Miguel Ângelo Martins Bernardo

Licenciado em Bioquímica

Development of a one-pot glycosylation method for the synthesis of MMP precursors

Dissertação para obtenção do Grau de Mestre em
Química Bioorgânica

Orientadora: Rita Ventura, Dra., ITQB

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Acknowledgments

A wise person once told me: ”people are what matter the most”. Indeed, I find it hard with each day not to agree. In some way, this work had the support of many people to whom I would like to express my sincere gratitude:

First of all, I want to thank Dra. Rita Ventura for giving me the opportunity to do my master thesis in her group and to learn a lot with this challenging project. I was fortunate to work in a creative free and collaborative environment under her supervision. I hope you continue to invest in people like you did with me.

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Last but not least to my parents for the much needed support and love, thank you for always believing in me.

Abstract

A one-pot glycosylation method was developed to simplify the synthesis of precursors of 3-*O*-methyl-mannose polysaccharides (MMPs) required for biosynthetic studies on mycobacteria. The unusual intracellular polysaccharides, proposed to modulate the formation of important building blocks for components of their cell wall, could uncover new and effective treatments against these threatening drug-resistent pathogens. Reacting trichloroacetyl and *N*-trichloroacetylcarbamate donor mannosides of temperature controlled reactivity, allowed the synthesis of the desired trimannoside (26%) with an estimated $\alpha\alpha$-selectivity greater than 99%, under activation by the same Lewis acid. Although the problem of enhancing both reactivity and selectivity still remains to address, interesting promising solutions are proposed which have the potential to be developed as an approach to a viable new method to supply larger MMPs synthetic targets. Furthermore, the scope of these less commonly used leaving groups as well the applications of the presented protecting strategies for combined donor and acceptor glycosides, essential for the synthesis of other oligo and polysaccharides, are discussed.

Keywords: mycobacteria; MMPs; one-pot reaction; glycosylation; trisaccharide.

Resumo

Desenvolveu-se um método de glicosilação *one-pot* que simplifica a síntese de precursores de polissacáridos de 3-*O*-metil-manose (PMMs), importantes para estudos biosintéticos em micobactérias. Os raros polissacáridos intracelulares intervêm na formação de componentes estruturais da sua parede celular, podendo por isso ajudar a descobrir novos tratamentos eficazes contra estes patogénios perigosos e resistentes a antibióticos. Com ativação do mesmo ácido de *Lewis*, a reação de dadores tricloroacetilo e *N*-tricloroacetilcarbamato, de reatividade controlada por temperatura, permitiu a síntese do trimanósido desejado (26%) com uma seletividade $\alpha\alpha$ estimada superior a 99%. Face ao problema de melhorar a seletividade e a reactividade do sistema de glicosilação, são propostas soluções promissoras com potencial para serem desenvolvidas como abordagem para um método novo e viável, capaz de fornecer alvos de PMMs sintéticos de maior tamanho. Também são discutidas as aplicações tanto destes grupos de saída menos utilizados, bem como a estratégia de proteção desenvolvida para aceder a glicósidos dador e aceitador combinados, essenciais à síntese de outros oligo e polissacáridos.

Palavras-chave: micobactérias; PMMs; reação one-pot; glicosilação; trissacárido.

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Abbreviations

$[\alpha]_D$ specific rotation

δ chemical shift

Ac acetyl (CH_3CO-)

Ar aryl

ATR attenuated total reflectance

ax axial

BDA benzaldehyde dimethyl acetal

Bn benzyl ($C_6H_5CH_2$-)

br broad

Bu butyl (C_4H_9-)

Bz benzoyl (C_6H_5CO-)

c concentration

calcd calculated

cat. catalytic

COSY correlation spectroscopy

C_q quaternary carbon

CSA 10-Camphorsulfonic acid

d doublet

DBU 1,8-diazobicycloundec-7-ene

DCM dichloromethane (CH_2Cl_2)

DDQ 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

DIPEA diisopropylethylamine

DMAP 4-dimethylaminopyridine

DMF dimethylformamide

E^+ electrophile

eq equivalent or equatorial

ESI electron spray ionisation

Et ethyl (C_2H_5-)

Exp. experiment

FTIR Fourier transform infrared spectroscopy

h hour

Hex Hexane

HMBC Heteronuclear Multiple-Bond Correlation Spectroscopy

HMQC Heteronuclear Multiple-Quantum Correlation

Hz hertz

IR infra-red

J coupling constant

LG leaving group

m meta

M molecular ion

m multiplet or mass

min minutes

m.p. melting point

Me methyl (CH_3-)

MS mass spectroscopy

NMR nuclear magnetic resonance

NOESY Nuclear Overhauser Effect Spectroscopy

Nu nucleophile

o ortho

p para

P protecting group

Ph phenyl (C_6H_5-)

PMB *p*-methoxybenzyl ($C_7H_7CH_2$-)

ppm parts per million

ps pseudo-singlet

q quartet

R generic group

r.t. room temperature

s singlet

sat. saturated

SET single electron transfer

st stretching

t tert

t triplet

T temperature

TBS *tert*-butyldimethylsilyl (tBuMe$_2$Si-)

Tf trifluoromethanesulfonyl (CF_3SO_2-)

THF tetrahydrofuran

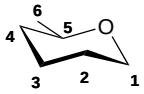
TLC thin layer chromatography

TMS trimethylsilyl (Me_3Si-) or tetramethylsylane ($SiMe_4$)

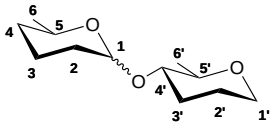
z charge

Numeration

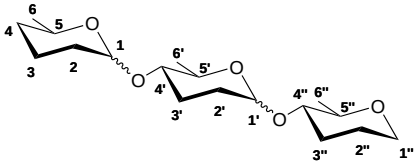
Monosaccharides



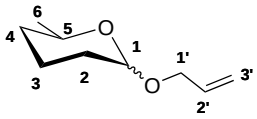
Disaccharides



Trisaccharide



Allyl glycosides



1 — Introduction

1.1 A world of carbohydrates

Carbohydrates are ubiquitous in biological and biochemical systems, from cellulose that provides structural support in plants all the way to glycans present on the surface of red blood cells, defining their different types (*i.e.* ABO antigens). Marx's[1] interesting article stresses the need for developing tools which can easily study the astonishing structural complexity of carbohydrates, in comparison with other biomolecules, whether to understand certain pathologies, finding new materials or answering fundamental questions like, how protein glycosylation can affect cellular physiology. Undoubtedly, synthetic chemists have critical contribution to the field of glycoscience supplying structurally well-defined carbohydrates and developing general methods to access them, leading to tomorrow's autonomous and expeditious systems. Indeed, it is an exciting time to synthesise sugars knowing the applications that will come forth to enhance our lives!

1.2 Mycobacteria and PMPS

Mycobacterium is a diverse and clinically relevant genus of bacteria best known for being the causative agents of tuberculosis (TB) and leprosy (*M. tuberculosis* and *M. leprae*, respectively). In 2016, TB alone have claimed globally around 1.7 million human lives, making this disease a serious threat to public health. The appearance of mycobacteria resistant to all classes of antibiotics compromise treatment even in developed countries (accounting for 5% of the recorded cases) with access to expensive treatments.[2] The vast majority of over 150 Mycobacterium species [3] are referred as nontuberculous mycobacteria (NTM) which cause other less life-threating diseases, including lymphadenitis and pulmonary disease resembling TB. Nevertheless, the high infectious potential of NTM make immunocompromised (*e.g.* HIV victims) and immunosuppressed patients greatly susceptible to these illnesses, thus NTM being also an important concern.[4] Mycobacteria pathogenesis is intimately associated with its unique lipid-rich thick cell wall and to distinct metabolic pathways. Moreover, the high hydrophobicity combined with drug efflux transporters (*i.e.* membrane proteins that reduce intracellular drug accumulation) gives them a natural resistance to antimicrobial agents.[5] In this context, whether to combat drug-resistant TB or fight-off NTM diseases it is imperative to find novel targets for the design of new and effective drugs.

Mycobacteria produce two classes of unusual intracellular polymethylated polysaccharides (PMPS): 6-*O*-methylglucose lipopolysaccharides (MGLPs) and 3-*O*-methylmannose poly-

saccharides (MMPs). While MMPs are restricted to fast-growing species, like *M. segmatis*, MGLPs can be found in both fast and slow-growing bacteria, being MGLPs the only PMPS present in *M. tuberculosis*.[6] Structurally, MMPs are composed of 10-13 α-(1→4) linked 3-*O*-methyl-mannose units terminated at the non-reducing end by a single α-(1→4) linked unmethylated mannose and at the reducing end by α-methyl aglycon. On the other hand, more complex MGLPs are made of 15-20 α-(1→4) linked D-glucose or 6-*O*-methyl-D-glucose units with a 3-*O*-methyl-glucose at the non-reducing end. The reducing end composed of diglucosylglycerate (DGG; α-glucosyl-(1→6)-α-glucosyl-(1→2)-glycerate) having its second glucose α-(1→4) linked to the MGLP main chain, containg two additional branching β-(1→3)-linked glucoses (Figure 1.1).

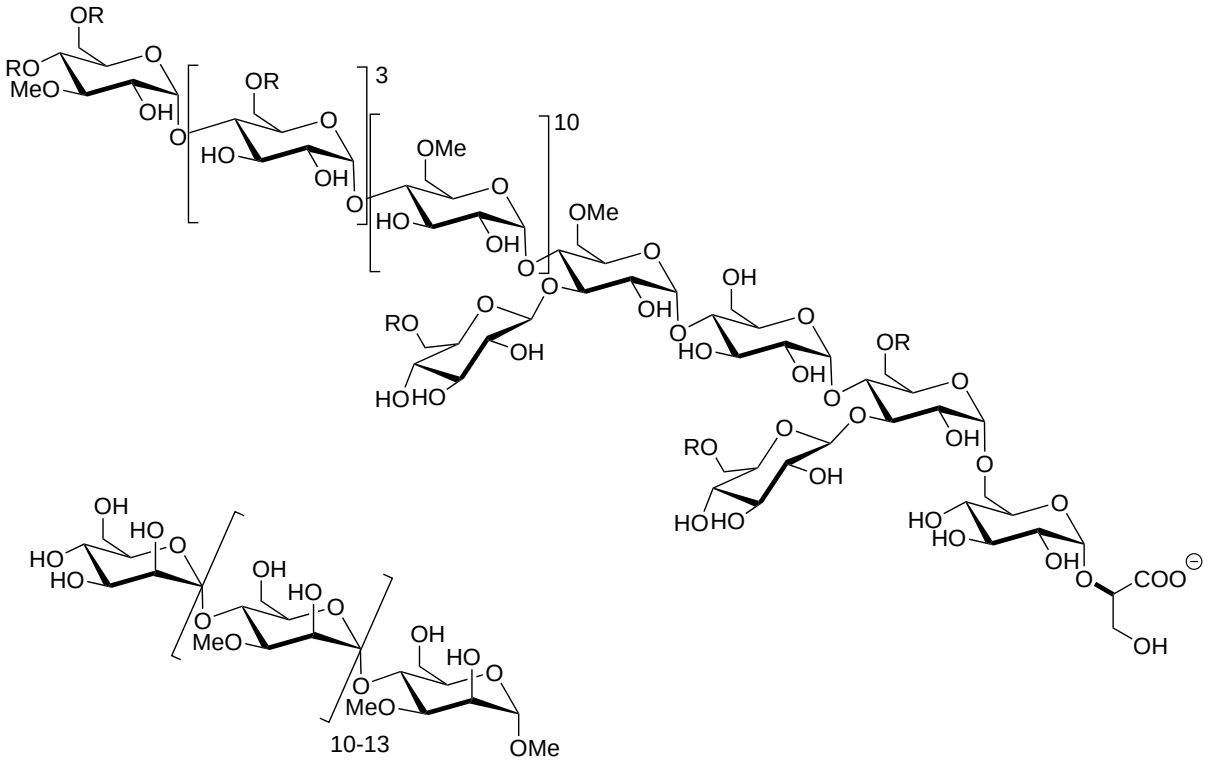


Figure 1.1: MGLPs and MMPs structure determined respectively from *M. bovis* and *M. segmatis* cell extracts.[7, 8]

Their biological role is still not yet fully understood, however *in vitro* these molecules are proposed to modulate the synthesis of medium chain fatty acids, which are essential building blocks for components of the mycobacteria cell wall, including, large chain mycolic acids.[6, 9] This process is possible due to the α-(1→4) linking pattern that gives these molecules a tendency to assume a helical conformation with inward facing methyl groups, forming a non-polar cavity capable of accommodating fatty acids. Currently, most anti-mycobacterial drugs in use or being developed target enzymes involved in the assembly of the mycobacteria cell envelope

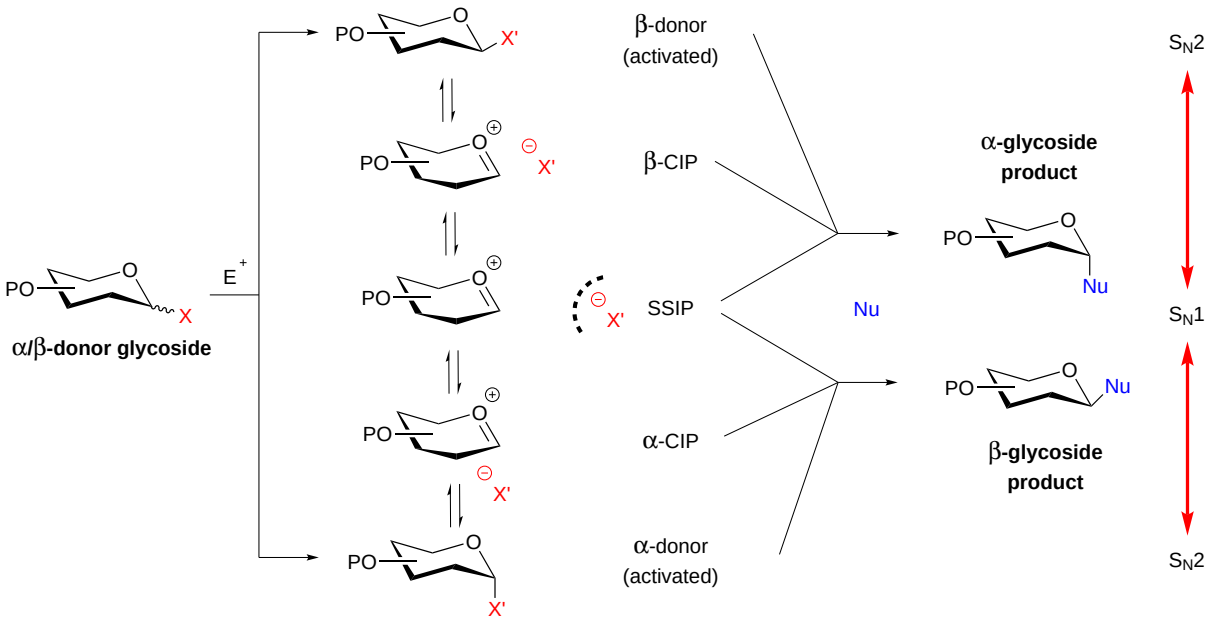
and its components.[10-12] For this reason, the enzymes involved in the biosynthesis of PMPS are potentially attractive drug targets that can disrupt the formation of mycolic acids, major components responsible for hydrophobicity and impermeability [4], thus compromising cell wall assembly and integrity.

1.3 The glycosylation reaction

Carbohydrate synthesis is invariably related to the stereoselective formation of a glycosidic linkage between its constituting monosaccharide units, named glycosylation reaction. While nature can achieve this flawlessly through enzymes, some carbohydrates still cannot be easily accessed even with modern synthetic methods. The glycosylation reaction involves nucleophilic substitution of a leaving group at the anomeric center of a glycosyl donor in the presence of a promoter. According to Crich[13, 14], this complex phenomenon cannot be simply described by pure unimolecular (S_N1) or bimolecular (S_N2) reactions, but instead being a "continuum of mechanisms" including also reactions with both features (Scheme 1.1). Hosoya *et al.*[15] have proven theoretically that leaving group displacement gives rise to a series of equilibrating reactive oxocarbenium ion intermediates which lead to different stereochemical outcomes. The incoming nucleophile can either attack the face opposite to the one shielded by the contact ion pair (CIP) - S_N2-like mechanism with an "exploded transition state" [13] - or both faces of the solvent separated ion pair (SSIP; *i.e.* free oxocarbenium ion) - S_N1-like mechanism. Although in rare cases concerted S_N2 reactions are possible, weakly nucleophilic glycosyl acceptors are more likely to follow ion intermediate pathways.[16, 17] For a given transformation, the exact place on the continuum, and hence the stereoselectivity of the process, is defined by the donor-nucleophile pair and reaction conditions.

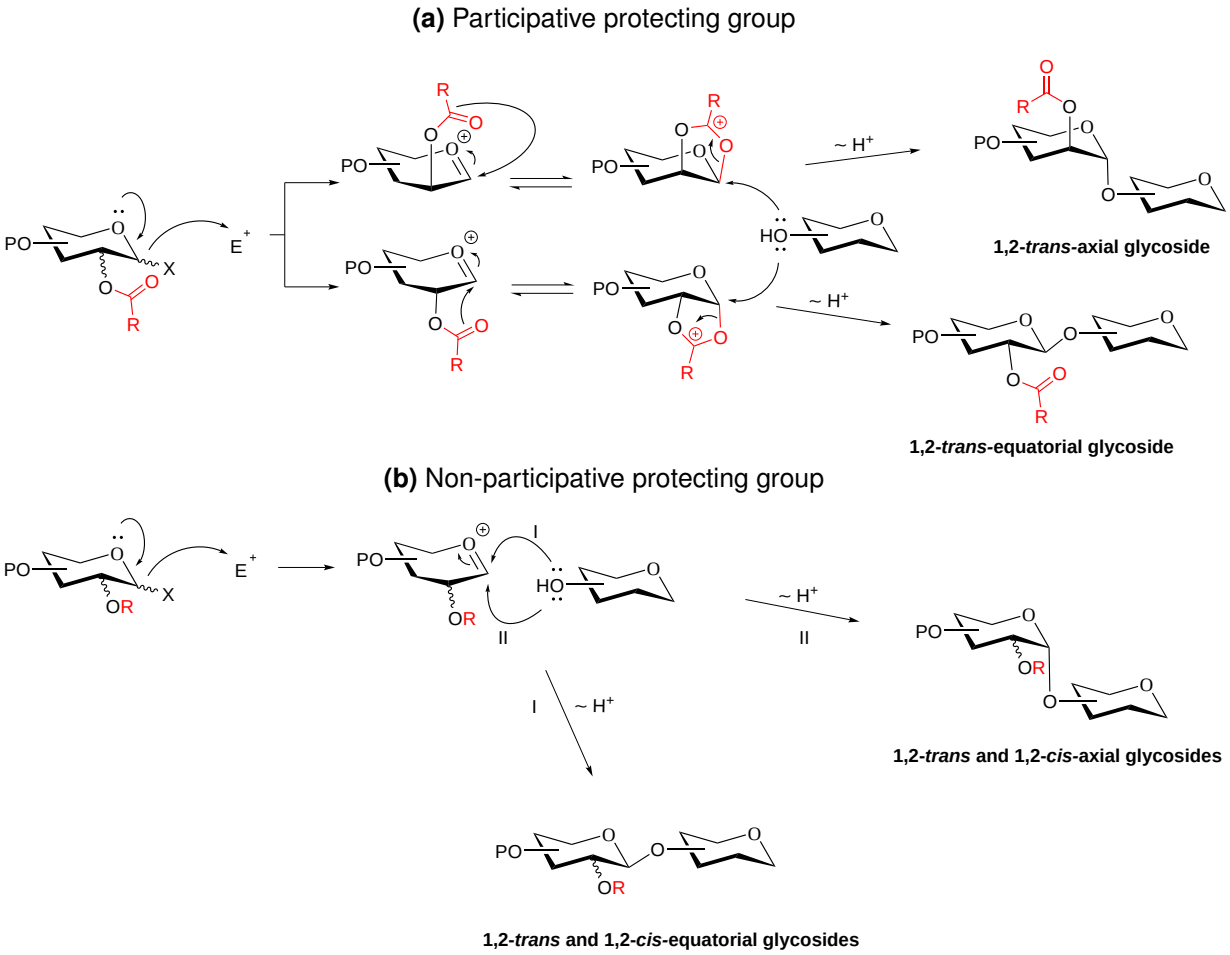
To afford the desired glycoside product, one must consider several factors that influence the stereochemical outcome of such reactions, including:

- Nature of the protecting groups
- Reactivity of the glycosyl donor, acceptor and promoter
- Solvent
- Steric hindrance of the acceptor
- Temperature at which the reaction takes place
- Additives



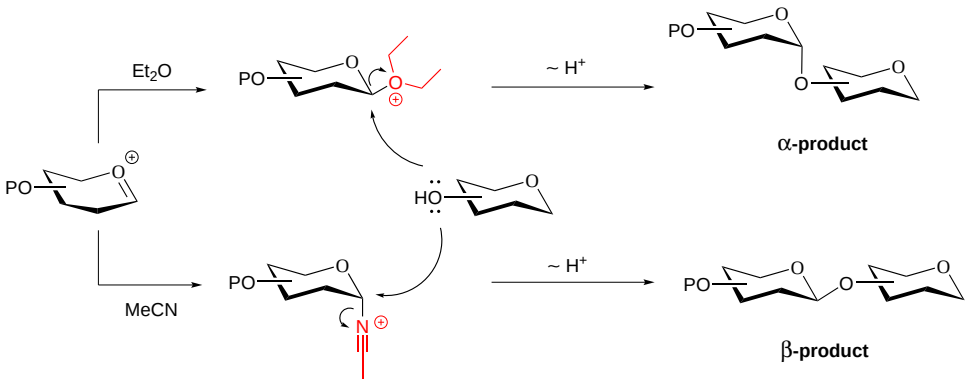
Scheme 1.1: Adapted full spectrum of glycosylation mechanisms.[13, 14, 18] X' = LG, counter ion derived from LG or promoter, additive.

Protecting groups have influence on the donor glycoside reactivity, being important its careful choice. It follows that glycosyl donors bearing electron-withdrawing protecting groups (*e.g.* acyl functions) are less reactive - "disarmed" - than those with electron-donating substituents (*e.g.* alkyl and aryl functions) - "armed" -, since the reduced electron density diminishes reactivity of the leaving group, attacking the electrophilic promoter, and suppresses the formation of oxocarbenium ion intermediates.[16] The highest impact on the stereoselectivity comes from the protective groups positioned on C-2, adjacent to the anomeric center. Acyl-type protective groups (*e.g.* OAc, OBz, 2-phthalimido) can donate their lone pair of electrons to help stabilize the developed positive charge at the anomeric carbon, forming an acyloxonium ion intermediate, shielding one face from the nucleophile, and thus affording mainly 1,2-*trans* glycosides. Conversely, ether-type nonparticipating substituents (*e.g.* OMe, OBn) allow the nucleophile to approach either face of the oxocarbenium ion intermediate, reducing stereocontrol to afford mixtures of 1,2-*cis* and 1,2-*trans* glycosides (Scheme 1.2). While 1,2-*trans* selectivity can be generally achieved with this effect, selective formation of 1,2-*cis* glycosides is far more complicated requiring specific methods.[16, 19] Furthermore, participation of substituents remote to the anomeric center can be considered to have less importance for the stereochemical outcome, since non-unanimous and controversial reports about observed changes in stereoselectivity cannot distinguish between electron-withdrawing and participation effect when esters replace ethers at C-3, C-4 and C-6 positions.[20]



Scheme 1.2: Effects of C-2 protecting groups in the glycosylation reaction.

Solvents also influence stereoselectivity, being charge separation and the extent of hydrogen bonding with the acceptor, which affect its effective steric bulk, through variation of polarity, the most immediate. In general, polar solvents favor formation of β-glycoside products, while non-polar solvents have the opposite effect.[16] Moreover, some solvents can also participate in a more direct manner by adduct formation with the glycosyl donor. Excluding the effects of neighbor group participation, acetonitrile can trap the glycosyl cation to generate α-nitrilium ion intermediates, affording mainly β-glycosides after nucleophilic displacement. On the contrary, diethyl ether leads to the formation of β-diethyl oxonium-ion intermediates, directing the synthesis towards α-products (Scheme 1.3). It is noteworthy to consider that acetonitrile use fails to achieve selectivity for 1,2-*cis*-equatorial glycosides (*e.g.* β-mannosides).[21]



Scheme 1.3: Adduct formation through solvent participation.

Temperature can direct selectivity in kinetically controlled reactions where composition can be shifted through increase of temperature from the kinetic to the thermodynamic product. Generally, the α-product is more stable due to the anomeric effect.[16, 17]

1.4 Objectives

For this work, the aim was to develop a general sequential one-pot synthetic method that could afford different size oligosaccharides which are precursors of MMPs (Figure 1.2), for biosynthetic studies in mycobacteria.

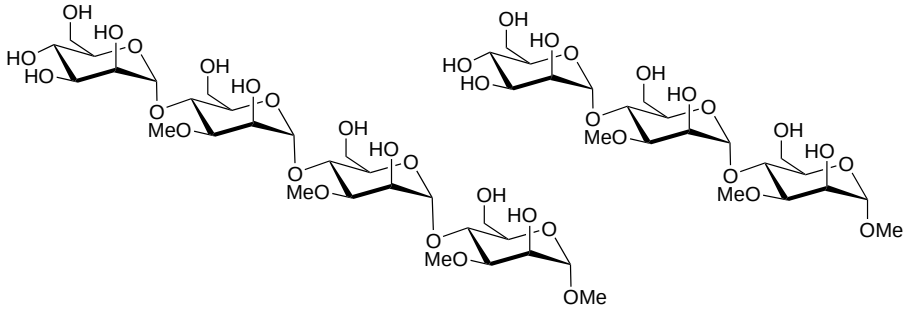
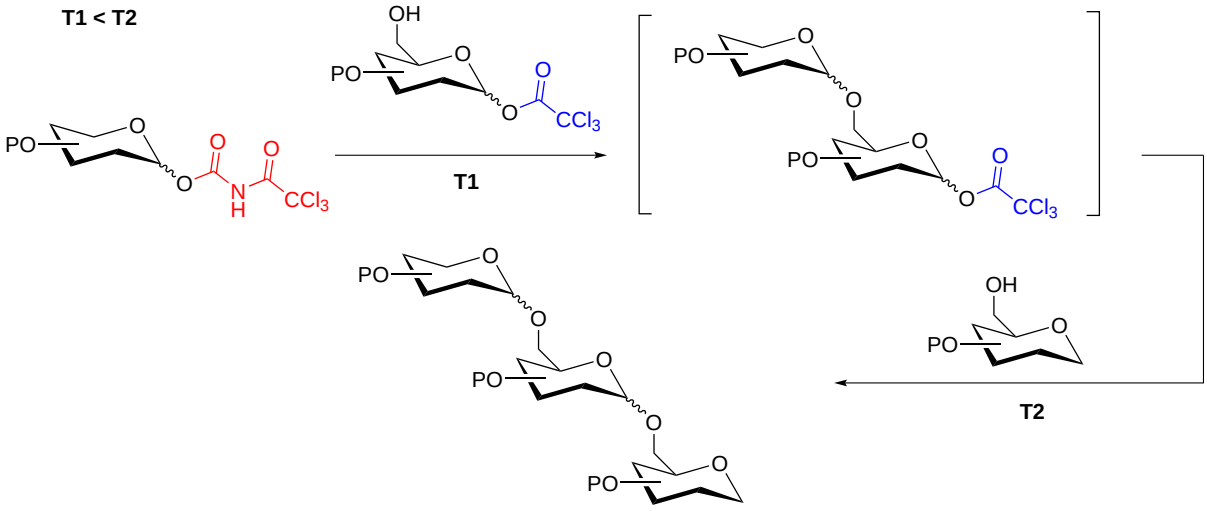


Figure 1.2: Synthetic targets corresponding to precursors of MMPs.

2 — Results and discussion

2.1 Glycosylation strategy

Among the various glycosylation strategies currently available, one-pot methods stand out in comparison with the traditional linear chain elongation of the oligosaccharide, due to reduced number of steps and avoiding time-consuming and tedious isolation of intermediates. Procedures include: chemoselective, orthogonal and pre-activation based strategies.[16, 22] The first two, are dependent on the use of either glycosyl donors with different reactivity, tuned through the protective groups (*i.e.* armed-disarmed), or different leaving groups which can be activated selectively in the presence of the others. And the later, relies on *in situ* conversion of the glycosyl donor to a reactive intermediate, in the absence of the acceptor with the same latent anomeric group. Considering the structural restrictions of the synthetic targets, imposed by the 3-OMe groups, there is not much room to tune reactivity of the donor glycosides, neither finding several orthogonal leaving groups seems an easy task, especially for the larger tetramannoside. The pre-activation based strategy would be a viable alternative, however, an interesting one-pot system of Shirahata *et al.*[23] caught our attention for its apparent simplicity. Their successful approach in affording α-1→6 linked trisaccharides uses two leaving groups of tunable reactivity through temperature change, being the *N*-trichloroacetylcarbamate more reactive than the trichloroacetyl moiety, which can be activated by the same promoter (Scheme 2.1). As a proof of concept, we decided to apply this glycosylation method for the synthesis of the desired trimannoside having more challenging α-1→4 glycosidic bonds. Also, we are interested in finding out the scope for these less frequently used leaving groups.



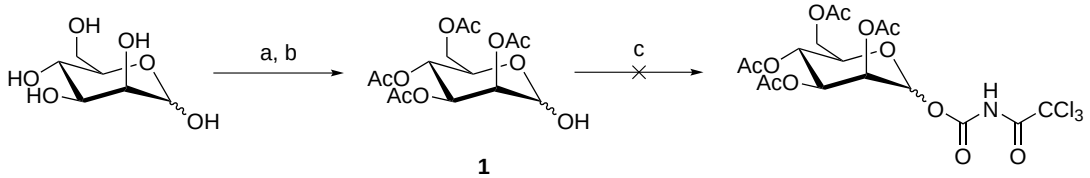
Scheme 2.1: Shirahata's sequential one-pot glycosylation.

2.2 Block synthesis

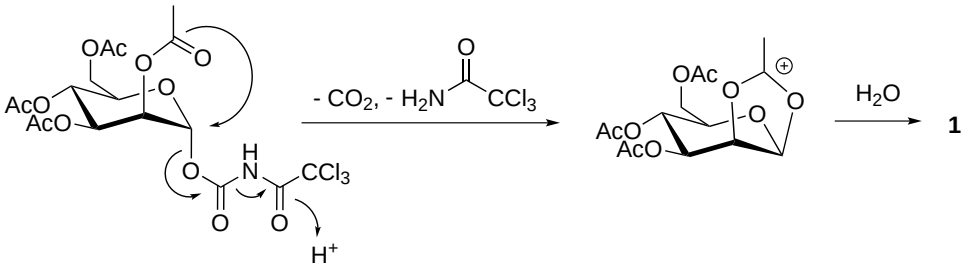
A great deal of work comes from adequate protection of the monosaccharide building blocks, using regioselective reactions to differentiate the various hydroxyl groups of similar reactivity, to transform them into suitable glycosyl donors or acceptors. Without exception, we also designed protecting sequences to afford the required materials for glycosylation.

2.2.1 Development of a suitable first glycosyl donor

Considering the absence of methylation on trimannoside's non-reducing end, a direct protection with the same group was chosen for preparation of the first donor. Our initial choice was to synthesise a peracetylated carbamate glycosyl donor (Scheme 2.2). D-mannose was completely acetylated and the anomeric hydroxyl group deprotected using hydrazine acetate for regioselective de-acetylation, resulting in compound **1**. Nevertheless, carbamate formation was unsuccessful as the product was readily hydrolysed, probably due to neighbor acyl group participation which displaces the leaving group at *anti* position (Scheme 2.3).



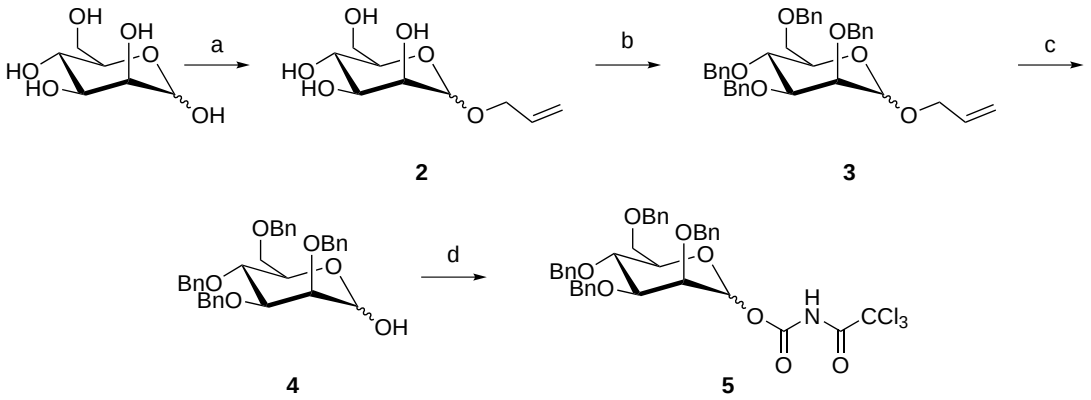
Scheme 2.2: Failed synthesis of a peracetyl carbamate donor. Reaction conditions: a) Ac_2O, DMAP, pyridine, 0°C $\rightarrow$ r.t., overnight; b) hydrazine acetate, DMF, r.t., 5h, 97% (overall yield, 2 steps); c) trichloroacetyl isocyanate, DCM, 0°C $\rightarrow$ r.t.; 2h.



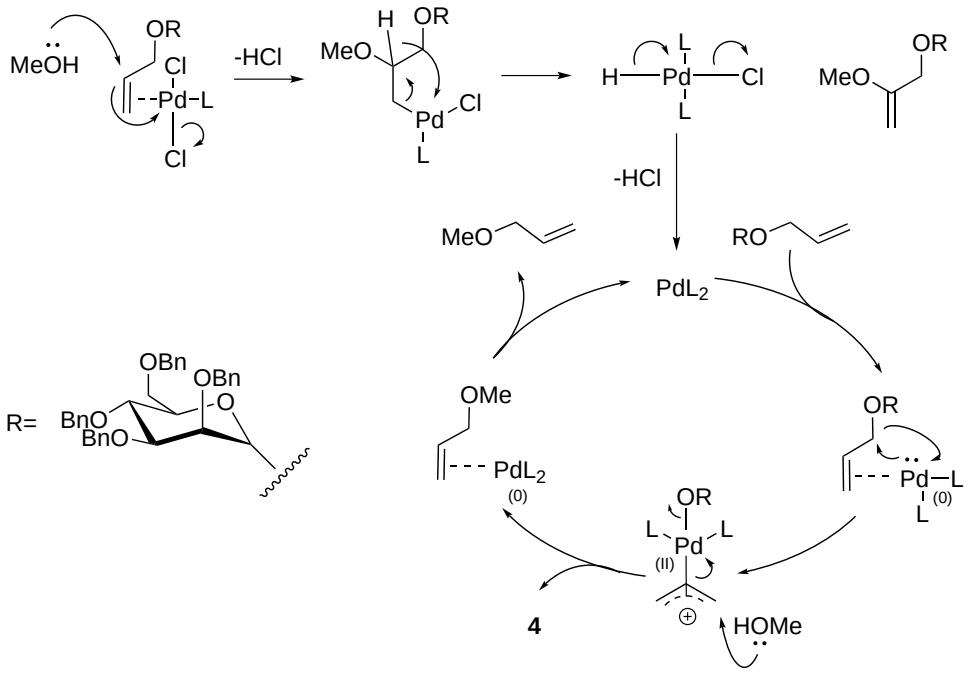
Scheme 2.3: Proposed mechanism for acetyl group induced decomposition of the *N*-trichloroacetyl carbamate leaving group.

As an alternative, perbenzylated carbamate glycosyl donor **5** was instead synthesised (Scheme 2.4). D-mannose underwent a Fisher glycosylation[24] reaction with allyl alcohol to form the orthogonal anomeric-protected allyl mannoside **2**. After benzylation of the remaining hydroxyl

groups, compound **3** was converted to hemiacetal **4** through a palladium-catalysed Tsuji-Trost[25] type substitution reaction (Scheme 2.5), beginning the catalytic cycle after *in situ* alkene reduction of $PdCl_2$. Reaction of **4** with the reactive trichloroacetyl isocyanate reagent gave carbamate **5**, being stable enough for spectroscopic characterisation. The presence of the broad NH (8.4 ppm) and downfield-shifted anomeric proton (6.3 ppm) signals along with the observed quaternary carbons (157.5, 148.2, 91.6 ppm) and carbonyl infrared bands (1791, 1726 cm^{-1}) are consistent for installation of the leaving group moiety.



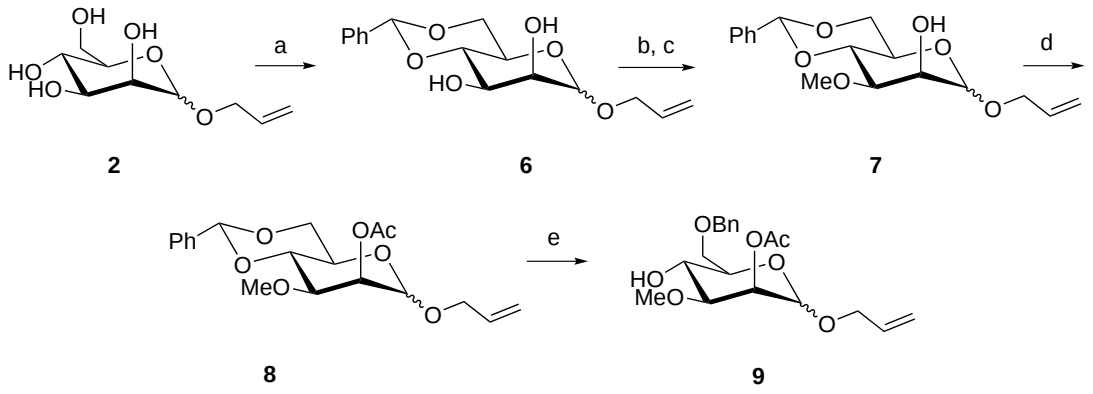
Scheme 2.4: Synthesis of carbamate donor **5**. Reaction conditions: a) allyl alcohol, CSA, reflux, overnight, 84%; b) BnBr, NaH, DMF, 0°C → r.t., overnight, 78%; c) $PdCl_2$, MeOH, r.t.; 4h, 93%; d) trichloroacetyl isocyanate, DCM, 0°C → r.t.; 40 min, 81%.



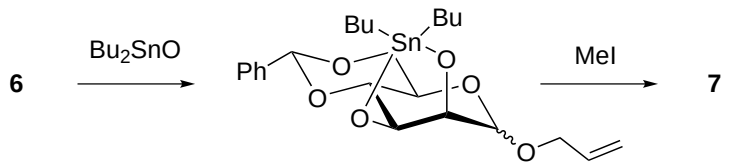
Scheme 2.5: Proposed mechanism for formation of compound **4**.

2.2.2 Development of a suitable glycosyl donor and acceptor

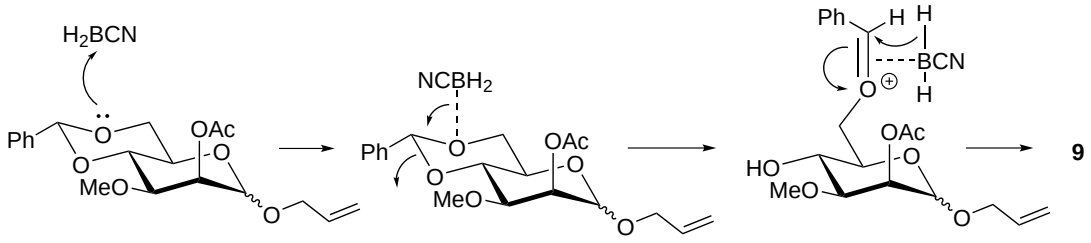
The restrictions imposed by the 3-OMe group and the required free 4-OH for an ambivalent glycosyl donor and acceptor, necessarily complicated the protecting sequence for its synthesis (*i.e.* increased the number of steps). At first, we chose to make a block with 2-OAc, for α-selectivity, and use a temporary orthogonal protecting group, masking the 4-OH during introduction of the trichloroacetyl leaving group. Our efforts begun with the synthesis of intermediate **9** (Scheme 2.6) based on a modified strategy of Liao and Lu.[26] The 4 and 6-OH of compound **2** were regioselectively protected by acid catalysed benzylidene acetal formation with BDA. Although the reaction conditions afforded only the 6-membered acetal product **6**, a lot of starting material was left to react explaining the low yield (43%). Tin-mediated alkylation of **6** formed mannoside **7** methylated exclusively at 3-OH. Precise mechanism of this transformation is unknown but it is agreed [27] that formation of a stannylene intermediate (Scheme 2.7), through a combination of steric and electronic factors, is responsible for the observed regioselectivity. After acetylation of the remaining 2-OH, benzylidene **8** suffered regioselective reductive ring opening towards the 6 position, in the presence of cyanoborohydride and HCl, to give the desired intermediate **9** with free 4-OH. In this case, the *in situ* generated electrophilic borane forms a complex at the acetal most electron rich 6-O, thus leading to the observed regioselectivity [28] (Scheme 2.8).



Scheme 2.6: Synthesis of intermediate **9**. Reaction conditions: a) $PhCH(OMe)_2$, CSA, THF, reflux, 5h, 43%; b) Bu_2SnO, MeOH, reflux, 1.5h; c) MeI, DMF, 50°C, overnight, 80% (overall yield, 2 steps); d) Ac_2O, DMAP, pyridine, 0°C, 45 min, 92%; e) $NaBH_3CN$, $HCl-Et_2O$, THF, 0°C, 70%.



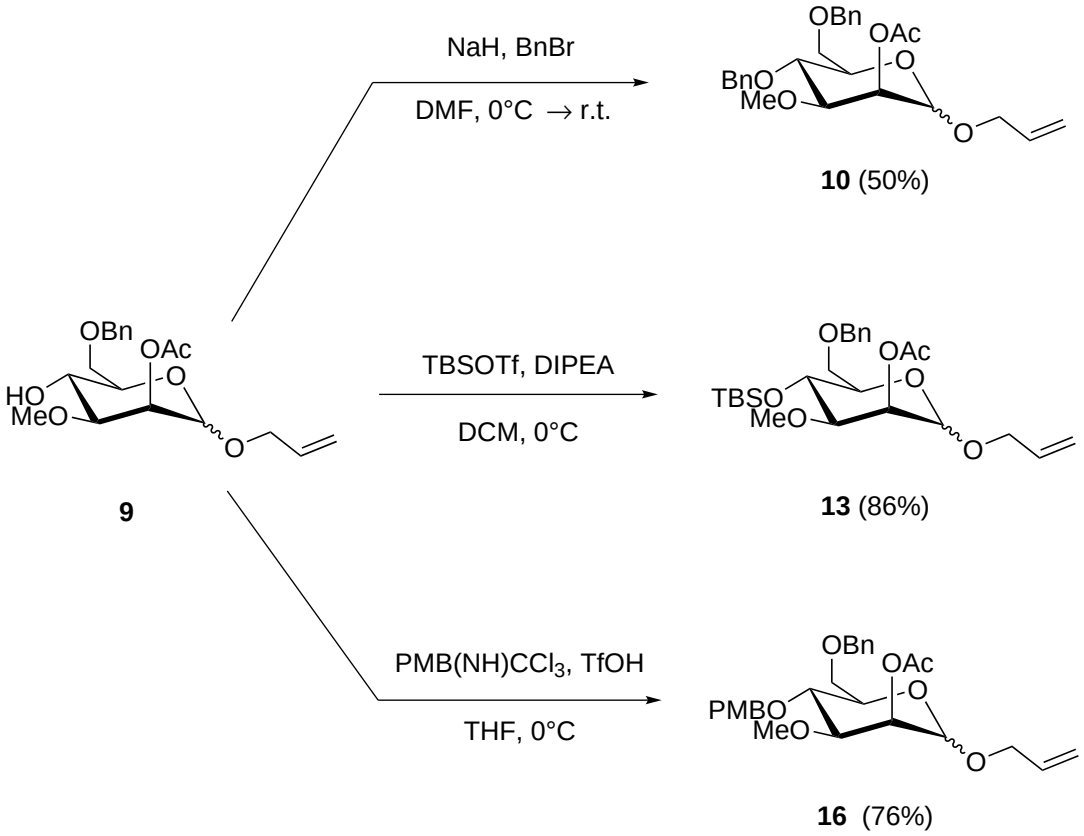
Scheme 2.7: Stannylene intermediate responsible for regioselective alkylation of compound **6**.



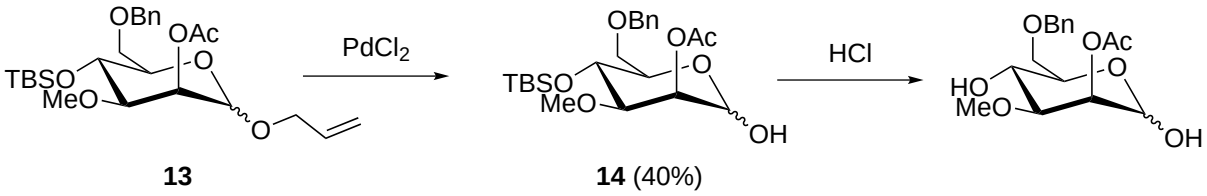
Scheme 2.8: Proposed mechanism for formation of intermediate **9**.

Following the mentioned strategy, we tried to find a temporary protecting group for the 4-OH (Scheme 2.9). Suspecting its low reactivity, a probing benzylation reaction was carried out to decide on a suitable reactive system. After the incomplete conversion of **9** into **10** (50%), we chose the TBS silyl protecting group, introduced successfully with the corresponding reactive triflate reagent under basic conditions, affording silylated mannoside **13**. Surprisingly, the TBS group showed low tolerance to the acidic conditions of the Tsuji-Trost deallylation, with the competing hydrolysis side reaction affecting formation of compound **14** (Scheme 2.10). Furthermore, decreasing the reaction time did not improve the yield beyond the obtained 40%. Therefore, to not compromise the yield at the last steps, we changed the protecting group. PMB, with better tolerance to acidic reaction conditions, was instead introduced using the synthesised corresponding trichloroacetimidade **15** under triflic acid catalysis, affording glycoside **16**. It should be noted that THF, commonly dried in our lab, was a good replacement for dioxane used in a typical procedure.[29]

Lastly, the most challenging part of the synthesis was the deprotection of the 4-OH after introduction of the trichloroacetyl leaving group (Scheme 2.11). Taking in account the previous discussed results, the acetyl group of compound **16** was changed to a benzyl one, leading to mannoside **17**. Despite the successful removal of the allyl group, conversion of compound **18** into ester **19** using Shirahata's procedure [23] caused appreciable hydrolysis of the PMB ether which, in turn, did the same for the trichloroacetyl leaving group. We addressed this problem by increasing the equivalents of pyridine and carrying out the reaction at 0°C with DMAP catalysis, effectively avoiding the observed side reactions. As a side note, the direct use of HCl or its generating counterparts could be viable alternatives to trifilic acid removal of PMB ethers in

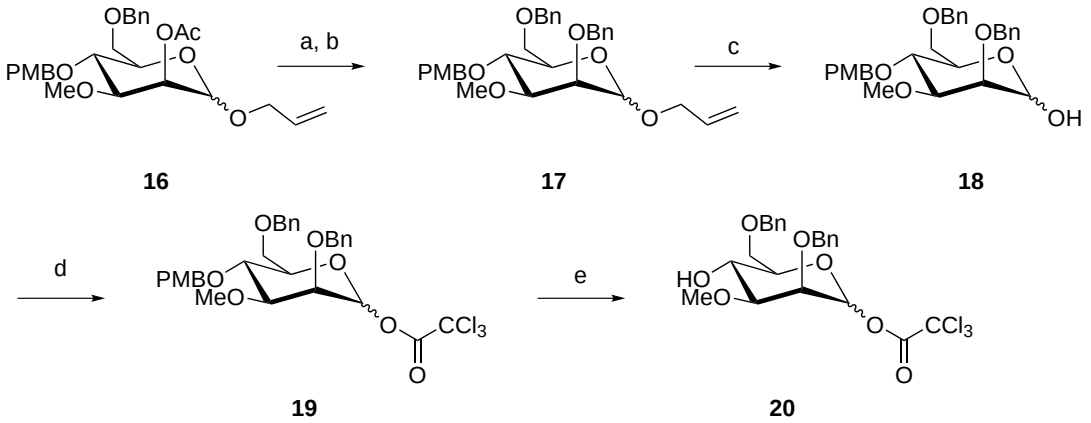


Scheme 2.9: Finding the ideal temporary protecting group for intermediate **9**.

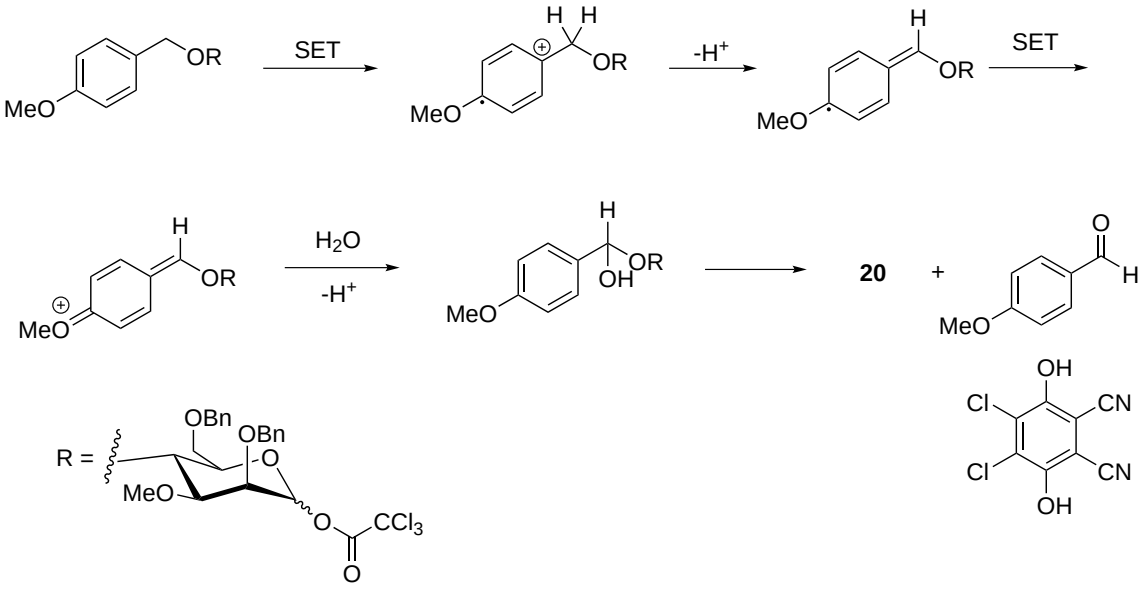


Scheme 2.10: Hydrolysis side reaction of TBS protecting group.

the absence of acid labile functions.[30] The acid decomposition of the thrichloroacetyl moiety, limited the conditions to remove the 4-OPMB. We opted for neutral DDQ oxidation [31] (Scheme 2.12) which successfully gave the desired glycosyl donor and acceptor **20**. Since this reaction is very messy, simple silica pad filtration is not enough for purification requiring silica column to purify the product, where it suffers some inevitable decomposition. Also the sensibility of the trichloroacetyl group to water contributed to a lower yield (57%), even with quick sodium sulfate drying. Carbonyl band (1770 cm^{-1}) in the FTIR spectrum, downfield-shifted anomeric proton (6.3 ppm) and respective quaternary carbons (160.0 and 89.7 ppm), in the ^{1}H and ^{13}C spectra respectively, confirmed that the ester group remained intact.



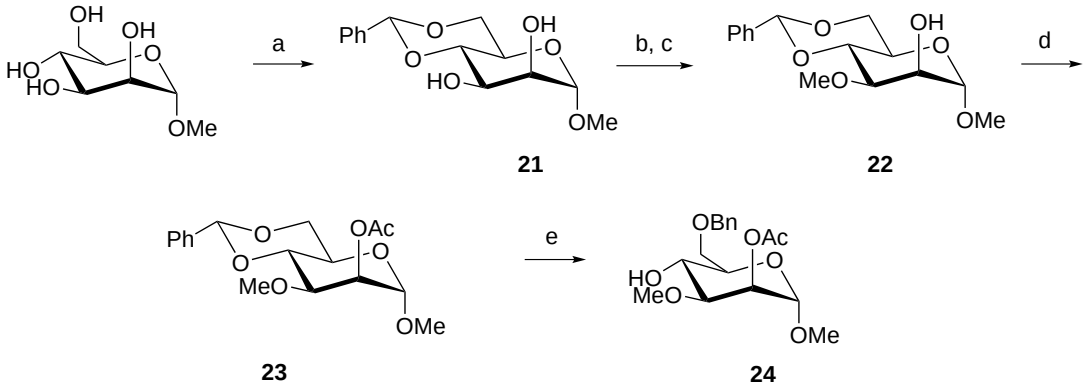
Scheme 2.11: Synthesis of the glycosyl donor and acceptor **20**. Reaction conditions: a) NaOMe, MeOH, 0°C → r.t., 3h; 99% b) NaH, BnBr, DMF, 0°C → r.t., 1h, 92%; c) $PdCl_2$, MeOH, r.t., 4h, 90%; d) trichloroacetyl chloride, DMAP, pyridine, DCM, 0°C, 30 min, 90%; e) DDQ, DCM/H_2O (95:5), 80 min, 57%.



Scheme 2.12: Proposed mechanism for oxidation of compound **19**.

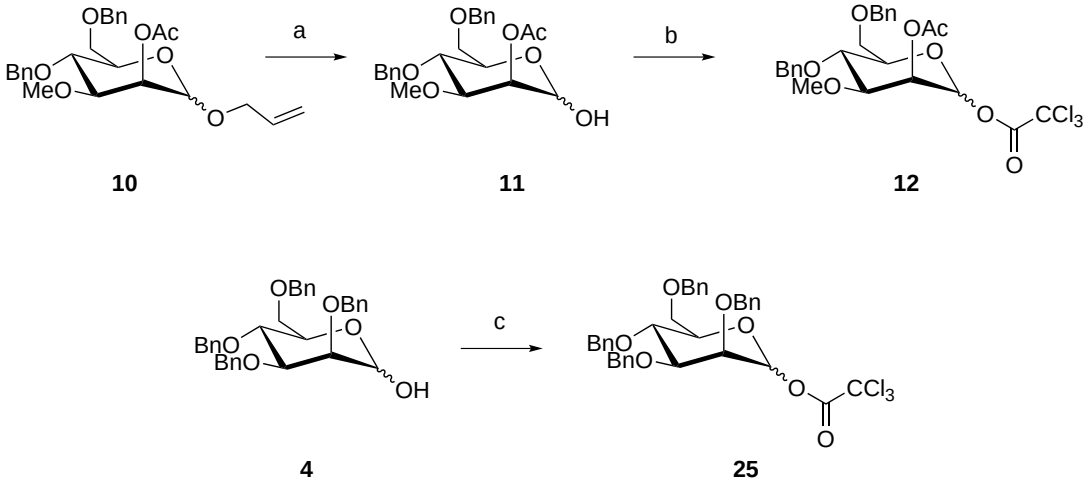
2.2.3 Synthesis of the last glycosyl acceptor

Starting from commercially available and unexpensive methyl α-D-mannopyranoside, the glycosyl acceptor **24** was synthesised using the previous discussed strategy for intermediate **9** (Scheme 2.13). Hsu's [32] procedure, which uses the stronger fluoroboric acid, afforded benzylidene **21** with good yield (82%). Unfortunately, when applied to the allyl protected analogue **2**, the original substrate of this reaction, poorer results were obtained with the yield comparable to the alternative CSA catalysed procedure. In our case, the competing side reaction of 5-membered acetal formation, with 2 and 3-OH, was more extensive.



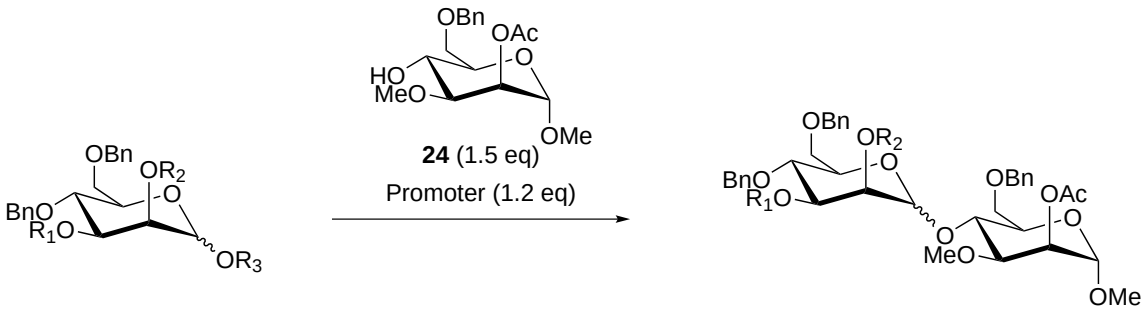
Scheme 2.13: Synthesis of last glycosyl acceptor **24**. Reaction conditions: a) $PhCH(OMe)_2$, HBF_4-Et_2O, DMF, r.t., 2h, 82%; b) Bu_2SnO, MeOH, reflux, 2h; c) MeI, DMF, 50°C, overnight, 81% (overall yield, 2 steps); d) Ac_2O, DMAP, pyridine, 0°C, 1h, 84%; e) $NaBH_3CN$, $HCl-Et_2O$, THF, 0°C, 62%.

2.3 Optimisation of the reaction conditions for the one-pot procedure



Scheme 2.14: Synthesis of trichloroacetyl glycosyl donors **12** and **25**. Reaction conditions: a) $PdCl_2$, MeOH, r.t., 4h, 62%; b) trichloroacetyl chloride, pyridine, DCM, r.t., 2h, quantitative; c) trichloroacetyl chloride, pyridine, DCM, r.t., 2.5h, quantitative.

Before applying the synthesised glycosylation materials in the one-pot method, we first optimised the reaction conditions in order to obtain the best stereo and chemoselectivities (Table 2.1). Initially, compounds **4** and **10** were converted through already discussed reactions to the corresponding trichloroacetyl glycosyl donors, respectively, **25** and **12** (Scheme 2.14), to be used along with carbamate donor glycoside **5** in the glycosylation reaction with acceptor **24** (Scheme 2.15).



5 (R_1=R_2=Bn, R_3= *N*-Trichloroacetyl carbamate)
12 (R_1=Me, R_2=Ac, R_3= Trichloroacetyl)
25 (R_1=R_2=Bn, R_3= Trichloroacetyl)

26 (R_1=R_2=Bn)
27(R_1=Me, R_2=Ac)

Scheme 2.15: Optimisation of one-pot method conditions.

Although we observed good α-selectivity with 2-OAc participation for donor **12**, diminished leaving group reactivity also affected the yield of disaccharide **27** (Entry 1). Following this line of thought, more reactive benzylated trichloroacetyl donor **25** was employed, furnishing disaccharide **26** with better yield but poorer stereoselectivity (Entry 2). Replacing the solvent for diethyl ether, slowed the reaction and decreased its yield, to give a marginal improvement on selectivity (Entry 3). Curiously, promoter change for boron trifluoride substantially increased the amount of disaccharide **26-α**, while maintaining reactivity of the trichloroacetyl group (Entry 4). The same observation was extended to carbamate donor **5**, however less efficient in its activation (Entry 8). After the failed attempt at improving selectivity towards **26-α** product with a solvent mixture (Entry 9), we focused only in controlling chemoselectivity of the leaving groups through temperature, using TMSOTf as a promoter for both donors. A great difference in reactivity was found at -20°C (Entries 5-7), being the temperature selected for the first step of the one-pot glycosylation, whereby reactivity of the trichloroacetyl donor is reduced in favor of the carbamate one. Moreover, we observe that an increase in reactivity at r.t. is accompanied with lower selectivity, apparently meaning that stereocontrol cannot be reconciled with reactivity on the second step for the trichloroacetyl leaving group (Entries 2 and 6). Nevertheless, we decided not to lower its reactivity with the 2-OAc group, since it already greatly impacts the global yield of the one-pot reaction. Hence the 2-OBn mixed glycosyl donor and acceptor **20** was chosen as the “middle” building block to access the desired trisaccharide. Additionally, the boron trifluoride/trichloroacetyl system could be an alternative to 2-OAc participation of trichloroacetimidate mannoside donors, previously used by our group, allowing formation of α-1→4 bonds on perbenzylated mannoside donors at r.t.

Table 2.1: Optimisation studies for the one-pot glycosylation reaction.

Entry	Glycosyl donor	Solvent	Promoter	T (°C)	Time (h)	Yield (%) [a]	α/β [b]
1	12	DCM	TMSOTf	r.t.	1.5	32	8.6:1
2	25	DCM	TMSOTf	r.t.	1.5	53	4.8:1
3	25	Et_2O	TMSOTf	r.t.	24 [c]	21	5.6:1
4	25	DCM	BF_3-Et_2O	r.t.	1.5	56	17:1
5	25	DCM	TMSOTf	0	1.5	47	4.1:1
6	25	DCM	TMSOTf	-20	1.5	27	8.2:1
7	5	DCM	TMSOTf	-20	2	73	8.5:1
8	5	DCM	BF_3-Et_2O	0	2	44	30:1
9	5	DCM/Et_2O (95:5)	TMSOTf	0	2	72	8.6:1

[a] Glycosyl acceptor **24** was recovered. [b] Determined by NMR. [c] The reaction did not seem to proceed after 3h.

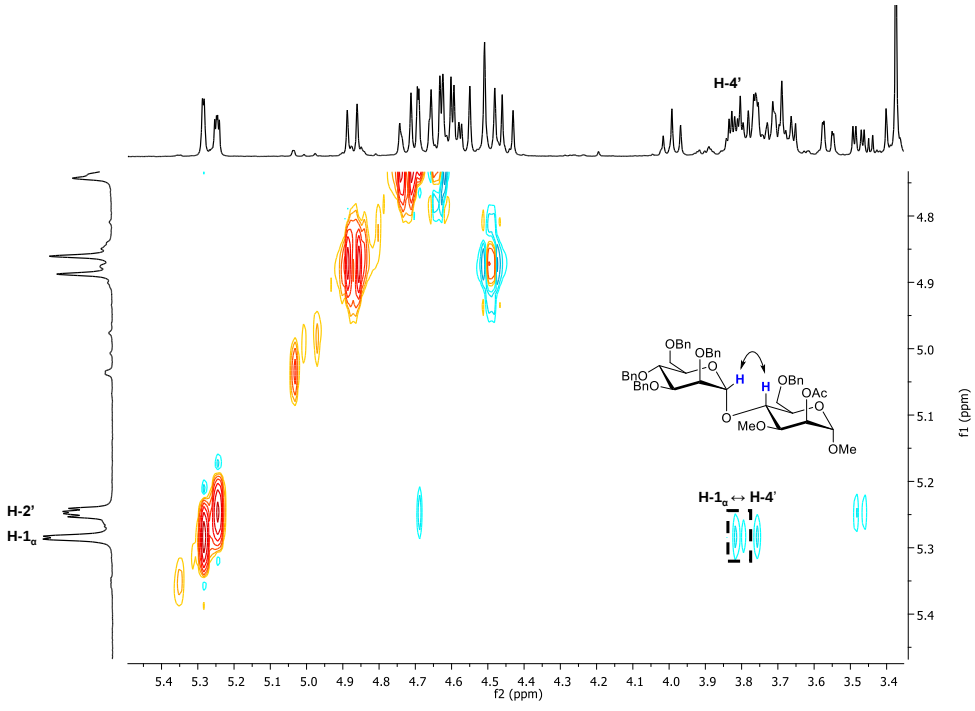


Figure 2.1: 2D-NOESY experiment of disaccharide **26**.

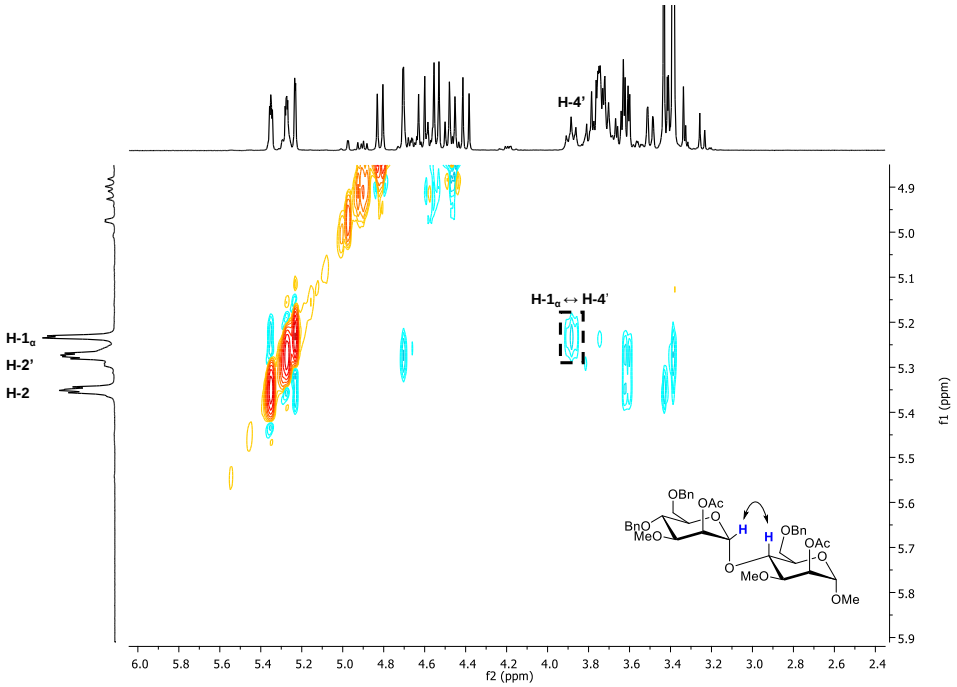


Figure 2.2: 2D-NOESY experiment of disaccharide **27**.

The small vicinal coupling constants between H-1 and H-2 at around 2 Hz, characteristic for equatorial position, and the found correlation between H-1 and H-4 in 2D-NOESY experiments (Figures 2.1 and 2.2) confirmed that the glycosylation reactions afforded mainly the α-disaccharides.

2.4 One-pot glycosylation reaction

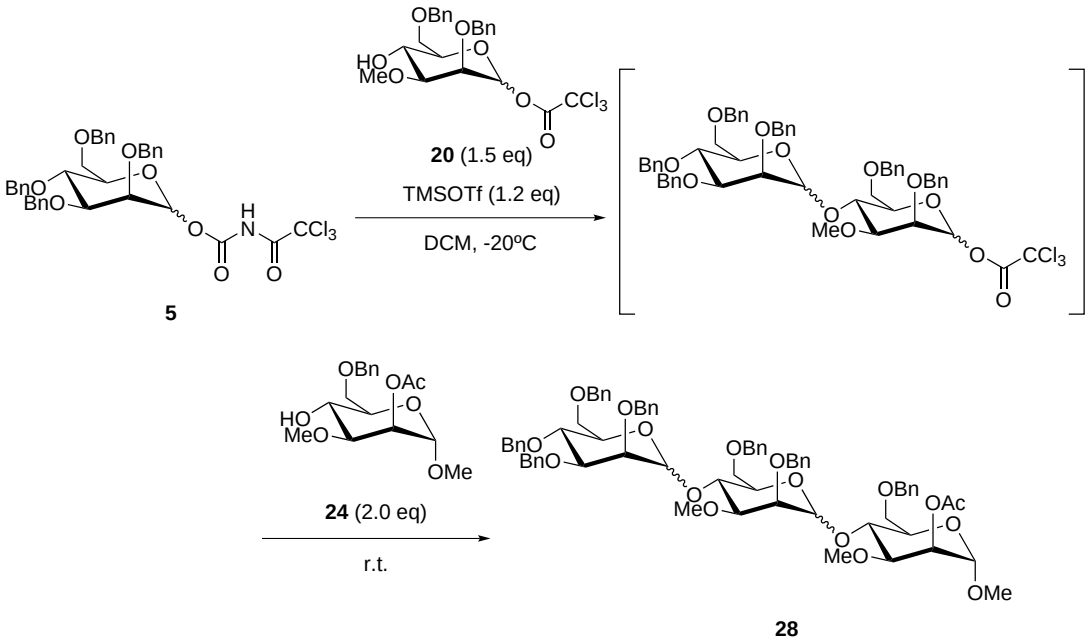
Having set the temperature conditions for the one-pot reaction (Table 2.2; Scheme 2.16), we decided to react 1.5 equivalents of glycoside **20** to ensure consumption of carbamate donor **5** and 2 equivalents of acceptor **24** to push for conversion of the less reactive *in situ* generated trichloroacetyl intermediate. Our successful first attempt gave the expected trimannoside **28** (15%; entry 1), being disaccharide **26** also isolated (26%), meaning donor **5** was not fully consumed in the first step. Therefore, when the first step was extended 10 min, trimannoside **28** was obtained with higher yield (26%; entry 2) and without formation of byproduct **26**. The difficulty to achieve completely anhydrous conditions translated in slight decomposition of donors **5** and **20** before promoter addition, thus limiting the obtained yields for both experiments. Additionally, the glycosylation conditions (*i.e.* r.t. and stoichiometric amount of promoter) allowed silylation of acceptor **24**, however we dismissed this side reaction because its non silylated counterpart was also recovered. Overall the one-pot procedure was a success, being fairly

close to the combined yield (39%) of the isolated steps. As previously discussed, mannose has a low reactive 4-OH which requires a highly reactive leaving group to introduce α-1$\rightarrow$4 bonds with good yields, that is why Shirahata's procedure can achieve a higher yield (52%) [23] as they form bonds with more reactive primary 6-OHs.

Table 2.2: One-pot glycosylation experiments.

Entry	1st step time (min)	2nd step time (min)	Yield (%)	$\alpha\alpha$ (%)[b]
1	10	100 [a]	15	>99
2	20		26	

[a] The reaction did not proceed after this time. [b] Determined by NMR



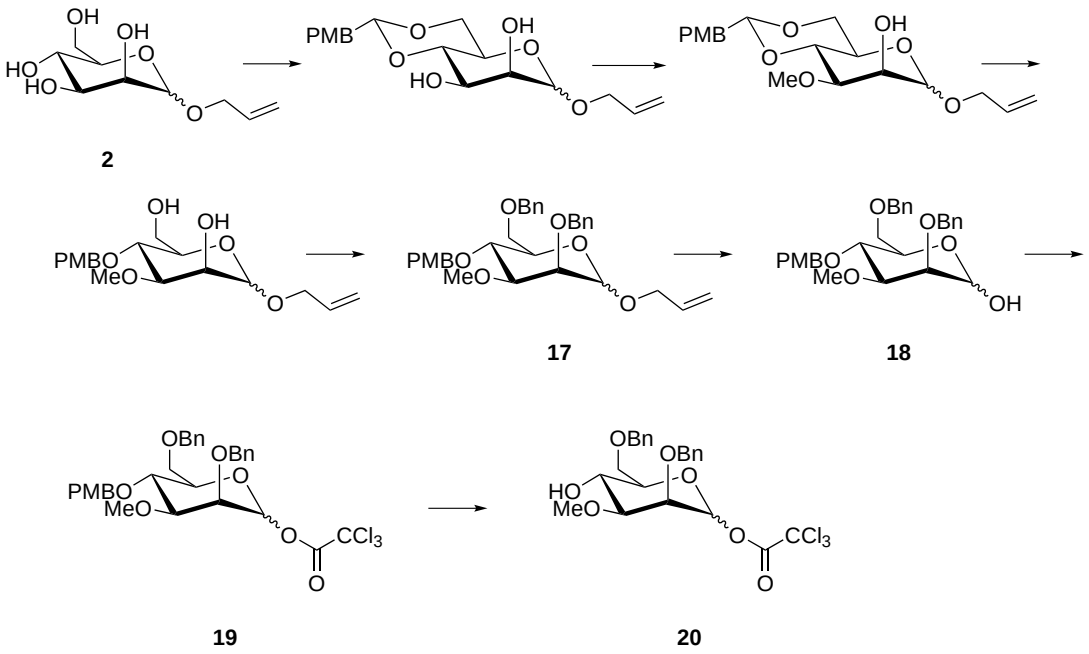
Scheme 2.16: One-pot glycosylation.

By analysing trisaccharide's **28** NMR spectra, we could conclude that α-anomeric selectivity was very high in both glycosidic bonds. No minor peaks were detected that would indicate the presence of other isomers. In this way, the anomeric selectivity was considered to be higher than 99%. Nevertheless, this result should be confirmed by HPLC. The use of an acceptor with a more hindered secondary alcohol for 1→4 bond formation might explain the observed higher selectivity in comparision with Shirahata and co-workers (70%) [23], as they form 1→6 bonds with an acceptor having a less hindered primary alcohol. We and other authors have seen in the past that the nature of the glycosyl acceptor has indeed a large influence in the stereochemical outcome of the glycosylation reaction.[16, 17]

2.5 Future improvements

The selectivity/reactivity paradox is not an easy problem to address. To increase the formation of trisaccharide **28** we could use a dehydrative approach where mannoside **4** is *in situ* converted to carbamate **5**, which would then be used immediately in the one-pot reaction. This would greatly reduce the observed decomposition of this unstable donor associated with storage and handling before the reactions. Another obvious way to enhance reactivity would be to use a more nucleophilic acceptor, perhaps by changing to an electron-donating 2-OBn group on glycosyl acceptor **24**. Lastly, should TMSOTf not interfere with boron trifluoride activation of the trichloroacetyl group, α-selectivity could be achieved by the use of these two promoters, employing the later in the second step. Given more time to follow through with this challenging project, we are confident that these improvements could be successful to further optimise the one-pot method to afford the MMPs' precursors.

Furthermore, the synthesis of compound **20** could be simplified since we now know that 2-OBn group is required (Scheme 2.17). The 4 and 6-OH of allyl mannoside **2** could be protected with the *p*-methoxybenzylidene acetal. After installation of the required 3-OMe moiety and regioselective acetal ring opening towards the 4-ether, the remaining free hydroxyls would be benzylated to afford compound **17** with 4-OPMB group already in place, then proceeding with the last 3 steps to afford the desired product. Thereby, allowing the synthetic route of compound **20** to be shortened 4 steps.



Scheme 2.17: Hypothetical simplified synthetic route of glycoside **20**.

3 — Conclusion

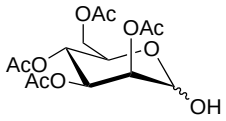
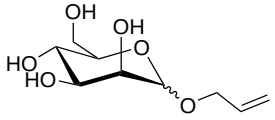
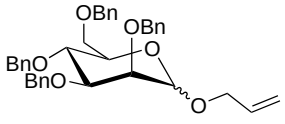
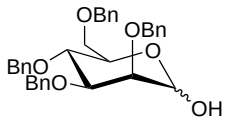
The aim of this work was to develop a one-pot glycosylation method to simplify the synthesis of MMPs' precursors. As a proof of concept, Shirahata's strategy was successfully applied, using two different leaving groups with their reactivity tuned by different temperatures, to afford the desired trimannoside **28**. While the reactivity/selectivity problem still remains to be addressed, promising and interesting solutions were proposed. If successfully applied, this method has the potential of becoming a new simpler alternative to more conventional one-pot glycosylation methods. In that case, future works could aim to more changeling synthesis of larger precursors, including, the tetramannoside which we would like to have synthesised. In the process, the scope of these less commonly used leaving groups was studied; the trichloroacetyl leaving group activated by boron trifluoride was found to be a good system to form α-1$\rightarrow$4 bonds for single step glycosylations of perbenzylated mannoside donors at r.t. Furthermore, we overcame the main problems related to orthogonal protection of the "middle" building block with the developed protecting strategy, opening the possibility of different combined glycosyl donors and acceptors to be supplied for other one-pot glycosylation methods involving formation of 1$\rightarrow$4 glycosidic bonds, commonly found in natural oligo and polysaccharides.

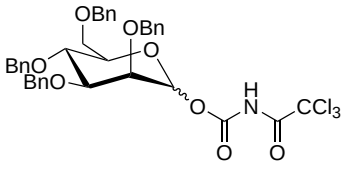
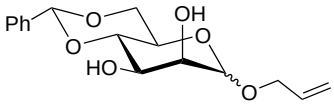
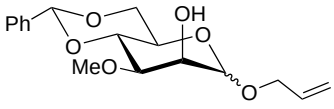
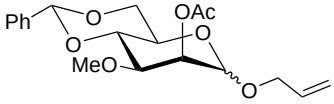
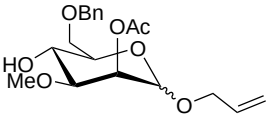
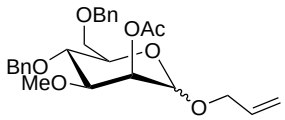
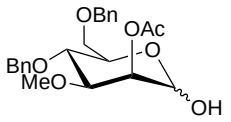
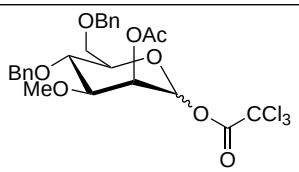
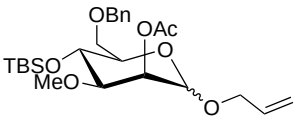
4 — Experimental part

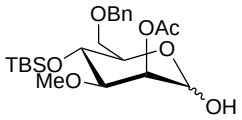
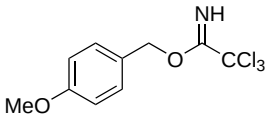
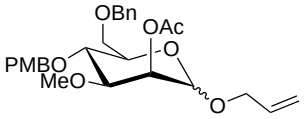
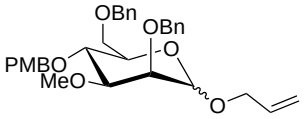
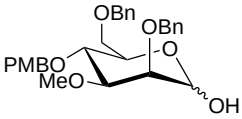
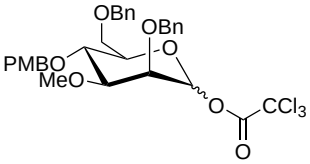
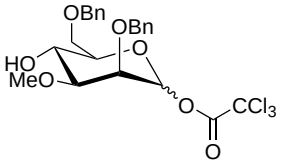
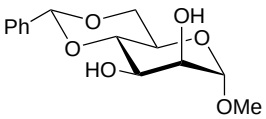
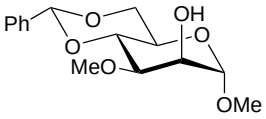
4.1 General

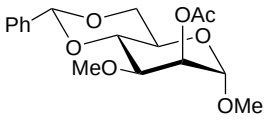
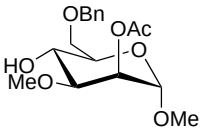
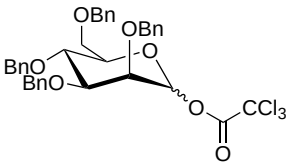
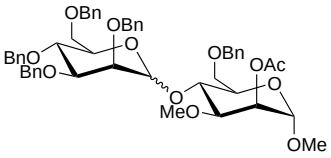
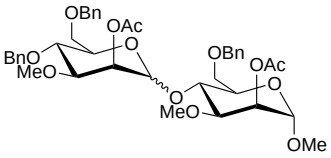
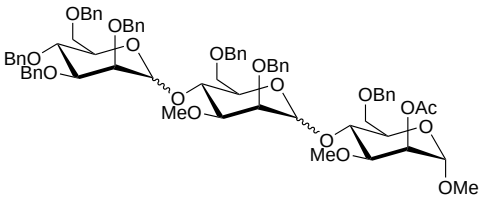
All chemicals were of reagent grade used without further purification, unless otherwise noted. Solvents were dried according to established methods.[33] All reactions were carried out under argon atmosphere, except when dried solvents were not used. Sigma-Aldrich pre-activated 4Å powdered molecular sieves were employed in glycosylation reactions. Analytical TLC: aluminum-backed silica gel Merck 60 F_{254}. Flash chromatography was performed on Kieselgel 60 (0.032-0.063 mm particle size). Preparative TLC employed silica gel Merck GF_{254}. Infrared (IR) spectra were obtained using a commercial ATR-FTIR spectrophotometer and are in cm^{-1}. Specific rotations were measured using an automatic polarimeter and are reported as follows: $[\alpha]_D$ (c=g/100mL; solvent). Melting points were determined with a capillary apparatus. MS was recorded on a commercial apparatus (ESI source). NMR spectra were obtained at 400 MHz (^{1}H) and 101 MHz (^{13}C) using $CDCl_3$ as solvent, unless otherwise stated. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS and coupling constants in hertz (Hz). Water pre-saturation experiments were performed for ^{1}H spectra using D_2O as solvent. Peak assignments of all compounds were carried out with the help of 2D NMR experiments as COSY and HMQC. When required, HMBC and NOESY were also used.

Graphic index of compounds

Exp.	Structure	Name and Number	Page
1		2,3,4,6-tetra-*O*-acetyl-α/β-D-mannopyranose 1	36
2		Allyl α/β-D-mannopyranoside 2	36
3		Allyl 2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranoside 3	37
4		2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranose 4	37

5		2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranosyl *N*-trichloroacetylcarbamate 5	38
6		Allyl 4,6-*O*-benzylidene-α/β-D-mannopyranoside 6	38
7		Allyl 4,6-*O*-benzylidene-3-*O*-methyl-α/β-D-mannopyranoside 7	39
8		Allyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α/β-D-mannopyranoside 8	40
9		Allyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranoside 9	40
10		Allyl 2-*O*-acetyl-4,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranoside 10	41
11		2-*O*-acetyl-4,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranose 11	41
12		2-*O*-acetyl-4,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranosyl trichloroacetate 12	42
13		Allyl 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-α/β-D-mannopyranoside 13	42

14		2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-α/β-D-mannopyranose 14	43
15		4-Methoxybenzyl-2,2,2-trichloroacetimidate 15	43
16		Allyl 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*p*-methoxybenzyl-3-*O*-methyl-α/β-D-mannopyranoside 16	44
17		Allyl 2,6-di-*O*-benzyl-4-*O*-*p*-methoxybenzyl-3-*O*-methyl-α/β-D-mannopyranoside 17	44
18		2,6-di-*O*-benzyl-4-*O*-*p*-methoxybenzyl-3-*O*-methyl-α/β-D-mannopyranose 18	45
19		2,6-di-*O*-benzyl-4-*O*-*p*-methoxybenzyl-3-*O*-methyl-α/β-D-mannopyranosyl trichloroacetate 19	46
20		2,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranosyl trichloroacetate 20	46
21		Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside 21	47
22		Methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside 22	47

23		Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside 23	48
24		Methyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 24	48
25		2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranosyl trichloroacetate 25	49
26		Methyl 2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 26	49
27		Methyl 2-*O*-acetyl-4,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 27	50
28		Methyl 2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 28	51

4.2 Synthesis experiments

General acetylation procedure

Acetic anhydride (1.5 eq per OH group) and DMAP (cat. amount) were added to a solution of starting material (1.0 eq) in dry pyridine (1.1 mL/mmol for penta-acetylation; 2.2 mL/mmol for mono-acetylation) at 0°C. The reaction mixture was allowed to warm to r.t. and stirred. Then it was carefully quenched at 0°C with $NaHCO_3$ aqueous solution (sat.) and extracted with DCM. The collected organic phases were dried with anhydrous $MgSO_4$ and evaporated to dryness. The desired acetylated product was obtained and purified accordingly.

General benzylation procedure [34]

Distilled benzyl bromide (1.3 eq per OH group) was added to a solution of starting material (1.0 eq) in dry DMF (8.6 mL/mmol). Then, powdered sodium hydride (1.6 eq per OH group) was carefully added at 0°C. The resulting mixture was allowed to warm to r.t. and vigorously stirred. Afterwards, the reaction was carefully quenched with methanol at 0°C. Water was added and the resulting mixture extracted with Et_2O. The collected organic phases were dried with anhydrous Na_2SO_4 and evaporated to dryness. The benzylated product was obtained after purification by chromatography.

General deallylation procedure

$PdCl_2$ (0.2 eq) was added to a solution of the allyl protected compound (1.0 eq) in dry methanol (5.7 mL/mmol). The resulting mixture was vigorously stirred and then filtered through a pad of silica and celite rinsed with ethyl acetate. After proper purification of the filtrate, the desired unprotected product was obtained.

Development of a suitable first glycosyl donor

2,3,4,6-tetra-*O*-acetyl-α/β-D-mannopyranose 1

Two step synthesis

Following the general acetylation procedure D-mannose (2.005 g, 11.13 mmol) reacted overnight affording the desired penta-acetyl mannoside (4.603 g, quantitative). Then it was resuspended in dry DMF (13 mL) to which hydrazine acetate (1.130 g, 12.27 mmol) was added. After stirring for 5 h, the reaction mixture was quenched with $NaHCO_3$ aqueous solution (sat.) and extracted with DCM. The collected organic phases were dried with anhydrous Na_2SO_4 and evaporated to dryness. Purification of the resulting crude by flash column chromatography (Hex/AcOEt 1:1), afforded product **1** (3.755 g, 97% overall yield, α/β=22:1) as a colourless oil.

^{1}H NMR δ: 5.43 (1H, dd, $J_{3,4}$=10.0, $J_{3,2}$=3.1, H-3), 5.34-5.28 (2H, m, H-2, H-4), 5.26 (1H, dd, $J_{1,OH}$=3.9, $J_{1,2}$=1.4, H-1$_\alpha$), 5.23 (1H, ps, H-1$_\beta$), 4.29-4.22 (2H, m, H-5, H-6), 4.17-4.12 (1H, m, H-6), 2.93 (1H, d, $J_{OH,1}$=4.0, OH), 2.17 (3H, s, Me Ac), 2.14 (3H, s, Me Ac), 2.11 (3H, s, Me Ac), 2.09 (3H, s, Me Ac), 2.08 (3H, s, Me Ac), 2.00 (3H, s, Me Ac). ^{13}C NMR δ: 170.11 (C=O Ac), 169.99 (C=O Ac), 169.93 (C=O Ac), 169.76 (C=O Ac), 92.29 (C-1), 69.83 (C-2), 68.65 (C-3, C-5), 66.14 (C-4), 62.54 (C-6), 20.91 (Me Ac), 20.80 (Me Ac), 20.73 (Me Ac), 20.70 (Me Ac). IR: 3436 (OH st), 1738 (C=O st), 1216 (C-O st).

Allyl α/β-D-mannopyranoside 2

To a solution of D-mannose (10.035 g, 55.70 mmol) dissolved in distilled allyl alcohol (75 mL) was added racemic CSA (132 mg, 0.57 mmol). The mixture was heated to reflux and stirred overnight. Afterwards, the solvent was removed and the resulting crude flushed through a silica gel column (DCM/MeOH 9:1 → 8:2). Product **2** (10.334 g, 84%, α/β=11:1) was afforded as a colorless oil.

^{1}H NMR (D_2O) δ: 5.89 (1H, ddt, $J_{2',3'}$=17.2, $J_{2',3'}$=11.1, $J_{2',1'}$=5.8, H-2'), 5.28 (1H, dq, $J_{3',2'}$=17.2, ^2J= $J_{3',1'}$=1.4, H-3'), 5.20 (1H, dd, $J_{3',2'}$=10.5, ^2J=1.1, H-3'), 4.83 (1H, d, $J_{1,2}$=1.6, H-1$_\alpha$), 4.62 (1H, ps, H-1$_\beta$), 4.16 (1H, dd, ^2J=12.7, $J_{1',2'}$=5.4, H-1'), 3.99 (1H, dd, ^2J=12.8, $J_{1',2'}$=6.2, H-1'), 3.87 (1H, dd, $J_{2,3}$=3.4, $J_{2,1}$=1.5, H-2), 3.81 (1H, dd, ^2J=12.1, $J_{6,5}$=1.1, H-6), 3.72 (1H, dd, $J_{3,4}$=9.5, $J_{3,2}$=3.5, H-3), 3.69-3.64 (1H, m, H-6), 3.58-54 (1H, m, H-4, H-5). ^{13}C NMR (D_2O) δ:

133.13 (C-2'), 118.36 (C-3'), 99.01 (C-1), 72.81, 70.52 (C-3), 69.95 (C-2), 68.04 (C-1'), 66.71, 60.80 (C-6). IR: 3415 (OH st).

Allyl 2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranoside 3

Following the general benzylation procedure compound **2** (1.808 g, 8.21 mmol) reacted overnight to afford, after purification by flash column chromatography (Hex/AcOEt 95:5 → 9:1), the desired tetra-benzyl mannoside **3** (3.628 g, 78%, α/β>30:1) as a light yellow oil.

^{1}H NMR δ: 7.38-7.25 (18H, m, Ar), 7.17-1.14 (2H, m, Ar), 5.84 (1H, ddt, $J_{2',3'}$=17.1, $J_{2',3'}$=11.5, $J_{2',1'}$=5.5, H-2'), 5.21 (1H, dq, $J_{3',2'}$=17.5, ^2J=$J_{3',1'}$=1.5, H-3'), 5.14 (1H, dd, $J_{3',2'}$=10.5, ^2J=1.2, H-3'), 4.92 (1H, d, $J_{1,2}$=1.7, H-1), 4.88 (1H, d, ^2J=10.7, CH_2 Bn), 4.73 (2H, ABq, ^2J=12.0, CH_2 Bn), 4.67 (1H, d, ^2J=12.0, CH_2 Bn), 4.62 (2H, s, CH_2 Bn), 4.54 (1H, d, ^2J=12.2, CH_2 Bn), 4.50 (1H, d, ^2J=11.0, CH_2 Bn), 4.16 (1H, dd, ^2J=13.0, $J_{1',2'}$=5.1, H-1'), 3.99 (1H, t, $J_{4,3}$=$J_{4,5}$=9.0, H-4), 3.96-3.91 (2H, m, H-1', H-2), 3.81-3.70 (4H, m, H-3, H-5, H-6). ^{13}C NMR δ: 138.56 (Ar C_q), 138.47 (Ar C_q), 138.43 (Ar C_q), 138.37 (Ar C_q), 133.80 (C-2'), 128.34 (Ar CH), 128.31 (Ar CH), 128.28 (Ar CH), 128.00 (Ar CH), 127.82 (Ar CH), 127.74 (Ar CH), 127.63 (Ar CH), 127.58 (Ar CH), 127.56 (Ar CH), 127.52 (Ar CH), 127.45 (Ar CH), 117.21 (C-3'), 97.08 (C-1), 80.26 (C-2), 75.16 (CH_2 Bn), 74.98 (C-4), 74.66 (C-3), 73.34 (CH_2 Bn), 72.57 (CH_2 Bn), 72.16 (CH_2 Bn), 71.89 (C-5), 69.28 (C-6), 67.80 (C-1'). IR: 1091 (C-O st).

2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranose 4

Following the general deallylation procedure compound **3** (1.200 g, 2.12 mmol) reacted for 4 h to afford, after purification by flash column chromatography (Hex/AcOEt 8:2 → 7:3), product **4** (1.069 g, 93%, α/β>30:1) as a colourless oil.

^{1}H NMR δ: 7.38-7.26 (18H, m, Ar), 7.17-7.15 (2H, m, Ar), 5.27 (1H, dd, $J_{1,OH}$=3.3, $J_{1,2}$=1.9, H-1), 4.88 (1H, d, ^2J=10.8, CH_2 Bn), 4.74 (2H, ABq, ^2J=12.5, CH_2 Bn), 4.63 (2H, s, CH_2 Bn), 4.58 (1H, d, ^2J=10.1, CH_2 Bn), 4.50 (1H, d, ^2J=10.6, CH_2 Bn), 4.01 (1H, ddd, $J_{5,4}$=9.7, $J_{5,6}$=5.8, $J_{5,6}$=2.4, H-5), 3.96 (1H, dd, $J_{3,4}$=9.1, $J_{3,2}$=2.8, H-3), 3.89 (1H, t, $J_{4,3}$=$J_{4,5}$=9.2, H-4), 3.81 (1H, t, $J_{2,1}$=$J_{2,3}$=2.3, H-2), 3.74-3.67 (2H, m, H-6), 2.51 (1H, d, $J_{OH,1}$=3.3, OH). ^{13}C NMR δ: 138.47 (Ar C_q), 138.37 (Ar C_q), 138.31 (Ar C_q), 138.16 (Ar C_q), 128.57 (Ar CH), 128.53 (Ar CH), 128.37 (Ar CH), 128.34 (Ar CH), 128.16 (Ar CH), 127.99 (Ar CH), 127.93 (Ar CH), 127.87

(Ar CH), 127.66 (Ar CH), 127.64 (Ar CH), 127.62 (Ar CH), 127.58 (Ar CH), 92.87 (C-1), 79.67 (C-3), 75.17 (C-4), 75.08 (CH_2 Bn), 74.71 (C-2), 73.41 (CH_2 Bn), 72.73 (CH_2 Bn), 72.23 (CH_2 Bn), 71.82 (C-5), 69.05 (C-6). IR: 3415 (OH st), 1087 (C-O st).

2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranosyl *N*-trichloroacetylcarbamate 5 [35, 36]

Trichloroacetyl isocyanate (31 µL, 0.25 mmol) was added to a solution of compound **4** (121 mg, 0.22 mmol) in dry DCM (2.3 mL) at 0°C. The reaction mixture was allowed to warm to r.t. and stirred for 40 min. Then it was quickly filtered through a pad of silica rinsed with a solvent mixture of Hex/AcOEt (7:3). Glycosyl donor **5** was afforded (176 mg, quantitative, α/β=9:1) as a white solid.

^{1}H NMR δ: 8.36 (1H, br s, NH), 7.40-7.38 (2H, m, Ar), 7.34-7.25 (16H, m, Ar), 7.18-7.16 (2H, m, Ar), 6.27 (1H, d, $J_{1,2}$=1.9, H-1$_\alpha$), 5.66 (1H, ps, H-1$_\beta$), 4.88 (1H, d, ^2J=10.5, CH_2 Bn), 4.76 (2H, s, CH_2 Bn), 4.67-4.58 (3H, m, CH_2 Bn), 4.53 (2H, ABq, ^2J=11.0, CH_2 Bn), 4.10 (1H, t, $J_{4,3}$=$J_{4,5}$=9.5, H-4), 3.92-3.86 (2H, m, H-3, H-5), 3.84 (1H, t, $J_{2,1}$=$J_{2,3}$=2.4, H-2), 3.77 (1H, dd, ^2J=10.5, $J_{6,5}$=4.3, H-6), 3.71 (1H, dd, ^2J=11.0, $J_{6,5}$=1.8, H-6). ^{13}C NMR δ: 157.45 (C=O Carbamate), 148.16 (C=O Trichloroacetyl), 138.06 (Ar C_q), 138.02 (Ar C_q), 137.98 (Ar C_q), 137.52 (Ar C_q), 128.60 (Ar CH), 128.50 (Ar CH), 128.45 (Ar CH), 128.41 (Ar CH), 128.39 (Ar CH), 128.09 (Ar CH), 128.05 (Ar CH), 127.97 (Ar CH), 127.92 (Ar CH), 127.84 (Ar CH), 127.82 (Ar CH), 127.67 (Ar CH), 95.28 (C-1), 91.60 (CCl_3), 78.72, 75.29 (CH_2 Bn), 75.03, 73.98 (C-4), 73.57 (CH_2 Bn), 73.28 (C-2), 72.84 (CH_2 Bn), 72.39 (CH_2 Bn), 68.70 (C-6). IR: 1791 (C=O st), 1726 (C=O st), 1097 (C-O st). M.p.= 80.3-83.2°C.

Development of a suitable combined glycosyl donor and acceptor

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

Racemic CSA (cat. amount; pH=1) was added to a mixture of allyl mannoside **2** (3.601 g, 16.35 mmol) and BDA (4.95 mL, 32.98 mmol) in dry THF (11 mL). After heating to reflux and stirring for 4.5 h, the reaction mixture was quenched with $NaHCO_3$ aqueous solution (sat.) and extracted with AcOEt. The collected organic phases were dried with anhydrous Na_2SO_4 and evaporated to dryness. Crude purification by flash column chromatography (Hex/AcOEt 2:3), afforded benzylidene **6** (2.209 g, 43%, α/β=29:1) as a white solid.

^{1}H NMR δ: 7.49-7.47 (2H, m, Ar), 7.38-7.35 (3H, m, Ar), 5.89 (1H, ddt, $J_{2',3'}$=17.1, $J_{2',3'}$=10.7, $J_{2',1'}$=5.6, H-2'), 5.54 (1H, s, CH Benzylidene), 5.28 (1H, dq, $J_{3',2'}$=17.3, ^2J=$J_{3',1'}$'=1.5, H-3'), 5.20 (1H, dd, $J_{3',2'}$=10.3, ^2J=1.2, H-3'), 4.85 (1H, d, $J_{1,2}$=1.0, H-1$_\alpha$), 4.54 (1H, ps, H-1$_\beta$), 4.25 (1H, dd, ^2J=9.1, $J_{6,5}$=3.1, H-6), 4.18 (1H, ddt, ^2J=12.9, $J_{1',2'}$=5.2, $J_{1',3'}$=1.3 H-1'), 4.05 (1H, m, H-3), 4.00-3.95 (2H, m, H-1', H-2), 3.90 (1H, t, $J_{4,3}$=$J_{4,5}$=8.9, H-4), 3.86-3.77 (2H, m, H-5, H-6), 3.09 (1H, br s, OH) 3.04 (1H, br s, OH). ^{13}C NMR δ: 137.20 (Ar C$_q$), 133.48 (C-2'), 129.32 (Ar CH), 128.39 (Ar CH), 126.31 (Ar CH), 117.78 (C-3'), 102.28 (CH Benzylidene), 99.44 (C-1), 78.92 (C-4), 70.97 (C-2), 68.79 (C-6), 68.60 (C-3), 68.26 (C-1'), 63.20 (C-5). IR: 3222 (OH st), 1095 (C-O st), 1066 (C-O st). M.p.= 128.2-130.1°C.

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

Two step synthesis

Dibutyltin oxide (2.208 g, 8.87 mmol) was added to a solution of compound **6** (2.528 g, 8.20 mmol) in dry methanol (30 mL). The mixture was stirred vigorously and heated to reflux for 1.5 h, until it became clear. Then, the solvent was removed and the crude dried under vacuum. The stannylene intermediate was dissolved in dry DMF (30 mL) and methyl iodide was added (2.6 mL, 5 eq). The resulting solution was stirred and heated at 50°C overnight. Afterwards, the solvent was evaporated and the crude taken up in DCM. The resulting solution was filtered, concentrated and applied to a column of silica gel (Hex/AcOEt 9:1 → 8:2 → 7:3 → 5:5) affording the desired methyl mannoside **7** (2.115 g, 80%, α/β=18:1) as a yellow oil.

^{1}H NMR δ: 7.51-7.48 (2H, m, Ar), 7.39-7.33 (3H, m, Ar), 5.91 (1H, ddt, $J_{2',3'}$=17.2, $J_{2',3'}$=11.5, $J_{2',1'}$=5.4, H-2'), 5.59 (CH Benzylidene), 5.32 (1H, dq, $J_{3',2'}$=17.2, ^2J=$J_{3',1'}$=1.5, H-3'), 5.23 (1H, dq, $J_{3',2'}$=10.3, ^2J=$J_{3',1'}$=1.2, H-3'), 4.96 (1H, ps, H-1$_\beta$), 4.93 (1H, d, $J_{1,2}$=1.2, H-1$_\alpha$), 4.26 (1H, dd, ^2J=9.7, $J_{6,5}$=3.3, H-6), 4.20 (1H, ddt, ^2J=13.0, $J_{1',2'}$=5.1, $J_{1',3'}$=1.4, H-1'), 4.12 (1H, dd, $J_{2,3}$=3.6, $J_{2,1}$=1.3, H-2), 4.04-3.98 (2H, m, H-1', H-4), 3.90-3.80 (2H, m, H-5, H-6), 3.71 (1H, dd, $J_{3,4}$=9.5, $J_{3,2}$=3.6, H-3), 3.55 (3H, s, OMe), 2.70 (1H, br s, OH). ^{13}C NMR δ: 137.50 (Ar C$_q$), 133.50 (C-2'), 129.00 (Ar CH), 128.24 (Ar CH), 126.15 (Ar CH), 117.95 (C-3'), 101.77 (CH Benzylidene), 99.19 (C-1), 78.75 (C-4), 77.34 (C-3), 69.20 (C-2), 68.87 (C-6), 68.25 (C-1'), 63.33 (C-5), 58.68 (OMe). IR: 3431 (OH st), 1118 (C-O st), 1087 (C-O st).

Allyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α/β-D-mannopyranoside 8

Following the general acetylation procedure compound **7** (4.237 g, 13.14 mmol) reacted for 1 h affording, after purification by flash column chromatography (Hex/AcOEt 9:1 → 8:2 → 7:3), the acetylated mannoside **8** (4.400 g, 92%, α/β=27:1) as colourless oil.

^{1}H NMR δ: 7.51-7.49 (2H, m, Ar), 7.39-7.33 (3H, m, Ar), 5.91 (1H, ddt, $J_{2',3'}$=17.2, $J_{2',3'}$=10.8, $J_{2',1'}$=5.8, H-2'), 5.61 (1H, s, CH Benzylidene), 5.38 (1H, dd, $J_{2,3}$=3.5, $J_{2,1}$=1.4, H-2), 5.32 (1H, dq, $J_{3',2'}$=17.2, ^2J=$J_{3',1'}$=1.5, H-3'), 5.24 (1H, dd, $J_{3',2'}$=10.3, ^2J=1.2, H-3'), 4.92 (1H, d, $J_{1,2}$=1.4, H-1$_\beta$), 4.84 (1H, d, $J_{1,2}$=1.4, H-1$_\alpha$), 4.27 (1H, dd, ^2J=9.5, $J_{6,5}$=4.0, H-6), 4.19 (1H, ddt, ^2J=12.8, $J_{1',2'}$=5.3, $J_{1',3'}$=1.4, H-1'), 4.04-3.98 (2H, m, H-1', H-4), 3.90 (1H, td, $J_{5,4}$=$J_{5,6ax}$=9.8, $J_{5,6eq}$=4.1, H-5), 3.86-3.80 (2H, m, H-3, H-6), 3.50 (s, OMe β), 3.46 (3H, s, OMe α), 2.16 (3H, s, Me Ac α), 2.12 (s, Me Ac β). ^{13}C NMR δ: 170.20 (C=O Ac), 137.34 (Ar C$_q$), 133.21 (C-2'), 129.01 (Ar CH), 128.24 (Ar CH), 126.18 (Ar CH), 118.20 (C-3'), 101.86 (CH Benzylidene), 97.84 (C-1), 78.56 (C-4), 75.83 (C-3), 69.29 (C-2), 68.73 (C-6), 68.44 (C-1'), 63.89 (C-5), 58.44 (OMe), 21.02 (Me Ac). IR: 1745 (C=O st), 1230 (C-O st ester), 1093 (C-O st), 1066 (C-O st).

Allyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranoside 9

$NaBH_3CN$ (9.1 g, 0.14 mol) was added to a stirred solution of compound **8** (4.343 g, 12.03 mmol) in dry THF (45 mL) at 0°C. Then, freshly prepared HCl (1 M in Et_2O) was added portion-wise (125 mL; 5 mL portions). Upon stirring for an additional 30 min, the solvent was removed. The resulting crude was re-suspended in water and extracted with DCM. The collected organic phases were dried with anhydrous Na_2SO_4 and evaporated to dryness. Crude purification by flash column chromatography (Hex/AcOEt 9:1 → 7:3 → 6:4 → 5:5) afforded product **9** (3.036 g, 70%, α/β= 18:1) as a colorless oil.

^{1}H NMR δ: 7.37-7.28 (5H, m, Ar), 5.90 (1H, ddt, $J_{2',3'}$=17.2, $J_{2',3'}$=10.9, $J_{2',1'}$=5.6, H-2'), 5.32-5.27 (2H, m, H-3', H-2), 5.21 (1H, dq, $J_{3',2'}$=17.3, ^2J=$J_{3',1'}$=1.3, H-3'), 4.87 (1H, d, $J_{1,2}$=1.5, H-1$_\alpha$), 4.83 (1H, d, $J_{1,2}$=1.5, H-1$_\beta$), 4.62 (2H, ABq, ^2J=12.1, CH_2 Bn), 4.19 (1H, ddt, ^2J=12.8, $J_{1',2'}$=5.2, $J_{1',3'}$=1.2, H-1'), 4.00 (1H, ddt, ^2J=12.6, $J_{1',2'}$=5.9, $J_{1',3'}$=1.2, H-1'), 3.89 (1H, t, $J_{4,3}$= $J_{4,5}$=9.3, H-4), 3.83-3.77 (3H, m, H-5, H-6), 3.57 (1H, dd, $J_{3,4}$=9.2, $J_{3,2}$=3.4, H-3), 3.44 (s, OMe β), 3.42 (3H, s, OMe α), 2.52 (1H, br s, OH), 2.15 (s, Me Ac β), 2.11 (3H, s, Me Ac α). ^{13}C NMR δ: 170.38 (C=O Ac), 138.13 (Ar C$_q$), 133.41 (C-2'), 128.38 (Ar CH), 127.65 (Ar CH), 127.56 (Ar

CH), 117.94 (C-3'), 97.06 (C-1), 79.31 (C-3), 73.57 (CH_2 Bn), 71.12 (C-5), 69.90 (C-6), 68.27 (C-1'), 67.61 (C-4), 67.42 (C-2), 57.49 (OMe), 20.99 (Me Ac). IR: 3438 (OH st), 1745 (C=O st), 1234 (C-O st ester), 1074 (C-O st), 1049 (C-O st).

Allyl 2-*O*-acetyl-4,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranoside 10

Following the general benzylation procedure compound **9** (84 mg, 0.23 mmol) reacted for 1 h affording, after purification by preparative TLC (Hex/AcOEt 7:3), product **10** (53 mg, 50%, α/β=22:1) as a colourless oil.

^{1}H NMR δ: 7.37-7.26 (10H, m, Ar), 7.21-7.19 (2H, m, Ar), 5.88 (1H, ddt, $J_{2',3'}$=17.3, $J_{2',3'}$=10.9, $J_{2',1'}$=5.8, H-2'), 5.32-5.23 (2H, m, H-3', H-2), 5.19 (1H, dd, $J_{3',2'}$=10.5, ^2J=1.1, H-3'), 4.89 (1H, d, $J_{1,2}$=1.4, H-1), 4.84 (1H, d, ^2J=11.0, CH_2 Bn), 4.69 (1H, d, ^2J=12.0, CH_2 Bn), 4.51 (1H, d, ^2J=12.0, CH_2 Bn), 4.48 (1H, d, ^2J=11.0, CH_2 Bn), 4.16 (1H, dd, ^2J=12.9, $J_{1',2'}$=5.2, H-1'), 3.98 (1H, dd, ^2J=12.8, $J_{1',2'}$=6.0, H-1'), 3.80-3.66 (5H, m, H-3, H-4, H-5, H-6), 3.44 (3H, s, OMe α), 3.35 (s, OMe β), 2.15 (3H, s, Me Ac α), 1.98 (s, Me Ac β). ^{13}C NMR δ: 170.08 (C=O Ac), 138.47 (Ar C_q), 138.19 (Ar C_q), 133.43 (C-2'), 128.34 (Ar CH), 128.32 (Ar CH), 127.84 (Ar CH), 127.76 (Ar CH), 127.63 (Ar CH), 127.58 (Ar CH), 117.85 (C-3'), 96.79 (C-1), 80.07, 75.11 (CH_2 Bn), 74.46, 73.40 (CH_2 Bn), 73.31, 68.85 (C-6), 68.48 (C-2), 68.19 (C-1'), 57.59 (OMe), 21.11 (Me Ac). IR: 1741 (C=O st), 1234 (C-O st ester), 1053 (C-O st).

2-*O*-acetyl-4,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranose 11

Following the general deallylation procedure compound **10** (52 mg, 0.12 mmol) reacted for 4 h affording, after purification by preparative TLC (Hex/AcOEt 1:1), product **11** (30 mg, 62%, α/β=18:1) as a colourless oil.

^{1}H NMR δ: 7.36-7.28 (10H, m, Ar), 7.22-7.20 (2H, m, Ar), 5.32 (1H, dd, $J_{2,3}$=3.3, $J_{2,1}$=1.9, H-2), 5.25 (1H, dd, $J_{1,OH}$=3.7, $J_{1,2}$=1.7, H-1), 4.84 (1H, d, ^2J=11.4, CH_2 Bn), 4.64 (1H, d, ^2J=12.1, CH_2 Bn), 4.53 (1H, d, ^2J=12.4, CH_2 Bn), 4.48 (1H, d, ^2J=11.1, CH_2 Bn), 4.04 (1H, dt, $J_{5,4}$=9.3, $J_{5,6}$=3.7, H-5), 3.78 (1H, dd, $J_{3,4}$=9.3, $J_{3,2}$=3.2, H-3), 3.74-3.69 (3H, m, H-4, H-6), 3.44 (3H, s, OMe α), 3.34 (s, OMe β), 2.67 (1H, d, $J_{1,OH}$=3.7, OH), 2.15 (3H, s, Me Ac α), 2.05 (s, Me Ac β). ^{13}C NMR δ: 170.48 (C=O Ac), 138.42 (Ar C_q), 138.14 (Ar C_q), 128.37 (Ar CH), 128.35 (Ar CH), 127.94 (Ar CH), 127.84 (Ar CH), 127.70 (Ar CH), 127.65 (Ar CH), 92.47 (C-1), 79.54 (C-

3), 75.02 (CH_2 Bn), 74.62 (C-4), 73.48 (CH_2 Bn), 71.24 (C-5), 69.26 (C-6), 68.62 (C-2), 57.59 (OMe), 21.11 (Me Ac). IR: 3404 (OH st), 1737 (C=O st), 1236 (C-O st ester), 1053 (C-O st).

2-*O*-acetyl-4,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranosyl trichloroacetate 12

Trichloroacetyl chloride (7 µL, 0.06 mmol) was added to a mixture of compound **11** (17 mg, 0.04 mmol) and dry pyridine (7 µL, 0.08 mmol) in dry DCM (0.6 mL). After reacting for 1.5 h, more trichloroacetyl chloride (2 µL, 0.02 mmol) was added and stirring continued for another 30 min. Then, the reaction mixture was quickly filtered through a pad of silica rinsed with a solvent mixture of Hex/AcOEt (8:2), affording glycosyl donor **12** (41 mg, quantitative, α/β=17:1) as a colourless oil.

^{1}H NMR δ: 7.34-7.28 (10H, m, Ar), 7.23-7.21 (2H, m, Ar), 6.22 (1H, d, $J_{1,2}$=1.9, H-1$_\alpha$), 5.82 (1H, d, $J_{1,2}$=1.9, H-1$_\beta$), 5.41 (1H, dd, $J_{2,3}$=3.6, $J_{2,1}$=2.0, H-2), 4.84 (1H, d, ^2J=10.7, CH_2 Bn), 4.68 (1H, d, ^2J=12.1, CH_2 Bn), 4.52 (1H, d, ^2J=10.4, CH_2 Bn), 4.50 (1H, d, ^2J=12.1, CH_2 Bn), 3.99-3.89 (2H, m, H-4, H-5), 3.82 (1H, dd, $J_{3,4}$=10.9, $J_{3,2}$=3.6, H-3), 3.74-3.69 (2H, m, H-6), 3.47 (3H, s, OMe α), 3.35 (s, OMe β), 2.19 (3H, s, Me Ac α), 2.04 (s, Me Ac β). ^{13}C NMR δ: 170.37 (C=O Ac), 138.52 (Ar C_q), 138.22 (Ar C_q), 128.38 (Ar CH), 128.36 (Ar CH), 127.92 (Ar CH), 127.83 (Ar CH), 127.74 (Ar CH), 127.62 (Ar CH), 96.51 (C-1), 88.22 (CCl_3), 82.53 (C-3), 75.42 (CH_2 Bn), 74.76 (C-4), 73.52 (CH_2 Bn), 72.16 (C-5), 69.53 (C-6), 68.89 (C-2), 57.63 (OMe), 21.15 (Me Ac). IR: 1742 (C=O st), 1224 (C-O st ester), 1047 (C-O st), 816 (C-Cl st).

Allyl 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-α/β-D-mannopyranoside 13

Distilled TBSOTf (0.21 mL, 0.96 mmol) was added dropwise to a mixture of compound **9** (162 mg, 0.44 mmol) and dry DIPEA (0.22 mL, 1.26 mmol) in dry DCM at 0°C. After stirring for 40 min, the reaction was quenched with $NaHCO_3$ aqueous solution (sat.) and extracted with DCM. The collected organic phases were dried with anhydrous $MgSO_4$ and evaporated to dryness. Purification of the resulting crude by flash column chromatography (Hex/AcOEt 9:1) afforded the desired silyl mannoside **13** (193 mg, 86%, α/β= 9:1) as a colourless oil.

^{1}H NMR δ: 7.36-7.28 (5H, m, Ar), 5.92 (1H, ddt, $J_{2',3'}$=17.1, $J_{2',3'}$=11.2, $J_{2',1'}$=5.2, H-2'), 5.33-5.27 (2H, m, H-3', H-2), 5.21 (1H, dd, $J_{3',2'}$=10.4, ^2J=1.1, H-3'), 4.86 (1H, d, $J_{1,2}$=1.1, H-1$_\alpha$),

4.82 (1H, d, $J_{1,2}$=1.2, H-1$_\beta$), 4.60 (2H, ABq, ^2J=12.0, CH_2 Bn), 4.21 (1H, dd, ^2J=13.0, $J_{1',2'}$=5.0, H-1'), 4.00 (1H, dd, ^2J=13.0, $J_{1',2'}$=6.0, H-1'), 3.81-3.74 (3H, m, H-4, H-5, H-6), 3.68 (1H, dd, ^2J=10.8, $J_{6,5}$=5.0, H-6), 3.43 (1H, dd, $J_{3,4}$=9.1 $J_{3,2}$=3.3, H-3), 3.30 (3H, s, OMe), 2.12 (s, Me Ac β), 2.10 (3H, s, Me Ac α), 0.92 (s, ^{t}Bu TBS β), 0.82 (9H, s, ^{t}Bu TBS α), 0.06 (3H, s, Me TBS), 0.01 (3H, s, Me TBS). ^{13}C NMR δ: 170.45 (C=O Ac), 138.47 (Ar C_q), 133.64 (C-2'), 128.25 (Ar CH), 127.42 (Ar CH), 117.81 (C-3'), 96.83 (C-1), 79.84 (C-3), 73.23 (CH_2 Bn), 72.76 (C-5), 69.59 (C-6), 68.21 (C-1'), 67.89 (C-4), 67.69 (C-2), 56.75 (OMe), 25.96 (3xMe ^{t}Bu), 21.02 (Me Ac), 18.25 (^{t}Bu C_q), -4.06 (Me TBS), -5.17 (Me TBS). IR: 1747 (C=O st), 1232 (C-O st ester), 1107 (C-O st), 1056 (C-O st), 835 (Si-O-C bend).

2-*O*-acetyl-6-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-3-*O*-methyl-α/β-D-mannopyranose 14

Following the general deallylation procedure compound **13** (100 mg, 0.23 mmol) reacted for 1 h and 20 min affording, after purification by preparative TLC (Hex/AcOEt 7:3), product **14** (37 mg, 40%, α/β=7:3) as a colourless oil.

^{1}H NMR δ: 7.38-7.30 (5H, m, Ar), 5.34 (1H, dd, $J_{2,3}$=3.6, $J_{2,1}$=1.8, H-2), 5.22 (1H, ps, H-1), 4.60 (2H, ABq, ^2J=12.2, CH_2 Bn), 4.03 (1H, dt, $J_{5,4}$=$J_{5,6}$=8.4, $J_{5,6}$=1.4, H-5), 3.79 (1H, dd, ^2J=10.2, $J_{6,5}$= 1.5, H-6), 3.67 (1H, t, $J_{4,3}$=$J_{4,5}$=9.1, H-4), 3.59 (1H, dd, ^2J=10.6, $J_{6,5}$= 8.3, H-6), 3.49 (1H, dd, $J_{3,4}$=8.9, $J_{3,2}$=3.2, H-3), 3.32 (3H, s, OMe), 2.17 (s, Me Ac β), 2.12 (3H, s, Me Ac α), 0.85 (s, ^{t}Bu TBS β), 0.82 (9H, s, ^{t}Bu TBS α), 0.07 (3H, s, Me TBS), 0.01 (3H, s, Me TBS). ^{13}C NMR δ: 170.43 (C=O Ac), 137.84 (Ar Cq), 128.37 (Ar CH), 127.93 (Ar CH), 127.70 (Ar CH), 92.40 (C-1), 79.28 (C-3), 73.31 (CH_2 Bn), 72.20 (C-5), 69.93 (C-6), 68.28 (C-4), 68.04 (C-2), 56.80 (OMe), 25.94 (3xMe ^{t}Bu), 21.04 (Me Ac), 18.19 (^{t}Bu Cq), -4.05 (Me TBS), -5.20 (Me TBS). IR: 3427 (OH st), 1747 (C=O st), 1240 (C-O st ester), 1078 (C-O st), 1053 (C-O st), 835 (Si-O-C bend).

4-Methoxybenzyl-2,2,2-trichloroacetimidate 15

Distilled trichloroacetonitrile (1.1 mL, 10.97 mmol) and distilled DBU (0.14 mL, 0.94 mmol) were added to a solution of 4-methoxybenzyl alcohol (1.2 mL, 9.47 mmol) in dry DCM (20 mL) at 0°C. After stirring at r.t. for 5 h, the resulting mixture was concentrated and quickly filtered through a pad of silica rinsed with a solvent mixture of Hex/AcOEt (8:2), affording trichloroacetimidate **15** (2.397 g, 87%) as a yellow liquid.

^{1}H NMR δ: 8.36 (1H, s br, NH), 7.37 (2H, d, ^3J=8.1, Ar(*o*)), 6.91 (2H, d, ^3J=8.0, Ar(*m*)), 5.27 (2H, s, CH_2 PMB), 3.81 (3H, s, OMe). ^{13}C NMR δ: 162.63 (C=NH), 159.71 (Ar(*p*)), 129.71 (Ar(*o*)), 127.50 (PMB C_q), 113.91 (Ar(*m*)), 88.81 (CCl_3), 70.69 (CH_2 PMB), 55.28 (OMe). IR: 1660 (N=C st), 802 (C-Cl st).

Allyl 2-*O*-acetyl-6-*O*-benzyl-4-*O*-*p*-methoxybenzyl-3-*O*-methyl-α/β-D-mannopyranoside 16

Distilled TfOH (28 μL, 0.32 mmol) was added dropwise to a stirred mixture of compound **9** (0.850 g, 2.32 mmol), trichloroacetimidate **15** (0.96 mL, 4.64 mmol) and molecular sieves in dry THF (33 mL) at 0°C. The mixture was stirred for 30 min and then it was quenched with $NaHCO_3$ aqueous solution (sat.) and extracted with DCM. The collected organic phases were dried with anhydrous Na_2SO_4 and evaporated to dryness. Purification by flash column chromatography (Hex/AcOEt 9:1 → 85:15) afforded product **16** (0.860 g, 76%, α/β>30:1) as colourless oil.

^{1}H NMR δ: 7.37-7.27 (5H, m, Ar), 7.12 (2H, d, ^3J=8.6, Ar(*o*)), 6.82 (2H, d, ^3J=8.7, Ar(*m*)), 5.87 (1H, ddt, $J_{2',3'}$=17.4, $J_{2',3'}$=10.8, $J_{2',1'}$=5.6, H-2'), 5.30-5.24 (2H, m, H-3', H-2), 5.18 (1H, dd, $J_{3',2'}$=10.4, ^2J=1.2, H-3'), 4.88 (1H, d, $J_{1,2}$=1.8, H-1), 4.75 (1H, d, ^2J=10.3, CH_2 PMB), 4.69 (1H, d, ^2J=12.0, CH_2 Bn), 4.51 (1H, d, ^2J=12.0, CH_2 Bn), 4.40 (1H, d, ^2J=10.3, CH_2 PMB), 4.16 (1H, ddt, ^2J=12.9, $J_{1',2'}$=5.1, $J_{1',3'}$=1.1 H-1'), 3.98 (1H, ddt, ^2J=12.9, $J_{1',2'}$=6.2, $J_{1',3'}$=1.2 H-1'), 3.81-3.68 (8H, m, H-3, H-4, H-5, H-6, OMe), 3.45 (3H, s, OMe), 2.15 (3H, s, Me Ac). ^{13}C NMR δ: 170.53 (C=O Ac), 159.22 (Ar(*p*)), 138.28 (Bn C_q), 133.43 (C-2'), 130.62 (PMB C_q), 129.56 (Ar(*o*)), 128.33 (Ar CH), 127.78 (Ar CH), 127.59 (Ar CH), 117.82 (C-3'), 113.77 (Ar(*m*)), 96.77 (C-1), 80.08, 74.78 (CH_2 PMB), 74.13, 73.38 (CH_2 Bn), 71.32, 68.84 (C-6), 68.51 (C-2), 68.15 (C-1'), 57.63 (OMe), 55.29 (OMe), 21.12 (Me Ac). IR: 1740 (C=O st), 1242 (C-O st ester), 1087 (C-O st), 1033 (C-O st).

Allyl 2,6-di-*O*-benzyl-4-*O*-*p*-methoxybenzyl-3-*O*-methyl-α/β-D-mannopyranoside 17

Two step synthesis

NaOMe (1.06 mL, 1 M in MeOH, 0.6 eq) was added to a solution of compound **16** (0.857 g, 1.76 mmol) in dry MeOH at 0°C. After stirring for 1 h, the reaction was allowed to warm to r.t. and stirred for another 2 h. Then the resulting mixture was quenched with NH_4Cl aqueous so-

lution (sat.) and extracted with DCM. The collected organic phases were dried with anhydrous Na_2SO_4 and evaporated to dryness, affording the desired alcohol (0.780 g, 1.75 mmol, 99%). Afterwards, it was subjected to the general benzylation procedure, reacting for 1 h. Purification by flash column chromatography (Hex/AcOEt 95:5 $\rightarrow$ 9:1 $\rightarrow$ 85:15) afforded product **17** (0.856 g, 92%, α/β>30:1) as a colorless oil.

^{1}H NMR δ: 7.40-7.27 (10H, m, Ar), 7.13 (2H, d, ^3J=8.6, Ar(*o*)), 6.81 (2H, d, ^3J=8.5, Ar(*m*)), 5.84 (1H, ddt, $J_{2',3'}$=17.2, $J_{2',3'}$=10.4, $J_{2',1'}$=5.3, H-2'), 5.22 (1H, dq, $J_{3',2'}$=17.2, ^2J=$J_{3',1'}$=1.4, H-3'), 5.14 (1H, dd, $J_{3',2'}$=10.3, ^2J=1.1, H-3'), 4.92 (1H, d, $J_{1,2}$=1.7, H-1), 4.78 (1H, d, ^2J=10.1, CH_2 PMB), 4.74 (2H, ABq, ^2J=12.6, CH_2 Bn), 4.67 (1H, d, ^2J=12.0, CH_2 Bn), 4.54 (1H, d, ^2J=12.0, CH_2 Bn), 4.42 (1H, d, ^2J=10.1, CH_2 PMB), 4.15 (1H, dd, ^2J=12.8, $J_{1',2'}$=5.1, H-1'), 3.93 (1H, dd, ^2J=12.8, $J_{1',2'}$=6.2, H-1'), 3.87 (1H, t, $J_{4,3}$=$J_{4,5}$=9.1, H-4), 3.82 (1H, dd, $J_{2,3}$=3.4, $J_{2,1}$=1.7, H-2), 3.78 (3H, s, OMe), 3.76-3.69 (3H, m, H-5, H-6), 3.64 (1H, dd, $J_{3,4}$=9.4, $J_{3,2}$=3.2, H-3), 3.44 (3H, s, OMe). ^{13}C NMR δ: 159.19 (Ar(*p*)), 138.51 (Bn C_q), 138.36 (Bn C_q), 133.82 (C-2'), 130.84 (PMB C_q), 129.62 (Ar(*o*)), 128.31 (Ar CH), 128.29 (Ar CH), 127.79 (Ar CH), 127.73 (Ar CH), 127.58 (Ar CH), 127.44 (Ar CH), 117.37 (C-3'), 113.75 (Ar(*m*)), 96.93 (C-1), 81.97 (C-3), 74.70 (CH_2 PMB), 74.62 (C-4), 74.01 (C-2), 73.31 (CH_2 Bn), 72.56 (CH_2 Bn), 71.73 (C-5), 69.28 (C-6), 67.82 (C-1'), 57.74 (OMe), 55.28 (OMe). IR: 1087 (C-O st), 1033 (C-O st).

2,6-di-*O*-benzyl-4-*O*-*p*-methoxybenzyl-3-*O*-methyl-α/β-D-mannopyranose 18

Following the general deallylation procedure compound **17** (0.856 g, 1.60 mmol) reacted for 4 h affording, after purification by flash column chromatography (Hex/AcOEt 85:15 $\rightarrow$ 75:25 $\rightarrow$ 65:35), product **18** (0.710 g, 90%, α/β=12:1) as a colourless oil.

^{1}H NMR δ: 7.40-7.28 (10H, m, Ar), 7.13 (2H, d, ^3J=8.8, Ar(*o*)), 6.81 (2H, d, ^3J=8.7, Ar(*m*)), 5.27 (1H, dd, $J_{1,OH}$=3.5, $J_{1,2}$=1.9, H-1), 4.78 (1H, d, ^2J=11.1, CH_2 PMB), 4.74 (2H, ABq, ^2J=12.4, CH_2 Bn), 4.57 (2H, ABq, ^2J=12.4, CH_2 Bn), 4.42 (1H, d, ^2J=10.6, CH_2 PMB), 3.97 (1H, ddd, $J_{5,4}$=9.8, $J_{5,6}$=6.3, $J_{5,6}$=2.0, H-5), 3.83 (1H, dd, $J_{2,3}$=3.2, $J_{2,1}$=1.9, H-2), 3.79 (3H, s, OMe), 3.76-3.63 (4H, m, H-3, H-4, H-6), 3.53 (s, OMe β) 3.45 (3H, s, OMe α), 2.62 (1H, br s, OH). ^{13}C NMR δ: 159.19 (Ar(*p*)), 138.34 (Bn C_q), 138.24 (Bn C_q), 130.71 (PMB C_q), 129.64 (Ar(*o*)), 128.34 (Ar 2xCH), 127.93 (Ar CH), 127.83 (Ar CH), 127.64 (Ar CH), 127.55 (Ar CH), 113.75 (Ar(*m*)), 92.75 (C-1), 81.49 (C-3), 74.75 (C-4), 74.59 (CH_2 PMB), 74.04 (C-2), 73.37 (CH_2 Bn), 72.72 (CH_2 Bn), 71.70 (C-5), 69.65 (C-6), 57.76 (OMe), 55.29 (OMe). IR: 3402 (OH st), 1080

(C-O st).

2,6-di-*O*-benzyl-4-*O*-*p*-methoxybenzyl-3-*O*-methyl-α/β-D-mannopyranosyl trichloroacetate 19

Trichloroacetyl chloride (63 µL, 0.54 mmol) and DMAP (cat. amount) were added to a mixture of compound **18** (183 mg, 0.36 mmol) and dry pyridine (0.28 mL, 3.24 mmol) in dry DCM (9.5 mL) at 0°C. The mixture was stirred for 30 min and then it was quickly filtered through a pad of silica rinsed with a solvent mixture of Hex/AcOEt (7:3), affording product **19** (213 mg, 90%, α/β=12:1) as a colorless oil.

^{1}H NMR δ: 7.43-7.28 (10H, m, Ar), 7.14 (2H, d, ^3J=8.6, Ar(*o*)), 6.83 (2H, d, ^3J=8.6, Ar(*m*)), 6.28 (1H, d, $J_{1,2}$=2.0, H-1$_\alpha$), 5.80 (1H, d, $J_{1,2}$=1.3, H-1$_\beta$), 4.79 (2H, ABq, ^2J=12.3, CH_2 Bn), 4.77 (1H, d, ^2J=10.4, CH_2 PMB), 4.66 (1H, d, ^2J=11.9, CH_2 Bn), 4.51 (1H, d, ^2J=11.9, CH_2 Bn), 4.46 (1H, d, ^2J=10.4, CH_2 PMB), 4.04 (1H, t, $J_{4,3}$=$J_{4,5}$=9.3, H-4), 3.91-3.86 (2H, m, H-2, H-5), 3.79-3.76 (4H, m, H-6, OMe), 3.69 (1H, dd, ^2J=11.2, $J_{6,5}$=1.7, H-6), 3.61 (1H, dd, $J_{3,4}$=9.2, $J_{3,2}$=3.1, H-3), 3.44 (3H, s, OMe). ^{13}C NMR δ: 160.78 (C=O Trichloroacetyl), 159.16 (Ar(*p*)), 137.48 (Bn C_q), 137.34 (Bn C_q), 130.30 (PMB C_q), 129.80 (Ar(*o*)), 128.48 (Ar CH), 128.34 (Ar CH), 127.96 (Ar CH), 127.85 (Ar CH), 127.81 (Ar CH), 127.58 (Ar CH), 113.84 (Ar(*m*)), 97.02 (C-1), 89.28 (CCl_3), 81.13 (C-3), 75.21 (C-5), 74.87 (CH_2 PMB), 73.44 (C-4), 73.42 (CH_2 Bn), 73.08 (CH_2 Bn), 72.78 (C-2), 68.43 (C-6), 58.04 (OMe), 55.29 (OMe). IR: 1770 (C=O st), 1246 (C-O st ester), 1087 (C-O st), 823 (C-Cl st).

2,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranosyl trichloroacetate 20

DDQ (150 mg, 0.66 mmol) was added to compound **19** (210 mg, 0.33 mmol) in DCM (6.6 mL) and water (0.4 mL). The mixture was vigorously stirred for 1 h and 20 min. Then anhydrous Na_2SO_4 was added and the resulting mixture was quickly filtered through a pad of silica and celite rinsed with AcOEt. Purification by flash column chromatography (Hex/AcOEt 85:15 → 75:25) afforded the desired combined glycosyl donor and acceptor **20** (98 mg, 57%, α/β>30:1) as a colourless oil.

^{1}H NMR δ: 7.42-7.29 (10H, m, Ar), 6.32 (1H, d, $J_{1,2}$=1.8, H-1), 4.76 (2H, ABq, ^2J=12.1, CH_2 Bn), 4.61 (2H, ABq, ^2J=12.0, CH_2 Bn), 4.16 (1H, td, $J_{4,3}$=$J_{4,5}$=9.4, $J_{4,OH}$=1.4 H-4), 3.95-9.91

(2H, m, H-2, H-5), 3.81 (1H, dd, ^2J=10.4, $J_{6,5}$=4.7, H-6), 3.77 (1H, dd, ^2J=10.4, $J_{6,5}$=3.9, H-6), 3.48 (1H, dd, $J_{3,4}$=9.6, $J_{3,2}$=3.0, H-3), 3.33 (3H, s, OMe), 2.71 (1H, d, $J_{OH,4}$=1.6, OH). ^{13}C NMR δ: 159.96 (C=O Trichloroacetyl), 137.85 (Ar C_q), 137.23 (Ar C_q), 128.53 (Ar CH), 128.41 (Ar CH), 128.10 (Ar CH), 127.99 (Ar CH), 127.71 (Ar 2xCH), 96.82 (C-1), 89.65 (CCl_3), 80.32 (C-3), 74.79 (C-5), 73.66 (CH_2 Bn), 73.09 (CH_2 Bn), 71.45 (C-2), 69.53 (C-6), 66.89 (C-4), 57.50 (OMe). IR: 3423 (OH st), 1770 (C=O st), 1230 (C-O st ester), 1078 (C-O st), 823 (C-Cl st).

Synthesis of the last glycosyl acceptor

Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside 21 [32]

HBF_4 (2 mL, 53% wt. in Et_2O, 0.56 eq) was added dropwise to a mixture of methyl α-D-mannopyranoside (5.033 g, 25.92 mmol) and BDA (4.6 mL, 30.65 mmol) in dry DMF (74 mL). Upon addition, the reaction mixture was stirred for an additional 2 h. Then it was quenched with dry Et_3N (10 mL, 3.0 eq) and evaporated to dryness. Purification by flash column chromatography (Hex/AcOEt 3:2) afforded benzylidene **21** (6.029 g, 82%) as a white solid.

^{1}H NMR δ: 7.50-7.47 (2H, m, Ar), 7.39-7.36 (3H, m, Ar), 5.56 (1H, s, CH Benzylidene), 4.74 (1H, d, $J_{1,2}$=1.1, H-1), 4.28 (1H, dd, ^2J=9.6, $J_{6,5}$=3.6, H-6), 4.05 (1H, dd, $J_{3,4}$=9.2, $J_{3,2}$=3.4, H-3), 4.00 (1H, dd, $J_{2,3}$=3.6, $J_{2,1}$=1.1, H-2), 3.91 (1H, t, $J_{4,3}$=$J_{4,5}$=9.1, H-4), 3.87-3.79 (2H, m, H-5, H-6), 3.39 (3H, s, OMe), 2.80 (1H, br s, OH) 2.78 (1H, br s, OH). ^{13}C NMR δ: 137.25 (Ar C_q), 129.33 (Ar CH), 128.37 (Ar CH), 126.29 (Ar CH), 102.32 (CH Benzylidene), 101.27 (C-1), 78.93 (C-4), 70.87 (C-2), 68.86 (C-6), 68.66 (C-3), 62.91 (C-5), 55.13 (OMe). IR: 3222 (OH st), 1099 (C-O st), 1068 (C-O st). $[\alpha]_D$= +41.5 (c=1.10; MeOH) M.p.= 106.3-107.5°C.

Methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside 22

Two step synthesis

Experiment 7 procedure was applied to benzylidene **21** (7.206 g, 25.53 mmol) but with the reaction time of the first step increased to 2 h. Purification by flash column chromatography (Hex/AcOEt 8:2 → 7:3 → 5:5 → 4:6) afforded methyl mannoside **22** (6.105 g, 81%) as a yellow foam.

^{1}H NMR δ: 7.50-7.48 (2H, m, Ar), 7.39-7.34 (3H, m, Ar), 5.59 (1H, s, CH Benzylidene), 4.79 (1H, d, $J_{1,2}$=1.3, H-1), 4.28 (1H, dd, ^2J=10.3, $J_{6,5}$=3.7, H-6), 4.11 (1H, m, H-2), 4.00 (1H, t, $J_{4,3}$=$J_{4,5}$=9.4, H-4), 3.89-3.79 (2H, m, H-5, H-6), 3.67 (1H, dd, $J_{3,4}$=9.5, $J_{3,2}$=3.3, H-3), 3.54 (3H, s, OMe), 3.40 (3H, s, OMe), 2.54 (1H, d, $J_{OH,2}$=1.4, OH). ^{13}C NMR δ: 137.46 (Ar C_q), 129.00 (Ar CH), 128.24 (Ar CH), 126.16 (Ar CH), 101.82 (CH Benzylidene), 101.03 (C-1), 78.64 (C-4), 77.30 (C-3), 69.04 (C-2), 68.91 (C-6), 63.09 (C-5), 58.60 (OMe), 55.05 (OMe). IR: 3456 (OH st), 1054 (C-O st), 1051 (C-O st). $[\alpha]_D$= -43.0 (c=0.81; DCM).

Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside 23

Following the general acetylation procedure methyl mannoside **22** (6.090 g, 20.79 mmol) reacted for 1 h affording, after purification by flash column chromatography (Hex/AcOEt 8:2 → 7:3 → 6:4), product **23** (5.840 g, 84%) as a colourless foam.

^{1}H NMR δ: 7.50-7.48 (2H, m, Ar), 7.38-7.33 (3H, m, Ar), 5.61 (1H, s, CH Benzylidene), 5.35 (1H, dd, $J_{2,3}$=3.5, $J_{2,1}$=1.5, H-2), 4.68 (1H, d, $J_{1,2}$=1.4, H-1), 4.28 (1H, dd, ^2J=10.4, $J_{6,5}$=8.6, H-6), 3.99 (1H, t, $J_{4,3}$=$J_{4,5}$=9.5, H-4), 3.88-3.81 (2H, m, H-5, H-6), 3.78 (1H, dd, $J_{3,4}$=9.9, $J_{3,2}$=3.7, H-3), 3.44 (3H, s, OMe), 3.40 (3H, s, OMe), 2.16 (3H, s, Me Ac). ^{13}C NMR δ: 170.21 (C=O Ac), 137.34 (Ar C_q), 129.03 (Ar CH), 128.25 (Ar CH), 126.20 (Ar CH), 101.90 (CH Benzylidene), 99.72 (C-1), 78.48 (C-4), 75.81 (C-3), 69.15 (C-2), 68.79 (C-6), 63.68 (C-5), 58.39 (OMe), 55.17 (OMe), 21.00 (Me Ac). IR: 1747 (C=O st), 1230 (C-O st ester), 1083 (C-O st), 1076 (C-O st). $[\alpha]_D$= -84.3 (c=0.70; DCM).

Methyl 2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 24

Experiment 9 procedure was applied to compound **23** (5.708 g, 16.87 mmol). Purification by flash column chromatography (Hex/AcOEt 9:1 → 7:3 → 6:4 → 5:5) afforded glycosyl acceptor **24** (3.500 g, 62%) as a colourless oil.

^{1}H NMR δ: 7.36-7.28 (5H, m, Ar), 5.29 (1H, dd, $J_{2,3}$=3.3, $J_{2,1}$=1.7, H-2), 4.72 (1H, d, $J_{1,2}$=1.5, H-1), 4.62 (2H, ABq, ^2J=12.0, CH_2 Bn), 3.87 (1H, td, $J_{4,3}$=$J_{4,5}$=9.1, $J_{4,OH}$=1.8, H-4), 3.80-3.73 (3H, m, H-5, H-6), 3.52 (1H, dd, $J_{3,4}$=9.2, $J_{3,2}$=3.3, H-3), 3.41 (3H, s, OMe), 3.39 (3H, s, OMe), 2.52 (1H, d, $J_{OH,4}$=1.8, OH), 2.11 (3H, s, Me Ac). ^{13}C NMR δ: 170.52 (C=O Ac), 138.12 (Ar C_q), 128.39 (Ar CH), 127.65 (Ar CH), 127.58 (Ar CH), 98.98 (C-1), 79.29 (C-3), 73.58 (CH2

Bn), 70.94 (C-5), 69.93 (C-6), 67.56 (C-4), 67.28 (C-2), 57.41 (OMe), 55.09 (OMe), 20.97 (Me Ac). IR: 3448 (OH st), 1745 (C=O st), 1234 (C-O st ester), 1076 (C-O st), 1049 (C-O st). $[\alpha]_D$= -35.5 (c=1.19; DCM).

Development of a sequential one-pot glycosylation

2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranosyl trichloroacetate 25

Experiment 12 procedure was applied to compound **4** (54 mg, 0.10 mmol) with the exception that more trichloroacetyl chloride (0.5 eq) was added after 1.5 h and 2 h of reaction, with a total reaction time of 2.5 h. Glycosyl donor **25** was afforded (113 mg, quantitative, α/β=10:1) as a colourless oil.

^{1}H NMR δ: 7.42-7.38 (2H, m, Ar), 7.35-7.27 (16H, m, Ar), 7.20-7.18 (2H, m, Ar), 6.23 (1H, d, $J_{1,2}$=1.5, H-1$_\alpha$), 5.84 (1H, d, $J_{1,2}$=1.3, H-1$_\beta$), 4.88 (1H, d, ^2J=10.6, CH_2 Bn), 4.76 (2H, ABq, ^2J=12.2, CH_2 Bn), 4.66 (1H, d, ^2J=12.0, CH_2 Bn), 4.63 (2H, ABq, ^2J=11.7, CH_2 Bn), 4.54 (1H, d, ^2J=10.6, CH_2 Bn), 4.51 (1H, d, ^2J=12.0, CH_2 Bn), 4.15 (1H, t, $J_{4,3}$=$J_{4,5}$=9.3, H-4), 3.91-3.84 (2H, m, H-3, H-5), 3.80 (1H, dd, ^2J=11.0, $J_{6,5}$=4.3, H-6), 3.75 (1H, t, $J_{2,3}$=$J_{2,1}$=2.7, H-2), 3.70 (1H, dd, ^2J=11.3, $J_{6,5}$=1.3, H-6). ^{13}C NMR δ: 159.96 (C=O Trichloroacetyl), 138.12 (Ar C_q), 138.02 (Ar C_q), 137.87 (Ar C_q), 137.50 (Ar C_q), 128.50 (Ar 2xCH), 128.45 (Ar CH), 128.36 (Ar CH), 128.20 (Ar CH), 128.02 (Ar CH), 127.98 (Ar CH), 127.95 (Ar CH), 127.93 (Ar CH), 127.88 (Ar CH), 127.84 (Ar CH), 127.61 (Ar CH), 96.99 (C-1), 89.54 (CCl_3), 78.41 (C-3), 75.40 (C-5), 75.38 (CH_2 Bn), 73.92 (C-4), 73.78 (C-2), 73.47 (CH_2 Bn), 73.09 (CH_2 Bn), 72.64 (CH_2 Bn), 68.44 (C-6). IR: 1768 (C=O st ester), 1232 (C-O st ester), 1093 (C-O st), 825 (C-Cl st).

Methyl 2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 26

Typical glycosylation procedure using:

2,3,4,6-tetra-O-benzyl-α/β-D-mannopyranosyl N-trichloroacetylcarbamate

A mixture of donor **5** (38 mg, 0.05 mmol), acceptor **24** (23 mg, 0.07 mmol) and molecular sieves in dry DCM (2 mL) was vigorously stirred for 30 min. Distilled TMSOTf (11 µL, 0.06 mmol) was added at -20°C and stirring continued for 2 h. Then the reaction was quenched with $NaHCO_3$ aqueous solution (sat.) and extracted with DCM. The collected organic phases were dried with

anhydrous Na_2SO_4 and evaporated to dryness. Purification by preparative TLC (Hex/AcOEt 3:2) afforded disaccharide **26** (33 mg, 73%, α/β=9:1) as a colourless oil.

2,3,4,6-tetra-O-benzyl-α/β-D-mannopyranosyl trichloroacetate

A mixture of donor **25** (30 mg, 0.04 mmol), acceptor **24** (18 mg, 0.05 mmol) and molecular sieves in dry DCM (2 mL) was vigorously stirred for 30 min. Distilled TMSOTf (10 µL, 0.05 mmol) was added and stirring continued for 1.5 h. Then the reaction was quenched with $NaHCO_3$ aqueous solution (sat.) and extracted with DCM. The collected organic phases were dried with anhydrous Na_2SO_4 and evaporated to dryness. Purification by preparative TLC (Hex/AcOEt 3:2) afforded disaccharide **26** (20 mg, 53%, α/β=5:1) as a colourless oil.

^{1}H NMR δ: 7.38-7.24 (20H, m, Ar), 7.20-7.16 (2H, m, Ar), 5.29 (1H, d, $J_{1,2}$=1.7, H-1$_\alpha$), 5.25 (1H, dd, $J_{2',3'}$=3.4, $J_{2',1'}$=1.9, H-2'), 5.04 (1H, d, $J_{1,2}$=1.4, H-1$_\beta$), 4.87 (1H, d, ^2J=10.8, CH_2 Bn), 4.73 (1H, d, ^2J=12.5, CH_2 Bn), 4.69 (1H, d, $J_{1',2'}$=1.7, H-1'), 4.64 (2H, d, ^2J=9.7, CH_2 Bn), 4.61 (1H, d, ^2J=12.3, CH_2 Bn), 4.58 (1H, d, ^2J=11.6, CH_2 Bn), 4.57 (1H, d, ^2J=12.1, CH_2 Bn), 4.50 (2H, d, ^2J=11.4, CH_2 Bn), 4.45 (1H, d, ^2J=12.1, CH_2 Bn), 3.99 (1H, t, $J_{4,3}$=$J_{4,5}$=9.4, H-4), 3.84-3.78 (3H, m, H-5, H-4', H-6'), 3.77-3.73 (2H, m, H-2, H-3), 3.72-3.65 (3H, m, H-6, H-5', H-6'), 3.56 (1H, dd, ^2J=10.6, $J_{6,5}$=1.5, H-6), 3.48 (1H, dd, $J_{3',4'}$=9.2, $J_{3',2'}$=3.2, H-3'), 3.37 (3H, s, OMe), 3.18 (3H, s, OMe), 2.10 (Me Ac). ^{13}C NMR δ: 170.38 (C=O Ac), 138.64 (Ar C_q), 138.57 (Ar C_q), 138.51 (Ar C_q), 138.47 (Ar C_q), 138.38 (Ar C_q), 128.34 (Ar CH), 128.28 (Ar 2xCH), 128.24 (Ar 2xCH), 128.01 (Ar CH), 127.81 (Ar CH), 127.64 (Ar CH), 127.56 (Ar 2xCH), 127.54 (Ar CH), 127.46 (Ar CH), 127.44 (Ar 2xCH), 127.38 (Ar CH), 99.84 (C-1), 98.47 (C-1'), 79.99 (C-3'), 79.86 (C-5), 75.26 (C-2), 75.10 (CH_2 Bn), 74.77 (C-4), 74.03 (C-4'), 73.42 (CH_2 Bn), 73.28 (CH_2 Bn), 72.91 (C-3), 72.21 (CH_2 Bn), 71.99 (CH_2 Bn), 70.78 (C-5'), 69.83 (C-6'), 69.26 (C-6), 67.33 (C-2'), 56.85 (OMe), 55.07 (OMe), 21.00 (Me Ac). IR: 1745 (C=O st), 1453 (C-H bend methyl), 1234 (C-O st ester), 1076 (C-O st), 1049 (C-O st).

Methyl 2-*O*-acetyl-4,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 27

A mixture of donor **12** (19 mg, 0.03 mmol), acceptor **24** (14 mg, 0.04 mmol) and molecular sieves in dry DCM (1.2 mL) was vigorously stirred for 30 min. Distilled TMSOTf (7 µL, 0.04 mmol) was added and stirring continued for 1.5 h. Then the reaction was quenched with $NaHCO_3$ aqueous solution (sat.) and extracted with DCM. The collected organic phases were

dried with Na_2SO_4 and evaporated to dryness. Purification by preparative TLC (Hex/AcOEt 3:2) afforded disaccharide **27** (8 mg, 32%, α/β=8.6:1) as a colourless oil.

^{1}H NMR δ: 7.37-7.27 (24H, m, Ar), 7.22-7.20 (6H, m , Ar), 5.35 (1H, dd, $J_{2,3}$=3.4, $J_{2,1}$=2.0, H-2), 5.29 (1H, d, $J_{1,2}$=1.9, H-1$_\beta$), 5.27 (1H, dd, $J_{2',3'}$=3.4, $J_{2',1'}$=1.7, H-2'), 5.23 (1H, d, $J_{1,2}$=1.8, H-1$_\alpha$), 4.82 (1H, d, ^2J=10.8, CH_2 Bn), 4.70 (1H, d, $J_{1',2'}$=1.6, H-1'), 4.62 (1H, d, ^2J=12.1, CH_2 Bn), 4.54 (2H, ABq, ^2J=11.9, CH_2 Bn), 4.47 (1H, d, ^2J=10.8, CH_2 Bn), 4.40 (1H, d, ^2J=12.1, CH_2 Bn), 3.89 (1H, t, $J_{4',3'}$=$J_{4',5'}$=9.4, H-4'), 3.81-3.66 (6H, m, H-4, H-5, H-6, H-5', H-6'), 3.61 (2H, dd, $J_{3,4}$=$J_{3',4'}$=9.0, $J_{3,2}$=$J_{3',2'}$=3.2, H-3, H-3'), 3.50 (1H, dd, ^2J=10.8, $J_{6',5'}$=1.2, H-6'), 3.43 (3H, s, OMe), 3.39 (3H, s, OMe), 3.38 (3H, s, OMe), 2.12 (3H, s, Me Ac), 2.08 (3H, s, Me Ac). ^{13}C NMR δ: 170.35 (C=O Ac), 170.18 (C=O Ac), 138.57 (Ar C_q), 138.41 (Ar C_q), 138.31 (Ar C_q), 128.30 (Ar CH), 128.28 (Ar CH), 128.24 (Ar CH), 127.86 (Ar CH), 127.76 (Ar CH), 127.61 (Ar CH), 127.54 (Ar CH), 127.38 (Ar CH), 99.50 (C-1), 98.55 (C-1'), 80.02 (C-3), 79.90 (C-3'), 75.06 (CH_2 Bn), 74.28 (C-4), 73.62 (C-4'), 73.42 (CH_2 Bn), 73.32 (CH_2 Bn), 72.37 (C-5), 70.68 (C-5'), 69.61 (C-6), 68.82 (C-6'), 68.32 (C-2), 67.51 (C-2'), 57.50 (OMe), 57.22 (OMe), 55.10 (OMe), 21.10 (Me Ac), 20.97 (Me Ac). IR: 1743 (C=O st), 1452 (C-H bend methyl), 1232 (C-O st ester), 1076 (C-O st), 1049 (C-O st).

Methyl 2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-methyl-α/β-D-mannopyranosyl-(1→4)-2-*O*-acetyl-6-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside 28

A mixture of donor **5** (21 mg, 0.028 mmol), donor/acceptor **20** (22 mg, 0.042 mmol) and molecular sieves in dry DCM (1 mL) was vigorously stirred for 30 min. Distilled TMSOTf (6 µL, 0.034 mmol) was added at -20°C and stirring continued for 20 min. After adding acceptor **24** (23 mg, 0.068 mmol, in 0.5 mL of DCM), the reaction mixture was allowed to warm to r.t. and stirred for 1 h and 40 min. Then it was quenched with $NaHCO_3$ aqueous solution (sat.) and extracted with DCM. The collected organic phases were dried with anhydrous Na_2SO_4 and evaporated to dryness. Purification by preparative TLC (Hex/AcOEt 65:35) afforded trisaccharide **28** (9 mg, 26%, αα>99%) as a colourless oil.

^{1}H NMR δ: 7.40-7.23 (24H, m, Ar), 7.21-7.18 (6H, m, Ar), 5.30 (1H, d, $J_{1,2}$=0.9, H-1), 5.27-5.26 (2H, m, H-1', H-2"), 4.89 (1H, d, ^2J=10.9, CH_2 Bn), 4.76 (1H, d, ^2J=12.6, CH_2 Bn), 4.70 (1H, d, $J_{1",2"}$=1.7, H-1"), 4.68-4.63 (3H, m, CH_2 Bn), 4.60 (2H, s, CH_2 Bn), 4.58-4.56 (1H, m, CH_2 Bn), 4.53-4.49 (2H, m, CH_2 Bn), 4.46-4.43 (4H, m, CH_2 Bn), 4.02 (1H, t, $J_{4,3}$=$J_{4,5}$=9.4, H-4), 3.95

(1H, t, $J_{4',3'}$=$J_{4',5'}$=9.1 Hz, H-4'), 3.87-3.80 (4H, m, H-2, H-3, H-6"), 3.78-3.72 (3H, m, H-5, H-2', H-4"), 3.71-3.67 (4H, m, H-6, H-6', H-5', H-5"), 3.63 (1H, dd, ^{2}J=11.9, $J_{6,5}$=4.6, H-6), 3.56-3.52 (2H, m, H-6', H-3"), 3.38 (3H, s, OMe), 3.36-3.33 (1H, m, H-3'), 3.25 (3H, s, OMe), 3.13 (3H, s, OMe), 2.10 (3H, s, Me Ac). ^{13}C NMR δ: 170.42 (C=O Ac), 138.73 (Ar C_q), 138.64 (Ar C_q), 138.57 (Ar C_q), 138.51 (Ar C_q), 138.46 (Ar C_q), 138.41 (Ar C_q), 138.28 (Ar C_q), 128.31 (Ar CH), 128.27 (Ar CH), 128.23 (Ar CH), 128.20 (Ar CH), 128.00 (Ar CH), 127.85 (Ar CH), 127.66 (Ar CH), 127.55 (Ar CH), 127.53 (Ar CH), 127.49 (Ar CH), 127.46 (Ar CH), 127.43 (Ar CH), 127.41 (Ar CH), 127.30 (Ar CH), 99.84 (C-1), 99.68 (C-1'), 98.46 (C-1"), 81.35 (C-3'), 80.02 (C-3"), 79.94 (C-3), 75.13 (CH_2 Bn), 75.09 (C-2), 74.82 (C-4), 74.42 (C-4'), 74.33 (C-4"), 73.44 (CH_2 Bn), 73.33 (CH_2 Bn), 73.32 (CH_2 Bn), 73.11 (C-2'), 72.88 (C-5"), 72.30 (C-5), 72.11 (CH_2 Bn), 71.97 (CH_2 Bn), 71.90 (CH_2 Bn), 70.96 (C-5'), 67.26 (C-2"), 56.88 (OMe), 56.51 (OMe), 55.07 (OMe), 21.00 (Me Ac). IR: 1728 (C=O st), 1452 (C-H bend methyl), 1238 (C-O st ester), 1056 (C-O st), 1041 (C-O st). MS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{72}H_{81}NaO_{17}$ 1241.4128; Found 1241.5452.

Bibliography

1 Marx, V. *Nat. Methods* **(**2017), 14, 667.

2 WHO. *Tuberculosis key facts*.

3 King, H. C.; Khera-Butler, T.; James, P.; Oakley, B. B.; Erenso, G.; Aseffa, A.; Knight, R.; Wellington, E. M.; Courtenay, O. *PLoS One* (2017), 12, 1.

4 Nunes-Costa, D.; Maranha, A.; Costa, M.; Alarico, S.; Empadinhas, N. *Glycobiology* (2017), 27, 213.

5 Rossi, E. De; Aínsa, J. A.; Riccardi, G. FEMS *Microbiol. Rev.* (2006), 30, 36.

6 Jackson, M.; Brennan, P. J. *J. Biol. Chem.* (2009), 284, 1949.

7 Maitra, S. K.; Ballou, C. E. *J Biol Chem.* (1977), 252, 2459.

8 Tuffal, G.; Albigot, R.; Rivière, M.; Puzo, G. *Glycobiology* (1998), 8, 675.

9 Maranha, A.; Moynihan, P. J.; Miranda, V.; Lourenço, E. C.; Nunes-Costa, D.; Fraga, J. S.; Pereira, P. J. B.; Macedo-Ribeiro, S.; Ventura, M. R.; Clarke, A. J.; Empadinhas, N. *Sci. Rep.* (2015), 5, 1.

10 Jackson, M.; McNeil, M. R.; Brennan, P. J. *Future Microbiol.* (2013), 8, 855.

11 Chetty, S.; Ramesh, M.; Singh-Pillay, A.; Soliman, M. E. S. *Bioorganic Med. Chem. Lett.* (2017), 27, 370.

12 Caño-Muñiz, S.; Anthony, R.; Niemann, S.; Alffenaar, J. C. *Clin. Microbiol. Rev.* (2018), 31, 1.

13 Bohé, L.; Crich, D. C. R. *Chimie.* (2011), 14, 3.

14 Bohé, L.; Crich, D. *Carbohydr. Res.* (2015), 403, 48.

15 Hosoya, T.; Takano, T.; Kosma, P.; Rosenau, T. *J. Org. Chem.* (2014), 79, 7889.

16 Demchenko, A. V. *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*; Wiley, (2008).

17 Mydock, L. K.; Demchenko, A. V. *Org. Biomol. Chem.* (2010), 8, 497.

18 Van der Vorm, S.; Hansen, T.; Overkleeft, H. S.; Van der Marel, G. A.; Codée, J. D. C. *Chem. Sci.* (2017), 8, 1867.

19 Nigudkar, S. S.; Demchenko, A. V. *Chem. Sci.* (2015), 6, 2687.

20 Lourenço, E. C. *Synthesis of New Enzyme Stabilisers Inspired by Compatible Solutes of Hyperthermophilic Microorganisms*; ITQB, (2013).

21 Crich, D.; Patel, M. *Carbohydr. Res.* (2006), 341, 1467.

22 Kulkarni, S. S.; Wang, C.-C.; Sabbavarapu, N. M.; Podilapu, A. R.; Liao, P.-H.; Hung, S.-C. *Chem. Rev.* (2018), 118, 8025.

23 Shirahata, T.; Kojima, A.; Teruya, S.; Matsuo, J. I.; Yokoyama, M.; Unagiike, S.; Sunazuka, T.; Makino, K.; Kaji, E.; Omura, S.; Kobayashi, Y. *Tetrahedron* (2011), 67, 6482.

24 Davis, B. G.; Fairbanks, A. J. *Carbohydrate Chemistry*; Oxford University Press, (2002).

25 Trost, B. M.; Van Vranken, D. L. *Chem. Rev.* (1996), 96, 395.

26 Liao, W.; Lu, D. *Carbohydr. Res.* (1996), 296, 171.

27 David, S.; Hanessian, S. *Tetrahedron* (1985), 41, 643.

28 Ohlin, M.; Johnsson, R.; Ellervik, U. *Carbohydr. Res.* (2011), 346, 1358.

29 Skaanderup, P. R.; Poulsen, C. S.; Hyldtoft, L.; Jørgensen, M. R.; Madsen, R. *Synthesis (Stuttg)* (2002), 12, 1721.

30 Jung, M. E.; Koch, P. *Tetrahedron Lett.* (2011), 52, 6051.

31 Kocienski, P. J. *Protecting Groups*; Thieme, (2005).

32 Hsu, M. C.; Lee, J.; Kishi, Y. *J. Org. Chem.* (2007), 1931.

33 Armarego, W. L. F.; Chai, C. L. L. *Purification of Organic Chemicals*; Elsevier, (2009).

34 Tennant-Eyles, R. J.; Davis, B. G.; Fairbanks, A. J. *Tetrahedron Asymmetry* (2000), 11, 231.

35 Matsuo, J. I.; Shirahata, T.; Omura, S. *Tetrahedron Lett.* (2006), 47, 267.

36 Jayakanthan, K.; Vankar, Y. D. *Carbohydr. Res.* (2005), 340, 2688.