Benzylidene Acetals as Protecting Groups

Experiment 4

Submitted by:

CHM2123 - COO

Demonstrator:

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Introduction

Part A

The goal of Part A of this experiment is to create a protected glucose by means of transglycosylation; this is done by forming a benzylidene acetal at the C4 and C6 alcohols of glucose (Magnan). Chemical synthesis can be difficult during some reactions when molecules that contain multiple functional groups, such as glucose, are involved; more than one product can be formed if the conditions allow for more than one function group to react (Ogilvie). Forming the acetal groups at those specific allows for reactivity to occur at other positions by preventing the reactivity of the two alcohols previously mentioned. In other words, using a protecting group, such as acetals, prevents side reactions from occurring (Magnan). The protecting groups can then be removed in a separate step once the reaction is complete to restore the original function of the molecule (Ogilvie).

Acetals are formed when either an aldehyde or a ketone reacts with an alcohol in the presence of an acidic catalyst. To form the protecting groups, the benzaldehyde dimethyl acetate is first protonated, which results in the formation of oxonium. The mixed acetal is then formed once a hydroxyl group on the glucose trap the oxonium. The acetyl-protected molecule is produced once methanol is eliminated and another hydroxyl group traps the oxonium ion (Magnan).

Part A of this experiment is accomplished by heating the reaction flask at reflux, and using thin layer chromatography to monitor the progression of the reaction as well as to verify that the reaction is completed. Once the reaction was completed, three extractions were performed by using water to wash the organic layer. Once the collected organic layers were dried with Na_2SO_4 , hexanes were added to force the product to precipitate. Vacuum filtration was then performed, followed by a TLC to verify the purity of the product (Magnan).

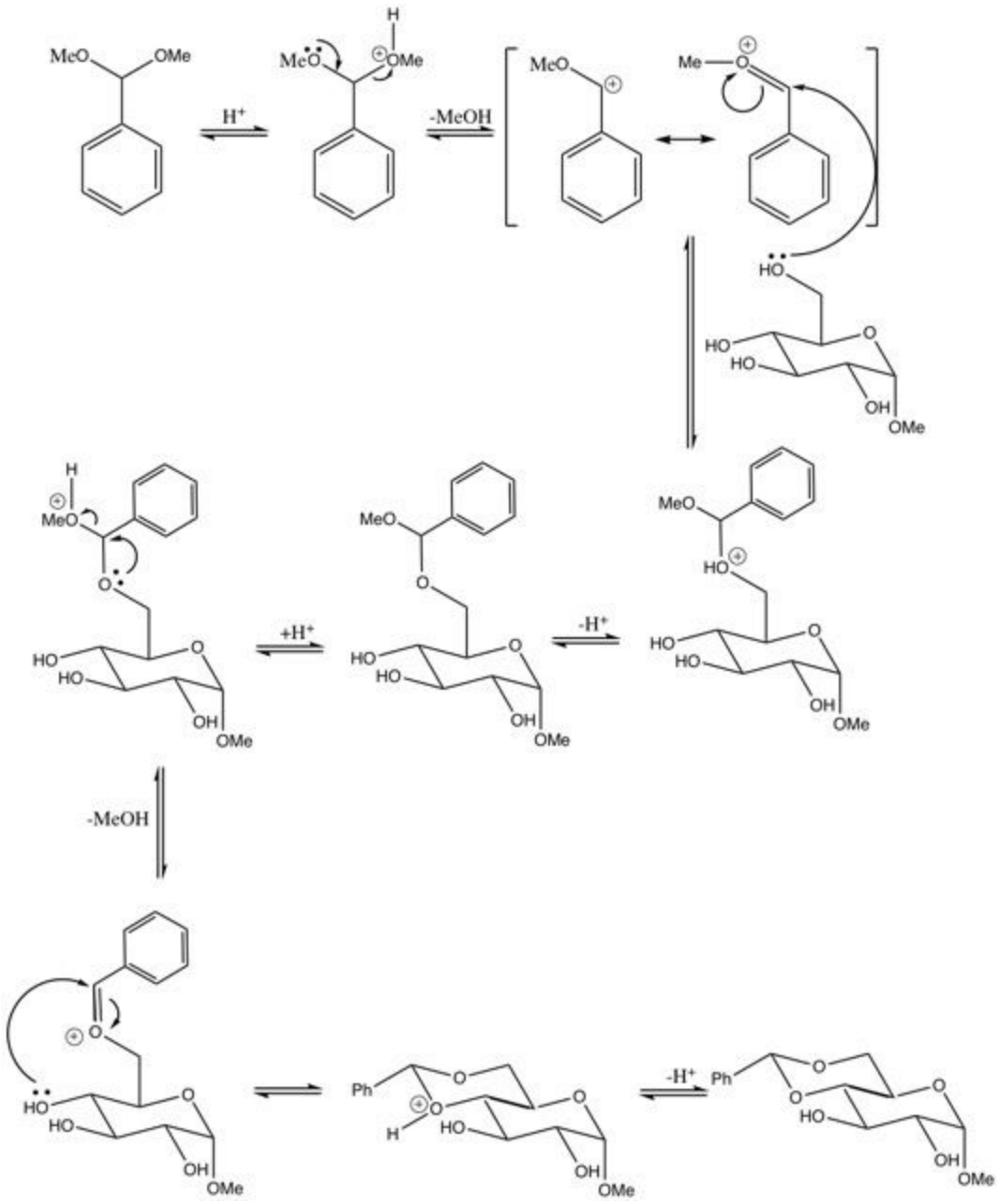
Part B

The goal of Part B of the experiment was to test different substances and assess the amount of reducing sugars of each substance; the substances tested were sucrose, glucose, milk, and cola. A reducing sugar is a sugar which contains either free aldehyde or alpha-hydroxy-ketone groups. Sugars which contain hemi-acetals are all reducing sugars; this means monosaccharides, a few disaccharides, oligosaccharides, and polysaccharides are reducing sugars (Magnan).

To verify the amount of reducing sugars in each substance, the Benedict test was used. A few drops of Benedict's reagent were placed in each substance tested. Once heated, the Benedict solution indicates the presence of reducing sugars by changing the colour of the solution from blue to green, yellow, orange, or red, depending on the concentration of reducing sugars; a green solution indicates a low concentration where as a red solution indicates a high concentration. Benedict's reagent is cupric citrate solution. When a sample containing the reagent is heated, reducing sugars in the sample are converted to enediols which then reduce the Cu (II) in the Benedict's reagent, resulting in the formation of Cu_2O and the reddish colour of the sample tested (Magnan).

Mechanism

Figure 1: Formation of benzylidene acetal, using an acid catalyst



Mechanism taken from CHM 2123 Lab manual. *Experiment 4: Benzylidene Acetals as Protecting Groups (figure 4.5)*

Table of Reagents

Part A

Table 1: Reagents used in Part A

Reagent	Molar Mass (g/mol)	Quantity	Density (g/mL)	Mmol	Equivalents
Methyl-α-D-glucopyranoside	194.18	0.5 g	----	2.57	6
Tetrahydrofuran	72.11	10 mL	0.889	123	286
Benzaldehyde dimethyl acetal	152.19	0.70 mL	1.014	4.66	11
10-Camphorsulfonic acid	232.29	0.10 g	----	0.43	1

Part B

Table 2: Reagents used in Part B

Reagent	Molar Mass (g/mol)	Quantity	Density (g/mL)	Mmol	Equivalents
Sucrose	342.30	0.1 g	----	0.292	1
Glucose	180.16	0.1 g	----	0.555	1.9
HCl	36.46	Few drops	1.18	----	----
Water	18.02	2 mL	1	110	377

Procedure

Refer to CHM 2123 Lab manual. *Experiment 4: Benzylidene Acetals as Protecting Groups*
Modification:

- Part A step 3: heated at reflux for more than 30 minutes

Observations

Part A

Table 3: Observations for Part A

Key Steps	Observations
Reflux	● Reflux occurred slowly
Extraction	● Aqueous layer is at the bottom; organic layer is on the bottom ● Aqueous layer is clear, colourless ● Organic layer is slightly cloudy, colourless
Adding hexanes	● No precipitation of product occurred

Part B

Table 4: Observations for Part B

Key Step	Observations
Adding Benedict's reagent to test tubes	● Solution containing sucrose is bright blue and transparent ● Solution containing glucose is bright blue and transparent ● Milk is blue, cloudy, and opaque ● Cola is light blue
After heating the solutions	● Sucrose solution remained blue. No change ● Glucose solution has turned slightly orange ● Milk has turned slightly yellow ● Cola has turned a dark green/brownish colour. Precipitation of red solid has appeared
Addition of HCl to sucrose solution	● Solution bubbled upon addition of HCl ● Colour of solution has remained blue

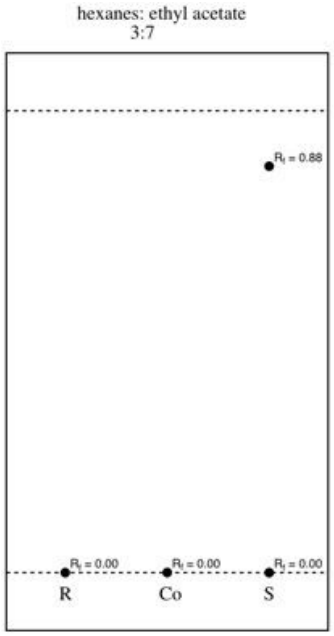
Results

Mass of Product	0.04g

Melting Point of Product	183.4 ºC

Percent Yield	2.42%

TLC

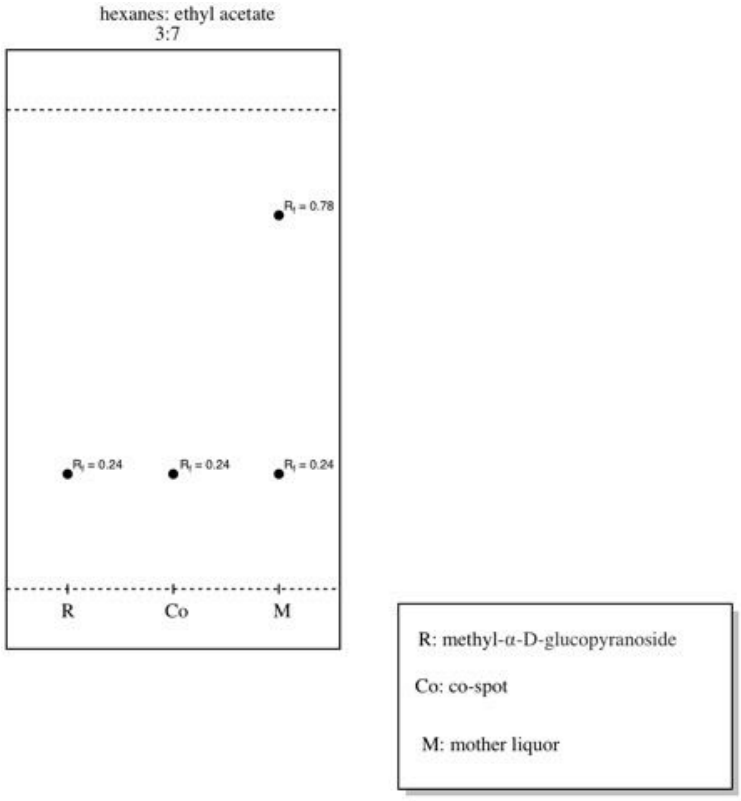


R: methyl-α-D-glucopyranoside

Co: co-spot

S: sample

Position	**Number of spots**	**R_f**
Lower	3	0
Higher	1	0.88



Position	Number of spots	R_f
Lower	3	0.24
Higher	1	0.78

Calculations

$\underline{R_f}$

$R_f = D_{Spot} / D_{Solvent\ front}$

$= 3.7\ cm / 4.2\ cm$

$= 0.88$

Percent Yield

$n_{product} = m_{product}/M_{product}$

$= 0.04\ g / (228.29 g/mol)$

$n_{product} = 0.0001752\ mol$

$n_{starting\ material} = n_{Methyl\text{-}\alpha\text{-}D\text{-}glucopyranoside} + n_{Benzaldehyde\ dimethyl\ acetal}$

$= 0.00257\ mol + 0.00466\ mol$

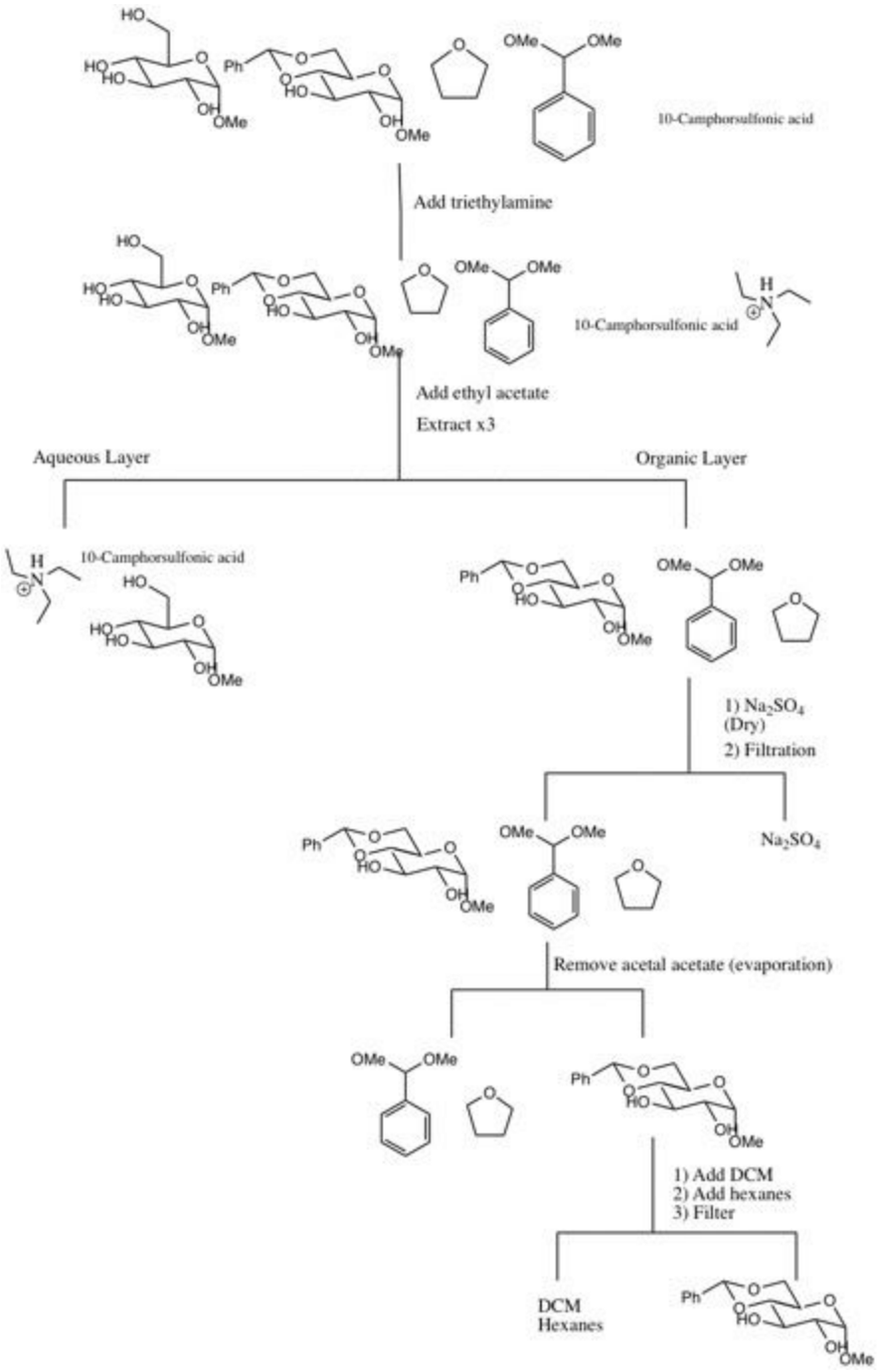
$= 0.00723\ mol$

Percent yield= (moles of product/moles of starting material) x 100

= (0.0001752 mol/0.00723 mol) x 100

= 2.42 %

Flow Chart


10-Camphorsulfonic acid
Add triethylamine
10-Camphorsulfonic acid
Add ethyl acetate
Extract x3
Aqueous Layer
Organic Layer
10-Camphorsulfonic acid
1) Na_2SO_4 (Dry)
2) Filtration
Na_2SO_4
Remove acetal acetate (evaporation)
1) Add DCM
2) Add hexanes
3) Filter
DCM
Hexanes

Discussion

Part A

The goal of Part A of this experiment is to create a protected glucose by forming a benzylidene acetal at the C4 and C6 of glucose. After adding the methyl-α-D-glucopyranoside to the round bottom flask, tetrahydrofuran (THF) was added to dissolve the solid; THF is a solvent that was used to create the necessary anhydrous conditions for this experiment. It is crucial that the condition remains anhydrous due to the fact that the equilibrium leading to the formation of acetals only weakly favours the products.The presence of water would shift towards the reactants, resulting in little to no product formation. 10-camphorsulfonic acid was then added to the round bottom flask to act as a catalyst (Magnan). The formation of hemi-acetals can form in neutral conditions but the use of an acid will help accelerate the rate of the reaction. The acid, however, is necessary to allow the hemi-acetal to undergo further reaction to become an acetal (Ashenhurst). Finally, some benzaldehyde dimethyl acetal is added, which will provide the protective acetal group.

The solution in the round bottom flask was then heated to reflux. The reflux was occurring slowly; the solution was heated at reflux for longer than indicated in the lab manual.

Once the solution has cooled, some triethylamine was added to the solution to help in the separation of the compounds present. Since triethylamine is basic, it will deprotonate the compounds. The compounds that become ionic after the addition of the triethylamine will become more soluble in the aqueous phase instead of the organic phase (Schaller). Then, the solution with the triethylamine has been transferred to a separatory funnel. Ethyl acetate was also added to the separatory funnel. Ethyl acetate is not soluble in water and formed the organic layer.

Three extractions were then performed, where the organic phase was washed with water before each extraction. The organic phase was the top layer and appeared colourless and slightly cloudy. The aqueous phase was on the bottom and appeared colourless and clear.

Once the extraction was complete, $Na_2SO_4$was added to the organic phase to dry it. Hexanes were then added to the organic phase to force the product to precipitate out. The product was able to precipitate out when adding the hexanes because the protected sugar is polar, where as the hexanes are nonpolar. The remaining benzaldehyde dimethyl acetal remains in the solution (Magnan).

The first TLC plate was taken to ensure that the reaction was complete before proceeding to the extraction. This TLC plate indicates that the reaction was incomplete. The spot representing the final product has an $R_f = 0.88$; a spot with this R_f value is not found in the lane of the starting materials. However, both the lane for the starting materials and the lane for the final product have a spot with the same R_f value ($R_f = 0$); this means that there was some of the starting material present in the reaction flask at the end of the reflux.

The second TLC plate was taken to test the purity of the product. The spot representing the product has an $R_f = 0.78$; this dot is only found in the lane of the mother liquor. However, an extra dot with an $R_f = 0.24$ is found in the mother liquor lane as well as the starting material lane; this signifies that the starting material was present in the mother liquor with the final product. The product may not be pure because, according to the first TLC plate, the reaction was not complete before proceeding to the extraction.

On both TLC plates, a spot is from the co-spot lane;this may signify that the co-spot was not sufficiently spotted.

A yield of 2.42% has been obtained. A possible explanation for this low yield would be that there has been some contamination; there could have been some water on the glassware. As previously mentioned, the presence of water during this reaction would shift the equilibrium to favour the starting materials over the products, leading to a low yield.To avoid this issue, the glassware should be assembled and used as soon as it comes out of the oven; leaving the glassware exposed to the air for too long can cause an accumulation of water on the glassware. It's also important to note that every time the product is transferred to different glassware, there will be some loss of product.

A melting point of 183.4 ºC has been obtained; the theoretical melting point of 4,6-O-benzylidene-α-D-glucopyranoside is 164-165 ºC (Magnan). The significant difference between the theoretical and experimental melting point indicates that the product that was obtained is not 4,6-O-benzylidene-α-D-glucopyranoside and/or, it may indicate the presence of impurities.

Part B

The goal of Part B of this experiment was to determine the reducing sugar content of samples of different substances. The substances tested are the following: sucrose, glucose, milk, and cola. The Benedict's reagent was used to determine if samples contained reducing sugars. Upon the addition of the Benedict's reagent to each solution, the sucrose and glucose solutions turned blue and were transparent; the milk and the cola turned blue but were opaque in appearance.

After heating each sample, changes in colour were observed in most of the sample. The sucrose did not change colour and remained blue; the blue colour of the sucrose solution indicates that there are no reducing sugars present. The glucose solution has turned slightly orange; the orange colour of the glucose solution indicates that there is a moderate amount of reducing sugars present. The milk had a slight yellow tint to it, meaning that there was only a small amount of reducing sugars present. The cola had a dark green/brown colour to it, in addition to red precipitate present on the bottom of the test tube; this indicated that the cola is rich in reducing sugars.

These results are consistent with what, theoretically, should be observed. Glucose is a monosaccharide and, as mentioned before, all monosaccharides are reducing sugars. Milk contains lactose, which is a disaccharide that contains a hemi-acetal, meaning lactose is a reducing sugar (Ashenhurst); knowing this, it is logical that the milk had a positive Benedict's test. Sodas contain

sweeteners including glucose, fructose, and sucrose. The cola had a positive Benedict's test because fructose and glucose are both monosaccharides and thus, both reducing sugars (Fontenot).

The sucrose did not have a positive Benedict's test because it is not a reducing sugar; it does not contain a free ketone or aldehyde, and therefore does not have a hemi-acetal (Kelly). When HCl was added to the sucrose solution after it has been heated, no colour change occurred; the solution bubbled and remained blue. In theory, the sucrose solution should have changed from blue to yellow. Adding HCl causes hydrolysis of sucrose, which would divide the disaccharide into two monosaccharides, glucose and fructose (Libretexts). The presence of the detached monosaccharides trigger a positive Benedict's test because both individual units are reducing sugars.

The colour changes observed, except for that of the cola, were very slight; most of the solutions were still blue with the colour change being present near the bottom of the test tube. A possible explanation for this would be that each of the solutions should have been heated for longer.

IR Analysis

Spectrum A seems to correspond to benzaldehyde dimethyl acetal. Sharp peaks near the 1200 mark indicated the presence of the C-O bonds branching off of the benzene ring. The C-H bonds found in the two methyl groups are are shown by the peaks near the 3000 mark. Finally, the C=C bonds in the benzene ring are shown by the peaks near the 1500 mark. With all of these characteristics shown, it is possible to conclude that spectrum A displays benzaldehyde dimethyl acetal.

Spectrum B seems to correspond to 4,6-O-benzylidene-α-D-glucopyranoside. The peaks in the 3300-3500 range indicate the presence of the alcohol groups. The peaks near the 3000 point show the presence of the C-H bonds in the ring. The peaks near the 1600 depict the presence of the C=C found in the phenyl groups branching off of the main ring. The element which concludes that the spectrum shows the protected sugar is the presence of the alcohol groups.

Works Cited

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