SYNTHESIS OF ARABINOGALACTANS AS PROBES FOR FUNCTIONAL GLYCOMIC STUDIES OF THE PLANT CELL WALL

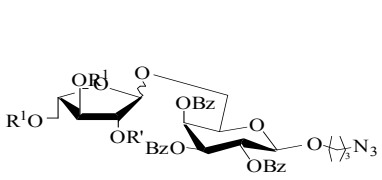
by

MONIQUE SHANTAE PHILLIPS

(Under the Direction of Geert-Jan Boons)

ABSTRACT

There is increasing that plant cell walls play important roles in the biology of plants, but little is known about their makeup at the cellular and subcellular levels. In this study, fragments of Type II arabinogalactan oligosaccharides **1-5** and the portion of extensin **6**, were synthesized to define the binding specificities of monoclonal antibodies for functional genomics studies in order to investigate the changes in plant cell walls during development and differentiation.



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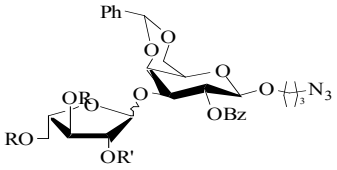

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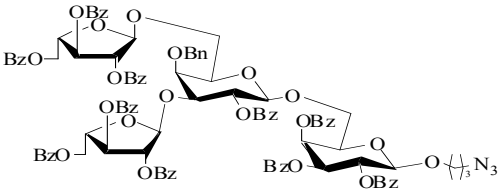

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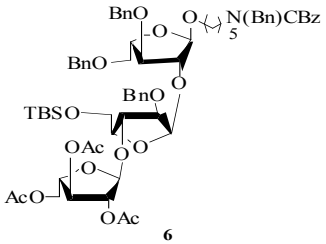

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Figure 1.0. Synthetically prepared Arabinofuranosides

Compounds **1-6** were synthesized by a building block strategy using appropriately protected monosaccharides, which can be used to assemble other Type II arabinogalactan oligosaccharides.

INDEX WORDS: Carbohydrate, Glycosylation, Arabinogalactan, Plant cell wall.

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SYNTHESIS OF ARABINOGALACTANS AS PROBES FOR FUNTIONAL GLYCOMIC STUDIES OF THE PLANT CELL WALL

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The University of Georgia
May 2008

DEDICATION

“Nothing in the world can take the place of persistence. Talent will not; nothing is more common than unsuccessful [people] with talent. Genius will not; unrewarded genius is almost a proverb. Education will not; the world is full of educated derelicts. Persistence and determination alone are omnipotent. The slogan "press on" has solved and always will solve the problems of the human race.”

--- Calvin Coolidge (30th President of the United States)

"Success is the ability to go from one failure to another with no loss of enthusiasm."

--- Sir Winston Churchill

To my parents

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LIST OF ABBREVIATIONS

Å ..... Angstrom

Ac ..... Acetyl

Ac_2O ..... Acetic Anhydride

AgOTf ..... Silver triflate

Ara*f* ..... Arabinofuranose

BF_3•OEt_2 ..... Boron tirfluoro diethyl etherate

Bn ..... Benzyl

Bz ..... Benzoyl

BzCl ..... Benzoyl Chloride

t-BuOH ..... tertiary butanol

C ..... Celsius

$CDCl_3$ ..... deuterochloroform

CD_3OD ..... methanol-d_4

DCM ..... dichloromethane

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

DMAP ..... *N,N*-dimethylaminopyridine

DMF ..... *N,N*-dimethylaminoformamide

DTBMP 2,6 di-*tert*-butyl-4-methylpyridine

DTBP ..... 2,6 di-*tert*-butylpyridine

Et ..... ethyl

EtOAc .....Ethyl acetate

EtOH .....Ethanol

Et_3SiH ..... Triethylsilane

Fmoc .....9-fluorenylmethoxycarbonyl chloroformate

Gal .....Galactose

h ..... Hours

Hz ..... Hertz

IDCP .....iodonium dicollidine perchlorat

m .....multiplet

MALDI .....Matrix Assisted Laser Disorption Ionization

m/z .....mass to charge ratio

min .....minutes

MeOH ..... methanol

mmol .....millimole

MS .....Molecular sieves

NIS .....*N*-iodosuccinimide

NaOMe .....Sodium methoxide

Nap 2-naphthylmethyl

NMR .....Nuclear Magnetic Reasonance

Ph ..... phenyl

Pd/C ..... Palladium on charcoal

PMB .....*para*-methoxybenzyl

py .....pyridine

q ..... quartet

RG-I ..... Rhamnogalacturonan-I

s .....singlet

SPh ..... thiophenyl

t .....triplet

TBAB ..... Tetrabutylammonium bromide

TBAF .....Tetrabutylammonium fluoride

TBAI .....Tetrabutylammonium iodide

TBS ..... *Tert*-butyldimethylsilyl chloride

TCA ..... Trichloroacetimidate

TEMDA .....*N,N,N′,N′*-Tetramethylethylenediamine

TFA .....trifluoroacetic acid

TfOH ..... triflouromethane sulfonate acid

THF .....Tetrahydrofuran

TLC .....Thin layer chromatography

TMSOTf ..... trimethylsilyl triflouromethane sulfonate

p-TsOH .....*p*-tolunesulfonic acid

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1 Plant Cell Walls

1.1.1 Overview

One major factor that distinguishes plant cells from animal cells is the presence of walls, which lie outside the cell membrane and provide a vital source of rigidity enabling plants to stand upright and harvest light energy from the sun. In addition to providing strength, the walls are flexible to accommodate growth and resist turgor pressure. Cell walls perform numerous functions such as determining and maintaining plant shape, regulating growth, storing carbohydrates, protecting the plant from foreign organisms, and mediating cell-to-cell interactions. While all plants have primary cell walls of similar organization and chemical composition, some plants form a secondary, more rigid wall after completion of growth.

Much has been accomplished in the elucidation of the organization of the primary cell wall and many models have been proposed; however, none of these models have been confirmed. The makeup of these models have not changed for several years, and their role is to provide hypotheses on the macromolecular organization of the cell wall (Figure **1.1**).[1,2]

Primary cell walls contain interconnected matrices composed mainly of polysaccharides (90%) and proteins (10%), most of which are glycoproteins. The major polysaccharide components in the primary wall are hemicelluloses, pectin, and cellulose microfibers.[3] Cellulose is made up of crystalline β-1,4 linked glucosides while hemicelluloses are branched

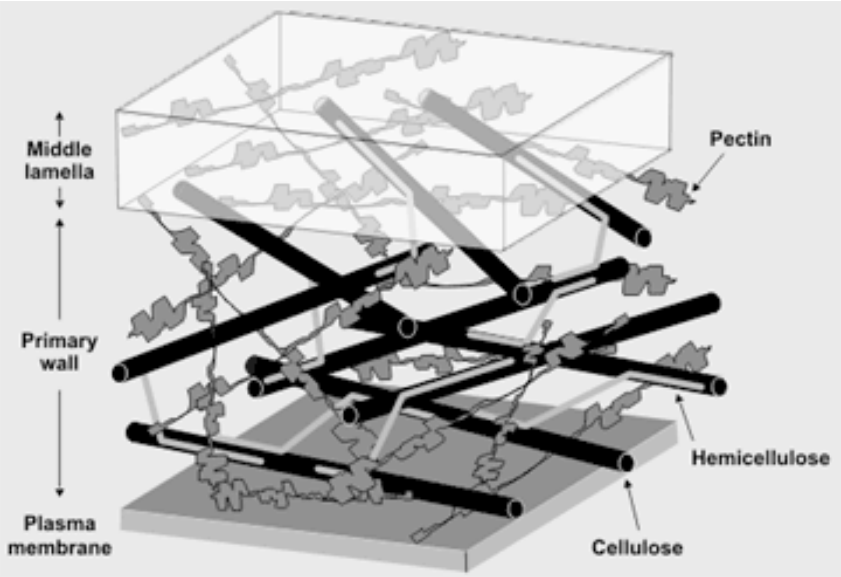

Figure **1.1.** Primary Plant Cell Wall[1]
Reproduced with permission of Complex Carbohydrate Research Center

polysaccharides with a β-1,4 linked hexoside backbone. Xyoglucan is a common form of hemicellulose found in many plants; its backbone of β-1,4-glucopyranose residues is stable and branched at the C-6 position of glucose. Xyloglucan also possesses a regular pattern of unbranched glucosyl residues, which allows it to fold into the proper conformation to interact with cellulose. Other hemicelluloses found in the primary wall include arabinoxylan, glucomannan, and galactomannan. Cellulose and hemicellulose form a strong network that is embedded in the pectin matrix. It is proposed that pectins complex with cations to allow cross linking. Pectin in the cell wall contains α-1,4-galacturonic acid, and the three classes of polysaccharides in the pectin matrix are homogalacturonan (HG), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II).

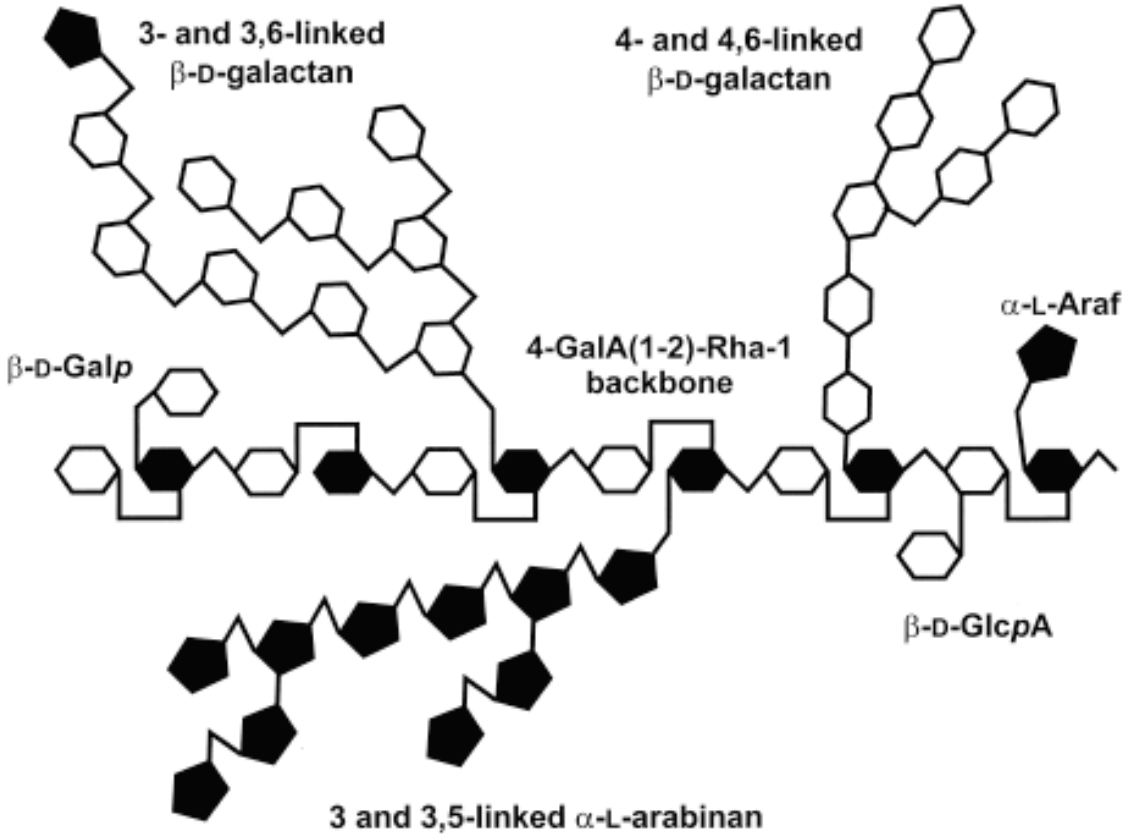

Figure **1.2.** Model of the major structural features of Rhamnogalacturonan I.[9]

Homogalacturonans are composed of linear chain α-1,4 galactosyluronic acid residues and express varying patterns and degrees of methyl esterfication. Moreover, HG can be partially O-acetylated at C-3 or C-2 depending on the plant source.[4] The rhamnogalacturonan I family of pectic polysaccharides have a backbone of [→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→] is large (Mr approximately 200,000) and found in almost all higher plants.[5] The Gal*p*A residues usually do not have side chains, but are often *O*-acetylated on C-2 and/or C-3.[6] However, 20-80% of the Rha*p* residues have side chains in which linear and branched α-L-arabinofuranosyl (Ara*f*) and/or β-D-galactopyranosyl (Gal*p*) residues predominate, but other residues may be present (Figure **1.2**).[7,8] The number of side chains and their lengths vary amongst plant sources.[9] Unlike RG-I

and HG, the RG-II is highly conserved and has a backbone of seven or more α 1,4-GalpA residues with four branching sites, of which only one side chain appears to vary between species.[10] Additionally, RG-II contains several rare sugars such as apiose.[11]

Primary cell walls also contain an assortment of hydroxyproline-rich glycoproteins (HRGPs) that fall into the following three categories: suspension culture cells (arabinogalactans), cells that are very tightly associated to the cell wall (e.g. extensins), and loosely associated with cells (e.g. peroxidases). These glycoproteins are believed to play important roles in plant growth and development.

1.1.2 Arabinogalactans

The arabinogalactan proteins (AGPs) are a family of highly glycosylated hydroxyproline-rich glycoproteins widely distributed in higher plants, where they occur in intercellular spaces, cell walls, plasma membranes and some cytoplasmic vesicles. AGPs are grouped according to the composition of the core protein. "Classical" AGPs span Hyp, Ala, Ser, Thr, and Gly as the major amino acid constituents, and "nonclassical" AGPs have different core proteins, such as Hyp-poor.[12, 13] AGPs are believed to play important roles in plant growth and development. Models for the molecular organization of AGPs are based upon the observed shapes during transmission electron microscopic imaging and include the wattle blossom model, twisted hairy rope model and modified wattle blossom model. In the twisted hairy rope model, the polysacharride chain wraps around the core protein, whereas the polysaccharide chains are folded into globular units in the wattle blossom model. In addition to the polysaccharide chains folded into globular units, the modified wattle blossom model has oligoarabinoside side chains.[14] AGPs are water soluble and are comprised predominately of carbohydrates, with only 1-10% of their weight as protein. The carbohydrate moieties consist mainly of arabinosides and

galactosides, with only small amounts of other monosaccharides, and can be grouped into three main structural types: Type I, β-1,4-linked D-galactopyranose backbone; Type II, arabino-3,6-galactans; and Type III, cell wall glycoproteins containing arabinose and galactose.[15] Type II arabinogalactans are structurally similar to the side-chains of rhamnogalcturonan I. While the exact structure of the arabinogalactans and side-chains of rhamnogalcturonan I are unknown, some basic structural features have already been determined.[16] Both have cores consisting of the 1,3-linked β-Gal residue with (1,6) β-Gal-(1,3)β-Gal residues substituted on the side chains. Many of these side chains have a non-reducing terminal Ara*f* residue attached at the C-3 and/or C-6 position of the galactoside residues. Additionally, these side chains may contain carbohydrate residues composed of 1,5 linked α-Ara*f* residues and/or a non-reducing terminal L-rhamnoside, L-fucoside, D-glucuronic acid, or 4-*O*-Me-D-glucuronic acid.

1.1.3 Extensin

Extensin is a highly insoluble HRGP that is believed to play important roles in structural strength, growth, and defense. Currently, it is unclear how extensin is attached to other cell wall components. Two popular hypotheses exist that explain extensin attachment. One claims that extensin is tightly associated to the cell wall by covalent bonds, and the other proposes that it is cross-linked by bonds between the peptide, forming a separate network that complements the cellulose framework. Recently, the latter hypotheses has become more popular. The presence of isoditrosine is believed to account for its insolubility. It is easy to differentiate extensins from the extractable arabinogalactan proteins because they contain shorter oligosaccharide chains and less alanine. The composition of extensins varies from species to species, usually comprised of 33-50% protein, of which 41% is hydroxyproline. The galactosides present are believed to be α-D-galactoside-linked to serine, most of the hydroxyproline residues are arabinofuranosylated, and

90% of the carbohydrate moieties are arabinose. Akiyama *et al.* found that the structures of the carbohydrate side chains of Hyp are either tri- or tetra- Arabinosides.[17, 18] The proposed structure of a portion of extensin in dicot cell walls has a tetra-arabinoside attached to the hydroxyproline residue, with all arabinofuranose residues β-linked except for the α-linked non-reducing terminal arabinofuranose residue (Figure **1.3**).[19]

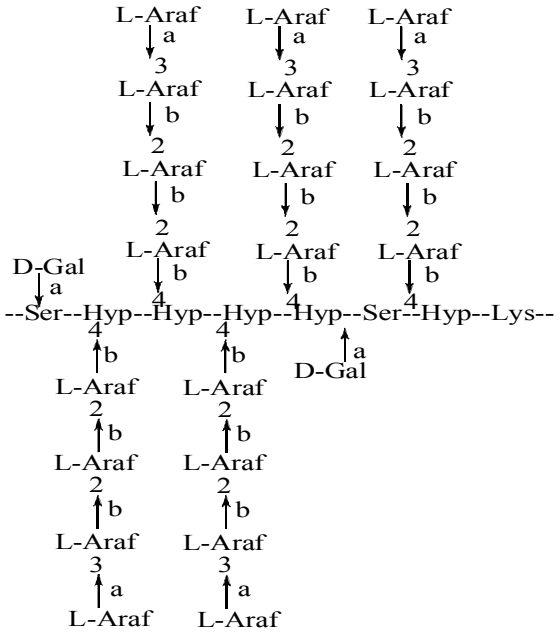

Figure **1.3.** Proposed structure of a portion of extensin.[17]
Modified Akiyma *et al.*

1.1.4 Conclusion

The exact nature, orientation and organization of the primary cell wall are not yet well understood. Therefore, more research aimed at a better understanding of the makeup of plant cell walls at the cellular and subcellular level is crucial. It has been estimated that over 2000 genes play a role in cell wall synthesis, deposition, and function.[20] Yet, only a few genes have been identified and very little is known about their corresponding enzymes. Molecular genetics approaches for studying the primary cell walls of plants have experienced limited success. Since cell wall biosynthesis is not template driven, it is more difficult to link cell wall polysaccharides

to specific genes, preventing an understanding of how these genes lead to a diverse array of polysaccharides. Moreover, plants are able to compensate for missing genes in order to maintain cell wall functionality, and these compensatory mechanisms further complicate the study of the plant cell wall using molecular approaches such as RNAi.[21, 22] Furthermore, chemical approaches for studying the cell wall lack the sensitivity necessary to detect small changes. The use of monoclonal antibodies provides an excellent alternative to the limitations experienced in the aforementioned methods.

1.2 Monoclonal Antibodies

The ability of antibodies to bind with great specificity to epitopes makes them attractive tools for research. Kohler, Milstein and Jerne invented the process for producing monoclonal antibodies in 1975.[23] Monoclonal antibodies are produced by removing B-cells from the spleen or lymph node of a mouse or human that have been subjected to the antigen of interest. Next, the antibody forming B-cells are fused with myeloma cancer cells to form hybridoma cells, which can be maintained and grown in an animal or cell culture (Figure **1.4**).[24] Hybridoma cells are clones of a single parent cell can grow indefinitely and secrete a single antibody having specificity for a single epitope.

The sensitivity and specifity of monoclonal antibodies has been exploited in the study of primary cell wall composition and organization at the cellular and subcellular levels, and used to monitor changes as the cells develop.[25, 26] Studies have shown that the composition of the walls not only differs among cell types, but differs in the wall of a single cell possessing sub-domains with different glycoconjugates. Antibody studies also suggest that the carbohydrate epitopes change during development. Moreover, monoclonal antibody work has provided insight into possible functions of AGPs, which are believed to have functions in cell development, cell-to-

cell interactions, and cell differentiations. Current findings show the appearance and disappearance of cell-specific AGP epitopes during development. The number of monoclonal antibodies that bind to plant cell wall carbohydrate structures is increasing, but of those only a few have fully characterized binding epitopes.[27]

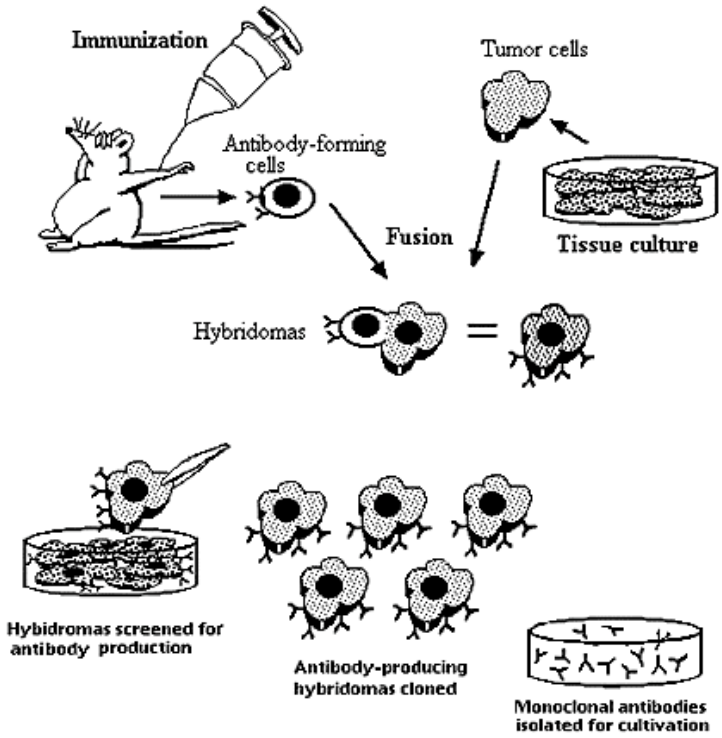

Figure **1.4.** Production of Monoclonal Antibodies[24]
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http://www.accessexcellence.org/RC/VL/GG/monoclonal.php

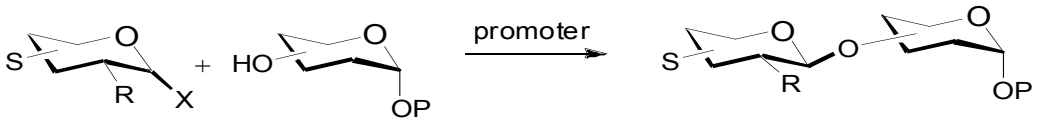
The lack of pure and structurally well-defined carbohydrates has complicated the elucidation of the binding specification of monoclonal antibodies elicited against plant cell wall fragments. Biological methods for extracting pure oligosaccharides are inefficient, often resulting in low yields and microheterogeneity. Fortunately, organic synthesis enables the

production of fully characterized, pure, homogeneous carbohydrates and glycoconjugates, as well as the production of non-naturally occuring carbohydrate moieties.[28]

1.3. Stereoselective Glycosylation

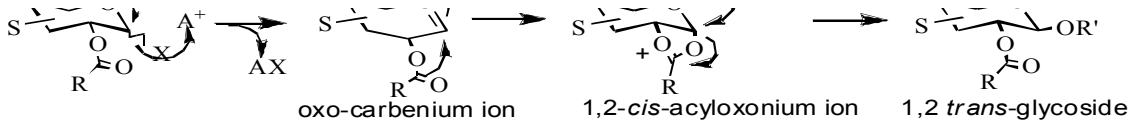
1.3.1 An Overview

A number of factors influence the α/β ratio in glycosylation: the structure of the C-2 protecting group, orientation of the C-2 substituent, protecting groups, leaving group, promoter, solvent, temperature, and pressure. Recently, many advances have been made in the synthesis of complex carbohydrates. However, there are still no universal reaction conditions for carbohydrate synthesis and their construction remains time-consuming.[29, 30] Arguably with respect to carbohydrate synthesis the most notable advancement has been the introduction of glycosyl donors, or leaving groups, which are more stable and can be activated under milder conditions. However, it still remains synthetically challenging to stereoselectively introduce a glycosidic linkage. As often during synthesis mixtures of α/β-anomers, which are difficult to separate, are formed. If the anomers are not separated, the product cannot be used in biological studies. Therefore, it is essential to develop methods for stereoselective glycosylations. Advancements have been made in the stereoselective synthesis of 1,2-*trans* glycosides and their synthesis has become fairly routine as they can easily be obtained through neighboring group participation of a 2-*O*-acyl protecting group on the glycosyl donor.



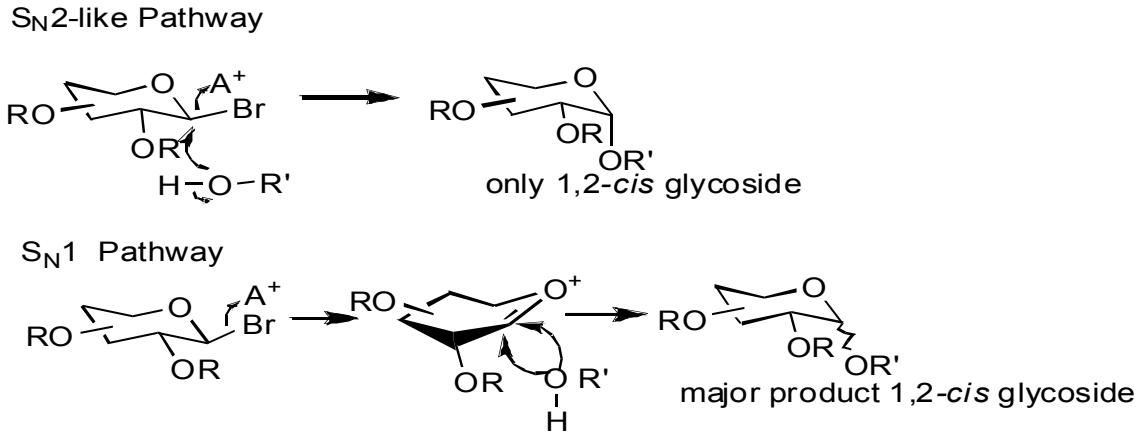
Scheme **1.1** General Approach for Chemical Glycosylation

The selection of the glycosyl donor's C-2 protecting group is essential for a stereochemical outcome. In general, the use of a participating 2-*O*-acyl protecting group yields 1,2-*trans* glycosides (Scheme **1.2**). The anomeric leaving group is activated by a promoter (Lewis acid), and as it departs it leaves an oxa-carbenium ion intermediate. The 2-*O*-acyl protecting group participates in and forms a more stable 1,2-*cis*-acyloxonium ion intermediate, after which the acetoxonium ion is subjected to nucleophillic attack at the anomeric center by an acceptor (alcohol) to give a 1,2-*trans*-glycoside.



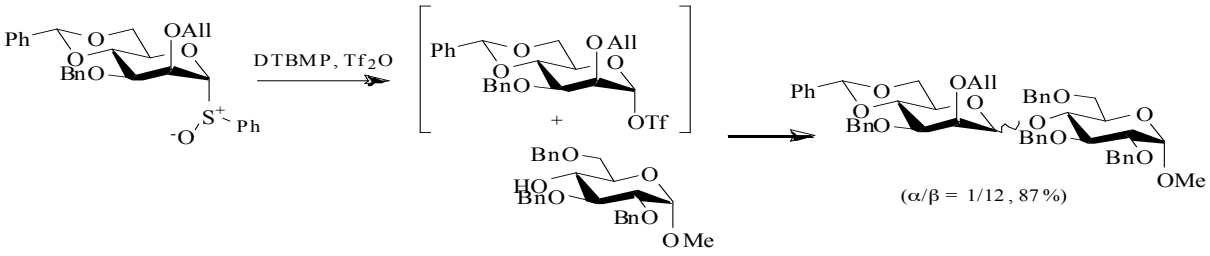
Scheme **1.2**. Neighboring Group Participation

1,2-c*is* Glycosides are more challenging to synthesize and often give mixtures of anomers and require a non-participating functionality such as benzyl ether or azide at the C-2 position. Theoretically, 1,2-*cis* glycosides can be synthesized through an S_N2 mechanism from a 1,2-*trans* glycosyl donor, but often the glycosyl acceptors are weak nucleophiles, which causes the reaction to proceed through an S_N1 mechanism, yielding a mixture of *cis* and *trans*-products (Scheme **1.3**). Due to the anomeric effect the α-product, in this case the 1,2-*cis* product will be favored. Other factors such as the nature of the solvent greatly influence the anomeric outcome of these glycosylations.[31] Relatively nonpolar solvents suppress oxa-carbenium ion formation, thereby increasing the α-selectivity of the glycosylation.



Scheme **1.3.** 1,2-*cis* Glycoside Synthesis

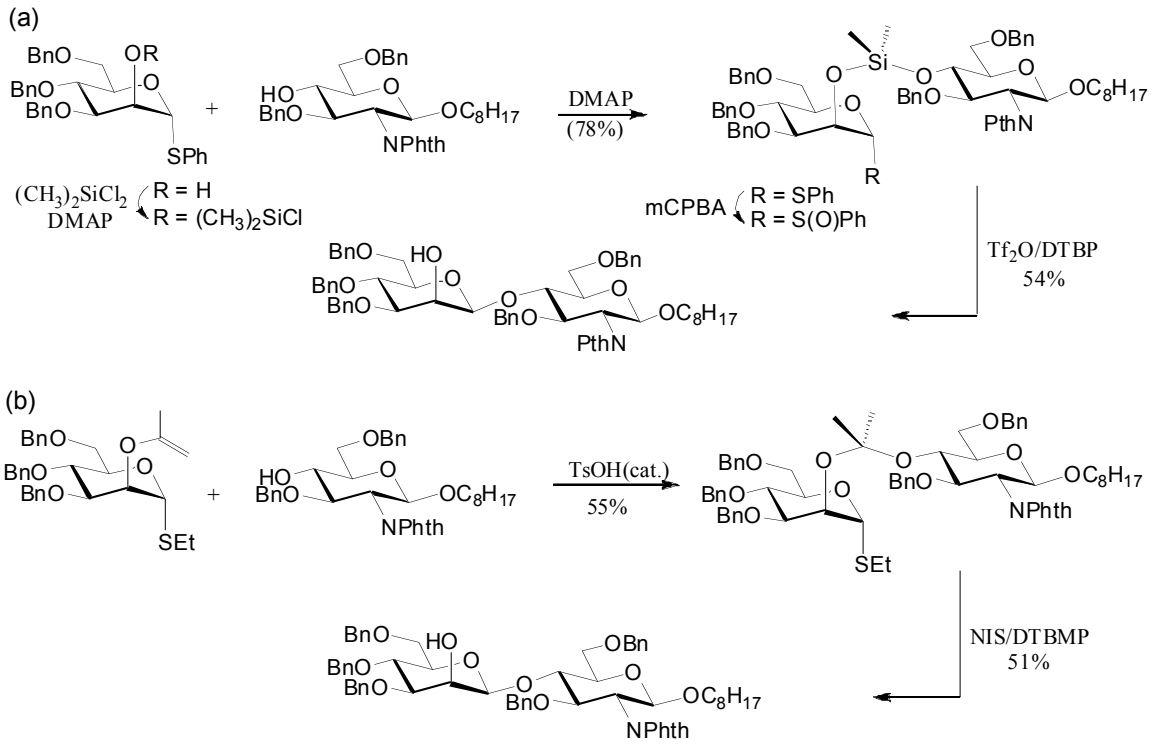
1,2-c*is* mannosides are exceptionally challenging to synthesize due to sterics and electronic factors. For example, the C-2 substituent in mannose is axial, causing nucleophillic attack from the β-face to be unfavorable. Nevertheless, synthesis of 1,2-*cis* mannosides has been accomplished by forming an α-triflate, which is stabilized by the endo-anomeric effect, after which the triflate is displaced yielding the 1,2-*cis* mannoside. Surprisingly, this method requires a 4,6-*O*-benzylidene acetal for increased selectivity (Scheme **1.4**).[32]



Scheme **1.4**. Glycosidation of Intermediate α-Triflates

Intramolecular aglycon delivery is another method by which 1,2-*cis* mannosides can be synthesized in a highly stereoselective manner. The first intramolecular glycosylation was reported by Hindsgaul in 1991 and works by linking a silicon or acetal tether to the C-2 position

of the mannosyl donor followed by activation of the anomeric center, which causes aglycon delivery to the β-face of the glycosyl donor (Scheme **1.5**).[33-37] This method, although stereospecific, is not an attractive method for the synthesis of large oligosaccharides.

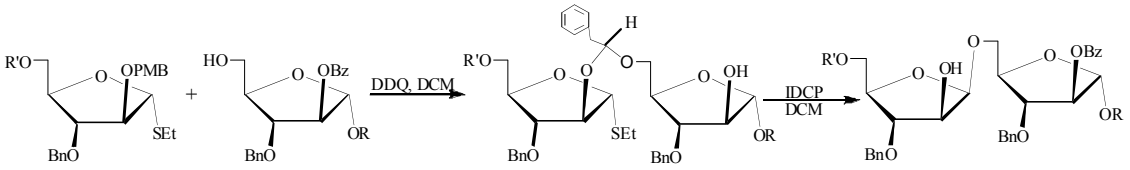


Scheme **1.5.** Intramolecular Aglycon Delivery

1.3.2 Furanoside Synthesis

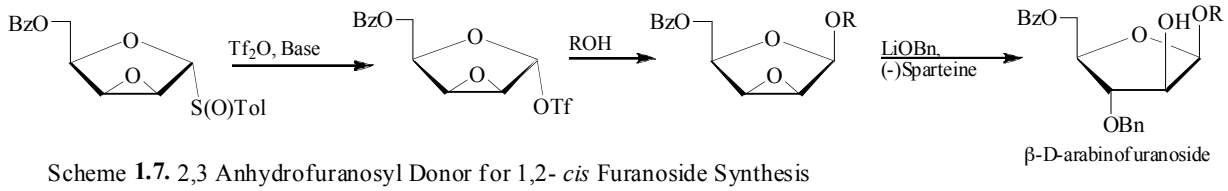
Stereoselective introduction of 1,2-*trans* furanosides is straightforward and can be achieved by neighboring group participation, much like the synthesis of 1,2-*trans* pyranosides; however, the synthesis of 1,2-*cis* furanosides is more challenging and has been considered analoguous to the synthesis of mannopyranosides, but less attention has been given to the stereoselective synthesis 1,2-*cis* furanoside.[38] Consequently, some of the methods employed to form 1,2-*cis* mannopyranosides, such as tethering, have been successful in the formation of 1,2-*cis* furanosides. Although tethering is stereospecific, it is not reasonable for the synthesis of polysaccharides. Moreover, the weak anomeric effect of furanosides makes it difficult to exploit

the *in-situ* anomerization used for gluco-type pyranosides.[39, 40] Furthermore, furanosides are flexible and can adapt various conformers, which allows them to glycosylate through several different transition states.[38] Until now, methods for the stereoselective introduction of 1,2-*cis* furanosides have been indirect and time consuming. For example, in intramolecular aglycon delivery the glycosyl acceptor is tethered to the C-2 hydroxyl via an acetal before glycosylation (Scheme **1.6**).[41, 42] The initial task of tethering together two sugars presents a formidable challenge. After tethering, the activation of the leaving group gives rise to a 1,2-*cis* glycoside.



Scheme **1.6.** Intramolecular Aglycon Delivery for 1,2 Furanoside Synthesis

Another example of the stereoselective introduction of 1,2-*cis* furanosides is the use of 2,3-anhydrofuranosyl donors, in which an S_N2-like attack of the acceptor to the triflate intermediate yields a product with the appropriate configuration. Afterwards, the oxirane ring is regioselectively opened by a benzyl alkoxide in the presence of (-)-sparteine to give a β-arabinoside (Scheme **1.7**).[43, 44]



Scheme **1.7.** 2,3 Anhydrofuranosyl Donor for 1,2- *cis* Furanoside Synthesis

1.3.3 A Novel Approach for β-Arabinofuranoside Synthesis

It is known that rigid, well-organized transition states, in which pre-existing stereochemical elements give rise to differential interactions with the substrate, enable greater

selectivity.[45] Furthermore, it has been shown that glycosylation transition states possess substantial double bond character between the endo-cyclic oxygen and C-1, affording the oxygen, C-1, C-2 and C-4 in the same plane. Therefore, the oxa-carbenium ions of L-furanosides can adopt two possible low energy conformations, specifically envolops,^{3}E or E_3, (Figure **1.5**).[46-48] The geometries of these conformations have been optimized by computational studies. Analysis of the Newman projections suggests that attack on the α-face is preferred in the ^{3}E conformer because substituents are staggered. Conversely, the E_3 conformer attack on the β is preferred.[49-51] In conclusion, high β-selectivity can be achieved if the furanosyl oxa-carbenium ion is locked in the E_3 conformer.[52]

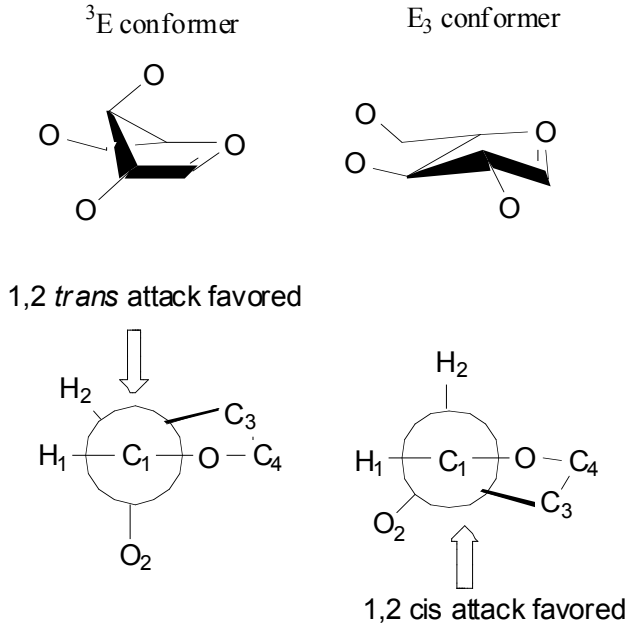
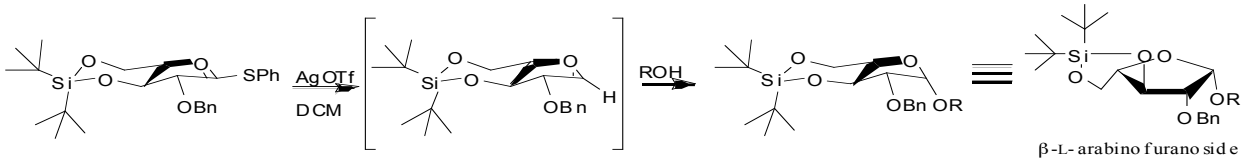


Figure **1.5.** Low Energy Conformation of L-Furanosides
modified from Zhu et al. JACS (2006), 128, (36), 11948-11957.

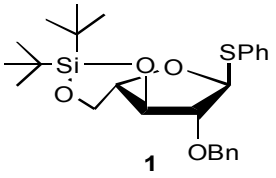
Recently, Boons exploited this analysis and developed a method for the stereoselective introduction of β-arabinofuranosides whereby an arabinosyl donor is locked in an E_3 conformation by protection of the C-3 and C-5 by a six-membered ring silyl specifically, the 3,5-*O*-(di-*tert*-butylsilane)-protecting group. In this conformation, the oxa-carbenium ion has C-5

and O-3 in a pseudoequatorial orientation, in which nucleophillic attack from the α face is disfavorable.[48] β-selectivity was achieved upon reaction of this glycosyl donor when a variety of acceptors were used. Recently, a study by Crich showed that both the activation method and donor were important for selectivity when using the method proposed by Boons (Scheme **1.8**).[53]

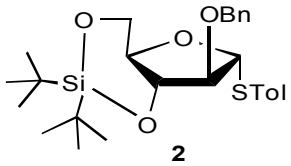


Scheme **1.8.** Formation of β-L-arabinofuranoside by locked donor.

A recent paper by Nacario *et al*. reports an X-ray study of of *p*-tolyl 2-*O*-benzyl-3,5-*O*-(di-*tert*-butylsilanediyl)-1-thio-α-D-arabinofuranoside and compares these results to the computational work described above for phenyl 2-*O* benzyl-3,5-*O*-(di-*tert*-butylsilanedilyl)-1-thio-α-L-arabinofuranoside (Figure **1.9**).[54] These results only differ slightly with the computational findings. This study showed that the furanose ring of **2** is locked into the E_4 conformation in which C4, and the six-member ring is in a distorted chair conformation. Whereas Zhu reported that the furanose ring of **1** is in the E_3 conformation and the six membered ring is in a chair conformation.



α-L-arabinofuranoside



α-D-arabinofuranoside

Figure **1.6.** X-ray study

1.4. Conclusions

Arabinose is one of the most common sugars found in the furanose form in nature. The L-version is very common in plants and occurs in both the α- and β-forms. α-Arabinofuranosides are readily synthesized through neighboring group participation. Conversely, β-arabinofuranaosides are more challenging to synthesize and until recently the approaches for their synthesis has been indirect. The method reported by Boons using the 3,5-*O*-(di-*tert*-butylsilane)-protecting group provides a direct way to synthesize β-arbinofuranosides and was employed to synthesize arabinogalactans which can be used for epitope characterization of monoclonal antibodies. These antibodies can then be used in studying of the functional genomics of the plant cell wall.

1.5. Project Objectives

The overall goal of the project is to provide a large, diverse, well characterized library of monoclonal antibodies for functional genomics studies. Synthetic structures can lead to well defined monoclonal antibodies, which will enable the investigation of changes in plant cell walls during development and differentiation. These findings could lead to a better understanding of the cellular and subcellular organization of cell walls and the functions of cell wall carbohydrates. In this study, a range of arabinogalactans and a fragment of extensin have been synthesized to define the binding specificities of the monoclonal antibodies.

The specific aims of this project are the following:

1) chemical synthesis of partial structures of Type II arabinogalactan oligosaccharides, **1-5**; and
2) chemical synthesis of a fragment of the side chain of extensin, **6**.

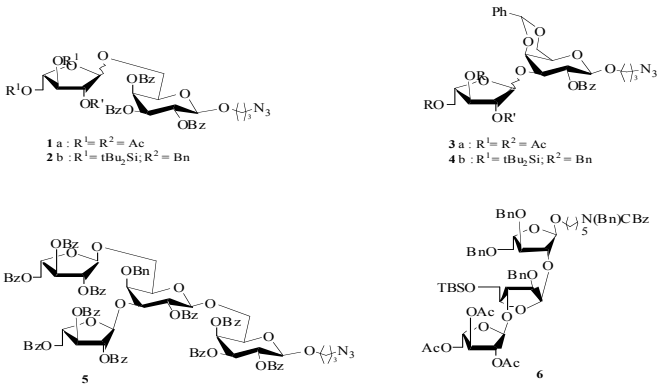


Figure 1.7. Synthetically prepared Arabinofuranosides

1.6 References

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CHAPTER 2

SYNTHESIS OF FRAGEMENTS OF TYPE II ARABINOGALACTAN

2.1 Introduction

It has been estimated that more than 2000 genes encode for proteins involved in the synthesis and maintinence of the polysaccharides found in the plant cell wall.[1] However, functions have been elucidated for only a very few genes. The elucidation of genes is important because a detailed knowledge of the makeup of the plant cell wall is essential to the development of agro-energy/material crops. Molecular genetics and chemical approaches for studying the plant cell wall lack the sensitivity necessary to monitor composition and organization at the cellular and subcellular levels during growth and development.[2, 3] The use of monoclonal antibodies provides an excellent alternative to the limitations experienced in the aforementioned methods.[4] Of the monoclonal antibodies produced, only a few have characterized binding specificities, making synthetic chemistry a vehicle whereby well-defined carbohydrates can be obtained for use in monoclonal antibody production and characterization.[5-7]

The arabinogalactan proteins (AGPs) are a family of highly glycosylated hydroxyproline-rich glycoproteins and are widely distributed in higher plants. AGPs share many of the structural features with the side chain of rhamnogalacturan I (RG-I).[8, 9] Both contain galactoside residues with arabinofuranoside residue attached at the 3- and/or 6- positions to the nonreducing terminal.[10] For this reason, arabinogalactans were selected as targets for monoclonal antibody production. Herein, we report the synthesis of disaccharides **1-4** and tetrasaccharide **5**, which have Araf residues 6- (**1** and **2**), 3- (**3** and **4**) and 3/6- (**5**) linked to the nonreducing terminal of a

galactose residue (Figure **2.1**). An artificial aminopropyl spacer was attached to the oligosaccharides to facilitate conjugation to carrier proteins to enable screening of monoclonal antibody epitope binding specificity.

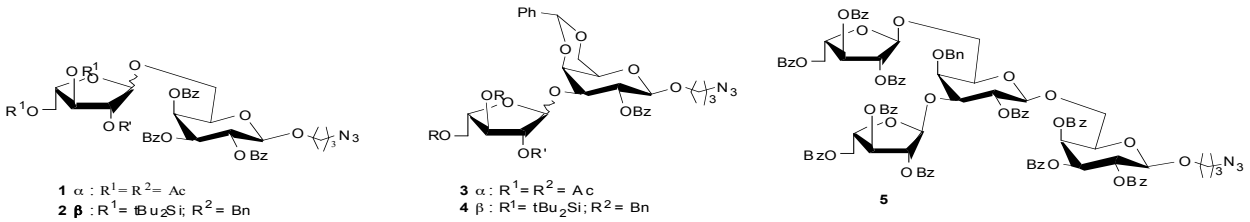


Figure 2.1 Synthetically Prepared Arabinogalactans

2.2 Results and Discussion

A building block approach was used to prepare arabinogalactans **1-5** (Figure **2.1**).[11] It is easy to envision the synthesis of **1** from donor **6** and accepter **8**. The 2-*O* acetyl protecting group on donor **1** will ensure desired α selectivity by neighboring group participation (Figure **2.3**). Acceptor 8 could easily be prepared by protecting group manipulations as the primary alcohol can be selective protected and deprotected. The chemical synthesis of **2** is more challenging due to the 1,2-*cis* linkages, which are difficult to introduce in a stereoselective manner. Therefore, the method developed by Boons for the stereoselective introduction of β-arabinofuranosides was employed, a method wherein the C-3 and C-5 of arabinose are locked into a six-membered ring that blocks nucleophillic attack from the α-face by the 3,5-*O*-(di-*tert*-butylsilane)-protecting group. Therefore, donor **7** was used and coupled with acceptor **8** (Figure **2.2**).

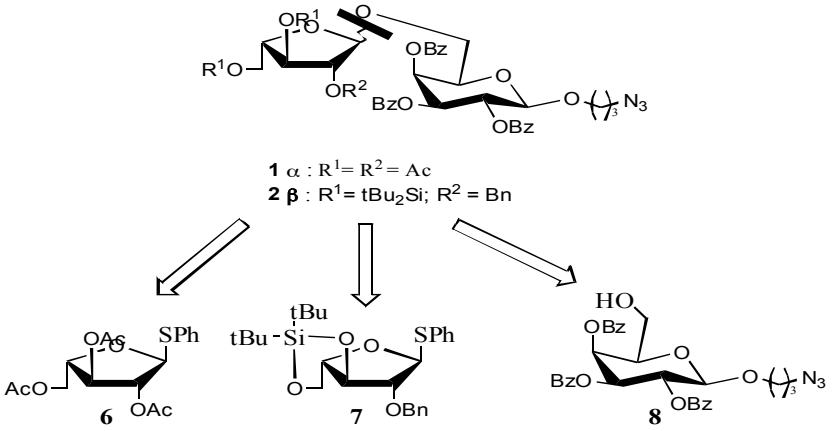
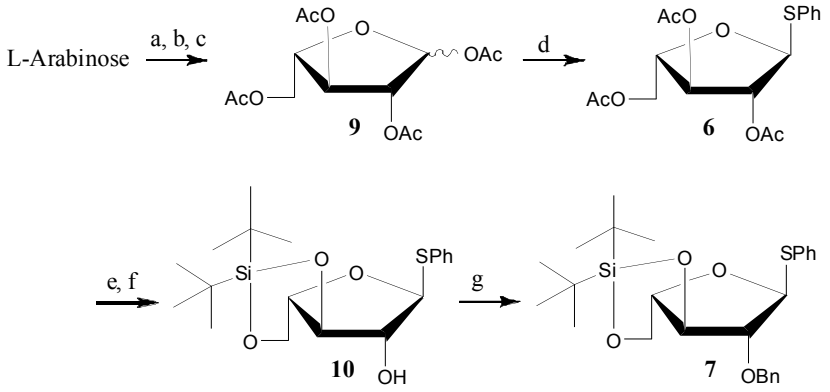


Figure **2.2.** Retrosynthetic Analysis of Targets **1** and **2**

Compounds **6** and **7** were prepared from commercially available L-arabinose by a one-pot, three-step reaction beginning with acid catalyzed ring opening and subsequent locking of the pyranose into the furanose ring conformation followed by acetylation with acetic anhydride in pyridine (83%, over 3 steps, Scheme **2.1**).[12] Compound **9** was then converted to thioarabinosyl donor **6** by treatment with thiophenol in the presence of boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) in 90% yield.[13] Compound **7** was conveniently prepared from **6** by removal of acetyl esters by treatment under Zemplen conditions (sodium methoxide in methanol, quant.), followed by treatment of a triol intermediate with di-*tert*-butylsilane bis (trifluoromethanesulfonate) in the presence of 2,6 lutidine in a mixture of DCM and DMF to give compound **10** in a 76% yield over two steps.[14] Then, the C-2 hydroxyl of **10** was benzylated with benzyl bromide in the presence of NaH in THF to give **7** (71%).

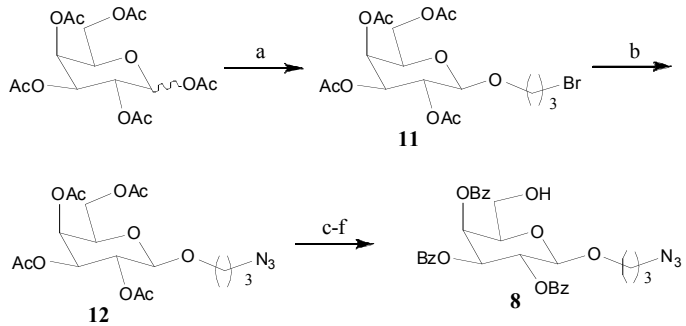
Synthesis of accepter **8** began with the installation of the linker. It was achieved by reaction of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactose with 3-bromo-1-propanol in the presence of boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) (Scheme **2.2**). Purification of the crude mixture

afforded intermediate **11** (58%). The bromide of **11** was displaced by an azido group to give **12** by heating under reflux in a 2:1 mixture of acetone and water in the presence of NaN_3 (98%).



Scheme 2.1. Reagents and conditions: a) 1.06 M Methanolic HCl, MeOH b) Ac_2O, py, DMAP c) H_2SO_4, Ac_2O, AcOH, 0 ^oC, 83% over 3 steps d) PhSH, BF_3OEt_2, DCM, 0 ^oC, 90% e) NaOMe/MeOH, f) $Bu_2Si(OTf)_2$, 2,6-lutidine, DCM:DMF, 5:1, v/v, 0 ^oC, 76% over 2 steps g) BnBr, NaH, TBAI, DCM, 0 ^oC, 71%

Compound **12** was treated under Zemplen conditions (sodium methoxide/methanol) to remove the acetyl esters. The C-6 hydroxyl of the resulting compound was regioselectively protected using tritylchloride (TrCl) in pyridine (80%). The C-2, C-3, and C-4 hydroxyl groups were converted into benzoyl (Bz) esters by treatment with benzoyl chloride (BzCl) in pyridine and subsequently the trityl group was removed by treatment with $FeCl_3 \cdot 6H_2O$ to afford **8** (69%).



Scheme 2.2. Reagents and conditions: a) 3-bromo-1-propanol, BF_3OEt_2, DCM, 0 ^oC, 58% b) NaN_3, acetone:water, 2:1, v/v, reflux, quant c) NaOMe/MeOH, d) TrCl, DMAP, py, 83 ^oC, e) BzCl, DMAP, py, 0 ^oC, f) $FeCl_36H_2O$, DCM, 69 % over three steps.

One could image that the synthesis of **3** and **4** would use the same donors **6** and **7**. Additionally, acceptor **13** with a C-3 free hydroxyl could be employed (Figure **2.3**).

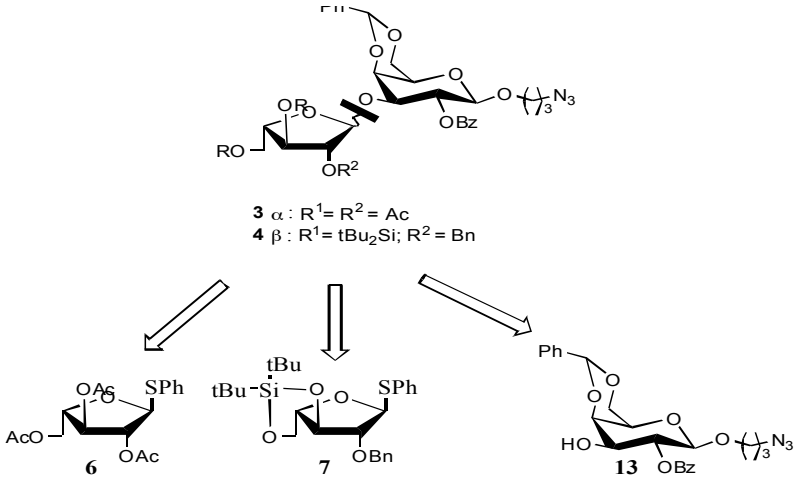
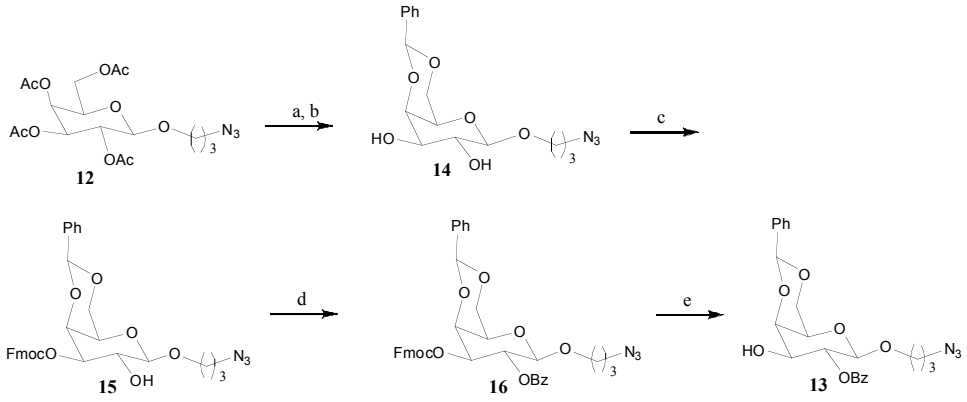


Figure **2.3.** Retrosynthetic Analysis of Target **3** and **4**

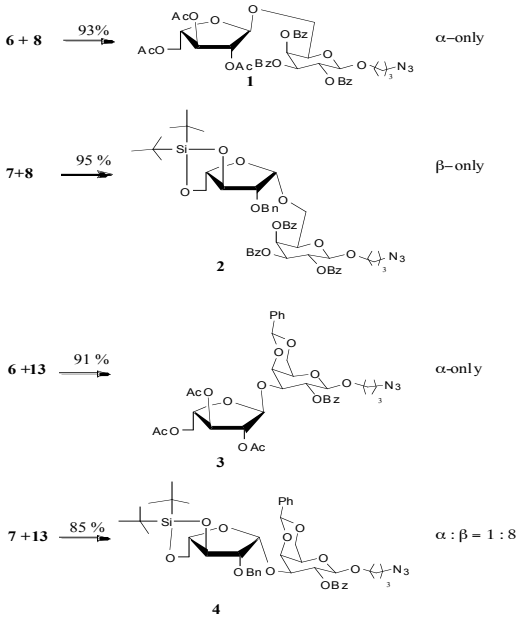
The preparation of acceptor **13** commenced with deacetylation of the esters of **12** via Zemplen conditions (Scheme **2.3**), after which treatment with benzaldehyde dimethylacetal in the presence of catalytic *para*-toluenesulfonic acid- (*p*-TsOH) afforded **14** (83%). This was regioselectively protected by treatment of **14** with one equivalent of 9-fluorenylmethoxycarbonyl chloroformate (FmocCl) in pyridine to afford **15** (73%). Subsequent treatment of **15** with benzoyl chloride (BzCl) in pyridine gave **16** (85%) followed by selective deprotection of Fmoc at C-3 with triethylamine gave accetor **13** (83%).



Scheme 2.3. Reagents and conditions: a) NaOMe/MeOH, b) $PhCH(OMe)_2$, p-TsOH, CHCN, 83 % c) FmocCl, py, 0°C, 73% d) BzCl, py, 0°C, 85% e) Et_3N, DCM, 83 %.

NIS/AgOTf-mediated glycosylation of donors **6** and **7** were employed to synthesize disaccharides **1**, **2**, **3**, and **4**.[15] Both the glycosylation of donor **6** with primary acceptor **8** and secondary acceptor **13** gave the α-anomer stereoselectively due to the neighboring group participation of the C-2 acyl functionality.

Glycosylation of donor **7** with primary acceptor **8** gave the β-anomer exclusively in excellent yield (95 %, Scheme **2.4**). However, glycosylation of donor **7** with secondary acceptor **13** gave a mixture of anomers. It is important to note that the β selectivity (β/α 8:1) was an improvement over selectivity that occurred in the absence of the 3, 5-*O*-di-*tert*-butylsilane protecting group (β/α 3:1). In general, the anomeric configuration of arabinofuranosides was established by a combination of chemical shifts and coupling constant data. β-ararbinofuranosides are characterized by $^{3}J_{\text{H-1,H-2}}$ = 4-5 Hz and δ(C-1) 97-104 ppm whereas, α-arabinofuranosides are characterized by $^{3}J_{\text{H-1,H-2}}$ = 1-3 Hz and δ(C-1) 104-110 ppm.



Scheme **2.4.** Glycosidation reactions with donors **6** and **7**. Reagents and conditions: NIS, AgOTf, 4 A molecular sieves, DCM, -30 ºC

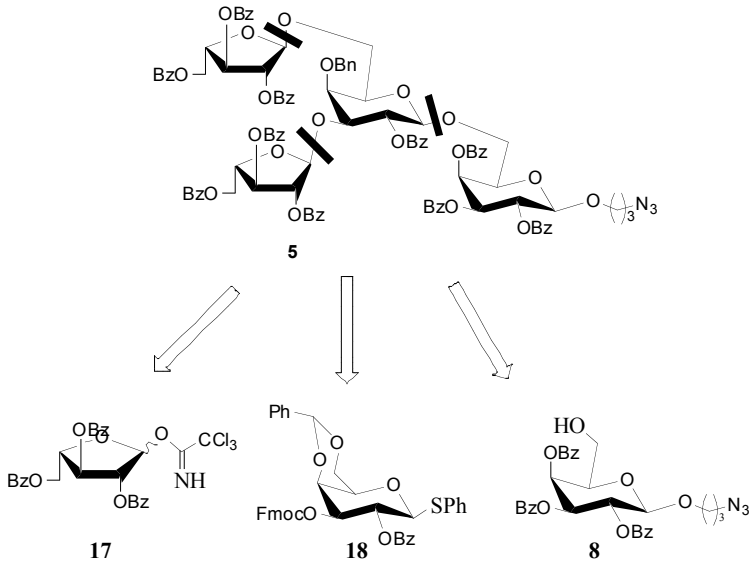
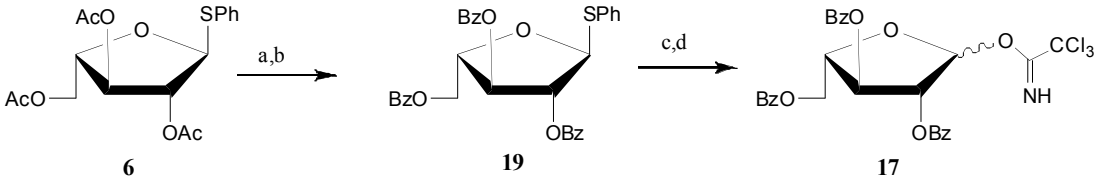


Figure **2.4.** Retrosynthetic Analysis of Target **5**

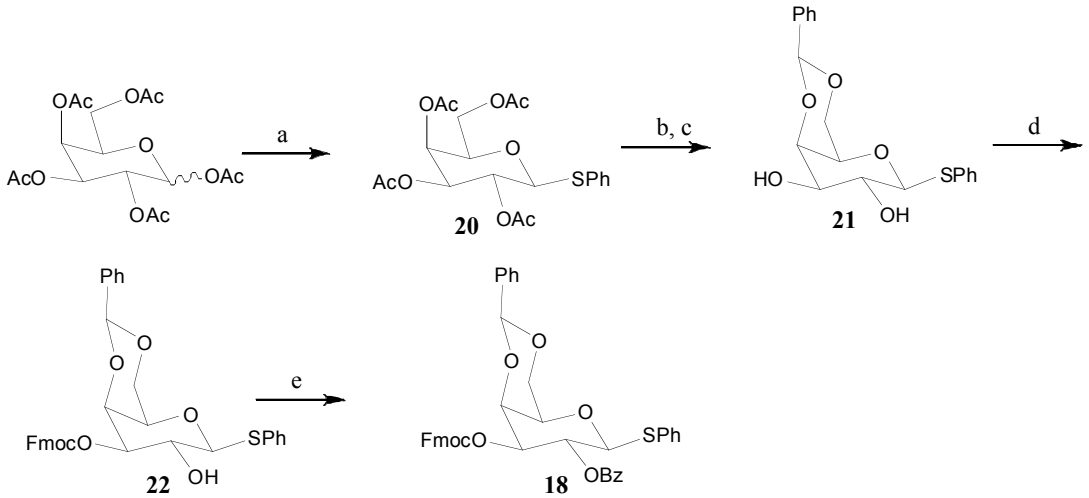
The preparation of tetrasaccharide **5** could be achieved from donors **17** and **18**, and acceptor **8** (Figure **2.4**). Donor **17** was prepared from donor **6** by deacetylation followed by benzoylation to give **19** (92%). Hemiacetal formation with NBS followed by treatment with trichloroacetonitrile in the presence of DBU gave **17** (57%) (Scheme **2.5**).



Scheme **2.5.** Reagents and conditions: a) NaOMe/MeOH, b) BzCl, py, 0 ^{o}C, 85%, c) NBS, 9:1 acetone/water v/v, d) CCl_3CN, DBU

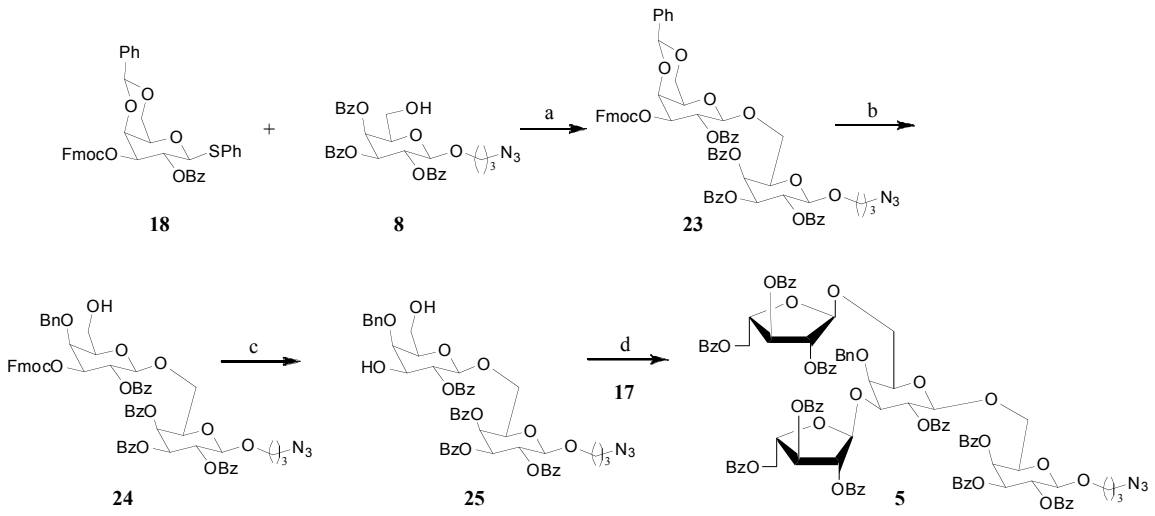
The preparation of thiogalactosyl donor **18** commenced with installation of the thiophenyl glycoside by reaction of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactose with thiophenol in the presence of $BF_3 \cdot Et_2O$ to afford **20** (98%, Scheme **2.6**). Subsequent removal of the acetyl esters under

Zemplen conditions and treatment with benzaldehyde dimethylacetal in the presence of catalytic *para*-toluenesulfonic acid- (*p*-TsOH) afforded a 4,6-*O*-benzylidene acetal protecting group to give **21** (84%, over two steps). Regioselective protection of the C-3 hydroxyl group of **21** over the less reactive hydroxyl at the C-2 position was achieved using one equivalent of a soft chloride electrophile, 9-fluorenylmethoxycarbonyl chloroformate (FmocCl, 1.1 equiv.) in pyridine to give **22** (73%), subsequent treatment of **22** with benzoyl chloride (BzCl) in pyridine gave glycosyl donor **18** (84%).



Scheme 2.6 Reagents and conditions: a) PhSH, BF_3OEt_2, DCM, 0 °C, 98% b) NaOMe/MeOH, c) $PhCH(OMe)_2$, p-TsOH, CH_3CN, 84% over 2 steps d) FmocCl, py, 0 °C, 73% e) BzCl, py, 0 °C, 84%.

Reaction of glycosyl donor **9** with acceptor **10** (Scheme **2.7**) using NIS/AgOTf as the promoter system gave disaccharide **23** (79 %). Access to the C-6 hydroxyl for further galactosylation was achieved by two methods, specifically chemoselective opening of the benzylidene acetal of **23** could be achieved using Et_3SiH and $PhBCl_2$ or $BH_3THF$and Bu_2BOTf to give **24** (60 %).[16, 17] The C-3 Fmoc was then selectively removed with triethylamine to afford **25** (86 %), which was glycosylated with donor **9** by a TMSOTf mediated glycosylation to afford tetrasaccharride **5** (90%).[18]



Scheme 2.7. Reagents and conditions a) NIS, AgOTf, 4 A molecular sieves, DCM, -30°C, b) BH_3THF, Bu_2BOTf, DCM, 0°C, c) Et_3N, DCM, d) TMSOTf, 4 A molecular sieves, DCM, -60°C.

2.3 Conclusions

The Boons method for the stereoselective introduction of a β-arabinofuranoside whereby an arabinosyl donor is locked in a conformation in which nucleophillic attack from the α-face is disfavored is practical for the preparation of the target compounds. Milligram quantities of arabinogalactans were prepared and will be used for epitope characterization of monoclonal antibodies of plant cell wall.

2.4 Experimental Section

General methods and material. Chemicals were purchased from Fluka and Aldrich and used without further purification. Molecular sieves were activated in a microwave oven (1.5 min, 3 times) and further dried *in vacuo*. Dichloromethane was distilled from CaH_2 and stored over 4Å molecular sieves. All the reactions were performed under anhydrous conditions under an atmosphere of Argon and monitored by TLC on a Kieselgel 60 F_{254} (Merck). Detection was performed by examination under a 254 nm UV light, charring with 10% sulfuric acid in

methanol, or charring with cerium ammonium molybdate. Flash chromatography was performed on silica gel (Merck, mesh 70-230), and Iatrobeads (60 μm) were purchased from Bioscan. Extracts were concentrated under reduced pressure at <40 °C (bath). ^{1}H NMR (1D, 2D) and ^{13}C spectra were recorded on a Varian Merc 300 spectrometer and Varian 500 MHz spectrometers equipped with Sun workstations. For ^{1}H and ^{13}C spectra recorded in $CDCl_3$, chemical shifts are given in ppm relative to solvent peaks (^{1}H = 7.24; ^{13}C, 77.0) as an internal standard for protected compounds, unless otherwise noted. Negative ion matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were recorded on VOYAGER-DE Applied Biosystems using dehydroxybenzoic acid in acetonitrile as an internal calibration matrix.

1,2,4,5-tetra-*O*-acetyl-α/β-arabinofuranose (9). L-Arabinose (25 g, 170 mmol) was dissolved in methanol (250 mL) and freshly prepared 1.06 M methanolic HCl (67.7 mL, prepared by dropwise addition of AcCl (4.7 mL, 66 mmol) to MeOH (63 mL) at 0 °C) was added dropwise over a period of 15 min under an atmosphere of argon. The reaction mixture was stirred at room temperature until it became clear (3 h), at which point it was neutralized by the addition of pyridine (25 mL) and the resulting mixture was concentrated to dryness. The residue was co-evaporated twice with a mixture of pyridine/toluene (50 mL, 1:1) and the resulting residue was dried *in vacuo* (30 min). The residue and catalytic DMAP was dissolved in pyridine (125 mL) and the resulting solution was cooled (0 °C) and placed under an atmosphere of argon, followed by the addition of acetic anhydride (50 mL) over a period of 15 min. The reaction mixture was stirred under an atmosphere of argon (3 h) after which it was concentrated *in vacuo.* The residue was dissolved in ethyl acetate (200 mL) and the resulting solution was washed with water (200 mL), after which the aqueous layer was extracted with EtOAc (50 mL). The combined extracts were washed successively with water (200 mL), aq. $NaHCO_3$ (2 x 150 mL),

water (150 mL), and brine (100 mL), and dried ($MgSO_4$) and concentrated *in vacuo*. The resulting syrup was used directly without further purification and was dissolved in glacial acetic acid (150 mL) and treated with acetic anhydride (38 mL) at room temperature. The reaction mixture was then cooled (0 °C) and concentrated sulfuric acid (10 mL) was added dropwise over a period of 10 min, after which the reaction mixture was allowed to warm to room temperature and stirring was continued for 14 h. The solution was then poured over crushed ice (250 g) and the resulting suspension was stirred until the ice melted. After the addition of water (200 mL) the resulting solution was back washed with CH_2Cl_2 (2 x 150 mL). The combined extracts were washed successively with water (3 x 200 mL), $NaHCO_3$ (2 x 150 mL), water (100 mL) and brine (100 mL), and the organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give an oily residue, which was purified by flash silica gel column chromatography (hexane/EtOAc, 3:1 → 1:1, v:v) to give **9** (46.5 g, 88%) as a syrup. R_F = 0.46 (hexane/EtOAc, 1:1) ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.36 (1H, $H_{1\beta}$), 6.19 (1H, $H_{1\alpha}$), 5.35 and 5.20 and 5.04 (2H, H_2 and H_3), 4.36 (d, 2H, H_5), 4.21 (1H, H_4), 2.14- 2.06 (4s, 12H, COC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.25-169.2 (CH_3CO), 99.5 ($C_{1\alpha}$), 93.8 ($C_{1\beta}$), 82.6 ($C_{4\alpha}$), 80.7 ($C_{2\alpha}$), 79.9 ($C_{4\beta}$), 77.0 ($C_{3\alpha}$), 75.5 ($C_{2\beta}$), 75.0 ($C_{3\beta}$), 64.6 ($C_{5\beta}$), 63.2 ($C_{5\alpha}$), 20.9 (CH_3CO). MALDI-MS: *m/z* 341.31 $[M+Na]^+$. Calcd for $C_{13}H_{18}O_9$ 341.266.

Phenyl 2,3,5-tri-*O*-acetyl-1-thio-α-L-arabinofuranose (6). 1,2,4,5-Tetra-*O*-acetyl-α/β-arabinofuranose (6.00 g, 18.9 mmol) was dissolved in CH_2Cl_2 (250 mL) and placed under an atmosphere of argon and cooled (0 °C). PhSH (2.41 mL, 23.6 mmol) and BF_3OEt_2 (2.77 mL, 22.1 mmol) were added dropwise. Next, the reaction mixture was allowed to warm to room temperature and stirred for 18 h, after which it was neutralized with Et_3N (5.3 mL, 38 mmol) and concentrated *in vacuo*. The residue was diluted with EtOAc (200 mL) and then washed

successively with water (70 mL) and brine (70 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was purified by flash silica gel column chromatography (hexane/EtOAc, 3:1 → 2:1, v:v) to afford **6** (6.25 g, 90%) as a colorless syrup: R_F = 0.56 (hexane/EtOAc, 1.7:1, v:v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50 (m, 2H, Ar), 7.30 (m, 3H, Ar), 5.53 (br s, 1H, H_1), 5.28 (br s, 1H, H_2), 5.08 (d, J = 5.1 Hz, 1H, H_3), 4.48 (m, 1H., H_4), 4.40 (dd, J = 12.0, 3.6 Hz, 1H, H_{5a}), 4.28 (dd, J = 12.0, 5.4 Hz, 1H, H_{5b}), 2.13, 2.10, 2.09 (3s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.65, 170.15, 169.72, 133.69, 132.24, 132.10, 129.24, 129.16, 127.97, 91.03, 81.66, 80.28, 77.33, 62.96, 20.92, 20.90(x2); MALDI-MS: *m/z* 391.3 $[M+Na]^+$. Calcd for $C_{17}H_{20}O_7S$ 391.0930.

Phenyl 3,5-*O*-(di-*tert*-butylsilanediyl)-1-thio-α-L-arabinofuranose (10). NaOMe (30%) in methanol was added to a solution of phenyl 2,3,5-tri-*O*-acetyl-1-thio-α-L-arabinofuranose (4.00 g, 12.6 mmol) in methanol and the reaction was allowed to stir at room temperature for 2h at pH 9, after which it was neutralized with weakly acidic (Amberlite IRC-50) resin for 30 min. Filtration of the suspension and concentration of the filtrate *in vacuo* gave an intermediate (2.60 g, 99%), which was dissolved in a mixture of DCM (50 mL) and DMF (10 mL), cooled (0 °C) and then 2,6 lutidine (5.00 mL, 42.92 mmol) and di-*tert*-butlylsiyl bis-(trifluoromethatnesulfonate) (5.48 mL, 15.02 mmol) were added. The reaction mixture was stirred for 1 h at 0 °C, and then for an additional 1 h at room temperature, after which it was quenched with water, concentrated *in vacuo*, diluted with EtOAc (50, mL), and washed successively with water (20 mL), and brine (20 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (hexane/EtOAc, 10:1 → 6:1, v:v) to give **10** (3.14 g, 77%) as a white solid: R_F = 0.43 (hexane/EtOAc, 5.5:1, v:v). ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.51 (m, 2H, Ar), 7.30 (m, 3H, Ar),

5.32 (d, J = 6.0 Hz, 1H, H_1), 4.34 (m, 2H, H_4, H_{5a}), 4.15 (m, 1H, H_3), 4.02 (dd, *J* = 9.5, 7.5 Hz, 1H, H_2), 3.94 (m, 2H, H_4, H_{5b}), 2.53 (d, *J* = 3.0 Hz, 1H, OH), 1.06 (s, 9H, ^{t}Bu), 0.99 (s, 9H, ^{t}Bu). ^{13}C NMR (75 MHz, $CDCl_3$): δ 134.53, 131.65 (x2), 129.25 (x2), 127.77, 91.33, 81.48, 80.97, 73.92, 67.56, 27.72, 27.39, 22.89, 20.37. MALDI-MS: *m/z* 404.49 $[M+ Na]^+$. Calcd for $C_{19}H_{30}O_4SSi$ 405.1634

Phenyl 2-*O*-Benzyl 3,5-*O*-(di-*tert*-butylsilanediyl)-1-thio-α-L-arabinofuranose (7). BnBr (0.38 mL, 3.23 mmol), NaH (60%, 94 mg) and catalytic TBAI were added to a solution of **10** (1.00 g, 2.61 mmol) in CH_2Cl_2 (35 mL) and the reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was quenched by the addition of MeOH, and the resulting solution was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (35 mL) and washed sequentially with 1N HCl (10 mL), $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*, and the resulting residue was purified by flash silica gel column chromatography (hexane/EtOAc, 50:1 → 10:1, v:v) to give **7** (871 mg, 71%) as a pale yellow oil. R_F = 0.65 (hexane/EtOAc 8:1, v:v). ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.41-7.27 (m, 10H, Ar), 5.43 (d, $J_{1,2}$ = 5.1 Hz, 1H, H_1), 4.79 (AB, *J* = 12.0 Hz, 2H, PhCH_2), 4.34 (q-like, $J_{4,5a}$ = 5.0 Hz, 1H, H_{5a}), 4.15 (m, $J_{2,3}$ = 6.5 Hz, $J_{3,4}$ =10 Hz, 1H, H_3), 4.03-3.97 (m, $J_{4,5}$ = 10.0 Hz, 3H, H_2, H_4, H_{5b}), 1.08 (s, 9H, ^{t}Bu), 0.99 (s, 9H, ^{t}Bu). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.9, 134.9, 131.5, 129.2, 128.7, 128.3, 128.2, 127.6, 90.2, 87.1, 81.6, 74.0, 72.5, 67.6, 27.8, 27.4, 22.9, 20.4. MALDI-MS: *m/z* 495.21 $[M+Na]^+$. Calcd for $C_{26}H_{36}O_4SSi$ 495.2104.

3-Bromopropyl 2,3,4, 6-tetra-acetyl β-D-galactopyranoside (11). 3-bromo-1-propanol (2.5 mL) and BF_3OEt_2 (3.6 mL) were added to a cooled solution (0 °C) of 2,3,4,6-terta-*O*-acetyl D-galactopyranose (10.06 g, 25.78 mmol) in CH_2Cl_2 (102 mL). The solution was allowed to warm to room temperature and after stirring for 18 h the reaction was quenched with aqueous $NaHCO_3$.

The residue was dissolved in EtOAc and successively washed with $NaHCO_3$ (40 mL) and brine (40 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* after which the residue was purified by flash silica gel column chromatography (hexane/EtOAc, 3:1 → 1:1) to give **11** (7.04 g, 58%) as a clear, colorless syrup. R_F = 0.62 (hexane/EtOAc, 2:1, v:v). ^{1}H NMR (300 MHz, $CDCl_3$); δ 5.40 (d, 1H, H_4), 5.20 (t, *J* = 9.6, 8.7 Hz, 1H, H_2), 5.03 (dd, *J* = 10.5 Hz, 1H, H_3), 4.48 (*J* = 7.8 Hz, 1H, H_1), 4.12 (m, 2H, O*CH*$_2$CH$_2$CH$_2$Br), 3.99 (dd, *J* = 5.1 Hz, 1H, H_{6a}), 3.92 (t, *J* = 6.3, 1H, H_{6b}) 3.69 (dt, *J* = 4.2 Hz, 1H, H_5), 3.48 (m, *J* = 5.7 Hz, OCH$_2$CH$_2$*CH*$_2$Br), 2.15 (s, OAc), 2.08 (s, OAc), 2.05 (s, OAc, CH$_2$*CH*$_2$CH$_2$Br), 1.98 (s, OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.48, 170.33, 170.21, 169.69, 101.64, 70.92, 70.76, 68.93, 67.40, 67.11, 62.37, 32.32, 30.26, 20.90, 20.77, 20.74, 20.67; MALDI-MS: *m/z* 491.11 $[M+Na]^+$. Calcd for $C_{17}H_{24}BrO_{10}$ 491.04.

3-Azidopropyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (12). Sodium azide (4.89 g, 75.01 mmol) was added to a solution of **11** (7.04 g, 15.00 mmol) in a mixture of acetone (200 mL) and water (100 mL) and the resulting reaction mixture was refluxed at 70 °C for 18 h, after which it was concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (70 mL) and washed successively with water (30 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* after which the residue was purified by flash silica gel column chromatography (hexane/EtOAc, 2:1, v:v) to give **12** (6.45 g, quantitative). R_F = 0.62 (hexane/EtOAc, 2:1, v:v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.37 (dd, *J* = 3.4, 0.9 Hz, 1H, H_4), 5.17 (dd, *J* = 10.4, 7.9 Hz, 1H, H_2), 4.99 (dd, *J* = 10.5, 3.4 Hz, 1H, H_3), 4.44 (d, *J* = 7.9 Hz, 1H, H_1), 4.19-4.07 (m, 2H, H_6), 3.97-3.87 (m, 2H, H_5, OCH$_2$*CH*$_2$CH$_2$ N$_3$), 3.62-3.54 (m, 1H, O*CH*$_2$CH$_2$CH$_2$ N$_3$) 3.37-3.32(m, 2H, OCH$_2$CH$_2$*CH*$_2$N$_3$), 2.13 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.93-1.72 (m, 2H, OCH$_2$*CH*$_2$CH$_2$N$_3$), ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.48,

170.33, 170.21, 169.69, 101.64, 70.92, 70.76, 68.93, 67.40, 67.11, 62.37, 32.32, 30.26, 20.90, 20.77, 20.74, 20.67; MALDI-MS: *m/z* 453.15 $[M+Na]^+$. Calcd for $C_{17}H_{24}N_3O_{10}$ 453.13.

3-Azidopropyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (8). NaOMe (30%) in methanol was added to a solution of **12** (6.45 g, 15.0 mmol) in methanol followed by stirring of the reaction mixture at room temperature (2 h) at pH 9. The resulting intermediate was used in the next step, in which TrCl (3.18 g, 11.40 mmol) and (DMAP) were added to a stirred solution of the deacetylated intermediate (2.00 g) in pyridine (50 mL). The reaction mixture was warmed (83 °C) and stirred (18 h), after which it was concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (60 mL) and washed successively with 1 N hydrochloric acid (15 mL), $NaHCO_3$ (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified by flash silica gel column chromatography (hexane/EtOAc, 1:1 → 0:1, v:v) to give a white foam that was used directly in the next step without further characterization. BzCl (2.15 mL, 18.6 mmol) was added dropwise to a cooled (0 °C) solution of the above intermediate (2.00 g, 3.95 mmol) in pyridine (35 mL). The resulting reaction mixture was stirred at room temperature for 18 h and then concentrated *in vacuo* to give a residue which was dissolved in EtOAc (70 mL). The resulting solution was washed successively with 1 N aqueous HCl (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was immediately treated with $FeCl_3 \cdot 6H_2O$ (2.56 g, 9.48 mmol) in CH_2Cl_2 (80 mL) at room temperature for 18 h. The reaction mixture was filtered through a pad Celite and the filtrate was concentrated *in vacuo* after which the residue was dissolved in CH_2Cl_2 (80 mL) and washed successively with water (30 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified by flash silica gel column chromatography (hexane/EtOAc, 3:1 → 1:3, v:v)

to give **8** (1.57 g, 69%) as a white amorphous solid. R_F = 0.35 (hexane/EtOAc 1:1, v:v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, *J* = 7.8 Hz, 2H, Ar), 8.00 (d, *J* = 7.8 Hz, 2H, Ar), 7.83 (d, *J* = 7.5 Hz, 2H, Ar), 7.64 (d, *J* = 7.2 Hz, 1 H, Ar), 7.54-7.44 (m, 6H, Ar), 7.27 (s, 2H, Ar), 5.84 (1H, H_4), 5.60 (dd, *J* = 11.1 Hz, 1H, H_3), 4.82 (d, *J* = 7.8 Hz, 1H, H_1), 4.14 (d, *J* = 7.5 Hz, 1H, H_2), 4.05 (m, 2H), 3.85 (dd, *J* = 12.0, 6.6 Hz, 1H, H_5), 3.70 (m, 2H, O*CH*$_2$$CH_2CH_2N_3$), 3.30 (m, 2H, OCH_2CH_2*CH*$_2$$N_3$), 1.82 (m, 2H, OCH_2*CH*$_2$$CH_2N_3$). ^{13}C NMR (75 MHz, CDCl3): δ 167.05, 165.76, 165.61, 134.07, 133.61, 133.58, 130.35, 129.99, 129.91, 129.47, 128.90, 128.72, 128.57, 101.96, 74.33, 71.98, 70.19, 69.23, 66.94, 60.84, 48.07, 29.22; MALDI-MS: *m/z* 598.11 $[M+Na]^+$. Calcd for $C_{30}H_{29}N_3O_9$ 598.19.

3-Azidopropyl 4,6-*O*-benzylidene-β-D-galactopyranoside (14). NaOMe (30%) in methanol was added to a solution of **12** (1.80g, 4.17mmol) in methanol and the reaction mixture allowed to stir at room temperature for 2 h at pH 9, after which it was neutralized with weakly acidic (Amberlite IRC-50) resin for 30 min. Filtration of the suspension and concentration of the filtrate *in vacuo* gave an intermediate which was dissolved in acetonitrile (25 mL), followed by the addition of $PhCH(OMe)_2$ (0.87 g, 5.7 mmol) and p-TsOH (0.58 g, 3.0 mmol). The reaction mixture was stirred for 18 h at room temperature, after which it was quenched with triethylamine and concentrated *in vacuo*. The resulting residue was subjected to flash silica gel chromatography (hexane/EtOAc, 1:1 → 1:5) to give **14** (1.11 g, 83%) as a white solid. R_F = 0.33 (EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38 (t, 4H, Ar), 7.28-7.17 (m, 3H, Ar), 5.52 (s, 1H, Ph*CH*), 4.75 (dd, *J* = 9.6, 3.0 Hz, 1H, H_3), 4.58 (d, *J* = 9.3 Hz, 1H, H_1), 4.30 (d, *J* = 8.4 Hz, 1H, H_2), 4.07 (dd, *J* = 12.0, 6.6 Hz, 1H, H_5), 3.68 (m, 2H, O*CH*$_2$$CH_2CH_2N_3$), 3.47 (m, 2H, OCH_2CH_2*CH*$_2$$N_3$), 1.93 (m, 2H, OCH_2*CH*$_2$$CH_2N_3$).

3-Azidopropyl 4,6-*O*-benzylidene-3-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-galactopyranoside (15). FmocCl (0.59 g, 2.3 mmol) was added to a cooled (0 °C) and stirred solution of **14** (0.72 g, 2.07 mmol) in dry pyridine (10 mL). The reaction mixture was stirred at 0 °C for 3.5 h, after which it was quenched with MeOH and concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (25 mL) and was washed successively with 1 N HCl, water (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was purified by flash silica gel column chromatography (hexane/EtOAc, 3:1 → 1:1, v:v) to afford **15** (1.01 g, 85 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (m, 2H, Ar), 7.60 (t, *J* = 7.5 Hz, 2H, Ar), 7.52 (t, *J* = 3.6 Hz, 2H, Ar) 7.38 (t, 4H, Ar), 7.28-7.17 (m, 3H, Ar), 5.52 (s, 1H, Ph*CH*), 4.75 (dd, *J* = 9.6, 3.0 Hz, 1H, H_3), 4.58 (d, *J* = 9.3 Hz, 1H, H_1), 4.30 (d, *J* = 8.4 Hz, 1H, H_2), 4.07 (dd, *J* = 12.0, 6.6 Hz, 1H, H_5), 3.68 (m, 2H, O$CH_2$$CH_2$$CH_2N_3$), 3.47 (m, 2H, $OCH_2CH_2CH_2N_3$), 1.93 (m, 2H, $OCH_2CH_2CH_2N_3$).

3-Azidopropyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-galactopyranoside (16). BzCl (0.56 mL, 4.0 mmol) was added over a period of 10 min to a cooled (0 °C) and stirred solution of **15** (1.00 g, 1.74 mmol) in dry pyridine (15 mL). The resulting reaction mixture was allowed to warm to room temperature and stirred for 6 h, after which it was quenched with MeOH and concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (20 mL) and washed successively with 1 N HCl (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was immediately subjected to silica gel column chromatography (hexane/EtOAc, 3:1 → 1:1, v:v) to give **16** (1.68 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (d, *J* = 7.5 Hz, 2H, Ar), 7.68-7.22 (m, 19H, Ar), 7.12 (t, *J* = 7.5 Hz, 1H, Ar), 7.06 (t, 7.5 Hz, 1H, Ar), 5.74 (t, *J* = 10.5, 7.8 Hz, 1H, H_2), 5.58 (s, 1H, Ph*CH*), 5.03 (dd, *J* = 10.2, 3.3 Hz, 1H, H_3), 4.69 (d, *J* = 7.8 Hz, 1H, H_1), 4.51

(d, J = 3.6 Hz, 1H, H_4), 4.40 (d, J = 12.3 Hz, 1H, H_{6a}), 4.30 (d, J = 7.5 Hz, 2H), 4.13 (d, 1H), 4.03 (m, 2H, O$CH_2$$CH_2CH_2N_3$ 3.61 (s, 1H, H_5) 3.24 (m, 2H, $OCH_2CH_2CH_2N_3$), 1.83 (m, 2H, $OCH_2CH_2CH_2N_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.92, 154.26, 142.92, 142.78, 140.92, 137.28, 13315, 129.57, 129.335, 128.87, 128.32, 127.99, 127.63, 127.59, 126.91, 126.18, 124.92, 124.87, 119.75, 100.97, 100.71, 100.65, 75.32, 73.01, 68.96, 68.66, 66.10, 65.86, 47.70, 46.21, 28.70.

3-Azidopropyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (13). Et_3N (9.00 mL, 10.3 mmol) was added to a cooled solution (0 °C) of **22** (0.70 g, 1.0 mmol) dissolved in CH_2Cl_2 (35 mL). The resulting reaction mixture was allowed to warm to room temperature and stirred for 6 h, after which it was concentrated *in vacuo*. The residue was subjected to flash silica gel column chromatography (hexane/EtOAc, 2:1 → 1:1, v:v) to give **13** (0.32 g, 65%), as a white solid. R_F = 0.29 (hexane/EtOAc 1:1, v:v). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.98, 137.31, 133.01, 129.62, 129.49, 128.98, 128.22, 128.01, 126.19, 101.03, 100.98, 100.76, 75.31, 72.61, 71.17, 68.67, 66.35, 65.86, 47.85, 28.23.

General procedure for the synthesis of disaccharides, 1, 2, 3, and 4. A mixture of thioglycoside (0.16 mmol) and an alcohol (0.10 mmol) in DCM (10 mL) was stirred at room temperature in the presence of 4Å molecular sieves (500 mg) for 30 min. After the mixture was cooled to -30 °C, NIS (54 mg, 0.24 mmol) followed by a solution of AgOTf (21 mg, 80 μmol) in toluene (0.2 mL) were added. The reaction mixture was warmed slowly to room temperature and stirring continued for 15 min. The reaction was quenched by the addition of Et_3N or pyridine. The suspension was diluted with EtOAc (50 mL) and filtered through a pad of Celite, and the filtrate was washed successively with 10% $Na_2S_2O_3$ (10 mL) and brine (20 mL). The

organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was purified on column chromatography to afford the corresponding disaccharides.

3-Azido propyl 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl -(1→6)- 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95 (d, *J* = 7.2 Hz, 2H, Ar), 7.90 (d, *J* = 7.5 Hz, 2H, Ar), 7.70 (d, *J* = 7.2 Hz, 2H, Ar), 7.53 (t, *J* = 7.2 Hz, 1H, Ar), 7.47-7.29 (m, 6H, Ar), 7.19 (t, 1H, Ar), 5.90 (s, 1H, H_{4gal}), 5.73 (t, *J* = 9.9, 8.1 Hz, 1H, H_{2gal}), 5.52 (dd, *J* = 10.5 Hz, 1H, H_{3gal}), 5.05 (d, *J* =12 Hz, 2H, H_{6gal}), 4.85 (d, 1H, H_{1ara}), 4.78(d, 7.5 Hz, 1H, H_{1gal}), 4.15 (2d, 2H, H_{3ara}, H_{5gal}), 4.02 (2d, 2H, H_{2ara}, H_{5ara}) 3.92 (t, *J* = 8.7, 7.8 Hz, 2H, O*CH*$_2$CH$_2$CH$_2$N$_3$), 3.67 (m, 2H, $H_{5'ara}$, H_{4ara}), 3.27 (t, 2H, OCH$_2$CH$_2$*CH*$_2$N$_3$), 2.06 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.85 (s, 3H, OAc), 1.75 (m, 2H, OCH$_2$*CH*$_2$CH$_2$N$_3$; ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.59, 170.40, 169.54, 165.61, 165.52, 165.21, 133.46, 133.29, 133.17, 129.87, 129.64, 129.62, 129.23, 129.04, 128.88, 128.56, 128.41, 128.22, 105.39, 101.76, 80.83, 80.86, 76.70, 72.16, 71.94, 69.67, 67.81, 66.65, 64.27, 63.04, 60.32, 28.94, 20.97, 20.66, 20.58; MALDI-MS: *m/z* 856.52 $[M+Na]^+$, calcd for $C_{42}H_{44}N_3O_{15}$ 856.25.

3-Azido propyl 2-*O*-benzyl-3,5-*O*-(di-*tert*-butylsilanediyl)-β-L-arabinofuranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95 (d, *J* =7.2 Hz, 2H, Ar), 7.90 (d, *J* = 7.5 Hz, 2H, Ar), 7.70 (d, *J* = 7.2 Hz, 2H, Ar), 7.53 (t, *J* = 7.2 Hz, 1H, Ar), 7.47-7.29 (m, 6H, Ar), 7.19 (t, 1H, Ar), 5.90 (s, 1H, H_{4gal}), 5.73 (t, *J* = 9.9, 8.1 Hz, 1H, H_{2gal}), 5.52 (dd, *J* = 10.5 Hz, 1H, H_{3gal}), 5.05 (d, *J* = 12 Hz, 2H, H_{6gal}), 4.85 (d, 1H, H_{1ara}), 4.78(d, *J* = 7.5 Hz, 1H, H_{1gal}), 4.15 (2 d, 2H, H_{3ara}, H_{5gal}), 4.02 (2d, 2H, H_{2ara}, H_{5ara}) 3.92 (t, *J* = 8.7, 7.8 Hz, 2H, O*CH*$_2$CH$_2$CH$_2$N$_3$), 3.67 (m, 2H, $H_{5'ara}$, H_{4ara}), 3.27 (t, 2H, OCH$_2$CH$_2$*CH*$_2$N$_3$), 2.06 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.85 (s, 3H, OAc), 1.75 (m, 2H, OCH$_2$*CH*$_2$CH$_2$N$_3$), 0.91 (s, 9H, ^{t}Bu), 0.79 (s, 9H, ^{t}Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.59, 170.40, 169.54, 165.61, 165.52,

165.21, 133.46, 133.29, 133.17, 129.87, 129.64, 129.62, 129.23, 129.04, 128.88, 128.56, 128.41, 128.22, 105.39, 101.76, 80.83, 80.86, 76.70, 72.16, 71.94, 69.67, 67.81, 66.65, 64.27, 63.04, 60.32, 28.94, 20.97, 20.66, 20.58.

3-Azido propyl 2,3,5-tri-*O*-acetyl-1-thio-α-L-arabinofuranosyl -(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (3). R_F = 0.16 (hex/EtOAc, 2:1, v:v). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07 (d, *J* = 7.5 Hz, 2H, Ar), 7.57 (t, *J* = 7.5 Hz, 1H, Ar), 7.50 (d, *J* = 7.0 Hz, 2H), 7.45 (t, *J* = 7,5 Hz, 2H), 7.29-7.19 (m, 8H, Ar), 5.60 (dd, *J* = 10.0, 8.5 Hz, 1H, H_{gal-2}), 5.54 (s, 1H, Ph*CH*), 5.18 (d, *J* = 5.0 Hz, 1H, H_{ara-1}), 4.65 (d, *J* = 8.0 Hz, 1H, H_{gal-1}), 4.60 (AB peak, *J* = 12.0 Hz, 2H, PhCH_2), 4.37 (m, 2H), 4.15 (t, *J* = 9.0 Hz, 1H), 4.10 (m, 2H), 4.00 (m, 2H), 3.89 (dd, *J* = 9.0, 5.0 Hz, 1H), 3.56 (m, 2H), 3.49 (t-like, *J* = 9.0 Hz, 2H), 3.23 (m, 2H), 1.78 (m, 2H),; ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.9, 138.1, 137.4, 133.0, 130.0, 129.7, 128.9, 128.4, 128.12, 128.11, 127.7, 127.3, 126.3, 101.4, 101.1, 97.2 (C_{ara-1}), 80.5, 77.3, 74.5,74.2 72.3, 70.9, 69.2, 68.2, 66.7, 65.7, 48.0, 29.0, 27.2, 27.1, 22.3, 20.0.

3-Azidopropyl 2-*O*-benzyl-3,5-*O*-(di-*tert*-butylsilandiyl)-β-L-arabinofuranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (4). R_F = 0.16 (hex/EtOAc, 2:1, v:v). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07 (d, *J* = 7.5 Hz, 2H, Ar), 7.57 (t, *J* = 7.5 Hz, 1H, Ar), 7.50 (d, *J* = 7.0 Hz, 2H), 7.45 (t, *J* = 7,5 Hz, 2H), 7.29-7.19 (m, 8H, Ar), 5.60 (dd, *J* = 10.0, 8.5 Hz, 1H, H_{gal-2}), 5.54 (s, 1H, Ph*CH*), 5.18 (d, *J* = 5.0 Hz, 1H, H_{ara-1}), 4.65 (d, *J* = 8.0 Hz, 1H, H_{gal-1}), 4.60 (AB peak, *J* = 12.0 Hz, 2H, PhCH_2), 4.37 (m, 2H), 4.15 (t, *J* = 9.0 Hz, 1H), 4.10 (m, 2H), 4.00 (m, 2H), 3.89 (dd, *J* = 9.0, 5.0 Hz,1H), 3.56 (m, 2H), 3.49 (t-like, *J* = 9.0 Hz, 2H), 3.23 (m, 2H), 1.78 (m, 2H), 0.91 (s, 9H, ^{t}Bu), 0.79 (s, 9H, ^{t}Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.9, 138.1, 137.4, 133.0, 130.0, 129.7, 128.9, 128.4, 128.12, 128.11, 127.7, 127.3, 126.3, 101.4, 101.1, 97.2

($C_{ara\text{-}1}$), 80.5, 77.3, 74.5,74.2 72.3, 70.9, 69.2, 68.2, 66.7, 65.7, 48.0, 29.0, 27.2, 27.1, 22.3, 20.0; MALDI-MS: *m/z* 841.2 $[M+Na]^+$, 857.7 $[M+K]^+$, calcd for $C_{43}H_{55}N_3O_{11}Si$ 840.361.

Phenyl 2,3,5-tri-*O*-benzoyl-1-thio-α-L-arabinofuranose (19). NaOMe (30%) in methanol was added to a solution of **6** (1.30 g, 3.53 mmol) in methanol and the reaction mixture allowed to stir at room temperature for 2 h at pH 9, after which it was neutralized with weakly acidic (Amberlite IRC-50) resin for 30 min. Filtration of the suspension and concentration of the filtrate *in vacuo* gave an intermediate which was dissolved in cooled (0 °C) pyridine (8 mL) and BzCl (2.1 mL, 17 mmol) was added dropwise and stirred 6 h. After which it was quenched with MeOH and concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (20 mL) and washed successively with 1 N HCl (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was immediately subjected to silica gel column chromatography (hexane/EtOAc, 3:1 → 1:1) to give **19** (1.8 g, 92%) as a white foam. R_F = 0.33 (hexane/EtOAc, 4:1, v:v).

2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl 2,2,2-trichloroacetimidate (17). NIS (0.92 g, 4.9 mmol) was added to a solution of **19** in Acetone:H_2O (9:1) and stirred overnight and concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (20 mL) and washed successively with NaS_2O_3 (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was immediately subjected to silica gel column chromatography (hexane/EtOAc, 3:1 → 1:1) to give **17** (0.85 g, 57%). ^{1}H NMR (300 MHz, CDCl3): δ 8.73 (s, 1H, NH), 8.13-7.24 (m, 15H, Ar), 6.67 (s, 1H, H_1), 5.81 (s, 1H, H_2), 5.68 (d, 1H, J = 3.3 Hz, H_3), 4.86-4.73 (m, 3H, $H_{4,5a,5b}$).

Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (20). PhSH (2.82 g, 25.6 mmol) and BF_3OEt_2 (3.40 g, 24.0 mmol) were added to a cooled (0 °C) solution of 1,2,3,4,6 penta- *O*-

acetyl galactopyranose (8.00 g, 20.5 mmol) dissolved in CH_2Cl_2 under an atmosphere of argon. The reaction mixture was allowed to warm to room temperature, and after stirring for 18 h the reaction was quenched with Et_3N and concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (100 mL), and washed successively with $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was subjected to flash silica gel column chromatography (hexane/EtOAc, 4:1 → 2:1, v:v) to give **10** (8.88 g, 98%) as a white amorphous solid. R_F = 0.33 (hexane/EtOAc, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50 (m, 2H, Ar), 7.30 (m, 3H, Ar), 5.40 (d, *J* = 2.4 Hz, 1H, H_4), 5.21 (t, *J* = 10.2 Hz, 1H, H_2), 5.05 (dd, *J* = 3.6 Hz, 1H, H_3), 4.70 (d, *J* = 10.2 Hz, 1H, H_1), 4.13 (m, 2H, H_6), 3.92 (t, 1H, H_5), 2.10, 2.08, 2.02, 1.96 (4s, 12H, OAc); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.2, 170.04, 169.89, 169.29, 132.51, 132.36, 128.78, 128.05, 86.45, 71.92, 67.19, 61.54, 20.69, 20.49, 20.46.

Phenyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (21). NaOMe (30%) in methanol was added to a solution of **20** (8.88 g) in methanol and the reaction was stirred at room temperature (2 h) at pH 9 to give an intermediate, which was then neutralized with weakly acidic (Amberlite IRC-50) resin for 30 min. Filtration of the suspension and concentration of the filtrate *in vacuo* gave an intermediate that was used in the next step, after which $PhCH(OMe)_2$ and p-TsOH (2.8 g, 15 mmol) were added to a stirred solution of deacetylated intermediate in acetonitrile (60 mL). The reaction mixture was stirred for 18 h at room temperature, and then neutralized by the addition of triethylamine diluted with toluene and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed subsequently with 1M HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified using flash silica gel column chromatography

(hexane/EtOAc, 2:1 → 1:4, v:v) to afford **21** (5.43 g, 82%) as a white, amorphous solid. R_F = 0.38 (hexane/EtOAc, 1:4). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50-7.25 (m, 10H, Ar), 5.53 (s, 1H, Ar), 4.50 (m, 1H, H_1), 4.40 (dd, 1H, J = 12.5, 1.6 Hz, H_6) 3.75-3.68 (m, 2H, $H_{2,3}$), 3.56 (dd, 1H, J = 2.8, 1.5 Hz , H_5), 2.63-2.57 (m, 2H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.2, 134.2, 129.8, 128.7, 126.9, 101.9, 87.4, 75.8, 74.2, 70.5, 69.7, 69.2; MALDI-MS: *m/z* 383.08 $[M+Na]^+$. Calcd for $C_{19}H_{20}O_5SNa$ 383.0922.

Phenyl 4,6-*O*-benzylidene-3-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-galactopyranoside (22). FmocCl (1.21 g, 4.67 mmol) was added to a cooled (0 °C) and stirred solution of **21** (1.53 g, 4.24 mmol) in pyridine (20 mL). The reaction mixture was stirred at 0 °C for 3.5 h, after which it was quenched with MeOH and concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (25 mL) and washed successively with 1 N HCl, water (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography (hexane/EtOAc, 4:1 → 1:1, v:v) to afford **22** (1.70 g, 73%) as a white amorphous solid. R_F = 0.48 (hexane/EtOAc, 2:1, v:v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (m, 4H, Ar), 7.55 (t, J = 6.9, 5.7 Hz, 2H, Ar), 7.42-7.17 (m, 12H, Ar), 5.48 (s, 1H, PhCH), 4.75 (dd, J = 9.6, 3.0 Hz, 1H, H_3), 4.58 (d, J = 9.3 Hz, 1H, H_1), 4.39 (m, 4H), 4.23 (t, J = 7.2 Hz, 1H), 4.03 (t, J = 10.5 Hz, 2H), 3.58 (s, 1H, H_5); ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.84, 143.58, 143.31, 141.50, 141.49, 137.93, 133.95, 130.62, 129.37, 129.30, 128.54, 128.39, 128.14, 127.45, 127.41, 126.72, 125.44, 120.29, 101.09, 87.51, 78.81, 73.54, 70.42, 69.93, 69.36, 65.81, 46.87; MALDI-MS: *m/z* 605.7 $[M+Na]^+$. Calcd for $C_{34}H_{30}O_7S$ 604.1712.

Phenyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-galactopyranoside (18). BzCl (0.77 mL, 6.7 mmol) was added to a cooled (0 °C) and stirred

solution of **22** (1.70 g, 2.92 mmol) in pyridine (20 mL) over a period of 10 min. The resulting reaction mixture was allowed to warm to room temperature and stirred for 18 h, after which it was quenched with MeOH and concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (20 mL) and washed successively with 1 N HCl (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was immediately subjected to silica gel column chromatography (hexane/EtOAc, 4:1 → 1:1) to give **18** (1.68 g, 84%) as a white foam. R_F= 0.33 (hexane/EtOAc, 3:1, v:v). ^{1}H NMR (300 MHz, CDCl3): δ 8.06 (d, *J* = 7.5 Hz, 2H, Ar), 7.68-7.22 (m, 19H, Ar), 7.12 (t, *J* = 7.5 Hz, 1H, Ar), 7.01 (t, 7.5 Hz, 1H, Ar), 5.69 (t, *J* = 9.9 Hz, 1H, H_2), 5.54 (s, 1H, Ph*CH*), 5.08 (dd, *J* = 9.9, 3.3 Hz, 1H, H_3), 4.92 (d, *J* = 9.6 Hz, 1H, H_1), 4.51 (d, *J* = 3.6 Hz, 1H, H_4), 4.43 (d, *J* = 12.3 Hz, 1H, H_{6a}), 4.25 (d, *J* = 7.5 Hz, 2H), 4.07 (m, 2H), 3.67 (s, 1H, H_5); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.05, 154.65, 143.37, 143.27, 141.38, 137.70, 134.05, 133.56, 131.52, 130.19, 129.90, 129.42, 129.09, 128.48, 128.44, 128.07, 128.02, 127.36, 126.82, 125.43, 125.36, 120.18, 120.17, 101.31, 85.56, 77.80, 73.59, 70.56, 69.99, 69.33, 67.64, 46.65; MALDI-MS: *m/z* 709.21 $[M+Na]^+$. Calcd for $C_{41}H_{34}O_8S$ 709.1974.

3-Azidopropyl 2-*O*-Benzoyl-4,6-*O*-benzylidien-3-*O*-(9-fluorenylmethozycarbonyl)-β-D-galactopyranosyl-(1 → 6)-2,34-tri-*O*-benzoyl-β-D-galactopyranoside (23). A mixture of glycosyl donor **18** (600 mg, 0.9 mmol), acceptor **8** (440 mg, 0.76 mmol), and powdered 4 Å molecular sieves (0.9 g) in CH_2Cl_2 (15 mL) was stirred at room temperature for 30 min and then cooled to -20 °C. Next, NIS (260 mg 1.16 mmol) followed by a solution of AgOTf (100 mg, 0.4 mmol) in toluene (0.8 mL) were added. The reaction mixture was warmed slowly to room temperature, stirred for 20 min, and then quenched by the addition of pyridine. The suspension was diluted with EtOAc (50 mL) filtered through a pad of Celite, and the filtrate was washed

successively with 10% $Na_2S_2O_3$ (8 mL) and brine (8 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue which was purified by silica gel column chromatography (hex/EtOAc, 5:1 → 1:1) to give **23** (691 mg, 79 %) as a white foam. R_F = 0.61 (hex/EtOAc, 1:1, v:v) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (t, *J* = 7.5 Hz, 4H, Ar), 7.93 (d, *J* = 7.8 Hz, 2H, Ar), 7.75 (d, *J* = 8.1 Hz, 2H, Ar), 7.69-7.19 (m, 23 H, Ar), 7.11 (t, *J* = 7.5 Hz, 1H, Ar), 7.01 (t, *J* = 7.5 Ha, 1H, Ar), 5.87 (d, *J* = 3.3 Hz, 1H, H_4), 5.78 (dd, *J* = 9.9, 8.4 Hz, 1H, H_2), 5.65 (t, *J* = 9.2 Hz, 1H, $H_{2'}$), 5.57 (s, 1H, Ph*CH*), 5.48 (dd, *J* = 10.2, 3.3 Hz, $H_{3'}$), 5.02 (dd, *J* = 10.5, 3.3 Hz, 1H, H_3), 4.74 (d, *J* = 7.8 Hz, 1H, H_1), 4.57 (d, *J* 7.8 Hz, 1H, $H_{1'}$), 4.49 (d, *J* = 3.0 Hz, 1H, $H_{4'}$), 4.43 (d, *J* = 12.6 Hz, 1H, H_{6a}), 4.28 (d, *J* = 7.5 Hz, 2H), 4.18 (m, 1H), 4.08 (m, 3H), 3.84 (dd, *J* = 10.5, 7.5 Hz, 1H), 3.61 (m, 2H, H5), 3.26 (m, 1H, H_{6b}), 3.07 (t, *J* = 6.9 Hz, 2H, $CH_2CH_2N_3$), 1.53 (m, 2H, $CH_2CH_2N_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.81, 165.64, 165.52, 165.10, 154.71, 143.31, 143.19, 121.37, 137.59, 133.48, 133.38, 130.27, 130.5, 129.96, 129.86, 129.78, 129.52, 129.34, 129.09, 128.78, 128.66, 128.44, 127.98, 127.29, 126.59, 125.35, 125.29, 120.15, 101.46, 101.16, 75.72, 73.68, 73.40, 71.97, 70.56, 70.09, 69.22, 68.56, 66.54, 66.49, 48.04, 46.62, 28.97. MALDI-MS *m/z* 1174.3673 $[M+Na]^+$, calcd for $C_{65}H_{57}N_3O_{17}$ 1174.3688.

3-Azidopropyl 2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-(9-fluorenylmethoxycarbonyl)-β-D-galactopyranosyl-(1 → 6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (24). BH_3·THF (1.0 M in THF, 5 mL, 5 mmol) and Bu_2BOTf (1.0M in CH_2Cl_2, 0.5 mL, 0.5 mmol) were added successively to a stirred and cooled (0 °C) solution of **23** (507 mg, 0.44mmol) in CH_2Cl_2 (15 mL). After stirring for 2 h, the reaction mixture was concentrated to a small volume, diluted with EtOAc (30 mL) and washed successively with saturated aqueous $NaHCO_3$ (15 mL) and brine (15 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica

gel column chromatography (hex/EtOAc, 2:1 → 1:1) to give **24** (305 mg, 60%) as a white foam: R_F = 0.52 (hex/EtOAc, 1:1, v:v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (d, *J* = 6.9 Hz, 4H, Ar), 7.95 (d, *J* = 7.2 Hz, 2H, Ar), 7.76 (d, *J* = 7.8 Hz, 2H, Ar), 7.69-7.09 (m, 25 H, Ar), 5.89 (br, s, 1H, H_4), 5.75 (t, *J* = 8.7 Hz, 1H, $H_{2'}$), 5.68 (t, *J* = 98.4 Hz, 1H, H_2), 5.51 (d-like, *J* = 10.5 Hz, 1H, H_3), 5.03 (d, *J* = 10.8 Hz, 1H, $H_{3'}$), 4.85 and 4.54 (AB, *J* = 11.5 Hz, 2H, Ph*CH*$_2$), 4.66 (d, *J* = 8.1 Hz, 1H), 4.63 (d, *J* = 8.4 Hz, 1H), 4.30 (m, 2H), 4.08 (m, 3H), 3.83 (m, 2H), 3.61 (m, 3H), 3.33 (m, 1H), 3.10 (m, 2H, CH_2*CH*$_2$$N_3$), 1.58 (m, 2H, *CH*$_2$$CH_2N_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.81, 165.64, 165.52, 165.10, 154.71, 143.31, 143.19, 121.37, 137.59, 133.48, 133.38, 130.27, 130.5, 129.96, 129.86, 129.78, 129.52, 129.34, 129.09, 128.78, 128.66, 128.44, 127.98, 127.29, 126.59, 125.35, 125.29, 120.15, 101.46, 101.16, 75.72, 73.68, 73.40, 71.97, 70.56, 70.09, 69.22, 68.56, 66.54, 66.49, 48.04, 46.62, 28.97. MALDI-MS: *m/z* 1176.3878 $[M+Na]^+$, calcd for $C_{65}H_{59}N_3O_{17}$ 1176.3845.

3-Azidopropyl 2-*O*-Benzoyl-4-*O*-benzyl-β-D-galactopyranosyl-(1 → 6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (25). Et_3N (2.60 mL, 1.87 mmol) was added to a cooled solution (0 °C) of **24** (0.10 g, 0.10 mmol) dissolved in CH_2Cl_2 (16 mL). The resulting reaction mixture was allowed to warm to room temperature and stirred for 6 h, then concentrated *in vacuo*. The residue was subjected to silica gel column chromatography (hex/EtOAc, 2:1 → 1:2, v:v) to give **25** (81.6 mg, 86%) as a white amorphous solid. R_F = 0.16 (1:1, hexane/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (d, *J* = 4.5 Hz, 4H, Ar), 7.93 (d, 2H, Ar), 7.75 (d, *J* = 7.5 Hz, 2H, Ar), 7.63-7.21 (m, 17 H, Ar), 5.89 (d, 1H, H_4), 5.67 (dd, 1H) 5.50 (dd, 1H), 5.29 (dd, 1H), 4.77 (dd, 2H), 4.64 (d, *J* = 8.0 Hz, 1H), 4.57 (d, *J* = 8.0 Hz, 1H), 4.11 (dd, 1H), 4.02 (dd, 1H), 3.81 (m, 4H), 3.70 (m, 1H), 3.56 (m, 1H), 3.37 (m, 2H), 3.10 (m, 2H, CH_2*CH*$_2$$N_3$), 1.58 (m, 2H, *CH*$_2$$CH_2N_3$).

3-Azido propyl 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl -(1→6)- 2-O-benzoyl-4,-O-benzyl – β-D-galactopyranosyl-(1 → 6)-2,3,4-tri-O benzoyl-β-D-galactopyranosyl (5) A mixture of thioglycoside (0.16 mmol) and an alcohol (0.10 mmol) in DCM (10mL) was stirred at room temperature in the presence of 4Å molecular sieves (500 mg) for 30 min. After the mixture was cooled to -30 °C, NIS (54 mg, 0.24 mmol) followed by a solution of AgOTf (21 mg, 80 μmol) in toluene (0.2 mL) were added. The reaction mixture was warmed slowly to room temperature, and stirring continued for 15 min. The reaction was quenched by the addition of Et_3N or pyridine. The suspension was diluted with EtOAc (50 mL) and filtered through a pad of Celite, and the filtrate was washed successively with 10% $Na_2S_2O_3$ (10 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was purified on column chromatography to afford **(5)**. R_F = 0.16 (1:1, hexane/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (d, *J* = 4.5 Hz, 4H, Ar), 7.93 (d, Hz, 2H, Ar), 7.75 (d, *J* = 7.5 Hz, 2H, Ar), 7.63-7.21 (m, 17 H, Ar), 5.89 (d, 1H, H-4), 5.67 (dd, 1H) 5.50 (dd, 1H), 5.29 (dd, 1H), 4.77 (dd, 2H), 4.64 (d, *J* = 8.0 Hz, 1H), 4.57 (d, *J* = 8.0 Hz, 1H), 4.11 (dd, 1H), 4.02 (dd, 1H),), 4.65 (d, *J* = 8.0 Hz, 1H, $H_{gal\text{-}1}$), 4.60 (AB peak, *J* = 12.0 Hz, 2H, Ph*CH*$_2$), 4.37 (m, 2H), 4.15 (t, *J* = 9.0 Hz, 1H), 4.10 (m, 2H), 4.00 (m, 2H), 3.89 (dd, *J* = 9.0, 5.0 Hz,1H), 3.56 (m, 2H), 3.49 (t-like, *J* = 9.0 Hz, 2H), 3.23 (m, 2H),3.81 (m, 4H), 3.70 (m, 1H), 3.56 (m, 1H), 3.37 (m, 2H), 3.10 (m, 2H, CH_2*CH*$_2N_3$), 1.58 (m, 2H, *CH*$_2CH_2N_3$); ^{13}C NMR (75 MHz, $CDCl_3$); δ 165.81, 165.64, 165.52, 165.10, 154.71, 143.31, 143.19, 121.37, 137.59, 133.48, 133.38, 130.27, 130.5, 129.96, 129.86, 129.78, 129.52, 129.34, 129.09, 128.78, 128.66, 128.44, 127.98, 127.29, 126.59, 125.35, 125.29, 120.15, 101.46, 101.16, 75.72, 73.68, 73.40, 71.97, 70.56, 70.09, 69.22, 68.56, 66.54, 66.49, 48.04, 46.62, 28.97.

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CHAPTER 3

SYNTHESIS OF A FRAGMENT OF A SIDE CHAIN OF EXTENSIN

3.1 Introduction

Extensins are highly insoluble hydroxyproline-rich glycoproteins (HRGPs) that are very tightly associated to the cell wall and are named according to their supposed important role in cell elongation. Hydroxyproline is the dominant amino acid in extensin. It is easy to differentiate extensins from the extractable arabinogalactan proteins because they contain shorter oligosaccharide chains and less alanine. Extensins are composed of 50% carbohydrate and 50% protein, of which 90% is arabinose and 41% is hydroxyproline, respectively. The galactosides present are believed to be α-D-galactosides linked to serine, and most of the hydroxyproline residues are arabinofuranosylated. The proposed structure of a portion of extensin in dicot cell walls has a tetra-arabinoside attached to the hydroxyproline residue, with all arabinofuranose residues β-linked except for the non-reducing terminal arabinofuranose residue, which is α-linked.[1] A portion of this oligosaccharide was synthesized for monoclonal antibody characterization (Figure **3.1**). Monoclonal antibodies offer an excellent vehicle to monitor the changes in plant cell walls at the cellular and subcellualar levels during growth and differentiation.

3.2 Results and Discussion

In general, the anomeric configuration of arabinofuranosides is established by a combination of chemical shifts and coupling constant data. β-ararbinofuranosides are characterized by $^{3}J_{\text{H-1,H2}}$ = 4-5 Hz and δ(C-1) 97-104 ppm whereas, α-arabinofuranosides are

characterized by $^{3}J_{H\text{-}1,H\text{-}2}$ = 1-3 Hz and δ(C-1) 104-110 ppm. Peak assignment was confirmed by 2D NMR analysis (HSQC and COSY). From the retrosynthetic analysis of the targeted trisaccharide of extensin shown in figure **3.1**, it is easy to envisage its possible synthesize from arabinofuranoside donors **6**, **7**, and **25**.

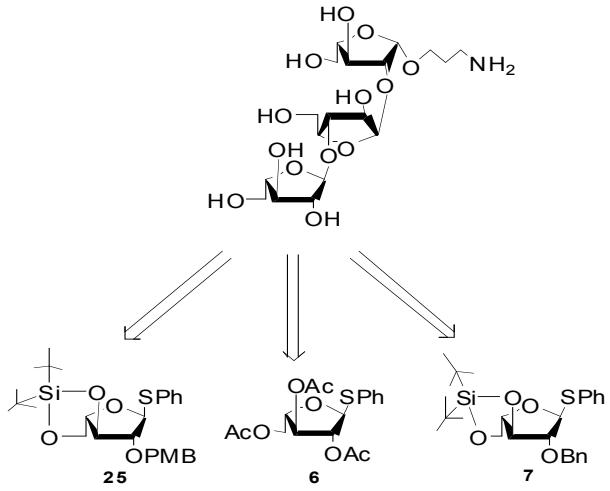
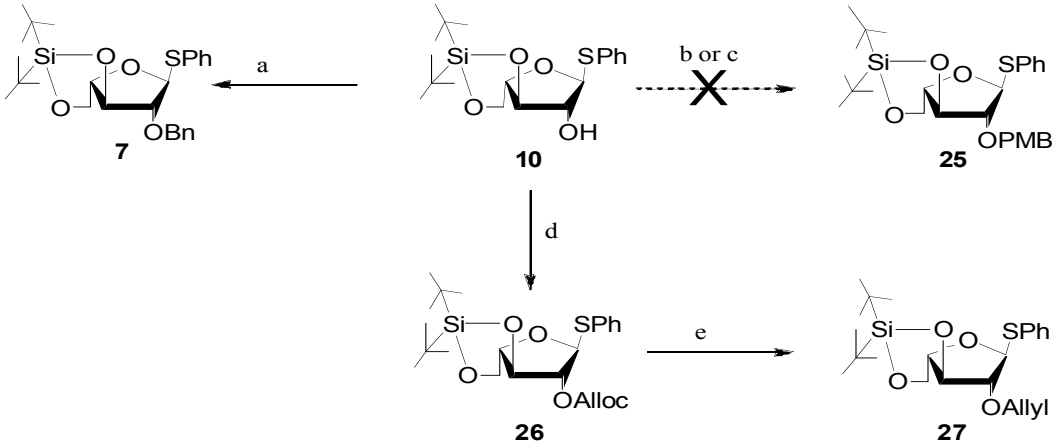


Figure. **3.1.** Retrosynthetic Analysis of Targeted Trisaccharide αAra*f* (1,3)β Ara*f* (1,2)βAra*f*

The 3,5-*O*-(di-*tert*-butylsilane)-protecting group in compounds **7** and **25** directs the stereochemistry of their glycosylation to give a β-linkage, whereas the C-2 acyl protecting group in compound **6** will enable selective synthesis of an α-linkage by neighboring group participation. *para*-Methoxyl benzyl (PMB) ether was chosen as the C-2 protecting group for **25** because it can be removed in the presence of the other protecting groups present in the molecule. However, attempts to protecti the C-2 hydroxyl in **10** with a PMB group using *para*-methoxyl benzyl chloride (PMBCl) was unsuccessful. In the first attempt to synthesize **25,** tetrabutylammonium iodide (TBAI) was used as the catalyst, but the reaction failed. Tetrabutlyammonium bromide (TBAB) was employed with the assumption that the failure was due to the incompatibility of

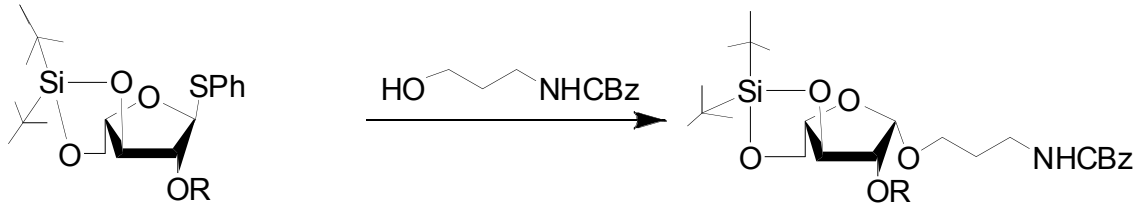
iodine and PMBCl. However, after switching to TBAB the reaction continued to fail. This outcome was perplexing, especially considering that benzyl bromide, another ether protecting group, was added to **10** using the same methodology. Nevertheless, additional atempts were made using silver oxide as the activator, but these conditions also failed, forcing the revision of the synthetic strategy (Scheme **3.1**).



Scheme **3.1.** C-2 Protection. Reagents and Conditions. a). BnBr, TBAI, THF, NaH, 0 °C rt, 85%; b) PMBCl, TBAI, THF, NaH, 0 °C rt; c). Ag_2O, DMF; d). Alloc, TEMDA, 90%; e). Ph_3P, Pd $(OAc)_2$, 98%.

An allyl ether was chosen as an alternative to the PMB ether as the C-2 protecting group. Thus, compound **27** was formed by the addition of allylchloroformate to **10** in the presence of *N,N,N′,N′*-Tetramethylethylenediamine (TEMED) to give **26** (90%), followed by the decarboxylation of **26** with triphenylphosphine (Ph_3P) and palladium acetate ($Pd(OAc)_2$) (Scheme **3.1**, 98 %).[2] Donors **7** and **27** were glycosylated to the linker using NIS/AgOTf and NIS/TfOH-mediated glycosylations (Scheme **3.2** and Table **3.1**) in order to evaluate which conditions were optimal to produce the arabinofuranoside β-linked to the linker; however, none these conditions gave the desired selectivity.[3] The best selectivity was obtained using NIS/AgOTf. This finding is analogous to the findings of Crich and co-workers when the

Boons's method was employed and in this study it was concluded that not just the protecting group, but the method of activation is important for β-selectivity.[4] In lieu of these findings, another stategy was implemented.



7: R = Bn
27: R = Allyl

28: R = Allyl
29: R = Bn

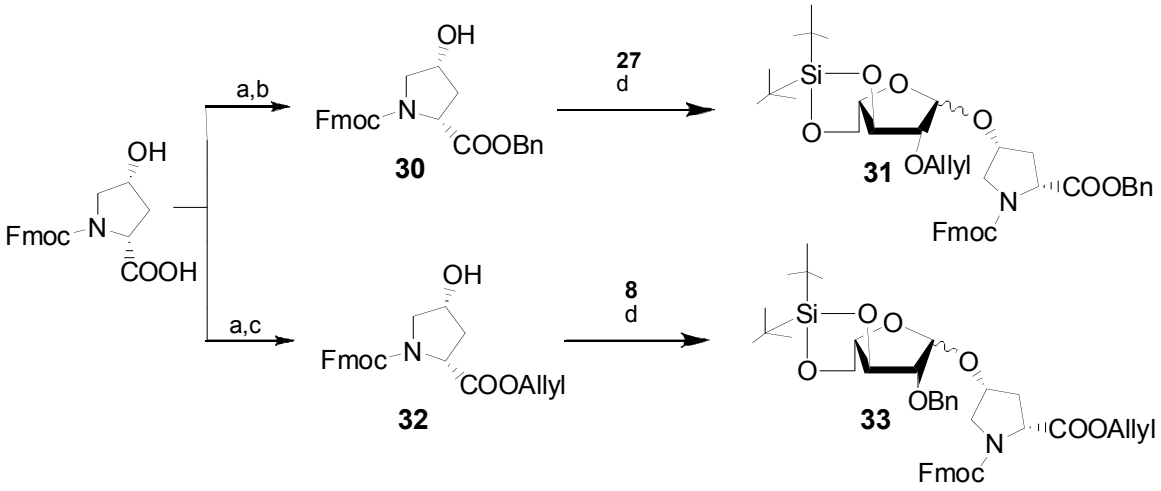
Scheme **3.2.** Glycosylations with donors **7** and **27**

R	CONDITIONS	% YEILD	BETA:ALPHA	COMMENTS
Allyl **27**	-40 °C - 0 °C, NIS, TfOH	77%	~1.5:1	Lost during purification
Bn **7**	-40 °C- 0°C, NIS, TfOH	82%	2:1	n/a
Allyl **27**	-40 °C- rt, NIS, TfOH	61%	1.3:1	Did not purify
Bn **7**	-40 °C-rt, NIS, TfOH	92%	2:1	Purify w/ prep TLC
Allyl **27**	-30 °C, NIS, AgOTf	64%	4:1	n/a
Bn **7**	-30 °C, NIS, AgOTf	66%	~4:1	n/a

Table **3.1.** Glycosylations with Linker

Taking clues from the natural compound it was decided to attach the arabinose directly to the hydroxyproline (Hyp). It was hypothesized that this glycosylation would have a higher β-selectivity due to the sterics produced by a bulkier acceptor and lower glycosyl reactivity by secondary hydroxyl. Therefore, the carboxylic acid of Hyp was protected as the benzyl **30** **(**69%), or allyl ester **32** (70%).[5] The protected hydroxyprolines were then glycosylated with

thiolphenyl donors **27** and **8**, respectively, using NIS/AgOTf as the activator to give **31** (43%) and **33** (47%) (Scheme **3.3**). Unfortunately, NMR analysis of the products revealed that both **31** and **33** behave as conformational isomers in which most signals in the spectra were doubled which would make the analysis of the di- and tri-saccharide challenging. Furthermore, the reaction proceeded in a low yield, which may be a result of the sterically congested ring conformations of the Hyp-OH acceptor. Therefore, this approach was abandoned.

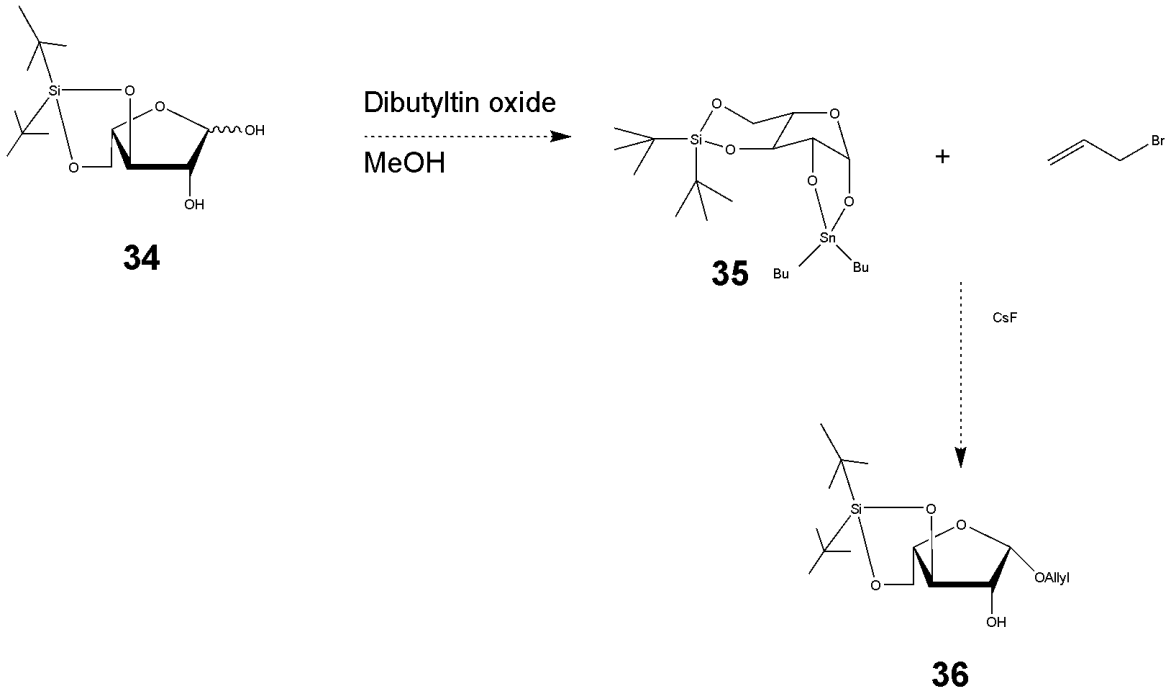


Scheme **3.3.** Reagents and conditions. a) $CsCO_3$/MeOH, b) AllylBr, DMF, 70%, c) AllylBr, DMF, 69% d) NIS/AgOTf, -30°C- rt

The undesired selectivity, poor yields, and production of conformational isomers of previous approaches led to another modification of the synthetic strategy. Kovac *et al.* reported that β–mannopyranosides could be stereoselectively synthesized by a stannylene acetal approach.[6] Since β-arabinofuranoside synthesis have a similar anomeric configuration as β-mannopyranosides and many methods for the stereoselective synthesis of β-mannopyranosides have been successfully applied to the synthesis of β-arabinofuranosides, the stannylene approach was attempted (Schmee **3.4**). The reaction mixture of numerous attempts to form and isolated **36**

showed several spots by TLC analysis. The products were not isolated or analyzed. However, a ublication by Lowary and co-workers reported the formation of desired β-arabinofuranosides

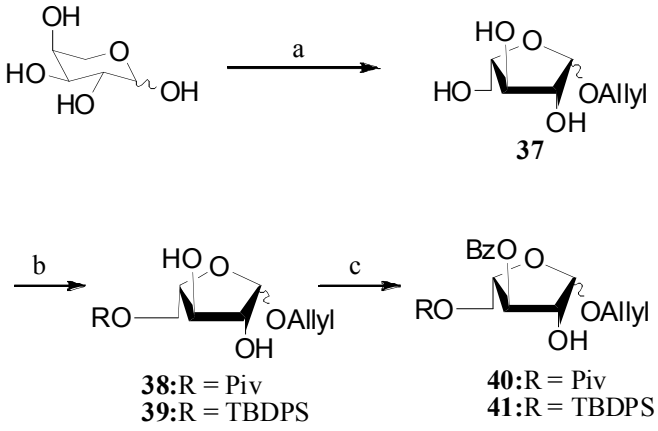
with no α-arabinofuranoside formation, but substantial amounts of the 2-O-alkylated lactols.[7] Although, the products of **36** were not analyzed, it can be assumed that the results are similar to the findings of Lowary.



Scheme **3.4.** Proposed Synthesis of β-Arabinofuranosides by the Stannylene Acetal Approach

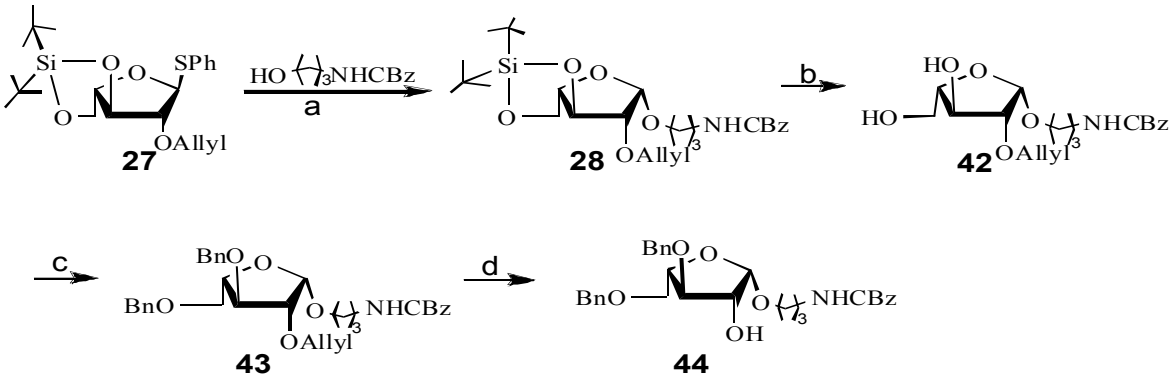
As an alternative to previous synthetic strategies for forming the desired trisaccharide, a Fischer glycosylation of L-arabinose with allyl alcohol to form **37** was proposed (Scheme **3.5**).[8] In a first attempt, a 3:1 β:α mixture of **37** was formed and the anomers were separated by first acetylating and then separated by silica gel chromotography. The C-5 hydroyl was protected with either pivaloyl chloride (PivCl) or *tert*-butyldiphenylsilyl chloride (TPDMSCl) to give **38** and **39**, respectively. Next selective benzoylation of C-3 was attempted to form **40** and **41,** respectively. The benzoylation however, was not selective and resulted in monobenzylation of

C-3 or C-2, along with dibenzoylation (Scheme **3.5**). Attempts to reproduce the Fischer glycosylation on larger scale not only resulted in α : β furanosides, but α : β pyranosides.

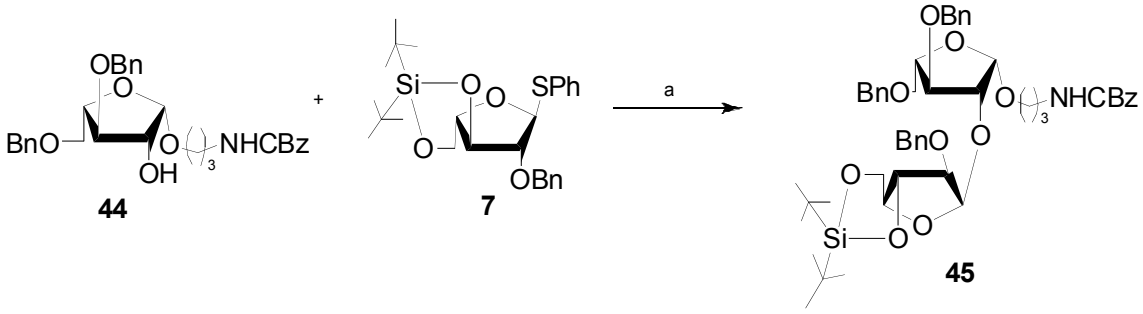


Scheme **3.5**. a) H_2SO_4, $CaSO_4$, allyl alcohol, 40 ^oC b) PivCl, py or TBDPSCl, imidazole, DMF c) BzCl, py

Due to the inability of any of the alternative strategies to produce the target compound, the Boons method with the C-2 allyl protecting group was revisited (Scheme **3.6**) and the α : β mixture of **28** was separated by column chromatography using toluene and ethyl acetate as the eluent. Compound **28** was desilylated using tetrabutylammonium fluoride (TBAF) to produce **42** (56%). The treatment to **42** with benzyl bromide in the presence of tetrabutylammonium iodide (TBAI) and sodium hydride (NaH) afforded **43** (85%). Then, compound **43** was treated with Pd/C in EtOH:water:1N HCl (5:2:0.02, v:v:v) to produce acceptor **44** (Scheme **3.6**). Glycosylation of acceptor **44** with donor **7** in the presence of NIS/AgOTf failed to produced disaccharide **45** and gave a mixture of spots (Scheme **3.7**). Therefore, the resynthesis of acceptor **44** was attempted on a larger scale, but during the glycosylation to form **28** addition of the linker across the allyl double bond was observed. Therefore, once again this approach was abandoned and the search for a non-participating protecting group for C-2 commenced.



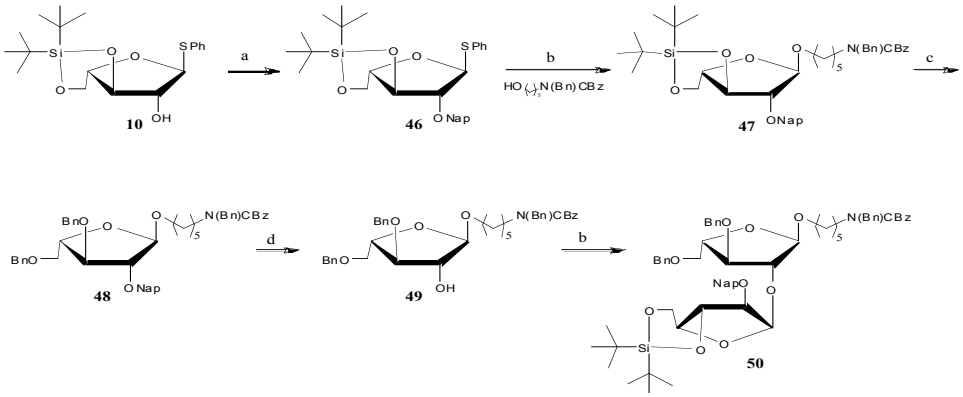
Scheme **3.6**. a) NIS, AgOTf, 64%; b) TBAF, 56%; c) BnBr, NaH, TBAI, 85%; d) Pd/C, EtOH:water: 1 N HCl, 5:2:0.02, v:v:v.



Scheme **3.7**. a) NIS, AgOTf

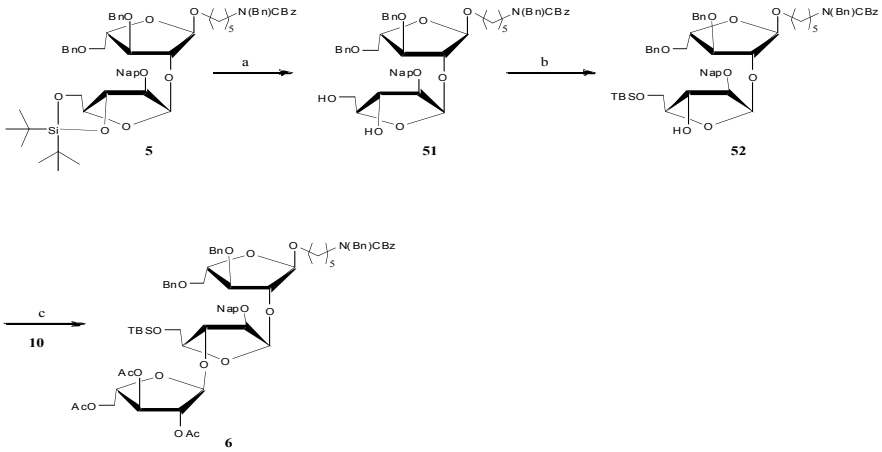
The 2-naphthylmethyl (NAP) ether was chosen as the C-2 protecting group because it is versatile and can readily be removed with 2,3-dichlor-5,6-dicyano-1,4-benzoquinone (DDQ) in CH_2Cl_2.[9, 10] Compound **46**, was synthesized by treatment of **10** with 2-napthylmethyl bromide in the presence of NaH and TBAI (87%). NIS/MeOTf and NIS/AgOTf-mediated glycosylation of **46** with *N*-Benzyl-*N*-benzyloxycarbonyl-5-aminopropyl-ol and *N*-Benzyl-*N*-benzyloxycarbonyl-5-aminopentyl-ol linkers were tested. NIS/AgOTf mediated glycosylation of **46** with the pentyl linker showed the best results and was used to synthesize **47** (41%). Treatment of **47** with TBAF

in THF afforded **48** (85%). The C-2 hydroxyl of **48** was deprotected by treatment with DDQ in CH_2Cl_2:H_2O (10:1, v:v) to afford **49** (80%).[10] NIS/AgOTf-mediated glycosylation of **46** and **49** afforded disaccharide **50** (95%).



Scheme **3.8.** a) NapBr, NaH, TBAI, DCM, 0°C to rt, 88%; b) NIS, AgOTf, CH_2Cl_2, 50%; c) TBAf, THF; BnBr, NaH, TBAI, 76% over two steps; d) DDQ, 20:1, CH_2Cl_2:H_2O, v:v, 80%

Compound **50** was then treated with TBAF in THF to give diol **51** (71%). The C-5 position of **51** was selectively silylated by treatment with *tert*-butyldimethylsilyl chloride (TBSCl) and imidazole in THF and afforded **52** (58%). Acceptor **52** was then glycosylated with donor **10** to afford target compound **6** (Scheme **3.9**).



Scheme **3.9.** a) TBAf, THF b) TBSCl, imidazole, THF; c) NIS, AgOTf, CH_2Cl_2, -30°C to rt; d) TBAf, THF; DDQ.

3.3 Conclusions

Synthesis of this portion of extensin proved extremely challenging, but it served as an excellent to examine the scope and limitations of the Boons's methodology. From this study we can conclude that the Boons method does work, but much research is still needed in the stereoselective introduction of β-arabinofuranosides.

3.4 Experimental Section

General methods and material. Chemicals were purchased from Fluka and Aldrich and used without further purification. Molecular sieves were activated in microwave oven (1.5 min, 3 times) and further dried *in vacuo*. Dichloromethane was distilled from CaH_2 and stored over 4Å molecular sieves. All the reactions were preformed under anhydrous conditions under an atmosphere of Argon and monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was by examination under 254 nm UV light, charring with 10% sulfuric acid in methanol, or charring with cerium ammonium molybdate. Flash chromatography was performed on silica gel (Merck, mesh 70-230). Iatrobeads (60 μm) were purchased from Bioscan. Extracts were concentrated under reduced pressure at <40 °C (bath). ^{1}H NMR (1D, 2D) and ^{13}C spectra were recorded on a Varian Merc 300 spectrometer and Varian 500 MHz spectrometers equipped with Sun workstations. For ^{1}H and ^{13}C spectra recorded in $CDCl_3$ unless otherwise noted, chemical shifts are given in ppm relative to solvent peaks (^{1}H = 7.24; ^{13}C, 77.0) as internal standard for protected compounds. Negative ion matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were recorded on VOYAGER-DE Applied Biosystems using dehydroxybenzoic acid in acetonitrile as an internal calibration matrix.

Phenyl 2-*O*-allylchloroformate-3,5-*O*-(di-*tert*-butylsilanediyl)-1-thio α-L-arabinofuranoside (26). Allyl chloroformate (1.0 g, 2.6 mmol) and *N,N,N′,N′*-Tetramethethylethylenediamine

(TEMED, 0.24 g, 2.1 mmol) were added to a solution of **14** in DCM (40 mL). The reaction was stirred under an atmosphere of argon for 2 h. Upon completion of reaction, the reaction mixture was diluted with DCM (30 mL) and washed successively with 1N hydrochloric acid (15 mL), saturated sodium bicarbonate (15 mL), and brine (15 mL), before drying over magnesium sulfate. After concentrating *in vacuo*, the resulting syrup was purified by flash silica gel chromatography (hexane: EtOAc, 20:1→ 10:1, v:v) to give compound **26** (1.1 g, 90 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45 (2H, Ar), 7.21 (3H, Ar), 5.91 (m, J = 6.3 Hz, 1H, H_7), 5.36-5.22 (3H, H_8 and H_1), 5.04 (dd, J = 5.1 Hz, 1H, H_2), 4.63 (d, J = 5.7 Hz, 2H, H_6), 4.28 (dd, J = 3.6 Hz, 1H, H_{5a}), 4.10 (t, J = 8.7 Hz, 1H, H_3), 3.92 (2H, H_4, H_{5b}), 0.91 (s, 9H, ^{t}Bu) 0.97 (s, 9H, ^{t}Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ134.25, 131.69, 131.28, 129.17, 127.54, 117.93, 90.20, 87.31, 81.86, 73.81, 71.41, 67.49, 29.94, 27.92, 27.66, 27.30, 22.82, 20.33.

Phenyl 2-*O*-allyl-3,5-*O*-(di-*tert*-butylsilanediyl)-1-thio α-L-arabinofuranoside (27).

Palladium (II) acetate (10 mg, 0.05 mmol) and triphenylphosphine (65 mg, 0.25 mmol) were added to a heated (60 °C) solution of **26** (1.1 g, 2.4 mmol) in benzene. After CO_2 evolution was complete (approx. 15 min) the reaction mixture was purified through a short flash silica gel column (hexane/EtOAc, 50:1→ 10:1, v:v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42(2H, Ar) 7.20 (3H, Ar), 5.89 (m, J = 5.7 Hz, 1H, H_7), 5.82-5.15 (3H, H_8, H_1), 4.28 (t, 1H, H_{5a}), 4.20 (2H, H_6), 4.00 (t, 1H, H_3), 3.98-3.84 (3H, H_4, H_{5b}, H_2), 0.99 (s, 9H, ^{t}Bu), 0.92 (s, 9H, ^{t}Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 134.92, 134.26, 132,06, 131.22, 129.08, 129.02, 127.54, 117.92, 90.21, 87,31, 81.39, 73.82, 71.41, 67.50, 27.75, 27.68, 27.39, 27.33, 22.83, 20.33.

N-Benzyloxycarbonyl-3-aminopropyl 2-*O*-allyl-3,5-*O*-(di-*tert*-butlysilanediyl)- β-L-arabinofuranoside (28). A mixture of thioglycoside (0.16 mmol) and alcohol (0.10 mmol) in CH_2Cl_2 (10mL) was stirred at room temperature in the presence of 4Å molecular sieves (500 mg)

for 30 min. After the mixture was cooled to -30 °C, NIS (54 mg, 0.24 mmol) followed by a solution of AgOTf (21 mg, 80 μmol) in toluene (0.2 mL) were added. The reaction mixture was warmed slowly to room temperature, and stirring was continued for 15 min. The reaction was quenched by the addition of Et_3N or pyridine. The suspension was diluted with EtOAc (50 mL) and filtered through a pad of Celite, and the filtrate was washed successively with 10% $Na_2S_2O_3$ (10 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was purified on column chromatography to afford **28** (64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (5H, Ar), 5.89 (m, J = 5.7 Hz, 1H, H_7),, 5.62 (s, 1H, NH), 5.28-5.05 (m, 1H, H_8), 5.02 (s, 1H, H_{12}), 4.91 (1H, H_1), 4.27-4.09 (4H, H_{3b}, H_6, H_{5a}), 3.88-3.75 (3H, H_2, H_{5b}, H_{9a}), 3.58 (m, 1H, H_4), 3.48 (1H, H_{9b}), 3.97-3.23 (2H, H_{11}), 1.73 (2H, H10), 0.99 (s, 9H, ^{t}Bu), 0.92 (s, 9H, ^{t}Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.77, 134.64, 128.65, 128.16, 118.10, 100.78, 87.86, 80.79, 79.10, 71.30, 68.56, 67.74, 39.68, 27.71, 27.36, 27.31, 22.76, 20.28.

N-Benzyloxycarbonyl-3-aminopropyl 2-*O*-allyl -β-L-arabinofuranoside (42). Tetrabutyl ammonium fluoride (TBAF, 781 μL in 1.0 M THF) and AcOH (one drop) were added to a solution of **28** (523 mg, 1.00 mmol) in THF (10 mL). The reaction mixture was stirred at rt for 24 h, after which a little pyridine was added and the solution was removed *in vacuo*. The resulting residue was dissolved in DCM (15 mL) was with water (2 x 15 mL) and then dried ($MgSO_4$). After filtration and evaporation of solvent, the residue was subjected to flash silica gel chromatography (hexane:EtOAc, 1:1 → 1:2) to afford diol intermediate (215 mg, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (5H, Ar), 5.87 (m, J = 6.3 Hz, 1H, H_7), 5.68 (s, 1H, NH), 5.15 (dd, J = 17.4 Hz, 2H, H_8), 5.05 (s, 2H, H_{12}), 4.87 (d, J = 4,5 Hz, 1H, H_1), 4.26 (t, J = 6.6 Hz, 1H, H_3), 4.07 (d, J = 5.7 Hz, 2H, H_6), 3.86-3.77 (3H, H_2, H_4, H_{9a}), 3.66 (2H, H_5), 3.44 (1H, H_{9b}), 3.27 (2H, H_{11}), 1.75(2H, H_{10}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.00, 136.90, 134.68, 128.70,

128.52, 128.31, 128.26, 118.19, 100.60, 84.33, 82.49, 74.14, 71.92, 66.87, 66.79, 63.56, 39.12, 29.67.

N-Benzyloxycarbonyl-3-aminopropyl 3,5-*O*-benzyl-2-*O*-allyl-β-L-arabinofuranoside (43). Benzyl bromide (140 μL, 1.2 mmol), 60 % NaH (20 mg, 1.4 mmol) and catalytic TBAI were added to a cooled (0 °C) solution of diol intermediate in THF (10 mL) for 1 hr. Then stirred for 2 h at rt. Then stirred at 50 °C for 2 h, after which the reaction mixture was quenched by the addition of MeOH (2 mL) and concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (15 mL) and washed subsequently with 1M HCl (5 mL), saturated aqueous $NaHCO_3$ (5 mL), and brine (5 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified using flash silica gel column chromatography (hexanes:EtOAc, 10:1 → 3:1, v:v) to afford **43** (70 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28 (15H, Ar), 5.84 (m, J = 6.3 Hz, 1H, H_7), 5.49 (s, 1H, NH), 5.18 (dd, J = 10.8, 17.4 Hz, 2H, H_8), 5.06 (s, 2H, H_{12}), 4.92 (d, J = 3.6 Hz, 1H, H_1), 4.54 (dd, J = 12 Hz, 4Hz OBn), 4.09-4.01 (5H, H_2, H_3, H_4, H_6), 3.77 (1H, H_{9a}), 3.48 (d, J = 5.7 Hz, 2H, H_5), 3.42 (1H, H_{9b}), 3.34-3.14 (2H, H_{11}) 1.71 (2H, H_{10}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.36, 138.16 134.62, 128.67, 128.60, 128.57, 128.27, 128.19, 128.07, 127.93, 127.89, 117.94, 100.87, 84.22, 83.21, 80.47, 73.50, 72.63, 72.28, 71.71, 66.85, 66.64, 39.66, 31.15, 29.92, 29.30.

N-Benzyloxycarbonyl-3-aminopropyl 3,5-*O*-benzyl-β-L-arabinofuranoside (44). Pd/C (10%, 1.0 times weight of starting material) was added to a solution of **43** in EtOH: water: 1 N HCl, (5:2:0.02, v:v:v) under an atmosphere of Ar. The reaction mixture was stirred 32 h until TLC analysis (3:1, hexane:EtOAc, v:v) indicated completion of the reaction. The reaction mixture was filtered through polytetrafluoroethene (PTFE) syringe filter (diameter 25 mm, pore size 0.2 mm), which was further washed with pyridine and EtOH. The solvents were co-evaporated with

toluene. The residue was dried *in vacuo* and used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (15H, Ar), 5.01 (2H, H_7), 4.83 (1H, J = 4.8 Hz, H_1), 4.70-4.39 (dd, 4H, OBn), 4.15 (s, 1H, H2), 4.02 (dd, J = 4.8 Hz, H_4), 3.82 (t, J = 6.3 Hz, 1H, H_3), 3.73 (dd, J = 3.9 Hz, 1H, H_{6a}), 3.47-3.38 (3H, $H_{5a,b}$, H_{6b}), 3.14 (2H, H_8), 1.65 (2H, H_9); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.73, 138.26, 138.08, 136.88, 128.75, 128.61, 128.34, 128.16, 128.06, 127.97, 127.93, 101.93, 84.30, 80.73, 73.56, 73.45, 72.24, 71.53, 66.87, 66.69, 66.03, 38.41, 29.93, 29.69.

N-Benzyloxycarbonyl-3-aminopropyl 2-*O*-benzyl-3,5-(di-tert-butylsilanediyl)-β-L-arabinofuranosyl (1 → 2) 3,5-*O*-benzyl-β-L-arabinofuranoside (45). A mixture of thioglycoside (0.16 mmol) and an alcohol (0.10 mmol) in DCM (10mL) was stirred at room temperature in the presence of 4Å molecular sieves (500 mg) for 30 min. After the mixture was cooled to -30 °C, NIS (54 mg, 0.24 mmol) followed by a solution of AgOTf (21 mg, 80 μmol) in toluene (0.2 mL) were added. The reaction mixture was warmed slowly to room temperature, and stirring was continued for 15 min. The reaction was quenched by the addition of Et_3N or pyridine, but it failed to give desired product.

Phenyl 2-*O*-(naphthyl)methyl 3,5-*O*-(di-tert-butylsilanediyl)-1-thio-α-L-arabinofuranose (46). NapBr (172 mg, 0.78 mmol), NaH (60%, 39 mg) and catalytic TBAI were added to a solution of **10** (0.25 g, 0.65 mmol) in CH_2Cl_2 (10 mL) and the reaction mixture was stirred at 0 °C for 2 h, then stirred at room temperature for an addition 3 h. The reaction mixture was quenched by the addition of MeOH, and the resulting solution was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (25 mL) and washed sequentially with 1N HCl (5 mL), $NaHCO_3$ (5 mL), water (5 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*, and the resulting residue was purified by flash silica gel column chromatography (hexane/EtOAc, 50:1 → 10:1, v:v) to give **46** (294 mg, 87%) as a pale yellow

oil. R_F = 0.65 (hexane/EtOAc 8:1, v:v). ^{1}H NMR (300 MHz, $CDCl_3$); δ 7.90-7.85 (m, 4H, Ar), 7.59-7.44 (m, 5H, Ar), 7.27-7.24 (m, 3H, Ar), 5.51 (d, $J_{1,2}$=5.4 Hz, 1H, H-1), 5.00 (AB, J= 12.0 Hz, 2H, NapCH_2), 4.34 (q-like, $J_{4,5a}$ = 4.2 Hz, 1H, H_{5a}), 4.47 (m, $J_{2,3}$ = 6.9 Hz, $J_{3,4}$ = 9.3 Hz, 1H, H_3), 4.09-3.80 (m, 3H, H_2, H_4, H_{5b}), 1.08 (s, 9H, ^{t}Bu), 0.99 (s, 9H, ^{t}Bu). ^{13}C NMR (75 MHz, $CDCl_3$): δ 135.2, 134.7, 133.6, 133.4, 131.5, 129.2, 128.5, 128.3, 128.0, 127.6, 127.3, 126.2, 126.2, 90.2, 86.9, 81.6, 74.0, 72.5, 67.6, 27.8, 27.4, 22.9, 20.4. MALDI-MS: *m/z* 388.66[M+Na]$^+$. Calcd for 388.15.

N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-*O*-(naphthyl)methyl -3,5-*O*-(di-*tert*-butlysilanediyl)-β-L-arabinofuranoside (47). A mixture of thioglycoside (1.00 g, 1.91 mmol) and an alcohol (0.82 mg, 2.49 mmol) in DCM (50 mL) was stirred at room temperature in the presence of 4Å molecular sieves (500 mg) for 2 h. After the mixture was cooled to -30 °C, NIS (1.03 g, 1.03 mmol) followed by AgOTf (390 mg, 1.53 mmol) were added. The reaction mixture was stirred at -30 °C for 10 min then warmed slowly to room temperature, and stirring was continued for 15 min. The reaction was quenched by the addition of pyridine and concentrated *in vacuo*. The suspension was diluted with EtOAc (100 mL) and filtered through a pad of Celite, and the filtrate was washed successively with 10% $Na_2S_2O_3$ (20 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was purified on column chromatography to afford **47** (580 mg, 41%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83-7.78 (m, 4H, Ar), 7.50-7.43 (m, 3H, Ar), 7.30-7.26 (m, 10H, Ar), 5.17 (s, 1H, $H_{7linker}$), 4.86 (q-like with s inside, J = 12.3 Hz, 3H, H_1, NapCH_2), 4.48 (s, 2H, NCH_2Bn), 4.33 (d, J = 3.6 Hz, 1H, $H_{1alinker}$), 4.12 (m, 1H, H_4), 3.99-3.91 (3H, H_3, H_2, $H_{1blinker}$), 3.60 (1H, $H5_a$), 3.25 (3H, H_{5b}, $H_{5linker}$), 1.52 (4H, $H_{2linker}$, $H_{4linker}$), 1.26 (2H, $H_{3linker}$), 1.08 (s, 9H, ^{t}Bu), 0.99 (s, 9H, ^{t}Bu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.14, 133.26, 128.76, 128.35, 128.12, 128.06, 127.91, 127.00,

126.28, 126.13, 107.28 ($H_1\alpha$), 88.07, 81.71, 73.98, 72.36, 67.85, 67.39, 50.74, 27.72, 27.34, 23.53, 22.85.

N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-*O*-(naphthyl)methyl-3,5-*O*-benzyl-β-L-arabinofuranoside (48). Tetrabutyl ammonium fluoride (TBAF, 594 μL in 1.0 M THF) was added to a solution of **47** (560 mg, 0.76 mmol) in THF (20 mL). The reaction mixture was stirred at rt for 12 h, after which the solution was removed *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (25 mL) was with water (2 x 10 mL), brine (5mL) and then dried ($MgSO_4$). After filtration and evaporation of solvent, the residue was subjected to flash silica gel chromatography (hexane:EtOAc, 1:1 → 1:2) to afford diol intermediate (340 mg, 75%) which was used in the next step without characterization. Benzyl bromide (180 μL, 1.60 mmol), 60 % NaH (53 mg, 1.33 mmol) and catalytic TBAI were added to a cooled (0 °C) solution of diol intermediate in THF (15 mL) for 1 hr. Then stirred for 2 h at rt. Then stirred at 50 °C for 10 h, after which the reaction mixture was quenched by the addition of MeOH (5 mL) and concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (25 mL) and washed subsequently with 1M HCl (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (5 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified using flash silica gel column chromatography (hexanes:EtOAc, 6:1 → 3:1, v/v) to afford **48** (351 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (4H, Ar), 7.46 (3H, Ar), 7.26 (20H, Ar), 5.17 (s, 1H, $H_{7linker}$), 5.04 (s, 1H, H_1), 4.68-4.47 (8H, BnCH_2, NapCH_2), 4.18 (m, 1H, H_4), 4.04 (1H, H_2,), 3.95(1H, H_3), 3.62 (3H, H_{5a}, $H_{1linker}$), 3.22 (3H, H_{5b}, $H_{5linker}$), 1.53 (4H, $H_{2linker}$, $H_{4linker}$), 1.26 (2H, $H_{3linker}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.35, 138.15, 135.28, 133.46, 133.27, 128.76, 128.67, 128.56, 128.43, 128.13, 128.05, 128.00, 127.92, 127.81, 127.51,

126.98, 126.41, 126.22, 126.06, 106.39 (C_1 α), 88.75, 83.75, 80.70, 73.64, 72.37, 70.00, 67.61, 67.37, 60.62, 50.41, 47.35, 46.41, 29.45, 28.15, 27.73, 23.63, 21.29, 14.44.

N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 3,5-*O*-benzyl-α-L-arabinofuranoside (49). DDQ (113mg, 0.50 mmol) was added to a stirred solution of **48** in CH_2Cl_2:H_2O (20:2, v:v). The reaction mixture was stirred at rt for 4 h, after which it was quenched with aq. $NaHCO_3$ and washed successively with water (3 x 8 mL) and brine (8 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (hexane/EtOAc, 1:1, v:v) to give **49** (214 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (20H, Ar), 5.17 (s, 1H, $H_{7linker}$), 5.04 (s, 1H, H1), 4.68 (2H, Bn$CH_{2linker}$), 4.47 (4H, BnCH_2), 4.23 (br s, 1H, H_4), 4.12 (1H, H_2,), 3.95(1H, H_3), 3.65 (2H, H_{5a}, $H_{1alinker}$), 3.50(1H, $H_{1blinker}$) 3.34(1H, H_{5b}) 3.17 (2H, $H_{5linker}$), 1.54 (4H, $H_{2linker}$, $H_{4linker}$), 1.26 (2H, $H_{3linker}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.16, 137,32, 128.79, 128.74, 128.60, 128.28, 128.13, 128.10, 128.05, 127.98, 127.48, 109.30, 85.45, 83.36, 78.29, 74.00, 72.15, 70.06, 67.54, 67.37, 50.71, 50.42, 47.37, 46.41, 29.39, 28.11, 27.66, 23.58, 14.44.

N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-*O*-(naphthyl)methyl-3,5-*O*-(di-*tert*-butlysilanediyl)-β-L-arabinofuranosyl (1 → 2) 3,5-*O*-benzyl-α-L-arabinoside (50). A mixture of thioglycoside **46** (0.38 mmol) and alcohol **49** (0.31 mmol) in DCM (10mL) was stirred at room temperature in the presence of 4Å molecular sieves (400 mg) for 2 h. After the mixture was cooled to -30 °C, NIS (166 mg, 0.74 mmol) followed by AgOTf (166 mg, 0.25 mmol) were added. The reaction mixture was stirred at -30 °C for 5 min then warmed slowly to room temperature, and stirring was continued for 15 min. The reaction was quenched by the addition of pyridine. The suspension was diluted with EtOAc (50 mL) and filtered through a pad of Celite, and the filtrate concentrated *in vacuo*. The remaining residue was diluted with EtOAc

washed successively with 10% $Na_2S_2O_3$ (10 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was purified on column chromatography (hexane:EtOAc, 3:1, v:v) to afford disaccaharide **50** (310 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75-7.71 (m, 4H, Ar), 7.45-7.37 (m, 3H, Ar), 7.22-7.16 (m, 20H, Ar) 5.09 (2H, $H_{7linker}$), 4.96(d, $J_{1,2}$ =5.4 Hz, 1H, $H_{1\beta}$), 4.89(3, H1α, NapCH_2), 4.62-4.21 (6H BnCH_2), 4.19 (2H, $H_{4\alpha}$, $H_{4\beta}$), 4.05 (2H, $H_{2\alpha}$, $H_{2\beta}$), 3.90 (2H, $H_{3\alpha}$, $H_{3\beta}$), 3.77(1H, $H_{5\beta a}$), 3.51(3H, $H_{5\beta b}$, $H_{5\alpha}$), 3.14 (2H, $H_{5linker}$), 1.46 (4H, $H_{2linker}$, $H_{4linker}$), 1.18 (2H, $H_{3linker}$), 1.00 (s, 9H, ^{t}Bu), 0.93 (s, 9H, ^{t}Bu). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.36, 138.19, 135.53, 133.43, 133.29, 129.50, 129.23, 128.74, 128.67, 128.55, 128.51, 128.41, 128.12, 128.03, 127.94, 127.86, 127.80, 127.46, 127.08, 126.36, 126.21, 106.53 (C-1α) 100.17 (C-1β), 87.31, 83.41, 80.83, 80.68, 78.68, 73.93, 73.56, 72.23, 72,13, 70.15, 68.64, 67.64, 67.35, 28.50, 27.74, 27.39, 23.59, 22.84, 20.33.

N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl -2-*O*-(naphthyl)methyl β–L-arabinofuranosyl (1 → 2) 3,5-*O*-benzyl-α-L-arabinoside (51). Tetrabutyl ammonium floride (TBAF, 222 μL in 1.0 M THF) was added to a solution of **50** (295 mg, 0.28 mmol) in THF (15 mL). The reaction mixture was stirred at rt for 12 h, after which the solution was removed *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (25 mL) was with water (2 x 10 mL), brine (5mL) and then dried ($MgSO_4$). After filtration and evaporation of solvent, the residue was subjected to normal silica gel chromatography (hexane:EtOAc, 5:1 → 3:1 → 1:1) to afford diol intermediate **51** (181 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82-7.79 (m, 4H, Ar), 7.49-7.44 (m, 3H, Ar), 7.31-7.15 (m, 20H, Ar) 5.15 (2H, $H_{7linker}$), 5.02 (1H, $H_{1\beta}$), 4.92(3, $H_{1\alpha}$), 4.77 (2H, NapCH_2), 4.60-4.45 (6H BnCH_2), 4.24 (1H, $H_{2\alpha}$), 4.11 (2H, $H_{3\alpha}$, $H_{3\beta}$), 3.96(1H, $H_{2\beta}$), 3.84(1H, $H_{4\alpha}$), 3.70-3.51 (4H, $H_{4\beta}$, $H_{5\beta}$, $H_{5\alpha a}$), 3.21 (2H, $H_{5\alpha b}$, $H_{5linker}$), 2.13 (2H, OH), 1.48 (4H, $H_{2linker}$, $H_{4linker}$), 1.26 (2H, $H_{3linker}$). ^{13}C NMR (75 MHz, $CDCl_3$): 138/19, 138.12, 138.07, 135.31,

133.43, 133.35, 128.76, 128.67, 128.62, 128.56, 128.15, 128.01, 128.04, 128.01, 127.93, 127.50, 127.07, 126.52, 126.38, 126.10, 106.33 (C-1α), 99.90 (C-1β), 86.51, 84.52, 83,32, 82.19, 80.83, 73.63, 73.46, 73.11, 72.39, 69.85, 67.74, 67.40, 62.77, 29.46, 23.57.

N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2-*O*-(naphthyl)methyl 5-*tert*-butyldimethylsilyl-β-L-arabinofuranosyl (1 → 2) 3,5-*O* benzyl-α-L-arabinoside (52). *tert*-Butyldimethylsilyl chloride (32 μL, 0.19 mmol) and imidazole (29 mg, 0.43 mmol) were added to a stirred solution of **51** in 8 mL THF: DMF(1:1, v:v). The reaction mixture was stirred at room temperature for 5 h, then quenched with MeOH and concentrated *in vacuo*. The resulting residue was diluted with EtOAc (40 mL) and then washed sucessively with water (3 x 10 mL), and brine (10 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was purified by normal column chromatography (hexane/EtOAc, 3:1) to afford **52** (101 mg, 58%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82-7.79 (m, 4H, Ar), 7.49-7.44 (m, 3H, Ar), 7.31-7.15 (m, 20H, Ar) 5.15 (2H, $H_{7linker}$), 5.02(1H, H1β), 4.92(3, $H_{1α}$), 4.77 (2H, NapCH_2), 4.60-4.45 (6H BnCH_2), 4.24 (1H, $H_{2α}$), 4.11 (2H, $H_{3α}$, $H_{3β}$), 4.04 (2H, $H_{5β}$,), 3.96(1H, $H_{2β}$), 3.84(1H, $H_{4α}$), 3.70-3.51 (2H, $H_{4β}$, $H_{5αa}$), 3.21 (2H, $H_{5αb}$, $H_{5linker}$), 2.13 (1H, OH), 1.48 (4H, $H_{2linker}$, $H_{4linker}$), 1.26 (2H, $H_{3linker}$), 1.00 (s, 9H, ^{t}Bu), 0.25 (s, 6H, SiCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 138.19, 138.12, 138.07, 135.31, 133.43, 133.35, 128.76, 128.67, 128.62, 128.56, 128.15, 128.01, 128.04, 128.01, 127.93, 127.50, 127.07, 126.52, 126.38, 126.10, 106.33 (C-1α), 99.90 (C-1β), 86.51, 84.52, 83,32, 82.19, 80.83, 73.63, 73.46, 73.11, 72.39, 69.85, 67.74, 67.40, 62.77, 30.6, 29.46, 25.9, 23.57.

N-Benzyl-N-benzyloxycarbonyl-5-aminopentyl 2,3,5-*tri*-*O*-acetyl-α-L-arabinosyl (1 → 3) 2-*O*-(naphthyl)methyl 5 *tert*-butyl(dimethylsilyl)-β-L-arabinofuranosyl (1 → 2) 3,5-*O*-benzyl-α-L-arabinofuranoside (6). A mixture of thioglycoside **10** (0.10 mmol) and alcohol **52** (0.09

mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature in the presence of 4Å molecular sieves (200 mg) for 2 h. After the mixture was cooled to -30 °C, NIS (40 mg, 0.18 mmol) followed by AgOTf (40 mg, 0.06 mmol) were added. The reaction mixture was stirred at -30 °C for 5 min then warmed slowly to room temperature, and stirring was continued for 15 min. The reaction was quenched by the addition of pyridine. The suspension was diluted with EtOAc (50 mL) and filtered through a pad of Celite, and the filtrate concentrated *in vacuo*. The remaining residue was diluted with EtOAc washed successively with 10% $Na_2S_2O_3$ (10 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give a residue, which was purified on column chromatography (hexane:EtOAc, 2:1, v:v) to afford ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82-7.79 (m, 4H, Ar), 7.49-7.44 (m, 3H, Ar), 7.31-7.15 (m, 20H, Ar) 5.15 (2H, $H_{7linker}$), 5.02(1H, $H_{1\beta}$), 4.92(3, $H_{1\alpha}$), 4.85 (1H, $H_{1'\alpha}$) 4.77 (2H, NapCH_2), 4.60-4.45 (6H BnCH_2), 4.24 (1H, $H_{2\alpha}$), 4.15 (d, 1H, $H_{3'\alpha}$), 4.11 (2H, $H_{3\alpha}$, $H_{3\beta}$), 4.04 (4H, $H_{5\beta}$, $H_{2'\alpha}$, $H_{5'\alpha a}$), 3.96(1H, $H_{2\beta}$), 3.84(1H, $H_{4\alpha}$), 3.70-3.51 (4H, $H_{4\beta}$, $H_{5\alpha a}$, $H_{5'\alpha b}$, $H_{4'\alpha}$), 3.21 (2H, $H_{5\alpha b}$, $H_{5linker}$), 2.13 (1H, OH), 2.06(s, 3H, OAc), 2.01 (s, 3H, OAc), 1.48 (4H, $H_{2linker}$, $H_{4linker}$), 1.26 (2H, $H_{3linker}$), 1.00 (s, 9H, ^{t}Bu), 0.25 (s, 6H, SiCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 170.40, 138.19, 138.12, 138.07, 135.31, 133.43, 133.35, 128.76, 128.67, 128.62, 128.56, 128.15, 128.01, 128.04, 128.01, 127.93, 127.50, 127.07, 126.52, 126.38, 126.10, 106.33 (C-1α), 105.38 (C-1'α), 99.90 (C-1β), 86.51, 84.52, 83,32, 82.19, 80.86, 80.83, 76.70, 73.63, 73.46, 73.11, 72.39, 69.85, 67.74, 67.40, 63.04, 62.77, 30.6, 29.46, 25.9, 23.57, 20.97, 20.66, 20.58.

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CHAPTER 4

CONCLUSIONS

4.1 Summary

Increasing evidence shows that primary cell walls play important roles in the biology of plants. The structural complexities of the cell wall polysaccharides indicate that substantial energy and nutrient resources are utilized in the synthesis, modification and maintenance of the cell wall during growth and development. Therefore, a more thorough understanding of the makeup of plant cell walls at the cellular and subcellular level is essential to understanding how these components interact. Molecular genetic approaches for studying the primary cell walls of plants have experienced limited success, as the lack of pure and structurally well-defined carbohydrates and glycoconjugates presents a formidable challenge in glycobiology. In some cases, well-defined carbohydrates can only be obtained by organic synthesis. Organic synthesis allows for the production of fully characterized, pure, homogeneous carbohydrates and glycoconjugates. In this study, a range of arabinogalactans were synthesized to define the binding specificities of the monoclonal antibodies for functional genomics studies in order to investigate the changes in plant cell walls during development and differentiation. Figure **1** depicts the fragments of Type II arabinogalactan oligosaccharides which have been synthesized.

Compounds **1-5** were synthesized by a building block strategy using appropriately protected monosaccharides, which can be used to assemble other Type II arabinogalactan oligosaccharides. Traditionally, the formation of the 1,2-*cis* furanoside has been a formidable challenge; therefore, the methodology developed by the Boons's Group for the stereoselective

introduction of β-arabinofuranosides was employed. In this method, the C-3 and C-5 are locked into a six-membered ring by the 3,5-*O*-(di-*tert*-butylsilane)-protecting group, which causes nucleophillic attack from the α-face to be disfavorable. The use of this method along with traditional neighboring group participation lead to the achievement of greater α:β stereoselectivity was achieved. All oligosaccharides were prepared as aminopropyl glycosides to facilitate conjugation of the compounds to proteins, biotin or solid surfaces. These oligosaccharides were fully deprotected for use in biological studies.

In addition to the synthesis of fragments of Type II arabinogalactan oligosaccharides, a fragment of the side chain of extensin, **6** has been synthesized. Extensin is a highly insoluble hydroxyproline-rich glycoprotein that is named according to its supposed role in cell elongation. The synthesis of 6 was more challenging then the synthesis of 1-5 because it was harder to control the stereochemical outcome when adding the linker to arabinofuranose.