ABSTRACT

SYNTHESIS OF A DIISOBUTYRATE VARIENT OF A SIMPLIFIED GRACILIN A DERIVATIVE TOWARD INCREASED SERUM STABILITY

Diego Antonio Valdes

Director: Professor Daniel Romo

Gracilin A, a natural product of the marine sponge, *Spongionella Gracilis*, has shown promising neuroprotective and immunosuppressive effects. The targeted derivatives exemplify their use as a potential treatment for breast cancer in comparison to cyclosporine A which is known to interact with cyclophilins A and D. In addition, gracilin A has a potential role in inhibiting the cyclophilin A calcineurin pathway to increase sensitivity to necrosis as a potential cell death pathway for breast cancer. The diisobutyrate variant of a simplified gracilin A derivative was targeted for synthesis and subsequent study in a serum stability assay. It was hypothesized that by increasing the sterics around the ester functionality of the bis-acetixy furan of this gracilin A derivative, this derivative, will have greater stability in serum, due to the decreased rate of hydrolysis compared to previously synthesized derivatives of gracilin A.

APPROVED BY DIRECTOR OF HONORS THESIS

_____

Dr. Daniel Romo, Department of Chemistry

APPROVED BY THE HONORS PROGRAM

_____

Dr. Elizabeth Corey, Director

DATE: _____

SYNTHESIS OF A DIISOBUTYRATE VARIENT OF A SIMPLIFIED GRACILIN A DERIVATIVE TOWARD INCREASED SERUM STABILITY

A thesis submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

By

Diego Antonio Valdes

Waco, Texas

July 2021

TABLE OF CONTENTS

TABLE OF FIGURES

ACKNOWLEDGMENTS

I would like to thank Dr. Romo for allowing me to be a part of the Baylor Underdraduate MiniPharma Program. Thank you to the biological assays team and the information they provided for the completion of this thesis. Thank you to Dr. Ken Hull and the modeling group for identifying synthetic targets to pursue. Finally, thanks to Dr. Haoran Xue for mentoring me and guiding me through this project.

CHAPTER ONE

Introduction to Gracilins

The gracilins are natural products isolated from the marine sponge, *Spongionella gracilis,* that were found to exhibit neuroprotective and immunosuppressive effects. Previous studies done led the way for the production of several gracilin A derivatives using a retrosynthesis strategy developed by the Romo group (Abbasov et. al., 2019).

The Romo group and MiniPharma Team have recently targeted and synthesized a variety of gracilin A derivatives in order to determine which variations of the gracilin A molecule have the greatest binding affinity for cyclophilin D. In addition, these variants are also being targeted for the selectivity between cyclophilins A and D. The compound that I am attempting to synthesize has a binding score of -91 for cyclophilin D and -61 for cyclophilin A, indicating potential cyclophilin D selectivity. The MiniPharma team is working to synthesize this specific target in order to test the target's stability in serum and so that the biological assays team may perform studies to determine neuroprotective and immunosuppressive effects of certain derivatives. In addition, the biological assays team current focus is on the cyclophilin proteins role in cancer, which is discussed in the following chapter. This thesis will discuss the biological activity focusing on cancer and how to increase sensitivity to necrosis as a potential for breast cancer treatment.

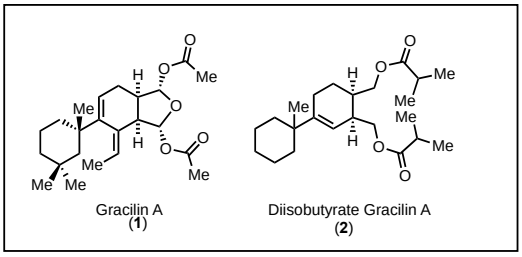


Figure 1: Gracilin A and Diisobutyrate Gracilin A

The targeted di-isobutyrate variant of gracilin A (**2**, Fig. 1) was sought as it was anticipated to have increased serum stability

studies due to ester group variations. It was proposed that by increasing sterics around the ester functionality, (as seen in (Fig. 1)) the rate of hydrolysis should decrease, increasing the molecules half-life in serum.

This hypothesis is premised on the notion that esterases in blood that would typically have the ability to cleave the small alkyl esters, such as methyl or ethyl, will hydrolyze the designed diisobutyl group more slowly, due to increased bulk (UCLA Chem). The longer the molecule maintains its structural integrity, the longer the molecule's half-life, and as a result the more effective the molecule might be as a potential drug lead.

Compounds with greater selectivity for cyclophilin D have greater potential to be developed as treatments for neuroprotection (Abbasov et. al., 2019). These scores are determined by the molecular modeling team using MOE software. Although the team is concerned with the docking scores of my compound the most important aspect of my compound is determining the rate at which esterase's would cleave the compound in serum.

CHAPTER TWO

Cancer Biology as it relates to Gracilins

Toward investigating the potential of gracilin A as an anticancer agent, the Romo Group is collaborating Taube lab (Baylor, Dept. of Biology). Currently the MiniPharma Biological Assay Team is focused on the inhibition of the cyclophilin A calcineurin pathway to increase sensitivity to necrosis as a cell death pathway for potential breast cancer treatment. Competitive inhibition of the calcineurin pathway increases intracellular calcium. Through the use of thapsigargin induced cell necrosis the biological assays team is able to measure differences in cell death. The cells being compared are those with vehicle controls versus those induced with FK506, which is the competitive inhibition of the calcineurin pathway to increase intracellular calcium (Tong et. al, 2015). In addition, these cells are also being compared to those whose necrosis is induced using thapsigargin, and they are being compared to cells given both, FK506 and thapsigargin (Janssen et. al., 2009).

Prior to the research described in the previous paragraph the MiniPharama Biological Assay Team was researching cyclophilin A and cyclophilin D and their role in apoptosis and necrosis, the two mechanisms of cell death. Apoptosis is programmed cell death and necrosis is unplanned cell death due to uncontrolled external conditions (Panalawa, 2017). In relation to apoptosis these cyclophilin proteins are tumor suppressing, therefore the team did not feel the need to investigate this pathway further. However, relating to necrosis, unplanned cell death, these cyclophilin proteins act as oncogenes for breast cancer. This means that these proteins promote properties similar to those exhibited in cancer cells, such as the evasion of cell death and uncontrolled cell growth typical of

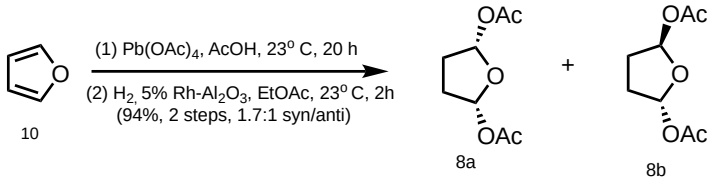
most cancer cells (Zhu, 2018). Through further investigation the biological assays team was able to determine that cyclophilin D is an oncogene for estrogen receptor positive cells (Machida et. al., 2006). What this means is that cells of this type have receptors that, when estrogen binds to these receptors, allow the cell to grow in an uncontrolled fashion. Typically, an anti-estrogen hormone is used to block cell growth and treat this variety of breast cancer. An anti-estrogen hormone would be an endocrine hormone of some kind. The most common treatment for estrogen receptor positive breast cancer is through the use of selective estrogen receptor modulators, which bind to estrogen receptors on cancer cells preventing estrogen from binding and promoting cell growth (Mayo Clinic, 2020).

Furthermore, the biological assays team found that the protein cyclophilin A is an oncogene for triple negative breast cancer cells (Nigro et. al., 2013). A triple negative breast cancer cell is one that does not have any of the typical surface receptors for the common hormones in other breast cancer types such as, estrogen, progesterone, and human epidermal growth factor 2 or HER2 (CDC, 2020). This means that physicians are left with fewer treatment options, like the previously described anti-estrogen hormone treatment used for estrogen receptor positive cells. However, chemotherapy is still an effective option. Unfortunately, those afflicted with triple negative breast cancer typically have the lowest rate of survival compared to the other classes of breast cancer that do have surface level receptors for some variety of hormone (CDC, 2020).

CHAPTER THREE

Prior Syntheses of Bis-acetoxyfuranoses and their Relation to this Synthetic Strategy

A number of analogues of gracilin A are synthesized so that their structure activity relationship can be studied and determined. However, we targeted the gracilin A derivative (**2**, Fig. 1) in order to study its stability in human plasma as described above.

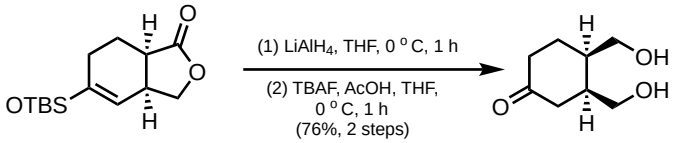


Scheme 1: Synthesis of a Highly Simplified Analogue

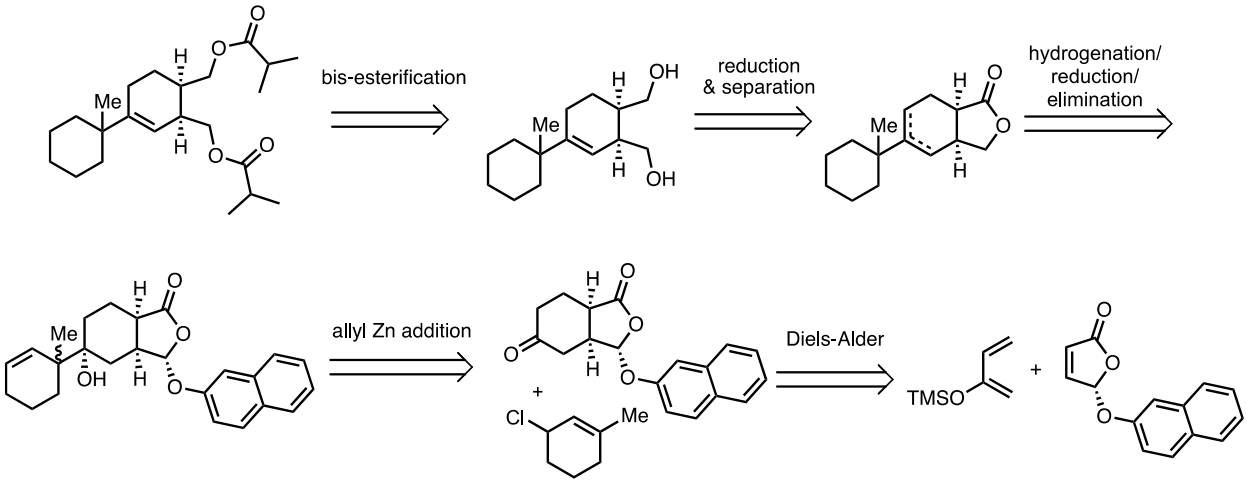
The targeted gracilin A derivative is premised on the bis-acetoxy derivative (2) initially identified through the application of pharmacophore directed retrosynthesis applied to gracilin A (Abbasov, et. al., 2019. Our synthesis strategy toward this derivative is premised on the prior synthesis of the bis-acetocyfuranose (Scheme 1). From the simple bis-acetoxyfuranose (**10**) a two-step oxidation/hydrogenation sequence was performed which delivered the bis-acetoxyfuran.

The proposed synthesis, following from previous work specifically involves reduction of a bicyclic lactone (**15a)** to a diol intermediated (**16**) (Scheme 2). The conditions of the reaction in comparison as well are very similar. The reduction is performed using lithium aluminum hydride in tetrahydrofuran at 0^{o}C for one hour (Scheme

2). These conditions are the exact same as the first part of the final step in my synthesis shown in scheme 4.



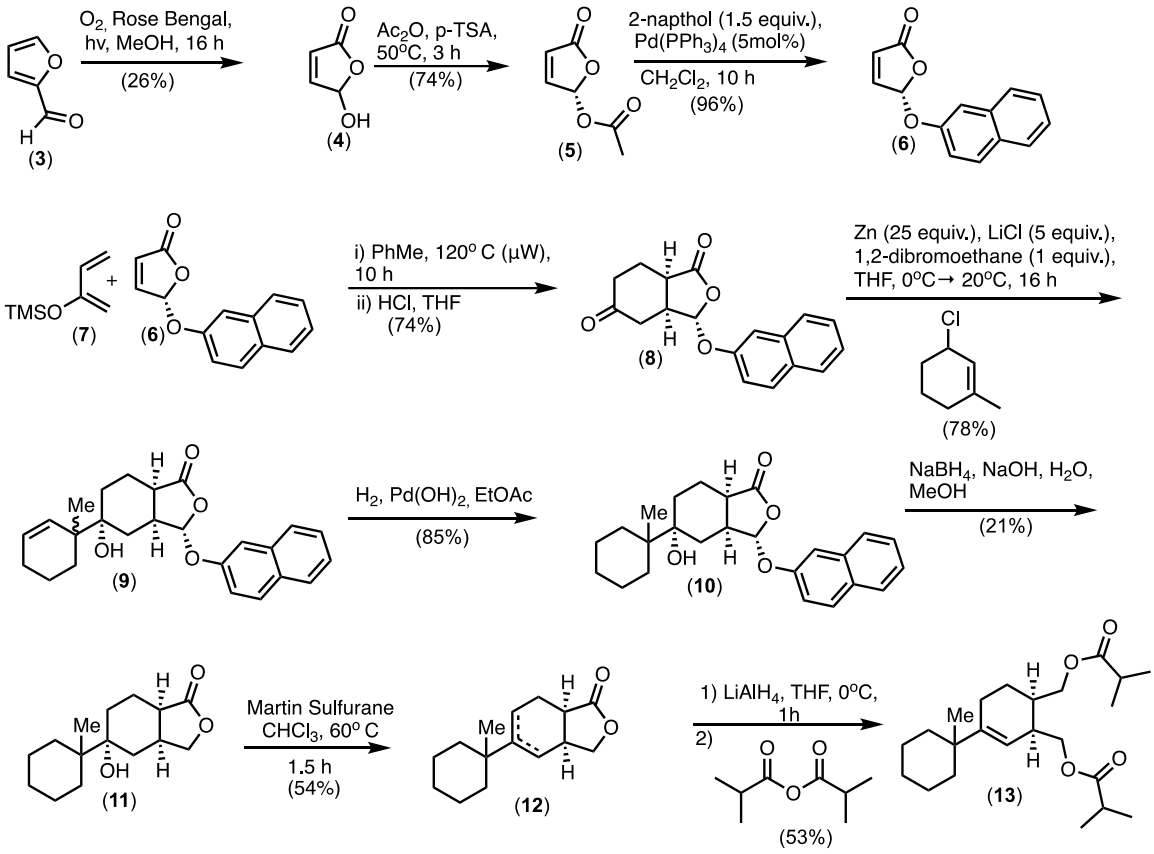
Scheme 2: Assembly of Bicyclic Core



Scheme 3: Retrosynthesis

Key steps of the synthesis have been included in the above retrosynthesis (Scheme 3). The first step shown in the retrosynthesis is the bis-esterification to yield the diisobutyrate derivative of gracilin A, the final product of synthesis. Following that is a reduction using lithium aluminum hydride and separation to yield the dimethanol product. A hydrogenation/reduction/elimination reaction yields the reduced lactone, lacking the naphthol group of the previous compound. An allyl zinc addition is then performed using a 3-chloro-1-methylcyclohex-1-ene compound which had been synthesized prior. This yields the desired cyclohexane group attached to larger bicyclic structure. A Diels-Alder

reaction is performed followed by an acid work up using hydrochloric acid in tetrahydrofuran provides the desired bicyclic structure, which allows the entire synthesis to move forward.



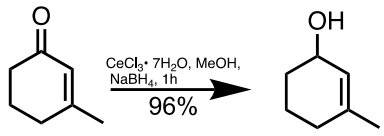
Scheme 4: Complete Forward Synthesis

In comparison with my synthesis scheme (Scheme 4) it is easy to see the similarities in technique and methods used to derive both my own target molecule and the originally derived gracilin A analog (Abbasov et. Al, 2019). My synthesis began with the distillation of furfural. It is important that it is distilled using a heating block and a stir bar, and to ensure that the hydroquinone was not allowed to dry out. O_2 was then bubbled gently into a mixture of menthanol and Rose Bengal under a bright light for 16 hours providing the

desired substrate (**3**). Then the free hydroxyl group (4) was acetylated using pTSA and acetic anhydride. The next step in the synthesis was the addition of the naphthol group using palladium as a catalyst in order to prepare the dienophile (**6**) for the upcoming Diels-Alder reaction. Following the addition of the naphthol group was a Diels-Alder reaction performed under microwave conditions followed by an acid workup to yield the desired product (**7**).

In order to perform the next step in my synthesis the ketone seen in scheme 5 was reduced to an alcohol using sodium borohydride and methanol. I found that separation in methanol is difficult to see, so brine was used to assist the separation. The alcohol was then chlorinated using NCS to yield the desired reagent for my synthesis. The dimethyl sulfide and substrates were added under nitrogen atmosphere. To test the completion of the chlorination a complete mini workup was performed and an NMR was taken to confirm the desired product was formed.

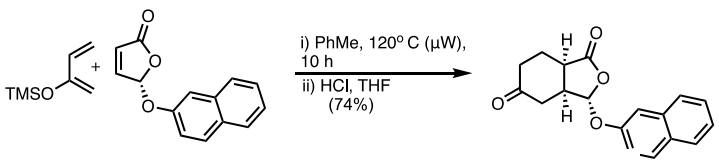
Scale up synthesis of intermediates within my synthesis such as the reduction of 3-methylcyclohex-2-enone to an alcohol using sodium borohydride and methanol (Scheme 5) provides immediate access to the desired substrates required to complete the synthesis. By doing large scale production of this alcohol, I am able to utilize this intermediary molecule much more quickly and perform a greater number of reactions in the lab.



Scheme 5: Synthesis of Alcohol Reagent

Using the previously generated chloride reagent a 1,2 addition of the chlorination product was performed yielding the desired tricyclic structure (**9**, Scheme 4). Once the

tricyclic structure was formed the alkene was to hydrogenated using an H_2 balloon and palladium hydroxide on carbon as a catalyst. The crude product (**10**, Scheme 4) was then carried over to the next step without further purification. In order to move closer to the desired synthetic target the naphthol group needed to be removed. This was done using sodium borohydride and methanol in order to yield the desired lactone (**11**, Scheme 4). The product was not UV active, so in order to confirm the completion of the reaction a Hanessian stain was used for TLC visualization. Dehydration of the hydroxyl group was achieved using Martin Sulfurane in chloroform. Martin Sulfurane had to be weighed carefully in a glove box and then put into a vial since it is very hydroscopic. The substrate had to be added, then Martin Sulfurane and then chloroform. Once they were added the flask will be purged with nitrogen three times. The starting material (**11**) did not stain on TLC with $KMnO_4$ but the product (**12**) did, which made tracing this reaction straight-forward using this stain. The product of the dehydration (**12**) was reduced to a diol using $LiAlH_4$ in THF. In order to test completion a TLC was taken. I noted that the starting material could not be seen on TLC by UV, and the product could be visualized using a Hanessian stain. The diol was converted to the diisobutyrate variant of the gracilin A derivative using DMAP and acid anhydride to yield the diisobutyrate derivative of gracilin A (**13**).



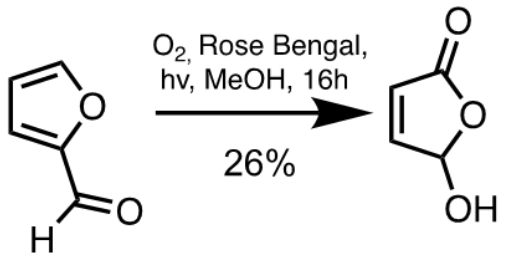
Scheme 6: Diels-Alder Reaction

Another intermediary target that proved highly useful was the product of a Diels-Alder reaction (Scheme 6). This Diels-Alder reaction was performed using a TMSO protected diene and the previously synthesized dienophile. Again, the large-scale production of this product allowed me to perform a great number of reactions without having to start the synthesis from scratch. Due to the limitation of performing this reaction in a microwave the largest scale this reaction could be performed on was the 2-gram scale. This would yield about 1.5-grams of product. This technique of using intermediary targets is not unique to my synthesis. As previously mentioned, this technique is demonstrated in the original pharmacophore directed retrosynthesis of gracilin A. Using techniques and known reaction sequences from prior synthesis of natural products I was able to more effectively attempt to synthesize my molecule.

After completion of the synthetic route the diisobutyrate variant of a gracilin A derivative is to be used in future serum stability assays. Although I was able to complete the entire synthesis route the amount of final product was too little to be effectively utilized in serum stability assays. In the future the Baylor MiniPharma Team will continue to attempt to synthesize variants of the gracilin A derivative. The synthetic route (Scheme 4) will be repeated by future members of the Baylor MiniPharma Team to yield a greater amount of the desired variant so that it may be used in serum stability assays in the future.

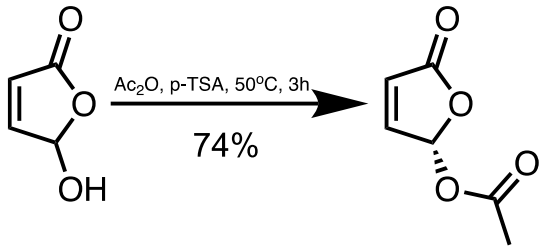
CHAPTER FOUR

Experimental Procedures



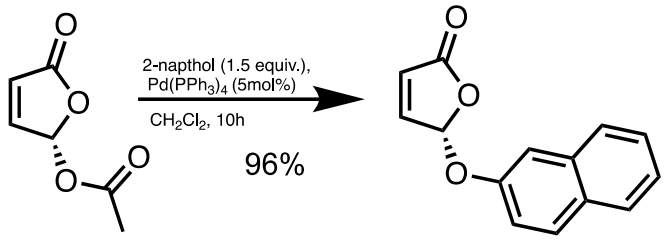
Scheme 7: Oxidation of Furfural

5-hydroxyfuran-2(5*H*)-one. In a 250ml round bottom flask furfural was distilled at a reduced pressure from hydroquinone before use. A 3-neck 1L round bottom flask with a stir bar and condenser was charged with rose bengal (4.2g 4.3mmol 0.2% mol/eq.), furfural (205g, 2133.6mmol, 0.002 eq.), and methanol(967mL). Oxygen was bubbled into the solution using a fritted glass tube. Fans were used to keep the reaction below 33^{o} C and tungsten lamps were used to irradiate the reaction. TLC was used to monitor the reaction progress (70% EtOAc:Hexanes with $KMnO_4$ stain). Once the reaction was complete it was concentrated via rotary evaporation and high vacuum. 30mL of chloroform was added to the reaction mixture and it was left in dry ice (about -78^{o} C) overnight to for the product to crash out.



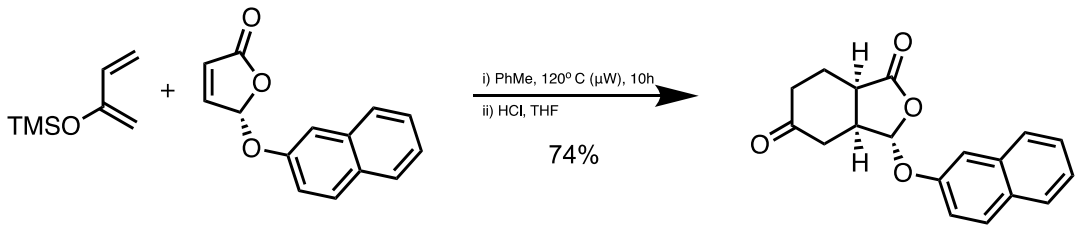
Scheme 8: Acetylation of Alcohol

5-oxo-2,5-dihydrofuran-2-yl acetate. In a 250mL flask round-bottom flask, with a stir bar and condenser 5-hydroxy-2(5H)-furanone (28.4g, 0.000228mmol, 1 equiv.) was added followed acetic anhydride (30.04mL, 317.8mmol, 1.13equiv.) and p-TSA monohydrate (0.097g, 0.0000005mmol, 0.0018 equiv.) and then the reaction mixture was heated to 50° C. The reaction was monitored by TLC (70% EtOAc:Hexanes with $KMnO_4$ stain). After about 3 hours the reaction was complete and the mixture was distilled under house, then high vacuum to remove acetic anhydride and acetic acid (Distillation head temp. 30°C. The receiver should be cooled by dry ice to ensure acid doesn't enter the cold trap). Product was distilled at 75° C to give 21g of a pale-yellow liquid.



Scheme 9: Naphthol Substitution

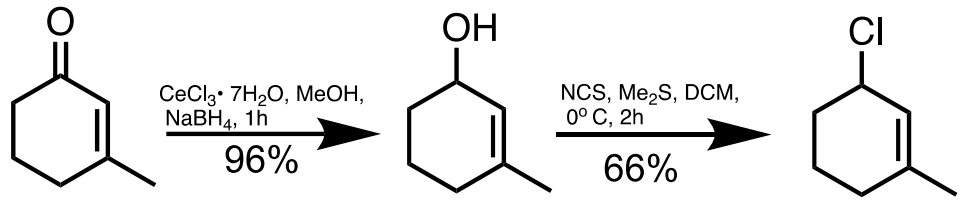
5-(naphthalen-2-yloxy) furan-2(5*H*)-one. A 500mL round-bottom flask was charged with DCM (120mL), 2-napthol (3.04g, 21.1mmol, 1.5 equiv.), and $Pd(PPh_3)_4$(0.8g, 0.765, 0.05 equiv.). 5-oxo-2,5-dihydrofuran-2-yl acetate (2g, 14.1mmol, 1.0 equiv.) was dissolved in about 20mL of DCM and then added via cannula from a separate round bottom flask. The reaction was then placed under N_2 and allowed to stir until TLC (30% EtOAc:Hexanes) indicated a complete reaction. After about 15 hours the reaction was concentrated with rotary evaporation then purified using automatic column chromatography (0 to 60% EtOAc:Hexanes). Following the column, the product was concentrated via rotatory evaporation and high vacuum to yield 1.92g of the desired product.



Scheme 10: Diels-Alder Reaction

(3*R*,3a*R*,7a*S*)-3-(naphthalen-2-yloxy) tetrahydroisobenzofuran-1,5(3*H*,4*H*)-dione. 5-(naphthalen-2-yloxy) furan-2(5*H*)-one (1g, 4.41mmol, 1 equiv.) and 1-(trimethylsiloxy)-

1,3-butadiene (1.92mL, 11.025mmol, 2.50 equiv.) and toluene(3.51mL) were added to a microwave test tube purged with N_2. It was placed in the microwave for 10 hours at 120°C. After TLC (25% EtOAc:Hexanes) indicated a complete reaction THF (10mL) and HCl (10mL) was added to the test tube and it was allowed to stir for 30 min vigorously. Then the mixture was transferred to a 125 mL separatory funnel. The aqueous phase was extracted with MTBE (3x20mL). After separation the organic phase was washed with $NaHCO_3$ (15mL sat. aqueous) and brine (20mL). The organic phase was dried over magnesium sulfate, filtered, and concentrated. It was then purified via automatic column chromatography (10 to 100% EtOAc:Hexanes). The product was then concentrated. After concentration DCM (8.14mL) was added to the solid. Then hexanes (8.14mL) was added and the mixture was left to sit out for recrystallization. Then the solid was filtered out and washed with a 20% DCM:hexanes solution, finally yielding 0.74g. The mother liquor was collected and recrystallized again. This time with DCM and hexanes (3mL each).

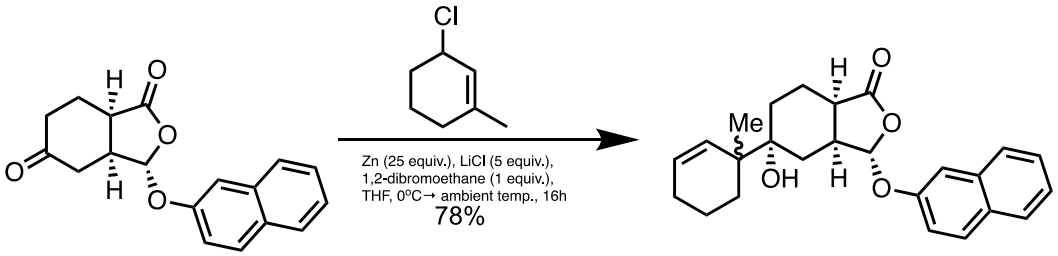


Scheme 11: Reduction of the Ketone and Reagent Generation

3-methylcyclohex-2-en-1-ol. 3-methylcyclohex-2-enone (5.0g, 45.0mmol, 1.0 equiv.) was added to a solution of cerium chloride heptahydrate (1.68g, 45.0mmol, 1.0 equiv.) in MeOH(82mL). Under ice bath cooling $NaBH_4$ (1.70g, 45.0mmol, 1.0 equiv.) was added slowly and the reaction was allowed to stir about 1 hour at room temperature. Once TLC (50% EtOAc:Hexanes) indicated a complete reaction it was quenched with D.I. water

(10mL) and extracted in a separatory funnel with EtOAc (3x15mL). The organic phase was washed with brine, dried over sodium sulfate and concentrated to yield 4.8g of an oil.

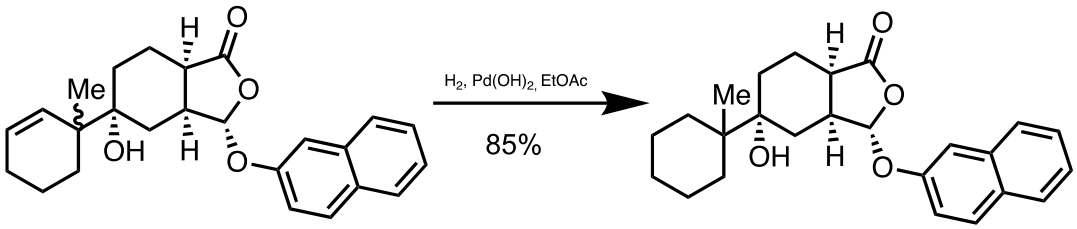
3-chloro-1-methylcyclohex-1-ene. NCS (2.48g, 18.6mmol 1.10 equiv.) was added to a 200mL round bottom flask with DCM (33.0mL). It was then cooled to 0º C and dimethyl sulfide (1.61mL, 21.9mmol, 1.30 equiv.) was added dropwise. 3-methylcyclohex-2-en-1-ol (2.00mL, 16.9mmol, 1.00 equiv.) was added dropwise with vigorous stirring. Reaction stirred at 0º C for about 3 hours. After completion the reaction mixture was concentrated and then 50mL of pentane was added. Any remaining solid was washed with pentane. Then the solution was added to a separatory funnel and 10mL of brine was added. Pentane (2x35mL) was used to extract. The organic layer was collected, dried over magnesium sulfate and concentrated yielding 1.32g.



Scheme 12: Coupling Reaction

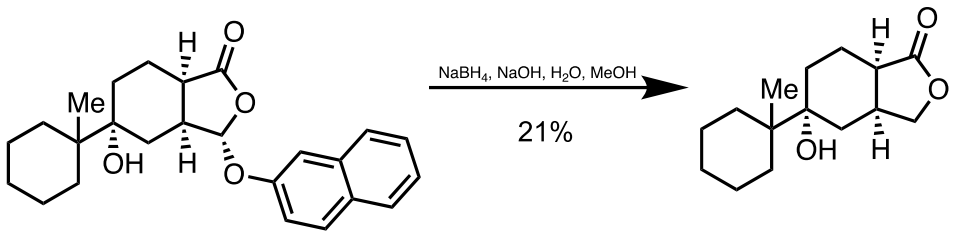
(3*R*,3a*R*,5*S*,7a*S*)-5-hydroxy-5-(1-methylcyclohex-2-en-1-yl)-3-(naphthalen-2-yloxy) hexahydroisobenzofuran-1(3*H*)-one. Zinc powder (1.38g, 21.1mmol, 25 equiv.) and LiCl (179mg, 4.22mmol, 5.00 equiv.) were added to a 100mL round bottom flask and dried with a heat gun under high vacuum(2x). Upon the final cooling, THF (11.7mL) was added and the resulting solution was stirred vigorously. 1,2-dibromoethane (73.0µL, 0.84mmol, 1.0 equiv.) was added to the solution and heated with a heat gun to initiate a gentle reflux. The solution was then cooled to room temperature and then to 0º C and 250mg, 0.84mmol, 1.0 equiv.) (3R,3aR,7aS*)*-3-(naphthalen-2-yloxy) tetrahydroisobenzofuran-1,5(3H,4H*)*-

dione was added as a solution in THF(1.50mL). 3-chloro-1-methylcyclohex-2-en-ol (990μL, 7.59mmol, 9.0 equiv.) was added over 5 hours via syringe pump as a solution in THF(2.40mL). The reaction was allowed to rise to room temperature as it stirred overnight. After TLC indicated a complete reaction (25% EtOAc:Hexanes) the mixture was quenched with NH_4Cl sat. solution(60mL). The aqueous phase was separated and extracted with EtOAc (3x60mL). The organic layers were combined and washed with brine (120mL), dried over magnesium sulfate and concentrated. It was the purified via automatic column chromatography (0 to 40% EtOAc:Hexanes) yielding about 0.7g of material.



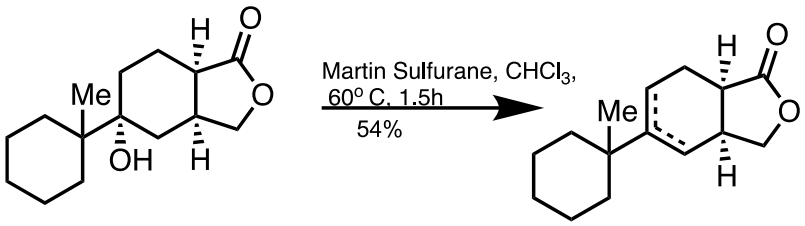
Scheme 13: Hydrogenation of the Alkene

(3*R*,3a*R*,5*S*,7a*S*)-5-hydroxy-5-(1-methylcyclohexyl)-3-(naphthalen-2-yloxy) hexahydroisobenzofuran-1(3*H*)-one. (3*R*,3a*R*,5*S*,7a*S*)-5-hydroxy-5-(1-methylcyclohex-2-en-1-yl)-3-(naphthalen-2-yloxy) hexahydroisobenzofuran-1(3*H*)-one (200mg, 0.8mmol, 1.0 equiv.), $Pd(OH)_2/C$ (20% weight)(22.47mg, 0.16mmol, 0.2 equiv.), and EtOAc(16.2mL) were added to a 100mL round bottom flask and then capped with a rubber stopper. Then an H_2 balloon was attached via needle through the rubber stopper and the flask was evacuated with vacuum and hydrogen 3 times. Then the reaction was left to stir overnight. To check reaction, progress an NMR was taken of a small 2mL sample. After completion the reaction was filtered over celite and concentrated yielding 0.17g of product. The product was used in the next step without further purification.



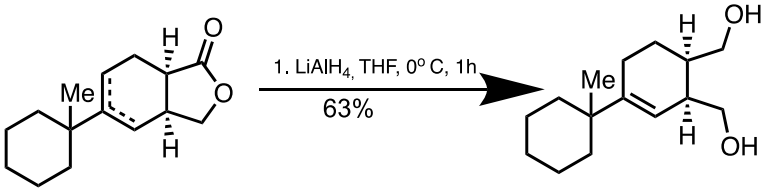
Scheme 14: Removal of the Naphthol Group

(3a*R*,5*S*,7a*S*)-5-hydroxy-5-(1-methylcyclohexyl) hexahydroisobenzofuran-1(3*H*)-one. Sodium borohydride (75.4mg, 2.0mmol, 2.,5 equiv.) was added to a stirring aqueous solution of NaOH (1M pre-made) (4.0mL, 4.0mmol, 5 equiv.) under air atmosphere. After stirring for 5 minutes **(**3*R*,3a*R*,5*S*,7a*S*)-5-hydroxy-5-(1-methylcyclohexyl)-3-(naphthalen-2-yloxy) hexahydroisobenzofuran-1(3*H*)-one (200mg, 0.8mmol, 1 equiv.) was added as a solution in MeOH(20mL). The mixture was left to stir and monitored with TLC (20% EtOAc:Hexanes). After TLC indicated a complete reaction the mixture was cooled to 0º C and concentrated HCl(1mL) was added to the mixture. It was then stirred for 1 hour. It was then extracted with EtOAc (30mL) and brine (15mL). Then the organic phase was extracted with brine(1x15mL) to remove all water. Then the aqueous layer was extracted with EtOAc(2x15mL) to remove all organic product. The organic phase was then dried over magnesium sulfate, filtered, and concentrated. Then the mixture was purified via automatic column chromatography (10 to 80% EtOAc:Hexanes) yielding 0.042g of the desired product.



Scheme 15: Dehydration via Martin Sulfurane

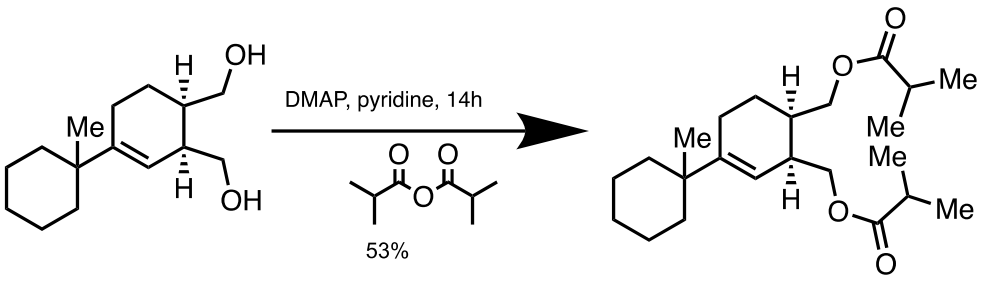
(3aS,7aR)-5-(1-methylcyclohexyl)-3a,4,7,7a-tetrahydroisobenzofuran-1(3H)-one A 50 ml round bottom flask was charged with the above substrate, Martin Sulfurane, and $CHCl_3$. The reaction mixture was then placed on a heating block at 60°C and left to stir under N_2 atmosphere. The reaction progress was monitored by TLC (50% EtOAc:Hexanes). Once the TLC indicated a complete reaction the flask and reaction mixture were allowed to cool to room temperature. Then the reaction was concentrated and purified via auto flash chromatography (5-30% EtOAc:Hexanes). The product was then concentrated via rotary evaporation and high vacuum to give 0.05g of material.



Scheme 16: Reduction to a Diol

((3*S*,4*R*)-1'-methyl-[1,1'-bi(cyclohexan)]-1-ene-3,4-diyl)dimethanol. THF (1.50mL) was added to a 25mL round bottom flask already containing the starting substrate (27.0mg, 0.12mmol, 1.0 equiv.). $LiAlH_4$ (0.15mL, 0.30mmol, 2.50 equiv.) was added dropwise following the THF. The addition of all of these reagents occured at 0°C using an ice bath.

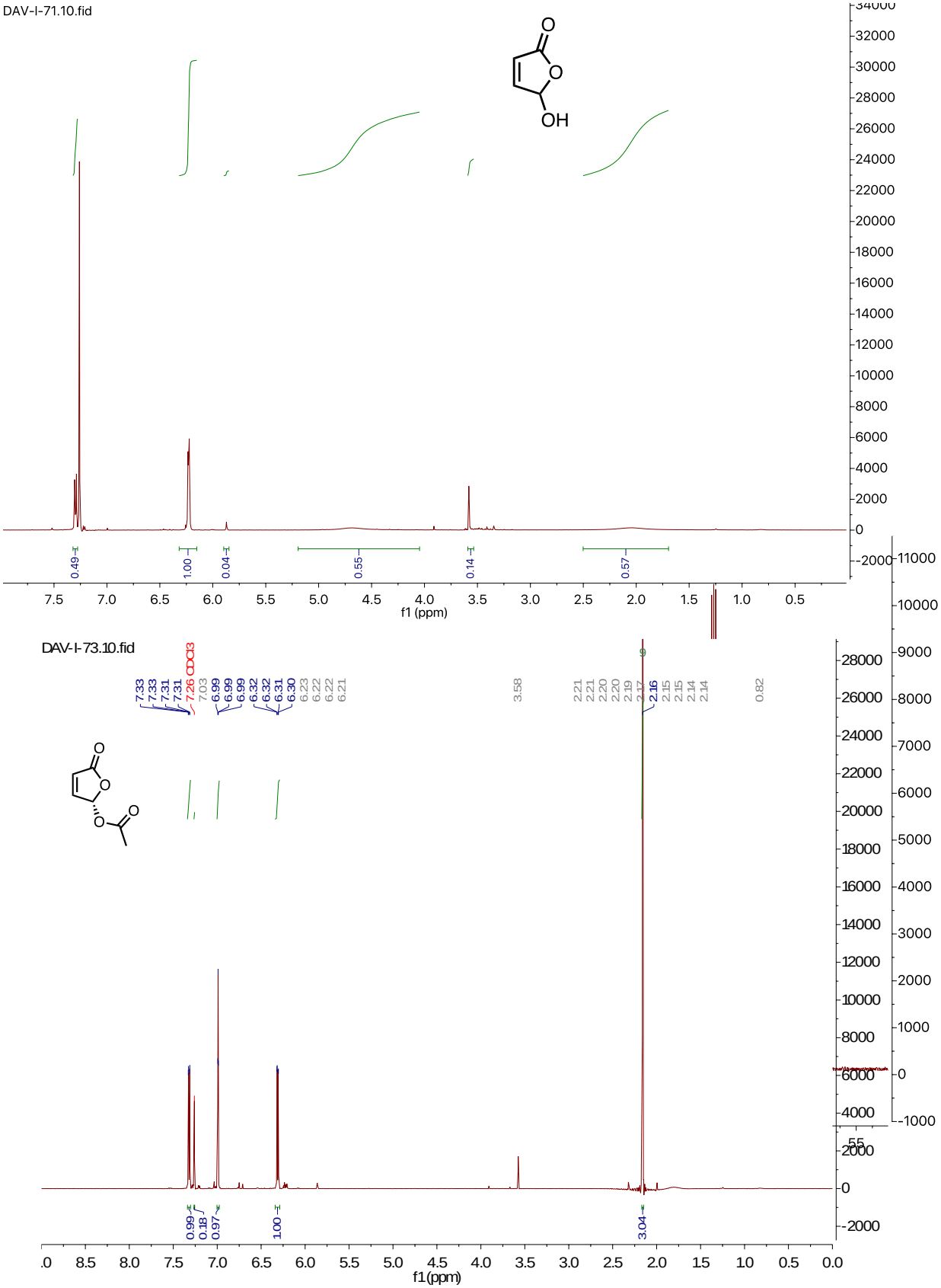
The reaction mixutre was left to stir at 0^{o}C slowing warming to room temperature over the course of one hour. A TLC (35% EtOAc:Hexanes) was taken after an hour in order to onsure that all of the starting material had been used. Upon consumption of the starting material the reaction was cooled to 0^{o}C and quenched with 12μL of water, 12μL of 25% NaOH, and 30μL of water in that order. The ice bath was removed and the mixture was warmed to ambient temperature. After about 30 minutes the mixture was filtered through a pipette with a cotton filter and magnesium sulfate. The substance was concentrated using rotary evaporation and moved to the next step without further purification.

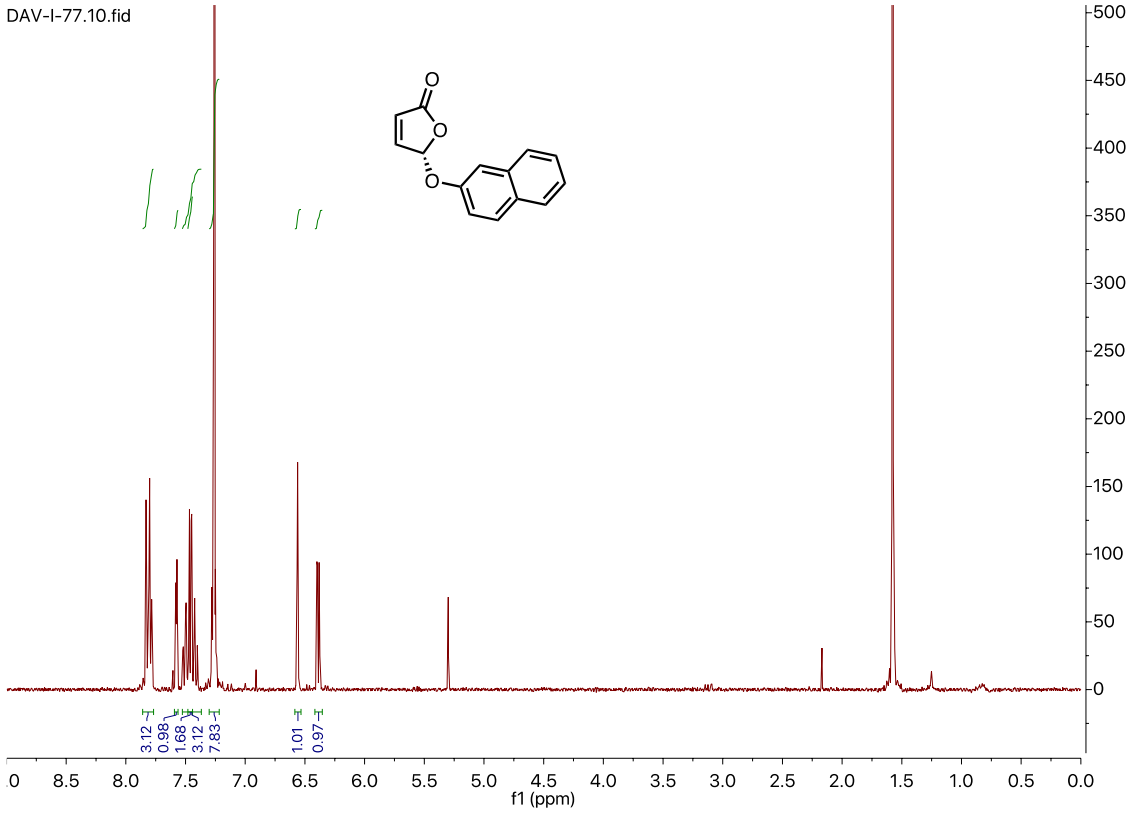


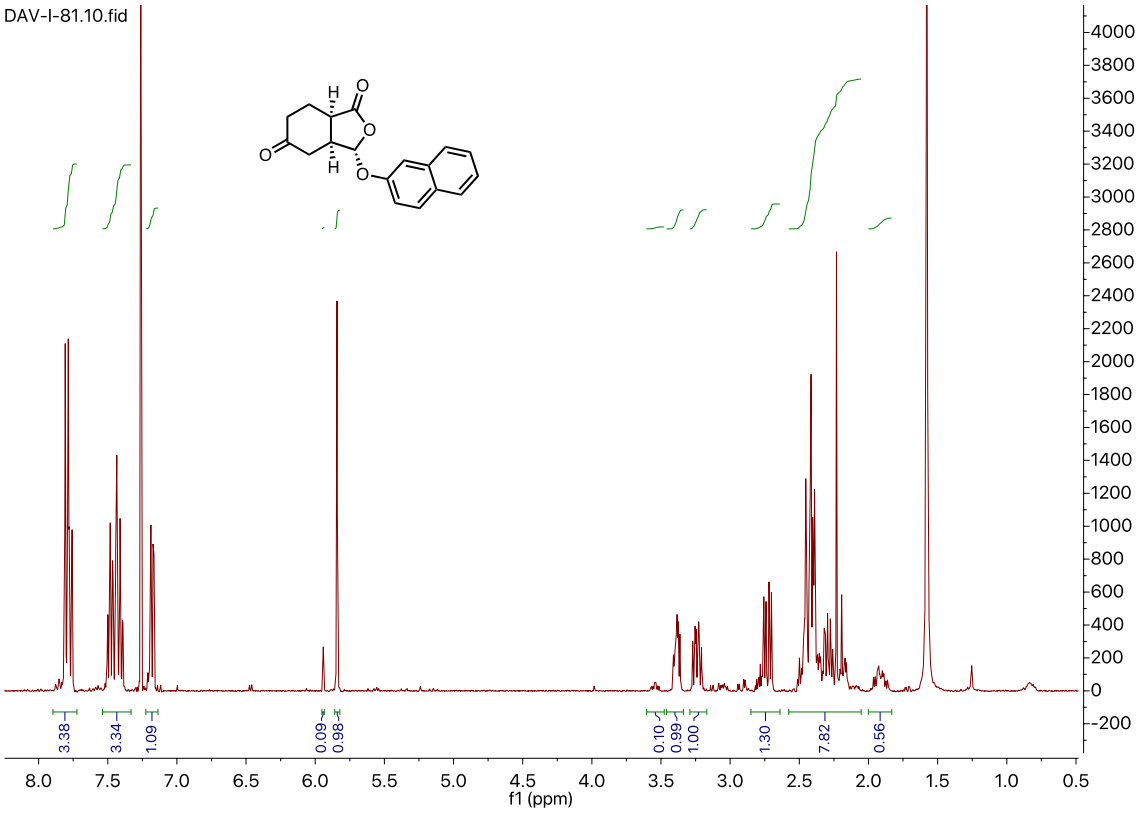
Scheme 17: Yielding the Desired Compound

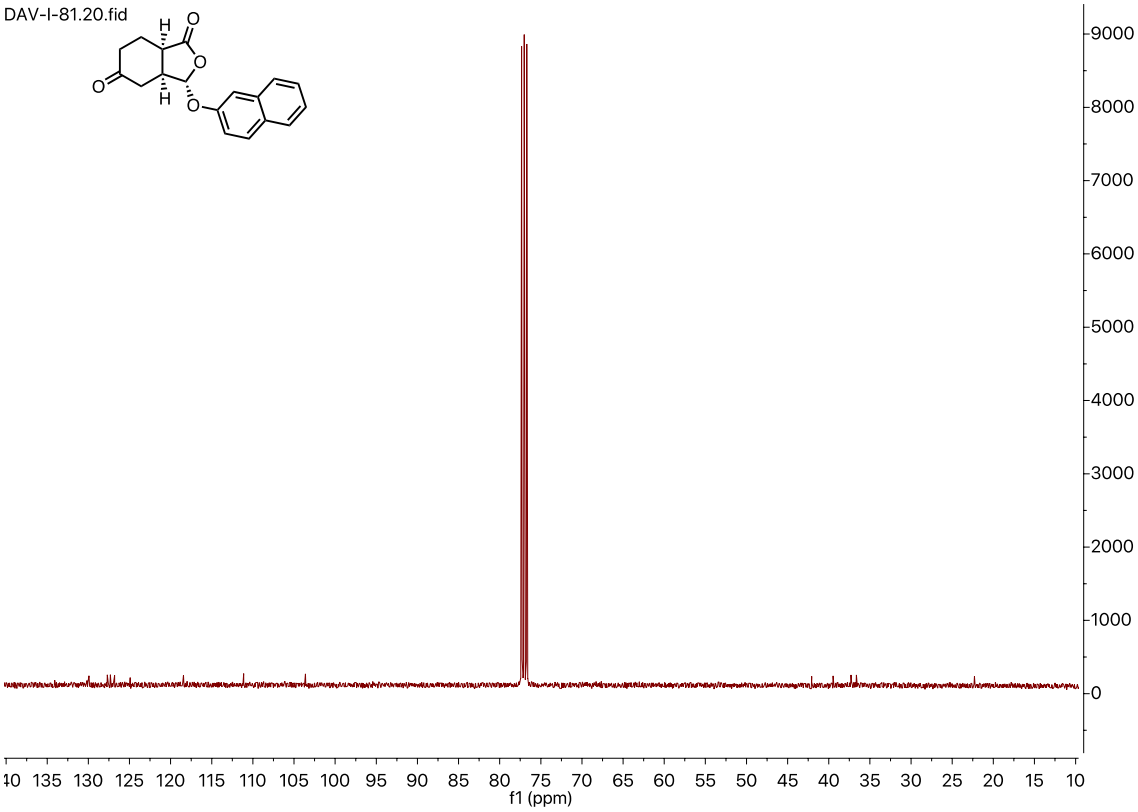
((3*S*,4*R*)-1'-methyl-[1,1'-bi(cyclohexan)]-1-ene-3,4-diyl)bis(methylene)bis(2-methylpropanoate). To the previously generated diol, **(**(3*S*,4*R*)-1'-methyl-[1,1'-bi(cyclohexan)]-1-ene-3,4-diyl)dimethanol, was added DMAP (2.0mg, 12.0μmol, 0.10 equiv.) followed by isobutyric anhydride (212.2μL, 1.28mmol, 10.0equiv.) and pyridine (1.0mL) at 0^{o}C. Then the ice bath was removed and the reaction stirred for 14 hours. Once TLC (25% EtoAc:Hexanes) indicated a complete reaction $NaHCO_3$ was added to the reaction mixture for extraction. The aqueous layer was extracted with EtOAc (4x 10mL). The organic layer was washed with brine (10mL). Then it was dried over

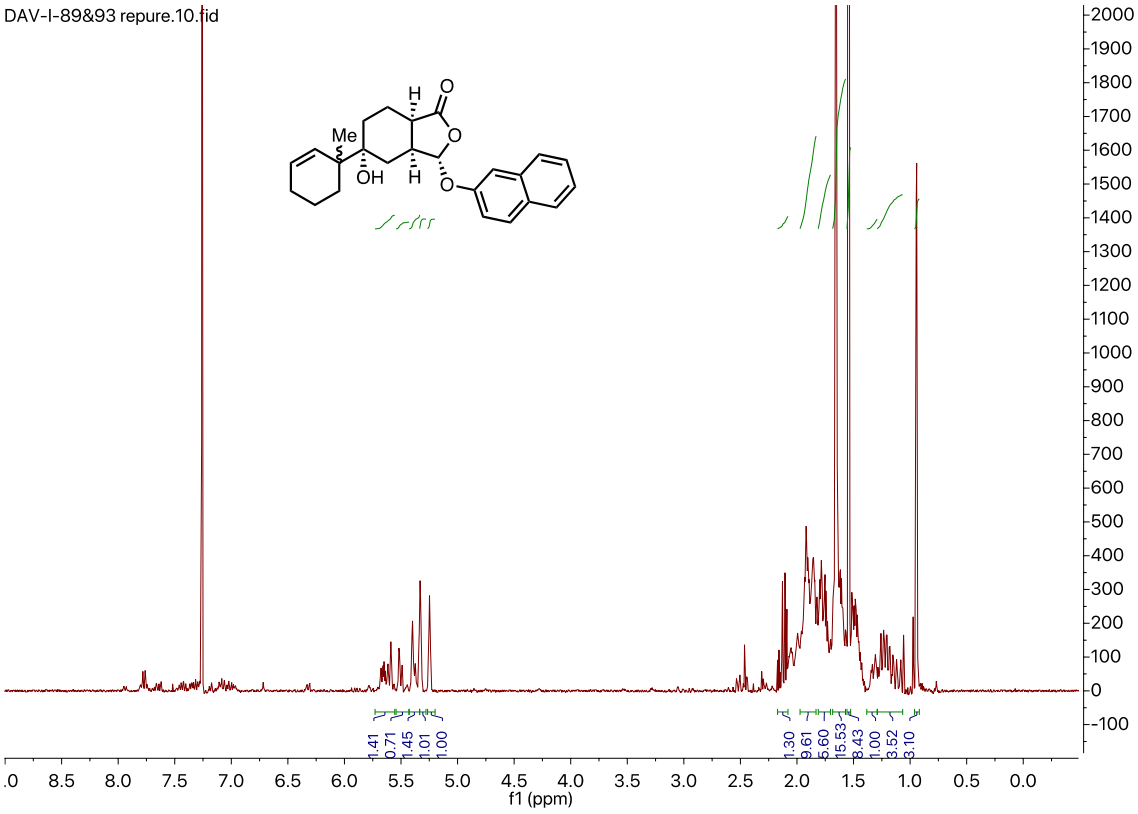
$MgSO_4$, filtered and concentrated using rotary evaporation, yielding 0.009g of the final product.

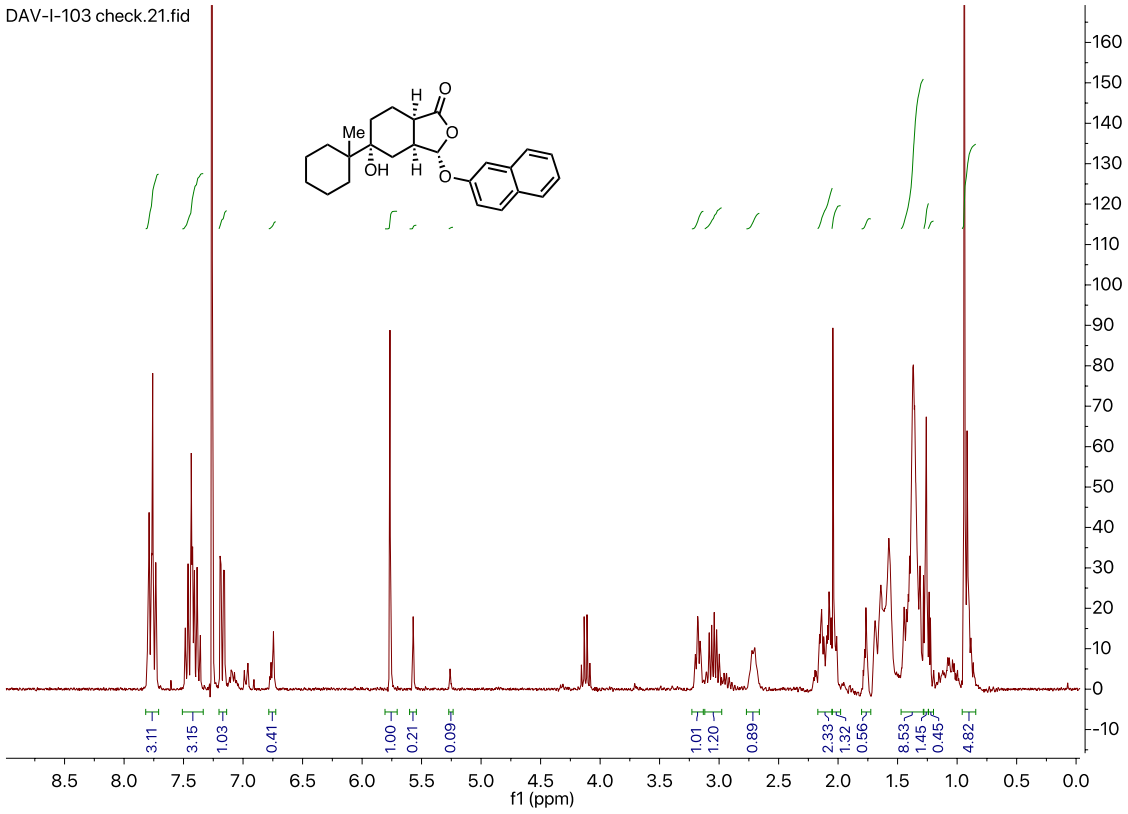

DAV-I-71.10.fid
O
O
OH
0.49
1.00
0.04
0.55
0.14
0.57
f1 (ppm)
DAV-I-73.10.fid
7.33
7.33
7.31
7.31
7.26 CDCl3
7.03
6.99
6.99
6.99
6.32
6.32
6.31
6.30
6.23
6.22
6.22
6.21
3.58
2.21
2.21
2.20
2.20
2.19
2.17
2.16
2.15
2.15
2.14
2.14
0.82
O
O
O
O
0.99
0.18
0.97
1.00
3.04
f1(ppm)

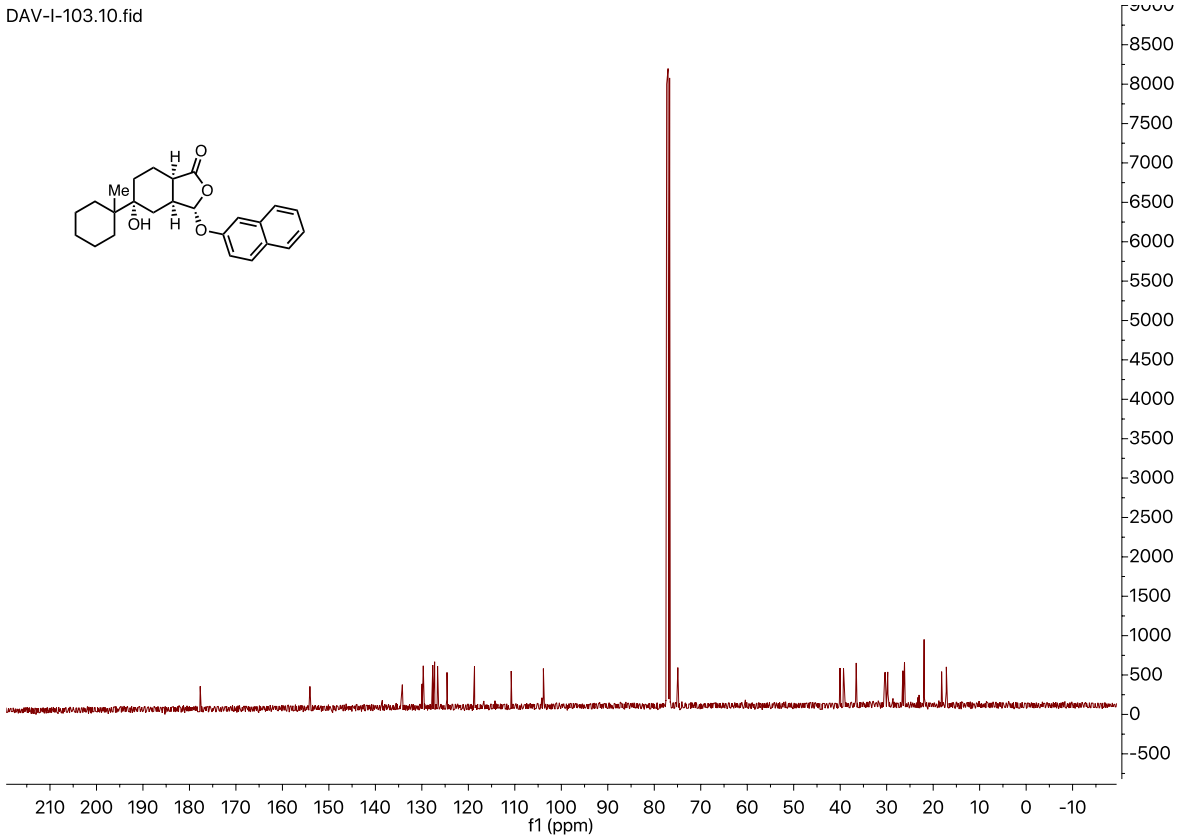

DAV-I-77.10.fid
O
O
O
3.12
0.98
1.68
3.12
7.83
1.01
0.97
500
450
400
350
300
250
200
150
100
50
0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

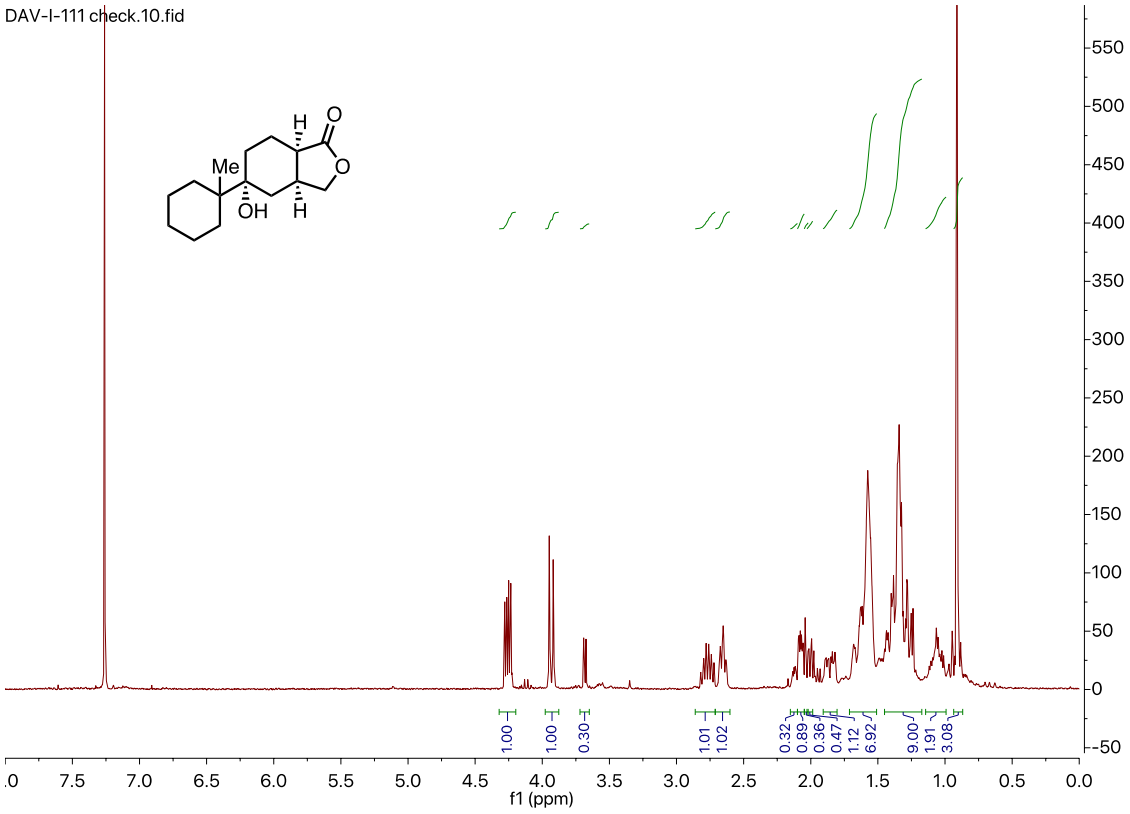

DAV-I-81.10.fid
H
O
O
O
H
O
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
3.38
3.34
1.09
0.09
0.98
0.10
0.99
1.00
1.30
7.82
0.56
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

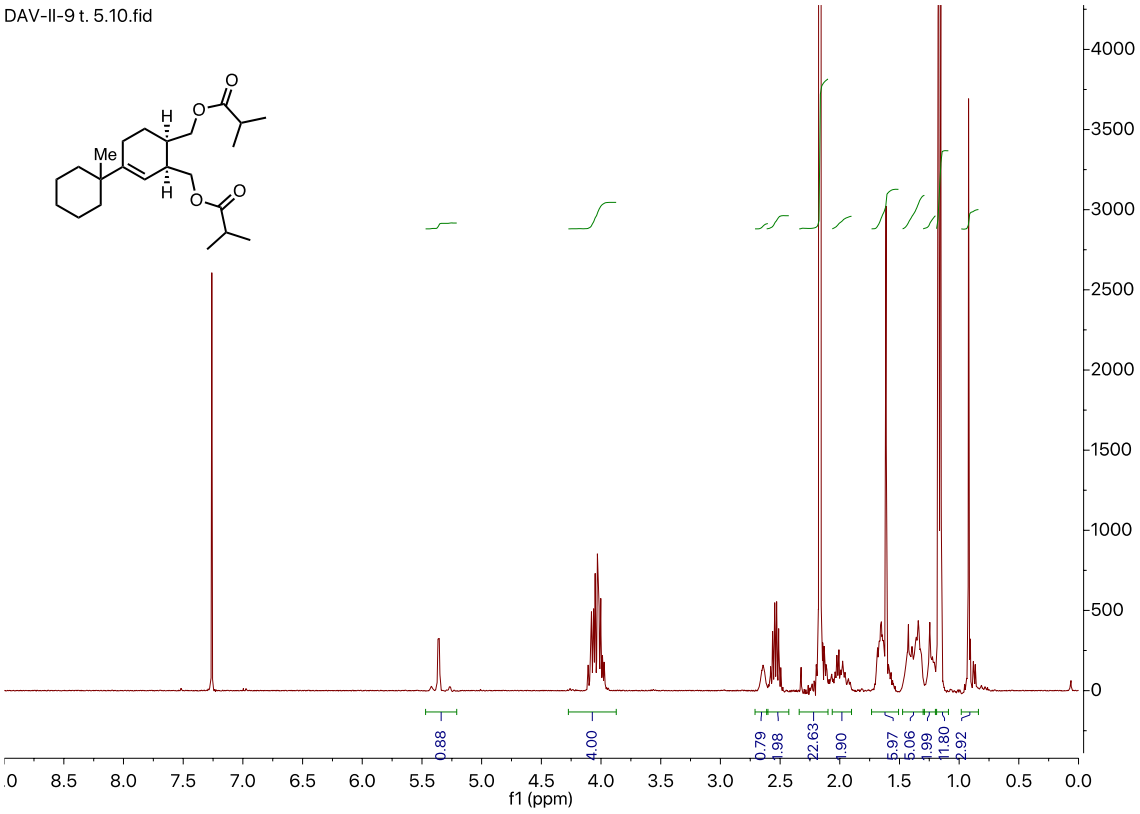

DAV-I-81.20.fid
H
O
O
O
H
O
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
40 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10
f1 (ppm)


DAV-I-89&93 repure.10.fid
Me
OH
H
O
O
H
O
1.41
0.71
1.45
1.01
1.00
1.30
9.61
5.60
15.53
8.43
1.00
3.52
3.10
f1 (ppm)


DAV-I-103 check.21.fid
Me
OH
H
O
O
H
O
3.11
3.15
1.03
0.41
1.00
0.21
0.09
1.01
1.20
0.89
2.33
1.32
0.56
8.53
1.45
0.45
4.82
f1 (ppm)


DAV-I-103.10.fid
H
O
Me
O
OH
H
O
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


DAV-I-111 check.10.fid
H
O
Me
O
OH
H
550
500
450
400
350
300
250
200
150
100
50
0
-50
1.00
1.00
0.30
1.01
1.02
0.32
0.89
0.36
0.47
1.12
6.92
9.00
1.91
3.08
.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


DAV-II-9 t. 5.10.fid
Me
H
H
O
O
O
O
4000
3500
3000
2500
2000
1500
1000
500
0
0.88
4.00
0.79
1.98
22.63
1.90
5.97
6.06
1.99
11.80
2.92
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

BIBLIOGRAPHY

Abbasov, M. E., Alvariño, R., Chaheine, C. M., Alonso, E., Sánchez, J. A., Conner, M. L., Alfonso, A., Jaspars, M., Botana, L. M., & Romo, D. (2019). Simplified immunosuppressive and neuroprotective agents based on gracilin A. *Nature Chemistry*, *11*(4), 342–350. https://doi.org/10.1038/s41557-019-0230-0

Caggiano, V., Bauer, K., & Parise, C. (2011). Survival of triple-negative and HER2-positive breast cancer by AJCC stage. *Journal of Clinical Oncology*, *29*(15_suppl), 1078–1078. https://doi.org/10.1200/jco.2011.29.15_suppl.1078

Centers for Disease Control and Prevention. (2020, September 14). *Triple-Negative Breast Cancer*. Centers for Disease Control and Prevention. https://www.cdc.gov/cancer/breast/triple-negative.htm.

Hormone Therapy for Breast Cancer Fact Sheet. National Cancer Institute. (2021, July 7). https://www.cancer.gov/types/breast/breast-hormone-therapy-fact-sheet.

Hormones and the Endocrine System. Johns Hopkins Medicine. (2021). https://www.hopkinsmedicine.org/health/conditions-and-diseases/hormones-and-the-endocrine-system.

Janssen, K., Horn, S., Niemann, M. T., Daniel, P. T., Schulze-Osthoff, K., & Fischer, U. (2009). Inhibition of the ER Ca2+ pump FORCES MULTIDRUG-RESISTANT CELLS deficient IN Bak and Bax Into necrosis. *Journal of Cell Science*, *122*(24), 4481–4491. https://doi.org/10.1242/jcs.055772

Machida, K., Ohta, Y., & Osada, H. (2006). Suppression of Apoptosis By CYCLOPHILIN D Via stabilization of Hexokinase II Mitochondrial binding in cancer cells. *Journal of Biological Chemistry*, *281*(20), 14314–14320. https://doi.org/10.1074/jbc.m513297200

Mayo Foundation for Medical Education and Research. (2020, February 5). *What your breast cancer type means*. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/breast-cancer/in-depth/breast-cancer/art-20045654.

Oskarsson, T., Nagorny, P., Krauss, I. J., Perez, L., Mandal, M., Yang, G., Ouerfelli, O., Xiao, D., Moore, M. A., Massagué, J., & Danishefsky, S. J. (2010). Diverted Total Synthesis Leads to the Generation of Promising Cell-Migration Inhibitors for Treatment of Tumor Metastasis: In vivo and Mechanistic Studies on the Migrastatin Core Ether Analog. *Journal of the American Chemical Society*, *132*(9), 3224–3228. https://doi.org/10.1021/ja9101503

Panawala, L. (2017). Difference Between Apoptosis and Necrosis. *Pediaa*. https://doi.org/http://www.rnlkwc.ac.in/pdf/study-material/zoology/DifferenceBetweenApoptosisandNecrosis_DefinitionProcessFunctionComparison-1.pdf

Steric Hindrance. Illustrated glossary of organic chemistry - steric hindrance. (n.d.). http://www.chem.ucla.edu/~harding/IGOC/S/steric_hindrance.html.

Tong, M., & Jiang, Y. (2015). FK506-Binding proteins and their diverse functions. *Current Molecular Pharmacology*, *9*(1), 48–65. https://doi.org/10.2174/1874467208666150519113541

Zhu, P., Hu, S., Jin, Q., Li, D., Tian, F., Toan, S., Li, Y., Zhou, H., & Chen, Y. (2018). Ripk3 promotes er stress-induced necroptosis in cardiac ir injury: A mechanism involving calcium overload/xo/ros/mptp pathway. *Redox Biology*, *16*, 157–168. https://doi.org/10.1016/j.redox.2018.02.019