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TESI DI DOTTORATO IN SCIENZE CHIMICHE
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SINTESI DI MONO ED OLIGOSACCARIDI DI ORIGINE BATTERICA

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Abbreviations

DNA	Deoxyribonucleic acid
Glc*p*	Glucopyranose
Gal*p*	Galactopyranose
Gal*p*NAc	2-acetamido-2-deoxy-D-galactose
NMR	Nuclear Magnetic Resonance
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
$BF_3.OEt_2$	Boron trifluoride-ethyl etherate
TsOH	Toluene-*p*-sulfonic acid
NIS	*N*-iodosuccinimide
IDCP	Iodonium dicollidine perchlorate
NBS	*N*-Bromosuccinimide
DMTST	Dimethyl(thiomethyl)sulfonium trifluoromethanesulfonate
Tf_2O	Trifluoromethanesulfonic anhydride
TfOH	Trifluoromethanesulfonic acid
AgOTf	Silver trifluoromethanesulfonate
MeCN	Acetonitrile
Bu_3SnH	Tributylstannane
DCM	Dichlorometane
EtOH	Ethanol
Ac_2O	Acetic anhydride
NaOMe	Sodium methoxide
MeOH	Methanol
CSA	(±)-Camphor-10-sulfonic acid (β)
DMF	*N,N*-Dimethylformamide
AllBr	Allyl bromide
AcOH	Acetic acid
DMSO	Dimethyl sulfoxide
THF	Tetrahydrofuran
$NH_2OMe{\cdot}HCl$	Methoxyamine hydrochloride
Cl_3CCN	Trichloroacetonitrile
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene

BnBr	Benzyl bromide
Py	Pyridine
$CF_3C(NPh)CCl$	*N*-Phenyltrifluoroacetoimidoyl chloride
DME	1,2-Dimethoxyethane
AW-MS	Acid washed molecular sieves
Pd/C	Palladium on carbon
$BnSO_2Cl$	Benzylsulphonyl chloride
Bu_2SnO	Dibutyltin oxide
TBAB	Tetrabutylammonium bromide
$(PhO)_2POCl$	*O,O*-Diphenylphosphorochloridate
DMAP	4-(Dimethylamino)pyridine
TLC	Thin layer chromatography
ESI-MS	Electrospray Ionization Mass Spectroscopy
Ph_2SO	Diphenyl sulfoxide
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
$MeC(OMe)_3$	Trimethyl orthoacetate
EtSH	Ethyl thiol
AcCl	Acetyl chloride
DIPEA	*N,N'*-Diisopropylethyldiamine
LevOH	Levulinic acid
BzCl	Benzoyl chloride
1D	Monodimensional
2D	Bidimensional
COSY	Correlation Spetroscopy
TOCSY	Total Correlation Spectroscopy
NOESY	Nuclear Overhauser Exchange Spectroscopy
HSQC	Heteronuclear Single Quantum Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HPLC	High Pressure Liquid Cromatography
Da	Dalton
NaOAc	Sodium Acetate
t-BuOK	Potassium *tert*-butoxide
MeI	Methyl iodide
PMBCl	4-Methoxybenzyl chloride

CAN	Ceric ammonium nitrate
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
Et_2O	Diethyl ether
DMP	2,2-Dimethoxypropane

1. INTRODUCTION

1.1 Carbohydrates

Carbohydrates are the most aboundant group of natural products. They are found in nature as monomers, oligomers or polymers; furthermore they can be components of biopolymers (glycolipids, glycoproteins, proteoglycans) and other naturally occurring substances. In this last case, they play a role in conferring physical, chemical and biological properties to their carrier molecules. It has been known for a long time their role as energy and biosynthetic resources (glycolysis, etc.), skeletal component (chitin), and key structural elements in the formation of biological architectures (2-deoxyribose for DNA). Carbohydrates and related structures are also involved in biochemical and bioorganic processes such as the molecular recognition for the transmission of biological informations. Indeed, it is reported that the presence of sugars modifies the biological activities and absorption of all drugs. Again, human blood groups are differentiated by relatively simple changes in oligosaccharide structures (**Figure 1.1**).[1]

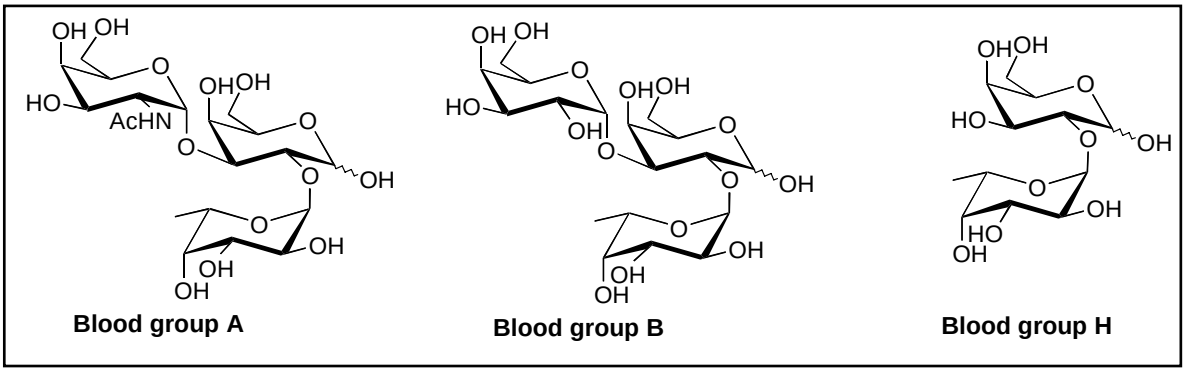


Figure 1.1: Human blood groups oligosaccharide structures

It is also well established that protein- and lipid-bound saccharides play a role in a number of cellular processes such as cell-cell recognition, and cell-external agent interactions.[2] These interactions can start both advantageous biological events and damaging disease processes; for instance, they can stimulate fertilization cell-growth and differentiation (embryogenesis) and immune response, as well as, inflammation, viral and cancer metastasis bacterial infections.[1]

1.2 Bacteria

The term “bacteria” has traditionally been generally applied to all microscopic, single celled prokaryotes. Although this term remains in everyday use, the scientific nomenclature changed after the discovery that prokaryotic life actually consists of two very different lines of evolution. Originally called Eubacteria and Archeabacteria, these evolutionary domains are now called Bacteria and Archea.

Bacteria play important roles in the cycling of nutrients in the environment, and many important steps in the nutrient cycle are catalyzed exclusively by bacteria, such as the fixation of nitrogen from the atmosphere. Bacteria are also important in numerous industrial processes, such as wastewater treatment and industrial production of antibiotic. Besides, bacteria are significant to human health, as they are the causative agent of many infectious and disease, including cholera and tuberculosis. Bacteria display a wide diversity of shapes and sizes. Despite this diversity, each bacteria species tends to display a characteristic morphology, which is the basis for their classification. Many bacterial species exist simply as single cells, while others tend to associate in diploids (pairs) or can even form complex associations (in natural environment most bacteria are found associated with surfaces in biofilms).

1.2.1 Bacterial cell structure[3]

A bacterial cell is bound by a lipid membrane, or plasma membrane, which encompasses the contents of the cell (cytoplasm) and holds nutrients, protein and other essential molecules within the cell. The cell membrane is surrounded by a cell wall which maintains the overall shape of a bacterial cell and is essential for the survival of the bacteria. Basically, bacterial cell walls are composed of peptidoglycans (carbohydrate polymers cross-linked by proteins). However, there are two different arrangement of the cell wall in bacteria and this difference is the base for the most simple and familiar bacteria classification: some bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acid; they retain a purple colour when stained with a dye known as crystal violet and are, therefore, known as Gram-positive (after the Danish bacteriologist who developed this staining procedure). In contrast, other bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by an outer lipid membrane containing lipopolysaccharides (LPS) and lipoproteins; they do not stain purple with crystal violet and are known as Gram-negative (**Figure 1.2**).

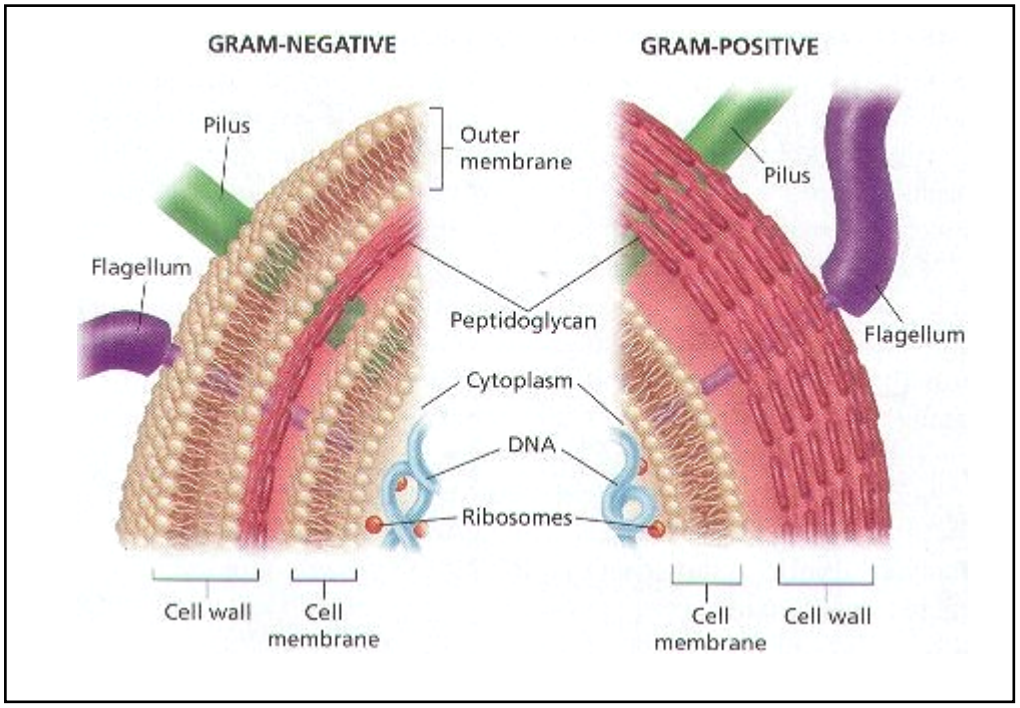


Figure 1.2: Schematic section of Gram-negative and Gram-positive bacterial cell structure

Most bacteria have the Gram-negative cell wall. Gram-negative bacteria cells have a cellular compartment, called periplasmic space, containing enzymes and other proteins that help digest and move nutrients into the cell. Surrounding the cell wall of many bacteria, there is the capsule, a layer of polysaccharides (sometimes proteins) which protects the bacterial cell from environmental aggressions (phagocytosis by eukaryotic cells) and plays a role in bacterial attachment to surfaces and biofilms formation.

Superficial structures are directly involved in cell-cell and cell-environment interactions. During an infection, these structures are in contact with host cells and can interact with their immunity system. In Gram-negative bacteria, lipopolysaccharides (LPS) are key molecules for these functions as they cover roughly 75% of the outer membrane.

1.3 LPS: a little of hystory

In the eighteenth century the association of fever and desease-producing substances with unhygienic conditions was referred to pyrogenic materials, putrid poisons or toxins. Around the end of the century, a Danish pathologist named Panum reported a non-volatile water-soluble, pyrogenic toxin obtained from putrid matter. Later, with the pure-culture techniques developed by Koch it was possible to show that specific bacteria cause different diseases. From the same laboratory in 1892, Pfeiffer reported that the agent of cholera, *Vibrio cholerae*, produced a pyrogenic, non-secreted

toxin that was heat-stabile, in addition to a secreted, heat-labile toxin. He called it "endotoxin", a term still used today for the lipopolysaccharides, amphiphilic macromolecules that were found to constitute them.[4] LPS are responsible for manifestation of infections caused by Gram negative bacteria; they are released as consequences of lyse, death or reproduction of microbic cells into the host organism. This means that their presence is a sign of a bacterial proliferation.[5] The introduction of extraction methods,[6] the amphipathic nature of LPSs, and, consequently, the possibility to separate a lipid region after a weak acid hydrolysis, gave rise the elucidation of a general architecture consisting of two or three regions.[7]

1.3.1 LPS: structure

From the bacterial membrane outwards, a LPS structure comprises a lipid moiety, called Lipid A, connected with an oligosaccharide core region by means a Kdo (3-deoxy-oct-2-ulosonic acid) residue. Bacterial LPSs can show a rough or a smooth appearance depending on sugar composition. More precisely, rough-type bacteria produce LPSs containing an oligosaccharide core of approximately 10 monosaccharides, whereas LPS produced by smooth-appearing bacteria are enriched of a polysaccharidic portion called O-Chain (**Figure 1.3**).

Lipid A is considered the endotoxic component of a LPS and it is locked into the bacterial membrane by means of hydrophobic and electrostatic interactions. A common general lipid A structure can be described consisting of a biphosphorylated β-(1→6)-linked glucosamine disaccharide. Aminic and hydroxyl functions present on position 2, 3, 2', 3' are generally acylated by fatty acids (primary fatty acids) which may be themselves substituted with fatty acids ester-linked (secondary fatty acids).[8] Even though Lipid A is considered the most conservative element of the LPS there are some structural variations related to acyl and phosphate groups.

Core is a complex oligosaccharide where it is possible to distinguish a fraction made up of common sugar (Glc*p*, Gal*p*, Glc*p*NAc, Gal*p*NAc), called outer core, and an internal fraction, called inner core, composed of more peculiar sugars such as L-*glycero*-D-*manno*-heptose and Kdo. In smooth-type bacteria core region is, generally, a diminutive portion of the entire sugar moiety so that its characterization is not always easy to be accomplished.

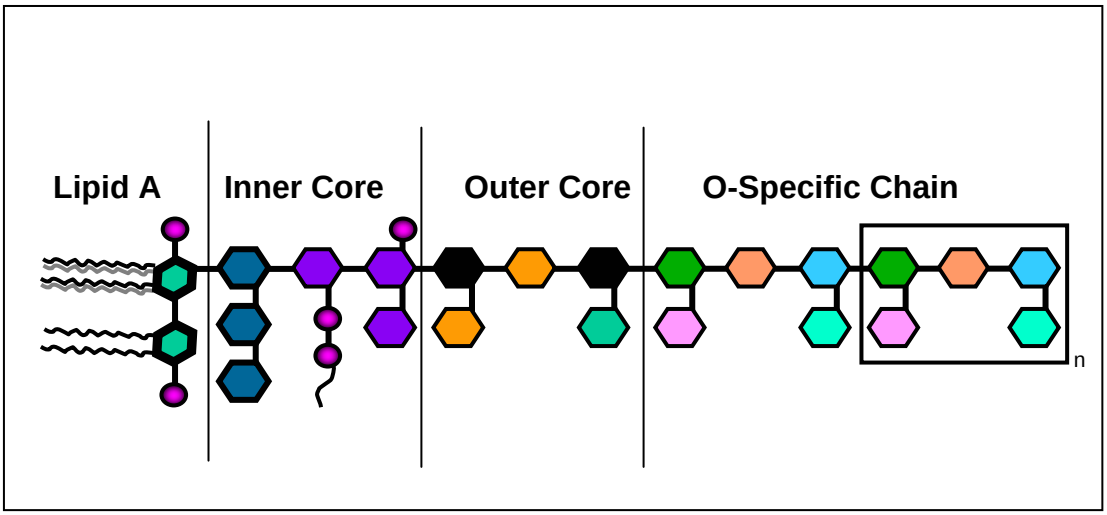


Figure 1.3: Schematic structures of LPS

O-Chain is a polysaccharide made up of repeating subunits and each subunit consists of one to eight sugar residues; the length of an O-Chain is variable from bacterium to bacterium but it can be stated that in an O-Chain there may be up to 50 identical sub-units and they can be linear or branched. Subunits are polymerised and adjoined to the core during the biosynthesis. Sometimes the polymerisation can be interrupted by a substituent on the last sugar of the chain; for instance, the O-Chain of *Vibrio cholera* serotype O1 Ogawa shows an *O*-methyl substituent on the terminal perosamine residue which is not present in the O1 Inaba serotype.[9] Considering synthetic aspects (substitution and configuration of sugars, different possibilities of glycosidic linkage) and genetic capacities of different organisms, nature gives a wide range of unique O-chain structures showing its well known greatness. Indeed, O-chain is considered the most structurally changeable portion of a LPS. As the O-chains extend outward from the bacterial cell surface up to 10 nm, they are exposed to the cell-cell environment and thus involved in the defence system of a potential host. The O-chain is also called "O-antigen" because of its antigenic activity mirrored in specific recognition antigen-antibody; definitely, the O-chain can be considered a finger-print for Gram-negative bacteria, determining the specificity of each bacterial serotype. Further, pathogenic effects of bacteria may be directly related to the nature of the O-Chain; this is, for example, the case of the O-chains of *Proteus* whose acidic group are suspected to contribute its capacity to form stones in bladder or kidney.[10]

1.3.2 LPS: biological activity

During an infection, the bacterial O-Chains are in direct contact with the host. Once in the host-cells, LPSs prompt production of molecules responsible to eradicate bacterial infection and consecutively to wipe out bacterial cells.[11] However, if there is a massive presence of bacterial cells,

over-production of antibacterial molecules can prompt negative effects on the guest leading even to lethal septic shock. Again, one of the studied functions of LPSs is shielding bacteria from some antibiotic effects; actually rough-type strains showed a relative higher sensitivity to antibiotics compared to smooth-type strains.[12]

1.3.3 LPS from phytopathogenic bacteria

Even though O-Chain polysaccharide is the most variable portion of LPS, O-chains from phytopathogenic bacteria show a quite regular pathway in their structures. Structural studies showed that they are almost exclusively constituted of a small range of monosaccharide.[13] A more interesting information is that the repeating subunits consist of linear backbones made up, mainly, of D- and L- rhamnose residues, very frequently connected by α-(1→2) and α-(1→3) linkages; very often single monosaccharides, within a small range, are present as branch on the linear backbone (**Figure 1.4**).

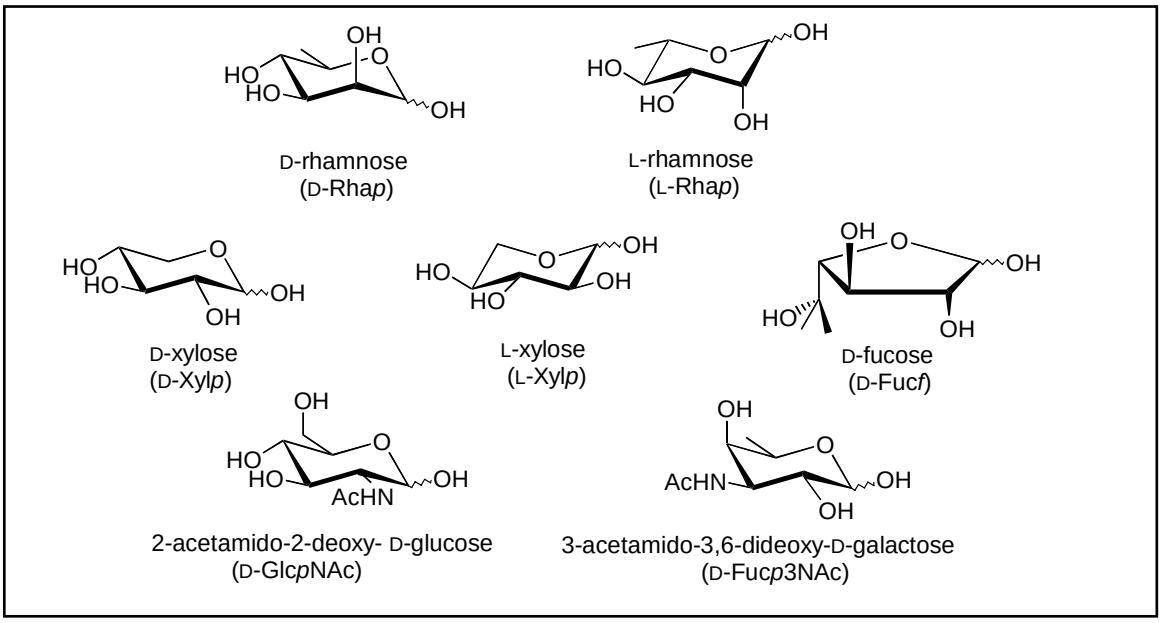


Figure 1.4: Monosaccharides consisting *O*-chain from phytopathogenic bacteria.

An evidence of these structural restrictions lies in the fact that almost 80% of O-chains from 35 different phytopathogenic strains of *Pseudomonas syringae* and *Xanthomonas campestris* species show just the four following structurally different linear backbones:

→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→

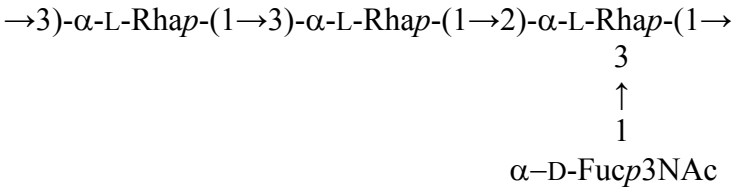
→3)-α-D-Rha*p*-(1→3)-α- D-Rha*p*-(1→2)-α-D-Rha*p*-(1→2)-α- D-Rha*p*-(1→

→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→

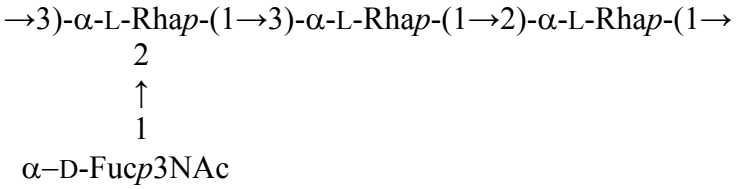
→3)-α-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→2)-α-D-Rha*p*-(1→

In most cases, these O-chains differentiate each other, simply by bearing different branch monosaccharides on the same position or the same branch monosaccharide on different positions of an identical linear backbone; few examples are shown below:

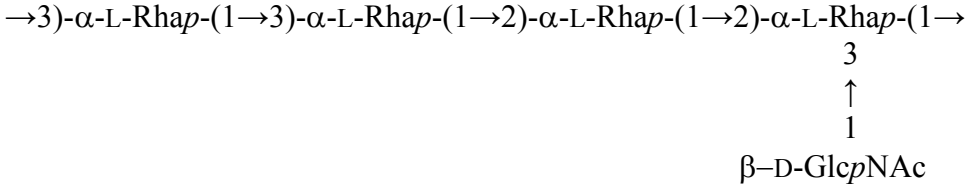
Pseudomonas syringae pv. *savastanoi* ITM 519 (VII)[14]



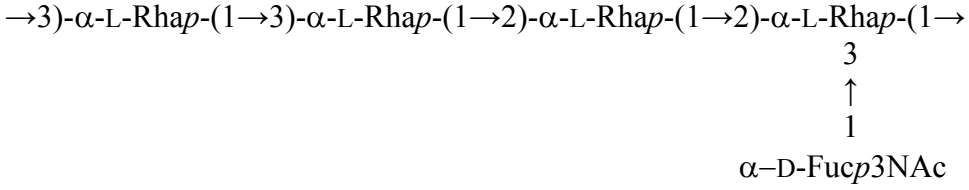
Pseudomonas syringae pv. *tabaci* IMV 223 (VII)[15]



Pseudomonas syringae pv. *ribicola* NVPPB 1010 (VIII)[16]



Pseudomonas syringae pv. *holci* IMV 8300 (I)[17]



One of the most interesting aspects of LPS-phytopathogenic O-chains is the absence of any direct relationship between O-chain structures and bacteria-host recognition; this is illustrated, for

instance, by three different strains of *P. savastanoi* pv. *savastanoi* which show the same O-chain structure of *P. syringae* pv. *tomato* albeit the former attack olive and oleander and the latter are pathogenic for tomatoes. Even though bacterial human- and plant-pathogens share a common LPS structure, host cells have different recognition and defence system. Indeed, biological functions and molecular mechanisms have been quite widely investigated for human pathogenic bacteria but still very little is known about LPS-plant interactions. Actually, the most confirmed proposed mechanism for plant defence activation by LPS is based on recognition of peculiar structures of the pathogen (PAMPs, standing for pathogen-associated molecular patterns) which are crucial in its growth within the host;[18] this mechanism is analogue to the innate immunity system of animals.[19] Certainly, one of the most widely studied effects of LPSs on plants cells is the ability, induced by avirulent bacteria, to prevent the hypersensitive response (HR), a programmed cell death response.[20] A recent work showed that the Lipid A moiety may be partially responsible for LPS perception by plant cells;[21] however, oligosaccharides have also been proved to play key roles in plant innate immunity. More precisely, biological tests of oligosaccharides **A**, **B** and **C** (**Figure1.5**) on *Arabidopsis thaliana* leaves were performed;[22] the results of these tests evidence that oligosaccharides **A**, **B** and **C** were effective in inducing *PR-1* gene expression, a plant immune response, and in suppressing HR.

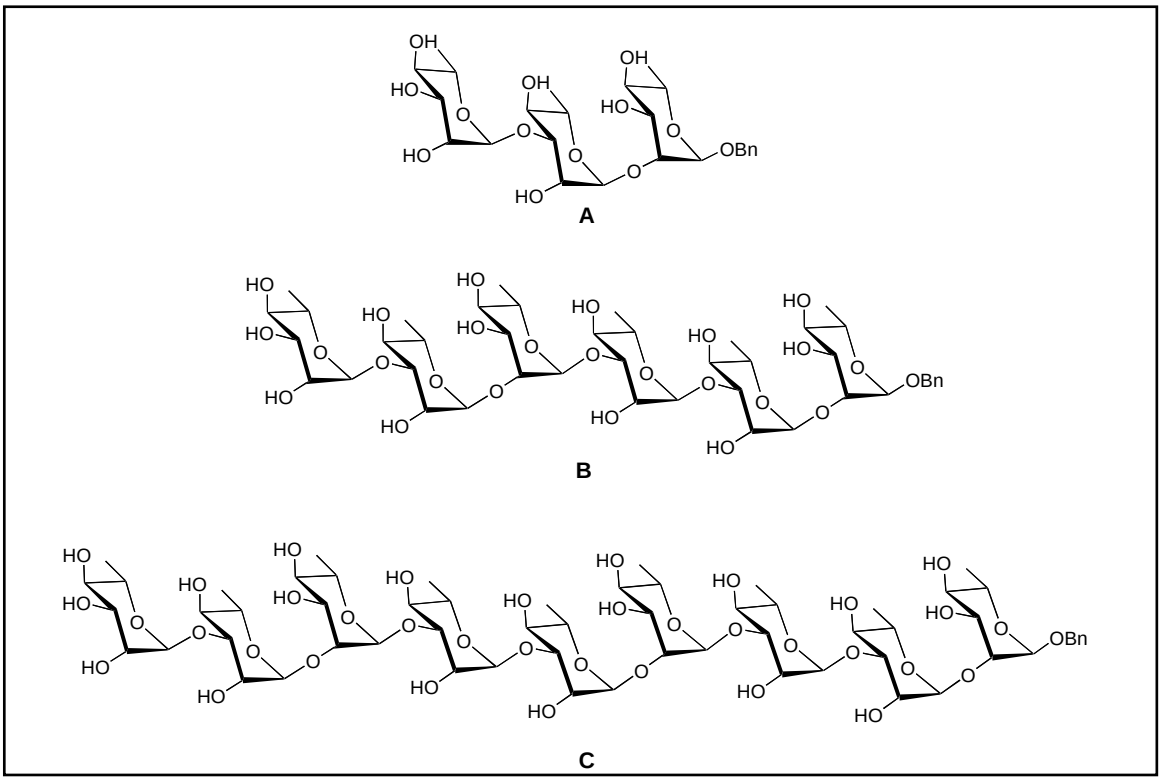


Figure 1.5: Oligosaccharides tested on *Arabidopsis thaliana* leaves

Once inoculated with aqueous solution of oligosaccharides, plant leaves were treated with different phytopathogenic Gram-negative bacteria. HR suppression was pointed out and it was manifested in decreasing expanse of yellow spots on plant leaves (**Figure 1.6**).

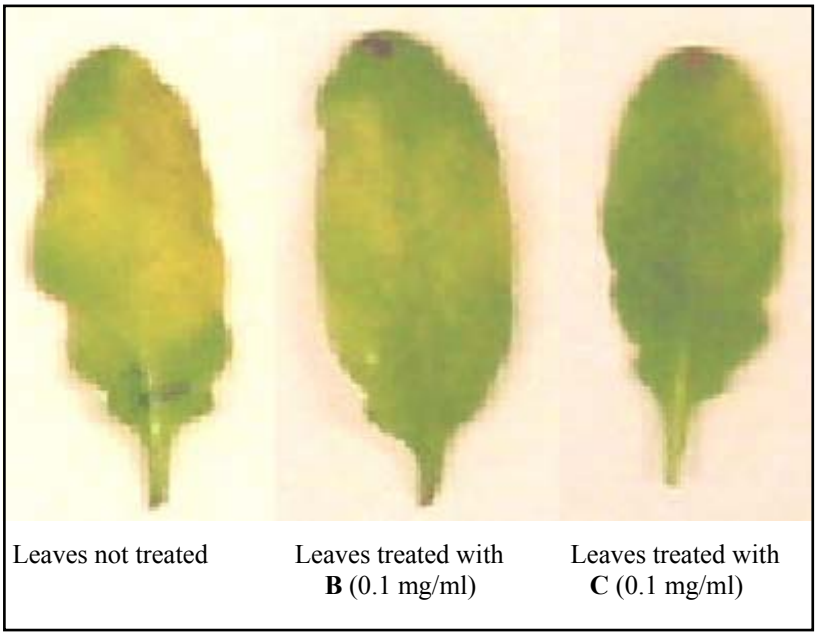


Figure 1.6: Comparing biological activity of **B** and **C** on *Arabidopsis thaliana* leaves

The plant response depends on the length of the chain and, in contrast with the case of oligosaccharides **A** and **B**, HR suppression induced by oligosaccharide **C** was not concentration-dependent. A similar response was observed in *PR-1* gene expression. Additionally, combination of NMR and molecular modelling elucidated coiled structures for oligosaccharides **B** and **C**, proposing them as PAMPs in plant-bacteria recognition (**Figure 1.7**).

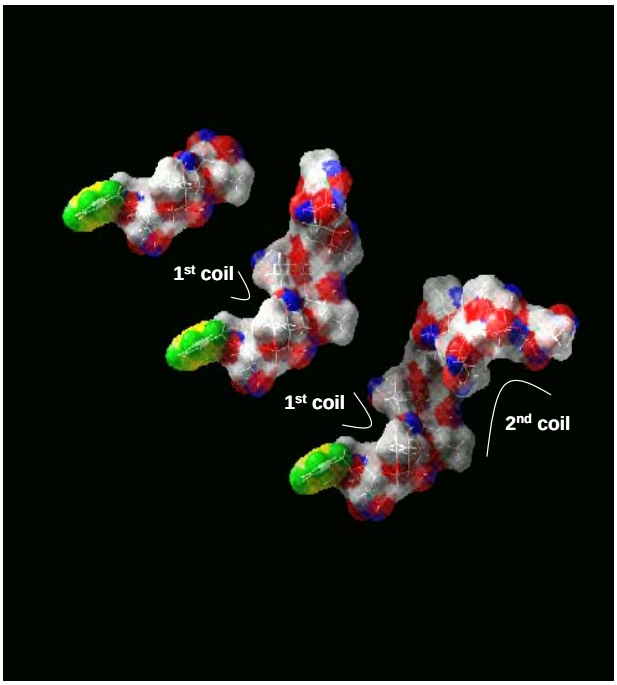


Figure 1.7: 3D structures of **A**,**B** and **C**

Even though this is an helpful demonstration that short oligosaccharides can prompt plant immune-response, still more has to be done in description of molecular mechanisms involved in plant-LPSs interaction. Beside a detailed determination of the minimal oligosaccharide fraction involved in host-guest interaction processes, this work would require the synthesis of adequate quantity of them. In this matter, my Ph.D. project concerned the synthesis of model oligosaccharides related to phytopathogenic bacteria O-Chains mimicking natural structures to employ, in future, in biological experiments.

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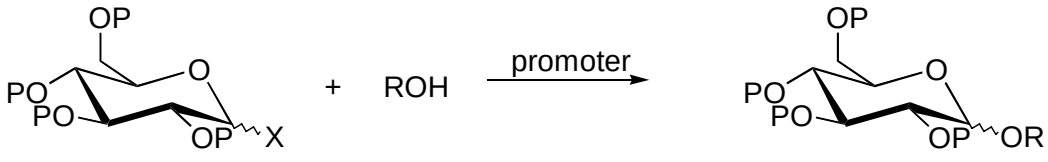
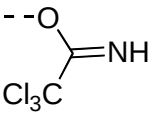
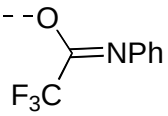
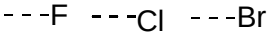
2. SYNTHETIC ASPECTS

2.1 *O*-Glycosylations

The key step in the synthesis of oligosaccharides is the building of a glycosidic linkage by means of an *O*-glycosylation reaction; this involves the coupling between a glycosyl donor (a sugar bearing a suitable leaving group at the anomeric carbon) and a glycosyl acceptor (a sugar containing a free hydroxyl group); a promoter agent is used to activate the donor in building the new bond. Very often dehydrating agents are used to avoid competitive reaction by water. The following **Scheme 2.1** shows a generic glycosylation reaction and a list of the most used glycosyl donors and activating agents:



DONOR	X	PROMOTER
Glycosyl trichloroacetimidate		TMSOTf, $BF_3{\cdot}OEt_2$, TsOH
Glycosyl N-phenyl trifluoroacetimidate		TMSOTf, $BF_3{\cdot}OEt_2$, TsOH
Thioglycoside		NIS/TfOH, IDCP, NBS, DMTST
Glycosyl sulfoxide		Tf_2O, TfOH
Glycosyl halide		AgOTf, Ag_2O, $SnCl_2/AgClO_4$

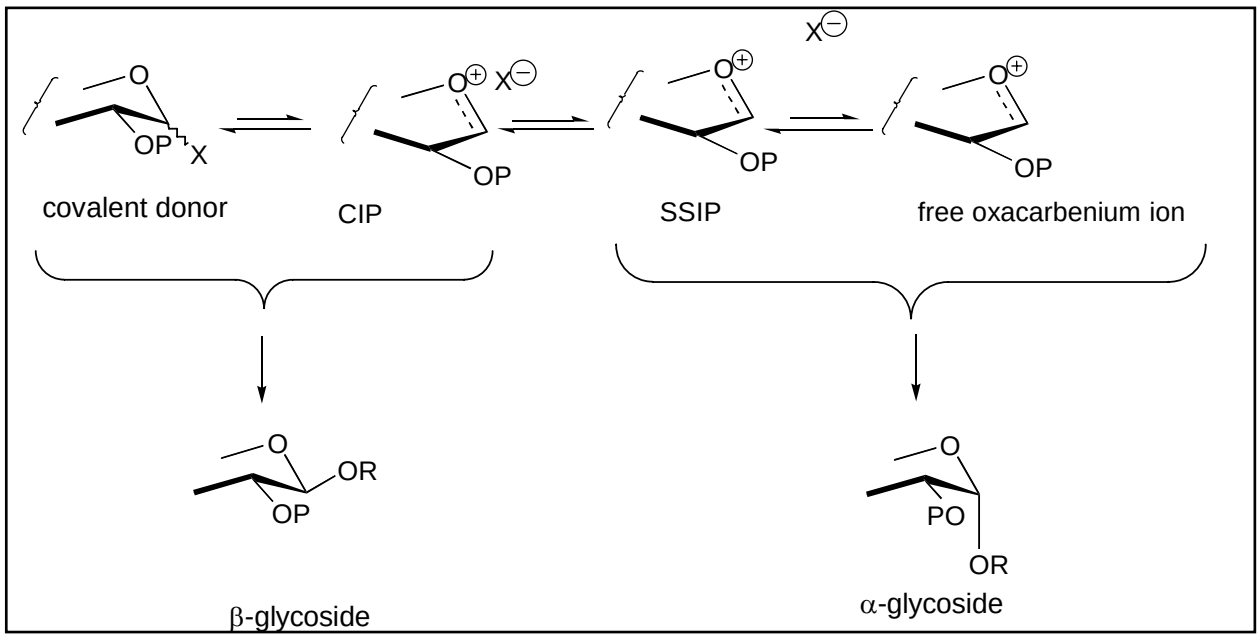
Scheme 2.1: Generic scheme of a glycosylation reaction and the most used glycosyl donors and promoters

Because of the number of factors involved in the reaction and the instability of the intermediates a glycosylation process is complex to describe.
Besides chemical synthesis, more recently enzyme-catalyzed procedures have been developed but they are still limited to certain types of glycosidic linkages at a preparative useful scale.

2.2 Regioselectivity and stereoselectivity in glycosylations

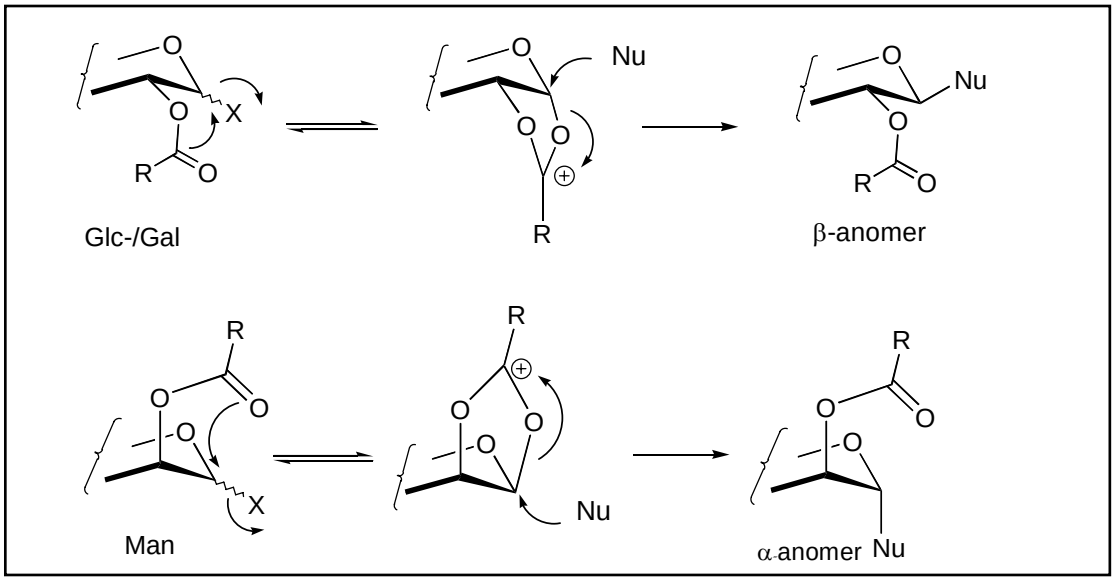
The efficiency of an *O*-glycosylation reaction requires high yield, regioselectivity and stereoselectivity but these aims are not very easy to achieve. Indeed, carbohydrate building blocks are able to exist in more than one form (pyranose and furanose forms, α and β anomers) and so it is important to control which form is available for the reactions. Again, carbohydrates have a number of alcoholic functions whose reactivity does not follow any universally applicable rules, but usual generalization for which primary>secondary>tertiary and equatorial>axial. Thus, there is the need to isolate them, alternatively, in a reactive form to avoid mixture of products and so to render the reaction as regioselective as possible. This goal can be pursued by using suitable protecting groups which mask temporarily OH-functions. This involves a number of chemical manipulations so it is essential that the protecting groups are introduced and removed with excellent yields; furthermore, they need to leave unaffected other parts of the molecule and must be stable in succeeding reaction conditions. Besides this aspect, protecting groups can also fine-tune reactivity of both donor and acceptor moieties by means of their electronic density. This is true also for protecting groups on the anomeric position of the acceptor: for instance, thioglycoside acceptors are, generally, less nucleophile then their corresponding *O*-glycosides.[1]
Stereoselective problems also need to be considered: the formation of a new bond between two carbohydrate units can afford two anomeric isomers. Conventionally, in the D-series, the α–anomer is the one in which the aglycone projects downwards in Haworth formula; α–L compounds have the aglycone projecting upwards in Haworth formula. The β form have the opposite configuration at the anomeric centre, i.e. the aglycone projects upwards and downwards for β–D and β–L compounds, respectively. A simplified mechanism of a glycosylation reaction showing α- and β-anomers formation is the one reported in **Scheme 2.2**: by action of an activating agent, a transient contact ion pair (CIP), or a solvent separated ion pair (SSIP), is formed. In conditions which favour the CIP formation, such as unpolar solvent, the nucleophilic acceptor might attack on the tight ion pair in a S_N2-like pathway; more likely, due to the anomeric effect (polar substituents such as halide, OR, or SR derivatives prefer an axial orientation),[2] the reaction proceeds, mainly, through

the SSIP leading to the α-linked glycoside which is, actually, the main product coming from any oxacarbenium ion.[3]



Scheme 2.2: Abbreviated glycosylation mechanism

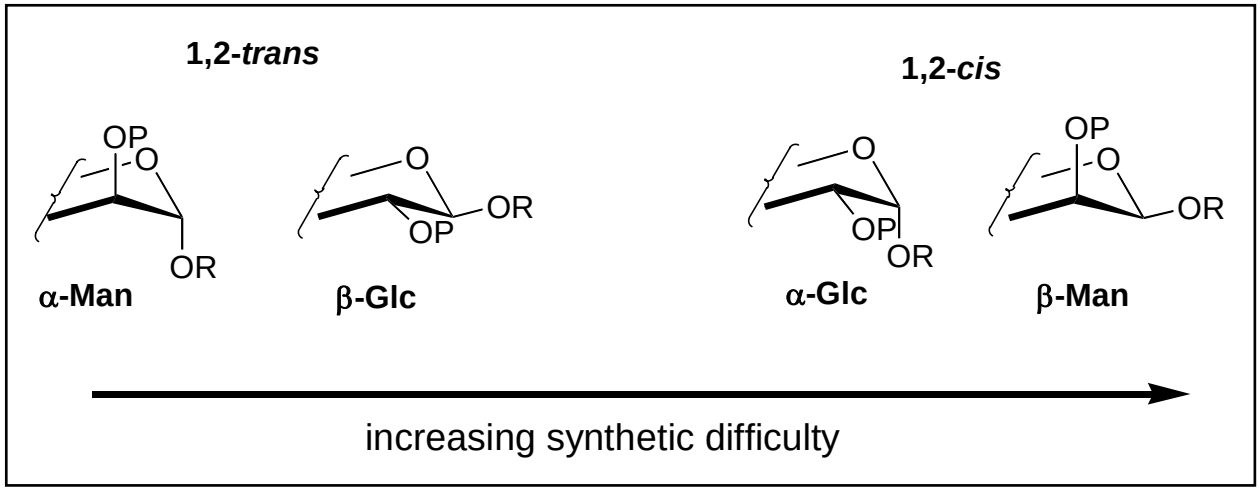
As the first step is the rearrangement of the sugar and the development of a positive charge, the outcome of a glycosylation will be affected by any factor which can influence changing in sugar-conformation.[4] Stereoselectivity can be controlled by the presence of a neighbouring participating group (anchimeric assistance).[5] Generally, a carbonyl functionality at *C*-2 of the glycosyl-donor (ester, carbonate, phtalimide, *etc*…) can support the departure of the leaving group attacking the incipient oxonium ion and forming the thermodynamically more stable acyloxonium species (**Scheme 2.3**); the position of the equilibrium will depend upon the nucleophilicity of the carbonyl oxygen.[6] The attack of the acceptor will occur *anti* to the carbonyl bond resulting in generation of the 1,2-*trans* glycoside. This tool to direct the stereoselectivity will lead to a β-configurated anomeric bond using a Glc/Gal-configurated glycosyl donor; if the glycosyl donor has a Rha/Man-configuration it will result in an α-configurated anomeric bond.



Scheme 2.3: Anchimeric assistance in glycosylation reaction

In the case that some steric barrier will occur between donor and acceptor, disfavouring reaction on the dioxonium species, the reaction will proceed through the incipient oxonium ion with concomitant loss of stereoselectivity.[7]

Due to combination of the anomeric effect (which favours α configuration) and neighbouring participation groups (which favours 1,2-*trans* adduct) the generic stereoselective outcome of a glycosylation reaction can be illustrated in the following **Scheme 2.4**.

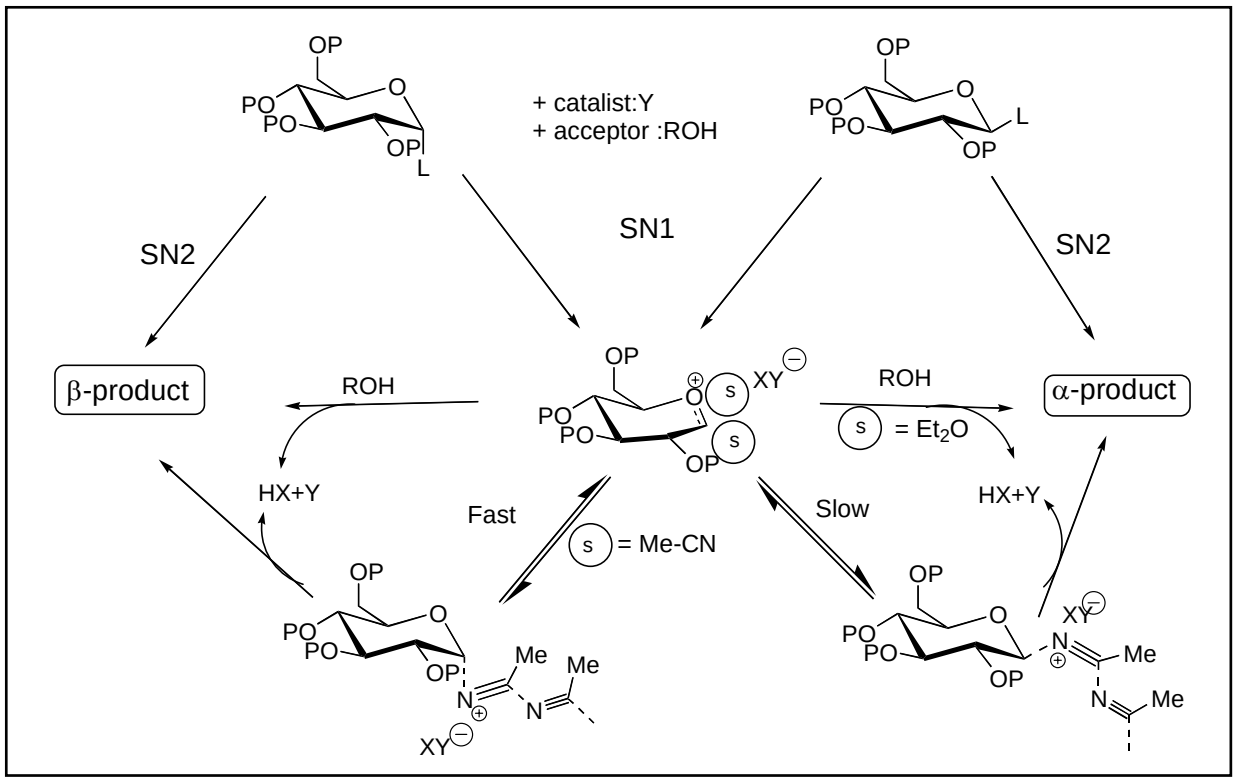


Scheme 2.4: Increasing synthetic difficulty in stereoselective glycosylation

However, electron-withdrawing protecting groups, such as acyl-type protecting group, depress anomeric reactivity (as compared with ether groups) on ion-pair formation, translating this effect on the rate of the reaction; this kind of protected glycosyl donors is named "disarmed".[8] In contrast, the rate of the reaction may be accelerated using a glycosyl-donor with an ether-type protecting group at *C*2; this kind of glycosyl donor are indicated as "armed".

On the other hand, not all the time it is possible to functionalize the *C*-2 of the donor with a participating protecting group. This is the case when 1,2-*cis* glycoside formation is reuired. Thus, stereoselectivity can be controlled by choosing a suitable reaction solvent: [9] with non-polar solvents a S_N2 mechanism is preferred (nucleofilic attack by the acceptor proceeds with inversion of configuration at the anomeric position of the donor) while in polar solvents the oxonium ion is better solvated and the reaction proceeds following a S_N1 pathway (causing loss of stereoselectivity). Deeply speaking, diethyl ether can form a more stable β-orientated diethyl oxonium ion whose stability is due to the exo-anomeric effect. [10] This is the tendency of positively charged substituents at *C*-1 of a pyranose ring to adopt the equatorial configuration; acceptor, then, will attack on the solvated ion to give the α-linked glycoside (**Scheme 2.5**).

On the other hand, nitriles as participating solvents are able to activate, selectively, highly reactive glycosyl donors towards β-linked glycosides formation. The reaction occurs through the formation of a nitrilium-nitrile-conjugate intermediate: the fast α-intermediate formation providing the β-glycoside precedes the formation of the thermodynamically more stable β-intermediate which furnishes the α-glycoside; additionally the equilibrium between α and β nitrilium-ions is quite slow so the accumulation of the β-configurated glycoside is favoured.



Scheme 2.5: Participating solvents in glycosylations

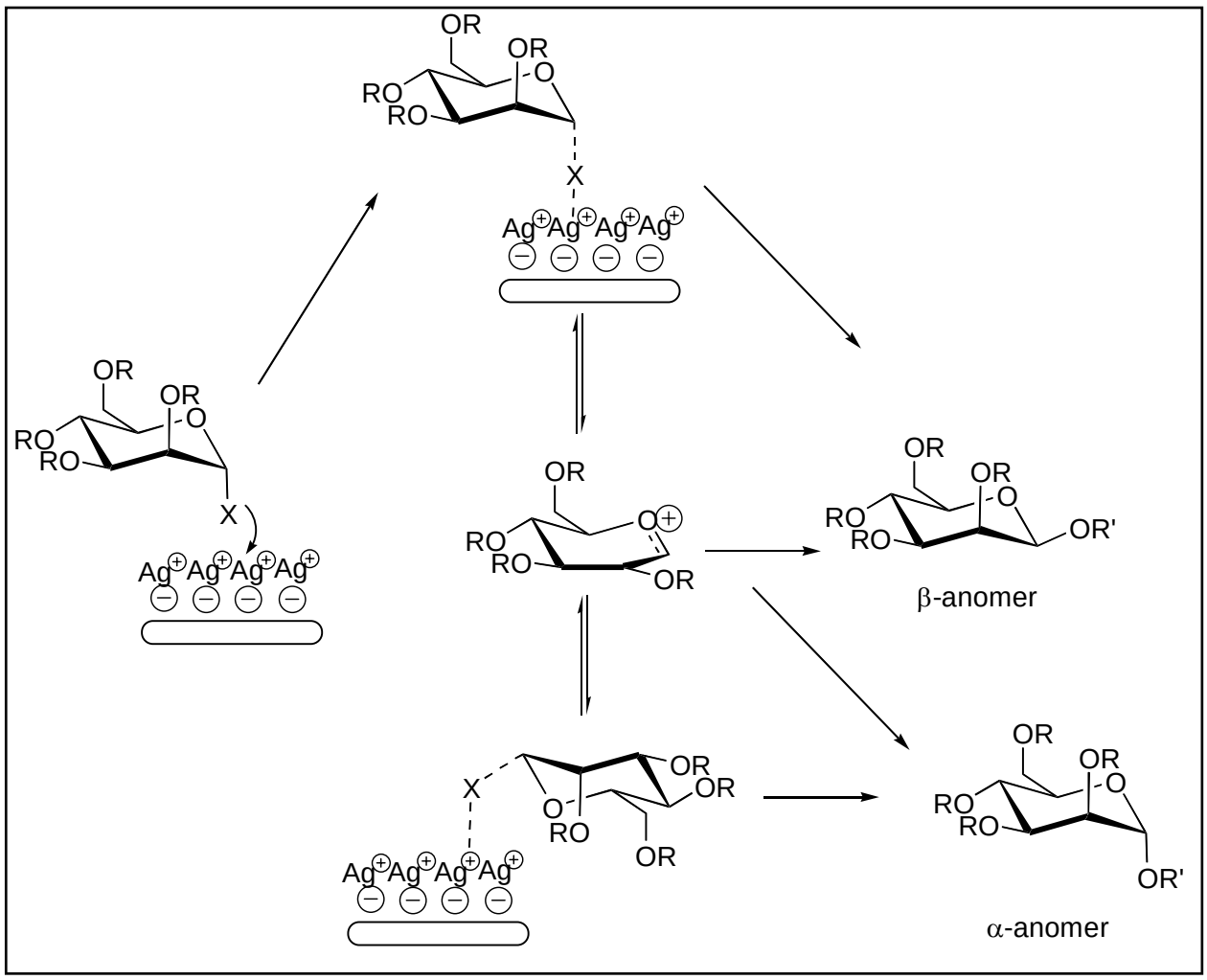
Other factors affecting stereoselectivity include long-range participation, protecting groups at *C*-6,[11] temperature,[12] etc.

2.3 β-Mannosylations and β-rhamnosylations

As showed in **Scheme 2.4** the β-mannosides are the most difficult glycosides to be synthesized. However, D-mannose is a common constituent of naturally occurring oligosaccharides, playing an important role in many biological processes[13] and although the most common linkage is the α-configurated one, a significant amount of the β-counterpart is found.[14] This creates the cue for its synthesis which is still a challenging goal for two main reasons: besides the anomeric effect already described in the previous paragraph, which favours the formation of α-mannosides, 1,2-*cis* arrangement of the equatorial aglycone and the axial functionality at *C*-2 in β-mannopyranosides shows a repulsive steric effect. Again, once formed, the β-mannoside linkage might anomerize in acidic conditions. Other complications in the synthesis of β-mannoside linkages are due to difficulties of α and β anomers characterization: actually, they are not easily differentiable by their $^{3}J_{H\text{-}1,H2}$ coupling constant because of the *gauche* relationship between the H-1 and H-2 protons in both anomers.

As mannose and 6-deoxy-mannose (rhamnose) share structural relationship they can also share similar behaviour. However, synthesis of β-rhamnoside is more complex than the synthesis of β-mannoside due to the 6-deoxy function which influences the conformation of the intermediates affecting directly the glycosylation mechanism. Herein some of the common strategies used to build β-mannosides/β-rhamnosides are reported.

The first direct β-selective mannosylation was performed in 1961 by Gorin and Perlin[15] who condensed a 2,3-*O*-carbonate–protected mannosyl bromide with a highly reactive primary alcohol under silver oxide conditions. Later several examples reporting the same effect have been collected in literature.[16] The effect is usually explained by a combination of two factors: the carbonate destabilizes the potential oxacarbenium ion disfavouring a S_N1-like mechanism and, silver cations on the surface of insoluble catalysts absorb the bromide preferentially on the α-side: the acceptor is therefore forced to attack in a S_N2-like fashion from the β-side. The mechanism of silver insoluble catalyst is described in **Scheme 2.6**.

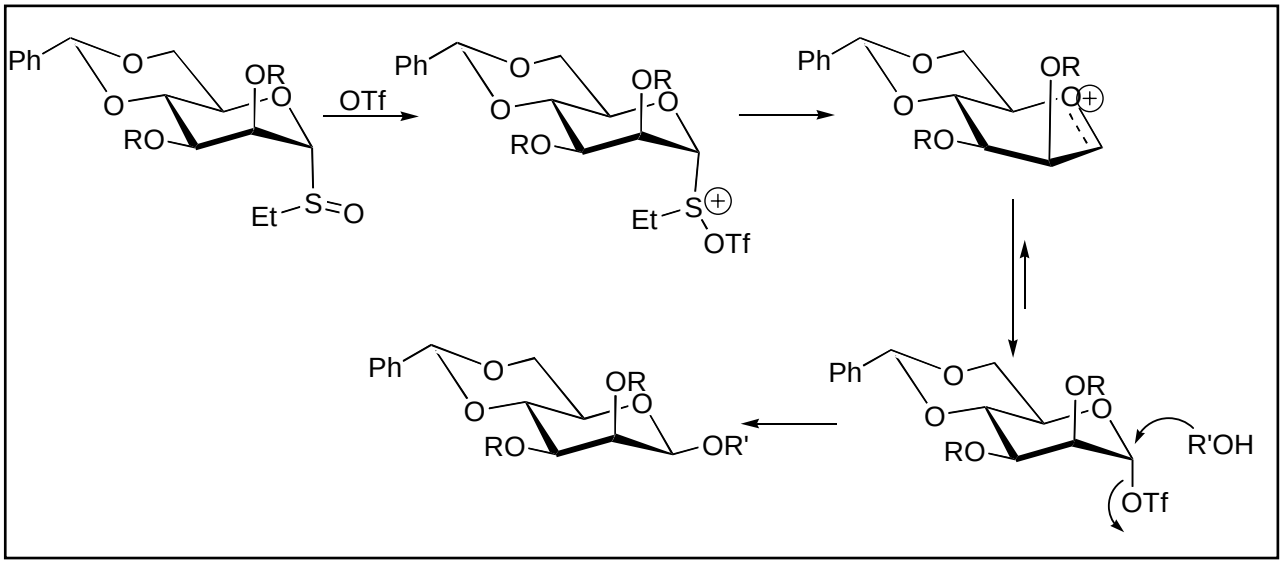


Scheme 2.6: Mechanism proposed for silver insoluble catalyst mediation in glycosylations

Actually, the proposed mechanism involves the equilibrium between α- and β-ion pairs: when the acceptor is not sufficiently reactive, the surface-bound glycosyl donor is liberate into the solution to give the more reactive oxacarbenium ion which can couple with the acceptor to give preferentially the α-glycoside. The oxacarbenium can also associate again with the negatively-charged surface; in this case the reaction proceeds in a non-selective way giving α- and β-ion pairs. Thus, it is so clear that to minimize the stereochemical scrambling of the anomeric centre, a not sterically hindered and highly reactive acceptors are required. The stereochemical outcome of this strategy is also affected by the protecting groups on the glycosyl donor: as the success of this protocol requires a rapid nucleophilic displacement, mannosyl donors having acyl protecting groups (disarmed) should be less β-selective.

High β-stereoselectivity can also be induced by torsional effects, as described by Crich[17] who used a triflic anhydride-mediated activation of 4,6-*O*-benzilidene-protected mannosyl donors to afford β-mannosidic linkage with high stereocontrol. It has been proved by NMR analysis that the reaction proceeds *via* an α-mannosyl triflate which is generated *in situ* after activation of the donor by triflic

anhydride. Successive addition of the acceptor provide the formation of β-mannoside by a S_N2-like process. The success of this stereocontrol depends on the order of addition of the reactants, the steric hindrance at *C*2 of the donor, the solvent as well as the presence of a 4,6-benzylidene group. In point of fact, deeper investigation revelead that 4,6-benzylidene group is crucial to reach high β-selectivity. This may be rationalized saying that the energy of the oxacarbenium ion is higher than the one related to covalently bound triflate; this is due to twist and torsional strain on the acetal ring imposed by the sofa conformation of the intermediates (**Scheme 2.7**). This effect is not present in the per-ether protected systems.



Scheme 2.7: β–Mannosylation reaction *via* glycosyl-triflate

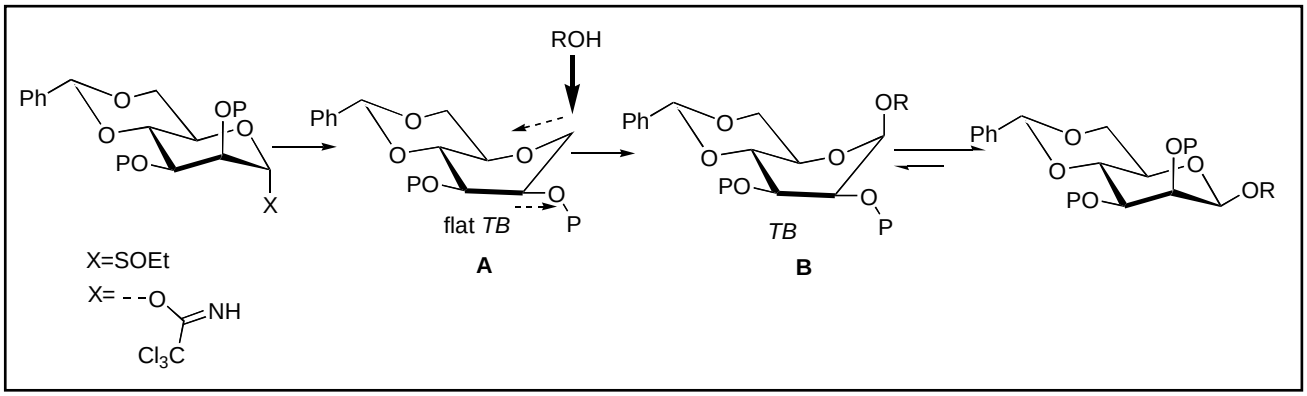
In order to profit by 4,6-*O*-benzylidene-mediated stereocontrol even on rhamnose series, Crich and co-workers developed different synthetic pathways involving a 4,6-*O*-benzylidene-protected mannosyl donor and consecutive cleavage of the benzylidene acetal to get the deoxy function. He introduced the (4,6-*O*-[α-(2-(2-iodophenyl)ethylthiocarbonyl)benzylidene] group as a surrogate for the 4,6-*O*-benzylidene group which is highly β-directing in mannosylations. This benzylidene is then removed in a reductive radical fragmentation, by using Bu_3SnH, to obtain β-D-rhamnopyranoside systems.[18] The radical fragmentation allows to bypass the NBS-based cleavage of 4,6-*O*-benzylidene ring, which is an established tool in carbohydrate chemistry[19] but not compatible with benzyl and allyl-type protecting groups because of competitive cleavage of this groups.[20] More recently, based on the chemistry of cyano groups applied on synthesis by Rychnovsky,[21] Crich and co-workers launched a new-generation of 4,6-*O*-benzylidene-protected glycosyl donors bearing a 4,6-*O*-[1-cyano-2-(2-iodophenyl)ethylidene] group.[22] Its introduction undergoes by using mild conditions, over short reaction times, and it is compatible with a wide

variety of other protecting groups. Additionally, its removal is performed by tin-mediated radical fragmentation providing β-D-rhamnopyranosides in high yields and stereoselectivity.

On the basis of torsional effects on stereoselectivity and Gorin and Perlin's experience, Crich and co-workers attempted the synthesis of a β-mannosydic linkage invoking the combination of both 2,3-*O*-carbonate and 4,6-*O*-benzylidene protecting group on a thiomannosyl donor.[23] However, only α-anomers were obtained under such homogeneous conditions, in contrast to the expectation that the two protecting groups would reinforce each other providing for a highly β-selective system. Further investigations performed on rhamnosyl donors highlighted that shifting the carbonate group to the 3,4-positions, a positive β-directing effect was achieved in both homogeneous and heterogeneous conditions; this effect come up from the combination of the electron-withdrawing nature of the carbonate which destabilizes the anomeric oxacarbenium ion and its inability to take part in neighbouring participation due to its cyclic nature.

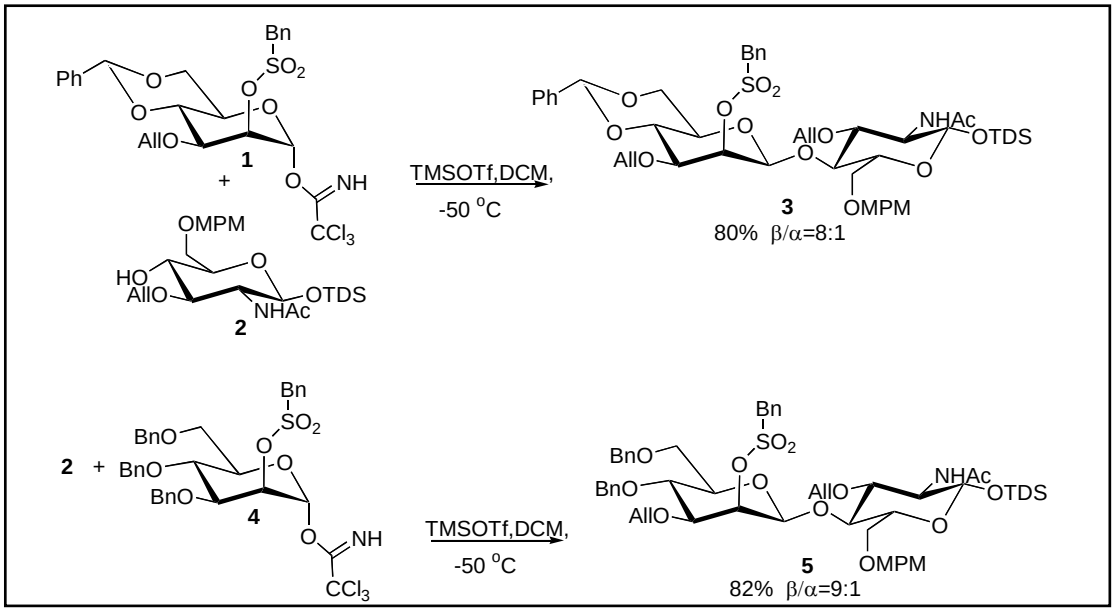
However, there are also several other non-requiring 4,6-*O*-benzylidene protecting group successful protocols for more direct synthesis of β-mannosides/β-rhamnosides. Among these, a strategy employing 2-*O*-sulfonate ester protecting group has been demonstrated to be β-stereoselective in glycosylation of L-rhamno-pyranosyl chlorides by Schuerch and co-workers;[24] they demonstrated that the 2-*O*-sulfonyl group is able to stabilize an α-mannosyl sulfonate, obtained *in situ* from the chloride, and therefore to direct the glycosylation towards β-mannosyl adducts. Successively this strategy has been applied to thioglycosides by Crich and co-workers.[25,3]

In order to better define the role of non-participating 2-*O*-sulfonyl groups, Schmidt and co-workers speculated on reactivity of 2,3-di-*O*-alkyl-4,6-*O*-benzylidene-protected mannosyl donors with various acceptors obtaining high β-stereoselectivity in glycosylations. To rationalize the good results, they postulated a mechanism where anomeric stereocontrol is caused by a conformational effect enforced by the 4,6-*O*-benzylidene group on the pyranosyl ring, which favours generation of a flattened twist-boat conformation **A** as the intermediate (**Scheme 2.8**).[26] Because of stereoelectronic and steric reasons **A** will be preferentially attacked from the β side, which gives a twist-boat intermediate **B** that equilibrates to the $^4C_1$ conformer. Non-participating strongly electron-withdrawing groups P at 2-*O* atom should facilitate β-mannopyranoside formation because generation of the twist-boat intermediate **A** would gain from a strong dipole effect.



Scheme 2.8: Mechanistic proposal for preferred β-mannopyranoside formation. TB = twist-boat conformation.

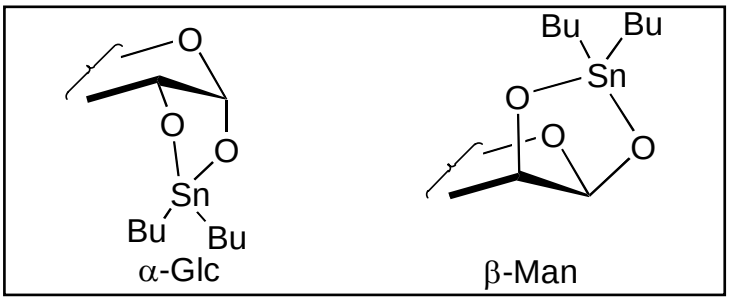
However, this approach requires an excellent leaving group at the glycosyl donor and a good strong electron-withdrawing 2-*O*-protecting group such as benzylsulfonyl group which is easily installed and removed compatibly with other ether-based protecting groups. They found that coupling 3-*O*-allyl-2-*O*-benzylsulfonyl-4,6-*O*-benzylidene-protected mannopyranosyl trichloroacetimidate **1** with 4-*O*-unprotected glucosamine derivative **2** provides mainly β(1-4)-linked disaccharide. This approach was also tested without a 4,6-*O*-benzylidene ring giving even better results showing that the presence of a 4,6-*O*-benzylidene is not crucial (**Scheme 2.9**).



Scheme 2.9: Examples of β-mannopyranoside formation reported by Schmidt

A totally different approach for stereospecific building of β-mannopyranosyl and β-rhamnopyranosyl linkages is the one first discovered by Srivastava and Schuerch in 1979,[27] successively developed by Hodosi and Kováč in 1996,[28] called "glycosylation *via* locked anomeric

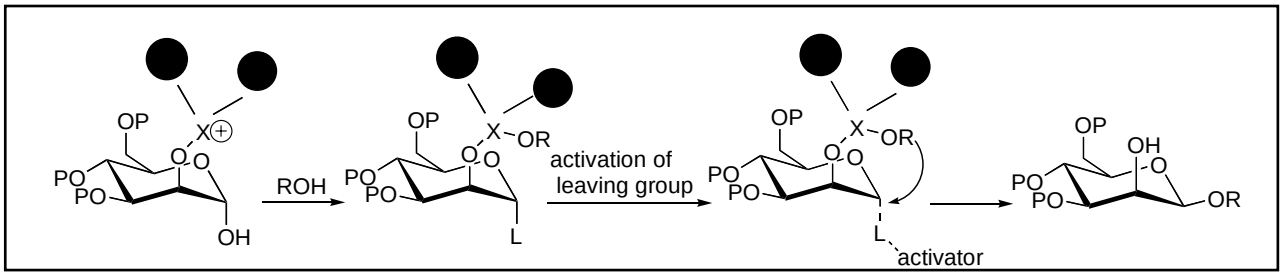
configuration“. The role of glycosyl donor and acceptor are reversed: 1,2-*O*-*cis*-stannylene sugar acetals, as powerful nucleophile displaces, *via* S_N2 process, good leaving groups on activated acceptors; indeed, five-membered cyclic dibutylstannylene acetals formed on vicinal *cis*-axial-equatorial pair of hydroxyl groups selectively enhance the nucleophilicity of the equatorial oxygen in *O*-alkylation reactions.[29] This method does not involve any formation of the oxacarbenium ion as intermediate and the stereospecific formation of 1,2-*cis*-glycosidic linkage occurs because 1,2-*O*-stannylene acetals of sugars favour the *cis*-arrangement around the anomeric centre (**Scheme 2.10)**.



Scheme 2.10: 1,2-*cis* stannylene acetal arrangement

Sometimes epimerization can occur at *C*-2 during dibutylstannylene complex formation; this process can be minimized by conducting the acetalation under milder conditions (diluted solution and low temperature).[30] Alternatively, one can profit by this to obtain rare sugars.[31] Another problem which can occur by using this strategy is the isomerization of the acetal ring from 1,2-position to 2,3-position that gives formation of undesired disaccharide; this can be prevented by using 3-*O*-protected analogues. Besides these disadvantages, one of the great aspects of this method is that specific protection of hydroxyl groups on the glycosyl donor is not necessary. This procedure achieves excellent stereocontrol with both primary and secondary triflates, however it is more effective with rhamnose than with mannose.

One of the most elegant-considered protocol to achieve selective β-mannosylations/β-rhamnosylations is “intramolecular aglycone delivery” method (IAD). It is based on an intramolecular glycosylation which has initially been employed in the stereocontrolled synthesis of a β-*C*-mannoside[32] and then extended to the synthesis of *O*-linked β-mannosides. This strategy makes use of a β-manno-configurated donor bearing the acceptor already linked to an acetal at *C*-2 **(Scheme 2.11**). Successive activation of the donor releases the aglycone which is structurally disposed to direct S_N2-like substitution at the anomeric centre affording then the β-mannoside.



Scheme 2.11: Theoretical approach[33] of intramolecular aglycone delivery method

However, these described herein are just few of the numerous methods developed in the last decades to solve out the challenging problem of β-selective mannosylations and rhamnosylations, which is still outstanding for carbohydrate chemists.

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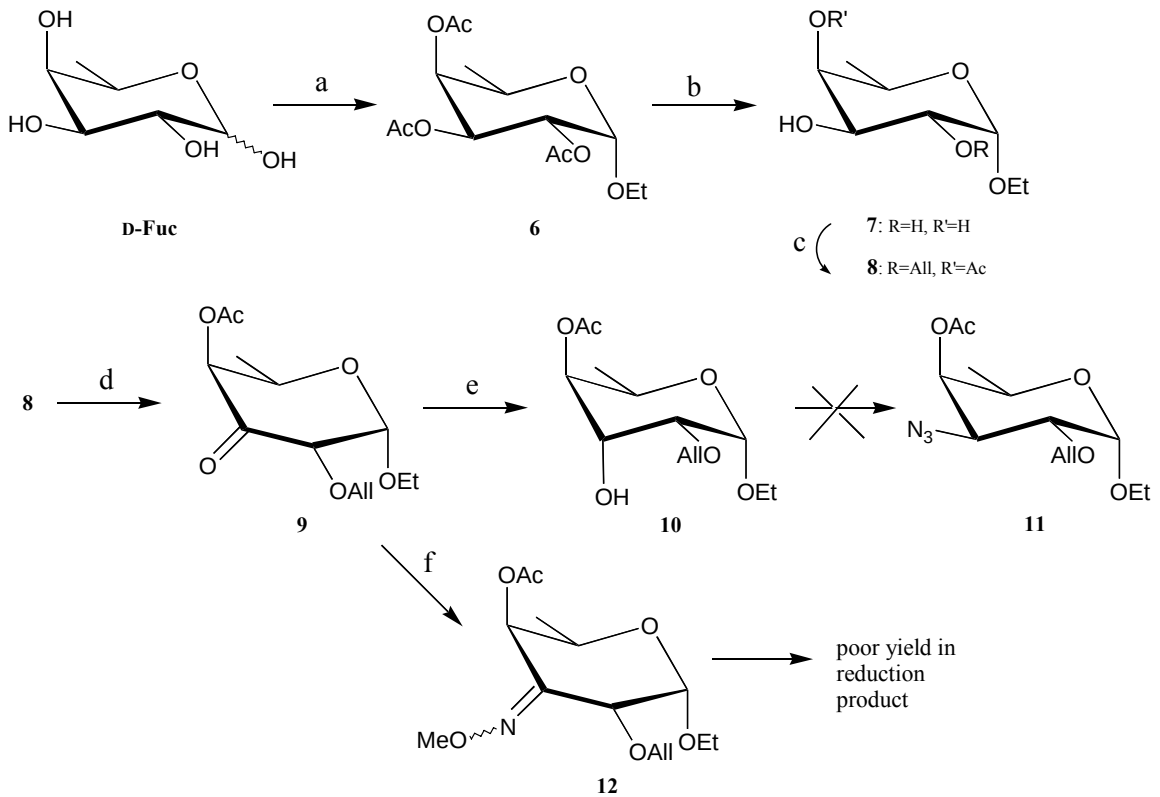
3. DISCUSSION AND RESULTS

In this section first syntheses of building blocks and three oligosaccharide repeating units from phytopathogenic LPS O-chains are described. It is noteworthy that all the syntheses described herein have never been accomplished before and the strategies employed make use of orthogonal protecting group patterns, which could allow, in the future, the further elongation of the targets.

3.1 Synthesis of 3-acetamido-3,6-dideoxy-D-galactopyranosyl donors and related tests of glycosylation[1,2]

The first structure to be considered was the 3-acetamido-3,6-dideoxy-D-galactopyranose (D-Fuc*p*3-NAc) which is one of the most frequently sugar unit found in phytopatogenic Gram-negative bacterial O-chains (see **Figure 1.3**).[3] An interesting aspect of its natural distribution is that it is always present with an α-configurated linkage imposing some structural restriction in its synthesis. At the first time two conventional routes to convert an alcoholic function to an amino one were chosen but they proved to be unsuccessful. The initial idea was to perform a S_N2 displacement of an epimerized activated hydroxyl function at *C*3, to get the azido-sugar with the right configuration, by using sodium azide as nucleophile. In order to obtain the deoxygulose derivative (antiarose), the planned strategy involved the stereoselective reduction of a 3-keto derivative. Thus, starting from D-fucose, intermediate **6** was obtained by a sequence of Fischer glycosylation, acetylation and α-anomerization with $FeCl_3$[4] (**Scheme 3.1**). The enrichment of the anomeric mixture with the α-anomer would favour the subsequent stereoselective reduction of the 3-keto derivative: reasonably, the steric hindrance of the axial ethyl group at the anomeric position would force the hydride anion to attack from the pseudo-equatorial side of the carbonyl. Thus, after deacylation of **6** to obtain triol **7,** the synthetic strategy would require the selective protection of the hydroxyl functions at position *C*2 and *C*4 of the sugar; a useful procedure to obtain regioselectively and orthogonally protected building blocks is the one developed by Binkley and co-workers[5] and improved by Field and co-workers.[6] This procedure involves several reaction steps (orthoesterification, alkylation, orthoester hydrolysis) in a single pot and purification only at the final stage by chromatography. The strategy significantly reduces the time for making building blocks, and overall yields are higher compared to

those obtained by stepwise reactions. Thus, a one-pot sequence of orthoesterification, allylation and orthoester regioselective opening afforded alcohol **8** in 51% yield over three steps. Moffatt oxidation conditions[7] on **8** afforded the ketone derivative **9** which was then reduced with $NaBH_4$ at 0 ºC to obtain the deoxygulose sugar **10** in 55% yield and excellent stereoselectivity. Compound **10** was then activated as triflate which was treated with sodium azide; unfortunately, this reaction produced a complex mixture in which elimination products prevailed instead of the azide substituted D-fucose. Actually, difficulties in nucleophilic displacement of 3-OTf-gulose derivatives have been reported,[8,9] and they have been solved by using a 4,6-*O*-benzylidene ring[8] and/or a 2-*O*-acyl protecting group.[9] Both methods, which minimize elimination reactions, nevertheless were considered to be not very useful for our scope, due to the impossible installation of a 4,6-*O*-benzylidene on a 6-deoxysugar and the necessity of avoiding the use of 2-*O*-acyl protecting group in the synthesis of a D-Fuc*p*3NAc donor so to favour α-stereoselectivity in subsequent glycosylations.

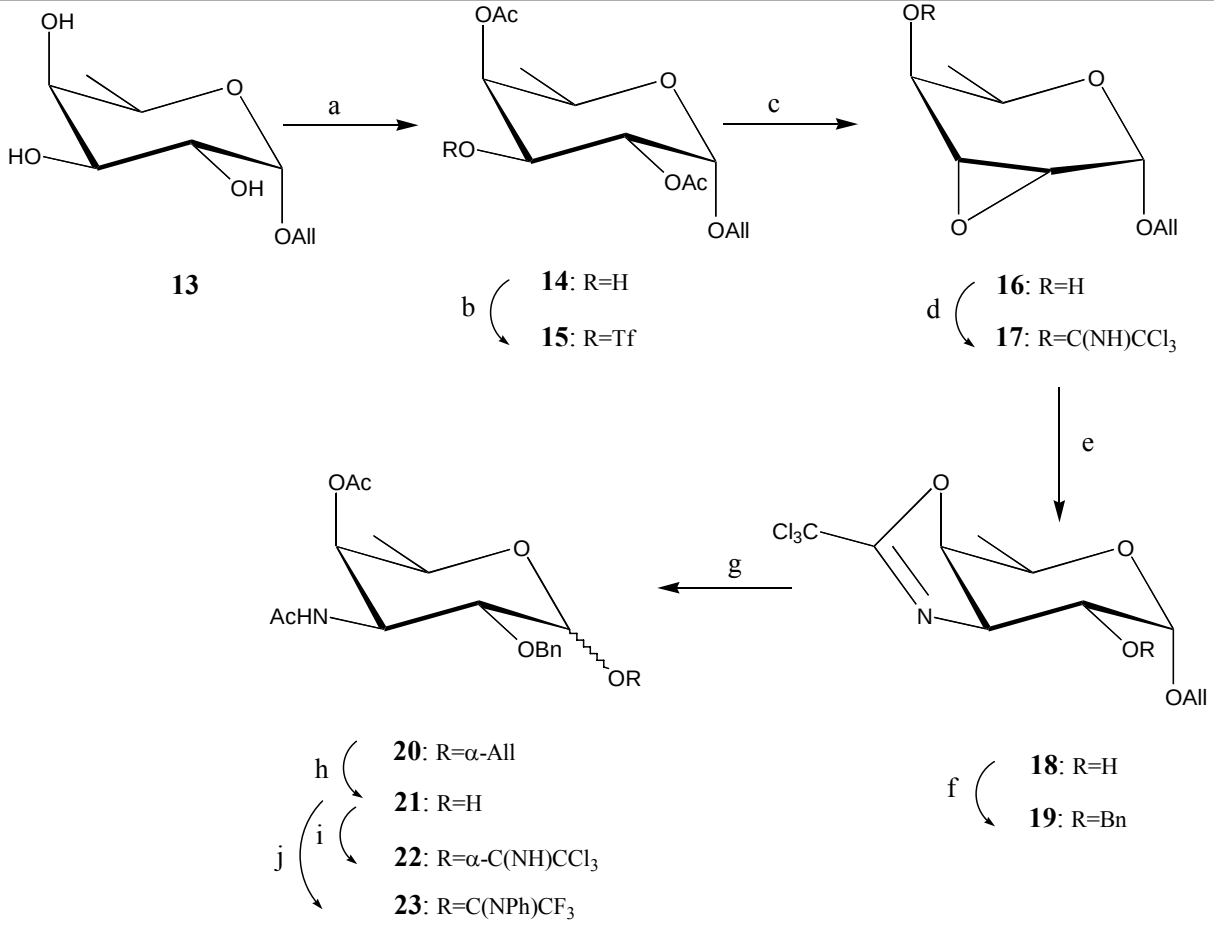


***Reagents and conditions**:* (a) i. EtOH, Amberlist-15 (H^+), reflux, ii. Ac_2O, pyridine, rt, iii. $FeCl_3$, CH_2Cl_2, rt, 56% over three steps; (b) NaOMe, MeOH, rt, 87%; (c) i. trimethyl orthoacetate, CSA, DMF, 40°C, ii. NaH, AllBr, rt, iii. 80% AcOH, rt, 51% over three steps; (d) 2:1 DMSO/Ac_2O, rt; (e) $NaBH_4$, 9:1 THF/MeOH, 0°C, 55% over two steps from **3**; (f) $NH_2OMe.HCl$, 64% over two steps from **3**.

Scheme 3.1: Ineffective attempts in synthesis of D-Fuc*p*3-NAc building block

Trying to use anyway the keto-intermediate **9**, we decided to convert it into its *O*-methyloxime derivative to be successively reduced.[10] Thus, compound **9** was treated with $NH_2OMe{\cdot}HCl$ giving intermediate **12** on which, unfortunately, reduction conditions were not successful to obtain the desired 3-amino compound.

In carbohydrate chemistry, formation of trichloromethyl ozaxolines has already been experimented on allylic trichloroacetimidate[11] and bis(trichloroacetimidate) since 1983.[12] Even though formation of a trichloromethyloxazoline by means of an intramolecular cyclization has been accomplished on open-chain compounds,[13] its application on saccharidic compounds is not widely reported.[14] Thus, we thought to use this tool to put an amino function with the right configuration at fucose-*C*3 by means of an intramolecular cyclization of a 2,3-epoxytrichloroacetimidate derivative.



***Reagents and conditions**:* (a) i. trimethyl orthoacetate, CSA, DMF, 40°C, ii. Ac_2O, pyridine, rt, iii. AcOH 80%, rt, 58% over three steps; (b) Tf_2O, 1:1 CH_2Cl_2/py, 0°C; (c) NaOMe, MeOH, rt; (d) Cl_3CCN, DBU, CH_2Cl_2, 0°C; (e) silica gel (0.063-0.200mm), $CHCl_3$, 45°C, *in vacuo*, 64% over four steps from **9**; (f) BnBr, NaH, DMF, rt, 68%; (g) i. 1M HCl, THF, rt, ii. Ac_2O, py, rt, 63% over two steps; (h) $PdCl_2$, 1:1 CH_2Cl_2/MeOH, rt, 84% (α:β=1:1.5 as determined by ^1H NMR analysis); (i) Cl_3CCN, DBU, CH_2Cl_2, 0°C, 53%; (j) $CF_3C(NPh)CCl$, NaH, molecular sieves 4Å, CH_2Cl_2, 0°C, 67% (α:β=3:1 as determined by ^1H NMR analysis).

Scheme 3.2: Synthesis of D-Fuc*p*3-NAc donor

According to a published procedure, the commercially available D-galactose was converted into allyl α-D-fucopyranoside **13,**[15] which then underwent an one-pot sequence of three reactions to obtain the alcohol **14** regio- and stereo selectively (**Scheme 3.2**). After activation of **14** by triflation, Zemplèn conditions afforded the 2,3-epoxyalcohol **16** which was subsequently converted to the epoxytrichloroacetimidate **17** by treatment with Cl_3CCN and DBU. The crucial step of the strategy was the intramolecular cyclization of **17** which was simply performed by adsorption on silica gel at 45 °C in vacuo, bypassing Lewis acids catalysis which is generally required in the mechanism.[13,14,16] Purification of the product, gave the oxazoline derivative **18** in 64% yield (after four steps from **14)**. To satisfy the structural requirements of the target and to favour subsequent α-glycosylations, **18** was then protected with a benzyl group (non-participating protecting group) at *C*2 and with an acetyl group at *C*4, after acid hydrolysis of the fused oxazolinic ring; 4-*O*-acyl protecting group is, actually, supposed to be supportive in α-glycosylations by invoking a long range participation effect.[17] Anomeric deallylation of **20** afforded the hemiacetal **21** which was converted into two different glycosyl donors afterwards. Schmidt and co-workers[18] already reported the effectiveness of trichloroacetimidate (TCAI) as kind of glycosyl donor to achieve high α-selectivity in fucosylations. Thus, treatment of **21** with CH_3CN and DBU gave the triclhoroacetimidate **22** in moderate 53% yield. Since few years similar trihaloacetimidate-type donors, *N*-phenyl trifluoroacetimidates (TFAI), have been exploited in successful glycosylations.[19] Thus, we decide to activate hemiacetal **21** as TFAI-donor by treatment of **21** with $CF_3C(NPh)Cl$ and NaH[20] obtaining **23** with a superior yield (67%).

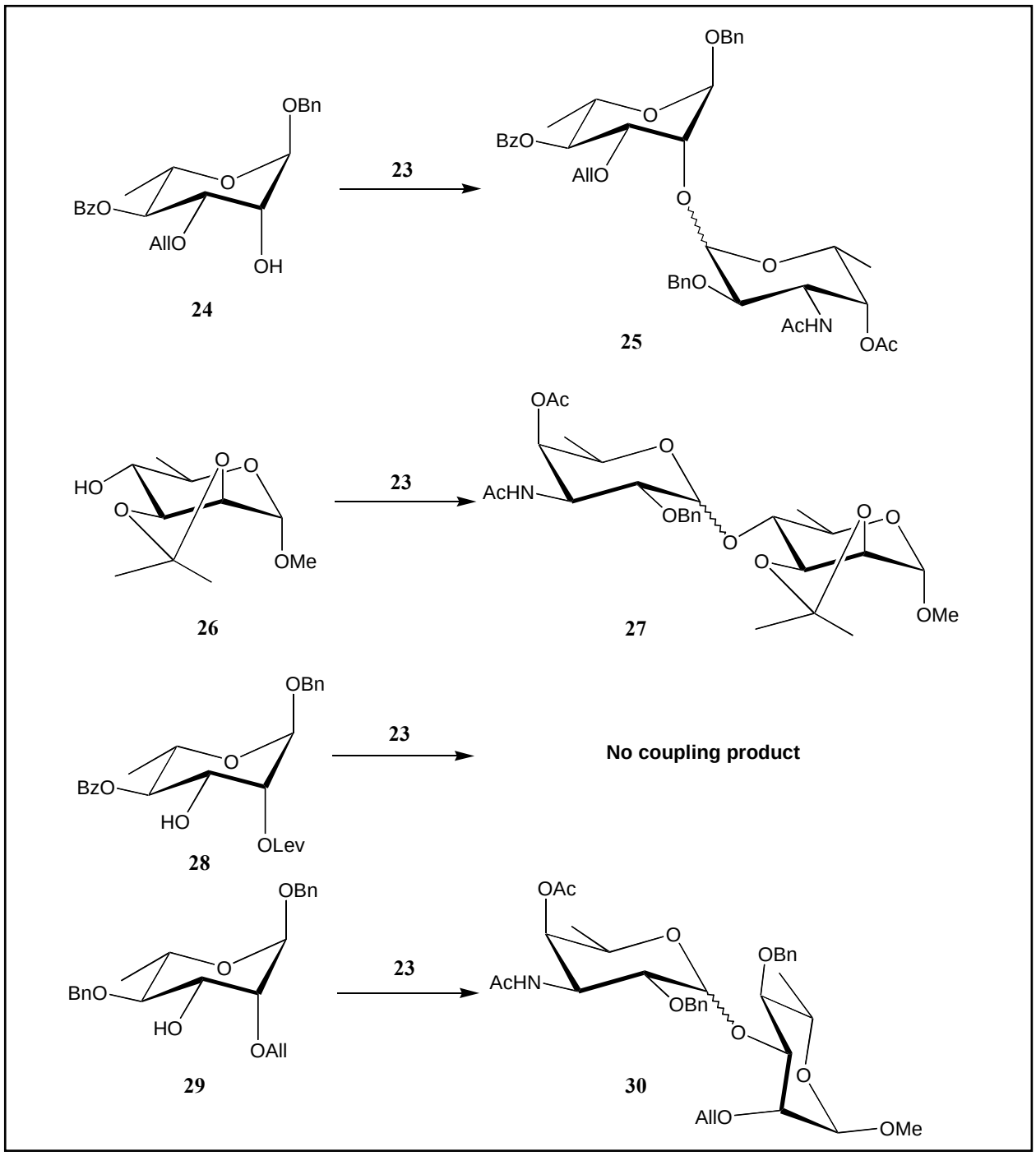
Both glycosyl donors **22** and **23** were tested in their reactivity towards some L-and D-rhamnosyl acceptors in order to open a route to synthesize α-D-Fuc*p*3NAc containing repeating units of O-chain from phytopathogenic bacteria. Trying to synthesize structures as similar as possible to the natural ones, we chose rhamnosyl acceptor **24**[21], **26**[22], **28**[23]and **29**[24] (**Scheme 3.3**) as the resulting disaccharides might be suitable building blocks for the synthesis of different D-Fuc*p*3NAc containing oligosaccharides related to phytopatogenic O-chains. As showed in **Table 3.1** donor **22** was not effective in glycosylation conditions when coupled with acceptors **24** and **26**: actually, no coupling product formation was observed activating **22** in mild conditions ($BF_3{\cdot}OEt_2$) even using stoichiometric amounts of activator in DCM at reflux; on the other hand, a catalytic amount (0.01eq.) of the stronger activator TMSOTf afforded only traces of disaccharide and the decomposition of the donor in few minutes even working at very low temperature (-50 °C).

Entry	Acceptor	Donor	Solvent	Activator	Yield[a] (α/β)	Product
1	**24**	**22** (2.0 eq)	CH_2Cl_2	TMSOTf	traces	**25**
2	**26**	**22** (2.0 eq)	CH_2Cl_2	TMSOTf	traces	**27**
3	**24**	**22** (2.0 eq)	CH_2Cl_2	$BF_3.OEt_2$	no product	////
4	**24**	**23** (2.0 eq)	CH_2Cl_2	TMSOTf	65% (62:38)[b]	**25**
5	**26**	**23** (2.0 eq)	CH_2Cl_2	TMSOTf	61% (68:32)[b]	**27**
6	**26**	**23** (1.5 eq)	Dioxane/DME/toluene 4:1:1	TMSOTf	55% (88:12)[b]	**27**
7	**28**	**23** (1.5 eq)	Dioxane/DME/toluene 4:1:1	TMSOTf	no product	////
8	**29**	**23** (1.8 eq)	CH_2Cl_2	TMSOTf	70% (82:18)[c]	**30**
9	**29**	**23** (1.5 eq)	Dioxane/DME/toluene 4:1:1	TMSOTf	63% (89:11)[c]	**30**

[a] Isolated yield. [b] Measured by ^{1}H NMR. [c] Measured after separation of the two anomers.

Table 3.1: Glycosylations with D-Fuc*p*3N trihaloacetimidate donors

These results prompted us to focus our efforts on the donor **23**. In contrast with previous glycosylation tests, activation of **23** with 0.1 eq. of TMSOTf afforded the desired disaccharides in good yields and without significant glycosyl donor decomposition; however, α-selectivity was not gratifyingly. It is known that electron-donating solvents have a beneficial effect on α-selectivity in glycosylation reactions even if they might affect the yields.[25] Nevertheless, recently Adinolfi and co-workers exploited the effectiveness of an ether-based ternary mixture (dioxane/DME/toluene 4:1:1) as solvent in glycosylations involving armed *N*-phenyl trifluoroacetimidate donors affording stereoselectively 1,2-*cis*-adducts with good yields.[19c,26] We decided, therefore, to make use of this ternary mixture in coupling of **23** with **26**: α-selectivity was noticeably improved despite the negligible decreasing of the yield. However, activation of **23** in coupling with the 2-acyl protected acceptor **28** by using TMSOTf and the ternary mixture as solvent, did not afford any disaccharide product. On the other hand, satisfying results in stereoselectivity and yield were obtained by coupling donor **23** with the ether-type protected acceptor **29**. Definitely, the above results point out that **23** is an efficiently reactive glycosyl donor only towards armed acceptors and α-selectivity of glycosylation can be well controlled by using an α–directing ether-based ternary mixture as solvent for the reaction.



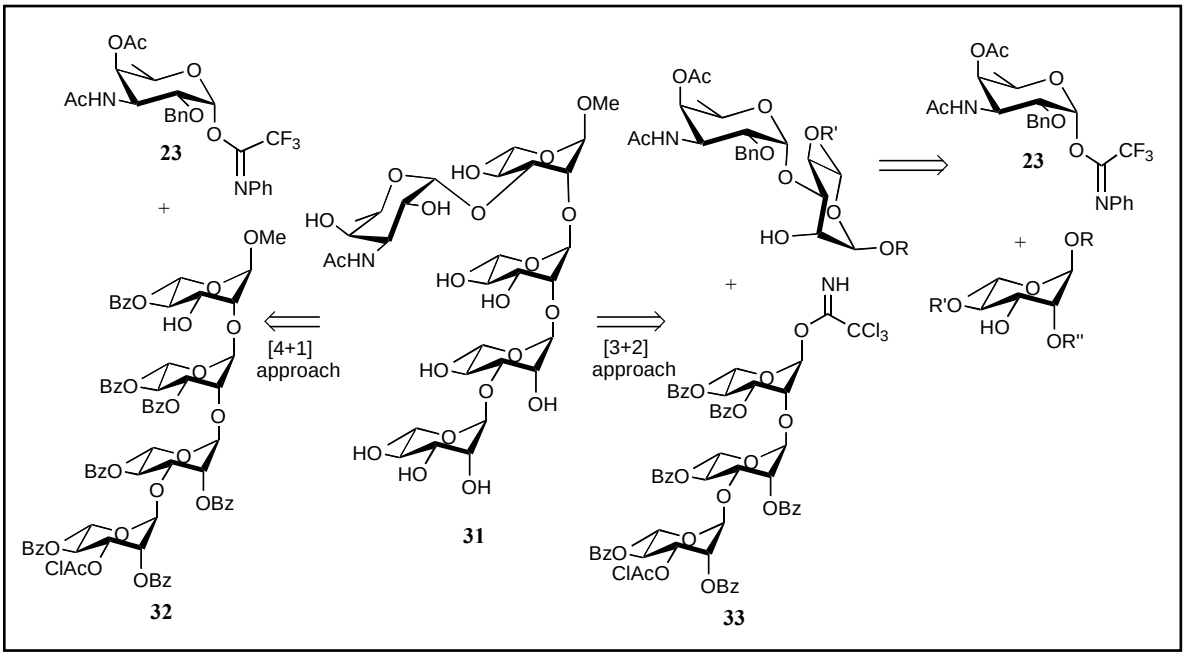
Scheme 3.3 Glycosyl acceptors and products of **Table 3.1** (reaction conditions of the glycosylations are described therein)

3.2 Synthesis of the repeating unit of the *O*-Chain from *Pseudomonas syringae* pv. *holci* IMV 8300[2]

The repeating unit of the major *O*-antigen component from *Pseudomonas syringae* pv. *holci,* a generic phtytopathogenic bacterium,[27] comprises a branched α-D-Fuc*p*3NAc-containing pentasaccharide rhamnanic chain:

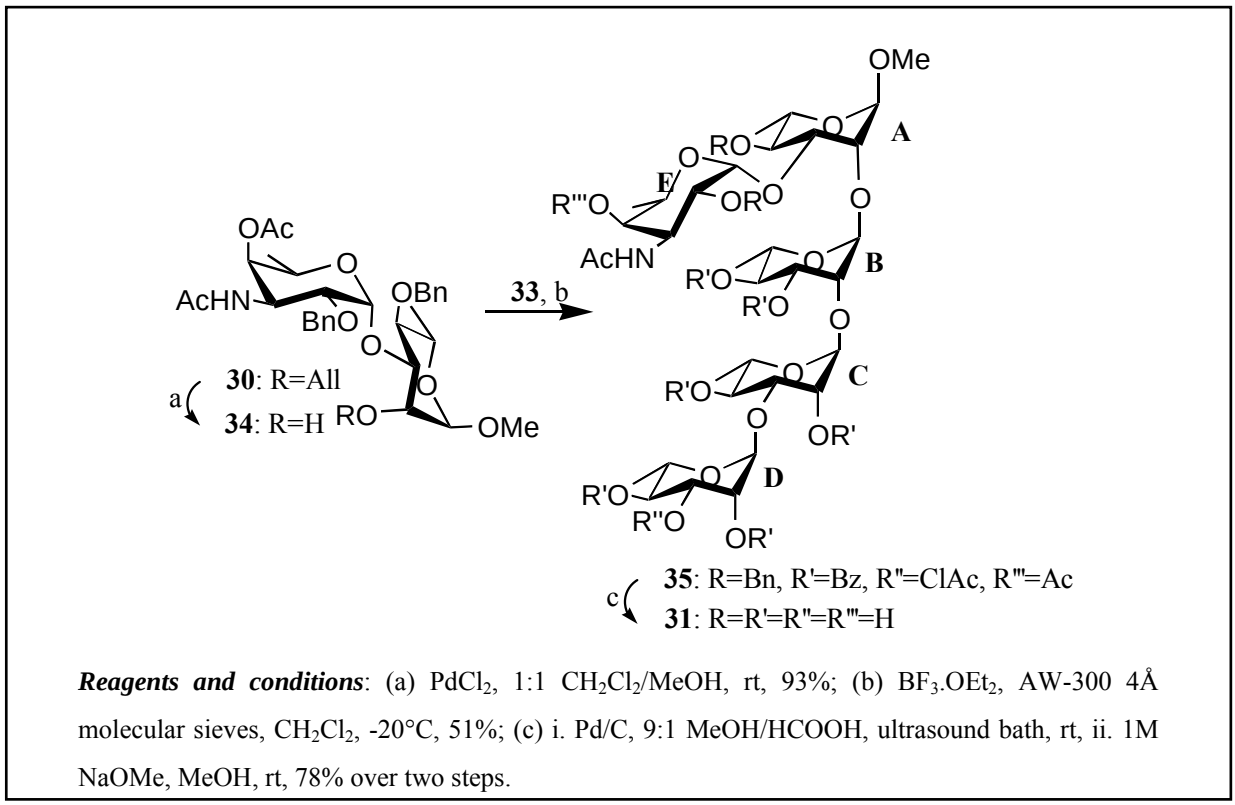
→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→
3
↓
1
α-D-Fuc*p*3NAc

This repeating unit is very similar to the one of the major *O*-antigen component from *Pseudomonas syringae* pv. *ribicola* NVPPB 1010: these two oligosaccharide chains differ each other in the type of side branch, which is a D-Glc*p*-NAc unit in *P. syringae* pv. *ribicola* related structure.[28] The synthesis of this last structure employed a [4+1] coupling highlighting that this strategy requires a large excess of amino sugar donor with a very poorly reactive benzoylated-protected tetrasaccharidic acceptor.[21] Based on this work and on optimized glycosylation conditions found for coupling the D-Fuc*p*3NAc-donor **23** with several rhamnosyl acceptors (see paragraph 3.1), we achieved easily the synthesis of the pentasaccharide showed above. Thus, as donor **23** was not easily synthesized we discarded the coupling between **23** and the linear rhamnosyl tetrasaccharide[21] and turned our attention to the alternative [3+2] approach (**Scheme 3.4**) .



Scheme 3.4: Retrosynthetic analysis of target **31**

As suggested from the target structure, the synthesis requires a 3-OH rhamnosyl acceptor bearing a temporary orthogonal protecting group at *C*2. Compound **30** was chosen for this purpose and as potential disaccharidic acceptor: **30** was treated with $PdCl_2$ affording **34** in 93% yield (**Scheme 3.5**). Even though 2-OH function on acceptor **34** might be considered poorly reactive because of its steric hindrance, the mild $BF_3{\cdot}OEt_2$ was strong enough to perform the coupling between **34** and the trisaccharide donor **33**[21] which was already stored in our laboratories. Moreover, no side product formation due to potential competitive nucleophilic attack by NHAc[29] was observed.



Reagents and conditions: (a) $PdCl_2$, 1:1 CH_2Cl_2/MeOH, rt, 93%; (b) $BF_3.OEt_2$, AW-300 4Å molecular sieves, CH_2Cl_2, -20°C, 51%; (c) i. Pd/C, 9:1 MeOH/HCOOH, ultrasound bath, rt, ii. 1M NaOMe, MeOH, rt, 78% over two steps.

Scheme 3.5: Affording the disaccharide acceptor **34** and target **31.**

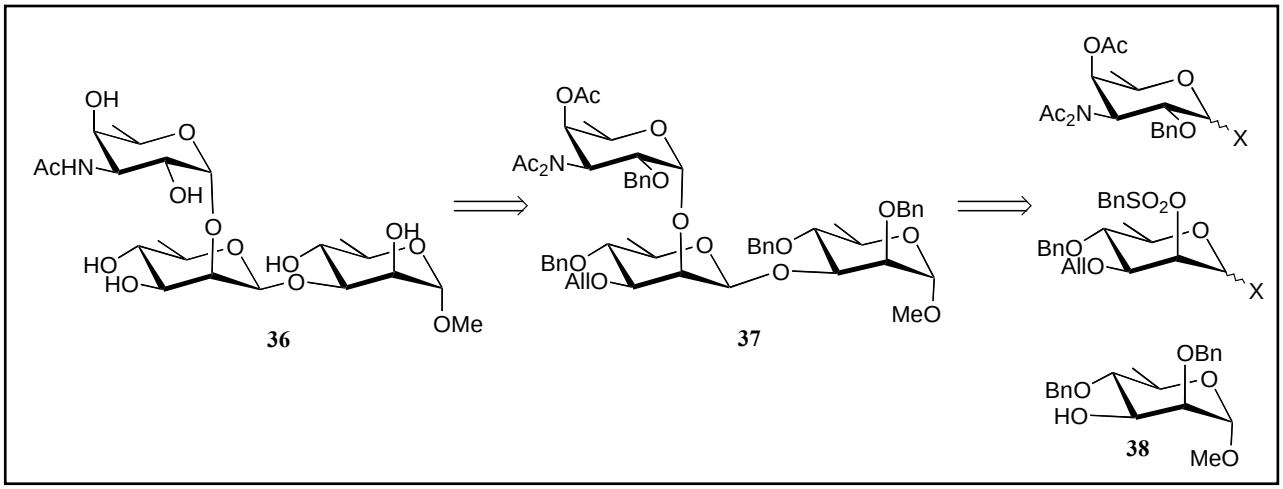
α-Configuration of the new glycosidic linkage was confirmed by the value of the heteronuclear $^1J_{C,H}$ coupling constant (173 Hz). The synthesis was completed by deprotection steps involving a debenzylation by transfer hydrogenation under Perlin conditions[30] and subsequent de-*O*-acylation under Zemplèn conditions; the target, as methyl glycoside, was obtained in 78% yield over the two last steps. It is noteworthy that this have been the first reported synthesis of an Fuc*p*3NAc containing oligosaccharide and is also useful to perform the oligomerization of the repeating unit.

3.3 Synthesis of the β-D-Rhamnosylated trisaccharide repeating unit of the O-Chain from *Xanthomonas campestris* pv. *campestris* 8004[31]

Xanthomonas campestris pv. *campestris* (*Xcc*) strain 8004 is a pathogen of cruciferous crops that is the causative agent of black rot, a disease of worldwide importance.[32] A very recent study has demonstrated the effectiveness of both Lipid-A and core extracted from this bacterium to be active in HR suppression triggering, moreover with two independent mechanisms.[33] The O-chain from *Xcc* 8004 shows a trisaccharide repeating unit consisting of a D-rhamnose disaccharide backbone with a 3-acetamido-3,6-dideoxy-D-galactopyranose (D-Fuc*p*3NAc) unit as branch:[34]

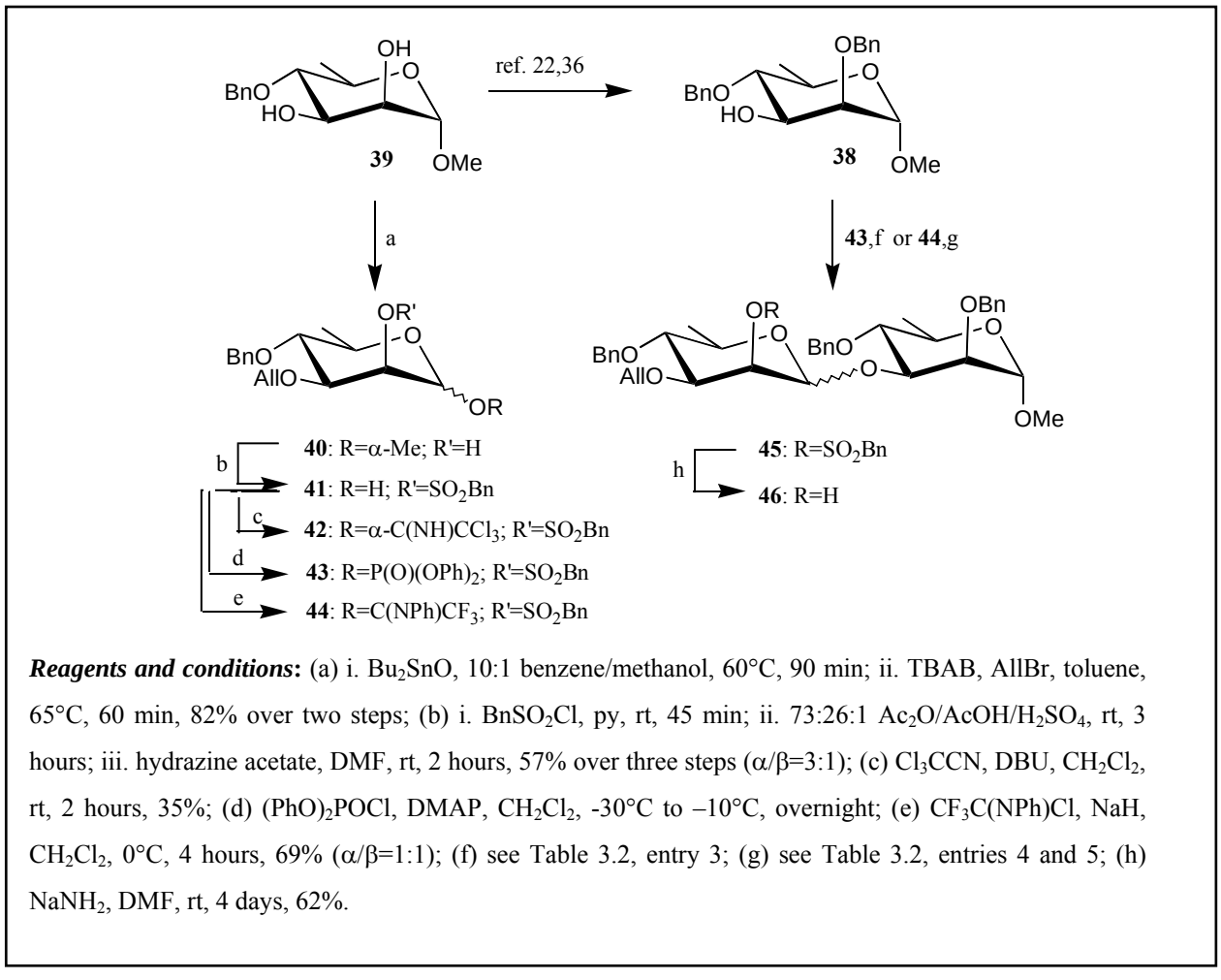
→3)-β-D-Rhap-(1→3)-α-D-Rhap-(1→
2
↑
1
α-D-Fuc*p*3NAc

Also from a synthetic point of view, this is one of the most interesting and challenging structure among phytopathogenic bacteria because of the presence of a β-rhamnosidic linkage, the "steric crowd" on the 1,2-*cis*-diglycosylated D-rhamnose unit and finally the non-commercial availability of both D-rhamnose and D-Fuc*p*3NAc. 1,2-*cis*-diglycosylated moiety present on the β-D-rhamnose unit clearly suggested a synthetic approach, in which the β-D-rhamnosidic linkage is firstly built up to give a rhamnose disaccharide with an orthogonal protecting-group pattern, that allows the selective deprotection on *O*-2_B position and the subsequent α-coupling with a suitable D-Fuc*p*3NAc donor. Retrosynthetic analysis is showed in the following **Scheme 3.6**:



Scheme 3.6: Retrosynthetic analysis of the methyl glycoside of the trisaccharide repeating unit of the *O*-chain from *Xcc* 8004

Among the several protocols developed to build β-rhamnosyl linkages we preferred to explore the possibility to apply a "non-benzylidene requiring" method of β-D-mannosylation to the D-rhamnose series: we decide to use a benzylsulfonyl group, already exploited by Schmidt (see chapter 2), as β-directing and temporary protecting group; beside, a benzylsulfonyl group can be very easily installed on a hydroxyl function and selectively cleaved in presence of ether-based protecting groups.[35] Thus, in order to prepare a suitable 2-*O*-benzylsulfonylated D-rhamnosyl donor, the known methyl 4-*O*-benzyl-α-D-rhamnopyranoside **39**[22] was regioselectively allylated at position *O*-3 with the stannylidene method giving **40** in 82% yield (**Scheme 3.7**) and this alcohol was then subjected to benzylsulfonylation with $BnSO_2Cl$ in pyridine without any intermediate chromatography; subsequent acetolysis and cleavage of the anomeric acetate gave the hemi-acetal **41** in 57% yield after three steps.



***Reagents and conditions*:** (a) i. Bu_2SnO, 10:1 benzene/methanol, 60°C, 90 min; ii. TBAB, AllBr, toluene, 65°C, 60 min, 82% over two steps; (b) i. $BnSO_2Cl$, py, rt, 45 min; ii. 73:26:1 $Ac_2O/AcOH/H_2SO_4$, rt, 3 hours; iii. hydrazine acetate, DMF, rt, 2 hours, 57% over three steps (α/β=3:1); (c) Cl_3CCN, DBU, CH_2Cl_2, rt, 2 hours, 35%; (d) $(PhO)_2POCl$, DMAP, CH_2Cl_2, -30°C to –10°C, overnight; (e) $CF_3C(NPh)Cl$, NaH, CH_2Cl_2, 0°C, 4 hours, 69% (α/β=1:1); (f) see Table 3.2, entry 3; (g) see Table 3.2, entries 4 and 5; (h) $NaNH_2$, DMF, rt, 4 days, 62%.

Schema 3.7: Synthesis of different suitable rhamnosyl donors used in formation of disaccaride acceptor **46**

Conversion of the hemi-acetal into trichloroacetimidate **42** surprisingly proceeded with low yield (35%). This result was explained with the high instability of compound **42**, which was degraded during the chromatographic purification, even when it was performed on a neutral alumina support. Despite this low stability, the coupling of **42** with the D-rhamnose acceptor **38**, which was synthesized in one step from **39** according to the known phase-transfer procedure,[22,36] was attempted, but the total consumption of the donor was observed giving no disaccharide product (**Table 3.2**). In order to have a glycosyl donor, that was effective in glycosylate **38** and not too fastly degradable, alternative glycosylation procedures were investigated. Gin dehydrative coupling[37] between hemi-acetal **41** and acceptor **38** was firstly tested, but it did not proceed at all: no product was detected by TLC analysis, even when the reaction was conducted for 2 days. Actually, ESI-MS analysis revealed the presence of a small peak related to disaccharide formation, which was quantified in less than 10% yield by NMR analysis. Since glycosyl phosphates are known to be β-directing glycosyl donor,[38] hemi-acetal **41** was converted into the diphenyl-phosphate donor **43** by treatment with diphenyl chlorophosphate in CH_2Cl_2 at –10°C in presence of DMAP.[39] Analogously to **42**, **43** demonstrated to be highly unstable by TLC analysis and chromatography on neutral alumina support, which did not allow the recover of any glycosyl phosphate. Thus, crude **43** (α/β=2.5:1) was directly subjected to glycosylation reaction without any chromatographic purification: upon coupling **43** and **44** with stoichiometric TMSOTf in CH_2Cl_2 at -78°C, the desired disaccharide **45** was obtained in 58% yield. The stereoselectivity of the coupling was quite low: the β-disaccharide **45β** was recovered in 31% yield, whereas the α- one in 27% yield. The configuration of the new glycosidic bond in **45α** and **45β** was ascertained by comparing the chemical shifts values of H-3_B and H-5_B, which are upfield shifted in **45β** (H-3: 3.34 ppm; H-5: 3.24 ppm) with respect to **45α** (H-3, H-5: 3.84 ppm). In order to enhance the yield of the coupling, a *N*-phenyl-trifluoroacetimidate was chosen as alternative leaving group on the anomeric position,[19a,b] since it leads to glycosyl donors that are more stable and sometimes also more effective in glycosylation reactions than trichloroacetimidate ones.[40,2] Hemi-acetal **41** was therefore treated with $CF_3C(NPh)CCl$ and NaH[20] to give **44**, after chromatography on neutral alumina, in a rather better yield (69%; α/β=1:1) than **42**. Coupling of **44** with **38** in CH_2Cl_2 at –25°C using catalytic TMSOTf gave **45** in excellent yield and acceptable ratio of anomeric glycosides (99%; α/β=2:3; 59% of isolated **45β**). A slight modification in the solvent mixture (addition of hexane in order to enhance the S_N2 character of the glycosyl acceptor attack on the supposed intermediate glycosyl triflate/oxacarbenium ion)[41] afforded **45** in slightly lower yield (**Table 3.2, entry 5)** and worst β *vs* α selectivity.

Entry	Donor	Acceptor	Solvent	Activator	Temperature	Yield[a] (α/β)[b]
1	**41**	**38**	CH_2Cl_2/toluene 3:1	Tf_2O/Ph_2SO/DTBMP	-78°C to rt	traces
2	**42**	**38**	CH_2Cl_2	TMSOTf	-50°C to rt	no product
3	**43**	**38**	CH_2Cl_2	TMSOTf	-78°C to -15°C	58% (1:1.1)
4	**44**	**38**	CH_2Cl_2	TMSOTf	-60°C to -25°C	99% (2:3)
5	**44**	**38**	CH_2Cl_2/hexane 1:1	TMSOTf	-50°C	95% (1.1:1)

[a] Isolated yield. [b] Measured after isolation of the two anomers

Table 3.2: Glycosylation reactions of acceptor **38**to give disaccharide **45**

Cleavage of the benzylsulfonyl protecting group on **45β** with sodium amide in DMF afforded the disaccharide acceptor **46** (62%).

The installation of the D-Fuc*p*3NAc unit was firstly attempted with the known *N*-phenyl-trifluoroacetimidate **23**, the sole efficient D-Fuc*p*3NAc donor reported to date (see paragraph 3.1): the glycosylation with TMSOTf in an α-stereodirecting ternary solvent mixture (4:1:1 dioxane/DME/toluene)[26] afforded the trisaccharide **47** in only 17% yield (**Table 3.3, entry 1**).

Entry	Donor	Acceptor	Solvent	Activator	Temperature	Yield	Product
1	**23**	**46**	dioxane/DME/toluene 4:1:1	TMSOTf	0°C to rt	17%	**47**
2	**51**	**46**	CH_2Cl_2/Et_2O 1:1	NIS/TfOH	-20°C	traces	**47**
3	**52α**	**46**	CH_2Cl_2/Et_2O 1:1	NIS/TfOH	-20°C	15%	**37**
4	**52β**	**46**	CH_2Cl_2/Et_2O 1:1	NIS/TfOH	-20°C	40% (55%)[a]	**37**

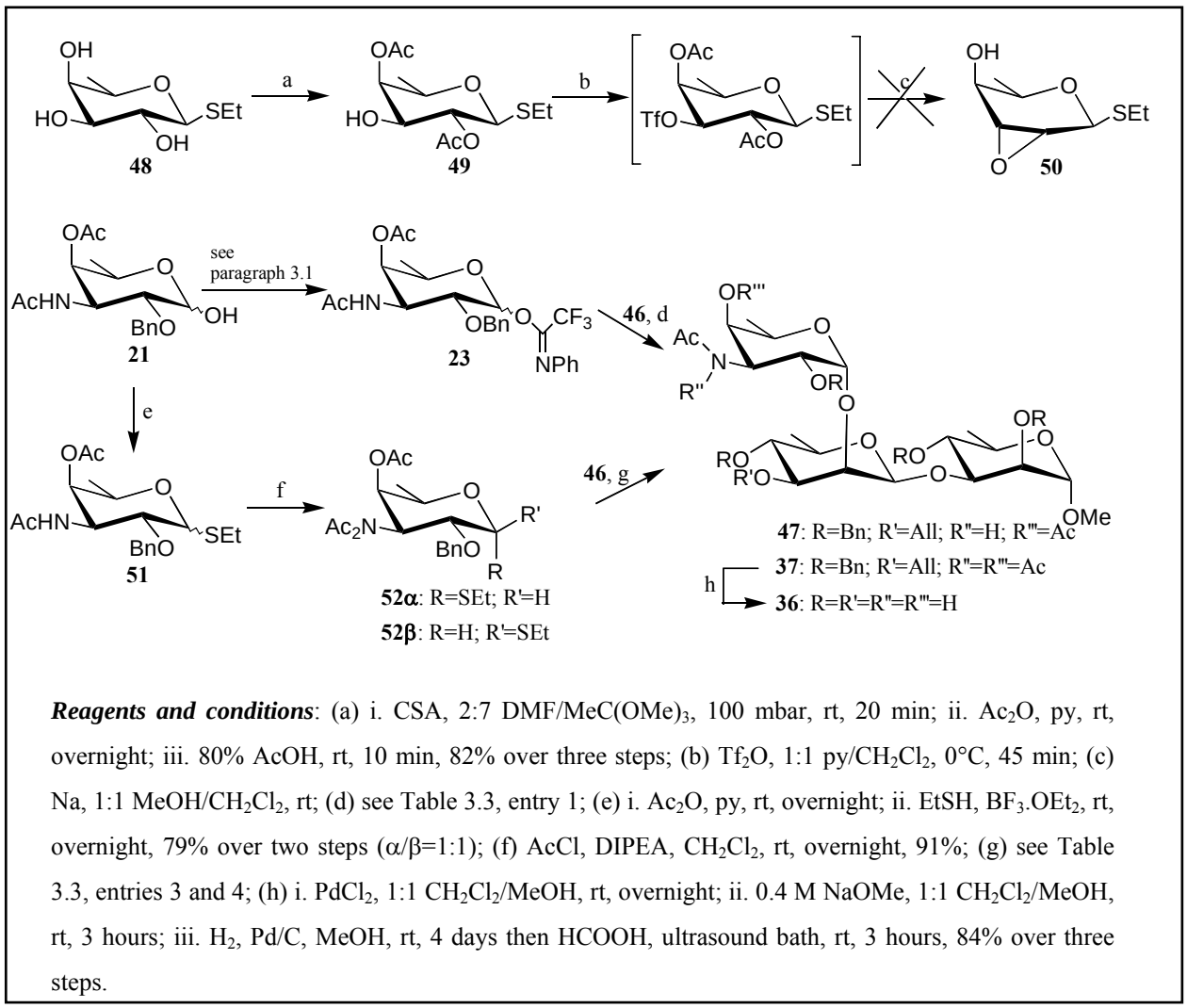
[a]Yield calculated on reacted acceptor

Table 3.3: Glycosylation reactions of disaccharide acceptor **46**

Not surprisingly, coupling between **23** and **46** proceeded with low yield: since **23** has been already demonstrated to glycosylate selectively armed acceptors (see paragraph 3.1), low yield in this case can be ascribed to the nature of the acceptor which might be considered "sterically disarmed".

A different D-Fuc*p*3NAc donor was therefore required: since thiofucosides have been already reported to act as efficient donors in glycosylations in which glycosyl trihaloacetimidates failed,[42] the synthesis of a D-Fuc*p*3NAc thioglycoside was attempted (**Scheme 3.8**). Thus, compound **48**[43] was subjected to a *one-pot* sequence of three reactions (orthoesterification, acetylation and orthoester regioselective opening; 82% over three steps) to afford the alcohol **49**. Unfortunately, the treatment of the triflate derivative of **49** with sodium in methanol gave a complex mixture, in which only traces of the desired 2,3-epoxide **50** were identified; the latter was required for the subsequent

insertion of the 3-amino functionality via the intramolecular cyclization of an α-epoxytrichloroacetimidate (see paragraph 3.1).



Reagents and conditions: (a) i. CSA, 2:7 DMF/$MeC(OMe)_3$, 100 mbar, rt, 20 min; ii. Ac_2O, py, rt, overnight; iii. 80% AcOH, rt, 10 min, 82% over three steps; (b) Tf_2O, 1:1 py/CH_2Cl_2, 0°C, 45 min; (c) Na, 1:1 MeOH/CH_2Cl_2, rt; (d) see Table 3.3, entry 1; (e) i. Ac_2O, py, rt, overnight; ii. EtSH, $BF_3.OEt_2$, rt, overnight, 79% over two steps (α/β=1:1); (f) AcCl, DIPEA, CH_2Cl_2, rt, overnight, 91%; (g) see Table 3.3, entries 3 and 4; (h) i. $PdCl_2$, 1:1 CH_2Cl_2/MeOH, rt, overnight; ii. 0.4 M NaOMe, 1:1 CH_2Cl_2/MeOH, rt, 3 hours; iii. H_2, Pd/C, MeOH, rt, 4 days then HCOOH, ultrasound bath, rt, 3 hours, 84% over three steps.

Scheme 3.8 Synthesis of fucosyl donors **52** and trisaccharide target **36**

For this reason, it was decided to install a thioalkyl group directly on a D-Fuc*p*3NAc building-block, whose position 3 is namely already aminated. Thus, hemiacetal **21** was acetylated and then treated with EtSH/$BF_3.OEt_2$ in CH_2Cl_2 to give the thioglycoside **51** (79%; α/β=1:1 as an inseparable mixture). The NIS/TfOH mediated coupling of this donor and acceptor **46** in 1:1 CH_2Cl_2/Et_2O afforded only traces of the desired α-trisaccharide **47 (Table 3.3)**; this result can be ascribed to the inhibitory effect of the NHAc group on the glycosyl donor, as it has already been reported.[44,29] Thus, in order to enhance the yield, compound **51** was converted into a more efficient D-Fuc*p*3NAc donor: it was treated with AcCl/DIPEA in CH_2Cl_2 to give the *N,N*-diacetylated thioglycoside **52** in a *ca*. 1:1 α/β mixture, which was then easily separated by standard silica gel chromatography (**52α**: 44%; **52β**: 47%). The α-anomer gave the α-trisaccharide **37** in 15% yield, whereas compound **52β**

afforded the same coupling product in higher yield (40%) together with a 27% recovery of unreacted acceptor **46** (55% yield based on reacted **46**) and 10% of compound **47**, whose formation is probably due to an acidic cleavage of the diamide function to NHAc group. The α-configuration of the newly formed glycosydic bond was ascertained by the $^{3}J_{H1\text{-}H2}$ value (3.4 Hz). In comparison with the NHAc group, the presence of a *N,N'*-diacetyl protecting group in **37** does not increase the number of the required deprotection steps, since conventional transesterification with NaOMe on a NAc_2 group retains one *N*-acetyl functionality, that occurs in the natural repeating unit of the *O*-antigen from *Xcc*. Thus, after a first de-*O*-allylation step with $PdCl_2$ in 1:1 MeOH/CH_2Cl_2, Zemplèn deacetylation and subsequent hydrogenolysis afforded the target-compound **36** (84%). Interestingly, hydrogenolysis with Pd/C in MeOH under H_2 atmosphere allowed the cleavage only of two benzyl groups, even after a prolonged period of several days; the complete debenzylation was however accomplished by transfer hydrogenation under Perlin conditions.[30]

It is noteworthy that the proposed synthetic approach yields the orthogonally protected trisaccharide building-block **37**, whose allyl protecting group could be regioselectively cleaved to give a trisaccharide acceptor. This one would allow its further elongation to higher oligosaccharide fragments of the *O*-antigen from *Xcc*, suitable, as **36**, for phytopathological structure-activity studies.

3.4 Synthesis of the repeating unit of the O-Chain from *Pseudomonas syringae* pv. *syringae* (*cerasi*) 435[45]

The O-Chain from *Pseudomonas syringae* pv. *cerasi* 435, a generic phytopatogenic bacteria,[46] consists of an α-linked D-rhamnose branched tetrasaccharide repeating unit, which is shown below:[47]

α-D-Rhap
1
↓
3
→3)-α-D-Rhap-(1→3)-α-D-Rhap-(1→2)-α-D-Rhap-(1→

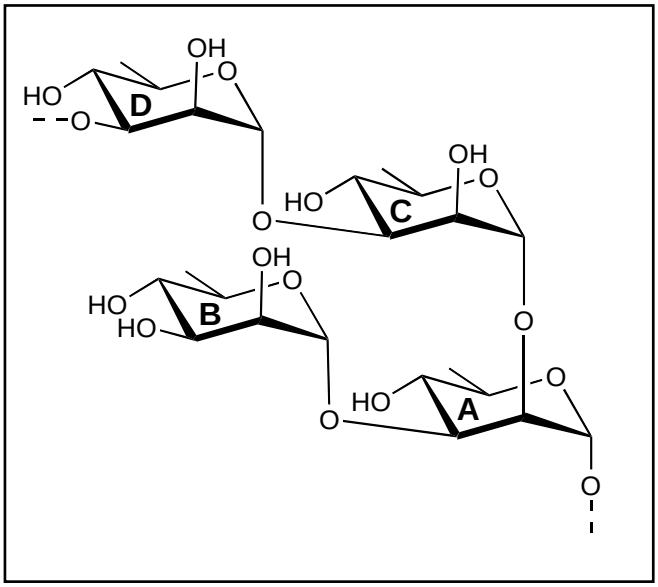
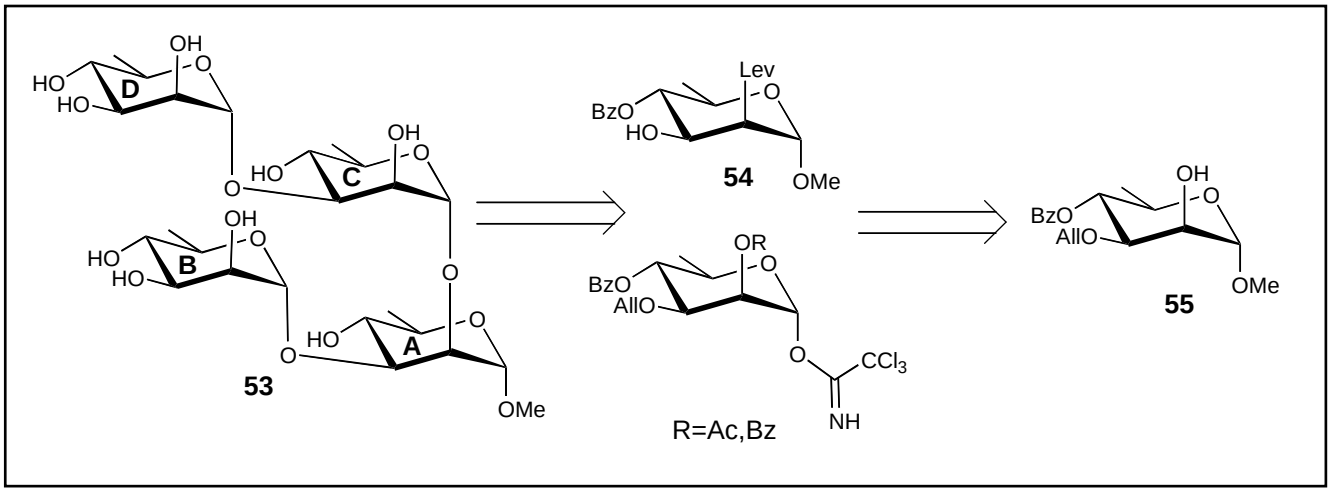


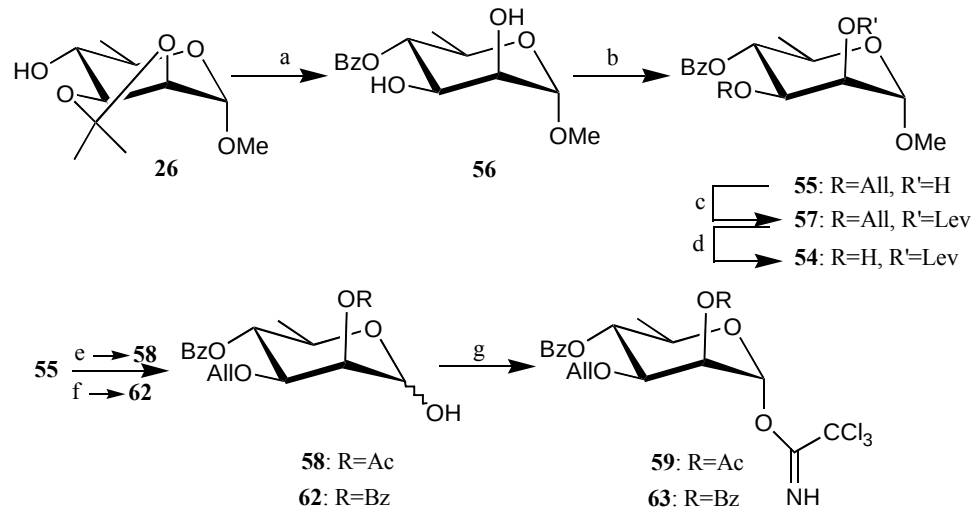
Figure 3.1: Repeating unit of the O-Chain from *Pseudomonas syringae* pv. *cerasi* 435

The structure suggests a strategy which makes use of a D-rhamnose building-block which would be suitably functionalized during the synthesis in order to convert it into both a glycosyl-donor and a glycosyl-acceptor. On purpose, we chose a D-rhamnose unit bearing a selectively removable 3-*O*-protecting group, such as methyl 3-*O*-allyl-4-*O*-benzoyl-α-D-rhamnopyranoside **55** (**Scheme 3.9**). Again, the "ambitious" aim to have an oligomerizable tetrasaccharide structure imposes the condition to employ an orthogonal protecting group pattern; more precisely, we need to differentiate residues B and D of the target relating to their 3-*O*-position. The following scheme shows retrosynthetic analysis of the target as α-methyl- glycoside:



Scheme 3.9: Retrosynthetic analysis of tetrasaccharide **53**

Benzoylation and ketal cleavage performed on the known methyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside **26**[48] afforded the diol **56** which was selectively allylated at 3-*O* position by the well-tested stannylene-mediated method (**Scheme 3.10**).[49] To synthesize the glycosyl acceptor, intermediate **55** was then converted into a 3-OH-rhamnosyl unit bearing a selectively removable protecting group at 2-*O* position. Levulinoyl protecting group was chosen as it is reported to be not prone to acyl migration[23] when it is adjacent to alcohol functions; on the contrary, in a first attempt a chloroacetyl group showed to be inconvenient for the same purpose because of its migration from 2-*O* to 3-*O* position after palladium-catalyzed deallylation. Thus, acceptor **54** was obtained from compound **55** by treatment with LevOH in the presence of *N*,*N*'-diisopropylcarbodiimide (DIPC) and 4-dimethylaminopyridine (DMAP) and subsequent deallylation with $PdCl_2$.

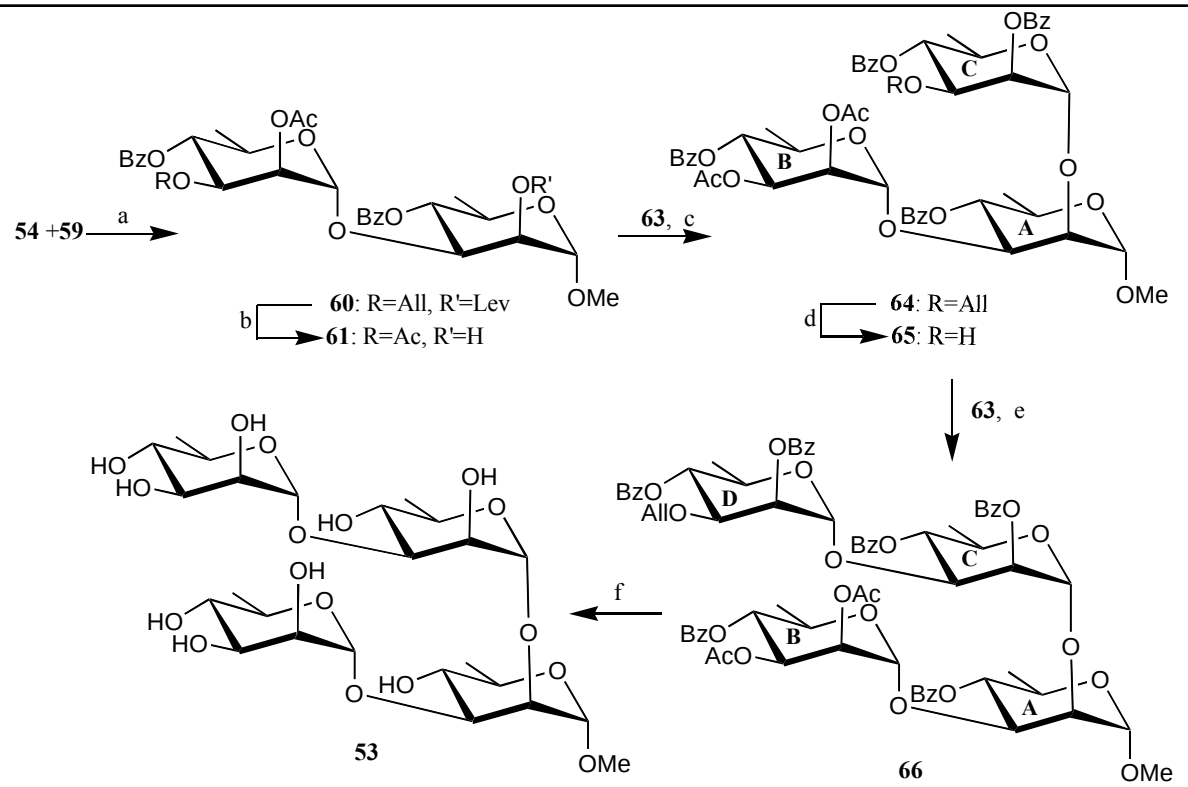


Reagents and conditions: (a) i: BzCl, pyridine, 0°C, 60 min; ii: 4:1 TFA/H_2O, rt, 20 min; 77%; (b) i: Bu_2SnO, 10:1 benzene/MeOH 0°C, 90 min; ii: Bu_4NBr, AllBr, toluene, 65°C, 2 hours; 88%; (c) LevOH, DIPC, DMAP, CH_2Cl_2, rt, 60 min, 79%; (d) $PdCl_2$, 3:2 MeOH/CH_2Cl_2, rt, overnight, 78%; (e) i: 100:40:1 Ac_2O/AcOH/H_2SO_4, rt, 30 min; ii: hydrazine acetate, DMF, rt, 40 min; 59% (α/β=3.5/1); (f) i: BzCl, pyridine, rt, 30 min; ii: 100:40:1 Ac_2O/AcOH/H_2SO_4, rt, 60 min; iii: hydrazine acetate, DMF, rt, 45 min; 58% (α/β=5:1); (g) Cl_3CCN, DBU, CH_2Cl_2, 0°C, 60 min; 72% for **59**, 55% for **63**.

Scheme 3.10: Synthesis of glycosyl donors **37** and **41**

To achieve a suitable glycosyl donor, 2-*O*-acyl-protected hemiacetal **58**, obtained from **55** by a one-pot sequence of two reactions, was converted into the trichloroacetimidate **59** by treatment with Cl_3CCN and DBU. The first coupling of the synthesis concerned donor **59** and acceptor **54** and was performed by $BF_3{\cdot}OEt_2$-mediated activation at -50 °C; it afforded disaccharide **60**(residue A and B of the target) in 86% yield (**Scheme 3.11**). Conversion of **60** into a glycosyl acceptor for the next coupling needed first the displacement of the allyl protecting group with a permanent one because of the requirement of oligomerizability explained above. Thus, compound **60** was treated with $PdCl_2$, acetylated and then deprived of Lev-group to afford alcohol **61** in a very satisfying 87% yield over three steps. However, deallylation of **60** produced a 3-*O*-acetylated side-product which would have been a problem in a later step of the synthesis, more precisely in the formation of a trisaccharide acceptor. For this reason, we prefered to use donor **63** instead than **59** to lengthen the chain, as benzoyl groups are reported to be less prone than acetates to acyl migration;[50] **63** was synthesized similarly to **59** but benzoylation of **55** was required before acetolysis and cleavage of the anomeric acetate. Probably, the presence of a benzoyl group decreases the reactivity of a glycosyl-donor more than an acetyl one as a stronger promoter, such as TMSOTf, was necessary to build the glycosidic linkage between **61** and **63**. Successively, trisaccharide **64** was converted into acceptor **65** which was then coupled with donor **63** in 70% yield.

The synthesis was finally ultimated by deprotecting tetrasaccharide **66** with a de-*O*-allylation first and a Zemplén deacylation afterwards.



Reagents and conditions: (a) $BF_3.OEt_2$, 4Å AW-300 MS, CH_2Cl_2, -50°C, 150 min; 86%; (b) i: $PdCl_2$, 2:1 MeOH/CH_2Cl_2, rt, 4 hours; ii: Ac_2O, pyridine, rt, overnight; iii: hydrazine acetate, 4:1 CH_2Cl_2/MeOH; 87%; (c) TMSOTf, 4Å AW-300 MS, CH_2Cl_2, -50°C, 3 hours; 71%; (d) $PdCl_2$, 3:2 MeOH/CH_2Cl_2, rt, 4 hours; 92%; (e) TMSOTf, 4Å AW-300 MS, CH_2Cl_2, -50°C, 90 min; 70%; (f) i: $PdCl_2$, 1:1 MeOH/CH_2Cl_2, rt, overnight; ii: 3.4M NaOMe, 1:1 MeOH/CH_2Cl_2, rt, overnight; 72%.

Scheme 3.11: Couplings in synthesis of target **53**

All configurations of glycosidic bonds were assigned by measuring heteronuclear $^1J_{C,H}$ coupling constant value in bi-dimensional NMR experiments. Besides, ^{1}H NMR spectrum of synthetic tetrasaccharide **53** was fairly completely coincident (Δppm = ±0.06) with the spectrum of the natural O-chain.[47] More appreciable differences in ^{13}C chemical shifts concern, obviously, positions involved in glycosylations within the natural oligosaccharide; more precisely, in synthetic structure $C3_D$ and $C1_A$ signals are highfield shifted (8.0 ppm and 1.3 ppm respectively) and $C2_A$ signal is downfield shifted in comparison with the values of the natural O-chain, due both to the absence of other linked subunit and the presence of a methoxyl as "storing" protecting group on the $C1_A$ position. The proposed synthetic approach allows the selectively deprotection of the tetrasaccharide so to convert it into a suitable building block for its oligomerization.

3.5 Experimental part

General methods. — ^{1}H (400 MHz; 200 MHz) and ^{13}C NMR (100 MHz; 50 MHz) spectra were respectively recorded on a Bruker DRX-400 or on a Varian XL-200 NMR, in $CDCl_3$ (internal standard, for ^{1}H: $CHCl_3$ at δ 7.26; for ^{13}C: $CDCl_3$ at δ 77.0) or in D_2O (internal standard, for ^{1}H and ^{13}C: $(CH_3)_2CO$ at δ 2.22 and at δ 31.5 respectively). Assignment of proton and carbon chemical shifts were based on 1D and 2D NMR experiments such as COSY, TOCSY, NOESY, HSQC and HMQC-COSY. IR spectra were recorded on a JASCO-FT/IR-430 spectrometer Positive ESI-MS spectra were recorded on a Finnigan LCQ-DECA ion trap mass spectrometer. Optical rotations were measured on a JASCO P-1010 polarimeter. Elementar analysis were performed on a Carlo Erba 1108 instrument. Purification of compound **37** was performed by HPLC with an Agilent 1100 series instrument (Phenomenex Proteo 90A C-18 column, 250x10 mm; 2:2:1 MeOH, CH_3CN, H_2O as eluent). Analytical thin layer chromatographies (TLC) were performed on aluminium plates precoated with Merck Silica Gel 60 F_{254} as the adsorbent. The plates were developed with 5% H_2SO_4 ethanolic solution and then heating to 130°C. Column chromatographies were performed on Kieselgel 60 (63-200 mesh). Gel filtration chromatographies were permorfed on a Sephadex G-10 column (1.0 x 20 cm) with H_2O as eluant. Solvents used were purchased from Fluka and not further purified before use.

Allyl 3-deoxy-4,3-(2-trichloromethyl-1-oxa-3-azaprop-2-eno)-α-D-*fucopyranoside* **(18)**: A solution of **14**(1.34 g, 4.61 mmol) in 1:1 CH_2Cl_2/pyridine (10 mL) was cooled at 0°C and then Tf_2O (1.6 mL, 9.7 mmol) was slowly added. The solution was stirred at 0°C for 40', after that the solution was diluted with CH_2Cl_2 (300 mL) and washed with 1M HCl (300 mL), 1M $NaHCO_3$ (300 mL) and water (300 mL). The organic layer was collected, dried and concentrated to afford an oily residue, that was dissolved in 2:1 MeOH/CH_2Cl_2 (21 mL) and treated with a 0.6 M solution of NaOMe in MeOH (12 mL) at rt. After 2 hours, the solution was diluted with CH_2Cl_2 (350 mL) and washed with water (350 mL). The organic layer was collected, dried and concentrated to afford an oily residue, that was then dissolved in CH_2Cl_2 (13 mL). The solution was cooled at 0°C and then treated with Cl_3CCN (4.5 mL, 44.8 mmol) and DBU (360 μL, 0.72 mmol). After 60' under stirring at 0°C, the solution was concentrated. Silica gel (0.063-0.200 mm) (5.6 g) was then added to the residue, the mixture was suspended in $CHCl_3$ (20 mL) and immediately concentrated *in vacuo* at 45°C. After 10' the solvent was completely evaporated and the solid residue was chromatographed (8:1 petroleum ether/EtOAc) to give **18** (965 mg, 64%) as a yellowish oil. $[\alpha]_D$= +31.7 (c=0.7,

CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.90 (m, 1H, OCH_2CH=CH_2), 5.28 (dd, 1H, J_{vic}= 17.2 Hz, J_{gem}=1.6 Hz, OCH_2CH=CH_2 *trans*), 5.21 (dd, 1H, J_{vic}= 10.4 Hz, J_{gem}=1.6 Hz, OCH_2CH=CH_2 *cis*), 4.85 (dd, 1H, $J_{4,3}$=9.8 Hz, $J_{4,5}$=1.6 Hz, H_4), 4.76 (d, 1H, $J_{1,2}$=4.4 Hz, H_1), 4.72 (dd, 1H, $J_{3,4}$=9.8 Hz, $J_{3,2}$=3.8 Hz, H_3), 4.40-4.28 (m, 3H, H_2, H_5, OCH_2CH=CH_2), 4.13 (m, 1H, OCH_2CH=CH_2), 3.08 (bs, 1H, OH), 1.29 (d, 3H, $J_{6,5}$=6.6 Hz, H_6); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 164.2 (*C*=N), 133.8 (OCH_2CH=CH_2), 117.5 (OCH_2CH=CH_2), 94.6 (C_1), 83.5 (C_4), 68.3, 66.9, 65.7, 64.8 (C_2, C_3, C_5, OCH_2CH=CH_2), 15.9 (C_6). ESI-MS for $C_{11}H_{14}Cl_3NO_4$ (*m/z*): M_r (calcd) 329.00, M_r (found) 351.88 $(M+Na)^+$.

*Allyl 2-O-benzyl-3-deoxy-4,3-(2-trichloromethyl-1-oxa-3-azaprop-2-eno)-α-*D*-fucopyranoside* **(19):**
A solution of **18** (903 mg, 2.74 mmol) in DMF (20 mL) was treated with BnBr (3.4 mL, 28.6 mmol) and NaH (60% oil suspension; 353 mg, 14.7 mmol). The solution was stirred at rt for 90 min, then diluted with CH_2Cl_2 (300 mL) and washed with water (300 mL). The organic layer was collected, dried and concentrated to give a residue, that, after chromatography (11:1 petroleum ether/EtOAc), afforded pure **19** (840 mg, 68%) as a yellowish oil. $[\alpha]_D$= +70.2 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3035, 2944, 1677, 1266 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.36 (m, 5H, H-Ar), 5.91 (m, 1H, OCH_2CH=CH_2), 5.32 (dd, 1H, J_{vic}= 17.2 Hz, J_{gem}=1.6 Hz, OCH_2CH=C*H*H *trans*), 5.20 (dd, 1H, J_{vic}= 10.4 Hz, J_{gem}=1.6 Hz, OCH_2CH=C*H*H *cis*), 4.85 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.76-4.69 (m, 3H, H_1, H_4, OC*H*HPh), 4.55 (dd, 1H, $J_{3,4}$=9.0 Hz, $J_{3,2}$=5.4 Hz, H_3), 4.30 (m, 2H, H_5, OC*H*HCH=CH_2), 4.05 (m, 1H, OC*H*HCH=CH_2), 3.77 (dd, 1H, $J_{2,3}$=5.4 Hz, $J_{2,1}$=4.0 Hz, H_2), 1.33 (d, 3H, $J_{6,5}$=6.6 Hz, H_6); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 164.1 (*C*=N), 138.0 (C_{ipso}), 134.0 (OCH_2CH=CH_2), 128.5 (C-Ar), 117.2 (OCH_2CH=CH_2), 96.1 (C_1), 84.7 (C_4), 74.0, 73.8, 68.7, 66.8, 64.2 (C_2, C_3, C_5, OCH_2CH=CH_2, OCH_2Ph), 16.1 (C_6). ESI-MS for $C_{18}H_{20}Cl_3NO_4$ (*m/z*): M_r (calcd) 419.05, M_r (found) 442.28 $(M+Na)^+$. Anal. calcd.: C 51.39, H 4.79, N 3.33. Found: C 51.55, H 4.70, N 3.32.

*Allyl 4-O-acetyl-3-acetamido-2-O-benzyl-α-*D*-fucopyranoside* **(20):**
To a solution of **19** (689 mg, 1.64 mmol) in THF (10 mL), 1M HCl was added (1.57 mL). The mixture was vigorously stirred at rt for 30 min, after that 1M $NaHCO_3$ (200 mL) was added. Stirring was continued for additional 10 min, then EtOAc (200 mL) was added. The organic layer was collected, dried and concentrated to afford an oily residue that was subsequently dissolved in pyridine (3 mL). The solution was treated with acetic anhydride (3 mL) and stirred at rt overnight. The solution was then concentrated and the residue dissolved in CH_2Cl_2 (100 mL) and extracted with 1M HCl (100 mL) and water (100 mL). The organic layer was collected, dried and

concentrated to afford a residue, that, after chromatography (3:2 petroleum ether/EtOAc), gave pure **20** (403 mg, 63%) as a white foam. $[\alpha]_D$= +126.8 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3055, 2951, 1735, 1664, 1259 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.36 (m, 5H, H-Ar), 5.897 (m, 1H, OCH_2CH=CH_2), 5.35-5.14 (m, 4H, H_4, NH, OCH_2CH=CH_2), 4.95 (d, 1H, $J_{1,2}$=2.7 Hz, H_1), 4.65 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.53 (m, 1H, H_3), 4.47 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.13 (m, 2H, H_5, OCH_2CH=CH_2), 3.97 (m, 2H, OCH_2CH=CH_2), 3.67 (dd, 1H, $J_{2,3}$=11.4 Hz, $J_{2,1}$=2.7 Hz, H_2), 2.06 (s, 3H, OAc), 1.79 (s, 3H, NAc), 1.03 (d, 3H, $J_{6,5}$=6.6 Hz, H_6); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.0 (2 *C*OCH_3), 138.0 (C_{ipso}), 133.7 (OCH_2CH=CH_2), 128.5 (C-Ar), 117.8 (OCH_2CH=CH_2), 95.4 (C_1), 73.1, 72.7, 71.6, 68.4, 64.8 (C_2, C_4, C_5, O*C*H_2CH=CH_2, O*C*H_2Ph), 48.2 (C_3), 23.0, 20.6 (2 CO*C*H_3), 16.1 (C_6). ESI-MS for $C_{20}H_{27}NO_6$ (*m/z*): M_r (calcd) 377.18, M_r (found) 400.37 $(M+Na)^+$. Anal. calcd.: C 63.64, H 7.21, N 3.71. Found: C 63.80, H 7.00, N 3.65.

4-O-Acetyl-3-acetamido-2-O-benzyl-D-*fucopyranose* **(21):**

A suspension of **20** (357 mg, 0.92 mmol) and $PdCl_2$ (27 mg, 0.15 mmol) in 1:1 CH_2Cl_2/MeOH (10 mL) was vigorously stirred at rt for 5 h. The mixture was filtered over a Celite pad, then diluted with CH_2Cl_2 (150 mL) and washed with 5M NaCl (150 mL). The organic layer was collected, dried and concentrated. The resulting residue was chromatographed (1:1 petroleum ether/EtOAc) to afford **21** (270 mg, 84%; α:β=1:1.5) as a yellowish oil. IR (thin film, NaCl) 3517, 3009, 1743, 1666, 1255 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 5.40 (d, 1H, $J_{1,2}$=3.6 Hz, H-1^{α}), 5.27 (d, 1H, $J_{4,3}$=2.8 Hz, H-4^{α}), 5.21 (d, 1H, $J_{4,3}$=2.8 Hz, H-4^{β}), 5.01-4.83 (m, 4H, H-1^{β}, NH^{α}, NH^{β}, OC*H*HPh), 4.76 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.68 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.53 (m, 2H, H-3^{α}, OC*H*HPh), 4.38 (q, 1H, $J_{5,6}$=6.6 Hz, H-5^{α}), 4.17 (m, 1H, H-3^{β}), 3.84 (q, 1H, $J_{5,6}$=6.6 Hz, H-5^{β}), 3.63 (dd, 1H, $J_{2,3}$=10.2 Hz, $J_{2,1}$=3.6 Hz, H-2^{α}), 3.33 (dd, 1H, $J_{2,3}$=10.2 Hz, $J_{2,1}$=7.2 Hz, H-2^{β}), 2.08 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.84 (s, 3H, NAc), 1.79 (s, 3H, NAc), 1.13 (d, 3H, $J_{6,5}$=6.6 Hz, H-5^{β}), 1.07 (d, 3H, $J_{6,5}$=6.6 Hz, H-5^{α}); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.0 (4 *C*OCH_3), 138.1 (C_{ipso}), 137.4 (C_{ipso}), 128.4 (C-Ar), 98.0 (C-1^{α}), 90.5 (C-1^{β}), 76.0, 73.7, 72.8, 72.1, 71.9, 70.2, 65.0, 60.4 (C-2^{α}, C-2^{β}, C-4^{α}, C-4^{β}, C-5^{α}, C-5^{β}, 2 O*C*H_2Ph), 52.0, 47.8 (C-3^{α}, C-3^{β}), 23.2 (2 *C*OCH_3), 21.0, 20.7 (2 CO*C*H_3), 16.5, 16.3 (C-6^{α}, C-6^{β}). ESI-MS for $C_{17}H_{23}NO_6$ (*m/z*): M_r (calcd) 337.15, M_r (found) 360.22 $(M+Na)^+$. Anal. calcd.: C 60.52, H 6.87, N 4.15. Found: C 60.89, H 6.80, N 4.00.

4-O-Acetyl-3-acetamido-2-O-benzyl-α-D-*fucopyranosyl trichloroacetimidate* **(22):**

Compound **21** (77 mg, 0.22 mmol) was dissolved in CH_2Cl_2 (3.0 mL) under an argon atmosphere and to the 0°C cooled solution Cl_3CCN (115 μL, 1.21 mmol) and DBU (3.3 μL, 6.6 μmol) were

added. The solution was stirred at 0°C for 4 h and then concentrated. The resulting residue was chromatographed (1:1 petroleum ether/EtOAc) over neutral alumina gel to afford **22** (57 mg, 53%) as a white foam. $[\alpha]_D$= +99.2 (c=0.8, CH_2Cl_2). IR (thin film, NaCl) 3022, 2979, 1739, 1671 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.60 (s, 1H, N*H*CCl_3), 7.36 (m, 5H, H-Ar), 6.60 (d, 1H, $J_{1,2}$=3.4 Hz, H_1), 5.41 (d, 1H, $J_{4,3}$=2.4 Hz, H_4), 5.02 (d, 1H, $J_{H,NH}$=6.6 Hz, NH), 4.75 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.55 (m, 1H, H_3), 4.46 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.33 (q, 1H, $J_{5,6}$=6.6 Hz, H_5), 3.81 (dd, 1H, $J_{2,3}$=11.4 Hz, $J_{2,1}$=3.4 Hz), 2.08 (s, 3H, OAc), 1.84 (s, 3H, NAc), 1.09 (d, 3H, $J_{6,5}$=6.6 Hz, H_6); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.1, 169.9 (2 *C*OCH_3), 161.4 (Cl_3CC=NH), 137.4 (C_{ipso}), 128.3 (C-Ar), 93.5 (C-1), 72.0, 71.9, 71.8, 67.8 (C_2, C_4, C_5, O*C*H_2Ph), 48.7 (C_3), 23.1, 20.6 (2 CO*C*H_3), 16.3 (C_6). ESI-MS for $C_{19}H_{23}Cl_3N_2O_6$ (*m/z*): M_r (calcd) 480.06, M_r (found) 513.30 $(M+Na)^+$. Anal. calcd.: C 47.37, H 4.81, N 5.81. Found: C 47.60, H 4.89, N 5.75.

*4-O-Acetyl-3-acetamido-2-O-benzyl-*D*-fucopyranosyl N-phenyl-trifluoroacetimidate* **(23):**

A mixture of **21** (251 mg, 0.72 mmol) and freshly powdered 4Å molecular sieves was suspended under argon in CH_2Cl_2 (5 mL) and cooled to 0°C under stirring. CF_3C(NPh)Cl (53 μL, 0.42 mmol) and NaH (60% oil suspension; 17 mg, 0.42 mmol) were added and stirred was continued at 0°C for 3 h, after that the mixture was filtered over Celite and the filtrate concentrated. Neutral alumina (Brockman grade 1) column chromatography (3:2 petroleum ether/EtOAc) on the residue, afforded **23** (277 mg, 74%; α:β=3:1) as a colourless oil. IR (thin film, NaCl) 3040, 1738, 1670, 1656, 1260 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) (α-anomer) δ 7.42-6.73 (H-Ar), 6.60 (m, 1H, H-1), 5.38 (d, 1H, $J_{4,3}$=2.4 Hz, H-4), 4.83 (m, 2H, NH, OC*H*HPh), 4.50 (m, 2H, H_3, OC*H*HPh), 4.28 (q, 1H, $J_{5,6}$=6.6 Hz, H-5), 3.75 (dd, 1H, $J_{2,3}$=10.2 Hz, $J_{2,1}$=3.4 Hz, H-2), 2.07 (s, 3H, OAc), 1.84 (s, 3H, NAc), 1.11 (dd, 3H, $J_{6,5}$=6.6 Hz, H-6); ^{13}C NMR ($CDCl_3$, 50 MHz) (α-anomer) δ 170.1, 169.9 (2 *C*OCH_3), 143.5, 137.4 (2 C_{ipso}), 129.3-119.4 (C-Ar), 92.5 (C-1), 72.3, 71.9, 71.8, 67.9 (C_2, C_4, C_5, O*C*H_2Ph), 48.5 (C_3), 23.2, 20.6 (2 CO*C*H_3), 16.4 (C_6). ESI-MS for $C_{25}H_{27}F_3N_2O_6$ (*m/z*): M_r (calcd) 508.18, M_r (found) 531.38 $(M+Na)^+$. Anal. calcd.: C 59.05, H 5,35; N. 5.51. Found: C 59.10, H 5.45, N 5.43.

General procedure for D-Fuc*p*3NAc couplings in CH_2Cl_2:

A mixture of donor **23** (37 mg, 0.074 mmol) and rhamnosyl acceptor (0.037 mmol) was coevaporated three times with toluene, the residue was then mixed with freshly powdered AW-300 4Å molecular sieves and suspended under argon in CH_2Cl_2 (1.0 mL). The mixture was cooled and stirred at 0°C, TMSOTf (1.2 μL, 7.4 μmol) was added and the temperature was allowed to gradually rise to rt. After completion of the reaction (TLC analysis), the mixture was neutralized by

adding pyridine. The mixture was then filtered over Celite and concentrated to give a residue, that was purified by column chromatography.

General procedure for D-Fuc*p*3NAc couplings in dioxane/toluene/DME 4:1:1 v/v/v:

A mixture of donor **23** (37 mg, 0.074 mmol) and rhamnosyl acceptor (0.049 mmol) was coevaporated three times with toluene, the residue was then mixed with freshly powdered AW-300 4Å molecular sieves and suspended under argon in 4:1 dioxane/toluene (1.5 mL). The mixture was cooled and stirred at 0°C, a 0.025 M DME solution of TMSOTf (0.3 mL , 7.4 μmol) was added and the temperature was allowed to gradually raise to rt. After completion of the reaction (TLC analysis), the mixture was neutralised by adding pyridine. The mixture was then filtered over Celite and concentrated to give a residue, that was purified by column chromatography.

*Benzyl (4-O-acetyl-3-acetamido-2-O-benzyl-*D*-fucopyranosyl)-(1→3)-3-O-allyl-4-O-benzoyl-α-*L*-rhamnopyranoside* **(25):**

See the general procedure for D-Fuc*p*3NAc couplings in CH_2Cl_2 and in dioxane/toluene/DME 4:1:1 v/v/v. IR (thin film, NaCl) 3025, 2980, 2933, 1744, 1666 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.11-7.26 (m, 30H, H-Ar), 5.73 (m, 2H, 2 OCH_2CH=CH_2), 5.50 (t, 1H, $J_{4,3}$=$J_{4,5}$=10.0 Hz, H-4_A^{β}), 5.41 (d, 1H, $J_{4,3}$=1.8 Hz, H-4_B^{α}), 5.35 (t, 1H, $J_{4,3}$=$J_{4,5}$=10.0 Hz, H-4_A^{α}), 5.20-5.10 (m, 5H, H-1_A^{β}, H-4_B^{β}, OC*H*HPh, 2 OCH_2CH=C*H*H *trans*), 5.08-4.97 (m, 4H, H-1_B^{α}, NH^{α}, 2 OCH_2CH=C*H*H *cis*), 4.91 (bs, 1H, H-1_A^{α}), 4.77 (m, 4H, NH^{β}, 3 OC*H*HPh), 4.65 (d, 1H, $J_{1,2}$=7.4 Hz, H-1_B^{β}), 4.61-4.50 (m, 6H, H-3_B^{α}, H-5_B^{α}, 4 OC*H*HPh), 4.12-4.03 (m, 5H, H-2_A^{α}, H-2_A^{β}, H-3_B^{β}, 2 OC*H*HCH=CH_2), 4.01-3.94 (m, 6H, H-3_A^{α}, H-3_A^{β}, H-5_A^{α}, H-5_A^{β}, 2 OC*H*HCH=CH_2), 3.78 (dd, 1H, $J_{2,3}$=10.3 Hz, $J_{2,1}$=3.4 Hz, H-2_B^{α}), 3.69 (q, 1H, $J_{5,6}$=6.4 Hz, H-5_B^{β}), 3.34 (dd, 1H, $J_{2,3}$=10.7 Hz, $J_{2,1}$=7.4 Hz, H-2_B^{β}), 2.078 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.84 (s, 3H, NAc), 1.79 (s, 3H, NAc), 1.29 (m, 6H, H-6_A^{α}, H-6_A^{β}), 1.01 (d, 3H, $J_{6,5}$=6.4 Hz, H-6_B^{α}), 0.98 (m, 6H, $J_{6,5}$=6.4 Hz, H-6_B^{β}); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.1, 169.9 (4 *C*OCH_3), 165.5, 165.4 (*C*OPh), 134.4-128.1 (2 OCH_2CH=CH_2, C-Ar), 116.7, 116.6 (2 OCH_2CH=*C*H_2), 105.8 (C-1_B^{β}), 98.7, 96.6, 96.4 (C-1_A^{α}, C-1_A^{β}, C-1_B^{α}), 76.9, 76.0, 75.8, 74.4, 74.2, 73.6, 73.5, 73.4, 72.6, 71.5, 71.3, 71.2, 70.9, 70.5, 69.1, 69.0, 68.9, 67.3, 66.7, 65.1 (C-2_A^{α}, C-2_A^{β}, C-2_B^{α}, C-2_B^{β}, C-3_A^{α}, C-3_A^{β}, C-4_A^{α}, C-4_A^{β}, C-4_B^{α}, C-4_B^{β}, C-5_A^{α}, C-5_A^{β}, C-5_B^{α}, C-5_B^{β}, 2 O*C*H_2CH=CH_2, 4 O*C*H_2Ph,), 23.0, 22.9, 20.6, 20.4 (4 CO*C*H_3), 17.6, 17.5, 16.3, 16.1 (C-6_A^{α}, C-6_A^{β}, C-6_B^{α}, C-6_B^{β}). ESI-MS for $C_{40}H_{47}NO_{11}$ (*m/z*): M_r (calcd) 717.31, M_r (found) 740.51 $(M+Na)^+$. Anal. calcd.: C 66.93, H 6.60, N 1.95. Found: C 67.10, H 6.47, N 1.99.

*Methyl (4-O-acetyl-3-acetamido-2-O-benzyl-*D*-fucopyranosyl)-(1→3)-2,3-O-isopropylidene-α-*L*-rhamnopyranoside* **(27):**

See the general procedure for D-Fuc*p*3NAc couplings in CH_2Cl_2 and in dioxane/toluene/DME 4:1:1 v/v/v. IR (thin film, NaCl) 3042, 1748, 1680, 1229 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.41-7.26 (m, 10H, H-Ar$^{\alpha,\beta}$), δ 5.73 (d, 1H, $J_{1,2}$=3.5 Hz, H-1$_B^{\alpha}$), 5.33 (d, 1H, $J_{4,3}$=2.2 Hz, H-4$_B^{\alpha}$), 5.14 (d, 1H, $J_{4,3}$=2.2 Hz, H-4$_B^{\beta}$), 4.86 (m, 5H, H-1$_A^{\alpha}$, H-1$_B^{\beta}$, NH$^{\alpha}$, 2 OC*H*HPh), 4.67-4.54 (m, 4H, H-1$_B^{\beta}$, H-5$_B^{\beta}$, NH$^{\beta}$, OC*H*HPh), 4.44 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.40 (m, 1H, H-3$_B^{\alpha}$), 4.31 (t, 1H, $J_{4,3}$= $J_{4,5}$=7.1 Hz, H-3$_A^{\beta}$), 4.24 (t, 1H, $J_{4,3}$= $J_{4,5}$=7.1 Hz, H-3$_A^{\alpha}$), 4.20-4.07 (m, 4H, H-2$_A^{\alpha}$, H-2$_A^{\beta}$, H-3$_B^{\beta}$, H-5$_B^{\alpha}$), 3.72 (m, 2H, H-5$_A^{\alpha}$, H-5$_A^{\beta}$), 3.65 (dd, 1H, $J_{2,3}$=11.4 Hz, $J_{2,1}$=3.5 Hz, H-2$_B^{\alpha}$), 3.56 (dd, 1H, $J_{4,5}$=9.9 Hz, $J_{4,3}$=7.1 Hz, H-4$_A^{\alpha}$), 3.51 (dd, 1H, $J_{4,5}$=9.9 Hz, $J_{4,3}$=7.1 Hz, H-4$_A^{\beta}$), 3.36 (m, 7H, H-2$_B^{\beta}$ OMe$^{\alpha}$, OMe$^{\beta}$), 2.08 (2s, 6H, 2 OAc), 1.85 (s, 3H, NAc), 1.76 (s, 3H, NAc), 1.57 (s, 6H, 2 CH_3), 1.36 (s, 6H, 2 CH_3), 1.34 (d, 3H, $J_{6,5}$=6.2 Hz, H-6$_A^{\alpha}$), 1.24 (d, 3H, $J_{6,5}$=6.6 Hz, H-6$_B^{\beta}$), 1.15 (d, 3H, $J_{6,5}$=6.2 Hz, H-6$_A^{\beta}$), 1.09 (d, 3H, $J_{6,5}$=6.6 Hz, H-6$_B^{\alpha}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.8 (*C*OCH$_3$), 137.6 (C$_{ipso}$), 126.5-125.7 (C-Ar), 104.2 (C-1$_B^{\beta}$), 97.9 C-1$_A^{\alpha}$, C-1$_A^{\beta}$), 94.5 (C-1$_B^{\alpha}$), 82.2, 78.4, 78.2, 76.4, 76.0, 75.8, 75.2, 73.8, 72.3, 72.1, 71.2, 70.5, 67.3, 65.0, 63.7 (C-2$_A^{\alpha}$, C-2$_A^{\beta}$, C-2$_B^{\alpha}$, C-2$_B^{\beta}$, C-3$_A^{\alpha}$, C-3$_A^{\beta}$, C-4$_A^{\alpha}$, C-4$_A^{\beta}$, C-4$_B^{\alpha}$, C-4$_B^{\beta}$, C-5$_A^{\alpha}$, C-5$_A^{\beta}$, C-5$_B^{\alpha}$, C-5$_B^{\beta}$, OC*H*HPh), 54.5 (OMe), 51.8 (C-3$_B^{\beta}$), 47.9 (C-3$_B^{\alpha}$), 27.8 (CH_3), 26.1 (CH_3), 22.9, 22.8, 20.6, 20.5 (4 CO*C*H$_3$), 17.8, 17.5, 16.3, 15.9 (C-6$_A^{\alpha}$, C-6$_A^{\beta}$, C-6$_B^{\alpha}$, C-6$_B^{\beta}$). ESI-MS for $C_{27}H_{39}NO_{10}$ (*m/z*): M_r (calcd) 537.26, M_r (found) 538.20 $(M+H)^+$. Anal. calcd.: C 60.32, H 7.31, N 2.61. Found: C 60.40, H 7.19, N 2.51.

*Methyl (4-O-acetyl-3-acetamido-2-O-benzyl-α-*D*-fucopyranosyl)-(1→3)-2-O-allyl-4-O-benzyl-α-*L*-rhamnopyranoside* **(30):**

See the general procedure for D-Fuc*p*3NAc couplings in CH_2Cl_2 and in dioxane/toluene/DME 4:1:1 v/v/v. (α-anomer) $[\alpha]_D$= +20.4 (c=0.6, CH_2Cl_2). IR (thin film, NaCl) 3019, 2956, 1747, 1665, 1253 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.42-7.27 (m, 10H, H-Ar), 5.87 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.24 (dd, 1H, J_{vic}= 17.3 Hz, J_{gem}=1.7 Hz, OCH$_2$CH=C*H*H *trans*), 5.21 (d, 1H, $J_{1,2}$=3.4 Hz, H-1$_B$), 5.14 (dd, 1H, J_{vic}= 10.2 Hz, J_{gem}=1.7 Hz, OCH$_2$CH=C*H*H *cis*), 5.07 (d, 1H, $J_{4,3}$=3.0 Hz, H-4$_B$), 4.86 (d, 1H, J_{gem}=10.5 Hz, OC*H*HPh), 4.81-4.60 (m, 5H, H-1$_A$, H-3$_B$, NH, 2 OC*H*HPh), 4.43 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.27-4.09 (m, 3H, H-5$_B$, OC*H*$_2$CH=CH$_2$), 4.05 (dd, 1H, $J_{3,4}$=8.7 Hz, $J_{3,2}$=2.7 Hz, H-3$_A$), 3.79 (bs, 1H, H-2$_A$), 3.71 (m, 2H, H-2$_B$, H-5$_A$), 3.61 (t, 1H, $J_{4,3}$= $J_{4,5}$=9.3 Hz, H-4$_A$), 3.33 (m, 3H, OMe), 2.04 (s, 3H, OAc), 1.79 (s, 3H, NAc), 1.37 (d, 3H, $J_{6,5}$=6.0 Hz, H-6$_A$), 0.78 (d, 3H, $J_{6,5}$=6.6 Hz, H-6$_B$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9 (*C*OCH$_3$), 138.1, 138.0 (2 C$_{ipso}$),

134.9 ($OCH_2CH=CH_2$), 129.6-127.7 (C-Ar), 117.2 ($OCH_2CH=CH_2$), 98.9 ($C\text{-}1_A$), 93.6 ($C\text{-}1_B$), 79.8, 75.5, 74.4, 72.9, 72.8, 72.2, 71.6, 68.1, 68.0, 65.1 ($C\text{-}2_A$, $C\text{-}2_B$, $C\text{-}3_A$, $C\text{-}4_A$, $C\text{-}4_B$, $C\text{-}5_A$, $C\text{-}5_B$, OCH_2Ph, $OCH_2CH=CH_2$), 54.7 (OMe), 48.1 ($C\text{-}3_B$), 23.1, 20.6 (2 $COCH_3$), 18.1, 15.9 ($C\text{-}6_A$, $C\text{-}6_B$). ESI-MS for $C_{34}H_{45}NO_{10}$ (*m/z*): M_r (calcd) 627.72, M_r (found) 650.50 $(M+Na)^+$. Anal. calcd.: C 65.05, H 7.23, N 2.23. Found: C 65.20, H 7.19, N 2.32

*Methyl (4-O-acetyl-3-acetamido-2-O-benzyl-α-*D*-fucopyranosyl)-(1→3)-4-O-benzyl-α-*L*-rhamnopyranoside* **(34):**

A mixture of compound **30-α** (56 mg, 0.089 mmol) and $PdCl_2$ (7.8 mg, 44 μmol) was suspended in 1:1 CH_2Cl_2/MeOH (2.0 mL) under vigorous stirring. After 4h the mixture was filtered on a Celite pad, then diluted with CH_2Cl_2 (25 mL) and washed with 5M NaCl (30 mL). The organic layer was collected, dried and concentrated to give an oily residue that, after column chromatography (1:2 petroleum ether/EtOAc) afforded **34 (**49 mg, 93%) as an oil. $[\alpha]_D$= +32.3 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3509, 3030, 1741, 1669, 1258 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.49-7.29 (m, 10H, H-Ar), 5.07 (d, 1H, $J_{4,3}$=3.0 Hz, $H\text{-}4_B$), 5.04 (bs, 1H, $H\text{-}1_A$), 4.80-4.71 (m, 4H, $H\text{-}1_B$, 3 OC*H*HPh), 4.53 (m, 1H, $H\text{-}3_B$), 4.44 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.06 (q, 1H, $J_{5,6}$=6.6 Hz, $H\text{-}5_B$), 3.99 (m, 2H, $H\text{-}2_A$, $H\text{-}3_A$), 3.75 (dq, 1H, $J_{5,4}$=9.3 Hz, $J_{5,6}$=6.0 Hz, $H\text{-}5_A$), 3.63 (dd, 1H, $J_{2,3}$=11.4 Hz, $J_{2,1}$=3.3 Hz, $H\text{-}2_B$), 3.52 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.3 Hz, $H\text{-}4_A$), 3.36 (s, 3H, OMe), 2.05 (s, 3H, OAc), 1.79 (s, 3H, NAc), 1.38 (d, 3H, $J_{6,5}$=6.0 Hz, $H\text{-}6_A$), 0.73 (d, 3H, $J_{6,5}$=6.6 Hz, $H\text{-}6_B$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9 (*C*OCH_3), 138.1, 137.1 (2 C_{ipso}), 128.7-127.5 (C-Ar), 99.9 ($C\text{-}1_A$), 93.3 ($C\text{-}1_B$), 79.3, 77.1, 75.5, 73.1, 73.0, 72.7, 67.6, 67.2 ($C\text{-}2_A$, $C\text{-}2_B$, $C\text{-}3_A$, $C\text{-}4_A$, $C\text{-}4_B$, $C\text{-}5_A$, $C\text{-}5_B$, 2 O*C*H_2Ph), 54.6 (OMe), 48.1 ($C\text{-}3_B$), 23.1, 20.5 (2 $COCH_3$), 17.9 ($C\text{-}6_A$), 15.8 ($C\text{-}6_B$). ESI-MS for $C_{31}H_{41}NO_{10}$ (*m/z*): M_r (calcd) 587.27, M_r (found) 610.00 $(M+Na)^+$. Anal. calcd.: C 63.36, H 7.03, N 2.38. Found: C 63.55, H 6.96, N 2.34.

*Methyl (2,4-di-O-benzoyl-3-O-chloroacetyl-α-*L*-rhamnopyranosyl)-(1→3)-(2,4-di-O-benzoyl-α-*L*-rhamnopyranosyl)-(1→2)-(3,4-di-O-benzoyl-α-*L*-rhamnopyranosyl)-(1→2)-[4-O-acetyl-3-acetylamino-2-O-benzyl-α-*D*-fucopyranosyl-(1→3)]-4-O-benzyl-α-*L*-rhamnopyranoside* **(35):**

A mixture of **34** (20 mg, 34.1 μmol) and **33**(66 mg, 50.8 μmol) was coevaporated three times with toluene, the residue was then mixed with freshly powdered AW-300 4Å molecular sieves, suspended under argon in CH_2Cl_2 (2.0 mL) and stirred at –20°C. $BF_3.OEt_2$ (3.2 μL, 25.4 μmol) was then added. After 24 hours, an additional aliquot of **33** (44 mg, 33.9 μmol) and $BF_3.OEt_2$ (2.1 μL, 16.9 μmol) was added. After an additional day the reaction was quenched with a drop of Et_3N. After filtration over a Celite pad, the mixture was concentrated to give a residue, that, after column

chromatography (1:1 petroleum ether/EtOAc), afforded **35** (30 mg, 51%) as a white foam. $[\alpha]_D$= +113.3 (c=1.0, CH_2Cl_2). IR (thin film, NaCl) 3052, 3028, 1739, 1656, 1249 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.18-7.10 (m, 40H), 5.83 (dd, 1H, $J_{3,4}$=9.6 Hz, $J_{3,2}$=2.8 Hz, H-3_B), 5.72 (d, 1H, $J_{2,3}$=3.0 Hz, H-2_C), 5.59 (t, 1H, $J_{4,3}$=$J_{4,5}$=10.0 Hz, H-4_B), 5.56 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.6 Hz, H-4_C), 5.44 (dd, 1H, $J_{3,4}$=9.8 Hz, $J_{3,2}$=2.8 Hz, H-3_D), 5.34 (m, 2H, H-1_B, H-4_D), 5.18 (m, 3H, H-1_D, H-1_E, H-2_D), 5.11 (d, 1H, $J_{4,3}$=2.8 Hz, H-4_E), 5.06 (bs, 1H, H-1_C), 4.90 (d, 1H, J_{gem}=10.8 Hz, OC*H*HPh), 4.87 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.84 (bs, 1H, H-1_A), 4.76 (d, 1H, J_{gem}=10.8 Hz, OC*H*HPh), 4.63 (m, 2H, H-3_C, NH), 4.50 (m, 1H, H-3_E), 4.42 (d, 1H, J_{gem}=12.0 Hz, OC*H*HPh), 4.34 (bs, 1H, H-2_B), 4.30-4.20 (m, 4H, H-5_B, H-5_C, H-5_D, H-5_E), 4.13 (dd, 1H, $J_{3,4}$=9.6 Hz, $J_{3,2}$=2.8 Hz, H-3_A), 4.08 (bs, 1H, H-2_A), 3.79-3.64 (m, 5H, H-2_E, H-4_A, H-5_A, CH_2Cl), 3.37 (s, 3H, OMe), 1.96 (s, 3H, OAc), 1.54 (s, 3H, NAc), 1.42 (d, 3H, $J_{6,5}$=6.0 Hz, H-6_A), 1.33 (m, 6H, H-6_B, H-6_C), 1.18 (d, 3H, $J_{6,5}$=6.2 Hz, H-6_D), 0.77 (d, 3H, $J_{6,5}$=6.6 Hz, H-6_E); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.9 (*C*OCH_2Cl), 165.4-164.5 (*C*OCH_3, *C*OPh), 138.2, 137.1 (2 C_{ipso}), 133.4-128.0 (C-Ar), 100.5 (C-1_B), 99.8 (C-1_C, $^1J_{C,H}$=173 Hz), 99.6 (C-1_A), 98.9 (C-1_E), 94.2 (C-1_E), 79.8 (C-4_A), 78.7 (C-2_B), 76.6 (C-2_A), 75.7 (C-3_A), 75.5 (O*C*H_2Ph), 74.4 (C-3_C), 73.5 (C-4_C), 72.7 (C-4_E), 72.2 (C-2_E), 72.0 (C-4_B), 71.8 (C-2_C), 71.5 (C-4_D, O*C*H_2Ph), 70.4 (C-3_B), 70.3 (C-3_D), 68.2 (C-5_A), 67.8-67.2 (C-5_B, C-5_C, C-5_D, C-5_E), 55.0 (OMe), 48.1 (C-3_E), 40.2 (CO*C*H_2Cl), 22.9, 20.4 (2 CO*C*H_3), 18.1 (C-6_A), 17.7 (C-6_B, C-6_C), 17.3 (C-6_D), 15.8 (C-6_E). ESI-MS for $C_{93}H_{96}ClNO_{29}$ (*m/z*): M_r (calcd) 1725.58, M_r (found) 1748.05 $(M+Na)^+$. Anal. calcd.: C 64.67, H 5.60, N 0.81. Found: C 64.57, H 5.80, N 0.75.

*Methyl α-*L*-rhamnopyranosyl-(1→3)-α-*L*-rhamnopyranosyl-(1→2)-α-*L*-rhamnopyranosyl-(1→2)-[3-acetamido-α-*D*-fucopyranosyl-(1→3)]-α-*L*-rhamnopyranoside* **(31):**

Compound **35** (13.0 mg, 7.54 μmol) was dissolved in 9:1 MeOH/HCOOH (2.0 mL) under argon. Pd/C (8 mg) was added and the mixture was kept in an ultrasound bath for 1 hour, after that it was filtered on a Celite pad and concentrated. The residue was dissolved in MeOH (2.0 mL) and NaOMe 1M in MeOH (250 μL) was added. After 48 hours the solution was neutralized with Amberlist-15 H^+, filtered and concentrated. The residue was purified by gel filtration to obtain **31** (4.7 mg, 78%) as a white wax. $[\alpha]_D$= +11 (c=0.3, D_2O). ^1H NMR (D_2O, 400 MHz) δ 5.12 (d, 1H, $J_{1,2}$=1.6 Hz, H-1_B), 5.04 (d, 1H, $J_{1,2}$=1.6 Hz, H-1_D), 5.02 (d, 1H, $J_{1,2}$=4.0 Hz, H-1_E), 4.93 (d, 1H, $J_{1,2}$=1.6 Hz, H-1_C), 4.83 (d, 1H, $J_{1,2}$=1.6 Hz, H-1_A), 4.33 (q, 1H, $J_{5,6}$=6.5 Hz, H-5_E), 4.25 (dd, 1H, $J_{3,2}$=11.1 Hz, $J_{3,4}$=2.9 Hz, H-3_E), 4.12 (m, 2H, H-2_A, H-2_C), 4.07 (dd, 1H, $J_{2,3}$=3.6 Hz, $J_{2,1}$=1.6 Hz, H-2_D), 4.04 (dd, 1H, $J_{2,3}$=3.6 Hz, $J_{2,1}$=1.6 Hz, H-2_B), 3.92-3.73 (m, 10H, H-2_E, H-3_A, H-3_B, H-3_C, H-3_D, H-4_E, H-5_A, H-5_B, H-5_C, H-5_D), 3.63 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.6 Hz, H-4_A), 3.52 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.5 Hz, H-4_C), 3.49 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.8 Hz, H-4_B), 3.46 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.8 Hz, H-4_D), 3.42 (s, 3H,

OMe), 2.05 (s, 3H, Ac), 1.35 (d, 3H, $J_{6,5}$=6.2 Hz, H-6_A), 1.29 (2d, 6H, $J_{6,5}$=6.2 Hz, H-6_B, H-6_D), 1.26 (d, 3H, $J_{6,5}$=6.2 Hz, H-6_C), 1.18 (d, 3H, $J_{6,5}$=6.6 Hz, H-6_E); ^{13}C NMR (D_2O, 100 MHz) δ 165.5 (CO*C*H$_3$), 102.4 (C-1_D), 101.8 (C-1_C), 100.5 (C-1_B), 99.4 (C-1_A), 94.5 (C-1_E), 78.5 (C-2_B), 78.0 (C-3_C), 75.0 (C-2_A), 74.0 (C-3_A), 72.1 (C-4_B), 72.0 (C-4_D), 71.3 (C-4_C), 70.4 (C-4_A), 70.3 (C-4_E), 70.1 (C-2_D), 70.0 (C-3_D), 69.9 (C-2_C), 69.8 (C-3_B), 69.2 (C-5_B), 69.0 (C-5_C), 68.9 (C-5_D), 68.3 (C-5_A), 66.8 (C-5_E), 65.5 (C-2_E), 54.9 (OMe), 51.1 (C-3_E), 22.0 (CO*C*H$_3$), 16.7-16.6 (C-6_A, C-6_B, C-6_C, C-6_D), 15.1 (C-6_E). ESI-MS for $C_{33}H_{57}NO_{21}$ (*m/z*): M_r (calcd) 803.34, M_r (found) 826.53 $(M+Na)^+$. Anal. calcd.: C 49.31, H 7.15, N 1.74. Found: C 49.49, H 7.10, N 1.85.

*Methyl 3-O-allyl-4-O-benzyl-α-*D*-rhamnopyranoside* **(40):**

Diol **39**[22] (1.337 g, 4.99 mmol) was dissolved in 10:1 benzene/methanol (34 mL) and Bu_2SnO (1.565 g, 6.29 mmol) was then added. After stirring at 60°C for 90 min, the solvent was evaporated. Bu_4NBr (1.609 g, 4.99 mmol) was added to the residue under argon. The mixture was suspended in toluene (22 mL), AllBr (4.63 mL, 54.8 mmol) was then added and stirring was conducted at 65°C for 60 min, after that the solvent was evaporated. A column chromatography (6:1 petroleum ether-ethyl acetate) on the residue afforded **40** (1.258 g, 82%) as a yellowish oil. $[\alpha]_D$= +54.0 (c=0.9, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.33 (m, 5H), 5.93 (m, 1H), 5.32 (dd, 1H, J_{vic}=17.0 Hz, J_{gem}=1.6 Hz), 5.20 (dd, 1H, J_{vic}=10.4 Hz, J_{gem}=1.6 Hz), 4.87 (d, 1H, J_{gem}=10.8 Hz), 4.69 (bs, 1H), 4.62 (d, 1H, J_{gem}=10.8 Hz), 4.16 (m, 2H), 3.99 (bd, 1H, $J_{2,3}$=3.0 Hz), 3.68 (m, 2H), 3.40 (t, 1H, $J_{4,3}$= $J_{4,5}$=9.6 Hz), 3.34 (s, 3H), 2.48 (s, 1H), 1.31 (d, 3H, $J_{6,5}$=6.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 138.4, 134.5, 128.3-127.7, 117.3, 100.0, 79.8, 79.6, 75.3, 70.9, 68.6, 67.0, 54.7, 17.9. ESI-MS for $C_{17}H_{24}O_5$ (*m/z*): M_r (calcd) 308.16, M_r (found) 331.39 $(M+Na)^+$. Anal. calcd.: C 66.21, H 7.84. Found: C 66.50, H 7.87

*3-O-Allyl-2-O-benzensulfonyl-4-O-benzyl-*D*-rhamnopyranose* **(41):**

Compound **40** (0.577 g, 1.87 mmol) was dissolved in pyridine (12 mL) and then $BnSO_2Cl$ (0.899 g, 4.71 mmol) was added. The solution was stirred for 45 min at rt, after that water (10 mL) was added. The mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was collected, dried and concentrated to give a brown oil, that was dissolved in Ac_2O (10 mL), cooled to 0°C and a 25:20:0.5 v/v/v mixture of Ac_2O/AcOH/H_2SO_4 (15 mL) was added. The solution was allowed to gradually warm to rt and after 3 hours it was diluted with CH_2Cl_2 and washed with water, 1M $NaHCO_3$ and water again. The organic layer was collected, dried and concentrated to give a residue, that was dissolved in DMF (5 mL). The solution was treated with hydrazine acetate (0.488 g, 5.10 mmol) and then stirred for 2 hours at rt, after that it was diluted with CH_2Cl_2, washed

with 5N NaCl, dried and concentrated. The residue was subjected to column chromatography (4:1 to 2:1 petroleum ether/ethyl acetate) to give **41** (0.478 g, 57%; α/β=6:1) as a yellowish oil. ^{1}H NMR ($CDCl_3$, 200 MHz) (α-anomer) δ 7.50-7.27 (m, 10H), 5.96 (m, 1H), 5.36 (dd, 1H, J_{vic}=17.2 Hz, J_{gem}=1.6 Hz), 5.23 (dd, 1H, J_{vic}=10.5 Hz, J_{gem}=1.6 Hz), 5.14 (d, 1H, $J_{1,2}$=1.6 Hz), 4.99 (dd, 1H, $J_{2,3}$=2.8 Hz, $J_{1,2}$=1.6 Hz), 4.92 (d, 1H, J_{gem}=11.2 Hz), 4.64 (d, 1H, J_{gem}=11.2 Hz), 4.54 (AB d, 1H, J_{gem}=14.6 Hz), 4.45 (AB d, 1H, J_{gem}=14.6 Hz), 4.23 (m, 2H), 3.91 (m, 2H), 3.37 (t, 1H, $J_{4,3}$= $J_{4,5}$=9.6 Hz), 1.28 (d, 3H, $J_{6,5}$=6.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) (α-anomer) δ 138.2, 134.2, 130.8-127.8, 117.7, 92.3, 79.8, 78.0, 75.6, 75.4, 71.5, 67.7, 57.4, 17.9. ESI-MS for $C_{23}H_{28}O_7S$ (*m/z*): M_r (calcd) 448.16, M_r (found) 471.41 $(M+Na)^+$. Anal. calcd.: C 61.59, H 6.29. Found: C 61.70, H 6.26.

*3-O-Allyl-2-O-benzensulfonyl-4-O-benzyl-*D*-rhamnopyranosyl trichloroacetimidate* **(42):**
Hemi-acetal **41** (0.459 g, 1.02 mmol) was dissolved under argon in CH_2Cl_2 (11 mL) and Cl_3CCN (0.565 mL, 5.60 mmol) and DBU (30 μL, 0.20 mmol) were sequentially added and the solution was stirred at rt for 2 hours, after that it was concentrated to give a residue, which, after neutral alumina (Brockman grade 1) column chromatography (10:1 petroleum ether/ethyl acetate) afforded **42** (0.214 g, 35%) as a colourless oil. $[\alpha]_D$= +6.1 (c=1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.65 (s, 1H), 7.44-7.20 (m, 10H), 6.14 (bs, 1H), 5.92 (m, 1H), 5.31 (dd, 1H, J_{vic}=17.2 Hz, J_{gem}=1.6 Hz), 5.19 (dd, 1H, J_{vic}=10.5 Hz, J_{gem}=1.6 Hz), 5.05 (bd, 1H, $J_{2,3}$=2.8 Hz), 4.89 (d, 1H, J_{gem}=11.1 Hz), 4.61 (d, 1H, J_{gem}=11.1 Hz), 4.52 (d, 1H, J_{gem}=14.7 Hz), 4.47 (m, 3H), 4.35-4.10 (m, 5H), 3.87 (m, 2H), 3.44 (m, 3H), 1.29 (d, 3H, $J_{6,5}$=6.2 Hz), 1.23 (d, 1H, $J_{6,5}$=6.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) 159.7, 137.7, 133.9, 130.7-127.5, 118.4, 95.0, 78.8, 76.5, 75.6, 75.4, 71.5, 70.9, 57.6, 17.7. Anal. calcd.: C 50.64, H 4.76, N 2.36. Found: C 50.58, H 4.73, N 2.37.

*3-O-Allyl-2-O-benzensulfonyl-4-O-benzyl-*D*-rhamnopyranosyl N-phenyl-trifluoroacetimidate* **(44):**
A mixture of **41** (0.544 g, 1.21 mmol) and freshly activated 4Å molecular sieves was suspended under argon in CH_2Cl_2 (25 mL) and cooled to 0°C. $CF_3C(NPh)Cl$ (195 μL, 1.58 mmol) and NaH (60% oil suspension; 86 mg, 2.14 mmol) were sequentially added. The mixture was stirred at 0°C for 4 hours after that it was filtered over a Celite pad and concentrated. Neutral alumina (Brockman grade 1) column chromatography (13:1 petroleum ether/ethyl acetate) on the residue afforded **44** (0.514 g, 69%; α:β=1:1) as a yellowish oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.45-6.79 (m, 15H), 6.08-5.90 (m, 3H), 5.79 (bm, 1H), 5.35 (2 dd, 2H, J_{vic}=17.4 Hz, J_{gem}=1.6 Hz), 5.21 (m, 3H), 5.11 (dd, 1H), 4.95 (d, 1H, J_{gem}=10.8 Hz), 4.91 (d, 1H, J_{gem}=10.8 Hz), 4.67 (d, 1H, J_{gem}=10.8 Hz), 4.62 (d, 1H, J_{gem}=10.8 Hz), 4.57 (d, 1H, J_{gem}=14.4 Hz), 4.47 (m, 3H), 4.35-4.10 (m, 5H), 3.87 (m, 2H),

3.44 (m, 3H), 1.29 (d, 3H, $J_{6,5}$=6.2 Hz), 1.23 (d, 1H, $J_{6,5}$=6.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 135.9, 130.9, 130.7, 128.7-123.7, 119.3, 119.2, 94.0, 93.4, 78.8, 78.7, 77.5, 75.6, 75.5, 75.1, 72.9, 71.7, 71.1, 70.6, 57.6, 17.7. ESI-MS for $C_{31}H_{32}F_3NO_7S$ (*m/z*): M_r (calcd) 619.19, M_r (found) 619.65 $(M+Na)^+$. Anal. calcd.: C 60.09, H 5,21; N 2.26. Found: C 60.16, H 5.22, N 2.25.

*Methyl (3-O-allyl-2-O-benzensulfonyl-4-O-benzyl-β-*D*-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-*D*-rhamnopyranoside* **(45):**

A mixture of acceptor **38** (0.182 g, 0.51 mmol) and donor **44** (0.362 g, 0.58 mmol) was coevaporated three times with toluene (5 mL). The residue was mixed with freshly activated AW-300 4Å molecular sieves and suspended under argon in CH_2Cl_2 (20 mL). The mixture was cooled to –60°C and a 80 μM solution of TMSOTf in CH_2Cl_2 (75 μL, 6.0 μmol) was added. The temperature was allowed to gradually rise to –25°C. After 4 hours the mixture was neutralized by adding Et_3N, then filtered over Celite and concentrated to give a residue, that after column chromatography (9:1 to 6:1 petroleum ether/ethyl acetate) afforded, as first eluted compound, **45α** (0.165 g, 41%) as a yellowish oil. $[\alpha]_D$= -6.8 (c=1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.40-7.24 (m, 20H), 5.91 (m, 1H), 5.27 (dd, 1H, J_{vic}=17.4 Hz, J_{gem}=1.6 Hz), 5.14 (dd, 1H, J_{vic}=10.6 Hz, J_{gem}=1.6 Hz), 5.07 (bd, 1H, $J_{2,3}$=2.7 Hz), 4.91 (d, 1H, J_{gem}=11.1 Hz), 4.80 (d, 1H, J_{gem}=10.8 Hz), 4.66 (d, 1H, $J_{1,2}$=1.5 Hz), 4.64 (bs, 1H), 4.62 (d, 1H, J_{gem}=11.1 Hz), 4.57 (d, 1H, J_{gem}=10.8 Hz), 4.42 (d, 1H, J_{gem}=14.1 Hz), 4.35 (d, 1H, J_{gem}=14.1 Hz), 4.17 (m, 1H), 4.02 (m, 2H, H-3_A), 3.84 (m, 2H), 3.70 (bd, 1H, $J_{2,3}$=2.7 Hz), 3.63 (dq, 1H, $J_{5,4}$=9.4 Hz, $J_{5,6}$=6.2 Hz), 3.57 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.4 Hz), 3.36 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.8 Hz), 3.30 (s, 3H), 1.26 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 138.4, 138.0, 134.3, 130.9, 128.7-127.8, 117.7, 99.1, 98.3, 80.5, 79.7, 78.4, 77.4, 77.3, 75.3, 75.2, 72.7, 71.3, 68.6, 67.9, 57.5, 54.7, 17.9. ESI-MS for $C_{44}H_{52}O_{11}S$ (*m/z*): M_r (calcd) 788.32, M_r (found) 810.91 $(M+Na)^+$. Anal. calcd.: C 66.98, H 6.64. Found: C 66.88, H 6.62.

As second eluted compound **45β** (0.237 g, 59%) was recovered as a yellowish oil. $[\alpha]_D$= -22.1 (c=0.8, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.40-7.25 (m, 20H), 5.99 (m, 1H), 5.39 (dd, 1H, J_{vic}=17.4 Hz, J_{gem}=1.8 Hz), 5.23 (dd, 1H, J_{vic}=10.5 Hz, J_{gem}=1.8 Hz), 5.04 (d, 1H, $J_{2,3}$=2.1 Hz), 4.97 (d, 1H, J_{gem}=10.8 Hz), 4.92 (d, 1H, J_{gem}=10.8 Hz), 4.79 (d, 1H, J_{gem}=12.0 Hz), 4.75 (bs, 1H), 4.65 (d, 1H, J_{gem}=12.0 Hz), 4.61 (d, 1H, J_{gem}=10.8 Hz), 4.52 (d, 3H), 4.44 (bs, 1H), 4.34 (dd, 1H, J_{gem}=14.4 Hz, J_{vic}=6.6 Hz), 4.11 (m, 2H, H-3_A), 3.75 (t, 1H, $J_{2,1}$=$J_{2,3}$=5.8 Hz), 3.63 (dq, 1H, $J_{5,4}$=9.6 Hz, $J_{5,6}$=6.2 Hz), 3.55 (t, 1H, $J_{4,5}$=$J_{4,3}$=9.6 Hz), 3.34 (m, 5H), 3.24 (dq, 1H, $J_{5,4}$=9.6 Hz, $J_{5,6}$=6.2 Hz), 1.28 (d, 6H, $J_{6,5}$=6.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 138.8, 138.3, 134.3, 131.0, 130.9, 128.6-127.4, 117.7, 99.1, 96.8, 80.0, 79.9, 79.7, 78.1, 75.9, 75.7, 74.0, 72.8, 72.1, 71.2, 67.7, 57.9,

54.9, 18.2, 17.9. ESI-MS for $C_{44}H_{52}O_{11}S$ (*m/z*): M_r (calcd) 788.32, M_r (found) 810.91 $(M+Na)^+$. Anal. calcd.: C 66.98, H 6.64. Found: C 67.11, H 6.58.

*Methyl (3-O-allyl-4-O-benzyl-β-*D*-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-*D*-rhamnopyranoside* **(46):**

A mixture of **45** (0.229 g, 0.29 mmol) and $NaNH_2$ (127 mg, 3.26 mmol) were suspended in DMF (5 mL) and stirred at rt. After 24 and 48 hours additional aliquots of $NaNH_2$ (127 mg, 3.26 mmol) were added. After 4 days methanol (30 mL) and then, dropwise, AcOH (3 mL) were added. The mixture was concentrated to give a residue, that was dissolved in CH_2Cl_2, washed with 1M $NaHCO_3$ and 5M NaCl, dried and concentrated. Column chromatography (6:1 petroleum ether/EtOAc) on the residue afforded **46** (0.114 g, 62%) as a yellowish oil. $[\alpha]_D$= -11.1 (c=1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.39-7.26 (m, 15H), 5.98 (m, 1H), 5.34 (dd, 1H, J_{vic}=17.2 Hz, J_{gem}=1.6 Hz), 5.21 (dd, 1H, J_{vic}=10.4 Hz, J_{gem}=1.6 Hz), 4.95 (d, 1H, J_{gem}=10.8 Hz), 4.90 (d, 1H, J_{gem}=10.8 Hz), 4.77 (d, 1H, J_{gem}=12.4 Hz), 4.72 (d, 1H, $J_{1,2}$=1.9 Hz), 4.61-4.56 (m, 3H), 4.28 (bs, 1H), 4.21 (m, 2H), 4.11 (m, 1H), 3.88 (d, 1H, $J_{2,3}$=3.0 Hz), 3.73-3.64 (m, 2H), 3.56 (t, 1H, $J_{4,3}$=$J_{4,5}$=8.9 Hz), 3.45 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.3 Hz), 3.33 (s, 3H), 3.28 (dd, 1H, $J_{3,4}$=9.3 Hz, $J_{3,2}$=3.0 Hz), 3.19 (dq, $J_{5,4}$=9.3 Hz, $J_{5,6}$=6.2 Hz), 1.35 (d, 3H, $J_{6,5}$=6.2 Hz), 1.27 (d, 3H, $J_{6,5}$=6.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 138.5, 138.0, 134.8, 134.7, 128.3-127.5, 117.2, 98.6, 97.1, 81.4, 79.7, 75.5, 75.0, 74.7, 72.5, 71.6, 70.5, 68.8, 67.5, 54.7, 18.1, 17.9. ESI-MS for $C_{37}H_{46}O_9$ (*m/z*): M_r (calcd) 634.31, M_r (found) 657.47 $(M+Na)^+$. Anal. calcd.: C 70.01, H 7.30. Found: C 69.90, H 7.30.

*Ethyl 2,4-di-O-acetyl-1-thio-β-*D*-fucopyranoside* **(49)**

Triol **48** (0.775 g, 3.72 mmol) was dissolved in 2:7 v/v DMF/$MeC(OMe)_3$ (9.0 mL), CSA (80 mg, 0.34 mmol) was then added and the solution was evacuated at 100 mbar for 20 min, after that pyridine (7.0 mL) and Ac_2O (7.0 mL) were sequentially added. The solution was stirred overnight at rt, then coevaporated four times with toluene (10 mL each). The residue was dissolved in 80% AcOH and the solution was stirred at rt for 10 min, after that it was coevaporated two times with toluene (5 mL each). The residue was subjected to column chromatography (5:2 petroleum ether/ethyl acetate) to give **49** (0.893 g, 82%) as a white solid. $[\alpha]_D$= -2.4 (c=1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.17 (dd, 1H, $J_{4,3}$=3.2 Hz, $J_{4,5}$=0.8 Hz), 4.97 (t, 1H, $J_{2,3}$= $J_{2,1}$=9.6 Hz), 4.37 (d, 1H, $J_{1,2}$=10.0 Hz), 3.79 (dd, 1H, $J_{3,2}$=9.6 Hz, $J_{3,4}$=3.2 Hz), 3.71 (dq, $J_{5,6}$=6.4 Hz, $J_{5,4}$=0.8 Hz), 2.67 (dq, 2H, J_{vic}=7.2 Hz, J_{gem}=3.2 Hz), 2.14, 2.07 (2s, 6H), 1.23 (t, 3H, J_{vic}=7.2 Hz), 1.16 (d, 3H, $J_{6,5}$=6.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.3, 171.0, 83.1, 73.4, 73.1, 72.4, 71.0, 24.1,

20.9, 20.8, 16.6, 14.7. ESI-MS for $C_{12}H_{20}O_6S$ (*m/z*): M_r (calcd) 292.10, M_r (found) 292.21 $(M+Na)^+$. Anal. calcd.: C 49.30, H 6.90. Found: C 49.35, H 6.85.

Ethyl 3-acetamido-4-O-acetyl-2-O-benzyl-1-thio-D-*fucopyranoside* **(51):**

Hemiacetal **21**[38] (100 mg, 297 μmol) was dissolved in pyridine (1.5 mL) and Ac_2O (2.0 mL) was added. The solution was stirred overnight at rt, then coevaporated two times with toluene (10 mL). The residue was dissolved in CH_2Cl_2, washed with 1M HCl and 0.2M $NaHCO_3$, dried and concentrated to give a residue, which was then dissolved in CH_2Cl_2 (2.0 mL) and treated with EtSH (25 μL, 0.34 mmol) and $BF_3.OEt_2$ (76 μL, 0.60 mmol). After stirring overnight at rt the mixture was diluted with CH_2Cl_2 (40 mL), washed with 1M KOH (50 mL) and water (50 mL), dried and concentrated. The residue was subjected to column chromatography (1:1 petroleum ether/ethyl acetate) to afford **51** (90 mg, 79%; α/β=1:1) as a white foam. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.36 (m, 10H), 5.57 (d, 1H, $J_{1,2}$=5.0 Hz), 5.33 (d, 1H, $J_{4,3}$=2.2 Hz), 5.20 (d, 1H, $J_{4,3}$=2.4 Hz), 5.02-4.87 (m, 2H), 4.86 (d, 1H, J_{gem}=11.4 Hz), 4.78 (d, 1H, J_{gem}=12.0 Hz), 4.57 (m, 2H), 4.46 (q, 1H, $J_{5,6}$=6.0 Hz), 4.38 (d, 1H, J_{gem}=12.0 Hz), 4.33-4.16 (m, 2H), 3.87 (dd, 1H, $J_{2,3}$=11.2 Hz, $J_{2,1}$=5.0 Hz), 3.76 (q, 1H, $J_{5,6}$=6.6 Hz), 3.397 (t, 1H, $J_{2,3}$=$J_{2,1}$=9.8 Hz), 2.79, 2.58 (2q, 4H, J_{vic}=6.8 Hz), 2.09, 2.08 (2s, 6H), 1.82, 1.73 (2s, 6H), 1.33 (t, 6H, J_{vic}=6.8 Hz), 1.13 (d, 3H, $J_{6,5}$=6.0 Hz), 1.07 (d, 3H, $J_{6,5}$=6.6 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.1-169.9, 137.6, 137.4, 128.4-128.0, 85.8, 82.8, 75.4, 74.1, 73.6, 72.6, 72.2, 72.1, 71.2, 64.9, 53.3, 49.8, 25.2, 23.6, 23.0, 20.6, 16.7, 16.1, 14.9, 14.8. ESI-MS for $C_{19}H_{27}NO_5S$ (*m/z*): M_r (calcd) 381.16, M_r (found) 404.36 $(M+Na)^+$. Anal. calcd.: C 59.82, H 7.13, N 3.67. Found: C 59.89, H 7.19, N 3.68.

Ethyl 3,3-Diacetamido-4-O-acetyl-2-O-benzyl-1-thio-D-*fucopyranoside* **(52):**

Compound **51** (71 mg, 186 μmol) was dissolved under argon in CH_2Cl_2 (2.0 mL). This solution was treated with DIPEA (148 μL, 0.86 mmol) and then, dropwise, with AcCl (183 μL, 2.58 mmol). The solution was stirred overnight at rt, then diluted with CH_2Cl_2, washed with 1M $NaHCO_3$, dried and concentrated. The residue was subjected to column chromatography (5:1 to 3:1 petroleum ether/ethyl acetate) to give, as first eluted compound, **52α** (35 mg, 44%) as a white foam. $[\alpha]_D$= +20.3 (c=2.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.30 (m, 5H), 5.55 (d, 1H, $J_{1,2}$=4.0 Hz), 5.11 (d, 1H), 4.68 (m, 2H), 4.58 (d, 1H, J_{gem}=10.6 Hz), 4.47 (q, 1H, $J_{5,6}$=6.6 Hz), 4.26 (d, 1H, J_{gem}=10.6 Hz), 2.51 (q, 2H, J_{vic}=7.2 Hz), 2.21 (s, 6H), 2.12 (s, 3H), 1.27 (t, 3H, J_{vic}=7.2 Hz), 1.15 (d, 3H, $J_{6,5}$=6.6 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.9, 171.3, 137.1, 128.3-127.9, 83.6, 71.6, 71.3, 70.8,

65.7, 57.9, 27.0, 23.7, 21.0, 16.1, 14.9. ESI-MS for $C_{21}H_{29}NO_6S$ (*m/z*): M_r (calcd) 423.17, M_r (found) 446.41 $(M+Na)^+$. Anal. calcd.: C 59.55, H 6.90, N 3.31. Found: C 59.56, H 6.87, N 3.29.

As second eluted compound was recovered **52β** (37 mg, 47%) as a white foam. $[\alpha]_D$= -45.3 (c=0.9, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.30 (m, 5H), 5.12 (bs, 1H), 4.99 (d, 1H, J_{gem}=11.0 Hz), 4.50 (m, 3H), 4.33 (d, 1H, J_{gem}=11.0 Hz), 3.82 (q, 1H, $J_{5,6}$=6.0 Hz), 2.79 (dq, 2H, J_{vic}=7.6 Hz, J_{gem}=2.0 Hz), 2.23 (s, 6H), 2.10 (s, 3H), 1.33 (t, 3H, J_{vic}=7.6 Hz), 1.19 (d, 3H, $J_{6,5}$=6.0 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 174.2, 171.5, 138.1, 128.3-127.4, 87.2, 74.4, 73.7, 73.5, 72.0, 62.5, 27.2, 25.0, 20.9, 16.6, 14.9. ESI-MS for $C_{21}H_{29}NO_6S$ (*m/z*): M_r (calcd) 423.17, M_r (found) 446.39 $(M+Na)^+$. Anal. calcd.: C 59.55, H 6.90, N 3.31. Found: C 59.59, H 6.86, N 3.28.

*Methyl (3,3-diacetamido-4-O-acetyl-2-O-benzyl-α-*D*-fucopyranosyl)-(1→2)-(3-O-allyl-4-O-benzyl-β-*D*-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-*D*-rhamnopyranoside* **(37):**

A mixture of acceptor **46** (19.7 mg, 31.0 μmol) and donor **52β** (28 mg, 66 μmol) was coevaporated three times with toluene (1 mL). The residue was mixed with freshly activated AW-300 4Å molecular sieves and suspended under argon in 1:1 v/v CH_2Cl_2/Et_2O (800 μL). NIS (16 mg, 71 μmol) was then added under argon, the mixture was cooled to –20°C and a 0.60 mM solution of TfOH in CH_2Cl_2 (20 μL, 12 μmol) was added. After 90 min stirring at –20°C, the mixture was filtered over Celite, diluted with CH_2Cl_2, washed with 10% $Na_2S_2O_3$ and 1M $NaHCO_3$, dried and concentrated. The residue was then subjected firstly to column chromatography (6:1 petroleum ether/ethyl acetate) and then to HPLC (Phenomenex Proteo 90A C-18 column, 250x10 mm; eluent: MeOH/CH_3CN/H_2O 2:2:1) to afford a first eluted fraction, containing **46** (5.4 mg, 27%), and a second fraction, which contained **37** (12.3 mg, 40%) as a white foam. $[\alpha]_D$= +5 (c=0.3, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39-7.10 (m, 20H), 5.92 (m, 1H), 5.76 (d, 1H, $J_{1,2}$=3.4 Hz), 5.29 (d, 1H, J_{gem}=18.0 Hz), 5.21 (d, 1H, J_{gem}=10.4 Hz), 5.13 (bs, 1H), 5.04 (m, 2H), 4.85 (q, 1H, $J_{5,6}$=6.4 Hz), 4.75 (d, 1H, J_{gem}=12.0 Hz), 4.71 (m, 2H), 4.64 (d, 1H, J_{gem}=12.0 Hz), 4.52 (m, 2H), 4.39 (m, 2H), 4.28 (d, 1H, J_{gem}=11.1 Hz), 4.24-4.07 (m, 4H), 3.67 (m, 2H), 3.49 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.5 Hz), 3.44 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.3 Hz), 3.32 (s, 3H), 3.24 (m, 2H), 2.11 (s, 3H), 2.01 (s, 6H), 1.35 (d, 3H, $J_{6,5}$=6.0 Hz), 1.28 (d, 3H, $J_{6,5}$=6.2 Hz), 1.16 (d, 3H, $J_{6,5}$=6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) 174.3, 171.5, 138.8, 138.5, 138.3, 138.0, 134.5, 130.8-126.9, 117.6, 98.9, 98.4, 95.8, 83.2, 80.6, 80.5, 75.1, 75.0, 73.3, 72.5, 72.4, 72.2, 72.1, 72.0, 71.9, 71.5, 70.2, 68.2, 65.7, 56.7, 54.7, 23.8, 21.0, 18.5, 17.9, 16.2. ESI-MS for $C_{56}H_{69}NO_{15}$ (*m/z*): M_r (calcd) 995.47, M_r (found) 1018.50 $(M+Na)^+$. Anal. calcd.: C. 67,52; H. 6,98; N. 1,41; Found: C 67.44, H 7.02, N 1.40.

*Methyl 3-acetamido-α-*D*-fucopyranosyl-(1→2)-β-*D*-rhamnopyranosyl-(1→3)-α-*D*-rhamnopyranoside* **(36):**

To a solution of **37** (8.6 mg, 8.6 μmol) in 1:1 CH_2Cl_2/MeOH (400 μL) $PdCl_2$ (0.6 mg, 3.4 μmol) was added and the mixture was vigorously stirred at rt overnight, after that it was filtered over a Celite pad, diluted with CH_2Cl_2, washed with 5N NaCl, dried and concentrated. The residue was then dissolved in 1:1 CH_2Cl_2/MeOH (800 μL) and treated with a 0.4 M methanolic solution of NaOMe (30 μL). After 3 hours stirring at rt the solution was neutralized with Amberlist-15 (H^+), filtered and concentrated. The residue was dissolved in MeOH (1.5 mL) and then added to a suspension of 10% Pd/C (catalyst amount) in MeOH (0.5 mL). After stirring at rt for 4 days under a hydrogen atmosphere, HCOOH (100 μL) was added and the mixture was kept in an ultrasound bath for 3 hours, after that it was filtered on Celite and concentrated to give **36** (3.7 mg, 84% yield). $[\alpha]_D$= +26 (c=0.2, H_2O). ^{1}H NMR (D_2O, 600 MHz) δ 5.18 (d, 1H, $J_{1,2}$=3.6 Hz, H-1_C), 4.81 (s, 1H, H-1_B), 4.74 (s, 1H, H-1_A), 4.54 (q, 1H, $J_{5,6}$=6.4 Hz, H-5_C), 4.27 (dd, 1H, $J_{3,2}$=9.5 Hz, $J_{3,4}$=3.2 Hz, H-3_C), 4.14 (bs, 1H, H-2_A), 4.12 (d, 1H, $J_{2,3}$=3.0 Hz, H-2_B), 3.92 (dd, 1H, $J_{3,4}$=9.8 Hz, $J_{3,2}$=3.0 Hz, H-3_A), 3.87 (dd, 1H, $J_{2,3}$=9.5 Hz, $J_{2,1}$=3.6 Hz, H-2_C), 3.72 (m, 3H, H-3_B, H-4_C, H-5_A), 3.53 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.5 Hz, H-4_A), 3.50 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.3 Hz, H-4_B), 3.41 (m, 4H, H-5_B, OMe), 2.06 (s, 3H, NHAc), 1.33 (d, 3H, $J_{6,5}$=6.2 Hz, H-6_A), 1.31 (d, 3H, $J_{6,5}$=6.2 Hz, H-6_B), 1.18 (d, 3H, $J_{6,5}$=6.4 Hz, H-6_C); ^{13}C NMR ($CDCl_3$, 150 MHz) 174.4 (NH*C*OCH$_3$), 100.5 (C-1_A), 100.1 (C-1_C), 96.6 (C-1_B), 78.7 (C-2_B), 77.0 (C-3_A), 73.5 (C-3_B), 72.5 (C-4_B), 72.4 (C-5_B), 70.5 (C-4_A), 70.3 (C-4_C), 68.5 (C-5_A), 67.1 (C-2_A, C-5_C), 66.6 (C-2_C), 54.7 (OMe), 51.2 (C-3_C), 22.0 (NHCO*C*H$_3$), 16.7, 16.6 (C-6_A, C-6_B), 15.3 (C-6_C). ESI-MS for $C_{21}H_{37}NO_{13}$ (*m/z*): M_r (calcd) 511.23, M_r (found) 533.71 $(M+Na)^+$. Anal. calcd.: C. 49.31; H. 7.29; N. 2.74; Found: C 48.79, H 7.47, N 2.67.

*Methyl 4-*O*-benzoyl-α-*D*-rhamnopyranoside* **(56)**: To a 0°C cooled solution of **26** (0.869 g, 4.02 mmol) in pyridine (5.8 mL), BzCl (1.3 mL, 10.9 mmol) was added and the mixture was stirred at 0°C for an hour, after that CH_2Cl_2 (20 mL) was added. The mixture was washed with 0.5 M HCl. The organic phase was collected, dried and concentrated to afford a brown residue, that was subsequently suspended in 4:1 TFA/H_2O (9 mL) and stirred at rt. After 20' the mixture was concentrated to give a residue, that, after silica gel chromatography (2:1 petroleum ether/ethyl acetate), afforded **56** (0.878 g, 77%) as a white foam. $[\alpha]_D$ +114.5 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.10-7.41 (m, 5H, H-Ar), 5.04 (t, $J_{4,3}$=$J_{4,5}$=9.8 Hz, 1H, H-4), 4.77 (bs, 1H, H-1), 4.14-3.91 (m, 3H, H-2, H-3, H-5), 3.42 (s, 3H, OMe), 1.30 (d, $J_{6,5}$=6.2 Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 167.5 (C=O), 133.5 (C_{ipso}), 129.8-128.5 (C-Ar), 100.4 (C-1), 70.8, 70.6, 70.4 (C_2,

C_3, C_4), 65.5 (C_5), 55.1 (OMe), 17.7 (C_6). ESI-MS for $C_{14}H_{18}O_6$ (*m/z*): M_r (calcd) 282.11, M_r (found) 305.25 $(M+Na)^+$. Anal. calcd: C. 59.57; H. 6.43. Found: C. 59.25; H. 6.53.

Methyl 3-O-*allyl-4*-O-*benzoyl-α*-D-*rhamnopyranoside* **(55)**: A mixture of **56** (0.878 g, 3.14 mmol) and Bu_2SnO (0.978 g, 3.91 mmol) was suspended in 10:1 benzene/methanol (23 mL) and then heated and stirred at 60°C. After 90' solvent was removed. The residue was mixed under argon atmosphere with Bu_4NBr (0.995 g, 3.21 mmol) and the solid mixture suspended in toluene (12 mL). Allyl bromide (2.91 mL, 34.1 mmol) was added and the mixture was stirred at 65°C. After 2 hours the residue was concentrated. Silica gel chromatography (7:1 petroleum ether/ethyl acetate) of the residue afforded **55** (2.76 mmol, 88%) as a yellowish oil. $[\alpha]_D$ +42.0 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.10-7.40 (m, 5H, H-Ar), 5.72 (m, 1H, OCH_2CH=CH_2), 5.28 (t, $J_{4,3}$=$J_{4,5}$=9.8 Hz, 1H, H-4), 5.16 (bd, J_{vic}=17.4 Hz, 1H, OCH_2CH=CH_2 *trans*), 5.07 (bd, J_{vic}=10.2 Hz, 1H, OCH_2CH=CH_2 *cis*), 4.78 (bs, 1H, H-1), 4.16-3.89 (m, 4H, H-2, H-5, OCH_2CH=CH_2), 3.81 (dd, $J_{3,4}$=9.8 Hz, $J_{3,2}$=3.4 Hz, 1H, H-3), 3.41 (s, 3H, OMe), 1.25 (d, $J_{6,5}$=6.4 Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 165.7 (C=O), 134.1 (OCH_2CH=CH_2), 133.1 (C_{ipso}), 129.7-128.4 (C-Ar), 117.7 (OCH_2CH=CH_2), 100.2 (C-1), 76.6 (C-3), 73.1, 71.0, 68.7, 66.1 (C-2, C-4, C-5, OCH_2CH=CH_2), 55.0 (OMe), 17.5 (C-6). ESI-MS for $C_{17}H_{22}O_6$ (*m/z*): M_r (calcd) 322.14, M_r (found) 345.39 $(M+Na)^+$. Anal. calcd: C. 63.34; H. 6.88. Found: C. 63.45; H. 6.99.

Methyl 3-O-*allyl-4*-O-*benzoyl-2*-O-*levulinoyl-α*-D-*rhamnopyranoside* **(57)**: To a solution of **55** (0.499 g, 1.55 mmol) in CH_2Cl_2 (11 mL), levulinic acid (1.0 mL, 8.64 mmol), DMAP (0.120 g, 0.98 mmol) and then DIPC (1.6 mL, 10.2 mmol) were added. The mixture was stirred at rt for 60', after that it was filtered over a Celite pad, washed with water, dried and concentrated to afford a brown residue. Silica gel chromatography (6:1 petroleum ether/ethyl acetate) of the residue afforded **57** (0.516 g, 79%) as a yellowish oil. $[\alpha]_D$ -3.3 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.08-7.40 (m, 5H, H-Ar), 5.65 (m, 1H, OCH_2CH=CH_2), 5.30 (dd, $J_{2,3}$=3.2 Hz, $J_{2,1}$=1.6 Hz, 1H, H-2), 5.22 (t, $J_{4,3}$=$J_{4,5}$=10.0 Hz, 1H, H-4), 5.13 (bd, J_{vic}=17.4 Hz, 1H, OCH_2CH=CH_2 trans), 5.02 (bd, J_{vic}=10.4 Hz, 1H, OCH_2CH=CH_2 cis), 4.67 (bs, 1H, H-1), 4.12-3.81 (m, 4H, H-3, H-5, OCH_2CH=CH_2), 3.39 (s, 3H, OMe), 2.82-2.62 (m, 4H, CH_2CH_2), 2.20 (s, 3H, CH_3CO), 1.25 (d, $J_{6,5}$=6.2 Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 206.3 (CH_3C=O), 171.9 (C=O Lev), 165.6 (C=O Bz), 134.3 (OCH_2CH=CH_2), 133.1 (C_{ipso}), 130.0-128.4 (C-Ar), 117.2 (OCH_2CH=CH_2), 98.7 (C-1), 74.4, 73.1, 70.5, 69.0, 66.4 (C-2, C-3, C-4, C-5, OCH_2CH=CH_2), 55.1 (OMe), 38.0, 29.8, 28.2 (CH_2CH_2, CH_3C=O), 17.6 (C-6). ESI-MS for $C_{22}H_{28}O_8$ (*m/z*): M_r (calcd) 420.18, M_r (found) 443.40 $(M+Na)^+$. Anal. calcd: C. 62.85; H. 6.71. Found: C. 62.95; H. 6.78.

Methyl 4-O-*benzoyl-2*-O-*levulinoyl-α*-D-*rhamnopyranoside* **(54)**: Compound **57** (0.487 g, 1.16 mmol) was dissolved in 3:2 MeOH/CH_2Cl_2 (20 mL) and $PdCl_2$ (82 mg, 0.46 mmol) was added. The mixture was stirred at rt overnight, after that it was filtered over a Celite pad, washed with 5N NaCl, dried and concentrated. Silica gel chromatography (4:1 petroleum ether/ethyl acetate) afforded **54** (0.346 g, 78%) as a white foam. $[\alpha]_D$ +30.8 (*c* 0.9, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.12-7.41 (m, 5H, H-Ar), 5.19 (dd, $J_{2,3}$=3.2 Hz, $J_{2,1}$=1.6 Hz, 1H, H-2), 5.09 (t, $J_{4,3}$=$J_{4,5}$=10.0 Hz, 1H, H-4), 4.69 (bs, 1H, H-1), 4.23-3.88 (m, 2H, H-3, H-5), 3.40 (s, 3H, OMe), 2.86-2.65 (m, 4H, C*H*$_2$C*H*$_2$), 2.22 (s, 3H, C*H*$_3$CO), 1.27 (d, $J_{6,5}$=6.2 Hz, 3H, H-6).. ^{13}C NMR (50 MHz, $CDCl_3$): δ 207.0 (CH_3*C*=O), 172.2 (C=O Lev), 166.7 (C=O Bz), 133.3 (C_{ipso}), 129.8-128.4 (C-Ar), 98.4 (C-1), 75.3, 72.7, 68.7, 66.1 (C-2, C-3, C-4, C-5), 55.2 (OMe), 38.3, 29.8, 28.3 (*C*H$_2$*C*H$_2$, *C*H$_3$C=O), 17.6 (C-6). ESI-MS for $C_{19}H_{24}O_8$ (*m/z*): M_r (calcd) 380.15, M_r (found) 403.37 $(M+Na)^+$. Anal. calcd: C. 59.99; H. 6.36. Found: C. 60.18; H. 6.29.

2-O-acetyl-3-O-allyl-4-O-benzoyl-D-*rhamnopyranose* **(58)**: Compound **55** (1.337 g, 4.15 mmol) was dissolved in Ac_2O (10 mL). To this solution 25:20:0.5 v/v/v Ac_2O/AcOH/H_2SO_4 (18 mL) was added. The solution was stirred for 30'at rt, then water (5.0 mL) was dropwise added and stirring was continued for additional 10', after that the solution was diluted with CH_2Cl_2 (400 mL). After successive washings with water, 1M $NaHCO_3$ and then with water again, the organic layer was collected, dried and concentrated. The residue was dissolved in DMF (15 mL) and then hydrazine acetate (0.295 g, 3.11 mmol) was added. After 40' stirring at rt, the solution was diluted with CH_2Cl_2 (400 mL) and washed with 5N NaCl, dried and concentrated to give a residue, that after silica gel chromatography (5:1 petroleum ether/ethyl acetate), afforded **58** (0.858 g, 59%; α/β=3.5/1) as a yellowish oil. ^{1}H NMR (200 MHz, $CDCl_3$; α-anomer): δ 8.09-7.43 (m, 5H, H-Ar), 5.68 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.36 (dd, $J_{2,3}$=3.3 Hz, $J_{2,1}$=1.8 Hz, 1H, H-2), 5.28 (t, $J_{4,3}$=$J_{4,5}$=9.9 Hz, 1H, H-4), 5.22 (bs, 1H, H-1), 5.16 (dd, J_{vic}=17.4 Hz, J_{gem}=1.5 Hz, 1H, OCH$_2$CH=C*H*$_2$ *trans*), 5.05 (dd, J_{vic}=10.2 Hz, J_{gem}=1.5 Hz, 1H, OCH$_2$CH=C*H*$_2$ *cis*), 4.21 (dq, $J_{5,4}$=9.9 Hz, $J_{5,6}$=6.2 Hz, 1H, H-5), 4.17-3.94 (m, 3H, H-3, OC*H*$_2$CH=CH$_2$), 2.24 (s, 3H, C*H*$_3$CO), 1.25 (d, $J_{6,5}$=6.2 Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$; α-anomer): δ 170.3 (C=O Ac), 166.5 (C=O Bz), 134.1 (OCH$_2$*C*H=CH$_2$), 133.2 (C_{ipso}), 129.8-128.5 (C-Ar), 117.3 (OCH$_2$CH=*C*H$_2$), 93.0 (C-1), 74.4, 73.2, 70.4, 70.2, 66.9 (C-2, C-3, C-4, C-5, O*C*H$_2$CH=CH$_2$), 20.4 (*C*H$_3$C=O Ac), 17.7 (C-6).. ESI-MS for $C_{18}H_{22}O_7$ (*m/z*): M_r (calcd) 350.14, M_r (found) 373.27 $(M+Na)^+$. Anal. calcd: C. 61.71; H. 6.33. Found: C. 61.88; H. 6.20.

*2-O-acetyl-3-O-allyl-4-O-benzoyl-α-*D*-rhamnopyranosyl trichloroacetimidate* **(59)**: To a 0°C cooled solution of **58** (0.429 g, 1.23 mmol) in CH_2Cl_2 (10 mL), Cl_3CCN (0.610 mL, 6.08 mmol) and DBU (0.105 mL, 0.703 mmol) were added under argon atmosphere. After 60' stirring at 0°C, the solution was concentrated at 20°C. Silica gel chromatography (14:1 petroleum ether/ethyl acetate) of the residue afforded **59** (0.435 g, 72%) as a yellowish oil. $[\alpha]_D$ +14.4 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.73 (s, 1H, NH), 8.10-7.44 (m, 5H, H-Ar), 6.25 (d, $J_{1,2}$=2.0 Hz, H-1), 5.68 (m, 1H, OCH_2CH=CH_2), 5.51 (dd, $J_{2,3}$=3.2 Hz, $J_{2,1}$=2.0 Hz, 1H, H-2), 5.37 (t, $J_{4,3}$=$J_{4,5}$=10.0 Hz, 1H, H-4), 5.16 (dd, J_{vic}=17.4 Hz, J_{gem}=1.6 Hz, 1H, OCH_2CH=CH_2 *trans*), 5.08 (dd, J_{vic}=10.2 Hz, J_{gem}=1.6 Hz, 1H, OCH_2CH=CH_2 *cis*), 4.23-3.90 (m, 4H, H-3, H-5, OCH_2CH=CH_2), 2.21 (s, 3H, CH_3C=O Ac), 1.30 (d, $J_{6,5}$=6.2 Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.1 (C=O Ac), 165.6 (C=O Bz), 159.9 (C=NH), 134.0 (OCH_2CH=CH_2), 133.3 (C_{ipso}), 129.8-128.5 (C-Ar), 118.0 (OCH_2CH=CH_2), 95.1 (C-1), 77.2 (C-3), 72.2, 70.9, 69.7, 67.4 (C-2, C-4, C-5, OCH_2CH=CH_2), 20.9 (CH_3C=O), 17.6 (C-6). ESI-MS for $C_{20}H_{22}Cl_3NO_7$ (*m/z*): M_r (calcd) 493.05, M_r (found) 516.41 $(M+Na)^+$. Anal. calcd: C. 48.55; H. 4.48; N. 2.83. Found: C. 49.00; H. 4.44; N.2.78.

*Methyl (2-*O*-acetyl-3-*O*-allyl-4-*O*-benzoyl-α-*D*-rhamnopyranosyl)-(1→3)-4-*O*-benzoyl-2-*O*-levulinoyl-α-*D*-rhamnopyranoside* **(60)**: A suspension of acceptor **54** (0.243 g, 0.64 mmol), imidate **59** (0.413 g, 0.84 mmol) and freshly powdered 4Å HW-300 molecular sieves in CH_2Cl_2 (15 mL) was stirred at -50°C under argon atmosphere. $BF_3.OEt_2$ (32 μL, 0.25 mmol) was added and the mixture was kept at -50°C for 150 min, after that it was filtered on a Celite pad and washed with 1M $NaHCO_3$ and water. The organic layer was collected, dried and concentrated. Silica gel chromatography (3:1 petroleum ether/ethyl acetate) of the residue afforded **60** (0.389 g, 86%) as a white foam. $[\alpha]_D$ -21.4 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.10-7.41 (m, 10H, H-Ar), 5.52 (m, 1H, OCH_2CH=CH_2), 5.34 (t, $J_{4,3}$=$J_{4,5}$=9.8 Hz, 1H, H-4_A), 5.27 (dd, $J_{2,3}$=3.2 Hz, $J_{2,1}$=1.6 Hz, 1H, H-2_A), 5.13 (t, $J_{4,3}$=$J_{4,5}$=10.0 Hz, 1H, H-4_B), 5.02-4.85 (m, 3H, H-2_B, OCH_2CH=CH_2), 4.69 (bs, 1H, H-1_A), 4.67 (bs, 1H, H-1_B), 4.26 (dd, $J_{3,4}$=10.2 Hz, $J_{3,2}$=3.2 Hz, 1H, H-3_A), 3.96 (m, 2H, H-5_A, H-5_B), 3.84-3.63 (3H, H-3_B, OCH_2CH=CH_2), 3.41 (OMe), 2.77 (m, 4H, CH_2CH_2), 2.21 (s, 3H, CH_3C=O Lev), 1.92 (s, 3H, CH_3C=O Ac), 1.29 (d, $J_{6,5}$=6.2 Hz, 3H, H-6_A), 1.19 (d, $J_{6,5}$=6.2 Hz, 3H, H-6_B). ^{13}C NMR (50 MHz, $CDCl_3$): δ 206.6 (CH_3C=O), 171.8 (C=O Lev), 169.6 (C=O Ac), 165.7 (C=O Bz), 134.1 (OCH_2CH=CH_2), 133.4, 133.0 (2 C_{ipso}), 129.8-128.3 (C-Ar), 117.0 (OCH_2CH=CH_2), 99.8 (C-1_B, $^1J_{C,H}$=173 Hz), 98.3 (C-1_A), 75.3, 74.0, 73.2, 72.9, 71.6, 70.5, 68.8, 67.5, 66.5 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, OCH_2CH=CH_2), 55.2 (OMe), 37.8, 29.8, 28.1 (CH_2CH_2, CH_3C=O Lev), 20.7 (CH_3C=O Ac), 17.5, 17.4 (C-6_A, C-6_B). ESI-MS for

$C_{37}H_{44}O_{14}$ (*m/z*): M_r (calcd) 712.27, M_r (found) 735.49 $(M+Na)^+$. Anal. calcd: C. 62.35; H. 6.22. Found: C. 62.50; H. 6.34.

Methyl (2,3-di-O-*acetyl-4*-O-*benzoyl-α*-D-*rhamnopyranosyl)-(1→3)-4*-O-*benzoyl-α*-D-*rhamnopyranoside* **(61)**: To a solution of **60** (0.350 g, 0.49 mmol) in 2:1 MeOH/CH_2Cl_2 (8.6 mL), $PdCl_2$ (18 mg, 0.10 mmol) was added and the mixture was stirred vigorously at rt for 4 hours. The mixture was then filtered over a Celite pad, diluted with CH_2Cl_2 (100 mL) and washed with 5N NaCl. The organic layer was collected, dried and concentrated to afford a brownish residue, that was dissolved in pyridine (4.0 mL) and then Ac_2O (4.0 mL) was added to the mixture. The solution was stirred at rt overnight, then it was concentrated, diluted with CH_2Cl_2 (100 mL) and washed with 1M HCl (100 mL) and 1M $NaHCO_3$ (100 mL). The organic layer was collected, dried and concentrated. The residue was dissolved in 4:1 CH_2Cl_2/MeOH (8.0 mL) and then hydrazine acetate (65 mg, 0.68 mmol) was added. The solution was stirred 4 hours at rt, then it was concentrated to give a residue, that, after silica gel chromatography (4:1 petroleum ether/ethyl acetate) afforded **61** (0.278 g, 87%) as a white foam. $[\alpha]_D$ +0.9 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.09-7.40 (m, 10H, H-Ar), 5.47-5.35 (m, 2H, H-4_A, H-3_B), 5.23 (t, $J_{4,3}$=$J_{4,5}$=9.6 Hz, 1H, H-4_B), 5.02 (dd, $J_{2,3}$=3.6 Hz, $J_{2,1}$=1.6 Hz, 1H, H-2_B), 4.94 (d, $J_{1,2}$=1.6 Hz, 1H, H-1_B), 4.76 (bs, 1H, H-1_A), 4.23-4.05 (m, 3H, H-2_A, H-3_A, H-5_B), 3.93 (dq, $J_{5,4}$=9.8 Hz, $J_{5,6}$=6.2 Hz, 1H, H-5_A), 3.43 (s, 3H, OMe), 1.90 (s, 3H, C*H*$_3$C=O), 1.79 (s, 3H, C*H*$_3$C=O), 1.26 (m, 6H, H-6_A, H-6_B). ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.5, 169.2 (2 C=O Ac), 165.6, 165.4 (2 C=O Bz), 133.4, 133.2 (2 C_{ipso}), 129.8-128.3 (C-Ar), 100.3, 99.1 (C-1_A, C-1_B), 77.2 (C-3_A), 73.0, 71.4, 70.8, 69.7, 68.4, 67.4, 66.3 (C-2_A, C-2_B, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B), 55.1 (OMe), 20.5, 20.4 (2 *C*H$_3$C=O Ac), 17.5, 17.4 (C-6_A, C-6_B). ESI-MS for $C_{31}H_{36}O_{13}$ (*m/z*): M_r (calcd) 616.22, M_r (found) 639.31 $(M+Na)^+$. Anal. calcd: C. 60.38; H. 5.88. Found: C. 60.55; H. 6.00.

3-O-allyl-2,4-di-O-benzoyl-D-*rhamnopyranose* **(62)**: Compound **55** (0.982 g, 3.05 mmol) was dissolved in pyridine (5.0 mL) and then BzCl (0.710 mL, 6.14 mmol) was added. The mixture was stirred for 30', water (20 mL) was then added. After 10' additional stirring it was diluted with CH_2Cl_2 (100 mL) and washed with 0.5M HCl. The organic layer was collected, dried and concentrated to give a residue that was suspended in Ac_2O (7.5 mL). 25:20:0.5 v/v/v Ac_2O/AcOH/H_2SO_4 (12.5 mL) was added and the solution was stirred for 60' at rt. Water (5.0 mL) was then dropwise added and after 5' additional stirring the mixture was diluted with CH_2Cl_2 (300 mL) and washed with 5N NaCl, 1M $NaHCO_3$ and water. The organic layer was collected, dried and concentrated to give a yellowish oil, that was dissolved in DMF (15 mL). Hydrazine acetate (0.331

g, 3.46 mmol) was added and the mixture was stirred for 45' at rt, after that it was diluted with CH_2Cl_2 (400 mL) and washed with 5N NaCl, dried and concentrated. Silica gel chromatography (5:1 petroleum ether/ethyl acetate) of the residue afforded **62** (0.733 g, 58%; α/β=5:1) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$; α-anomer): δ 8.14-7.42 (m, 10H, H-Ar), 5.66 (m, 1H, OCH_2CH=CH_2), 5.58 (dd, $J_{2,3}$=3.3 Hz, $J_{2,1}$=1.8 Hz, 1H, H-2), 5.43 (t, $J_{4,3}$=$J_{4,5}$=9.8 Hz, 1H, H-4), 5.38 (bs, 1H, H-1), 5.15 (dd, J_{vic}=17.2 Hz, J_{gem}=1.6 Hz, 1H, OCH_2CH=CH_2 *trans*), 5.03 (dd, J_{vic}=10.4 Hz, J_{gem}=1.6 Hz, 1H, OCH_2CH=CH_2 *cis*), 4.27 (dq, $J_{5,4}$=9.8 Hz, $J_{5,6}$=6.2 Hz, 1H, H-5), 4.17-3.91 (m, 3H, H-3, OCH_2CH=CH_2), 1.30 (d, $J_{6,5}$=6.2 Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$; α-anomer): δ 165.9, 165.7 (2 C=O), 134.3 (OCH_2CH=CH_2), 133.3, 133.1 (2 C_{ipso}), 130.0-128.4 (C-Ar), 117.4 (OCH_2CH=CH_2), 92.5 (C-1), 73.9, 73.3, 70.7, 70.0, 66.8 (C-2, C-3, C-4, C-5, OCH_2CH=CH_2), 17.8 (C-6). ESI-MS for $C_{23}H_{24}O_7$ (*m/z*): M_r (calcd) 412.15, M_r (found) 435.38 $(M+Na)^+$. Anal. calcd: C. 66.98; H. 5.87. Found: C. 66.74; H. 5.99.

*3-O-allyl-2,4-di-O-benzoyl-α-*D*-rhamnopyranosyl trichloroacetimidate* **(63)**: To a 0°C cooled solution of **62** (0.728 g, 1.77 mmol) in CH_2Cl_2 (13 mL), Cl_3CCN (0.890 mL, 8.88 mmol) and DBU (0.152 mL, 1.02 mmol) were added under argon atmosphere. After 60' stirring at rt, the solution was concentrated at 20°C. Silica gel chromatography (12:1 petroleum ether/ethyl acetate) of the residue afforded **63** (0.539 g, 55%) as a white foam. $[\alpha]_D$ -50.3 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.77 (s, 1H, NH), 8.12-7.39 (m, 10H, H-Ar), 6.38 (d, $J_{1,2}$=2.0 Hz, H-1), 5.72 (dd, $J_{2,3}$=3.4 Hz, $J_{2,1}$=2.0 Hz, 1H, H-2), 5.67 (m, 1H, OCH_2CH=CH_2), 5.49 (t, $J_{4,3}$=$J_{4,5}$=10.0 Hz, 1H, H-4), 5.13 (dd, J_{vic}=17.4 Hz, J_{gem}=1.6 Hz, 1H, OCH_2CH=CH_2 *trans*), 5.04 (dd, J_{vic}=10.2 Hz, J_{gem}=1.6 Hz, 1H, OCH_2CH=CH_2 *cis*), 4.27-3.92 (m, 4H, H-3, H-5, OCH_2CH=CH_2), 1.33 (d, $J_{6,5}$=6.0 Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 165.6, 165.4 (2 C=O Bz), 159.8 (C=NH), 134.0 (OCH_2CH=CH_2), 133.4, 133.2 (2 C_{ipso}), 129.9-128.4 (C-Ar), 117.9 (OCH_2CH=CH_2), 95.1 (C-1), 73.9, 72.4, 70.9, 69.7, 68.0 (C-2, C-3, C-4, C-5, OCH_2CH=CH_2), 17.7 (C-6). ESI-MS for $C_{25}H_{24}Cl_3NO_7$ (*m/z*): M_r (calcd) 555.06, M_r (found) 578.17 $(M+Na)^+$. Anal. calcd: C. 53.93; H. 4.34; N. 2.52. Found: C. 54.10; H. 4.24; N.2.73.

*Methyl (3-*O*-allyl-2,4-di-*O*-benzoyl-α-*D*-rhamnopyranosyl)-(1→2)-[2,3-di-*O*-acetyl-4-*O*-benzoyl-α-*D*-rhamnopyranosyl-(1→3)]-4-*O*-benzoyl-α-*D*-rhamnopyranoside* **(64)**: A suspension of acceptor **61** (0.271 g, 0.44 mmol), imidate **63** (0.342 g, 0.62 mmol) and freshly powdered 4Å AW-300 molecular sieves in CH_2Cl_2 (16 mL) was stirred at -50°C under argon atmosphere. TMSOTf (1.1 μL, 6.1 μmol) was added and the mixture was kept at -50°C. After 60' other TMSOTf (9.0 μL, 50 μmol) was added and stirring was continued for additional 2 hours, after that the reaction was

quenched by adding a drop of Et_3N. The mixture was then filtered on a Celite pad and concentrated. Silica gel chromatography (6:1 petroleum ether/ethyl acetate) of the residue afforded **64** (0.315 g, 71%) as a white foam. $[\alpha]_D$ -59.0 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15-7.29 (m, 20H, H-Ar), 5.76 (m, 1H, OCH_2CH=CH_2), 5.63 (dd, $J_{2,3}$=3.0 Hz, $J_{2,1}$=1.6 Hz, 1H, H-2_B), 5.50 (m, 2H, H-4_A, H-3_C), 5.42 (t, $J_{4,3}$=$J_{4,5}$=9.8 Hz, 1H, H-4_B), 5.30 (dd, J_{vic}=17.2 Hz, J_{gem}=1.4 Hz, 1H, OCH_2CH=CH_2 *trans*), 5.26 (t, $J_{4,3}$=$J_{4,5}$=9.9 Hz, 1H, H-4_C), 5.22 (d, $J_{1,2}$=1.6 Hz, 1H, H-1_B), 5.15 (dd, J_{vic}=10.2 Hz, J_{gem}=1.4 Hz, 1H, OCH_2CH=CH_2 *cis*), 5.05 (dd, $J_{2,3}$=3.2 Hz, $J_{2,1}$=1.6 Hz, 1H, H-2_C), 4.97 (d, $J_{1,2}$=1.6 Hz, 1H, H-1_C), 4.88 (d, $J_{1,2}$=1.5 Hz, 1H, H-1_A), 4.28-4.11 (m, 6H, H-3_A, H-3_C, H-5_A, H-5_C, OCH_2CH=CH_2), 4.06 (dd, $J_{2,3}$=3.0 Hz, $J_{2,1}$=1.6 Hz, 1H, H-2_A), 3.93 (dq, $J_{5,4}$=9.8 Hz, $J_{5,6}$=6.2 Hz, 1H, H-5_B), 3.42 (s, 3H, OMe), 1.94 (s, 3H, CH_3C=O), 1.71 (s, 3H, CH_3C=O), 1.34 (d, $J_{6,5}$=6.2 Hz, 3H, H-6_B), 1.31 (d, $J_{6,5}$=6.2 Hz, 3H, H-6_C), 1.27 (d, $J_{6,5}$=6.2 Hz, 3H, H-6_A). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3, 169.0 (2 C=O Ac), 165.9-165.4 (4 C=O Bz), 134.1 (OCH_2CH=CH_2), 133.1-133.0 (4 C_{ipso}), 129.9-128.4 (C-Ar), 118.2 (OCH_2CH=CH_2), 100.0, 99.9, 99.7 (C-1_A, C-1_B, C-1_C, $^1J_{C,H}$=173 Hz, $^1J_{C,H}$=173 Hz, $^1J_{C,H}$=174 Hz), 79.6 (C-2_A), 77.2 (C-3_A), 73.9, 73.3, 73.2, 71.5, 71.0, 70.1, 70.0, 68.5, 67.7, 67.6, 66.7 (C-2_B, C-2_C, C-3_A, C-3_B, C-3_C, C-4_A, C-4_B, C-4_C, C-5_A, C-5_B, C-5_C, OCH_2CH=CH_2), 55.2 (OMe), 20.7, 20.4 (2 CH_3C=O), 17.8-17.7 (C-6_A, C-6_B, C-6_C). ESI-MS for $C_{54}H_{58}O_{19}$ (*m/z*): M_r (calcd) 1010.36, M_r (found) 1033.70 $(M+Na)^+$. Anal. calcd: C. 64.15; H. 5.78. Found: C. 64.00; H. 5.72.

Methyl (2,4-di-O-*benzoyl-α*-D-*rhamnopyranosyl)-(1→2)-[2,3-di*-O-*acetyl-4*-O-*benzoyl-α*-D-*rhamnopyranosyl-(1→3)]-4*-O-*benzoyl-α*-D-*rhamnopyranoside* **(65)**: A solution of **64** (0.297 g, 0.29 mmol) in 3:2 MeOH/CH_2Cl_2 (10 mL) was treated with $PdCl_2$ (16 mg, 90 μmol). After 7 hours stirring at rt, the mixture was filtered on a Celite pad, diluted with CH_2Cl_2 (100 mL) and extracted with 5M NaCl. The organic layer was collected, dried and concentrated. The residue was subjected to silica gel chromatography (5:1 petroleum ether/ethyl acetate) to afford **65** (0.260 g, 92%) as a white foam. $[\alpha]_D$ -48.0 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16-7.37 (H-Ar), 5.54 (m, 2H, H-2_B, H-3_C), 5.42 (m, 2H, H-4_A, H-4_B), 5.27 (t, $J_{4,3}$=$J_{4,5}$=9.8 Hz, 1H, H-4_C), 5.21 (d, $J_{1,2}$=1.9 Hz, 1H, H-1_B), 5.08 (dd, $J_{2,3}$=3.2 Hz, $J_{2,1}$=1.8 Hz, 1H, H-2_C), 5.00 (d, $J_{1,2}$=1.8 Hz, 1H, H-1_C), 4.86 (d, $J_{1,2}$=1.6 Hz, 1H, H-1_A), 4.58 (dd, $J_{3,4}$=9.8 Hz, $J_{3,2}$=3.4 Hz, 1H, H-3_B), 4.33-4.25 (m, 3H, H-3_A, H-5_B, H-5_C), 4.06 (dd, $J_{2,3}$=3.2 Hz, $J_{2,1}$=1.6 Hz, 1H, H-2_A), 3.92 (dq, $J_{5,4}$=9.6 Hz, $J_{5,6}$=6.0 Hz, 1H, H-5_A), 3.43 (s, 3H, OMe), 1.89 (s, 3H, CH_3C=O), 1.70 (s, 3H, CH_3C=O), 1.35-1.24 (m, 9H, H-6_A, H-6_B, H-6_C). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 169.0 (2 C=O Ac), 166.4, 165.6, 165.5, 165.2 (4 C=O Bz), 133.1-133.0 (4 C_{ipso}), 129.7-128.2 (C-Ar), 99.4-99.2 (C-1_A, C-1_B, C-1_C), 79.2 (C-2_A), 75.6 (C-3_A), 74.4, 73.6, 73.2, 71.2, 69.7, 68.5, 67.9, 67.6, 67.5, 66.4 (C-2_B, C-2_C, C-3_A, C-3_B, C-3_C,

C-4_A, C-4_B, C-4_C, C-5_A, C-5_B, C-5_C), 54.9 (OMe), 20.4, 20.3 (2 *C*H_3C=O), 17.6-17.5 (C-6_A, C-6_B, C-6_C). ESI-MS for $C_{51}H_{54}O_{19}$ (*m/z*): M_r (calcd) 970.33, M_r (found) 971.02 $(M+H)^+$. Anal. calcd: C. 63.09; H. 5.61. Found: C. 63.09; H. 5.58.

Methyl (3-O-*allyl-2,4-di*-O-*benzoyl-α*-D-*rhamnopyranosyl)-(1→3)-(2,4-di*-O-*benzoyl-α*-D-*rhamnopyranosyl)-(1→2)-[2,3-di*-O-*acetyl-4*-O-*benzoyl-α*-D-*rhamnopyranosyl-(1→3)]-4*-O-*benzoyl-α*-D-*rhamnopyranoside* **(66)**: A suspension of acceptor **65** (0.212 g, 0.22 mmol), imidate **63** (0.165 g, 0.30 mmol) and freshly powdered 4Å AW-300 molecular sieves in CH_2Cl_2 (10 mL) was stirred at -50°C under argon atmosphere. TMSOTf (0.54 μL, 3.0 μmol) was added and the mixture was kept at -50°C. After 90' the reaction was quenched by adding a drop of Et_3N. The mixture was then filtered on a Celite pad and concentrated. Silica gel chromatography (7:1 petroleum ether/ethyl acetate) of the residue afforded **66** (0.210 g, 70%) as a white foam. $[\alpha]_D$ -82.0 (*c* 1.0, CH_2Cl_2);. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06-7.34 (H-Ar), 5.67 (dd, $J_{2,3}$=3.2 Hz, $J_{2,1}$=1.6 Hz, 1H, H-2_C), 5.64 (t, $J_{2,3}$=9.8 Hz, 1H, H-4_C), 5.44 (m, 2H, H-3_B, H-4_A), 5.33 (d, $J_{2,1}$=1.6 Hz, 1H, H-1_C), 5.30-5.23 (m, 5H, H-1_C, H-1_D, H-2_D, H-4_B, H-4_D), 5.08 (dd, $J_{2,3}$=3.2 Hz, $J_{2,1}$=1.6 Hz, 1H, H-2_B), 4.96 (d, $J_{2,1}$=1.6 Hz, 1H, H-1_B), 4.87 (m, 2H, H-1_A, $OCH_2CH=CH_2$ *trans*), 4.77 (dd, J_{vic}=10.2 Hz, J_{gem}=1.4 Hz, 1H, $OCH_2CH=CH_2$ *cis*), 4.59 (dd, $J_{3,4}$=9.8 Hz, $J_{3,2}$=3.2 Hz, 1H, H-3_C), 4.29 (dd, $J_{3,4}$=9.8 Hz, $J_{3,2}$=3.3 Hz, 1H, H-3_A), 4.23 (m, 2H, H-5_B, H-5_C), 4.08 (m, 2H, H-2_A, H-5_D), 3.93 (m, 1H, H-5_A), 3.83 (dd, $J_{3,4}$=9.8 Hz, $J_{3,2}$=3.4 Hz, 1H, H-3_D), 3.75 (m, 1H, $OCH_2CH=CH_2$), 3.62 (m, 1H, $OCH_2CH=CH_2$), 3.43 (s, 3H, OMe), 1.34 (m, 6H, H-6_A, H-6_C) 1.26 (d, $J_{6,5}$=6.2 Hz, 3H, H-6_B), 1.15 (d, $J_{6,5}$=6.2 Hz, 3H, H-6_D). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 168.6 (2 C=O Ac), 166.0, 165.8, 165.3, 165.2, 165.0, 164.9 (6 C=O Bz), 134.0 ($OCH_2CH=CH_2$), 133.4-132.8 (6 C_{ipso}), 129.8-128.1 (C-Ar), 117.2 ($OCH_2CH=CH_2$), 100.0, 99.7, 99.6, 99.2 (C-1_A, C-1_B, C-1_C, C-1_D, $^1J_{C,H}$=173 Hz, $^1J_{C,H}$=173 Hz, $^1J_{C,H}$=173 Hz, $^1J_{C,H}$=174 Hz), 79.0 (C-2_A), 76.4, 74.9, 73.8, 73.3, 72.9, 72.4, 71.5, 70.2, 70.0, 69.2, 68.4, 67.7, 67.6, 67.3, 66.9 (C-2_B, C-2_C, C-2_D, C-3_A, C-3_B, C-3_C, C-3_D, C-4_A, C-4_B, C-4_C, C-4_D, C-5_A, C-5_B, C-5_C, C-5_D, $OCH_2CH=CH_2$), 55.2 (OMe), 20.6, 20.2 (2 *C*H_3C=O), 17.8-17.5 (C-6_A, C-6_B, C-6_C, C-6_C). ESI-MS for $C_{74}H_{76}O_{25}$ (*m/z*): M_r (calcd) 1364.47, M_r (found) 1387.43 $(M+Na)^+$. Anal. calcd: C. 65.09; H. 5.61. Found: C. 66.01; H. 5.48.

Methyl α-D-*rhamnopyranosyl-(1→3)-α*-D-*rhamnopyranosyl)-(1→2)-[α*-D-*rhamnopyranosyl-(1→3)]-α*-D-*rhamnopyranoside* **(53)**: A solution of **66** (30 mg, 22 μmol) in 1:1 MeOH/CH_2Cl_2 (1.5 mL) was treated with $PdCl_2$ (4.0 mg, 22 μmol). After stirring overnight at rt, the mixture was filtered on a Celite pad, diluted with CH_2Cl_2 (25 mL) and extracted with 5M NaCl (25 mL). The organic layer was collected, dried and concentrated. The residue was dissolved in 1:1

MeOH/CH_2Cl_2 (1.0 mL) and treated with a 3.4 M methanolic solution of NaOMe (0.1 mL). The solution was stirred overnight at rt, then Amberlist-15 (H^+) was added to adjust the pH value to 6. The mixture was filtered and concentrated to give a residue, that was purified by gel filtration on a G-10 (Sephadex) column using water as eluant, to obtain **53** (9.8 mg, 72%) as a white foamy solid. $[\alpha]_D$ +38.3 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, D_2O): δ 5.06 (bs, 2H, H-1_B, H-1_D), 4.96 (d, 1H, $J_{1,2}$=1.7 Hz, 1H, H-1_C), 4.78 (d, 1H, $J_{1,2}$=1.6 Hz, 1H, H-1_A), 4.06 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{2,3}$=1.6 Hz 1H, H-2_B), 4.04 (m, 2H, H-2_C, H-2_D), 4.00 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{2,3}$=1.6 Hz 1H, H-2_A), 3.89-3.85 (m, 2H, H-3_A, H-3_C), 3.82-3.76 (m, 2H, H-3_B, H-5_C), 3.74-3.65 (m, 4H, H-3_D, H-5_A, H-5_B, H-5_D), 3.59 (t, 1H, $J_{4,3}$= $J_{4,5}$=9.6 Hz, 1H, H-4_A), 3.55 (t, 1H, $J_{4,3}$= $J_{4,5}$=9.6 Hz, 1H, H-4_C), 3.47 (m, 2H, H-4_B, H-4_D), 3.41 (s, 3H, OMe), 1.30 (m, 12H, H-6_A, H-6_B, H-6_C, H-6_D). ^{13}C NMR (100 MHz, D_2O):[28] δ 103.4 (C-1_B, C-1_D), 103.0 (C-1_C), 100.6 (C-1_A), 79.5 (C-2_A), 78.5 (C-3_A, C-3_C), 73.3 (C-4_A, C-4_B), 73.2 (C-4_D), 73.0 (C-4_C), 71.5 (C-2_B), 71.3 (C-2_C), 71.2 (C-2_D), 71.1 (C-3_B, C-3_D), 70.4 (C-5_A, C-5_B, C-5_C, C-5_D), 56.0 (OMe), 18.4-18.3 (C-6_A, C-6_B, C-6_C, C-6_D). ESI-MS for $C_{25}H_{44}O_{17}$ (*m/z*): M_r (calcd) 616.26, M_r (found) 639.58 $(M+Na)^+$. Anal. calcd: C. 48.70; H. 7.19. Found: C. 48.81; H. 7.17.

3.6 References

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4. SYNTHESIS OF A RHAMNOGALACTURONAN II FRAGMENT

To gain deeper acknowledgments on synthesis of rhamnosyl linkages, I spent my last year Ph.D. working in the laboratories of *Centre for Carbohydrate Chemistry (CCC)* (University of East Anglia, Norwich, UK) directed by Prof. R.A. Field. Among several projects, Field and co-workers are motivating in gain an insight into structural and functional knowledge of pectic polysaccharides of the plant cell wall. In this chapter a description of plant cell wall and its component has been reported. Additionally, herein a revised synthesis of a tetrasaccharide fragment from a pectic polysaccharide of the plant cell wall is described.

4.1 Plant cell wall

When pathogens attack a plant it has several passive (size of the pore too small for viruses) and active (death of the cell under attack) means of defence, and almost all of them involve the cell wall; actually, by affecting cell wall properties, plants are more likely to be vulnerable to viruses and pathogens. The cell wall, an essential component of higher plants, is also known to be involved in cell stability, shape, and development, as well as in the protection of the cell against pathogens.[1] Cellulose, hemicellulose and pectins are part of its components forming a compact network (**Figure 4.1**) which contributes to the various chemical and structural properties of the whole cell wall.

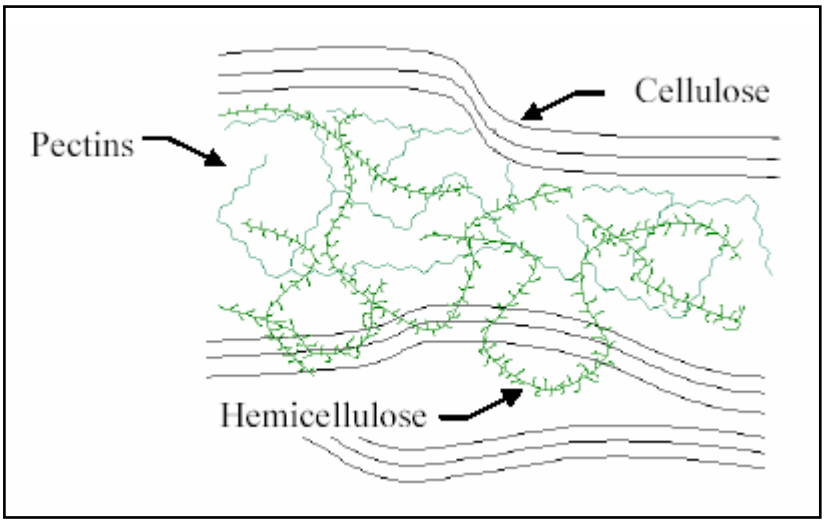

Figure 4.1: Model of a primary plant cell wall

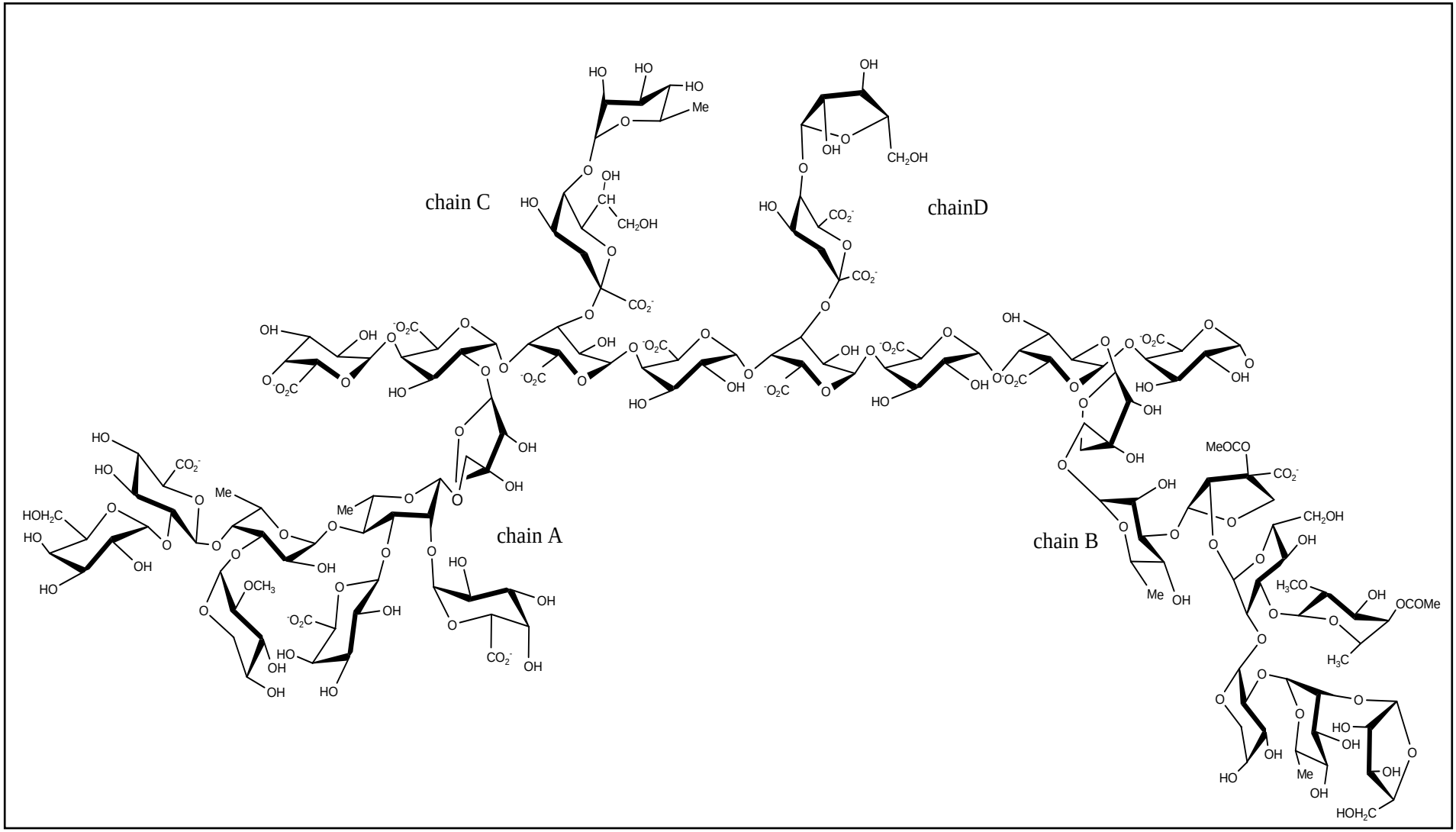

Figure 4.2 Rhamnogalacturonan II structure

Cellulose is composed of (1→4)-β-D-glucans forming a sheet-like structure and gives the mechanical strength to the plant cell wall.[2] Hemicelluloses are flexible heterogeneous polysaccharides binding to the (1→4)-β-D-glucans. In contrast to cellulose that is crystalline, strong and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. The combination of both cellulose and hemicellulose in a tight network accounts for the structural support provided by the wall during the growth.[2] The term "pectin" is employed to cover a group of acidic heteropolysaccharides consisting of linear chains of D-galacturonic acids which are α-(1→4)-linked. The precise role of pectins is still uncertain; however, the pectic network of the primary cell wall is clearly a target for specific developmental modifications such as cell wall swelling and softening during fruit ripening, and cell separation during leaf and fruit abscission, pod dehiscence and root cap cell differentiation.[3] The pectins are divided into three major more or less complex polysaccharides containing galacturonic acids and neutral sugars within their structure. Homogalacturonan (HG), and rhamnogalacturonan I (RG-I) are two of the pectins which are defined as relatively simple structurally[4,1b] whereas rhamnogalacturonan II (RG-II), is considered to be a very complex highly branched polysaccharide (Figure 3c).[1b,4,5] From a compositional and structural point of view, HG has a relatively simple primary structure of 1,4-linked α-D-Gal*p*A which are partially esterified, while the RG-I backbone consists of a repeating sequence of 1,4-linked α-D-Gal*p*A-(1→2)-α-L-Rha*p* disaccharide residues. In contrast to HG and RG-I which are notable for their heterogeneity, RG-II has a highly conserved structure throughout the plant kingdom. Not all details of this structure have yet been elucidated.

4.2. Rhamnogalacturonan

Rhamnogalacturonan-II (RG-II) is considered to be the most complex of the pectins and, in contrast to HG and RG-I which are other pectic polysaccharides, has a highly conserved structure throughout the plant kingdom. It is a low molecular mass (5-10kDa) pectic polysaccharide with a backbone composed of 1,4-linked α-D-Gal*p*A residues to which four oligosaccharide side chains are attached (**Figure 4.2**). The order in which the side chains are attached to the backbone has still to be unambiguously determined, and so their assignment is arbitrary. Nevertheless, it has been shown that two of the backbone Gal*p*A residues are substituted at *C*3 with two structurally different disaccharides, and two octasaccharides are attached to *C*2 of two other backbone Gal*p*A residues. Monomeric RG-II yields, upon hydrolysis, more than 12 different monosaccharides, including the rarely observed aceric acid and apiose, the only two-branched sugars known to exist in plant polysaccharides, and 2-*O*-methylxylose and 2-*O*-methylfucose.[6]

The complexity and occurrence of RGII suggest that it might have functions other than being a simple structural component of the cell wall.

4.2.1. Rhamnogalacturonan-II dimer

Inside the pectin matrix, recent studies have established that RG-II exists predominantly as a dimer resulting from a 1:2-borate-diol ester linkage which involves two apiofuranosyl residues belonging to the side chain called *side chain A* of two different RG-II units (**Figure 4.3**).[7] The specificity of this cross-linkage suggests that there are precise structural requirements (nature of monosaccharides within the structure of the polysaccharide, for instance)[8] for dRG-II formation, and this may explain why the structure of RG-II is highly conserved in plants.[9,7a]

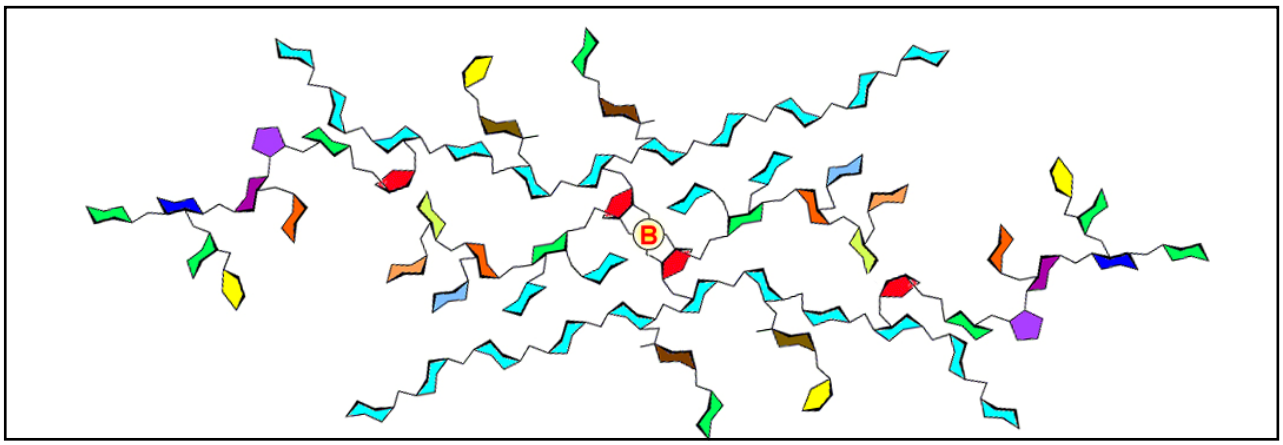

Figure 4.3: RGII dimer cross-linked via borate diester between apiose residues of the side chain A

This RG-II dimer accounts for more than 80% of RG-II in plants[10] and contributes to the coordination bonding of pectic chains and Ca^{2+}.[11] Furthermore, 1:2-borate cross-linked RG-II has been isolated from wine[9a] and its presence was confirmed in other fruit-derived products including juices. As an example, the dRG-II concentration corresponds to about 150 mg/L in red wine and 50 mg/L in white wine.[12] This high concentration, despite the fact that RG-II accounts for less than 5% in the primary cell wall, is explained by its resistance to a wide variety of enzymes. Studies revealing the specificity of the 1,2-diolester cross linkage,[9b,8b] enabled a better understanding of plant morphogenesis; thus, it is well clear the importance of studying specificity in dimer formation and, obviously, structural arrangement of fragment A. This would require synthesis of large oligosaccharide fragments modelling side-chain A of RG-II by means of orthogonal strategy leading to higher and higher structure.

4.3 Revised synthesis of a tetrasaccharide from RGII side chain A

One of the ongoing research project in the *CCC* at the University of East Anglia (UEA) is the synthesis of RGII. In a previous work the synthesis of the tetrasaccharide **67** α-D-GalA-(1→2)-[β-D-GalA-(1→3]-[α-L-Fuc-(1→4)]-α-L-Rha-OMe (**Figure 4.4**), a fragment of RG II side chain A, has been reported.[13]

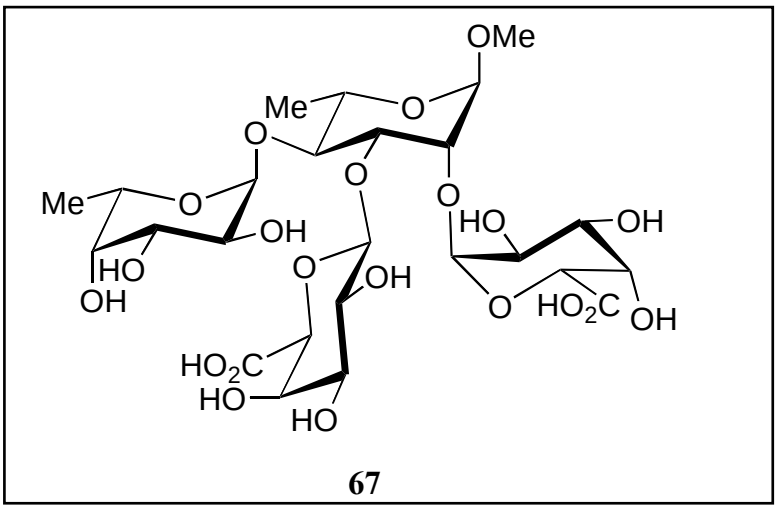

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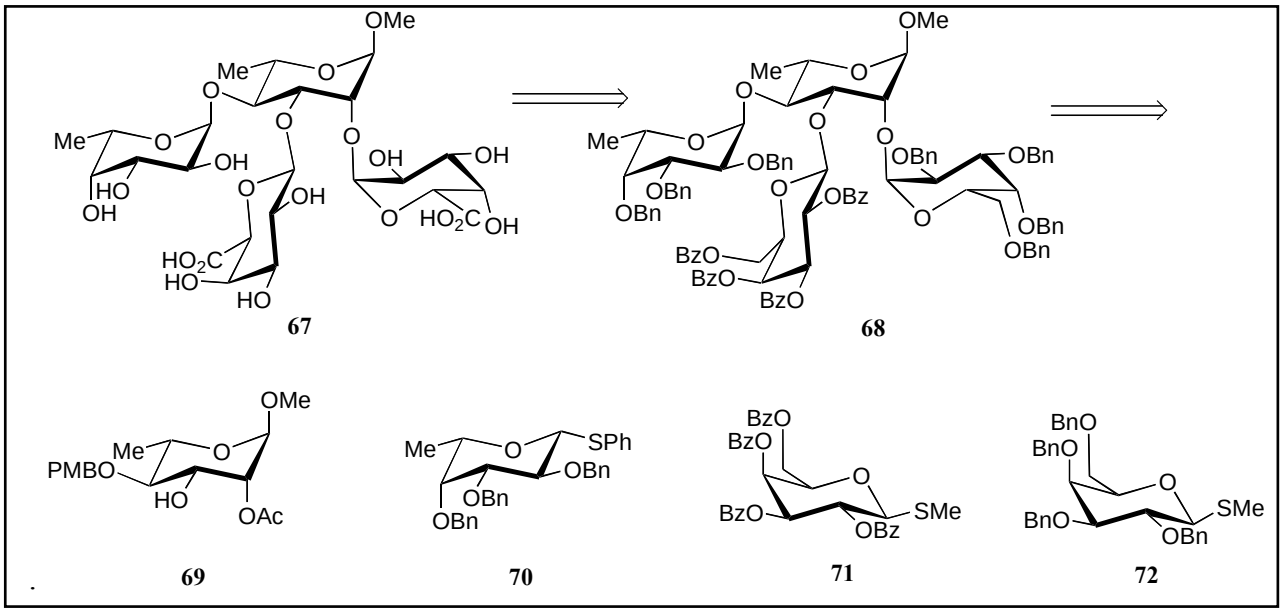
Figure 4.4: Tetrasaccharide fragment from RGII side chain A

The strategy used in this work involves iterative glycosylations of the orthogonally protected α-methyl rhamnoside acceptor **69** followed by oxidations carried out on galactosyl moieties; the retrosynthetic analysis is shown in the following scheme (**Scheme 4.1**).

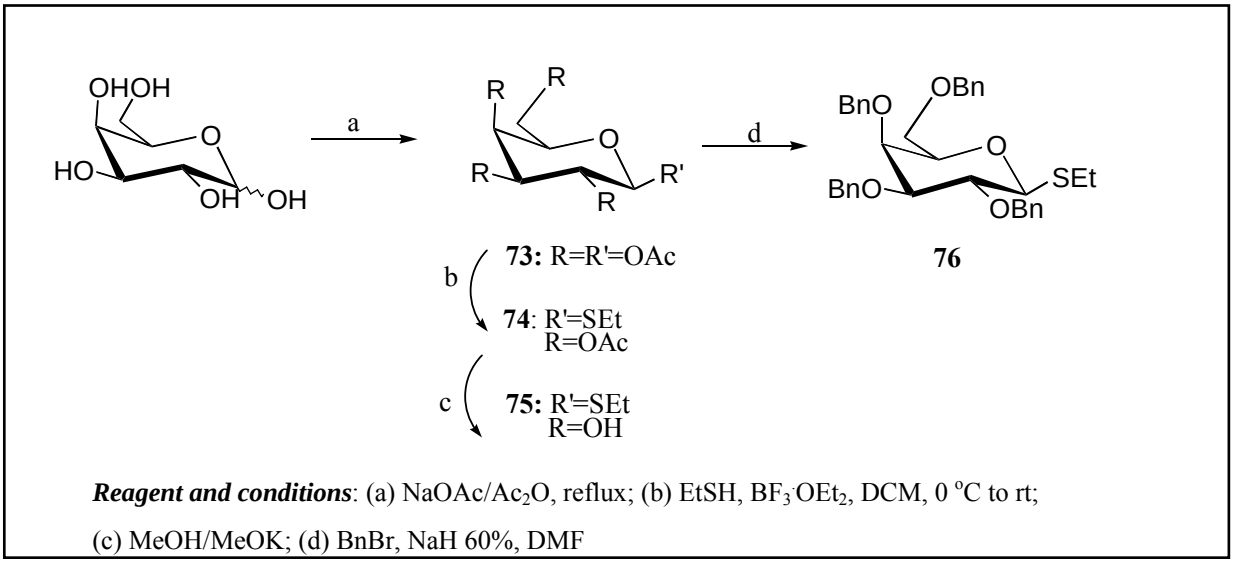


Scheme 4.1: Retrosynthesis of side chain A tetrasaccharide fragment from RGII

Nevertheless a revised synthesis of this tetrasaccharide has been considered; it involves the incorporation of a per-benzylated galactosyl residue at *C*-3 rhamnoside. This variation would render the synthesis more straightforward bypassing the de-benzoylation reaction in the deprotecting step. Furthermore building of 1,2-*trans*-anomeric linkage using an armed donor would be a challenging goal. Moreover the new synthesis would involve the use of a β-rhamnosyl acceptor whose configuration is the one naturally occurring. However glycosylation attempts were carried out using first the α-configurated rhamnosyl acceptor which is more straightforward to synthesize.

4.3.1 Synthesis of galactosyl donor

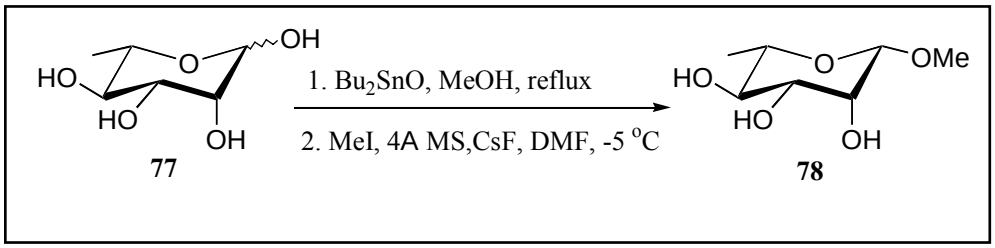
Thioglycoside was synthesised using the traditional method by Lewis acid-mediated activation of per-acetylated glycosides in the presence of a thiol.[14] The 1,2-*trans*-glycosides are the main products because of the anchimeric assistance from the acetyl group. Anyway this reaction has to be carried out carefully because anomerization can occur when performing the reaction for a long time. The per-*O*-acetylated galactose **73** starting material was converted into its thioglycoside counterpart **74** as described above. According to the procedure reported in the previous work by Field and co-workers[13] the thioglycoside was deprotected by a Zemplén reaction to obtain the compound **75**, then benzylated by BnBr and NaH 60% (**Scheme 4.2**).The benzylation was confirmed by the presence of CH_2 peaks of benzyl groups in the ^{1}H NMR spectrum.



Scheme 4.2: Synthesis of an armed perbenzylated galactosyl donor

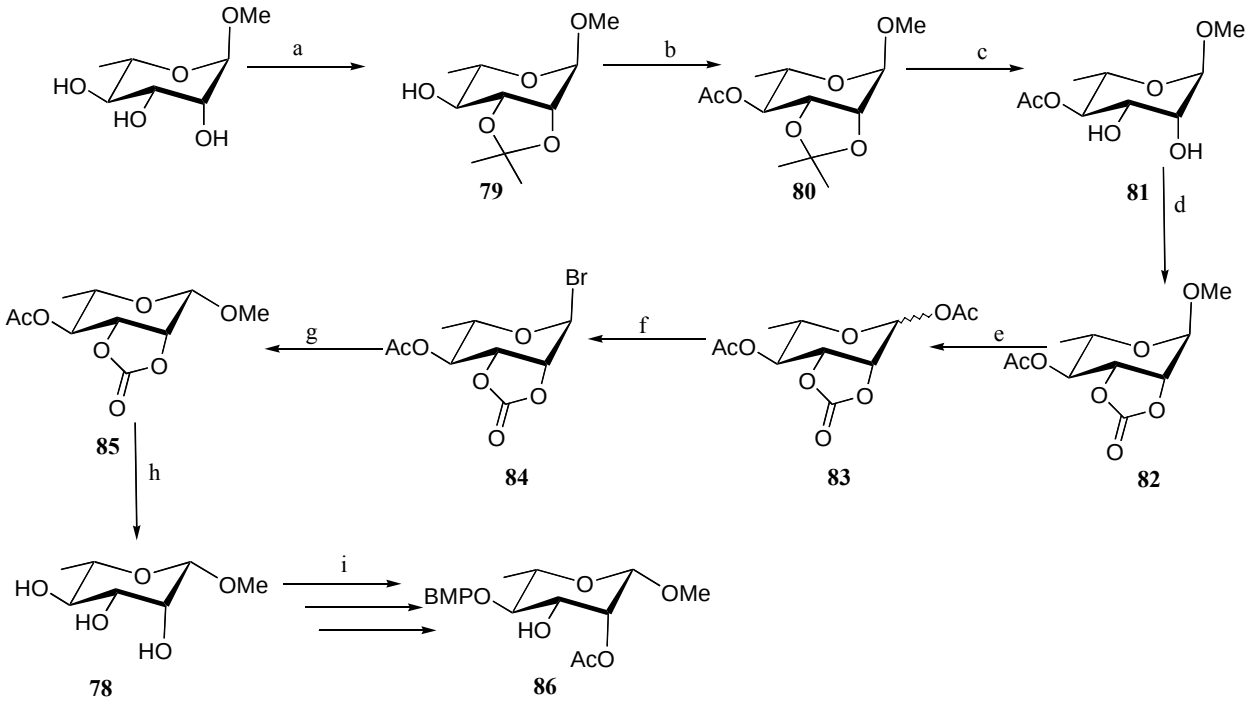
4.3.2 Synthesis of β-rhamnosyl acceptor

A first attempt to synthesize an *O*-Me-β-rhamnoside acceptor was performed using a 1,2-*cis*-stannylene acetal-based procedure[15] (**Scheme 4.3**), but the reaction gave us a mixture of compounds within there was not the desired compound.



Scheme 4.3: Failed attempt of synthesis of *O*-Me-β-rhamnoside *via* stannylene acetal-based procedure

Thus, it was decided to achieve the β-configuration by conversion of an *O*-Me-α-rhamnoside building block using a more classic protocol by means of a multi-step synthesis. The synthetic strategy employed on this purpose is shown in the following **Scheme 4.4.**



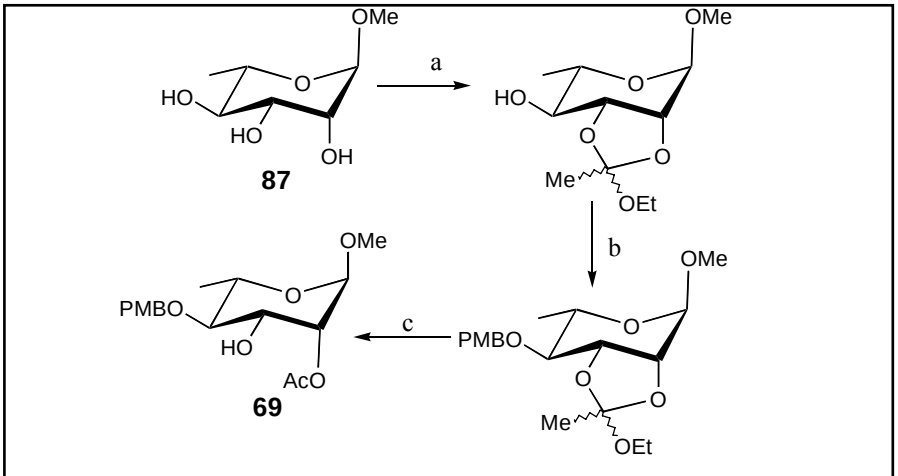
Reagents and conditions: a) DMP, TsOH, DMF; b) 1:3 Ac_2O, Py; c) 4:1 TFA/H_2O, 45% over three steps; d) $(Cl_3CO)_2CO$, DCM, Py, -70 °C ; e) Ac_2O, H_2SO_4, 90% over two steps; f) 33% HBr/AcOH; g) $Hg(CN)_2$, MeOH, CH_3CN, 79% over two steps; h) MeOH/MeOK; i) (i) $MeC(OEt)_3$, CSA, CH_3CN; (ii) PMBCl, NaH, CH_3CN; (iii) 80% aq AcOH, 20% over three steps

Scheme 4.4: Multi-step synthesis of *O*-Me-β-rhamnoside acceptor

Starting from *O*-Methyl α-L-rhamnopyranoside, Br-α-rhamnoside **84** was synthesized by means a sequence of protection/deprotection steps.[16] The most crucial step in the chosen route was the conversion of bromide **84** to the *O*-Me-β-rhamnoside acceptor. Thus, with the compound **84** in hands, several attempts of β-methylation were performed using different conditions. The best result was obtained using $Hg(CN)_2$[17] as promoter. The synthesis proceeded as described in the previous work using the one-pot method[18,19] described in paragraph 3.1; protection step of 4-OH with a *p*-methoxybenzyl protecting group was performed in between the formation of the orthoester and its ring-opening leading to compound **86** with a free equatorial hydroxyl group on *C*-3 and two differentially protected hydroxyl groups on 2-OH and 4-OH(PMB and Ac), thus, creating a difference in reactivity between the groups on the ring. However, this reaction gave us compound **86** with a very poor yield (20%) and needed to be optimized. Since compound **86** was quite "precious" we decided to carry glycosylation tests on its α-analogue; thus the synthesis of compound **69** was performed according to the procedure from previous work.[13]

4.3.3 Synthesis of α-rhamnosyl acceptor[13]

Starting from the commercially available methyl rhamnoside **87,** the α-glycosyl acceptor **69** was synthesised by means of the one-pot protocol previously described which yields 70% of protected rhamnosyl acceptor in this case (**Scheme 4.5**).

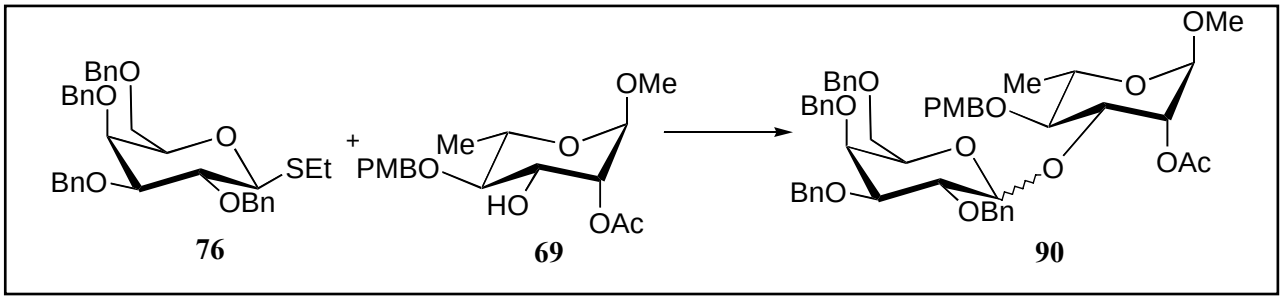


Reagents and conditions: a) $MeC(OEt)_3$, *p*TsOH, CH_3CN; b) PMBCl, NaH, CH_3CN; c) 80% aq. AcOH, 70% over three steps

Scheme 4.5: One-pot procedure on synthesis of *O*-Me-α-rhamnoside

4.3.4 Towards the synthesis of the tri-substituted rhamnoside

In the previous work the coupling between rhamnosyl acceptor **69** and thiogalactosyl donor **72** was considered. Unfortunately poor yields and stereselectivities were observed using NIS/TfOH (or NIS/TMSOTf) as promoter in a range of different solvents. In this work new attempts of glycosylation are reported, in order to improve yield and β-stereoselectivity of the 1,2-*trans* linked disaccharide. In order to have a wide range of tests where to pick up the best result from, several attempts of 1,2-*trans* glycosylation were performed in the present work by using different alonium ion-based activation methods; furthermore the efficiency of propionitrile as participating solvent favouring the β-stereoselectivity, as reported by Schmidt and co-workers,[20] was tested and it was compared with the most used CH_3CN. Thus, the efficiency of NIS/TfOH as promoter in coupling **76** and **69,** was tested again slightly changing the molar ratio and the temperature.



Scheme 4.6: Coupling of galactosyl donor **76** and α-rhamnosyl acceptor **69**

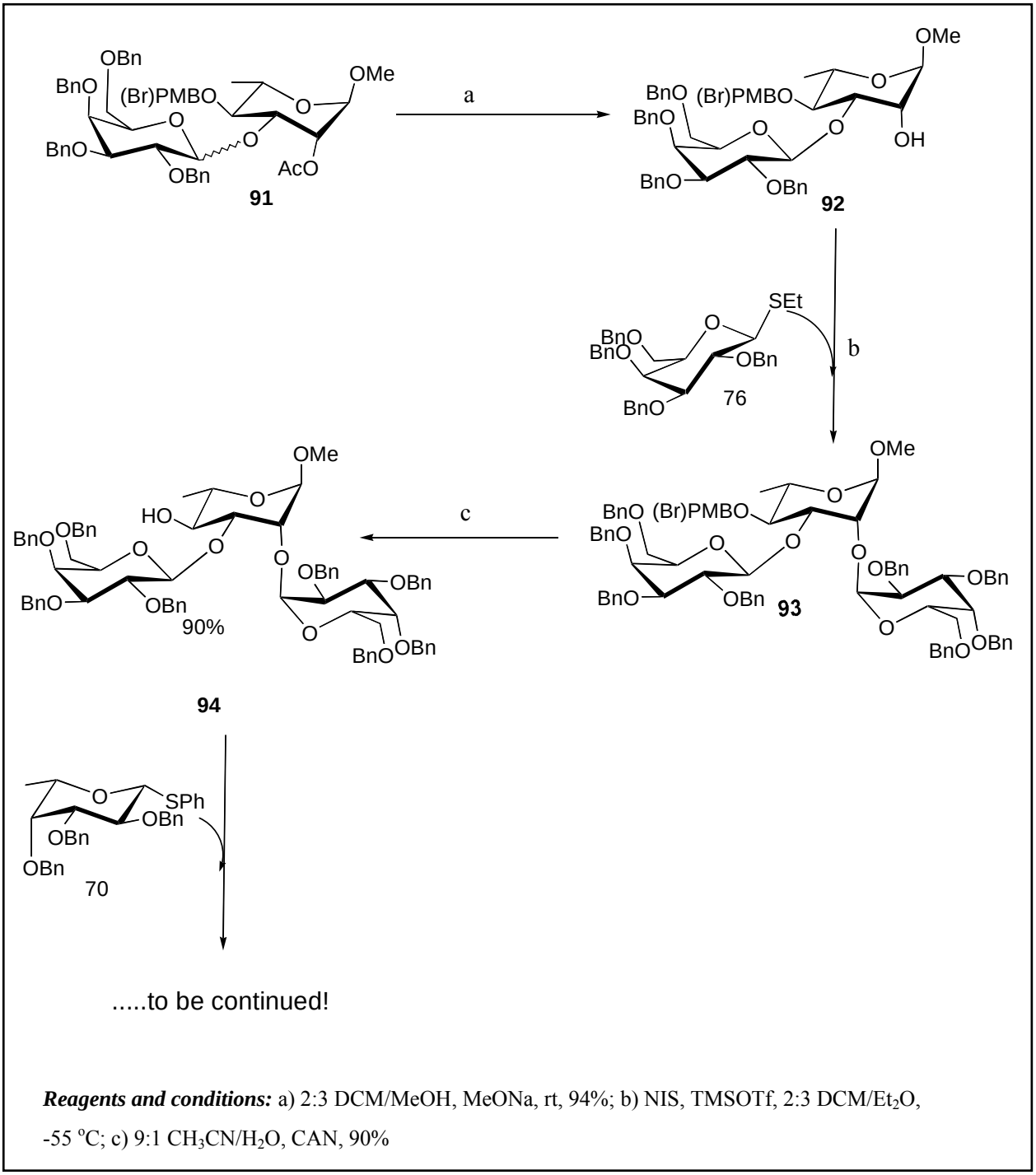
Unfortunately, these variations were unsuccessfull affording the disaccharide **90,** as anomeric mixture, with poor yield and stereoselectivity in both nitriles as solvents. The nature of the triflate promoter and its effect on the anomeric selectivity was kept considering. As reported by Lowary and co-workers,[21] thioglycosyl donors are activated with NIS/AgOTf. In fact, coupling between donor **76** and acceptor **69** using the last mentioned promoter provided disaccharide **90** with better stereocontrol in both nitrilic solvents. NBS has been reported to be a convenient activating agent for thioglycosyl donor; besides, NBS is less expansive and more stable than NIS. Nicolaou and co-workers[22] and Sasaki and co-workers[23] used *N*-bromosuccinamide (NBS) alone, or in combination with TfOH, to activate phenyl thioglycosides; Li and co-workers[24] found that NBS together with a catalytic amount of Me_3SiOTf was effective for the activation of both phenyl- and ethyl-thioglycosides with satisfying β-stereocontrol. In our case, combination of NBS with Me_3SiOTf first and AgOTf later, yields a brominated anomeric mixture of the desired disaccharide in both nitrilic solvents, with a β/α ratio about 3. The anomeric ratios were based on the integration of the -OCH_3 group or acetyl group of the rhamnosyl moiety at 3.2 or 2.0 ppm respectively. Trying to gain

better in stereoselectivity, we exploited the use of the combination I_2/DDQ in coupling **76** and **69**. Iodine itself is a mild and effective activator of thioglycosides. Whilst iodine alone is able to activate reactive glycosyl donors, less reactive donors are activated when iodine is used together with DDQ, a versatile single electron oxidising agent;[25] on the other hand, DDQ alone is not effective in activation of thioglycosides demonstrating that the combination of two reagents is key. The mechanism of activation by I_2/DDQ is not yet completely understood but the more credible hypothesis is that DDQ oxidises iodide, generated in the activation process, to iodine, so preventing glycosyl iodide and favouring α-glycosyl nitrilium ion formation. Unfortunately, this glycosylating condition was not effective in couplings **76** and **69.** All the results of the coupling are shown in **Table 4.1**.

ENTRY	PROMOTER	SOLVENT	T (^{o}C)	YIELD (%)	β/α ratio
1	NIS/TfOH	6.7:1 CH_3CN/DCM	0	41	1.6
2	NIS/TfOH	Pr-CN	-35	47	1.8
3	NIS/AgOTf	CH_3CN	-35 to -20	71	2.7
4	NIS/AgOTf	Pr-CN	-50 to -20	58	2.9
5	NBS/Me_3SiOTf	CH_3CN	-35 to -20	70	3.6
6	NBS/Me_3SiOTf	Pr-CN	-50 to -20	51	3.7
7	NBS/AgOTf	CH_3CN	-35	25	1.1
8	I_2/DDQ	CH_3CN	-35	31	1.5

Table 4.1: Glycosylation conditions and results in coupling galactosyl donor **76** and α-rhamnosyl acceptor **69**

The best compromise in yield and stereoselectivity was afforded activating donor **76** by NBS/Me_3SiOTf (entry 5, **Table 4.1**). However, not surprisingly, MS analysis revealed the presence of a bromine atom on the coupling product, as it is reported by Li and co-workers.[24] The value of mass peak, indicating just one atom of bromine, and the difference in reactivity between benzyl and *p*-methoxybenzyl protecting group suggest us to hypothesize the presence of bromine on the aromatic ring of the latter. To be sure this would not have affected the further steps of the synthesis, we attempted on disaccharide anomeric mixture a preventing test of removal the brominated protecting group in the same conditions used to remove a not brominated one. The good outcome of the test encouraged us to proceed as in the previous work.



Scheme: Synthesis of trisaccharide **94**

Thus, the anomeric mixture of **91** was treated with MeONa affording the disaccharide alcohol **92** as a single anomer after chromatography (**Scheme 4.7**). Consecutively acceptor **92** was coupled with donor **76** in an α-stereoselectivity-favouring mixture of solvents; the formation of the trisaccharide was ascertained by MS spectrum. Compound **93** was treated with CAN in 9:1 CH_3CN/H_2O affording the 3-OH-rhamnoside-acceptor which was ready to be coupled with fucosyl donor **70** in the same condition used in the previous work published by Field and co-workers.[13]

4.4 Experimental part

All solvents were used as purchased, except for CH_2Cl_2, which was freshly distilled from CaH_2, and stored over 4 Å molecular sieves. Cation-exchange resin (Amberlite IR-120, H^+ form Fluka) was pre-washed with water and dry MeOH before use. Thin layer chromatography (TLC) was performed on aluminium-backed, pre-coated silica gel plates (Silica Gel 60 F_{254}, Merck) which were developed by immersion in a 5% ethanolic solution of H_2SO_4, followed by heating to 200ºC. Column chromatography was performed on silica gel (40 – 70 μm, BDH-Merck). Evaporation of solvents was performed under reduced pressure at 25 – 40ºC. Reagents and dry solvents were added *via* syringes through septa. ^{1}H and ^{13}C NMR spectra were recorded at 24ºC with a Varian Gemini 2000 spectrometer at 300 and 75 MHz, respectively, or with a Varian Unity Plus spectrometer at 400 and 100 MHz, respectively, using $CDCl_3$ or D_2O as solvents. Resonance assignments for compound **91-94** were not made for all signals but only for diagnostic peaks. Accurate mass electrospray ionisation (ESI) mass spectra were obtained on a Finnigan MAT 900 XLT mass spectrometer at the EPSRC Mass Spectrometry Service Centre, Swansea.

*Ethyl 2,3,4,6-tetra-O-benzyl-1-thio-β-*D*-galactopyranoside* **(76).** Compound **75**[13,14] was dissolved in DMF (50 cc) and then NaH (60% dispersion in mineral oil, 2.530 g, 58.73 mmol) was added. The mixture was kept under stirring for 30 min and then BnBr (4.9 eq, 58.73 mmol) was added at 0 ^{o}C. The mixture was kept under stirring at room temperature over night. MeOH (45 cc) was then added and the mixture was allowed to stir for 10 min. The mixture was partitioned between EtOAc and H_2O. The organic layers were dried by $MgSO_4$, filtered and concentrated.
Column chromatography (7:1 hexane-ethyl acetate) of the residue afforded **76** as a white oil (86.7%, yield calculated over the last two steps): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.95 (d, 1H, J_{gem} = 11.6 Hz , OCH_2Ph), 4.87 (d, 1H, J_{gem} = 10.4 Hz, OCH_2Ph), 4.79 (d, 1H, J_{gem} = 10 Hz, OCH_2Ph), 4.62 (d, 1H, J_{gem} = 12 Hz, OCH_2Ph), 4.45 (m, 4H, H-1, OCH_2Ph), 3.95 (d,1H, J = 2.8 Hz, H-4), 3.83 (t, 1H, J = 9.6 Hz, H-2), 3.59 (m, 4H, H-3, H-5, H-6, H-6'), 2.78 (dq, 2H, SCH_2CH$_3$), 1.30 (t, 3H, J = 7.2 Hz, SCH$_2$CH_3).

*Methyl 2-O-acetyl-4-O-(4-methoxybenzyl)-β-*L*-rhamnopyranoside* **(86).** A solution of compound **78**[17] (8.30 mmol, 1.478 g) and triethylorthoacetate (106 mmol 12.8 eq) in CH_3CN was stirred for few minutes at room temperature and a catalytic amount of CSA was added. The mixture was stirred until TLC (7:1 DCM/MeOH) showed disappearance of starting material (over night), and the

solution was neutralized with Et_3N. NaH (60% dispersion in mineral oil, 1.302 g, 32.5 mmol) was added to the mixture and after 15 min of stirring 4-methoxybenzyl chloride (7.5 mL, 49.8 mmol,) was added carefully. After one night the reaction is quenched by adding MeOH (7.5 mL) and the mixture concentrated. The residue was dissolved in EtOAc and washed with H_2O, dried by $MgSO_4$, filtered and concentrated. Column chromatography (5:2 hexane-ethyl acetate) of the residue gave **86** (16%, yield calculated over three steps).

*Methyl (2,3,4,6-tetra-O-benzyl-*D*-galactopyranosyl)-(1→3)-2-O-acetyl-4-O-paramethoxybenzyl-α-*D*-rhamnopyranoside* **(90) .**

Protocol with NIS/TfOH: A solid mixture of compound **76** (1.46 eq, 0.22 mmol, 128 mg) and compound **69**[13] (0.15 mmol, 51 mg) was codistilled by toluene and dried. Molecular sieves (4 Å, 0.13 g) were added and the mixture was suspended in dry solvent (2.5cc) and kept under stirring for 30 min at room temperature under N_2. The mixture was cooled at 0 ^oC (or -50 ^oC) and NIS (0.33 mmol, 1.5 eq) and TfOH (0.033 mmol) were added under N_2. The mixture was allowed to stir at the desired temperature for 1h.Then the mixture was neutralized with Et_3N, dilueted with CH_2Cl_2, filtered through celite, washed with 10% aq $Na_2S_2O_3$ solution and H_2O. The organic layers were dried by $MgSO_4$, filtered and concentrated.

Protocol with NIS/AgOTf: A solid mixture of compound **76** (1.33 eq, 0.22 mmol, 128 mg) and compound **69**[13] (0.16 mmol, 56 mg) were codistilled by toluene and dried. Molecular sieves (4 Å, 0.15 g) were added and the mixture was suspended in dry solvent (2.5cc) and kept under stirring for 30 min at room temperature under N_2. The mixture was cooled at -40 ^oC and NIS (0.25 mmol, 1.25 eq) and AgOTf (0.07 mmol) were added under N_2. The mixture was allowed to stir in a range of temperature between -40 ^oC and -20 ^oC for 1h. Then the mixture was neutralized with Et_3N, diluted with CH_2Cl_2, filtered through celite, washed with 10% aq $Na_2S_2O_3$ solution and H_2O.The organic layers were dried by $MgSO_4$, filtered and concentrated.

Protocol with NBS/Me_3SiOTf: A solid mixture of compound **76** (1.33 eq, 0.22 mmol, 128 mg) and compound **69** (0.16 mmol, 56 mg) was codistilled by toluene and dried. Molecular sieves (4 Å, 0.15 g) were added and the mixture was suspended in dry solvent (2.5cc) and kept under stirring for 30 min at room temperature under N_2. The mixture was cooled at -40 ^oC and NBS (0.4 mmol, 2 eq) and Me_3SiOTf (0.04 mmol) were added under N_2. The mixture was allowed to stir in a range of temperature between -40 ^oC and -20 ^oC for 1h. Then the mixture was neutralized with Et_3N, diluted with CH_2Cl_2, filtered through celite, washed with aq $NaHCO_3$, $NaHSO_3$ and H_2O.The organic layers

were dried by $MgSO_4$, filtered and concentrated. The anomeric ratios β/α are based on the integration of OCH_3 group or acetyl group of the rhamnosyl moiety at 3.2 or 2.0 ppm respectively.

Protocol with NBS/AgOTf: A solid mixture of compound **76** (1.33 eq, 0.22 mmol, 128 mg) and compound **69** (0.16 mmol, 56 mg) were codistilled by toluene and dried molecular sieves (4 Å, 0.15 g) were added and the mixture was suspended in dry solvent (2.5cc) and kept under stirring for 30 min at room temperature under N_2. The mixture was cooled at -40 ^oC and NBS (0.4 mmol, 2 eq) and AgOTf (0.07 mmol, 0.35 eq) were added under N_2. The mixture was allowed to stir in a range of temperature between -40 ^oC and -20 ^oC for 1h. Then the mixture was neutralized with Et_3N, diluted with CH_2Cl_2, filtered through celite, washed with aq $NaHCO_3$, $NaHSO_3$ and H_2O.The organic layers were dried by $MgSO_4$, filtered and concentrated. The anomeric ratios β/α are based on the integration of OCH_3 group or acetyl group of the rhamnosyl moiety at 3.2 or 2.0 ppm respectively.

Protocol with I_2/DDQ: A solid mixture of compound **76** (1.25 eq, 0.22 mmol, 128 mg) and compound **69** (0.18 mmol, 60 mg) was dissolved in dry solvent (2.5cc), dried molecular sieves (4 Å, 0.15 g) were added and the reaction mixture was stirred under nitrogen for 30 min. I_2 (1.5 eq) and DDQ (1.5 eq) were added. When the reaction was complete, the mixture was cooled to 0 ^oC, neutralized with Et_3N, filtered through celite and concentrated.

Brominated compound **92:** Anomeric mixture of brominated compound **91** (1.462 g, 1.579 mmol) was suspended in 2:3 DCM/MeOH (15 mL) and 1 M MeONa solution was added until basic pH. The mixture was kept under stirring at room temperature. After 4.5 h a bit more of 1 M MeONa solution was added. After 7 h the mixture was neutralized by DOWEX H^+ until pH 7, filtered and concentrated. Column chromatography (6:1 toluene-EtOAc) afforded **92** (1.308 g, 94%). The disappearance of a signal at δ= 2.19 ppm in ^1H NMR spectrum was diagnostic of the absence of acetyl group. ESI-MS found *m/z* 900.90 $[M + NH4]^+$.

Brominated trisaccharide **93:** A solution of disaccharide **92 (**1.0 g, 1.131 mmol**)** and ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside **76** (2 eq, 1.323 g, 2.262 mmol) in 30 mL of dry DCM-Et_2O (2:3) was stirred with molecular sieves 4 Å (1 g) for 2 h at room temperature. After addition of NIS (2.262 mmol, 500 mg) the mixture was cooled to -55 ^oC and TMSOTf (0.19 mmol 34.38 μl) was added. The mixture was stirred for 75' at -55 ^oC, neutralized with Et_3N , then allowed to warm

at room temperature and diluted with DCM. The organic solution was washed with 10% aqueous $Na_2S_2O_3$ solution, water, aqueous $NaHCO_3$ solution, and brine, then dried and concentrated. Column chromatography (7:1, toluene-EtOAc) afforded **93** (1.420 g 88%). ESI-MS found *m/z* 1422.56 $[M + NH4]^+$.

*Methyl 2-O-(2,3,4,6-tetra-O-benzyl-α-*D*-galactopyranosyl)-3-O-(2,3,4,6-tetra- O-benzyl-β-*D*-galactopyranosyl)-α-*L*-rhamnopyranoside* (**94**). A solution of trisaccharide **93** (0.25 g, 0.177 mmol) and $(NH_4)_2Ce(NO_3)_6$ (0.58 g, 1.066 mmol) in CH_3CN-H_2O (9:1 3 ml) was stirred at room temperature for 1 h, diluted with DCM, washed with water, aqueous $NaHCO_3$ solution and brine, dried and concentrate. Column chromatography (5:1 toluene-EtOAc) afforded **94** (195 mg 90%). The disappearance of a signal at δ= 2.99 ppm in ^1H NMR spectrum was diagnostic of the absence of *p*-methoxybenzyl group. ESI-MS found *m/z* 1241.47 $[M + NH4]^+$.

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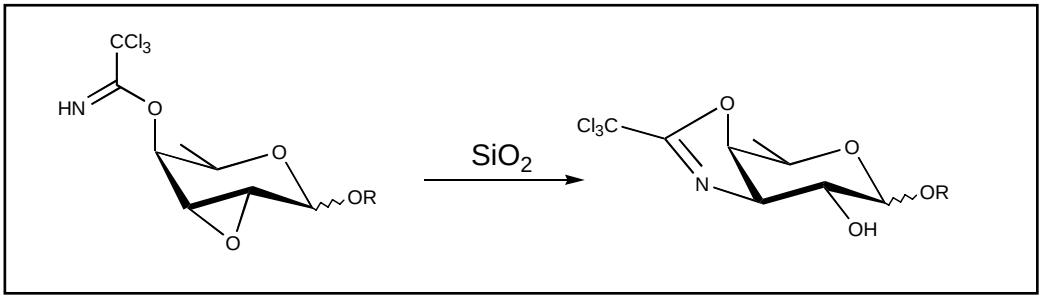
5. CONCLUSIONS

The most widespread molecules in living systems are, undoubtedly, carbohydrates which can exist also as glycoconjugates (glycolipids, glycoproteins, proteoglycans). Their role in cell-cell recognitions and interactions is one of the most studied aspects of carbohydrates. Among these molecules, lipopolysaccharides (LPS), which occur on the cell surface of Gram-negative bacteria, are key structures in cell-cell interactions and are directly involved in pathogenesis of Gram-negative bacterial infections.[1] It is interesting that bacterial human- and plant-pathogens share a common LPS architecture even though host cells have different recognition and defence system. Indeed, biological functions and molecular mechanisms have been quite widely investigated for human pathogenic bacteria but still very little is known about LPS-plant interactions. In this field, a recent work[2] highlighted that synthetic oligorhamnans[3] induce biological activity in *Arabidopsis thaliana* leaves which were treated with different phytopathogenic Gram-negative bacteria once inoculated with aqueous solution of synthetic oligosaccharides and the plant response depends on the length of the chain. Nevertheless, still more has to be done in description of molecular mechanisms involved in plant-LPSs interaction. Based on this results, in order to gain deeper in structure-activity relationship of bacterial O-chains, my Ph. D. project concerned the synthesis of model oligosaccharides related to phytopathogenic bacterial O-chains mimicking natural structures to employ, in future, in biological experiments. Structural studies[4] on different strains of *Pseudomonas syringae* and *Xanthomonas campestris* species show that their O-chains consist of linear rhamnans bearing different branch monosaccharides. Thus, I focused my work on the synthesis of branched rhamnan structures, which would be ready, as building blocks, for successive glycosylations to higher oligosaccharides to be emploied in biological studies. The first synthesized building-block was the amino-sugar 3-acetamido-3,6-dideoxy-D-galactopyranose (D-Fuc*p*3N),[5] which is always α-linked in bacterial O-chains. This synthesis, which is the first one reported in literature, was based on the conversion of a 2,3-epoxitrichloroacetimidate to trichloromethiloxazoline by means of an intramolecular cyclization, in order to insert a latent amino function with the right configuration at *C*3 of the sugar (**Scheme 5.1**). The glycosylation reaction involving D-Fuc*p*3N donors was studied with several rhamnosyl acceptors and the best yields and stereoselectivities were obtained using a *N*-phenyl-trifluoroacetimidate glycosyl donor in a ternary ether-based mixture as solvent.[6]

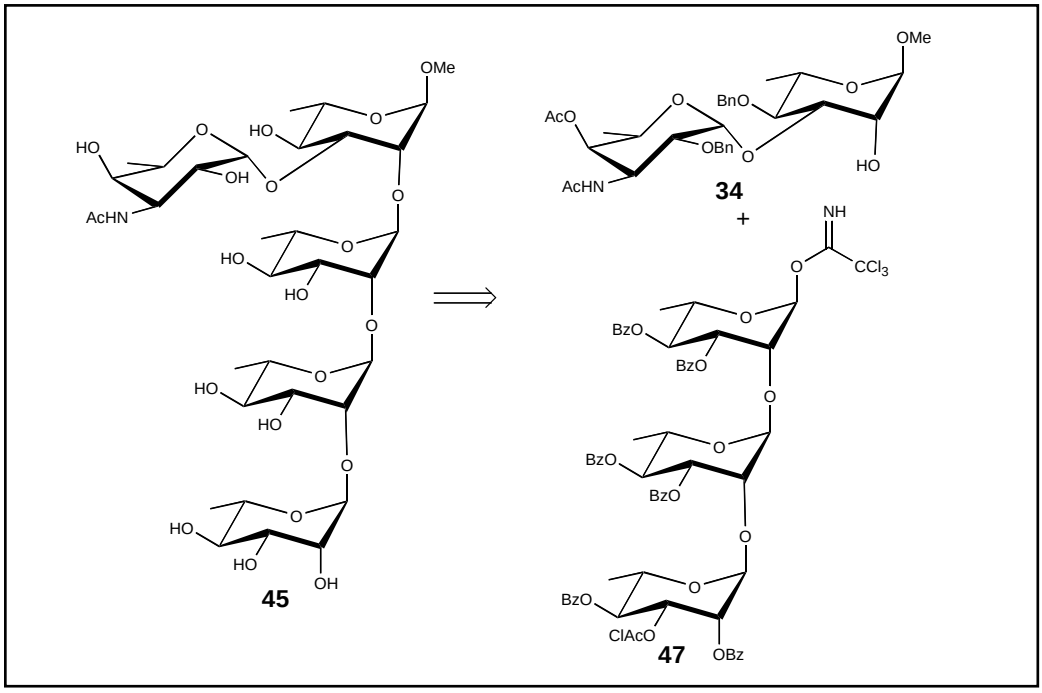


Scheme 5.1: Key step in synthesis of D-Fuc*p*3NAc N-phenyl-trifluoroacetimidate.

The optimization of glycosylation conditions opened the access to synthetic α-D-Fuc*p*3NAc containing oligorhamnans and they were applied on the synthesis of O-chains repeating units from *Pseudomonas syringae* pv *holci* IMV 8300[6] and *Xanthomonas campestris* pv. *campestris* 8004,[7] which have never been reported before. The former structure is a D-Fuc*p*3NAc-branched tetrasaccharide oligorhamnan:[8]

α-D-Fuc*p*3NAc
1
↓
3
→3)- α-L-Rha*p*-(1→3)- α-L-Rha*p*-(1→2)- α-L-Rha*p*-(1→2)- α-L-Rha*p*-(1→

The synthetic strategy make use of a (3+2) approach, coupling a trisaccharide donor and a disaccharide acceptor (**Scheme 5.2**):

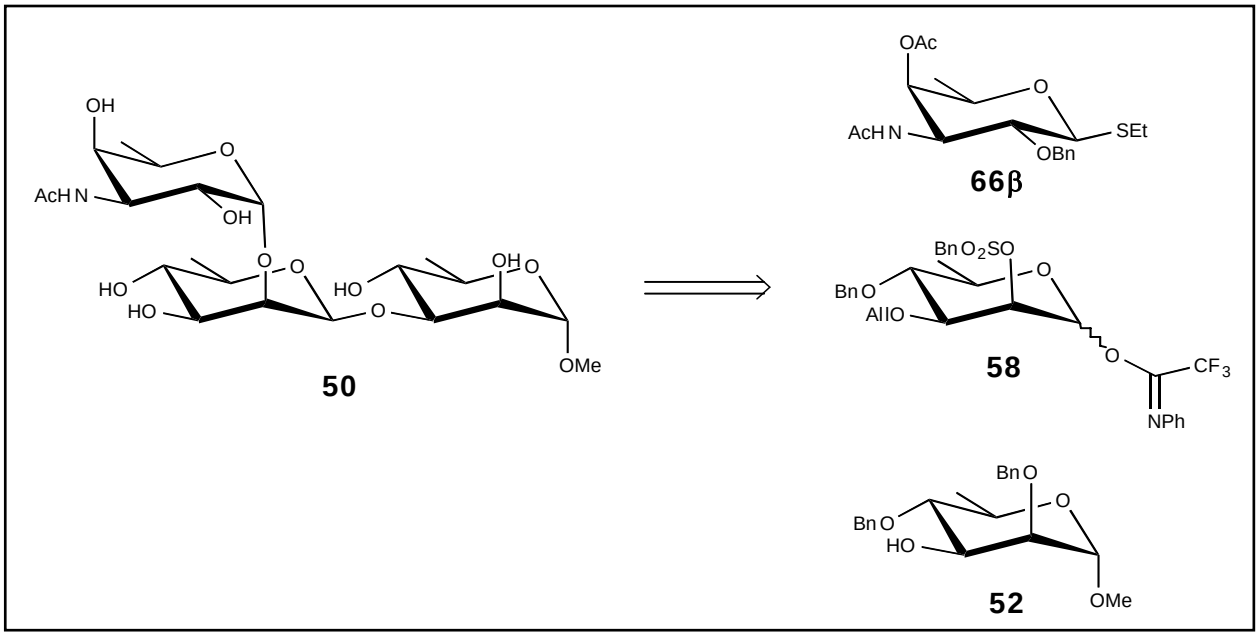


Scheme 5.2: Retrosynthesis of the pentasaccharide repeating unit of the major O-chain component from *Pseudomonas syringae* pv. *holci* IMV 8300.

The O-chain repeating unit from *Xanthomonas campestris* pv. *campestris* 8004 displays a D-Fuc*p*3NAc residue on a linear rhamnosyl disaccharide:[9]

→3)-β-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→
2
↑
1
α-D-Fuc*p*3NAc

Its synthesis has been less easy than the previous one: the presence of a β-linked rhamnose disaccharide and the non-commercially availability of its constituents rendered the synthesis quite challenging. The trisaccharide as methyl glycoside was obtained by exploiting a strategy whose key steps were the sequential β-rhamnosylation of a 2-*O*-benzylsulfonyl-*N*-phenyltrifluoroacetimidate donor, debenzylsulfonylation, and coupling with a D-Fuc*p*3NAc thioglycoside donor (**Scheme 5.3**).



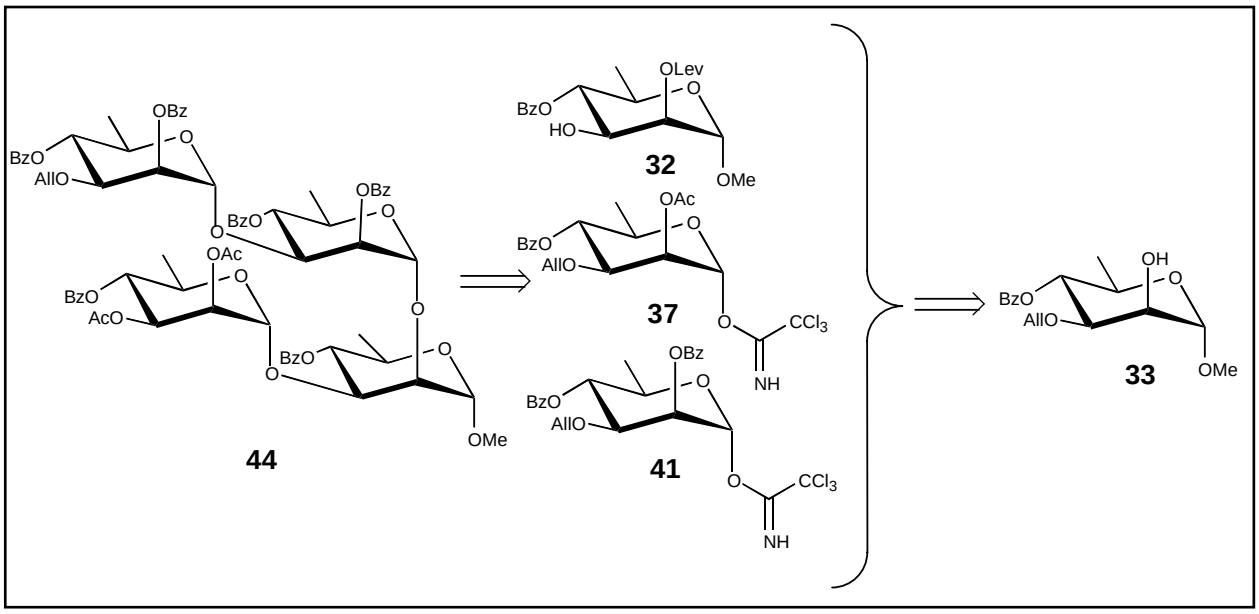
Scheme 5.3: Retrosynthesis of the β-D-rhamnosylated trisaccharide repeating unit of the O-chain from *Xanthomonas campestris* pv. *campestris* 8004.

NMR analysis performed on synthetic oligosaccharides confirmed the structure of natural O-chains by means of comparison of the chemical shift of the signals.

Successively, the synthesis of a D-rhamnose branched tetrasaccharide has been accomplished;[10] it is the O-chain repeating unit from the bacterium *Pseudomonas syringae* pv. *cerasi* 435:[11]

α-D-Rha*p*
1
↓
3
→3)- α-D-Rha*p*-(1→3)- α-D-Rha*p*-(1→2)- α-D-Rha*p*-(1→

The synthetic strategy used was based on the synthesis of just one rhamnosyl building block (methyl 3-*O*-allyl-4-*O*-benzoyl-α-D-rhamnopyranoside) converted alternatively into both a glycosyl acceptor and two different glycosyl trichloroacetimidate donors (**Scheme 5.4**).



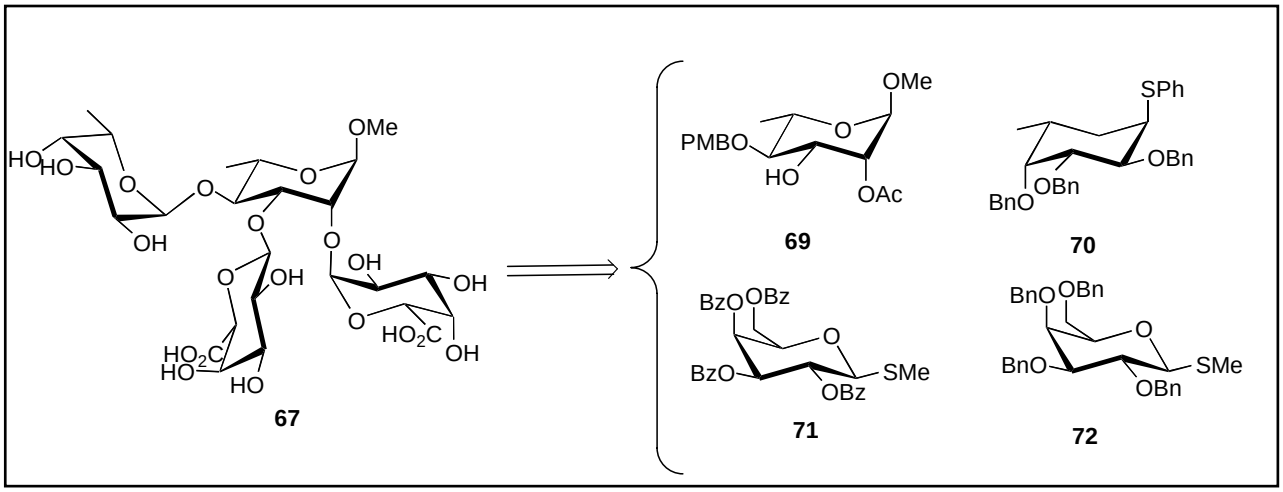
Scheme 5.4: Retrosynthesis of branched tetrasaccharide repeating unit of the O-chain from *Pseudomonas syringae* pv. *cerasi* 435

It is worthy to remember, once again, that all the synthetic approaches used, involve protecting group pattern which could allow the oligomerization of the structures to obtain even higher oligosaccharides suitable for phytopathological structure-activity studies.

In order to increase my acknowledgments on rhamnose chemistry, I worked eight months in the laboratories of *Centre for Carbohydrate Chemistry (CCC)* (University of East Anglia, Norwich, UK), directed by Prof. R. A. Field, where one of the ongoing project is the synthesis of rhamnogalacturonan II (RGII), a pectic polysaccharide of the plant cell wall.[12] My contribute to this work has dealt with the optimization of the synthesis of a tetrasaccharide fragment of RGII:

α-D-GalA-(1→2)-[β-D-GalA-(1→3]-[α-L-Fuc-(1→4]-α-L-Rha-OMe

The previous work[13] involved consecutively couplings of an orthogonally protected α-rhamnosyl acceptor with three different glycosyl donors (**Scheme 5.5**).



Scheme 5.5: Retrosynthesis of **67**, tetrasaccharide fragment of rhamnogalacturonan II (RGII).

The variation to the previous work was based on the use of a perbenzylated galactosyl moiety, as permanently protected galactosyl donor despite to the requirement of anchimeric assistance to build the β-linkage. Different activation conditions and participating solvents have been exploited in order to achieve a better yield and stereoselectivity in building the β-linkage at *C*3 of the α-rhamnosyl acceptor. Attempts of synthesis of a β-rhamnosyl acceptor, whose anomeric configuration is the natural occurring one, have been unsuccessfully performed and glycosylation tests have been carried on its α-anomer. Using just one type of galactosyl donors would render the synthesis more straightforward, reducing the number of deprotection steps. Besides, this would open the way for a more ambitious project which involves the incorporation of the tetrasaccharide, as building block, into an higher fragment structurally closer to the RGII.

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Published papers

1. Emiliano Bedini, Alfonso Iadonisi, Antonella Carabellese, Michelangelo Parrilli
   First preparative synthesis of a 3-acetamido-3,6-dideoxy-D-galactopyranose glycosyl donor via intramolecular cyclization of an epoxytrichloroacetimidate
   Tetrahedron Lett. **2004**, *45*, 4445-4448.

2. Emiliano Bedini, Antonella Carabellese, Maria Michela Corsaro, Cristina De Castro, Michelangelo Parrilli
   Synthesis of a D-rhamnose branched tetrasaccharide, repeating unit of the O-chain from *Pseudomonas syringae* pv. *syringae* (*cerasi*) 435
   Carbohydr. Res. **2004**, *339*, 1907-1915.

3. Emiliano Bedini, Antonella Carabellese, Marialuisa Schiattarella, Michelangelo Parrilli
   First synthesis of an α-D-Fuc*p*3NAc containing oligosaccharide:
   a study on D-Fuc*p*3NAc glycosylation
   Tetrahedron **2005**, *61*, 5439-5448.

4. Emiliano Bedini, Antonella Carabellese, Gaspare Barone, Michelangelo Parrilli
   First synthesis of the β-D-rhamnosylated trisaccharide repeating unit of the O-antigen from *Xanthomonas campestris* pv. *campestris* 8004
   J. Org. Chem. **2005**, *70*, 8064-8070.

5. Emiliano Bedini, Antonella Carabellese, Daniela Comegna, Cristina De Castro, Michelangelo Parrilli
   Synthetic oligorhamnans related to the most common O-chain backbone from phytopathogenic bacteria
   Tetrahedron **2006**, *62*, 8474-8483.

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I want to spend a think for prof. Gaspare Barone whose office I shared for writing my thesis; I will not forget our conversation about classic music!

And lastly, I would like to express my thanks to my parents for their help during all those years. a they are so sweet!!!