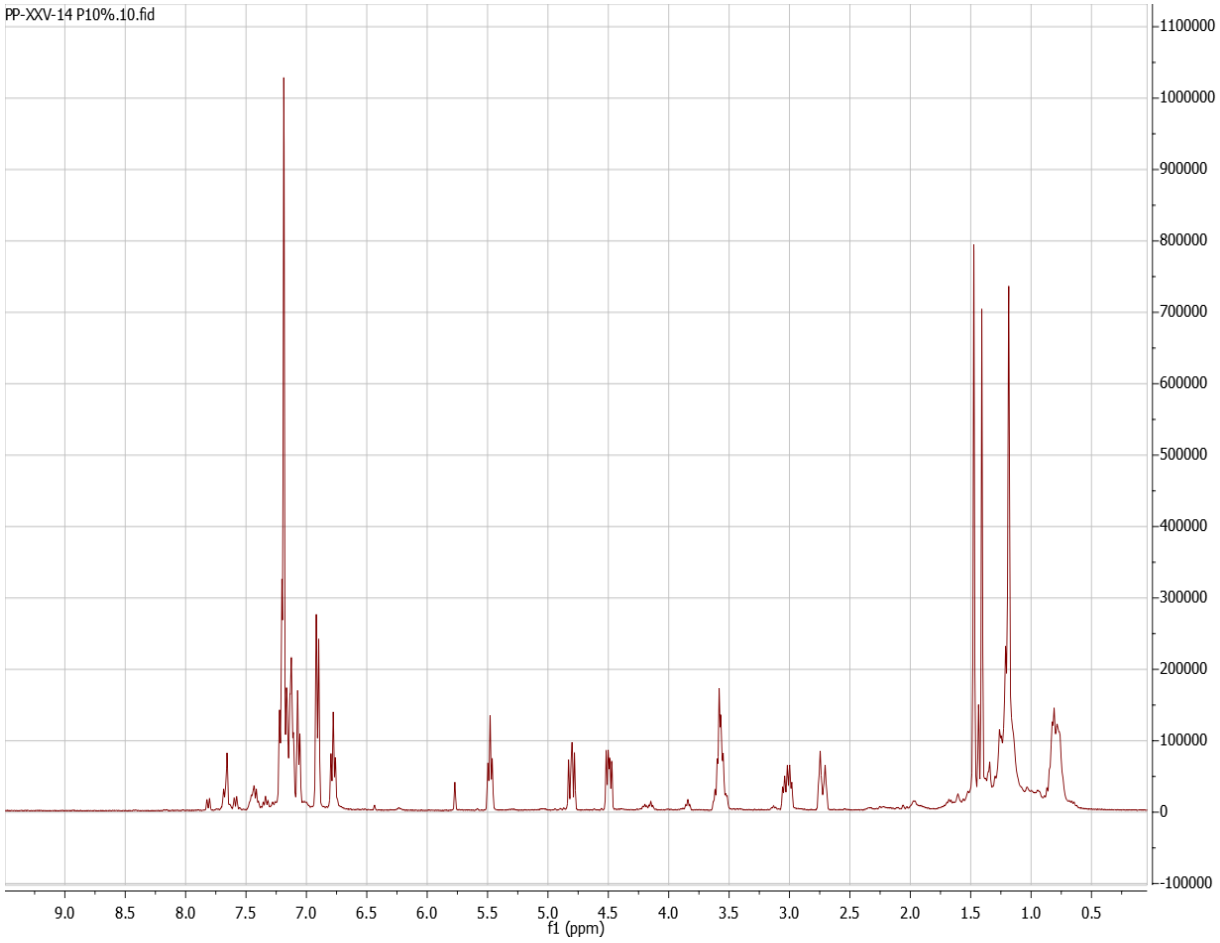


Figure A3

Carbon NMR of 1-(nitromethyl)-2-phenyl-1,2,3,4-tetrahydroisoquinoline

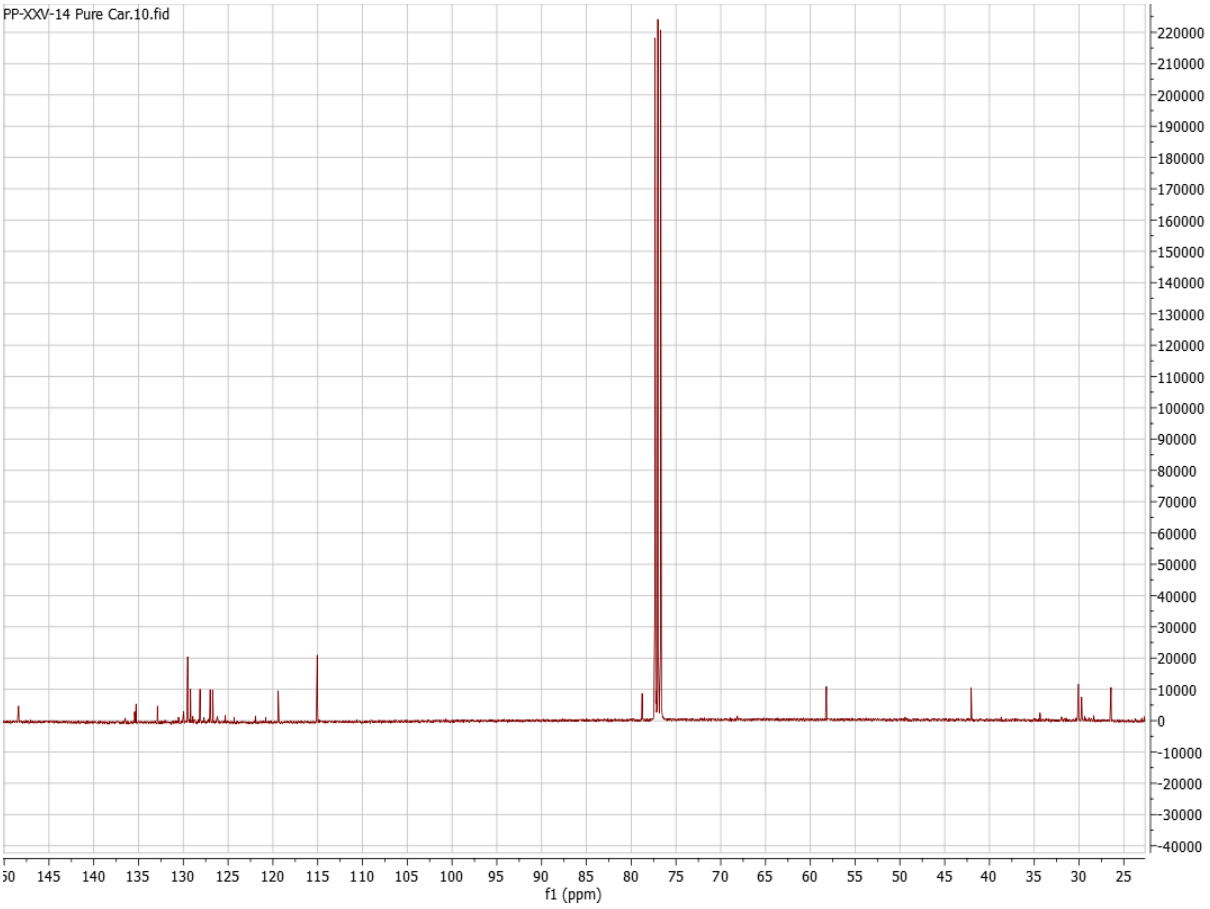


Figure A4

Bacterial-mediated oxidation of 2-phenyl-1,2,3,4-tetrahydroisoquinoline

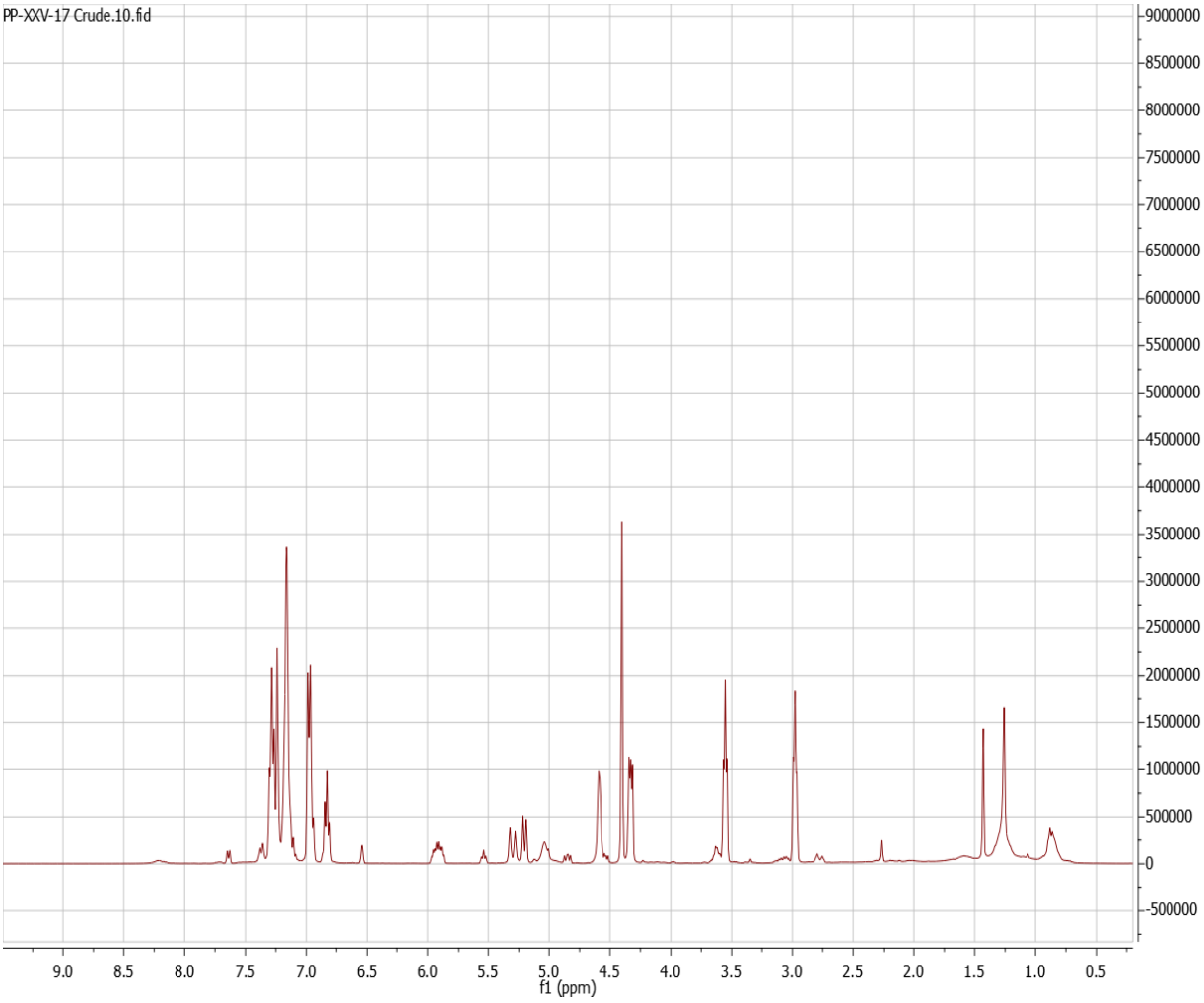
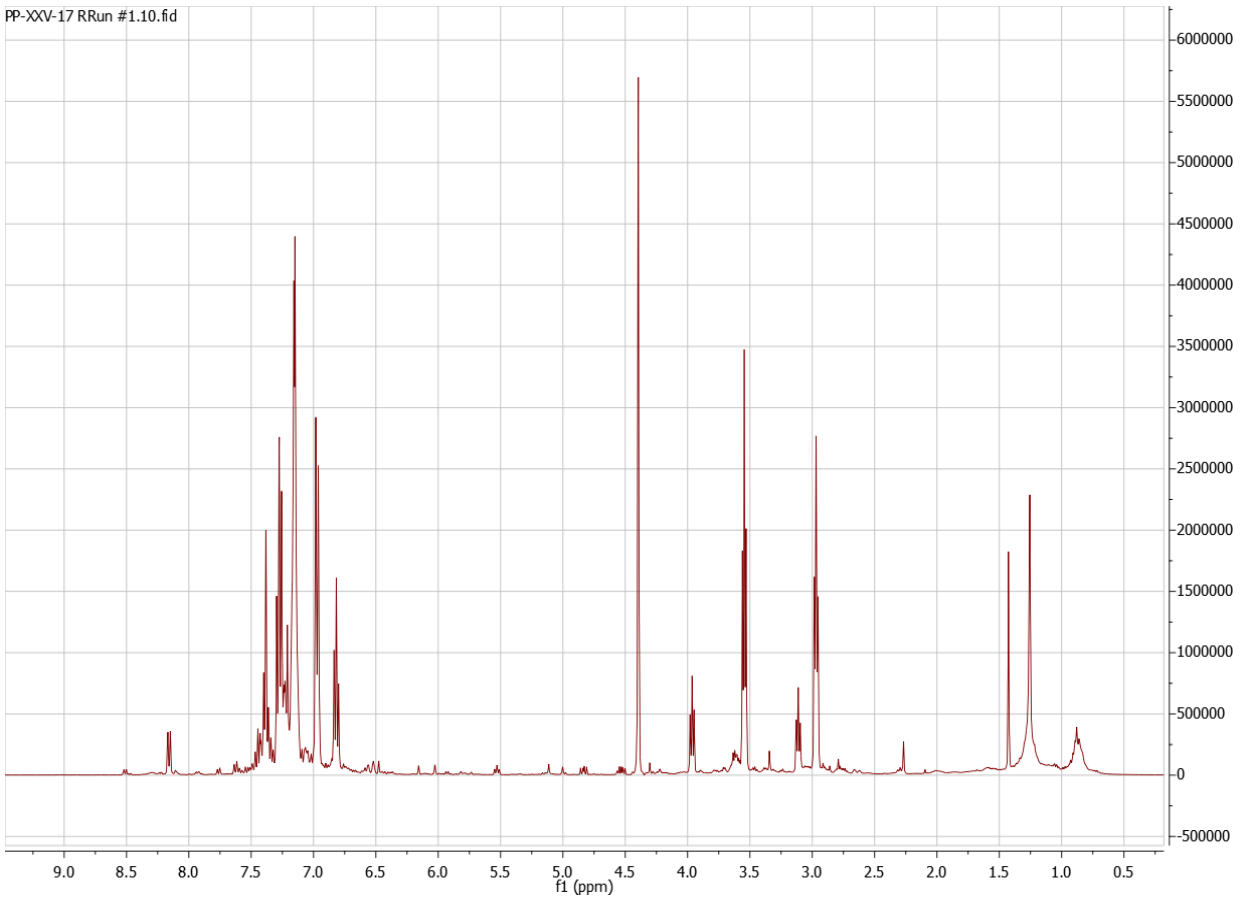


Figure A5

NMR from Early Bacterial-Mediated Experiment



Note. Proton NMR from one of the first bacterial-mediated photoredox experiments. At ppm's of 3.2 and 4.0 the new alpha oxidation product can be seen.

Figure A6

Proton NMR of a crystallized sample of 2-phenyl-1,2,3,4-tetrahydroisoquinoline

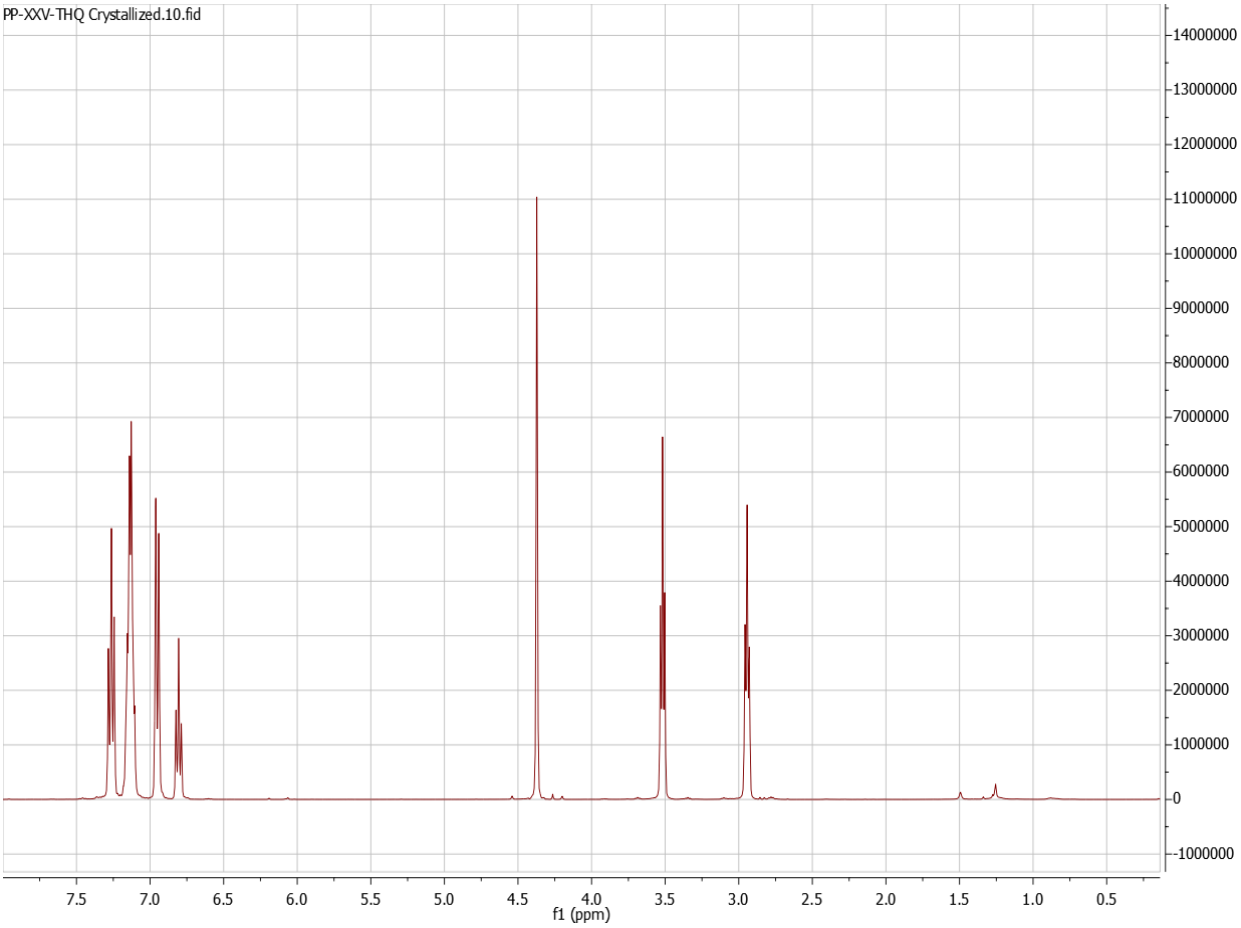


Figure A7

Carbon NMR of crystallized sample of 2-phenyl-1,2,3,4-tetrahydroisoquinoline

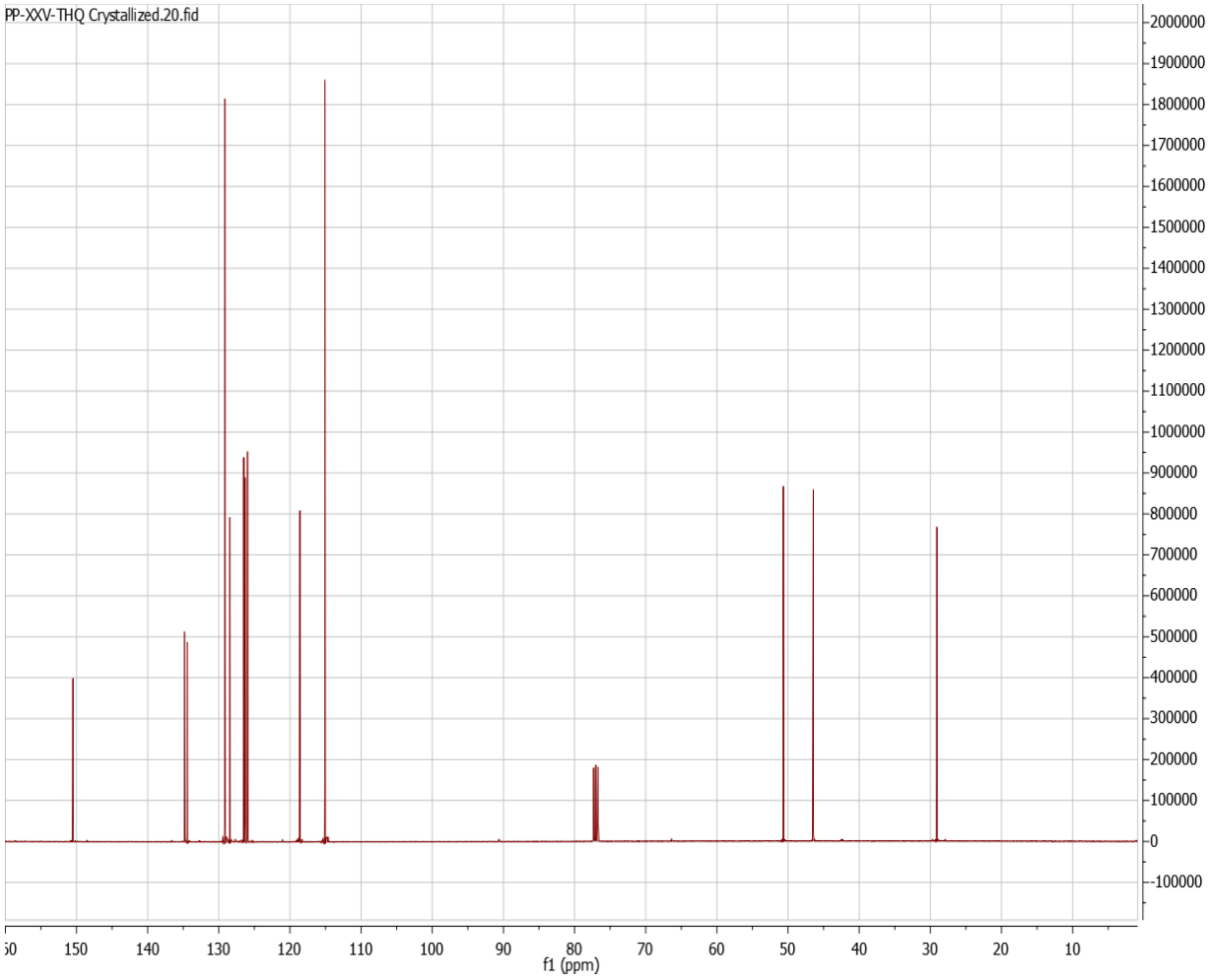
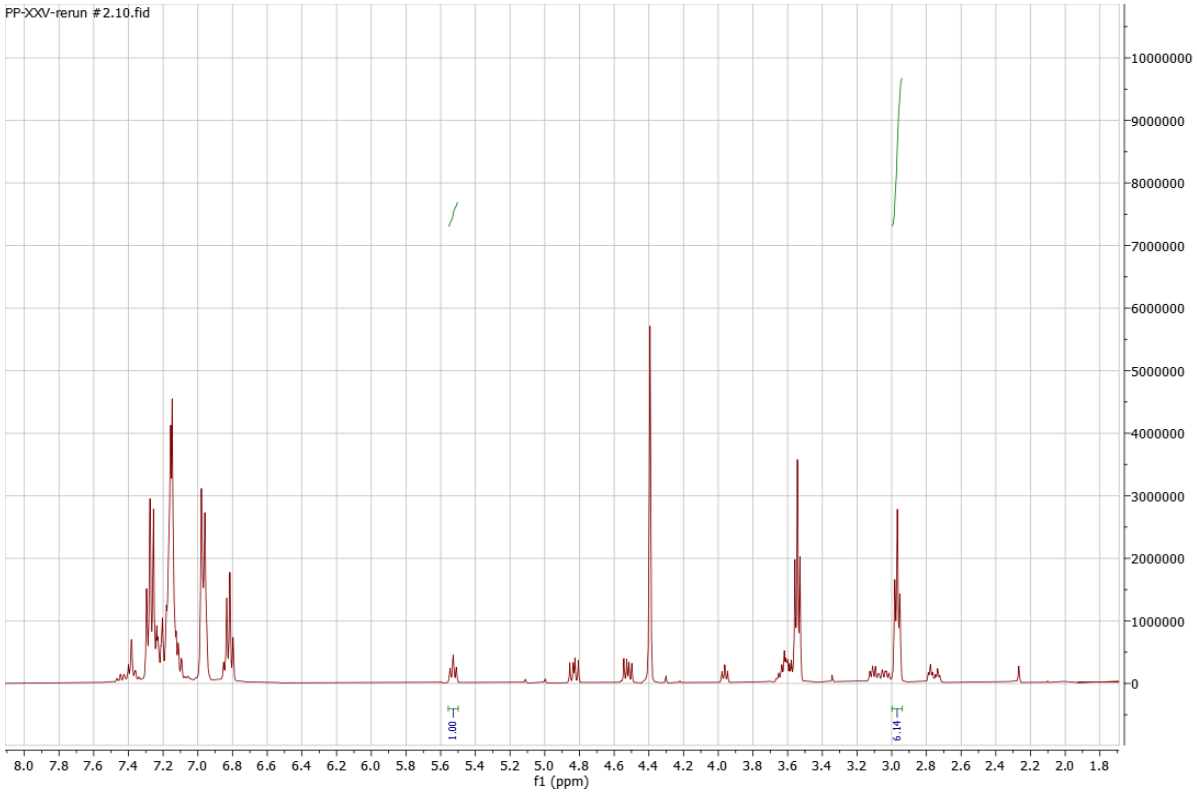


Figure A8

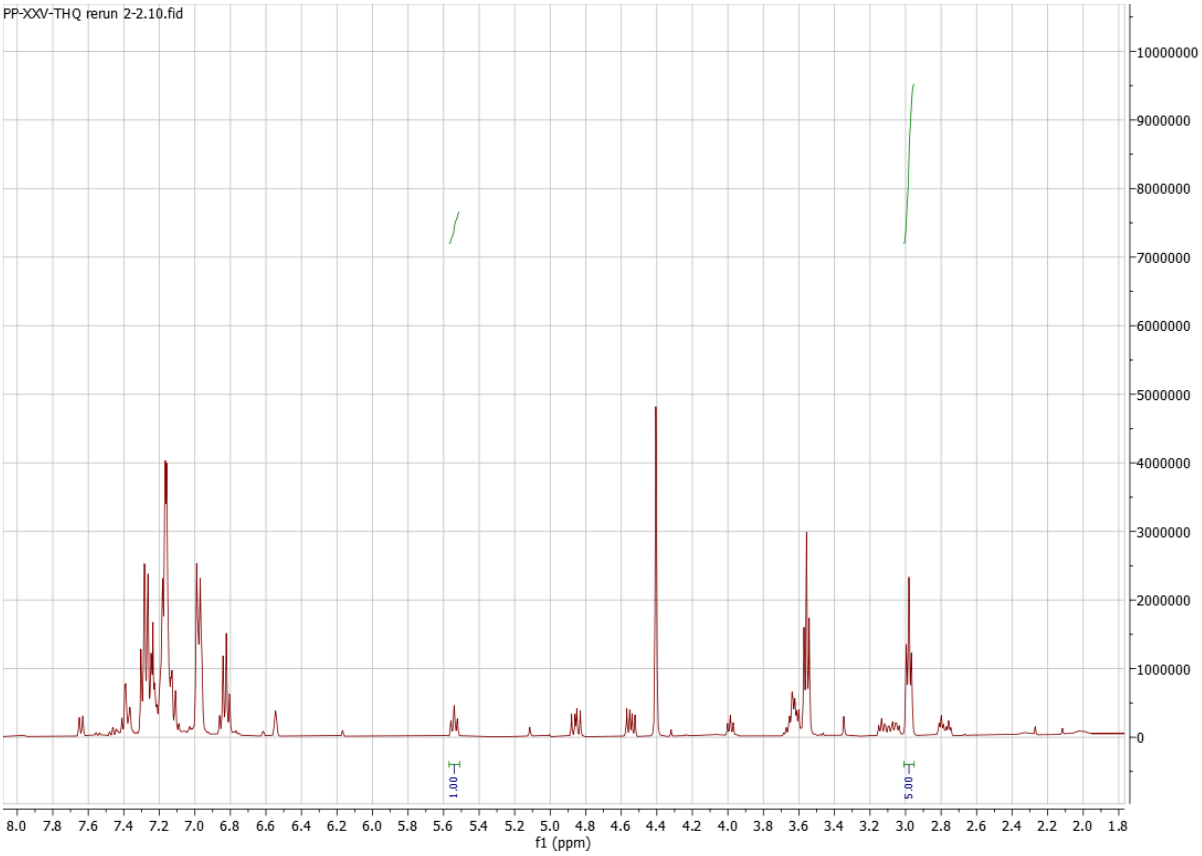
First Recycling Bacterial Experiment



Note. Proton NMR of the 1st recycling run in a series of bacterial-mediated experiments. Through calculation of ppm shifts between product and starting material this experiment yielded a 8% conversion to product.

Figure A9

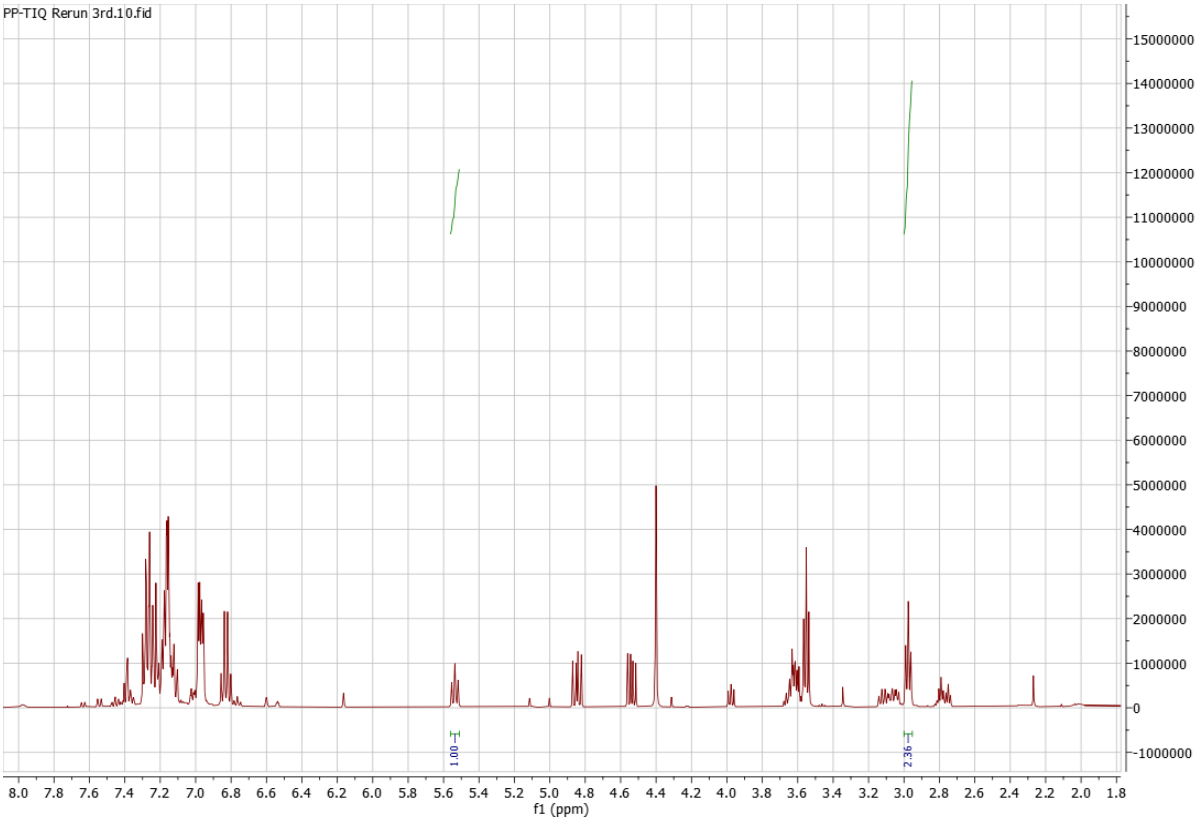
Second Recycling Bacterial Run



Note. Proton NMR of the 2nd recycling run, following (SI. N8), of a bacterial-mediated experiment. Through calculation of ppm shifts between product and starting material this experiment yielded a 2% conversion to product. Totaling up to a 10% conversion.

Figure A10

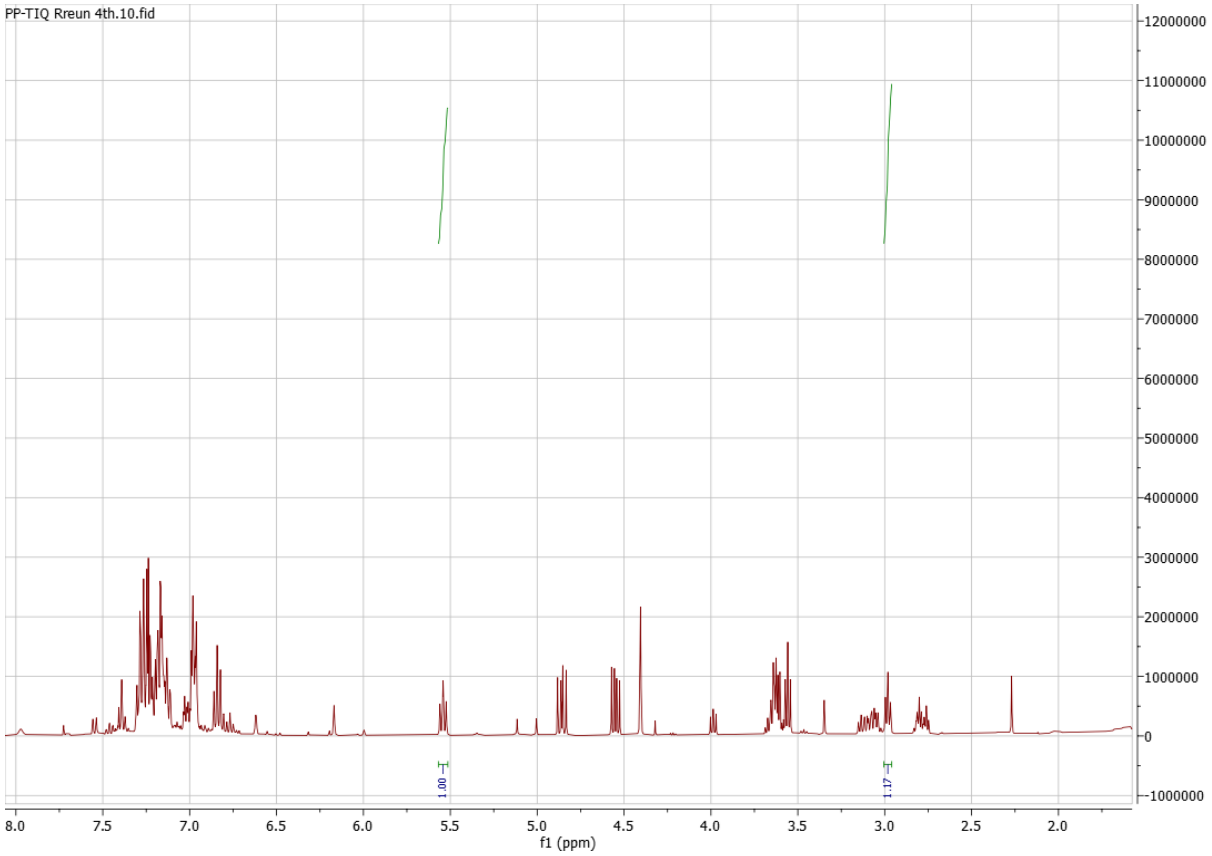
Third Recycling Bacterial Run



Note. Proton NMR of the 3rd recycling run, following (SI. N9), of a bacterial-mediated experiment. Through calculation of ppm shifts between product and starting material this experiment yielded a 11% conversion to product. Totaling up to a 21% conversion.

Figure A11

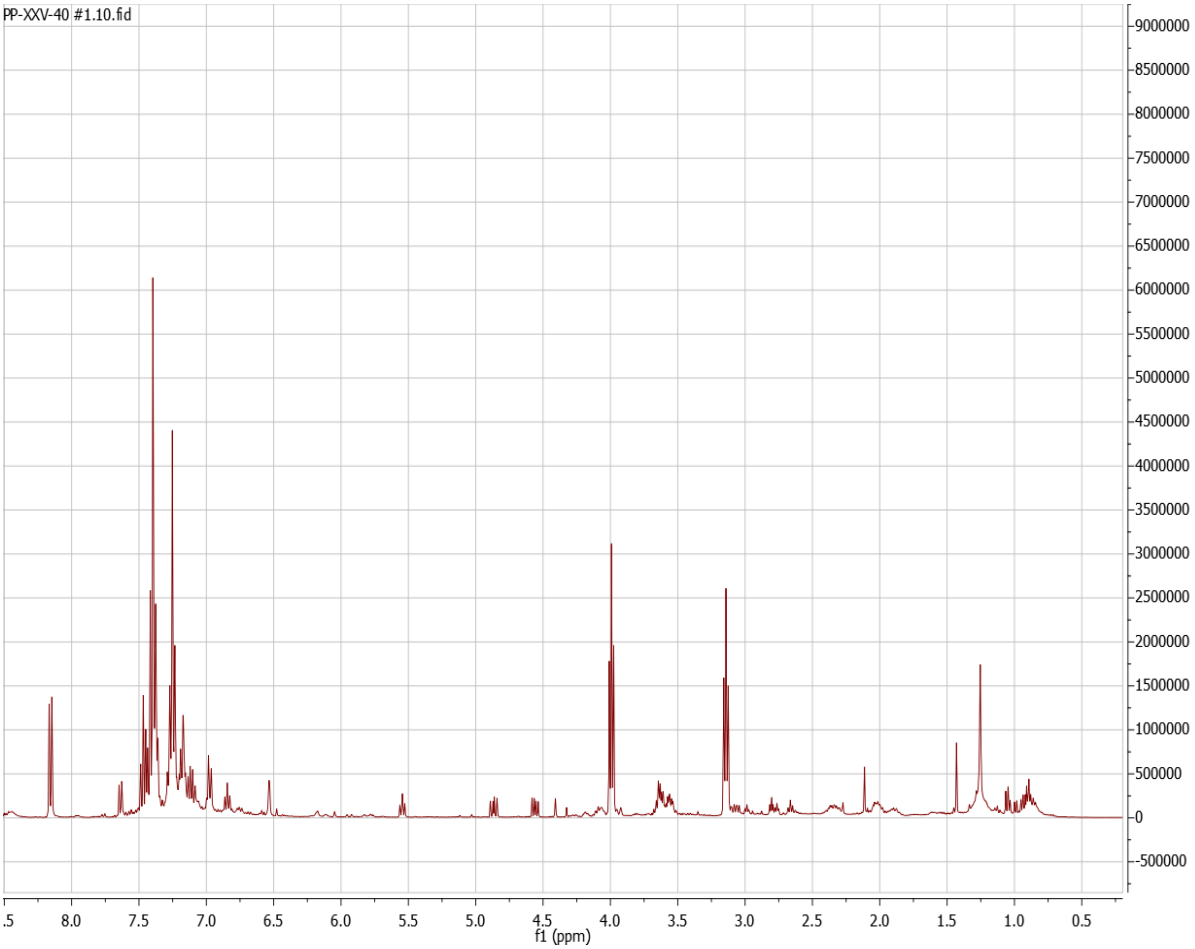
Fourth Recycling Bacterial Run



Note. Proton NMR of the 4th recycling run, following (SI. N10), of a bacterial-mediated experiment. Through calculation of ppm shifts between product and starting material this experiment yielded a 22% conversion to product. Totaling up to a 43% conversion

Figure A12

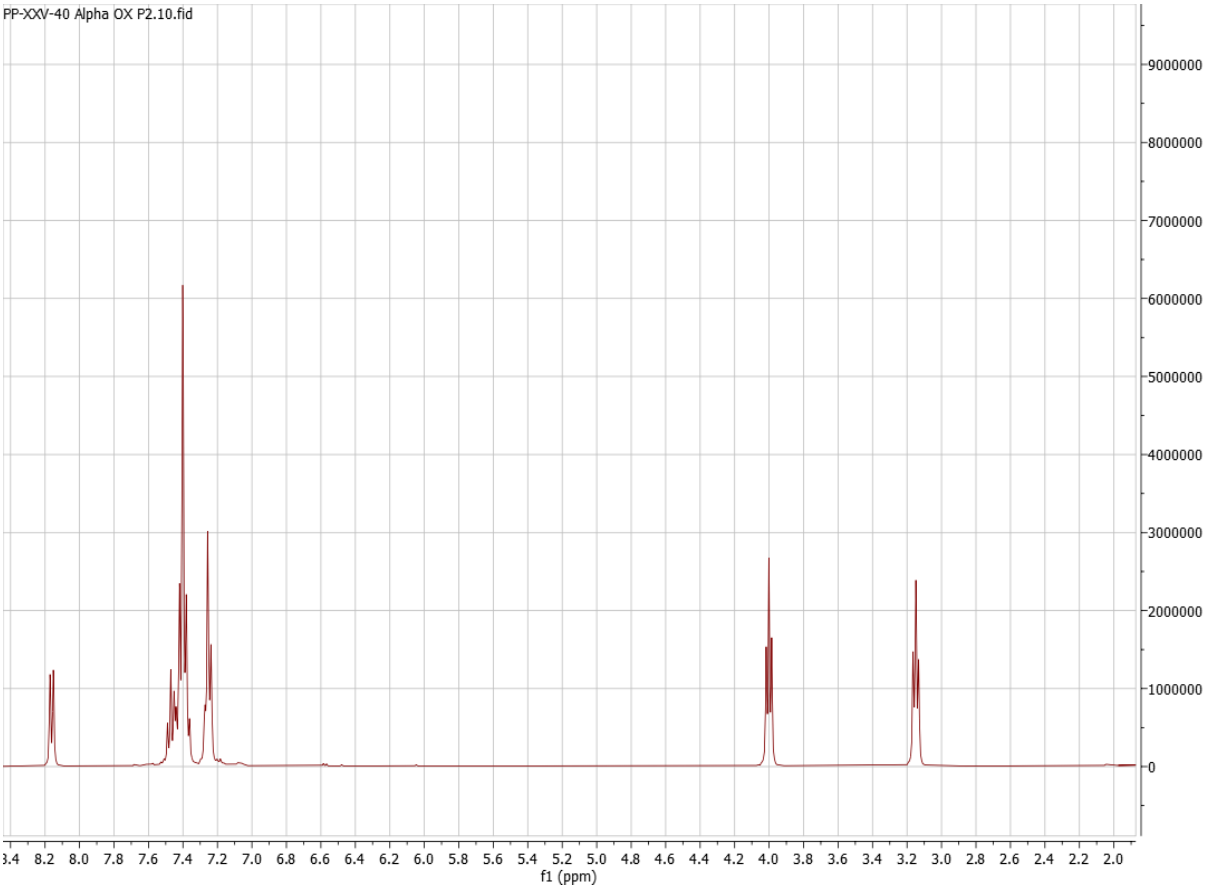
.Bacterial-Mediated Synthesis of 2-phenyl-3,4-dihydroisoquinolin-1(2H)-one



Note. Crude proton NMR of the first bacterial-mediated photoredox experiment focused solely on the production of 2-phenyl-3,4-dihydroisoquinolin-1(2H)-one.

Figure A13

Pure NMR of Alpha Oxidation Product



Note. Proton NMR of pure alpha oxidation product from bacterial-mediated photoredox reaction. Note the triplet shift normally at 4.4ppm to signal the nitromethane addition is missing, confirming the new alpha redox product (2-phenyl-3,4-dihydroisoquinolin-1(2H)-one).

Figure A14

Carbon of Pure Alpha Oxidation Product

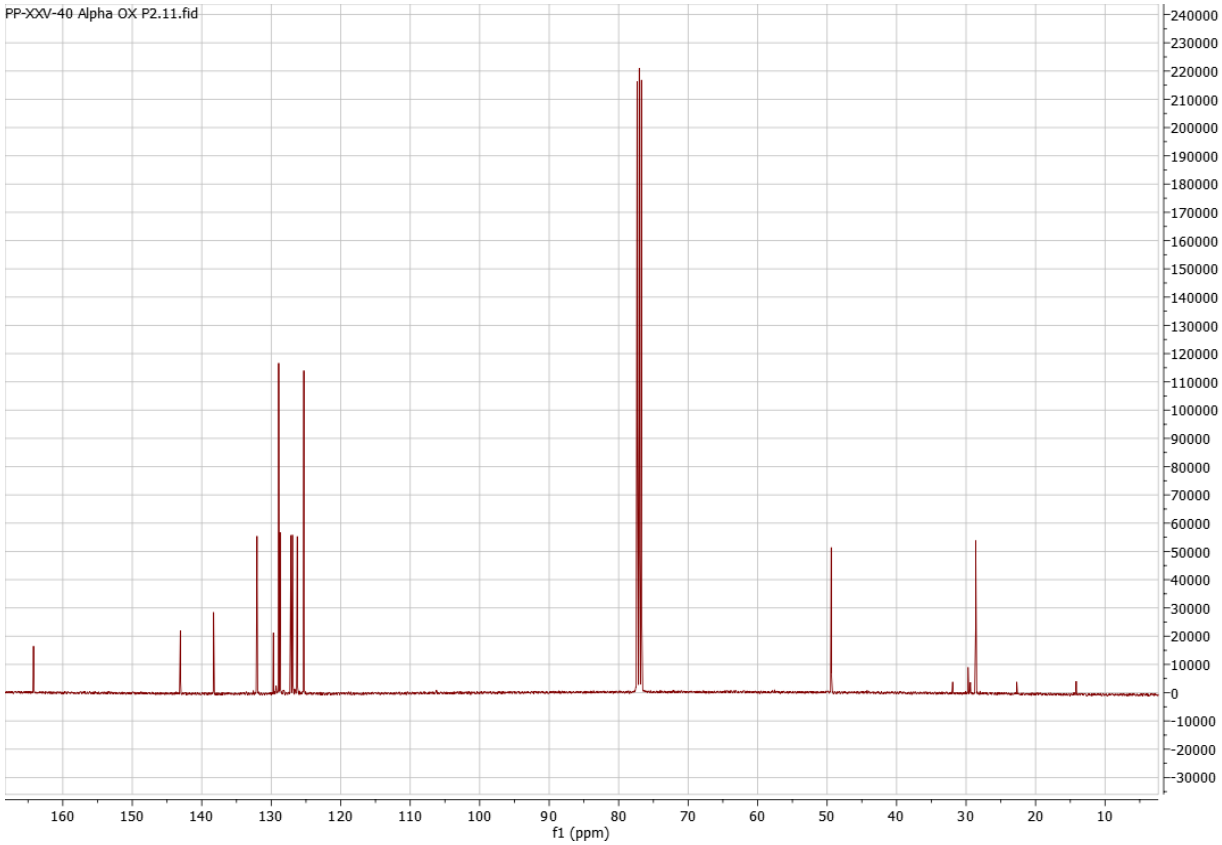
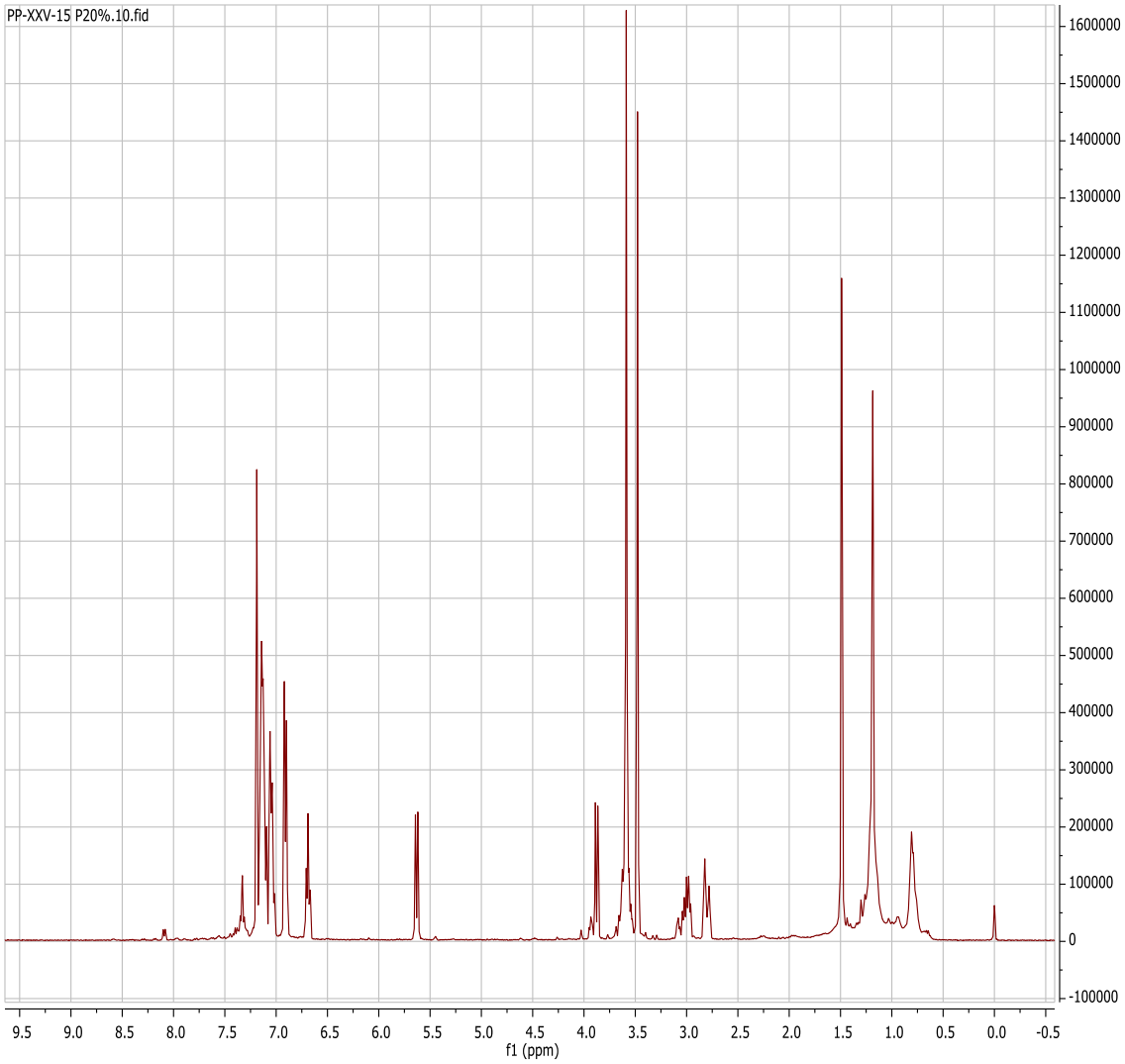


Figure A15

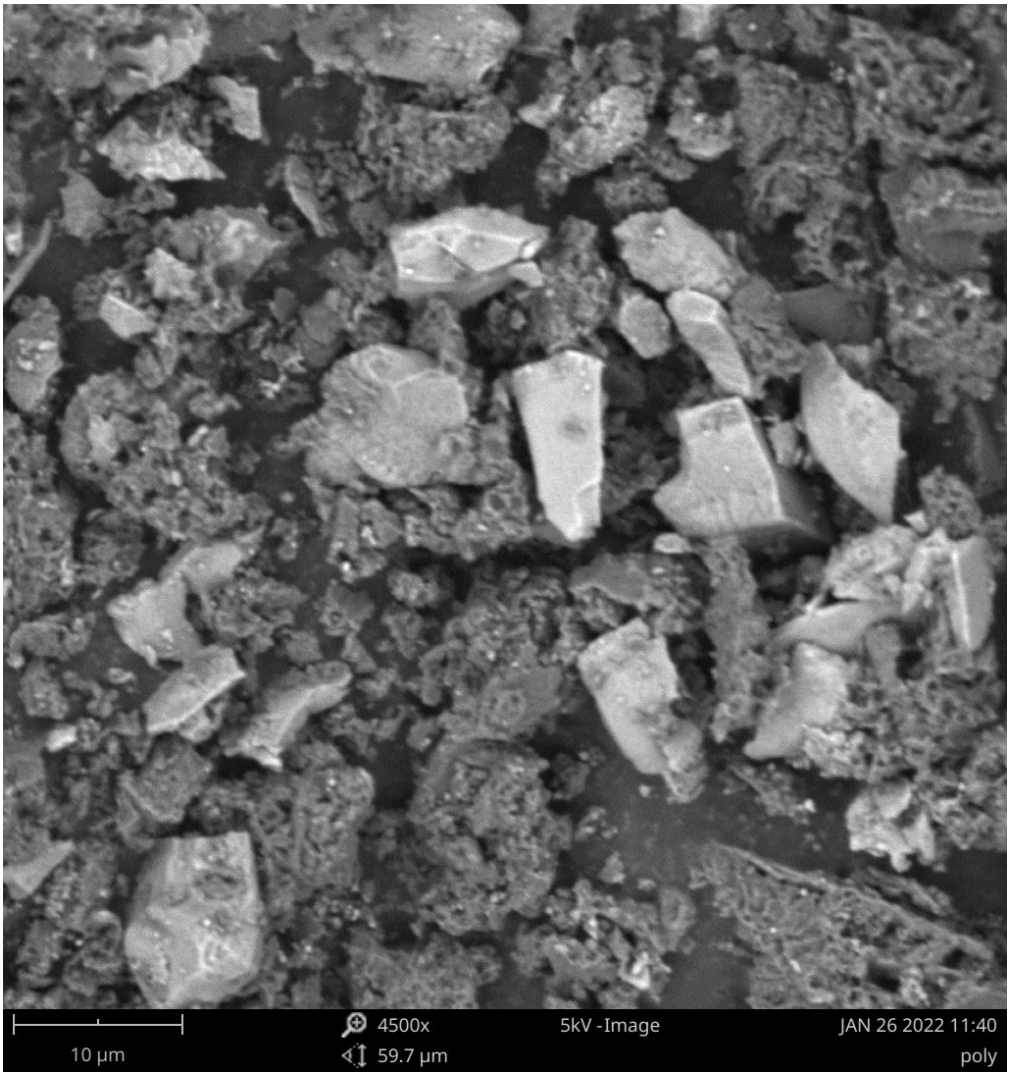
Synthesis of Malonate Product via Bacterial Photocatalysis



Note. Crude proton NMR of the bacterial-mediated photoredox experiment focused on the production of dimethyl-2-(2-phenyl-1,2,3,4-tetrahydroisoquinoline-1-yl)malonate

Figure A16

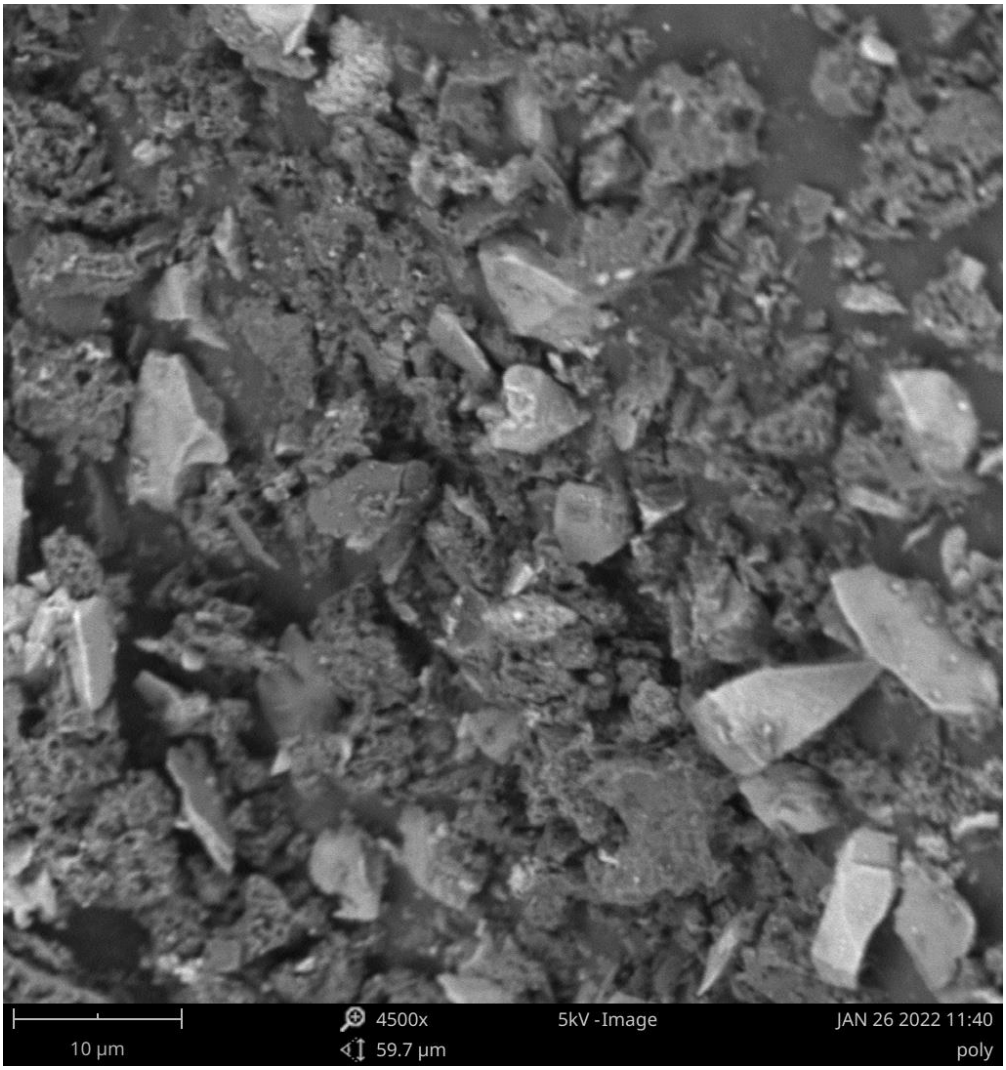
SEM of Graphitic Carbon Nitride



Note. Scanning electron microscope image of mesoporous graphitic carbon nitride. (Thank you to Matt Jackson, AMT, for helping provide the SEM data)

Figure A17

Second SEM of Graphitic Carbon Nitride



Note. Scanning electron microscope image of mesoporous graphitic carbon nitride.

(Thank you to Matt Jackson, AMT, for helping provide the SEM data)