Synthesis of Oligosaccharides for Interaction Studies with Various Lectins

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Abstract

In this thesis, the syntheses of oligosaccharides for interaction studies with various lectins are described. The first section reports the syntheses of tetra-, tri- and disaccharides corresponding to truncated versions of the glucosylated arm of $Glc_1Man_9(GlcNAc)_2$, found in the biosynthesis of N-glycans. The thermodynamic parameters of their interaction with calreticulin, a lectin assisting and promoting the correct folding of newly synthesised glycoproteins, were established by isothermal titration calorimetry. In the second section, a new synthetic pathway leading to the same tetra- and trisaccharides is discussed. Adoption of a convergent strategy and of a different protecting group pattern resulted in significantly increased yields of the target structures. The third section describes the syntheses of a number of monodeoxy-trisaccharides related to the above trisaccharide Glc-α-(1→3)-Man-α-(1→2)-Man-α-OMe. Different synthetic approaches were explored and the choice of early introduction of the deoxy functionality proved the most beneficial. In the last section, the synthesis of spacer-linked LacNAc dimers as substrates for the lectins galectin-1 and -3 is presented. This synthesis was realized by glycosidation of a number diols with peracetylated LacNAc-oxazoline. Pyridinium triflate was tested as a new promoter, affording the target dimers in high yields. This promoter in combination with microwave irradiation gave even higher yields and also shortened the reaction times.

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List of Papers

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I. Interaction of Substrate with Calreticulin, an Endoplasmic Reticulum Chaperone

Mili Kapoor, Honnappa Srinivas, Eaazhisai Kandiah, Emiliano Gemma, Lars Ellgaard, Stefan Oscarson, Ari Helenius and Avadhesha Surolia

J. Biol. Chem., **2003**, *278*, 6194-6200

II. Synthesis of the tetrasaccharide α-D-Glc*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p* recognised by Calreticulin/Calnexin

Emiliano Gemma, Martina Lahmann and Stefan Oscarson

Submitted for publication in Carbohydr. Res.

III. Efficient Synthesis of Spacer-linked Dimers of *N*-Acetyllactosamine Using Microwave-assisted Pyridinium Triflate-promoted Glycosylations with Oxazoline Donors

Halasayam Mohan, Emiliano Gemma, Katinka Ruda and Stefan Oscarson

Synlett, **2003**, 1255-1256

IV. Synthesis of monodeoxy analogues of the trisaccharide α-D-Glc*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*

Emiliano Gemma, Martina Lahmann and Stefan Oscarson

Preliminary Manuscript

Papers not discussed in this thesis

Mutational Analysis Provides Molecular Insight into the Carbohydrate-Binding Region of Calreticulin: Pivotal Roles of Tyrosine-109 and Aspartate-135 in Carbohydrate Recognition

Mili Kapoor, Lars Ellgaard, Jayashree Gopalakrishnapai, Christiane Schirra, Emiliano Gemma, Stefan Oscarson, Ari Helenius and Avadhesha Surolia

Biochemistry, **2004**, *43*, 97-106

Atomic Mapping of the Interactions between the Antiviral Agent Cyanovirin-N and Oligomannosides by Saturation-Transfer Difference NMR

Corine Sandström, Olivier Berteau, Emiliano Gemma, Stefan Oscarson, Lennart Kenne, and Angela M. Gronenborn

Biochemistry, **2004**, *43*, 13926-13931

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List of Abbreviations[#]

AcCl	Acetyl Chloride
AcOH	Acetic Acid
AgOTf	Silver Triflate (Trifluoromethanesulfonate)
AIBN	α,α'-Azobisisobutyronitrile
Asn	Asparagine
CNX	Calnexin
ER	Endoplasmic Reticulum
cod	Cyclooctadiene
CRT	Calreticulin
CSA	Camphorsulfonic Acid
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
DMTST	Dimethyl(methylthio)sulfonium Triflate (Trifluoromethanesulfonate)
Gal	Galactose
Glc	Glucose
GlcII	Glucosidase II
GlcNAc	*N*-Acetylglucosamine, 2-Acetamido-2-deoxy-glucose
Im_2CS	1,1'-Thiocarbonyldiimidazole
ITC	Isothermal Titration Calorimetry
LacNac	*N*-Acetyllactosamine, Gal-β-(1→4)-GlcNAc
Man	Mannose
MeOTf	Methyl Triflate (Trifluoromethanesulfonate)
NIS	*N*-Iodosuccinimide
PyOTf	Pyridinium Triflate (Trifluoromethanesulfonate)
PyOTs	Pyridinium Tosylate (*p*-Toluenesulfonate)
TBDPS	*tert*-Butyldiphenylsilyl
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TMSCl	Trimethylsilyl Chloride
TMSI	Trimethylsilyl Iodide
TMSOTf	Trimethylsilyl Triflate (Trifluoromethanesulfonate)
TsCl	Tosyl (*p*-Toluenesulfonyl) Chloride
UGGT	UDP-Glucose Glycoprotein Glucosyltransferase (UDP = Uridine Diphosphate)

[#]Unless otherwise indicated, all monosaccharides are assumed to be in the D-configuration and in the pyranose form (six-membered ring).

1 General Introduction

1.1 Biology of Carbohydrates

The historical definition of a carbohydrate is "a hydrate of carbon" and the term carbohydrate describes the main feature of this class of compounds, namely a carbon chain rich in hydroxyl groups.[1] Carbohydrates are mainly known to the general public as a dietary component, providing us with the energy for many biochemical processes. However, over the last few decades, it has been revealed that carbohydrates play other vitally important roles in biological systems. Indeed, these molecules are implicated in a number of biological events, *e.g.* in intercellular recognition, bacterial and viral infection processes, the fine tuning of protein structure, the inflammation event and some aspects of cancer.[2]

Monosaccharides are the smallest carbohydrate unit. When they are covalently bound to each other, they form a macromolecule that goes under the name of glycan. Most glycans are attached to a protein or a lipid, forming a glycoconjugate. Because of the high number of functionalities on a monosaccharide, glycans possess a very large variety of structures with regard to the type of linkage or branching. Major classes of glycans of eukaryotic cells can be defined depending on the way they are linked to, and the nature of, the aglycon (a protein or a lipid):

- N-glycans, where the glycan is attached to the protein backbone via an asparagine residue
- O-glycans, where the glycan is attached to the protein backbone via a hydroxyl group of an amino acid residue (mainly serine or threonine)
- Glycosphingolipids, where the glycan is attached to the lipid moiety ceramide (composed of a long-chain base and a fatty acid)
- Glycophospholipid anchor, where the glycan bridges a protein with a fatty acid anchored into the membrane

The biosynthesis of these glycans occurs mainly within the endoplasmic reticulum (ER) and Golgi apparatus, where, in a complicated sequence of enzymatic reactions, the glycoconjugates are assembled in a stepwise fashion.

A remarkable feature of glycoproteins is the phenomenon of *microheterogeneity*. This term describes the occurrence of structural modifications of the glycan part of a glycoprotein synthesised by a particular type of cell, going from one cell to another or within the same cell at different stages of development. Such different structures of a glycoconjugate are called glycoforms. This phenomenon greatly complicates the task of isolation and identification of a certain glycan structure from natural sources.

The biological roles of glycans can be roughly divided into two major classes: they can have structural and modulatory functions or be specifically recognized by a receptor, generally a protein (lectin). This receptor may belong either to the same organism or to exogenous agents such as viruses, bacteria or parasites.[3]

1.2 Chemistry of Carbohydrates

Synthetic carbohydrate chemistry has been an active area of chemical research for more than a century. However in the recent past, along with the numerous discoveries from the rapidly expanding field of glycobiology, it has gained growing attention.[4] In fact, the ability of synthetic carbohydrate chemists to provide well-defined and purified structures in large amounts has become a precious tool for biologists. Neoglycoproteins based on synthetic oligosaccharides can be utilized to study immunological properties and to develop vaccines.[5] Synthetic glycodendrimers, *i.e.* macromolecules displaying a large number of carbohydrate residues at their periphery, are useful in investigating multivalent interactions with proteins.[6] Simpler oligosaccharides can be employed for studying their interaction with a receptor at the molecular level. Additional advantages of synthetic carbohydrates are the possibility of making non-natural derivatives or analogues, and the possibility of simple introduction of labels for biological measurements.[7,8]

The key reaction in oligosaccharide synthesis is the glycosidation reaction. In this reaction, a bond is formed between the anomeric center of a saccharide and an alcohol (usually an hydroxyl from another sugar molecule). Generally, one needs a leaving group at the anomeric carbon of the so-called donor, which, under the appropriate reaction conditions can be released from the donor, creating a highly reactive carbocationic intermediate, the oxacarbenium ion (Figure 1.1).

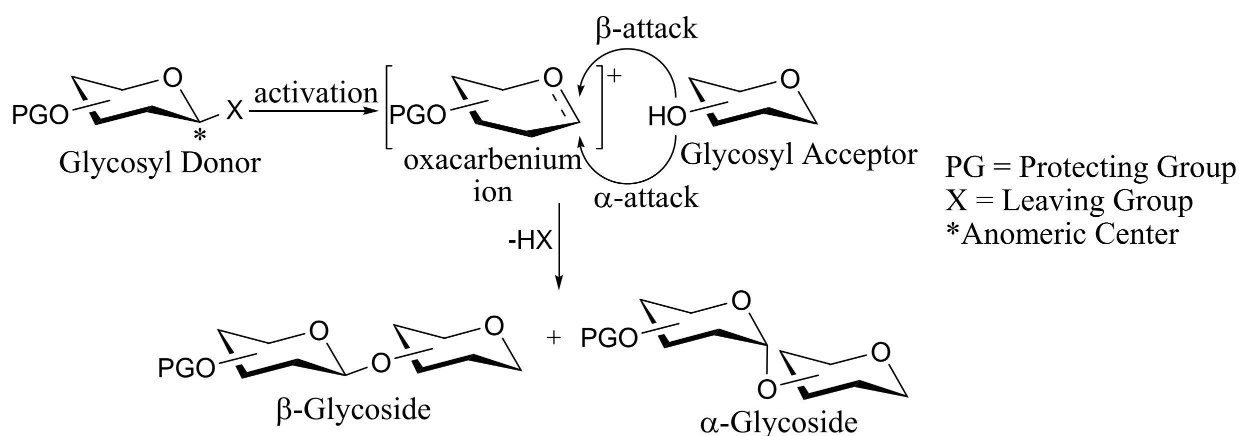


Figure 1.1 The glycosidation reaction

A nucleophilic attack by a hydroxyl group of a saccharidic residue (glycosyl acceptor) then follows. Given the planar geometry of the previously anomeric carbon of the oxacarbenium ion, this attack can take place from either side of the plane defined by the ring, resulting in two different diastereomeric products, namely the α- and β-glycosides. A successful synthesis of an oligosaccharide should employ glycosidation reactions that are stereoselective. For this purpose, one could take advantage of an enzyme (either a glycosyltransferase or a glycosidase), which ensures complete stereoselectivity in most cases. Glycosyltransferases display high regiospecificity and produce glycosides in nearly quantitative yields. However, these enzymes are difficult to isolate and only a few are commercially available. Other drawbacks to consider with the use of glycosyltransferases are the requirements of nucleotide sugars as donors, as well as their often high substrate-specificity. Nevertheless, cloning of bacterial glycosyltransferases might pave the way for a wider application of enzymes in oligosaccharide synthesis.[9] Chemical methodologies have also been developing remarkably over the last two decades and represent a viable approach to the synthesis of complex carbohydrates.[4,10] As discussed above, a classical chemical glycosidation reaction is realized by activation of a fully protected glycosyl donor in the presence of a suitably protected glycosyl acceptor. Glycosyl donors often encountered are thioglycosides, glycosyl halides and trichloroacetimidates (Figure 1.2).

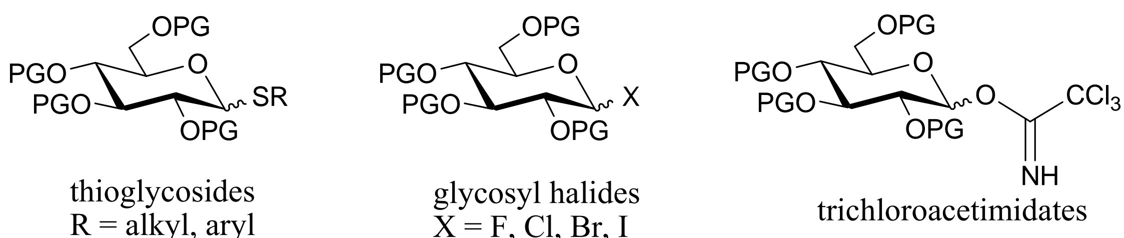


Figure 1.2 Common glycosyl donors

By activating these donors with a suitable promoter (in most cases a Lewis acid), their residue at the anomeric position turns into a good leaving group, thus allowing the reaction with the acceptor. One of the main factors influencing the stereochemical outcome of a glycosidation reaction is the nature of the protecting group at C-2 (*i.e.* in the immediate vicinity of the anomeric carbon). If this group contains an ester functionality like an acetate or a benzoate, it stabilizes the intermediate oxacarbenium ion by formation of an acyloxonium ion. This phenomenon, which is known as neighbouring group participation, directs the attack of the incoming nucleophile so that usually only the 1,2-*trans*-glycoside is formed (Figure 1.3).

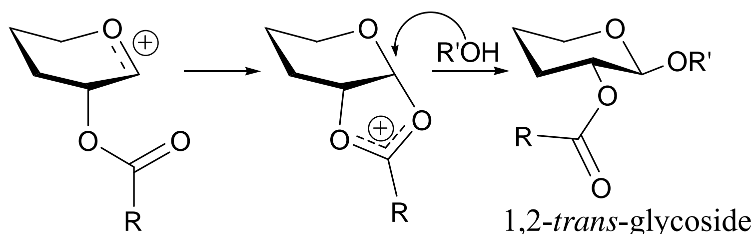


Figure 1.3 Neighbouring group participation

When the protecting group at C-2 is not capable of such assistance (like an ether), the stereoselectivity of the glycosidation becomes much less predictable. In this case a whole range of factors come into play in determining the α:β ratio, like the type of leaving group, type of promoter, protecting group pattern and also solvent[11,12] and temperature. Creation of a 1,2-*cis*-interglycosidic linkage is evidently a challenging task in oligosaccharide synthesis. One of the most established methods for highly stereoselective introduction of 1,2-*cis*-interglycosidic linkages is that developed by Lemieux and co-workers, the *in situ* anomerisation (or halide-assisted) procedure.[13] According to this protocol, a glycosyl bromide with a non-participating group at C-2 is reacted with an acceptor in the presence of a tetraalkyl ammonium bromide salt (Figure 1.4).

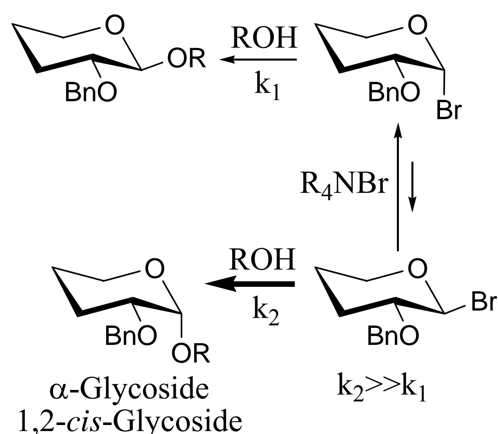


Figure 1.4 *In situ* anomerisation glycosidation

The glycosyl bromide is mainly present as the more stable α anomer (because of the so-called anomeric effect)[14] and the ammonium salt catalyses its conversion to the β stereoisomer. Because of the instability of the β bromide, this promptly reacts with the acceptor in an S_N2-like fashion, thus affording the α-glycoside.

A special case is represented by glycosidation involving mannose donors (Figure 1.5). In mannose the 2-OH is oriented axially instead of equatorially (as in glucose or galactose). Regardless of whether the group at C-2 is participating or not, the 1,2-*trans*-glycoside (α-glycoside) will be formed preferentially.[15] This is probably due to the fact that, in the absence of neighbouring group participation, the anomeric effect becomes predominant and thus the α-linked product more favoured. Therefore, creation of a β-mannopyranosidic linkage represents a major challenge for synthetic carbohydrate chemists.[15,16]

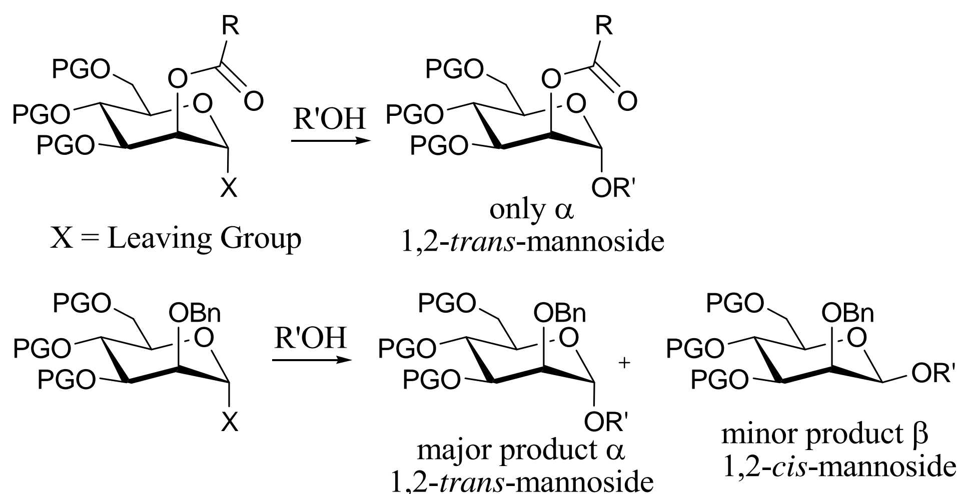


Figure 1.5 Glycosidation employing mannose donors

2 Synthesis of Oligosaccharides for Binding Studies with Calreticulin, a Lectin-like Molecular Chaperone (Paper I)

2.1 Introduction

2.1.1 Lectins

Lectins (from the Latin legere, to select or to choose) are non-enzymatic proteins that specifically recognize a carbohydrate residue. They are found in most organisms (animals, plants and microbes) and exert a wide range of important biological functions. One class of animal lectins, the galectins (specific for glycans containing a terminal galactose), has been found to be involved in various processes, including cell adhesion, cell-growth regulation, apoptosis, inflammation, tumor growth and metastasis formation.[17] Other animal lectins, found in the endoplasmic reticulum and Golgi apparatus, are important in the biosynthesis of glycoproteins.[18,19] Microbial lectins (bacterial adhesins and viral hemagglutinins) are also widespread and microorganisms exploit their interaction with cell-surface glycans to enter and colonize the tissues of a host animal.[20] One example is urinary tract infection by uropathogenic *E. coli*: adhesion of *E. coli* to the urothelial surface is mediated, for example, by binding of a bacterial lectin (on the tip of type 1 fimbriae) to high-mannose glycoproteins present on urothelial cells.[20] Interestingly, Tamm-Horsfall glycoprotein, which is the most abundant protein in mammalian urine, seems to interact with these pathogens via its high-mannose glycan and thereby prevents infection.[21] Another lectin that has attracted widespread interest is cyanovirin-N (CV-N). This protein, isolated from a cyanobacterium, has been found to bind to the highly glycosylated surface envelope proteins of human immuno-deficiency virus (HIV). In particular, this lectin shows high affinity for N-linked high mannose oligosaccharides on the virus protein.[22,23]

2.1.2 Calreticulin

N-glycoproteins of eukoaryotic cells are synthesised within the endoplasmic reticulum (ER) and Golgi apparatus. Their biosynthesis starts with the transfer *en bloc* of a large oligosaccharide to the side chain of an asparagine residue (Asn) of the nascent polypeptide. Within the endoplasmic reticulum (ER), the nascent glycoprotein then enters a cyclic pathway of crucial importance, which

will lead to the proper folding of the polypeptide chain. This cycle is called the calnexin/calreticulin (CNX/CRT) cycle and is schematically depicted in Figure 2.1.

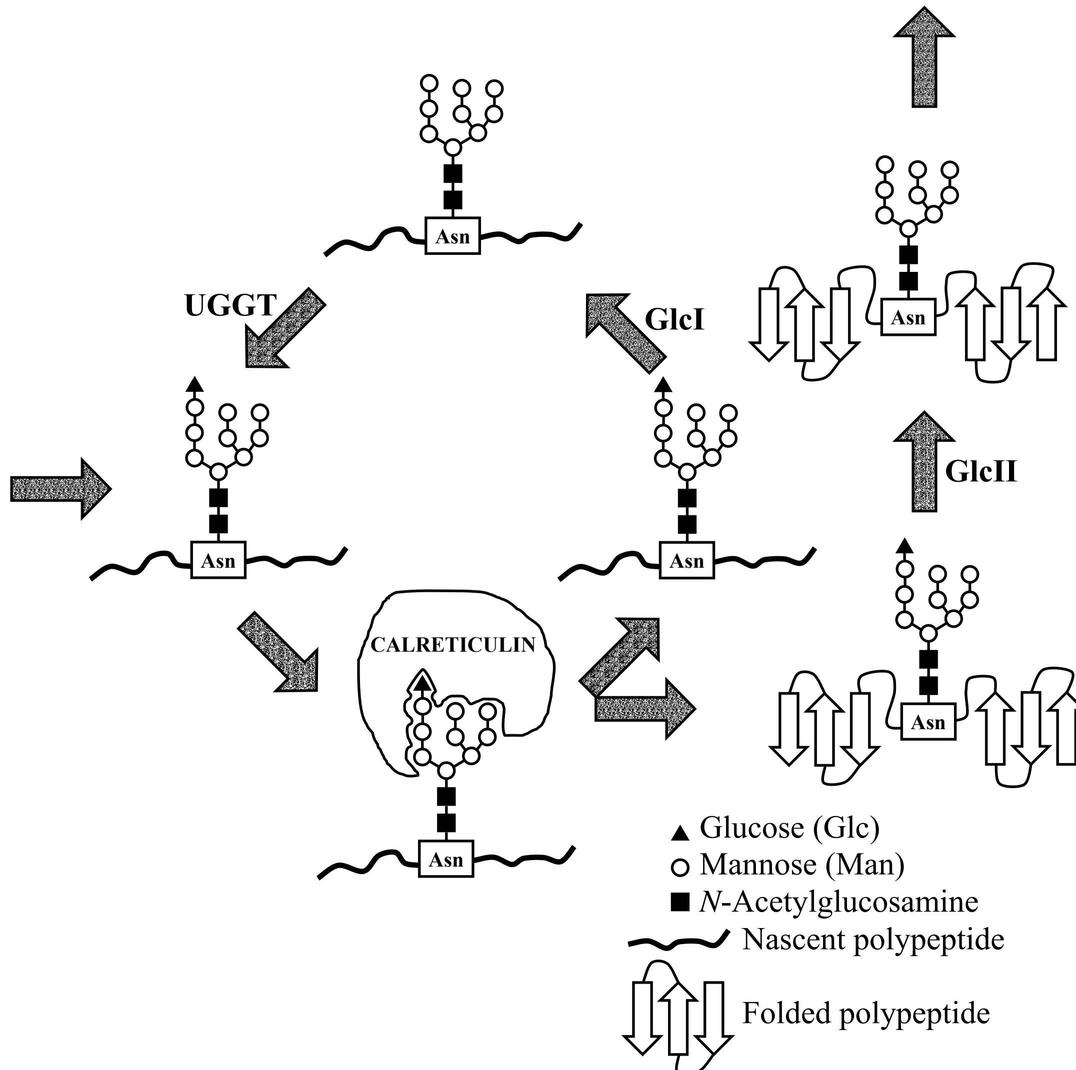


Figure 2.1 Schematic depiction of the calnexin-calreticulin cycle

The main actors of this pathway are two homologous proteins, calnexin and calreticulin. Calnexin is the membrane-bound counterpart of calreticulin, which is a soluble protein in the lumen of the ER. The main feature of these proteins is that they are lectins. In fact, the high-mannose type glycan $Glc_1Man_9(GlcNAc)_2$ is bound by such a lectin, which then acts as a molecular chaperone, assisting and promoting the correct folding of the peptide part of the glycoprotein, and giving it its functional conformation. After release from the chaperone, the terminal glucose residue on the glycan is removed by a hydrolytic enzyme (glucosidase II, GlcII) and the correctly folded protein leaves the ER. If the deglucosylated glycoprotein is not correctly folded, another enzyme present in

the lumen of the ER (UDP-glucose glycoprotein glucosyltransferase, UGGT) reglucosylates the glycan. The glycan is now again $Glc_1Man_9(GlcNAc)_2$ and can pass through the calnexin/calreticulin cycle once more and the whole process is repeated until the glycoprotein has the correct folding. In other words, UGGT works as a protein-folding sensor.

Calnexin and calreticulin show high similarity with regard to their amino acid sequences. Moreover it has been shown that both CNX and CRT recognize the same carbohydrate determinant. This high-mannose type chain has a terminal glucose residue on one branch. The presence of this glucose unit is crucial for binding to the lectin. However, it is now clear that these two chaperones bind to different glycoprotein substrates and this difference might be ascribed to their topology (as mentioned earlier, CNX is membrane-bound, whereas CRT is a soluble protein).[18,19]

The crucial importance of the interaction between these lectins and glycoproteins makes studies at the molecular level a worthwhile task. In particular calreticulin, whose crystal structure is still not available, represents an interesting field of investigation. For this purpose, the syntheses of tetra- (**14**), tri- (**18**), and disaccharides (**22**) corresponding to truncated versions of the glucosylated arm of $Glc_1Man_9(GlcNAc)_2$ were accomplished (Fig. 2.2).

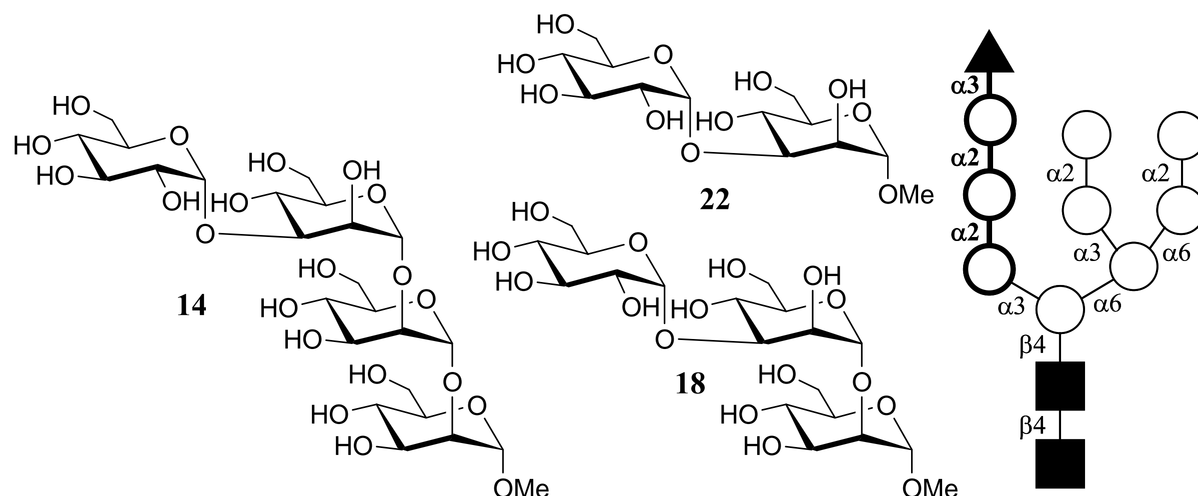


Figure 2.2 Synthesised oligosaccharides related to the glucosylated arm of $Glc_1Man_9(GlcNAc)_2$

Interaction of the synthetic substrates with calreticulin was investigated by isothermal titration microcalorimetry. The synthesis of the oligosaccharides is described in the following sections.

2.2 Previous syntheses

Tetrasaccharide **14** was previously synthesised by Matta *et al.*[24] and Monneret *et al.*[25] The first author's chosen synthetic approach was a linear one, where mannose and glucose derivatives were introduced one at a time starting from the reducing end mannose. In the other case, a convergent strategy was adopted and the tetrasaccharide was realized by condensation of two disaccharides. The di- and trisaccharide were also synthesised previously by Monneret *et al.*[26]

2.3 Synthetic strategy

Since early attempts in our laboratory to produce the tetrasaccharide in a 2 + 2 block-fashion failed, we planned the synthesis of the target oligosaccharides using a linear strategy. Ethyl thioglycosides were chosen as donors in the following glycosidations because of their stability and robustness during protecting group manipulations.[27] Employment of a 4,6-*O*-benzylidene acetal on mannose derivatives would also be advantageous, since interglycosidic linkages were to be introduced at either C-2 or C-3 (see compounds **1**[28] and **2**[29] in Figure 2.3). Introduction of the terminal α-linked glucose unit was planned to be achieved by use of a glucosyl donor with a non-participating group at C-2 (such as the perbenzylated donor **3**[30], Figure 2.3). Activation of donor **3** with dimethyl(methylthio)sulfonium triflate (DMTST)[31] in Et_2O should predominantly give the α-linked product, as Et_2O is known to favor formation of α-glycosides.[32]

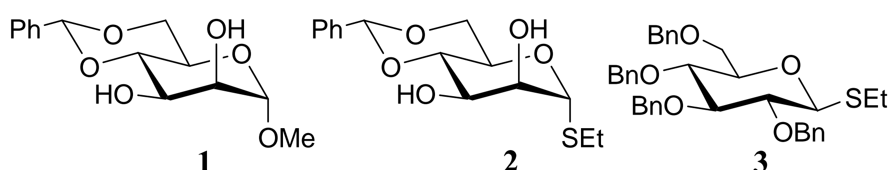


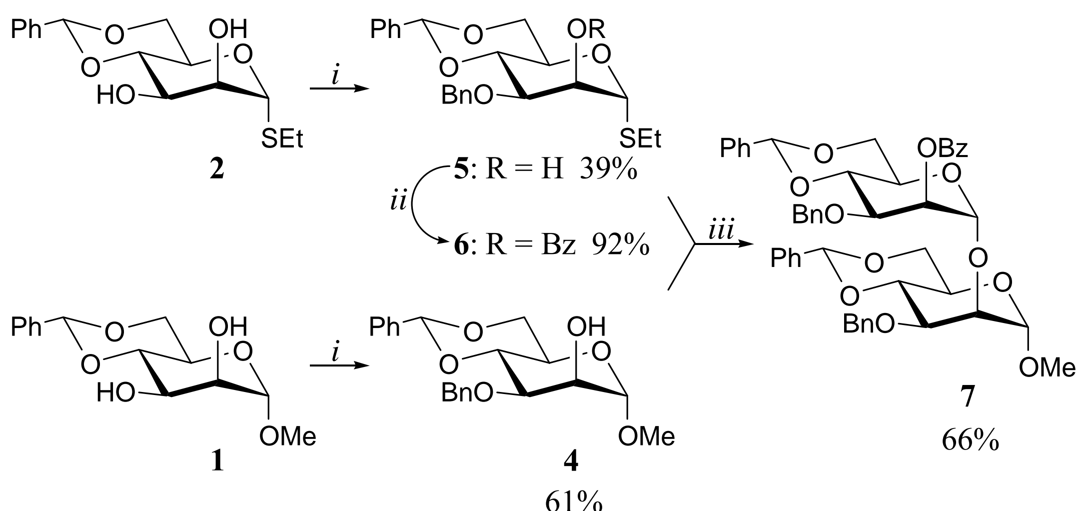
Figure 2.3 Building blocks for the synthesis of the target oligosaccharides

2.4 Results and Discussion

2.4.1 Synthesis of the Tetrasaccharide

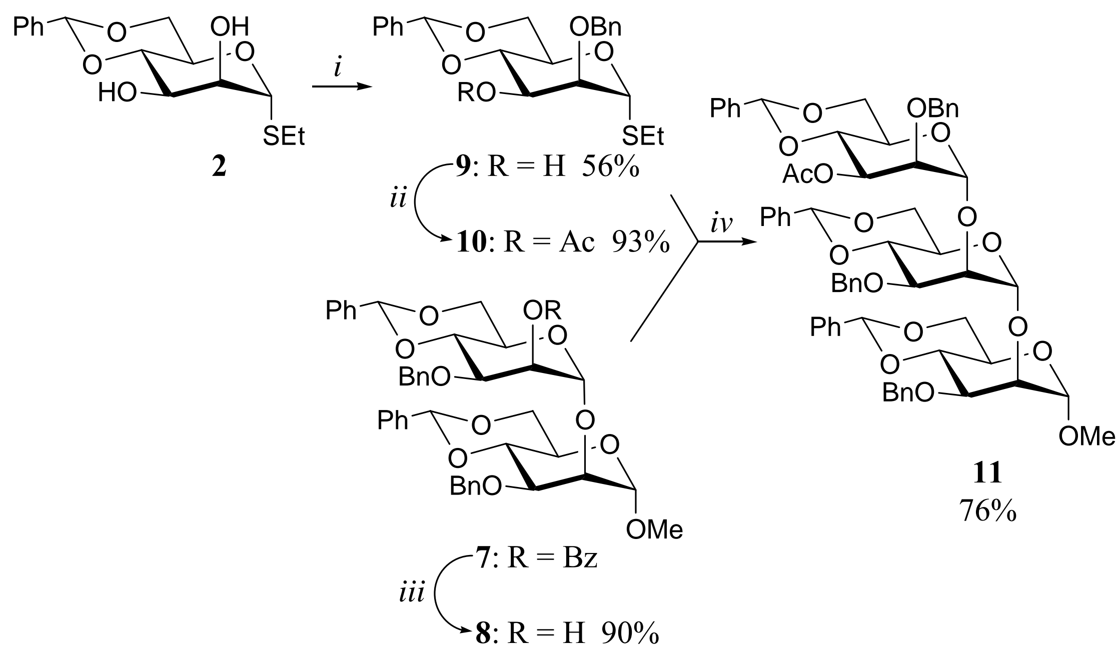
Tetrasaccharide **14** was built up starting from mannose derivatives **1** and **2** (Scheme 2.1). Methyl glycoside **1** and thioglycoside **2** were regioselectively protected at C-3 with a benzyl ether via alkylation of the stannylidene acetal formed upon treatment with Bu_2SnO, affording **4**[33] and **5**[34] with a free hydroxyl group at C-2.[35] Product **5** was subsequently benzoylated in order to obtain the

fully protected donor **6**. The presence of a participating group at C-2 was desirable for introduction of a 1,2-*trans*-glycoside in the glycosidation. Even though this is not a crucial requirement for the formation of α-mannopyranosides, in this specific case, a 2-*O*-ester group also served nicely as a temporary protecting group. A benzoate was preferred to an acetate to minimize the risk of orthoester formation.[36] Donor **6** and acceptor **4** were then coupled together, yielding the disaccharide **7**. The reaction worked best with *N*-iodosuccinimide (NIS)/silver trifluoromethanesulfonate (AgOTf) as promoter[37] and dichloromethane (DCM) as solvent.



Scheme 2.1 *i*) 1.Bu_2SnO, 2.BnBr; *ii*) BzCl, pyridine; *iii*) NIS/AgOTf, DCM.

Thioglycoside **2** was also selectively benzylated at O-2 under phase-transfer conditions (Scheme 2.2).[29] Acetylation of product **9**[29] gave donor **10**, which was subsequently reacted with acceptor **8**[25], obtained from **7** after removal of the benzoate under Zemplén deacylation conditions[38]. Glycosidation was performed again using NIS/AgOTf as promoter in DCM, to give exclusively the α-linked trisaccharide **11** in 76% yield.



Scheme 2.2 *i*) 5% aq. NaOH, BnBr, *n*-Bu_4NHSO_4 (cat.), DCM; *ii*) Ac_2O, pyridine; *iii*) NaOMe, MeOH; *iv*) NIS/AgOTf, DCM.

Trisaccharide **11** was then deacetylated with sodium methoxide, thus generating the acceptor for the final glycosidation (**12**, Scheme 2.3). Donor **3** was activated by DMTST in diethyl ether to enhance α-selectivity of the reaction. Indeed, the product with α-linked terminal glucose was predominant, even if contaminated with the corresponding β stereoisomer (α/β = 6.9:1). Separation of the α/β mixture was possible by high performance liquid chromatography (HPLC). The desired tetrasaccharide **13**[25] was isolated in 48% yield. The fully protected **13** was then deprotected in a single step by catalytic hydrogenolysis, affording target tetrasaccharide **14**.

Scheme 2.3 *i*) NaOMe, MeOH; *ii*) DMTST, Et_2O; *iii*) H_2, Pd/C.

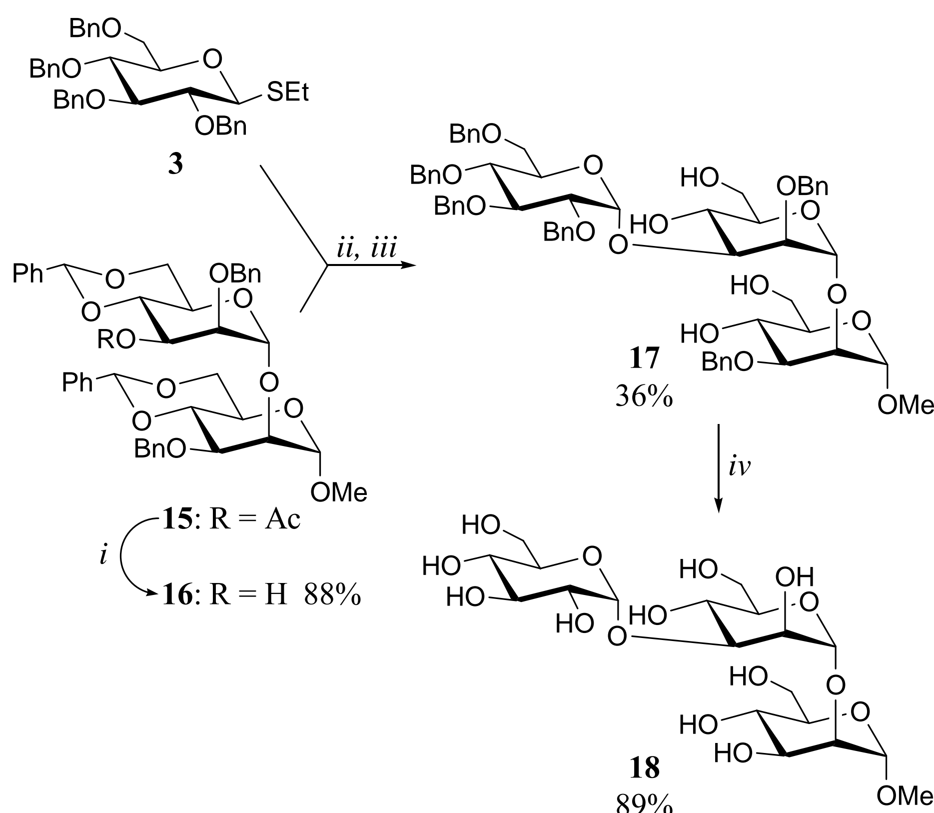
2.4.2 Synthesis of the Trisaccharide

Trisaccharide **18** was assembled in a similar fashion to the tetrasaccharide. At first, donor **10** and acceptor **4** were coupled together in a NIS/AgOTf-mediated glycosidation reaction (Scheme 2.4).

Scheme 2.4 *i*) NIS/AgOTf, DCM.

Compound **15**[26] was then deacetylated, furnishing the 3'-OH disaccharide **16**[26] in 88% yield (Scheme 2.5). Introduction of a glucose residue on **16** was realized

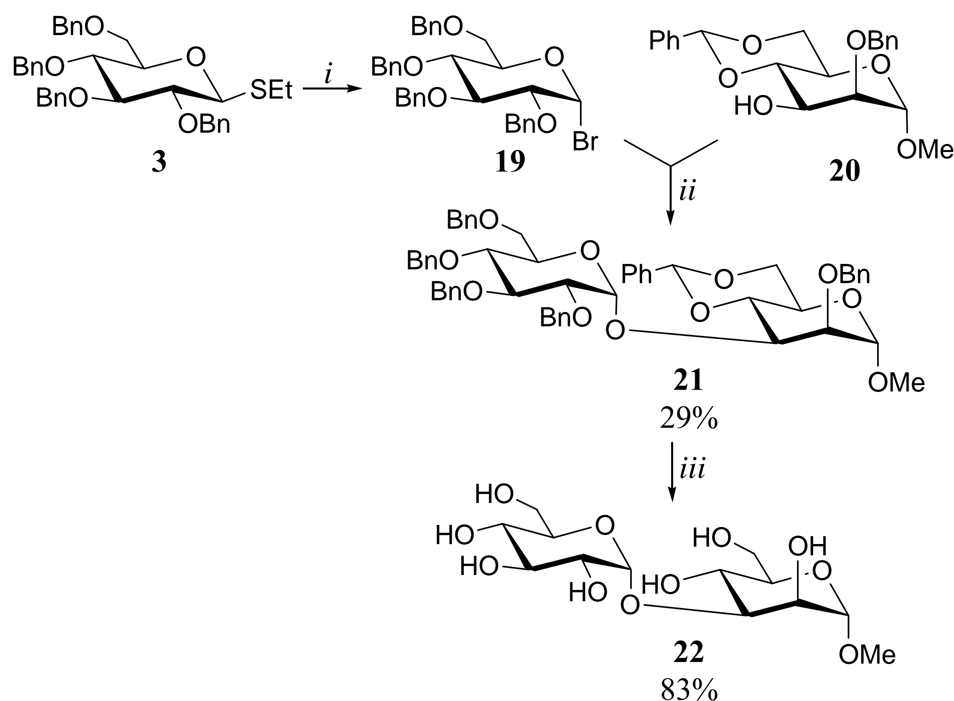
by reaction with **3** in the presence of DMTST in diethyl ether. Along with the α-linked trisaccharide, some β-linked product was also formed. Anticipating that separation of these two stereoisomers would be easier after chemical modification, the 4,6-benzylidene groups were removed by treatment with aqueous acetic acid. After HPLC, the desired trisaccharide **17** was eventually isolated in 36% yield for two steps. Lastly, **17** was fully deprotected by catalytic hydrogenation to afford the target trisaccharide **18**[26].



Scheme 2.5 *i*) NaOMe, MeOH; *ii*) DMTST, Et_2O; *iii*) 60% aq. AcOH, 70 °C; *iv*) H_2, Pd/C.

2.4.3 Synthesis of the Disaccharide

Donor **3** was converted into the corresponding glucosyl bromide **19** by treatment with bromine in DCM (Scheme 2.6).[39] The crude bromide was then coupled to acceptor **20**[40] (obtained from diol **1** after phase-transfer catalyzed benzylation) employing *in situ* anomerization conditions. The reaction yielded disaccharide **21** as a single isomer, albeit in a low yield (29%). Reductive cleavage of all protecting groups by H_2-Pd/C afforded the target disaccharide **22**.



Scheme 2.6 *i*) Br_2, DCM, 0 °C; *ii*) Et_4NBr, DCM; *iii*) H_2, Pd/C.

2.5 Biological Results

Isothermal titration microcalorimetry (ITC) is a quantitative technique that provides a direct estimate of the binding constants (K_b) and changes in enthalpy of binding ($\Delta H_b°$) as well as the stoichiometry of the interaction.[41] By ITC, the parameters of the interaction between synthetic substrates **14**, **18** and **22** and calreticulin were established. From the ITC data obtained, it emerged that the stoichiometry of the calreticulin-sugar interactions is unambiguously 1. The terminal glucose α-1,3 linked to mannose was found to be necessary for the binding. Moreover, the binding of the trisaccharide is 25-fold stronger than that of the disaccharide and the tetrasaccharide binds twice as strongly as the trisaccharide. This finding suggests that the binding site of calreticulin consists of a number of subsites, each of which is able to accommodate a hexopyranosyl unit of the tetrasaccharide.

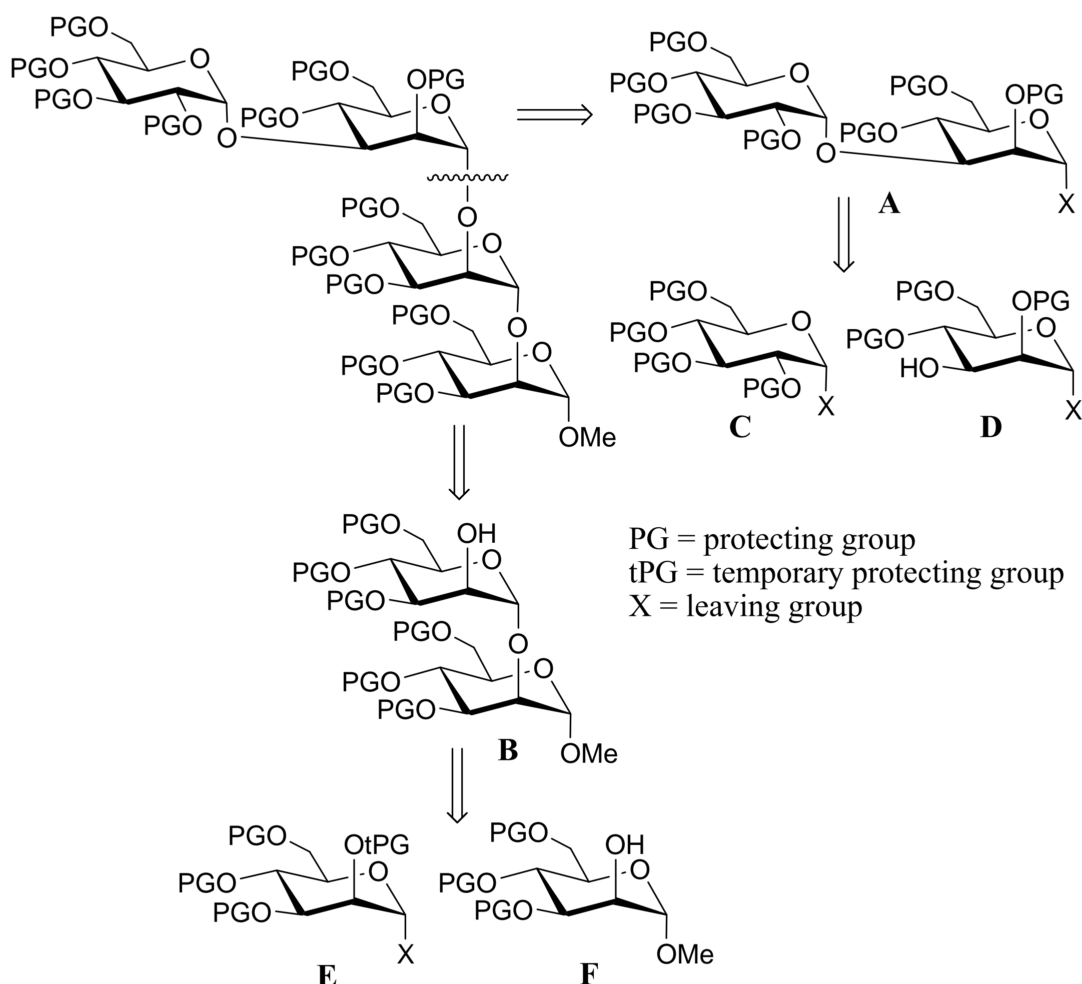
3 Improved Synthesis of the Tetrasaccharide Glc-α-(1→3)-Man-α-(1→2)-Man-α-(1→2)-Man-α-OMe (Paper II)

3.1 Introduction

Tetrasaccharide **14** (see Fig. 2.2) proved to be a valuable substrate for calreticulin and mutants of the same lectin.[42] For this reason, larger amounts of the tetrasaccharide were required. Even though the synthesis described in the previous chapter was satisfactory, it did present some limitations in terms of synthetic strategy and the moderate yields in some steps. Therefore, development of a more expedious and efficient synthetic pathway would be desirable. In this chapter a new synthetic approach to tetrasaccharide **14** and trisaccharide **18** is described.

3.2 Synthetic Strategy

One disadvantage of the previously described synthesis was its linearity. In fact, the need for additional protection and deprotection steps makes this approach rather lengthy. Hence a more convergent strategy was chosen and two disaccharidic residues **A** and **B** were to be used as building blocks for construction of the tetrasaccharide (Scheme 3.1). Disaccharide **A** would be obtained by condensation of donors **C** and **D**, *i.e.* **C** needs to be chemoselectively activated in the presence of **D**. Building block **B** was retrosynthetically disconnected into mannosides **E** and **F**. Donor **F** should possess a temporary protecting group at O-2 to allow later coupling between **A** and **B**. This should also preferably be a participating group, in order to ensure formation of an α-glycosidic bond in disaccharide **B**. With regard to the protecting group strategy, it was decided to avoid employment of 4,6-*O*-benzylidene acetal as protecting group for the mannose derivatives. One reason for that is the difficult introduction of such a protecting group, since mannose has a 2,3-*cis*-diol and the 2,3;4,6-di-*O*-benzylidene product is also formed in the reaction.[43] Moreover, cyclic acetal groups on a glycosyl donor are also deactivating in glycosidation reactions. This is believed to be due to the increased rigidity they confer on the sugar ring, which thereby hinders formation of the intermediate oxacarbenium ion.[44,45]



Scheme 3.1 Retrosynthetic analysis of tetrasaccharide **14**

3.3 Construction of the Glc-α-(1→3)-Man Building Block

Disaccharide **A** was built by conjunction of glycosyl donors **C** and **D** (Scheme 3.1). We decided to use glucosyl iodide **23**[46] for **C** and thioglycoside **24**[47] for **D** (Figure 3.1).

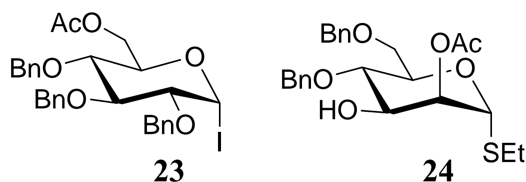
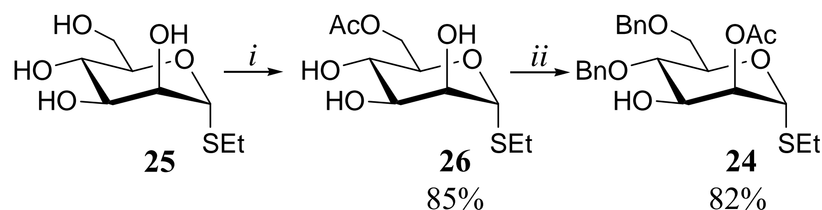


Figure 3.1 Selected derivatives for construction of the Glc-α-(1→3)-Man building block

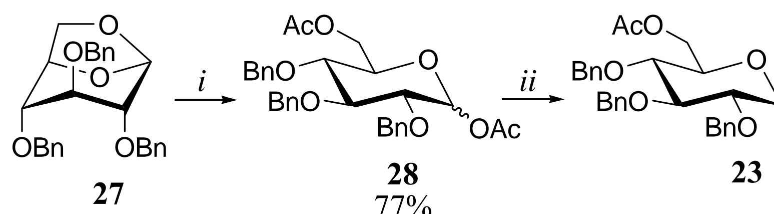
Acceptor **24** was prepared according to Scheme 3.2. Mannoside **25**[48] was selectively acetylated at the primary alcohol by using a sterically hindered amine and by performing the reaction at low temperature.[49] A cyclic 2,3-orthoacetate was then introduced on **26**, following a standard procedure.[50] The purpose of having an acetate at 6-OH is to block this position temporarily, ensuring that no

4,6-orthoester is formed in the reaction. The crude orthoester intermediate was subsequently benzylated at positions 4 and 6 and then subjected to acid-catalysed ring-opening of the 2,3-orthoester, thus affording the 2-*O*-acetylated compound **24**.[51] The nearly quantitative yield of the last three reactions permits the execution of this series of steps in a one-pot fashion, without purification of the intermediates. In this way, rapid access to acceptor **24**, having a free hydroxyl at position 3 and devoid of a 4,6-*O*-benzylidene acetal, was gained.



Scheme 3.2 *i*) AcCl, 2,4,6-collidine, -35 °C; *ii*) 1. $CH_3C(OEt)_3$, CSA; 2. NaH, BnBr, DMF; 3. 1 N aq. HCl.

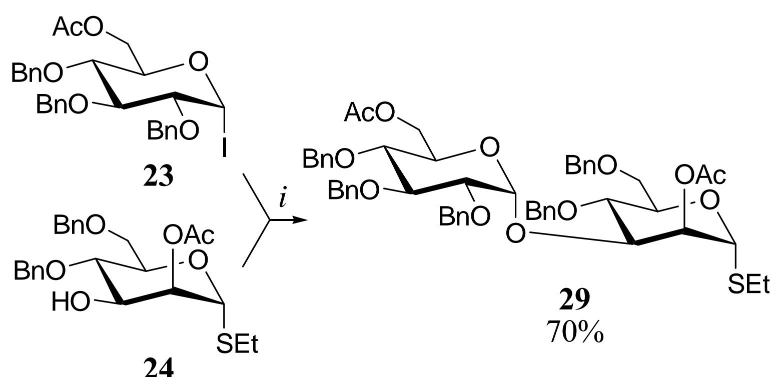
Glucosyl donor **23** was prepared from 1,6-anhydro-glucopyranoside **27**[52] (Scheme 3.3). Acetolysis with trifluoroacetic acid in acetic anhydride afforded the 1,6-di-acetate **28**.[53,54] The anomeric acetate was then directly converted into the corresponding iodide by reaction with iodotrimethylsilane (TMSI), yielding donor **23**[46], which was used in the following step without further purification.[55]



Scheme 3.3 *i*) TFA, Ac_2O; *ii*) TMSI, DCM, 0 °C.

Next, donor **23** and acceptor **24** were coupled together. At first, the coupling was attempted under *in situ* anomerisation conditions with tetrabutylammonium iodide, since it had previously been shown that, under these reaction conditions, donor **23** is able to produce α-glycosides in good yields.[46] However, despite promising initial results, this reaction proved to be irreproducible. We then turned our attention to a recently published paper in which a new method for activation of glycosyl iodides is described.[56] According to this work, phosphine oxides, in particular triphenylphosphine oxide, constitute good promoters for glycosyl iodides, giving excellent yields and high α-stereoselectivity. The reaction is believed to proceed via a reactive glycosyl phosphonium iodide,

formed by reaction between the glycosyl iodide and phosphine oxide. Indeed, triphenylphosphine oxide-promoted coupling of **23** and **24** did provide the desired disaccharide **29** (Scheme 3.4). Only the α-linked product was detected and isolated from the reaction in 70% yield. The presence of a 6-*O*-acetate on the glucose donor seems to be beneficial, not only in stabilizing the reactive iodide but also by enhancing the α-selectivity of the glycosidation reaction.



Scheme 3.4 *i*) Ph_3PO, DCM.

3.4 Construction of the Man-α-(1→2)-Man-α-OMe Building Block

Dimannoside building block **B** was to be obtained with a free 2'-OH on the non-reducing end mannose, where the glucose-containing disaccharide **A** were to be incorporated (Scheme 3.1). On the other hand, the two mannose units of **B** was also to be attached through the 2-OH of the reducing end mannose. Hence, the synthesis of this disaccharide was designed to take advantage of its symmetry, and a common precursor to both mannose derivatives was identified in the 1,2-orthoester **30**[57] (Figure 3.2).

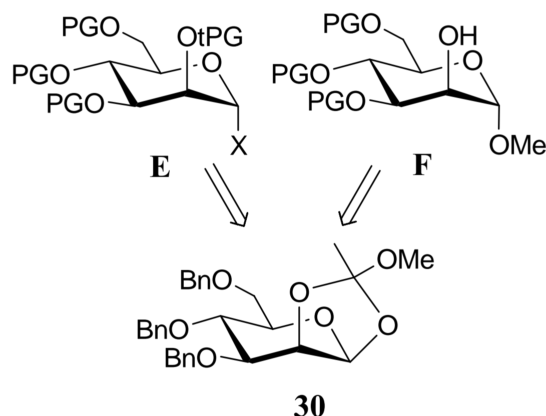
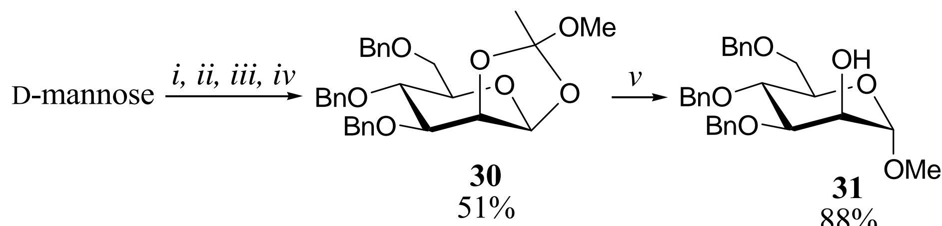


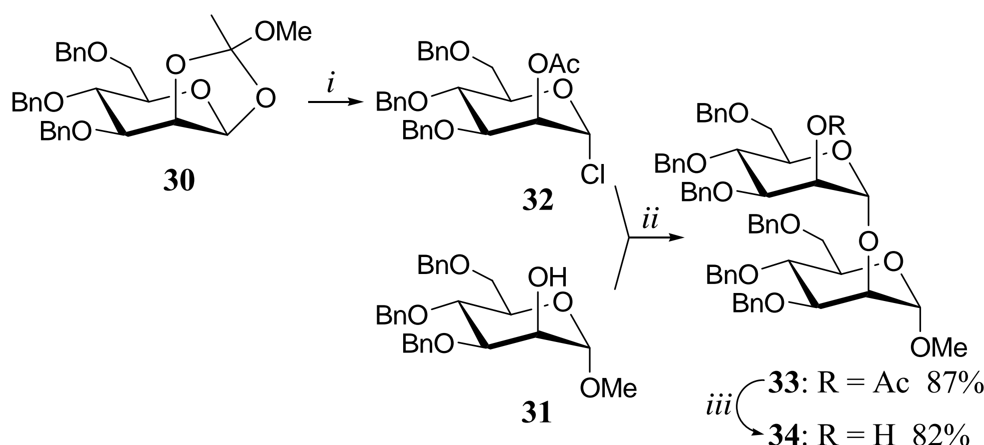
Figure 3.2 Common precursor to **E** and **F**.

Compound **30**, which is readily available from D-mannose in 4 steps, was converted into methyl mannoside **31**[57] by refluxing in methanolic hydrochloric acid (Scheme 3.5).[57,58] Using this procedure, formation of the methyl glycoside and cleavage of the 2-*O*-acetate take place in the same reaction.



Scheme 3.5 *i*) Ac_2O, $HClO_4$; *ii*) PBr_3, H_2O; *iii*) 2,4,6-collidine, MeOH; *iv*) KOH, BnBr, toluene, reflux; *v*) 5% HCl in MeOH, reflux.

With acceptor **31** in hand, derivative **30** had to be transformed into a suitable donor. This was possible by reaction with trimethylsilyl chloride in DCM (Scheme 3.6).[59] The corresponding glycosyl chloride **32**[60] was thus obtained and used directly in the following Koenigs-Knorr glycosidation together with acceptor **31**.[61] Product **33**[60] was then subjected to methanolysis to liberate the 2'-position, providing the desired disaccharide **34**[60] ready for further elongation.

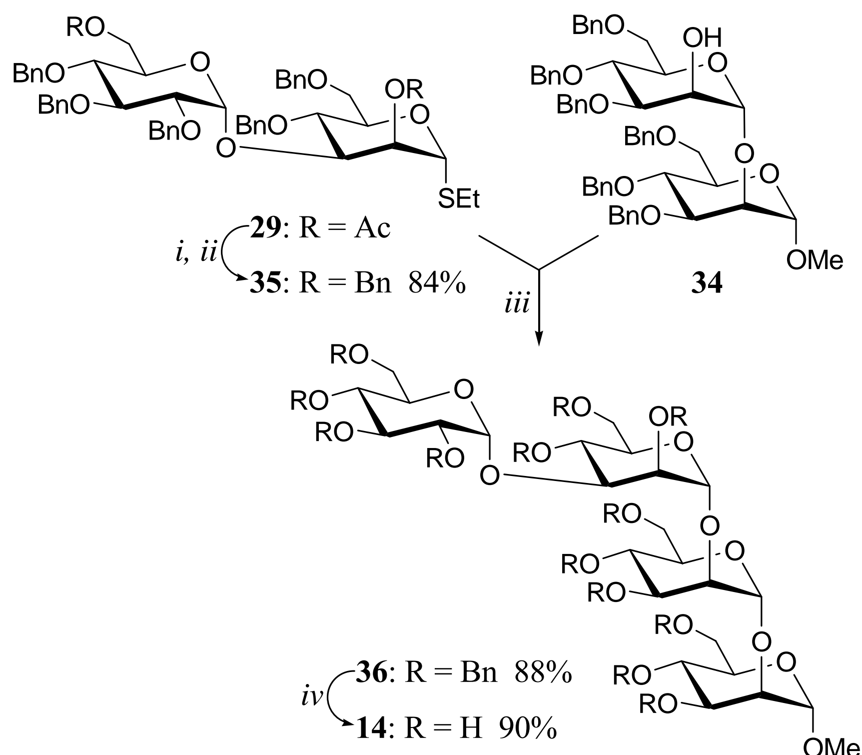


Scheme 3.6 *i*) TMSCl, DCM, 0 °C; *ii*) AgOTf, DCM; *iii*) NaOMe, MeOH.

3.5 Final Glycosidation and Deprotection

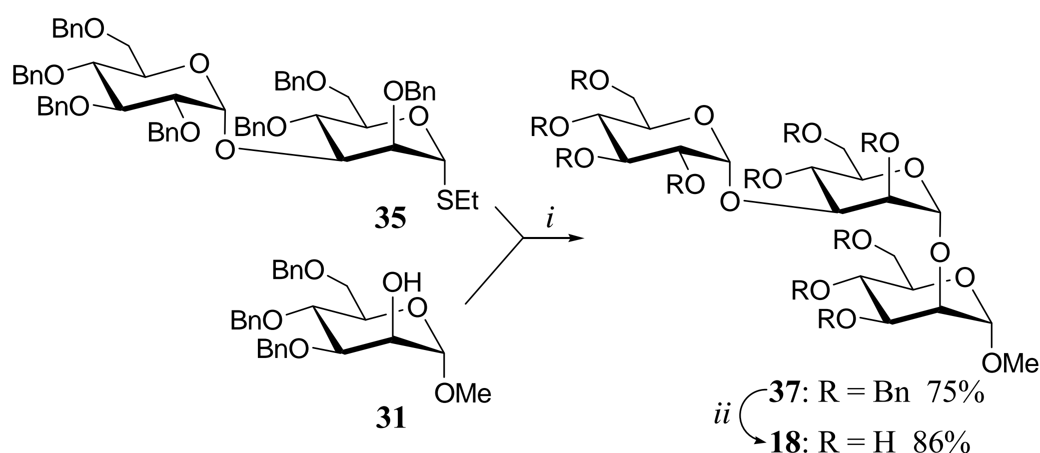
Donor **29** and acceptor **34** had now to be coupled together. At first, direct reaction of **29** and **34** was attempted using NIS/AgOTf as promoter. Unfortunately, the reaction failed to give any product. Anticipating that the acetate groups on donor **29** were responsible for this, they were exchanged with benzyl groups instead (Scheme 3.7). Gratifyingly, fully benzylated **35** proved to

be a much better donor and its coupling with **34** furnished tetrasaccharide **36** in high yield. Finally, hydrogenolysis of **36** afforded **14**.



Scheme 3.7 *i*) NaOMe, MeOH; *ii*) NaH, BnBr, DMF; *iii*) NIS/AgOTf, DCM; *iv*) H_2, Pd/C, MeOH.

In the same way, donor **35** was also coupled to acceptor **31**, giving trisaccharide **37** (Scheme 3.8), which was fully deprotected by hydrogenolysis to afford target trisaccharide **18**.



Scheme 3.8 *i*) NIS/AgOTf, DCM; *ii*) H_2, Pd/C, MeOH.

3.6 Concluding Remarks

In conclusion, a new synthetic pathway leading to tetrasaccharide **14** was developed. Compared to the approach described in the previous chapter, this new one is advantageous for several reasons. The synthetic strategy is convergent, thus diminishing the total number of steps. Moreover, the glycosidation reactions involved in this approach are generally characterized by higher yield and stereoselectivity than those employed in the previous one. The overall yield going from monosaccharidic building blocks to protected tetrasaccharide is raised from 19% in the previous approach to 52% in the present one.

4 Synthesis of Deoxy-Trisaccharides for Binding Studies with Calreticulin (Appendix A, Paper IV)

4.1 Introduction

The ITC results of the interaction between calreticulin and the synthesised di-, tri- and tetrasaccharides (see Chapter 2) provided information about the size of the glycan recognized by this lectin. In fact, one of the most remarkable results was the significant (25-fold) increase in binding going from di- to trisaccharide. However, additional studies were required to gain a more detailed picture of this interaction at the molecular level. In order to understand which hydroxyl groups on the carbohydrate residues are involved in hydrogen bonding to calreticulin, various monodeoxy (*i.e.* lacking one hydroxyl) analogues of trisaccharide Glc-α-(1→3)-Man-α-(1→2)-Man-α-OMe (**38-44,** Figure 4.1) were to be synthesised.

Glc-α-(1→3)-Man-α-(1→2)**-3-Deoxy-Man-α-OMe** (**38**)

Glc-α-(1→3)-Man-α-(1→2)-**4-Deoxy-Man-α-OMe** (**39**)

Glc-α-(1→3)-**4-Deoxy-Man-**α-(1→2)-Man-α-OMe (**40**)

Glc-α-(1→3)-**6-Deoxy-Man-**α-(1→2)-Man-α-OMe (**41**)

2-Deoxy-Glc-α-(1→3)-Man-α-(1→2)-Man-α-OMe (**42**)

3-Deoxy-Glc-α-(1→3)-Man-α-(1→2)-Man-α-OMe (**43**)

6-Deoxy-Glc-α-(1→3)-Man-α-(1→2)-Man-α-OMe (**44**)

Figure 4.1 Target deoxy trisaccharides

4.2 Synthesis of the Glc-α-(1→3)-Man-α-(1→2)-Deoxy-Man-α-OMe Trisaccharides

4.2.1 Synthetic Strategy

Target trisaccharides **38** and **39** both contain a deoxy functionality on the reducing end mannose. Therefore, a convenient synthetic approach would feature coupling of an appropriate deoxy acceptor with a common Glc-α-(1→3)-Man donor. Advantageously, such a disaccharidic donor (**35**, Figure 4.2) had

been obtained in a previous synthesis and could be used in the present one as well (see Chapter 3).

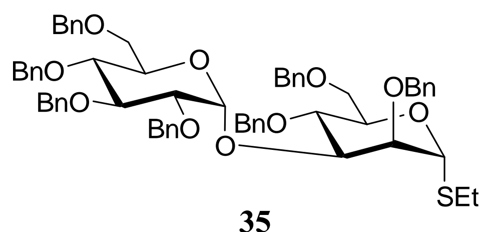
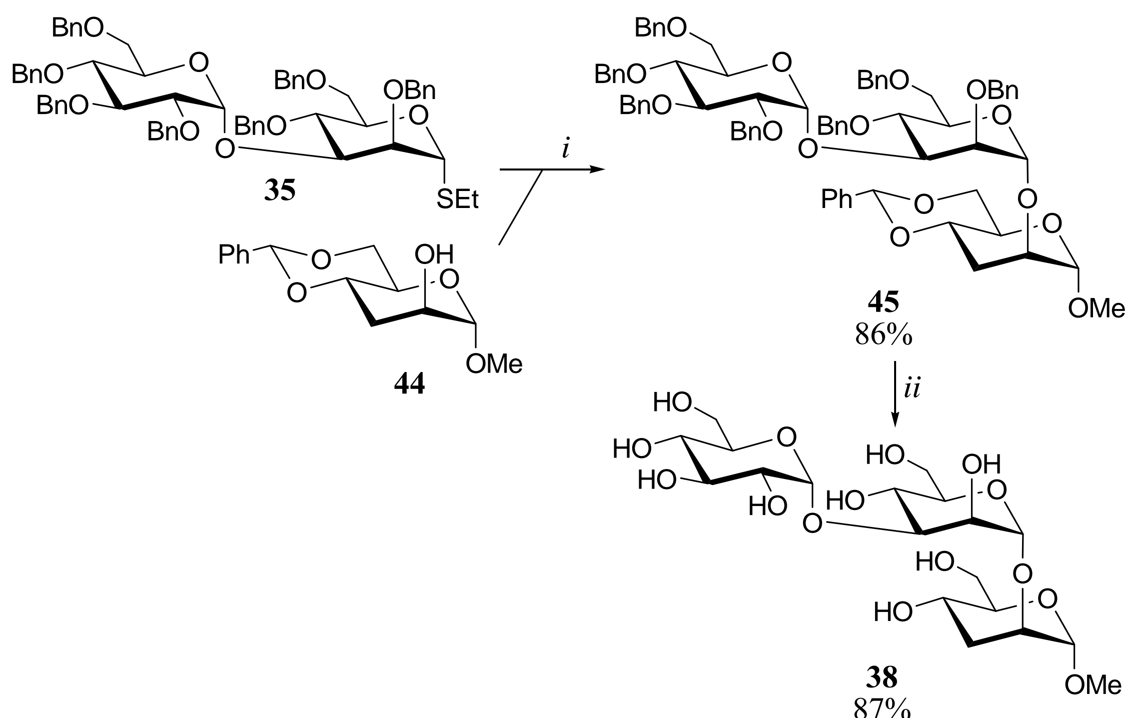


Figure 4.2 Glc-α-(1→3)-Man donor

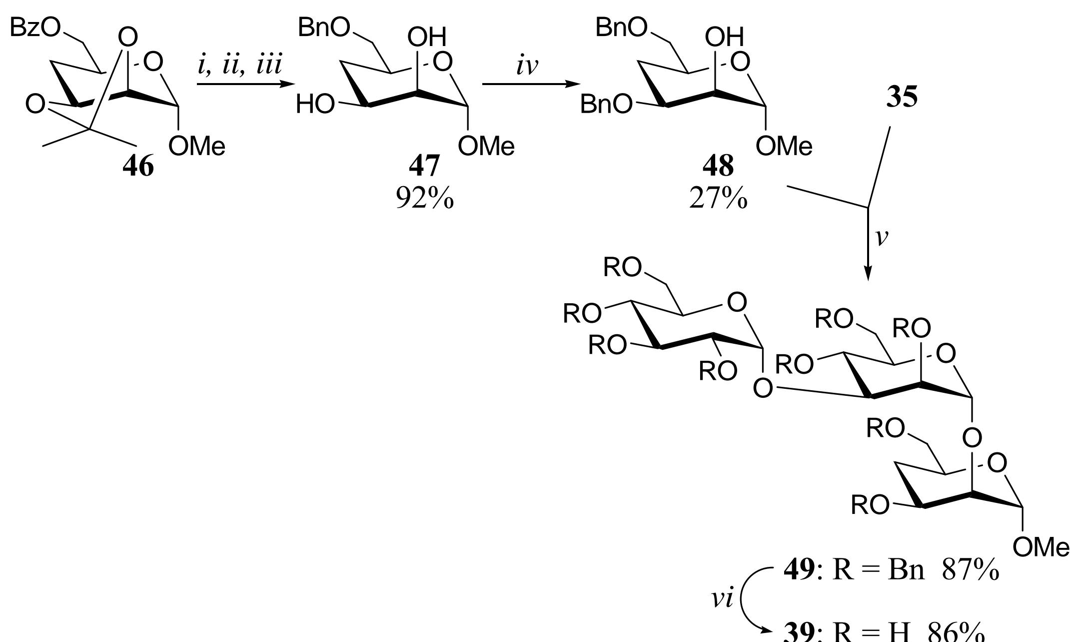
4.2.2 Assembly and Deprotection of the Trisaccharides

The 3-deoxy-Man acceptor was represented by the known 4,6-*O*-benzylidene derivative **44**[62] (Scheme 4.1). This was reacted with donor **35** in a NIS/AgOTf-mediated glycosidation, which readily afforded trisaccharide **45** in 86% yield. Catalytic hydrogenolysis of **45** furnished the first target trisaccharide, **38**.



Scheme 4.1 *i*) NIS/AgOTf, DCM; *ii*) H_2, Pd/C, MeOH/1 N aq. HCl.

4-Deoxy-Man acceptor **48** was obtained from **46**[63] (Scheme 4.2). Benzylation at position 6 and removal of the 2,3-*O*-isopropylidene acetal gave diol **47**, which was converted into acceptor **48** by selective tin-mediated benzylation at the equatorial 3-OH. NIS/AgOTf-promoted coupling of acceptor **48** with donor **35** gave the fully protected trisaccharide **49**. Target trisaccharide **39** was obtained after catalytic hydrogenation of **49** in 86% yield.



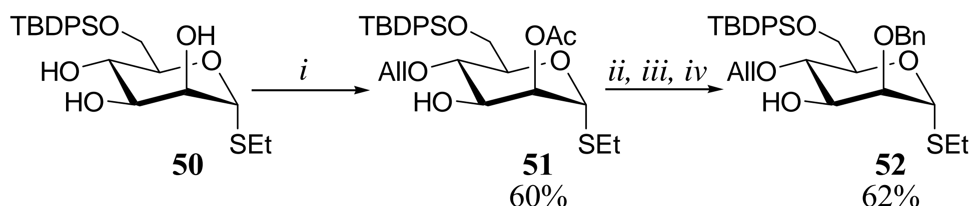
Scheme 4.2 *i*) NaOMe, MeOH; *ii*) NaH, BnBr, DMF; *iii*) 80% aq. TFA; *iv*) 1. Bu_2SnO, MeOH; 2. BnBr, DMF; *v*) NIS/AgOTf, DCM; *vi*) H_2, Pd/C, MeOH-EtOAc 5:1.

4.3 Synthesis of the Glc-α-(1→3)-Deoxy-Man-α-(1→2)-Man-α-OMe Trisaccharides

4.3.1 An Unsuccessful Approach

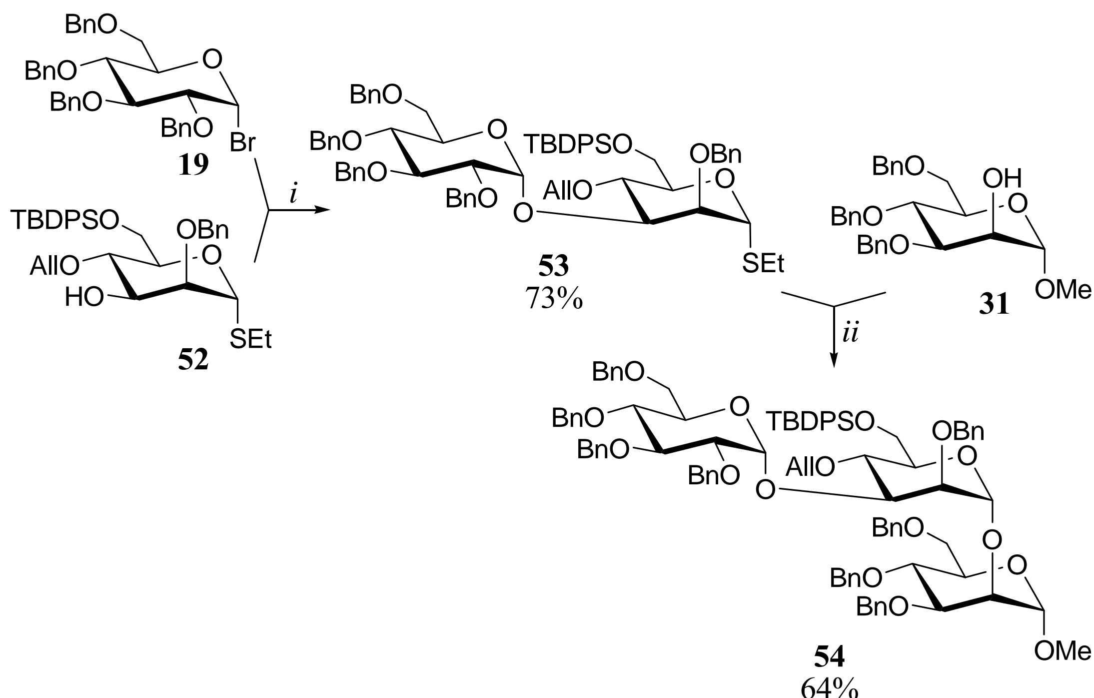
Our first approach to the trisaccharides, lacking a hydroxyl group on the middle mannose unit (either at position 4 or 6), involved introduction of the deoxy functionality at a later stage of the synthetic route (instead of having deoxy-monosaccharides from the outset, as in the preceding case). This should be made possible by employing a mannose derivative with orthogonal protecting groups at positions 4 and 6, which could be selectively removed, thereby leaving the corresponding hydroxyl accessible for deoxygenation. The advantage of this approach was the possibility of obtaining both trisaccharides from a single trisaccharide precursor. In our attempt, compound **52** was designated as the middle mannose building block (Scheme 4.3). Its synthesis was accomplished starting from ethyl 6-*O*-*t*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside (**50**)[64], since introduction of this bulky protecting group is very selective for the primary hydroxyl.[65] A 2,3-orthoacetate was then introduced on **50**, and using the same one-pot procedure presented in Chapter 3 (See Scheme 3.2), allylated at position 4. An acidic wash prompted opening of the cyclic orthoester, thus affording acetate **51**. The 2-*O*-acetate was then replaced with a benzyl group (**52**), by

removal of the ester, introduction and reductive opening of a 2,3-*O*-benzylidene acetal, thus leaving position 3 available for elongation with an α-glucose moiety.[66,67]



Scheme 4.3 *i*) 1. $CH_3C(OEt)_3$, CSA; 2. NaH, AllBr, DMF; 3. 1 N aq HCl; *ii*) NaOMe, MeOH; *iii*) $PhC(OMe)_2$, CSA, DMF; *iv*) $NaCNBH_3$, HCl/Et_2O, THF.

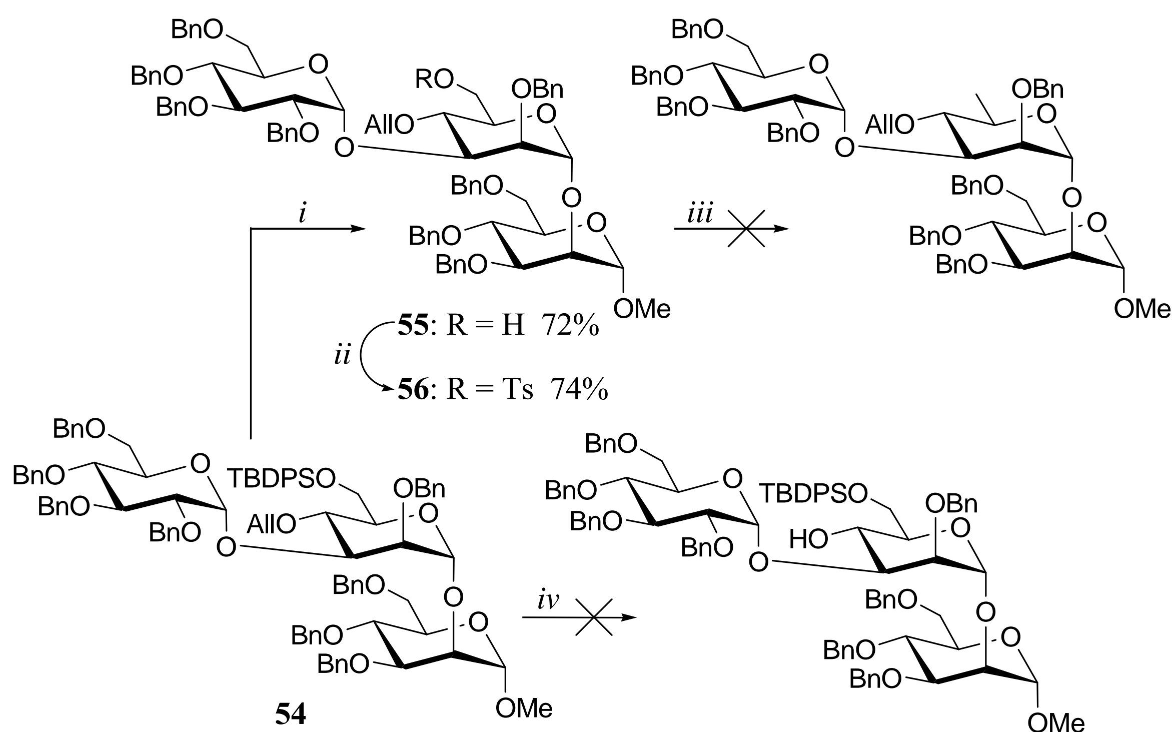
Perbenzylated glucose bromide **19** was now coupled to acceptor **52** under *in situ* anomerisation conditions, successfully providing desired disaccharide **53** as a single isomer in 73% yield (Scheme 4.4). Disaccharide **53** was subsequently reacted with methyl mannoside **31** to give trisaccharide **54**. Methyl triflate was preferred to NIS/AgOTf as a promoter for this glycosidation because of the possible interference of NIS with the allyl group on donor **53**.[32]



Scheme 4.4 *i*) Et_4NBr, DCM; *ii*) MeOTf, DCM.

Next, the 6'-*O*-silyl or the 4'-*O*-allyl protecting groups on **54** were to be orthogonally cleaved and the resulting alcohol deoxygenated in order to obtain the two target monodeoxy trisaccharides of this series (as fully benzylated derivatives). First, the 6'-*O*-*t*-butyldiphenylsilyl group was removed by

treatment with Bu_4NF in THF (**55**, Scheme 4.5). The 6'-position was subsequently tosylated, giving **56** in 74% yield. Hydride displacement of this tosylate by reaction with $LiAlH_4$ was unfortunately not successful, resulting merely in decomposition of the starting material. Attempts to introduce a 4'-deoxy function on **54** were just as discouraging. In fact, removal of the 4'-*O*-allyl group was tried both with 1,5-cyclooctadiene-bis-[methyldiphenylphosphine]-iridium hexafluorophosphate[68]/-NIS-water[69] and with SmI_2/water/pyrrolidine[70], but without success. This was possibly due to the steric hindrance imposed by the bulky silyl group adjacent to the 4'-position.

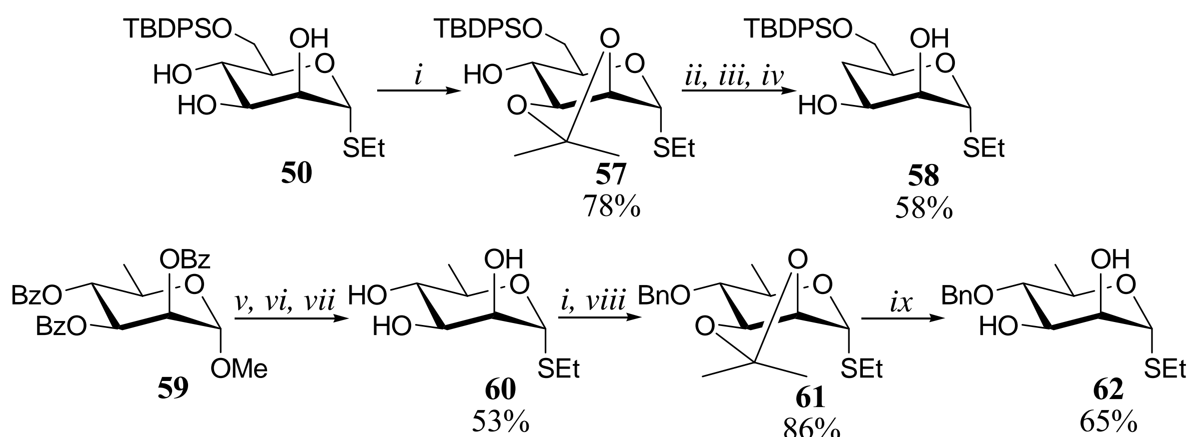


Scheme 4.5 i) 1 M Bu_4NF in THF; ii) TsCl, pyridine; iii) $LiAlH_4$, Et_2O; iv) $[Ir(cod)(PMePh_2)_2]^+ PF_6^-$, NIS/water or SmI_2/water/pyrrolidine.

4.3.2 A New Approach

Since introduction of the deoxy functionality at the trisaccharide level failed, we chose to employ deoxy monosaccharidic derivatives for incorporation into the trisaccharide. Accordingly, 4- and 6-deoxy thiomannosides **58** and **62**[71] were synthesised (Scheme 4.6). 4-deoxy **58** was realized by deoxygenation of 2,3-*O*-isopropylidene derivative **57**[72]. This was first converted into the 4-*O*-thiocarbonylimidazole ester by reaction with 1,1'-thiocarbonyldiimidazole in 1,2-dichloroethane (DCE) and then reduced with tributyltin hydride in benzene, employing AIBN as radical initiator.[73] Treatment of this intermediate with a

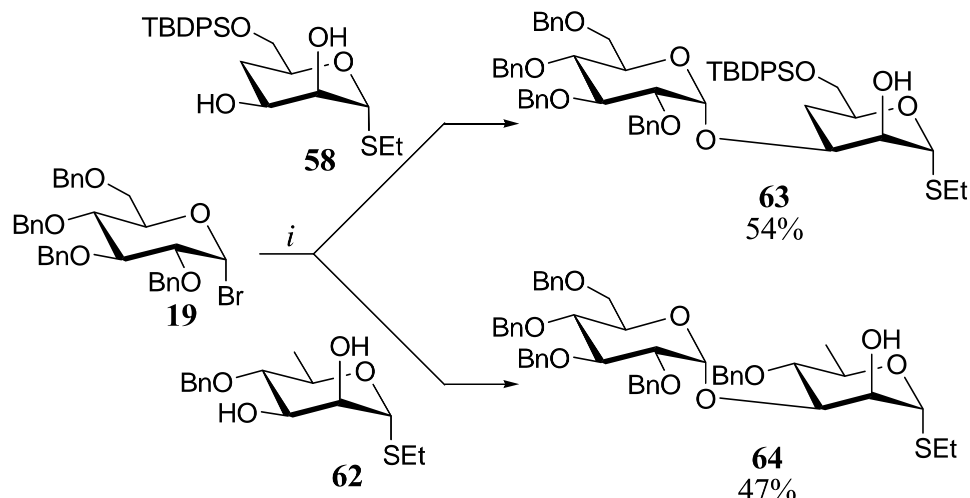
mixture of AcOH, water and TFA (10:2:1 v/v/v) at 0 °C afforded 2,3-diol **58** in 58% overall yield. 6-Deoxy thiomannoside **62** was prepared from the corresponding methyl glycoside **59**[74] (available in our lab). Standard acetolysis[75] of **59** gave the anomeric acetate, which was converted to ethyl thioglycoside **60**[76] by reaction with EtSH/$BF_3 \cdot Et_2O$[77] and subsequent methanolysis. A 2,3-*O*-isopropylidene group was introduced by reaction with acetone and 2-methoxypropene, catalyzed by CSA, and subsequent benzylation of position 4 yielded **61**[71] (86%). Finally, treatment with 90% aqueous TFA resulted in hydrolysis of the isopropylidene acetal and the 2,3-diol **62** was obtained in 65% yield.



Scheme 4.6 *i*) 2-methoxypropene, acetone, CSA; *ii*) Im_2CS, DCE; *iii*) Bu_3SnH, AIBN, benzene; *iv*) AcOH-H_2O-TFA 10:2:1 (v/v/v), 0 °C; *v*) Ac_2O-AcOH (1:2), conc H_2SO_4; *vi*) EtSH, $BF_3 \cdot Et_2O$, DCM; *vii*) NaOMe, MeOH; *viii*) NaH, BnBr, DMF; *ix*) 90% aq. TFA.

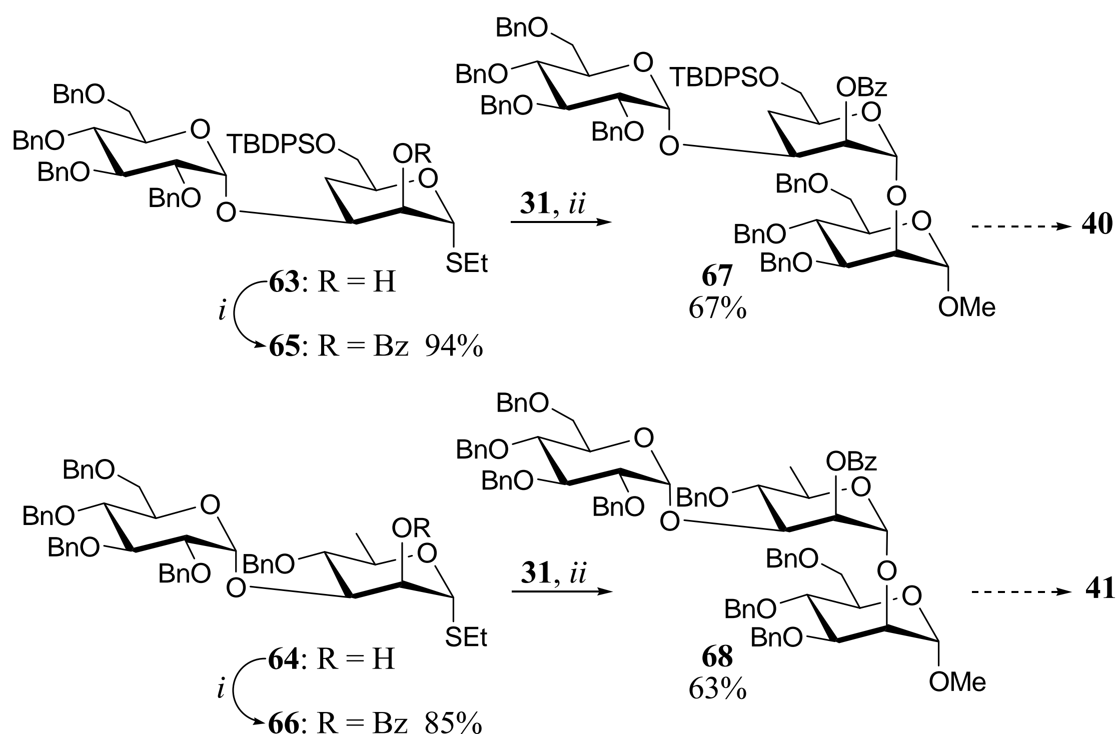
Since an α-glucose residue had to be attached to the 3-OH of the middle monodeoxy mannose unit, the 2-OH of **58** and **62** was to be preferably protected before glycosidation. Hence in initial attempts, 2-*O*-protected **58** and **62** (with an acetyl, benzoyl or benzyl group) were employed as acceptors under *in situ* anomerisation conditions with Bn_4Glc-α-Br (**19**). However, these acceptors did not provide the desired disaccharidic product, with the exception of the 2-*O*-benzyl derivatives where the products were obtained after prolonged reaction times (1-2 weeks) and only in moderate yields. Earlier experiments reporting regioselective 3-O-glycosidation of 2,3-unprotected mannose acceptors,[78,79] made us try the same reaction with diols **58** and **62** as acceptors (Scheme 4.7). Indeed, the 3-*O*-linked disaccharide was obtained in both couplings (**63** and **64**) in good yield (54% for **63** and 47% for **64**). The reactions were surprisingly fast (36-40 hours) and showed high regioselectivity (3-*O*-linked disaccharide/2-*O*-

linked disaccharide ratio was 3.4 for the 4-deoxy acceptor and 2.6 for the 6-deoxy acceptor) and complete stereoselectivity.



Scheme 4.7 *i*) Et_4NBr, DCM.

Disaccharides **63** and **64** were then benzoylated at O-2 and the resulting products (**65** and **66**) used as donors in NIS/AgOTf-promoted glycosidations to the reducing-end mannose residue **31** (Scheme 4.8). Accordingly, fully protected versions of the target trisaccharides were obtained as **67** and **68**. Deprotection of these should afford target structures **40** and **41**.



Scheme 4.8 *i*) BzCl, pyridine; *ii*) NIS/AgOTf, DCM.

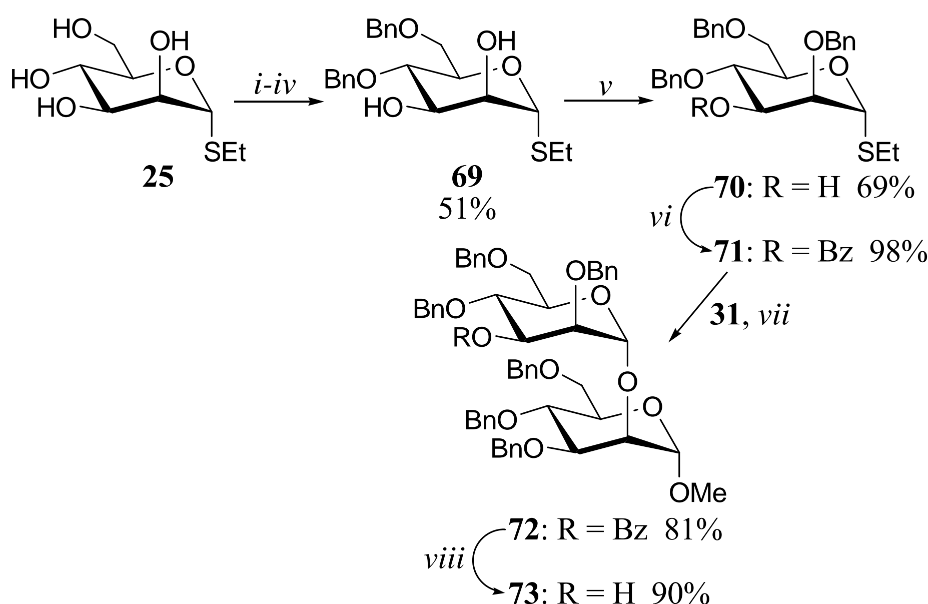
4.4 Synthesis of the Deoxy-Glc-α-(1→3)-Man-α-(1→2)-Man-α-OMe Trisaccharides

4.4.1 Synthetic strategy

An obvious synthetic approach to the target trisaccharides with 2-, 3- or 6-deoxy-glucose residues (**42**, **43** and **44**) was to begin with assembly of the two mannose units, since this fragment of the molecule is conserved in all of the three target structures. This disaccharidic building block should allow elongation at the 3'-position, where the various glucose derivatives were to be introduced.

4.4.2 Synthesis of the Man-α-(1→2)-Man-α-OMe Building Block

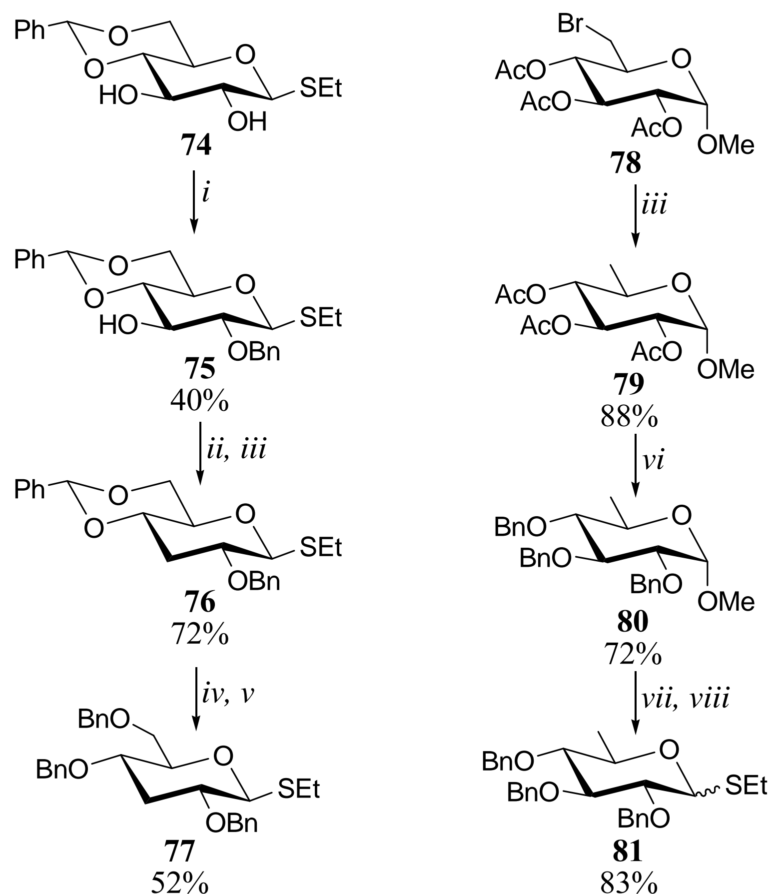
The assembly Man-α-(1→2)-Man-α-OMe building block **73** was realized by condensation of mannosides **71** and **31** (Scheme 4.9). Synthesis of **71** was performed starting from ethyl thiomannoside **25**. Following a standard procedure, **25** was transformed into 2,3-diol **69**[47] in four steps.[80] Benzylation of **69** under phase transfer catalysis conditions afforded 2-*O*-benzyl protected **70**[81] in 69% yield, which was then benzoylated to give donor **71**[44]. Disaccharide **72** was obtained by NIS-AgOTf-promoted glycosidation of **31** with **71** in high yield (81%). Debenzoylation of **72** under Zemplén's conditions gave the desired 3'-OH disaccharide **73**, to be employed as acceptor in the subsequent glycosidations.



Scheme 4.9 *i*) 2,2-dimethoxypropane/acetone (1:1), CSA; *ii*) 90% aq. AcOH; *iii*) NaH, BnBr, DMF; *iv*) 90% aq. TFA; *v*) 5% aq. NaOH, BnBr, *n*-Bu_4NHSO_4, DCM; *vi*) BzCl, pyridine; *vii*) NIS/AgOTf, DCM; *viii*) NaOMe, MeOH.

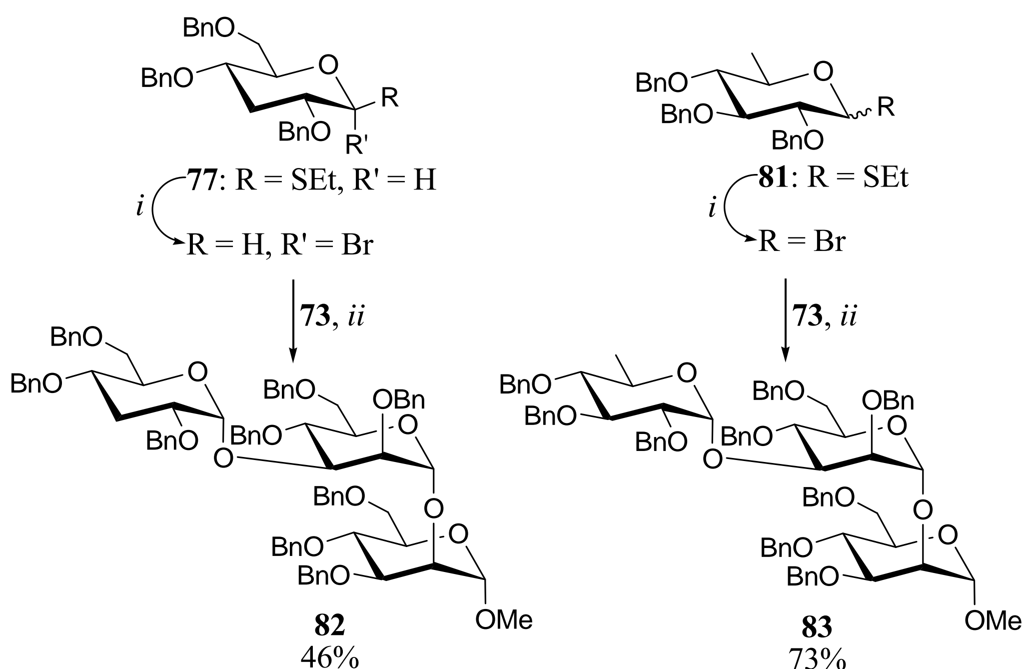
4.4.3 Synthesis of the 3- and 6-Deoxy-Glc-α-(1→3)-Man-α-(1→2)-Man-α-OMe Trisaccharides

With acceptor **73** in hand, the monodeoxy-glucose donors were now to be prepared. 3-deoxy thioglucoside **77**[82] was prepared starting from ethyl 4,6-*O*-benzylidene-thio-β-glucopyranoside **74**[29] (Scheme 4.10). Benzylation of **74** under phase-transfer catalysis conditions gave the 2-*O*-benzyl protected **75**[29]. Attempts to introduce a thiocarbonyl imidazole ester at position 3 by reaction with 1,1'-thiocarbonyldiimidazole failed, even when using a large excesses of the reagent or a different solvent (THF instead of DCE) or catalyst (DMAP instead of imidazole)[83]. For this reason, alcohol **75** was reacted with carbon disulfide and methyl iodide in the presence of sodium hydride, thus affording the corresponding xanthate. By treatment with tributyltin hydride in benzene using AIBN as radical initiator, this was deoxygenated and 3-deoxy derivative **76** obtained in 72% overall yield from **75**.[73] The 4,6-*O*-benzylidene acetal was then reductively opened[84] and, after benzylation, perbenzylated **77**was obtained. The 6-deoxy donor **81**[82] was prepared starting from 6-bromo glucoside **78**[85], which was converted to 6-deoxy derivative **79**[85] by reduction with tributyltin hydride/AIBN. Benzylation with KOH and benzyl bromide in toluene gave **80**[86] in 72% yield. This methyl glucoside was converted to the corresponding ethyl thioglucoside **81** in two steps: first **80** was subjected to acetolysis[87], and the resulting acetate treated with $BF_3 \cdot Et_2O$ and ethanethiol affording thioglucoside **81** in 83% yield.



Scheme 4.10 *i*) 5% aq. NaOH, BnBr, *n*-Bu_4NHSO_4, DCM; *ii*) NaH, CS_2, CH_3I, DMF; *iii*) Bu_3SnH, AIBN, benzene; *iv*) 1 M BH_3 in THF, Bu_2BOTf, 0 °C; *v*) NaH, BnBr, DMF; *vi*) KOH, BnBr, toluene, reflux; *vii*) Ac_2O-AcOH (1:2), conc H_2SO_4, 0 °C; *viii*) $BF_3{\cdot}Et_2O$, EtSH, DCM.

By treatment with bromine, thioglucosides **77** and **81** were transformed into the corresponding bromides, which were employed as donors in the glycosidations with acceptor **73** under *in situ* anomerisation conditions (Scheme 4.11). Only the α-linked products were obtained in 73% yield for 6’’-deoxy-trisaccharide **83** and in 46% for 3’’-deoxy-trisaccharide **82**. These trisaccharides should afford target structures **43** and **44** after complete deprotection by hydrogenolysis.



Scheme 4.11 *i*) Br_2, DCM; *ii*) Et_4NBr, DCM.

4.5 Attempts Towards the Synthesis of 2-Deoxy-Glc-α-(1→3)-Man-α-(1→2)-Man-α-OMe

The only trisaccharide left to assemble was the one where the deoxy function is positioned at C-2 of the terminal glucose. Synthesis of 2-deoxy-glycosides is not trivial. In fact, the absence of the 2-substituent limits the possibility of neighbouring group participation and makes stereoselective formation of the glycosidic linkage more difficult.[88] One common method for introduction of a 2-deoxy-glycoside is by electrophilic activation of glycals. The nature of the electrophile influences the stereochemical outcome of the glycosidation.[89] For example, NIS (I^+) mainly leads to formation of the *trans*-diaxial addition product and has been used to introduce α-linked 2-deoxy-glucose (or mannose), which is obtained after reduction of 2-iodo-α-mannoside (Figure 4.3).[90]

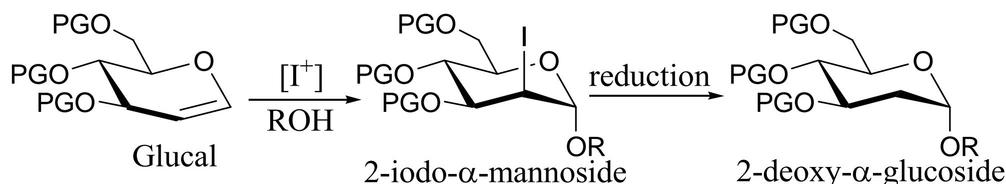
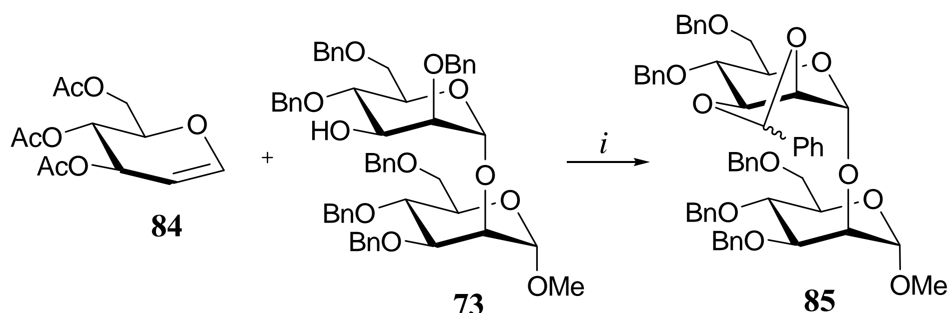


Figure 4.3 NIS-promoted glycosidation of glucal

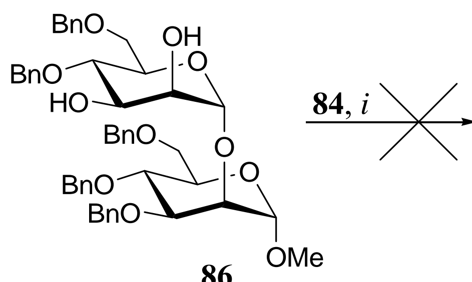
Hence, coupling of 3,4,6-tri-*O*-acetyl-D-glucal (**84**) and disaccharide acceptor **73** in the presence of NIS was tried (Scheme 4.12). However, the major product isolated from this reaction was not the expected trisaccharide but, surprisingly,

the 2,3-*O*-benzylidene derivative **85**. A similar result has been previously observed by Bols and Madsen.[91]



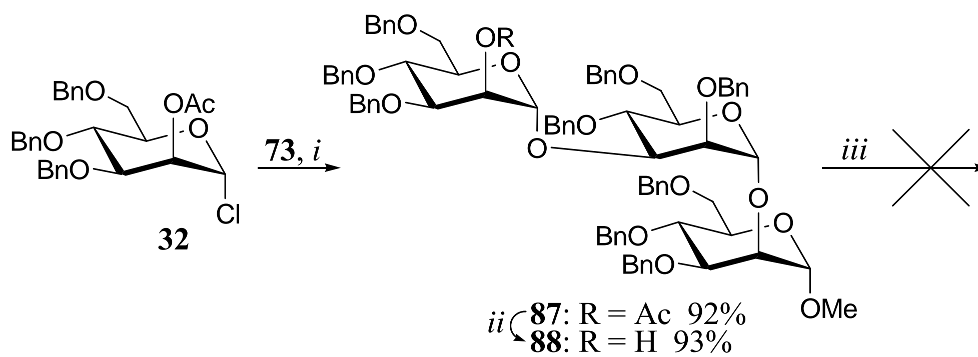
Scheme 4.12 *i*) NIS, CH_3CN.

Previous work in our laboratory had shown that NIS-promoted glycosidation of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside with **84** is highly regioselective for position 3 and the corresponding 3,4,6-tri-*O*-acetyl-2-iodo-α-D-mannopyranosyl-(1→3)-4,6-*O*-benzylidene-α-D-mannopyranoside is formed in good yield.[79] Therefore, disaccharide **86** was synthesised and tested in the same coupling with **84** (Scheme 4.13). Unfortunately, this reaction resulted in a complex mixture, which was not possible to resolve by chromatography.



Scheme 4.13 *i*) NIS, CH_3CN.

Eventually, a different approach was chosen, namely to deoxygenate the 2’’-position at the trisaccharide level.[92] For this purpose, mannosyl chloride **32** was coupled to **73** by AgOTf promotion, giving **87** in 92% yield (Scheme 4.14). Following deacetylation afforded the corresponding trisaccharide with the 2’’-position unprotected (**88**). However, reaction of **88** with 1,1’-thiocarbonyldiimidazole failed to give the 2’’-*O*-thiocarbonylimidazole derivative which was to be deoxygenated.



Scheme 4.14 *i*) AgOTf, DCM; *ii*) NaOMe, MeOH; *iii*) Im_2CS, DCE, reflux.

5 Synthesis of Spacer-linked Dimers of *N*-Acetyllactosamine Using Microwave Heating and Oxazoline Donors (Paper III)

5.1 Introduction

As mentioned earlier, galectins are an important class of animal lectins. There is strong evidence that galectins play crucial roles in many biological processes, for example in regulation of immunity and inflammation, progression of cancer, and in some developmental processes.[17] As their name suggests, these lectins are characterized by affinity for terminal β-galactose residues. Fourteen members of this family have been identified and named with numbers (galectin-1 to -14). Galectin-1 and -3 are those which have been most thoroughly studied with regard to their carbohydrate binding specificities.[93] According to these studies, such galectins show specificity for *N*-acetyllactosamine (LacNAc, Gal-β-(1→4)-GlcNAc) residues. Polylactosamine chains, which are commonly found on the surface of cells, are recognized by these galectins with high affinity.[94] Structurally, galectin-1 and -3 possess one single carbohydrate recognition domain (CRD) per polypeptide chain. However, they usually occur as dimers (galectin-1) or, in some cases, oligomers (galectin-3).[95]

With the purpose of studying cross-linking of these lectins, spacer-linked *N*-acetyllactosamine dimers **89-92** were needed and their synthesis was undertaken (Figure 5.1).

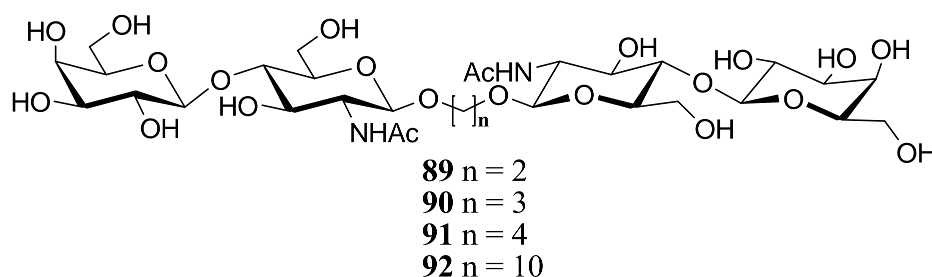
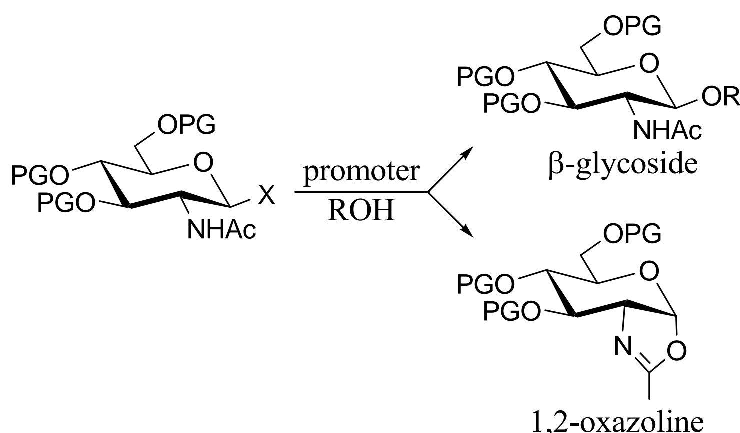


Figure 5.1 Target LacNAc-dimers

5.2 Synthetic Strategy

2-Acetamido sugars are very common in nature and are present in many biologically important glycans. For this reason, many glycosyl donors have been developed for their efficient introduction. Usually, these donors are equipped with a protecting group on the 2-amino function (*e.g.* phthalimido,

trifluoroacetyl), which is eventually replaced with the desired acetate after glycosidation. This is because glycosyl donors with a 2-acetamido function are usually poor donors. In fact, they generally show low solubility in solvents commonly employed in glycosidation reactions. Moreover, under slightly acidic conditions they give rise to the corresponding 1,2-oxazoline derivative, a byproduct which is normally obtained (to a variable extent) in glycosidation reactions with 2-acetamido donors (Scheme 5.1).[96]



Scheme 5.1 Typical glycosidation with a 2-acetamido donor. PG = protecting group, X = leaving group.

Attempts have been made to use the oxazoline itself as donor, but because of its low reactivity, elevated temperatures and prolonged reaction times are required, especially when the acceptor is a secondary alcohol.[96] On the other hand, the advantages of using 1,2-oxazoline as donor are its easy preparation and that the 2-acetamido function is directly obtained in the glycosidation product. Since relatively reactive acceptors were to be used in the present synthesis, 1,2-oxazoline derivative **93**[97] was designated as a viable donor (Figure 5.2).

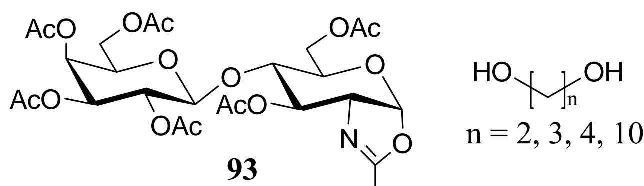
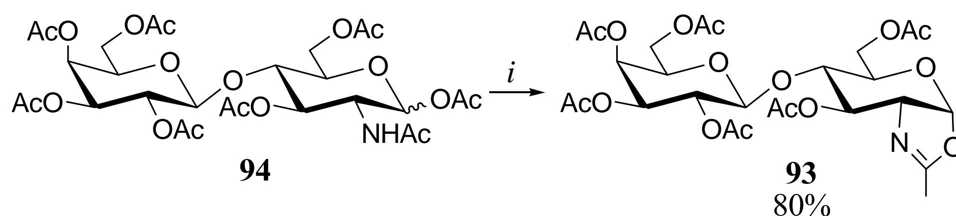


Figure 5.2 Donor and acceptors for synthesis of the LacNAc-dimers.

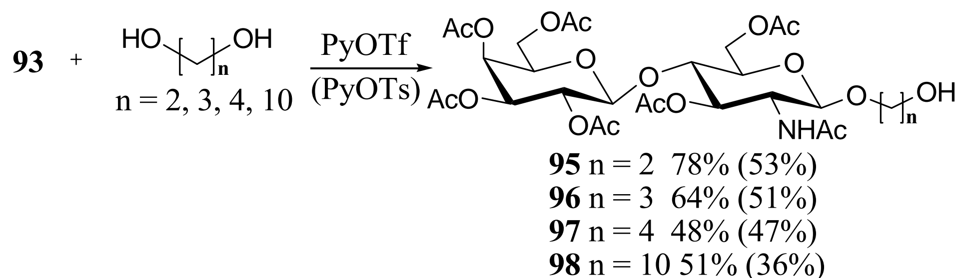
5.3 Synthesis of the LacNAc-Dimers

Conversion of peracetylated **94**[98] into **93** was performed effectively by treatment with trimethylsilyl triflate (TMSOTf) in 1,2-dichloroethane (Scheme 5.2).[99]



Scheme 5.2 *i*) TMSOTf, DCE, 50 °C.

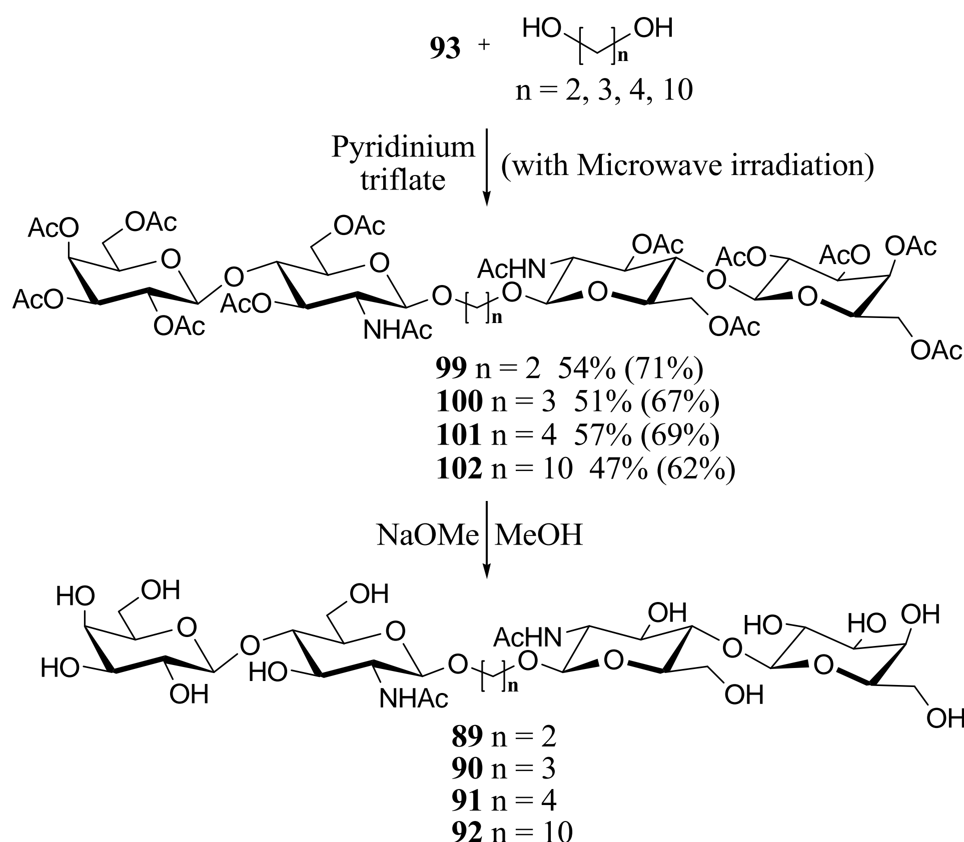
Currently, several promoters are available for effective activation of 1,2-oxazolines. Sulfonate salts are the most established ones, $CuCl_2$ being a more recent discovery.[100,101] In initial attempts, pyridinium *p*-toluenesulfonate (pyridinium tosylate) was used to promote coupling of **93** with the different diols (Scheme 5.3). With this promoter, only the glycoside monomers could be obtained in satisfactory yield (36-53%). Despite the use of a large excess of the oxazoline donor, only minor amounts of the dimeric products were observed. These were obtained by repeated glycosidation of monomers **95-98**, although only in low yield (15-20% overall). In a search for a better promoter, we reasoned that pyridinium triflate would be an attractive candidate, since many Lewis acids employed in glycosidation reactions contain trifluoromethanesulfonate (triflate) as counter anion. Indeed, pyridinium triflate-mediated glycosidation employing 1.1 equivalents of **93** with the diol acceptors furnished the corresponding monomer glycosides **95-98** in higher yields.



Scheme 5.3 Glycosidation promoted by pyridinium triflate or by pyridinium tosylate (yields in brackets). Reaction conditions: 1.1 equivalents of donor, 1 equivalent of diol, 1.1 equivalents of promoter, DCE, reflux.

Even more pleasingly, when an excess of oxazoline donor **93** was employed (2.2 equivalents), dimers **99-102** were formed in good yields (Scheme 5.4). However, relatively long reaction times were still required (around 3 hours). Therefore, it was decided to try this reaction with microwave heating. This technique is becoming increasingly popular among organic chemists to speed up chemical reactions. Numerous examples of organic reactions profiting from

microwave irradiation have been reported.[102] However, application of this technique in carbohydrate chemistry has been rather limited and examples of microwave-assisted glycosidation reactions are particularly rare.[103] Nevertheless, when pyridinium triflate-promoted glycosidation of oxazoline **93** was subjected to microwave irradiation, considerably higher yields of the linker-spaced dimeric products were obtained. Moreover, the reaction times were reduced to less than 20 minutes. Treatment with sodium methoxide in MeOH afforded the unprotected LacNAc-dimers **89-92** in nearly quantitative yields. These were then used in galectin binding studies.



Scheme 5.4 Pyridinum triflate-promoted glycosidation of oxazoline **93** with (yields in brackets) and without microwave irradiation. Reaction conditions: 2.2 equivalents of donor, 1 equivalent of diol, 2.2 equivalents of PyOTf, DCE, reflux (80 °C in a sealed vessel when microwave heating was applied).

5.4 Concluding Remarks

In conclusion, pyridinium triflate was discovered as a novel efficient promoter for 1,2-oxazolines. Indeed, glycosidation of LacNAc-oxazoline with diols of variable chain length using pyridinium triflate gave better results than the known pyridinium tosylate-promoted reaction. In addition, combination of pyridinium

triflate with microwave irradiation resulted in very good conversion of LacNAc-oxazoline into the desired spacer-linked dimers. This is one of the first examples of microwave-assisted glycosidation.

Interaction of the synthesised substrates with galectin-1 and galectin-3 was investigated by ITC. Neither lectin showed any significant enhancement of affinity for the dimeric substrates as compared to monomeric LacNAc.[104]

Appendix A

Experimental Section

The numbering refers to the compounds discussed in Chapter 4.

General methods

DCM was distilled from calcium hydride. Organic solutions were concentrated under reduced pressure at <45 °C (bath temperature). NMR spectra were recorded at 400 MHz for ^{1}H and at 100 MHz for ^{13}C. Chemical shifts are reported relative to $CHCl_3$ [δ_H 7.26, δ_C (central of triplet) 77.0] or to acetone as internal standard (D_2O). TLC was performed on silica gel 60 F254 with detection by charring with 8% sulfuric acid. Silica gel (0.040-0.063 mm) was used for column chromatography.

Methyl 6-*O*-benzyl-4-deoxy-α-D-*lyxo*-hexopyranoside (**47**). Methyl 6-*O*-benzoyl-2,3-*O*-isopropylidene-4-deoxy-α-D-*lyxo*-hexopyranoside **46** (300 mg, 0.93 mmol) was dissolved in MeOH (10 ml) and a catalytic amount of 1M NaOMe in MeOH was added. After being stirred overnight, the reaction was neutralized with Dowex 50 (H^+) ion exchange resin. The resin was filtered away, the solvent removed at reduced pressure and the residue dried under vacuum. The crude residue was dissolved in DMF (4 ml) and sodium hydride (74 mg of 60% dispersion in mineral oil, 1.86 mmol) added at 0 °C. After 10 min, benzyl bromide (167 μl, 1.39 mmol) was added and the ice-bath removed. Once the reaction was completed (~ 2 hours), ice was poured into the reaction. The mixture was then extracted with EtOAc, which in turn was washed with water, dried with Na_2SO_4 and concentrated. The crude product was dissolved in 80% aqueous TFA (10 ml). The solution was stirred at rt for 2 hours and subsequently coevaporated with toluene several times. The resulting residue was purified by silica gel chromatography (DCM-MeOH 30:1) to give **47** (230 mg) in 92% yield. ^{1}H NMR ($CDCl_3$): δ 1.63 (q, 1H, H-4b, J = 12 Hz), 1.75 (m, 1H, H-4a), 2.11 (d, 1H, O*H*, J = 8.5 Hz), 2.15 (d, 1H, O*H*, J = 7 Hz), 3.38 (s, 3H, OCH_3), 3.51 (dd, 1H, H-6b, $J_{6b,6a}$ = 10 Hz, $J_{6b,5}$ =4 Hz), 3.55 (dd, 1H, H-6a, $J_{6a,6b}$

= 10 Hz, $J_{6a,5}$ = 6Hz), 3.73 (m, 1H, H-2), 3.88-4.01 (m, 2H, H-3, H-5), 4.58 (2d, 2H, CH_2Ph, J = 12 Hz), 4.78 (d, 1H, H-1, $J_{1,2}$ = 1.1 Hz), 7.25-7.34 (m, 5H, H-Aromatic).

Methyl 3,6-di-*O*-benzyl-4-deoxy-α-D-*lyxo*-hexopyranoside (**48**). Diol **47** (225 mg, 0.84 mmol) was dissolved in MeOH (5 ml) with dibutyltin oxide (245 mg, 0.98 mmol) and heated to 50 °C for three hours. The solvent was removed under reduced pressure and the residue dissolved in DMF (5 ml). Benzyl bromide (235 μl, 1.96 mmol) was added and the solution heated to 110 °C for 12 hours. Saturated aq. $NaHCO_3$ was poured into the mixture, which was extracted with EtOAc. The organic phase was washed with 1N HCl and water and dried over $MgSO_4$. After concentration, silica gel chromatography (toluene-EtOAc 6:1) afforded **48** (80 mg, 27%). ^{1}H NMR ($CDCl_3$): δ 1.77 (m, 2H, H-4b, H-4a), 2.40 (bs, 1H, O*H*), 3.37 (s, 3H, OCH_3), 3.50 (dd, 1H, H-6b, $J_{6b,6a}$ = 10 Hz, $J_{6b,5}$ = 4 Hz), 3.60 (dd, 1H, H-6a, $J_{6a,6b}$ = 10 Hz, $J_{6a,5}$ = 6Hz), 3.83 (m, 1H, H-5), 3.91 (m, 2H, H-2, H-3), 4.44-4.69 (m, 4H, CH_2Ph), 4.81 (d, 1H, H-1, $J_{1,2}$ = 1.5 Hz), 7.28-7.35 (m, 10H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 28.5 (C-4), 54.9 (OCH_3), 66.7, 67.4, 70.1, 72.9, 73.0, 73.5 (C-2, C-3, C-5, C-6, CH_2Ph), 101.1 (C-1), 127-129, 138.1, 138.3 (C-Aromatic).

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-deoxy-α-D-*lyxo*-hexopyranoside (**49**). Acceptor **48** (60 mg, 0.17 mmol) and ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside **35** (145 mg, 0.14 mmol) were dissolved in DCM (2 ml) and the solution stirred for 10 min in the presence of powdered 4Å molecular sieves under N_2. NIS (44 mg, 0.2 mmol) was added and thereafter a catalytic amount of AgOTf. After 45 min the reaction was neutralized with Et_3N, filtered over a Celite pad and concentrated. Silica gel chromatography of the residue (toluene-EtOAc 15:1) gave **49** (160 mg, 87%). ^{1}H NMR ($CDCl_3$): δ 1.81 (m, 2H, H-4b, H-4a), 3.32 (s, 3H, OCH_3), 3.52 (m, 3H), 3.71 (m, 4H), 3.99 (m, 8H), 4.21 (m, 1H), 4.35 (m, 2H), 4.44-4.68 (m, 13H), 4.78-4.97 (m, 4H), 5.15 (2bs, 2H), 5.34 (d, 1H, J = 2 Hz), 7.05-7.40 (m, 45H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 29.3 (C-4), 54.8 (OCH_3), 67.9,

68.3, 69.7, 70.3, 70.9, 72.2, 72.8, 73.3, 73.5, 73.9, 74.6, 74.8, 75.6, 77.1, 77.7, 79.7, 79.8, 81.9 (C-2-3, C-5-6, C-2'-6', C-2''-6'', *C*H_2Ph), 98.4, 99.1, 101.1 (C-1, C-1', C-1''), 127-129, 138.0, 138.4, 138.5, 138.6, 138.7, 138.9 (C-Aromatic).

Methyl α-D-glucopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-4-deoxy-α-D-*lyxo*-hexopyranoside (**39**). Compound **49** (70 mg, 0.053 mmol) was dissolved in MeOH-EtOAc 5:1 (v/v, 10 ml). A catalytic amount of Pd/C was then added and the reaction stirred under 1 atm H_2. After two days, the catalyst was filtered away and the solution concentrated. The residue thus obtained was dissolved in distilled water, applied on a 600 mg C18 MAXI-CLEAN cartridge (purchased from Alltech) and eluted with water, to give pure **39** (23 mg, 86%). ^{1}H NMR (D_2O): δ 1.67 (m, 2H, H-4b, H-4a), 3.36 (s, 3H, OC*H_3*), 3.53 (dd, 1H, J = 10 Hz, J = 4 Hz), 3.59-3.89 (m, 13H), 3.95 (m, 1H), 4.07 (m, 1H), 4.20 (bs, 1H), 4.98 (d, 1H, J = 1.4 Hz), 5.00 (d, 1H, J = 1.5Hz), 5.23 (d, 1H, J = 4 Hz). ^{13}C NMR (D_2O): δ 29.6 (C-4), 54.8 (O*C*H_3), 60.8, 61.1, 64.2, 65.2, 66.4, 69.3, 69.8, 70.0, 71.9, 72.5, 73.0, 73.5, 76.4, 78.4 (C-2-3, C-5-6, C-2'-6', C-2''-6''), 100.2, 100.5, 102.1 (C-1, C-1', C-1'').

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside (**45**). Donor **35** (145 mg, 0.14 mmol) and methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside **44** (50 mg, 0.19 mmol) were dissolved in DCM (1 ml) and the solution stirred for 15 min in the presence of powdered 4Å molecular sieves under N_2. NIS (48 mg, 0.21 mmol) was then added and thereafter a catalytic amount of AgOTf. The reaction was neutralized with Et_3N after 30 min and filtered through a Celite pad and this solution was washed with $Na_2S_2O_3$, water and concentrated. The crude material was chromatographed on a silica gel column (toluene-EtOAc 30:1→15:1) affording trisaccharide **45** (150 mg, 86%). ^{1}H NMR ($CDCl_3$): δ 2.05 (m, 2H, H-3b, H-3a), 3.32 (s, 3H, OC*H_3*), 3.56 (m, 3H), 3.64-3.96 (m, 9H), 4.12 (m, 3H), 4.24 (m, 1H), 4.42-4.68 (m, 11H), 4.78-4.97 (m, 3H), 5.04 (d, 1H, J = 1.8 Hz), 5.12 (m, 2H), 5.56 (s, 1H, C*H*Ph), 7.12-7.52 (m, 40H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 29.8 (C-3), 54.8 (O*C*H_3), 68.8, 69.5, 69.6, 71.1, 71.2, 72.4, 73.1, 73.4, 73.6, 74.1, 74.6, 74.8,

74.9, 75.7, 77.9, 78.2, 79.9, 81.9 (C-2, C-4-6, C-2'-6', C-2''-6'', *C*H_2Ph), 99-100 (C-1, C-1', C-1''), 102.1 (*C*HPh), 126-129, 137.7, 137.8, 138.3, 138.4, 138.4, 138.6, 138.8, 138.8 (C-Aromatic).

Methyl α-D-glucopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-3-deoxy-α-D-*arabino*-hexopyranoside (**38**). **45** (140 mg, 0.11 mmol) was dissolved in MeOH (5 ml) and a catalytic amount of Pd/C was added. 20 µl 1N HCl were also added and the reaction stirred under H_2 (1 atm). After 3 hours the catalyst was removed by filtration and the solvent evaporated. Purification on a 600 mg C18 MAXI-CLEAN cartridge (purchased from Alltech) afforded **38** (50 mg, 87%). ^{1}H NMR (D_2O): δ 1.74 (m, 1H, H-3b), 2.21 (dt, 1H, H-3a, J = 13 Hz, J = 4 Hz), 3.39 (t, 1H, J = 9 Hz), 3.42 (s, 3H, OC*H_3*), 3.55 (dd, 1H, J = 10 Hz, J = 4 Hz), 3.63 (m, 1H), 3.69-3.98 (m, 13H), 4.11 (bs, 1H), 4.78 (bs, 1H), 4.99 (d, 1H, J = 1.4 Hz), 5.24 (d, 1H, J = 4 Hz). ^{13}C NMR (D_2O): δ 30.1 (C-3), 54.7 (O*C*H_3), 60.9, 61.1, 61.3, 61.8, 66.3, 69.9, 70.4, 71.9, 72.5, 73.0, 73.1, 73.4, 73.7, 78.4 (C-2, C-4-6, C-2'-6', C-2''-6''), 98.4, 98.5, 100.5 (C-1, C-1', C-1'').

Ethyl 4-deoxy-6-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-*lyxo*-hexopyranoside (**58**). Ethyl 6-*O*-*t*-butyldiphenylsilyl-2,3-*O*-isopropylidene-1-thio-α-D-mannopyranoside **57** (270 mg, 0.54 mmol) was dissolved in 1,2-dichloroethane (10 ml) together with 1,1'-thiocarbonyldiimidazole (191 mg, 1.07 mmol) and the reaction was refluxed for 10 hours. Thereafter the solvent was removed under reduced pressure and the brown residue was chromatographed on a silica gel column (toluene-EtOAc 6:1). The product was dissolved in benzene (5 ml) and Bu_3SnH (2.60 mmol, 700 µl) was added. The mixture was degassed and put under N_2 atmosphere. AIBN (~20 mg) was then added, the solution refluxed for 10 min and concentrated. The residue was partitioned in pentane-acetonitrile and the acetonitrile layer washed twice with pentane. After concentration of the acetonitrile layer, the crude product was dissolved in of AcOH-H_2O-TFA 10:2:1 (v/v/v, 13 ml) and the solution kept at 0 °C for 2 hours. The reaction mixture was then coevaporated with toluene several times and the resulting residue chromatographed on a silica gel column (toluene-EtOAc 5:1) affording **58** (140 mg, 58%). ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 9H, C(C*H_3*)$_3$), 1.26 (t, 3H, SCH_2C*H_3*), 1.59 (q, 1H, H-4b, J = 12 Hz), 1.80 (m, 1H,

H-4a), 2.06 (d, 1H, O*H*, J = 8.5 Hz), 2.24 (d, 1H, O*H*, J = 6.5 Hz), 2.52-2.67 (m, 2H, SCH_2CH_3), 3.63 (dd, 1H, H-6b, $J_{6b,6a}$ = 10.6 Hz, $J_{6b,5}$ = 5 Hz), 3.73 (dd, 1H, H-6a, $J_{6a,6b}$ = 10.6 Hz, $J_{6a,5}$ = 6 Hz), 3.83 (m, 1H), 3.96 (m, 1H), 4.23 (m, 1H), 5.33 (d, 1H, H-1, $J_{1,2}$ = 1.1 Hz), 7.34-7.45 (m, 6H, H-Aromatic), 7.65-7.67 (m, 4H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 14.8 (SCH_2*C*H_3), 19.3 (*C*(CH_3)$_3$), 24.7 (S*C*H_2CH_3), 26.9 (C(*C*H_3)$_3$), 31.5 (C-4), 66.3, 66.4, 69.1, 70.7 (C-2-3, C-5-6), 84.1 (C-1), 127.8, 129.8, 133.4, 135.7 (C-Aromatic).

Ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-deoxy-6-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-*lyxo*-hexopyranoside (**65**). Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside **3** (264 mg, 0.45 mmol) was dissolved in DCM (1 ml) and bromine (0.68 mmol, 35 μl) added at 0 °C. The reaction was stirred under N_2 for 1 hour and concentrated. The crude glucosyl bromide was added to a solution of acceptor **58** (90 mg, 0.20 mmol) and Et_4NBr (104 mg, 0.49 mmol) in DCM (1 ml) and DMF (100 μl). The reaction mixture was stirred at rt in the presence of 4Å powdered molecular sieves for 36 hours. This was then directly applied on a silica gel column and eluted with toluene-EtOAc 15:1→10:1, thus yielding product **63** (105 mg, 54 %). **63** was thereafter dissolved in pyridine (5 ml) and BzCl (18 μl, 0.15 mmol) added. The reaction was stirred overnight, concentrated and coevaporated with toluene several times. The residue was purified by silica gel chromatography (toluene-EtOAc 30:1), affording disaccharide **65** (110 mg, 94%). ^{1}H NMR ($CDCl_3$): δ 1.07 (s, 9H, C(C*H_3*)$_3$), 1.29 (t, 3H, SCH_2C*H_3*), 1.92 (d, 1H, H-4b, J = 12 Hz), 2.08 (q, 1H, H-4a, J = 12 Hz), 2.60-2.69 (m, 2H, SC*H_2*CH_3), 3.45-3.87 (m, 8H), 4.08 (m, 1H), 4.26 (m, 1H), 4.38 (t, 2H, C*H_2*Ph, J = 12 Hz), 4.57 (t, 2H, C*H_2*Ph, J = 12 Hz), 4.65-4.82 (m, 4H, C*H_2*Ph), 4.96 (d, 1H, H-1’, $J_{1',2'}$ = 4 Hz), 5.35 (bs, 1H, H-2), 5.52 (s, 1H, H-1), 7.03 (m, 2H, H-Aromatic), 7.22-7.45 (m, 23H, H-Aromatic), 7.54 (t, 2H, H-Aromatic), 7.67-7.71 (m, 4H, H-Aromatic), 8.06 (d, 3H, H-Aromatic), 8.18 (d, 1H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 15.0 (SCH_2*C*H_3), 19.4 (*C*(CH_3)$_3$), 25.4 (S*C*H_2CH_3), 26.9 (C(*C*H_3)$_3$), 29.1 (C-4), 66.6, 68.2, 69.6, 70.9, 72.3, 73.0, 73.5, 74.6, 75.6, 80.2, 81.6 (C-2-3, C-5-6, C-2’-6’, *C*H_2Ph), 82.4 (C-1), 96.4 (C-1’), 127.5-128.9, 129.7, 129.9, 130.3, 130.7, 133.1, 133.4, 133.6, 134.6, 135.7, 135.8, 138.0, 138.5, 138.9 (C-Aromatic), 165.7 (*C*=O).

Ethyl 4-*O*-benzyl-1-thio-α-D-rhamnopyranoside (**62**). Ethyl 4-*O*-benzyl-2,3-*O*-isopropylidene-1-thio-α-D-rhamnopyranoside **61** (700 mg, 2.07 mmol) was dissolved in 90% aqueous TFA (10 ml) and stirred for 10 min. The solvent was coevaporated with toluene and the resulting solid recrystallized from petroleum ether-EtOAc. Pure **62** (400 mg, 65%), was thus obtained as white needles. ^{1}H NMR ($CDCl_3$): δ 1.27 (t, 3H, SCH_2CH_3), 1.34 (d, 3H, H-6, $J_{6,5}$ = 6.2 Hz), 2.40 (bs, 1H, O*H*), 2.52-2.66 (m, 3H, SCH_2CH_3, O*H*), 3.37 (t, 1H, H-4, $J_{4,3}$ = $J_{4,5}$ = 9 Hz), 3.86 (d, 1H, H-3, $J_{3,4}$ = 9 Hz), 4.01 (bs, 1H, H-2), 4.07-4.11 (m, 1H, H-5), 4.73 (2d, 2H, CH_2Ph, J = 12 Hz), 5.22 (d, 1H, H-1, $J_{1,2}$ = 1.1 Hz), 7.30-7.36 (m, 5H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 15.0 (SCH_2CH_3), 18.0 (C-6), 25.1 (SCH_2CH_3), 67.8, 72.0, 72.7, 75.0, 82.0 (C-2-5, CH_2Ph), 83.7 (C-1), 128-129, 138.3 (C-Aromatic).

Ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-D-rhamnopyranoside (**66**). **3** (396 mg, 0.68 mmol) was dissolved in DCM and bromine (1.03 mmol, 53 μl) added at 0 °C. The reaction was stirred under N_2 for 1 hour and concentrated. The crude glucosyl bromide was added to a solution of acceptor **62** (100 mg, 0.33 mmol) and Et_4NBr (156 mg, 0.73 mmol) in DCM (1 ml) and DMF (100 μl). After 40 hours of stirring at rt in the presence of powdered 4Å molecular sieves, the reaction mixture was directly applied on a silica gel column and eluted (toluene-EtOAc 10:1). Disaccharide **64** (130 mg, 47%) thus obtained was thereafter benzoylated by addition of BzCl (28 μl, 0.23 mmol) to a solution in pyridine (3 ml) and stirred at rt (1 day). The solvent was then removed and the residue coevaporated with toluene before being chromatographed on a silica gel column (toluene-EtOAc 40:1), affording **66** (125 mg, 85%). ^{1}H NMR ($CDCl_3$): δ 1.26-1.35 (m, 6H, SCH_2CH_3, H-6), 2.65 (m, 2H, SCH_2CH_3), 3.28 (bs, 2H), 3.53 (dd, 1H, J = 10 Hz, J = 4 Hz), 3.63-3.74 (m, 3H), 3.94 (t, 1H, J = 10 Hz), 4.10 (dd, 1H, J = 9.5 Hz, J = 3.5 Hz), 4.16 (m, 1H), 4.27-4.37 (2d, 2H, CH_2Ph, J = 12 Hz), 4.51-4.59 (2d, 2H, CH_2Ph, J = 12 Hz), 4.66-4.74 (m, 4H, CH_2Ph), 4.87 (d, 1H, CH_2Ph, J = 12 Hz), 4.99 (d, 1H, H-1', $J_{1',2'}$ = 3.5 Hz), 5.23 (d, 1H, CH_2Ph, J = 12 Hz), 5.38 (bs, 1H, H-1), 5.48 (bs, 1H, H-2), 6.95-8.05 (m, 30H, H-Aromatic). ^{13}C NMR

($CDCl_3$): δ 15.2 (SCH_2CH_3), 18.1 (C-6), 26.0 (SCH_2CH_3), 67.8, 68.6, 71.4, 73.2, 73.5, 74.6, 75.0, 75.4, 75.6, 77.2, 79.6, 79.9, 80.8, 81.7, 81.8 (C-1-5, C-2'-6', *C*H_2Ph), 99.6 (C-1'), 127-130, 133.3, 137.9, 138.3, 138.6, 138.7, 138.8 (C-Aromatic), 166.0 (Ph*C*=O).

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-deoxy-6-*O*-*tert*-butyldiphenylsilyl-α-D-*lyxo*-hexopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (67). Methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside **31** (100 mg, 0.21 mmol) and donor **65** (110 mg, 0.10 mmol) were dissolved in DCM (4 ml) and the solution was stirred for 30 min under N_2 in the presence of 4Å powdered molecular sieves. NIS (35 mg, 0.15 mmol) was then added and thereafter a catalytic amount of AgOTf. The reaction was neutralized by dropwise addition of Et_3N after 2 hours and the mixture directly applied on a silica gel column, eluted with toluene-EtOAc 20:1. Trisaccharide **67** (101 mg, 67%) was thus isolated. ^{1}H NMR ($CDCl_3$): δ 1.08 (s, 9H, $C(CH_3)_3$), 1.78 (d, 1H, H-4b', J = 12 Hz), 2.05 (q, 1H, H-4a', J = 12 Hz), 3.29 (s, 3H, OCH_3), 3.51 (m, 3H), 3.66-3.94 (m, 10H), 4.08 (m, 2H), 4.26 (m, 2H), 4.40 (d, 1H, CH_2Ph, J = 11 Hz), 4.51-4.80 (m, 12H), 4.89 (d, 1H, CH_2Ph, J = 11 Hz), 5.04 (d, 1H, H-1'', $J_{1'',2''}$ = 4 Hz), 5.38 (d, 1H, H-1', $J_{1',2'}$ = 1.1 Hz), 5.50 (bs, 1H, H-2'), 7.05 (m, 2H, H-Aromatic), 7.14-7.43 (m, 42H, H-Aromatic), 7.54 (t, 1H, H-Aromatic), 7.71 (m, 4H, H-Aromatic), 8.08 (d, 1H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 19.4 ($C(CH_3)_3$), 26.9 ($C(CH_3)_3$), 28.7 (C-4'), 54.7 (OCH_3), 66.8, 68.1, 69.6, 69.7, 70.2, 70.9, 71.4, 71.6, 71.9, 72.7, 73.3, 73.4, 73.8, 74.6, 74.9, 75.2, 75.5, 79.9, 80.0, 81.6 (C-2-6, C-2'-3', C-5'-6', C-2''-6'', *C*H_2Ph), 96.1, 99.6, 100.0 (C-1, C-1', C-1''), 127.4-128.5, 129.7, 129.9, 130.4, 132.9, 133.5, 133.6, 135.7, 135.8, 138.1, 138.5, 138.5, 138.7, 139.0 (C-Aromatic), 165.8 (Ph*C*=O).

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (68). **31** (100 mg, 0.21 mmol) and donor **66** (110 mg, 0.12 mmol) were dissolved in DCM (3 ml) and the solution was stirred for 15 min under N_2 in the presence of 4Å powdered molecular sieves. NIS (40 mg, 0.18 mmol) was then added and thereafter a catalytic amount of AgOTf. The reaction was neutralized by dropwise addition

of Et_3N after 1 hour and the mixture directly applied on a silica gel column, eluted with toluene-EtOAc 15:1. Trisaccharide **68** (100 mg, 63%) was thus isolated. ^{1}H NMR ($CDCl_3$): δ 1.33 (d, 3H, H-6c'-a', J = 6 Hz), 3.35 (s, 3H, OCH_3), 3.51 (dd, 1H, J = 10 Hz, J = 3 Hz), 3.63-3.86 (m, 6H), 3.90-3.99 (m, 3H), 4.04 (bs, 1H), 4.23 (d, 1H, CH_2Ph, J = 12 Hz), 4.28 (dd, 1H, J = 9 Hz, J = 3 Hz), 4.38 (d, 1H, CH_2Ph, J = 12 Hz), 4.49-4.79 (m, 12H), 4.87 (m, 2H), 5.11 (d, 1H, H-1'', $J_{1'',2''}$ = 4 Hz), 5.22 (d, 1H, CH_2Ph, J = 12 Hz), 5.30 (d, 1H, H-1', $J_{1',2'}$ = 1.8 Hz), 5.61 (bs, 1H, H-2'), 6.97 (m, 2H, H-Aromatic), 7.15-7.48 (m, 40H, H-Aromatic), 7.60 (t, 1H, H-Aromatic), 8.07 (d, 2H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 18.4 (C-6'), 54.8 (O*C*H_3), 67.9, 68.3, 69.5, 71.4, 71.7, 72.1, 72.9, 72.9, 73.3, 73.4, 74.3, 74.6, 75.0, 75.2, 75.6, 77.3, 77.6, 79.6, 79.8, 79.9, 81.8 (C-2-6, C-2'-5', C-2''-6'', *C*H_2Ph), 98.4, 99.3, 100.0 (C-1, C-1', C-1''), 127.4-128.6, 129.9, 130.1, 133.2, 138.0, 138.3, 138.5, 138.7, 138.8, 138.8 (C-Aromatic), 165.7 (Ph*C*=O).

Methyl 3-*O*-benzoyl-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**72**). Ethyl 3-*O*-benzoyl-2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside **71** (255 mg, 0.43 mmol) and acceptor **31** (180 mg, 0.39 mmol) were dissolved in DCM (2 ml) and stirred for 10 min in the presence of 4Å powdered molecular sieves under N_2. NIS (144 mg, 0.64 mmol) and a catalytic amount of AgOTf were then added and the reaction continued for 30 min, whereafter it was neutralized by dropwise addition of Et_3N. The mixture was directly applied on a silica gel column, eluted with toluene-EtOAc 15:1. Disaccharide **72** (315 mg, 81%) was thus obtained. ^{1}H NMR ($CDCl_3$): δ 3.28 (s, 3H, OCH_3), 3.73-3.94 (m, 7H), 4.09-4.14 (m, 4H), 4.35 (d, 1H, CH_2Ph, J = 12 Hz), 4.47-4.72 (m, 10H), 4.83 (bs, 1H, H-1), 4.86 (d, 1H, CH_2Ph, J = 12 Hz), 5.24 (bs, 1H, H-1'), 5.56 (dd, 1H, H-3', $J_{3',4'}$ = 8.5 Hz, $J_{3',2'}$ = 3.5 Hz), 7.05-7.40 (m, 30H, H-Aromatic), 7.45 (t, 2H, H-Aromatic), 7.58 (t, 1H, H-Aromatic), 8.05 (d, 2H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 54.7 (O*C*H_3), 69.3, 69.6, 71.8, 72.0, 72.6, 72.7, 73.4, 73.5, 73.9, 74.2, 74.6, 75.0, 75.3, 76.1, 80.3 (C-2-6, C-2'-6', *C*H_2Ph), 99.7 (C-1, C-1'), 127.5-128.8, 129.9, 130.1, 132.9, 138.0, 138.1, 138.3, 138.4, 138.5, 138.7 (C-Aromatic), 165.6 (Ph*C*=O).

Methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (73). **72** (310 mg, 0.31 mmol) was dissolved in MeOH (10 ml) and a catalytic amount of 1M NaOMe in MeOH was added. After 3 days the reaction was neutralized with Dowex 50 (H^+) ion exchange resin and the solvent removed under reduced pressure. Silica gel column chromatography (toluene-EtOAc 6:1) afforded **73** (250 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 2.29 (d, 1H, O*H*, J = 9 Hz), 3.26 (s, 3H, OC*H₃*), 3.65-3.85 (m, 8H), 3.89-3.94 (m, 2H), 4.03 (dt, 1H, J = 9 Hz, J = 3.5 Hz), 4.09 (bs, 1H), 4.27 (d, 1H, CH_2Ph, J = 12 Hz), 4.47-4.57 (m, 5H, CH_2Ph), 4.62-4.73 (m, 4H, CH_2Ph), 4.78 (d, 1H, J = 1.8 Hz), 4.86 (d, 2H, CH_2Ph, J = 12 Hz), 5.24 (bs, 1H), 7.19-7.38 (m, 30H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 54.7 (O*C*H_3), 69.3, 69.5, 71.4, 71.6, 71.8, 72.4, 72.7, 73.4, 74.7, 75.1, 75.2, 78.2, 80.2 (C-2-6, C-2'-6', *C*H_2Ph), 98.7, 99.9 (C-1, C-1'), 127.5-128.5, 137.9, 138.4, 138.4, 138.5, 138.6, 138.6 (C-Aromatic).

Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-deoxy-1-thio-β-D-ribo-hexopyranoside (76). Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside **75** (320 mg, 0.80 mmol) was dissolved in DMF (5 ml) and sodium hydride (65 mg of 60% dispersion in mineral oil, 1.62 mmol) added. The reaction was stirred at rt for 10 min and carbon disulfide (100 μl, 1.68 mmol) added. After additional 10 min, methyl iodide (100 μl, 1.61 mmol) was added and the reaction continued for 30 min. Ice was poured into the reaction mixture, which was extracted with EtOAc. The organic phase was washed with water twice and concentrated. The residue was chromatographed on a silica gel column, eluted with toluene-EtOAc 50:1. The resulting product was dissolved in benzene (10 ml) and Bu_3SnH (400 μl, 1.50 mmol) was added. The mixture was degassed and put under N_2 atmosphere. AIBN (~20 mg) was then added, the solution refluxed for 10 min and concentrated. The residue was partitioned in pentane-acetonitrile and the acetonitrile layer washed twice with pentane and concentrated. Purification by silica gel chromatography (toluene-EtOAc 30:1) afforded **76** (220 mg) in 72% yield. ^{1}H NMR ($CDCl_3$): δ 1.31 (t, 3H, SCH_2CH_3), 1.68-1.79 (m, 1H, H-3b), 2.53-2.58 (m, 1H, H-3b), 2.69-2.80 (m, 2H, SCH_2CH_3), 3.38-3.56 (m, 3H, H-2, H-4, H-5), 3.72 (t, 1H, H-6b, $J_{6b,6a}$ = $J_{6b,5}$ = 10 Hz), 4.31 (dd, 1H, H-6a, $J_{6a,6b}$ =

10 Hz, $J_{6a,5}$ = 4.5 Hz), 4.54 (d, 1H, H-1, $J_{1,2}$ = 9 Hz), 4.64-4.72 (2d, 2H, CH_2Ph, J = 12 Hz), 5.48 (s, 1H, C*H*Ph), 7.22-7.47 (m, 10H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 15.1 (SCH_2*C*H_3), 25.0 (S*C*H_2CH_3), 36.1 (C-3), 69.2, 72.3, 73.4, 75.4, 76.0 (C-2, C-4-6, *C*H_2Ph), 87.0 (C-1), 101.7 (*C*HPh), 126.2, 128.0, 128.1, 128.4, 128.5, 129.2, 137.3, 137.8 (C-Aromatic).

Methyl 2,4,6-tri-*O*-benzyl-3-deoxy-α-D-ribo-hexopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**82**). Ethyl 2,4,6-tri-O-benzyl-3-deoxy-1-thio-α-D-ribo-hexopyranoside **77** (120 mg, 0.25 mmol) was dissolved in DCM and bromine (0.38 mmol, 20 µl) added at 0 °C. The reaction was stirred under N_2 for 1 hour and concentrated. The crude glucosyl bromide was added to a solution of acceptor **73** (120 mg, 0.13 mmol) and Et_4NBr (60 mg, 0.38 mmol) in DCM (1 ml) and DMF (100 µl). After 2 weeks of stirring at rt in the presence of powdered 4Å molecular sieves, the reaction mixture was filtered through a Celite pad and concentrated. Silica gel column chromatography (toluene-EtOAc 20:1) afforded **82** (80 mg, 46%). ^{1}H NMR ($CDCl_3$): δ 1.96 (m, 1H, H-3b''), 2.34 (m, 1H, H-3a''). 3.25 (s, 3H, OC*H_3*), 3.41-3.64 (m, 3H), 3.71-3.81 (m, 6H), 3.89-4.10 (m, 6H), 4.26-4.64 (m, 19H), 4.84 (m, 2H), 5.21 (bs, 2H), 7.15-7.36 (m, 45H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 31.0 (C-3''), 54.7 (O*C*H_3), 68.4, 69.4, 69.7, 70.5, 70.8, 71.3, 71.7, 71.8, 71.9, 72.3, 73.3, 73.3, 73.5, 73.7, 74.4, 75.1, 75.2, 75.3, 77.8, 80.1 (C-2-6, C-2'-6', C-2'', C-4''-6'', *C*H_2Ph), 97.9, 99.3, 100.0 (C-1, C-1', C-1''), 127.0-128.4, 138.2, 138.3, 138.4, 138.5, 138.5, 138.6, 138.9 (C-Aromatic).

Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**83**). Ethyl 2,3,4-tri-*O*-benzyl-6-deoxy-1-thio-α/β-D-glucopyranoside **81** (133 mg, 0.28 mmol) was dissolved in DCM and bromine (0.42 mmol, 22 µl) added at 0 °C. The reaction was stirred under N_2 for 1 hour and concentrated. The crude glucosyl bromide was added to a solution of acceptor **73** (130 mg, 0.15 mmol) and Et_4NBr (65 mg, 0.41 mmol) in DCM (1 ml) and DMF (100 µl). After 2 weeks of stirring at rt in the presence of powdered 4Å molecular sieves, the reaction mixture was filtered through a Celite pad and concentrated. Silica gel column

chromatography (toluene-EtOAc 20:1) afforded **83** (140 mg, 73%). ^{1}H NMR ($CDCl_3$): δ 1.17 (d, 3H, H-6'', J = 6 Hz), 3.13 (t, 1H, J = 9 Hz), 3.28 (s, 3H, OC*H*$_3$), 3.49 (dd, 1H, J = 10 Hz, J = 3.5 Hz), 3.71-3.77 (m, 5H), 3.81-4.06 (m, 6H), 4.12 (bs, 1H), 4.19 (m, 1H), 4.44-4.70 (m, 14H), 4.76-4.93 (m, 5H), 5.08 (m, 2H), 5.23 (s, 1H), 7.11-7.39 (m, 45H, H-Aromatic). ^{13}C NMR ($CDCl_3$): δ 18.2 (C-6''), 54.8 (O*C*H$_3$), 67.2, 67.4, 69.4, 69.7, 71.7, 72.0, 72.5, 72.7, 73.3, 73.4, 73.9, 74.5, 74.9, 75.1, 75.2, 75.6, 78.1, 80.2, 80.2, 81.7, 84.2 (C-2-6, C-2'-6', C-2''-5'', *C*H$_2$Ph), 98.6, 99.1, 100.1 (C-1, C-1', C-1''), 127.1-129.1, 138.3, 138.4, 138.5, 138.5, 138.7, 138.8, 138.9 (C-Aromatic).

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