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Carbohydrate Synthesis

- Modifications on Cellobiose

Master's thesis in Material Chemistry

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Carbohydrate synthesis – *Modifications on Cellobiose*

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Abstract

Polysaccharides have been widely used as drug-carriers, especially of hydrophobic drugs, by attaching them to the polymer chain. One polysaccharide that has shown to target damaged cells is pullulan, constructed by the repeating unit maltriose, meaning 3 maltose units. Cellobiose is a disaccharide very similar in structure to maltose. The main focus of this thesis was to see whether cellobiose could be modified and possibly be used as a drug carrier. By selectively protecting one of the primary alcohols, a drug could be attached on the other primary alcohol, giving a more homogenous compound. Using *tert*-butyldimethylsilyl as a protecting gave first indications of a non-selective reaction. Instead using Dowex as a catalyst at 80°C for 24h in methanol gave a selective protection of the primary alcohol. Further testing should be done to optimize a scale-up reaction. Also selective oxidation could be performed on one of the primary alcohols and to learn more about oxidation, tests on micro- and nano- crystalline cellulose were successfully performed.

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1. Introduction

Polysaccharides are natural polymers formed in nature by either photosynthesis, by bacteria or from fungi. Every polymer consists of repeating monosaccharides in the chain and can either have a linear or branched structure. [1][2] Most common polysaccharides are based on glucose units, where the only functional groups are hydroxyl molecules. Cellulose and pullulan are examples of such polysaccharides as seen in figure 1. Examples of polysaccharides with different functional groups are chitosan and chitin, see figure 1. [1].

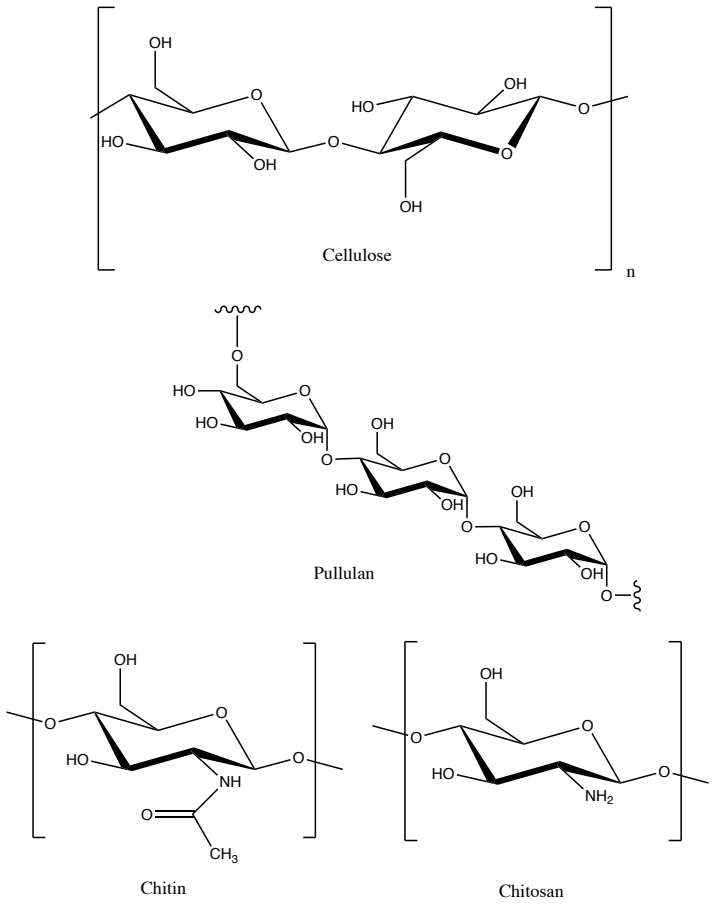


Figure 1. Repeating units of some natural polysaccharides. [Adapted from 1]

These natural polymers are some of the most popular biomaterials for controlled drug-released in the human body. A hydrophilic polymer-matrix is used to attach drugs to the polymer and this makes it possible to design the release rate of drugs *in vivo*. Instead of taking five or six tablets a day, it can be reduced to one single dose by using appropriate drug carriers. [3]

Hydroxyl-, amino- and carboxylic acid groups are normal occurring functional groups on polysaccharides. These side groups make it possible to conduct chemical modification and change the properties of the polymer, for example increase the hydrophobicity. [2] By increased hydrophobicity, drug delivery systems have been developed, where hydrophobic molecules (water insoluble drugs) are transported through micelle carrier systems in the body. [1]

One polysaccharide with special properties is pullulan, which consist of the repeating unit maltotriose. Each maltose units are connected by the α- (1→4) bond and the repeating units are attached through the primary alcohols. (α- (1→6) bond.) It has been reported that pullulan has high selectivity when it comes to target infected cells/tissues and release cytotoxic molecules. Several studies have thus been performed to use pullulan as a drug carrier. [2]

By attaching cholesterol to the backbone of Pullulan, different drugs can be attached to the polymer. Cholesterol bearing pullulan (CHP) is both a hydrophobic molecule (cholesterol group) and hydrophilic (pullulan chain). (See figure 2) In aqueous solutions CHP forms spherical hydrogel particles and this makes it possible to use the CHP as a drug carrier. (Hydrogel particles swell in presence with water) Studies on several drugs have been done, such as attaching insulin to the CHP. The study showed increased stability and higher biological activity compared to normal insulin injected in the blood. [2]

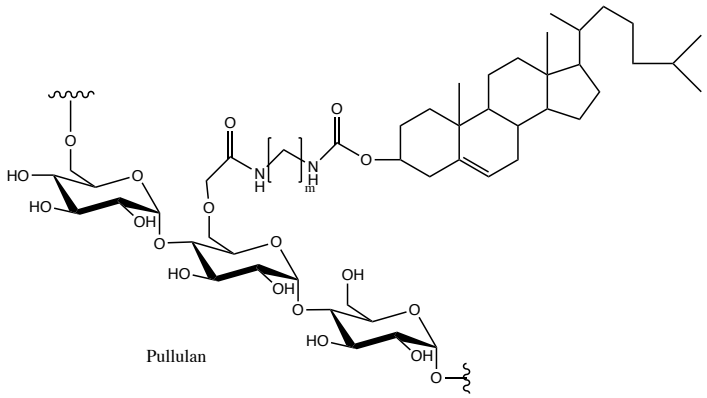


Figure 2. Illustration of cholesterol bearing pullulan (CHP) [Adapted from 2]

The backbone of Pullulan can also be modified to attach hydrophobic drugs. By modifying pullulan using carboxylate groups, the molecule becomes negatively charge, making it pH and ionic strength sensitive. Reports has shown nanoparticles of carboxymethyl pullulan being able to form hydrazone bonds with hydrophobic drugs, enhancing the drug delivery to tumour cells, compared to the drug itself. One example of such drugs is doxorubicin, a drug especially effective on cancer cells in the liver. By lowering the pH, more of the drug could be released from the drug carrier. [2]

Another material used in the pharmaceutical area is cellulose. By modifying the backbone, different properties of the material has been observed. For example, some of the hydroxyl groups of cellulose have been replaced by propylene oxide forming hydroxypropyl cellulose (HPC). By combing with ethyl cellulose EC (another modified derivate) the drug release of hydrophilic drugs could be controlled. The water-soluble HPC swells in water and EC traps the water in the matrix, slowing the drug release of a drug attached to the backbone of the polymer. [3]

Compared to synthetic polymers, natural polysaccharides show very low or no toxicity and also high biocompatibility for the human body. Synthetic polymers consist of monomers and additives are often added. Natural polysaccharides are not considered toxic since the degradation of the polymers does not give toxic side products. It's possible to either attach drugs directly on the polymer chain or modify the backbone to change the properties of the molecule. [1][3]

1.1 Hypothesis

Cellulose consists of several glucose units and two of those units together are referred to as cellobiose. The repeating unit in pullulan is maltotriose and has very similar structure to cellobiose. Cellobiose could have similar properties when it comes to target damaged cells and be a suitable drug carrier. Since it's modified from start, the drugs could also be attached in a controlled manner getting a more homogeneous substance, instead of a randomly ordered one with a lot of drugs on the chain.

1.1.1 Aim

The aim of this thesis was to study the possibility of modifying cellobiose in different manners and study its reactivity. The first goal was to see the possibility to protect one of the primary alcohols and attach a drug or similar compounds to the other primary alcohol. This would give a drug loading ratio 1:1 between drug and cellobiose, giving a more homogenous substance. Another possibility would be to investigate if one of the primary alcohols could be oxidized. (While the other one is protected)

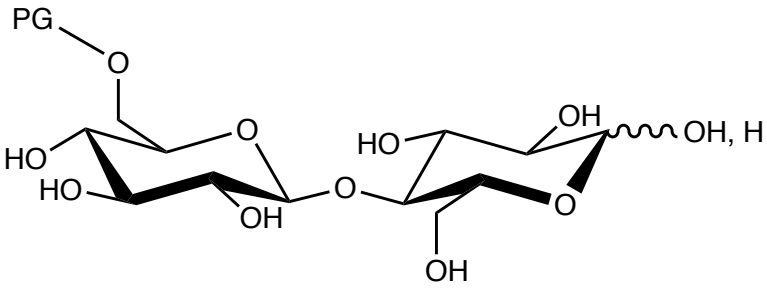


Figure 3. The aim of the thesis was to look whether it was possible to selectively protect one of the primary alcohols with a protecting group (PG).

If both primary alcohols are protected, several cellobiose units could be attached together via the primary alcohols, giving α- (1→6) bonds. (As previously seen in pullulan). The second step would be protecting the remaining hydroxyl groups, thereafter remove the protecting groups on the primary alcohols. See figure 4 for a summary on how to link several cellobiose units. This may in the future give a molecule with similar application as pullulan.

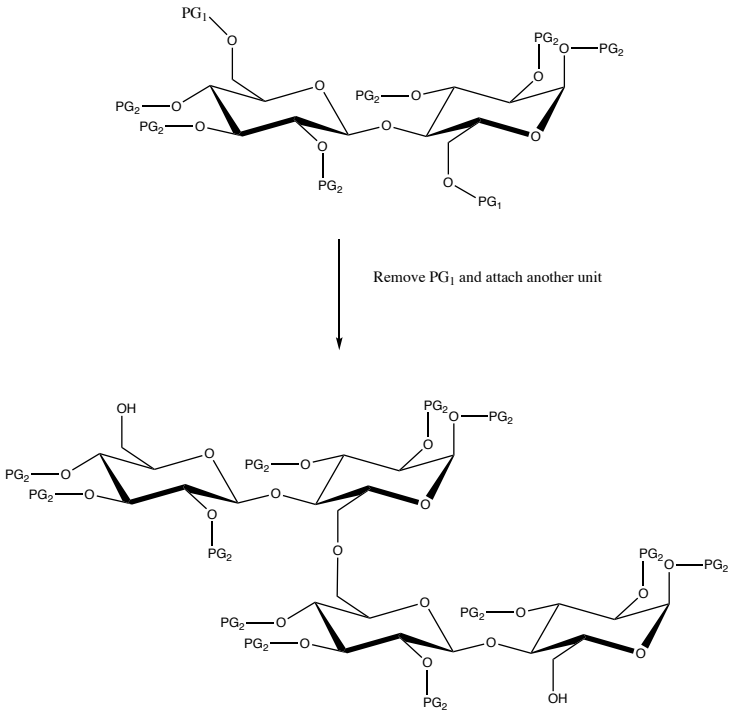


Figure 4. Proposed way on how to connect two cellobiose units using two different protecting groups. (PG_1 and PG_2).

2. Theory

2.1 Cellulose

Cellulose is a biodegradable polymer found naturally in the cell walls of wood and plants. It's considered a linear homo-polymer consisting of *D*-anhydroglucopyranose units (AGU), which are linked together with β-(1→4)-glycoside bonds. Every second AGU is turned 180 degrees and two AGUs form a cellobiose unit, smallest repeating unit in the polymer. (See figure 5) [4]

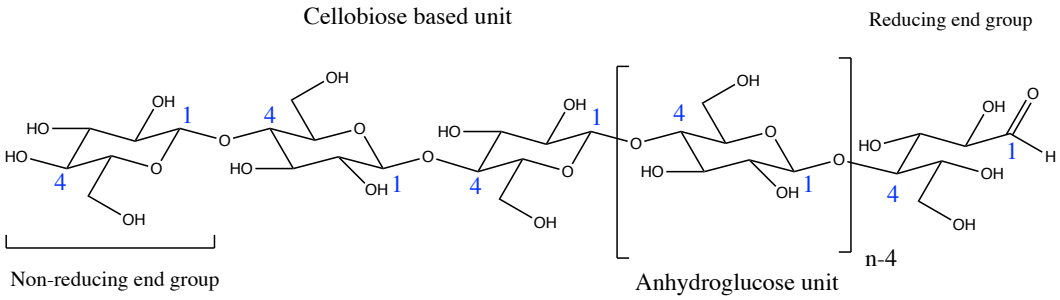


Figure 5. Structure of cellulose. Blue numbers show the β- (1→4)-glycoside bonds and n is equal to the degree of polymerization. (Amount of AGUs.) [Adapted from 4]

Cellulose consists of three different AGUs. The internal AGU is the main component of the polymer chain, which is linked with the β-bonds. Each internal AGU has three hydroxyl groups, one primary alcohol at C-6 and two secondary alcohols at C-2 and C-3. All these sites are possible to undergo chemical modification. (See figure 6) The second form of AGU is the reducing end group, which is an open form of the ring and contains either a free hemi-acetal or aldehyde at the C-1. (Figure 5 shows the aldehyde form.) The third form of AGU is the non-reducing end with a hydroxyl group at the C-4 position. [4] [5]

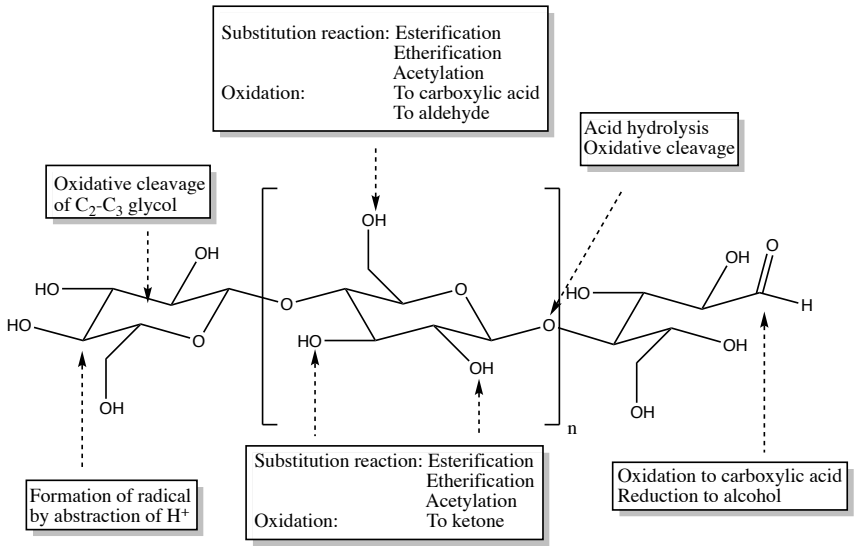


Figure 6. Illustration of the three different types of AGUs and possible chemical modifications on the different parts. [Adapted from 3]

The number of AGU, also known as the degree of polymerization (DP), differs between materials. Native cotton has range of DP up to 12000 and cellulose polymer in wood fibres can have up to 10000. After extraction and purification through pulping, wood pulp have a DP between 600-1200. [4]

2.1.1 Cellobiose

As previously mentioned, two AGU is also known as the disaccharide cellobiose. A total of eight hydroxyl groups are present, where two are primary alcohols. One of the units can be seen as a non-reducing end and the other as a reducing end, as similar to the cellulose structure. [4] In solution two anomeric forms exist α and β, which are at an equilibrium ratio of 38:62. (See figure 7). [6]

The two units are linked through a β-(1→4)-glycoside bond. Comparing with Maltose, another disaccharide with the same molecular formula, where the two monomers are linked through a α-(1→4)-glycoside bond, as seen in figure 7. This slight difference in configuration gives cellobiose its own properties. [6]

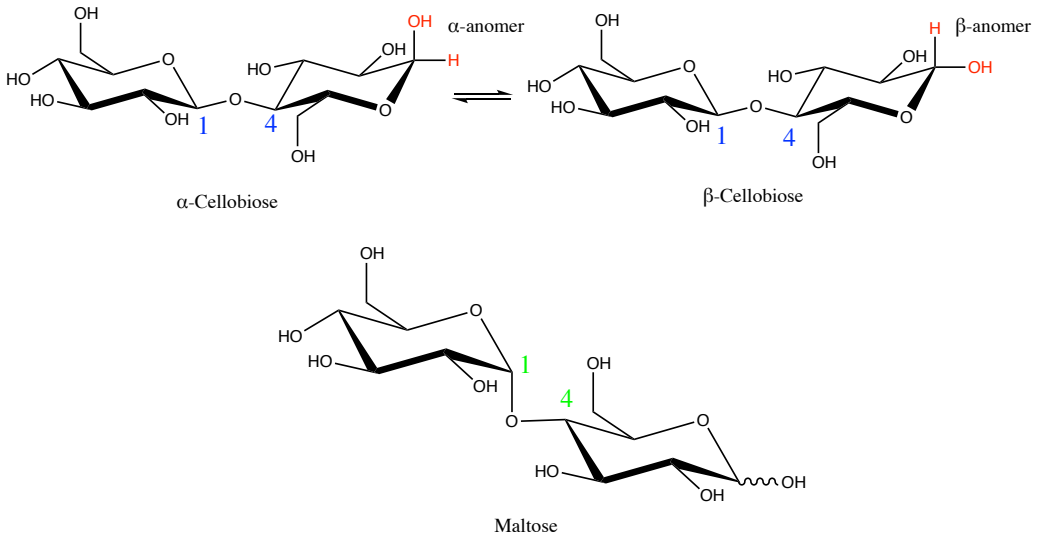


Figure 7. Structure of cellobiose anomers, which are in equilibrium in solution. Maltose has a α-bond compared to cellobioses' β-bond. [Adapted from 6]

To obtain cellobiose, wood or other rich cellulose materials undergo enzymatic hydrolysis. Endoglucanase is an enzyme that randomly cleaves the β -(1→4)-glycoside bonds forming a glucan chain, with no reducing end. (The chain differs in length) The 2nd enzyme Cellobiohydrolase cleaves the chain forming the dimer cellobiose. Further enzymatic work can cleave cellobiose into glucose. (See figure 8). Cellulose, and thus cellobiose, is produced in very large scales, making it a good source for green new materials. To understand how it reacts could open the possibility to more applications.

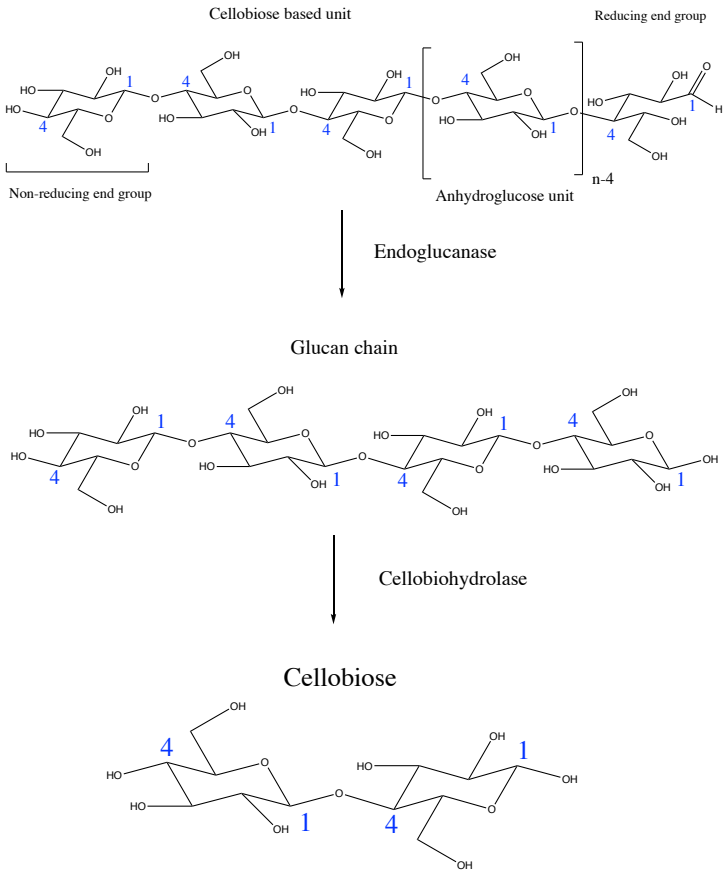


Figure 8. Illustration on how to obtain cellobiose from cellulose using enzymes [7]

2.1.1.1 Intra-molecular bonds and structure of cellobiose

Cellobiose does not have inter-molecular bonds between the molecules, only intra-molecular hydrogen bonds exists, specifically between the O-5 oxygen and the O-3’ hydroxyl group, as seen in figure 9. There is an O-6 to O-3’ hydrogen bond as well which is not as strong. Cellulose has both inter- and intra-molecular hydrogen bonds, between the chains and between each monomer [8]

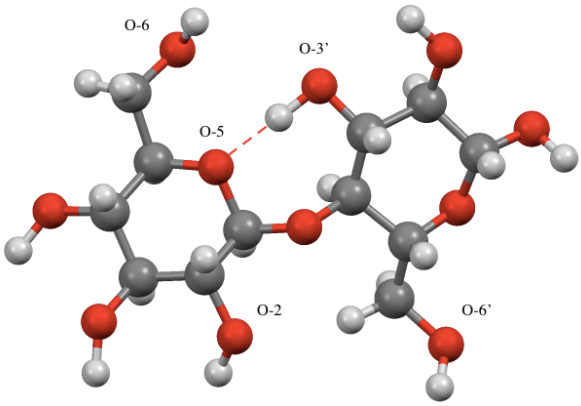


Figure 9. Crystal structure of cellobiose showing the strongest hydrogen bond between O-5 and O-3’. [Adapted from 9]

In cellulose the O-6’ oxygen form a second hydrogen bond with the O-2, which are none existent in cellobiose. The conformation of the hydroxymethyl-group determines whether there’s a second hydrogen bond or not. The different conformations can be viewed in figure 10. [8]

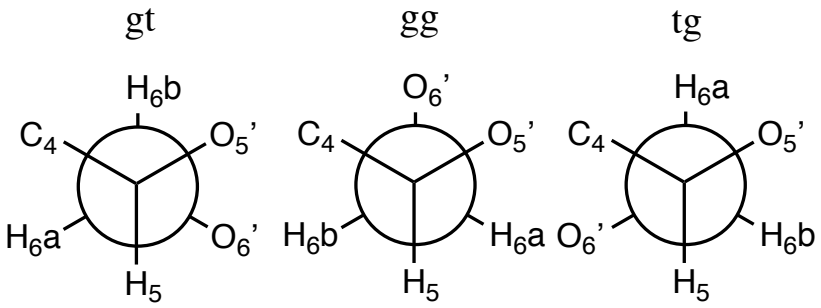


Figure 10. The conformation of the hydroxymethyl group of the reducing end. The Newman projects are viewed from the top of the C5-C6 bond and the angle of the bond is different; gauche-trans (+65°), gauche-gauche (-65°) and trans-gauche (±180°). [Adapted from 8]

The dominant conformation for cellobiose, both α and β, is gt or gg. The oxygen (O-6’) does not interact with O-2 since it’s pointing away, meaning no second hydrogen bond is formed. [8] Comparing with crystalline cellulose I structure, where the conformation of every second AGU is tg, two hydrogen bonds exist, giving the molecule a more rigid structure. [10]

By looking at something called radial distribution function (RDF), the coordination number/hydrogen-bonding interactions can be estimated. Cellulose has intermolecular hydrogen bonds between the O-6 and O-3 between each chain. RDF calculations showed that there was very little interaction between cellobiose molecules, only 1% of the cellobiose molecules interact with each other. [8]

With the presence of water, each hydroxyl-group accepts 0.9-1 hydrogen bonds from water and the O-5 – O3’ hydrogen bond is non-existing in water. In aq. solutions the bond was non-existing while in DMSO, the bond was shown to exist. [9] Instead the O-5 forms a strong hydrogen bond with water, with coordination number of 0.46 meaning about 50% of times the hydrogen bond is populated. RDF calculations have also shown this to be a stronger bond and therefore the intra-molecular bond is broken. [8]

2.2 *tert*-butyldimethylsilyl – protecting group

In order to protect the hydroxyl groups, a suitable compound is *tert*-butyldimethylsilyl (TBDMS). It's stable under several different conditions and can easily be removed with a fluoride ion or treatment with acid. See figure 11 for the structure. [11]

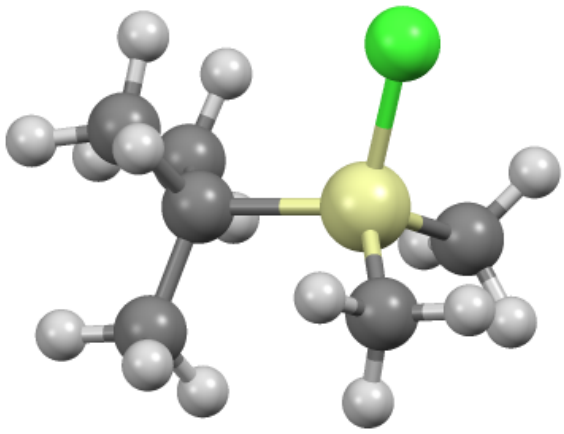


Figure 11. 3D-structure showing the atoms in the protecting group. Green = Cl and yellow = Si.

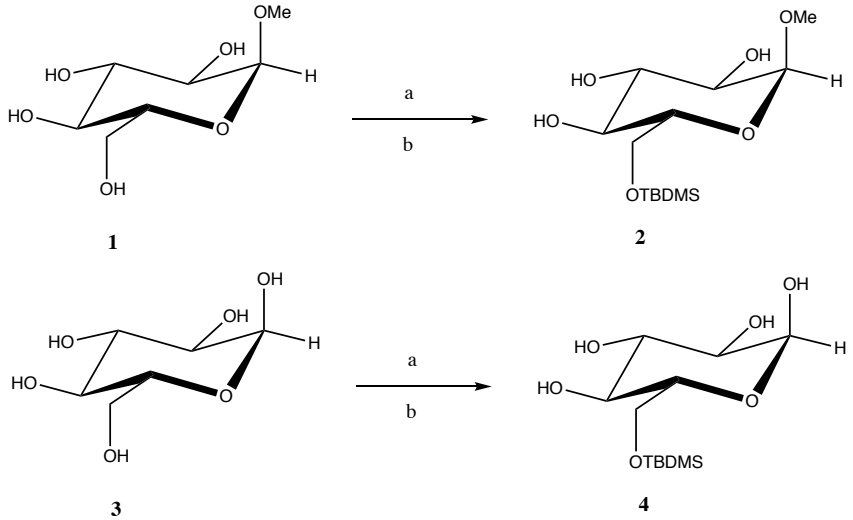
The chloride anion is removed and the silyl group attach to the oxygen in the hydroxyl group, replacing the hydrogen. [11] As seen in the figure the protecting group is quite bulky so depending on the stereochemistry of the targeted molecule, the protection may not be plausible.

A huge advantage of this protecting group is that it has shown good selectivity towards primary hydroxyl groups in different carbohydrate derivatives. Selective studies on secondary hydroxyl groups have also been conducted, the difference being the amount used. The mole equivalent determines which group is being protected, where an estimate of 1.1 mole equivalent have been used to protect primary hydroxyl groups in carbohydrates. [11]

3. Experimental

General; Thin layer chromatography was conducted on silica sheets (Merck, Silica gel 60 F_{254}) and visualized by dipping in a $KMnO_4$-solution and thereafter heating the plate. Instrument used to obtain Nuclear magnetic resonance (NMR) spectra were performed on a Varian MR-400 spectrometer. (^{1}H NMR 400 MHz and ^{13}C at 101 MHz.) NMR spectra were analysed using the software MestreNova and FTIR spectra using the software KnowItAll.

3.1 *tert*-Butyldimethylsilyl- chloride reactions on model substrates



Scheme 1. Reactions conducted on model substrates. Reagents and conditions: a; DMF, DIPEA, 4-Pyrrolidinopyridine, rt b; THF, N-methylimidazole, Iodine, rt

3.1.1 *tert*-Butyldimethylsilyl-α-*D*-glucopyranoside (2a)

1 mmol of methyl-α-*D*-glucopyranoside **1** (0.20 g) was mixed with 3 ml DMF, 2 ml *N,N*-Diisopropylethylamine (DIPEA) and 4-Pyrrolidinopyridine-catalyst was added. The solution was set in an ice bath before adding 1.1 mole eq. of TBDMS-Cl (165.8 mg) and before being left to stir overnight at room temperature. Dichloromethane was added and washed with 10% aqueous NH_4Cl, dried with Na_2SO_4 and the solvent was evaporated. The residue was purified using flash chromatography (5% Methanol/DCM). It resulted in 89.5 mg of compound **2**. (0.29 mmol, 29 %). R_f= 0.30;

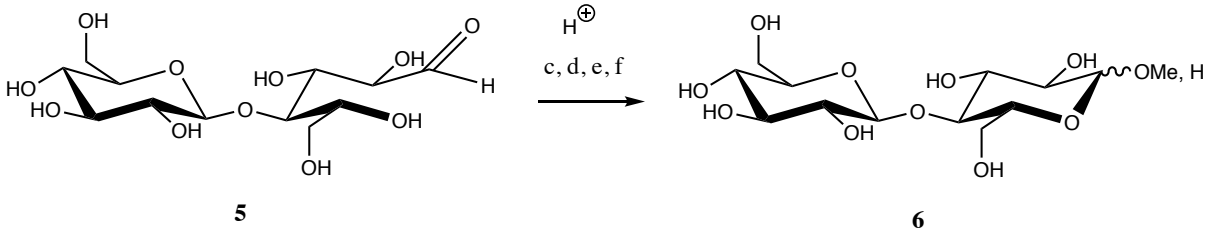
3.1.2 tert-Butyldimethylsilyl-α-*D*-glucopyranoside (2b)

1 mmol of methyl-α-*D*-glucopyranoside (0.20 g) was mixed with 3 ml THF, iodine 0.34 g (2.67 mmol) and 3.28 mmol (0.25 ml, 0.27 g) N-methylimidazole. 1.1-mole eq. of TBDMS-Cl (165.8 mg) was added before leaving the solution stirring overnight. (Added in an ice bath). The solvent was evaporated before dissolving in EtOAc and washed with concentrated $Na_2S_2O_3$ (aq). Organic phase was dried (Na_2SO_4) and the solvent was evaporated. The residue was purified using flash chromatography (5% Methanol/DCM) yielding 56.9 mg of compound **2**. (18 mmol, 18 %) R_f = 0.3.

3.1.3 tert-Butyldimethylsilyl-glucose (4ab)

The same reactions as described above were performed with 1 mmol of glucose **3** (0.18 g) instead of **1**. These reactions were not successful. After flash chromatography, (5% Methanol/DCM) the protecting group (TBDMS) was separated from the glucose **3**.

3.2 Methylation of cellobiose



5 **6**

Scheme 2. Reaction conditions of the methylated cellobiose: 10 ml anhydrous methanol at 80°C with different acetic mediums c: Amberlite d: Dowex e: H_2SO_4 f: H_2SO_4-silica

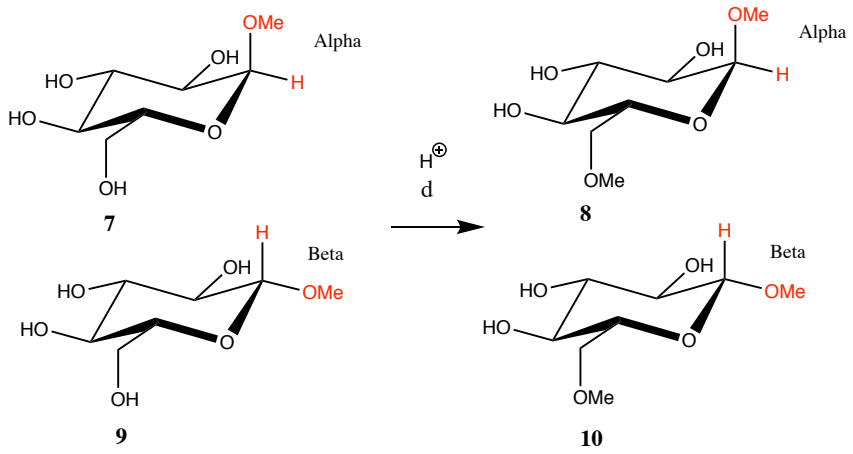
3.2.1 Methyl-Cellobiose (6c,d)

1 mmol (0.3423 g) of cellobiose **(5)** was mixed with 10 ml anhydrous methanol and 1 gram of ion-exchange resin (Amberlite H^+ respectively Dowex H^+). The mixture was heated under agitation at 80°C (in a sealed vial) for 24 hours until TLC, 20% methanol/DCM, showed conversion of product (R_f = 0.3). The resin was filtered off and washed with methanol. The solvent was evaporated and purified by column chromatography

3.2.2 Methyl-Cellobiose (6e,f)

1 mmol (0.342 g) of cellobiose **(5)** was mixed with 10 ml anhydrous methanol and 0.7 g $CaSO_4$. (2:1 ratio between $CaSO_4$ and cellobiose) H_2SO_4 acid (95 %) respectively H_2SO_4-silica was added until pH was in the range of 2-3. The mixture was heated under agitation to 80°C and reaction status was checked with TLC. (20% Methanol/DCM). The H_2SO_4 sample was left for 5 hours before purifying, while the silica based was left for 24 hours. After cooling down, $Ca(OH_2)$ was added until pH reached 7-8. The mixture was filtered and washed with methanol before evaporating the solvent. The residue was purified with column chromatography.

3.2.3 Methylation on model substrates. 6OMe-methyl- α/β -D-glucopyranoside (8d, *10d*)



*Scheme 3. Methylation of methyl- α -D-glucopyranoside 7 and methyl- β -D-glucopyranoside **9** under conditions d: Dowex in 10 ml anhydrous methanol at 80°C for 24h.*

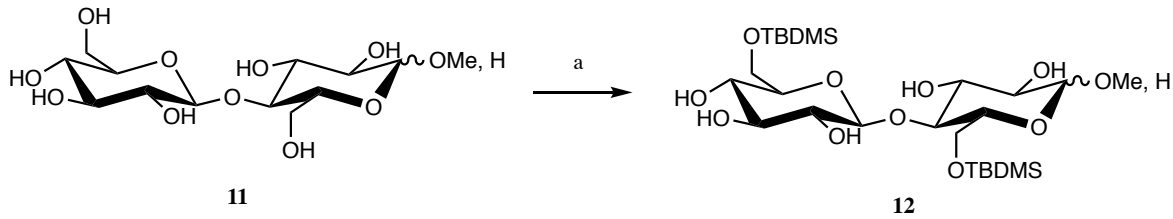
3.2.3 6OMe-methyl- α / β -D-glucopyranoside (8d, 10d)

1 mmol (0.194 g) of respective methylated glucose **(7** and **9)** was heated to 80°C for 24h together with 10 ml anhydrous methanol and 1 g Dowex. (TLC was used to monitor the reactions, 5% Methanol/DCM) The solutions were filtered and washed with anhydrous methanol before evaporating the solvent.

3.2.4 Methyl-Cellobiose (6d) – Big batch

2.0 gram (5.84 mmol) of cellobiose **(5)** was mixed with 58 ml anhydrous methanol and 6 gram of Dowex. (Same ratio as in the small-scale reaction) The reaction was heated under agitation to 80°C for 24h. The reaction was monitored using TLC. (20% Methanol/DCM). The resin was filtered off and washed with anhydrous methanol before evaporating the solvent

3.3 Protecting me-cellobiose with TBDMS group

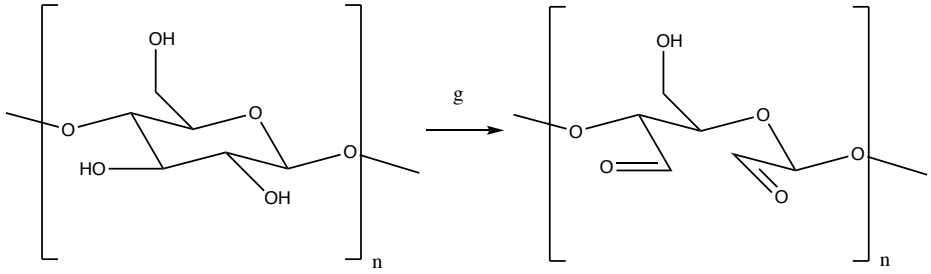


Scheme 4. Protecting the primary alcohols on the methylated cellobiose with TBDMS-Cl. Reaction conditions a: DMF, DIPEA, 4-Pyrrolidinopyridine, rt.

3.3.1 tert-Butyldimethylsilyl-Methyl-Cellobiose (12)

200 mg of compound **11** (0.56 mmol) was mixed with 1 ml DMF and 1 ml of DIPEA before adding a small amount of the catalyst 4-Pyrrolidinopyridine. 1.1 mole eq. of TBDMS-Cl was added at 0°C. The reaction was left stirred overnight at rt. The product was extracted with DCM and 10% aqueous NH_4Cl. The organic phase was dried with Na_2SO_4 before removing the solvent. The remaining residue was purified using flash chromatography. (5% Methanol/DCM). $R_f = 0.3$.

3.4 Oxidation of cellulose



Cellulose **13** 2,3-Di-Aldehyde-Cellulose **14**

Scheme 5. Oxidation of both micro- and nano- crystalline cellulose under conditions g: H_2O, $NaIO_4$, acetic acid at 45°C for 4 hours.

3.4.1 Oxidation of microcrystalline cellulose (14)

1.15 gram of microcrystalline cellulose (mcc) was mixed with 20 ml of distilled water and 1 gram of $NaIO_4$ was dissolved in 5 ml of distilled water before mixing both solutions. (Strived to have a 4% consistency solution). The pH was lowered to around 4 using a 36% acetic acid solution. The reaction vessel was covered in aluminium foil before heating the solution to 45°C. Samples for UV-Vis measurements were taken at 0 hours and after 4 hours. Dialysis was conducted for 2 days before freeze-drying the material. A thin film, 1.12 g, was obtained which underwent FTIR measurements.

3.4.1 Oxidation of nanocrystalline cellulose (14)

The nanocrystalline cellulose (CNC) used was already in solution so the dry weight content was determined to be 5.74 %. 20 g of the CNC was weighed up giving 1.148 g dry weight content. The remaining 19 gram was assumed to be 19 ml of water. 1 gram of $NaIO_4$ was dissolved in 6 ml of distilled water, which gave a 4% consistency solution. Both solutions were mixed and a sample for UV-VIS measurements was taken. pH was lowered to around 4 and the reaction was left for 4 hours in the dark. Too much stirring splashed mixture to the vessel walls so additional distilled water was added to remove mixture from the walls. UV-Vis was taken after 4 hours. After dialysis for 2 days and freeze-drying, a foam like material was obtained, 1.05 g. Part of the resulting material can be seen in figure 12. The material underwent FTIR measurements.

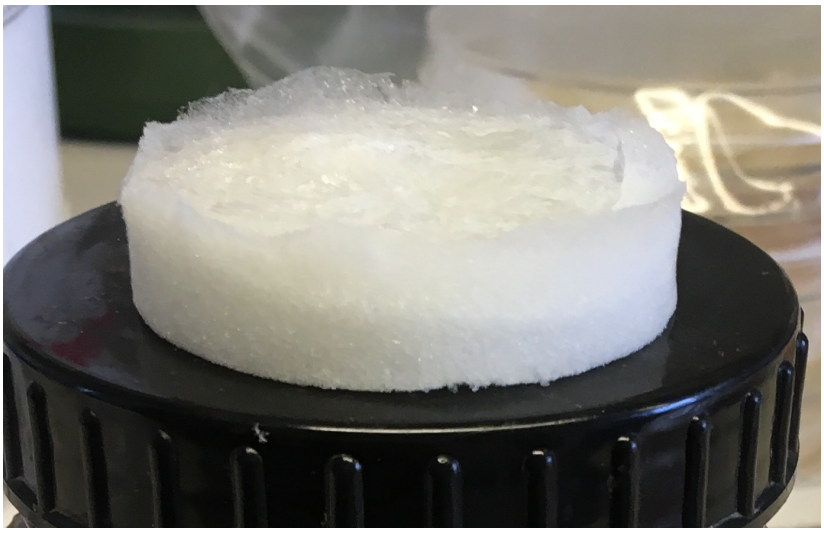


Figure 12. Oxidized CNC resulted in a foam like material.

4. Results and discussion

4.1 Reactions on model substrates

Protecting the primary alcohol was first performed on model substrates, compounds similar in structure to cellobiose, to get a better understanding of the reaction. The protection was successful on methyl-α-*D*-Glucopyranoside **1** using both reactions a and b. The kinetics of the reactions was not taken into consideration, rather the selectivity of the TBDMS-group. By looking at COSY-spectra and HSQC the primary alcohol could be seen protected.

Same attempt to protect the primary alcohol of cellobiose **(5)** and thereafter glucose **(3)** with TBDMS-Cl was conducted using both methods (a and b). Both syntheses were unsuccessful. This could be due to the fact in aqueous solutions the carbohydrates obtain a reducing end group. The methyl group makes it non-reducing and this could possibly affect the stereochemistry in the molecule and explain why it only works on the methylated carbohydrate. Experiments conducted under inert gas and dried- glassware and solvents were also conducted without giving a successful reaction.

4.2 Methylation of cellobiose

Therefore attempts to methylate the reducing end group of cellobiose were performed. Four different acetic mediums were used (d,e,f,g) and the reactions were first conducted at 80°C for 24h. Dowex and Amberlite took longer time to react, as observed by TLC. After 24h the samples were purified and NMR-analysis showed more methyl peaks than just α and β. More peaks could indicate that not only was the first hydroxyl group methylated but also one of the primary alcohols. Summary of the first results are seen in table 1.

Table 1. Results of the methylation of cellobiose. C6 refers to the primary alcohol on the reducing end. Each number equals the protons of the peak.

Catalyst	Solvent	Temp/time	α	β	C6	Yield
Dowex	MeOH	80°C/24h	3.04	2.16	5.10	39.29%
Amberlite	MeOH	80°C/24h	5.08	2.16	5.60	58.03%
H_2SO_4-Silica	MeOH	80°C/24h	2.80	1.92	3.49-C6’	44.89%
H_2SO_4	MeOH	80°C/24h	-	-	-	233.1%
Amberlite	MeOH	80°C/16h	3.37	2.27	-	27.7%

As seen in the table, running the reactions for 24h gave a third methyl peak, most likely one of the primary alcohols. According to literature the methyl group from α -Me-Cellobiose should have a shift at 3.40 ppm while β-Me-Cellobiose at 3.53 ppm. [12] The additional peak seen in table 1 had a shift below 3.40 ppm in the range of 3.3-3.35 ppm. To identify this peak, the same reaction (d) was conducted on methyl-α-*D*-glucopyranoside and methyl-β-*D*-glucopyranoside.

The resulting study showed methyl-α peak at 3.40 ppm and methyl-β at 3.55 ppm. Another methyl peak can be viewed at 3.3 ppm and 3.35 ppm. The shift differs at little bit on the methyl-α but the conclusion can be drawn that the methyl group on the primary alcohol has a lower shift than the α peak at 3.40 ppm.

Thus, the proposed structure of the Dowex and Amberlite reactions for 24h can be seen in figure 13.

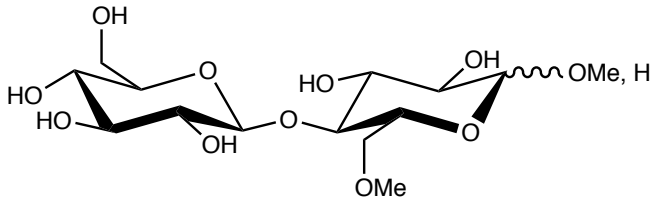


Figure 13. Proposed product from running the reaction with Dowex and Amberlite at 80°C for 24h at a small scale. This included both methylation of the reducing end and the closest primary alcohol.

The proposed structure was not found in the literature. The most similar one was methylation of the reducing end of maltose as well as the primary alcohol. According to the report the methylation of the reducing end had a shift at 3.55 ppm while the methyl group on the primary alcohol had a shift at 3.40 ppm. (Did not tell whether it was α or β) [13] Since maltose and cellobiose are two different molecules, the shifts could differ and therefore not have the exact same shifts.

When using sulphuric acid as the catalyst a yield of 233.1 % indicated impurities. Crystallisation using water was successful but NMR did not show any wanted products. Could have been residues from the work-up. The second attempt of the same reaction gave 0.5 gram of crude product. After purification, NMR analysis showed that the disaccharide had been cleaved. The reaction conditions could have been too acidic, instead the spectrum showed 4 methyl peaks, possibly resulting in two glucose units with both primary alcohols methylated. Using sulphuric-based materials as the acetic medium was excluded from further testing due to the difficulty to obtain certain pH. One single drop of the sulphuric acid changed the pH drastically when doing small-scale reactions.

The reaction with H_2SO_4-particles attached on silica gave both a methyl α peak at 3.42 ppm, β at 3.60 ppm and additional methyl peak at 3.76 ppm. The closest primary alcohol is less affected by steric hindrance so the possibility to methylate it is more likely. (The primary alcohol further away has an angle pointing inwards towards the second glucose unit, as seen in figure 9) Further testing is needed to identify the peak at 3.76 ppm.

To only obtain α - and β- methyl cellobiose the reaction time was reduced to 16h. When performing the reaction with Amberlite for 16h, showed a successful reaction of the methylated cellobiose. Only two methyl peaks were present, indicating α and β products. (Shifts at 3.40 ppm and 3.55 ppm, see appendix A1) The yield of the reaction was 27%, as seen in table 1.

The reaction gave low yield (27%, 200 mg) but enough starting material to continue with the next step of the reaction process. Previous methylation of maltose, under the same conditions, had a yield of 29.6%. [14] The bad yield of the reaction may be due to fact that cellobiose doesn't dissolve in methanol. Instead a cloudy, emulsion type solution appears and when agitation stops, a white powder sediments on the bottom. In order to increase the yield, small amount of additional solvents were added.

To get a more selective methylation of the reducing end of the disaccharide, water molecules may possibly protect the primary alcohols. Cellobiose has intra-molecular hydrogen bonds, which don't exist in an aqueous solution. Instead each hydroxyl group and specially the O-5 oxygen's coordinate with water. The water acts as a steric hindrance on the primary alcohols since the reducing end won't have any hydrogen bonds. [8]

Table 2. Additional amount of solvent was added to increase the yield of the α and β configuration. Numbers show amount of protons. These reactions were not purified.

Catalysis	Solvent	Temp/time	α	β	C6
Amberlite	MeOH/0.5 ml H_2O	80°C/8h	3.96	3.21	-
Amberlite	MeOH/1ml H_2O	80°C/8h	2.04	1.28	-
Dowex	MeOH/1ml H_2O	80°C/24h	3.04	0.50	-
Amberlite	MeOH/DMF	80°C/8h	3.05	2.36	9.62
Amberlite	MeOH/CH_3CN	rt/24h	-	-	-

Adding water increased the solubility of cellobiose, a much more clear solution was seen and less white powder sediments when stopping the stirring. As seen in table 2, NMR analysis showed increased yield of the α and β, except when adding DMF. DMF does not form hydrogen bonds so the primary alcohols are not protected. This may support the fact that the water molecules interact with the hydroxyl groups on the primary alcohols and hinder them from being methylated.

4.3 TBDMS-Me-Cellobiose (7)

Only one of the protection reactions were conducted on the methylated cellobiose, (reaction a), and it was successful with a yield of 30%. By looking at the NMR spectrum, both primary alcohols seem to have been protected. When purifying the crude product, difficulties in removing the DMF emerged. Small traces of the solvent remained in the mixture despite running it twice on the column. Since only one reaction was conducted, it's hard to tell whether there's a possibility on solely protecting one of the primary alcohols. Not many tests were performed due to low amount of the methylated cellobiose.

4.4 Results oxidation of cellulose

Sodium periodate ($NaIO_4$) is an oxidizing agent which can cleave the C2-C3 bond and turn their hydroxyl-groups into aldehydes. Looking at the consumption of $NaIO_4$ by UV-Vis light monitored the reactions. $NaIO_4$ is a light sensitive compound, which can otherwise degrade if being exposed to light. Both the reaction vessel and sample vials were covered with aluminium foil and the lights in the fume hood were turned off. UV-Vis measurements were measured directly after taking the sample, minimizing the risk of degradation. Figure 14 and 15 shows the results of the UV-Vis measurements.

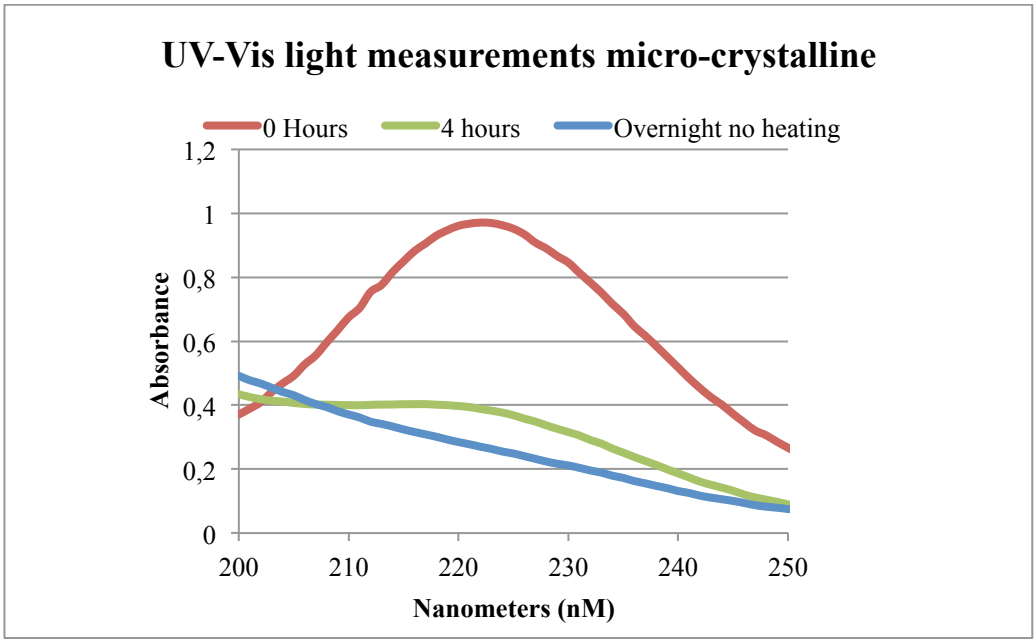


Figure 14. Consumption of $NaIO_4$. Between 0 and 4 hours the solution was heated at 45°C, while overnight it stayed in room temperature.

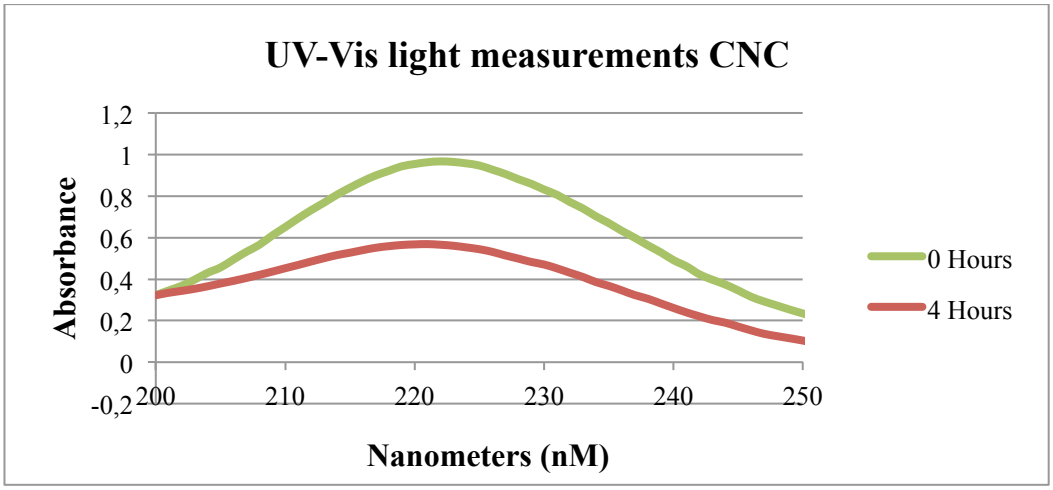


Figure 15. Consumption of $NaIO_4$ when oxidizing nano-crystalline cellulose.

The wavelength where $NaIO_4$ absorbs the light was measured at 220 nM. The figures clearly show a decrease in these regions meaning that the reactions have consumed the oxidizing agent. This doesn't necessarily mean that it has all reacted with the cellulose. Some may have been degraded when diluting the samples and also the acetic acid could have been oxidized. Table 3 summarizes the reaction conditions and the consumption of $NaIO_4$ in per cent.

Table 3. The amount $NaIO_4$ consumed was calculated by the difference between 0 hours and the last sample.

Cellulose	Oxidating Agent	Temp/time	pH	$NaIO_4$ consumed
CNC	$NaIO_4$	45°C/4h	4	58%
Micro	$NaIO_4$	45°C/4h	4	79%

4.4.1 FTIR results

The resulting oxidized materials underwent Fourier-transform infrared spectroscopy (FTIR) measurements, which were compared to non-modified. The non-modified microcrystalline was already in solid form while the CNC was dissolved in water. The dry weight content was determined to be 5.74%, so 20 g of CNC solution (1 g dry weight) was freeze-dried together with the modified CNC. To avoid loss of product when freeze-drying, plastic beakers were covered with aluminium foil with small holes at the top.

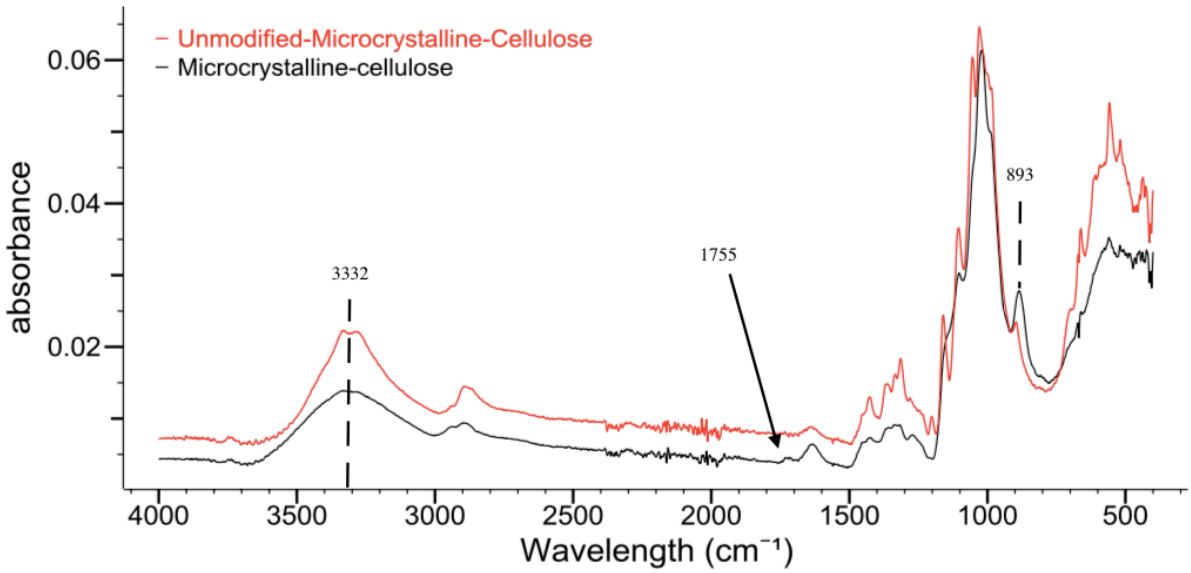


Figure 16. FTIR-spectrum showing divided plots between oxidized and non-modified microcrystalline cellulose. The black arrow points at small carbonyl peak at 1755 cm^{-1}

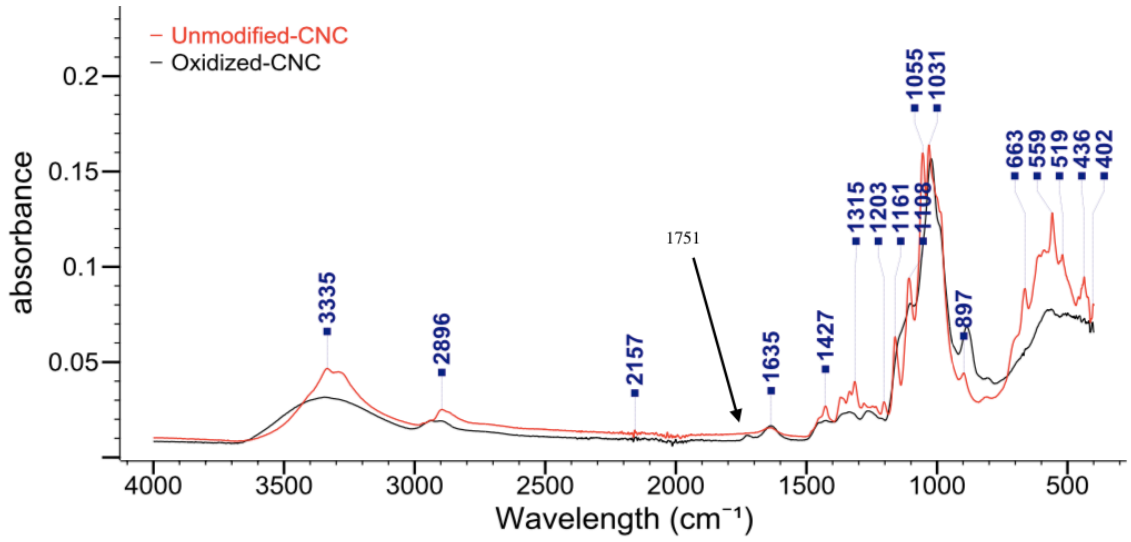


Figure 17. FTIR spectrum of CNC oxidation compared to non-modified CNC. The most significant peak can be seen by the black arrow showing the carbonyl peak at 1751cm^{-1}

Both FT-R spectra show the same results, meaning a successful oxidation of the hydroxyl-groups. At around 1750 cm^{-1} there are small peaks indicating absorption of the carbonyl groups. The low carbonyl peaks may be explained that only the surface of the crystalline cellulose been oxidized. In the hydroxyl-region at 3335 cm^{-1} a decrease is observed between modified and non-modified, indicating a consumption of the hydroxyl-groups into their corresponding aldehyde groups. At 890 cm^{-1} there's a much stronger hemiacetal vibration on the modified material, which would indicate decreased crystallinity. [15]

The decrease in crystallinity is due to the lowered pH, which makes it possible for the oxidizing agent to reach the hydroxyl groups at the surface. To understand how much of the crystallinity has decreased, an XRD analysis could be performed. Also to get a quantitative measure on how much of the aldehyde groups have formed, additional reactions could be performed, for example Cannizzario reaction, to monitor the reaction. [15]

The decrease in hydroxyl groups could be due the presence of water. But since the compounds were freeze-dried for 5 days and the amount of product was equal to the resulting product, 1 g of starting material gave 1 g of product, being water in the sample is rather unlikely.

5. Conclusion and future work

Protecting reactions conducted on cellobiose were not successful. The open ring, reducing end, may lower the reactivity of the molecule therefore the reducing end was closed using a methyl-group. At small-scale, only the reducing end was methylated at 80°C for 16h using amberlite as catalyst. Longer reaction time methylated the closest primary alcohol. (At 80°C for 24h using dowex). To avoid methylation of the primary alcohols and increase solubility of cellobiose, a small amount of water was added. The purification was quite easy in a small-scale reaction but would it be scaled-up, it might be more difficult to remove the water.

The aim was to solely protect one of the primary alcohols and was actually achieved by methylating one of the primary alcohols. Doing the reaction at a large scale (Dowex at 80°C for 24h) gave very low yield and further work would be optimizing the reaction conditions. Although this thesis presents a one-pot synthesis to both methylate the reducing end and one of the primary alcohols.

A TBDMS reaction was conducted on methyl-cellobiose, giving protection on both the primary alcohols. Further testing could be done if more starting material could be obtained, but at a first glance, the reaction appears not to be selective. It would makes sense since the only difference is one of the glucose units is turned 180°.

Regarding the oxidation, the crystalline celluloses were successfully oxidised. To only oxidise one of the primary alcohols, different conditions should be applied, since this method only cleaves the C2-C3 bond.

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Nicolas Axelsson Gothenburg June 2019

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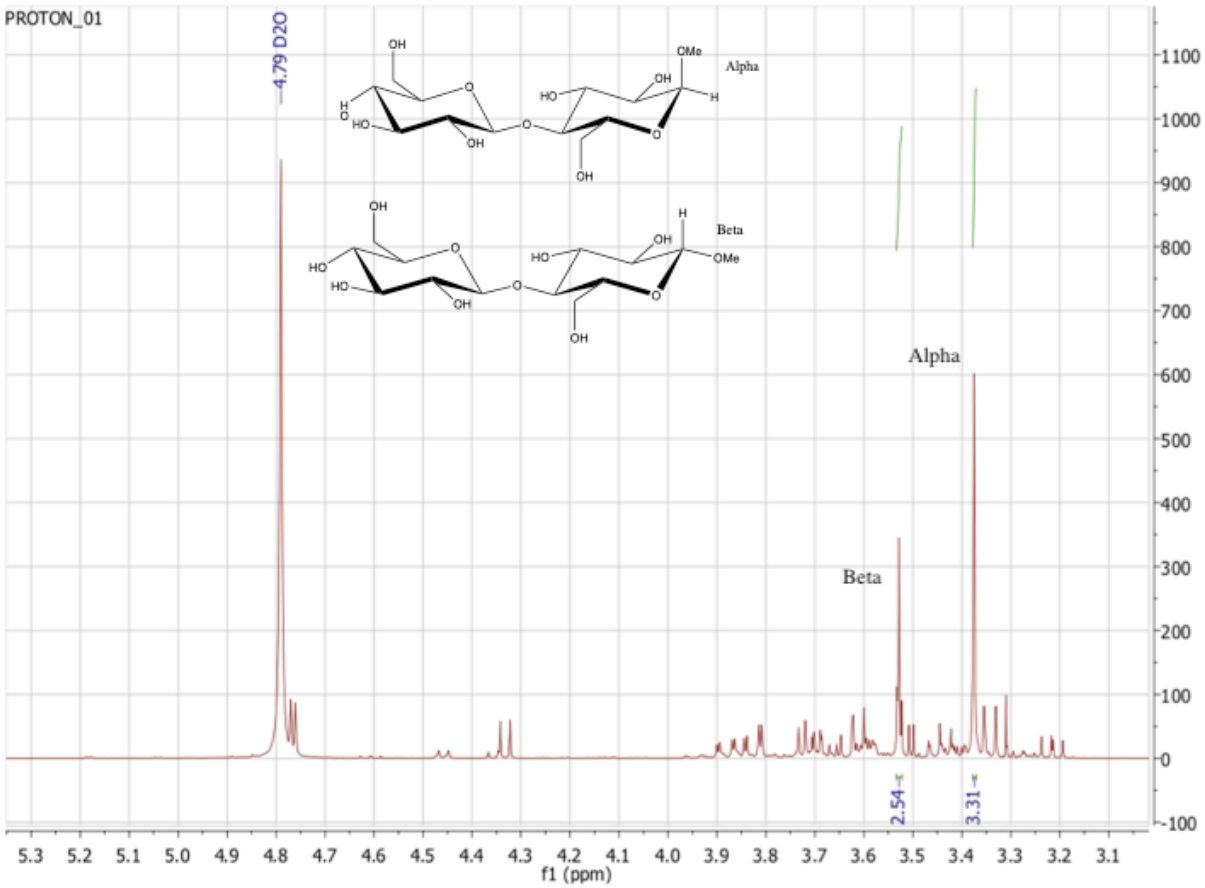
Appendix A

A.1 NMR spectrum Methyl-Cellobiose (6c,d)



Appendix A NMR-spectrum of Methyl-Cellobiose. β-methyl peak at 3.54 ppm and α-methyl peak at 3.38 ppm.