NEW ANTIMALARIAL AND ANTITUBERCULOSIS DRUGS

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NEW ANTIMALARIAL AND ANTITUBERCULOSIS DRUGS

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I. ABSTRACT

The non-mevalonate biosynthetic pathway has only recently been mapped and identified as a potential target for the development of novel antibiotics. With the current problems in regard to antibiotic resistance, the discovery of new compounds that are active medicinally against bacterial infections like malaria and tuberculosis, that cause the death of approximately two million people worldwide yearly, is imperative. The goal of this project was aimed at the synthesis and evaluation of methylerythritol cyclodiphosphate (MEcPP) and 4-hydroxy-3-methyl-2-(E)-butenyl-4-diphosphate (HMBPP) analogs as potential therapeutic agents against bacterial infections such as malaria, tuberculosis, and related infections. In bacteria and plants, the non-mevalonate biosynthetic pathway is utilized to produce crucial isoprenoid building blocks. This pathway is appealing as a target for the development of new antibiotics because it is absent in humans, likely resulting in reduced toxicity and side effects. Particularly, MEcPP analogs may be especially selective due to the unique 8-membered cyclic pyrophosphate moiety in MEcPP. An MEcPP analog has never been synthesized before, and this project intended to prepare and evaluate both cyclic and acyclic MEcPP and HMBPP mimics. The goal of this project was to take steps toward filling the gaps in current knowledge that may allow for the production of antibiotics, specifically those of medicinal relevance with activity on resistant bacterial strains.

II. ACKNOWLEDGMENTS

I would like to sincerely thank Dr. Jean-Luc Montchamp. This project would not have been possible without his help and guidance through every step. Dr. Montchamp provided the idea of working on research in this area and given me an opportunity that has allowed me to develop my technical and cognitive research skills, as well as gain a firsthand appreciation and emphasis on the importance of research. I would also like to thank Karen Winters for giving her time, skills, and advice throughout the course of this project, including the time she spent teaching the technical skills and setups necessary for research. Further, I would like to thank Dr. Sergei Dzyuba and Dr. Mikaela Stewart for their time and service on my project committee. Lastly, I would like to thank the Research and Creative Activities Fund (RCAF) Committee for the generous grant that provided the funds to make this research project possible.

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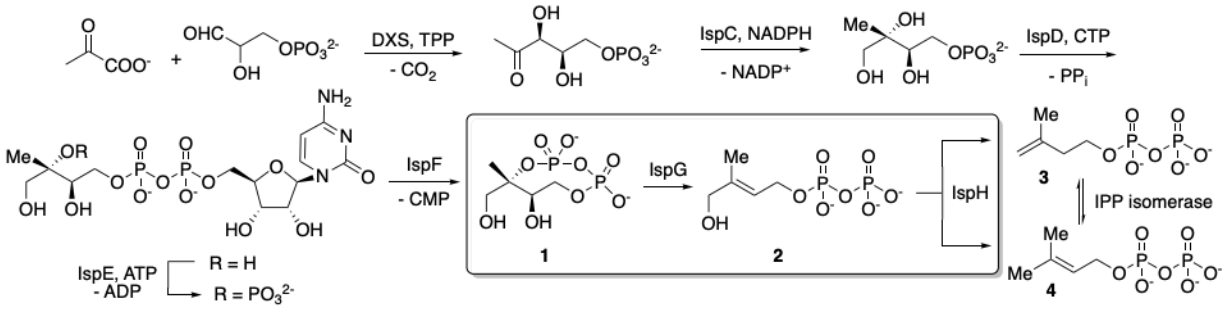
LIST OF ABBREVIATIONS

^{1}H NMR	*Proton Nuclear Magnetic Resonance*
DCM	*Dichloromethane*
DMF	*Dimethylformamide*
Eq or Equiv	*Equivalent*
Et_3B	*Triethylborane*
EtOAc	*Ethyl acetate*
H_3O^+	*Hydronium (acid)*
HMBPP	*4-hydroxy-3-methyl-2-(E)-butenyl-4-diphosphate*
Hrs	*Hours*
K_2CO_3	*Potassium bicarbonate*
$KMnO_4$	*Potassium permanganate*
Me_4Si	*Tetramethylsilane*
MEcPP	*Methylerythritol cyclodiphosphate*
MeOH	*Methanol*
MEP	*Non-mevalonate pathway*
$MgSO_4$	*Magnesium sulfate*
N_2	*Under Nitrogen gas*
NaH_2PO_2	*Sodium hypophosphite*
NaOH	*Sodium hydroxide*
NCS	*N-chlorosuccinimide*
NH_4Cl	*Ammonium chloride*
PCC	*Pyridinium chlorochromate*
R	*Any group*
R.t.	*Room temperature*
TEMPO	*(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl or (2,2,6,6-Tetramethylpiperidin-1-yl)oxidanyl*
THF	*Tetrahydrofuran*
TLC	*Thin layer chromatography*

III. INTRODUCTION

Biosynthesis of isoprenoid precursors via the non-mevalonate pathway is opportunistic in designing and synthesizing novel antibiotics and antiparasitic agents for medical use, specifically in the cases of tuberculosis and malaria. The non-mevalonate (methylerythriol-4-phosphate or MEP) (Scheme 1) pathway was only recently discovered. This pathway is compelling because it



Scheme 1. The non-mevalonate pathway for isoprenoid precursor biosynthesis (methylerythritol cyclodiphosphate (MEcPP) **1**, 4-hydroxy-3-methyl-2-(*E*)-butenyl-4-diphosphate (HMBPP) **2**, isopentenyl diphosphate (IPP) **3**, dimethylallyl diphosphate (DMAPP) **4**).

does not exist in humans – allowing for selective inhibition in the pathogen.[1] The isoprenoids that ultimately result from this pathway are converted into critical lipid metabolites and therefore are crucial for cell growth.[2] The MEP pathway is used by bacteria, parasites, and plants, which indicates its utility in tuberculosis and malaria cases. The World Health Organization (WHO) estimates that in 2019, 10 million people worldwide had tuberculosis, resulting in 1.5 million deaths. TB is one of the top ten causes of death, and the leading cause of death from a single infectious agent – even higher than HIV/AIDS. In regard to malaria, the WHO estimates 228 million cases in 2019, resulting in 405,000 deaths.[3] The WHO further states that "multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat". TB is also the leading killer of HIV-infected people. Due to increased resistance in a variety of pathogens including the aforementioned, drugs must continuously be developed to target them effectively.

The MEP pathway is a promising source of opportunity in novel drug development. There have been very few inhibitors of the non-mevalonate pathway reported thus far, and those that have been were reviewed in 2013.[4,5] According to a literature survey, there is a scarcity of inhibitors for the MEP pathway.

The goal of this research was to synthesize analogs of the metabolites MEcPP (1) (Figure 1) and HMBPP (2) (Figure 2), the substrates of the IspG and IspH enzymes respectively, in hopes to achieve novel bioactivity. The specific goal analog is boxed in Figure 1.

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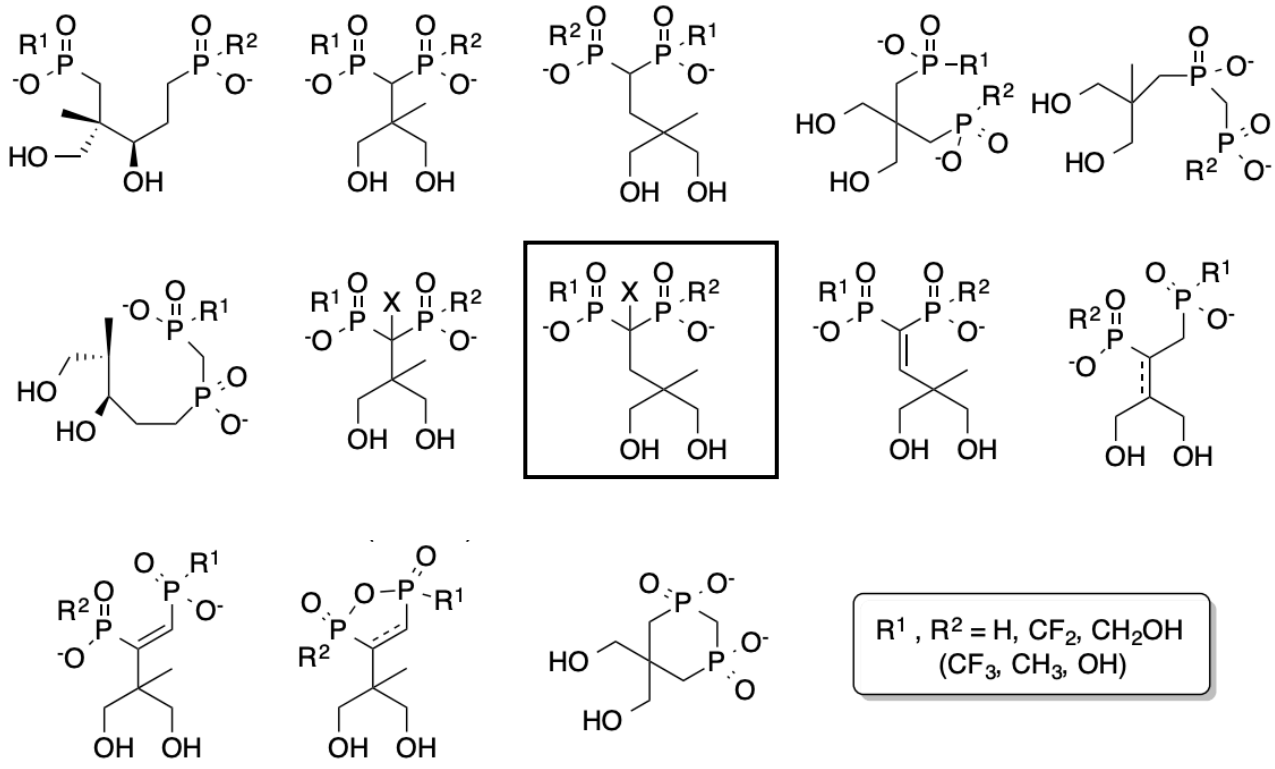


Figure 1. Various potential MEcPP analogs.

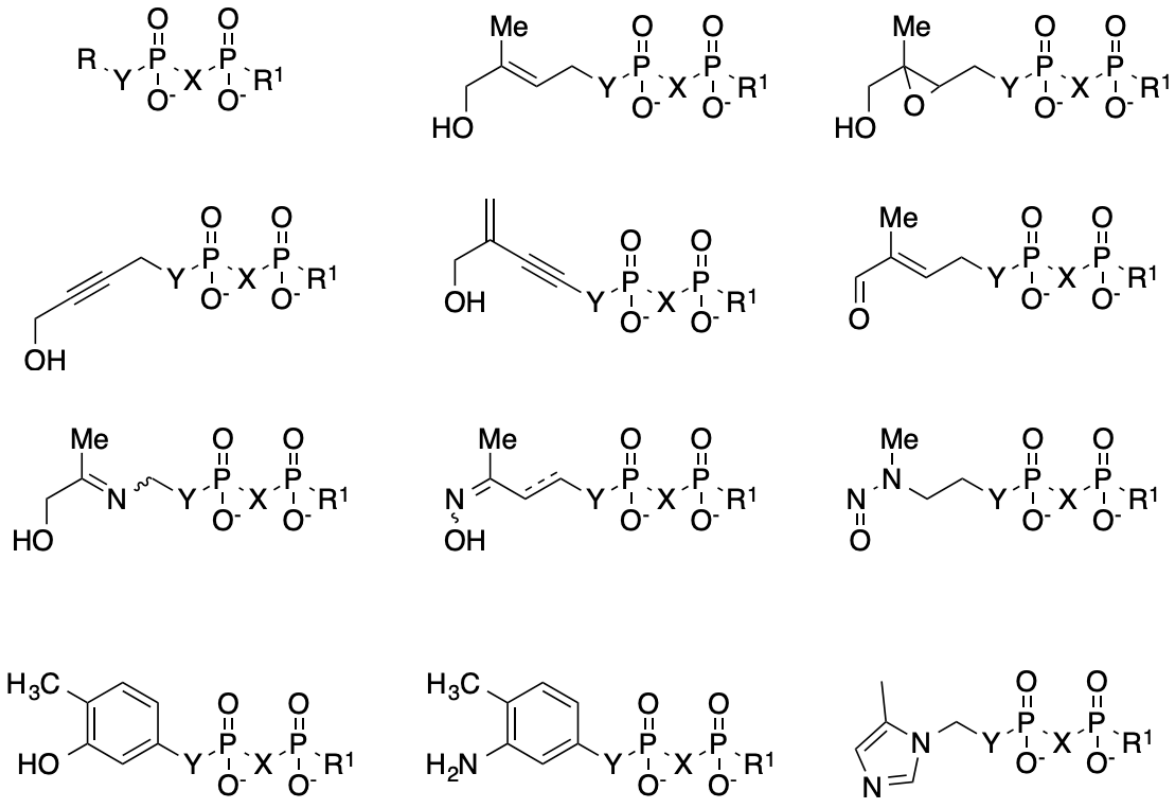
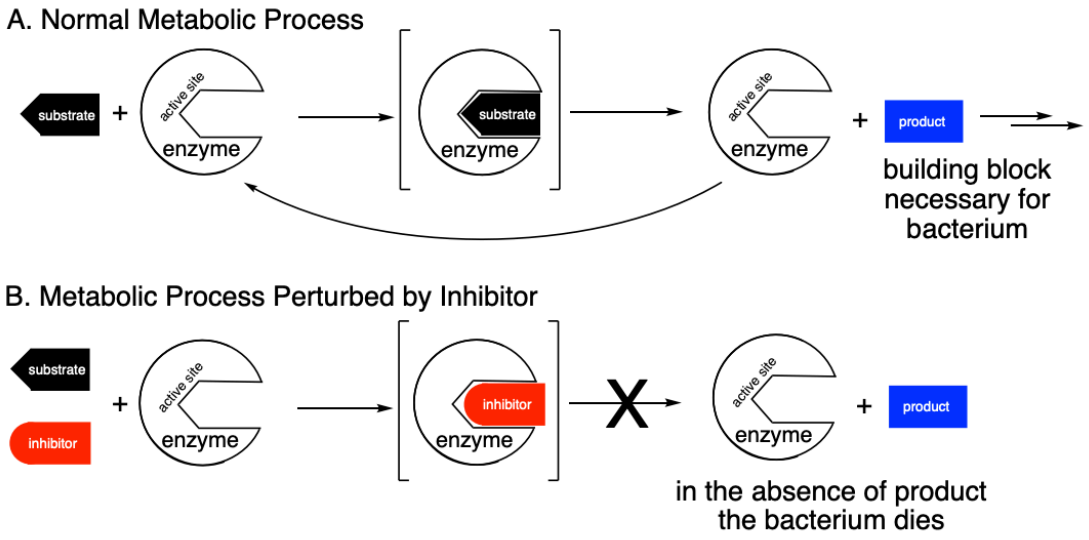


Figure 2. Various potential HMBPP analogs.

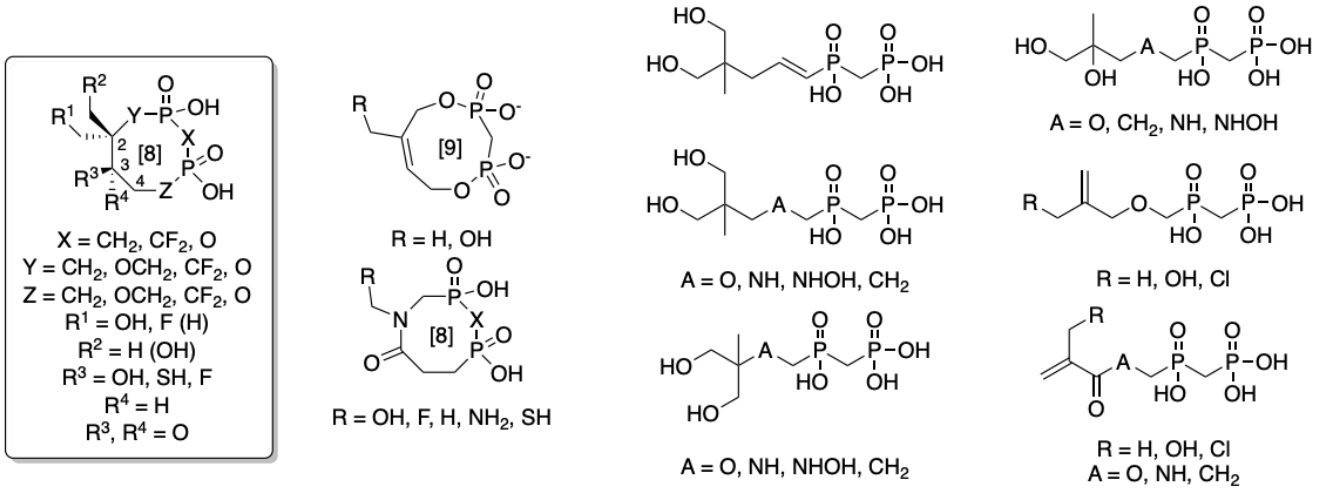
Blocking the machinery that creates these substrates will lead to bacterial death because of their necessary role in bacterial growth. No cyclic pyrophosphate analog of MEcPP has ever been prepared for the inhibition of the IspG enzyme. Similarly, there have not been any analogs of acyclic HMBPP tested to inhibit the enzyme IspH. The more similar the synthesized compound to the natural metabolite, the higher the likelihood of strong inhibition of the enzymes involved. The goal is to make the inhibition strong enough to restrict the organism's (bacteria or parasite) ability to produce key molecules necessary for survival. This will result in death of the organism. The inhibitor interacts with the enzyme in a way that prevents it from carrying out its function – processing its normal substrate. Therefore, there will not be enough product made to support the



Scheme 2. Blockade of a metabolic pathway with an inhibitor that looks like the substrate of an enzyme. The enzyme cannot perform its normal function or be recycled, therefore the resulting product is depleted ultimately leading to the organism's death.

organism's survival (Scheme 2). This mode of action – enzymatic inhibition – is the most common among all drugs. 47% of all current drugs inhibit enzymes.[6] Unlike many other drugs' enzymatic targets, humans do not possess the target (IspG and IspH). As a result, the compounds are much less likely to interfere with human pathways. This is beneficial in that possible toxicity or side-effects should be minimized.

The synthesis of a variety of compounds mimicking the overall structure of MEcPP and HMBPP is the ultimate goal of this ongoing research. Scheme 3 displays a few of the possible target structures. Because the cyclic targets are significantly more difficult to make, the focus was on the acyclic targets. Once synthesized, the compounds can be sent for testing against IspG and IspH in Professor Andrew Wiemer's laboratory at the University of Connecticut.



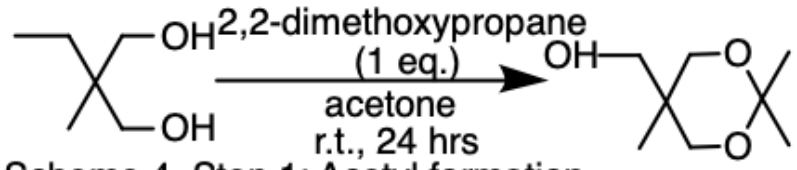
Scheme 3. Representative target molecules, analogs of MEcPP and HMBPP.

IV. RESULTS AND DISCUSSION

Although the target HMBPP analog was not obtained, progress was made in its synthesis. The first steps were accomplished in synthesizing this target molecule and subsequent steps were planned.

1. Acetal Formation

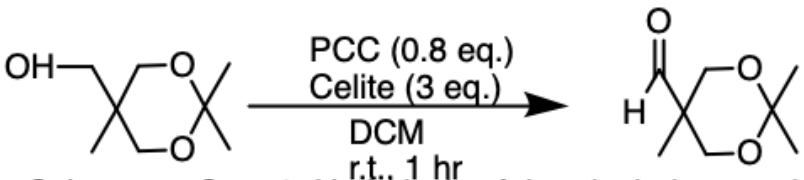
The first step of the synthesis involved a reaction between tris(hydroxymethyl)ethane and acetone/2,2-dimethoxypropane as seen in Scheme 4. The purpose of this step was a protection step. The ring formation limited the molecule to only one hydroxyl group available for reaction. This step was first carried out with only acetone initially and was unsuccessful. But, following the addition of the 2,2-dimethoxypropane to the procedure, this reaction resulted in 95 percent yield. The product was not purified because based on the ^{1}H NMR, the purity was sufficient to utilize in the next step.



Scheme 4. Step 1: Acetyl formation.

2. Hydrolysis of the Alcohol to an Aldehyde

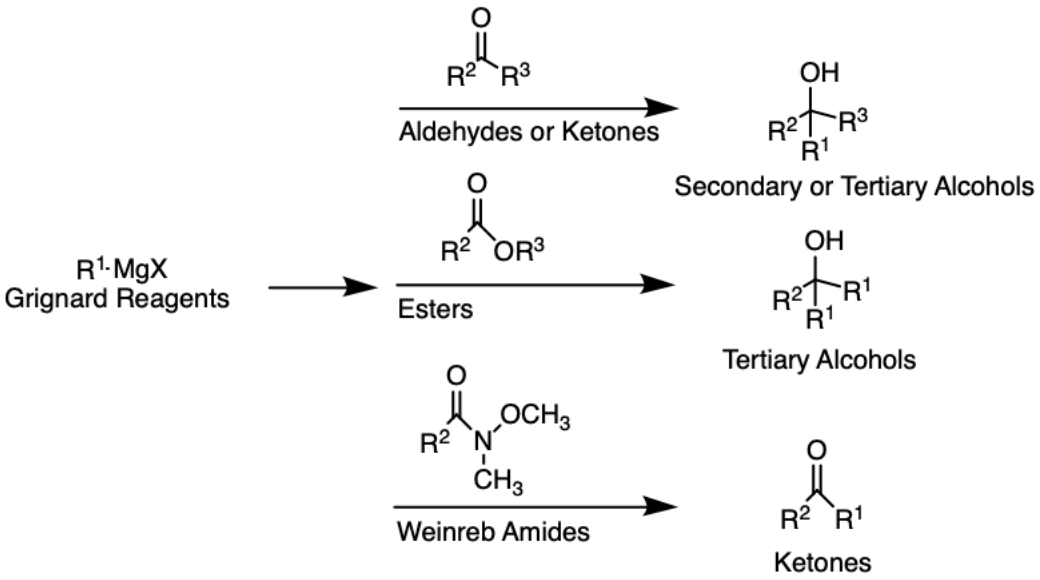
The next step in the synthesis involved oxidation of the hydroxyl group to yield the aldehyde, as shown in Scheme 5. The formation of the aldehyde is an important step because it acts as an intermediate for many reactions including the following step in this synthesis, alkylation. Scheme 5 shows the conditions that were utilized in the oxidation. This step was particularly difficult. Multiple methods of synthesis were attempted previous to the PCC/celite method. The different syntheses attempted included one utilizing N-chlorosuccinimide, dodecyl methyl sulfide, and triethylamine and one that included sodium hypochlorite and TEMPO. Both resulted in the aldehyde, but with low yields between 5 and 40 percent. Finally, the oxidation using a PCC/celite mixture in dichloromethane proved to be a successful synthesis. This reaction resulted in an 81 percent yield. Because the aldehyde is an intermediate in this sequence of reactions, and the ^{1}H NMR showed the product to be relatively clean, it was not purified and used directly in the next step.



Scheme 5. Step 2: Hydrolysis of the alcohol to an aldehyde.

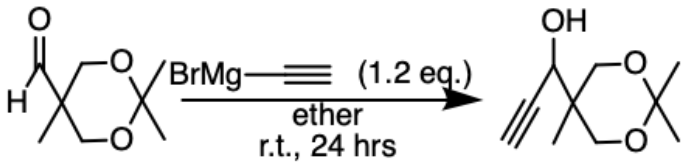
3. Alkylation

Following the aldehyde formation, an alkylation reaction was carried out to introduce another carbon-carbon bond into the molecule. Although a method utilizing lithium acetylide-ethylenediamine complex was attempted, the Grignard reaction proved to give a better yield. This reaction is used to introduce alkyl groups into molecules. It can be utilized with functional groups such as aldehydes and ketones (as in this reaction), esters, and Weinreb amides to yield secondary or tertiary alcohols, tertiary alcohols, and ketones respectively, as shown in Scheme 6.[7] The



Scheme 6. The Grignard Reaction

specific Grignard reagent shown in Scheme 7 was used due to the resulting product's utility in the next step of the synthesis discussed in the 'Future Work' section. This reaction resulted in a 70 percent yield.



Scheme 7. Step 2: Alkylation via the Grignard reaction.

The ^{1}H NMR spectra and data for the three products of each of the synthesis steps are found in the 'Experimental' section. Unfortunately, the remaining steps in this synthesis were not carried out due to Covid-19 and TCU's transition to online learning, but the planned remaining steps are found in the 'Conclusion and Future Work' section.

V. CONCLUSION AND FUTURE WORK

The goal of the synthesis of the target MEcPP analog did not occur, but the first three steps showed promise in achieving its synthesis in the future. The yields of the initial three steps were relatively high, which will be advantageous in continuing the synthetic scheme. Scheme 8 shows the overall synthesis of the target analog including both the first three steps that were successfully completed and the future experimental steps to be carried out.

The urgent need for antibiotics and antiparasitic agents against multidrug-resistant pathogens underlines the necessity to continue the work on this project. The non-mevalonate pathway for the biosynthesis of isoprenoid precursors (Scheme 1) present in bacteria, parasites, and plants offers opportunities for the synthesis of these medications against major diseases like tuberculosis and malaria. Further research should continue to focus on the synthesis not only on the analog that was the primary subject of this project, as well as another potential target via Wadsworth-Horner-Emmons olefination of the aldehyde (product in Scheme 5), shown in Figure

3; but also on other possible inhibitors – additional analogs of MEcPP, the substrate of the enzyme IspG and analogs of HMBPP, the substrate of the enzyme IspH, both of which play integral roles in the MEP pathway.

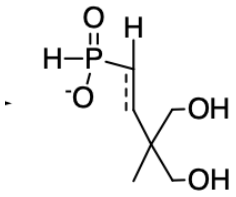
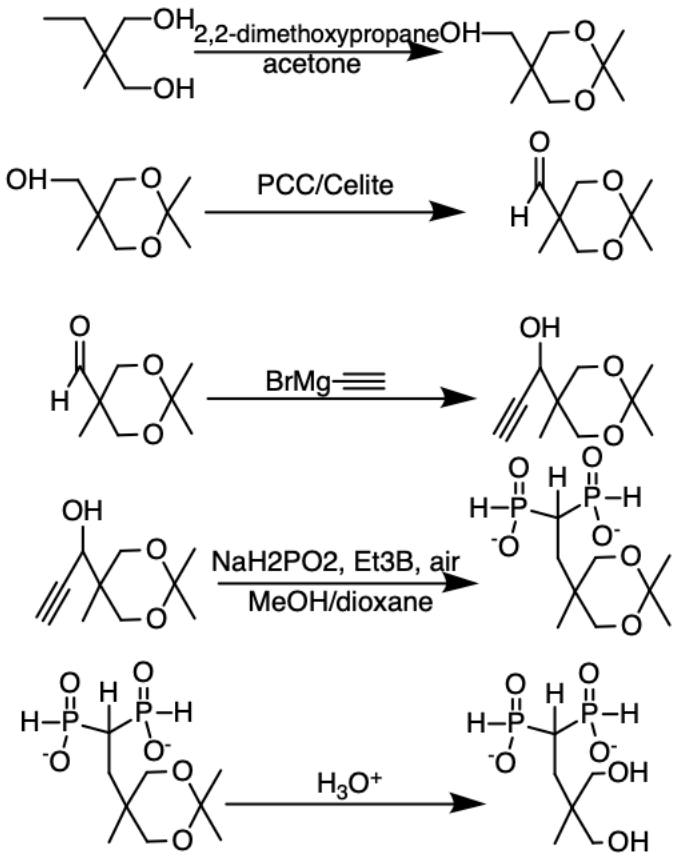


Figure 3. Additional potential target molecule though the Wadsworth-Horner-Emmons olefination of the aldehyde.



Scheme 8. Overall synthesis of the target MEcPP analog.

VI. EXPERIMENTAL SECTION

General Chemistry:

^{1}H NMR spectra were recorded on a 300-MHz Varian INOVA spectrometer or 400-MHz Bruker Avance spectrometer. ^{1}H NMR were acquired using $CDCl_3$, and chemical shifts are reported in part per million relative to internal Tetramethylsilane (Me_4Si, δ = 0.00 ppm). Flash chromatography was carried out on Silica Gel premium Rf grade (40−75 μm). Ethyl acetate/hexane mixtures or dichloromethane/acetone were used as the eluent for chromatographic purifications. TLC plates were visualized by UV or immersion in potassium permanganate solution (3 g $KMnO_4$, 20 g K_2CO_3, 5 mL 5% aq. NaOH and 300 mL of water) followed by heating.

Reagents and solvents:

All starting materials were purchased from commercial sources and used as received. The solvents were distilled under N_2 and dried according to standard procedures (DMF from $MgSO_4$; CH_3CN, toluene and dichloromethane from CaH_2).

First Step – Acetal Formation[8]

A 1000 mL round bottom flask containing acetone (64 equiv, 250 mL, 3404 mmol) was flushed with N_2. Next, 2,2-dimethoxypropane (1 equiv, 30 mL, 250 mmol), tris(hydroxymethyl)ethane (1 equiv, 30 g, 250 mmol), and p-Toluesnesulfonic acid (0.01 equiv, 0.48 g, 2.5 mmol) were added to the round bottom flask. The mixture was stirred for 24 hours in room temperature under N_2. Then, the reaction was quenched with K_2CO_3 (0.35 equiv, 12.0 g, 87 mmol) and the solvent was evaporated. Figure 4 shows the ^{1}H NMR of this product. The NMR indicated that the product was the intended molecule based on the peak data shown below, and because the spectrum is relatively clean, the product was pure enough to proceed with the next step.

[1]H NMR (400 MHz, Chloroform-d) δ 3.66 (d, J = 7.8 Hz, 2H), 3.64 – 3.63 (m, 2H), 3.58 (d, J = 12.0 Hz, 2H), 2.85 – 2.74 (m, 1H), 1.42 (s, 3H), 1.37 (s, 3H), 0.81 (s, 3H).

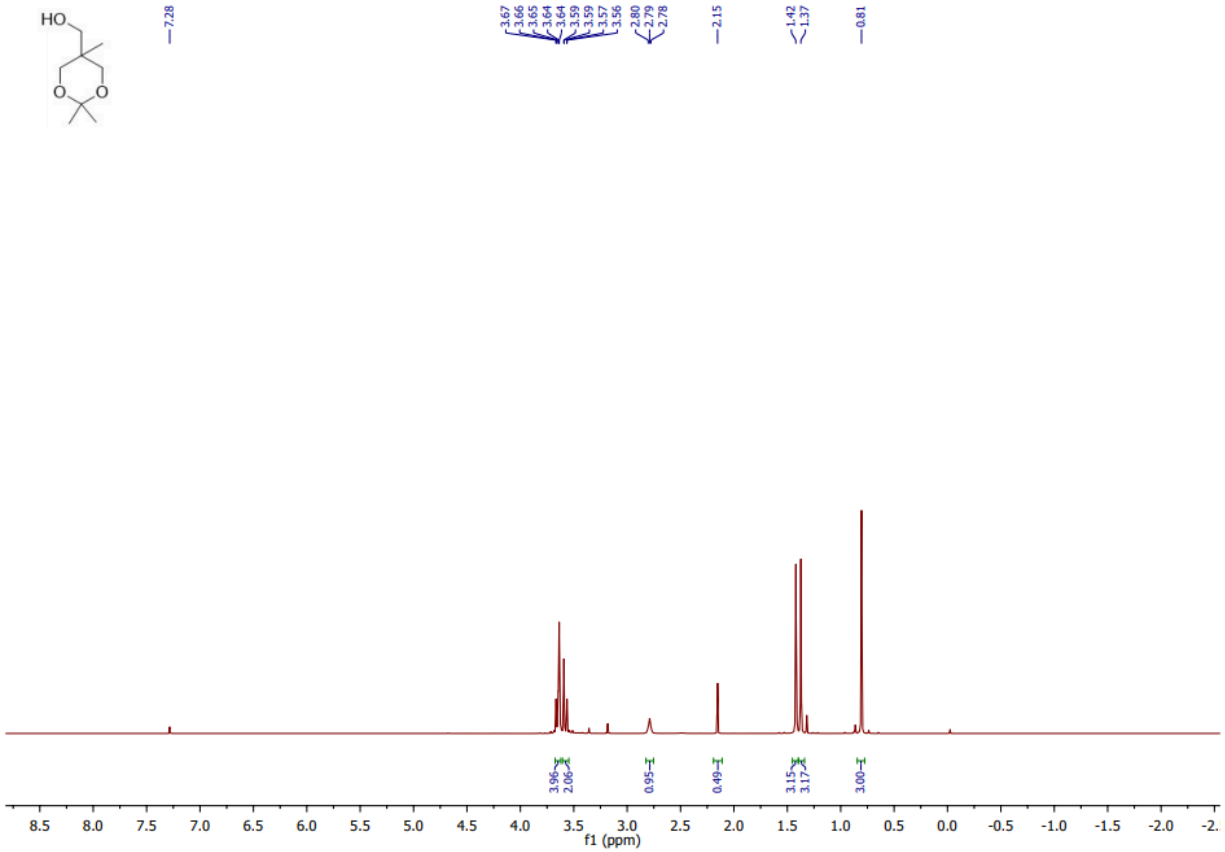


Figure 4. [1]H NMR of the product from the first step.

Second Step – Hydrolysis of the Alcohol to an Aldehyde

350 mL of DCM was added to a 1000 mL round bottom flask and flushed with N_2. The product from the first step (1 equiv, 13.8 g, 84 mmol) was then added and dissolved. The 50/50 by mass mixture of PCC (0.8 equiv, 15.1 g, 70 mmol) and celite (3 equiv, 15.1 g, 250 mmol) was slowly added to the round bottom flask. The mixture was stirred for 1 hour in room temperature under N_2. Then, the mixture was filtered to remove the precipitate and the solvent was evaporated. Figure 5 shows the [1]H NMR of this product. The NMR indicated that the product was the intended molecule

based on the peak data shown below, and because the spectrum is relatively clean, the product was pure enough to proceed with the next step.

^{1}H NMR (400 MHz, Chloroform-d) δ 9.74 (s, 1H), 4.04 (m, J = 11.9, 0.9 Hz, 2H), 3.81 – 3.63 (m, 2H), 1.41 (d, J = 0.7 Hz, 3H), 1.30 (d, J = 0.8 Hz, 3H), 0.82 (s, 3H).

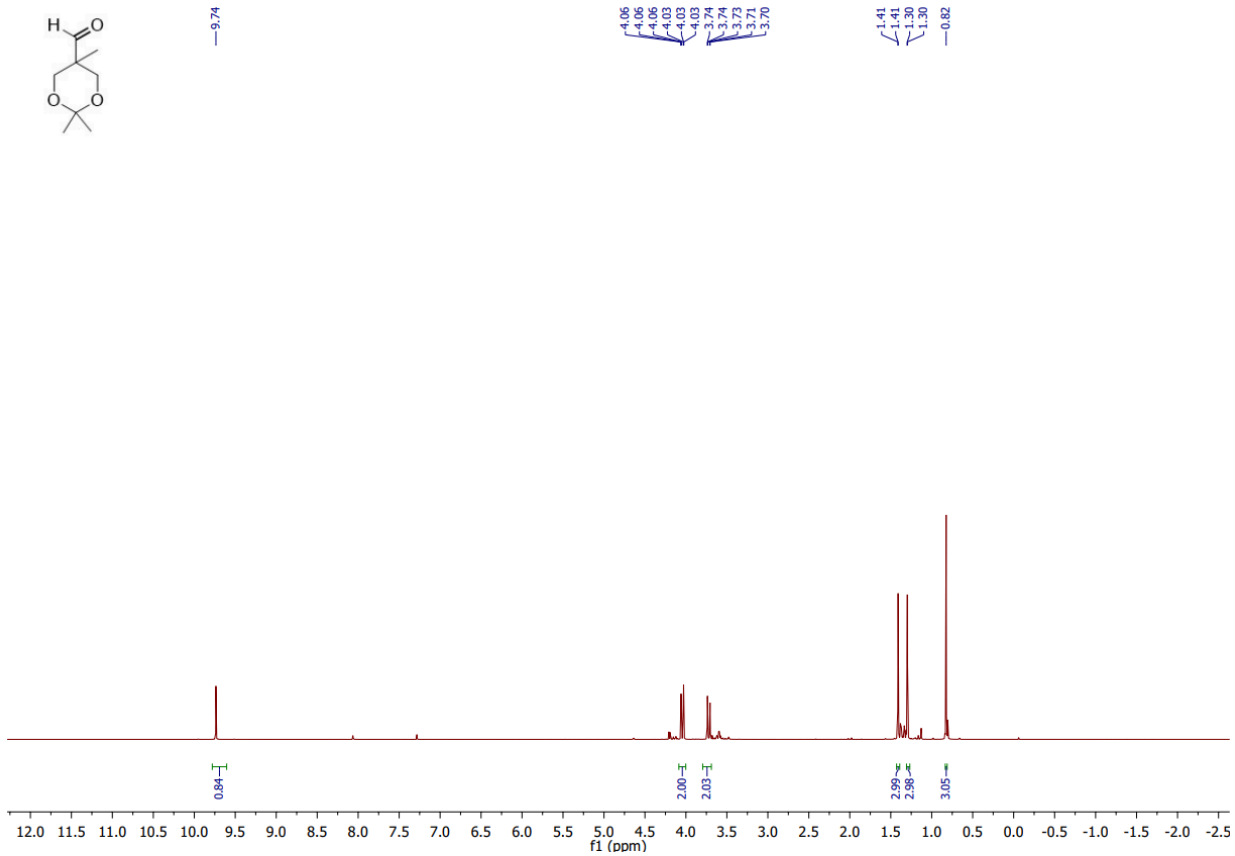


Figure 5. ^{1}H NMR of the product from the second step.

Third Step – Alkylation

50 mL of THF was added to a 500 mL round bottom flask and flushed with N_2. The product from the second step (1 equiv, 1 g, 6 mmol) was then added and dissolved. Next, the ethynylmagnesium bromide (0.5M in THF) (1.2 equiv, 14 mL, 7 mmol) was added slowly. The mixture was stirred

for 24 hours in room temperature under N_2. Then, the reaction was quenched with NH_4Cl and washed with ethyl acetate and brine three times. The organic layer was dried with $MgSO_4$, filtered, and evaporated to remove the solvent. Figure 6 shows the ^1H NMR of this product.

No data available.

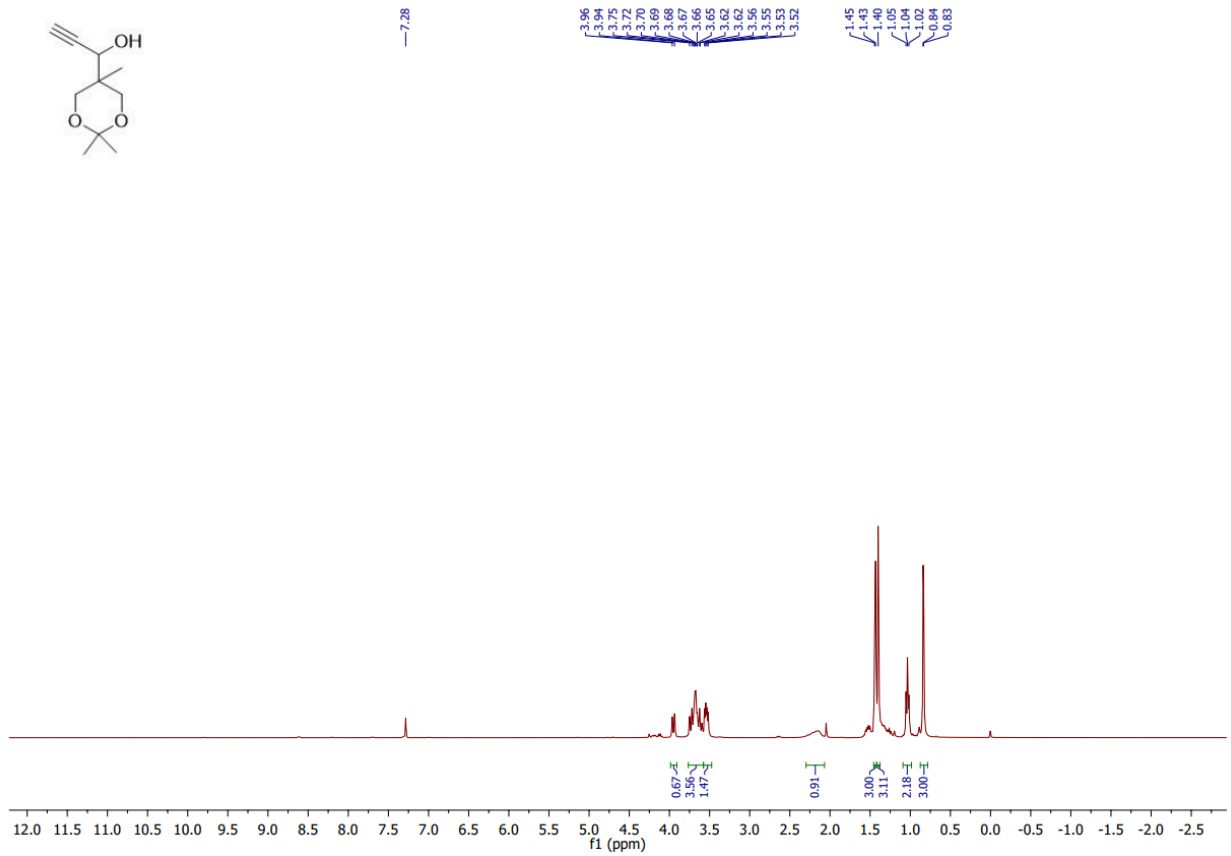


Figure 6. ^1H NMR of the product from the third step.

REFERENCES

[1] (a) “Druggability Of The Enzymes Of The Non-Mevalonate-Pathway”, Masini, T.; Kroezen, B. S.; Hirsch, A. K. H. Drug Discov. Today 2013, 18, 1256-1262. (b) “Isoprenoid precursor biosynthesis offers potential targets for drug discovery against diseases caused by apicomplexan parasites”, Hunter, W. N. Curr. Top. Med. Chem. 2011, 11, 2048-2059. (c) Hirsch, A. K. H.; Diederich, F. Chimia 2008, 62, 226- 230. (d) “The non-mevalonate pathway of isoprenoid precursor biosynthesis”, Hunter, W. N. J. Biol. Chem. 2007, 282, 21573-21577. (e) “Targeting Isoprenoid Biosynthesis for Drug Discovery: Bench to Bedside”, Oldfield, E. Acc. Chem. Res. 2010, 43, 1216-1226. (f) "The MEP Pathway: A New Target for the Development of Herbicides, Antibiotics and Antimalarial Drugs", Rodriguez-Concepción, M. Curr. Pharm. Des. 2004, 10, 2391-2400. (g) "The MEP Pathway and the Development of Inhibitors of Potential Anti-Infective Agents", Hale, I.; O'Neill, P. M.; Berry, N. G.; Odom, A.; Sharma, R. Med. Chem. Commun. 2012, 3, 418-433.

[2] (a) “Druggability Of The Enzymes Of The Non-Mevalonate-Pathway”, Masini, T.; Kroezen, B. S.; Hirsch, A. K. H. Drug Discov. Today 2013, 18, 1256-1262. (b) “Isoprenoid precursor biosynthesis offers potential targets for drug discovery against diseases caused by apicomplexan parasites”, Hunter, W. N. Curr. Top. Med. Chem. 2011, 11, 2048-2059. (c) Hirsch, A. K. H.; Diederich, F. Chimia 2008, 62, 226- 230. (d) “The non-mevalonate pathway of isoprenoid precursor biosynthesis”, Hunter, W. N. J. Biol. Chem. 2007, 282, 21573-21577. (e) “Targeting Isoprenoid Biosynthesis for Drug Discovery: Bench to Bedside”, Oldfield, E. Acc. Chem. Res. 2010, 43, 1216-1226. (f) "The MEP Pathway: A New Target for the Development of Herbicides, Antibiotics and Antimalarial Drugs", Rodriguez-Concepción, M. Curr. Pharm. Des. 2004, 10, 2391-2400. (g) "The MEP Pathway and the Development of Inhibitors of Potential Anti-Infective Agents", Hale, I.; O'Neill, P. M.; Berry, N. G.; Odom, A.; Sharma, R. Med. Chem. Commun. 2012, 3, 418-433.

[3] (a) 2018 WHO tuberculosis report: https://www.who.int/en/news-room/fact-sheets/detail/tuberculosis (accessed 04/10/20); (b) WHO 2019 malaria report: https://www.who.int/publications-detail/world-malaria-report-2019 (accessed 04/10/20)

[4] “Druggability Of The Enzymes Of The Non-Mevalonate-Pathway”, Masini, T.; Kroezen, B. S.; Hirsch, A. K. H. Drug Discov. Today 2013, 18, 1256-1262. (b) “Isoprenoid precursor biosynthesis offers potential targets for drug discovery against diseases caused by apicomplexan parasites”, Hunter, W. N. Curr. Top. Med. Chem. 2011, 11, 2048-2059.

[5] (a) “The isoprenoid-precursor dependence of Plasmodium spp.”, van der Meer, J.-Y.; Hirsch, A. K. H. Nat. Prod. Rep. 2012, 29, 721-728. (b) "Tuberculosis: The Drug Development Pipeline at a Glance", Villemagne, B.; Crauste, C.; Flipo, M.; Baulard, A. R.; Déprez, B.; Willand, N. Eur. J. Med. Chem. 2012, 51, 1-16. (c) "Molecular Players in Tuberculosis Drug Development: Another

Break in the Cell Wall", Squeglia, F.; Romano, M.; Ruggiero, A.; Berisio, R. Curr. Med. Chem. 2017, 24, 3954-3969. (d) "Recent Advances in Treatment for Tuberculosis: A Review", Padgilwar, S. S.; Manmode, R. S.; Sahare, A. Y.; Manish, K.; Manwar, J. V.; Warade, P. P.; Kumbhar, D. D. Int. J. Pharm. Sci. Rev. Res. 2016, 40, 162-172. (e) "Host Directed Therapies for Tuberculosis: Future Strategies for an Ancient Disease", Palucci, I.; Delogu, G. Chemotherapy 2018, 63, 172-180. (f) "Drug Discovery in Tuberculosis. New Drug Targets and Antimycobacterial Agents", Campaniço, A.; Moreira, R.; Lopes, F. Eur. J. Med. Chem. 2018, 150, 525- 545. (g) "Recent Therapeutic Approaches for the management of Tuberculosis: Challenges and Opportunities", Patil, K.; Bagade, S.; Bonde, S.; Sharma, S.; Saraogi, G. Biomed. Pharmacother. 2018, 99, 735-745.

[6] Ramsay, R., & Tipton, K. (2017). Assessment of Enzyme Inhibition: A Review with Examples from the Development of Monoamine Oxidase and Cholinesterase Inhibitory Drugs. *Molecules*, *22*(7), 1192.

[7] TCI America. (n.d.). TCI America. Retrieved from https://www.tcichemicals.com/en/us/product/name_reaction/Grignard_reaction.html

[8] Chesterman, J. P., Chen, F., Brissenden, A. J., & Amsden, B. G. (2017). Synthesis of cinnamoyl and coumarin functionalized aliphatic polycarbonates. Polymer Chemistry, 8(48), 7515–7528.