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Synthesis of Dicamba Glucosides for the Study of Environmental Dicamba Drift Effects on Soybeans

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Synthesis of Dicamba Glucosides for the Study of Environmental Dicamba Drift Effects on Soybeans

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of the requirements for the degree of
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by

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ABSTRACT

The most popular herbicide used for weed control has been glyphosate for many years in the Midwestern United States. Plants have begun to develop a resistance to glyphosate due to over use of the herbicide. This herbicide resistance has pushed farmers to turn to alternative herbicides such as dicamba and 2,4-D. Recently agrochemical companies have developed genetically modified crops that are resistant to herbicides such as dicamba. These modified crops allow farmers to spray their fields with dicamba without fear of crop damage. Farmers of non-genetically modified crops, however, suffer damage and loss of yield from herbicide drift effects of this spraying. We sought to prepare the dicamba glucosides, DCSA-glucoside, DCGA-glucoside, and 5-OH dicamba-glucoside standards for LC/MS/MS analysis. Pure samples of these glucosides will provide a reference point in which to study how genetically modified plants metabolize dicamba. Efforts to prepare these glucoside samples, will be discussed. Experiments done for the glucoside synthesis followed a Michael glycosylation type reaction using a glucosyl halide, aromatic phenolic compound, in the presence of a biphasic catalyst, tetrabutylammonium bromide. Reactions failed to yield desired products or were unable to be purified. Further investigation into other types of glycosylation reactions is necessary to continue synthesis of the desired glucosides.

ACKNOWLEDGMENTS

I would like to thank everyone who has helped me throughout my graduate career. First I want to thank my research professor Dr. Matt McIntosh for his guidance and support and for allowing me to join his research team. He has given me the opportunity to further my knowledge of synthetic organic chemistry and for his patience when research did not cooperate. I want to thank Dr. Cammy Willett for her patience and kindness as I struggle to make the compounds for her research. She is one of the most understanding people I have ever known. Even after failing to complete the synthesis of the compounds she needed, she was still willing to be a member on my graduate committee. I am grateful to her for her help and guidance on writing this thesis. I would like to thank Dr. Hassan Beyzavi for helping me with the research by helping me interpret NMR spectra and hypothesizing new approaches to the research. He is very helpful and generous with his chemicals and allowed me to use his equipment on a regular basis. I would like to thank Dr. Suresh "Kumar" Thallapuranam and his two postdocs Sanhita and Ravi for their help on this project. They have taken their very valuable time and given me a crash course on biochemical methods and procedures. Dr. Kumar has allowed me to attempt to accomplish this project using his equipment and chemicals simply to help me. He gains nothing through this and actually loses chemicals (money) and his postdocs time when teaching me. I am extremely grateful to him for this. I would also like to thank Dr. Susanne Striegler and her lab for supplying some of the chemicals necessary for this project. I was also able to gather needed information about carbohydrate synthesis from her. Dr. Striegler's graduate student Ifedi Orizu deserves to be mentioned as well he helped me gain a better understanding of the unpredictable nature of carbohydrate synthesis and also taught me much about how to identify carbohydrates on NMR. I want to thank Liz Williams who has acted as a surrogate mother to me these last

three years. She has been extremely kind in helping me sort through many problems I have faced as a graduate student. She is very blunt but it is this quality that was helpful to me. I can always trust her to be honest with me, even if it is brutally honest. Lastly I would like to thank my family and closest friends who provided moral support when I became discouraged. If not for them I would not have had the strength to make it this far. I would also like to thank my Lord Jesus Christ for all that he has done for me. If not for him, I would have given up and never have accomplished this task. I believe he will see this through and help me finish this project.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

CHAPTER 1: Dicamba

1.1: Introduction

Dicamba is the trade name given to the herbicide scientifically known as 3,6-dichloro-2-methoxybenzoic acid (Figure 1). As the name implies the molecule consists of a benzoic acid substituted with a methoxy group at C-2 and two chloro groups in the C-3 and C-6 positions.

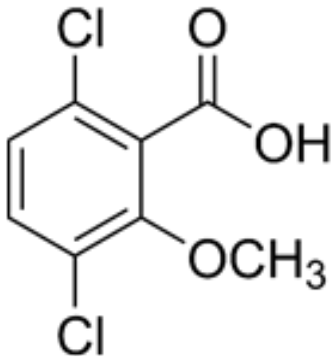


Figure 1: Structure of dicamba

Dicamba was developed in 1942 by Zimmermann and Hitchcock and has been produced and sold under various brand names such as Banvel®, Diablo®, Oracle™, and Vanquish® since the 1960's. Dicamba has since been used by farmers as a way to control broad leaf plant growth in their pasture lands and crops. It is useful for broad leaf plant control because it generally has no effect on the grass family of plants.[1,2]

Glyphosate has traditionally been one of the most widely used herbicide by farmers because it is considered to have more "flexibility and simplicity" of use than other types of herbicides.[25] Glyphosate is more commonly known by its commercial name Roundup and has an inhibitory type mode of action by which it kills plants.[24] It is called an amino acid synthesis inhibitor. ESPS (5-enolpyruvate shikimate-3-phosphate) is a key enzyme needed for aromatic

amino acid biosynthesis. Glyphosate kills the plant by inhibiting the activity of enzyme ESPS. Glyphosate's widespread use has created a significant drawback for the farming community. Many plants have developed a resistance to glyphosate in much the same way as microbes develop resistance to antibiotics. This resistance has forced farmers to turn to other well established herbicides such as dicamba and 2,4-D to control broad leaf weeds.[25]

Dicamba is considered moderately toxic if ingested and slightly toxic upon dermal exposure.[2] Dicamba's oral LD_{50} in rats is 1039 mg/kg of body weight, and a dermal LD_{50} of >2000 mg/kg in rabbits. 2,4-D is more toxic with an oral LD_{50} of 375 mg/kg in rats. Dicamba and 2,4-D are part of a class of herbicides called synthetic auxins and have a different mode of action than glyphosate. Synthetic auxins mimic the naturally occurring growth hormone Indole-3-acitic acid (IAA) which is the main auxin found in plants. Synthetic and naturally occurring auxins essentially cause the plant to grow abnormally and uncontrollably leading to its eventual death.[23,24] Many plants that have developed a resistance to or are naturally tolerant to glyphosate, are still susceptible to the growth regulators dicamba and 2,4-D. Dicamba and 2,4-D are two of very few effective products available for broadleaf weed control.[25] Agrochemical companies claim that synthetic auxins are less likely to develop resistance issues than other herbicides available.[25] This belief is met with opposition by many that claim the eventual outcome will be the same as with glyphosate.[25] Regardless of the eventual outcome of dicamba and 2,4-D resistance, recent years have seen an increase in synthetic auxin production and use. This increase of use has produced a different type of problem.[25]

Monsanto, an American agricultural biotechnology company, has recently developed a modified cultivar of soybeans and cotton known as Roundup Ready 2 Xtend® that are resistant to glyphosate and dicamba. These genetically engineered crops were developed by inserting

genes from soil bacteria (that have resistance to glyphosate and dicamba) into the crop's DNA. These modified cultivars allow for the farmer to plant and grow crops without fear of damage from dicamba or glyphosate that would kill a non-modified crop. This approach to the problem works well for farmers that use this new soybean, but due to a phenomena called "herbicide drift" can be detrimental to farmers not using the new soybean technolony.[2]

Dicamba drift occurs when a farmer sprays his dicamba resistant crop and some of the sprayed dicamba "drifts" and damages non-resistant crops. It is believed the volatile nature of dicamba is what allows for the drift effect as well as its water solubility and droplet drift. This has caused crop damage to farmers all over the Midwest. As much as 1 million acres of Monsanto's resistant soybeans were planted in 2016 and an estimated 200,000 acres of non-resistant soybeans in Arkansas, Missouri, and Tennessee were affected by dicamba drift.[2] The drift can cause neighboring vegetation, including crops, to experience damage such as leaf wrinkling and cupping and stunted growth (Figure 2).[1]

Figure 2: Crop Damage
(Photograph: J. Franklin Egan)

Dicamba drift is apparent from its high volatility, and droplet drift when being sprayed. Dicamba has a vapor pressure of $2.6x10^{-8}$ atm at 25 ºC but is sprayed during the summer at temperatures as high as 95 ºF (35 ºC). This increase in temperature increases the vapor pressure therefore increasing drift. The type of nozzle used when spraying also effects how much drift occurs. Synthetic auxins are difficult to clean from sprayers and are thus mistakenly sprayed on susceptible crops via contamination.[25] Dr. Cammy Willett's lab in the Crop, Soil, and Environmental Sciences department at the University of Arkansas is currently studying how and to what extent dicamba drift harms non-resistant crops. Dr. Willett's research requires that she have pure standards of certain glucosides, for LC/MS/MS analysis, that are known to be metabolized by soybean plants. This thesis demonstrates efforts put forth to synthesize the pure standards needed for Dr. Willett's research.

1.2: Dicamba Metabolites and Glucosides

Following plant exposure to dicamba, it is metabolized into 3,6-dichloro-2-hydroxybenzoic acid (DCSA) **1**, 2,5-dichloro-3,6-dihydroxybenzoic acid (DCGA) **2**, 2,5-dichloro-3-hydroxy-6-methoxybenzoic acid (5-OH dicamba) **3**, DCSA-glucoside **4**, DCGA-glucoside **5a**, **5b**, **5c**, and 5-OH dicamba-glucoside **6 (**Figure 3). Dicamba, DCSA, DCGA, and 5-OH dicamba are commercially available, but the corresponding glucosides must be synthesized. Once synthesized and purified these glucosides can be used as analytical standards for experiments quantifiying metabolite production following drift events under various environmental conditions.

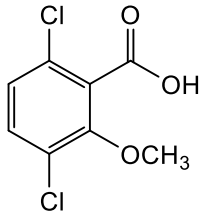
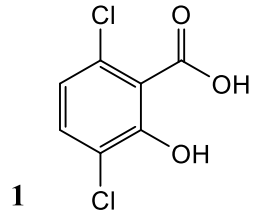
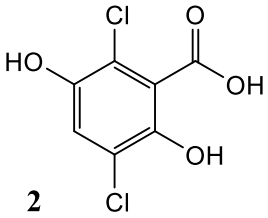
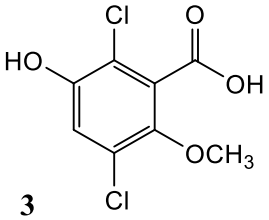
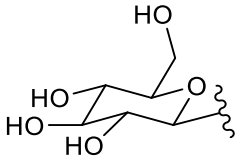
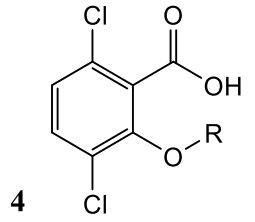
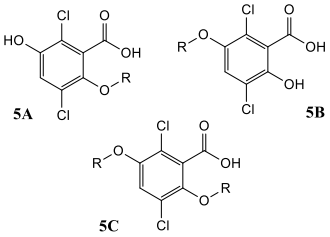
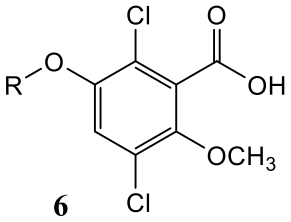
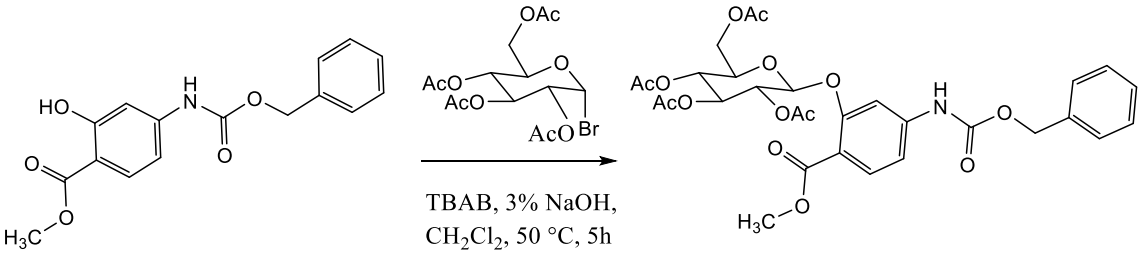
Dicamba	**DCSA**	**DCGA**	**5-OH dicamba**
	 **1**	 **2**	 **3**
-R	**DCSA Glucoside**	**DCGA Glucoside**	**5-OH Dicamba Glucoside**
	 **4**	 **5A** **5B** **5C**	 **6**

Figure 3: Dicamba Metabolites and Glucosides

The synthetic route chosen for the glycosylation reaction is a basic Michael glycosylation



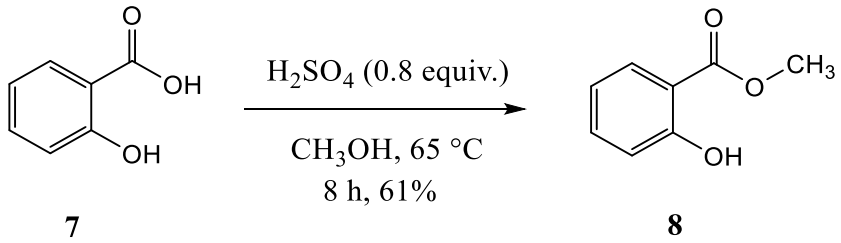
Scheme 1: Glycosylation of 4-(N-(benzyloxycarbonyl)amino)-2-hydroxybenzoate

reaction. A procedure was acquired from European Journal of Medicinal Chemistry in which they glucosylated 4-(N-(benzyloxycarbonyl) amino)-2-hydroxybenzoate using 2,3,4,6-Tetra-O-acetyl-alpha-D-glucopyranosyl bromide (Scheme 1).[9] This procedure was followed for the synthesis of the glucosides.

CHAPTER 2: Discussion and Experimental

2.1 Synthetic Organic Experiments

A)



7 **8**

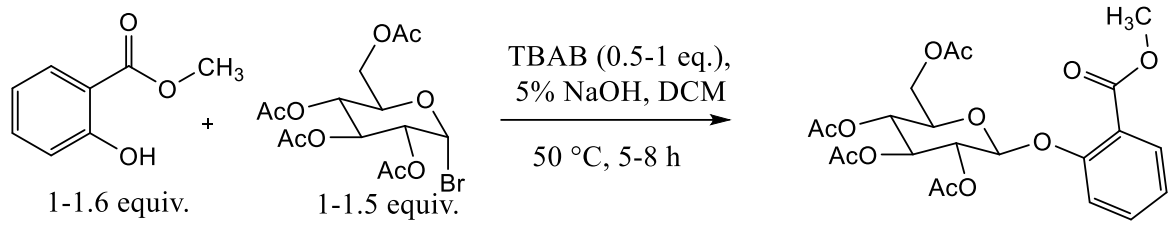
Scheme 2: Esterification of Salicylic Acid

Model Studies:

Salicylic acid was employed as model compound because it is inexpensive, and is similar in its structure to the compounds used to synthesize the metabolites.

The sugar could potentially react at the hydroxyl and/or carboxyl groups of salicylic acid. A phenol has a pKa of ~10 in water and benzoic acid has a pka of ~4.2 in water, both are deprotonated by the base NaOH. The glycosylation reaction is done in a NaOH solution, so both hydroxyl and carboxyl can be deprotonated during reaction. To avoid the reaction at the carboxylic acid of salicylic acid, a Fischer Esterification reaction was performed. Sulfuric acid catalyzed the esterification of salicylic acid to provide ester **8** with 61% yield. The crude product proved to be remarkably clean and no further purification was necessary (Scheme 2).

B)



1-1.6 equiv. 1-1.5 equiv.

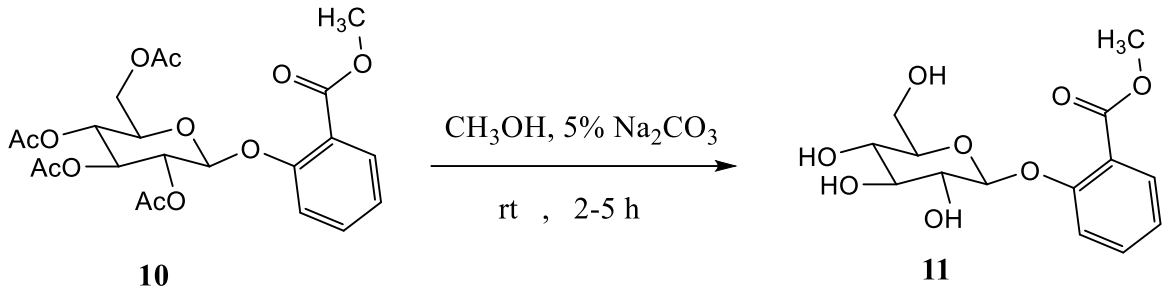
8 **9** **10**

Scheme 3: Glycosylation of Methyl Salicylate

The next step in the synthesis of the salicylic acid glucoside is to attach a glucose ring to the hydroxyl group of the newly formed methyl salicylate (Scheme 3). Acetobromo-α-D-glucose **9** is able to undergo a Michael glycosylation type reaction with **2** to substitute the bromo group with the methyl salicylate to afford **10**.[9] The glucoside used 1.6 equivalents of the methyl salicylate, 1 equivalent of the acetylated bromo glucose, and 0.5 equivalents of TBAB. The reaction time was varied between 5-8 hours and temperature was kept between 40-60 ºC. A single reaction was done with 1 equivalent of methyl salicylate, 1.5 equivalents of the acetylated glucosyl bromide, and 1 equivalent of TBAB at 45 ºC for 8 hours. Pure product was never isolated for any of these reactions so no accurate/pure yields exist for **10**. TBAB is a phase transfer catalyst and initially posed contamination issues. It was found that a prep TLC plate could be used to remove TBAB (tetra-N-butylammonium bromide) but is complicated because TBAB does not appear under UV light nor upon sulfuric acid staining and is therefore impossible to visualize on a TLC plate. This problem was solved by use of a short "plug" column that removed it from the product or by using 2 separate prep TLC plates (the first plate was run solely to remove the TBAB). These procedures, however, caused the loss of yield of **10**. It was

also discovered from ^{1}H-NMR that multiple products arose from the reaction and were extremely difficult to separate because of product overlap. Both α and β anomers are believed to be present in NMR as well as TBAB. Due to not isolating a pure glycosylation product, subsequent reactions were done with impure **10** and accurate identification impossible.

C-1)

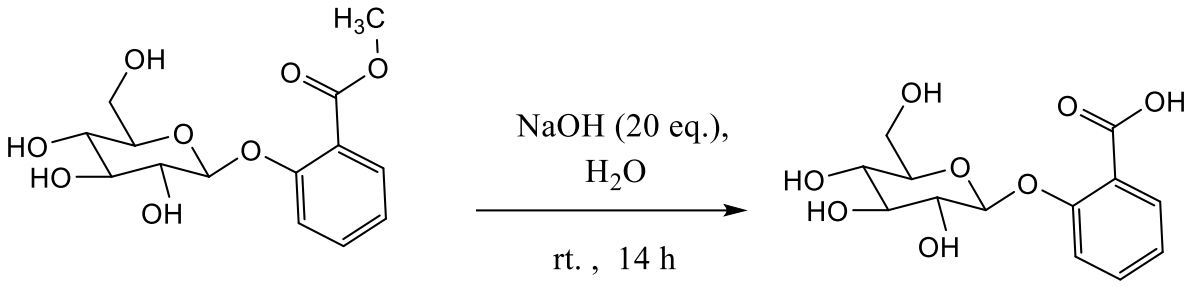


10 **11**

Scheme 4: Saponification and Hydrolysis of Methyl Salicyl Glycoside

Once the glycosylation was completed the next step was to deacetylate the sugar and hydrolyze the methyl ester (Scheme 4). But because of the extremely low yields of **11** and impure products from the glycosylation reaction, there was frequently not enough of **11** to do the subsequent saponification reaction. Reactions were done at room temperature with varying reaction times between 2-5 hours to synthesize **11**. The highest yield obtained was 16% and it was impure.

C-2)

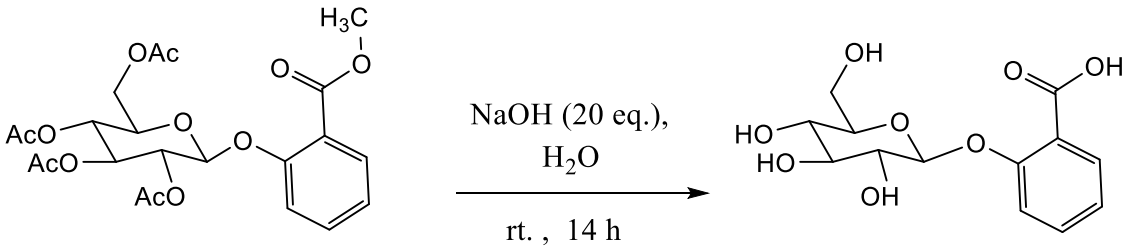


11 **12**

Scheme 5: Saponification and Hydrolysis of Methyl Salicyl Glycoside

A 10 mg sample of **11** was reacted with excess NaOH and H_2O (few drops) for 14 hours at room temperature (Scheme 5). ^{1}H-NMR confirmed product was present but was not purified.

C-3)



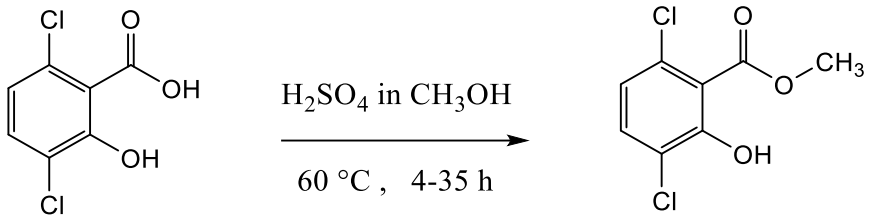
10 **12**

Scheme 6: Saponification and Hydrolysis of Methyl Salicyl Glycoside

It was theorized that it might be possible to do the complete hydrolysis with only one reaction instead of two in hopes of avoiding any further loss of product. **10** was allowed to react with an excess of NaOH in DI water for 14 hours at room temperature (Scheme 6). Residual water complicated product identification by ^{1}H-NMR. Water and silica gel problems posed the biggest issue in purification. Temperatures above 45˚C were avoided for drying the compound

because the glucoside is prone to decomposition above this temperature. So the only drying method employed was high vacuum for extended periods of time (up to 48 h).

D)



1 **13**

Scheme 7: Esterification of DCSA

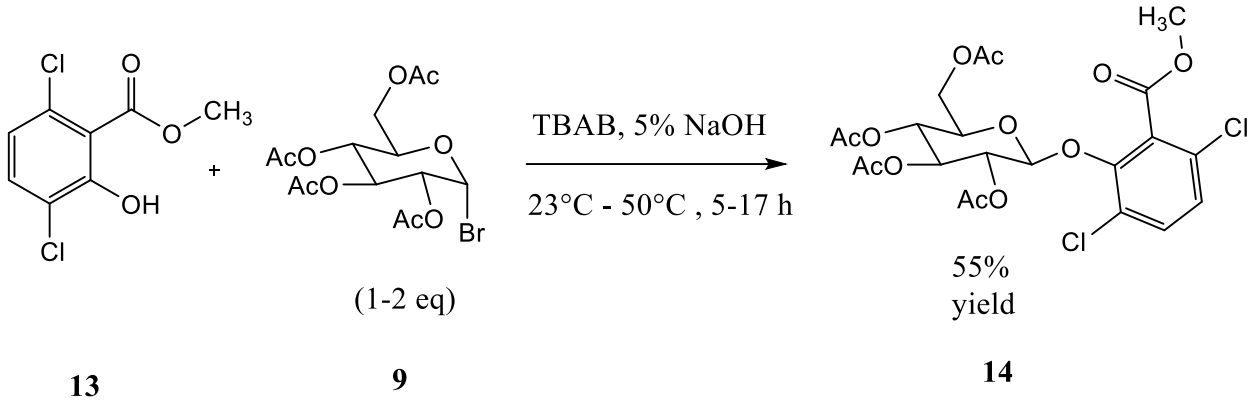
Table 1: DCSA Esterification Data

	H_2SO_4 eq.	Time (h)	Yield (%)
1	0.8	7-8	0
2	1	9	38
3	1	4	21
4	1	35	44
5	2	10	96.6

The same synthetic approach was used for the synthesis of the DCSA metabolite as was used with salicylic acid model. DCSA was protected at the carboxylic acid to force reaction at the carbon-2 hydroxyl group on the aromatic ring (Scheme 7). This esterification was successfully accomplished by heating DCSA under reflux with H_2SO_4 in methanol. Reaction time ranged from 4-35 hours. The amount of H_2SO_4 varied between 0.8-2 equivalents. The

highest yield (96.6%) was accomplished when 2 equivalents of H_2SO_4 was used . No reaction occurred using only 0.8 equivalents of H_2SO_4 (Table 1).

E-1)



13 **9** **14**

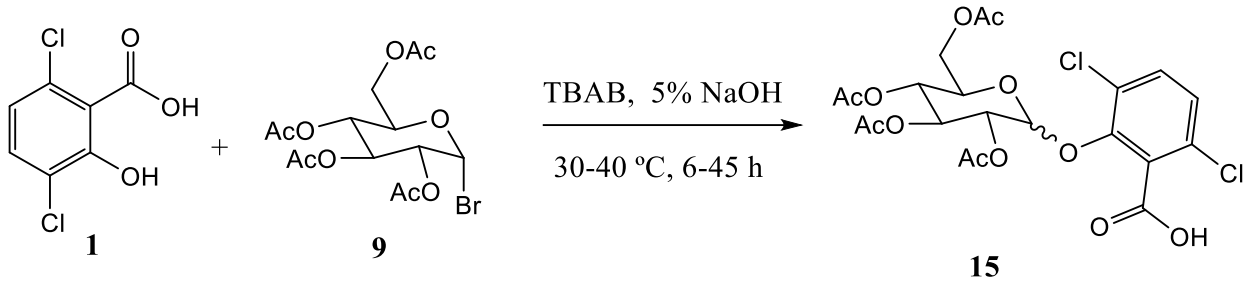
Scheme 8: Glycosylation of DCSA Methyl Ester

Table 2: Glycosylation of DCSA Methyl Ester Data

	Comp 3 eq.	Time (h)	Temp. (˚C)	Yield
1	1.5	8 h	50 ºC	55%
2	2	17 h	rt	23%
3	1.5	5 h	35-40 ºC	18%

The glycosylation of the DCSA methyl ester **13** was accomplished through the reaction of the glucosyl bromide, 5% NaOH solution, and TBAB (1 eq.) as phase transfer catalyst (Scheme 8)**.** Temperatures ranged from 23 ˚C to 50 ˚C and times ranged from 5-17 hours. The amount of **9** was varied from 1.5-2 equivalents (Table 2). These reactions had low to moderate yields but were successful in creating the glycosylation product **14**. However, this approach was revised when it became clear how difficult the saponification reaction of **14** would be.

E-2)



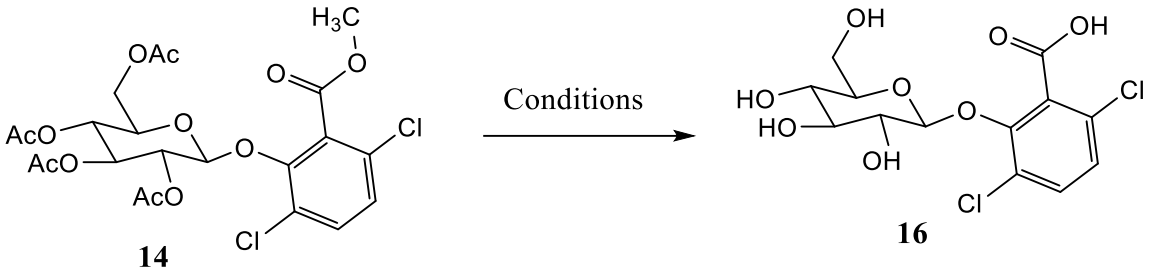
Scheme 9: Glycosylation of DCSA

Table 3: Glycosylation of DCSA Data

	Comp 3 eq.	Time	Notes
1	2	6 h	No Rxn
2	1	45 h	No Rxn

In an attempt to remove the need for a saponification reaction the synthetic approach was altered so that DCSA as the free acid would react with the glucosyl bromide to form **15** (Scheme 9). Neither of the 2 reactions appeared to produce any product based on the TLC (Table 3). It is unclear why the glycosylation occurred when the carboxylic acid was protected but no glycosylation occurred when there was no acid protection. This problem may be due to an increased number of reactive sites on the unprotected compound causing competition and thus lower/no yields.

F)



Scheme 10: Saponification and Hydrolysis of DCSA Methyl Ester Glycoside

Table 4: Saponification and Hydrolysis of DCSA Methyl Ester Glycoside

	Conditions	Notes
1	NaOH (excess) in DI water, rt, 15 h	
2	7M NH_3 in methanol, rt, 3 h	Methyl ester still present in NMR
3	1) 7M NH_3 in methanol, rt, 6 h 2) 0.2% NaOH in methanol, rt, 13 h	
4	0.2% NaOH in methanol, DI water (few drops), rt, 16 h	
5	1) NaOH (1.6 eq), methanol, DI water, EtOAc (few drops), rt, 4 h 2) 7M NH3 in methanol, rt, 4 h	No NMR

Saponification and hydrolysis of the DCSA methyl ester glucoside was attempted just as was done with the methyl salicylate glucoside (Scheme 10). 5 separate reactions were tried but **16** was never synthesized or recovered (Table 4). Based on ^{1}H-NMR analysis, the acetyl groups of the sugar portion were hydrolyzed to the hydroxyl groups but there was no saponification of the methyl ester.

2.2 Methods, Materials, and Select Spectra

General Methods and Materials

Reactions were done in standard glassware that had been washed in Alconox and air dried. All reagents were purchased from commercial sources and were used without further purification. Most reactions were done under standard conditions and select reactions were done under a nitrogen atmosphere. All reactions were monitored using thin layer chromatography (TLC) using glass-backed silica gel plates. TLC plate were visualized under UV light (for aromatic compounds) and by charring with 5% (v/v) H_2SO_4 in EtOH (for sugar compounds). Chemical separation was done on custom made prep TLC plates as well as commercial TLC plates. Column chromatography was performed on silica gel (230–450 mesh, Sorbent). NMR data were obtained using a 400 MHz Bruker NMR and a 300 MHz Bruker NMR. ^{1}H and ^{13}C NMR's were run in deuterated solvents and said solvents were used as the internal reference.

Experimental and Select Spectra:

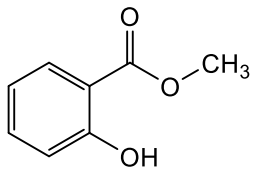


Figure 4: Methyl 2-hydroxybenzoate

Salicylic Acid (4.4 g, 1 equiv.) was put in a round bottom flask. Concentrated H_2SO_4 (1.4 mL, 0.8 equiv.) was dissolved in methanol (20 mL, 16 equiv.) and was poured into flask with the salicylic acid. Solution refluxed for 8 hours. Methanol was removed under vacuum and DI

water was used to dissolve solid/oil. Solution was transferred to a separatory funnel and water layer was washed 3 times with 30 mLs of ethyl acetate. Organic layer was then washed with 30mLs of $NaHCO_3$ solution and lastly 15 mLs of DI water. Solution was dried with $MgSO_4$ overnight. Crude product was filtered off $MgSO_4$ and ethyl acetate was removed under vacuum affording a relatively pure **8** (61%) (Figure 4).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.75 (s, 1H, H-1), 7.81 (m, 1H, H-2), 7.43 (m, 1H, H-3), 6.96 (d, 1H, H-4, $J_{1,2}$=8.4 Hz), 6.85 (m, 1H, H-5), 3.92 (s, 3H, H-6). (Figure 5)

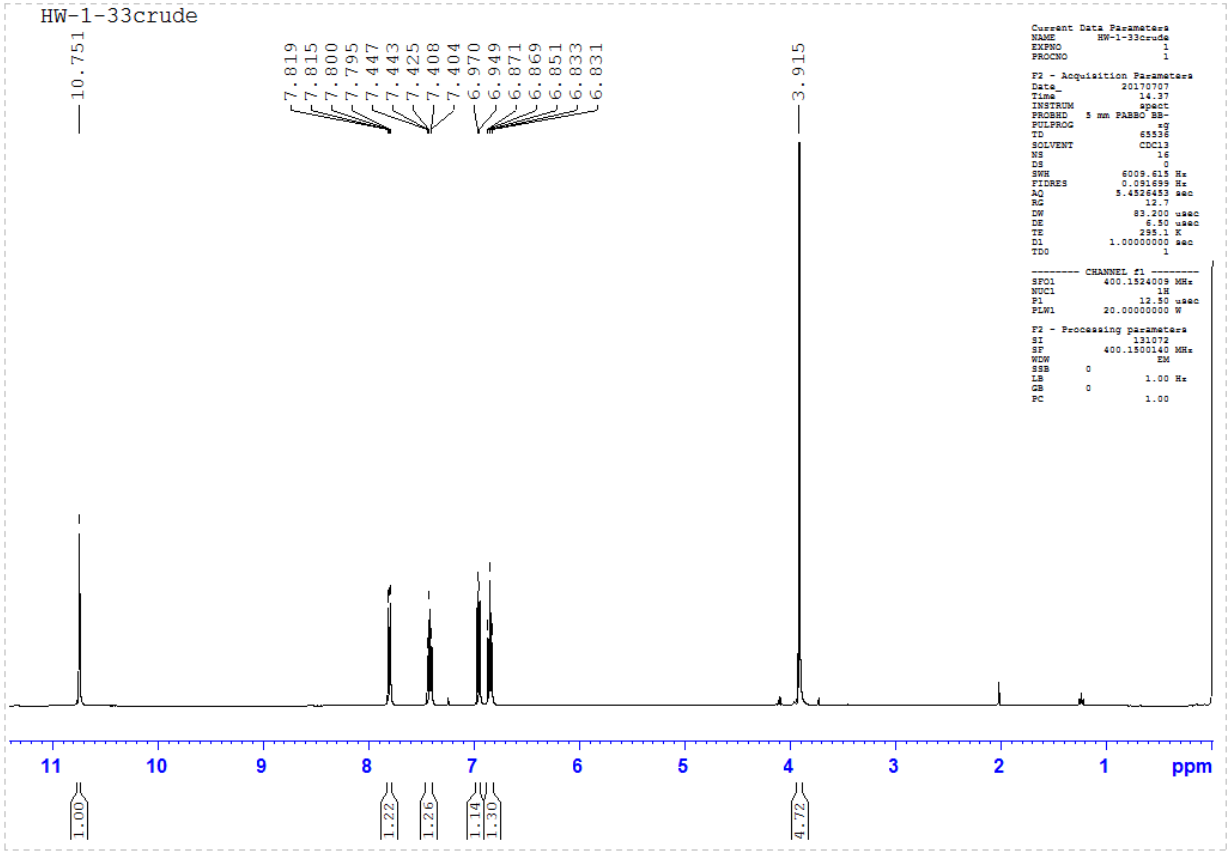


Figure 5: H[1]-NMR of Methyl 2-hydroxybenzoate

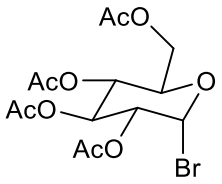


Figure 6: 2,3,4,6-Tetra-O-acetyl-alpha-D-glucopyranosyl bromide

9 was generously synthesized by the Streigler lab and was purified periodically due to decomposition. Compound was stored in freezer to minimize decomposition (Figure 6).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.62 (d, 1H, H-1, $J_{1,2}$=4.04 Hz), 5.57 (at, 1H, H-3, J=9.72 Hz), 5.17 (at, 1H, H-4, J=9.88 Hz), 4.85 (dd, 1H, H-2, $J_{2,3}$=5.92), 4.36-4.29 (m, 2H, H-6, H-5), 4.15-4.13 (m, 1H, H-7), 2.1123, 2.108, 2.0625, 2.0453 (4s, 4 x $OC(O)CH_3$). (Figure 7)

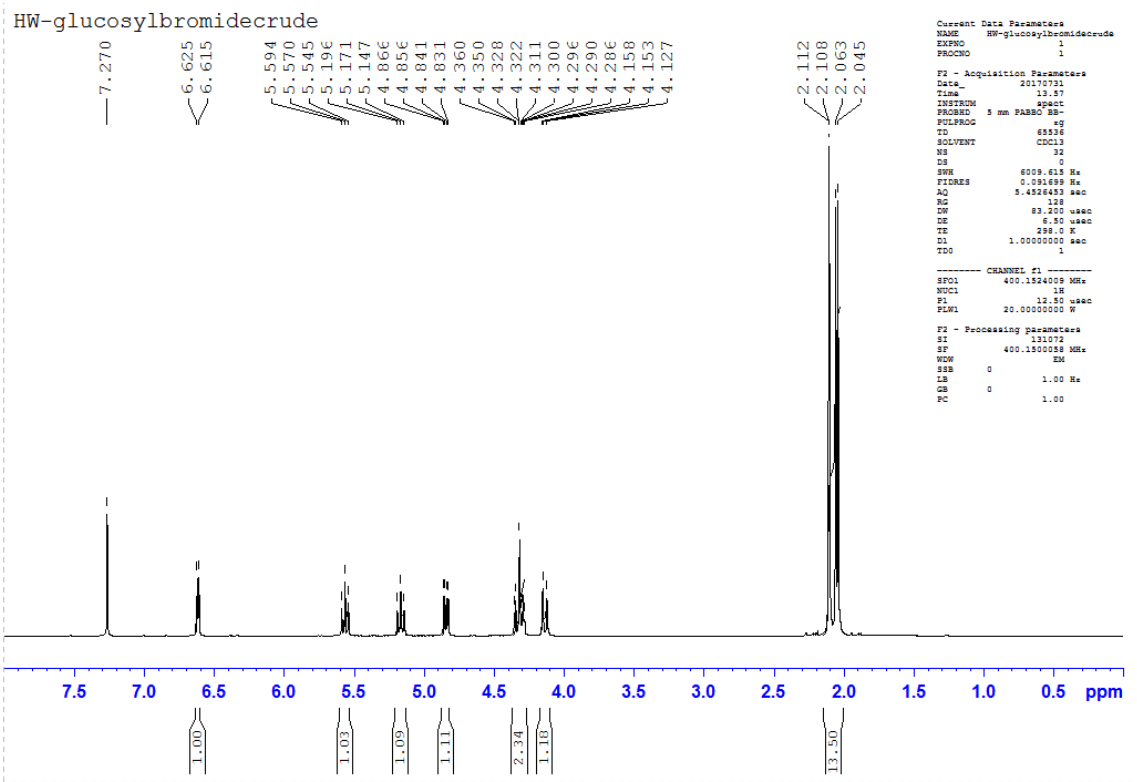


Figure 7: H[1]-NMR of 2,3,4,6-Tetra-O-acetyl-alpha-D-glucopyranosyl bromide

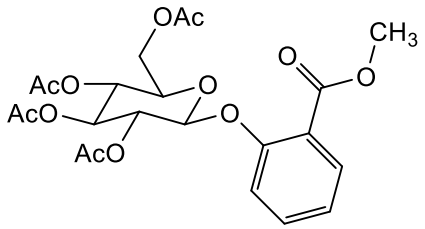


Figure 8: Methyl Salicylate ß-D-Glucose Tetraaacetate

Methyl Salicylate (0.1 g, 1.6 equiv.) and tetra- butyl ammonium bromide (0.07 g, 0.5 equiv.) were dissolved in 2 mL of dichloromethane. Then 1 mL of a 5% NaOH solution (v/v) was added and mixture stirred for approximately 30 minutes. **9** (0.17 g, 1 equiv.) was dissolved in 2 mL of dichloromethane and added dropwise to stirring mixture. Solution was allowed to react for 7 hours at 40-60 ˚C. Solution was cooled to room temperature then put in an ice bath. Solution was transferred to separatory funnel and organic layer was washed with two 2 mL portions of the 5% NaOH solution (v/v). The organic layer was then washed with three 2 mL portions of DI water. Dichloromethane was removed by evaporation and produced 0.074 g of crude product (Figure 8).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.75 (m, 1H, H-11), 7.45 (m, 1H, H-9), 7.14-7.10 (m, 2H, H-8, H-10), 5.56 (d, 1H, H-1, $J_{1,2}$=4.29 Hz), 5.38-5.09 (m, approx. 2H, H-6, H-7), 4.29 (dd, 1H, H-3, $J_{2,3}$=6.99 Hz), 4.18 (dd, 1H, H-2, $J_{2,3}$=9.84 Hz), 3.91-3.87 (m, 1H, H-4), 3.85 (s,3H, H-12), 2.07, 2.06, 2.05, 2.04 (4s, 4 x $OC(O)CH_3$). (Figure 9)

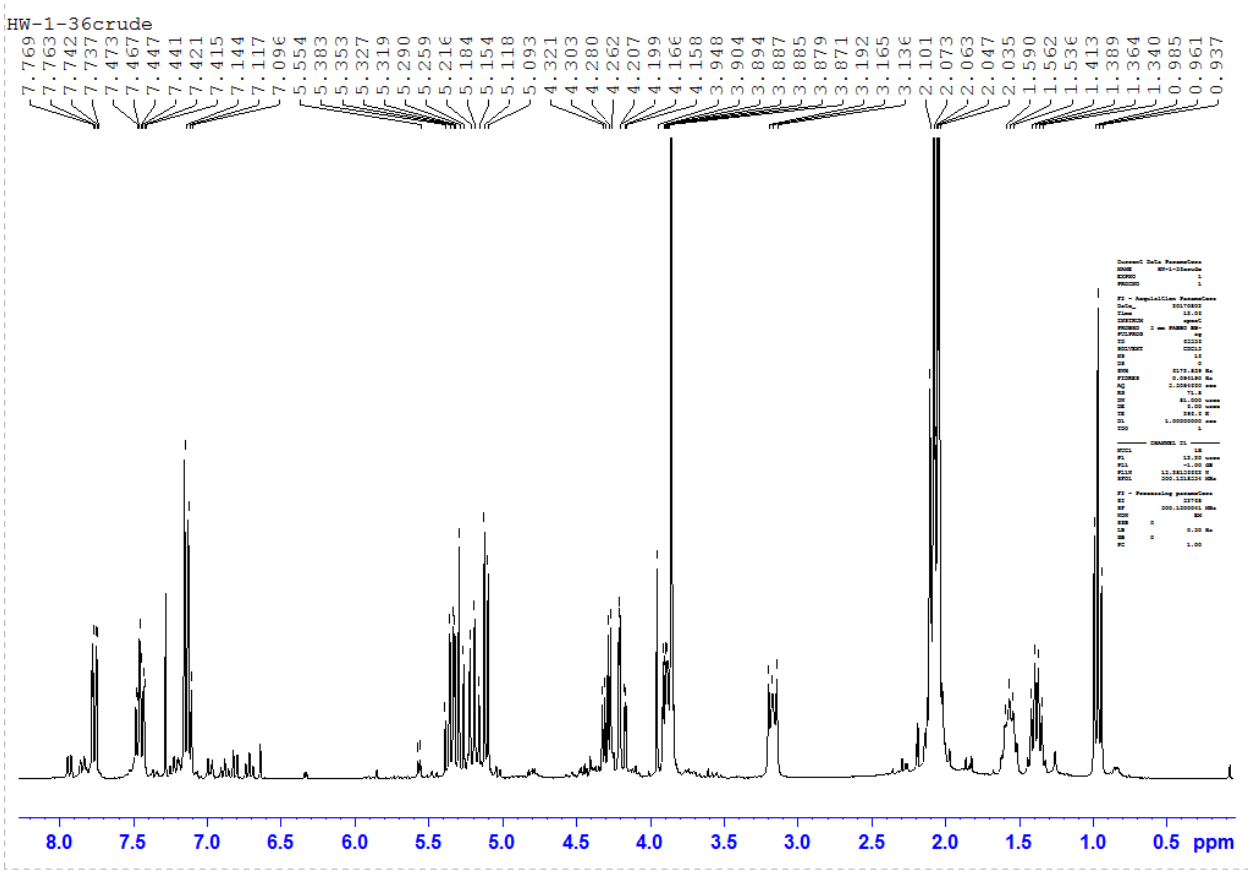


Figure 9: H^1-NMR of Methyl Salicylate ß-D-Glucose Tetraaacetate

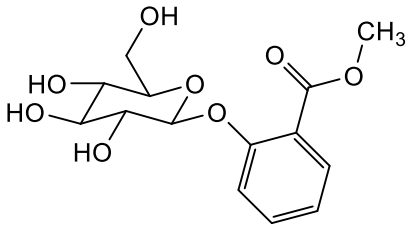


Figure 10: DCSA Methyl Ester Glucoside

Crude **10** (0.247 g) was dissolved in 2 mL of methanol and 0.5 mL of a 5% Na_2CO_3 solution was added dropwise. Solution was allowed to stir at room temperature for 2 hours. Solution was neutralized with trifluoroacetic acid. Solution was put in a separatory funnel and DI water was

added. Dichloromethane was used to extract compound from water layer. Purified using a Preparatory TLC (dichloromethane/10% methanol as solvent). **11** was found to be the second product on TLC plate and 0.022 g were isolated (16%) (Figure 10).

^{1}H NMR (400 MHz, D_2O) δ (ppm): 7.82 (m, 1H, H-10), 7.63 (m, 1H, H-8), 7.33 (d, 1H, H-7, $J_{1,2}$=8.44 Hz), 7.23 (at, 1H, H-9, J=7.58 Hz), 5.17 (d, 1H, H-1, $J_{1,2}$=7.20 Hz), 3.95-3.93 (m, 4H,

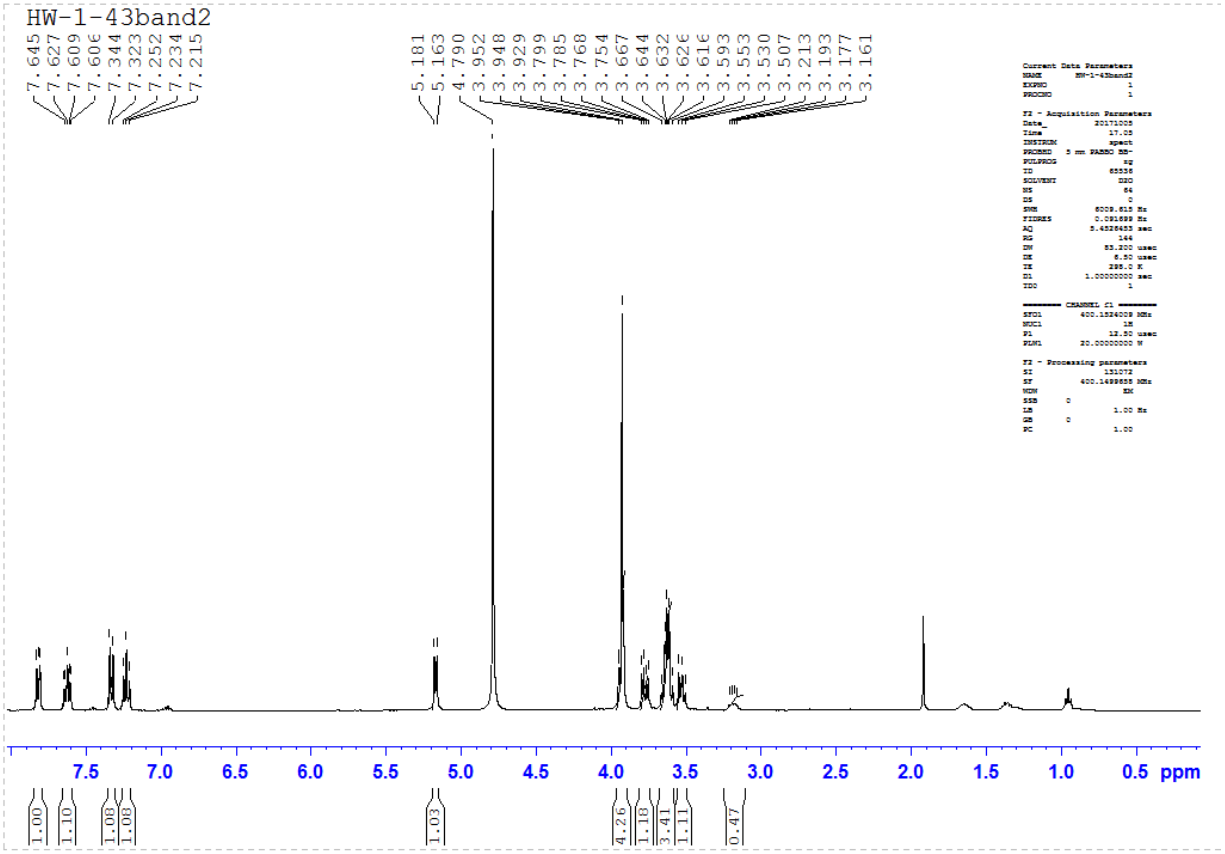


Figure 11: H[1]-NMR of DCSA Methyl Ester Glucoside

[1H,H-3], [3H, H-6]), 3.78 (dd, 1H, H-2, $J_{2,3}$=7.00 Hz), 3.67-3.59 (m, 2H, H-6, H-7), 3.53 (m, 1H, H-4), 3.19 (m, 1H, H-5). (Figure 11)

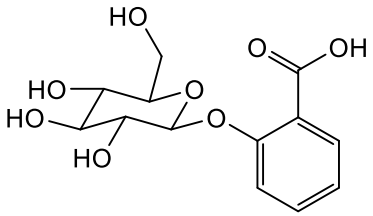


Figure 12: Salicylic Acid Glucoside

11 (10 mg, 1 eq.) (Figure 12) was dissolved in DI water and NaOH (0.025 g, 20 eq) and stirred at room temperature for 14 hours. Purified on preparatory TLC using dichloromethane/20% methanol as solvent.

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.82 (d, 1H, H-1), 7.28 (m, 1H, H-3), 6.77 (m, 2H, H-4, H-2), 5.49 (d, 1h, H-5), 3.67 (m, H7, H-6 – H-11). (Figure 13)

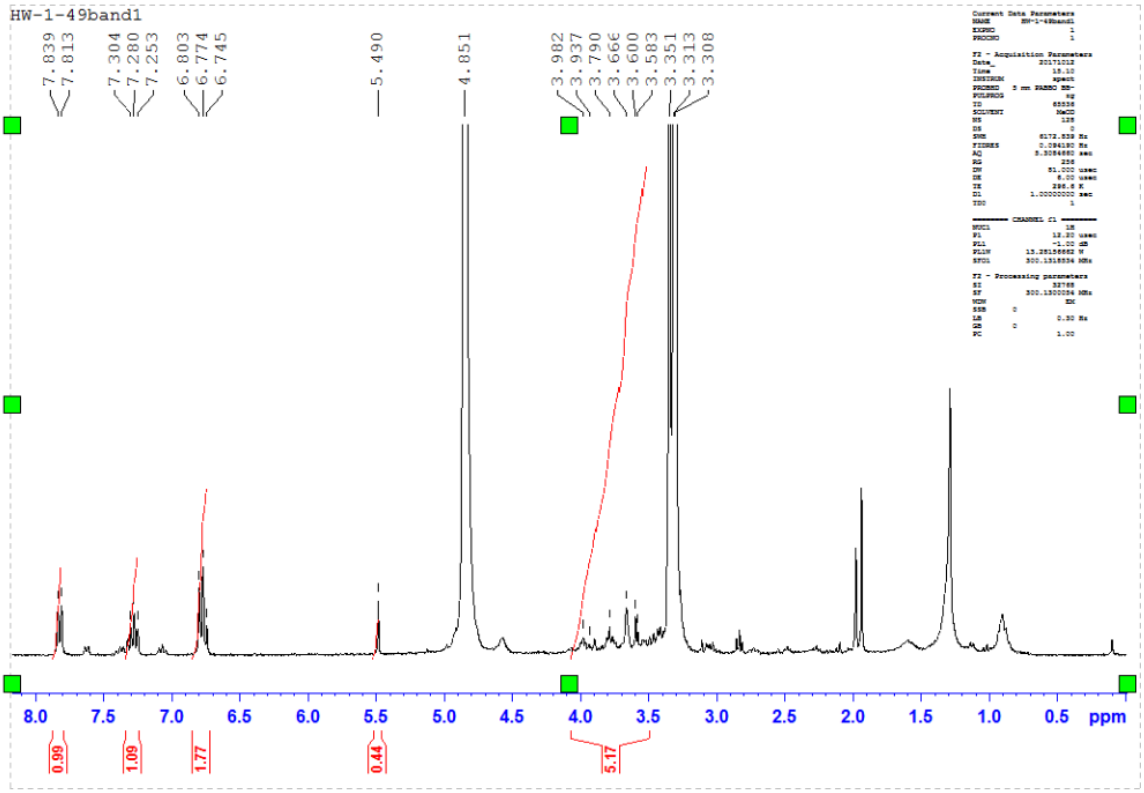


Figure 13: H^{1}-NMR of Salicylic Acid Glucoside

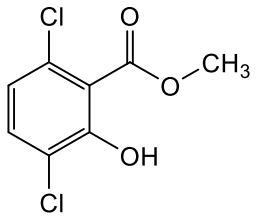


Figure 14: DCSA Methyl Ester

1 (0.4 g, 1 eq) was dissolved in excess methanol and H_2SO_4 (0.1 mL, 1 eq) was added. Solution stirred at room temperature for 14 hours with no reaction occurring based of TLC. Temperature was increased to 70 ˚C and stirred for an additional 21 hours. After reaction occurred the methanol was removed by vacuum and DI water was added to re-dissolve solid. Solution was transferred to a separatory funnel and three 5 mL portions of ethyl acetate were used to extract product from water layer. Ethyl Acetate washes were collected and washed with a saturated $NaHCO_3$ solution. Extracted organic layer from $NaHCO_3$/water layer and evaporated ethyl acetate under vacuum. A preparatory TLC was used to purify **13**. **13** was found to be the top product on the TLC plate. Ethyl acetate was used as solvent for purification. 0.189 g of **13** were recovered (44% yield) (Figure 14).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 11.42 (s, 1H, H-1), 7.33 (d, 1H, H-2, $J_{1,2}$=8.56), 6.86 (d, 1H, H-3, $J_{1,2}$=8.56), 3.97 (s, 3H, H-4). (Figure 15)

^{13}C NMR (400, $CDCl_3$) δ (ppm): 169.69 (C-1), 158.25 (C-2), 134.05 (C-3), 133.29 (C-4), 122.55 (C-5), 121.30 (C-6), 113.46 (C-7), 53.11 (C-8). (Figure 16)

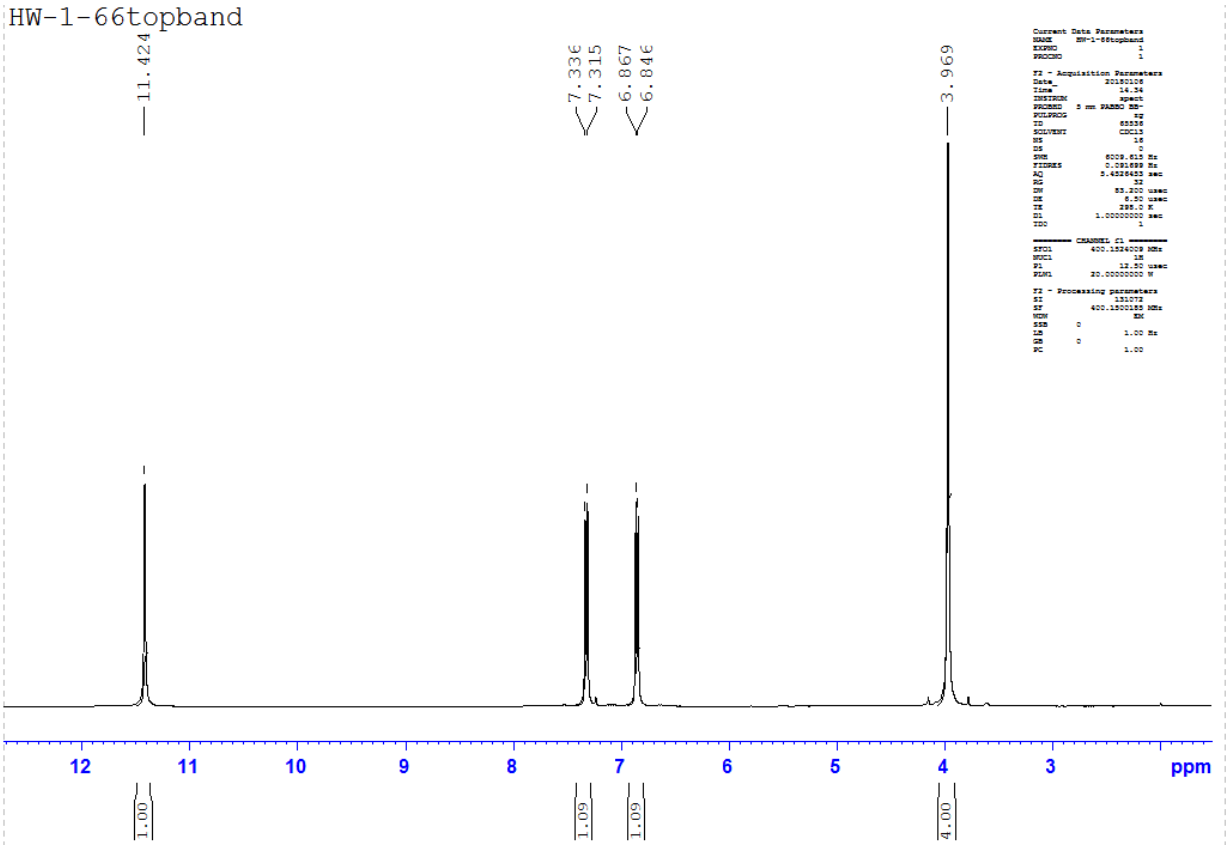


Figure 15: H^1-NMR of DCSA Methyl Ester

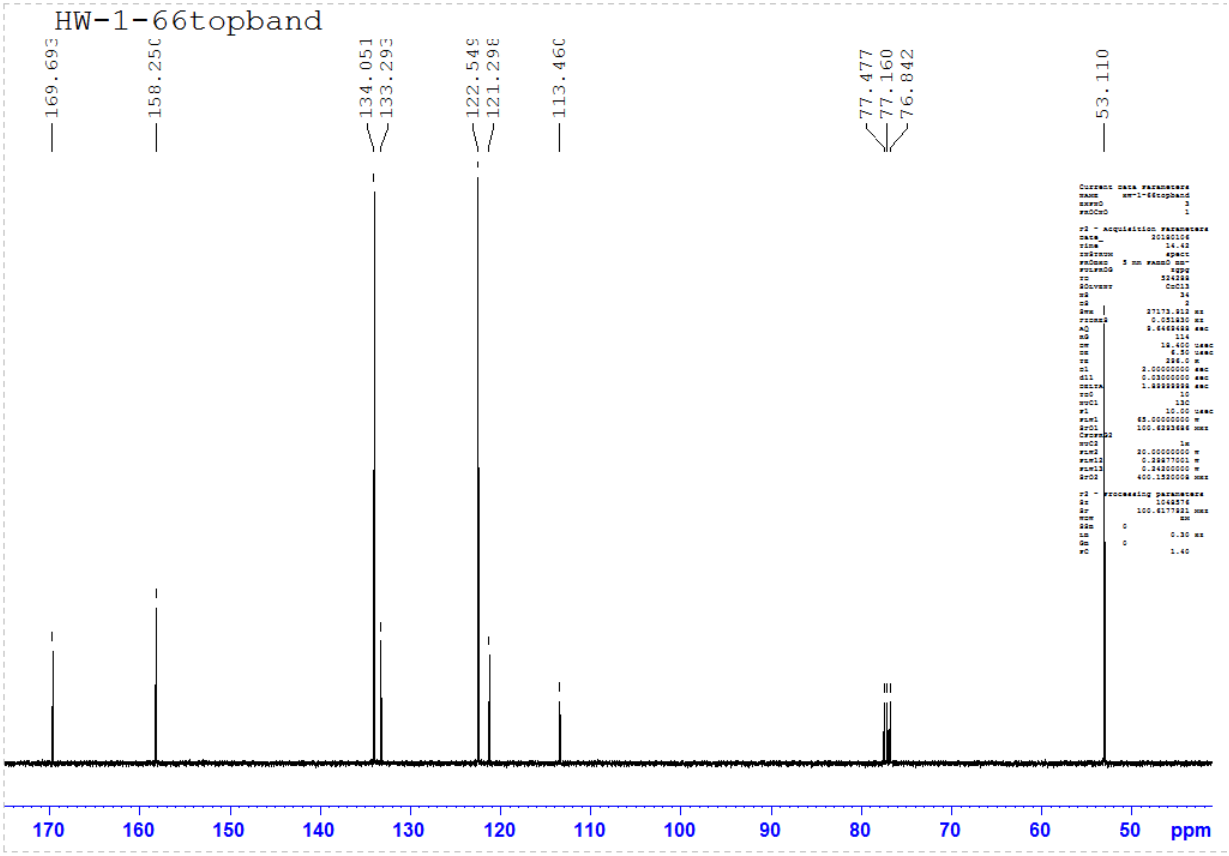


Figure 16: C^{13}-NMR of DCSA Methyl Ester

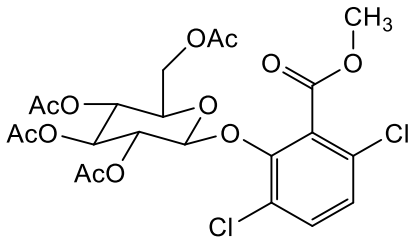


Figure 17: DCSA ß-D-Glucose Tetraacetate

13 (0.02 g, 0.5 eq) and tetra butyl ammonium bromide (0.03 g, 0.5 eq) were dissolved in 1 mL of dichloromethane. Then 0.5 mL of a 5% NaOH solution (v/v) was added and mixture stirred for approximately 30 minutes. **9** (0.075 g, 1 eq) was dissolved in 0.5 mL of dichloromethane and added dropwise to stirring mixture. Solution was allowed to react for 17 hours at room

temperature. Solution was put in an ice bath. Solution was transferred to separatory funnel and organic layer was washed with two 0.5 mL portions of the 5% NaOH solution (v/v). Organic layer was then washed with two 0.5 mL portions of DI water. Dichloromethane was removed by evaporation and produced 0.065 g of crude product. Purification was done using a series of 3 preparatory TLC plates. The first plate used ethyl acetate as solvent and top compound on the TLC plate was kept (this removed TBAB). The second plate used an ethyl acetate/hexanes 1:1 solvent system keeping the second product on this TLC plate. The third plate used dichloromethane as solvent and was allowed to run for 5 hours. Bottom product of this TLC plate was desired product. Yield was 0.011 g of product (23% yield) (Figure 17).

^{1}H NMR (400 MHz, C_6D_6) δ (ppm): 6.67 (d, 1H, H-9, $J_{1,2}$=8.68 Hz), 6.51 (d, 1H, H-10, $J_{1,2}$=8.72), 5.53 (at, 1H, H-3, J.=8.58 Hz), 5.39-5.29 (m, 2H, H-6, H-7), 4.95 (d, 1H, H-1, $J_{1,2}$=7.88 Hz), 4.25 (dd, 1H, H-2, $J_{2,3}$=8.56 Hz), 3.9 (m, 1H, H-4), 3.62 (s, 3H, H-11), 2.95 (ddd, 1H, H-5, $J_{4,5}$=3.8 Hz), 1.82, 1.74, 1.69, 1.69 (4s, 4 x OC(O)CH_3). (Figure 18)

^{13}C NMR (400, C_6D_6) δ (ppm): 169.96, 169.93, 169.02, 168.84 (C-7), 163.94 (C-8), 149.69 (C-9), 131.99 (C-10), 131.75 (C-11), 130.88 (C-12), 127.42 (C-13), 126.95 (C-14), 101.73 (C-1), 73.30 (C-5), 72.55 (C-3), 71.97 (C-2), 68.03 (C-4), 61.01 (C-6), 52.49 (C-15), 20.42, 20.28, 20.19, 20.10 (C-16). (Figure 19)

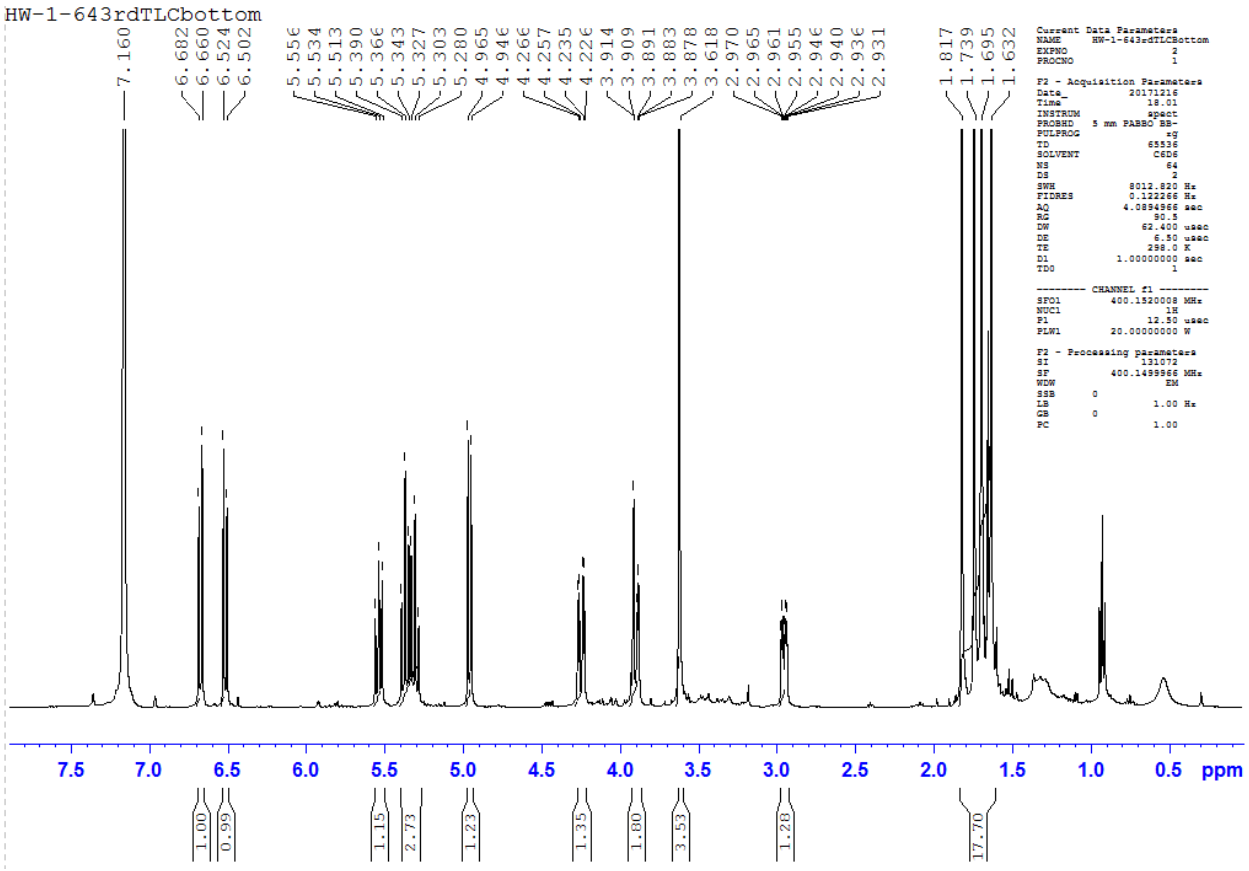


Figure 18: H^1-NMR of DCSA ß-D-Glucose Tetraacetate

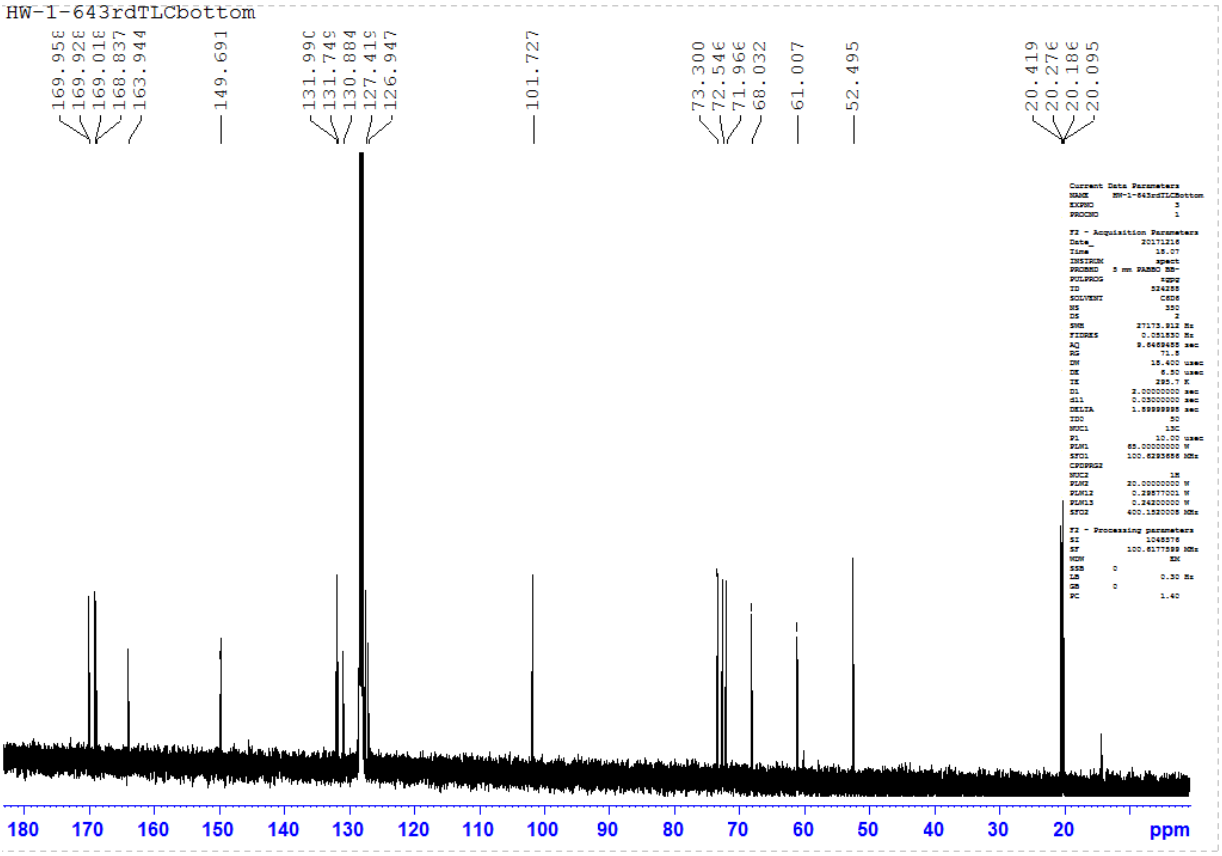


Figure 19: C^{13}-NMR of DCSA ß-D-Glucose Tetraacetate

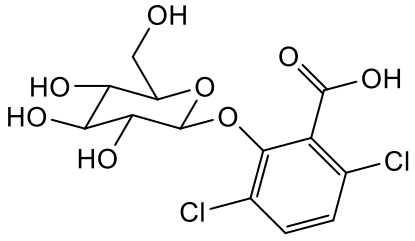


Figure 20: DCSA Glucoside

14 was dissolved in excess 7M NH_3 in methanol solution and allowed to react for 3 hours at room temperature. The 7M NH_3 in methanol solution was removed via vacuum and purification was done. A preparatory TLC was used with ethyl acetate as solvent. An NMR was run on the bottom product of the TLC plate. The NMR proved that the reaction was not completely

successful. The acetyl groups of the sugar were hydrolyzed but the methyl ester on the aromatic ring was still present in sample (Figure 20).

^{1}H NMR (400 MHz, MD_3OD) δ (ppm): 7.54 (d, 1H, H-9, $J_{1,2}$=8.72 Hz), 7.31 (d, 1H, H-10, $J_{1,2}$=8.72 Hz), 4.89 (dd, 1H, H-3, $J_{2,3}$=3.16 Hz), 3.93 (s, 3H, H-8), 3.77 (dd, 1H, H-2, $J_{2,3}$=9.48 Hz), 3.66-3.61 (m, 2H, H-6, H-7), 3.41 (dd, 1H, H-4, $J_{2,3}$=3.48 Hz), 3.19 (m, 1H, H-5). (Figure 21)

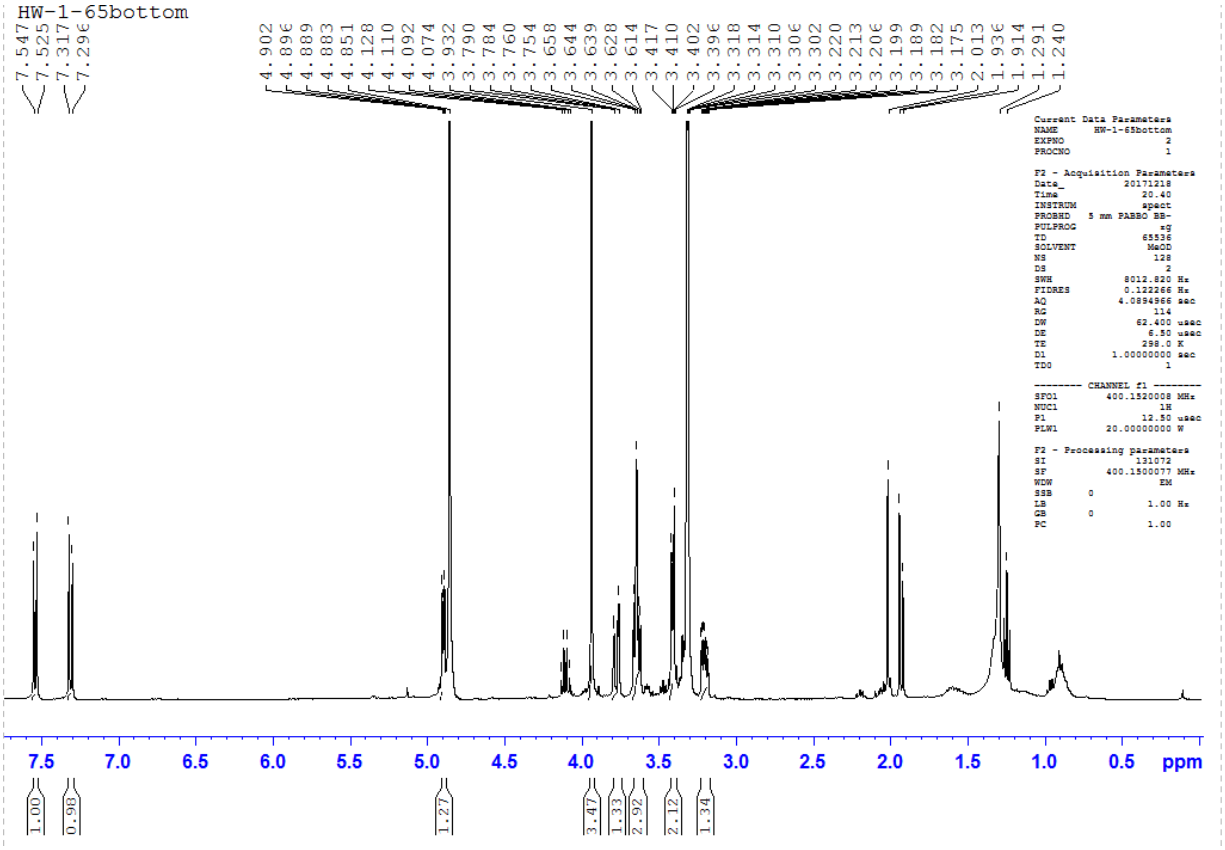


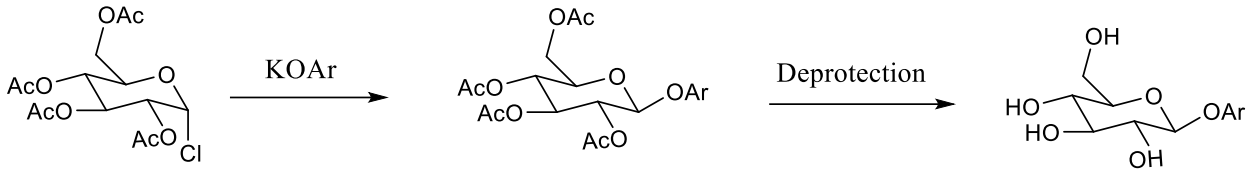
Figure 21: H[1]-NMR of DCSA Glucoside

CHAPTER 3: Results and Conclusion

The plan was to synthesize the glucosides of three different dicamba metabolites by attaching a glucose ring to the hydroxyl groups of the different metabolites. The first step was to use a model system that is similar to dicamba (salicylic acid). The carboxylic acid group of salicylic acid was protected by esterification in order to allow for glycosylation at only the hydroxyl group. This reaction was simple and provided a reasonably pure product with no additional purification necessary for **8**.

A tetra acetylated glucosyl bromide sugar was provided by the Striegler lab to do the Michael glycosylation reaction. All the free hydroxyl groups on the sugar were protected from reaction by using acetyl groups as protecting groups. This sugar contained an alpha bromine group on the aromatic carbon to act as leaving group during the glycosylation reaction. The glycosylation reaction was optimized but proved difficult to purify. TBAB is undetectable on TLC plate under UV light and upon sulfuric acid staining/burning. This problem was solved by using either a silica gel "plug" column or by using multiple preparatory TLC plates. Purification was further complicated by presence of alpha and beta anomers. It is believed (from 400 MHz ^{1}H-NMR) that the beta anomer was the predominate anomer produced with an α/β 1:4 ratio. The product **10** is crude and ^{1}H-NMR is complicated, but the alpha proton peak shows up as a doublet at ~6.35 ppm and the beta proton peak is visible at 5.56 ppm.[21] Pure product was not recovered and subsequent reactions were carried out with impure products. It is possible that product was never synthesized or that it decomposed. The NMR is complicated making accurate identification impossible.

The synthetic strategy for synthesizing the glucosides follows a traditional Michael glycosylation reaction (Scheme 11). It was initially believed that glycosylation reactions



occurred

Scheme 11: Original Michael Reaction

simply through an SN2 type mechanism based on studies done by Koenig and Knorr in 1901.

To reach the final salicylic acid metabolite it was necessary to hydrolyze the glucosyl acetyl groups as well as the ester protecting group. The plan was to accomplish this over two reaction steps. First would be the removal of the acetyl groups **11** followed be the ester de-protection **12**. It became clear however, that attempting to do two reactions/purifications that there was virtually no product left and that this procedure should be modified. Adjustments were made and complete hydrolysis was attempted over one step instead of the previous two steps. The fully hydrolyzed glucoside was synthesized but NMR is messy because of the presence of water and methanol in sample **12**.

With a working model, DCSA metabolite synthesis was begun. Using the same methodology as with salicylic acid, the carboxylic acid of DCSA was protected by transforming it into a methyl ester **13**. This reaction proceeded as planned and produced as much as a 96% yield. Product purity was established via NMR.

The glycosylation of the protected DCSA **14** was eventually successful with low to moderate yields (55% yield was the highest obtained) and was able to be purified. This method would have been fine were it not for the fact that the final product required the de-protection of

the ester. The hydrolysis of the DCSA-glucoside was very difficult **16**. Multiple attempts were made and I was able to hydrolyze the acetyl groups on the sugar with ease but could never manage to hydrolyze the ester.

Further work on this synthesis can be done to improve yields and purification. There are also other synthetic routes that may prove more beneficial. The Koenigs-Knorr glycosylation reaction is a possible alternative. This reaction glycosylates an alcohol in the presence of Ag_2CO_3. The Koenigs-Knorr glycosylation reaction employs the use of the 2-O-acyl group to increase selectively toward the alpha or beta product. The Helferich conditions may also be a viable route of synthesis. Beta-D-glucose pentaacetate is used as glucosyl donor in the presence of a lewis acid such as $BF_3 \cdot OEt_2$ to glycosylate various phenols. This route may eliminate the need for saponification of glycosylation product that has been a complication with current methods. Many other glycosylation reactions exist that may accomplish the synthesis of the glucosides.[22]

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