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Total Synthesis of E Stereoisomers of Bilirubin Oxidation Products

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TOTAL SYNTHESIS OF *E* STEREOISOMERS OF BILIRUBIN OXIDATION PRODUCTS

A Thesis

by

TESS C. RUIZ

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ABSTRACT

Ruiz, Tess C., Total Synthesis of *E* Stereoisomers of Bilirubin Oxidation Products. Master of Science (MS), December, 2021, 28pp., 5 figures, 10 schemes, references, 19 titles.

The purpose of the research was to synthesize the *E* stereoisomer of Bilirubin Oxidation Products that had not been synthesized through a total organic synthesis at the start of this project. There were numerous different procedures for the bromination of citraconic anhydride performed in an attempt to optimize yield and purity. A proposed scheme for the total synthesis of the *E* isomers was constantly updated and altered while the project was ongoing. The bioactivity of the compounds was to be tested if the project had been finished. This might have contributed to a greater understanding of their function and association with subarachnoid hemorrhage induced vasospasm.

ACKNOWLEDGMENTS

I appreciate Dr. Shizue Mito, chair of my thesis committee, because she gave me the opportunity to do research in her laboratory under her guidance. Her mentorship has allowed me to become a better chemist, both in the classroom and the laboratory. Only through her knowledge, patience, and willingness to assist was this project possible. The information and words of encouragement she provided me throughout my education was invaluable and has been essential to the completion of my master's degree.

I would also like to thank my laboratory colleagues for helping me improve my understanding of organic chemistry techniques, theories, and concepts. This laboratory allowed me to develop the skillset of a scientist.

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CHAPTER I

INTRODUCTION

1.1 Subarachnoid hemorrhage induced cerebral vasospasm

Subarachnoid hemorrhage (SAH) is a medical condition in which there is bleeding in the subarachnoid space. The subarachnoid space is an area between the arachnoid membrane and the pia mater that surrounds the brain.[1] It accounts for 5% of strokes, but it has a mortality rate of approximately 40%.[2] A majority of deaths attributed to this condition occur within 30 days.[3] A predictor of an adverse outcome is cerebral vasospasm (CV), a secondary condition, where there is constriction of intracranial blood vessels.[2] It typically occurs with a delayed onset of 3 to 14 days, and it peaks approximately a week after the initial SAH.[4] Cerebral vasospasm is prevalent in SAH patients, with up to 70% being affected. After SAH events, around 30% of patients also go on to have delayed cerebral ischemia (DCI). Both conditions are proposed to develop through multifaceted disease processes. These remain as challenging aspects of SAH, and they are largely responsible for its morbidity and mortality. Severe complications, such as neurological deficit, are present in 20-40% of SAH patients. [2]

1.2 SAH induced cerebral vasospasm treatment

The primary goal of treatment for SAH induced cerebral vasospasm and DCI is prevention because of the poor prognosis in patients who develop these conditions.[5] There are a wide array of medications that have been implemented in treatment of cerebral vasospasm, but more research needs to be conducted to prove their efficacy. A drug called nimodipine, shown in Figure 1, is the primary treatment currently used. Nimodipine has been shown to improve the outcome of patients through decreases in mortality and reduced incidences of DCI. However, there is no convincing evidence that it decreases the incidence of CV.[6]

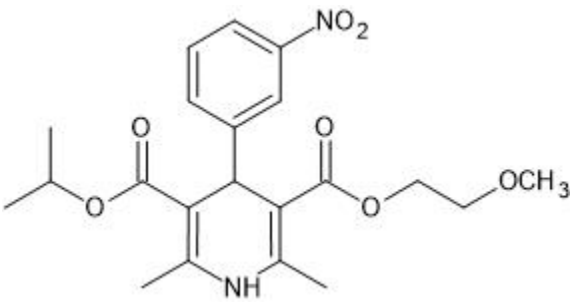


Figure 1: Structure of Nimodipine

Overall, there is a lack of effective treatment options, and there is no consensus on diagnostics, procedures, and medical management, despite recent advancements.[6] Research and clinical trials are ongoing to find pharmacological interventions and novel targets for treatment. Further understanding of the mechanisms of cerebral vasospasm and DCI are still needed before conventional new treatments are likely to be developed. If treatment is developed to target multiple aspects of these conditions, it may improve prevention and long-term outcomes.[5]

1.3 Bilirubin Oxidation Products

Bilirubin oxidation products (BOXes), shown in Figure 2, are compounds that are purported to be associated with subarachnoid hemorrhage (SAH) induced cerebral vasospasm in addition to other substances such as inhibitors of nitric oxide and vasoconstrictors. BOXes have been found in the cerebral spinal fluid (CSF) in patients that experience SAH, and some are capable of evoking vasoconstriction.[7]

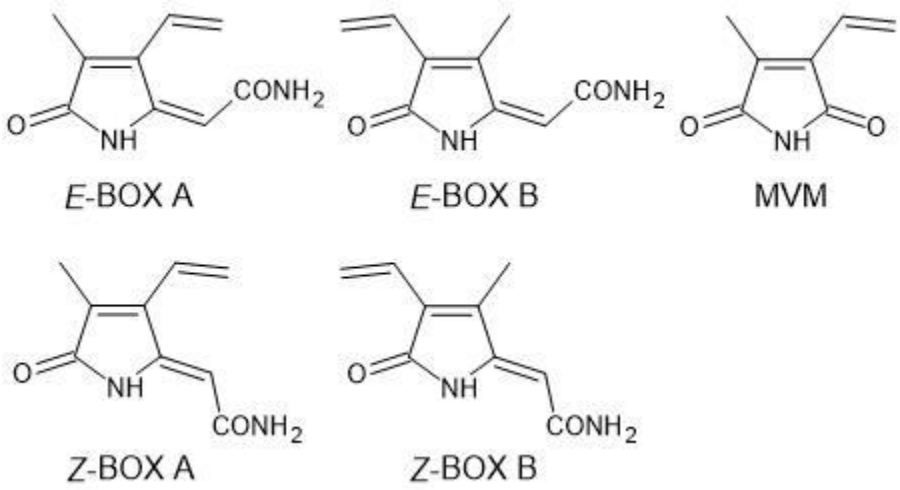


Figure 2: Bilirubin Oxidation Products and MVM

A proposed pathway for the formation of BOXes, shown in Figure 3, that they are produced when blood accumulates in CSF after SAH, and this leads to the formation of blood clots. In these blood clots, red blood cells undergoing lysis release heme. The enzymes heme oxygenase 1 and 2 (HO-1, HO-2) act on heme to form biliverdin, which is subsequently reduced by biliverdin reductase to form bilirubin. Bilirubin, biliverdin, and heme are oxidized by oxygen free radicals to form BOXes, among other products, such as MVM. This generally results in cerebral vasospasm, cellular damage, stress in neural tissue, and stress in vascular tissue.[7]

When examining the CSF from SAH patients with cerebral vasospasm, the CSF was found to have higher levels of bilirubin, BOXes, HO-1, and peroxidized lipids (an indicator of an oxidizing environment). Bilirubin production requires not only blood and HO-1; it also requires CSF. In SAH patients, bilirubin concentration increases in CSF over time because SAH blood remains in the CSF for a longer time. The chemical environment required to produce BOXes is present in the CSF of SAH patients with cerebral vasospasm. The HO-1 enzyme is inducible by hemorrhagic means, thus this isozyme is likely responsible for the conversion of heme to biliverdin after an incidence of SAH. A strong oxidizing environment for the production of BOXes may be present in cells such as macrophages containing peroxidizing vesicles. This may contribute to the difficulty in treating cerebral vasospasm. The delay in onset of cerebral vasospasm is believed to be a result of the time it takes for HO-1 to be induced. The rise of bilirubin concentrations and the oxidative stress conditions for BOXes to form are also likely related to the onset delay.[8]

The role of BOXes in the body is not well understood. Therefore, further research is necessary to elucidate more about their functions, formation, and mechanism of action. A better overview of BOXes may help develop treatment for those affected with SAH-induced cerebral vasospasm to improve quality of life and lower mortality rate.[7]

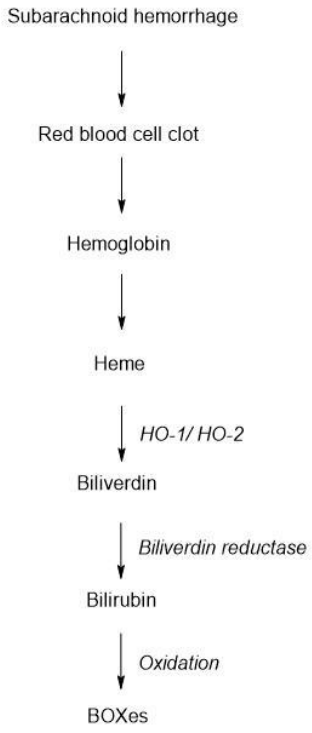
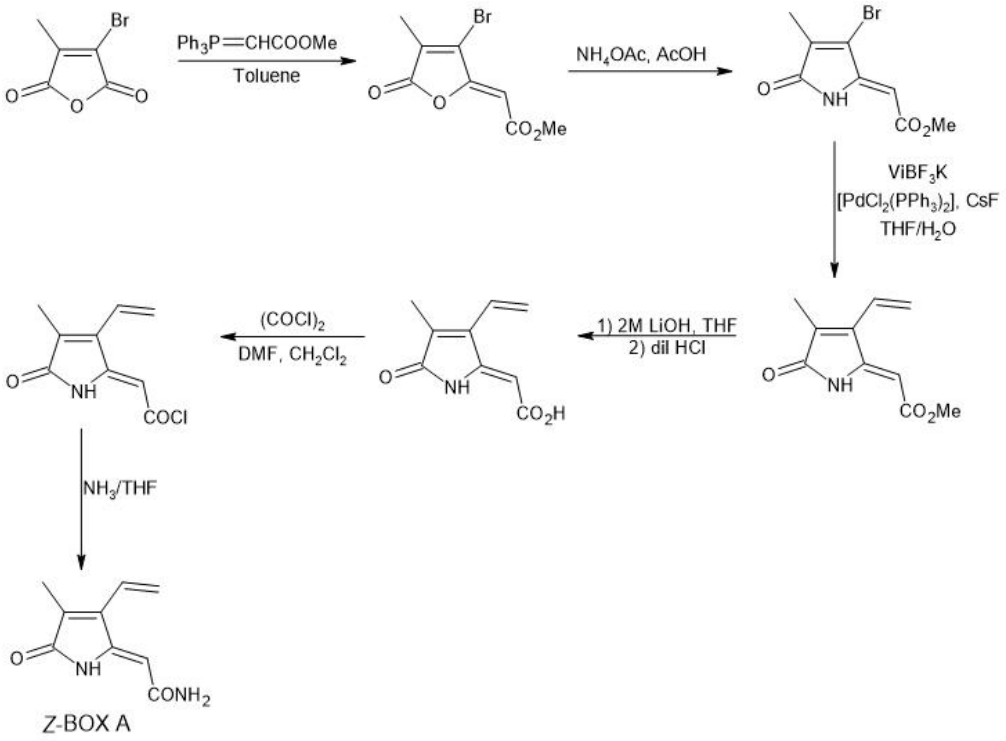


Figure 3: Formation of BOXes in the body

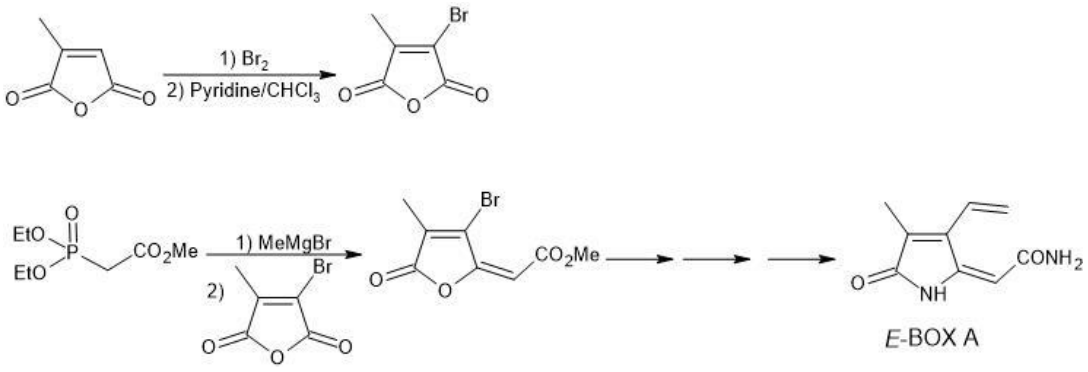
1.4 Production of BOXes

Bilirubin oxidation products are compounds of interest in this project because it has been a challenge to synthesize these molecules with high purity in sufficient quantities. The synthesis of exogenous BOXes began by lyophilization and oxidation of bilirubin with hydrogen peroxide, but the quantity was not consistent and contamination was evident.[7] At the start of this research project only the *Z* stereoisomers of BOX A and BOX B had been produced through total synthesis, shown in Scheme 1. The aim of this research was to develop a pathway for total synthesis of the *E* stereoisomer. The total synthesis of BOXes was part of a collaboration to access their function in lysosomal ion channels and transporters. While this project was ongoing, research regarding the synthesis of BOXes progressed. Now both *E* and *Z* stereoisomers of BOX A and BOX B can be made through total synthesis.[7]



Scheme 1: Synthetic pathway for *Z*-BOX A

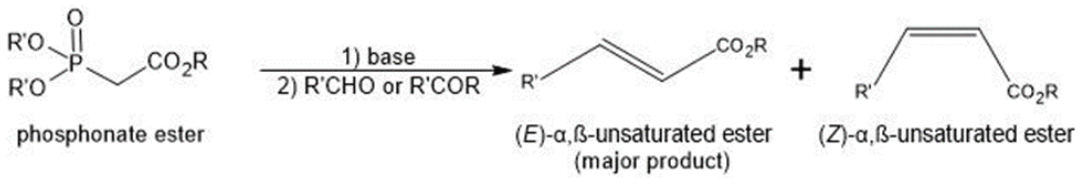
This project proposed the synthesis of the *E*-stereoisomers of BOXes, shown in Scheme 2, by utilizing a highly *E* selective Wadsworth-Emmons reaction on bromocitraconic anhydride in hopes of obtaining the desired stereochemistry. This is a novel use for this reaction because this experiment is performed on aldehydes, and to some extent ketones, but bromocitraconic anhydride is a cyclic anhydride. The rest of the synthetic pathway was adapted from the publication of the total synthesis of the *Z*-stereoisomer.[9] The bromination reaction for citraconic anhydride was obtained from literature to produce bromocitraconic anhydride as a starting material for the Wadsworth-Emmons reaction.



Scheme 2: Proposed synthetic pathway for *E*-BOX A

1.5 Wadsworth-Emmons Reaction

The Wadsworth-Emmons reaction, shown in Scheme 3, is a variation of the Wittig reaction. It utilizes a resonance stabilized phosphonate ester carbanion opposed to a Wittig reagent to react with an aldehyde or ketone to produce a mixture of *E*- and *Z*- α,ß-unsaturated ester that is selective for the *E* stereoisomer. When methylmagnesium bromide is utilized in base promotion, the reaction becomes a highly *E*-Selective Wadsworth-Emmons reaction. A variety of aldehydes reactants can be utilized in this highly selective reaction to obtain the corresponding *E*-α,ß-unsaturated ester with ratios of *E*:*Z* diastereoselectivity reaching up to 180:1 for some reactions with yields exceeding 88%. There are other Wadsworth-Emmons reactions that have high selectivity of the *E* stereoisomer. This includes those performed under Masamune-Roush conditions which utilizes lithium or magnesium halides and a tertiary amine base, but it results in low product yield. Therefore, it is preferable to use methylmagnesium bromide in olefination because it exhibits both a higher diastereoselectivity for the *E*-α,ß-unsaturated ester and there are higher yields of the product.[10]



Scheme 3: General Wadsworth-Emmons Reaction

CHAPTER II

REVIEW OF LITERATURE

2.1 Synthesis of Bilirubin Oxidation Products

Allan et al. reported a synthesis of bromocitraconic anhydride through a bromination reaction that did not utilize a catalyst or heating.[11] The reaction also differed from other bromination reactions for citraconic anhydride because it employed pyridine and chloroform. The intermediate was a mixture of trans- and cis- 2,3-dibromo-2-methyl succinic anhydride.[11] All other procedures obtained from literature and attempted for this reaction utilized aluminum bromide as a catalyst. These procedures employed heating to 120 °C for at least 12 hours, with one up to 16 hours. Most of the variations among these reactions included washing the reaction mixture with different solutions.[12, 13, 14] However, in the procedure by Nardi et al., recrystallization was performed in place of washing the organic layer.[14] In each of these articles where bromocitraconic anhydride was synthesized, the product was sufficiently pure to be used in the next reaction.[11, 12, 13, 14] When the reaction was performed for this project, these reactions did not result in a product that could be confirmed as bromocitraconic anhydride ^{1}H NMR. An adaption to Allan et al. procedure, resulted in the successful production of bromocitraconic anhydride.

The Highly *E* Selective Wadsworth-Emmons reaction by Claridge et al. performed the experiment on a range of straight-chain and branched aliphatic, substituted aromatic, and

base-sensitive aldehydes. They employed the use of various alkyl diethylphosphonoacetates and methylmagnesium bromide. A typical Wadsworth-Emmons reaction can also be conducted on ketones, but they tend to have lower stereoselectivity. This article did not implement ketone or various other functional groups such as anhydrides. The base for this particular Wadworth-Emmons reaction was a Grignard reagent methylmagnesium bromide.[10] This particular procedure was incorporated into the proposed pathway for the synthesis of BOXes in an effort to obtain E stereochemistry as the major product.

Klopfleisch et al. illustrates the first synthetic pathway for BOXes, although only the *Z* stereoisomers. In the first reaction of the pathway, bromocitraconic anhydride undergoes a Wittig reaction, and the product has *Z* stereochemistry. This remains consistent throughout the pathway.[9] The proposed pathway for this project adapted the pathway for this *Z* stereoisomer by trying to implement *E* stereochemistry into the BOX in the first reaction through a Wadsworth-Emmons reaction.[10] A procedure from Pérez-Venegas et al. detailing the synthesis of the the Wittig reagent found in the Klopfleishch et al. pathway was utilized in an attempt to carry out the synthesis of a *Z*-BOX.[9,15] The reasoning for conducting this reaction was to become familiar with the reactions shared with the proposed pathway for *E* -BOX A synthesis and to possibly devise alternative routes for synthesis of the *E*- stereoisomer at various steps.

2.2 Characterization of BOXes

Studies on BOXes have helped to characterize their chemical and biochemical properties. It has been established that BOXes photodegrade with a half-life of up to 10 hours, depending on the specific conditions. This may contribute to the reason they have been difficult to study, as they have characteristic instability in ambient light.[16] The UV light absorption profile, λ_{max}, and

the extinction coefficient for BOXes have also been examined utilizing various solvents.[17] Intermediates in the production of BOXes from bilirubin have been isolated, identified, and it is possible that other compounds exist in the pathway. These intermediates are also more prevalent in bile than BOXes, which calls for an assessment their presence and biological activity in various parts of the body.[18] Interestingly, although BOXes photodegrade as reported by Wurster et al., they can undergo *Z* to *E* photoconversion.[16, 19] They reisomerize to the *Z*-configuration over time because it is more thermodynamically favorable. It should also be noted that is not a complete photoconversion, thus *Z*-stereoismers are still present, but they can undergo purification to isolate the *E*-stereoisomers.[19] In addition, intermediates, such as those discovered by Ritter et al., can also undergo photoisomerization.[18,19] It is hypothesized that the photoconversion acts as a detoxification mechanism to prevent and treat DCI, but it requires further investigation .[19] Overall, a better comprehension of the chemical profile of BOXes would allow for greater understanding of their role in SAH, their presence in CSF, and other functions they may have in the body .

2.3 Progress in SAH, CV, and DCI

In Kiser et al., Triple-H therapy (hypertension, hypervolemia, and hemodilution) was a widely accepted treatment for CV and DCI, though variation of implementation was seen among institutions.[4] It is no longer recommended because there is no substantial evidence that this therapy led to improvement in SAH patient outcome. In addition to being an ineffective treatment option, this therapy has significant risks that include cardiac failure, cerebral edema, and electrolyte abnormalities.[5] Kiser et al. and Daou et al. articles were published six years apart, and this emphasizes the evolving treatment of CV and DCI shifting toward a preference of nimodipine for prevention and management.[4,5]

The evolution of knowledge on the connection between CV and DCI is illustrated in Li et al.[6] It emphasizes that there is strong correlation between the two conditions, but it may be imperfect. The theory is supported by some procedural studies, but this theory is called into question by CV and good long-term outcomes coinciding together. It also highlights that there are various other theories that try to define the relationship between these two conditions. It should also be noted that arterial vessel narrowing is not necessary or always sufficient to cause DCI. This shows moving towards a multifaceted understanding of the disease process behind both CV and DCI.[6]

A report by Chan et al. examined the admissions and mortality rates for subarachnoid hemorrhages from 2004 to 2015. The study found that both are on the decline, though mortality rates still remain high. [3] It is possible that this could be attributed to medical advancements in treatment, management, and diagnostics in SAH, CV, and DCI. As understanding of these conditions expands, it may open the possibility of new therapeutic options. It is anticipated that it will continue to decline.

CHAPTER III

EXPERIMENTAL PROCEDURES

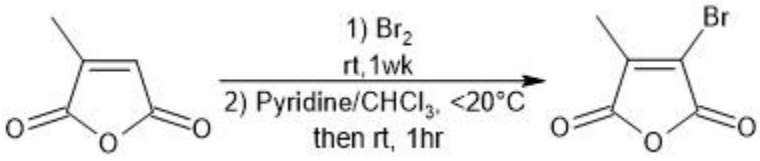
3.1 Materials

All materials are available from common commercial sources and were purchased from Sigma/Aldrich Chemical Company.

3.1.1 Nuclear Magnetic Resonance

NMR analysis was performed using a Bruker Avance 3 600 MHz

3.1.2 Synthesis of Bromocitraconic anhydride



Scheme 4: Bromination utilizing pyridine and chloroform rt

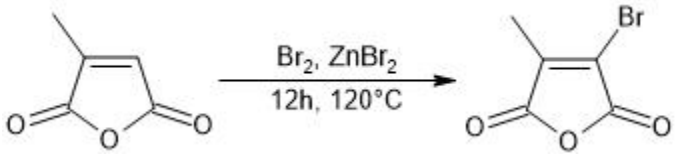
3.1.3 Reaction 1

A dry 50mL round-bottom flask equipped with a magnetic stir bar, citraconic acid anhydride (4.01 mL, 44.6 mmol), and bromine (2.51 mL, 49.06 mmol) was closed with a stopper then left to stir for 1 week at room temperature. After a week, excess bromine was removed by bubbling nitrogen through the reaction mixture followed by evaporation under reduced pressure. The crude product was dissolved in chloroform (22.3 mL) then cooled in an ice bath while

pyridine (3.61 mL, 44.6 mmol) was added dropwise over a period of 40 minutes. The temperature was maintained below 20 °C throughout this time. After the addition was complete, the mixture was allowed to stir at room temperature for an hour. The mixture was then washed with water 3 times and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure then dried in vacuo. The purity of the product was assessed through ^1H NMR.[11]

3.1.4 Reaction 2

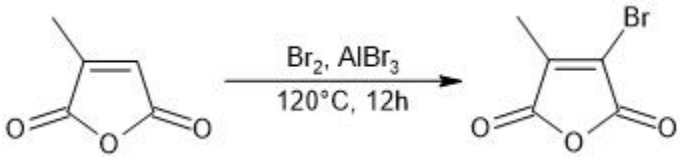
A dry 50mL round-bottom flask equipped with a magnetic stir bar, citraconic acid anhydride (4.01 mL, 44.6 mmol), and bromine (2.51 mL, 49.06 mmol) was closed with a stopper then left to stir for 1 week at room temperature. After a week, the mixture was washed with thiosulfate 3 times and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The crude product was dissolved in chloroform (22.3 mL), then cooled in an ice bath while pyridine (3.61 mL, 44.6 mmol) was added dropwise over a period of 40 minutes. The temperature was maintained below 20 °C throughout the addition process. After the addition was complete, the mixture was allowed to stir at room temperature for an hour. The mixture was then washed with water 3 times and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure then dried in vacuo. The purity of the product was assessed through ^1H NMR.[11]



Scheme 5: Bromination utilizing heat and zinc bromide 12h

3.1.5 Reaction 3

The zinc bromide catalyst was synthesized by combining zinc and bromine. A dry, pressurized, and sealed vial was equipped with a magnetic stir bar, citraconic acid anhydride (0.8 mL, 8.92 mmol), bromine (0.46 mL, 8.92 mmol), and zinc bromide (22.07 mg, 0.098 mmol). It was left to stir for 12 hours at 120 °C. After 12 hours, the mixture was cooled to room temperature then diluted with ethyl acetate. The mixture was filtered through celite, then it was washed twice with water and once with brine. It was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. It was then dried in vacuo. The purity of the product was assessed through ^1H NMR.[12]



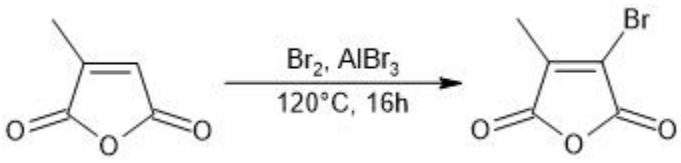
Scheme 6: Bromination utilizing heat and aluminum bromide 12h

3.1.6 Reaction 4

A dry, pressurized, and sealed vial was equipped with a magnetic stir bar, citraconic acid anhydride (0.8 mL, 8.92 mmol), bromine (0.46 mL, 8.92 mmol), and aluminum bromide (27 mg, 0.098 mmol). It was left to stir for 12 hours at 120 °C. After 12 hours, the mixture was cooled to room temperature, then diluted with ethyl acetate. The mixture was filtered through celite, then it was washed twice with water and once with brine. It was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. It was then dried in vacuo. The purity of the product was assessed through ^1H NMR.[12]

3.1.7 Reaction 5

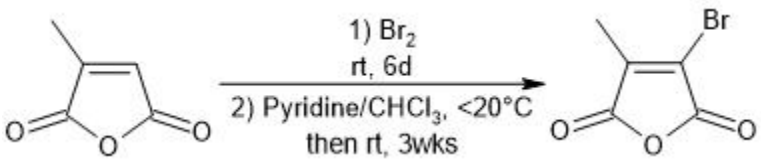
A dry, pressurized, and sealed vial was equipped with a magnetic stir bar, citraconic acid anhydride (0.8 mL, 8.92 mmol), bromine (0.46 mL, 8.92 mmol), and aluminum bromide (26 mg, 0.094 mmol). It was left to stir for 12 hours at 120 °C. After 12 hours, the mixture was cooled to room temperature then diluted with ethyl acetate. The mixture was washed with HCl 0.1% and brine. It was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. It was then dried in vacuo. The purity of the product was assessed through ^{1}H NMR.[13]



Scheme 7: Bromination utilizing heat and aluminum bromide 16h

3.1.8 Reaction 6

A dry, pressurized, and sealed vial was equipped with a magnetic stir bar, citraconic acid anhydride (0.8 mL, 8.92 mmol), bromine (0.46 mL, 8.92 mmol), and aluminum bromide (29 mg, 0.107 mmol). It was left to stir for 16 hours at 120 °C under nitrogen atmosphere. After 16 hours, the mixture was cooled to 0 °C. The solids were removed from the vial through recrystallization using toluene/heptane. The solids were washed with heptane and dried in vacuo. The purity of the product was assessed through ^{1}H NMR.[14]

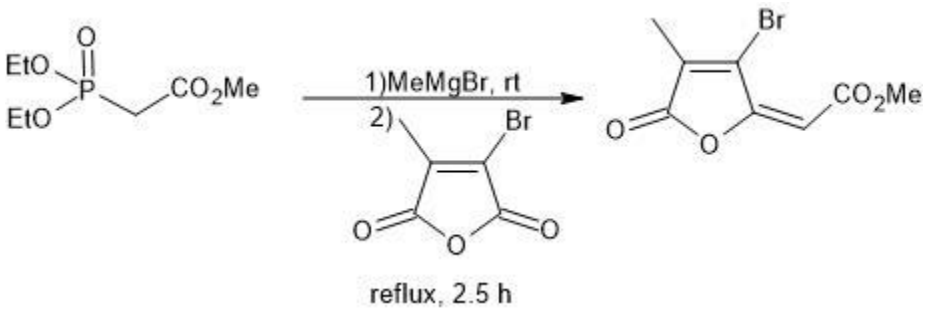


Scheme 8: Bromination at room temperature and no catalyst

3.1.9 Reaction 7

A dry, pressurized, and sealed vial was equipped with a magnetic stir bar, citraconic acid anhydride (8.97 mL, 100 mmol), and bromine (5.69 mL, 110 mmol). It was then left to stir for 6 days at room temperature. The crude product was dissolved in chloroform (50 mL) then cooled in an ice bath while pyridine (8.09 mL, 100 mmol) was added dropwise over a period of 40 minutes. The temperature was maintained below 20 °C throughout this time. After the addition was complete, the reaction mixture was transferred to an Erlenmeyer flask and left under the fume hood for 3 weeks. The purity of the product was assessed through ^{1}H NMR.

3.1.10 Synthesis of E alkene



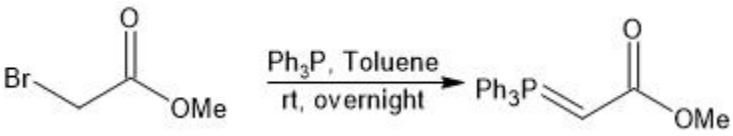
Scheme 9: Highly *E*-Selective Wadsworth-Emmons utilizing citraconic anhydride

3.1.11 Reaction 8

A dry, nitrogen-flushed 50 mL, two-neck, round-bottom flask equipped with a magnetic stir bar was charged with methyl diethylphosphonoacetate (0.14mL, 0.79 mmol) and anhydrous THF (10 mL). At room temperature under nitrogen atmosphere, methylmagnesium bromide (0.79 mmol) was added dropwise to the stirring mixture. After 15 minutes of stirring, the product from the bromination of citraconic anhydride reaction (97mg) was added. The reaction was heated to a reflux for 2.5 hours. Then, it was cooled to room temperature and quenched with

saturated aqueous NH_4Cl (4 mL). The mixture was then extracted with Et_2O three times, and the organic layer was washed with brine. It was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure before being dried in vacuo. The purity of the product was assessed through ^1H NMR.[10]

3.1.12 Synthesis of methyl (triphenylphosphoranyldiene) acetate (TPA)



Scheme 10: Synthesis of TPA a Wittig reagent

3.1.13 Reaction 9

A dry 50 mL round-bottom flask was equipped with a magnetic stir bar, triphenylphosphine (2.62 g, 0.01 mmol), methyl bromoacetate (1.03 mL, 0.01 mmol), and toluene (33 mL). It was closed with a stopper then left to stir overnight. After stirring the reaction, the mixture was filtered. The collected solid was then dissolved in water (66 mL) and basified to a pH of 9 using KOH (2M). The pH was then monitored with litmus paper. It was then extracted twice with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. It was then dried in vacuo. The purity of the product was assessed through ^1H NMR.[15]

3.2 Discussion

3.2.1 Bromination

The synthesis of bromocitraconic anhydride was attempted through seven different procedures. Thin-layer chromatography was used to monitor progress of the reactions. The purity

of the product from each reaction was assessed through ^{1}H NMR, but the product could not be confirmed due to the presence of contaminants in reactions 1 through 6. The crude product obtained from reactions 1 through 5 underwent flash column chromatography. An issue occurred when the products obtained from the fractions were assessed with ^{1}H NMR. This issue was that the presence of bromocitraconic anhydride could not be confirmed. It is hypothesized that the bromocitraconic anhydride, if present, became bound to the silica. Furthermore, even when ethyl acetate was run through the column it could not be confirmed. The literature of many of these reactions stated that the product obtained was sufficiently pure to be used in the next reaction, but that was not found to be the case when these reactions were performed. The crude product obtained from reactions 6 and 7 did not undergo column chromatography as a means of purification. In reaction 6, recrystallization was utilized as a purification technique. The synthesis of bromocitraconic anhydride was confirmed from reaction 7, but purification was not performed.

In reaction 1, bubbling nitrogen through the reaction mixture followed by evaporation under reduced pressure was utilized to remove excess bromine. However, it was evident that bromine was still present due to the dark reddish brown color of the mixture. As a consequence, reaction 2 utilized washing with thiosulfate to remove excess bromine, but bromine still remained present. In reaction 3, zinc bromide was utilized as a catalyst and the reaction mixture was heated to 120 °C for 12 hours. This is in contrast to reactions 1 and 2 which did not implement a catalyst or heat. Reaction 4 differed from reaction 3 because the catalyst was changed from zinc bromide to aluminum bromide. This difference was because this was the catalyst utilized in the literature. Reaction 5 differed from reaction 4 because 0.1% HCl was used to wash the reaction mixture instead of water. However, reactions 1 through 3 also utilized water

to wash the reaction mixture in addition to other procedural differences. Reaction 6 utilized a longer heating time of 16 hours, a nitrogen atmosphere, cooling to 0 °C after heating, and recrystallization. Reaction 6 did not implement washing the reaction mixture as reactions 1 through 5 had done. Reaction 7 did not implement heat, a catalyst, or washing the reaction mixture.

Each reaction was performed multiple times with modifications in an attempt to optimize the reaction. The heating time was increased to 16 hours for the reactions with a standard heating time of 12 hours. The amount of bromine was increased because it was evident that the bromine was affecting the rubber ring on the pressurized sealed vial, which allowed it to escape. The amount of catalyst was increased to allow more contact with the reactants. Reactions were performed both under nitrogen atmosphere and without it to see if this would influence the outcome. The reactions were scaled down from 44.6 mmol to 8.92 mmol of citraconic acid anhydride to conserve resources until a reaction could be performed successfully on a larger scale.

3.2.2 Wadsworth-Emmons

In reaction 8, the synthesis of the *E* stereoisomer was attempted with the crude products from reactions 1 and 2 to see if the reaction could be successfully performed assuming that bromocitraconic anhydride was present in an impure form. When the ^{1}H NMR was used to assess the product of the reaction, the starting materials were still present. This indicated that the reaction was unsuccessful. This experiment was performed twice, but due to an error, THF instead of anhydrous THF was utilized the first time. The highly selective *E* Wadsworth-Emmons reaction is typically done with aldehydes and ketones so utilizing an anhydride was a novel use for this particular reaction. This experiment has not been performed on product

confirmed to be bromocitraconic anhydride. It may be possible to synthesize the *E* stereoisomers of BOXes with this reaction, but it may require additional steps to introduce an aldehyde or ketone on the molecule before conducting the reaction.

3.2.3 Wittig reagent

This reaction was performed to produce a Wittig reagent that would be utilized to conduct the first reaction in the scheme for synthesis of *Z* stereoisomer of BOX A. The reason for conducting this reaction was to proceed with this pathway and become familiar with reactions also in the synthesis of *E* stereoisomer of BOX A. This would also allow for possible modifications to the scheme to obtain an *E* stereoisomer at a different step in the pathway. The purity of the product was assessed through ^{1}H NMR but the product could not be confirmed due to the presence of contaminants.

3.3 Spectra

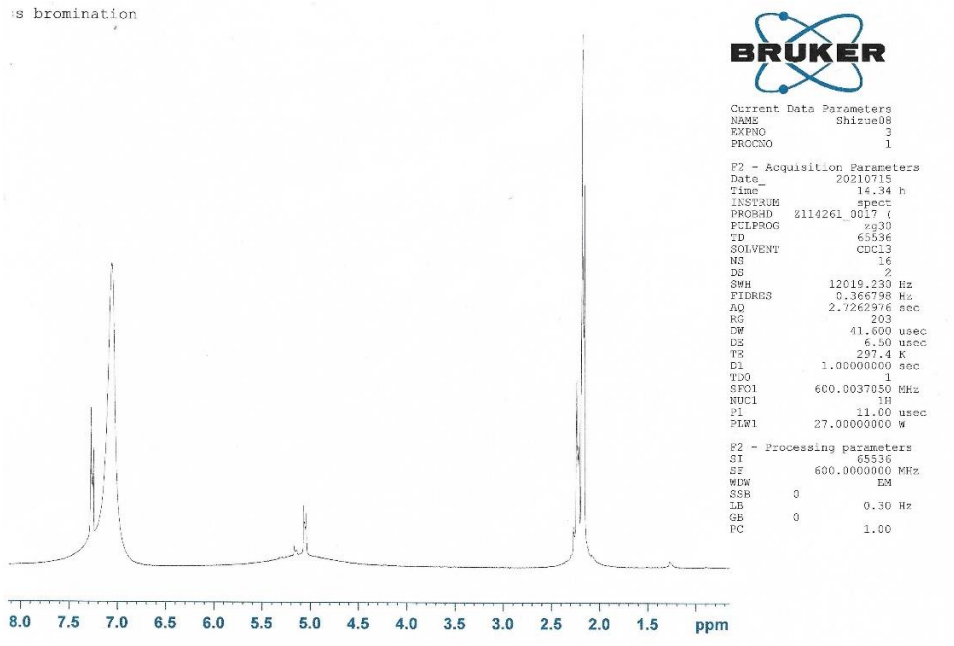


Figure 14: ^{1}H NMR for Intermediate of Bromocitraconic anhydride

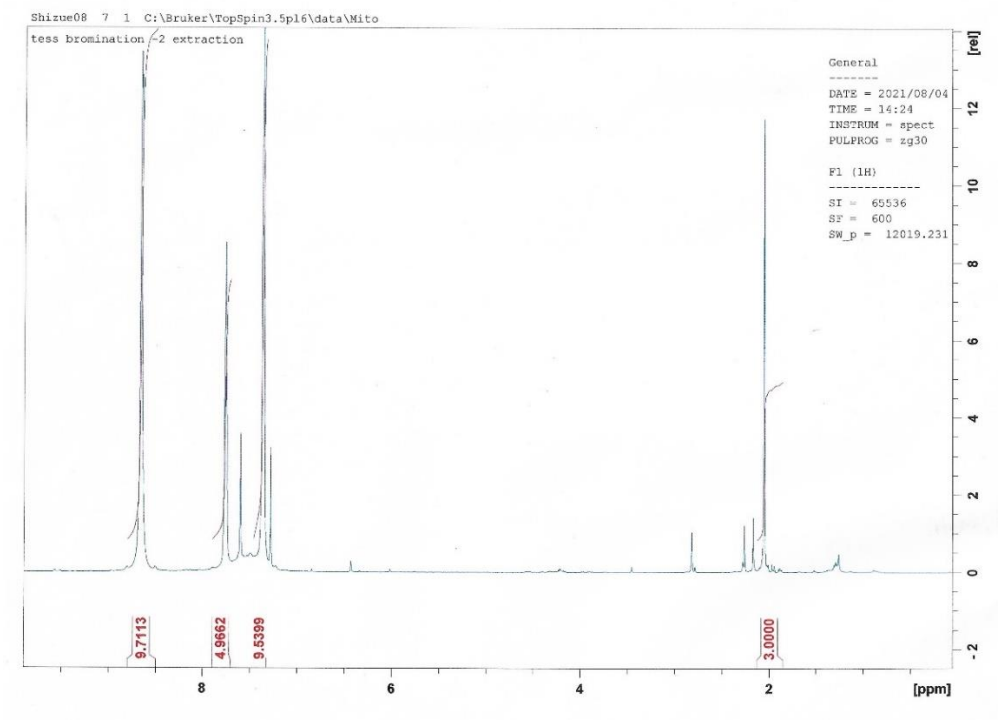


Figure 15: ^{1}H NMR for Bromocitraconic anhydride

3.3.1 Chemical Shift

Intermediate of Bromocitraconic anhydride ^{1}H NMR δ 5.03 (total 1 H, CHBr), and 2.19 (total 3 H, CH_3). Bromocitraconic anhydride is a solid ^{1}H NMR δ 2.17 (s, CH_3).[11]

CHAPTER IV

CONCLUSION

Bromocitraconic anhydride was successfully synthesized after numerous attempts of bromination, but the project will no longer be pursued due to existing publications illustrating a synthetic pathway for the *E* stereoisomers of BOX A and BOX B. It may be possible to synthesize the *E* stereoisomers of these BOXes with a Wadsworth-Emmons reaction, but further modifications to the synthetic pathway devised for this project may need to be implemented. The fact that both *E* and *Z* stereoisomers of BOXes can be produced through total synthesis paves the way for further research. This further research could improve understanding of their function, mechanisms, and targets. This could provide treatment options for SAH-induced cerebral vasospasm or other ailments with which BOXes may be associated.

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