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Synthesis and Evaluation of Modifications to the 6,7,6-fused Ring Depsidone Core

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Abstract

Depsidones are 6,7,6-fused tricyclic natural products with a wide range of antibacterial activity (MIC = 0.0825-8 ppm). The *Spiromastix sp.* fungus, which was recently discovered in the deep sea near Japan, has been found to produce 15 new depsidone molecules known collectively as Spiromastixones A-O. This work involves the synthesis of B ring analogs with varying size and electronic profiles, and their effects on depsidones antibiotic activity. The core depsidone structure has been successfully synthesized, albeit with low yields, using a 4 step synthetic sequence involving copper catalyzed etherification and then esterification for ring closure. Modifications to this structure are currently underway to exchange the ester for both an amide and a thioester, as well as increasing the size of the central ring. Once synthesized, each depsidone analog will be evaluated for antibiotic activity against both Gram-positive and Gram-negative bacteria. The assays are expected to show how changes to the central ring affect biological activity. Modifications to the ester and ether functional groups are targeted for their electronic interactions with the active site for the molecule, whereas lengthening carbon chains of the starting materials will enlarge the central ring and show whether changing the size of the molecule strongly affects its activity.

Keywords: Synthesis, Spiromastixones, Modification

1. Background

From the outset of their use, antibiotics have become an ever-growing necessity in the modern world. However, as the struggle to isolate and create drugs with new properties and effects continues, the bacteria have evolved and developed resistances to a majority of the currently prescribed antibiotics. Due to this constant atmosphere of change in the biological world, the pharmacological spectrum must adapt just as quickly to produce effective modifications of existing drugs which are able to combat the growing threat of antibiotic resistant diseases.

One effective method for determining what compounds have strong antibiotic properties is through studying compounds that are produced when some organism competes naturally with bacteria. Various chemical compounds, some of which are assumed to have antibacterial properties, are produced by the species in competition and can be elucidated using IR and NMR technology. One such species is a lichen, which is a composite organism of algae and/or cyanobacteria.[1] Lichens have been known as a medicinally useful organism for ages,[2] and are a relatively straightforward starting point for isolating and developing new drugs. It has been found experimentally that lichen products produced as secondary metabolites[2,3] possess effective antibacterial properties depending on environmental stimuli.[4]

Depsides are one class of compounds produced by lichens, which consist of two phenolic rings bound by an ester linkage[5] as shown in Figure 1. These compounds exhibit a variety of medicinal properties, such as anticancer[6], antioxidant, antiviral, and analgesic effects[7], but it is their cyclized derivatives, known as depsidones, which have been shown to be active antibacterial agents against Gram-positive bacteria such as *Staphylococcus aureus*.[2,4,8] This cyclization to form a depsidone from a depside is accomplished through an ether linkage created between the two

phenolic rings, forming a seven-membered ring between them (Figure 2).The formation of this second linkage appears to reduce steric interference caused by the hydroxy group from the depside and helps to increase the antibiotic activity of the other substitutions on the phenolic rings in the depsidone.

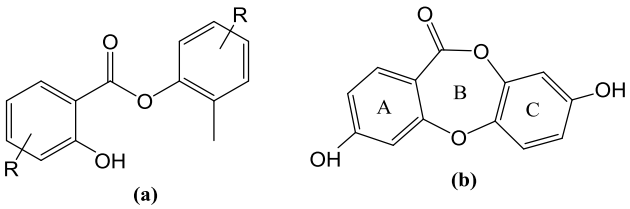


Figure 1. Depside (a) and depsidone (b) structures

Neither depsides nor depsidones are found in large quantities naturally outside of lichen production[9], therefore pathogenic bacteria may have not yet built up a resistance against them. Many structural variations of depsides and depsidones exist in nature, and while some have had their biological targets isolated, many targets are still unknown, especially in regard to their antibacterial activity.

Recently Niu, et al. isolated a new subset of depsidones, Spiromastixones A-O, from the *Spiromastix sp.* Fungus, and it was determined that the position and number of substituted chlorines on the rings greatly determined the effectiveness of the depsidone as an antibacterial agent.[8] For the purposes of this experiment, Spiromastixone (I) (Figure 2), whose minimum inhibitory concentration (MIC) was 2-4 µg/mL in tests against three different Gram-positive bacteria, was identified as a viable synthetic target for simple modification of its B-ring.[8] Potent activity of this compound compared to other depsidones in the series was attributed to the large number of chlorine substituents on the aromatic rings. In testing modified synthetic depsidone analogs, it was expected to be determined whether the central ring had any effect on the antibiotic activity of depsidone molecules.

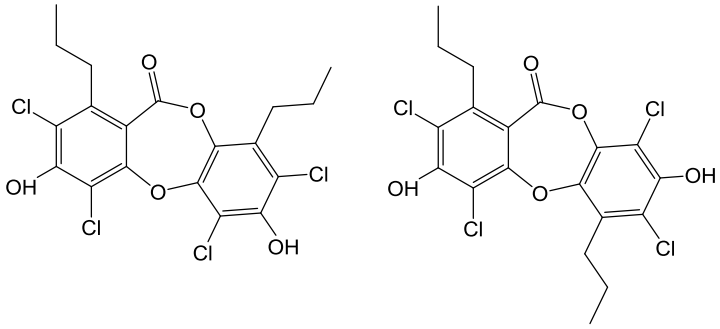


Figure 2. cis- and trans- isomers of Spiromastixone (I)

Seemingly the most effective method for synthesizing depsidones is to first produce the phenolic rings, then combine them through the ester linkage and finally cyclize to form the ether. There are two highly effective methods for doing this. The first of these is known as *para*-depside oxidative coupling[3] and is achieved through combination of β-orcinol derivatives,[10,11] the structure of which is shown in Figure 3. Although this is the proposed biosynthetic pathway (Figure 3) for most depsidones[3,12], it is has only been shown to work experimentally for a few compounds The second of these methods is known as a grisadienedione interconversion and involves transitioning through a pentacyclic center ring intermediate before forming the heptacyclic product.[13]

Both pathways are supported by the natural biosynthesis of depsidones, though neither seems to dominate.[3] Experiments on each are widely found in literature, with researchers in the Elix lab seemingly favoring the oxidative coupling method whereas others use a mixture of the two. Due in part to the quantity of research by Elix and collaborators used in this experiment, the oxidative coupling method appears more reproducible with many similar pathways to take for modification. This also appears to be the simplest path to take due to steric hindrance caused by the intermediate cyclic structure formed in the grisadienedione method.

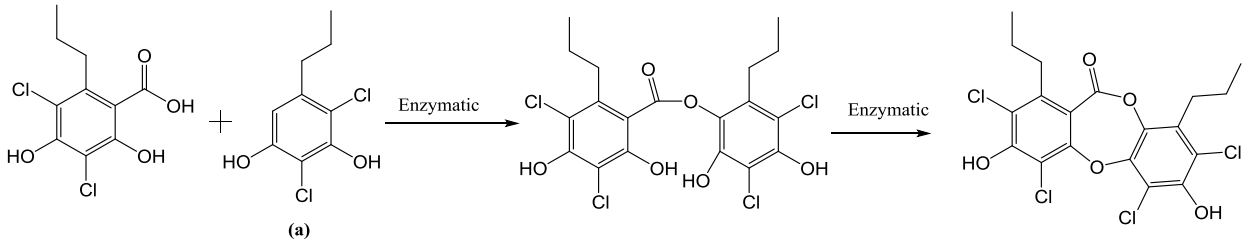


Figure 3. para-depside oxidative coupling biosynthetic pathway through β-orcinol derivatives (a) for depsidones[12]

The overall goal of this project was to successfully synthesize and modify B-ring analogs of the spiromastixone core structure in order to enhance its antibacterial properties. Synthesis pathways for the oxidative coupling and grisadienedione synthesis of Spiromastixone (I)[8] are shown in Figures 4 and 5 respectively. For the purposes of this experiment, the *para*-depside oxidative coupling method was chosen for reasons already stated, though the grisadienedione pathway was a reliable second option.

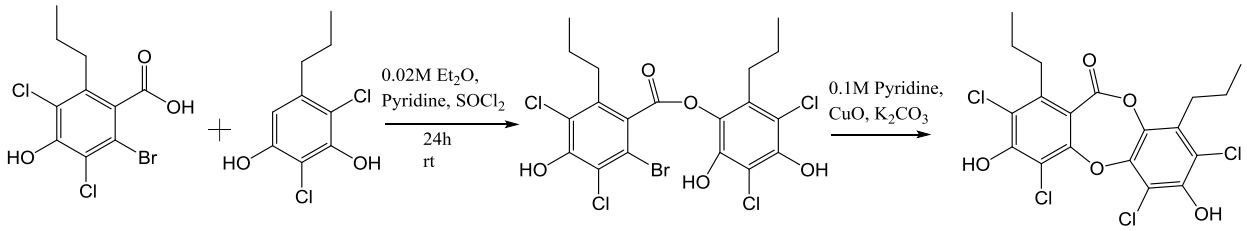


Figure 4: Proposed para-depside oxidative coupling synthesis pathway for Spiromastixone (I)[13]

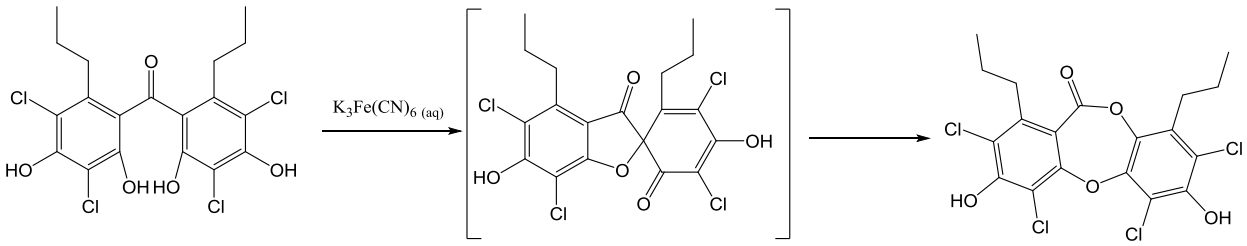


Figure 5. Proposed grisadienedione synthesis pathway for Spiromastixone (I)[13]

Modifications to the B-ring included both electronic and size changes in order to change activity with the target active site. The initial targets of these changes are shown in Figure 6. The electronic modifications were limited to substitution of the oxygen atoms for those of similar polarity, while steric modifications explored central ring sizes ranging from 7-13 membered systems. Each of the different analogs synthesized from these changes are in the process of being tested in bacterial assays for antibiotic properties. Although none of them contained the aromatic substituents, it was hypothesized that the central ring itself could contribute to the overall antibacterial quality of the depsidone compound, and shifting or changing any of its composition would have shown this effect.

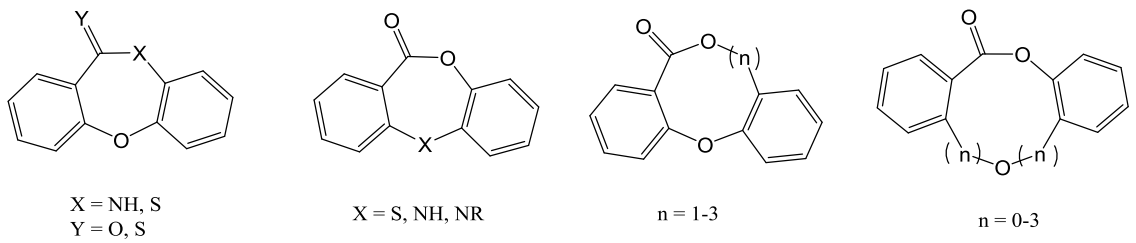


Figure 6. Modification of the central ring

2. Methods

2.1 General Procedure For Esterification Using Acetic Anhydride.

The carboxylic acid was combined with a phenol derivative in acetic anhydride (0.1 M) in a 1:1 molar ratio in a round bottom flask, which was warmed to 80 ˚C and stirred for 12 h. Upon completion, the reaction was diluted with ethyl acetate, washed with H_2O, washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The crude reaction was purified via normal phase column chromatography (SiO_2, 10% ethyl acetate/hexane).

2.2 General Procedure For The De-Protection Of Acetyl Alcohols.

Ester coupled products were combined with K_2CO_3 in a 1:4.25 molar ratio and dissolved in methanol (0.14 M) in a round bottom flask, which was stirred at 23 ˚C for 30 min. Upon completion, the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted ethyl acetate (3x). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure.

2.3 General Procedure For Intermolecular Esterification With Thionyl Chloride.

The carboxylic acid and phenol derivative were combined with thionyl chloride and anhydrous pyridine in a 1:1:3:3 ratio in THF (0.1 M), along with a catalytic amount of DMF (Scheme 1b), in a dried and purged (N_2(g) or Ar(g)) round bottom flask, which was stirred at 23 ˚C. After 2 h, anhydrous pyridine and the phenol derivative were and the reaction was allowed to stir for 24h. Upon completion, the reaction was diluted with ethyl acetate, washed with 1M HCl, washed with saturated aqueous $NaHCO_3$, washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified via normal phase column chromatography (SiO_2, 10% EtOAc/hexane).

2.4 General Procedure For Intramolecular Copper Coupling.

The carboxylic acid was combined with K_2CO_3 in a 1:2.5 molar ratio, along with a catalytic amount of CuO, in a dried and purged (N_2(g) or Ar(g)) round bottom flask, and these solids then were dissolved in anhydrous pyridine (0.1 M). This reaction was attached to a flame dried reflux condenser, submerged in a 115 °C oil bath, and allowed to stir for 48 h. Upon completion, the reaction was filtered through a fritted funnel and washed with ethyl acetate. The filtered reaction was poured into a separatory funnel, washed with saturated aqueous $NaHCO_3$, washed with 1M HCl, washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The crude reaction was purified via normal phase column chromatography (SiO_2, 10% EtOAc/hexane).

2.5 General Procedure For Methyl Ester Protection Of Benzoic Acids.

The carboxylic acid was dissolved in methanol (1.0 M) and a catalytic amount of H_2SO_4 in a round bottom flask, which was warmed to 65 ˚C and stirred for 12 h. Upon completion, the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate (2x). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure.

2.6 General Procedure For Intermolecular Copper Coupling.

The carboxylic acid was combined with a phenol derivative and K_2CO_3 in a 1:2:2.5 molar ratio, along with a catalytic amount of CuO, in a dried and purged (N_2(g) or Ar(g)) round bottom flask, and these solids were dissolved in anhydrous pyridine (0.1 M). This reaction was attached to a flame dried reflux condenser, submerged in a 115 ° C oil bath, and allowed stirred for 48 h. Upon completion, the reaction was filtered through a fritted funnel and washed with ethyl acetate. The filtered reaction was poured into a separatory funnel, washed with saturated aqueous $NaHCO_3$, washed with 1M HCl, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude reaction was purified via normal phase column chromatography (SiO_2, 10% EtOAc/hexane).

2.7 General Procedure For Methyl Ester De-Protection.

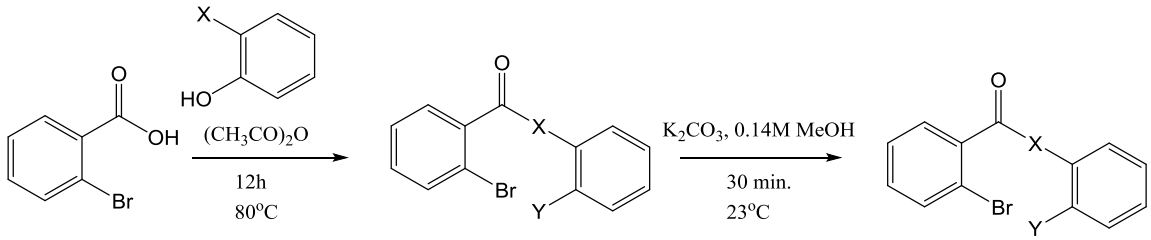
The benzoate derivative was dissolved in methanol (0.93 M) and 6M NaOH (0.59 M) in a round bottom flask, which was warmed to 60 ˚C and stirred for 1 h. Upon completion, the reaction was quenched with 1M HCl and extracted with ethyl acetate (2x). The organic layers were then combined, dried over Na_2SO_4, and concentrated under reduced pressure.

2.8 General Procedure For Intramolecular Esterification With Thionyl Chloride.

The carboxylic acid was combined with thionyl chloride and anhydrous pyridine in a 1:3:3 ratio in THF (0.1 M) along with a catalytic amount of DMF (Scheme 2) in a vial, which was warmed to 40 ˚C and stirred for 24 h. Upon completion, the reaction was diluted with ethyl acetate, washed with 1M HCl, dried over Na_2SO_4, and concentrated under reduced pressure. The crude reaction was purified via normal phase column chromatography (SiO_2, 10% EtOAc/hexane).

3. Results

The para-depside oxidative coupling method was first used to attempt synthesis of the desired B ring depsidone analogues (Scheme 1). For this initial process, coupling of the phenyl rings was preceded by benzyl protection of catechol in order to selectively protect one of the two alcohols as benzylation gave monobenzyl catechol in a 19% yield. This step proved inefficient due to the dibenzylated product and starting material both being left in solution after optimization. Due to the symmetric nature of catechol, however, it was determined that this step was unnecessary and it was discarded immediately.

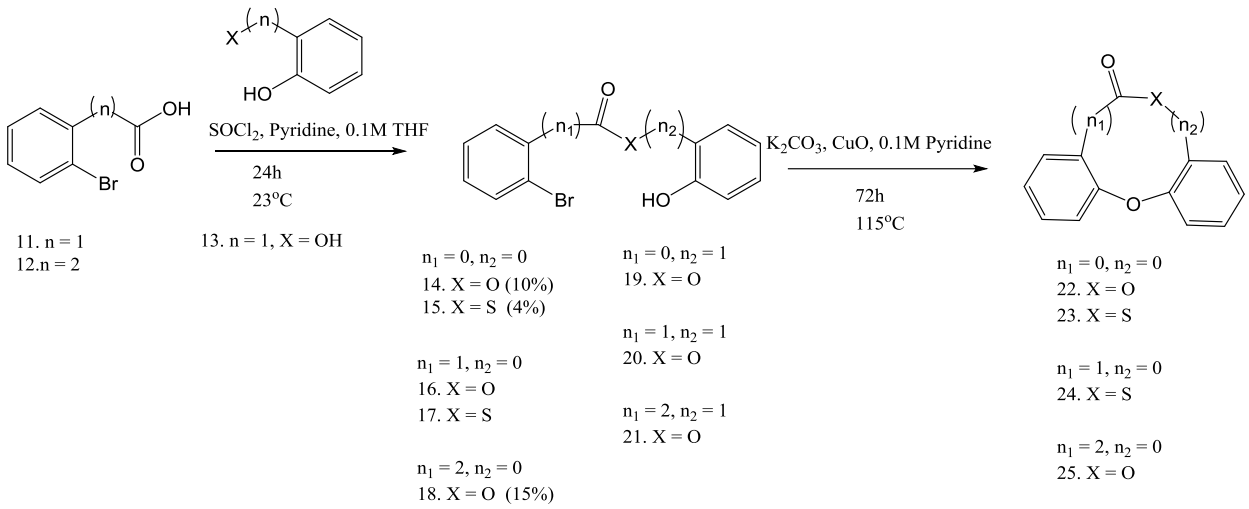


1. X = OH
2. X = NH_2
3. X = SH

4. X = O, Y = OAc (98%)
5. X = O, Y = NH_2
6. X = NH, Y = OAc (46%)
7. X = S, Y = OAc (28%)

9. X = NH, Y = OH (15%)
10. X = S, Y = OH (70%)

Scheme 1a: Para-depside oxidative coupling with acetic anhydride



Scheme 1b: Para-depside oxidative coupling with thionyl chloride

As shown in Scheme 1a, catechol (**1**) and 2-bromobenzoic acid were combined in acetic anhydride to give acylated diphenyl ester (**4**) where rather than obtaining the desired product (**14**) with a free hydroxyl, the oxygen was inadvertently protected. The desired product was obtained through reaction under basic conditions in aqueous solution in order to de-protect the alcohol. Modifications using 2-aminophenol (**2**) and 2-mercaptophenol (**3**) yielded acetylated products (**6** and **7**) at 46% and 28% respectively after one run of each, and normal phase columns were run on each reaction to isolate products. Product spots for the reaction of 2-aminophenol showed a 50:50 mixture of products **5** and **6**. While both products were collected and stored, yield was only determined for the amide product (**6**). After confirmation of structure through ^{1}H-NMR, de-protection of the acetylated hydroxyl groups was performed under basic conditions. Column separation was not required for analysis of these reactions, as thin layer chromatography (TLC) showed one spot lower than that of the starting materials. ^{1}H-NMR again confirmed the identity of the amide (**9**) and thioester (**10**) products, though at 15% and 70% respectively.

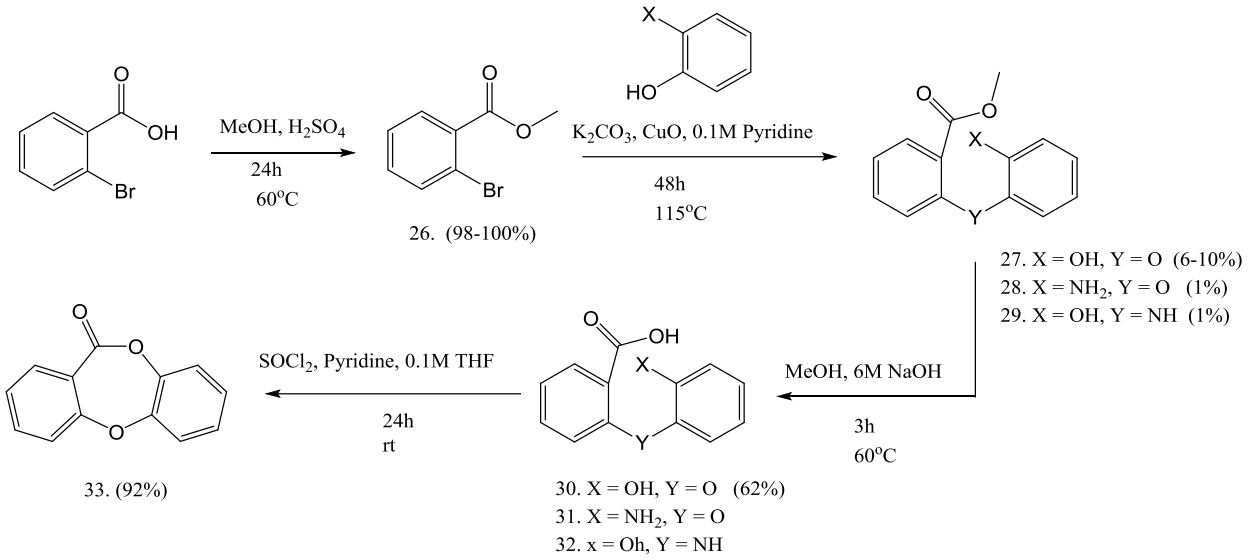
A modified version of the previous reactions (Scheme 1b) was performed in order to eliminate overprotection of the phenol starting products during ester coupling. Optimization for the process was accomplished under three different sets of conditions shown in Table 1.

Table 1. Reaction Conditions For Coupling Optimization

Reaction	$SOCl_2$	Pyridine	Catechol addition	Temperature
14A	3 equivalents	20 equivalents	After 5 hours	Room Temp.
14B	3 equivalents	10 equivalents	After 2 hours	50^0C
14C	3 equivalents	3 equivalents	After 2 hours	Room Temp.

Conditions used for Product **14**C were the best by yield production, though only at 10% yield, thus these conditions were used for the remainder of the reactions using this scheme. The only yields able to be obtained for these were for Products **14**, **15**, and **18**, and amounted to 10%, 4%, and 15% respectively. Other products were unable to be measured for yield due to unclear ^{1}H-NMR data. These yields were low, though gave enough product to continue and attempt ring closing. However, NMR data for the subsequent ether formations were all inconclusive and no conclusions could be drawn.

After encountering the issues seen through low yields for the para-depside oxidative coupling method, it was decided, this was ineffective for the purposes of the experiment. Instead, it was determined that coupling through the ether first would be more effective, as outlined by Deraeve et. al[14]. This process involved both a protection[15] and de-protection[16] reaction due to the highly reactive nature of the carboxylic acid, resulting in a longer overall synthesis scheme (Scheme 2). Protection of 2-bromobenzoic acid to form Product **26** worked at 98% and 100% yields the two times the reaction was carried out. This protection step was to ensure non-reaction of the carboxylic acid with the hydroxy group becoming de-protonated during ether formation.



Scheme 2: Proposed Synthesis through Ether Coupling

The ether coupling step was expected to occur at higher yields than in other processes due to the lessened steric hindrances caused by the intermolecular nature of the reaction rather than intramolecular. Filtration of the product through a fritted funnel before extraction was also undertaken in order to better visualize extraction layers and reduce interference in the column and data analysis by copper by-products. However, the reaction still occurred with yields in the range of 1-10%. A normal phase column was used to isolate products, and the product was determined to be that desired (**27**) through ^{1}H-NMR. De-protection of the carboxylic acid after ether coupling was also a straightforward process and gave the desired product (**30**), though only at a 62% yield, much lower than that of the original protection reaction. This was still a fairly good yield compared to other reactions within the experiment, however, and was sufficient to proceed to the final ring closure step. The reaction also showed only one product by TLC, indicating complete reaction. This reaction could easily be optimized to produce better yields, but this was not undertaken for the purposes of this project.

The final step of the synthesis process involved esterification to completely close the central ring, forming the desired product (**33**). TLC showed a completed reaction, and the identity of the product was confirmed through both ^{1}H-NMR and ^{13}C-NMR. Intramolecular esterification, however, was far more successful than the original intermolecular process, occurring at a 92% yield.

After the success of synthesizing the fully oxygenated central ring, modification to add nitrogen substituents was begun. The coupling of Product **26** with 2-aminophenol eluted two different spots by normal phase column chromatography, on which another column separation was performed for each. ^{1}H-NMR data for the two purified compounds revealed two desired products (**28** and **29**). However, these compounds could not be completely characterized due to rotomers of each causing multiple similar peaks on ^{1}H-NMR data. Yields for this reaction were extremely low (0.58% and 1.2%), partially due to the formation of two products rather than one, but further reactions were still carried out for complete synthesis and characterization.

De-protection of the coupled products was carried out in the vial used to collect them due to the small amount available. Due to this, the reactions were done at 40˚C over a period of 24 hours rather than heated to 60˚C for 3 hours. Extractions were also carried out in these vials using small amounts of 1N HCl and ethyl acetate with physical separation and filtration by pipette. The first product to elute from the Product **31** reaction formed an orange-brown solid upon de-protection, and the second product to elute from the Product **31** formed a darker brown product. This reaction occurred at high yields and proved successful by ^{1}H-NMR.

4. Future Work

Reactions were also started using scheme for Products **26-33** to create the analogs shown in Figure 7. Neither of the reactions proceeded past the ether coupling reaction, however, and were not completely analyzed for further reaction.

The steric analogs proved extremely difficult to isolate by column chromatography. Future work for the modification of depsidone compounds for use as antibiotics should first of all incorporate these targets, as well as those with modifications to add sulfur and steric modifications to different positions. Each analog synthesized should also be subjected to bacterial assays for both Gram-positive and Gram-negative bacteria. From this information, further possible work could be done to infer the identity of the biological target for the antibacterial activity of these molecules with changes in inhibition due to modifications.

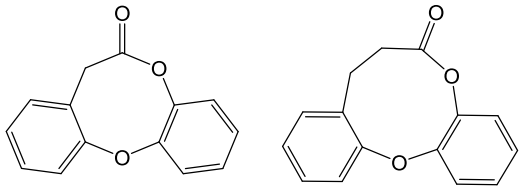


Figure 7: Current Analog Targets

5. Conclusion

The initial stages of this experiment were carried out in order to determine the most efficient method of synthesizing the depsidone core structure. Through multiple method modifications, this goal was accomplished with the successful synthesis of Product **33**. The individual steps for the final synthesis scheme worked at relatively high production yields except for the etherification coupling step, which significantly lowered the yield of the scheme as a whole. However, as the goal was to test successful products against bacterial assays, low yields were not a primary concern. From this, modifications were carried out to synthesize amine and amide modified analogs at similar yields. Future goals for this experiment include continued steric and electronic modification of the depsidone B-ring along with bacterial assays for successfully synthesized products.

6. Acknowledgements

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