André Oliveira Sequeira

Licenciado em Bioquímica

Synthesis of precursors of the rare 3-*O*-methylmannose polysaccharides present in Nontuberculous Mycobacteria

Dissertação para obtenção do Grau de Mestre em

Química Bioorgânica

Orientadora: Rita Ventura, Dra., Instituto de Tecnologia Química e Biológica António Xavier

Co-orientadora: Teresa Barros, Prof. Dra., Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa

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Agradecimentos

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Abstract

3-*O*-methylmannose polysaccharides (MMPs) are cytoplasmic carbohydrates synthesized by mycobacteria, which play important intracellular roles, such as for example in metabolism regulation. An important way to confirm if the inhibition of the synthesis of these polysaccharides will critically affect the survival of mycobacteria is the study of the biosynthetic pathways from these molecules on these microorganisms.

The purpose of this work is the efficient synthesis of three saccharides, which are rare cellular precursors from the biosynthesis of the mycobacterial polysaccharides, allowing its study. In order to obtain these molecules, a chemical strategy to connect two precursors was used. This process is called chemical glycosylation and its importance will be highlighted as an important alternative to enzymatic glycosylation.

The first objective was the synthesis of the disaccharides Methyl (3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-α-D-mannopyranoside and (3-*O*-Methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-(α/β)-D-mannopyranose. The mannose precursors were prepared before the glycosylation reaction. The same mannosyl donor was used in the preparation of both molecules and its efficient synthesis was achieved using a 8 step synthetic route from D-mannose. A different mannosyl acceptor was used in the synthesis of each disaccharide and their syntheses were also efficient, the first one a 4 step synthetic route from α-methyl-D-mannose and the second one as an intermediate from the synthesis of the mannosyl donor. The stereoselective preparation of these disaccharides was performed successfully.

The second and last objective of the proposed work was the synthesis of the tetrasaccharide methyl (3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranoside. The disaccharide acceptor and donor to be linked through a stereoselective glycosidic reaction had to be first synthesized. Several synthetic strategies were studied. Neither the precursors nor the tetrasaccharide were synthesized, but a final promising synthetic route for its preparation has been proposed.

Keywords

MMPs

Saccharides

Precursors

Chemical glycosylation

Resumo

Os Polissacáridos de 3-O-metil-manose (PMMs) são açúcares citoplasmáticos sintetizados pelas micobactérias, que desempenham funções intracelulares importantes, como por exemplo na regulação do metabolismo. Uma maneira importante de confirmar se a inibição da síntese destes polissacáridos vai afectar criticamente a sobrevivência das micobactérias é o estudo das vias biossintéticas destas moléculas nestes microrganismos.

O objectivo deste trabalho é a síntese eficiente de três sacáridos, que são precursores celulares raros da biossíntese dos polissacáridos das micobactérias, permitindo o seu estudo. De forma a se obter estas moléculas, uma estratégia química para ligar dois precursores foi usada. Este processo é denominado de glicosilação química e a sua importância vai ser destacada como uma importante alternativa à glicosilação enzimática.

O primeiro objectivo foi a síntese dos dissacáridos Metil-(3-*O*-metil-α-D-manopiranosil)-(1→4)-3-*O*-metil-α-D-manopiranosídeo e (3-*O*-Metil-α-D-manopiranosil)-(1→4)-3-*O*-metil-(α/β)-D-manopiranose. Os precursores da manose foram preparados antes da reacção de glicosilação. O mesmo doador de manosil foi usado na preparação de ambas as moléculas e a sua síntese eficiente foi alcançada usando uma estratégia com 8 passos, a partir da D-manose. Um diferente aceitador manosil foi usado na preparação de cada dissacárido e as suas sínteses foram também eficientes, o primeiro foi obtido de uma estratégia de síntese de 4 passos a partir da α-metil-manose e o segundo como um intermediário da síntese do doador manosil. A preparação estereoselectiva destes dissacáridos foi realizada com sucesso.

O segundo e último objectivo do trabalho proposto foi a síntese do tetrassacárido Metil--3-*O*-metil-α-D-manopiranosil-(1→4)-3-*O*-metil-α-D-manopiranosil-(1→4)-3-*O*-metil-α-D--manopiranosil-(1→4)-3-*O*-metil-α-D-manopiranosídeo. Os dissacáridos aceitador e doador a serem ligados por uma ligação glicosídica estereoselectiva tinham de ser sintetizados primeiro. Algumas estratégias de síntese foram estudadas. Nem os percursores nem o tetrassacárido final foram sintetizados, mas uma estratégia de síntese promissora para a sua formação foi proposta.

Palavras-Chave

PMMs

Sacáridos

Precursores

Glicosilação química

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Abbreviations and Symbols

ABdd – AB doublet of doublets

Ac – Acetyl

Ar - Aromatics

ATR-FTIR – Attenuated Total Reflectance-Fourier Transform Infra-red Spectroscopy

br – Broad

Bn – Benzyl

Bu - Butyl

COSY – Correlation Spectroscopy

^{13}C-NMR - Carbon-13 nuclear magnetic resonance

d – Doublet

DBU- 1,8-Diazabicycloundec-7-ene

dd – Doublet of doublets

ddd - Doublet of doublet of doublets

DIPEA – N,N-Diisopropylethylamine

DMAP - 4-Dimethylaminopyridine

DMF – Dimethylformamide

HMQC - Heteronuclear Multiple-Quantum Correlation

^{1}H-NMR – Proton nuclear magnetic resonance

IR – Infra-Red

J – Coupling constant

Me – methyl

m – Multiplet

Nu - Nucleophile

Ph – Phenyl

rt – Room temperature

s – Singlet

TBDMSOTf - tert-Butyldimethylsilyl triflate

THF – Tetrahydrofuran

TLC – Thin Layer Chromatography

TMSOTf - Trimethylsilyl trifluoromethanesulfonate

t – Triplet

TrCl – Trityl chloride

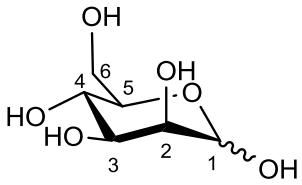
t-BuOK- Potassium tert-butoxide

UV – Ultraviolet

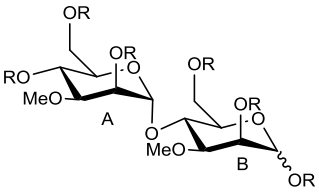
δ – Chemical shift

Δ - Reflux

Mannose carbon numeration:



Mannose disaccharide monomer identification:



CHAPTER 1

INTRODUCTION

1. Introduction

Organic synthesis is often connected with several biological fields because it allows the synthesis of several compounds, which can become potential drugs, or even assist the understanding of some cellular metabolic processes. Carbohydrates are present on most cells as glycoproteins, glycopeptides or polysaccharides, and they have important functions, such as cell-wall receptors during many biological processes. The objective of this work is the synthesis of three saccharides, which are cellular precursors for the biosynthesis of rare mycobacterial polysaccharides, 3-*O*-methyl-mannose polysaccharides (MMPs). The structures of these compounds are shown in Figure 1.1.

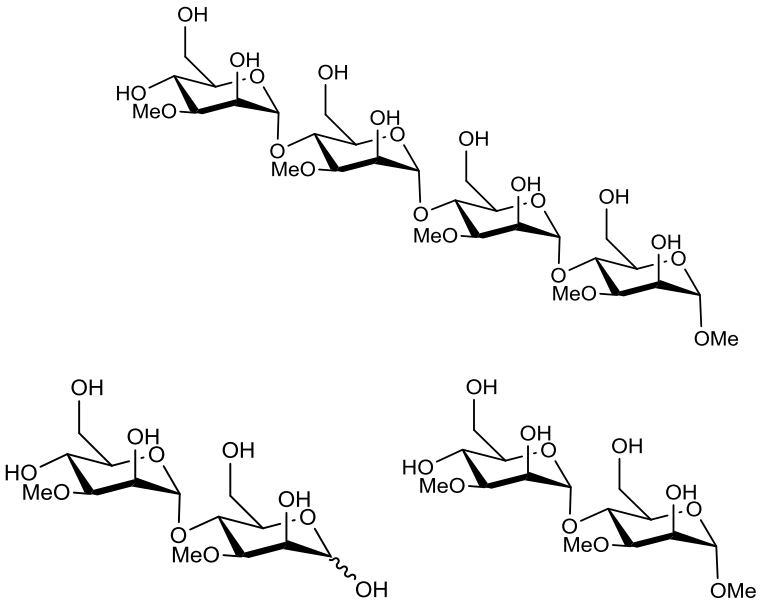


Figure 1.1 – The structure of the three MMP cellular precursors.

1.1 Location and biological function of MMPs

Mycobacterium is a genus of Actinobacteria, which includes pathogens known to cause serious diseases, including tuberculosis, leprosy, pulmonary disease resembling tuberculosis or lymphadenitis. Mycobacteria in general synthesize some types of cytoplasmic carbohydrates, polymethylpolysaccharides (PMPs), which play important roles in metabolism regulation, like for example lipid metabolism. Some of these microorganisms produce a class of PMPs, 3-*O*--methylmannose polysaccharides (MMPs). These are composed of 10-13 α-(1→4)-linked 3-*O*--methyl-D-mannoses.[1] The nonreducing end of these compounds is terminated by a single α-linked unmethylated D-mannose and the reducing end by an α-methyl aglycon.[1] The structures of MMPs are shown in Figure 1.2.

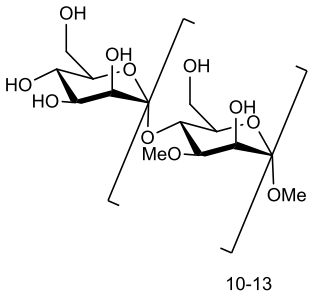


Figure 1.2 - The structure of mycobacterial MMPs.

In this work, these molecules have some particular interest, because of their important biological functions in mycobacteria. MMPs have been detected only in nontuberculous mycobacteria (NTM), like for example in *Mycobacterium smegmatis*.[1] These are the mycobacteria which can cause pulmonary disease resembling tuberculosis or lymphadenitis. One of the intracellular functions of MMPs is the formation of a stable 1:1 complex with long-chain fatty acids and acyl coenzyme A (acyl-CoA), because of the helically coiled conformation of this polysaccharide, which enables it to include the lipid in its interior in a specific orientation.[1] The 3-*O* methylation has an important role in the stabilization of the helical conformation of this polysaccharide and also enhances the direct interaction with lipids and acyl-CoA, because methyl groups are hydrophobic.[1] The formation of these stable complexes, allows MMPs to be used as intracellular lipid carriers, regulators of the fatty acid synthesis, because they can activate or inhibit the fatty acid synthetase complex (FAS-I), and regulators of the length of the fatty acid chain, due to the fact that they can also facilitate the release of the neo-synthesized fatty-acid chains from the FAS-I, terminating their elongation.[1][2]

Recent studies revealed that these molecules synthesized by these species of mycobacteria have a significant role as a potential antigen or target for new vaccines and drugs used in tuberculosis disease treatment and diagnosis.[1] Besides that, the synthesis of saccharide precursors that are MMP intermediates allows the study of their biosynthetic pathways on these microorganisms, because these molecules are going to be important in enzyme identification, characterization and functional validation. After studying this pathway it will be confirmed if the inhibition of the synthesis of MMP from *M. smegmatis* will critically affect the survival of these microorganisms.

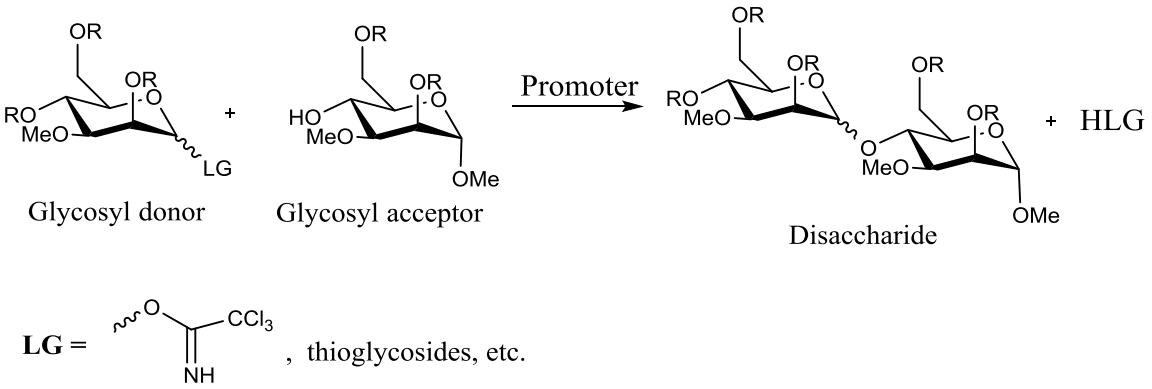
1.2 Chemical synthesis of mannose oligosaccharides

For the synthesis of any poly- or oligosaccharide, it is initially necessary to create the bond between two precursors (monosaccharides) in a process that is called glycosylation reaction (Scheme 1.1). After the formation of this disaccharide the compound may react again

with other saccharides, in the same process, making an oligosaccharide or a polysaccharide, depending on the number of monomers that the final molecule has.

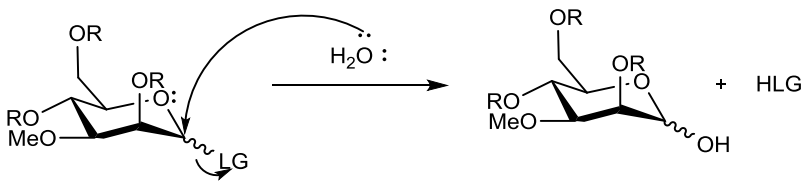
At the end of the nineteenth century, Emil Fischer and other chemists showed that the formation of this glycosidic bond could be done by a chemical process. However, these scientists also verified the complexity of the glycosylation reaction. After these first attempts, there was a huge development in the study of this chemical process, but only in the last twenty years the scientific community had reached a major advance of the methods used for this reaction.[3] The development of new strategies has not only allowed the access to novel types of glycosidic linkages but also led to the discovery of efficient strategies for the synthesis of several oligosaccharides and polysaccharides.

This important work allowed to understand that some crucial factors must be considered. The two precursors for this reaction must have special characteristics so that this reaction can actually happen. One of these precursors, the glycosyl donor, must have in its anomeric carbon a leaving group (LG). The second one, the glycosyl acceptor, must possess a free hydroxyl group so that he can react with the anomeric carbon of the donor, just like a nucleophile (Scheme 1.1).



Scheme 1.1 - The chemical glycosylation reaction between two monosaccharides.

This initial process will enable the growth of the oligosaccharide (or the polysaccharide).[4] There are some limitations in this kind of reaction. The glycosylation has to be, for example, carried out in anhydrous conditions, because of the formation of by-products that result from the hydrolysis of the glycosyl donor in the presence of water (Scheme 1.2).



Scheme 1.2 – Hydrolysis of the glycosyl donor.

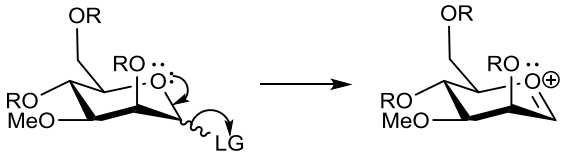
Besides the necessity of anhydrous conditions during the reaction (assured by adding molecular sieves to the reaction, performing it under an inert environment and using dry solvents) it is important to consider other factors such as:

- Regioselectivity, because only one hydroxyl group of the acceptor precursor has to react with the anomeric carbon of the donor;
- Stereoselectivity, because the product that is formed must be predominantly α or β;
- Efficiency, because alcohols are not good nucleophiles, so, many strategies are taken to improve the yield of the reaction, like for example a good leaving group at the donor.[4]

1.2.1 Mechanism

1.2.1.1 SN_1 reaction

Chemical glycosylation is a substitution reaction, because the acceptor, which has a free hydroxyl group, reacts, as a nucleophile, with the anomeric carbon of the donor, affording a glycosidic bond.[4] This reaction follows very often a unimolecular mechanism (SN_1),[4] mostly because sugar acceptors are very weak nucleophiles and the fact that the oxygen linked to the anomeric carbon has two non-bonding electron pairs that facilitate the departure of the LG, which is a very good leaving group [4]:



Scheme 1.3 – Departure of the leaving group and formation of the oxonium ion.

This interaction can be described as a $\mathbf{n} \rightarrow \boldsymbol{\sigma}^*$ donation. After the formation of this oxonium ion, the nucleophile can react with this intermediate. However, this nucleophilic attack can be made in two ways, giving two different products, β and α:

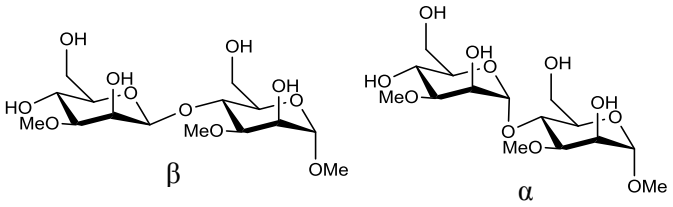


Figure 1.3 - Two different glycosylation products, the α- and the β-O-glycoside.

The formation of this oxonium ion, and the use of this very good LG is crucial for the efficiency of the glycosylation reaction, because, in most cases, it allows a unimolecular substitution reaction (SN_1), so, the anomeric carbon becomes more electrodeficient and more capable to be attacked even by a weak nucleophile, like the acceptor.[3] So, leaving groups are very important in the chemical glycosylation because most donors are too stable to undergo spontaneous glycosylation. Depending on the kind of glycosyl donor and final product, there are several types of leaving groups, such as halides, trichloroacetimidates, thioglycosides, acetates, phosphites, etc.[4] However, most leaving groups first have to be activated, before their departure from the molecule, during the glycosylation reaction. Promoters (activators) are used to form an activated species with the LG and that will eventually lead to its departure.[5]

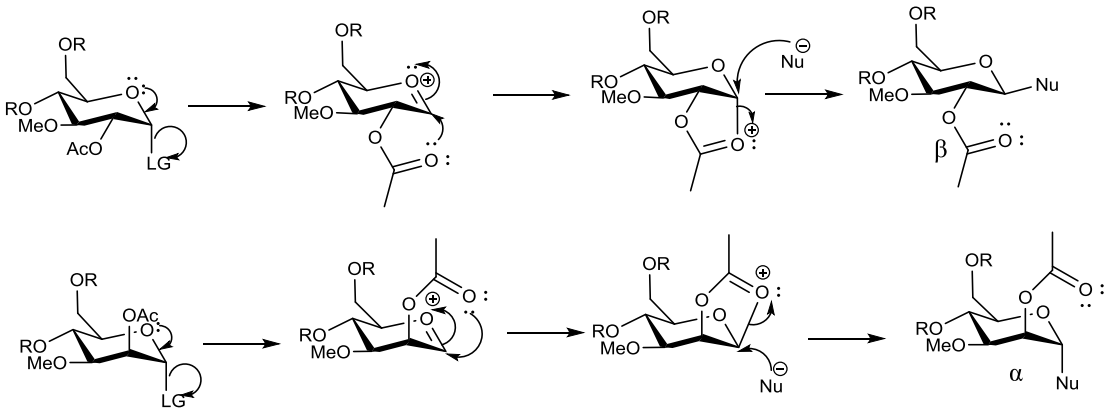
Other factors can increase the stereoselectivity of the product, such as the solvent, the protecting groups at 2-OH and other positions.

1.2.1.2 Protecting groups

In carbohydrate chemistry protecting groups like allyl ether, silyl ethers (TBDMS or TBDPS), acetals, benzyl ethers or the acetyl group, are used for the protection of sugar hydroxyls, allowing a regioselective reaction, since only one hydroxyl group of the acceptor is free to react with the anomeric carbon of the mannosyl donor. Besides that, one of the powerful strategies used to positively influence the stereoselectivity outcome of the reaction, is also the use of those protecting groups, like an ester or ether, at the neighbouring group (C-2 carbon).

Acetyl

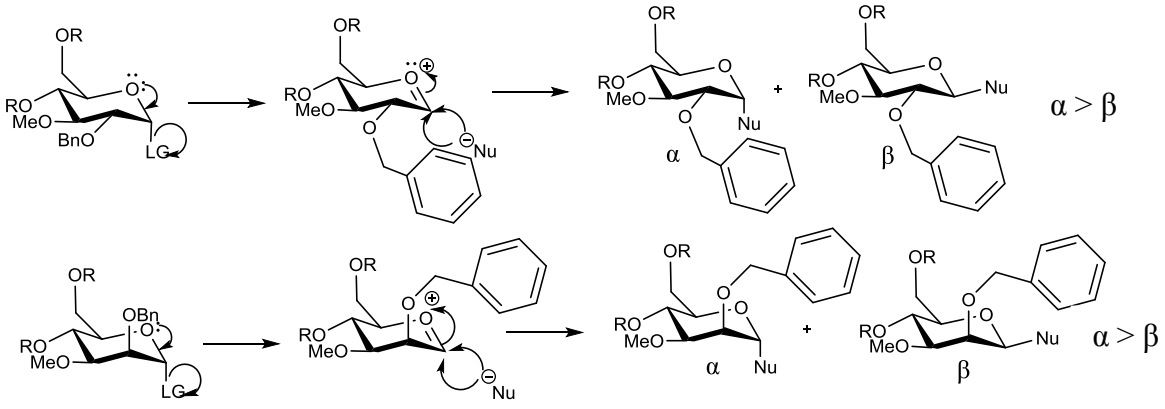
Acetyl is a very common protecting group used in carbohydrate chemistry. The existence of an acetyl protecting group at the 2-OH allows the nucleophilic attack on only one side of the molecule, because of the formation of an intermediate, the acyloxonium ion, that results from the attack of the acetate carbonyl oxygen to the anomeric carbon (neighbouring group participation). This cyclic oxonium ion can be opened by a bimolecular nucleophilic substitution (SN_2) reaction by the reacting nucleophile.[3,4] The new bond formed is also *trans* compared with the 2-OH (Scheme 1.4). With the acetyl protection at 2-OH, the 1,2-*trans* stereoselectivity is strongly favored.



Scheme 1.4 – Acetyl protection at 2-OH. Neighbouring group participation due to the formation of the acyloxonium ion – formation of 1,2-*trans* glycosides.

Benzyl ether

Benzyl ether is also used as a protecting group at 2-OH, but since there is not any neighbouring group participation, a mixture of anomers, which result from the nucleophilic attack on both sides of the molecule, are formed (Scheme 1.5). However, there is a slight stereochemical outcome for glycosyl donors with this nonparticipating group at 2-OH, due to the existence of an anomeric effect, which favors the α-product.[3] Even so, the fact that the glycosylation is irreversible, makes the role of the anomeric effect diminished.[3] Because of that, benzyl ether is often used as a neighbouring group in the chemical formation of β-mannosides, for example, but in this case there are other factors which influence the stereochemistry of the final product, like for example the solvent.[3]



Scheme 1.5 - Benzyl ether protection at 2-OH and neighbouring group non-participation.

Comparing to the effect of acetyl protection at 2-OH, the appearance of stereoselectivity is obviously less favored, because of the absence of a participating group.

1.2.1.3 Solvent effect

In a glycosylation reaction the solvent is another important factor which influences the stereoselectivity at the anomeric center of the final molecule. The use of polar solvents increases the formation rate of β-glycosides. Non-polar solvents, such as dichloromethane or toluene, are used in the synthesis of α-glycosides.[4]

This work will also highlight the importance of chemical glycosylation, which in this case can be an important alternative to enzymatic glycosylation, since the first one can solve many problems which enzymatic glycosylation cannot.

CHAPTER 2

RESULTS AND DISCUSSION

2. Results and discussion

As it was said before, the objective of this work is an efficient synthesis of three rare cellular precursors, which are saccharides used in the biosynthesis of rare mycobacterial polysaccharides - MMPs. Since the commercially available compounds are monosacharides, in order to obtain the desired products with good yields, some crucial factors on the glycosylation reaction must be considered, such as its regioselectivity, efficiency and stereoselectivity. Besides that, the desired compounds have to be methylated in specific positions, so the strategy of the synthesis also has to include good regioselective methylation steps.

2.1 Disaccharide synthesis

Two of the proposed objectives was the synthesis of the disaccharides methyl (3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-α-D-mannopyranoside and (3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-(α/β)-D-mannopyranose (Figure 2.1).

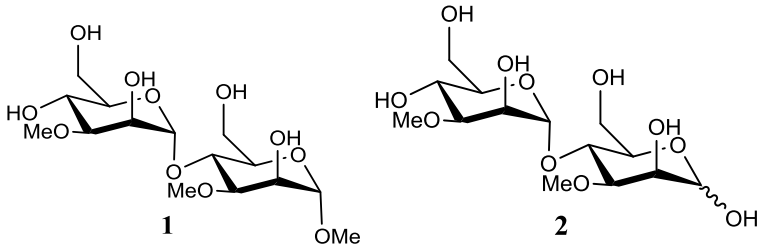


Figure 2.1 - The structure of methyl (3-O-methyl-α-D-mannopyranosyl)-(1→4)-3-O-methyl-α-D-mannopyranoside **1** and (3-O-methyl-α-D-mannopyranosyl)-(1→4)-3-O-methyl-(α/β)-D-mannopyranose **2**.

In order to fulfill the conditions mentioned above, the mannose precursors need to be prepared for the glycosylation reaction. To accomplish that, the synthetic strategies for the preparation of the mannosyl donor and acceptor were drawn.

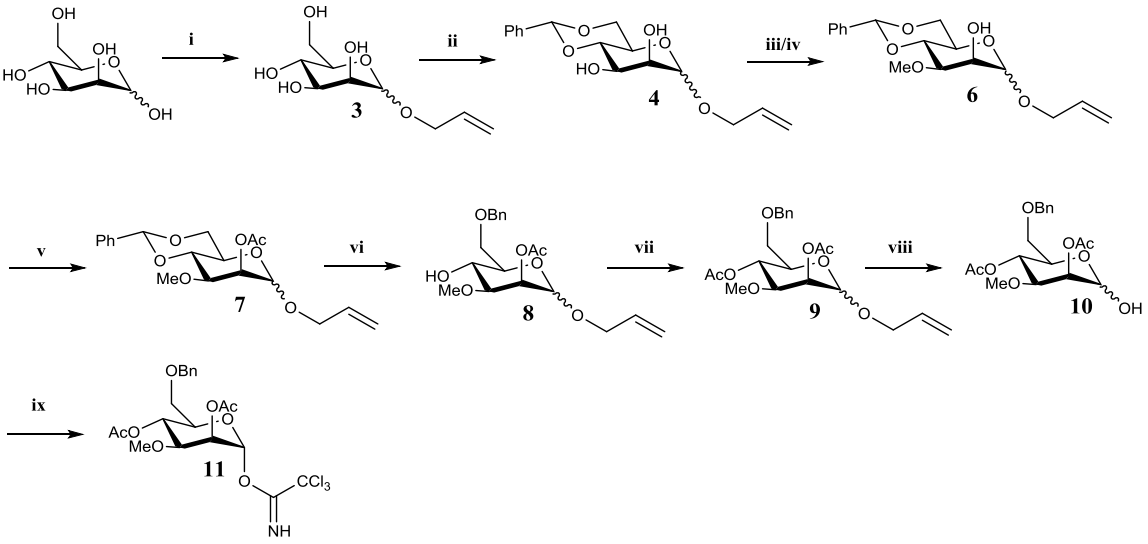
2.1.1 Monosaccharide glycosyl donor synthesis

D-mannose was used as starting material for the formation of the glycosyl donor. During this synthesis, the configuration of the anomeric carbon is not important. Only after the formation of the disaccharide, the formed glycosidic bond must have the right anomeric configuration.

An efficient synthetic pathway for the synthesis of a 3-*O*-methyl mannose glycosyl donor has been reported.[2] Benzyl ether was used as protecting group at 2-OH. However, as it was said before, an acetyl protecting group at 2-OH offers better stereochemical results on the glycosylation reaction. Besides that, this pathway includes an efficient 3-*O*-methylation step,

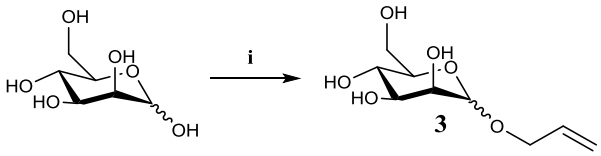
which facilitates one of the challenges mentioned above. In conclusion, a synthetic strategy based on the reported work [2] was drawn with some additional changes.

The proposed synthetic route is show below in Scheme 2.1.



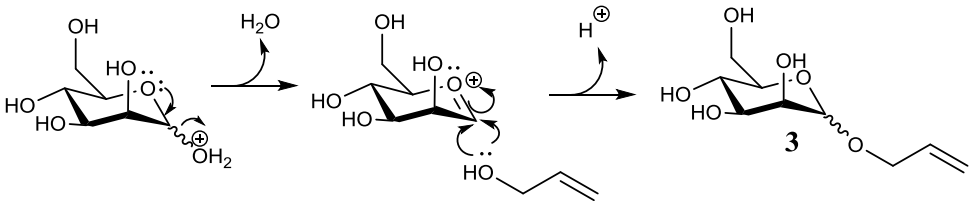
Scheme 2.1 – Synthetic strategy followed for the synthesis of the glycosyl donor **11**. Reagents and conditions: **i)** allylic alcohol, camphorsulfonic acid, Δ, overnight, 94%; **ii)** benzaldehyde dimethyl acetal, camphorsulfonic acid, THF, Δ, 4 hours: 30 minutes, 59 %; **iii)** dibutyltin oxide, methanol, Δ, 3 hours and **iv)** iodomethane, DMF, 50 ºC, overnight, 2 steps: 80 %; **v)** acetic anhydride, DMAP, 0ºC → rt, pyridine 2 hours; 91 %; **vi)**, sodium cyanoborohydride, hydrogen chloride in diethyl ether 1 M, THF, 0ºC, 81 %; **vii)** acetic anhydride, DMAP, pyridine, 0ºC → rt, 1 hour: 30 minutes; 88 %; **viii)** palladium (II) chloride, methanol, rt, 2 hours; 75 %; **ix)** DBU and trichloroacetonitrile, dichloromethane, 0ºC, 10 minutes, 76 %.

2.1.1.1 Allyl (α/β)-D-mannopyranoside 3 synthesis



Scheme 2.2 - Synthesis of allyl (α/β)-D-mannopyranoside **3** with **i)** allylic alcohol, camphorsulfonic acid, Δ, overnight, 94%.

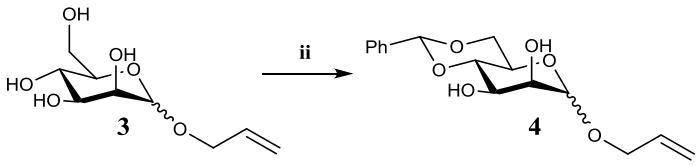
Liao et al synthetic route[2] uses allyl α-D-mannopyranoside as starting material, which is an expensive reagent. However, since a reported procedure [6] of D-mannose 1-*O*-allylation using allylic alcohol (as reagent and solvent) and camphorsulfonic acid as acid catalyst, under reflux, gives very good yields, it is not necessary to use allyl α-D-mannopyranoside as starting material. The reason why this allylation is regioselective is because camphorsulfonic acid, as a source of protons, catalyses the formation of the oxonium ion. After that, the allylic alcohol will attack the anomeric carbon on both sides of the molecule (Scheme 2.3).



Scheme 2.3 - Mechanism for the synthesis of allyl (α/β)-D-mannopyranoside **3.**

This reaction follows a unimolecular mechanism (SN_1), due to the fact that the acid catalyst and the oxygen linked to the anomeric carbon facilitate the departure of the leaving group. The resulting product is not only the allyl α-D-mannopyranoside, due to the nucleophilic attack on both sides of the molecule, which also leads to the formation of the allyl β-D-mannopyranoside. As it was said before, the configuration of the anomeric carbon will not be important during the synthesis of the glycosyl donor. The allyl ether as protecting group has been frequently used in carbohydrate research, mostly because it has great advantages in comparison with other protecting groups, such as the fact that it is a very stable group. Unfortunately the same advantages can sometimes bring disadvantages, as it will be seen further in this work. Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 94 %.

2.1.1.2 Allyl 4,6-*O*-benzylidene-(α/β)-D-mannopyranoside 4 synthesis

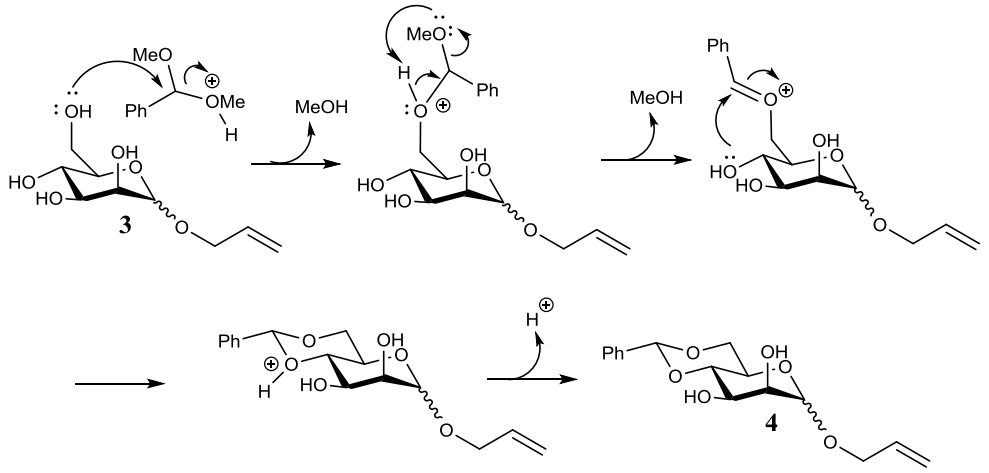


Scheme 2.4 - Synthesis of allyl 4,6-*O*-benzylidene-(α/β)-D-mannopyranoside **4** with **ii)** benzaldehyde dimethyl acetal, camphorsulfonic acid, THF, Δ, 4 hours: 30 minutes, 59 %.

The synthesis of **4** consists in a regioselective formation of a 4,6-*O* benzylidene acetal, catalyzed by camphorsulfonic acid, using benzaldehyde dimethyl acetal as reagent and THF as solvent, all stirred under reflux. The reason why this cyclic diol protection is 4,6-*O* regioselective, is due to the thermodynamic control on the reaction, which favors the formation of a six-membered benzylidene acetal ring, a very stable product.[7] The phenyl group is oriented in an equatorial orientation.

In this acid-catalysed acetalation, camphorsulfonic acid is used as the catalyst and it activates benzaldehyde dimethyl acetal.[8] The protonated methoxy group of the reagent can be displaced by the sugar primary hydroxyl group and gives a mixed acetal. The protonation of the second methoxy group and its further displacement gives the formation of an oxocarbenium ion. The second hydroxyl group from the molecule reacts with this ion, and gives the protonated

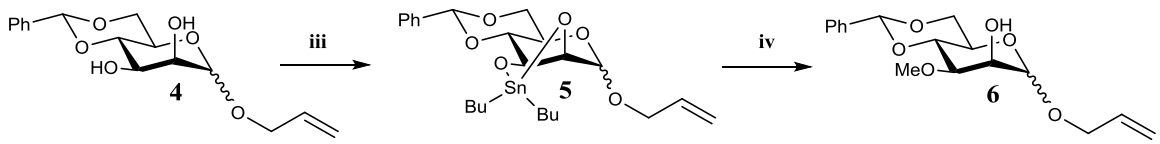
acetal, which after deprotonation (the catalyst is regenerated) results in the cyclic acetal (Scheme 2.5).



Scheme 2.5 - Mechanism for the synthesis of allyl 4,6-*O*-benzylidene-(α/β)-D-mannopyranoside **4**.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 59%. The use of benzylidene acetal as a protecting group in this synthetic strategy is important, because it has some advantages, such as the fact that it can be introduced in the molecule under acidic conditions, it protects the compound in the 4 and 6 positions, even with the rest of the positions available to be protected, and it can be regioselectively opened, as it will be seen further in this work.

2.1.1.3 Allyl 4,6-*O*-benzylidene-3-*O*-methyl-(α/β)-D-mannopyranoside 6 synthesis



Scheme 2.6 - Synthesis of allyl 4,6-O-benzylidene-3-O-methyl-(α/β)-D-mannopyranoside **6** with **iii)** dibutyltin oxide, methanol, Δ, 3 hours and **iv)** iodomethane, DMF, 50 ºC, overnight; 2 steps: 80 %.

The synthesis of **6** consists in a regioselective 3-*O* methylation. A two step described procedure[9] was applied to **4**, using more quantity of dibutyltin oxide. The reason why this reaction is 3-*O* regioselective is because of the dibutyltin oxide, which reacts with the mannopyranoside, under reflux in methanol, and forms the cyclic 2,3-*O*-di-butylstannylene intermediate **5**, shown in Figure 2.2.

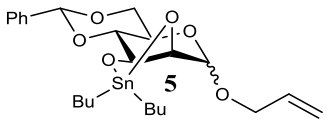
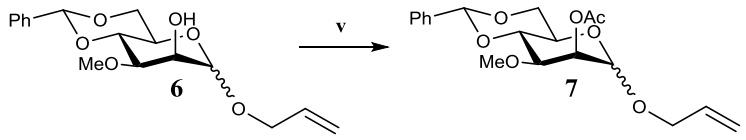


Figure 2.2 - Structure of allyl 4,6-*O*-benzylidene-2,3-*O*-dibutylstannylene-(α/β)-D-mannopyranoside **5**.

After the formation of the intermediate, iodomethane is added, in DMF at 50 ºC. The reagent is going to selectively methylate the equatorial hydroxyl group.[10] Despite some hypothesis,[11] it is not clear why reactions using organotin derivatives are regioselective, such as the mechanism. Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 80 %. *Hsu et al*[9] described procedure was efficient and one of the challenges of the synthesis was achieved.

The reason why the synthetic route for the synthesis of the glycosyl donor did not start with the methylation of **3**, as it was reported by *Liao et al*[2], is going to be explained further in this work.

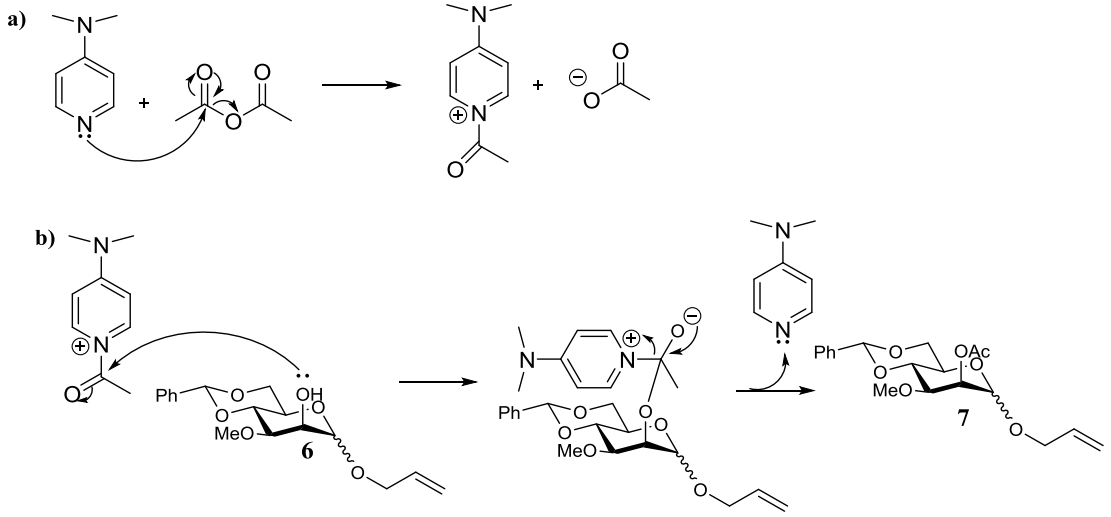
2.1.1.4 Allyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-(α/β)-D-mannopyranoside 7 synthesis



Scheme 2.7– Synthesis of allyl 2-O-acetyl-4,6-O-benzylidene-3-O-methyl-(α/β)-D-mannopyranoside **7** with **v)** acetic anhydride, DMAP, 0ºC → rt, pyridine 2 hours; 91 %.

The synthesis of **7** consists in an acetyl protection of 2-OH, using acetic anhydride as reagent, DMAP as catalyst, and pyridine as solvent.

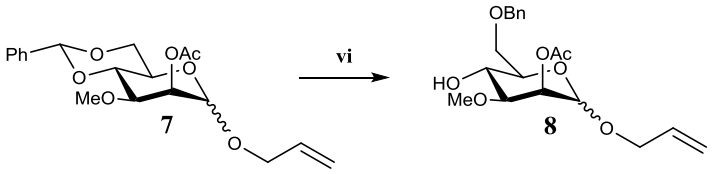
DMAP first reacts with acetic anhydride, and forms an acylpyridinium cation (Scheme 2.8**a**). The free hydroxyl from the sugar then reacts with the acylated catalyst to form the ester product[12] (Scheme 2.8**b**). Pyridine will neutralize the acetic acid formed.



Scheme 2.8 - **a)** Mechanism for the formation of the acylpyridinium cation; **b)** Mechanism for the synthesis of allyl 2-O-acetyl-4,6-O-benzylidene-3-O-methyl-(α/β)-D-mannopyranoside **7**.

This important step is going to influence, as it was said before, the stereoselectivity of the glycosylation reaction. Fortunately, the acetyl group can be introduced and removed in the molecule very easily, which justifies the fact that it is one of the most important protecting groups used in carbohydrate chemistry. It was important to proceed to this acetylation step with the molecule containing only one free hydroxyl, because it is not a regioselective reaction. Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 88 %.

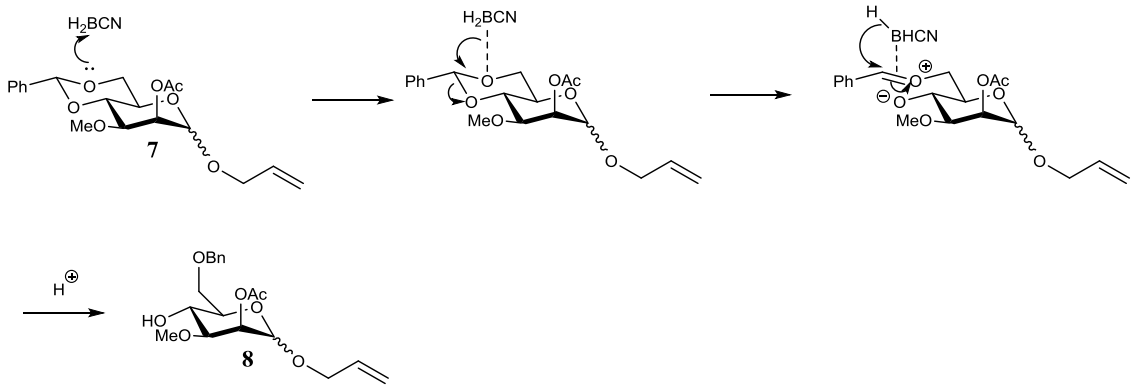
2.1.1.5 Allyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside 8 synthesis



Scheme 2.9 – Synthesis of allyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside **8** with **vi)**, sodium cyanoborohydride, hydrogen chloride in diethyl ether 1 M, THF, 0ºC, 81 %.

Benzylidene acetal can be either removed from the molecule, by for example acidic hydrolysis, or opened regioselectively using different methods.[13] The synthesis of **8** consists in a regioselective reductive opening of the benzylidene acetal from **7**. A described procedure [2] was applied to the compound, with some changes. **7** and sodium cyanoborohydride were dissolved in THF, and hydrogen chloride in diethyl ether was added portionwise, at 0ºC, until the reaction was finished.

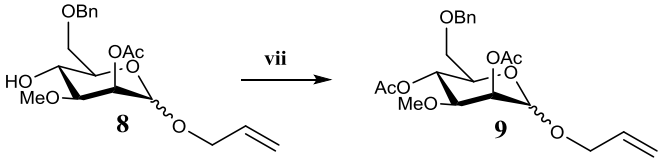
Hydrogen chloride acts in this reaction as a Brønsted acid and reacts with cyanoborohydride, to give H_2BCN and H_2. The formed borane, activated by the acid, is electrophilic enough to form an initial complex with the most electron rich oxygen of the acetal (6-O). The reason why the solution was added portionwise is because the acid must not be added in excess, as it can provoke the degradation of the molecule. This reaction proceeds through an oxocarbenium ion, which is reduced by the borane to give the pretended compound, following the proposed mechanism[13] (Scheme 2.10).



Scheme 2.10 – Mechanism for the synthesis of allyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside **8.**

The reason why this reaction has to use a large excess of sodium cianoborohydride (12 equivalents) is unknown. An experiment using less quantity of this compound (6 equivalents) was performed and afforded the expected product but with a lower yield (60 %). However, using the other conditions (12 equivalents), interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 81 %.

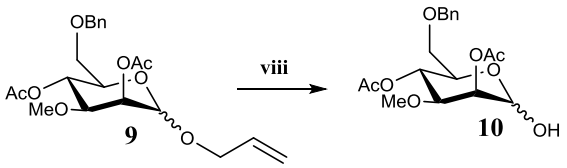
2.1.1.6 Allyl 2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside 9 synthesis



Scheme 2.11 – Synthesis of allyl 2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside **9** with **vii)** acetic anhydride, DMAP, pyridine, 0ºC → rt, 1 hour: 30 minutes; 88 %.

The synthesis of **9** consists in an acetylation of the 4-OH, using acetic anhydride, DMAP and pyridine. Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 88 %. It is very important to have all the hydroxyls protected, and the reason will be seen in the next steps of the synthetic route.

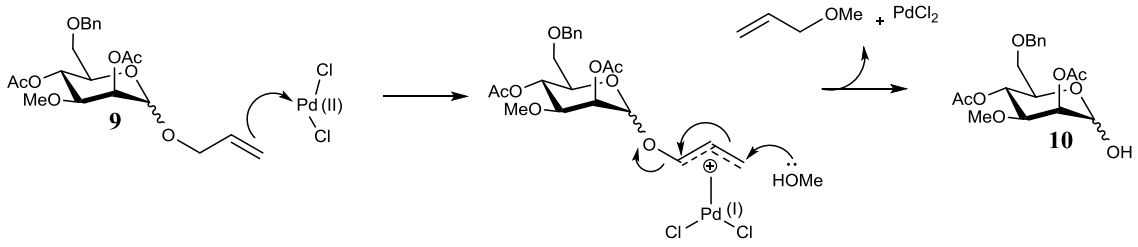
2.1.1.7 2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose 10 synthesis



Scheme 2.12 - Synthesis of 2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose **10** with **viii)** palladium (II) chloride, methanol, rt, 2 hours; 75 %.

The allyl ether has been frequently used as protecting group in carbohydrate research. In this synthetic strategy, it is used in the protection of the anomeric hydroxyl, because it is a very stable group, and it is only removed in certain conditions. The synthesis of **10** consists in the deallylation of **9**, using a described procedure [2] with palladium (II) chloride as catalyst, and methanol as reagent and solvent.

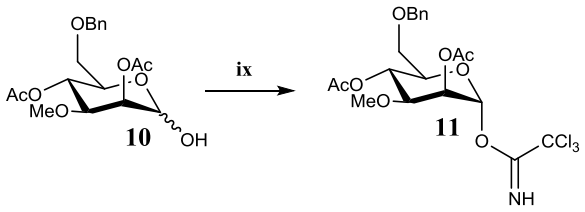
Palladium (II) chloride is the electrophile and reacts with the olefin from the allyl very easily, forming a complex. Then, methanol acts like a nucleophile, and attacks the olefin, provoking the departure of the leaving group, which in this case is the sugar itself. The proton from methanol is released and protonates the anomeric hydroxyl. Allyl methyl ether is formed after decomplexation and palladium (II) chloride is regenerated (Scheme 2.13).



Scheme 2.13 - Mechanism for the synthesis of 2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose **10**.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 75 %. In this case, the use of allyl ether protecting only one hydroxyl allows its selective removal without affecting the other protecting groups, which will be important in the next step.

2.1.1.8 (2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate 11 synthesis



Scheme 2.14 – Synthesis of (2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate **11** with **ix)** DBU and trichloroacetonitrile, dichloromethane, 0ºC, 10 minutes, 76 %.

The synthesis of **11**, consists in the conversion of the anomeric hydroxyl group into a trichloroacetimidate using DBU as catalyst and trichloroacetonitrile as reagent, added sequentially and dichloromethane as solvent, all stirred at 0ºC. As it was said before, besides the fact that the glycosyl donor must contain all the hydroxyls protected, the anomeric carbon needs a leaving group. The use of the trichloroacetimidate group as LG in carbohydrate chemistry was first developed by R. R. Schmidt,[5][14] and since then it has been often used.

In this base catalysed reaction, DBU first deprotonates the anomeric hydroxy group, which becomes more nucleophilic and attacks more easily the triple bond system present in the electron deficient trichloroacetonitrile. Then, DBU is regenerated, because it is deprotonated, to give the proton to the leaving group. The reason why the final product is only the α anomer, is due to the anomeric effect (thermodynamically the α anomer is more stable). In this way, the anomeric oxygen atom has been transformed into a good leaving group.

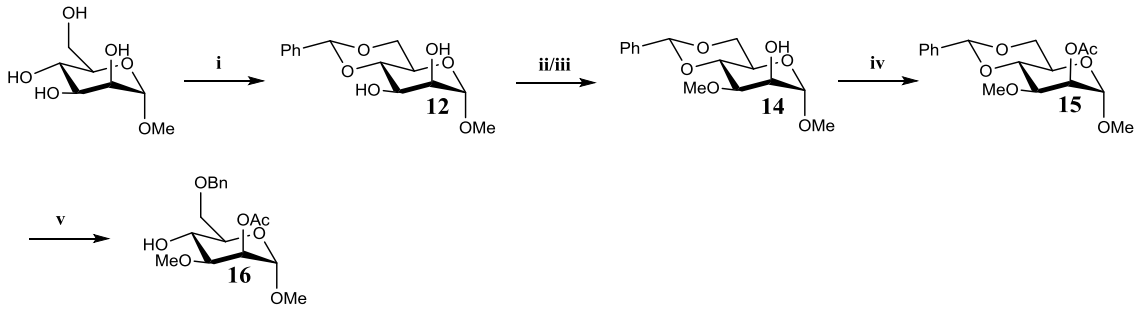
Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 76 % and $[\alpha]_D^{20}$ +38.8 (c 0.95, CH_2Cl_2). This synthesis was successful. This glycosyl donor can be used in the synthesis of both disaccharides.

2.1.2 Monosaccharide glycosyl acceptors synthesis

For the formation of the disaccharides **1** and **2** two different glycosyl acceptors were needed.

α-methyl-D-mannose was used as starting material for the preparation of the first glycosyl acceptor. An efficient synthetic route for the synthesis of a 3-*O* methyl-mannose glycosyl acceptor has been reported also by *Liao et al*[2]. Once again, benzyl ether was used as the protecting group at 2-OH. In this case, the acetyl group was chosen as protecting group at 2-OH not because it could influence the stereochemistry of the disaccharide but because of the

fact that the conditions used above for the acetylation gave very good yields. A synthetic strategy based on the reported work [2] was drawn with some additional changes. The proposed synthetic route is shown below in Scheme 2.15.

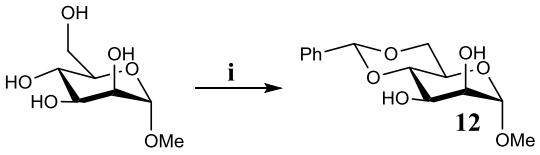


Scheme 2.15 – Synthetic strategy followed for the synthesis of the glycosyl acceptor **16**. Reagents and conditions: **i)** benzaldehyde dimethyl acetal, camphorsulfonic acid, THF, Δ, overnight, 50 %; **ii)** dibutyltin oxide, methanol, Δ, overnight and **iii)** iodomethane, DMF, 65 ºC, overnight, 2 steps: 50%; **iv)** acetic anhydride, DMAP, 0ºC → rt, pyridine 2 hours; 97%; **v)**, sodium cyanoborohydride, hydrogen chloride in diethyl ether 1 M, THF, 0ºC, 100 %.

This strategy is very similar to the previous one, mostly because of the necessity of a regioselective methylation step and the regioselective benzylidene opening step.

The second acceptor has already been synthesized, which is compound **8**. The strategy for the synthesis of glycosyl donor **11** (Scheme 2.1) was also drawn so that this intermediate could be obtained.

2.1.2.1 Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside 12 synthesis

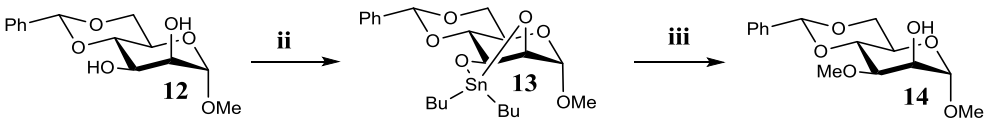


Scheme 2.16 – Synthesis of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside **12** with **i)** benzaldehyde dimethyl acetal, camphorsulfonic acid, THF, Δ, overnight, 50 %.

The synthesis of **12** consists in a regioselective formation of a 4,6-*O* benzylidene acetal, using the same conditions for the synthesis of **4**, but with the reaction time increased to overnight, because the compound is more polar and takes longer to dissolve in THF.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 50 %. This yield was not the expected for this reaction, which may be due to a problem of solubility of the starting material in THF. Since the reaction did not occur using DMF as solvent (a more polar solvent) and the use of benzylidene acetal as a protecting group in this synthesis is important, this result was accepted.

2.1.2.2 Methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside 14 synthesis



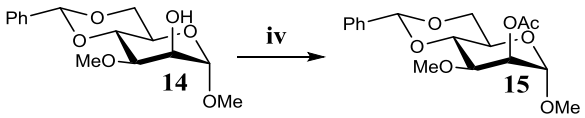
Scheme 2.17 – Synthesis of methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside **14** with **ii)** dibutyltin oxide, methanol, Δ, overnight and **iii)** iodomethane, DMF, 65 ºC, overnight; 2 steps: 50%.

The synthesis of **14** consists in a regioselective 3-*O* methylation. The conditions used for the synthesis of **6** were applied to **12**.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 33%. This yield was very low, comparing to the expected for this reaction, which can be related to the fact that the compound is more polar, and needs more reaction time and temperature to dissolve. In order to optimize it, the reaction time for **ii)** was increased to overnight, and for **iii)** the reaction temperature was increased to 65 ºC. Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 50 % (70% each step). In spite of the yield being better, it still was not the expected one. Even so, this methylation step is very important for the synthetic route and this result was acceptable.

The reason why, once again this synthetic strategy did not start with the methylation of α-methyl-D-mannose, as it was reported by *Liao et al*[2], is going to be explained further in this work.

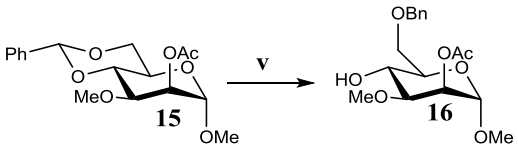
2.1.2.3 Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside 15 synthesis



Scheme 2.18 – Synthesis of methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside **15** with **iv)** acetic anhydride, DMAP, 0ºC → rt, pyridine 2 hours; 97%.

The synthesis of **15** consists in an acetylation of the 2-OH, using the conditions for the synthesis of **7**. Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 97%. This group will not influence the stereochemistry of the disaccharide, but the hydroxyl needed to be protected, so the acetyl group was a good choice, due to the high yield obtained.

2.1.2.4 Methyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 16 synthesis



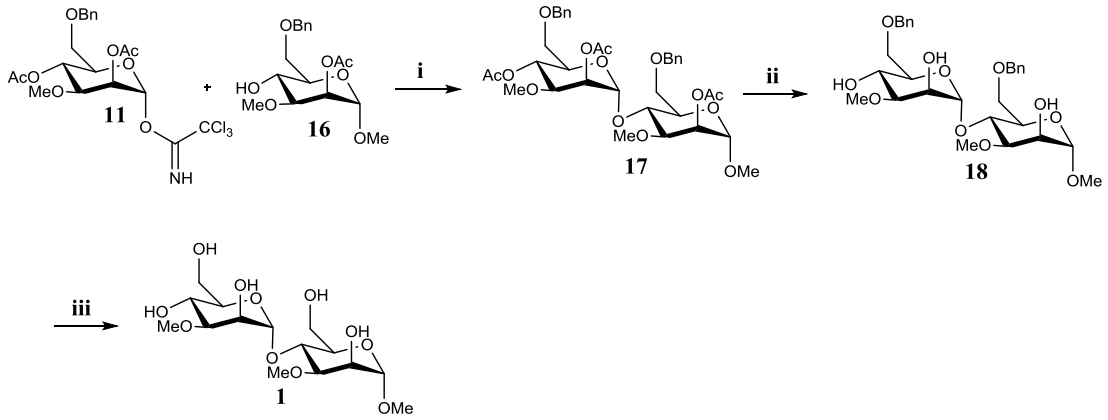
Scheme 2.19 – Synthesis of methyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside **16** with **v)**, sodium cyanoborohydride, hydrogen chloride in diethyl ether 1 M, THF, 0ºC, 100 %.

The synthesis of **16** consists in a regioselective reduction opening of the benzylidene acetal using the conditions for the synthesis of **8**. Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 100 %. The synthesis of this glycosyl acceptor was successful.

2.1.3 Glycosylation reaction and hydroxyl group deprotection

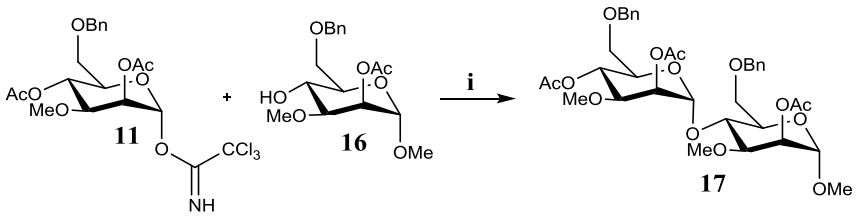
After the synthesis of the glycosyl donor and acceptors, the respective monosaccharides are ready for the glycosylation reaction. The glysosyl donor **11** has a leaving group in its anomeric carbon, and the other hydroxyls are all protected. The glycosyl acceptors **16** and **8** have all the hydroxyls protected, except for the 4-OH. After the formation of the disaccharide, the protecting groups have to be removed from the molecule. The deprotection steps have to be very efficient, and must not hydrolyze the molecule.

A synthetic route for the glycosylation reaction and further protecting group removal was proposed for the first disaccharide (Scheme 2.20).



Scheme 2.20 – Synthetic route followed for the synthesis of the disaccharide **1**. Reagents and conditions: **i)** TMSOTf, dichloromethane, -20 ºC, 30 minutes, 69 %; **ii)** sodium methoxide, methanol, rt, 2 hours: 30 minutes, 98%; **iii)** H_2/Pd/C 10%, ethyl acetate /ethanol 1:1, 50 psi, overnight, 100 %.

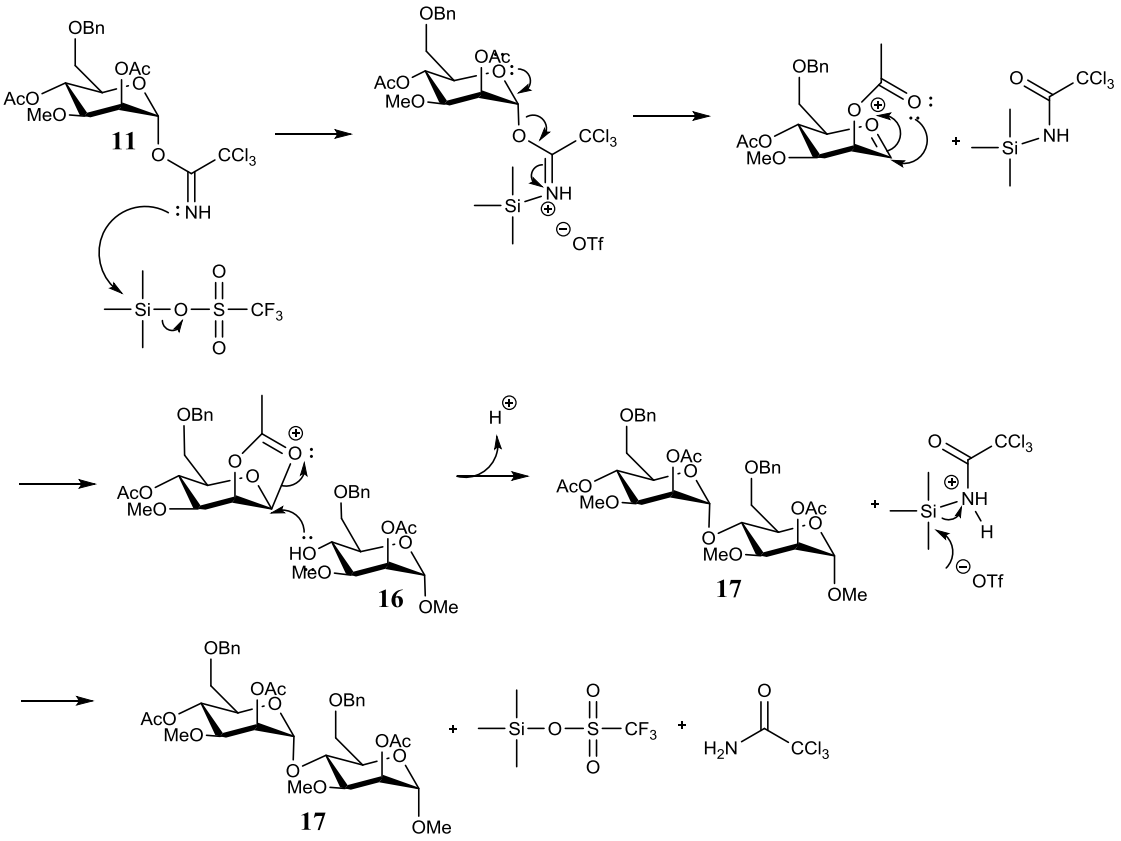
2.1.3.1 Methyl (2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 17 synthesis



Scheme 2.21 – Synthesis of methyl (2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside **17** with **i)** TMSOTf, dichloromethane, -20 ºC, 30 minutes, 69 %.

The synthesis of **17** consists in a glycosylation reaction between **11** and **16**, using TMSOTf as catalyst and dichloromethane as solvent, all stirred at -20ºC. As it was said before, most chemical glycosylation reactions need a catalyst, a promoter, to assist the departure of the leaving group. This catalyst can be either a Lewis or a Brønsted acid.[5]

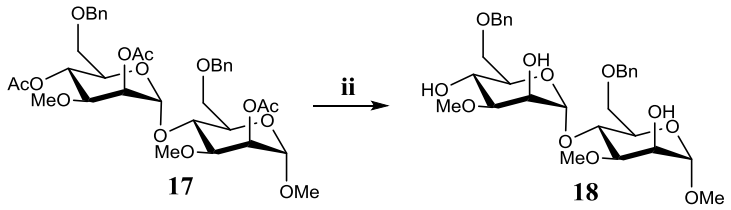
The use of *O*-glycosyl trichloroacetimidate donors has many advantages, such as the fact that they are easily prepared, sufficiently stable, the use of heavy metal salts as promoters can be avoided and they can be activated with catalytic amounts of Lewis acids, such as TMSOTf or $BF_3.OEt_2$.[14][15] The activation of this LG is initiated by coordination of TMSOTf to the nitrogen of the group. This LG now has conditions to depart from the molecule, using the oxygen adjacent to the anomeric carbon as driving force. The formation of the oxonium ion is followed by the attack of the acetate carbonyl oxygen to the anomeric carbon (neighbouring group participation) to form an intermediate, the acyloxonium ion. The glycosyl acceptor has now conditions to attack the intermediate to form a glycosidic bond. After the formation of the disaccharide, the proton liberated on the glycosidic bond formation reacts with the forming leaving group. The Lewis acid is released, becomes available for the next catalytic cycle, and also trichloroacetamide is formed (Scheme 2.22).[5][15]



Scheme 2.22 - Mechanism for the glycosylation reaction and synthesis of **17**.

Interpretation of the ^{1}H-NMR spectrum revealed only one doublet signal at δ 5.23 ppm, corresponding to an anomeric proton, which is the α anomeric proton from the glycosidic bond, due to the effect of the participating group. The absence of the signal corresponding to the β anomeric proton, indicates that the obtained product was the pretended compound, and not a mixture of anomers. A disaccharide with a yield of 69 % was obtained. After confirming that the obtained disaccharide was the pretended product the specific rotation of the compound was measured and $[\alpha]_D^{20}$ +50.4 (c 1.04, CH_2Cl_2) was obtained. Despite the solvent used in this reaction being dichloromethane, the main reason for this reaction to be stereoselective was due to the use of the acetate group at 2-OH, which strongly favors the formation of the α-glycosidic bond. After the formation of the disaccharide the protecting groups must be removed from the molecule.

2.1.3.2 Methyl (6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 18 synthesis



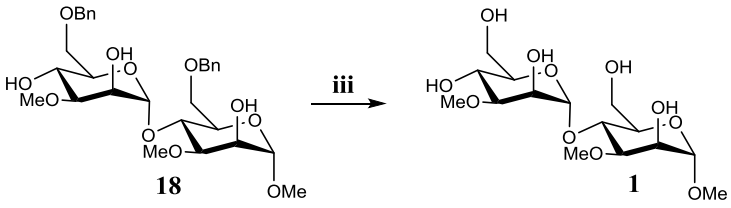
Scheme 2.23 - Synthesis of methyl (6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside **18** with **ii)** sodium methoxide, methanol, rt, 2 hours: 30 minutes, 98%.

After the formation of the glycosidic bond, it is important to find methods to remove the protecting groups on both monosaccharide precursors, without degrading the molecule. The synthesis of **18** consists in the deacetylation of **17**, using sodium methoxide as catalyst, and methanol as solvent.

The reaction first starts with a nucleophilic addition to the carbonyl from the acetate group by the methoxide ion, followed by the departure of the sugar, which becomes deprotected in that alcohol. With the formation of methyl acetate, there is not a possibility to regenerate the catalyst from this compound. Besides the fact that methanol is the solvent of the reaction, it also has an important role in the regeneration of the catalyst. Methanol is deprotonated and the methoxide ion is regenerated.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 98% and a $[\alpha]_D^{20}$ +55.4 (c 0.95, CH_2Cl_2). This yield reveals that removing first the acetyl groups from the molecule was a good choice.

2.1.3.3 Methyl (3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-α-D-mannopyranoside 1 synthesis



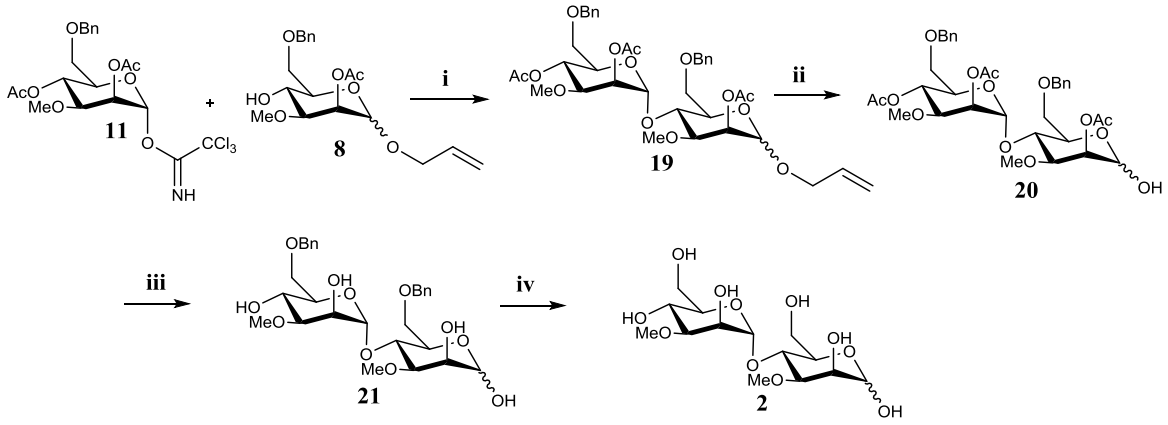
Scheme 2.24 – Synthesis of methyl (3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-α-D-mannopyranoside **1** with **iii)** H_2/Pd/C 10%, ethyl acetate /ethanol 1:1, 50 psi, overnight, 100 %.

The synthesis of **1** consists in the hydrogenation of **18**, in order to completely debenzylate the molecule, using Pd/C 10% as catalyst, and a ethyl acetate/ethanol 1:1 as a

mixture of solvents, shaken at 50 psi of hydrogen. One of the advantages of this hydrogenation method is the fact that the obtained product comes very pure, and a purification is not needed, so high yields can be obtained.

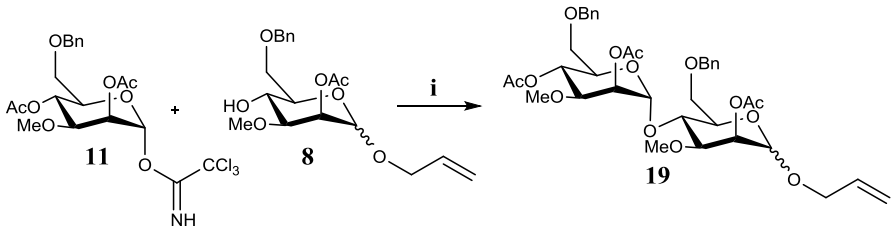
Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 100 % and $[\alpha]_D^{20}$ +67.5 (c 0.99, H_2O). This very good yield also indicates that first removing the acetyls and then the benzyl ether groups was a good choice. The synthesis of **1** was successful and efficient.

A route for the glycosylation reaction and further protecting group removal was proposed for the second disaccharide (Figure 2.25).



Scheme 2.25 – Synthetic route followed for the synthesis of the disaccharide **2**. Reagents and conditions: **i)** TMSOTf, dichloromethane, -20 ºC, 30 minutes, 77 %; **ii)** palladium (II) chloride, methanol, rt, 2 hours; 80%; **iii)** sodium methoxide, methanol, rt, 6 hours: 30 minutes, 78%; **iv)** H_2/Pd/C 10%, ethyl acetate /ethanol 5:1, 50 psi, 7 hours, 98 %.

2.1.3.4 Allyl (2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside 19 synthesis

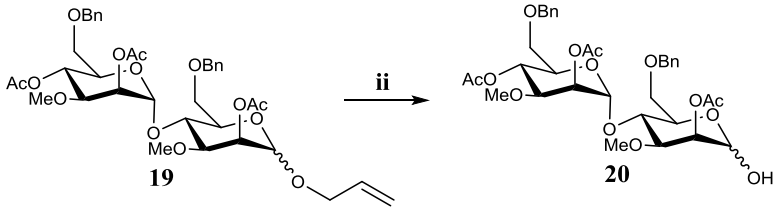


Scheme 2.26 – Synthesis of allyl (2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside **19** with **i)** TMSOTf, dichloromethane, -20 ºC, 30 minutes, 77 %.

The synthesis of **19** consists in a glycosylation reaction between **11** and **8**, using the same conditions as in the synthesis of **17**.

Interpretation of the ^{1}H-NMR spectrum revealed the presence of two doublet signals at δ 4.86 and 4.83 ppm corresponding to the anomeric protons from the allyl ether end, and a multiplet signal (δ 5.25-5.21 ppm) which contains the peak for the α anomeric proton from the newly formed glycosidic bond. Once again, the absence of the signal corresponding to the β anomeric proton from the glycosidic bond, indicates that the obtained product was the pretended compound. A disaccharide with a yield of 77 % (αα:αβ 9:1) was obtained. After confirming that the obtained disaccharide was the pretended product the specific rotation of the compound was measured and $[\alpha]_D^{20}$ +35.1 (c 1.05, CH_2Cl_2) was obtained. Once again the influence of the acetate at 2-OH was very important for the stereochemistry of the final compound.

2.1.3.5 (2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose 20 synthesis

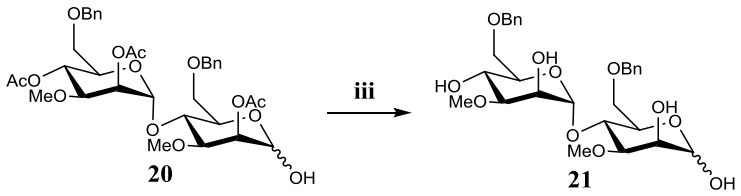


Scheme 2.27 – Synthesis of (2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose **20** with **ii)** palladium (II) chloride, methanol, rt, 2 hours; 80%.

Since the pretended disaccharide **19** has a free hydroxyl group in its reducing end, **8** could be used as the glycosyl acceptor in the glycosylation reaction. The allyl ether needed to be removed after the glycosylation reaction. The synthesis of **20** consists in the deallylation of **19**, using the same conditions as in the synthesis of **10**.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 80 % (αα:αβ 10:1) and $[\alpha]_D^{20}$ +39.8 (c 0.98, CH_2Cl_2).

2.1.3.6 (6-*O*-Benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose 21 synthesis

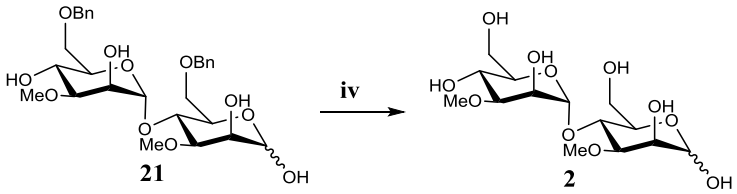


Scheme 2.28 – Synthesis of (6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose **21** with **iii)** sodium methoxide, methanol, rt, 6 hours: 30 minutes, 78%.

The synthesis of **21** consists in the deacetylation of **20** using the same conditions as in the synthesis of **18**, but with a longer reaction time. The work-up had to be different also, due to the fact that the product was more polar than **18**. Dowex-H^+ resin was added until neutral pH, so that the methoxide ion could be protonated, to form methanol, which is then evaporated, affording the pure product.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 78 % (αα:αβ 10:1) and a $[\alpha]_D^{20}$ +51.8 (c 0.95, CH_2Cl_2).

2.1.3.7 (3-*O*-Methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-(α/β)-D-mannopyranose 2 synthesis



Scheme 2.29 - Synthesis of (3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-(α/β)-D-mannopyranose **2** with **iv)** H_2/Pd/C 10%, ethyl acetate /ethanol 5:1, 50 psi, 7 hours, 98 %.

The synthesis of **2** consists in the hydrogenation of **21**, in order to debenzylate the molecule, using Pd/C 10% as catalyst, and ethyl acetate/ethanol 5:1 as mixture of solvents, shaken at 50 psi of hydrogen.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 98% (αα:αβ 2:1) and $[\alpha]_D^{20}$ +57.4 (c 0.96, MeOH). The synthesis of **2** was successful and efficient.

Since **2** has a free anomeric hydroxyl group, in solution this stereocenter can be interconverted in both anomeric forms due to mutarotation. **2** is found as a mixture of anomers.

2.2 Tetrasaccharide synthesis

The third and last of the proposed objectives was the synthesis of a tetrasaccharide, methyl (3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranoside **22** (Figure 2.3).

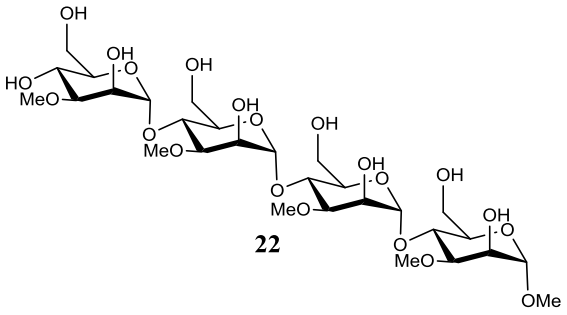


Figure 2.3 – The structure of methyl 3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranoside **22**.

For this synthesis, two disaccharides are needed, a disaccharide glycosyl donor and an acceptor (Figure 2.4).

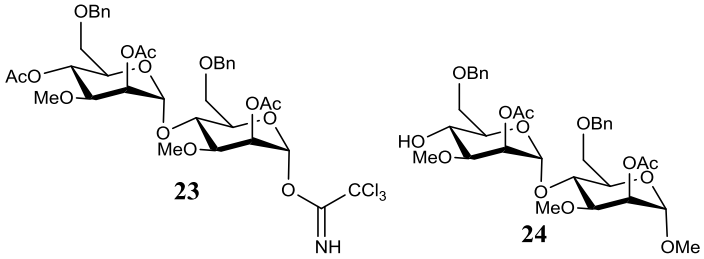
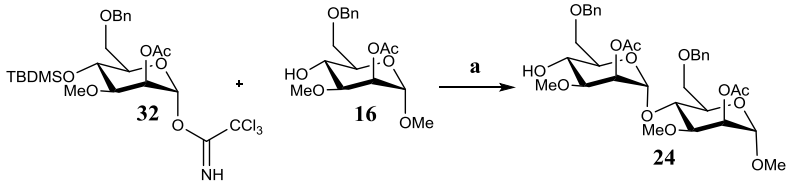


Figure 2.4 – The structure of the glycosyl donor (2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate **23** and the glycosyl acceptor methyl (2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside **24**.

The synthetic strategies for the preparation of the disaccharide glycosyl donor and acceptor were planned.

2.2.1 Disaccharide glycosyl acceptor synthesis

In order to obtain the disaccharide glycosyl acceptor, a glycosidic reaction between two mannose precursors was needed, as shown in Scheme 2.30.



Scheme 2.30 – Synthetic route followed for the synthesis of the disaccharide glycosyl acceptor **24**. Reagents and conditions: **a)** TMSOTf, dichloromethane, -20 ºC, 30 minutes.

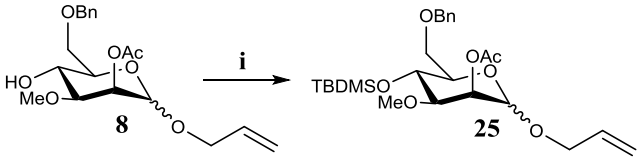
One of the advantages of having previously synthesized the disaccharide precursors is the use of some of its intermediates in the synthesis of this molecule. Intermediate **16** was used as glycosyl acceptor for this reaction, due to the fact that the final disaccharide is methylated on the α anomeric position and at 3-OH, and it has a free hydroxyl at 4-OH. The glycosyl donor

had to be synthesized because the final disaccharide precursor has a free hydroxyl, and there is not a selective method to remove one acetyl group from disaccharide **17**, at the pretended position.

A synthetic strategy for the synthesis of the glycosyl donor, using a different protecting group at 4-OH was proposed (Scheme 2.31).

Scheme 2.31 – Synthetic route proposed for the synthesis of the glycosyl donor **32**. Reagents and conditions: **i)** DIPEA, TBDMSOTf, dichloromethane, 0 ºC, 20 minutes, 89%.

2.2.1.1 Allyl 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranoside 25 synthesis



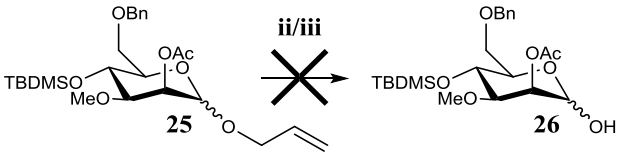
Scheme 2.32 - Synthesis of allyl 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranoside **25** with **i)** DIPEA, TBDMSOTf, dichloromethane, 0 ºC, 20 minutes, 89%.

The synthesis of **25** consists in the silylation of **8**, using DIPEA, TBDMSOTf and dichloromethane as solvent, stirred at -20ºC.

In this reaction, DIPEA first deprotonates the 4-OH, which becomes more nucleophilic, attacks more easily the silicon atom of TBDMSOTf and the triflate group departs from the molecule. DIPEA also neutralizes the triflic acid formed, which could remove the TBDMS group from **25**.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound with a yield of 89%. The use of this silyl ether as protecting group at 4-OH has great advantages, such as the fact that it is easily inserted on the molecule and can be selectively removed in the presence of the other protecting groups. In this case, this group is removed from the molecule, after the glycosylation reaction, allowing the formation of compound **24**.

2.2.1.2 Attempted synthesis of 2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose 26



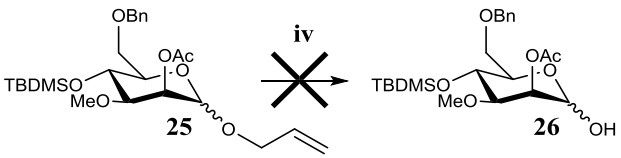
Scheme 2.33 – Attempted synthesis of 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose **26** with **ii)** bis(dibenzylideneacetone)palladium (0), 1,4-Bis (diphenyl-phosphino)butane, THF, rt, 15 minutes and **iii)** 1,3-dimethylbarbituric acid, THF, 60 ºC, overnight.

The conditions used for the synthesis of **10** could not be used in the synthesis of **26**, due to the acidic conditions of the reaction medium when methanol is deprotonated, which can remove the TBDMS group from the molecule. An alternative method for the deallylation of **25** was proposed.

This method consists in first activating bis(dibenzylideneacetone)palladium (0) to palladium(II) using 1,4-Bis(diphenylphosphino)butane in dry THF, all stirred at room temperature and then add it to a solution of **25** and 1,3-dimethylbarbituric acid in THF at the same temperature. After the formation of the complex between palladium(II) and the allyl group, 1,3-dimethylbarbituric acid, acts as a nucleophile just like methanol in the synthesis of **10**, attacks the olefin, promoting the departure of the sugar. In this case, protons are not released in the reaction medium, so there is less hypothesis for the silyl ether to be cleaved.

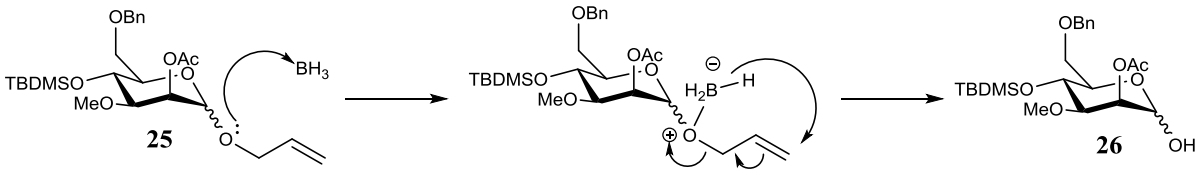
Using these conditions the starting material was not consumed after 30 minutes. The reaction temperature was increased to 60ºC and stirred for another 30 minutes. The starting material was not consumed, so the reaction time was increased to overnight. Even after overnight the starting material was not consumed. Other methods for the removal of the allyl group were tried.

2.2.1.3 Attempted synthesis of 2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose 26



Scheme 2.34 – Attempted synthesis of 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose **26** with **iv)** sodium borohydride, iodine, THF, 0 ºC, 3 hours and 20 minutes.

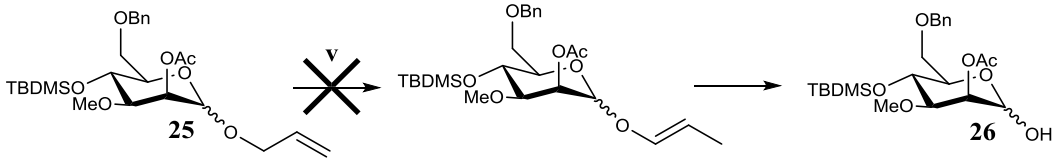
A method used in carbohydrates for the deallylation of **25** was the use of sodium borohydride and iodine, added sequentially at 0ºC in THF.[16] In this reaction, oxidation of this reagent with iodine in THF gives BH_3-THF, which can reduce the olefin from the allyl ether:



Scheme 2.35 - Mechanism for the synthesis of 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose **26** using the described reaction conditions.[16]

However, using the described conditions, after stirring the mixture for 20 minutes the starting material was not consumed. The mixture was stirred for more 3 hours and changes were not observed.

2.2.1.4 Attempted synthesis of 2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose 26

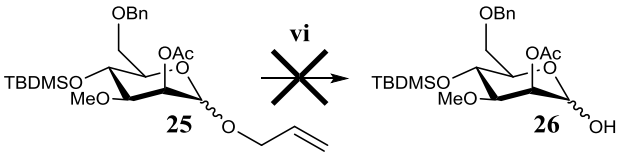


Scheme 2.36 – Attempted synthesis of 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose **26** with **v)** t-BuOK, DMF, 60 ºC, 1 hour.

Another way to remove this protecting group is by isomerization, allowing the formation of a prop-l-enyl group, which can be removed easily, using non-acidic conditions.[17] A described method[18] was applied to **25**, using t-BuOK in DMF at 60ºC. This reagent is a very strong base and is able to deprotonate the carbon adjacent to the double bond, allowing the isomerization. After the formation of the prop-l-enyl group, a non-acidic method could be performed for its removal, using iodine in THF/H_2O.

The reaction was stirred for 1 hour. Interpretation of the ^{1}H-NMR spectrum of the reaction mixture revealed that the obtained product was not the expected compound. Since the obtained compound was not the expected one, the step following the isomerization was not used. Other methods for the deallylation of this compound were attempted.

2.2.1.5 Attempted synthesis of 2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose 26



Scheme 2.37 – Attempted synthesis of 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose **26** with **vi)** acetic acid/H_2O (90 % v/v), sodium acetate, palladium (II) chloride, ethyl acetate, rt, overnight.

Another interesting method which is employed for the deallylation of carbohydrates, uses palladium (II) chloride, and a buffer solution (acetic acid/sodium acetate).[18] The buffer maintains the pH of the reaction medium constant, avoiding the removal of the silyl group. The other differences between this method and the one using palladium (II) chloride in methanol is the heterogeneous medium (H_2O/ethyl acetate), and a non-catalytic amount of palladium (II) chloride.

Before the formation of the complex, the acetic acid will act like an acidic catalyst and will first protonate the oxygen from the allyl ether. After the formation of the complex, instead of methanol, H_2O acts like a nucleophile and attacks the olefin, promoting the departure of the sugar and the formation of the hydroxyl group. The liberated proton will not induce the removal of the silyl group due to the presence of the acetate ion.

This described method was applied to **25**, with acetic acid/H_2O (90% v/v), sodium acetate and palladium (II) chloride being added sequentially at room temperature, and the reaction mixture was stirred overnight. The starting material was totally consumed. After the purification of the reaction mixture, interpretation of the ^{1}H-NMR spectrum revealed that the TBDMS group was still on the molecule and the signals of the allyl group disappeared. However, there was a difficulty in interpreting the ^{1}H-NMR spectrum, mainly because of two extra doublets, which appeared at δ 4.24 ppm and 4.13 ppm, and what it seems to be a mixture of two pairs of doublets (between δ 4.65 and 4.50 ppm) instead of the former ABdd at δ 4.60 ppm (in compound **25**), corresponding to the protons from the CH_2 of the benzyl groups (Figure 2.5).

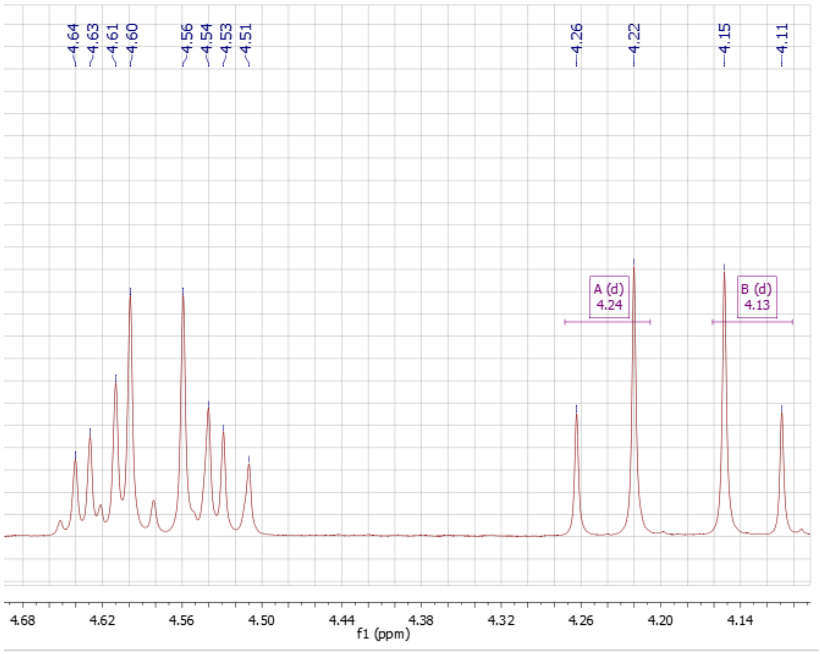


Figure 2.5 –[1]H-NMR spectrum, corresponding to the mixture of two pairs of doublets (between δ 4.65 and 4.50 ppm) and two doublets (at δ 4.24 ppm and 4.13 ppm).

The [1]H-NMR spectrum indicated a mixture of different compounds. In order to better identify and characterize the products, the sample was acetylated following the same procedure used in the preparation of compounds **7**, **9** and **15**. Two different products were obtained. One of the products was **27**, which is the pretended compound **26** acetylated, but only the α anomer (Figure 2.6). In this spectrum one ABdd at δ 4.59 ppm was present, corresponding to the protons from the CH_2 of the benzyl groups (Figure 2.7). The signal corresponding to the anomeric proton, due to the presence of the acetate group, is located at δ 6.03 ppm (Figure 2.7).

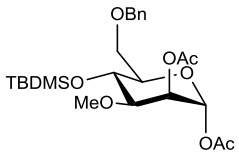


Figure 2.6 – The structure of 1,2-di-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-α-D-mannopyranose **27**.

In the other obtained product **28**, interpretation of the [1]H-NMR spectrum revealed one ABdd at δ 4.58 ppm and the presence of the two doublets at δ 4.24 and 4.13 ppm. The signal of the anomeric proton is located at δ 4.86 ppm (Figure 2.7), which reveals that the compound has not been acetylated at 1-OH. Interpretation of the **[13]C-APT** spectrum revealed a signal at δ 204.79 ppm (Figure 2.8), which indicates the presence of a ketone or an aldehyde in the molecule.

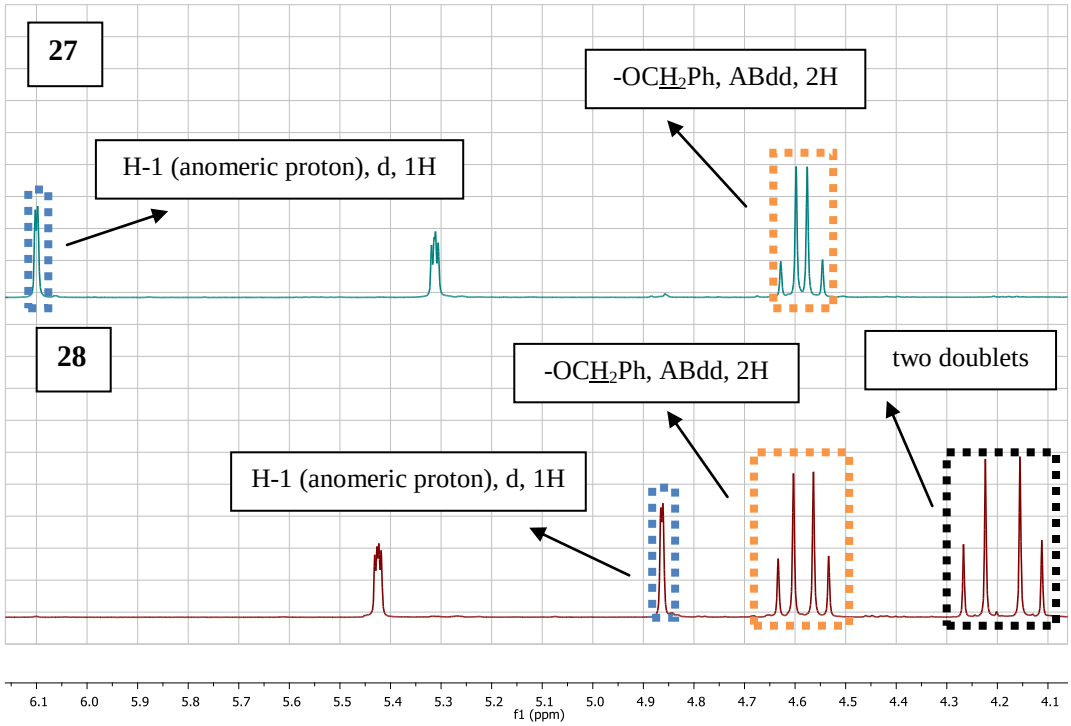


Figure 2.7 – ^{1}H-NMR spectra overlay from the two obtained compounds (between δ 6.2 and 4.0 ppm). The green spectrum is from compound **27** and the red one is from compound **28.**

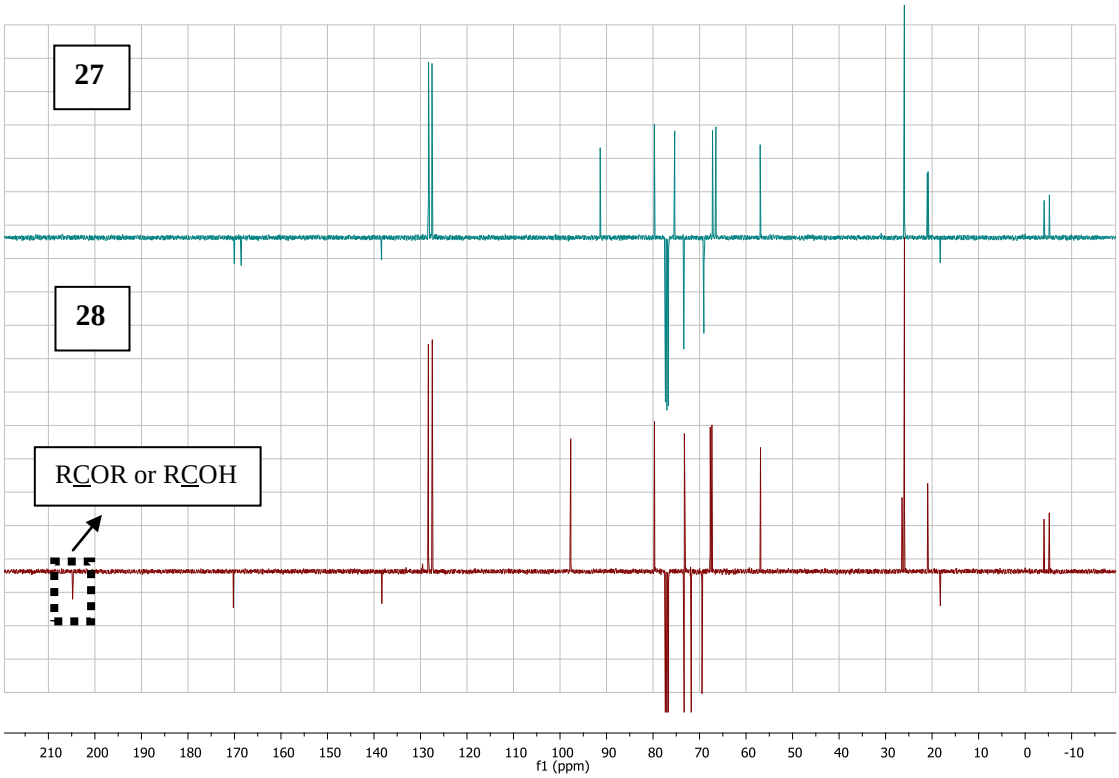


Figure 2.8 – ^{13}C-APT spectra overlay from the two obtained compounds (between δ 220.0 and -20.0 ppm). The green spectrum is from compound **27** and the red one is from compound **28**.

A reported work by *Lüning at al*[19] indicates that the deallylation using these conditions affords a byproduct, which is the formation of the Wacker oxidation product on the allyl group. With this oxidation of the olefin, two products can be formed:

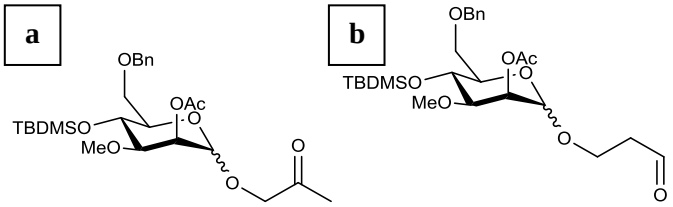
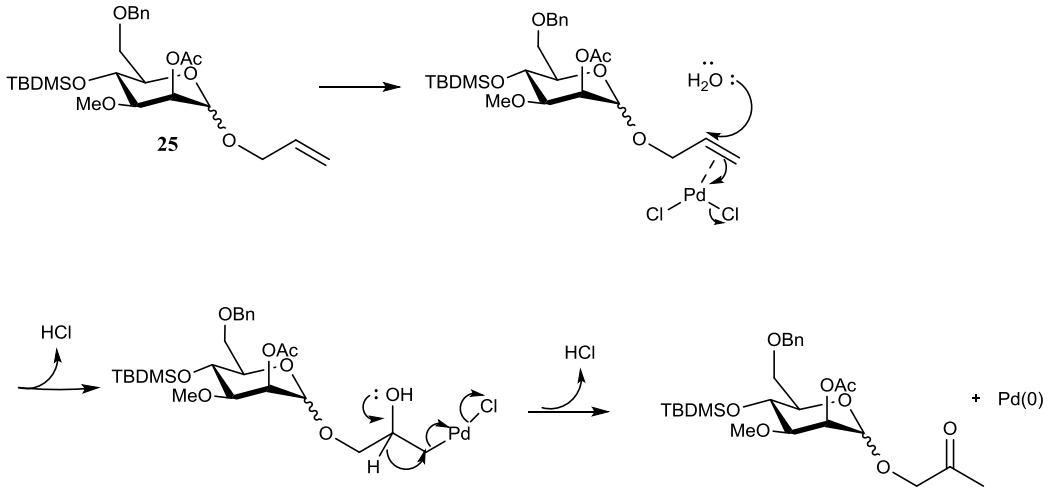


Figure 2.9 – Two possible products, which result from the Wacker oxidation of the allyl group.

With the interpretation of the NMR data and its comparison with the reported work by *Lüning at al*, it can be concluded that compound **28** results from the Wacker oxidation. In this case, the ketone was formed (**a**), because the two doublets at δ 4.24 and 4.13 ppm correspond to the protons from the CH_2 adjacent to the carbonyl. Besides that, the singlet signal from the other three protons adjacent to the ketone appear at δ 2.15 or at 2.10 ppm. The formation of this Wacker product brought some disadvantages, because it was impossible to separate the two compounds, without having to acetylate them. Besides that, the yield for the formation of **26** was low (48%), and for the Wacker product was 29%. The mechanism for this reaction is shown in scheme 2.38.



Scheme 2.38 – Mechanism for the Wacker oxidation.

The catalyst needed a certain quantity of oxidant (for example $CuCl_2$) to be regenerated. However since the quantity of palladium (II) chloride added is stoichiometric this was not necessary. Once again, only the α anomer was formed.

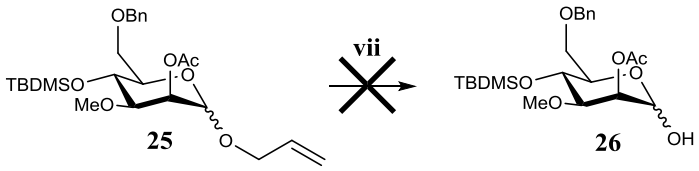
In order to avoid the formation of the Wacker product and to optimize this method, different reaction times and reagent quantities ($PdCl_2$) were studied.

Table 2.1 – Summary of the different experimental conditions used for the optimization of the synthesis of **26**.

	Reaction time	Quantity of $PdCl_2$	Starting material	26	28 (Wacker product)
Normal conditions	Overnight	1.5 equivalents	No	Yes	Yes
1	6 hours	1.5 equivalents	Yes	Yes	Yes
2	2 hours	1.5 equivalents	Yes	Yes	Yes
3	72 hours	0.2 equivalents	Yes	Yes	Yes

Shorter reaction times did not afford good results as well, neither less quantity of palladium (II) chloride. Since it was impossible to synthesize **26** without the parallel formation of **28** another method for the allylation was attempted.

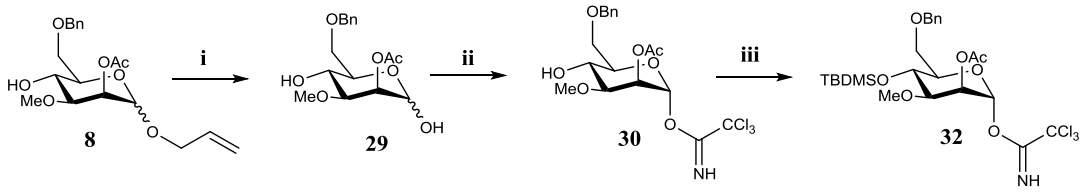
2.2.1.6 Attempted synthesis of 2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose 26



Scheme 2.39 - Attempted synthesis of 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose **26** with **vii)** (dimethyl sulfide)trihydroboron, THF, 0 ºC, 20 minutes.

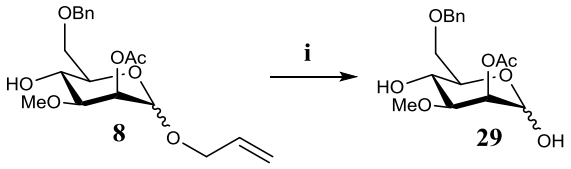
For the deallylation of **25** (dimethyl sulfide)trihydroboron, added at 0ºC in THF was tried.[16] This method is similar to the one used previously with sodium borohydride and iodine, but in this case the reagent does not need to be activated by the oxidation of iodine, since it is already in the BH_3 form. After 20 minutes, the starting material was totally consumed. However, the formation of several products was observed. Interpretation of the ^{1}H-NMR spectrum from the different products revealed none of them was the expected compound.

Since an efficient method to remove the allyl group was not found, other alternatives were considered. Another synthetic strategy was proposed:



Scheme 2.40 – Alternative synthetic route proposed for the synthesis of the glycosyl donor **32**. Reagents and conditions: **i)** acetic acid/H_2O (90 % v/v), sodium acetate, palladium (II) chloride, ethyl acetate, rt, overnight 73%; **ii)** DBU and trichloroacetonitrile, dichloromethane, 0ºC, 10 minutes, 36 %; **iii)** DIPEA, TBDMSOTf, dichloromethane, 0 ºC, 20 minutes, 74 %.

2.2.1.7 2-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose 29 synthesis

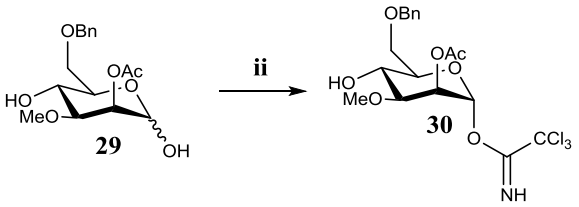


Scheme 2.41 – Synthesis of 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose **29** with **i)** acetic acid/H_2O (90 % v/v), sodium acetate, palladium (II) chloride, ethyl acetate, rt, overnight 73%.

In this reaction, in order to avoid the obstacles verified on the previous route, **8** was deallylated before being silylated at the 4-OH.

Using the conditions for the synthesis of **10**, interpretation of the ^{1}H-NMR spectrum indicated that the pretended compound was obtained, but with a yield of 22 %. This yield was not the expected and it was very low, so the other conditions using palladium (II) chloride and the buffer solution were experimented.[18] Compound **29** was obtained with a yield of 73%.

2.2.1.8 (2-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloro-acetimidate 30 synthesis



Scheme 2.42 – Synthesis of (2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloro-acetimidate **30** with **ii)** DBU and trichloroacetonitrile, dichloromethane, 0ºC, 10 minutes, 36 %.

The synthesis of **30** should consist in a regioselective trichloroacetimidation at the anomeric hydroxyl group. To accomplish that, a reported procedure[20] was applied for the synthesis of **30**, but instead of using Cs_2CO_3 as base, DBU was used. The difference between

this procedure and the one used in the synthesis of **11** is the different quantity of base and trichloroacetonitrile. In this case, less quantity of both reagents was needed, in order to avoid the trichloroacetimidation on both hydroxyls. However 10 minutes later the formation of two products was observed.

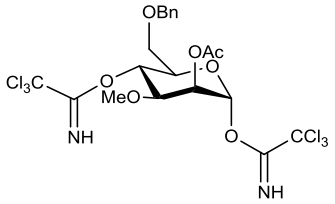
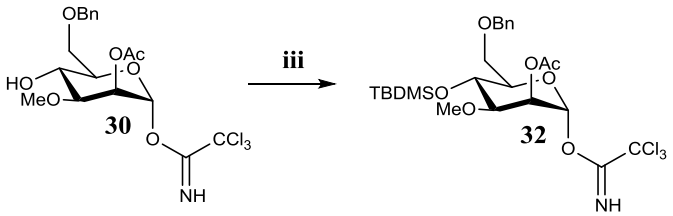


Figure 2.10 – The structure of (2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-1,4-*O*-α-D-mannopyranosyl)-di-trichloroacetimidate **31**.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained products were the pretended compound **30**, with a yield of 36% and **31** (Figure 2.10), with a yield of 59%. The yield for **30** unfortunately was not the expected, even with the changed conditions. However, **30** will be used further in this work in the synthesis of **32**.

2.2.1.9 (2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate 32 synthesis

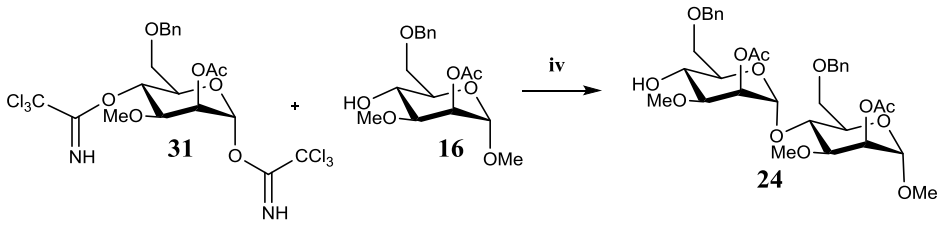


Scheme 2.43 – Synthesis of (2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate **32** with **iii)** DIPEA, TBDMSOTf, dichloromethane, 0 ºC, 20 minutes, 74 %.

Despite the yield obtained in the synthesis of **30** being very low, due to the formation of **31**, it could be possible that this reaction could be optimized by changing the parameters of the procedure, avoiding the formation of the secondary product.

30 was silylated at 4-OH in order to see if the previous reaction is the only one that needs to be optimized. Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound **32**, with a yield of 74 %. With this result, it can be concluded that this route could be successful if the previous trichloroacetimidation was a more efficient reaction.

2.2.1.10 Methyl (2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 24 synthesis

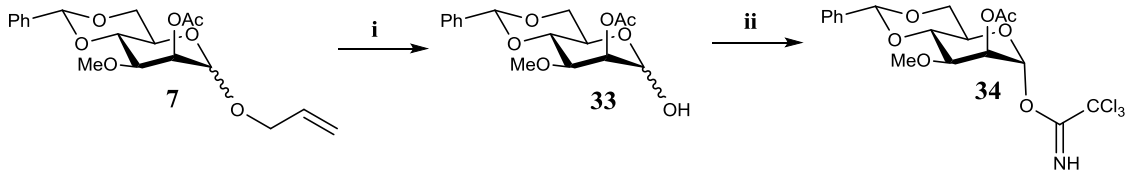


Scheme 2.44 - Synthesis of methyl (2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside **24** with **iv)** TMSOTf, dichloromethane, -20 ºC, 30 minutes, 18 %.

This alternative step consists in using **31** as the glycosyl donor in the glycosylation reaction to synthesize **24**. The same conditions as in the synthesis of **17** and **19** were used. If this reaction afforded a good yield, the regioselective step to synthesize **30** would not be needed. In this synthesis, after the glycosylation reaction, the trichloroacetimidate group protecting the 4-OH departs from the molecule, since it is a very unstable group and hydrolyses very easily.

However, interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound **24**, but with a low yield of 18%, which revealed that **31** was not a good glycosyl donor. Other possibilities were studied.

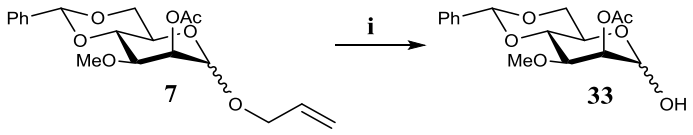
Since this synthetic strategy (Scheme 2.40) did not go as planned, another alternative was proposed:



Scheme 2.45 – Synthetic route proposed for the synthesis of the glycosyl donor **34**. Reagents and conditions: **i)** acetic acid/H_2O (90 % v/v), sodium acetate, palladium (II) chloride, ethyl acetate, rt, 5 hours, 78%; **ii)** DBU and trichloroacetonitrile, dichloromethane, 0ºC, 3 hours, 10%.

Instead of using compound **32**, **34** could be used as glycosyl donor. After the glycosylation reaction, the benzylidene group could be reduced to afford **24**.

2.2.1.11 2-*O*-Acetyl-4,6-*O*-benzylidene-3-*O*-methyl-(α/β)-D-mannopyranose 33

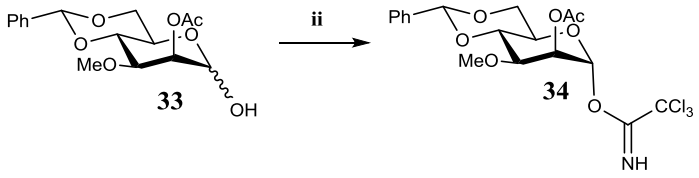


Scheme 2.46 – Synthesis of 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-(α/β)-D-mannopyranose **33** with **i)** acetic acid/H_2O (90 % v/v), sodium acetate, palladium (II) chloride, ethyl acetate, rt, 5 hours, 78%.

The synthesis of **33** consists in the deallylation of **7**, using palladium (II) chloride and the buffer solution[18], with the reaction time decreased to 5 hours. The conditions used for the synthesis of **10**, palladium (II) chloride and methanol, were not applied in this synthesis due to the presence of the benzylidene acetal, which is removed under acidic conditions.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound, with a yield of 78 %.

2.2.1.12 (2-*O*-Acetyl-4,6-*O*-benzylidene-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate 34



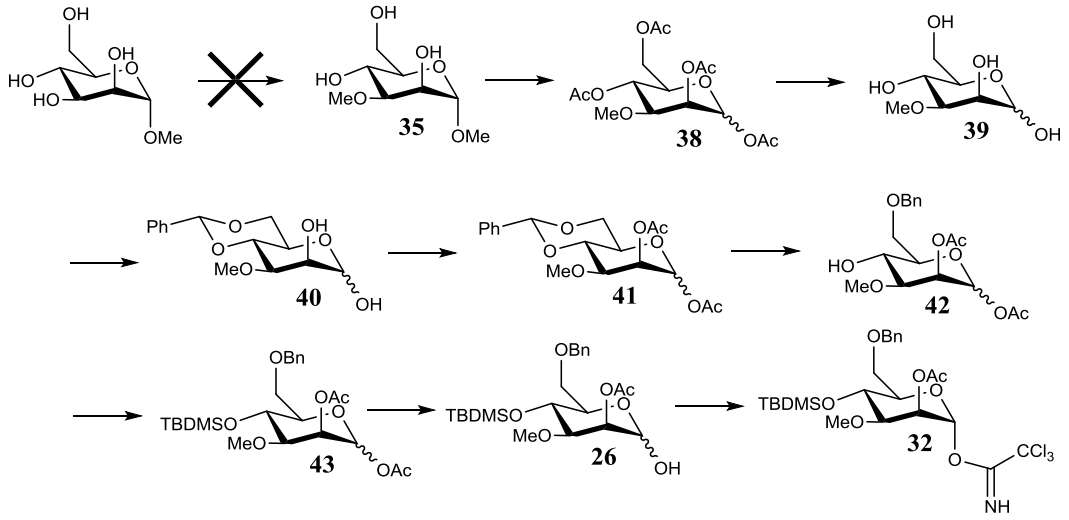
Scheme 2.47 – Synthesis of (2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate **34** with **ii)** DBU and trichloroacetonitrile, dichloromethane, 0ºC, 3 hours, 10 %.

The synthesis of **34** consists in the trichloroacetimidation of **33**, using the same conditions as in the synthesis of **11**, but with a longer reaction time.

However, interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound, with a very low yield of 10 %. With this result this route was not a good alternative.

One of the main obstacles in the synthesis of the glycosyl donor **32** was the use of allyl ether as protecting group at 1-OH. Even though the allyl ether is one of the most used protecting groups in carbohydrate chemistry, the fact that it is removed only under certain conditions brings some disadvantages when using other protecting groups, such as for example silyl ethers.

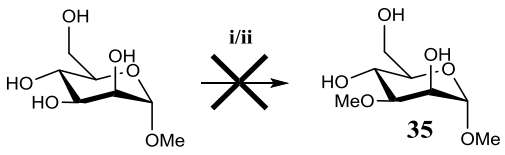
What if in this synthesis, another protecting group could be used at the anomeric position? The acetyl group for example could be a very good protecting group, since it is easily inserted and removed under some conditions which cannot remove other protecting groups or form secondary products. The only obstacle in the use of this protecting group, is that it has to be used also at 2-OH, due to the neighbouring group participation. So, a procedure for the regioselective removal of the acetyl group at the anomeric position has to be applied. A new strategy was proposed for the synthesis of glycosyl donor **32** (Scheme 2.48).



Scheme 2.48 – Alternative synthetic route followed for the synthesis of the glycosyl donor **32**.

A reported work[21] used α-methyl-D-mannose as starting material for the synthesis of 3-*O* methyl mannose, such as Liao and coworkers.[2] However this work[21] described a method to remove the anomeric methoxy group, by using a mixture of reagents (acetic anhydride, acetic acid and sulfuric acid) to afford an aggressive acetylation step, which can be useful in the proposed strategy. The reason why the benzylidene group has to be inserted in the molecule afterwards is due to the fact that it would be removed from the molecule with the aggressive acetylation step. Then, after 1-OH and 2-OH acetylation, the benzylidene ring can be regioselectively opened, in order to allow the 4-OH TBDMS protection. After the silyl ether protection, since there are two acetyl groups, and only one needs to be removed, some procedures can be used to accomplish that, such as the use of hydrazine acetate. [22]

2.2.1.13 Attempted synthesis of Methyl 3-*O*-methyl-α-D-mannopyranoside 35

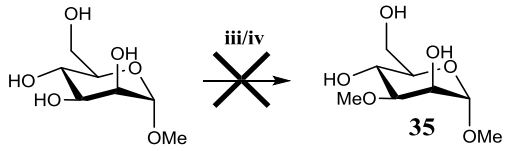


Scheme 2.49 – Attempted synthesis of methyl 3-*O*-methyl-α-D-mannopyranoside **35** with **i)** dibutyltin oxide, toluene, Δ, 3 hours; **ii)** iodomethane, TBAI, toluene, 70 ºC, 72 hours.

The reported 2 step method[21] for the regioselective 3-*O* methylation was applied to α-methyl-D-mannose using dibutyltin oxide on the first step and on the second step iodomethane and TBAI as reagents, and toluene as solvent on both steps. TBAI can stabilize the iodine atom, facilitating its departure from the iodomethane molecule, in order to increase the reaction rate.

However, interpretation of the ^{1}H-NMR spectrum, revealed that the reaction did not occur, probably because of a solubility problem. This starting material is a much more polar compound, so its solubility in tolune is lower and the formation of the 2,3-*O*-di-butylstannylene intermediate is not favored. Even if the stannylene intermediate is formed, this compound hardly dissolved in the solvent and even with 72 hours of reaction time, the reaction with iodomethane and TBAI is not favored.

2.2.1.14 Attempted synthesis of Methyl 3-*O*-methyl-α-D-mannopyranoside 35

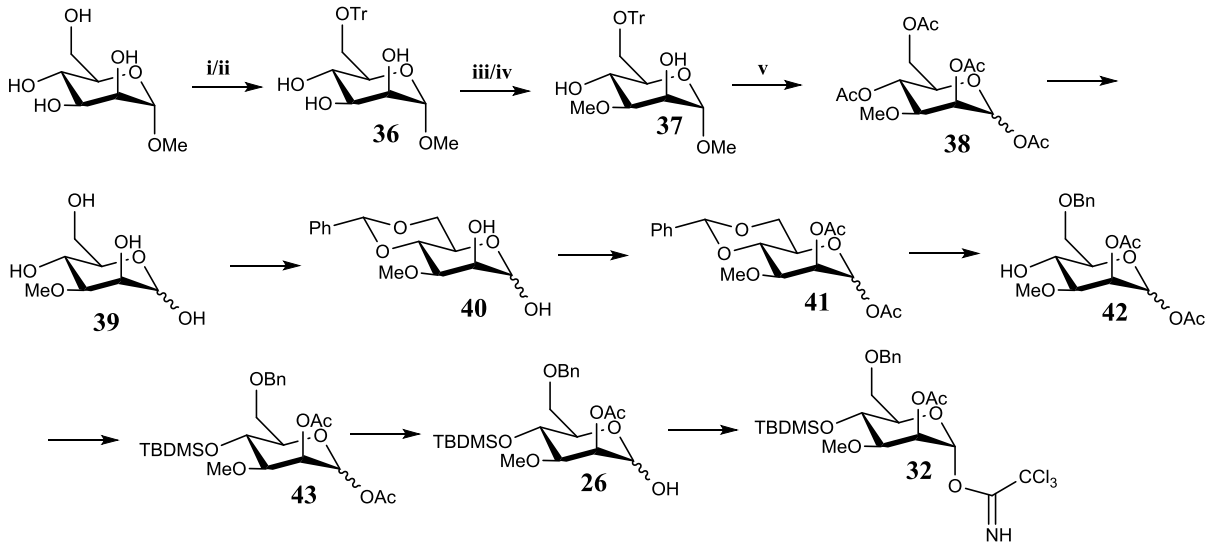


Scheme 2.50 – Synthesis of methyl 3-*O*-methyl-α-D-mannopyranoside **35** with **iii)** dibutyltin oxide, methanol, Δ, overnight; **iv)** iodomethane, DMF, 65 ºC, overnight.

The method applied on the synthesis of **14** was used in this step.

Once again, interpretation of the ^{1}H-NMR spectrum, revealed that the reaction did not occur, which also may be due to a solubility problem. The reason why during this work the synthesis of glycosyl donor **11** and acceptor **16** started first with the benzylidenation and then with the methylation step is mainly due to this result.

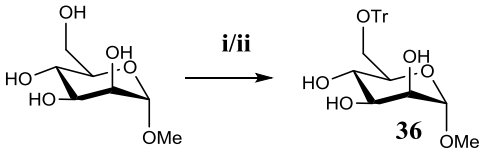
A way to solve this problem was to decrease the polarity of α-methyl-D-mannose by protecting one or several hydroxyls of the molecule. A new strategy for the synthesis of **32**, which included this important step, was drawn and proposed (Scheme 2.51)



Scheme 2.51 – Alternative route proposed for the synthesis of the glycosyl donor **32**. Reagents and conditions: **i)** TrCl, pyridine, rt, 24 hours; **ii)** TrCl, DMAP, pyridine rt, overnight; 2 steps: 100 %; **iii)** dibutyltin oxide, methanol, Δ, overnight and **iv)** iodomethane, DMF, 65 ºC, overnight; 2 steps : 68 %; **v)** acetic anhydride/acetic acid/sulfuric acid 105:45:1, v/v/v, rt, overnight, 80%.

Trityl was chosen as the protecting group, because it can be inserted and removed from the molecule very easily. This group will assist the 3-*O* methylation reaction and then will be removed in the aggressive acetylation step.

2.2.1.15 Methyl 6-*O*-trityl-α-D-mannopyranoside 36 synthesis



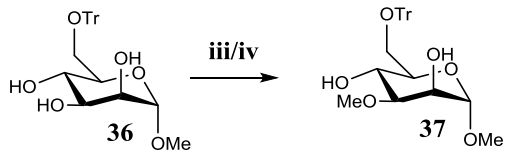
Scheme 2.52 – Synthesis of methyl 6-*O*-trityl-α-D-mannopyranoside **36** with **i)** TrCl, pyridine, rt, 24 hours; **ii)** TrCl, DMAP, pyridine rt, overnight; 2 steps: 100 %.

The synthesis of **36** consists in the tritylation of α-methyl-D-mannose, using a two step reaction,

In the first step TrCl is kept at rt with pyridine, in order to allow the departure of the chloride leaving group for the formation of the trityl carbocation. In the second step DMAP forms an activated species with the carbocation. The 6-OH group attacks the carbon, DMAP departs from the molecule, and **36** is formed. The catalyst is protonated but then is regenerated by pyridine. The reason why this tritylation is 6-*O* regioselective is due to the fact that this group is very bulky and selectively reacts with primary alcohols in carbohydrates.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound, with a yield of 100 %.

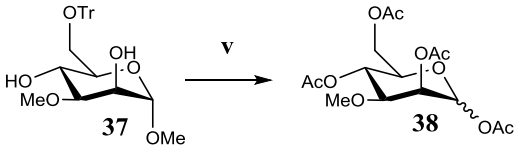
2.2.1.16 Methyl 3-*O*-methyl-6-*O*-trityl-α-D-mannopyranoside 37 synthesis



Scheme 2.53 – Synthesis of methyl 3-*O*-methyl-6-*O*-trityl-α-D-mannopyranoside **37** with **iii)** dibutyltin oxide, methanol, Δ, overnight; **iv)** iodomethane, DMF, 65 ºC, overnight; 2 steps : 68 %.

The same conditions as in the synthesis of **14** were applied to **36** and interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound, with a yield of 68 %. The use of trityl group to decrease the polarity of the compound was a very good choice, since the yield for the tritylation was very high (100 %) and the yield for the 3-*O*-methylation step was good.

2.2.1.17 1,2,4,6-Tetra-*O*-acetyl-3-*O*-methyl-(α/β)-D-mannopyranose 38 syn- thesis



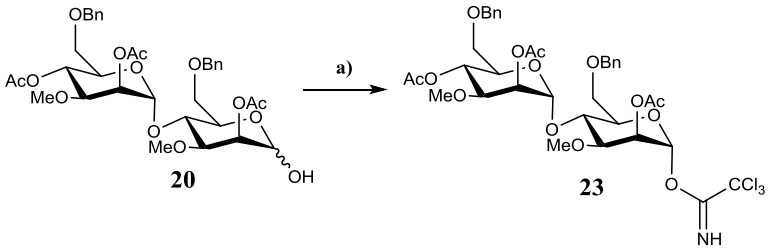
Scheme 2.54 – Synthesis of 1,2,4,6-tetra-*O*-acetyl-3-*O*-methyl-(α/β)-D-mannopyranose **38** with **v)** acetic anhydride/acetic acid/sulfuric acid 105:45:1, v/v/v, rt, overnight, 80 %.

The synthesis of **38** consists in the acetylation of **37** using a described procedure[21], with acetic anhydride and acetic acid as reagents and solvents, and sulfuric acid as catalyst, all stirred at rt. Besides the acetylation of the free hydroxyl groups, the anomeric methoxy group can be removed from the molecule when is protonated by the acidic catalyst, giving the formation of the oxonium ion. After that, the acetate ion attacks the anomeric carbon on both sides of the molecule, forming **38**.

Interpretation of the ^{1}H-NMR spectrum revealed that the obtained product was the pretended compound, with a yield of 80 %. The rest of the synthetic strategy could not be continued, but with these very good results, it is a very promising one.

2.2.2 Disaccharide glycosyl donor synthesis

One of the advantages of having synthesized first the disaccharide precursors was the use of some of its intermediates in the synthesis of the tetrasaccharide. **20** could be used in the synthesis of the disaccharide glycosyl donor, since it has a free anomeric hydroxyl group, ready to be trichloroacetimidated:



Scheme 2.55 – Synthesis of the disaccharide glycosyl donor **23**. Reagents and conditions: **a)** DBU and trichloroacetonitrile, dichloromethane, 0ºC, 10 minutes.

However, since the glycosyl acceptor disaccharide **23** could not be synthesized, this compound was not synthesized in this work.

CHAPTER 3

CONCLUSION

3. Conclusion

The three main objectives of this work were the efficient synthesis of three saccharides, which are cellular precursors for the biosynthesis of MMPs.

The two first sugars are disaccharides and to synthesize them a glycosyl donor and acceptor were needed. The same glycosyl donor **11** was used in the synthesis of both disaccharides. D-mannose was used as starting material. The synthesis was efficient, with individual yields equal or higher to 75 %, except for the benzylidenation step, which had a yield of 59%. However, this step was very important in the synthesis, because this acetal can be regioselectively opened and can facilitate the 3-*O* methylation, since it lowers the polarity of the sugar. Different glycosyl acceptors were used in the synthesis of each disaccharide, since they have structural differences - one has a reducing end and the other does not. The synthesis of the first glycosyl acceptor **16** used α-methyl-D-mannose as starting material. This was successful with individual yields higher than 95%, except for the benzylidenation and the methylation steps, which had yields of 50%. Once again, the acetalation step is important in this synthesis, so this reasonable yield was acceptable. The yield for the methylation step was also reasonable, since it is a 2 step reaction (70% yield each step), and it is a very important step for the synthesis. The second glycosyl acceptor **8** was one of the intermediates in the synthesis of the glycosyl donor **11**, so it was also successfully synthesized. The "building blocks" for the formation of both disaccharides were ready for the glycosylation reaction.

The synthesis of the first disaccharide **1**, using **11** and **16** as glycosyl donor and acceptor, respectively, was successful. The glycosylation reaction afforded esclusively the α anomer, which was the pretended product with a yield of 69%. The use of the acetyl group at 2-OH of the glycosyl donor was a good strategy to induce the formation of the pretended anomer, due to the participating group effect. The use of the trichloroacetimidate group as leaving group was also a good choice, since the yield of the glycosylation was good. After the formation of the glycosidic bond, the disaccharide was deprotected, to give the pretended compound **1**. The strategy used for the removal of the protecting groups was successful, with individual yields higher or equal than 98%.

The synthesis of the second disaccharide **2**, using **11** and **8** as glycosyl donor and acceptor, respectively, was also successful. The glycosylation reaction afforded exclusively the pretended α glycosidic bond, with a yield of 77 %. The use of the same reagents and the participating group at 2-OH were important for the outcome of the glycosylation reaction in terms of yield and stereoselectivity. Also, the removal of the protecting groups was successful, with individual yields higher or equal than 78%. Since the configuration of the anomeric carbon

from the reducing end can be interconverted due to a process called mutarotation, it was not relevant.

The third and last saccharide to be synthesised was a tetrasaccharide. Both disaccharide donor **23** and acceptor **24** needed to be first synthesized. **24** could be obtained from the glycosylation reaction between glycosyl donor **32** and the already synthesized acceptor **16**. **32** needed to have a silyl group at 4-OH, so that after the glycosylation reaction this group could be selectively removed, to form the disaccharide **24**. However, removing the allyl group with the molecule containing the 4-OTBDMS group was very difficult. The allyl group proved to be a good protecting group in the synthesis of **11**, but in the synthesis of **32** its constant use in carbohydrate research was questioned. Despite being removed from the molecule under certain conditions, sometimes those methods can form undesired secondary products. Some alternative synthetic strategies were proposed.

One of the proposed synthetic routes (Scheme 2.40), which deallylates the sugar before the formation of the silyl ether, gave good results, with individual yields higher or equal than 73%, except for the regioselective trichloroacetimidation step, which had a very low yield of 36%. Other alternative methods were attempted. In a future work, if some reactional conditions are found to increase this yield, this could be a very promising route for the synthesis of **32**.

Another promising strategy (Scheme 2.51), was proposed without the use of allyl ether as protecting group. The acetyl group was used as alternative, since there are methods which regioselectively remove this protecting group at the anomeric position. Unfortunately this strategy could not be continued in this work due to lack of time, and had to be stopped in the synthesis of **38** but with yields equal or higher than 68%. In a future work this strategy has great potential for the efficient synthesis of **32**.

Since the disaccharide **24** could not be formed, disaccharide **23** was not synthesized in this work. However, in a future work compound **20** could be trichloroacetimidated, to form **23**.

In general, this work had most of the objectives achieved. Even though the synthesis of the tetrasaccharide was not complete, some helpful tools for its chemical formation were developed. The reactions that did not go as the expected can guide a future work to not follow those. This work also successfully highlighted the importance of chemical glycosylation, in comparison with enzymatic glycosylation. It is very hard to purify a compound obtained from an enzymatic reaction, and the enzymatic reactions are usually performed in small scale, as they often need expensive co-factors. Moreover, the main objective of the synthesis of these compounds is to discover the enzymes which catalyse the formation of these glycosidic bonds, in order to characterize the synthesis of MMPs *in vivo*. So, since the enzymes which can

catalyse the formation of these saccharides have to be found, the only way to synthesize them is by chemical glycosylation.

CHAPTER 4

EXPERIMENTAL PART

4. Experimental part

4.1 General conditions

All reactions were carried out under an inert atmosphere (argon), except when the solvents were not dried. Air sensitive materials were handed in a Braun MB 150-Gl glove box. The synthesized compounds were purified by silica flash column chromatography or silica preparative TLC. Reactions were followed by Analytical TLC. The purity of synthesized compounds was also verified with Analytical TLC and the characterization of the same compounds was done by **^{1}H-NMR**, **^{13}C-NMR**, **^{13}C-APT**, 2D techniques (COSY and HMQC), IR spectroscopy and specific rotation, when applicable.

Analytical TLC was performed on aluminium-backed Merck 60 F_{254} silica gel plates. The spots corresponding to the products were identified by UV radiation (254 nm) and then immersed on a 5% phosphomolybdic acid solution in ethanol.

Silica preparative TLC in Silica gel Merck 60 F_{254}.

Silica flash column chromatography in Silica gel Merck 60.

^{1}H-NMR spectra were recorded on a Bruker 400 spectrometer and obtained at 400 MHz in $CDCl_3$ or D_2O. Chemical shifts are given in ppm, downfield from tetramethylsilane, for solutions in $CDCl_3$. Spectra in D_2O are pre-saturated on the water signal (4.7 ppm).

^{13}C-NMR spectra were recorded on a Bruker 400 spectrometer at 100.61 MHz in $CDCl_3$ or D_2O.

IR spectra were measured on a Nicolet 6700 ATR-FTIR spectrometer with a Zn-Se crystal.

Specific rotations ($[\alpha]^{20}_{D}$) were measured on a Perkin-Elmer D241 automatic polarimeter at the sodium D-line at 20 ºC, and reported as $[\alpha]_D$ (concentration in g/100 mL of solvent).

4.2 Solvent and Reagent Purification

All the used solvents were previously distilled in the laboratory.

Acetic Anhydride: distilled under reduced pressure.

Allyl alcohol: distilled at atmospheric pressure.

DBU: distilled under reduced pressure.

Dry Dichloromethane: previously distilled DCM was stirred with phosphorous pentoxide (drying agent) for 2 hours under reflux, being only distilled before its utilization.

DIPEA: distilled under reduced pressure, using calcium hydride as drying agent.

Dry DMF: to previously distilled DMF calcium hydride (drying agent) was added and the mixture was left overnight, followed by decantation from the drying agent and distillation under reduced pressure.

Dry Ethyl Ether: same procedure than THF and stored with sodium wire.

Dry Methanol: to 50-70 mL of previously distilled methanol 5g of magnesium turnings and iodine (0.5 g) were added, and it was refluxed until all the magnesium had been consumed. More methanol (1L) was added and the reflux was maintained for 2h.

Dry Pyridine: distilled twice at atmospheric pressure using potassium hydroxide as drying agent.

Dry THF: to previously distilled THF, sodium wire and benzophenone were added, and the mixture was refluxed under argon for several hours until the solvent turns deep blue in colour. Then the mixture was kept at low reflux, being only distilled before its utilization.

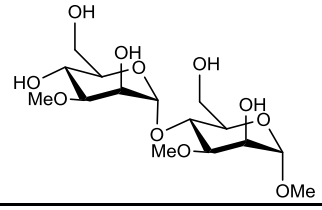
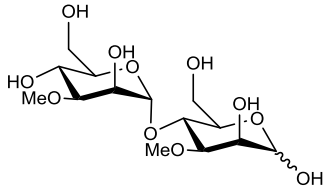
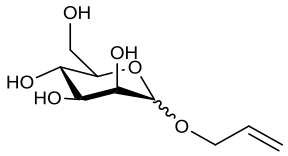
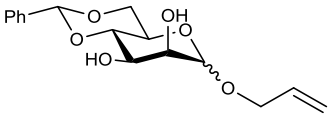
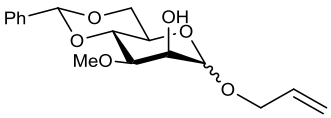
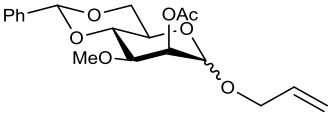
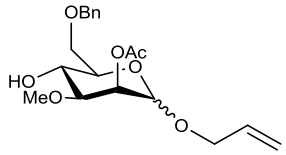
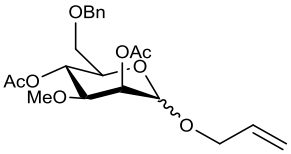
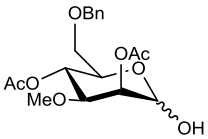
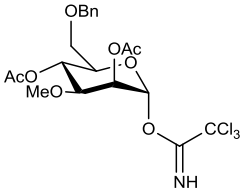
TMSOTf: distilled at atmospheric pressure.

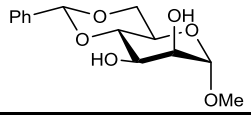
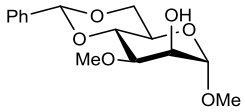
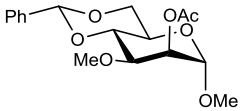
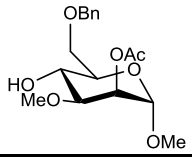
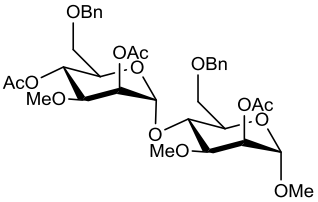
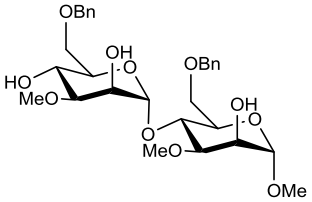
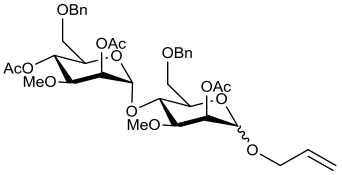
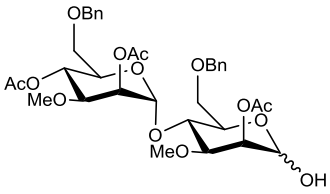
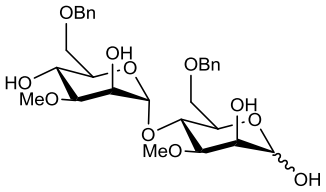
Dry Toluene: distilled at atmospheric pressure using sodium as drying agent, and stored with sodium wire.

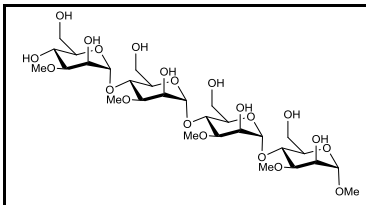
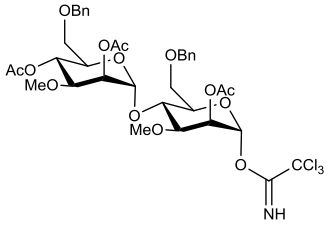
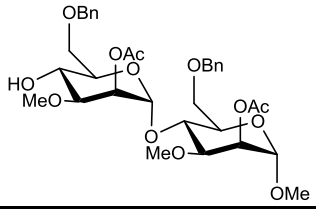
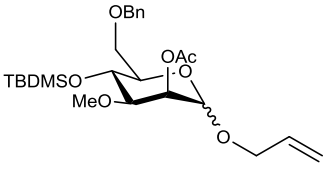
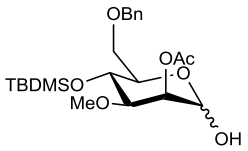
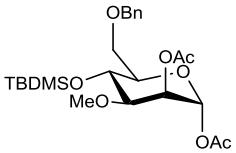
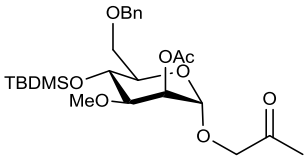
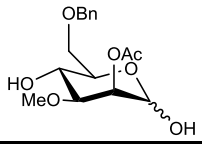
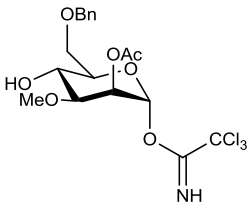
Trichloroacetonitrile: distilled at atmospheric pressure.

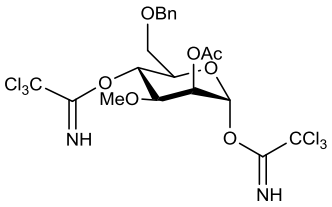
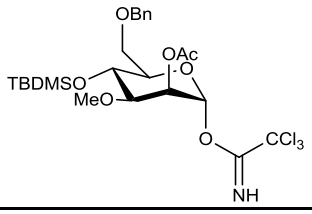
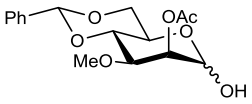
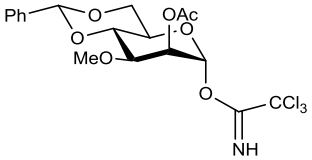
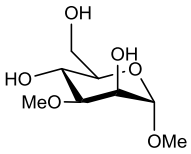
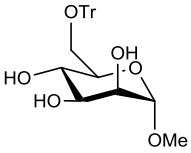
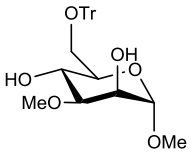
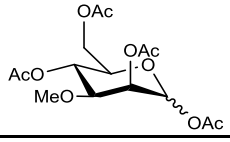
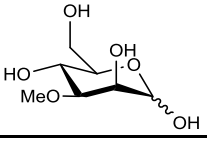
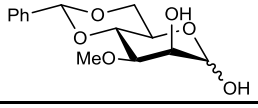
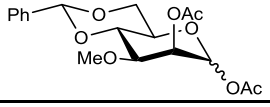
4.3 Compound list

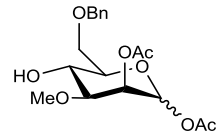
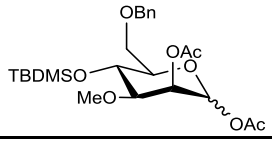
Table 4.1: Summary table of the synthesized compounds**.**

Compound	Nº	Name	Exp.	Page
	**1**	**Methyl (3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-α-D-mannopyranoside**	15	75
	**2**	**(3-*O*-Methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-(α/β)-D-mannopyranose**	19	79
	**3**	**Allyl (α/β)-D-mannopyranoside**	1	65
	**4**	**Allyl 4,6-*O*-benzylidene-(α/β)-D-mannopyranoside**	2	65
	**6**	**Allyl 4,6-*O*-benzylidene-3-*O*-methyl-(α/β)-D-mannopyranoside**	3	66
	**7**	**Allyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-O-methyl-(α/β)-D-mannopyranoside**	4	67
	**8**	**Allyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside**	5	68
	**9**	**Allyl 2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside**	6	69
	**10**	**2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose**	7	70
	**11**	**(2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate**	8	70

	**12**	**Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside**	9	71
	**14**	**Methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside**	10	72
	**15**	**Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside**	11	72
	**16**	**Methyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside**	12	73
	**17**	**Methyl (2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside**	13	73
	**18**	**Methyl (6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside**	14	74
	**19**	**Allyl (2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside**	16	76
	**20**	**(2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose**	17	77
	**21**	**(6-*O*-Benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose**	18	78

	**22**	**Methyl 3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranosyl-(1→4)-3-*O*-methyl-α-D-mannopyranoside**	-	-
	**23**	**(2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-manno-pyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate**	-	-
	**24**	**Methyl (2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-manno-pyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside**	30	87
	**25**	**Allyl 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranoside**	20	79
	**26**	**2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose**	21 22 23 24 26	80 81 82 84
	**27**	**1,2-di-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-α-D-mannopyranose**	25	82
	**28**	**1-(2-Oxopropyl)-2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-α-D-mannopyranoside**	25	82
	**29**	**2-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose**	27	84
	**30**	**(2-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-1-*O*-α-D-manno-pyranosyl)-trichloroace-timidate**	28	85

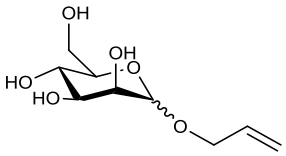
	**31**	**(2-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-1,4-*O*-α-D-mannopyranosyl)-di-trichloroacetimidate**	28	85
	**32**	**(2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate**	29	86
	**33**	**2-*O*-Acetyl-4,6-*O*-benzylidene-3-*O*-methyl-(α/β)-D-mannopyranose**	31	87
	**34**	**(2-*O*-Acetyl-4,6-*O*-benzylidene-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate**	32	88
	**35**	**Methyl 3-*O*-methyl-α-D-mannopyranoside**	33 34	89
	**36**	**Methyl 6-*O*-trityl-α-D-mannopyranoside**	35	90
	**37**	**Methyl 3-*O*-methyl-6-*O*-trityl-α-D-mannopyranoside**	36	90
	**38**	**1,2,4,6-Tetra-*O*-acetyl-3-*O*-methyl-(α/β)-D-mannopyranose**	37	91
	**39**	**3-*O*-Methyl-(α/β)-D-mannopyranose**	-	-
	**40**	**4,6-*O*-Benzylidene-3-*O*-methyl-(α/β)-D-mannopyranose**	-	-
	**41**	**1,2-di-*O*-Acetyl-4,6-*O*-benzylidene-3-*O*-methyl-(α/β)-D-mannopyranose**	-	-

	**42**	**1,2-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose**	-	-
	**43**	**1,2-di-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose**	-	-

4.4 Experimental Procedures

Experiment 1:

Allyl (α/β)-D-mannopyranoside 3



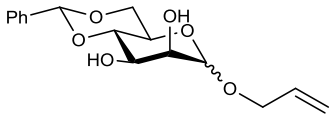
D-mannose (7.00 g, 0.039 mol) was dissolved in distilled allyl alcohol (46.7 mL, 0.687 mol). Then, camphorsulfonic acid was added (46.7 mg, 0.2 mmol). The mixture was refluxed and stirred overnight. TLC (9:1 dichloromethane-methanol) indicated that the reaction was completed. The solvent was evaporated over vacuum until dryness was achieved, and the mixture was concentrated. The reaction crude was applied to a column of silica gel (flash column chromatography) which was eluted with 9:1 dichloromethane-methanol to give **3** (7.57 g, 94%, α/β > 10:1), a colourless oil.

v_{max}/cm^{-1}: 3383.79 (O-H), 1647.0 (C=C), 1060.48 (C-O)

NMR data for the α-anomer (major anomer) in accordance to those described in the literature.[23]

Experiment 2:

Allyl 4,6-*O*-benzylidene-(α/β)-D-mannopyranoside 4



To a solution of **3** (7.57 g, 0.037 mol) in dry THF (25 mL), benzaldehyde dimethyl acetal (11.1 mL, 0.074 mol) and camphorsulfonic acid, in a catalytic amount, were added. The mixture was stirred and refluxed for 4 hours and 30 minutes. TLC (3:7 hexane-ethyl acetate) indicated that the reaction was completed. The mixture was neutralized and washed with an aqueous solution of sodium hydrogen carbonate (saturated) and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4, filtered and concentrated. Purification by recrystallization (9:1 hexane-ethyl acetate) afforded **4** (6.33 g, 59 %, α/β 5:1) as a white solid (Melting point: 148 ºC).

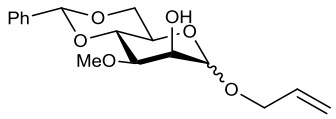
v_{max}/cm^{-1}: 3384.58 (O-H), 1647.06 (C=C), 1094.67-1027.72 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.51-7.48 (m, Ar), 7.41-7.35 (m, Ar), 5.98-5.86 (m, -$OCH_2C\underline{H}=CH_2$), 5.58 (s, -OC<u>H</u>Ph), 5.31 (dd, $^{3}J_{H\text{-}H}$ = 17.21 Hz, $^{2}J_{H\text{-}H}$ = 1.57 Hz, -$OCH_2CH=CH_{cis}\underline{H}_{trans}$), 5.23 (dd, $^{3}J_{H\text{-}H}$ = 10.34 Hz, $^{2}J_{H\text{-}H}$ = 1.36 Hz, -$OCH_2CH=C\underline{H}_{cis}H_{trans}$), 4.93 (1H, d, $^{3}J_{H\text{-}H}$ = 1.07 Hz, H-1_{α}), 4.64 (1H, d, $^{3}J_{H\text{-}H}$ = 1.01 Hz, H-1_{β}), 4.25, (dd, $^{2}J_{H\text{-}H}$ = 12.88 Hz, $^{3}J_{H\text{-}H}$ = 5.21 Hz, H-6_a), 4.18 (dd, $^{2}J_{H\text{-}H}$ = 12.60 Hz, $^{3}J_{H\text{-}H}$ = 4.39 Hz, -$OC\underline{H}_aH_bCH=CH_2$), 4.13-4.03 (m, H-2 and -$OCH_a\underline{H}_bCH=CH_2$), 4.03-3.77 (m, H-3,4,5,6_b), 2.97 (br s, -OH).

^{13}C-NMR ($CDCl_3$): δ 133.51 (-$OCH_2\underline{C}H=CH_2$), 129.01, 128.34 and 126.31 (Ar), 117.80 (-$OCH_2CH=\underline{C}H_2$), 102.22 (-O<u>C</u>HPh), 99.54 (C-1), 78.86 (C-4), 71.01 (C-2), 68.67 (C-3), 68.79 and 68.26 (-$O\underline{C}H_2CH=CH_2$ and C-6), 63.26 (C-5).

Experiment 3:

Allyl 4,6-*O*-benzylidene-3-*O*-methyl-(α/β)-D-mannopyranoside 6



To a solution of **4** (5.60 g, 0.019 mol) in dry methanol (25 mL), dibutyltin oxide (5.45 g, 0.022 mol) was added. The mixture was stirred and boiled under reflux for 3 hours. After 3 hours, the solvent was evaporated under vacuum and the mixture was dried, using a vacuum pressure pump. The reaction crude was dissolved in dry DMF (35 mL) and iodomethane (5.90 mL, 0.094 mol) was added. The mixture was heated at 50 ºC and stirred overnight. TLC (2:3 hexane-ethyl acetate) indicated that the reaction was completed. The solvent was first evaporated under vacuum until dryness was achieved. The mixture was dissolved in ethyl acetate and filtered. The solvent of the filtrate was removed under vacuum and the reaction mixture was purified. Purification by flash column chromatography, (eluent from 7:3 hexane-ethyl acetate to 1:1 hexane-ethyl acetate) afforded **6** (4.93 g, 80%, α/β > 10:1) as a yellowish oil.

ν_{max}/cm^{-1}: 3461.67 (O-H), 1646.98 (C=C), 1093.78-1034.83 (C-O)

NMR data for the α-anomer (major anomer):

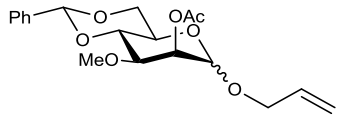
^{1}H-NMR ($CDCl_3$): δ 7.55-7.44 (2H, m, Ar), 7.42-7.29 (3H, m, Ar), 5.97-5.86 (1H, m, -$OCH_2C\underline{H}=CH_2$), 5.59 (1H, s, -O<u>C</u>HPh), 5.31 (1H, d, $^{3}J_{H\text{-}H}$ = 17.20 Hz, $^{2}J_{H\text{-}H}$ = 1.56 Hz, -$OCH_2CH=CH_{cis}\underline{H}_{trans}$), 5.23 (1H, d, $^{3}J_{H\text{-}H}$ = 10.37 Hz , $^{2}J_{H\text{-}H}$ = 1.36 Hz -$OCH_2CH=C\underline{H}_{cis}H_{trans}$), 4.94 (1H, s, $^{3}J_{H\text{-}H}$ = 1.25 Hz, H-1), 4.27 (1H, dd, $^{2}J_{H\text{-}H}$ = 8.78 Hz, $^{3}J_{H\text{-}H}$ = 3.01 Hz, H-6_a), 4.21 (1H, dd, $^{2}J_{H\text{-}H}$ = 12.88 Hz, $^{3}J_{H\text{-}H}$ = 5.23 Hz, -$OC\underline{H}_aH_bCH=CH_2$), 4.14-4.11 (1H, m, H-2), 4.05-

3.98 (2H, m, H-4 and -$OCH_a\underline{H}_bCH=CH_2$), 3.91-3.81 (2H, m, H-5 and H-6_b), 3.71 (1H, dd, $^3J_{H\text{-}H}$ = 9.52, $^3J_{H\text{-}H}$ = 3.41 Hz, H-3), 3.56 (3H, s, -$OC\underline{H}_3$), 2.58 (1H, s, -OH).

^{13}C-NMR ($CDCl_3$): δ 133.47 (-$OCH_2\underline{C}H=CH_2$), 129.01, 128.24 and 126.15 (Ar), 117.96 (-$OCH_2CH=\underline{C}H_2$), 101.79 (-O<u>C</u>HPh), 99.15 (C-1), 78.73 (C-4), 77.30 (C-3), 69.18 (C-2), 68.86 and 68.25 (-$O\underline{C}H_2CH=CH_2$ and C-6), 63.30 (C-5), 58.65 (-$O\underline{C}H_3$).

Experiment 4:

Allyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-O-methyl-(α/β)-D-mannopyranoside 7



To a solution of **6** (6.00 g, 0.019 mol) in dry pyridine (40 mL) at 0 ºC, distilled acetic anhydride (2.17 mL, 0.022 mol) and a catalytic amount of DMAP were added. The mixture was stirred at 0ºC for 5 minutes, allowed to warm room temperature and stirred for 2 hours. TLC (7:3 hexane-ethyl acetate) indicated that the reaction was completed. The mixture was washed and neutralized with water, and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4 and filtered. Ethyl acetate and pyridine were evaporated. Purification of the reaction crude, by flash column chromatography (7:3 hexane-ethyl acetate), afforded **7** (6.20 g, 91%, α/β > 10:1) as a colourless oil.

ν_{max}/cm^{-1}: 1746.45 (C=O), 1646.97 (C=C), 1091.51-1028.98 (C-O)

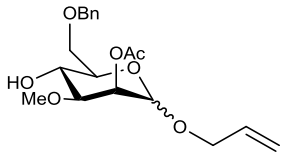
NMR data for the α-anomer (major anomer):

^{1}H-NMR ($CDCl_3$): δ 7.55-7.45 (2H, m, Ar), 7.40-7.31 (3H, m, Ar), 5.97-5.85 (1H, m, -$OCH_2C\underline{H}=CH_2$), 5.61 (1H, s, -O<u>C</u>HPh), 5.38 (1H, dd, $^3J_{H\text{-}H}$ = 3.39 Hz, $^3J_{H\text{-}H}$ = 1.49 Hz, H-2), 5.32 (1H, dd, $^3J_{H\text{-}H}$ = 17.2 Hz, $^2J_{H\text{-}H}$ = 1.38 Hz, -$OCH_2CH=CH_{cis}\underline{H}_{trans}$), 5.25 (1H, dd, $^3J_{H\text{-}H}$ = 10.39 Hz , $^2J_{H\text{-}H}$ = 0.95 Hz, -$OCH_2CH=C\underline{H}_{cis}H_{trans}$), 4.84 (1H, d, $^3J_{H\text{-}H}$ = 1.12 Hz, H-1), 4.27 (1H, dd, $^2J_{H\text{-}H}$ = 9.55 Hz, $^3J_{H\text{-}H}$ = 4.02 Hz, H-6_a), 4.19 (1 H, dd, $^2J_{H\text{-}H}$ = 12.74 Hz, $^3J_{H\text{-}H}$ = 5.31 Hz, -$OC\underline{H}_aH_bCH=CH_2$), 4.05-3.97 (2 H, m, H-4 and -$OCH_a\underline{H}_bCH=CH_2$), 3.90 (1H, ddd, $^3J_{H\text{-}H}$ = 10.12 Hz, $^3J_{H\text{-}H}$ = 10.12 Hz, $^3J_{H\text{-}H}$ = 4.37 Hz, H-5), 3.86-3.79 (2 H, m, H-3,6_b), 3.46 (3H, s, -$OC\underline{H}_3$), 2.16 (3H, s, -$OCOC\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 170.20 (-$O\underline{C}OCH_3$), 133.30 (-$OCH_2\underline{C}H=CH_2$), 129.01, 128.27 and 126.19 (Ar), 118.17 (-$OCH_2CH=\underline{C}H_2$), 101.86 (-O<u>C</u>HPh), 97.83 (C-1), 78.55 (C-4), 75.82 (C-3), 69.30 (C-2), 68.74 and 68.46 (-$O\underline{C}H_2CH=CH_2$ and C-6), 63.87 (C-5), 58.42 (-$O\underline{C}H_3$), 20.99 (-$OCO\underline{C}H_3$).

Experiment 5:

Allyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside 8



To a solution of **7** (2.74 g, 0.008 mol) in dry THF (25 mL) at 0 ºC, sodium cyanoborohydride (5.67 g, 0.090 mol) was added. The mixture was stirred at 0 ºC, and a solution of hydrogen chloride in dry diethyl ether 1 M (32 mL) was added portionwise (1 mL per portion) until the reaction was completed (TLC 6:4 hexane-ethyl acetate). The mixture was evaporated under vacuum, redissolved in water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4 and filtered. Purification by flash column chromatography (eluent from 7:3 hexane-ethyl acetate to 1:1 hexane-ethyl acetate) afforded **8** as a colourless oil (2.25 g, 81%, α/β 5:1).

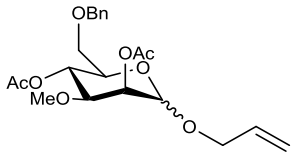
ν_{max}/cm^{-1}: 3467.07 (O-H), 1744.5 (C=O), 1646.98 (C=C), 1045.41 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.40-7.27 (m, Ar), 5.96-5.81 (m, -$OCH_2C\underline{H}=CH_2$), 5.33-5.24 (m, -$OCH_2CH=CH_a\underline{H}_b$ and H-2), 5.21 (dd, $^{3}J_{H\text{-}H}$ = 10.33 Hz, $^{2}J_{H\text{-}H}$ = 1.29 Hz, -$OCH_2CH=C\underline{H}_aH_b$), 4.87 (1H, d, $^{3}J_{H\text{-}H}$ = 1.56 Hz, H-1_α), 4.83 (1H, d, $^{3}J_{H\text{-}H}$ = 1.58 Hz, H-1_β), 4.66 (d, $^{2}J_{H\text{-}H}$ = 12.11 Hz, -$OC\underline{H}_aH_bPh$) , 4.58 (d, $^{2}J_{H\text{-}H}$ = 12.11 Hz, -$OCH_a\underline{H}_bPh$), 4.19 (dd, $^{2}J_{H\text{-}H}$ = 12.82 Hz, $^{3}J_{H\text{-}H}$ = 5.22 Hz, -$OC\underline{H}_aH_bCH=CH_2$), 4.00 (dd, $^{2}J_{H\text{-}H}$ = 12.87 Hz, $^{3}J_{H\text{-}H}$ = 6.22 Hz, -$OCH_a\underline{H}_bCH=CH_2$), 3.87 (dd, $^{3}J_{H\text{-}H}$ = 19.97 Hz, $^{3}J_{H\text{-}H}$ = 11.63 Hz, H-4) 3.82-3.73 (m, H-3_β,5, 6_a, 6_b), 3.57 (1H, dd, $^{3}J_{H\text{-}H}$ = 9.47 Hz, $^{3}J_{H\text{-}H}$ = 3.02 Hz, H-3_α), 3.44 (3H, s, -$OC\underline{H}_3$ β anomer), 3.42 (3H, s, -$OC\underline{H}_3$ α anomer), 2.15 (3H, s, -$OCOC\underline{H}_3$ β anomer), 2.11 (3H, s, -$OCOC\underline{H}_3$ α anomer).

^{13}C-NMR ($CDCl_3$): δ 170.36 (-$O\underline{C}OCH_3$), 133.40 (-$OCH_2\underline{C}H=CH_2$), 128.37, 127.64 and 127.56 (Ar), 117.94 (-$OCH_2CH=\underline{C}H_2$), 97.05 (C-1_α), 96.90 (C-1_β), 80.00 (C-3_β), 79.30 (C-3_α), 73.55 (-$O\underline{C}H_2Ph$), 71.13 (C-5_α), 71.06 (C-5_β), 69.87 (C-6'), 68.26 (-$O\underline{C}H_2CH=CH_2$), 67.56 and 67.41 (C-4 and C-2), 57.44 (-$O\underline{C}H_3$), 20.97 (-$OCO\underline{C}H_3$).

Experiment 6:

Allyl 2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside 9



To a solution of **8** (1.80 g, 0.005 mol) in dry pyridine (15 mL) at 0 ºC, distilled acetic anhydride (0.955 mL, 0.009 mol) and a catalytic amount of DMAP were added. The mixture was stirred at 0ºC for 5 minutes, allowed to warm room temperature and stirred for 1 hour and 30 minutes. TLC (7:3 hexane-ethyl acetate) indicated that the reaction was completed. The mixture was washed and neutralized with water, and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4 and filtered. Ethyl acetate and pyridine were evaporated. Purification of the reaction crude, by flash column chromatography (7:3 hexane-ethyl acetate), afforded **9** (1.76 g, 88 %, α/β 8:1) as a colourless oil.

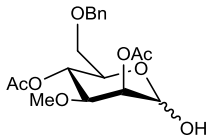
ν_{max}/cm^{-1}: 1743.29 (C=O), 1647.14 (C=C), 1040.02 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.35-7.27 (m, Ar), 5.96-5.85 (m, -$OCH_2C\underline{H}=CH_2$), 5.38-5.27 (m, -$OCH_2CH=C\underline{H}_aH_b$ and H-2), 5.25-5.15 (m, -$OCH_2CH=CH_a\underline{H}_b$ and H-4), 4.88 (1H, d, $^3J_{H\text{-}H}$ = 1.52 Hz, H-1_α), 4.85 (1H, d, $^3J_{H\text{-}H}$ = 1.51 Hz, H-1_β), 4.55 (2H, ABdd, $^2J_{H\text{-}H}$ = 11.92 Hz, -$OC\underline{H}_2Ph$), 4.21 (dd, $^2J_{H\text{-}H}$ = 12.91, $^3J_{H\text{-}H}$ = 5.28 Hz, -$OC\underline{H}_aH_bCH=CH_2$), 4.02 (dd, $^2J_{H\text{-}H}$ = 12.8, $^3J_{H\text{-}H}$ = 6.2 Hz, -$OCH_a\underline{H}_bCH=CH_2$), 3.93 – 3.87 (m, H-5), 3.67 (dd, $^3J_{H\text{-}H}$ = 9.75, $^3J_{H\text{-}H}$ = 3.40 Hz, H-3), 3.60 – 3.52 (m, H-6_a and H-6_b), 3.45 (3H, s, -$OC\underline{H}_3$ β anomer) 3.35 (3H, s, -$OC\underline{H}_3$ α anomer), 2.15 (3H, s, -$OCOC\underline{H}_3$ β anomer), 2.13 (3H, s, -$OCOC\underline{H}_3$ α anomer), 2.05 (3H, s, -$OCOC\underline{H}_3$ β anomer), 1.99 (3H, s, -$OCOC\underline{H}_3$ α anomer).

^{13}C-NMR ($CDCl_3$): δ 170.42 and 169.96 (-$O\underline{C}OCH_3$), 133.33 (-$OCH_2\underline{C}H=CH_2$), 128.30, 127.76 and 127.61 (Ar), 118.07 (-$OCH_2CH=\underline{C}H_2$), 96.76 (C-1_α), 96.42 (C-1_β), 77.04 (C-3), 73.54 (-$O\underline{C}H_2Ph$), 70.01 (C-5), 69.45 (C-6), 68.40 ($O\underline{C}H_2CH=CH_2$), 68.38 (C-4), 67.87 (C-2) 57.68 (-$O\underline{C}H_3$ α anomer), 57.51 (-$O\underline{C}H_3$ β anomer), 21.01 and 20.90 (-$OCO\underline{C}H_3$).

Experiment 7:

2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose 10



To a solution of **9** (1.76 g, 0.004 mol) in dry methanol (15 mL), palladium (II) chloride (0.153 g, 0.860 mmol) was added. The mixture was stirred at room temperature for 2 hours. TLC (3:2 hexane-ethyl acetate) indicated that the reaction was completed. The mixture was filtered through Celite, while washed with methanol. The filtrate was evaporated under vacuum. Purification of the reaction crude by flash column chromatography (eluent from 7:3 hexane-ethyl acetate to 1:1 hexane-ethyl acetate), afforded **10** (1.20 g, 75 %, α/β > 10:1) as a colourless oil.

ν_{max}/cm^{-1}: 3419.51 (O-H), 1743.65 (C=O), 1054.09 (C-O)

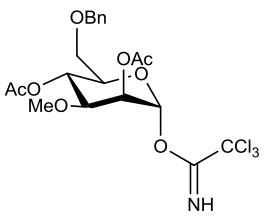
NMR data for the α-anomer (major anomer):

^{1}H-NMR ($CDCl_3$): δ 7.37-7.27 (5H, m, Ar), 5.34 (1H, dd, $^{3}J_{H-H}$ = 3.17, $^{3}J_{H-H}$ = 1.97 Hz, H-2), 5.24 (1H, d, $^{3}J_{H-H}$ = 1.51 Hz, H-1), 5.12 (1H, t, $^{3}J_{H-H}$ = 9.94 Hz, H-4), 4.55 (2H, s, -OC$\underline{H}_2$Ph), 4.15-4.09 (1H, m, H-5), 3.71 (1H, dd, $^{3}J_{H-H}$ = 9.73, $^{3}J_{H-H}$ = 3.32 Hz, H-3), 3.60 – 3.48 (2H, m, H-6_a and H-6_b), 3.35 (3H, s, -OC$\underline{H}_3$), 2.13 (3H, s, -OCOC$\underline{H}_3$) , 2.00 (3H, s, -OCOC$\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 170.42 and 170.09 (-O$\underline{C}$OCH$_3$), 128.39, 128.04 and 127.80 (Ar), 92.40 (C-1), 76.48 (C-3), 73.64 (-O$\underline{C}$H$_2$Ph), 69.89 (C-5), 69.62 (C-6), 68.36 (C-4), 68.05 (C-2), 57.71 (-O$\underline{C}$H$_3$), 21.03 and 20.90 (-OCO$\underline{C}$H$_3$).

Experiment 8:

(2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate 11



To a solution of **10** (1.20 g, 0.003 mol) in dry dichloromethane (15 mL) at 0ºC, distilled DBU (0.214 mL, 1.40 mmol) and distilled trichloroacetonitrile (1.63 ml, 0.016 mol) were added sequentially. The mixture was stirred for 10 minutes at 0ºC, allowed to warm room temperature and stirred for 2 h. TLC (7:3 hexane-ethyl acetate) indicated that the reaction was completed. The solvent was evaporated over vacuum. Purification of the reaction crude by flash column chromatography (eluent from 7:3 hexane-ethyl acetate to 3:2 hexane-ethyl acetate) afforded **11** (1.27 g, 76 %) as a colourless oil.

$[\alpha]_D^{20}$ +38.8 (c 0.95, CH_2Cl_2)

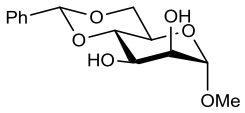
ν_{max}/cm^{-1}: 3316.9 (N-H), 1748.9 (C=O), 1045.4 (C-O).

^{1}H-NMR ($CDCl_3$): δ 8.76 (1H, s, -OC(N<u>H</u>)CCl_3), 7.36 – 7.27 (5H, m, Ar), 6.30 (1H, d, $^3J_{H-H}$ = 1.89 Hz, H-1), 5.52 (1H, dd, $^3J_{H-H}$ = 3.20, $^3J_{H-H}$ = 2.14 Hz, H-2), 5.32 (1H, t, $^3J_{H-H}$ = 10.01 Hz, H-4), 4.53 (2H, ABdd, $^2J_{H-H}$ = 11.88 Hz, -OC<u>H</u>$_2$Ph), 4.11 (1H, m, H-5), 3.72 (1H, dd, $^3J_{H-H}$ = 9.81, $^3J_{H-H}$ = 3.36 Hz, H-3), 3.59 (2H, d, $^3J_{H-H}$ = 4.11 Hz, H-6_a e H-6_b), 3.38 (3H, s, -OC<u>H</u>$_3$), 2.17 (3H, s, -OCOC<u>H</u>$_3$), 2.00 (3H, s, -OCOC<u>H</u>$_3$).

^{13}C-NMR ($CDCl_3$): δ 170.76, 169.98 and 168.07 (-O<u>C</u>OCH_3 and -O<u>C</u>(NH)CCl_3), 128.30, 127.85, 127.64 (Ar), 94.98 (C-1), 76.80 (C-3), 73.50 (-O<u>C</u>H_2Ph), 72.73 (C-5), 69.03 (C-6), 67.66 (C-4), 66.24 (C-2), 57.92 (-O<u>C</u>H_3), 20.90 and 20.88 (-OCO<u>C</u>H_3).

<u>Experiment 9</u>:

Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside 12

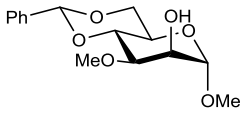


The procedure of Experiment 2 was applied to α-methyl-D-mannose (5.00 g, 0.026 mol), with the reaction time increased to overnight. TLC (2:3 hexane-ethyl acetate) indicated that the reaction was finished. Purification by recrystallization (9:1 hexane-ethyl acetate) afforded **12** (3.60 g, 50%) as a white solid.

IR and NMR data in accordance to those described in the literature.[24]

Experiment 10:

Methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside 14



The procedure of Experiment 3 was applied to compound **12** (3.60 g, 0.013 mol) with the first step reaction time increased to overnight, and in the second step the temperature was increased to 65ºC. TLC (2:3 hexane-ethyl acetate) indicated that the reaction was completed. Purification of the reaction crude, by flash column chromatography (eluent from 7:3 hexane-ethyl acetate to 1:1 hexane-ethyl acetate) afforded **14** (1.75 g, 50 %) as a yellowish oil.

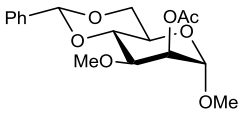
v_{max}/cm^{-1}: 3461.64 (O-H), 1095.18-1055.60 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.52-7.46 (2H, m, Ar), 7.38-7.33 (3H, m, Ar), 5.59 (1H, s, -OC<u>H</u>Ph), 4.79 (1H, s, $^{3}J_{H-H}$ = 0.94 Hz, H-1), 4.28 (1H, dd, $^{2}J_{H-H}$ = 9.19 Hz, $^{3}J_{H-H}$ = 3.54 Hz, H-6_a), 4.12-4.09 (1H, m, H-2), 4.00 (1H, t, $^{3}J_{H-H}$ = 9.28 Hz, H-4), 3.88-3.80 (2H, m, H-5 and H-6_b), 3.67, (1H, dd, $^{3}J_{H-H}$ = 7.47, $^{3}J_{H-H}$ = 2.03 Hz, H-3), 3.55 (3H, s, -OC<u>H</u>$_3$), 3.40 (3H, s, -OC<u>H</u>$_3$).

^{13}C-NMR ($CDCl_3$): δ 129.00, 128.24 and 126.17 (Ar), 101.83 (-O<u>C</u>HPh), 101.05 (C-1), 78.66 (C-4), 77.31 (C-3), 69.06 (C-2), 68.91 (C-6), 63.11 (C-5), 58.61 and 55.05 (-O<u>C</u>H_3).

Experiment 11:

Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside 15



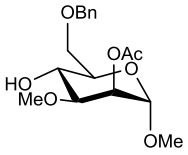
The procedure of Experiment 4 was applied to compound **14** (1.75 g, 5.90 mmol). TLC (7:3 hexane-ethyl acetate) indicated that the reaction was completed. Purification of the reaction mixture by flash column chromatography (7:3 hexane-ethyl acetate) afforded **15** (1.93 g, 97 %) as a colourless oil.

v_{max}/cm^{-1}: 3465.09 (O-H), 1747.32 (C=O), 1094.27-1060.10 (C-O)

NMR data in accordance to those described in the literature. [25]

Experiment 12:

Methyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 16



The procedure of Experiment 5 was applied to compound **15** (1.93 g, 5.70 mmol), with a solution of hydrogen chloride in dry diethyl ether 1 M (25 mL) being added portionwise (1 mL per portion). TLC (7:3 hexane-ethyl acetate), indicated that the reaction was completed. Purification of the reaction mixture by flash column chromatography (eluent from 7:3 hexane-ethyl acetate to 1:1 hexane-ethyl acetate) afforded **16** (1.94 g, 100 %) as a colourless oil.

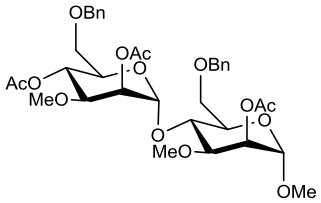
ν_{max}/cm^{-1}: 3446.2 (O-H), 1748.3-1724.8 (C=O), 1076.1 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.39-7.30 (5H, m, Ar), 5.31 (1H, dd, $^{3}J_{H-H}$ = 3.08, $^{3}J_{H-H}$ = 1.83 Hz, H-2), 4.72 (1H, d, $^{3}J_{H-H}$ = 1.66 Hz, H-1), 4.66 (1H, d, $^{2}J_{H-H}$ = 11.89 Hz, -OC$\underline{H}_a$H$_b$Ph), 4.59 (1H, d, $^{3}J_{H-H}$ = 11.90 Hz, -OCH$_a$$\underline{H}_b$Ph), 3.86 (1H, t, $^{3}J_{H-H}$ = 9.08 Hz, H-4), 3.81-3.69 (3H, m, H-5,6$_a$,6$_b$), 3.54 (1H, dd, $^{3}J_{H-H}$ = 9.38, $^{3}J_{H-H}$ = 3.21 Hz, H-3), 3.40 (3H, s, -OC$\underline{H}_3$), 3.39 (3H, s, -OC$\underline{H}_3$), 2.13 (3H, s, -OCOC$\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 170.41 (-O$\underline{C}$OCH$_3$), 128.60, 128.10 and 127.90 (Ar), 98.98 (C-1), 78.92 (C-3), 73.87 (-O$\underline{C}$H$_2$Ph), 70.21 (C-6), 70.11 (C-5), 68.25 (C-4), 67.02 (C-2), 57.04 and 55.29 (-O$\underline{C}$H$_3$), 20.90 (-OCO$\underline{C}$H$_3$).

Experiment 13:

Methyl (2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 17



To a solution of **11** (0.200 g, 0.391 mmol) and **16** (0.133 g, 0.391 mmol) in dry dichloromethane (4 mL), finely powdered molecular sieves (4 Å) were first added. The solution was stirred for 30 minutes at room temperature. At -20 ºC, distilled TMSOTf (71 μL, 0.391 mmol) was added and

the mixture was stirred at this temperature for another 30 minutes. TLC (3:2 hexane-ethyl acetate) indicated that the reaction was finished. The mixture was neutralized and washed with an aqueous solution of sodium hydrogen carbonate (saturated) and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered and concentrated. Purification by silica preparative TLC (3:2 hexane-ethyl acetate) afforded **17** (0.187 g, 69 %) as a colourless oil.

$[\alpha]_D^{20}$ +50.4 (c 1.04, CH_2Cl_2)

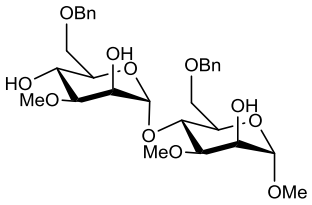
ν_{max}/cm^{-1}: 1746.15 (C=O), 1044.43 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.34-7.27 (10H, m, Ar), 5.37 (1H, dd, $^3J_{H-H}$ = 2.94 Hz, $^3J_{H-H}$ = 2.15 Hz, H-2_A), 5.29 (1H, dd, $^3J_{H-H}$ = 3.21 Hz, $^3J_{H-H}$ = 1.85 Hz, H-2_B), 5.23 (1H, d, $^3J_{H-H}$ = 1.75 Hz, H-1_A), 5.17 (1H, t, $^3J_{H-H}$ = 9.96 Hz, H-4_A), 4.71 (1H, d, $^3J_{H-H}$ = 1.62 Hz, H-1_B), 4.61-4.40 (4H, m, -OC$\underline{H}_2$Ph$_A$ and OC$\underline{H}_2$Ph$_B$), 3.91-3.83 (2H, m, H-4_B,5_A), 3.83-3.71 (3H, m, H-5_B,6_B,$6'_B$), 3.63 (1H, dd, $^3J_{H-H}$ = 9.09, $^3J_{H-H}$ = 3.30 Hz, H-3_B), 3.56 (1H, dd, $^3J_{H-H}$ = 9.77, $^3J_{H-H}$ = 3.23 Hz, H-3_A), 3.48-3.40 (5H, m, H-6_A, H-$6'_A$ and -OC$\underline{H}_3$), 3.39 (3H, s, -OC$\underline{H}_3$), 3.34 (3H, s, -OC$\underline{H}_3$), 2.10 (3H, s, -OCOC$\underline{H}_3$), 2.08 (3H, s, -OCOC$\underline{H}_3$), 1.98 (3H, s, -OCOC$\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 170.31, 170.11 and 169.90 (-O$\underline{C}$OCH$_3$), 128.27, 127.81 and 127.38 (Ar), 99.46 (C-1_A), 98.57 (C-1_B), 79.95 (C-3_B), 76.86 (C-3_A), 73.99, 70.85 and 70.73 (C-4_B,C-5_A and C-5_B), 73.53 and 73.29 (-O$\underline{C}$H$_2$Ph$_A$ and -O$\underline{C}$H$_2$Ph$_B$), 69.84 and 69.45 (C-6_A and C-6_B), 68.25 (C-4_A), 67.88 and 67.42 (C-2_A and C-2_B), 57.56, 57.18 and 55.13 (-O$\underline{C}$H$_3$), 21.01, 20.96 and 20.92 (-OCO$\underline{C}$H$_3$).

Experiment 14:

Methyl (6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 18



To a solution of **17** (0.245 g, 0.355 mmol) in dry methanol (1 mL), sodium methoxide (0.023 g, 0.426 mmol) was added. The mixture was stirred for 1 hour and 30 minutes at room temperature. TLC (1:4 hexane-ethyl acetate) indicated that the reaction was not completed. More quantity of sodium methoxide was added (0.011 g, 0.213 mmol) and after 1 h the reaction was completed. The mixture was washed with an aqueous solution of ammonium chloride

(saturated) and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4, filtered and concentrated. Purification by silica preparative TLC (100 % ethyl acetate) afforded **18** (0.196 g, 98 %) as a colourless viscous foam.

$[\alpha]_D^{20}$ +55.4 (c 0.95, CH_2Cl_2)

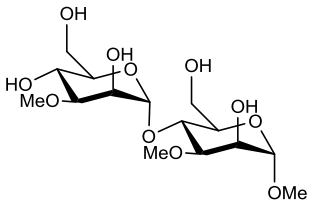
v_{max}/cm^{-1}: 3443.65 (O-H), 1043.25 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.35-7.27 (10 H, m, Ar), 5.30 (1 H, d, $^3J_{H\text{-}H}$ = 1.74 Hz, H-1), 4.81 (1 H, d, $^3J_{H\text{-}H}$ = 1.61 Hz, H-1), 4.61-4.43 (4H, m, -OC$\underline{H}_2$Ph$_A$ and OC$\underline{H}_2$Ph$_B$), 4.07-4.03 (2H, m, H-2,5), 3.93 (1H, t, $^3J_{H\text{-}H}$ = 9.12 Hz, H-4), 3.86 (1H, t, $^3J_{H\text{-}H}$ = 9.35 Hz, H-4), 3.77-3.71 (4H, m, H-2,5,6,6'), 3.63 (1H, dd, $^2J_{H\text{-}H}$ = 10.01 Hz, $^3J_{H\text{-}H}$ = 4.56 Hz, H-6), 3.58 (1H, dd, $^2J_{H\text{-}H}$ = 9.99 Hz, $^3J_{H\text{-}H}$ = 4.90 Hz, H-6'), 3.53 (1H, dd, $^3J_{H\text{-}H}$ = 8.97 Hz, $^3J_{H\text{-}H}$ = 3.34 Hz, H-3), 3.49 (3H, s, -OC$\underline{H}_3$), 3.43 (3H, s, -OC$\underline{H}_3$), 3.40 (3H, s, -OC$\underline{H}_3$), 3.37 (1H, dd, $^3J_{H\text{-}H}$ = 9.10 Hz, $^3J_{H\text{-}H}$ = 3.17 Hz, H-3).

^{13}C-NMR ($CDCl_3$): δ 128.40, 128.35, 127.75 (Ar), 100.47 and 99.03 (C-1$_A$ and C-1$_B$), 80.37 and 77.87 (C-3$_A$ and C-3$_B$), 73.43 and 73.34 (-O$\underline{C}$H$_2$Ph$_A$ and -O$\underline{C}$H$_2$Ph$_B$), 73.23 (C-4), 71.25 and 70.19 (C-2 and C-5), 69.96 and 69.63 (C-6$_A$ and C-6$_B$), 67.55 (C-4), 67.13 and 66.87 (C-2 and C-5), 57.59, 57.25 and 55.24 (-O$\underline{C}$H$_3$).

Experiment 15:

Methyl (3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-α-D-mannopyranoside 1



Compound **18** (0.295 g, 0,523 mmol) in ethyl acetate /ethanol 1:1 (6 mL), was hydrogenated overnight at 50 psi in the presence of Pd/C 10% (0.200 g). The mixture was filtered through Celite, while washed with methanol and water. The filtrate was evaporated under vacuum, which afforded **1** (0.201 g, 100%) as a colourless viscous foam.

$[\alpha]_D^{20}$ +67.5 (c 0.99, H_2O)

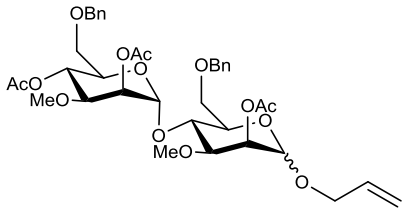
^{1}H-NMR (D_2O): δ 5.13 (1H, d, $^3J_{H\text{-}H}$ = 1.80 Hz, H-1), 4.72 (1H, d, $^3J_{H\text{-}H}$ = 1.73 Hz, H-1), 4.13 (1H, dd, $^3J_{H\text{-}H}$ = 2.86 Hz, $^3J_{H\text{-}H}$ = 2.17 Hz, H-2), 4.09 (1H, dd, $^3J_{H\text{-}H}$ = 3.12 Hz, $^3J_{H\text{-}H}$ = 1.98 Hz, H-2), 3.83-3.58 (8H, m, H-4$_A$,4$_B$,5$_A$,5$_B$, 6$_A$,6'$_A$, 6$_B$,6'$_B$), 3.53 (dd, $^3J_{H\text{-}H}$ = 9.09 Hz, $^3J_{H\text{-}H}$ = 3.26

Hz, H-3), 3.40 (1H, dd, $^{3}J_{H\text{-}H}$ = 9.13 Hz, $^{3}J_{H\text{-}H}$ = 3.09 Hz, H-3) 3.38-3.35 (6H, m, -OC$\underline{H}_3$), 3.33 (3H, s, -OCH_3).

^{13}C-NMR (D_2O): δ 101.39 and 100.66 (C-1_A and C-1_B), 80.99 and 79.72 (C-3_A and C-3_B), 73.73, 72.65, 70.99 and 65.45 (C-4_A, C-4_B , C-5_A, and C-5_B), 66.03 and 65.66 (C-2_A and C-2_B), 60.95 and 60.86 (C-6_A and C-6_B), 56.22, 56.12 and 54.81 (-O$\underline{C}H_3$).

Experiment 16:

Allyl (2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranoside 19



The glycosylation reaction of donor **11** (0.699 g, 1.40 mmol) and acceptor **8** (0.500 g, 1.40 mmol) was performed according to the procedure described in Experiment 13. TLC (3:2 hexane-ethyl acetate) indicated that the reaction was completed. Purification by flash column chromatography (3:2 hexane-ethyl acetate) afforded **19** (0.750 g, 77 %, αα/ αβ 9:1) as a colourless oil.

$[\alpha]_D^{20}$ +35.1 (c 1.05, CH_2Cl_2)

ν_{max}/cm^{-1}: 1746.63 (C=O), 1652.68 (C=C), 1047.17 (C-O)

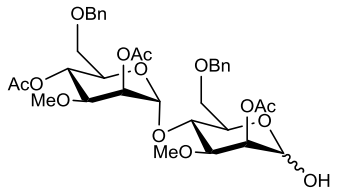
^{1}H-NMR ($CDCl_3$): δ 7.35-7.28 (m, Ar), 5.98-5.87 (m, -$OCH_2C\underline{H}$=CH_2), 5.39-5.37 (m, H-2_A), 5.34-5.28 (m, -OCH_2CH=C$\underline{H}_a$$H_b$ and H-2_B), 5.25-5.21 (m, -OCH_2CH=$CH_a\underline{H}_b$ and H-1_A), 5.17 (t, $^{3}J_{H\text{-}H}$ = 10.01 Hz, H-4_A), 4.86 (1H, d, $^{3}J_{H\text{-}H}$ = 1.21 Hz, H-$1_{B\alpha}$), 4.83 (1H, d, $^{3}J_{H\text{-}H}$ = 1.39 Hz, H-$1_{B\beta}$), 4.60-4.41 (m, -OC$\underline{H}_2$$Ph_A$ and OC$\underline{H}_2$$Ph_B$), 4.21 (dd, $^{2}J_{H\text{-}H}$ = 12.80, $^{3}J_{H\text{-}H}$ = 5.30 Hz, -OC$\underline{H}_a$$H_b$CH=$CH_2$), 4.02 (dd, $^{2}J_{H\text{-}H}$ = 13.02, $^{3}J_{H\text{-}H}$ = 6.19 Hz, -$OCH_a\underline{H}_b$CH=CH_2), 3.92 – 3.77 (m, H-4_B,5_A,5_B,6_B), 3.74 (dd, $^{3}J_{H\text{-}H}$ = 11.10 Hz, $^{3}J_{H\text{-}H}$ = 5.52 Hz, H-6'$_B$), 3.67 (dd, $^{3}J_{H\text{-}H}$ = 8.85, $^{3}J_{H\text{-}H}$ = 3.32 Hz, H-3_B), 3.57 (dd, $^{3}J_{H\text{-}H}$ = 9.77, $^{3}J_{H\text{-}H}$ = 3.12 Hz, H-3_A), 3.49-3.39 (m, H-6_A, H-6'$_A$ and -OC$\underline{H}_3$), 3.34 (s, -OC$\underline{H}_3$), 2.10 (s, -OCOC$\underline{H}_3$), 2.09 (s, -OCOC$\underline{H}_3$), 1.98 (s, -OCOC$\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 170.28, 170.10 and 168.04 (-O$\underline{C}$OCH_3), 133.44 (-$OCH_2\underline{C}H$=CH_2), 128.23, 127.81 and 127.36 (Ar), 118.04 (-OCH_2CH=$\underline{C}H_2$), 99.50 (C-1_A), 96.72 (C-1_B), 79.96 (C-3_B), 76.84 (C-3_A), 74.04, 70.85 and 70.92 (C-4_B, 5_A, 5_B), 73.53 and 73.26 (-O$\underline{C}H_2Ph_A$ and

O<u>C</u>$H_2$$Ph_B$), 69.83 and 69.47 (C-$6_A$ and C-6_B), 68.44 (O<u>C</u>H_2CH=CH_2), 68.28 (C-4_A), 67.69 and 67.56 (C-2_A and C-2_B), 57.57 and 57.19 (-O<u>C</u>H_3), 21.01, 20.95 and 20.92 (-OCO<u>C</u>H_3).

Experiment 17:

(2,4-di-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose 20



The procedure of Experiment 7 was applied to compound **19** (0.750 g, 1.05 mmol). TLC (1:1 hexane-ethyl acetate) indicated that the reaction was completed. Purification by flash column chromatography (eluent from 7:3 hexane-ethyl acetate to 1:1 hexane-ethyl acetate) afforded **20** (0.570 g, 80 %, αα/ αβ > 10:1) as a colourless viscous foam.

$[\alpha]_D^{20}$ +39.8 (c 0.98, CH_2Cl_2)

ν_{max}/cm^{-1}: 3418.6 (O-H),1743.5 (C=O), 1108.85-1044.3 (C-O)

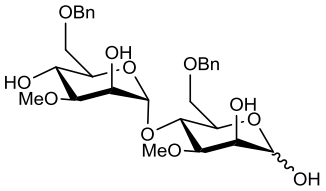
NMR data for the αα-anomer (major anomer):

^{1}H-NMR ($CDCl_3$): δ 7.35-7.27 (10H, m, Ar), 5.38 (1H, dd, $^3J_{H\text{-}H}$= 3.06 Hz, $^3J_{H\text{-}H}$= 2.06 Hz, H-2_A), 5.32 (1H, $^3J_{H\text{-}H}$= 3.09 Hz, $^3J_{H\text{-}H}$= 1.96 Hz, H-2_B), 5.22-5.21 (2H, m, H-1_A and H-1_B), 5.16 (1H, t, $^3J_{H\text{-}H}$ = 9.96 Hz, H-4_A), 4.57-4.42 (4H, m, -OC<u>H</u>$_2$$Ph_A$ and OC<u>H</u>$_2$$Ph_B$), 4.09-4.03 (1H, m, H-$5_B$), 3.85-3.78 (3H, m, H-$4_B$, 5_A,6_B), 3.73 – 3.66 (2H, m, H-3_B,6'$_B$), 3.55 (1H, dd, $^3J_{H\text{-}H}$ = 9.70 Hz, $^3J_{H\text{-}H}$ = 3.25 Hz, H-3_A), 3.44 (1H, dd, $^2J_{H\text{-}H}$ = 10.58 Hz, $^3J_{H\text{-}H}$ = 5.41 Hz, H-6_A), 3.40 (3H, s, -OC<u>H</u>$_3$), 3.38-3.33 (4H, m, H-6' $_A$ and -OC<u>H</u>$_3$), 2.10 (3H, s, -OCOC<u>H</u>$_3$), 2.09 (3H, s, -OCOC<u>H</u>$_3$), 2.00 (3H, s, -OCOC<u>H</u>$_3$).

^{13}C-NMR ($CDCl_3$): δ 170.31, 170.11 and 169.92 (-O<u>C</u>OCH_3), 128.28, 127.82 and 127.59 (Ar), 99.59 and 92.06 (C-1_A and C-1_B), 79.50 (C-3_B), 76.85 (C-3_A), 74.49 and 70.12 (C-4_B and C-5_A), 73.53 and 73.22 (-O<u>C</u>$H_2$$Ph_A$ and -O<u>C</u>$H_2$$Ph_B$), 70.92 (C-$5_B$), 70.09 (C-$6_B$), 69.32 (C-$6_A$), 68.16 (C-$4_A$), 67.67 (C-$2_A$ and C-2_B), 57.55 and 57.16 (-O<u>C</u>H_3), 20.96 and 20.90 (-OCO<u>C</u>H_3).

Experiment 18:

(6-*O*-Benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose 21



To a solution of **20** (0.470 g, 0.069 mmol) in dry methanol (2 mL), sodium methoxide (0.068 g, 1.25 mmol) was added. The mixture was stirred for 2 hours and 30 minutes at room temperature. TLC (100 % ethyl acetate) indicated that the reaction was not completed. More quantity of sodium methoxide was added (0.011 g, 0.213 mmol) and the mixture was stirred for more 4 hours. TLC with the same eluent indicated the reaction was completed. The mixture was diluted with methanol and Dowex-H^+ resin was added until neutral pH. The mixture was filtered and the filtrate concentrated. Purification by flash column chromatography (100 % ethyl acetate) of the filtrate afforded **21** (0.297 g, 78 %, αα/ αβ > 10:1) as a colourless viscous foam.

$[\alpha]_D^{20}$ +51.8 (c 0.95, CH_2Cl_2)

v_{max}/cm^{-1}: 3418.64 (O-H), 1101.36-1049.2 (C-O)

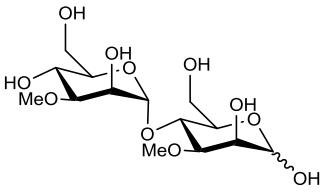
NMR data for the αα-anomer (major anomer):

^{1}H-NMR ($CDCl_3$): δ 7.36-7.27 (10 H, m, Ar), 5.29-5.21 (2 H, m, H-1_A and H-1_B), 4.60-4.43 (4H, m, -OC$\underline{H}_2$Ph$_A$ and OC$\underline{H}_2$Ph$_B$), 4.05 (1H, br s, H-2), 4.03-3.52 (10H, m, H-2,3,4_A,4_B,5_A,5_B, 6_A,$6'_A$, 6_B,$6'_B$), 3.48 (3H, s, -OC$\underline{H}_3$), 3.41 (3H, s, -OC$\underline{H}_3$), 3.35 (1H, dd, $^3J_{H\text{-}H}$ = 9.18 Hz, $^3J_{H\text{-}H}$ = 2.95 Hz, H-3).

^{13}C-NMR ($CDCl_3$): δ 128.43, 128.37, 127.76 (Ar), 101.17 and 93.79 (C-1_A and C-1_B), 81.38 and 80.68 (C-3_A and C-3_B), 73.63 and 73.41 (-O$\underline{C}$H$_2$Ph$_A$ and -O$\underline{C}$H$_2$Ph$_B$), 73.35 (C-4), 71.32 and 70.19 (C-2 and C-5), 70.28 and 69.90 (C-6_A and C-6_B), 67.73 (C-4), 67.06 and 66.99 (C-2 and C-5), 57.20 and 56.62 (-O$\underline{C}$H$_3$).

Experiment 19:

(3-*O*-Methyl-α-D-mannopyranosyl)-(1→4)-3-*O*-methyl-(α/β)-D-mannopyranose 2



Compound **21** (0.280 g, 0.509 mmol) in ethyl acetate/ethanol 5:1 (6 mL), was hydrogenated for 7 hours at 50 psi in the presence of Pd/C 10% (0.100 g). The mixture was filtered through Celite, while washed with methanol and water. The filtrate was evaporated under vacuum affording **2** (0.181 g, 98%, αα/ αβ 2:1) as a colourless viscous foam.

$[\alpha]_D^{20}$ +57.4 (c 0.96, MeOH)

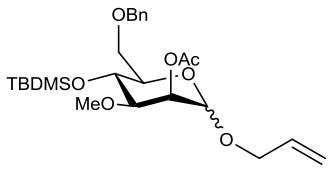
v_{max}/cm^{-1}: 3335.4 (O-H), 1041.2 (C-O)

^{1}H-NMR (D_2O): δ 5.18-5.11 (m, H-1_A and H-$1_{B\alpha}$), 4.81 (br s, H-$1_{B\beta}$), 4.16 (br s, H-2), 4.12 (br s, H-$2_{B\beta}$), 4.08 (br s, H-2), 3.86-3.55 (m, H-3,4_A,4_B,5_A,5_B, 6_A,$6'_A$, 6_B,$6'_B$), 3.51-3.45 (m, H-$3_{B\beta}$), 3.44-3.37 (m, H-3, -OC<u>H</u>$_{3A}$ and –OC<u>H</u>$_{3B}$).

^{13}C-NMR (D_2O): δ 101.36 and 93.78 (C-1_A and C-$1_{B\alpha}$), 93.66 (C-$1_{B\beta}$), 83.21 (C-$3_{B\beta}$), 80.70 and 79.69 (C-3_A and C-$3_{B\alpha}$), 73.70, 72.71, 70.91 and 65.45 (C-4_A, C-4_B , C-5_A, and C-5_B), 66.44 and 66.03 (C-2_A and C-2_B), 60.98 and 60.86 (C-6_A and C-6_B),56.18 and 56.08 (-O<u>C</u>H$_3$).

Experiment 20:

Allyl 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranoside 25



To a solution of **8** (0.475 g, 1.29 mmol) in dry dichloromethane (3 mL) at 0ºC, dry DIPEA (0.633 mL, 3.63 mmol) and TBDMSOTf (0.596 mL, 2.60 mmol) were added sequentially. The mixture was stirred for 20 minutes at 0ºC. TLC (4:1 hexane-ethyl acetate) indicated that the reaction was completed. The mixture was washed with an aqueous solution of sodium hydrogen carbonate (saturated) and extracted with dichloromethane. The organic layer was dried with

Na_2SO_4, filtered and concentrated. Purification of the reaction crude by flash column chromatography (eluent from 100 % hexane to 4:1 hexane-ethyl acetate) afforded **25** (0.560 g, 89 %, α/β 5:1) as a colourless oil.

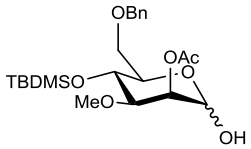
ν_{max}/cm^{-1}:1747.83 (C=O), 1647.34 (C=C), 1107.61-1058.19 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.38-7.27 (m, Ar), 5.97-5.87 (m, -$OCH_2C\underline{H}=CH_2$), 5.35-5.26 (m, -$OCH_2CH=CH_a\underline{H}_b$ and H-2), 5.21 (dd, $^3J_{H\text{-}H}$ = 10.42 Hz, $^2J_{H\text{-}H}$ = 1.50 Hz, -$OCH_2CH=C\underline{H}_aH_b$), 4.86 (1H, d, $^3J_{H\text{-}H}$ = 1.59 Hz, H-1_α), 4.82 (1H, d, $^3J_{H\text{-}H}$ = 1.69 Hz, H-1_β), 4.60 (ABdd, $^2J_{H\text{-}H}$ = 12.14 Hz, -$OC\underline{H}_2$Ph) , 4.21 (dd, $^2J_{H\text{-}H}$ = 12.99 Hz, $^3J_{H\text{-}H}$ = 5.23 Hz, -$OC\underline{H}_aH_bCH=CH_2$), 4.01 (dd, $^2J_{H\text{-}H}$ = 12.93 Hz, $^3J_{H\text{-}H}$ = 6.27 Hz, -$OCH_a\underline{H}_bCH=CH_2$), 3.88-3.66 (m, H-4,5,$6_a$,$6_b$), 3.44-3.40 (m, H-3), 3.32 (3H, s, -$OC\underline{H}_3$ β anomer), 3.30 (3H, s, -$OC\underline{H}_3$ α anomer), 2.11 (3H, s, -$OCOC\underline{H}_3$ β anomer), 2.10 (3H, s, -$OCOC\underline{H}_3$ α anomer), 0.92 (s, tert-butyl), 0.83 (s, tert-butyl), 0.06 (s, Si-$C\underline{H}_3$) and 0.01 (s, Si-$C\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 170.44 (-$O\underline{C}OCH_3$), 133.64 (-$OCH_2\underline{C}H=CH_2$), 128.26 and 127.43 (Ar), 117.80 (-$OCH_2CH=\underline{C}H_2$), 96.84 (C-1_α), 96.61 (C-1_β), 79.85 (C-3), 73.25 (-$O\underline{C}H_2$Ph), 72.77 (C-5), 69.60 (C-6_a and C-6_b), 68.21 (-$O\underline{C}H_2CH=CH_2$), 67.88 and 67.71 (C-4 and C-2), 56.75 (-$O\underline{C}H_3$), 25.98, 25.89 (tert-butyl), 21.02 (-$OCO\underline{C}H_3$), -4.05 and -5.16 (Si-$\underline{C}H_3$).

Experiment 21:

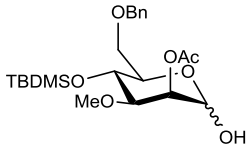
Attempted synthesis of 2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose 26



A solution of bis(dibenzylideneacetone)palladium (0) (5.98 mg, 0.01 mmol) and 1,4-Bis(diphenylphosphino)butane (0.044 g, 0.104 mmol) in dry THF (1 mL) was stirred at room temperature for 15 minutes. This mixture was then added to a stirred solution of **25** (0.050 g, 0.104 mmol) in dry THF, followed by addition of 1,3-dimethylbarbituric acid (0.032 g, 0.208 mmol). The solution was stirred at the same temperature for 30 minutes. TLC (4:1 hexane-ethyl acetate) indicated that the reaction did not occur. The mixture was stirred at 60 ºC for 30 minutes. After 30 minutes TLC with the same eluent indicated again that the reaction did not occur. The solution was stirred overnight at this temperature. TLC indicated that the starting material was not consumed.

Experiment 22:

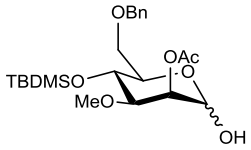
Attempted synthesis of 2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose 26



To a solution of **25** (0.050 g, 0.104 mmol) in dry THF at 0ºC, sodium borohydride (5.12 mg, 0.135 mmol) and iodine (1.32 mg, 0.005 mmol) were added sequentially. The mixture was stirred at 0ºC for 20 minutes. TLC (1:4 hexane-ethyl acetate) indicated that the reaction did not occur. The mixture was stirred for more 3 hours. TLC with the same eluent indicated that the starting material was not consumed.

Experiment 23:

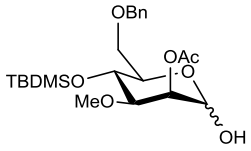
Attempted synthesis of 2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose 26



To a solution of **25** (0.025 g, 0.052 mmol) in dry DMF (1 mL) at 60 ºC, t-BuOK (0.012 g, 0.104 mmol) was added. The solution was stirred at this temperature for 1 hour. TLC (4:1 hexane-ethyl acetate) indicated that the initial product was totally consumed. Interpretation of the ^{1}H-NMR spectrum of the reaction mixture revealed that the obtained product was not the expected compound. A partially deprotected product was obtained.

Experiment 24:

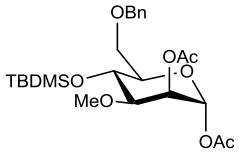
Attempted synthesis of 2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose 26



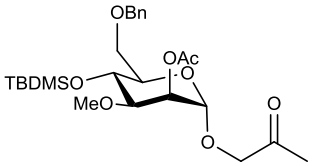
To a solution of **25** (0.075 g, 0.156 mmol) in distilled ethyl acetate, an aqueous solution of acetic acid (90 % v/v), sodium acetate (0.077 g, 0.936 mmol) and palladium (II) chloride (0.041 g, 0.234 mmol) were added sequentially. The mixture was stirred overnight at room temperature. TLC (4:1 hexane-ethyl acetate) revealed that the initial product was totally consumed. The reaction mixture was filtered through Celite, while washed with ethyl acetate. The filtrate was washed with an aqueous solution of sodium hydrogen carbonate (saturated) and extracted twice with dichloromethane. The combined organic layers were dried over Na_2SO_4 and concentrated. Purification by silica preparative TLC (7:3 hexane-ethyl acetate), afforded 0.053 g of a mixture of products.

Experiment 25:

1,2-di-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-α-D-mannopyranose 27



1-(2-Oxopropyl)-2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-α-D-mannopyranoside 28



The procedure of Experiment 6 was applied to the mixture of products afforded by Experiment 24 (0.053 mg, 0.120 mmol) with the reaction time increased to overnight. TLC (4:1 hexane-ethyl acetate) indicated that the reaction occurred in only one of the compounds. After the work-

up procedure, purification by silica preparative TLC (7:3 hexane-ethyl acetate) afforded product **27** (0.026 g, 35 %, 2 steps) and compound **28** (0.020 g, 29 %, 1 step) as colourless oils.

Compound **27**

v_{max}/cm^{-1}:1749.50 (C=O),1254.64 (C-Si), 1108.81-1025.71 (C-O)

NMR data:

^{1}H-NMR ($CDCl_3$): δ 7.29-7.19 (5H, m, Ar), 6.03 (1H, d, $^{3}J_{H-H}$ = 1.90 Hz, H-1), 5.24 (1H, dd, $^{3}J_{H-H}$ = 3.18 Hz, $^{3}J_{H-H}$ = 2.13 Hz, H-2), 4.59 (1H, ABdd, $^{2}J_{H-H}$ = 12.12 Hz, -OC$\underline{H}_2$Ph), 3.81 (1H, t, $^{3}J_{H-H}$ = 9.31 Hz, H-4), 3.71 (1H, ddd, $^{3}J_{H-H}$ = 9.50 Hz, $^{3}J_{H-H}$ = 4.35 Hz, $^{3}J_{H-H}$ = 2.43 Hz, H-5), 3.67- 3.62 (2H, m, H-6_a,6_b), 3.33 (1H, dd, $^{3}J_{H-H}$ = 9.04 Hz, $^{3}J_{H-H}$ = 3.33 Hz, H-3), 3.25 (3H, s, -OC$\underline{H}_3$), 2.06 (3H, s, -OCOC$\underline{H}_3$), 2.04 (3H, s, -OCOC$\underline{H}_3$), 0.76 (9H, tert-butyl), 0.07 (3H, s, Si-C$\underline{H}_3$) and 0.01 (3H, s, Si-C$\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 170.03 (-O$\underline{C}$OCH_3), 128.27, 127.53 and 127.44 (Ar), 91.36 (C-1), 79.70 (C-3), 75.34 (C-5), 73.38 (-O$\underline{C}H_2$Ph), 69.07 (C-6_a and C-6_b), 67.20 (C-4) , 66.49 (C-2), 56.97 (-O$\underline{C}H_3$), 25.95 (tert-butyl), 21.03 and 20.86 (-OCO$\underline{C}H_3$), -4.10, -5.22 (Si-$\underline{C}H_3$).

Compound **28**

v_{max}/cm^{-1}:1746.89 (C=O),1257.79 (C-Si), 1091.94 (C-O)

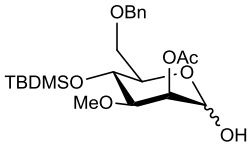
NMR data:

^{1}H-NMR ($CDCl_3$): δ 7.36-7.27 (5H, m, Ar), 5.42 (1H, dd, $^{3}J_{H-H}$ = 3.25 Hz, $^{3}J_{H-H}$ = 1.81 Hz, H-2), 4.86 (1H, d, $^{3}J_{H-H}$ = 1.60 Hz, H-1), 4.58 (2H, ABdd, $^{2}J_{H-H}$ = 12.10 Hz, -OC$\underline{H}_2$Ph), 4.24 (1H, d, $^{2}J_{H-H}$ = 17.27 Hz, -OC$\underline{H}_a$$H_bCOCH_3$), 4.13 (1H, d, $^{2}J_{H-H}$ = 17.26 Hz, -OC$H_a$$\underline{H}_bCOCH_3$), 3.81-3.62 (4H, m, H-4,5,6_a,6_b), 3.45 (1H, dd, $^{3}J_{H-H}$ = 8.52 Hz, $^{3}J_{H-H}$ = 3.33 Hz, H-3), 3.32 (3H, s, -OC$\underline{H}_3$), 2.15 (3H, s, -OCOC$\underline{H}_3$ or -OCH_2COC$\underline{H}_3$), 2.10 (3H, s, -OCOC$\underline{H}_3$ or -OCH_2COC$\underline{H}_3$), 0.84 (9H, tert-butyl), 0.08 (3H, s, Si-C$\underline{H}_3$) and 0.02 (3H, s, Si-C$\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 204.79 (-O$CH_2\underline{C}$OCH_3), 170.23 (-O$\underline{C}$OCH_3), 128.30, 127.50 and 127.46 (Ar), 97.69 (C-1), 79.70 (C-3), 73.30 (-O$\underline{C}H_2$Ph), 73.26 (C-5), 71.77 (-O$\underline{C}H_2$COCH_3), 69.47 (C-6_a and C-6_b), 67.70 and 67.34 (C-4 and C-2), 56.89 (-O$\underline{C}H_3$), 25.94 (tert-butyl), 26.46 and 20.95 (-OCO$\underline{C}H_3$ and -OCH_2COC$\underline{H}_3$), -4.07, -5.20 (Si-$\underline{C}H_3$).

Experiment 26:

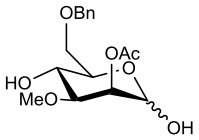
Attempted synthesis of 2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-(α/β)-D-mannopyranose 26



To a solution of **25** (0.100 g, 0.208 mmol) in dry THF at 0 ºC, (dimethyl sulfide)trihydroboron (52 μL, 0.104 mmol) was added. The mixture was stirred at this temperature for 20 minutes. TLC (7:3 hexane-ethyl acetate) indicated that the inicial product was totally consumed and the formation of several products. The crude was purified by silica preparative TLC (7:3 hexane-ethyl acetate). Interpretation of the ^{1}H-NMR spectrum from the different products revealed that none of them was the expected compound.

Experiment 27:

2-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-(α/β)-D-mannopyranose 29



The procedure of Experiment 24 was applied to compound **8** (0.153 g, 0.418 mmol). TLC (1:1 hexane-ethyl acetate) indicated that the reaction was completed. Purification by silica preparative TLC (1:1 hexane-ethyl acetate) afforded **29** (0.102 g, 73 %, α/β > 10:1) as a colourless oil.

ν_{max}/cm^{-1}: 3445.38 (O-H), 1747.76 (C=O), 1075.53 (C-O)

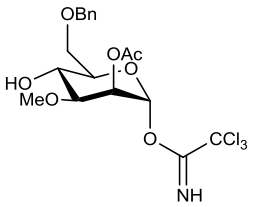
NMR data for the α-anomer (major anomer):

^{1}H-NMR ($CDCl_3$): δ 7.37-7.27 (5H, m, Ar), 5.28 (1H, dd, $^{3}J_{H\text{-}H}$ = 3.08 Hz, $^{3}J_{H\text{-}H}$ = 1.81 Hz, H-2), 5.18 (1H, d, $^{3}J_{H\text{-}H}$ = 1.43 Hz, H-1), 4.58 (2H, ddAB, $^{2}J_{H\text{-}H}$ = 12.04 Hz, -OC$\underline{H}_2$Ph), 4.05 (1H, ddd, $^{3}J_{H\text{-}H}$ =9.62 Hz,$^{3}J_{H\text{-}H}$ =6.90 Hz, $^{3}J_{H\text{-}H}$ = 2.65 Hz, H-5), 3.79 (1H, dd, $^{2}J_{H\text{-}H}$ =10.32 Hz, $^{3}J_{H\text{-}H}$ = 2.70 Hz, H-6_a), 3.72 (1H, t, $^{3}J_{H\text{-}H}$ = 9.61 Hz, H-4), 3.68 (1H, dd, $^{2}J_{H\text{-}H}$ =10.57 Hz, $^{3}J_{H\text{-}H}$ = 3.70 Hz, H-6_b), 3.58 (1H, dd, $^{3}J_{H\text{-}H}$ = 9.49 Hz, $^{3}J_{H\text{-}H}$ = 3.23 Hz, H-3), 3.40 (3H, s, -OC$\underline{H}_3$), 2.10 (3H, s, -OCOC$\underline{H}_3$).

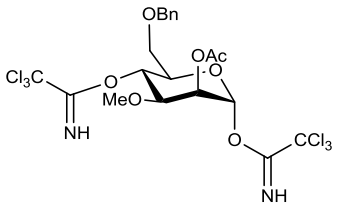
^{13}C-NMR ($CDCl_3$): δ 170.40 (-O<u>C</u>OCH_3), 128.42, 127.95 and 127.80 (Ar), 92.61 (C-1), 78.99 (C-3), 73.57 (-O<u>C</u>H_2Ph), 71.02 (C-5), 70.14 (C-6_a and C-6_b), 67.71 and 67.46 (C-4 and C-2), 57.38 (-O<u>C</u>H_3), 20.95 (-OCO<u>C</u>H_3).

Experiment 28:

(2-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate 30



(2-*O*-Acetyl-6-*O*-benzyl-3-*O*-methyl-1,4-*O*-α-D-mannopyranosyl)-di-trichloroacetimidate 31



The procedure of Experiment 8 was applied to compound **29** (0.042 g, 0.129 mol) using different equivalents of distilled DBU (1.9 μL, 0.013 mmol) and distilled trichloroacetonitrile (26 μL, 0.257 mmol). TLC (7:3 hexane-ethyl acetate) indicated the formation of two different compounds. Purification of the reaction crude, by silica preparative TLC (7:3 hexane-ethyl acetate) afforded **30** (0.022 g, 36 %) and **31** (0.047 g, 59 %) as colourless oils.

Compound **30**

^{1}H-NMR ($CDCl_3$): δ 8.72 (1H, s, -OC(N<u>H</u>)CCl_3), 7.37 – 7.27 (5H, m, Ar), 6.29 (1H, d, $^{3}J_{H\text{-}H}$ = 1.58 Hz, H-1), 5.48 (1H, dd, $^{3}J_{H\text{-}H}$ = 3.17 Hz, $^{3}J_{H\text{-}H}$ = 2.12 Hz, H-2), 4.65 (1H, d, $^{2}J_{H\text{-}H}$ = 12.00 Hz, -OC<u>H</u>$_2$Ph), 4.57 (1H, d, $^{2}J_{H\text{-}H}$ = 11.97 Hz, -OC<u>H</u>$_2$Ph), 4.06-3.97 (2H, m, H-4,6_a), 3.85-3.74 (2H, m, H-5,6_b), 3.63 (1H, dd, $^{3}J_{H\text{-}H}$ = 8.77 Hz, $^{3}J_{H\text{-}H}$ = 3.02 Hz, H-3), 3.45 (3H, s, -OC<u>H</u>$_3$), 2.15 (3H, s, -OCOC<u>H</u>$_3$).

^{13}C-NMR ($CDCl_3$): δ 169.99 and 168.32 (-O<u>C</u>OCH_3 and -O<u>C</u>(NH)CCl_3), 128.40, 127.72 and 127.65 (Ar), 95.35 (C-1), 79.11 (C-3), 73.78 (-O<u>C</u>H_2Ph), 73.60 (C-4), 69.51 (C-5), 67.10 (C-6), 65.85 (C-2), 57.78 (-O<u>C</u>H_3), 20.84 (-OCO<u>C</u>H_3).

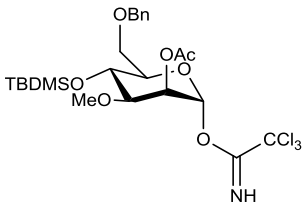
Compound **31**

^{1}H-NMR ($CDCl_3$): δ 8.78 (1H, s, -OC(N<u>H</u>)CCl_3), 8.55 (1H, s, -OC(N<u>H</u>)CCl_3), 7.35 – 7.27 (5H, m, Ar), 6.35 (1H, d, $^{3}J_{H\text{-}H}$ = 1.72 Hz, H-1), 5.62 (1H, t, $^{3}J_{H\text{-}H}$ = 10.02 Hz, H-4), 5.56 (1H, dd, $^{3}J_{H\text{-}H}$ = 3.29 Hz, $^{3}J_{H\text{-}H}$ = 2.04 Hz, H-2), 4.56 (2H, ABdd, $^{2}J_{H\text{-}H}$ = 12.40 Hz, -OC<u>H</u>$_2$Ph), 4.22 (1H, ddd, $^{3}J_{H\text{-}H}$ =10.22 Hz, $^{3}J_{H\text{-}H}$ = 3.68 Hz, $^{3}J_{H\text{-}H}$ =3.68 Hz, H-5), 3.84 (1H, dd, $^{3}J_{H\text{-}H}$ = 9.79 Hz, $^{3}J_{H\text{-}H}$ = 3.39 Hz, H-3), 3.67 (2H, d, $^{3}J_{H\text{-}H}$ = 3.73 Hz, H-6$_a$ e H-6$_b$), 3.41 (3H, s, -OC<u>H</u>$_3$), 2.18 (3H, s, -OCOC<u>H</u>$_3$).

^{13}C-NMR ($CDCl_3$): δ 170.55, 168.33 and 168.12 (-O<u>C</u>OCH_3 and -O<u>C</u>(NH)CCl_3), 128.27, 127.79 and 127.60 (Ar), 95.10 (C-1), 77.86 (C-3), 73.43 (-O<u>C</u>H$_2$Ph), 73.14 (C-5), 71.58 (C-4), 68.27 (C-6), 66.51 (C-2), 58.21(-O<u>C</u>H$_3$), 20.92 (-OCO<u>C</u>H$_3$).

Experiment 29:

(2-*O*-Acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate 32



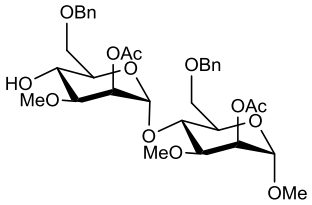
The procedure of Experiment 20 was applied to compound **30** (0.022 g, 0.047 mmol). TLC (4:1 hexane-ethyl acetate) indicated that the reaction was completed. Purification by silica preparative TLC (4:1 hexane-ethyl acetate) afforded **32** (0.020 g, 74 %) as a colourless oil.

^{1}H-NMR ($CDCl_3$): δ 8.69 (1H, s, -OC(N<u>H</u>)CCl_3), 7.39 – 7.27 (5H, m, Ar), 6.29 (1H, d, $^{3}J_{H\text{-}H}$ = 1.49 Hz, H-1), 5.50 (1H, dd, $^{3}J_{H\text{-}H}$ = 3.21 Hz, $^{3}J_{H\text{-}H}$ = 2.32 Hz, H-2), 4.59 (1H, s, -OC<u>H</u>$_2$Ph), 3.99-3.89 (2H, m, H-5,6$_a$), 3.79-3.71 (2H, m, H-4,6$_b$), 3.47 (1H, dd, $^{3}J_{H\text{-}H}$ = 8.36 Hz, $^{3}J_{H\text{-}H}$ = 3.22 Hz, H-3), 3.33 (3H, s, -OC<u>H</u>$_3$), 2.13 (3H, s, -OCOC<u>H</u>$_3$), 0.84 (9H, tert-butyl), 0.08 (3H, s, Si-C<u>H</u>$_3$) and 0.03 (3H, s, Si-C<u>H</u>$_3$).

^{13}C-NMR ($CDCl_3$): δ 170.40 and 167.89 (-O<u>C</u>OCH_3 and -O<u>C</u>(NH)CCl_3), 128.37, 127.92 and 127.69 (Ar), 97.70 (C-1), 79.30 (C-3), 73.32 (-O<u>C</u>H$_2$Ph), 72.24 (C-5), 69.93 (C-6), 68.28 and 68.06 (C-2 and C-4), 56.79 (-O<u>C</u>H$_3$), 25.92 (tert-butyl), 21.03 (-OCO<u>C</u>H$_3$), -4.06, -5.19 (Si-<u>C</u>H$_3$).

Experiment 30:

Methyl (2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranosyl)-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 24



The glycosylation reaction of donor **31** (0.047 g, 0.076 mmol) and acceptor **16** (0.026 g, 0.076 mmol) was performed according to the procedure described in Experiment 13, with the reaction time increased to overnight. TLC (1:1 hexane-ethyl acetate) indicated that the reaction was completed. Purification by silica preparative TLC (1:1 hexane-ethyl acetate) afforded **24** (0.011 g, 18 %, mostly the α anomer) as a colourless viscous foam.

v_{max}/cm^{-1}: 3420.22 (O-H), 1739.85 (C=O), 1072.58 (C-O)

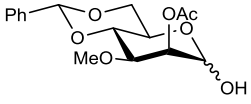
NMR data for the α anomer:

^{1}H-NMR ($CDCl_3$): δ 7.39-7.27 (10 H, m, Ar), 5.38 (1H, br s, H-2), 5.34-5.31 (1H, m, H-2), 5.28 (1H, br s, H-1), 5.23 (1H, br s, H-1), 4.66-4.53 (4H, m, -OC$\underline{H}_2$Ph$_A$ and OC$\underline{H}_2$Ph$_B$), 3.85-3.67 (m, H-3),3.63-3.56 (4H, m, H-3 and -OC$\underline{H}_3$), 3.42 (3H,s, -OC$\underline{H}_3$), 3.36 (3H, s, -OC$\underline{H}_3$), 2.15 (3H, s, -OCOC$\underline{H}_3$), 2.12 (3H, s, -OCOC$\underline{H}_3$).

^{13}C-NMR ($CDCl_3$): δ 128.46, 128.42 and 128.06 (Ar), 92.79 and 92.55 (C-1'$_A$ and C-1'$_B$), 80.91 and 78.87 (C-3$_A$ and C-3$_B$), 73.72 and 73.67 (-O$\underline{C}$H$_2$Ph$_A$ and -O$\underline{C}$H$_2$Ph$_B$), 71.16; 70.14 and 68.47 (C-6$_A$ and C-6$_B$), 69.15, 68.44; 67.97 and 67.54 (C-2$_A$ and C-2$_B$),67.45; 57.88, 57.85 and 57.41 (-O$\underline{C}$H$_3$), 21.67 and 20.97 (-OCO$\underline{C}$H$_3$).

Experiment 31:

2-*O*-Acetyl-4,6-*O*-benzylidene-3-*O*-methyl-(α/β)-D-mannopyranose 33



The procedure of Experiment 24 was applied to compound **7** (0.100 g, 0.274 mmol), with the reaction time decreased to 5 hours. TLC (1:1 hexane-ethyl acetate) indicated that the reaction

was completed. Purification by silica preparative TLC (1:1 hexane-ethyl acetate) afforded **33** (0.070 g, 78 %, α/β 6:1) as a colourless oil.

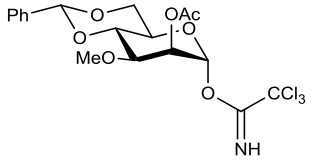
ν_{max}/cm^{-1}: 3382.32 (O-H), 1748.45 (C=O), 1101.41-1056.28 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.53-7.46 (m, Ar), 7.39-7.33 (m, Ar), 5.61 (s, -OC<u>H</u>Ph), 5.36 (dd, $^{3}J_{H-H}$ = 3.25 Hz, $^{3}J_{H-H}$ = 1.42 Hz, H-2), 5.16 (d, $^{3}J_{H-H}$ = 1.12 Hz, H-1$_{\alpha}$), 5.11 (d, $^{3}J_{H-H}$ = 1.11 Hz, H-1$_{\beta}$), 4.27-4.21 (m, H-6$_a$), 4.17-4.05 (m, H-5), 3.99 (t, $^{3}J_{H-H}$ = 9.58 Hz, H-4), 3.88-3.78 (m, H-3,6$_b$), 3.48 (3H, s, -OC<u>H</u>$_3$ β anomer), 3.46 (3H, s, -OC<u>H</u>$_3$ α anomer), 2.19 (3H, s, -OCOC<u>H</u>$_3$ β anomer) 2.16 (3H, s, -OCOC<u>H</u>$_3$ β anomer).

^{13}C-NMR ($CDCl_3$): δ 170.55 (-O<u>C</u>OCH$_3$), 129.01, 128.24 and 126.20 (Ar), 101.90 (-O<u>C</u>HPh), 93.45 (C-1), 78.59 (C-4), 75.40 (C-3), 69.69 (C-2), 68.77 (C-6), 63.78 (C-5), 58.46 (-O<u>C</u>H$_3$), 20.93 (-OCO<u>C</u>H$_3$).

<u>Experiment 32</u>:

(2-*O*-Acetyl-4,6-*O*-benzylidene-3-*O*-methyl-1-*O*-α-D-mannopyranosyl)-trichloroacetimidate 34



The procedure of Experiment 8 was applied to compound **33** (0.070 g, 0.216 mmol), with the reaction time increased to 3 hours. TLC (3:2 hexane-ethyl acetate) indicated that the reaction was completed. Purification by silica flash column chromatography (3:2 hexane-ethyl acetate) afforded **34** (0.010 g, 10 %) as a colourless oil.

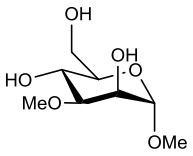
ν_{max}/cm^{-1}: 3337.85 (N-H), 1750.99 (C=O), 1093.79-1035.10 (C-O)

^{1}H-NMR ($CDCl_3$): δ 8.76 (1H, s, -OC(N<u>H</u>)CCl$_3$), 7.54-7.45 (2H, m, Ar), 7.41-7.33 (3H, m, Ar) 6.24 (1H, d, $^{3}J_{H-H}$ = 1.48 Hz, H-1$_{\alpha}$), 5.64 (1H, s, -OC<u>H</u>Ph), 5.54 (1H, dd, $^{3}J_{H-H}$ = 3.39 Hz, $^{3}J_{H-H}$ = 1.76 Hz, H-2), 4.33 (1H, dd, $^{2}J_{H-H}$ = 10.32 Hz, $^{3}J_{H-H}$ = 4.50 Hz H-6$_a$), 4.14-4.01 (2H, m, H-4,5), 3.90-3.82 (2H, m, H-3,6$_b$), 3.51 (3H, s, -OC<u>H</u>$_3$), 2.20 (3H, s, -OCOC<u>H</u>$_3$).

^{13}C-NMR ($CDCl_3$): δ 170.41, 167.78 (-O<u>C</u>OCH$_3$ and -O<u>C</u>(NH)CCl$_3$), 129.10, 128.27 and 126.11 (Ar), 101.82 (-O<u>C</u>HPh), 95.55 (C-1), 77.99 (C-4), 75.77 (C-3), 68.41 (C-6), 67.76 (C-2), 66.32 (C-5), 58.76 (-O<u>C</u>H$_3$), 20.87 (-OCO<u>C</u>H$_3$).

Experiment 33:

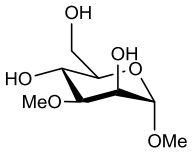
Attempted synthesis of Methyl 3-*O*-methyl-α-D-mannopyranoside 35



α-methyl-D-mannose (0.100 g, 0.515 mmol) was dissolved in dry toluene (5 mL). Then dibutyltin oxide (0.128 g, 0.515 mol) was added. The mixture was stirred and refluxed for 3 hours. TBAI (0.190 g, 0.515 mol) and iodomethane (0.097 mL, 1.54 mol) were added sequentially. The mixture was heated at 70 ºC and stirred for 72 hours. The solvent was first evaporated under vacuum. The mixture was dissolved in methanol and filtered. The solvent of the filtrate was removed under vacuum and the reaction mixture was purified by flash column chromatography, (Eluent from 12:1 dichloromethane-methanol to 9:1 dichloromethane-methanol) and interpretation of the obtained ^{1}H-NMR spectrum from the compound revealed that the reaction did not happen.

Experiment 34:

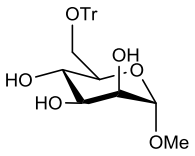
Attempted synthesis of Methyl 3-*O*-methyl-α-D-mannopyranoside 35



The procedure of Experiment 10 was applied to α-methyl-D-mannose (0.100 g, 0.515 mmol). Purification by silica preparative flash column chromatography (Eluent from 12:1 dichloromethane-methanol to 9:1 dichloromethane-methanol) and interpretation of the obtained ^{1}H-NMR spectrum from the compound revealed that the reaction did not happen.

Experiment 35:

Methyl 6-*O*-trityl-α-D-mannopyranoside 36



α-methyl-D-mannose (0.200 g, 1.03 mmol) was dissolved in dry pyridine. Then, TrCl (0.359 g, 1.29 mmol) was added, and the mixture was stirred at room temperature for 24 hours. After 24 hours more quantity of TrCl (0.287 g, 1.03 mmol) and DMAP (0.015 g, 0.124 mmol) were added. The mixture was stirred at the same temperature for 18 hours. TLC (1:4 hexane-ethyl acetate) indicated that the reaction was completed. Purification of the reaction crude, by flash column chromatography (1:4 hexane-ethyl acetate) afforded **36** (0.449 g, 100 %) as a colourless viscous foam.

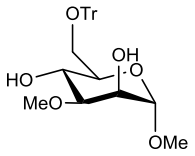
v_{max}/cm^{-1}: 3405.6 (O-H), 1056.01 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.47-7.43 (5H, m, Ar), 7.34-7.27 (10H, m, Ar), 4.72 (1H, br s, H-1), 3.92 (1H, br d, $^{3}J_{H-H}$ = 1.64 Hz, H-2), 3.79 (1H, dd, $^{2}J_{H-H}$ = 8.89 Hz, $^{3}J_{H-H}$ = 3.34 Hz, H-3), 3.72 (1H, t, $^{3}J_{H-H}$ = 9.08 Hz, H-4), 3.69-3.63 (1H, m, H-5), 3.47 (1H, dd, $^{3}J_{H-H}$ = 9.77 Hz, $^{3}J_{H-H}$ = 4.75 Hz, H-6_a), 3.41 (1H, dd, $^{3}J_{H-H}$ = 9.82 Hz, $^{3}J_{H-H}$ = 5.39 Hz, H-6_b), 3.38 (3H, s, -OCH_3).

^{13}C-NMR ($CDCl_3$): 128.59, 128.01 and 127.26 (Ar), 100.54 (C-1), 71.62 (C-3), 70.56 (C-4), 70.23 (C-2), 69.54 (C-5), 64.94 (C-6), 56.01 (-OCH_3).

Experiment 36:

Methyl 3-*O*-methyl-6-*O*-trityl-α-D-mannopyranoside 37



The procedure of Experiment 10 was applied to compound **36** (0.100 g, 0.515 mmol). TLC (2:3 hexane-ethyl acetate) indicated that the reaction was completed. Purification of the reaction crude, by flash column chromatography (2:3 hexane-ethyl acetate) afforded **37** (0.140 g, 68 %) as a yellowish viscous foam.

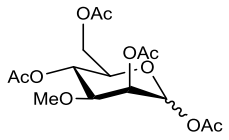
v_{max}/cm^{-1}: 3403.35 (O-H), 1057.30-1023.54 (C-O)

^{1}H-NMR ($CDCl_3$): δ 7.48-7.42 (5H, m, Ar), 7.31-7.20 (10H, m, Ar), 4.76 (1H, d, $^{3}J_{H\text{-}H}$ = 1.35 Hz, H-1), 4.02 (1H, br s, H-2), 3.76-3.70 (2H, m, H-4,5), 3.44 (3H, s, -OCH_3), 3.43-3.40 (3H, m, H-3,6_a,6_b), 3.39 (3H, s,-OCH_3).

^{13}C-NMR ($CDCl_3$): δ 128.65, 127.93 and 127.14 (Ar), 100.34 (C-1), 80.89 (C-3), 69.94 and 68.75 (C-4 and C-5), 69.77 (C-2), 64.89 (C-6), 57.34 (-OCH_3), 54.94 (-OCH_3).

Experiment 37:

1,2,4,6-Tetra-*O*-acetyl-3-*O*-methyl-(α/β)-D-mannopyranose 38



Compound **37** (0.140 g, 0.311 mmol) was dissolved in distilled acetic anhydride/acetic acid/sulfuric acid (105:45:1, v/v/v, 1.2 mL). The mixture was stirred overnight at room temperature. TLC (2:3 hexane-ethyl acetate) indicated that the reaction was completed. The mixture was neutralized and washed with an aqueous solution of sodium hydrogen carbonate (saturated) and extracted with dichloromethane. The organic layer was dried with Na_2SO_4, filtered and concentrated. Purification by flash column chromatography, (2:3 hexane-ethyl acetate) afforded **38** (0.090 g, 80%, α/β > 10:1) as a yellowish oil.

NMR data for the α anomer was in accordance with those described in the literature.[21]

CHAPTER 5

REFERENCES

5. References

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