Synthesis of Glycoalkaloids

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ABSTRACT: Glycoalkaloids are antibacterial and antifungal organic chemicals made by the solanum dulcamara family, also known as the bittersweet nightshades. These glycoalkaloid molecules are made of a combination of three different alkaloids: tomatidine, solasodine, solanidine, and various monosaccharide glycosidic units. Glycoalkaloids are understood to cause anti-viral, and anti-cancer effects, but it is still not known how the reaction pathway works. It is suggested that the change in the bolaamphiphile structure causes the membranes to thin and promote apoptosis in cells. Synthesizing specific glycoalkaloids to test in vitro can prove to be beneficial to the efficiency and development of new medicines and pest controls. Synthesizing glycoalkaloids will also allow for macro-scale tests. Glycoalkaloids can be synthesized using standard organic reactions. 10 major reactions need to be done to synthesis a glycoalkaloid. α-Chaconine is the target glycoalkaloid in this synthesis for its researched anti-cancer effects. The first 4 reactions were performed making 1-thio-β-D-glucose 2,3,4,6-tetraacetate, 1-thio-β-D-glucose, 1,2,3,4-tetraacetyl-L-rhamnose, and phenyl 4,6-O-benzylidene-1-thio-β-D-glucose. 1-Thio-β-D-glucose 2,3,4,6-tetraacetate reaction had a 77.5% yield and 1-thio-β-D-glucose reaction had a 92% yield. 1,2,3,4-tetraacetyl-L-rhamnose reaction was left in a syrup form and weight analysis has not yet been done. Phenyl 4,6-O-benzylidene-1-thio-β-D-glucose reaction was left in DCM and still needs to be separated from benzaldehyde dimethyl acetyl. These 4 reactions are a good start to synthesizing α-chaconine. Once α-chaconine is made, biological analysis can begin in vitro and can be used to study biological reactions and can make new and more efficient medicines and pest controls. Using and tweaking this procedure, different glycosidic units can be made allowing for unique and new glycoalkaloids to be synthesized and studied.

Introduction: Glycoalkaloids are organic compounds found in the plant species solanum dulcamara. This family is known as the bittersweet nightshades. Glycoalkaloids are understood to be toxic and act as antibacterial and antifungal compounds in the nightshades. This toxic property is also known to cause many biological implications such as medicine or pest control.[10] One implication is its ability to be an anti-cancer medicine.[9] Glycoalkaloids has been used in the past, but the specific mechanisms by which the glycoalkaloids destroy the cancer cells is not entirely known and has been an unapproved drug in 2004.[8] There still has not been a major break through but it is suspected that a change in the bolaamphiphile structure

will cause the membrane to thin and promote lipid flip-flop triggering cell death. Bolaamphiphilic compounds have two hydrophilic ends and a hydrophobic connection. It regulates cellular plasma and stabilization of membranes. Because glycoalkaloids have two distinct parts, it makes them act amphiphilic causing for this change in bolaamphiphile structure. These two parts of the glycoalkaloid comes out to be an alkaloid: a hydrophobic aglycone made from cholesterol, with at least one nitrogen atom, and a glycosidic unit made of different combinations of monosaccharides. (FIGURE 1)

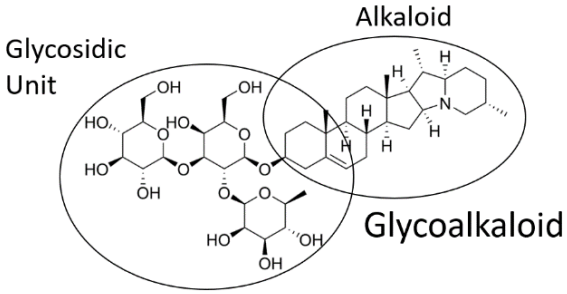


***FIGURE 1**, Glycoalkaloids are made from 2 main components. The alkaloids and the glycosidic unit. These two parts combine to make a glycoalkaloid.*

The alkaloid is known to be the functional part of the glycoalkaloid making it toxic, but the glycosidic unit gives allows for efficiency and reactivity.[9] Many alkaloids are already used in medicines as antimalarials, antiasthma, anticancer, cholinomimetic, vasodilator, antiarrhythmic, analgesic, antibacterial, and antihyperglycemic.[1,2,3,4,5,6,7] Alkaloids can also have the ability to be psychotropic and provide stimulant effects, much like cocaine caffeine, nicotine, and theobromine for recreation drugs use.[1,2,3,4,5,6,7] There are three major alkaloids found in the solanum genus, solasodine, solanidine, and tomatidine. (FIGURES 2,3,4) These alkaloids make up the toxic functionality of the glycoalkaloids and they all differ from each other. Tomatidine doesn't have a double bond solasodine has, and both tomatidine and solasodine have an oxygen incorporated into its rings, while solanidine doesn't.

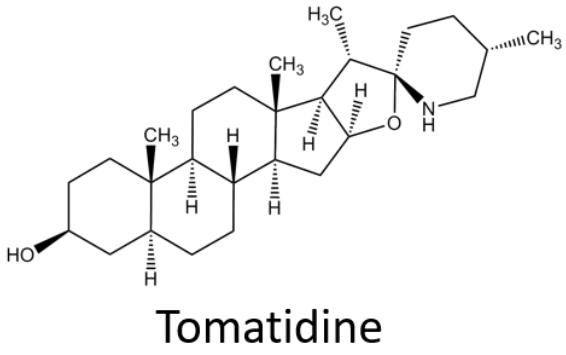


***FIGURE 2**, Tomatidine is an alkaloid that is found in many glycoalkaloids. This alkaloid is mainly found in tomatoes.*

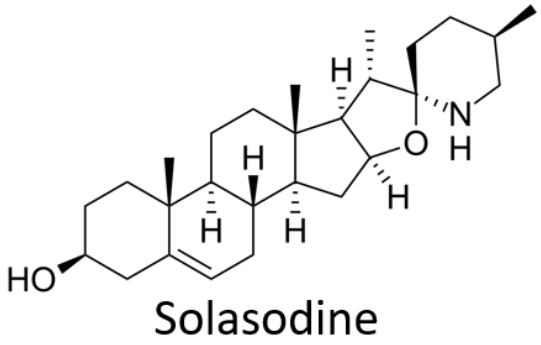


***FIGURE 3**, Solasodine is an alkaloid that is found in many glycoalkaloids. This alkaloid is found in all nightshades.*

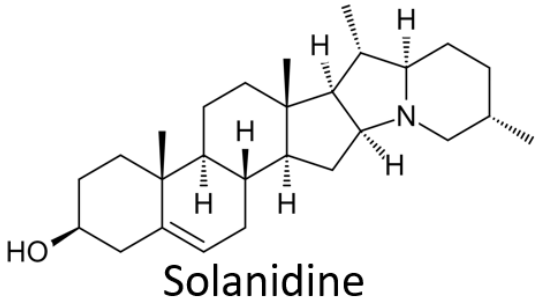


***FIGURE 4**, Solanidine is an alkaloid that is found in many glycoalkaloids. This alkaloid is found in all nightshades but can be found in high concentrations of young green potato skins.*

The monosaccharides glycosidic unit defines how well the glycoalkaloids works[9] and so the combinations of the alkaloids with the vast

number of glycosidic units have to be taken into consideration when synthesizing glycoalkaloids. α-Chaconine (FIGURE 5) is known to be a glycoalkaloid made from the alkaloid solanidine and the glycosidic unit made of two rhamnose and one glucose.

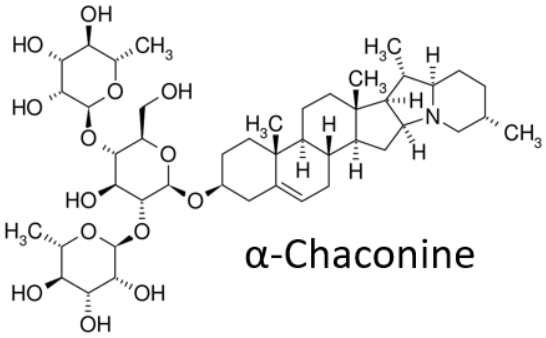


***FIGURE 5**, α-Chaconine is a glycoalkaloid with anti-cancer effects. This glycoalkaloid is the target glycoalkaloid to be synthesized.*

This structure is known to have an effective anti-cancer effects, even more so than α-solanine: solanidine connected to three glucose.

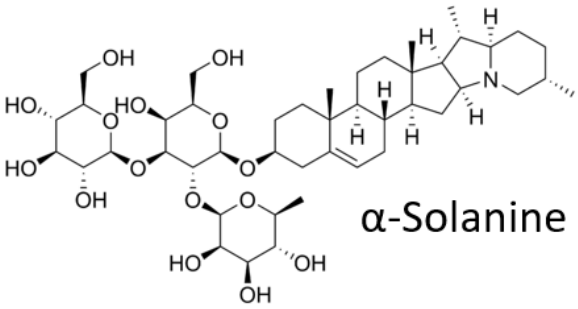


***FIGURE 6**, α-Solanine is a glycoalkaloid with anti-cancer effects. Its commonly found in young green potato skins.*

This is suggesting that the difference of a methyl group on the 5'C, which is the rhamnose structure, and the connection of the rhamnose to glucose at the 4'C makes the effective differences. This is seen in the chart below. (FIGURE 7)[9]

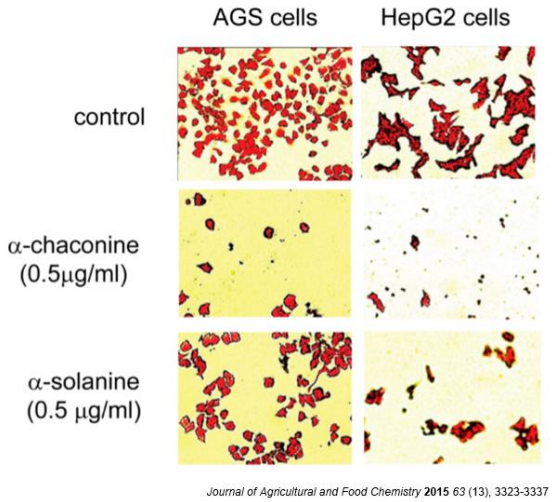


***FIGURE 7**, Glycosidic unit variation changes the effectiveness of its anti-cancer effects. Hep G2 and AGS cancer cells were treated in vitro with α-chaconine or with α-solanine for 48h and stained with sulforhodanine. Adapted from ref 9." Journal of Agricultural and Food Chemistry 2015."*

This chart shows the growth of AGS cancer cells and HepG2 cancer cells in vitro. Both α-chaconine and α-solanine showed anti-cancer growth, but α-chaconine showed to be more effective in both cancer cells. Being able to synthesize glycoalkaloids will allow its functionality to be further developed in vitro. Determining these functionalities, medicines and treatments using alkaloids can be further developed and improved with this knowledge. Organic synthesis is often done to make vast amounts of medicines and carbon-base compounds in large amounts. While it is possible to obtain glycoalkaloids from the plants themselves, it is also possible to synthesis glycoalkaloids in an organic lab. Doing so will allow large amounts of glycoalkaloids to be made. These amounts can then be used in vitro research to understand functionality. The organic synthesis will include hydroxylation, acetylation, protecting and deprotecting hydroxyl groups, activation of hydroxyl groups

with CCl_3CN, liquid separation, high-vacuum rotovap, recrystallization, and silica-gel column.[15,16,17,18,19] For analysis TLC (thin-layer chromatography), HPLC (High-pressure liquid chromatography), and NMR (nuclear magnetic resonance spectroscopy) was going to be used. TLC uses solvents to sperate compounds based off of there polarity, allowing for simple checking completion of reaction and contaminates. HPLC was to be used to check purity of the final glycoalkaloid. NMR was going to be used to check the structure and confirm it was the correct glycoalkaloid made. Glycoalkaloids can be made with commercially available reagents and with normal organic separation and extractions. Glycoalkaloid synthesis can then happen in basic organic labs and doesn't need expensive machines for it to be made.

Results and Discussion

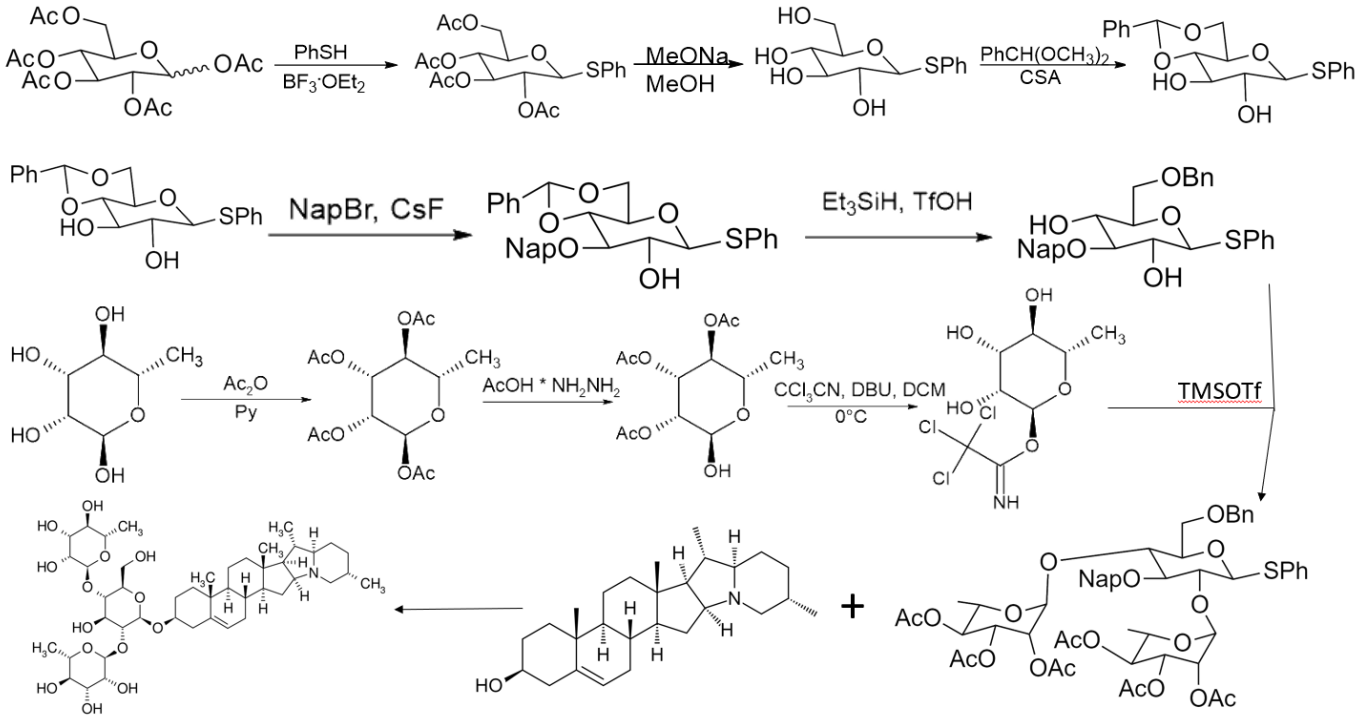
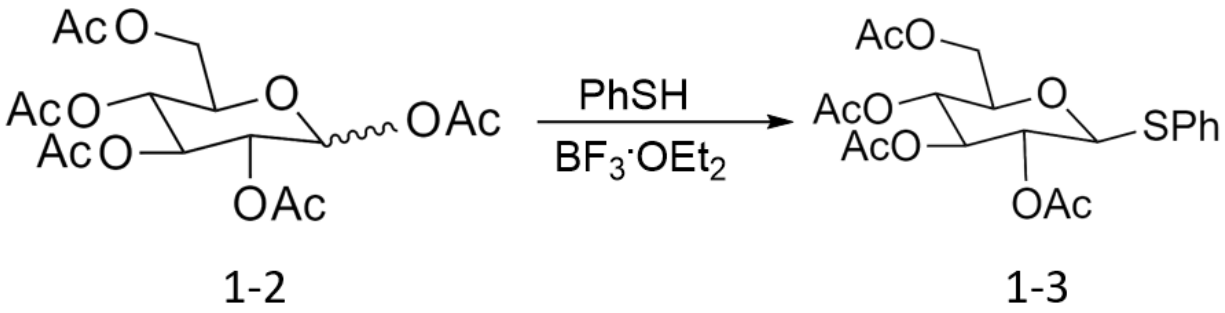


***FIGURE 8**, Glycoalkaloid Synthetic Route*

Experimental Procedure



1-2 1-3

1-Thio-β-D-glucose 2,3,4,6-Tetraacetate (1-3)

10.0695g of pentaacteyl-β-D-glucose **1-1** (25.8 mmol) diluted with DCM (20 mL). Covered and concentrated under N_2 using balloon syringe. Thiophenol (4.1 mL, 33.54 mmol) was added using syringe. This was stirred for 15 minutes sitting in an ice bath. Boron trifluoro etherate (10.7 mL, 77.4 mmol) was then added with syringe. Reaction ran over night and was then diluted with DCM, moved to separation reaction using $NaHCO_3$, brine, Na_2SO_4, and vacuum filtration keeping the DCM layer. This layer was then rotovap'd and 9.1724g (80.8%) solid was collect using 1:20 EA:Hex. TLC was ran determining impurity (FIGURE 9) and recrystallization (EA and Hex) was done. Rotovap was done again and the solid was rinsed with 1:100 EA:Hex. After drying 8.8006g (77.5%) of 1-thio-β-D-glucose 2,3,4,6-tetraacetate **1-3** was obtained and TLC determined pure (FIGURE 10).

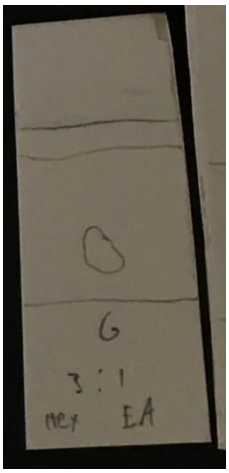


FIGURE 9, TLC impure

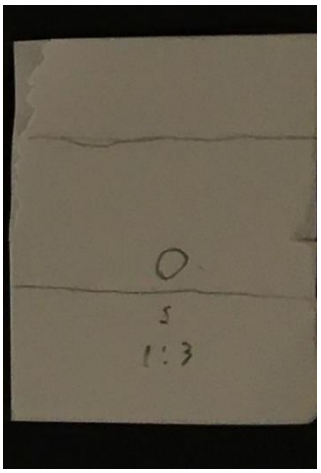
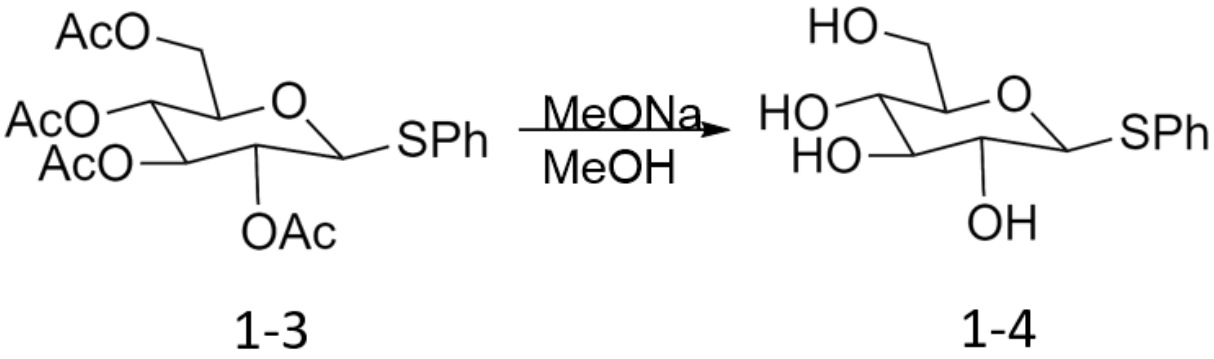


FIGURE 10, TLC pure



1-3 1-4

1-Thio-β-D-glucose (1-4)

The 1-thio-β-D-glucose 2,3,4,6-tetraacetate **1-3** (8.8006g) was dissolved using sodium methoxide in methanol. The pH was brought up to pH10. This was stirred for 1 hour and checked with TLC (FIGURE 11). The pH was then brought down to pH6 using amberlite (that was rinsed in methanol prior too but ran out) and amberlyst (making up for running out of amberlite). Once at pH6 the solution was stirred for 30min and checked with TLC (FIGURE 12). It was rotovap'd and 4.9332g (92%) of 1-thio-β-D-glucose **1-4** was collected.

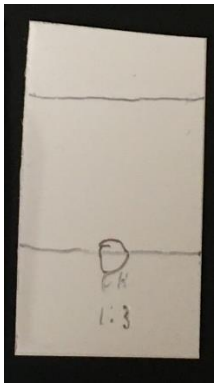


FIGURE 11, TLC pH10

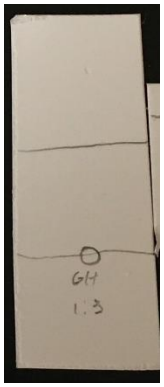
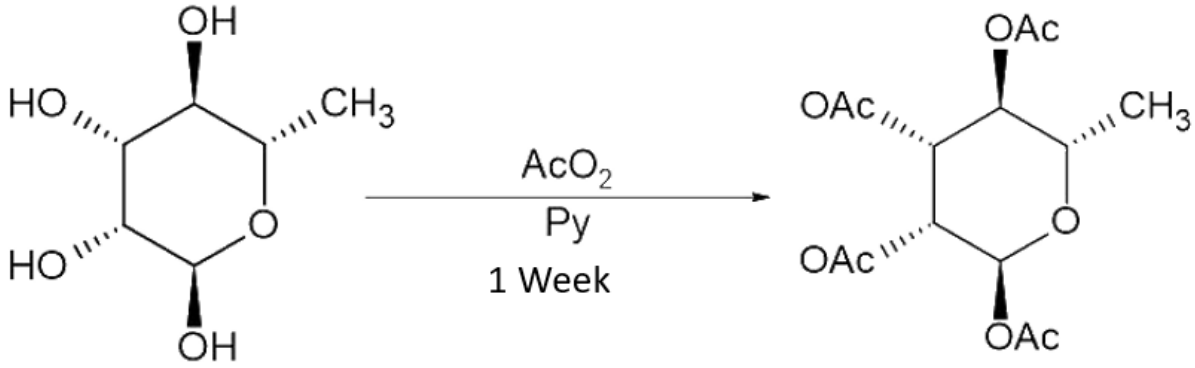


FIGURE 12, TLC pH6



2-1 2-2

1,2,3,4-Tetraacetyl-L-rhamnose (2-2)

Trial 1: Rhamnose **2-1** (5.1271g, 31.2mmol) was dissolved in pyridine (24.5mL, 303mmol) and acetic anhydride (25.0mL, 264mmol). Stirred at rt. for 9 days. Was then rotovap'd (40°C, 50 RPM). Syrup was made and diluted with DCM moving to separation reaction using $NaHCO_3$, brine, Na_2SO_4, and vacuum filtration keeping the DCM layer. TLC determined impure (FIGURE 13). Recrystallization was done.

Trial 2: Rhamnose **2-1** (10.2077g, 62.2mmol) was dissolved in pyridine (40mL, 495mmol) and acetic anhydride (45.0mL, 476mmol). Stirred at rt. for 14 days. Was then rotovap'd (40°C, 50 RPM). Initially it did not work, but after new oil was added to the vacuum pumped it worked. Syrup was made and diluted with DCM moving to separation reaction using $NaHCO_3$, brine, Na_2SO_4, and vacuum filtration keeping the DCM layer. TLC determined impure (FIGURE 14). 1 M HCl was then added in separation funnel with impure DCM layer. Dried with Na_2SO_4, and vacuum filtration keeping the new DCM layer. TLC determined pure (FIGURE 15). DCM layer was rotovap'd and came out as a 1,2,3,4-tetraacetyl-L-rhamnose **2-2** syrup.

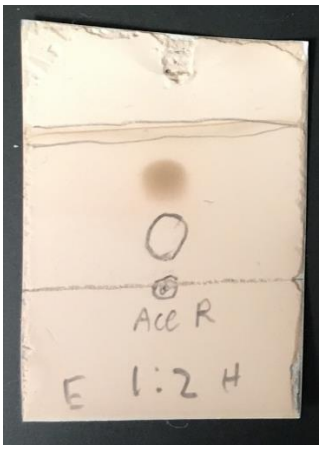


FIGURE 13, Trial 1, TLC impure

FIGURE 14, Trial 2, TLC impure

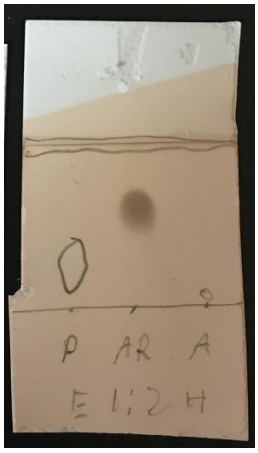
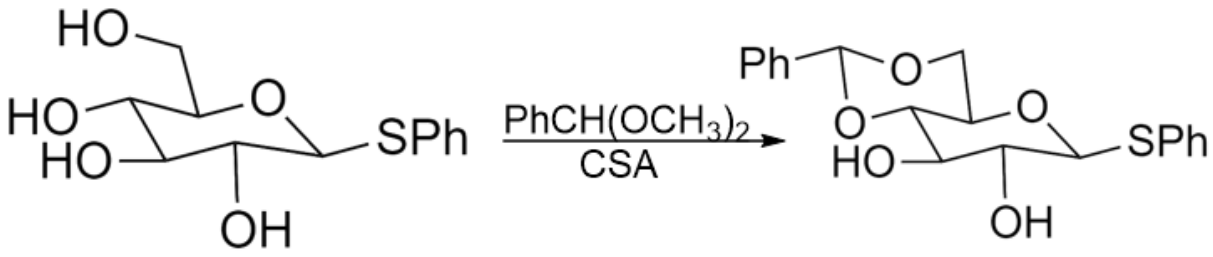


FIGURE 15, Trial 2, TLC pure



1-4 1-5

Phenyl 4,6-O-benzylidene-1-thio-β-D-glucose (1-5)

To 1-thio-β-D-glucose **1-4** (4.9332g, 18.4mmol) diluted in DMF (80mL); CSA (.9217g, 3.97mmol) and benzaldehyde dimethyl acetal (9.2mL, 61.3mmol) were added at rt. This was stirred in a hot water bath (50°C) for 20hr. TLC was done to check completion (FIGURE 16). This reaction was rotovap'd to get ride of DMF. The reaction was then diluted with DCM moving to separation reaction using $NaHCO_3$, brine, Na_2SO_4, and vacuum filtration keeping the DCM layer. Separation reaction of $NaHCO_3$ and brine

were done several times before drying with Na_2SO_4. TLC determined purity, collecting benzaldehyde dimethyl acetal and phenyl 4,6-O-benzylidene-1-thio-β-D-glucose **1-5** (FIGURE 17)

FIGURE 16, TLC impure

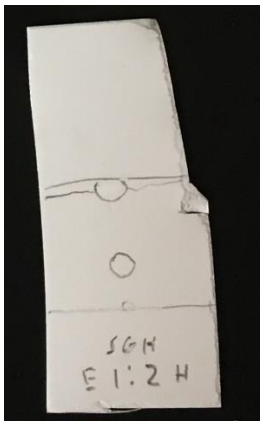


FIGURE 17, TLC impure

Discussion: There was successful synthesis of 1-thio-β-D-glucose 2,3,4,6-tetraacetate (**1-3**), 1-thio-β-D-glucose (**1-4**), and 1,2,3,4-tetraacetyl-L-rhamnose (**2-2**), but phenyl 4,6-O-benzylidene-1-thio-β-D-glucose (**1-5**) has still yet to be fully separated from its reaction. The next step is to perform a silica-gel column chromatography (using a 1:2 EA:Hex solvent) to sperate the benzaldehyde dimethyl acetyl and the product, phenyl 4,6-O-benzylidene-1-thio-β-D-glucose (**1-5**). After this a weight mass can be taken, and the reaction can continue to make phenyl 4,6-O-benzylidene-3-O-naphthalene-1-thio-β-D-glucose (**1-6**) using 2-(Bromomethyl) naphthalene and cesium fluoride as the catalyst. The first trial of 1,2,3,4-tetraacetyl-L-rhamnose (**2-2**) is still left with impurities believed to be pyridine, as it was not rinsed with 1M HCl to neutralize it and make it soluble in water. Trial 2 went much better when the 1M HCl was used but it was also left in a syrup form even after rotovap. This made it hard to do a weight mass analysis, but the next reaction can be done from the syrup to make 2,3,4-triaacetyl-L-rhamnose (**2-3**) using hydrazine acetate. Thiophenol was difficult to sperate when synthesizing 1-thio-β-D-glucose 2,3,4,6-tetraacetate (**1-3**) and column chromatography might be a better possibility for a higher percent yield. 77.5% is still ok for the reaction, but it could be better. Renewing the ice bath might also help the reaction. The deacetylation reaction making 1-thio-β-D-glucose (**1-4**) went well with a 92% reaction yield and the switch of using amberlite to using amberlyst with it seemed to work. The reaction making phenyl 4,6-O-benzylidene-1-thio-β-D-glucose (**1-5**) uses DMF and because of this there is potential to lose some product. Because of DMF's polarity it made it difficult to do a TLC and it made it difficult to sperate. DMF is polar and can bond to some water molecules this means its possible that during the separation process any DMF that was there could have carried some of the product into the water layer which was then put into waste. Perhaps a more efficient way would be to use acetonitrile. However, phenyl 4,6-O-benzylidene-1-thio-β-D-glucose (**1-5**) is unsoluable in acetonitrile and so a celite filtration would need to be done collecting the solid. This process would also get rid of the silica-gel column and hopefully improve reaction yield.

Summary: 4 reactions were performed. The first was adding a thiophenol group to β-D-glucose pentaacetyl using boron trifluoro etherate and thiophenol. This reaction had a 77.5% yield making 8.8006g 1-thio-β-D-glucose

2,3,4,6-tetraacetate (**1-3**). The second reaction was deacetylation creating 4.9332g 1-thio-β-D-glucose (**1-4**) with a reaction yield of 92%. The third reaction was an acetylation reaction of rhamnose. 1,2,3,4-tetraacetyl-L-rhamnose (**2-2**) was made but it was left in a syrup form. The fourth reaction went to synthesis phenyl 4,6-O-benzylidene-1-thio-β-D-glucose (**1-5**) using DMF, benzaldehyde dimethyl acetyl, and CSA. CSA and DMF was successfully separated using liquid separation with $NaHCO_3$, brine, Na_2SO_4, and vacuum filtration, but phenyl 4,6-O-benzylidene-1-thio-β-D-glucose (**1-5**) was still left in DCM and benzaldehyde dimethyl acetyl. These 4 reactions were a good start to synthesizing α-chaconine. 6 more reactions still need to be done. Once α-chaconine is made biological analysis can begin in vitro. This analysis can be used to help understand biological reactions and can make new and more efficient medicines or pest controls. Using and tweaking this procedure can provide different glycosidic units to be made allowing for different and new glycoalkaloids to be synthesized and tested.

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