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Natural Dibenz[*b,f*]oxepin Compound as a Potential Novel Antibacterial Agent: Progress Towards the Synthesis and Optimization of Empetroxepin A and B

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Abstract

The increase in multi-drug resistant strains of pathogenic bacteria has made the issue of bacterial resistance a global health concern. New classes of antibacterial drug compounds, able to work outside existing mechanisms of resistance, are needed to combat these infections. Natural product-based drug discovery is an effective method in the development of new classes of antibiotics due to the chemically unique structures characteristic of naturally occurring compounds. This study aims to develop a viable antibacterial drug using Empetroxepin A and B, novel dibenz[b,f]oxepin natural products, as the lead compounds. The natural products will be synthesized in seven steps from commercially available 3,4,5-trimethoxytoluene. To date, the first five steps have been completed successfully through a Wittig olefination of trimethylsilane-protected salicylaldehyde with the phosphonium salt generated from the toluene starting material. The desired phosphonium salt was synthesized through aromatic bromination and radical benzylic bromination of the starting material. High yields (68-99%) have been achieved on large scales for each of these steps. Hydrogenation of the alkene bridge formed by the Wittig olefination has also been completed though only low yields have been obtained to date. The remaining steps in the total synthesis include a copper oxide catalyzed etherification ring closure followed by selective deprotection of the methoxy substituents to give both Empetroxepin isomers A and B. Once the synthesis of the lead compounds has been completed, the synthetic route will be used to develop analogs for structure activity relationship studies to optimize the natural product's antibacterial activity.

1. Introduction

The discovery of penicillin in 1928 is widely considered to be one of the greatest scientific advancements of all time, revolutionizing modern medicine and sparking a worldwide era of antibiotic-focused drug development. This era, known as the "Golden Age" of antibacterial discovery began in 1940, with the approval of penicillin for use in humans, and continued through the mid-1960s.[1] During these two decades, the core structures of essentially all future antibacterial drugs were discovered from natural bioactive compounds. After 1962, however, the rapid rate of discovery of these novel classes of antibacterial compounds came to a halt as the approach to drug development shifted towards the optimization of existing compounds as the source of new drugs.[2]

The development of new antibiotics through modification of existing classes of antibacterial compounds allowed for the production of more potent drugs at a rapid and thus more profitable rate. These new drugs, while initially more effective, lacked unique structures and modes of action which allowed the targeted pathogenic strains of bacteria to easily develop resistance mechanisms.[1] Figure 1 shows the correlation between the rise of nosocomial infections of resistant pathogenic bacteria and the decrease in the number of novel antibiotics entering the market.[3] The increase in multi-resistance strains of pathogenic bacteria, some demonstrating resistance to every clinically available drug, has made the issue of bacterial resistance a global health concern.[4] To effectively fight the growing number of resistant

strains of bacteria, new antibacterial agents with chemically diverse structures and novel mechanisms of action are essential.[5]

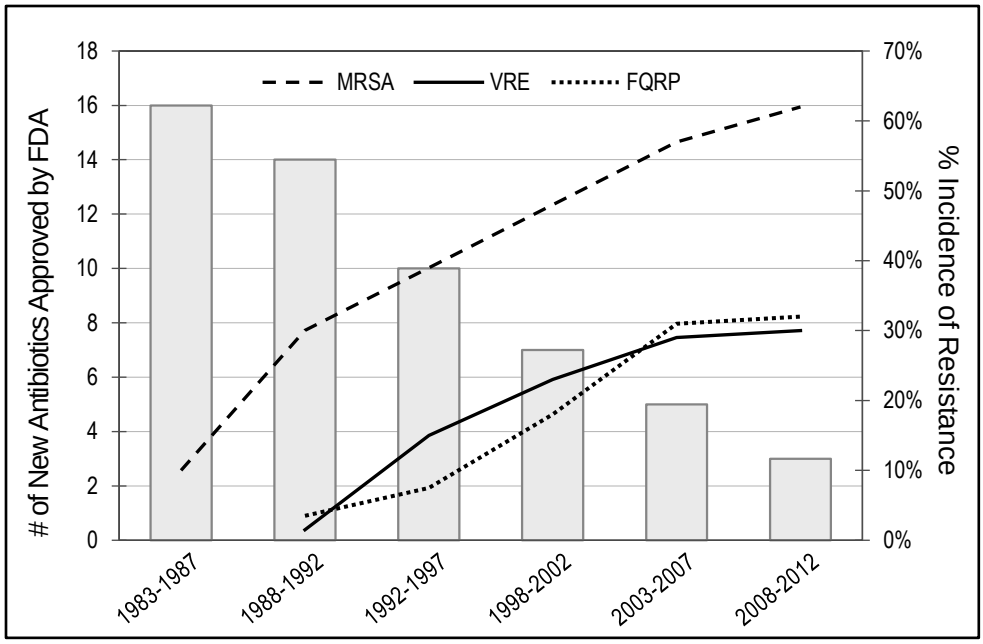


Figure 1: Trends in Antibiotic Development and Resistance.[3]

Two approaches exist for the development of novel antibacterial agents able to overcome the survival mechanisms used by resistant bacteria. The two approaches differ primarily through the use of either an existing synthetic antibiotic or a bioactive natural molecule as the lead compound (the structural starting point).[4] The use of existing synthetic compounds as drug leads is a faster and more cost-effective approach to drug discovery. This method, however, has a lower rate of success in developing novel agents due to the limited chemical diversity among existing antibiotic drug molecules.[2] Alternatively, natural antibacterial products, while more difficult and costly to use as lead compounds, have characteristically diverse and uncommon structures and are more likely to demonstrate a unique mechanism of action against pathogenic bacteria.[2] While the synthetic approach has dominated drug discovery for the last five decades, returning to the "Golden Age" practice of using natural products as lead drug compounds is the most promising method in the search for new classes of antibacterial agents needed to combat bacterial resistance.

The goal of this study is to develop a novel antibacterial agent from Empetroxepin (Figure 2), a recently isolated natural dibenze[*b,f*]oxepin compound, through synthesis, optimization, and biological evaluation, in an attempt to create a viable drug molecule effective against resistance strains of pathogenic bacteria.[2] Research in natural product-based drug discovery has become increasingly important with the rise of antibiotic resistance, yet this field remains relatively under-investigated.[1] This is primarily due to the fact that natural product-based drug development is not as economically favorable an approach as development via synthetic modification and thus lacks practicality for use by pharmaceutical companies. As a result, it is exceedingly important for this topic to be studied in the academic setting where research is not as restricted by the influence of economic gain.

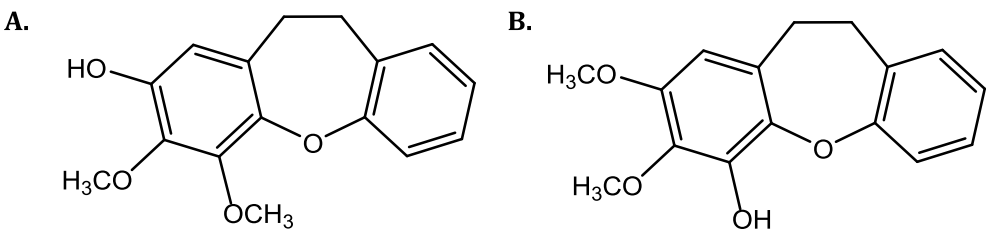


Figure 2. Structures A and B of Empetroxepin, a novel dibenze[b,f]oxepin compound.

2. Existing Research in Natural Product-Based Drug Development

Previous research in the field of natural product-based drug discovery is dominated by two focuses: development of antibacterial agents active against prominent multi-resistant bacteria and improving synthesis methods for structures common in bioactive natural products. The common multi-resistant bacteria under study in existing drug development research are methicillin-resistant and vancomycin-resistant pathogenic strains. Methicillin-resistant *Staphylococcus aureus*, commonly known as MRSA, is the primary focus of natural product antibiotic development. Vancomycin is the single antibiotic still active against MRSA and is therefore the only treatment available for infections caused by MRSA. The lack of diversity available for the treatment of MRSA infections increases the likelihood of *S. aureus* developing a resistance mechanism against vancomycin as well, leading to an untreatable strain of common pathogenic bacteria.[5]

Bottromycin (Figure 3), a naturally occurring protein synthesis inhibitor, has demonstrated significant activity against multi-resistant gram positive bacteria. A 2010 study carried out by Kobayashi et al., testing the antibacterial activity of various bottromycin analogs, revealed significant activity against MRSA with a minimum inhibitory concentration (MIC) of 1.0 µg/mL and vancomycin-resistant *Enterococci* (VRE) with an MIC of 0.5 µg/mL.[6] Analogs in which the methyl ester is replaced with a propyl ketone (Figure 3), increases stability of the compound in vivo while also increasing antibacterial activity to a level comparable to that of vancomycin.[6,7]

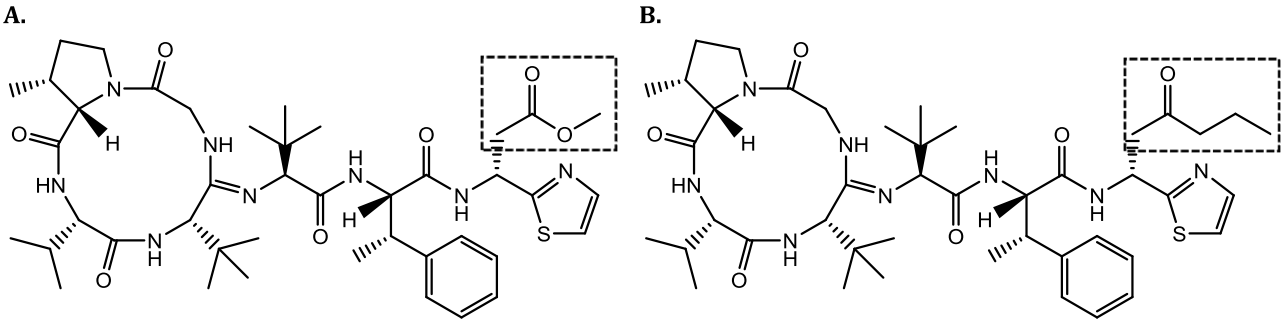


Figure 3: (A) Natural antibacterial agent Bottromycin A2 and (B) the more stable ketone analog.[7]

Pestalone is another naturally derived compound that demonstrates highly potent activity against both MRSA and VRE. In 2004, the total synthesis of pestalone was accomplished by Iijima et al., allowing for the compound's bioactivity to be tested. This study revealed MIC values of 37 ng/mL and 78 ng/mL for MRSA and VRE respectively.[8] Primary efforts following this study aim to increase the potency through analysis of structure-activity relationships (SAR) and compound optimization.[5]

One relatively common structural characteristic of naturally occurring antibacterial agents is the presence of an aromatic region, often consisting of a bicyclic or tricyclic structure. In the synthesis of tricyclic compounds, combretastatin A4 (Figure 4), a thoroughly studied natural anti-tumor agent, is often used as an intermediate

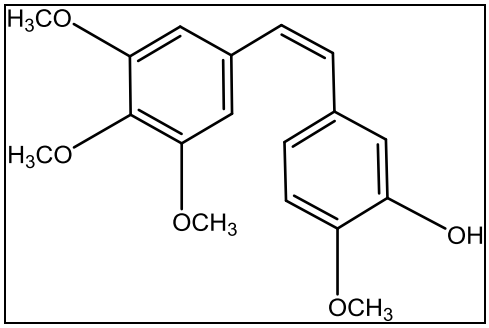


Figure 4: Structure of Combretastatin A4, a naturally occurring anti-tumor agent.

product, allowing the use of well-established methods of synthesis. Development of a simple and precise synthesis of various tricyclic compounds from a combretastatin A4 intermediate would allow for the total synthesis of many tricyclic natural products.[9,10] A 2011 study by Rouseaux et al. examined the use of palladium catalysts in the cyclization of combretastatin A4 into various tricyclic molecules including the core structures of depsidone, some flavonoids, chaconne derivatives, and dibenze-oxepin compounds such as Empetroxepin. The palladium-catalyzed synthesis of tricyclic compounds from combretastatin analogs substantially decreased the reaction time and gave products in high yields (Figure 5).[11]

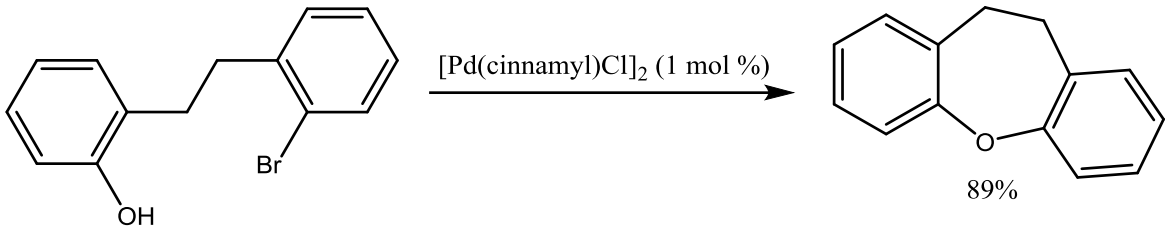


Figure 5: Palladium catalyzed cyclization in the formation of tricyclic compounds.[11]

In natural product-based drug discovery, the importance of selecting a viable lead compound is emphasized across the literature. A similar methodology is used in most studies to systematically select a natural product based on criteria that increases the feasibility of successfully developing a viable drug. An ideal compound will have a structure that is different enough from any existing antibacterial agent to ensure there is no crossover of the analogs, yet is similar enough in regards to specific structural characteristics with known links to bioactivity. Additionally, the structure must be simple enough to synthetically produce in a lab. The size and polarity of the lead compound is also an important consideration, with smaller, more polar molecules being more effective in vivo, though these aspects can be altered during optimization.

Empetroxepin, a recently discovered dibenz[b,f]oxepin natural product, was selected as the target lead compound in the development of a novel antibacterial drug, using the methodology described previously. The compound has sufficient structural similarities with known bioactive molecules such as depsidone, flavone, and chalcone (Figure 6), to suggest it too has some antibacterial activity. However, the unique structure of the central ring in Empetroxepin ensures enough dissimilarity between an optimized analog of this target molecule and existing bioactive molecule. Additionally, Empetroxepin was selected due to its small and relatively simplistic structure which makes it easier to synthetically recreate in a lab.

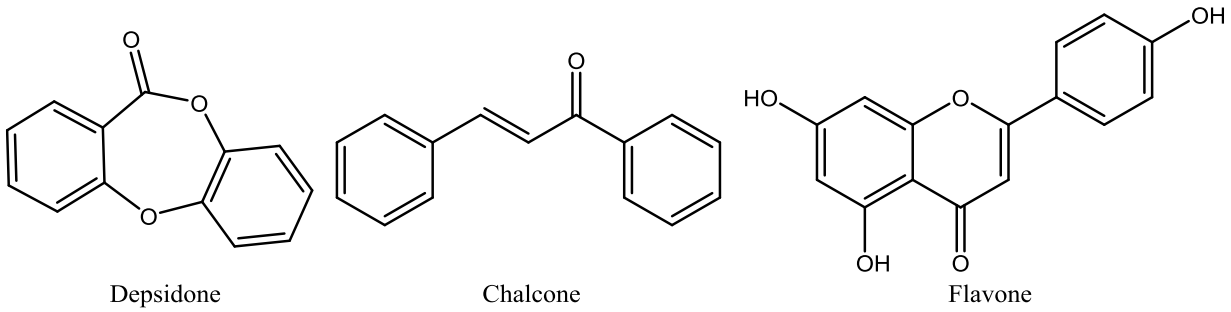
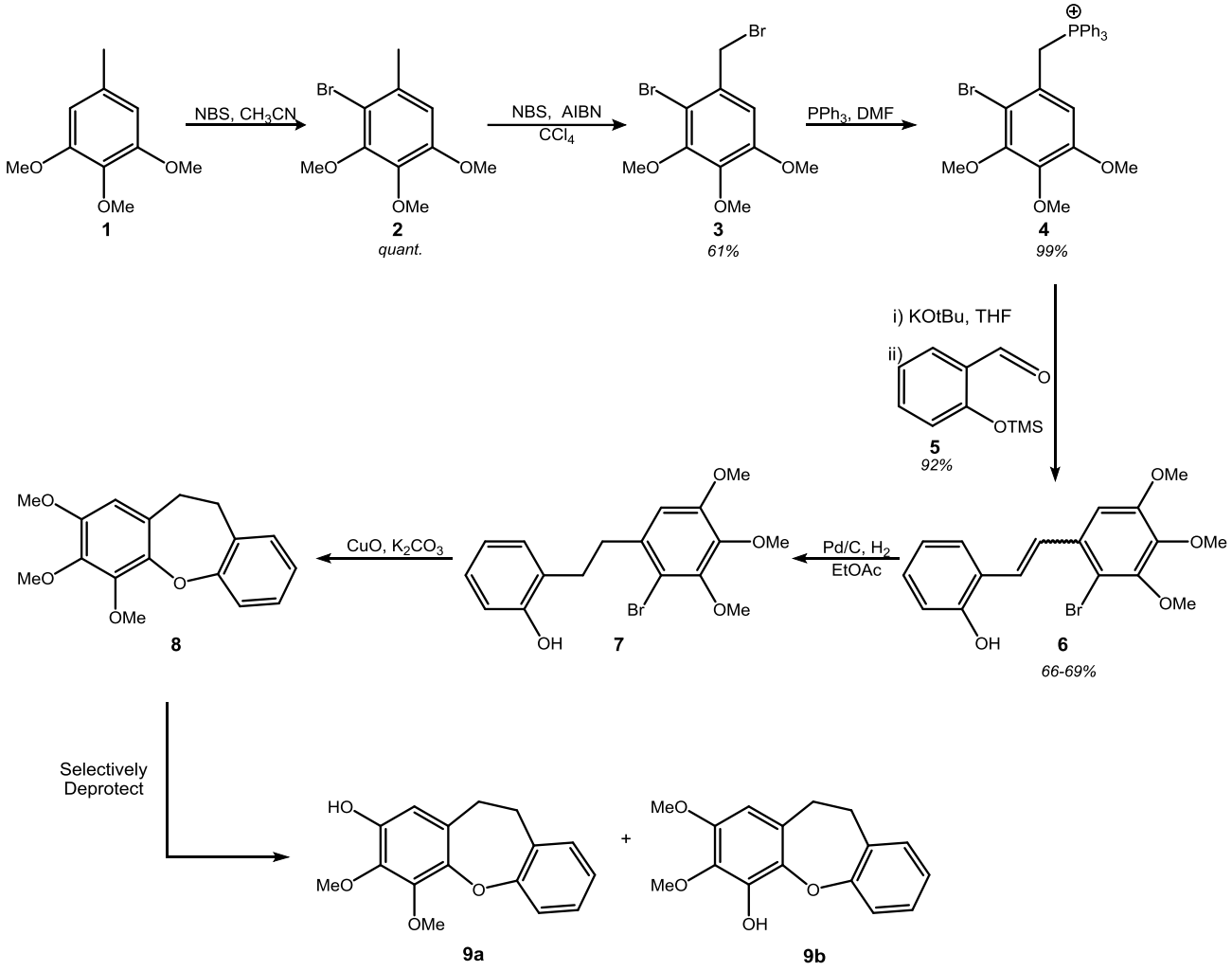


Figure 6: Common bioactive molecules with structural similarities to Empetroxepin.

The development of a novel antibiotic Empetroxepin derivative is accomplished through synthesis of the new natural compound followed by core structure optimization and biological evaluation. The synthesis of the target compound was designed based on existing synthesis methods for combretastatin A4 outlined by Rouseaux et al. combined with an etherification ring closure technique using copper oxide.[11, 12] Optimization of Empetroxepin, following total synthesis, will be attempted through systematically modifying the core structure and evaluating the effect of each modification on the antibacterial activity using an antibacterial assay.[13] Through this method, the most active form of the Empetroxepin can be derived.

3. Results and Discussion

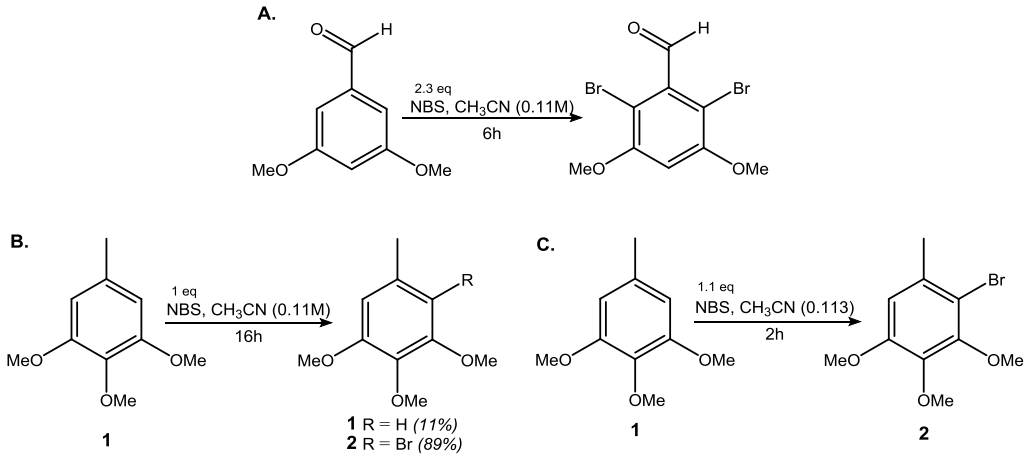
The overall strategy for the synthesis of Empetroxepin (Scheme 1) was designed based on three general goals: first, the synthesis of the phosphonium salt 4, then the Wittig olefination to form the combretastatin A4-like intermediate 5, and third, cyclization to form the Empetroxepin isomers 9a and 9b.



Scheme 1: Strategy for the overall synthesis of Empetroxepin isomers 9a and 9b.

3.1 Reaction 1: EAS Bromination

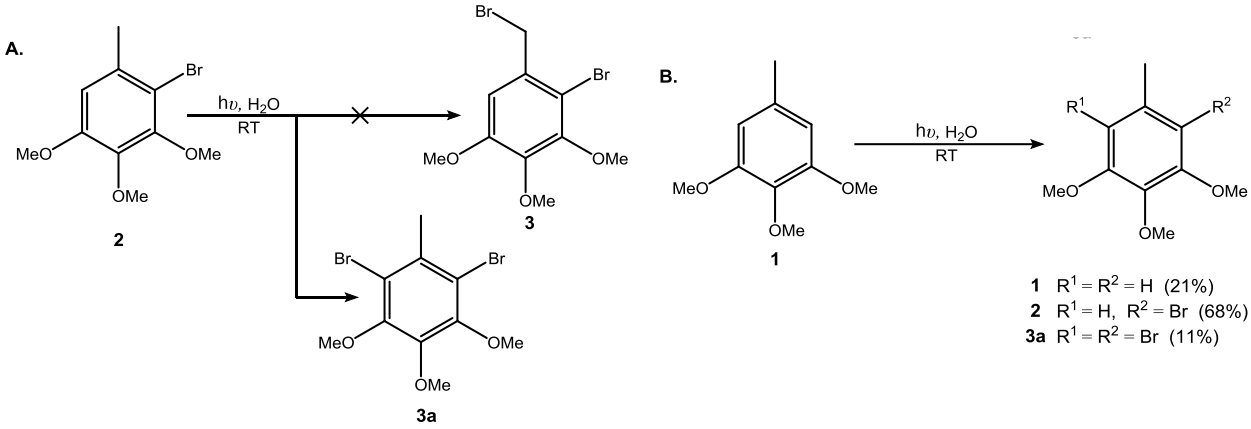
2-bromo-3,4,5-trimethoxy toluene (**2**) was synthesized from compound **1** using a modification of the bromination procedure described by Slavov et al. (Scheme 2). [14] Slavov et al. used a 2.3 molar equivalence of *N*-bromosuccinimide (NBS) for the di-bromination of the benzaldehyde compound. To achieve the single bromination of compound **1**, a 1.0 molar equivalence of NBS was used (method **A**) giving a mixed product of compound **1** and compound **2** in a 1:9 ratio. Increasing the amount of NBS to a 1.1 molar equivalence (method **B**) allowed for the complete conversion of compound **1** into compound **2**. The excess NBS resulted in a small amount of succinimide impurity in the product. Purification was not necessary as NBS is used in the next step of the synthesis scheme. This impurity however, prevented the determination of an accurate yield.



Scheme 2: Synthesis method used by Slavov et al (A).[14] Modified method used in synthesis of compound 2 with 1 eq NBS (B) and 1.1 eq NBS (C).

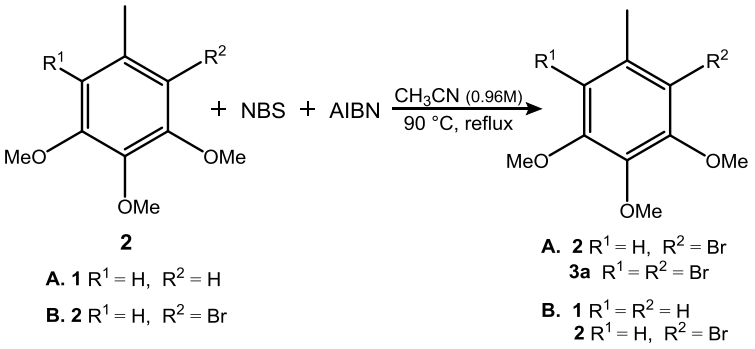
3.2 Reaction 2: Radical Benzylic Bromination

For the second step of the synthesis scheme, the radical benzylic bromination of compound **2** was first attempted using 30% hydrogen peroxide (2 eq) and 48% hydrobromic acid (HBr) (1.1 eq) with incandescent light as described by Podgorsek et al. (Scheme 3).[15] This approach proved unsuccessful in brominating the benzylic carbon, instead brominating the remaining aromatic carbon to form 2,6-dibromo-3,4,5-trimethoxy toluene (**3a**) with an 83% yield. This method of benzylic bromination was then attempted with compound **1,** which also proved unsuccessful, resulting in a mixture of compounds **1**, **2** (single aromatic bromination), and **3a** (double aromatic bromination) in a 2:6:1 ratio.



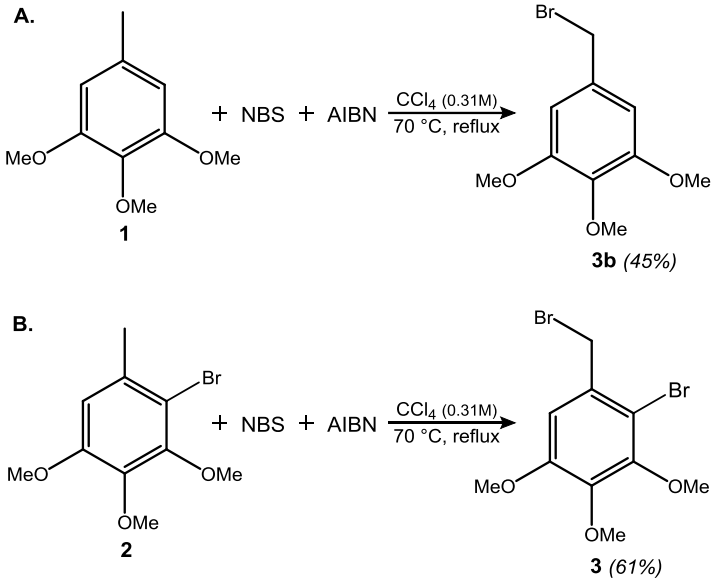
Scheme 3: Attempted radical benzylic bromination of compounds 2 (A) and 1 (B) using H_2O_2 (30%, 2eq) and HBr (48%, 1.1 eq).[15]

Radical benzylic bromination of compound **2** using the more common Wohl–Ziegler method with NBS as the bromine source and recrystallized azobisisobutyronitrile (AIBN) as the radical initiator was attempted next following the methods of Pieck et al (Scheme 4).[16] The method was unsuccessful in forming compound **3** through the benzylic bromination of compound **2**, instead giving a 1:5 ratio of starting material to compound **3a**. The method was attempted on the trimethoxy toluene (**1**) using recrystallized NBS, again proving unsuccessful with a 1:8.5 ratio of starting material (**1**) to compound **2**.



Scheme 4: Attempted benzylic bromination of compound 2 (A) and compound 1 (B) with 1.1 eq NBS (recrystallized for B) and 0.1 eq of recrystallized AIBN. Reaction times were 4h (A) and 21h (B).[16]

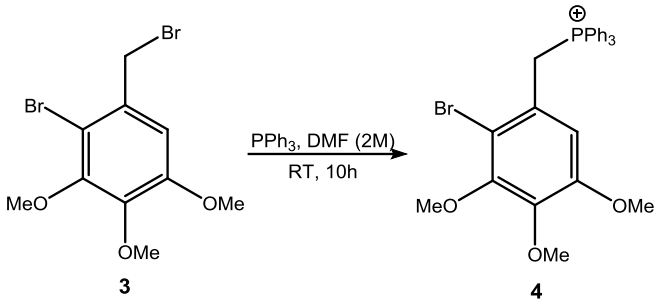
This reaction was then attempted using carbon tetrachloride as the solvent, based on the methods described by Heo et al. and Dai et al.[17, 18] The benzylic bromination of trimethoxy toluene (**1**) was successfully synthesized using 1.1 eq of NBS and 0.1 eq of recrystallized AIBN, resulting in a 45% yield of 3,4,5-Trimethoxybenzyl bromide, compound **3b** and unreacted starting material (Scheme 5). The successful benzylic Bromination technique was then attempted on compound **2** increasing the molar equivalence of NBS from 1.1 to 1.2 in an attempt to react more of the starting material and increase the yield. The increase in yield may also be attributed to the use of a new bottle of CCl_4 rather than the old and possibly contaminated solvent used in the first attempt.



Scheme 5: A. Benzylic bromination of toluene 1 (1.1 eq NBS, 0.1 eq recrystallized AIBN, 17h) resulting in a 45% yield of 3b and B. compound 2 (1.2 eq NBS, 0.1 eq recrystallized AIBN, 4h) resulting in a 61% yield. [17, 18]

3.3 Reaction 3: Triphenylphosphate salt Synthesis

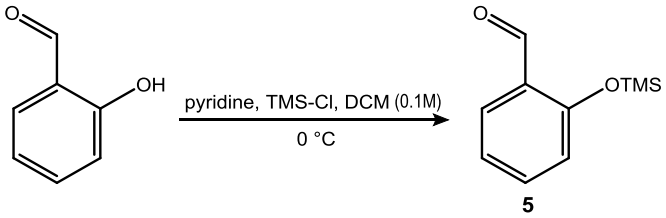
Formation of the triphenylphosphate salt from compound **3** was then carried out using 1 equivalence of triphenylphosphine (PPh_3) in DMF based on the method used by Rousseaux et al (Scheme 6).[11] The reaction was first tested on a small scale yielding 64% of the compound **4**. Scaling the reaction to >1.0g of starting material yielding 99% of compound **4**. The workup of the Triphenylphosphate salt **4** required a 3-part recrystallization which was difficult on the small scale used on the first attempt, the increase in yield is representative of the increase in efficiency of the workup when the reaction was performed on a larger scale.



Scheme 6: Formation of Triphenylphosphate salt (4) from compound 3. Attempt 1: 64% yield, attempt 2: 99% yield.

3.4 Reaction 4: Aldehyde Protection

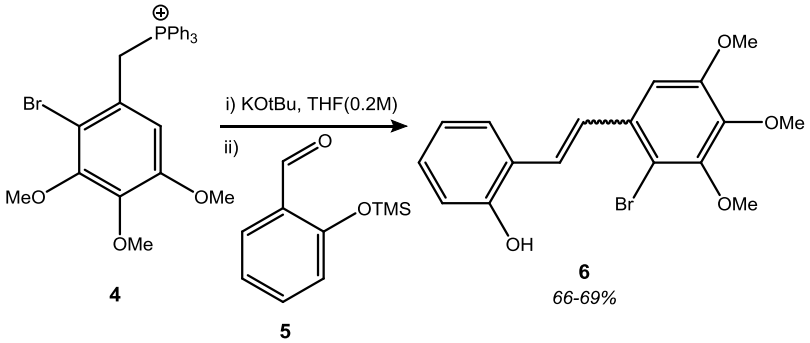
The alkene bridge was formed through a Wittig olefination of the Triphenylphosphate salt **4** and trimethylsilane (TMS) protected salicylaldehyde **5**. The aldehyde was protected using pyridine (2.5 eq) and TMS-Cl (2.1 eq) in anhydrous Dichloromethane (DCM) (Scheme 7). The crude product, which contained unreacted starting material, could not be purified due to the TMS protecting group falling off during purification through silica gel column chromatography. H^1-NMR revealed an 11:1 ratio of the desired protected aldehyde to the undesired unprotected aldehyde, which was used to calculate an approximate 85% yield of compound **5**.



Scheme 7: Trimethylsilane protection of salicylaldehyde giving aldehyde 5 with an approximate yield of 85%.

3.5 Reaction 5: Wittig Olefination

Two general methods exist for the synthesis of dibenze[*b,f*]oxepins: 1) through Wittig olefination forming the alkene bridge first followed cyclization to form the ether bridge and 2) formation of the ether bridge first followed by a McMurry coupling of the diaryl ether. The Wittig method was chosen as the foundation for the synthesis of Empetroxepin based on existing literature which favors this method in the synthesis of dibenze[*b,f*]oxepin compounds specifically. Wittig olefination was thus used to form an alkene bridge with the protected aldehyde **5** and the Triphenylphosphate salt **4** using KOtBu in THF based on the method used by Rousseaux et al (Scheme 8).[11] The



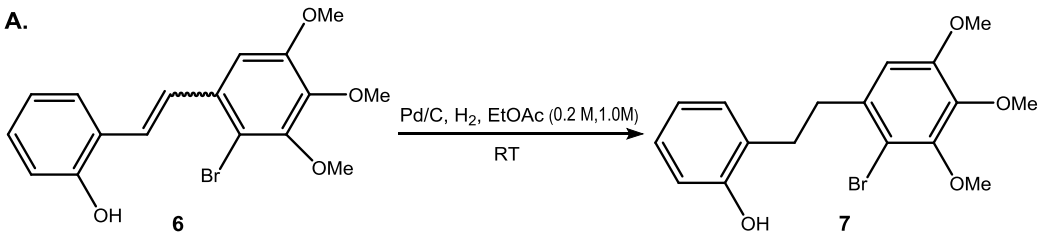
Scheme 8: Formation of the alkene bridge through a Wittig olefination of compounds 4 (1.2 eq) and 5 (1.0 eq) with KOtBu (1.4 eq) giving compound 6 with a yield of 66-69%.

reaction yielded 66-69% of alkene **6.** J-values for the two isomers were calculated from the H^1-NMR, allowing differentiation between the signals corresponding to the cis-isomer (J = 16 Hz) and the trans-isomer (J = 16.4 Hz). This revealed the ratio of cis:trans isomers as 1.3:1, or essentially equal. Silica gel column chromatography was used to remove excess aldehyde from the crude product while also allowing for the easy deprotection of the hydroxyl group.

3.6 Reaction 6: Hydrogenation of Alkene Bridge

Hydrogenation of the alkene bridge using activated Palladium on Carbon and Hydrogen gas in anhydrous Ethyl Acetate (EtOAc) was attempted with the method used by Rouseaux et al (Scheme 9). Lack of excess alkene **6** starting material required the reaction to be scaled down to a point where the mass of Pd/C was difficult to accurately measure and the volume of EtOAc needed was so low, evaporation of the solvent prevented the H_2 gas from being able to bubble through the reaction mixture for more than a few minutes. The majority of the crude product was the alkene **6** starting material. GCMS was used to confirm the presence of the hydrogenated compound **7** in the product but the amount was too low to quantify. Scaling the reaction up to 200 mg (attempt 3) of alkene **6** allowed for the two new compounds to be measured as 1.1 and 1.2 mg though it has not identified. Upon further synthesis of the alkene, the reaction was scaled up to 2 grams of this starting material (attempt 5) in an attempt to eliminate the problem of inadequate H_2 bubbling limited by the extensive evaporation of the solvent when present in such small volumes. Even with the necessary 10 minutes of H_2 bubbling in the large-scale reaction, the result was the same, with the crude product containing primarily unreacted alkene starting material.

A.



B.

Hydrogenation reaction attempts 1-5

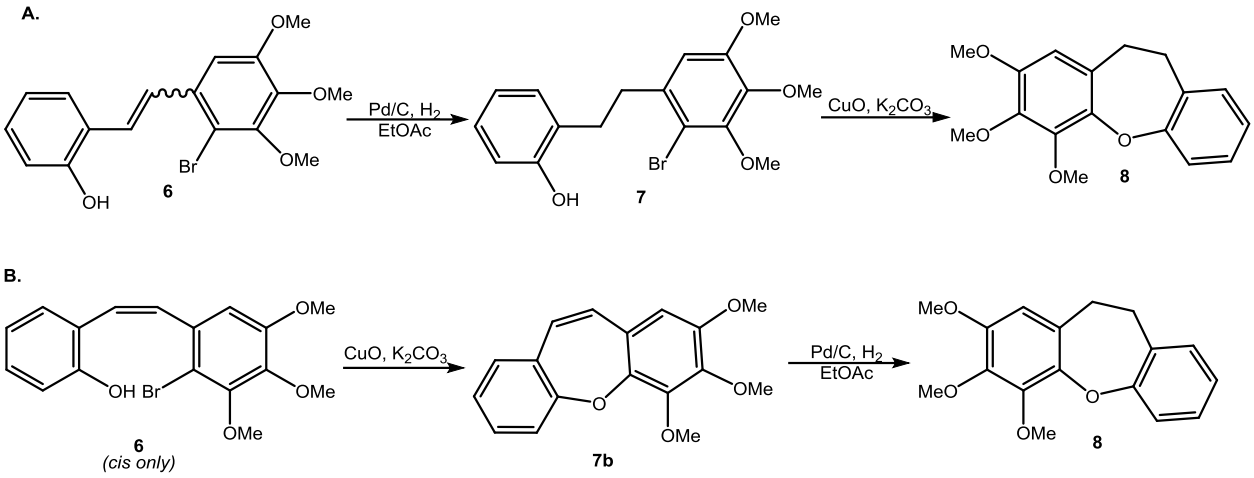
Attempt	Reagent	Yield	
1	Pd/C, 0.17 eq	N/A	SM majority of product
2	Pd/C, 0.1 eq	N/A	SM majority of product
3	Pd/C, 0.15 eq	N/A	172.3 mg SM 1.1 mg/1.2 mg unknown
4	Pd/C, 0.5 eq	N/A	SM majority of product
5	Pd/C, 0.5 eq	N/A	SM majority of product

Scheme 9: (A) Hydrogenation of the alkene bridge using a catalytic amount of activated Pd/C and H_2 gas in anhydrous ethyl acetate. The yield of 7 was too low to quantify. (B) Molar equivalencies and outcome of four hydrogenation reaction attempts.

3.7 Reaction 7: Etherification Ring Closure

Optimization of the alkene hydrogenation was not possible due to the likelihood that increasing the intensity would result in removal of the bromine substituent needed for cyclization. An alternative synthetic approach (Scheme 10b) was carried out in which cyclization of the cis alkene, in which the aryl bromide and aryl hydroxyl are oriented properly, was performed prior to hydrogenation of the double bond. Cyclization to form the ether bridge was achieved using potassium carbonate and a copper oxide catalyst in anhydrous pyridine. This reaction successfully cyclized the

cis alkene giving compound 7b with a 19% yield. TLC indicated the crude product contained the trans form of alkene 7 and an unknown less polar compound, suspected to be the dehalogenated form of the alkene starting material. While this etherification reaction was successful in cyclizing the cis alkene as the major product, it also transformed some of the cis starting material into the trans form, which was purified for use in the additional attempts of the original hydrogenation reaction (Scheme 10a).



Scheme 10: (A) Original post-Wittig reaction synthetic route. (B) Alternative post-Wittig reaction synthetic route in which cyclization is performed prior to hydrogenation of the alkene bridge.

8. Future Work

Key structural similarities of compound 7b to Empetroxepin suggests possible bioactivity of this precursor as a potential Empetroxepin derivative. The next step will therefore be to evaluate the antibacterial activity of compound 7b against *S. aureus* prior to hydrogenation of the alkene bridge.

The major focus of this study moving forward is optimization of the synthetic methods following the Wittig reaction. Upon the successful total synthesis of Empetroxepin A and B, the biological activity of these isomers will be tested. Some antibacterial activity is expected based on the findings by Li, et al. who discovered Empetroxepin.[19] The compound will then be optimized through structure activity relationship studies to develop the most active form of the lead compound, using the measured bioactivity of Empetroxepin as a basis for comparison. Optimization will focus first on ring A and the location and type of substituents resulting in the highest antibacterial activity.

9. Conclusion

The majority of the total synthesis of Empetroxepin isomers A and B has been successfully completed through the Wittig olefination with trimethylsilane protected salicylaldehyde and a triphenylphosphine salt. The salt was synthesized through aromatic bromination and radical benzylic bromination of commercially available 3,4,5-trimethoxy toluene. High yields have been achieved for each of these completed steps. Hydrogenation of the alkene bridge formed by the Wittig olefination has been completed but only in small yields. Cyclization through formation of an ether bridge was successfully performed on the cis-form of alkene 7. The remaining steps in the total synthesis of the tricyclic novel dibenze[*b,f*]oxepin compound is hydrogenation of the alkene bridge, followed by selective deprotection of the methoxy substiutents to form the two Empetroxepin isomers.

The synthesis scheme delineated by this study was the result of many various reactions and approaches, both successful and unsuccessful. This process of knowledge-directed trial and error has allowed for an effective synthetic route to be ascertained for dibenze[*b,f*]oxepin formation. An established synthetic route for highly substituted dibenze[*b,f*]oxepins will allow future studies on this class of natural compounds as potential drugs to be more easily achieved.

10. Experimental

***General*:** Infrared (IR) spectra were recorded on a Nicolet is16 Model Smart iTR. ^{1}H-NMR and ^{13}C-NMR spectra were obtained in $CDCl_3$ on an Oxford 800 MHz spectrometer, using $CDCl_3$ (^{1}H: 7.27 ppm, ^{13}C: 77.0 ppm) as an internal reference. Reactions were monitored using thin-layer chromatography performed on SiO_2 glass plates (eluted with 1:4 EtOAc-hexanes). All solvents and reagents were obtained from commercial suppliers and used without further purification with the exception of AIBN which was recrystallized before use.

Additional care was used when handling dichloromethane due to its carcinogenic properties. Caution was used when working with H_2O_2 and aromatic compounds, specifically while concentrating products, due to the possibility of unintentional formation of explosive organic peroxides. Na_2SO_3 was added to any reactions mixture that used 30% H_2O_2 in order to quench any remaining peroxide.

***2-Bromo-3,4,5-Trimethoxy toluene* (2), Method 1A:** 3,4,5-Trimethoxy toluene (**1**) (0.46 mL, 2.74 mmol, 1eq) was added via syringe to a flame dried 250 mL round bottom flask containing anhydrous acetonitrile (21 mL, 0.113M, 1eq) under a nitrogen atmosphere, and the mixture was cooled to 0 °C. While stirring at 0 °C, NBS (490 mg, 2.74 mmol, 1eq) was added in 5 portions (~100 mg each) at 10 min intervals. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was diluted with EtOAc (~50 mL) and washed with DI water (2 x 50 mL). The remaining organic extract was then washed with saturated aqueous NaCl (1 x 50 mL) and dried over Na_2SO_4. The EtOAc was evaporated under reduced pressure and the crude product was purified by column chromatography (SiO_2, EtOAc-hexanes, 1:4) to give 638 mg (89 % yield) of the title compound **2** as a highly viscous colorless liquid. IR: $\tilde{\nu}_{max}$ 2937, 2846, 1568, 1484, 1462, 1426, 1389, 1337, 1243, 1167, 1200, 1109, 1051, 1011, 969, 925, 829, 811 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.311 (s, 3H), 3.791 (s, 3H), 3.810 (s, 3H), 3.838 (s, 3H), 6.564 (s, 1H). ^{13}C-NMR ($CDCl_3$): δ 23.211, 56.045, 60.851, 61.095, 109.492, 110.773, 133.400, 141.028, 150.793, 152.281.

***2-Bromo-3,4,5-Trimethoxy toluene* (2), Method 1B:** 3,4,5-Trimethoxy toluene (**1**) (0.46 mL, 2.74 mmol, 1eq) was added via syringe to anhydrous acetonitrile (21 mL, 0.113M, 1eq) under a nitrogen atmosphere, and the mixture was cooled to 0 °C. While stirring at 0 °C, NBS (550mg, 3.02mmol, 1.1eq) was added in six portions (~100 mg each) in 10 min intervals. After stirring for 40 min, TLC analysis indicated the bromination reaction was complete. The workup for the reaction mixture was identical to that described for **method 1A**. ^{1}H-NMR of the crude product showed a small amount of succimide in an otherwise pure product of compound **2**. Further purification of the product was not needed. The additional 0.1 eq of NBS gave 733 mg of a slightly orange viscous liquid. ^{1}H-NMR ($CDCl_3$): δ 2.333 (s, 3H), 3.813 (s, 3H), 3.834 (s, 3H), 3.862 (s, 3H), 6.584 (s, 1H).

10.1 Reaction 2: Radical Benzylic bromination:

Method 2.1A: 2-bromo-3,4,5-Trimethoxy toluene (**2**) (0.22 mL, 1.15 mmol, 1 eq) was added to a small vial with a stir bar, followed by 30% H_2O_2 (0.235 mL, 2.298 mmol, 2 eq) and 48% HBr (0.143 mL, 1.26 mmol, 1.1 eq), all via syringe. The reaction became very exothermic with the addition of the HBr and the vial cap was vented. TLC analysis of the reaction mixture and starting material indicated the reaction was complete after 40 min. A 0.01M solution of Na_2SO_3 was prepared and ~2 mL was added to the reaction mixture to quench any excess peroxide. The reaction mixture was then diluted with water (~25 mL) and the crude product was extracted with dichloromethane (DCM) (2 x 25 mL). The combined organic layers were dried with sodium sulfate and then gravity filtered. DCM was evaporated under reduced pressure giving 322.2 mg (83% yield) of a highly viscous orange product. ^{1}H-NMR and ^{13}C-NMR were used to identify the product formed as pure 2,6-dibromo-3,4,5-Trimethoxytoluene (**3a**), corresponding to bromination of the aromatic carbon rather than the benzylic carbon. ^{1}H-NMR ($CDCl_3$): δ 2.554 (s, 1H), 3.873 (s, 6H), 3.904 (s, 3H). ^{13}C-NMR ($CDCl_3$): δ 24.066, 61.011, 61.446, 76.910, 77.230, 77.550, 115.373, 133.430, 145.667, 150.541.

Method 2.1B: 30% H_2O_2 (0.235 mL, 2.298 mmol, 2 eq) and 48% HBr (0.143 mL, 1.26 mmol, 1.1 eq) were added via syringe to a 50 mL pear-shaped flask with a stir bar and septa. A lamp with an 11-watt bulb was placed with the bulb ~10cm from the reaction flask. The bright orange mixture was allowed to stir for 30 minutes. 2-bromo-3,4,5-Trimethoxytoluene (**2**) (0.215 mL, 1.15 mmol, 1 eq) was then added, turning the clear orange mixture an opaque yellow. The reaction mixture was left to stir under the lamp for 15 h during which it turned from yellow to white. The reaction mixture was quenched with 2 mL of 0.01M Na_2SO_3 solution, diluted with water (~25 mL), and the crude product was extracted with DCM (3 x 25 mL). The combined organic layers were dried with sodium sulfate and gravity filtered. DCM was evaporated under reduced pressure and the crude product was identified by TLC and ^{1}H-

NMR as a 1:2.5 ratio of starting material (**2**) and the di-brominated toluene (**3a**). ^{1}H-NMR ($CDCl_3$): δ 2.347 (s, 3H), 2.548 (s, 3H), 3.831 (s, 3H), 3.847 (s, 3H), 3.869-3.875 (m, 6H), 3.901 (s, 3H), 3.906 (s, 3H), 6.596 (s, 1H).

Method 2.1C: 30% H_2O_2 (0.22 mL, 2.178 mmol, 2 eq) and 48% HBr (0.13 mL, 1.208 mmol, 1.1 eq) were added via syringe to a 50 mL pear-shaped flask with a stir bar and septa. A 40-watt light bulb was placed ~10cm from the reaction flask. After the H_2O_2-HBr mixture had stirred for 30 min, 3,4,5-Trimethoxytoluene (**1**) (0.185 mL, 1.098 mmol, 1eq) was added to the reaction flask, turning the color from clear orange to opaque yellow. The reaction mixture was left to stir for 8h during which it became clear and colorless. TLC analysis of the reaction mixture with the starting material **1**, compound **2**, and compound **3a**, showed the reaction had formed both the single brominated compound **2** and the double brominated compound **3a**. The workup for the reaction mixture was identical for that described for **method 2.1A**. ^{1}H-NMR analysis revealed the crude product consisted of a 2:6:1 ratio of compounds **1, 2,** and **3a**. ^{1}H-NMR ($CDCl_3$): δ 2.284 (s, 3H), 2.333 (s, 3H), 2.531 (s, 3H), 3.783 (s, 3H), 3.811(s, 6H) 3.812 (s, 3H), 3.826 (s, 3H), 3.850 (s, 6H), 3.855 (s, 3H), 3.879 (s, 3H), 6.366 (s, 2H), 6.581 (s, 1H).

Method 2.2A: NBS (187.5 mg, 1.053 mmol, 1.1 eq) and AIBN (16.00 mg, 0.0957, 0.1 eq) were added together to a 25 mL oven dried pear-shaped flask with a stir bar and column condenser, containing anhydrous CH_3CN (1.0 mL, 0.958M) under nitrogen. 2-bromo-3,4,5-Trimethoxy toluene (**2**) (0.18 mL, 0.957 mmol, 1 eq) was added via syringe to the reaction flask at the joint. The reaction mixture was heated to 90 °C and stirred under reflux for 4 hours, at which point the reaction flask was removed from heat and left to stir overnight. The CH_3CN was evaporated off with nitrogen giving 0.4388 g of crude product as a partially solid yellow product. TLC revealed the crude product as a mixture of starting material and compound **3a**, corresponding to aromatic bromination. H-NMR confirmed the presence of these two compounds in a 1:5 ratio. ^{1}H-NMR ($CDCl_3$): δ 2.532 (s, 3H), 2.552 (s, 3H), 3.830 (s, 3H), 3.847 (s, 3H), 3.87[1] (s, 6H), 3.876 (s, 3H), 3.901 (s, 3H), 6.597 (s, 1H).

Method 2.2B: 3,4,5-Trimethoxy toluene (0.23 mL, 1.37 mmol, 1eq) was added via syringe to a 10 mL pear shaped flask with a stir bar and column condenser containing anhydrous CH_3CN (1.4 Ml 0.958M) under Nitrogen. Recrystallized NBS (270.2 mg, 1.51 mmol, 1.1 eq) and recrystallized AIBN (27.00 mg, 0.137, 0.1 eq) were added together to the reaction flask at the joint, turning the reaction mixture a light yellow color. The reaction mixture was heated at to 90 °C and stirred under reflux for 21 hours. The CH_3CN was evaporated off with nitrogen revealing an orange/yellow solid. A crude ^{1}H-NMR showed the presence of the starting material (**1**) and compound **2**, corresponding to aromatic bromination rather than benzylic bromination. The crude product was purified via column chromatography (SiO_2, 10% EtOAc-hexanes). ^{1}H-NMR of the pure product confirmed the product as compound **2**.^{1}H-NMR ($CDCl_3$): δ 2.365 (s, 3H), 3.842 (s, 3H), 3.859 (s, 3H), 3.890 (s, 3H), 6.606 (s, 1H).

Method 2.3A: 3,4,5-Trimethoxy toluene (0.23 mL, 1.37 mmol, 1eq) was added via syringe to a 25 mL pear-shaped flask with a stir bar and column condenser containing CCl_4 (4.4 mL, 0.3067M) under argon. NBS (268.1 mg, 1.5l mmol, 1.1 eq) and recrystallized AIBN (23.00 mg, 0.137, 0.1 eq) were added together to the reaction flask at the joint, turning the reaction mixture from clear and colorless to opaque white. The mixture was heated at 77 °C under reflux for 17 hours, during which the mixture turned light yellow. Unwanted succimide solid was filtered from the reaction mixture and the remaining product was washed twice with a saturated aqueous sodium thiosulfate solution. The remaining aqueous solution was extracted twice with DCM and the combined organic layers were washed with NaCl brine and dried over sodium sulfate, and filtered. DCM was evaporated under reduced pressure. Crude NMR revealed a mixed product which was then purified via column chromatography (SiO_2, 10% EtOAc-hexanes) to give 170.8 mg (45% yield) of 3,4,5-Trimethoxybenzyl chloride (**3b**) as a white solid. ^{1}H-NMR ($CDCl_3$): δ 3.859 (s, 3H), 3.882 (s, 6H), 4.470 (s, 2H), 6.632 (s, 2H).

***2-bromo-1-(bromomethyl)-3,4,5-trimethoxybenzene (3)*: Method 2.3 B:** 2-Bromo-3,4,5-Trimethoxy toluene (0.78 mL, 4.24 mmol, 1eq) was added via syringe to a 50 mL pear-shaped flask with a stir bar and column condenser containing CCl_4 (13.4 mL, 0.321M) under argon. NBS (915 mg, 5.14 mmol, 1.2 eq) and recrystallized AIBN (72.5 mg, 0.442 mmol, 0.1 eq) were added together to the reaction flask at the joint, turning the reaction mixture opaque light yellow. The mixture was heated at 77 °C under reflux for ~4 hours, during which the mixture turned orange (t = 15 min) then back to pale yellow (t = 20 min). The reaction mixture was washed twice with a saturated aqueous sodium thiosulfate solution. The aqueous solution was extracted three times with DCM and the combined organic layers were washed with NaCl brine, dried over magnesium sulfate, and filtered. The crude product was concentrated under reduced pressure revealing an orange oil. TLC revealed a mixed product which was then purified via column chromatography (SiO_2, 5%, 10%, 20% EtOAc-hexanes) to give 871.3 mg (61% yield) of 2-bromo-1-(bromomethyl)-

3,4,5-trimethoxybenzene (**3**) as a white solid. ^{1}H-NMR ($CDCl_3$): δ 3.835 (s, 3H), 3.858 (s, 6H), 4.567 (s, 2H), 6.795 (s, 1H). ^{13}C-NMR ($CDCl_3$): δ 34.212, 56.190, 61.019, 61.126, 109.743, 111.193, 132.256, 143.401, 151.281,152.792.

10.2 Reaction 3: Formation of the Triphenylphosphate Salt

Compound 4: 2-bromo-1-(bromomethyl)-3,4,5-trimethoxybenzene (**3**) (1.137 g, 3.34 mmol, 1eq) was added to a 25 mL pear flask with stir bar under argon. Anhydrous DMF (1.7 mL, 2M) was added to the reaction flask via syringe followed by PPh_3 (891.6 mg, 3.40 mmol, 1.0 eq) turning the reaction mixture an opaque pale yellow. The reaction mixture was stirred at room temperature for ~16 hours turning an opaque white after 20 min. ~25 mL of toluene was added to the reaction mixture and the chalky white precipitate was filtered using a fritted funnel. The precipitate was dissolved in a minimal amount of DCM. The solution was precipitated again with EtOAc and the suspension was filtered with a fritted funnel giving 1.75 g (99% yield) of compound **4** as a white powdery solid. ^{13}C-NMR ($CDCl_3$): δ 31.000, 56.350, 60.828, 61.179, 112.146, 112.192, 113.657, 113.726, 116.739, 117.593, 122.186, 122.277, 130.104, 130.226, 134.292, 134.392, 135.208, 135.238, 143.248, 143.287, 150.831, 150.863, 152.800, 152.838.

10.3 Reaction 4: Aldehyde Protection

TMS protected salicylaldehyde (5)**:** Salicylaldehyde (1.75 mL, 16.4 mmol, 1eq) was added to a 500 mL round bottom flask with stir bar containing anhydrous DCM (160 mL, 0.1M) under nitrogen. Anhydrous pyridine (3.4 mL, 0.042 mol, 2.57 eq) and TMS-Cl (4.4 mL, .035 mol, 2.1 eq) were then added to the reaction flask via syringe. The reaction was cooled to 0 °C and stirred for 12 hours. The reaction mixture was washed times with deionized water and the combined aqueous layers were extracted three times with DCM. The combined organic layers were washed with NaCl brine, dried over sodium sulfate, filtered, and concentrated under deceased pressure to give 2.014 g of a clear colorless liquid. ^{1}H-NMR revealed the product was an 11:1 ratio of **compound 5** (84%) to salicylaldehyde.

10.4 Reaction 5: Wittif Olefination

Compound 6**:** Compound **4** (249.4 mg, 0.48 mmol, 1.2 eq) was added to a 10 mL pear flask with stir bar containing anhydrous THF (2.4 mL, 0.2M) under Argon and the reaction was cooled to 0 °C. KOtBu (69.5 mg, 0.57 mmol, 1.4 eq) was added to the reaction flask turning the white mixture a bright red-orange. The mixture was stirred at 0 °C for 30 min. Compound **5** (0.07 mL, 0.4 mmol, 1 eq) was added to the reaction flask via syringe turning the mixture bright yellow. The reaction was warmed to room temperature and stirred for 19 h. Deionized water was slowly added to the flask to dilute the reaction mixture which was then extracted three times with EtOAc. The combined organic layers were washed with NaCl brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a yellow-brown wax. TLC revealed the presence of excess aldehyde and the two desired isomers in the crude product which was then purified via column chromatography (SiO_2, 10% EtOAc-hexanes) to give 99.2 mg (69% yield) of the pure cis and trans isomers of compound **6.** ^{13}C-NMR ($CDCl_3$): δ 56.297, 61.111, 61.393, 105.456, 111.139, 116.136, 120.927, 124.520, 125.611, 127.335, 128.228, 129.174, 133.598, 142.722, 150.915, 152.830, 153.875.

10.5 Reaction 6: Hydrogenation

Compound 7 : Anhydrous EtOAc (0.55mL, 1.2 M) was added to a 10 mL pear flask with stir bar under Argon. Pd/C (11.0 mg, 0.103 mmol, 0.15 eq) was added to the reaction flask and Hydrogen gas was bubbled through the mixture for 5 min. 250.1 mg of compound **6** was dissolved in 0.7 mL of anhydrous EtOAc (0.7 mL, 1 M) and added to the reaction flask via syringe and the reaction was stirred for 19 hours at room temperature. The reaction mixture was filtered through a pipet filled with cotton (bottom layer), sand (middle layer), and celite (top layer), then washed twice with EtOAc and once with MeOH and concentrated under reduced pressure. TLC showed the presence of two products other than the starting material. The crude product which was then purified via column chromatography (SiO_2, 5%, 10%, 15%, 30% EtOAc-hexanes) to give 172.3 g of starting material and the two unknown products (1.1 mg and 1.2 mg).

10.6 Reaction 7: Etherification

Compound 7b : Anhydrous Pyridine (6.2 mL, 1.0 M) was added to a oven dried 20 mL Schleck flask with stir bar under Argon containing CuO (0.15 eq), K_2CO_3 (1.0 eq), and 250 mg (1.0 eq) of the cis alkene **6**. The reaction was stirred at 130 °C and for 48. The reaction mixture was filtered through celite, sand, and cotton. The filter was washed twice with diethyl ether. The product was further diluted with diethyl ether and washed three times with 1N HCL and saturated aqueous sodium bicarbonate. The aqueous layers were combined and extracted with ethyl ether. The combined organic layers were washed with saturated sodium chloride brine, dried over Magnesium Sulfate, and concentrated under reduced pressure. The crude product which was purified via column chromatography (SiO_2,10%, (1000 mL), 20%, (300 mL) 30% (250 mL) EtOAc-hexanes) to give the cyclized alkene product as a light red chalk with a 19% yield. ^{1}H-NMR ($CDCl_3$): δ 3.788 (s, 3H), 3.869 (s, 3H), 4.016 (s, 3H), 6.399 (s, 1H), 6.630, 6.602 (d, 1H), 6.686, 6.657 (d, 1H), 7.078, 7.099 (d, 1H), 7.125, 7.143 (d, 1H), 7.228, 7.251, 7.267 (t, 2H). ^{13}C-NMR ($CDCl_3$): δ 56.343, 61.484, 62.217, 106.173, 121.850, 125.062, 126.443, 129.326, 129.700, 129.898, 130.005, 130.882, 144.088, 144.484, 146.193, 150.168, 157.590.

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