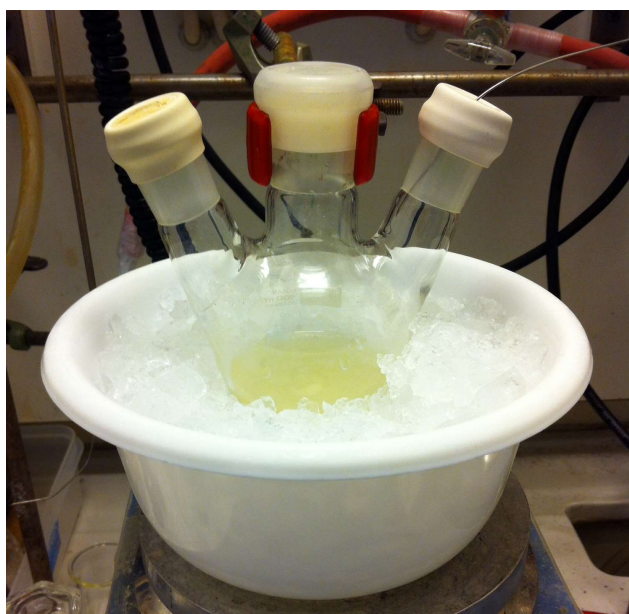




CHALMERS
UNIVERSITY OF TECHNOLOGY



Carbohydrate Synthesis

- Study on the synthesis of Methyl 2,3,6-tri-O-benzyl- α -D-Glucopyranoside

Bachelor of Science Thesis

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Carbohydrate synthesis – Study on the synthesis of Methyl 2,3,6-tri-O-benzyl- α -D-Glucopyranoside

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Cover: Picture from the synthesis work, found on page 14.

Abstract

Fossil fuels should be replaced with renewable biofuels for a sustainable future. A potential source is lignocellulosic biomass, which is made of lignin-carbohydrate complex. The problem lies in hydrolysing the complex with a profitable method. A potential method is using appropriate enzymes, which lower the production costs, but more knowledge about the complex is needed to increase the yield of raw material.

Model substrates resembling the structures in lignin-carbohydrate complex are synthesized, too potentially be used in enzymatic studies. This report takes a closer look on the potential model substrate Methyl 4-O-Methyl α -D-Glucuronide. The molecule requires several synthesis steps and intermediate molecules. One molecule is Methyl 2,3,6-tri-O-benzyl- α -D-Glucopyranoside, and the aim of this report was to study its synthesis steps. The first step was to apply protecting groups and this was successfully achieved with easy synthesis steps. The second step was to study how to reduce the acetal bond with regioselectivity. Two different methods were performed in a lab-scale whereas the most convenient one was studied in a larger scale.

The best method during lab-scale synthesis was to break the acetal bond with hydrogen chloride and sodium-cyanoborohydride. But it was hard to get the right conditions in a larger scale, not giving good results and an alternative synthesis step should be sought.

Sammanfattning

För en hållbar framtid bör fossila bränslen ersättas med förnybara bränslen. En potentiell källa är lignincellulosa material, som består av lignin-kolhydrat komplex. Problemet ligger i att hydrolysera komplexen med en lönsam metod, där ett alternativ är att använda lämpliga enzymer. Dock krävs det mer kunskap om lignin-kolhydrat komplex för att kunna bryta ner polymererna.

Modell substrat som liknar lignin-kolhydrat komplexen är syntetiserade, för att potentiellt användas i enzymatiska studier. Denna rapport tar en närmare titt på metyl 4-O-metyl α -D-Glukuronsyra, som kräver flera syntessteg att ta fram. En intermediär moleky i syntesen är metyl 2,3,6-tri-O-bensyl- α -D-Glukuronsyra, och syftet med denna rapport var att studera syntesstegen till denna intermediära moleky. Första steget var att applicera skyddsgrupper vilket utfördes med relativt enkla syntessteg. Andra steget bestod utav av reducera acetal bindningen med regioselektivitet. Två olika metoder utfördes i labbskala för att hitta den mest lämpliga att utföra i en större skala.

Den lämpligaste metoden i labbskala var att använda saltsyra och natrium-cyanoborohydrid. Men reaktionen var svår att utföra med dem rätta förhållanden i större skala, vilket bidrog med att syntesen inte lyckades och ett alternativ bör studeras.

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1. Introduction

The majority of today's motor vehicles are driven by fuel derived from petroleum. Petroleum-based fuels consist of hydrocarbons, causing environmental problems, such as air pollution and heating of planet earth, by greenhouse gas emissions. [1] For a sustainable future, renewable and more environmental friendly fuel-sources have to be found. A theory is replacing petroleum with fuel derived from plants. Both petroleum and plants consist of long hydrocarbon chains, whereas the later one uses photosynthesis to produce hydrocarbons. Fuel from plants has been derived, such as methanol and ethanol, and has successfully driven motor vehicles. [2]

Production of ethanol is the current leading biofuel and the most common raw materials are sugar canes, corn and soybean oil. Among the leading countries in the world of bioethanol production is Brazil, producing 24 billion litres in 2012/2013, mainly from sugar canes. Although the huge investment in bioethanol, the industry cannot yet compete with petroleum; the amount of bioethanol being produced is not enough. A debate has also risen whether the huge areas required to grow sugar canes, should be used to grow food instead. [3] [4] Another problem with the production of bioethanol is the high greenhouse gas emissions. The gas emissions are almost as high as from fossil fuels productions when including both the process and transport. [5]

The biggest problems Brazil faces are to increase the bioethanol yield and decrease gas emissions in the production, to be able to compete with petroleum. The debate over food contra fuel has created another obstacle. These problems have generated the so-called second-generation biomass, biomass not competing with actual food, because they grow in environments unsuitable for farmland. The biomass with most potential is lignocellulosic biomass consisting of complex polymers, cellulose, lignin and hemicellulose. One example of lignocellulosic biomass is trees and since we're moving towards a more digitalised society, trees can be used in biofuel production instead of producing paper. Lignocellulosic biomass is one of the best alternatives to replace petroleum, but it still has obstacles to overcome in order to find a cheaper process of biofuel production. [6]

General production of second-generation bioethanol consists of four steps: Pre-treatment, hydrolysis, fermentation and distillation. The purpose of the second step, hydrolysis, strives to break down polymers in the raw material (depolymerize) into sugars monomers, to enable fermentation. One way to hydrolyse is by breaking the polymers with acid, acid-hydrolysis, a procedure requiring high temperatures and can cause further breakdown of the monosaccharaides. The acidic conditions do also increase the risk of corrosive damages on the reactor, increasing production costs. Acid-hydrolysis is an expensive procedure and an alternative has to be found that works as good or even better. [7] [8].

An alternative to acid hydrolysis is to depolymerize the chains with enzymes, enzymatic hydrolysis. Suitable enzymes are used to cleave the polymers, which lower the production costs, by not requiring as much heat and giving higher monosaccharide yield. Enzymatic hydrolysis is used for example when depolymerizing cellulose into glucose, making it possible to produce bioethanol and other chemicals, such as sorbitol or lactic acid. To properly be able to compete with petroleum-based fuels, the area needs further technical improvement to increase the yields of biofuel. [6][8]

As pointed out above, the main components of lignocellulosic biomass are cellulose, lignin and hemicellulose. Cellulose polymers consist of glucose monomers linked together, while hemicellulose is made of several different sugar monomers. Lignin consists of different phenolic monomers, connected to the polymers in hemi- and cellulose by non- and covalent bonds. The covalent bonds are believed to prevent the enzymes efficiency and more knowledge about the bonds within the complex, could help increase conversion of bioethanol. [9] [10] There's also a possibility to fermentation of bioethanol from other monosaccharides, existing compounds in hemicellulose. [9]. The relationship between lignin and hemicellulose is viewed in this report, and how looking at model substrates could increase the understanding of the structure in lignin- carbohydrate complex.

Model substrates resembling lignin-carbohydrate compounds are synthesized to study how the structure of the molecule affects the enzymes activity. Glucuronide acids are common carbohydrates in hemicellulose and by synthesizing glucuronide acids with different structures and properties; more knowledge about the complex could be obtained.

1.1 Aim

This report takes a closer look on the synthesis of carbohydrate Methyl 4-O-Methyl α -D-Glucuronide, a potential model substrate to be used in enzymatic studies. Its whole reaction mechanism involves several steps, as shown in figure 1. [11]

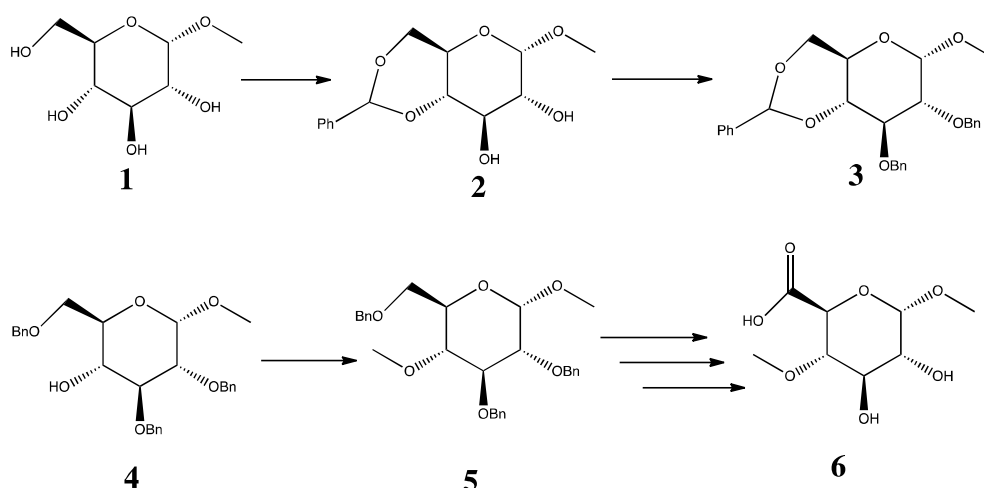


Figure 1. Reaction mechanism of the first five steps in the synthesis of Methyl 4-O-Methyl α -D-Glucuronide 6. Adapted from [11]

The aim of this report is to study the reaction between molecule 3 and target molecule 4, Methyl 2,3,6-tri-O-benzyl- α -D-Glucopyranoside. (See figure 2). First step will be to reach molecule 3 with well-known reagents, purify and identify each molecule for next synthesis step. The second step of the project will be to study different reagents and conditions to find the best reaction path.

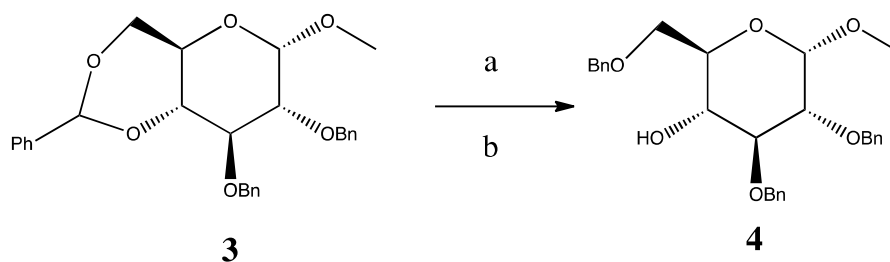


Figure 2. The main goal of this report is to study different reagents in order to reach the target molecule Methyl 2,3,6-tri-O-benzyl- α -D-Glucopyranoside, 4.

When testing different reagents, the reactions are first performed in a lab-scale and the best reaction is scaled up. Generally speaking, several parameters affect the up-scaling process. Besides good conversion, the starting material should be cheap and preferable, commercially available. Avoid reactions involving heating due to the increased costs of cooling and also avoid exothermic reactions, as control of the reaction will be crucial. Also try to avoid chemicals that are not environment friendly.

2. Lignocellulosic biomass

Lignocellulosic biomass is material non-digestive for us human beings, through the lack of appropriate enzymes in our bodies. Thus making lignocellulosic material out of competition with food or potential farmland for food, since the material are grown in different environments. Woody plants are seen as lignocellulosic biomass, having a stem growing above ground covered in bark, rich of carbohydrates. Example of lignocellulosic biomass: Trees, lianas and shrubs. Trees, for example, can be grown and harvested practically worldwide. Thanks to nature, lignocellulosic biomass is seen as a renewable source for production of biofuels. [12]

Nature produces about 200 billion metric tons of lignocellulosic biomass annually through photosynthesis, which is equivalent to 3×10^{18} kJ/ year. The annual energy consumption of the world is about a tenth of that energy [6]. The problem is extracting the wanted compounds to reduce the costs of biofuel production. Further knowledge about the structure in lignocellulosic material is needed, which can result in better technique and higher yields of material for fermentation. To get a better understanding of the relative unknown structures, model substrates, resembling the material in lignocellulosic biomass is synthesized.

2.1 Main components in lignocellulosic biomass

Cellulose is the main component in the cell wall of lignocellulosic plants. Cellulose consists of polymers of glucose monosaccharaides, connected together with β -(1 \rightarrow 4)-glycosidic bonds. (See figure 3) [13]

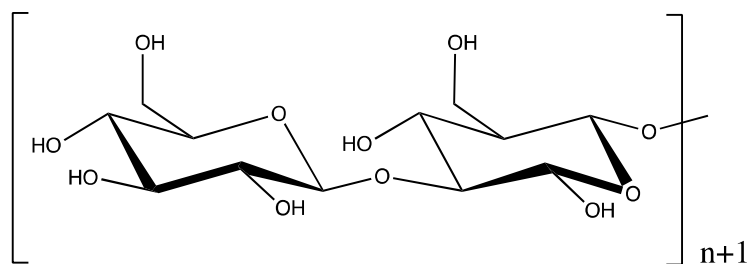


Figure 3. Structure of cellulose: polymers of glucose monosaccharaides. Adapted from [13]

Cellulose is the dominating compound in plants consisting of about 23-53% in lignocellulosic plants. The primary cell wall consists of about 6000 glucose monomers, while the secondary 13 000-16 000 units. The different polymer chains are bonded to each other by strong intra- or intermolecular hydrogen bonds and depending on the ordering of glucose, different regions could either be crystalline or amorphous. [13]

The main purpose of cellulose is to function as a protective layer in the cell wall, making it hard to break the strong hydrogen bonds. Carbohydrate such as starch, have weaker bonds between the molecules, making easier to depolymerize and produce biofuel from starch material. The amorphous regions in cellulose are preferred because of the looser packing, making it easier to depolymerize it. The challenge lies in either destroying the crystalline regions or only extracting the amorphous parts for biofuel production. [13]

Hemicellulose is the most complex component in the cell wall, connected to cellulose through hydrogen bonds and creating covalent bonds with lignin. The backbone of hemicellulose consists of four different types of polysaccharides: xylans, mannans, xyloglucans and mixed-linkage β – glucans, where xylan is the most common compound. Different types of carbohydrates are chained to the backbone, for example different structures of D-Glucuronic acid. (See figure 4) [13].

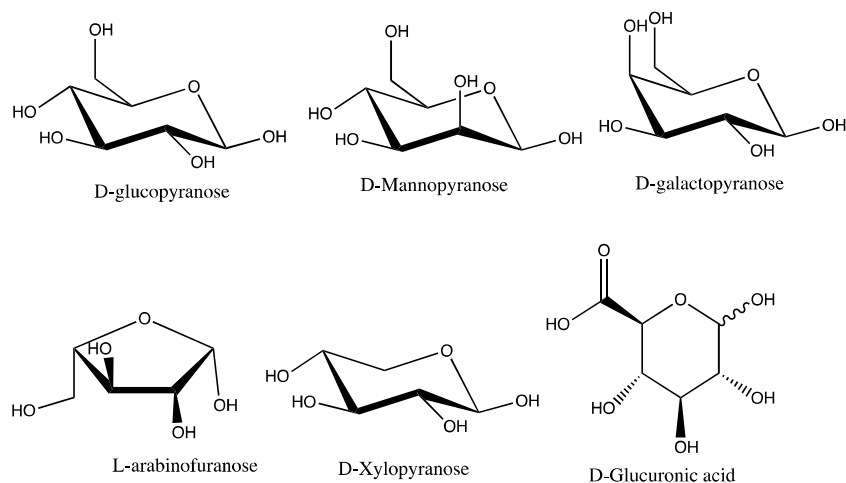


Figure 4. Different types of carbohydrates linked to the different polysaccharides in hemicellulose. D-Glucuronic acid is commonly chained to the xylan backbone. Adapted from [13]

Lignin consists of three different alcohols, *p*-coumaryl alcohol, coniferyl alcohol and sinaply alcohol, see figure 5. The structure of lignin is different from the cellulose by not being constructed by polymer chains, instead the phenolic monomers are linked together randomly by different bonds. [13]

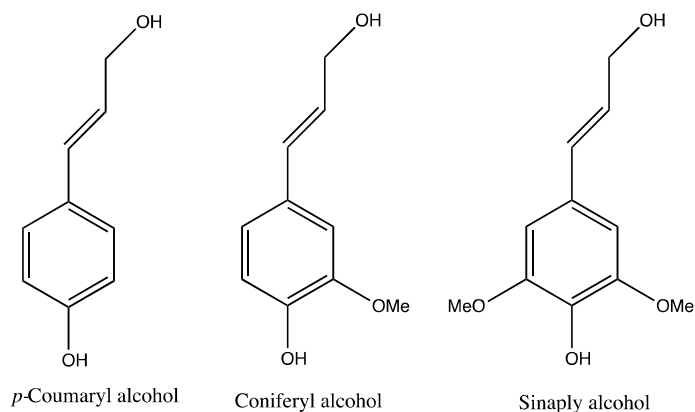


Figure 5. Main structural components in lignin. Adapted from [13]

2.1.1 Ligno-Carbohydrate complex

Lignin-carbohydrate complex (LCC) refers to the bonds between lignin and hemicellulose, cellulose in lignocellulosic biomass. In spruce, for example, an estimated 96% of LCC are bonds between hemicellulose and lignin, and roughly 4% between lignin and cellulose. The bonds in LCC are non- and covalent bonds and to understand the relationship between lignin and hemicellulose bonds, model substrates resembling their main components are synthesized. [10]

Figure 6 shows an example of model substrates, where compound **C** was used in enzymatic studies. Compound **A** and **B** were synthesized separately in order to give **C**.

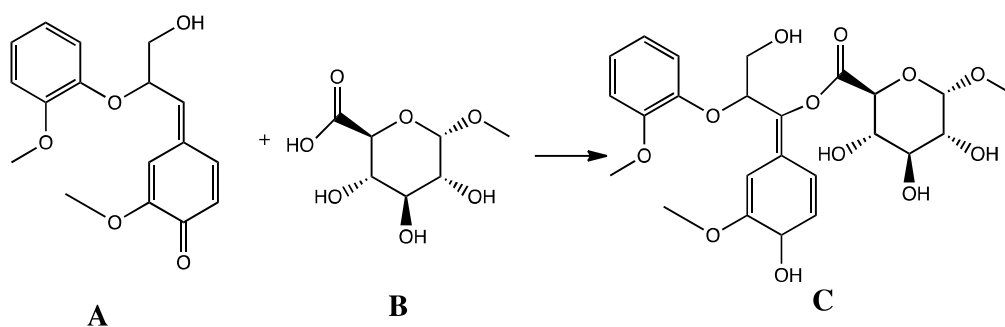


Figure 6. Example on model substrates used in an enzymatic study, resembling the structure in lignin-cellulose complex. Adapted from [10]

As seen in figure 6, compound **A** resembles the structure of lignin, containing its main components. Compound **B** is a type of glucuronic acid, which potentially exists in the xylan backbone in the hemicellulose structure. Compound **C** mimics how lignin and hemicellulose are linked with covalent bonds in lignocellulosic biomass. [10]

There are glucuronic acids with different structures, affecting the covalent bonds and the enzymes activity. Therefore different glucuronic acids are studied as potential model substrates. A potential model substrate is Methyl 4-O-Methyl α -D-Glucuronide (**6**); the molecule has potentially the same function as molecule **B**. More understanding about the bonds in LCC could help generate better conversion of biofuels, not only by fermentation of hexoses but also from pentoses in hemicellulose.

2.2 Protecting groups

During synthesis with several functional groups, the reagents will interact with all the potential groups and not only with the targeted group, causing problems in the synthesis. An example is if the start molecule consists of a ketone and an aldehyde group, the reagent could react with either double bond or oxygen. To reach the target molecule, one of the reactive functional groups has to be blocked. If the purpose is to alkylate the ketone, the aldehyde has to be temporally blocked with a so-called protecting group. A protecting group helps to neutralise a functional group, and the reagent could reach the target molecule, without interfering with the protected group. The protecting group can later be removed by different methods, turning back the group to its original state. Protecting groups should always be avoided if possible because of the extra amount of chemical reactions, first incorporating the group and removing it. The reactions also have to give high yields; otherwise the amount of material will decrease along the synthesis path. [14]

Examples of functional groups with high reactivity are alcohols, ketones, aldehydes and amines. In alcohols, the hydrogen (O-H), causes problems because its reactivity with other reagents. A solution is replacing the hydrogen with a protecting group, until the synthesis is completed. When working with protecting groups, synthesis with a low number of steps indicates a good reaction method.[14]

The first step of this report is to reach molecule **3**, and the starting material is methyl α -D-Glucopyranoside, which consists of several reactive alcohol groups. In the synthesis of my target molecule **4**, the 4-OH needs to be alkylated with a methyl group, so all the other alcohols have to be protected. The most common protecting groups used for alcohols are ether- and acetal groups. Both types of groups are easy to protect and remove, without reacting with reagents. Ester protecting groups are not as common due to the reactivity of the acyl carbon. [14]

The first step is applying a protecting group, capable of deprotecting 4-OH, whilst keeping the 6-OH protected in future synthesis. This is done by protecting the 4,6-diols with a benzaldehyde group. The start material, methyl α -D-glucopyranoside, reacts with benzaldehyde dimethyl acetal ($\text{PhCH}(\text{OMe})_2$) under acidic conditions, see figure 7. This reaction is possible because the 1,3-diol (4,6-diol) is more thermodynamically stable than any 1,2-diol bonds. [14] [15]

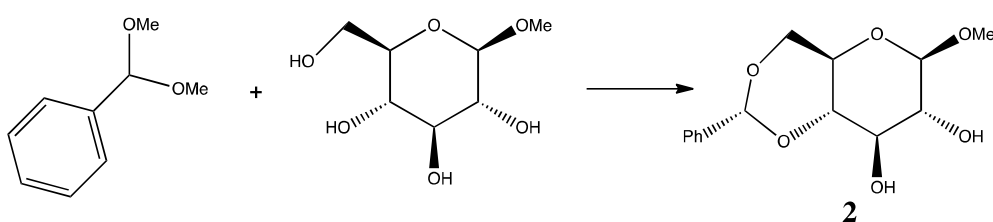


Figure 7. Protecting the 4,6-diols with benzaldehyde dimethyl acetal. Adapted from [16]

The remaining two alcohols are being protected with a common reagent, benzyl ether, and in this case, benzyl bromide. Benzyl ethers are very stable groups, being able to resist a lot of different reagents and conditions, for example pH 14 and hydrides. The reaction removes each proton and replaces them with a benzyl-protecting group, giving molecule **3**. [14] [15]

2.3 Methods for selective reduction of carbohydrates

It is necessary to reduce the acetal-protecting group with regioselectivity, in order to reach target molecule **4**. A number of methods exist, where generally an electrophile is needed to protonate a targeted molecule and reducing it later on [17]. Two different methods have been studied, first of all, the simplicity has been taken in mind and the requiring chemicals.

The first method studied was reducing the acetal with hydrogen chloride (HCl) and sodium cyanoborohydride (NaCNBH₃). Thanks due to the steric effects on the molecule; the electrophile (HCl) regioselectively targets the 4-OH and NaCNBH₃ reduces the molecule, see figure 8. This reaction contains very sensitive compounds and has to be carefully performed under inert gas and water free conditions. NaCNBH₃ is also a reactive compound, and a reaction shouldn't take more than 5-10 minutes, which is a huge advantage. Drawbacks; it's not an environment friendly compound and there is a risk of gas evolution when mixing with HCl. [17] [18] [19]

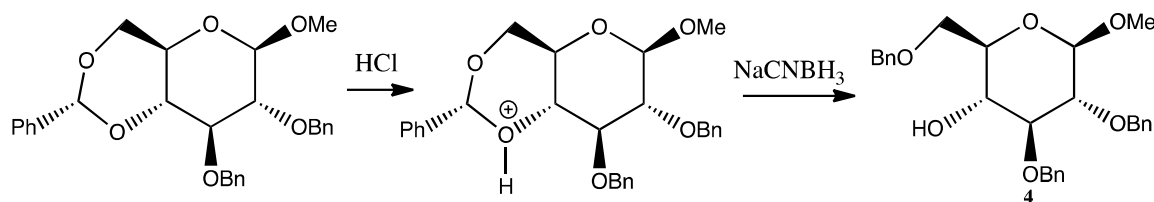


Figure 8. Reducing the compound with HCl and NaCNBH₃. Adapted from [16]

The second method is reducing the acetal with trifluoroacetic acid (TFA) and triethylsilane (TES). This combination is known for reducing acetals and ketals. TFA protonates the targeted molecule while TES reduces the molecule, working as the reducing agent, see figure 9. The target molecule **4**, usually predominates the reaction. Although to be certain, NMR analysis has to confirm the molecule. Both reagents are neither sensitive nor reactive and can cooperate at room temperature. The solvent used is dried dichloromethane, to avoid hydrolysis when removing the protecting group. The reaction itself is not complicated; being the easier method of the two studied ones. [17] [20]

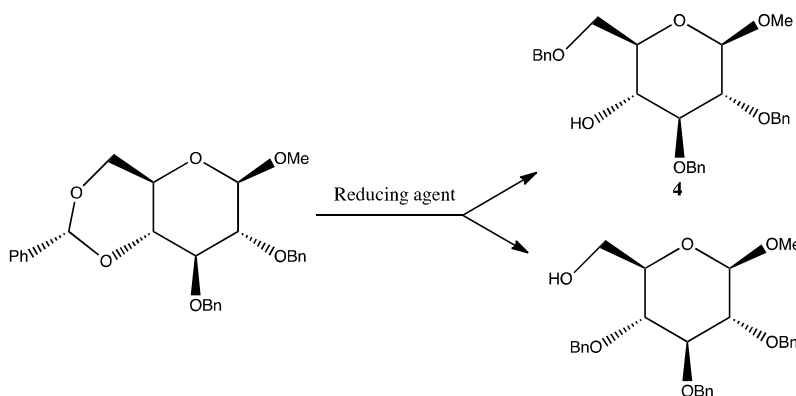


Figure 9. Reducing the acetal-protecting group with TFA and TES. [20]

Figure 10 demonstrates a summary on the synthesis leading to the seeking compound **4**.

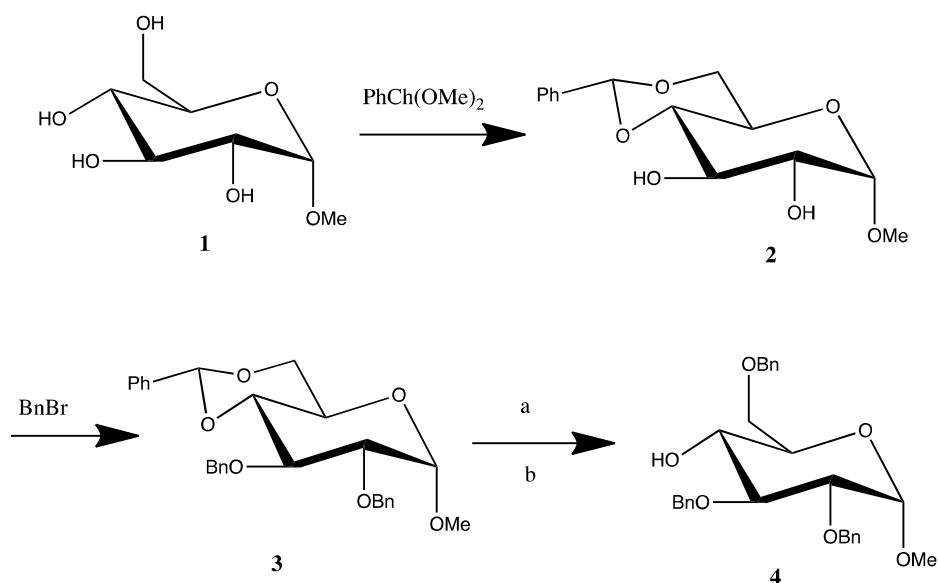


Figure 10 Reagents and conditions: *a*; HCl , NaBH_3CN , THF , 0°C *b*; TFA , TES , DCM , rt

3. Experimental

General; Reactions including water sensitive compounds were executed under inert gas (nitrogen gas) and in dried glassware (oven 150°C , $\approx 24\text{h}$). For example, wet solvents can lead to hydrolysis when removing protecting groups, and certain compounds, such as sodium hydride, increases the risk of fire or explosions in presence with water. Thin layer chromatography (TLC) was performed on silica sheets (Merck, Silica gel 60 F_{254}) and visualized with UV light. Nuclear magnetic resonance (NMR) spectra were acquired on Varian MR-400 spectrometer. ^1H NMR was performed at a frequency of 400 MHz and ^{13}C NMR at 101 MHz. The NMR spectra's were analysed on the software programme MestReNova.

3.1 Methyl 4,6-O-benzylidene-α-D-glucopyranoside (**2**)

Methyl α-D-Glucopyranoside **1** (8.65 g, 44.5 mmol) was dissolved in 100 ml dry acetonitrile. 15 g of silica and 1.5 g of H_2SO_4 -silica was added to the mixture. 17.4 ml of PhCH(OMe)_2 was added and the solution was left stirred at 60°C . After 2h, TLC showed complete conversion (2:3 n-hexane-EtOAc), 17.5 mL Et_3N was added and stirring continued for another 30 min. The pH-value was checked (not too acidic) and the solution was filtered through celite in vacuum, washed with acetonitrile and concentrated. It resulted in 13.28 g of crude product. Ethylacetate was found to be a potential crystallisation solvent. The crude product was diluted with EtOAc and re-concentrated, resulting in 6.0196 g (21.37 mml, 48 %) white solid material. (See figure 11) ^1H NMR (400 MHz, Chloroform-*d*) δ 7.68 – 7.30 (m, 6H), 5.53 (s, 1H), 4.79 (d, $J = 3.9$ Hz, 1H), 4.29 (dd, $J = 4.7$ Hz, 1H), 3.93 (t, $J = 9.2$ Hz, 1H), 3.87 – 3.67 (m, 3H), 3.70 – 3.37 (m, 3H), 3.46 (s, 3H), 3.19 – 3.07 (m, 1H), 1.44 – 1.32 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 137.00, 129.23, 128.30, 126.28 (Ph), 101.93 (CHPh), 99.74 (C-1), 80.88 (C-4), 77.19 (C-2), 72.88 (C-3), 68.91 (C-6), 62.35 (C-5), 58.06 (1-OMe), 55.57 (1-OMe), 8.63.

3.2 Methyl 2,3-di-O-benzyl-4,6-O-benzylidene- α -D-glucopyranoside (3)

To a solution of compound **2** (4.715 g, 16.74 mmol), 55 mL of dry DMF was added and stirred at 0 °C. NaH (1.95 g, 48.75 mmol, 60% dispersion in mineral oil) was added a little bit at the time (gas evolution). BnBr (3.5 mL, 29.3 mmol) was added and the reaction was left stirred at room temperature. After 3h TLC indicated complete consumption of start material. (8:1 *n*-hexane/EtOAc). 4.5 mL of 2-propanol was added and the mixture was left stirring for 1h. The solution was diluted with 200 mL EtOAc and extracted three times with 250 mL of water. (The combined aqueous phases should've been extracted with 100 mL of diethyl ether.) The organic phase was dried with MgSO₄, filtered and concentrated in vacuum. It resulted in 7.0449 g of crude product. The crude product was applied to a column of silica gel (10:1 *n*-hexane/EtOAc), yielding 2.2995 g (4.97 mmol, 29.69 %) of product **3** see figure 11. *R*_f 0.30; ¹H NMR (400 MHz, Chloroform-*d*) δ 7.61 – 7.21 (m, 16H), 5.58 (s, 1H), 4.86 (d, *J* = 5.0 Hz, 4H), 4.63 (d, *J* = 4.2 Hz, 1H), 4.30 (tt, *J* = 10.1, 9.9, 4.8, 4.2 Hz, 1H), 4.09 (t, *J* = 9.1 Hz, 1H), 3.87 (ddt, *J* = 14.1, 10.7, 4.7 Hz, 0H), 3.74 (dq, *J* = 20.9, 10.9, 10.2 Hz, 1H), 3.64 (t, *J* = 9.8, 9.2 Hz, 1H), 3.59 (dd, *J* = 9.4, 4.1 Hz, 1H), 3.43 (s, 3H), 1.40 – 1.25 (m, 4H).



Figure 11. Purified products **2** & **3**.

3.3 Methyl 2,3,6-tri-O-benzyl- α -D-glucopyranoside (4) a

Compound **3** (0.1 g, 0.22 mmol) combined with 0.17 g NaBH₃CN (2.7 mmol), was dissolved in 5 mL dry THF at room temperature. Hydrogen chloride in cyclopentyl methyl ether was added until the solution was acidic; it required 8 mL until pH-paper indicated pH-value around 2-3. TLC showed consumption of start material (10:1 *n*-hexane-EtOAc) after 30 min. The mixture was diluted with 50 mL dichloromethane and extracted with water and saturated aqueous sodium hydrogen carbonate. The organic phase was dried with MgSO₄, filtered and concentrated. The resulting yellow oil was purified with flash chromatography (2:1 *n*-hexane-EtOAc) resulting in 29 mg (0.0625 mmol, 29 %) of compound **4**.

3.4 Methyl 2,3,6-tri-O-benzyl- α -D-glucopyranoside (4) b

0.5 mL (6.4 mmol) trifluoroacetic acid was added dropwise to a solution consisting of compound **3** (0.1 g, 0.22 mmol) and 1 mL (6.4 mmol) triethylsilane at 0 °C. After the addition, the solution was warmed to room temperature and left stirred until TLC (10:1 *n*-hexane-EtOAc) indicated complete consumption of start material (24 h). The mixture was diluted with EtOAc, washed with saturated aqueous sodium hydrogen carbonate and brine. The organic phase was dried with MgSO₄, filtered

and concentrated. The crude product was applied to a column of silica gel (2:1 *n*-hexane-EtoAc), giving 0.30 mg (0.0646 mmol, 30 %) of compound **4**.

3.5 Methyl 2,3,6-tri-O-benzyl- α -D-glucopyranoside (**4**) a, Gram scale

The same procedure was done, only with different volumes. 1 g (2.2 mmol) of compound **3**, 1.7 g NaBH₃CN (27 mmol) was dissolved with 30 mL THF. It required 10 mL until pH-paper indicated pH-value 2-3. Also, due to the risk of gas-evolution when adding HCl, the reaction was performed at 0°C, see figure 12. The rest was performed as previous steps. The best reaction gave 0.15 g (0.323 mmol, 15 %) of molecule **4**. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.53 – 7.22 (m, 14H), 5.55 (s, 1H), 5.04 – 4.73 (m, 4H), 4.73 – 4.62 (m, 1H), 4.65 – 4.54 (m, 1H), 4.39 – 4.22 (m, 1H), 4.04 (t, *J* = 9.3 Hz, 1H), 3.89 – 3.34 (m, 7H), 3.40 (s, 2H), 1.32 – 1.19 (m, 3H), 0.91 – 0.81 (m, 2H).

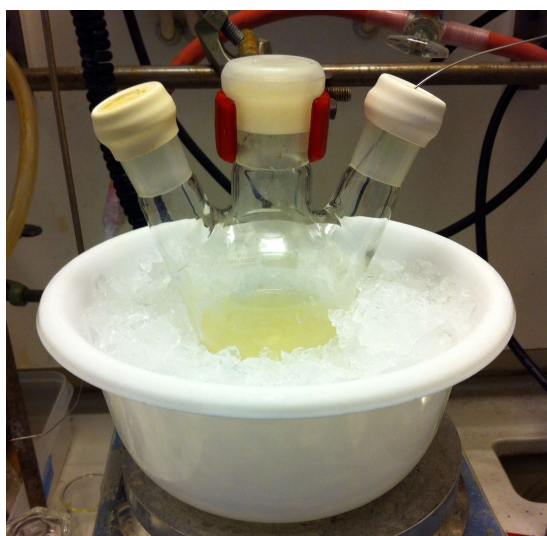


Figure 12, gram scale reaction performed at 0°C.

4. Results and discussion

When performing the reaction with benzylbromide (BnBr), synthesis from molecule **2** to **3**, the yield was only 29.69 %. There were some factors lowering the yield and could've been done better. The aqueous phases were supposed to be washed with diethyl ether to recover eventual product. The starting material, **2**, wasn't purified when the synthesis was performed, whereas the numbers aren't 100 % accurate. Also the procedure was only performed once, with a bit more experience, the yield could've higher. Although, the reaction gave 2.2995 g of product **3**, enough to study the next synthesis step.

Method **a** resulted in 29 mg (29 %) of molecule **4** and method **b** resulted in 30 mg (30 %). (Note: Ratio between molecule **3** and **4** equals to 1:1, calculated in weight.). When analysing the NMR-spectra of sample **b**, impurities were shown in the spectra, most likely to be ethylacetate, meaning that the purification wasn't done properly. There were generally difficulties to purify the small amounts of crude product. Loss of product could've occurred when transporting from different beakers and also losing product in the column. The amount of start material used in lab scale reactions could be increased for easier reprocessing of crude product in the future. Besides conversion, the reaction times were considered. Method **a** started to show consumption of start material after 30 min, while method **b** took about 24h. Both factors made me choose to scale up method **a**.

When performing method **a** at gram scale, the reaction was performed several times and the best conversion was about 15% (0.15 g, 0.323 mmol). When analysing NMR spectra's, several reactions showed only two benzyl groups, indicating that the environment could've been to acidic, removing the protecting groups. Besides water and air free conditions, the reaction requires accurate control of the pH-value. Not being able to get the right conditions might explain the low conversion.

Another factor affecting the bad conversion could've been the quality of NaCNBH₃. Information on the bottle said that the compound was recrystallized in 1997, making it possible to have been exposed to air under its 19 years and it might have reduced its reactivity. Normally NaCNBH₃ is a very reactive compound and the synthesis takes about 5-10 min before complete consumption of start material. [19] [20]. The NaCNBH₃ I used might have been the reason why it barely showed any consumption on the TLC plate when performing reactions in gram scale.

When scaling up a reaction, compounds not environmental friendly should be avoided. The idea was to find an alternative to NaCNBH₃, which has the same properties working as a reducing agent. But because the synthesis didn't succeed very well, a real attempt was never done. If method **a** is going to be a plausible and sustainable method, NaCNBH₃ should be replaced. If NaCNBH₃ is used in larger scales, the synthesis would generate bigger amount of waste, which can be expensive to get rid of. Also NaCNBH₃ forms the toxic gas, hydrogen cyanide, when reacting with hydrogen chloride. This could become an issue if performed at very large scales and something goes wrong, although this reaction doesn't strive to become commercialized.

5. Conclusion and future work

This project had two different milestones, first of all reach molecule **3** with relative known methods and thereafter study the next synthesis step, reaching target molecule **4**. Applying the protecting groups were easily performed, although the conversions weren't very high, it could get better with more experience. In the second step, method **a**, with HCl, NaCNBH₃ and THF, was decided to be studied. This reaction ended up being difficult to execute under the right conditions; the conversion wasn't good enough. Although the reaction seems as an easy and a potential synthesis route, an alternative should be sought. Method **b** could be studied even more as not much effort was put in this study. The reagents are not as sensible and the synthesis cooperates at milder conditions. Also if studying method **b**, the reaction time should be studied on how to lower it, to get a good synthesis route.

Besides studying the reaction even further, future work consist of reaching the model substrate Methyl 4-O-methyl α -D-Glucuronide **6**. Next step would be alkylate the 4-OH group thereafter removing the protecting groups and adding a carboxylic group to the 6-OH. Remaining synthesis steps could include investigating different reagents and conditions, finding the easiest method giving the best conversion.

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