UNIVERSITY OF ALBERTA

Arabinofuranose-Containing Oligosaccharides as Probes of the CS-35 Antibody Binding Pocket

by

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ABSTRACT

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis*, has reemerged as a global health problem over last deacade. There are antibiotics available for the treatment of TB and there is also a vaccine, the BCG vaccine, that can be used for the prevention of the disease. However, interest in the development of new methods for treatment and new vaccines has been renewed because of the emergence of drug resistant strains of *M. tuberculosis* and the limited efficacy of the BCG vaccine. Recently, one of the major antibodies generated against *M. tuberculosis* infection, CS-35, was shown to recognize a hexasaccharide motif that is a component of the cell wall polysaccharide, lipoarabinomannan. This hexasaccharide is therefore a possible epitope for the development of a novel TB vaccine. However, it was of interest to identify smaller, more easily accessible fragments of the glycan as possible vaccine epitopes. This thesis reports studies aimed at identifying the minimum oligosaccharide epitope that is bound by CS-35. Fragments of the hexasaccharide motif were synthesized and screened as ligands for CS-35 using FAC-MS (Frontal Affinity Chromatography-Mass Spectrometry). Among the synthesized oligosaccharides, only three, hexasaccharide **58**, pentasaccharide **59**, and tetrasaccharide **3**, showed binding affinity to the antibody. These results implicate the importance of two key residues, including one of the terminal β-arabinofuranosyl residues, in the interaction of the hexasaccharide with the monoclonal antibody CS-35.

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List of Abbreviations

Ab	antibody
AG	mycolyl-arabinogalactan
Ara*f*	arabinofuranosyl
BCG	Baciile Calmette-Guérin
Br	broad
Bn	benzyl
BnBr	benzyl bromide
Bz	benzoyl
d	doublet
DIAD	diisopropylazodicarboxylate
DMF	*N,N*-dimethylformamide
FAC/MS	frontal affinity chromatography/mass spectrometry
GCOZY	gradient coupling correlated spectroscopy
HMQC	heteronuclear multiple quantum coherence
Hz	hertz
J	coupling constant
LAM	lipoarabinomannan
mAb	monoclonal antibody
Man*p*	mannopyranosyl
mg	milligram
MHz	megahertz
mL	milliliter

mol	mole(s)
mmol	milimole(s)
MS	mass spectrometry
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
OD	optical density
Ph	phenyl
MPI	mannosylphosphatidyl-*myo*-inositol
PI	phosphatidylinositol
ppm	parts per million
s	singlet
SDS-PAGE	Sodium dodecyl sulfate – polyacrylamide gel electrophoresis
TB	tuberculosis
TBAF	tetrabutylammonium fluoride
THF	tetrahydrofuran
TLC	thin layer chromatography
Tr	triphenylmethyl
Tris	tris(hydroxymethyl)methylamine
UV	ultraviolet

Chapter 1

Introduction

1.1 Tuberculosis

Tuberculosis (TB) is the leading cause of death in the world from a single infectious agent, killing about 3 million individuals every year and accounting for 18.5% of all deaths of adults between 15 and 59.[73] One third of the world's population is infected with the causative agent, *Mycobacterium tuberculosis*, and at risk of developing the disease[1,2,3] in spite of the availability of some chemotherapies and the Bacille Calmette-Guérin (BCG) vaccine.[4]

Before the development of modern antibiotic therapy, tuberculosis was lethal in 50-60% of all cases.[1] However, the discovery of antibacterial and antitubercular agents such as streptomycin in 1944,[6] and isoniazid and pyrazinamide in 1952[7,8] led to effective chemotherapies, which contributed to decrease mortality rates associated with TB. There are, however, still difficulties in the treatment of tuberculosis, as strict adherence to a regimen of three or more antibiotics that must be taken for several months is required.[5] After 30 years of steady decline, tuberculosis has re-emerged in the mid-1980's as a public health problem. The emergence of HIV virus in the early 1980's, the increase in homeless populations, and the declining health care system in many developed countries

have played a major role in the resurgence of TB. Furthermore, the appearance of multi drug-resistant strains of *M. tuberculosis* have contributed to the resurgence of the disease and complicated its treatment.[1]

Although tuberculosis-like diseases can be caused by several mycobacterial species, the major causative agent is *M. tuberculosis,* which causes 90% of the cases of pulmonary tuberculosis. When this infection occurs, an immune response begins upon activation of $CD4^+$ T cells, which are required for immunity to tuberculosis. Within 2-6 weeks, large numbers of activated macrophages are induced, which suppress proliferation of the phagocytosed bacilli by killing them or by inhibiting their growth.[73] This process causes a granulomatous lesion called a tubercle consisting of a few small lymphocytes and a compact collection of activated macrophages. The massive activation of macrophages results in release of concentrated lytic enzymes that destroy nearby healthy cells, causing a lesion.

1.2 Mycobacteria

1.2.1 General Characteristics

Mycobacteria are gram-positive, non-motile, pleomorphic rods, distantly related to the actinomycetes. While a few mycobacteria are intracellular pathogens of animals and humans, most are found in water or soil. *M. tuberculosis*, along with *M. bovis*, *M.*

africanum, and *M. microti* all cause tuberculosis and they are members of the pathogenic tuberculosis species complex. Although several mycobacterial species can cause TB, *M. tuberculosis* is the principal causative agent in humans. *M. bovis* also causes TB but principally affect cattle and other livestock. However, this organism through the consumption of unpasteurized milk can also infect humans and this transmission can lead to the development of extrapulmonary TB. *M. avium* and *M. leprae* are also classified as human pathogens among the *Mycobacterium* genus. *M. avium* causes a TB-like disease especially prevalent in AIDS patients, and *M. leprae* is the causative agent of leprosy.

M. tuberculosis is an obligate aerobe growing most successfully in tissues with a high oxygen content, such as the upper lobes of the lungs where pathogens infect mononuclear phagocytes like macrophages. It has a slow generation time, 12-18 hours, that may contribute to its virulence.

1.2.2 The Mycobacterial cell wall

1.2.2.1 General feature in structure

There have been considerable interests in the structure of mycobacteria because it is highly complex and contains several chemically unique features, compared to those of other prokaryotes. For example, over 60% of the mycobacterial cell wall is lipid and

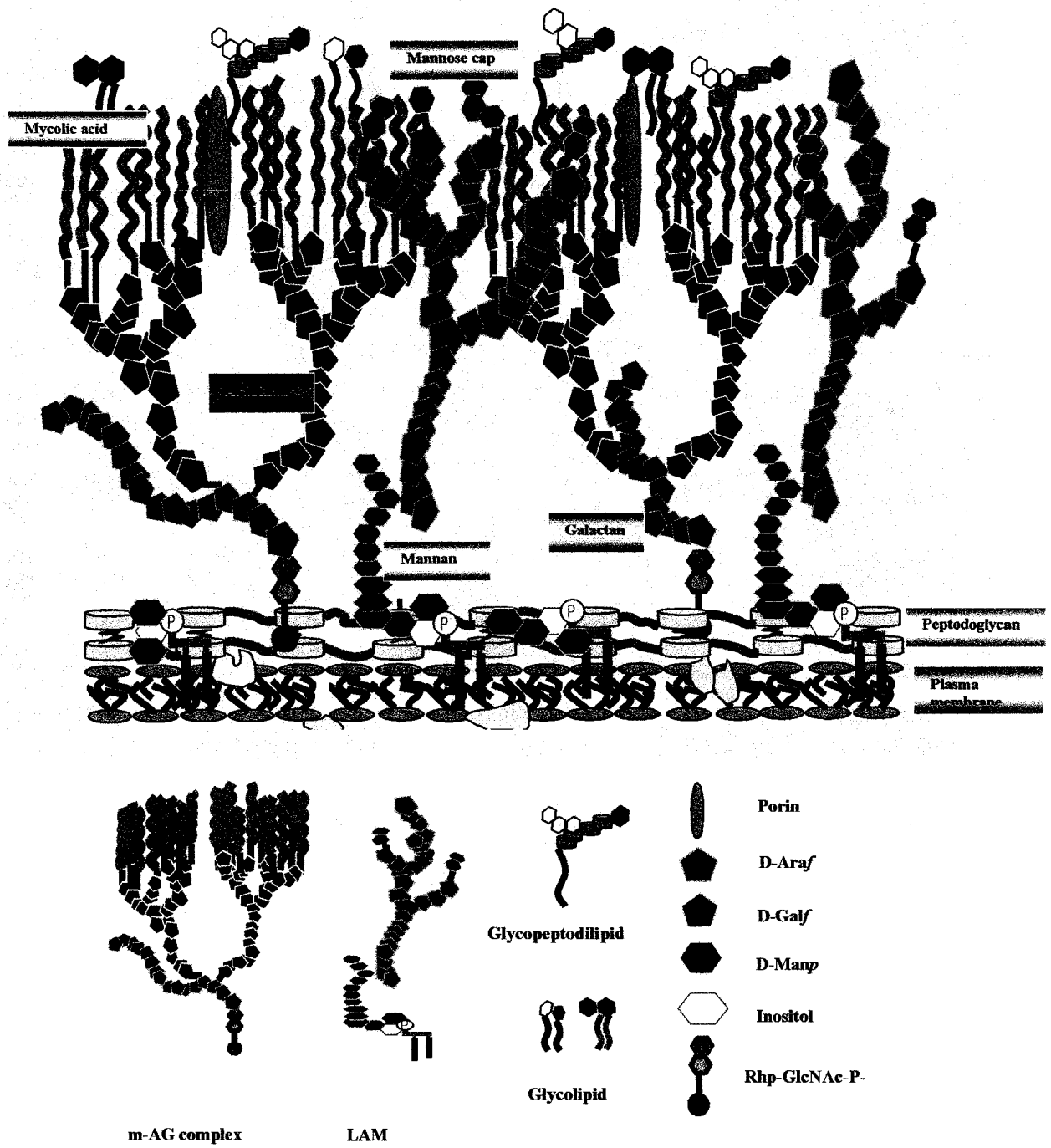


Figure 1.1. Schematic representation of the mycobacterial cell wall. The cell wall includes five major components: a plasma membrane, a peptidoglycan, m-AG complex, LAM, lipids and glycolipids.

this high concentration of lipid contributes to the impermeability to the bacteria, resulting in resistance to many antibiotics and the survival of the pathogen in macrophages.[9]

In general, the cell wall complex is divided into five major components (Figure 1.1):[10,11,12,13]

1) the plasma membrane

2) peptidoglycan

3) the mycolyl-arabinogalactan (AG) complex

4) lipoarabinomannan (LAM) and its truncated form, lipomannan

5) lipids and glycolipids noncovalently bound to the mycolate esters

1.2.2.2 Plasma membrane

The plasma membrane is a barrier between the cytosol and the periplasmic space and has a number of molecules crucial for the survival of the organism.[9] For example, a family of glycosylphosphoprenols that act as the sugar donor substrates for many of the glycosyltransferases that assemble cell wall polysaccharides are thought to be associated with the plasma membrane. The phospholipid bilayer beneath the peptidoglycan layer includes diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidylinositol (PI) and a family of phosphatidylinositolmannosides that anchor LAM to the plasma membrane.

1.2.2.3 Peptidoglycan

Peptidoglycan is a substance that forms a homogenous layer lying outside the plasma membrane, and serves a structural role, giving the bacteria shape and strength. The peptidoglycan of mycobacteria has a similar structure to that of other bacteria. It includes glycan chains composed of alternating *N*-acetylglucosamine and *N*-glycolylmuramic acid residues. Tetrapeptide side chains are attached to muramic acid, and the peptidoglycan is linked to arabinogalactan via phosphodiester bonds to a proportion of the muramic acid residues.[14,74] However, the peptidoglycan of mycobacteria is distinct in two ways. The muramic acid portions are *N*-glycolated rather than *N*-acetylated, and the peptide chains are composed of L-alanine, D-isoglutamine, *meso*-diaminopimelic acid (DAP) and D-alanine. Cross-links are found not only via usual linkages between DAP and D-alanine but also through the formation of linkages between two DAP residues.[9,13,14]

1.2.2.4 Mycolyl-arabinogalactan (mAG) complex

The mycolyl-arabinogalactan (mAG) complex is a polymer of galactofuranosyl and arabinofuranosyl residues covalently linked to peptidoglycan and capped at its non-reducing terminus by hydrophobic mycolic acid esters. Arabinogalactan (AG) consists of around 30 D-galactofuranose and 60 D-arabinofuranose residues bound to *O*-6 of muramic acid residues of peptidoglycan. The linkage to peptidoglycan is a diglycosyl

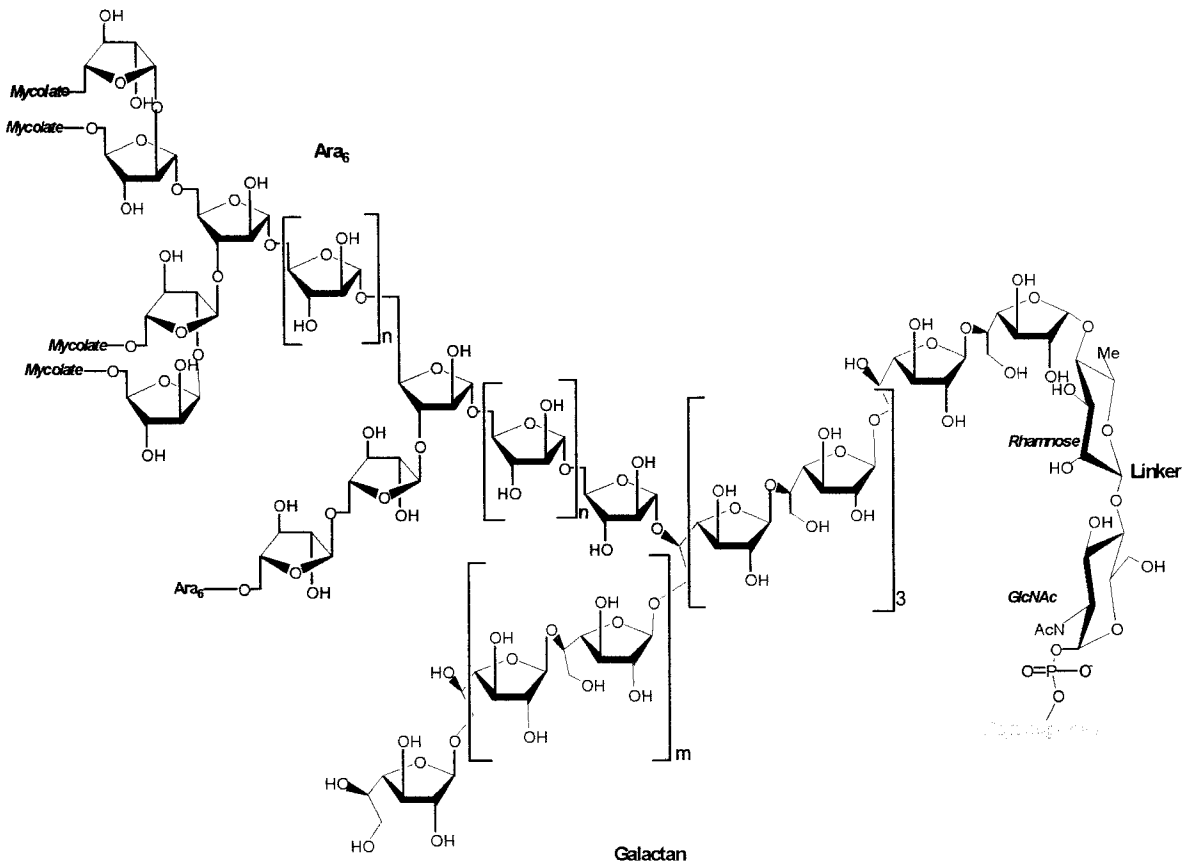


Figure 1.2. The structural model of mycobacterial mAG (This picture was modified from the reference : Lee, R. E. B.; Li, W.; Chatterjee, D; Lee, R. E. *Glycobiology.* 2005, 15, 139-151.)

phosphoryl bridge to a linker disaccharide made up of L-rhamnose and *N*-acetylglucosamine. The 4-position of rhamnose is attached to a linear chain of galactofuranose residues linked by alternating β-(1→5) and β-(1→6) linkages. Arabinofuranose chains, mainly linked by α-(1→5) linkages, are attached to this galactan backbone via the *O*-5 of some galactofuranosyl residues. Other linear arabinan chains are linked to the 3 position of some arabinofuranose residues of the arabinan. The

nonreducing end of this glycan is substituted with a branched hexasaccharide. (Figure 1.2)

The AG is covalently bound to branched chain lipids, mycolic acids, to form the mycolyl-arabinogalactan complex.[13,30] Mycolic acids are high molecular-weight α-alkyl, β-hydroxy fatty acids. Compared to mycolic acids from other actinomycetes, those in mycobacteria have the largest number of carbon atoms (70 to 90), longest α-branch (20 to 25 carbons) and include one or two groups of double bonds or cyclopropane and ketone, hydroxy, and methoxy functionalities.[14,15]

1.2.2.5 Lipoarabinomanan

Lipoarabinomannan (LAM), and its related precursors, lipomannan (LM) and mannosylphosphatidyl-*myo*-inositol (MPIs) are major lipoglycans of the mycobacterial cell wall. Structurally, LAM consists of three domains: a MPI anchor, a polysaccharide backbone, and a capping motif (Figure 1.3). LAM is noncovalently associated with the mycobacterial cytoplasmic membrane through their MPI anchor.[30,31] The polysaccharide backbone extends to the exterior of the cell wall structure and is terminated by capping motifs. LAM plays critical roles in the pathology of tuberculosis and in the modulation of the host response against the bacteria.[37,38] More specifically, LAM has been shown to inhibit T-cell proliferation,[2,6,17,18,19] interleukin-12 cytokine secretion by dendritic cells, protein kinase activity,[20,22] and synthesis of mRNA encoding IL-2, IL-5 and GM-CSF in

human T-cells as well as to interfere an interferon (IFN)-γ signaling in macrophages.[20,21,23] Furthermore, it was observed that LAM prevents the induction of the gene expression in macrophages,[24] the enhancement of production of tumor necrosis factor by mononuclear cells,[25,26,27,28] and the mediation of complement activation,[29] and scavenges toxic oxygen free radicals.[20]

1.2.2.5.1 MPI anchor

The MPI anchor serves as an attachment point of the LAM to the cell membrane. The polysaccharide backbone of the LAM extends from the *O*-6 position of myo-inositol to the extracellular space. The MPI anchor consists of an α-D-Manp unit linked to the *O*-2 position of an *sn*-glycero-3-phospho-(1-D-*myo*-inositol) core, and is multiacylated at the positions: 1 and 2 of the glycerol unit, position 6 of the Man*p* unit linked at *O*-2 of *myo*-inositol, and position 3 of the *myo*-inositol.[50,51] Acylation of LAMs occurs predominantly with tuberculostearic (10-methyl-octadecanoic) acids at the 1-position of glycerol and palmitic acids at the 2-position of glycerol, and to lesser extent by steric acid.

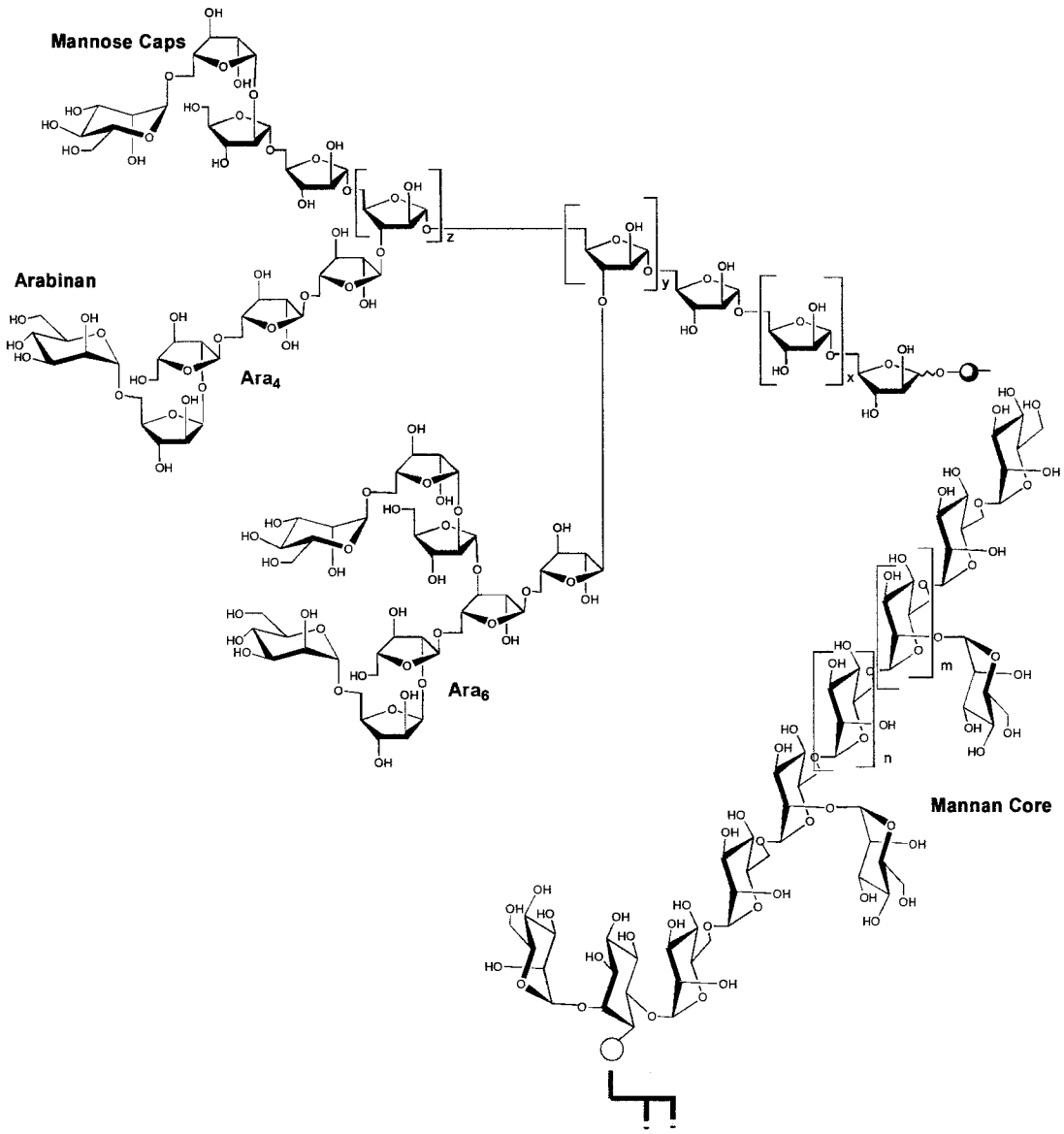


Figure 1.3. The structural model of mycobacterial **LAM** (This picture was modified from the reference : Lee, R. E. B.; Li, W.; Chatterjee, D; Lee, R. E. *Glycobiology.* 2005, 15, 139-151.)

1.2.2.5.2 Polysaccharide backbone

The polysaccharide is composed of two homopolysaccharides, D-mannan and D-arabinan. The mannan core includes 30-35 mannopyranosyl (Man*p*) residues from which extends an arabinan domain containing approximately 60 arabinofuranosyl (Ara*f*) residues. The mannan core is a linear structure composed of α-(1→6) linked residues occasionally substituted at *O*-2 by a single α-Man*p* unit. The degree of branching depends on the mycobacterial species. The arabinan consists of a linear α-(1→5) linked arabinofuranosyl backbone terminated by branched hexa-arabinofuranosides (Ara_6) and tetra-arabinofuranosides (Ara_4) (Figure 1.3).[37,40,47]

1.2.2.5.3 Capping motifs

LAMs can be grouped into three classes depending on the cap structures present at the nonreducing termini of the arabinosyl side-chains.[40,46] ManLAM, a structure generally found in slow-growing mycobacteria,[41,42,43] are terminated by mannose caps composed of mono-, α-(1→2)-di- and α-(1→2)-tri-mannopyranosyl units.[40,41] It has been found that these motifs are present on both linear Ara_4 and bi-antennary Ara_6 side chains in *M. tuberculosis* and *M. bovis* BCG, giving rise to all the possible combinations: $Manp_{(1\text{-}3)}Araf_4$ and $Manp_{(1\text{-}6)}Araf_6$. The dimannosyl motif is the most abundant among the mycobacteria species and the number of mannose caps varies depending on the species and strains.[40,48] In more recent papers, a new 5-methylthiopentose substituent has been

described attached to the mannose caps. PILAM, a second type of LAM found in fast growing mycobacteria is capped by phosphoinositide units.[45] The phosphoinositides caps are composed of *myo*-inositol-1-phosphate residues esterified at the *O*-5 of β-D-Ara*f* residues[40,41,45,49] and capped very weakly. The recently identified LAM, AraLAM, is devoid of both the manno-oligosaccharide and inositol phosphate caps.[47] The majority of mycobacteria have the ManLAM structure[27] and it is believed that these mannose-capped residues are involved in the initial stages of infection through interaction with mannose binding receptors on human cells.[13,32,33]

1.2.2.6 Lipids and glycolipids

Several lipids and glycolipids, referred to as "extractable lipids" are present at the periphery of the mycobacterial cell wall. These lipids associate with the cell membrane via noncovalent interaction to mycolic acid residues. Whereas several types of extractable lipids are present in mycobacteria, the frequency and exact structure of these compounds varies across the genus. Extractable lipids can be classified into four groups: glycopeptidolipids, lipooligosaccharides, phenolic glycolipids, and acylated trehalose derivatives. In glycolipids, the lipid portions are associated with mycolic acids, and the oligosacchride residues are exposed to the outer part of cell wall where they interact with the host immune system. Consequently, these oligosaccharides are believed to have important roles in eliciting immune responses. The presence and exact structure of these compounds varies across the geuns.[14]

The glycopeptidolipids have a common peptide, D-Phe-D-allo-Thr-D-Ala-L-alaninol, from which oligosaccharide and lipid residues extend. The hydroxy group of the alaninol is glycosylated with 3,4-di-*O*-methyl-L-rhamnose whereas the hydroxy group of the D-*allo*threonine is substituted with a highly complex oligosaccharide centered around a α-L-rhamnopyranosyl-(1→2)-6-deoxy-L-talopyranose moiety. The phenylalanine residue of the core peptide is *N*-acylated with a fatty acid. In contrast to glycopeptidelipids, lipooligosaccharides are composed of a carbohydrate structure directly attached to a lipid moiety. The lipooligosaccharides found in the mycobacterial cell wall contain a trehalose core, which is further glycosylated with various sugars on one glucosyl residue giving rise to complex oligosaccharide structures. Finally, phenolic glycolipids are composed of a hydrophobic phenol residue containing a C_{36} phenolic diol substituted by two molecules of a C_{34} fatty acid, mycocerosate. Highly deoxygenated and methylated oligosaccharides are attached to the lipid core, giving rise to a rather hydrophobic glycolipid.[14,15]

1.3 Current vaccines for TB prevention

For TB prevention, the Baciile Calmette-Guérin (BCG) vaccine has been the most widely used in the world. More than 80% of children receive the BCG vaccine against TB. BCG is an inexpensive, safe single-dose vaccine suitable for administration at birth and it often gives long-lasting immunity.[53] Despite these advantages, the use of the BCG vaccine for TB prevention has been controversial because of its inconsistent efficacy.

BCG vaccine was first developed in 1921 by Albert Calmette and Camille Guérin, using a live attenuated strain of *M. bovis* and has been extensively used since 1928.[54] There have been numerous controlled trials of BCG vaccine, yielding diverse and often contradictory results. For example, the protection imparted by BCG varies from 0 to 80% around the world. Furthermore, although BCG protects against severe forms of childhood TB, its protective efficacy declines during adolescence and the vaccine does not protect against pulmonary TB in adults. Consequently, the efficacy of the BCG vaccine has been reanalyzed.[54,55,60] The variation in protective efficacy has been attributed to several factors: strain differences in BCG preparations, genetic or nutritional differences in populations, and environmental influences such as sunlight exposure, poor cold-chain maintenance, exposure to environmental mycobacterial infections.[56,57,58] Because results from animal studies showed that prior contact with environmental mycobacteria interferes with the protection obtained by BCG vaccination, regional differences in environmental mycobacteria have been accepted as a main reason among the factors.[55] Therefore, the full potential of BCG might be achieved only in regions where the population is not heavily exposed to mycobacteria from the environment.[54,59]

1.4 Lipoarabinomanan based new vaccine generation for TB

The treatment of tuberculosis requires the use of a combination of several drugs, including isoniazid, rifampin, streptomycin, pyrazinamide, and etambutol. However, the intracellular growth of *M. tuberculosis* makes it difficult for drugs to reach the bacilli. Consequently, drug therapy needs a long term to eradicate the bacteria as well as much expense. Furthermore, TB has reappeared worldwide and the BCG vaccine has shown inefficiency for TB protection. Therefore, interest in developing new and improved TB vaccines has been renewed.

Antibodies produced against bacterial carbohydrate surface motifs have been shown to effect protective immunity against several bacterial infections. However, immunity against tuberculosis has been traditionally assumed mainly to rely not on humoral responses but on cell-mediated immune responses. Thus, antibody-mediated humoral responses often have been neglected on the basis of the results shown from earlier clinical studies.[36,75,76,77] Nevertheless, some early evidence indicated that mycobacterial carbohydrate antigens, such as lipoarabinomannan (LAM), elicit high antibody responses that might be beneficial to the infected host.[35,36] Among the components of LAM, Ara*f* residues that are part of the non-reducing end unit of the molecule have been implicated in binding to various receptors and are the cause of antigenicity of arabinogalactan (AG) and LAM. Also, it has been assumed that the arabinans of LAM and AG are major B-cell immunogens because the deacylated forms of LAM, such as arabinomannan, arabinogalactan lost their antigenicity after treatment

with arabinases. Therefore it is inferred that part of or the entire non-reducing termini motif is the major immunological epitope of AG and LAM as well as of the whole mycobacteria.[61]

In a recent study, it was found that an mAb (monoclonal antibody) CS-35, generated against M. leprae LAM recognizes a branched hexasaccharide (Figure 1.4) found on the non-reducing ends of LAM.[61] An antibody with a similar specificity indicated a protective effect in mice infected with TB.[2] Therefore, this hexasaccharide motif is a possible protective epitope for the development of a novel vaccine.

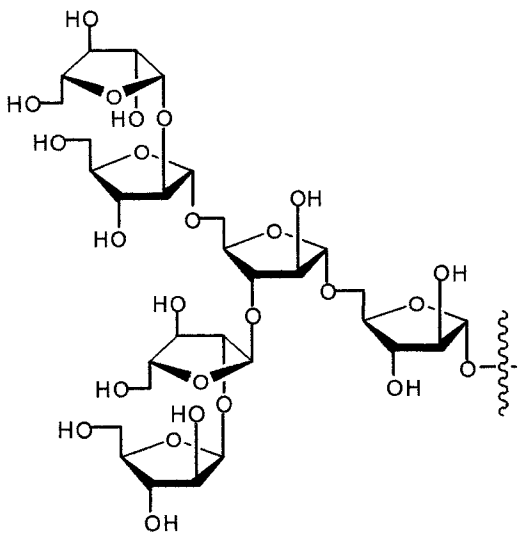


Figure 1.4. Hexasaccharide motif recognized by mAb CS-35

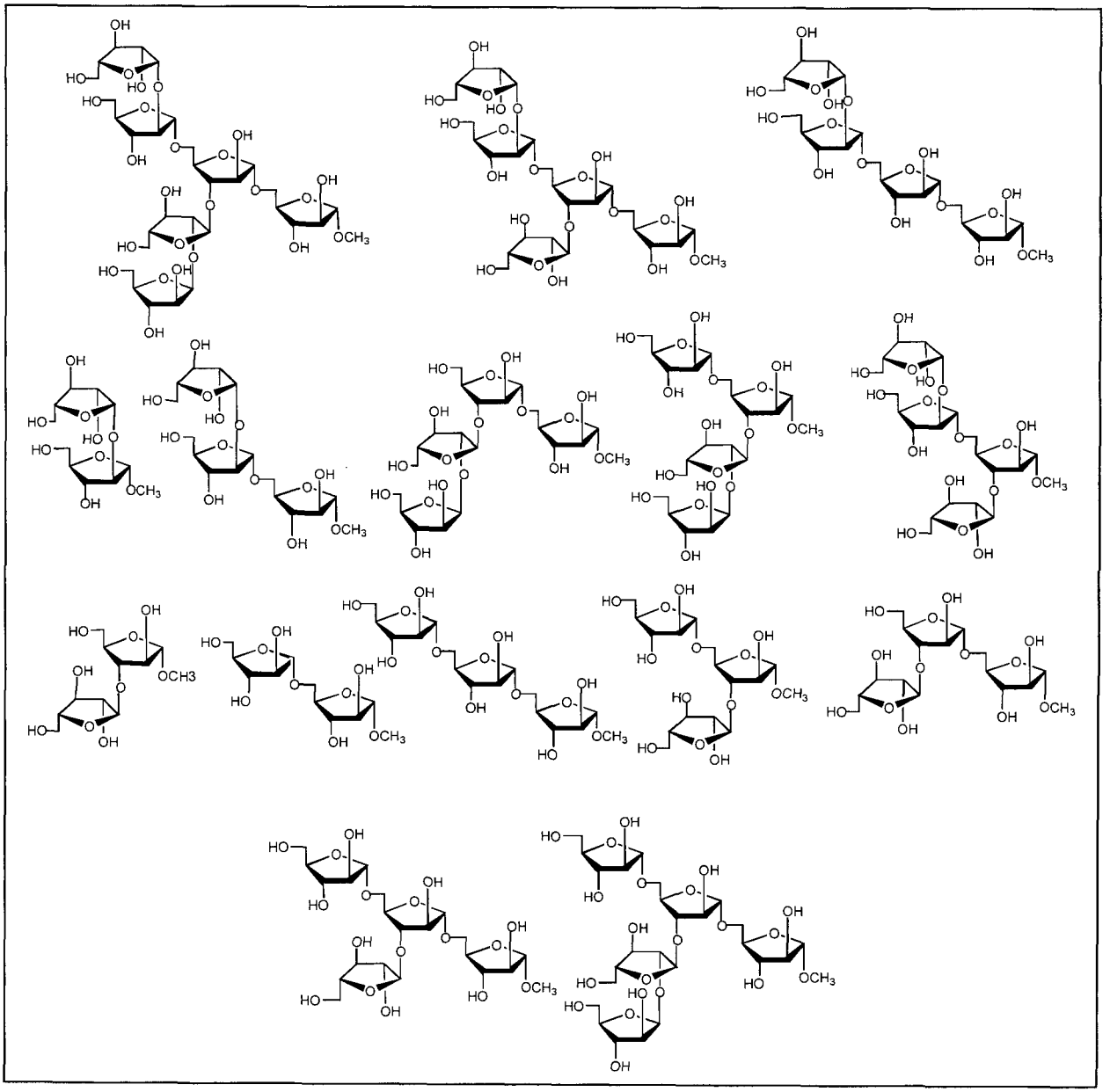


Figure 1.5. Hexasaccharide and synthetic fragments screened against CS-35 by FAC-MS

1.5 Scope of the research project

When considering haptens for potential vaccines against tuberculosis, the Ara6 hexasaccharide, or fragments of it, are reasonable starting points. Smaller fragments would obviously be more synthetically accessible and therefore an understanding of the interactions between CS-35 and this hexasaccharide would allow for the identification of the minimal structures required for binding. In addition, such investigations would provide key insights into how oligosaccharides containing furanose rings are bound by proteins, an area that has received essentially no attention. The goal of my project was to screen a panel of fragments of Ara6 (Figure 1.5) against the CS-35 antibody using Frontal Affinity Chromatography-Mass Spectrometry.[69,70,71] Many of the compounds to be screened were available from a previous group member, Dr. Haifeng Yin.[3] However, it was necessary to resynthesize some of these oligosaccharides (1-6) as some were lost when Dr. Lowary's group moved from The Ohio State University to The University of Aberta. This thesis describes the synthesis of **1-6** and the screening of the entire panel of glycans against the mAb CS-35, which resulted us in being able to define the binding epitope.

Chapter 2

Chemical synthesis

2.1 Introduction

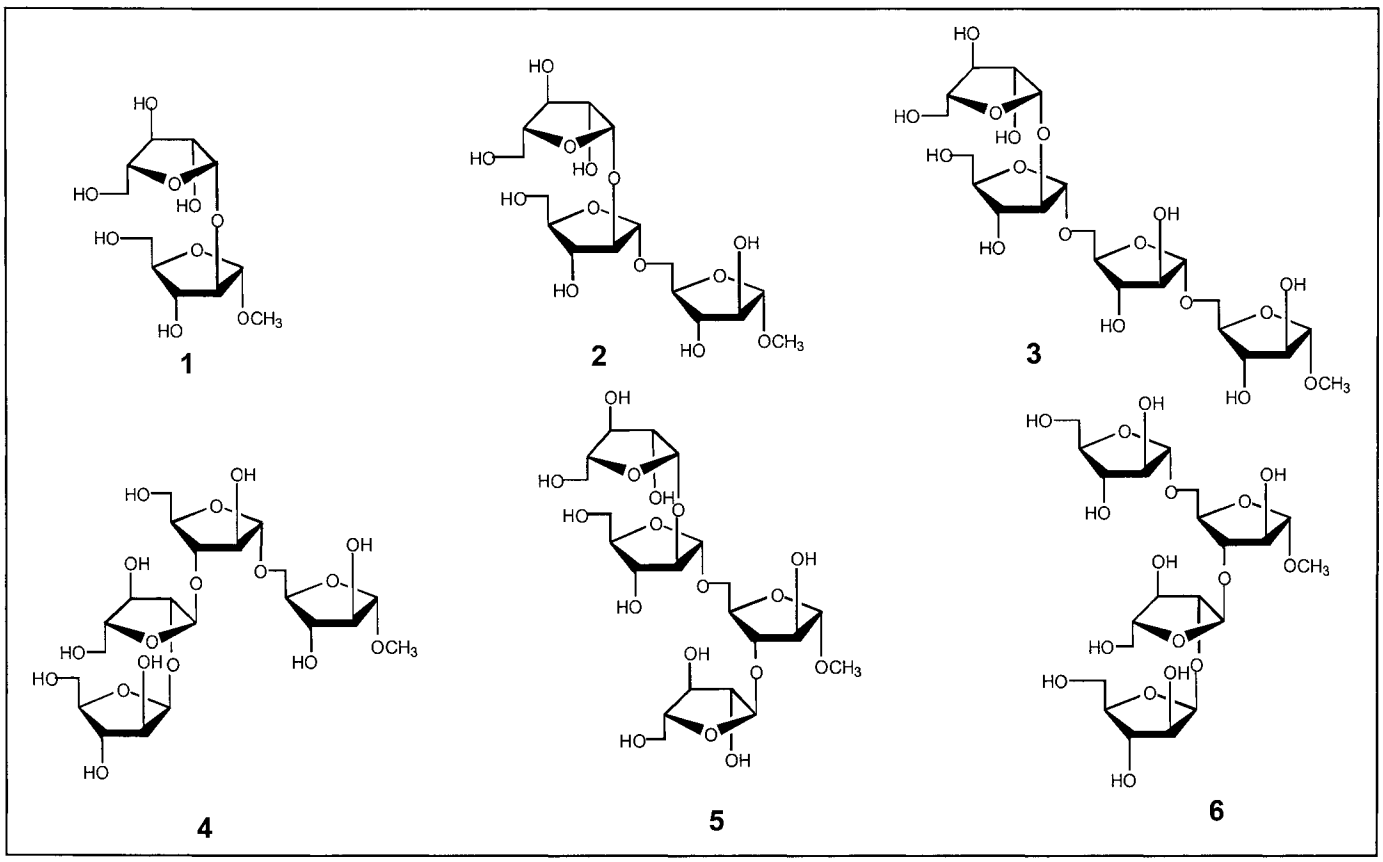


Figure 2.1. Synthetic targets

It has long been known that bactericidal and/or opsonic antibodies directed against capsular polysaccharide glycotopes protect against invasive diseases caused by encapsulated bacteria. Accordingly, vaccine development has focused on the elicitation

of these antibody specificities.[78] The polysaccharides, AG and LAM, in the mycobacterial cell wall are believed to play an important role in a number of immunological events occurring upon mycobacterial infection. Furthermore, in recent studies, interactions between a hexasaccharide component of LAM and mAb CS-35 were found. Therefore, this interaction prompted interest in using this hexasaccharide as a potential hapten for vaccine generations. To determine the minimum oligosaccharide fragments recognized by this mAb, oligosaccharide fragments of the hexasaccharide motif were synthesized.

2.2 Chemical synthesis

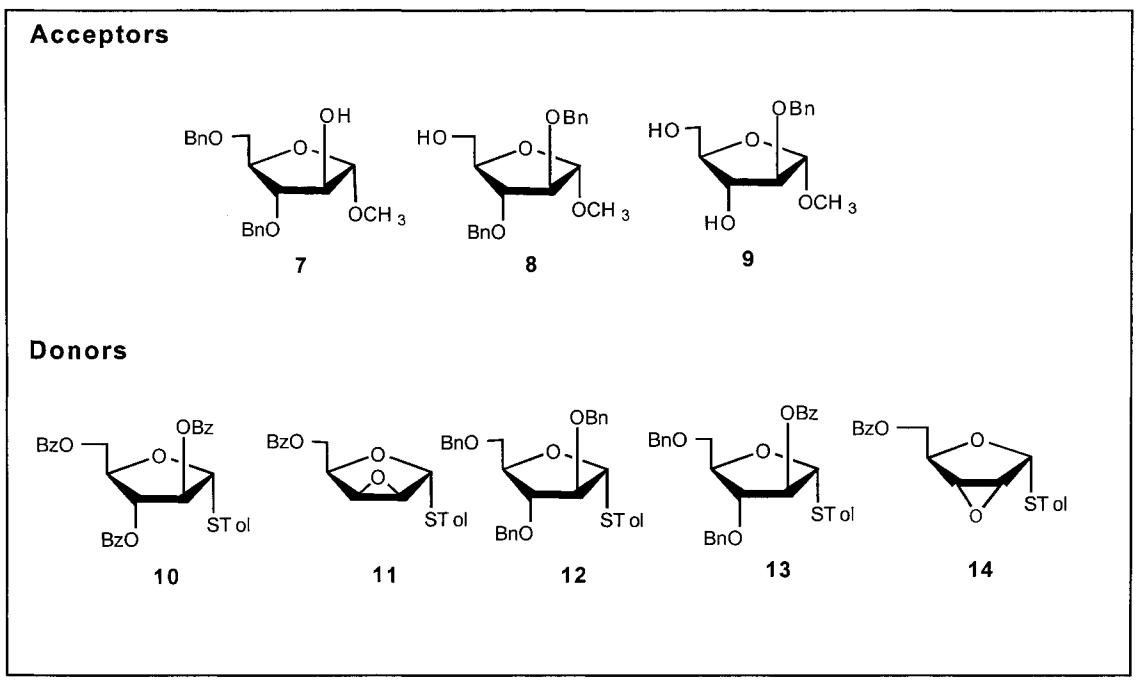
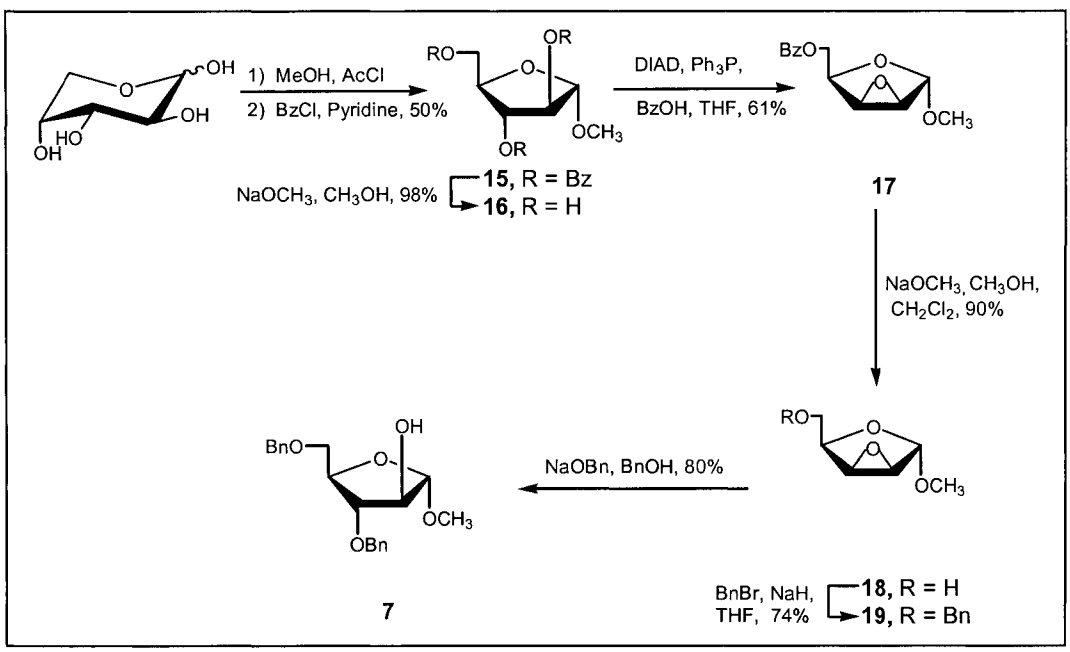


Figure 2.2. Building blocks used for the preparation of oligosaccharides

2.2.1 Preparation of building blocks

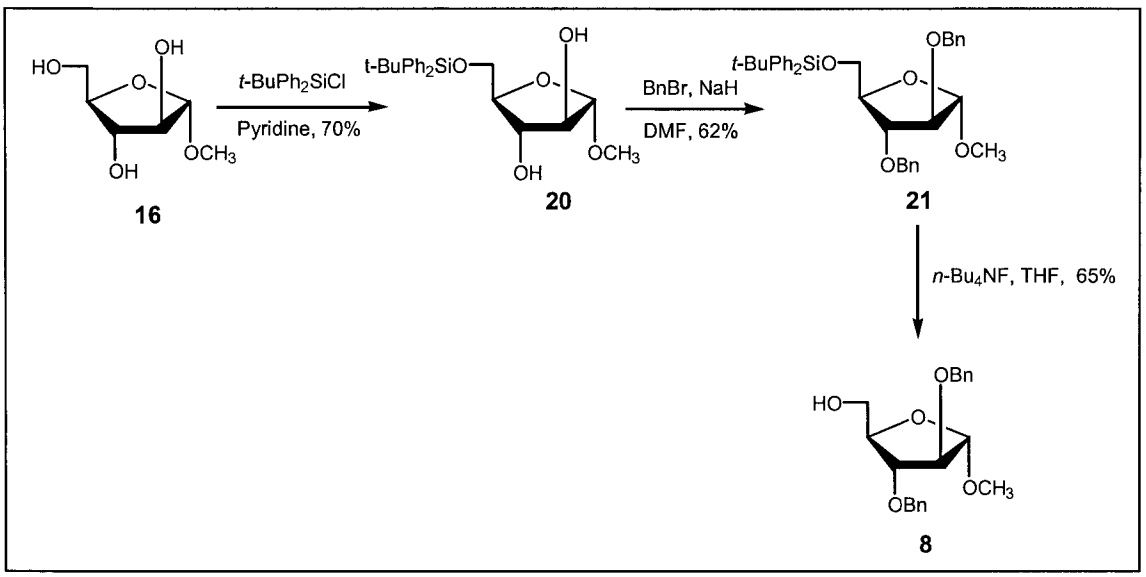
In designing the synthesis of the targets, we developed routes that required the eight monosaccharide building blocks shown in Figure 2.2. This includes the methyl glycoside acceptors **7-9** and five thioglycoside donors (**10-14**).

2.2.1.1 Preparation of acceptors 7-9



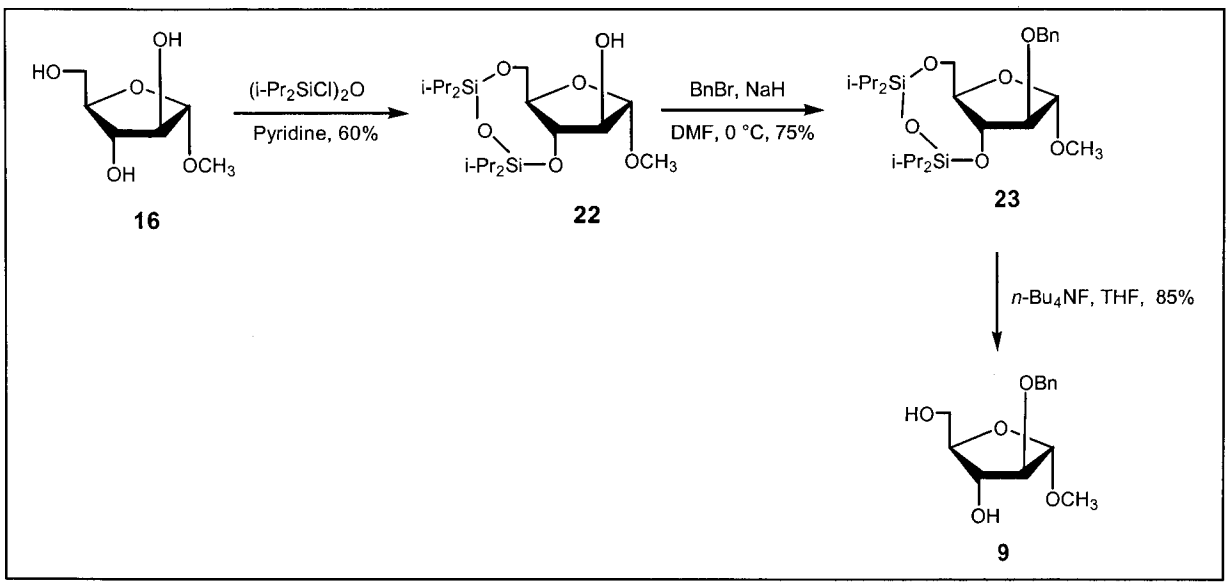
Scheme 2.1

The preparation of **7-9** was achieved from D-arabinose (Scheme 2.1). First, D-arabinose was converted to methyl D-arabinofuranoside under acid catalyzed condition with acetyl chloride and methanol, and then the free hydroxy groups were replaced with benzoate esters to afford **15** in 50% yield.[62] To obtain **7** methyl glycoside **15** was first debenzoylated with sodium methoxide yielding **16**. A Mitsunobu reaction was then conducted to afford the 2,3-anhydosugar methyl glycoside **17** in 61% yield. At this stage, the hydroxyl group at C-5 position was benzoylated to ease purification. Deprotection and subsequent benzylation gave compound **19** in 66% yield over two steps. Nucleophilic opening of epoxide **19** with sodium benzyloxide provided **7** as a dominant regioisomer, formed by the attack of the nucleophile at C-3 of the epoxide. The product **7** was obtained in 80% yield.



Scheme 2.2

Acceptor **8** was prepared from **16** (Scheme 2.2). The primary alcohol in **16** was first protected with a *t*-butyldiphenylsilyl group to provide **20** in 70% yield. Benzylation of the diol **20** in the presence of benzyl bromide and sodium hydride gave **21** in 62% yield. Finally, deprotection of the primary alcohol in **21** with tetrabutylammonium fluoride afforded **8** in 65% yield.

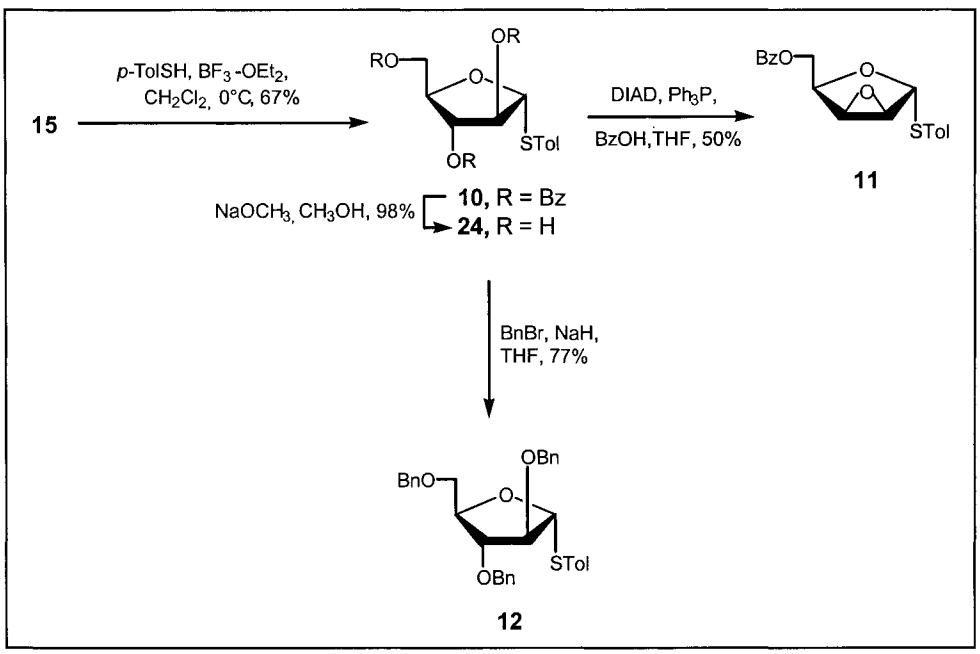


Scheme 2.3

The synthesis of **9** was achieved from methyl glycoside **16** in three steps (Scheme 2.3). First, the hydroxyl groups at 3 and 5 positions were protected with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine to give **22** in 60% yield. However, subsequent transformation of the hydroxyl group in **22** to a benzyl group in **23** created problems in obtaining **23** as the main product. At first, we followed the method that is

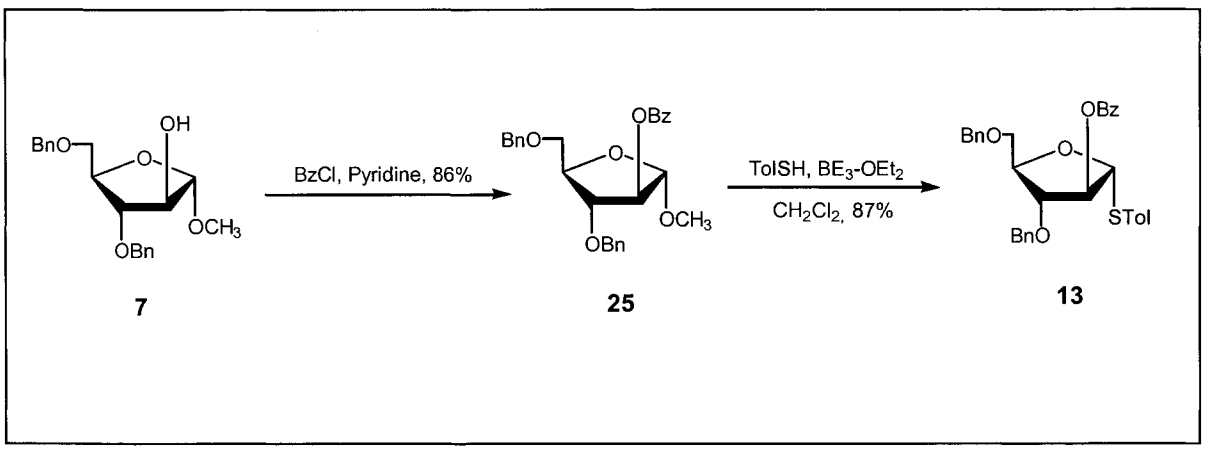
normally used for benzylation, using an excess amount of sodium hydride, but the reaction produced many by products. Because silyl protecting groups are base labile, undesired cleavage of the disilyl protecting group was observed as a side product under normal benzylation conditions. Therefore, a different strategy was required to benzylate **22**. Addition of small portions of base at time intervals and maintenance of the reaction temperature at 0 °C limited the formation of by-products and the desired products **23** was obtained as a main product. The removal of the disilyl group in **23** with tetrabutylammonium fluoride afforded **9** in 53% yield over two steps.

2.2.1.2 Preparation of donors 10-14



Scheme 2.4

The preparation of donors **11-12** is shown in Scheme 2.4. Methyl glycoside **15** was transformed into thioglycoside with *p*-thiocresol and borontrifluoride etherate to give **10** in 50% yield.[63] Deprotection of **10** gave **24,** which was then subjected to a Mitsunobu reaction with DIAD, benzoic acid, and triphenylphosphine to provide the 2,3-anhydrosugar thioglycoside **11** in 50% yield. Alternatively, treatment of **24** with benzyl bromide and sodium hydride provided **12** in 77% yield.[64]

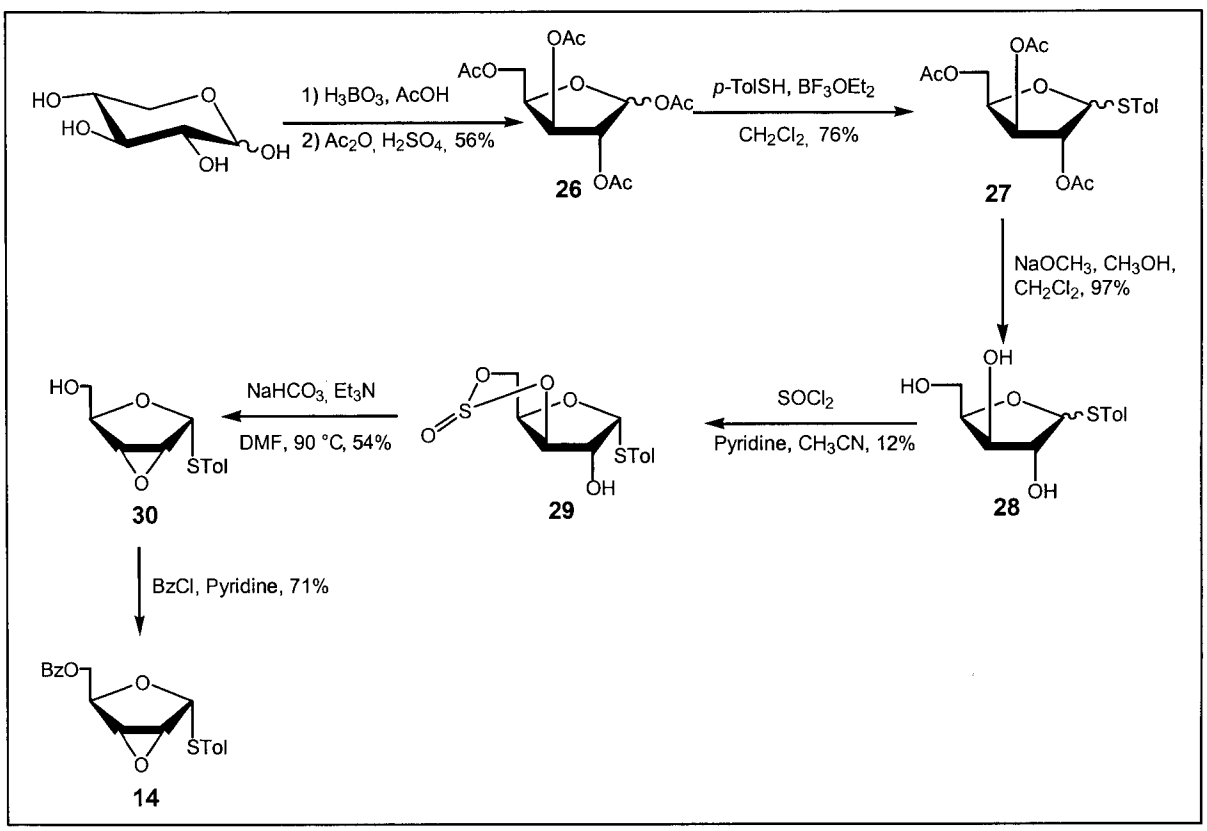


Scheme 2.5

The synthesis of **13** was achieved from methyl glycoside **7** in two steps. First, benzoylation of the hydroxy group afforded **25** in 87% yield. Subsequent reaction with *p*-thiocresol and borontrifluoride etherate yielded thioglycoside **13** in 40% yield (Scheme 2.5).

Thioglycoside **14** was prepared from commercially available D-xylose using a previously reported method (Scheme 2.6).[66] D-xylopyranose was converted to furanose

tetraacetate **26** in 56% yield by using a boric acid-mediated reaction.[61] Treatment of **26** with *p*-thiocresol in the presence of boron trifluoride etherate followed by deacetylation afforded **28** in 74% yield. Compound **28** was treated with thionyl chloride in a mixture of acetonitrile and pyridine, and the reaction afforded an α, β mixture of the 3,5-*O*-sulfinyl-D-xylofuranosyl thioglycoside. The anomers were separated at this stage to give pure **29**. Subsequently, **29** was treated with sodium bicarbonate and triethylamine in DMF at 90 °C to give the epoxide **30** in 54% yield. Protection of the alcohol in **30** with a benzoyl group afforded **14** in 71% yield.[68] (Scheme 2.6)



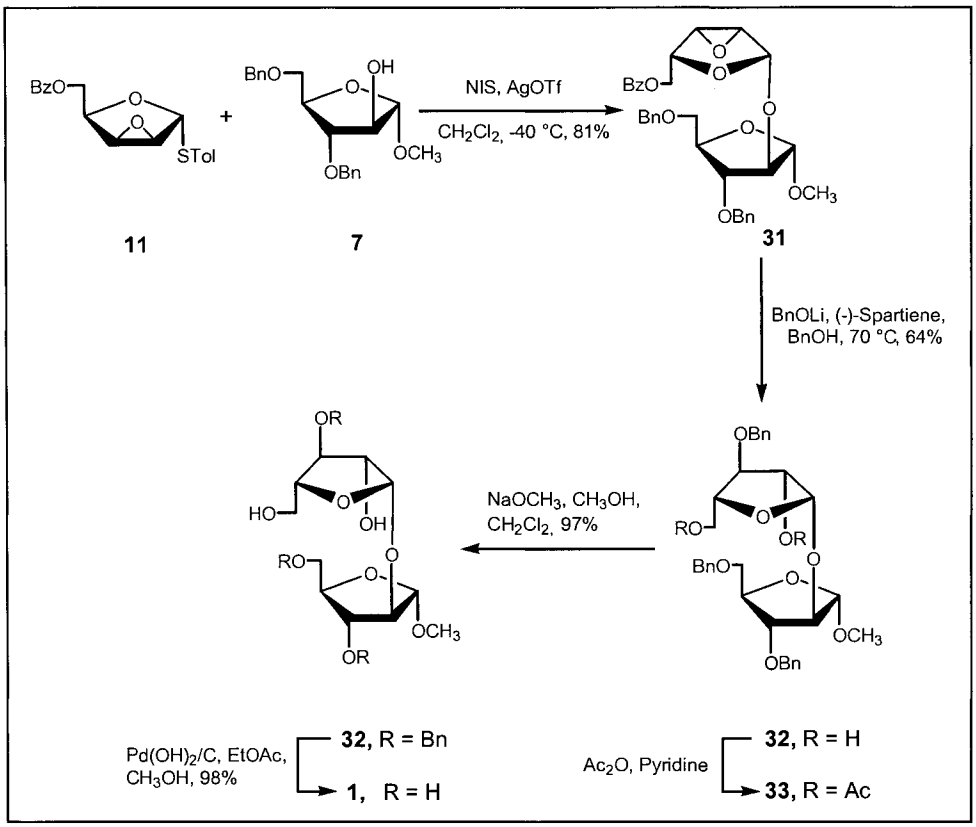
Scheme 2.6

2.2.2 Preparation of Oligosaccharides

Oligosaccharides **1-6** were obtained by coupling of the building blocks described above. All glycosylation reactions were conducted at -40 °C using *N*-iodosuccinimide and silver triflate as activators to yield ultimately oligosaccharides **1-6** in optimal yields. The stereochemistry of the newly formed glycosidic bonds was determined by NMR spectroscopy based on the coupling constant and ^{13}C chemical shift values, or by comparing with previously reported data.[3]

The ^{13}C chemical shift of the anomeric carbon of β-arabinofuranosyl residues appears upfield relative to the α-isomers. For example, compounds which have α-glycosidic bonds have ^{13}C chemical shifts between 109 and 105, while the compounds which have β-glycosidic bonds have ^{13}C chemical shifts around 100-103 ppm. Furthermore, the coupling constant difference between α and β-linkage in ^{1}H NMR spectrum supports the stereochemistry of two isomers. In the α anomer, the anomeric protons has a smaller coupling constant and appears as a singlet or a doublet with $J_{H1, H2}$ = 0-2.1 Hz. On the other hand, in the β-anomer, the coupling constants between H-1 and H-2, $J_{H1, H2}$, are 4.4 to 4.7 Hz, which are significantly larger than the values observed for the α anomer.

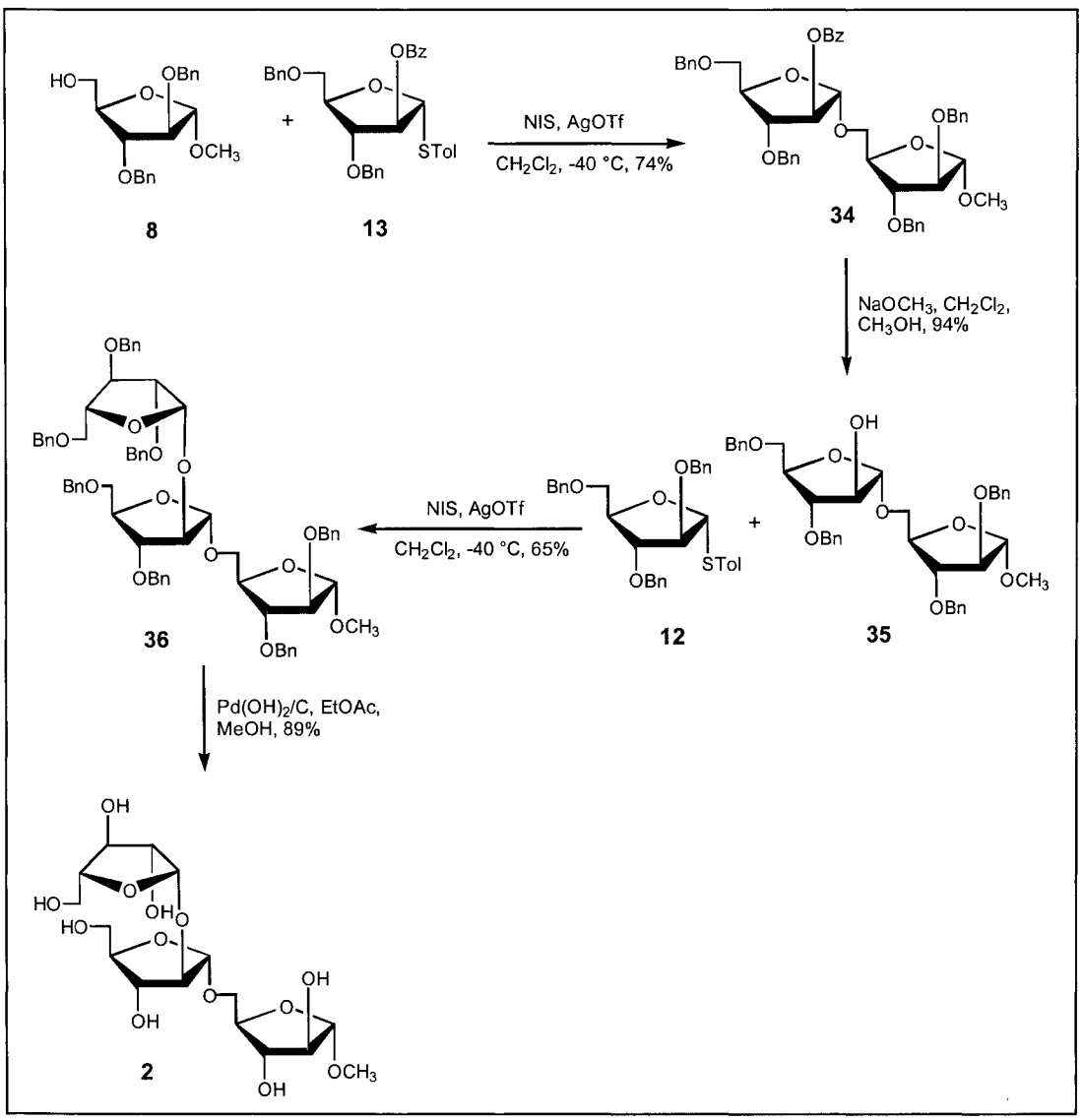
2.2.2.1 Synthesis of Disaccharide 1



Scheme 2.7

Disaccharide **1** was prepared from thioglycoside **11** and alcohol **7** in three steps (Scheme 2.7). In a previous report,[3] thioglycoside **11** was used as a donor, which was coupled with acceptor **15** to give the desired disaccharide **1** in two steps. With the expectation of better glycosylation stereoselectivity with the epoxide donors, **11** was coupled with alcohol **7** under the promotion of *N*-iodosuccinimide and silver triflate. The glycosylation reaction gave disaccharide **31** in 81% yield. Opening of the epoxide **31**

with lithium benzylate under the promotion of (-)-spartiene at 70 °C yielded a 4:1 mixture of both regioisomers with **32** as the major product in 64% yield. Before the hydrogenation of **32** to obtain **1**, **32** was acylated to separate isomers. After deacylation, **32** was hydrogenated with palladium hydroxide on carbon under hydrogen gas to give **1** in 98% yield.



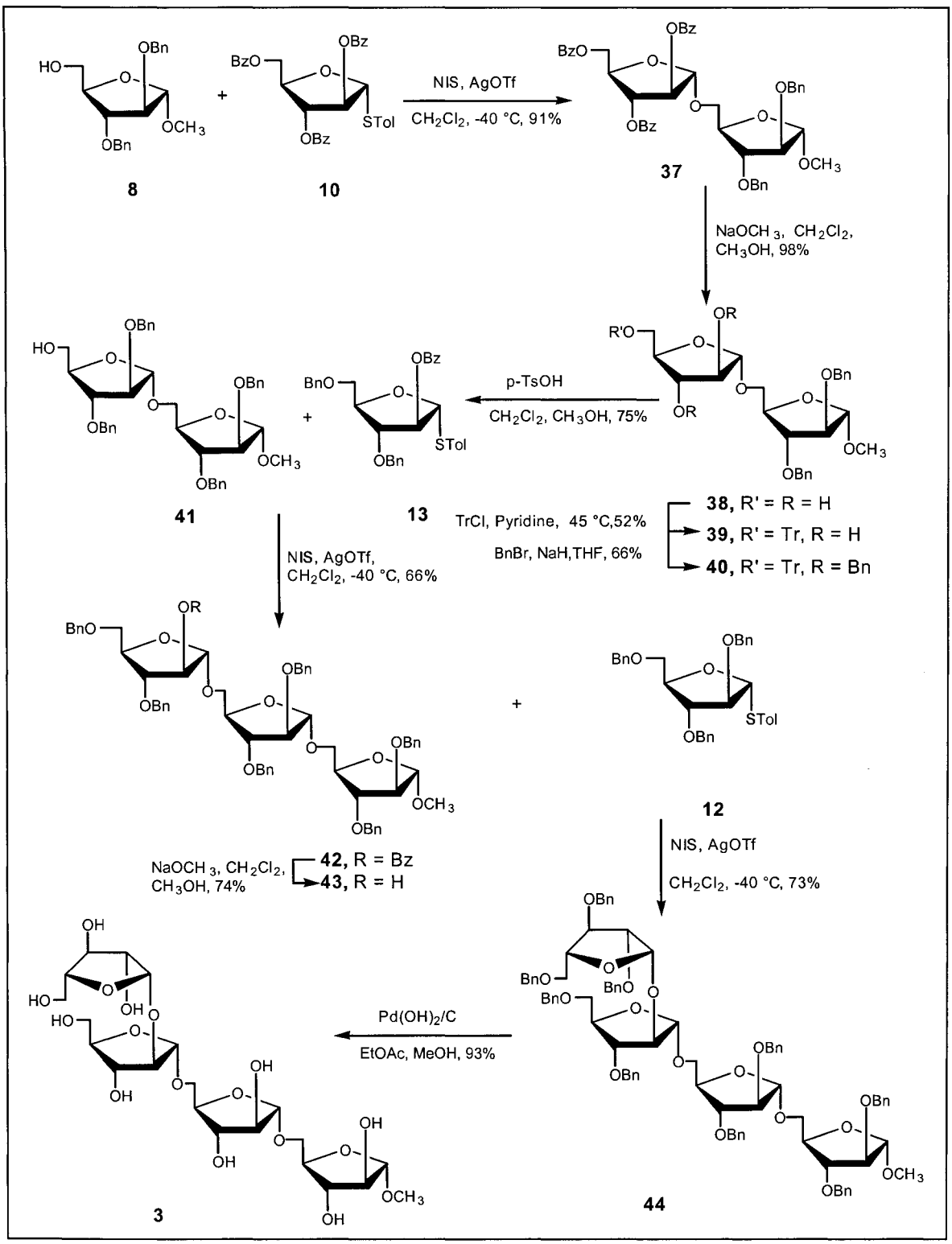
Scheme 2.8

2.2.2.2 Synthesis of Trisaccharide 2

The preparation of trisaccharide **2** is illustrated in Scheme 2.8. Assembly of the trisaccharide began with glycosylation of methyl glycoside **8** with thioglycoside **13** to provide disaccharide **34** in 74% yield. The disaccharide was then deacylated to give **35** in 94% yield. The β-arabinofuranosyl residue was introduced into disaccharide **35** by using thioglycoside **12** and *N*-iodosuccinimide and silver triflate to provide **36** in 65% yield. Trisaccharide **36** was then subjected to hydrogenation to give deprotected target **2** in 82% yield.

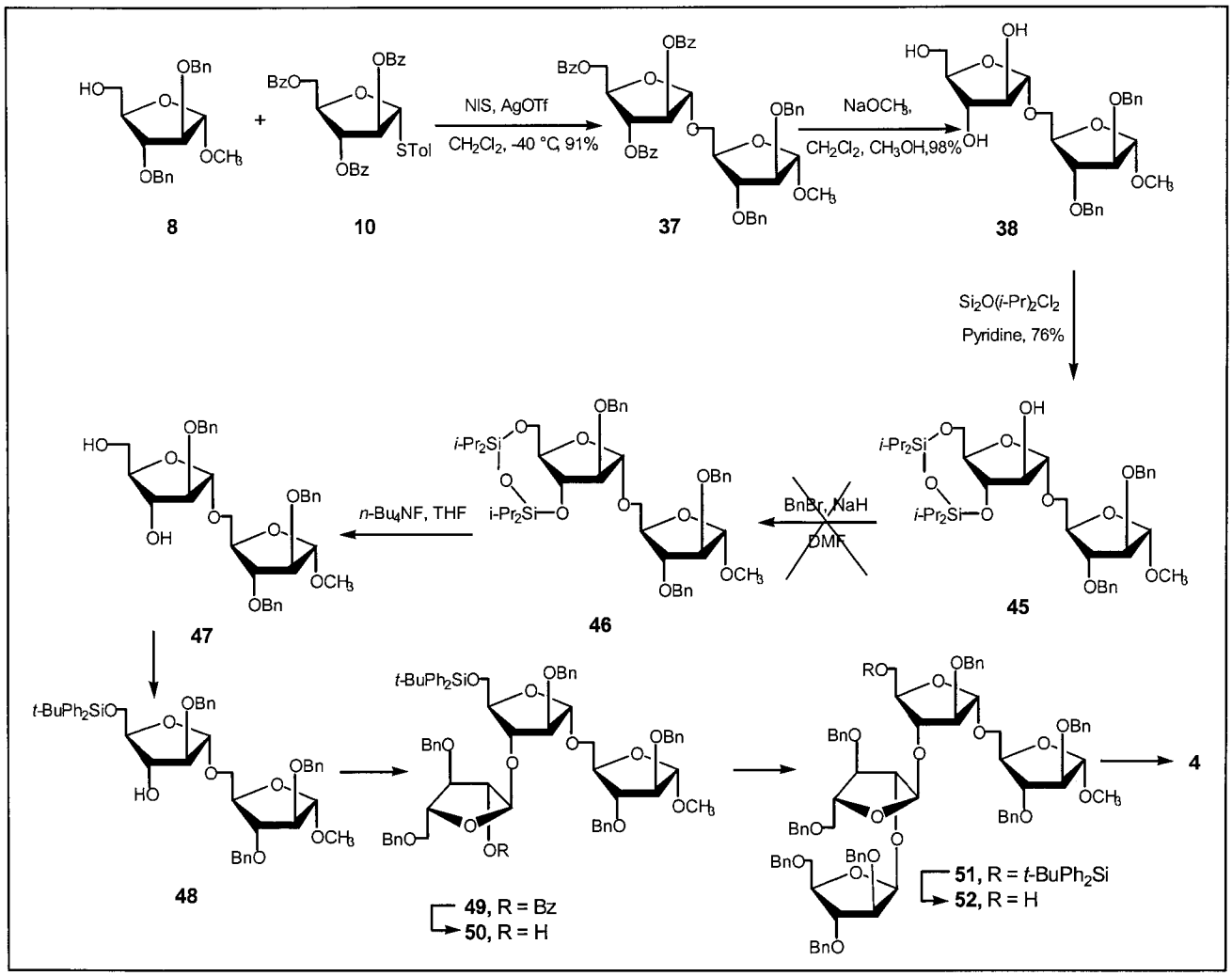
2.2.2.3 Synthesis of Tetrasaccharide 3

Tetrasaccharide **3** was synthesized as shown in Scheme 2.9. First, coupling of thioglycoside **10** and methyl glycoside **8** afforded disaccharide **37** in 91% yield. The three benzoyl groups were then removed by treatment with sodium methoxide giving triol **38** in 98% yield. The primary alcohol in **38** was protected with a trityl ether to give **39** in 52% yield, and the remaining secondary hydroxyl groups were benzylated to afford **40** in 66% yield. The trityl group was cleaved under acid catalysis giving alcohol **41** in 75% yield. Subsequent glycosylation of **41** with thioglycoside **13** gave **42** and removal of the benzoyl group upon treatment with sodium methoxide produced trisaccharide **43** in 58% overall yield. The β-arabinofuranosyl residue in the target was introduced via the reaction



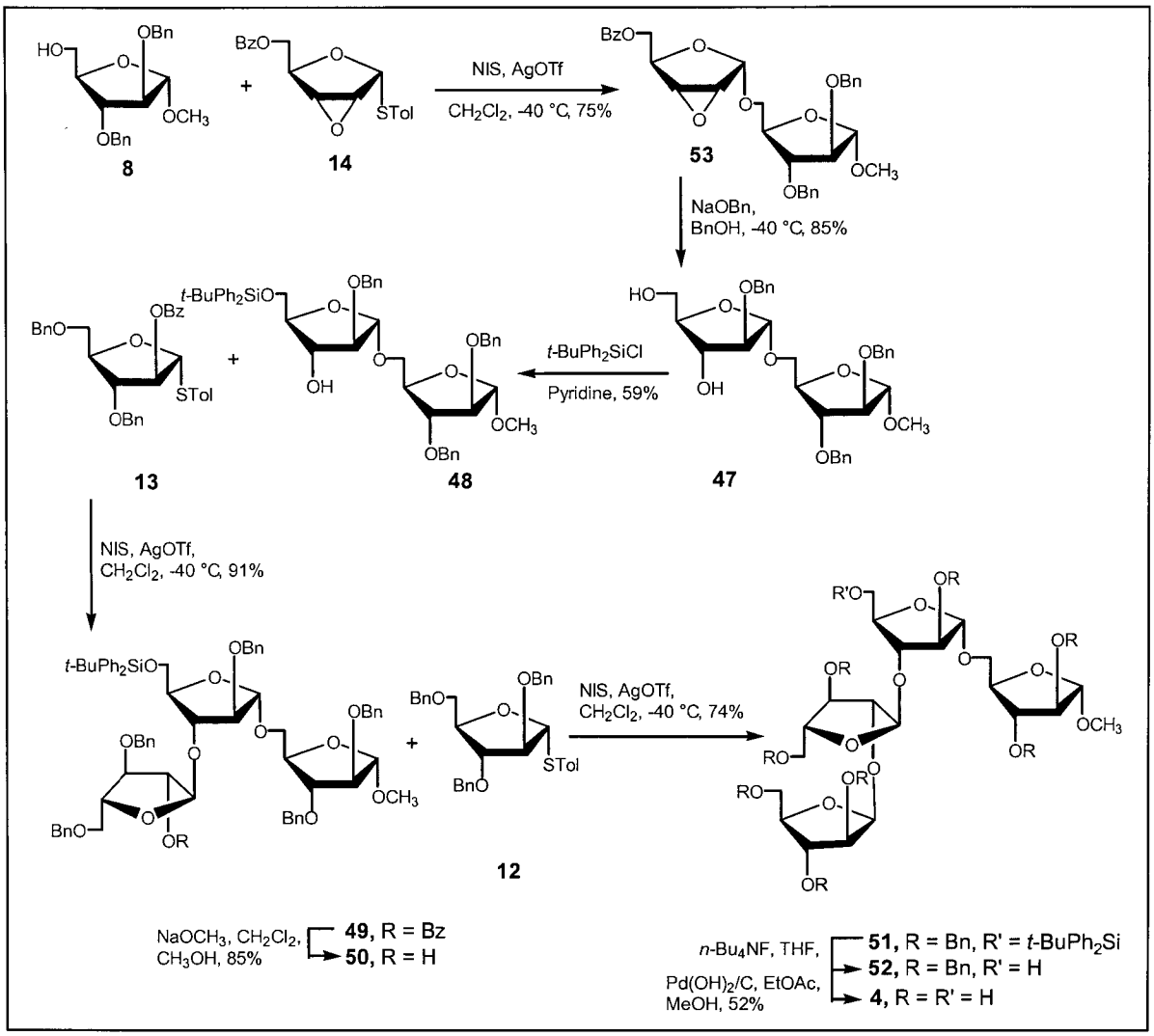
Scheme 2.9

of trisaccharide **43** with thioglycoside **12** to afford tetrasaccharide **44** in 73% yield. Deprotection by hydrogenation afforded **3** in 88% yield.



Scheme 2.10

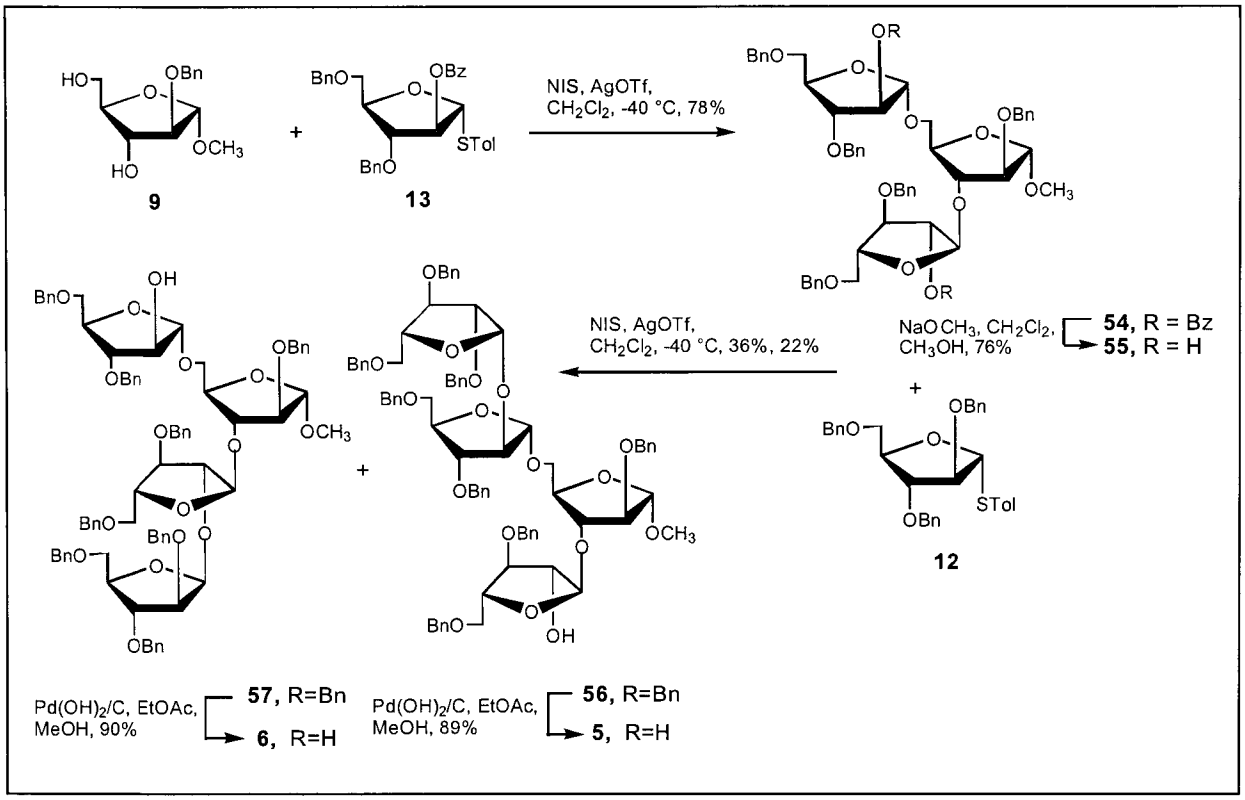
2.2.2.4 Synthesis of Tetrasaccharide 4



Scheme 2.11

Initial attempts to synthesize tetrasaccharide **4** began with the synthetic route previously described for this compound (Scheme 2.10).[3] As outlined in Scheme 2.10, tribenzoylated thioglycoside **8** and methyl glycoside **13** were coupled and the resulting disaccharide was deprotected to give triol **38**. Protection of O-3 and O-5 as a siloxane acetal gave **45,** which was subsequently subjected to benzylation to give **46**. However, the reaction for the conversion of the hydroxyl group at C-2′ into a benzyl ether did not afford the desired product. Instead, other desilyated products were formed in the reaction. Therefore, a more efficient route to prepare **47** was needed. Based on our success in the opening an epoxide in a similar disaccharide, we explored this method for the preparation of **4** (Scheme 2.11).[66] Firstly, epoxy thioglycoside **14** and methyl glycoside **8** were coupled to produce disaccharide **53** in 75% yield. Heating of disaccharide **52** with sodium benzylate in benzyl alcohol gave disaccharide **47** as the dominant product because steric hindrance of C-5 blocks attack of the nucleophile at C-3. The product, disaccharide **47**, was obtained in 65% yield over two steps. The primary alcohol in **47** was protected with *tert*-butyldiphenylsilyl chloride, and the reaction produced **48** in 59% yield. The secondary alcohol in **48** was then glycosylated with thioglycoside **13**. This reaction provided trisaccharide **49** in 91% yield, which was subsequently debenzoylated to afford **50** in 77% yield over two steps. The trisaccharide **50** was then reacted with thioglycoside **12** to form a β-arabinofuranosyl linkage to obtain the tetrasaccharide **51** in 74% yield. Cleavage of silyl protecting group using tetra-*n*-butylammonium fluoride and subsequent deprotection of the remaining hydroxy groups with palladium hydroxide on carbon gave tetrasaccharide **4** in 52% yield over two steps.

2.2.2.5 Synthesis of Tetrasaccharide 5, 6



Scheme 2.12

Tetrasaccharides **5** and **6** were prepared from a common trisaccharide intermediate **54** (Scheme 12). Glycosylation of alcohol **9** with an excess amount of the thioglycoside **13** provided trisaccharide **54** in 78% yield. Removal of benzoyl group followed by glycosylation with 1 equivalent of thioglycoside donor **12** gave a mixture of tetrasaccharides **56** and **57**. Tetrasaccharides **56** and **57** were separated by chromatography and were obtained in 36%, 22% yield respectively. The benzyl ether

protecting groups were removed from both products by hydrogenolysis with palladium hydroxide on carbon to afford tetrasaccharides **5** and **6** in 89%, 90% yield respectively. The structures of the compounds **5, 6** were proved by comparison to previous synthesized compounds.

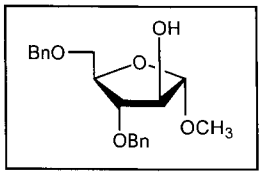
2.3 Experimental

General Methods

All chemical reagents were of analytical grade, used as supplied from Sigma-Aldrich, without further purification unless otherwise indicated. Solvents were distilled under an inert atmosphere from the appropriate drying agents before use. Unless stated otherwise, all reactions were carried out in oven-dried flasks under a positive pressure of argon. Molecular sieves were stored in an oven (140 °C) and cooled in vacuum prior to use. Analytical thin layer chromatography (TLC) was conducted on silica gel 60-F_{254} (0.25 mm, E. Merck). Plates were visualized under UV light or by charring with 10% H_2SO_4 in ethanol. In the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions when it was necessary. Organic solutions were concentrated under vacuum at < 40°C (bath). Organic solutions of crude products were dried over either anhydrous $MgSO_4$ or Na_2SO_4. Column chromatography was performed on silica gel 60 (40-60 μm, Silicycle) under medium pressure at a flow rate of 1-10 ml/min with an automatic pump. The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured on a polarimeter (Perkin-Elmer) at 22 ± 2 °C. ^{1}H NMR spectra were recorded on Varian INOVA 300, 400 or 500 MHz spectrometers. ^{1}H NMR chemical shifts are reported in δ (ppm) and referenced to either TMS (0.0, $CDCl_3$) or CD_3OD (3.30, CD_3OD). ^{13}C NMR spectra were recorded at 100 or 125 MHz and ^{13}C NMR chemical shifts are referenced to $CDCl_3$ (77.23, CD_3Cl_3) or CD_3OD (48.90, CD_3OD). ^{1}H data are reported as though they are first

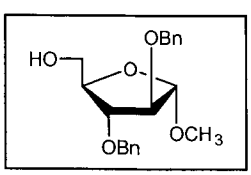
order. ^{1}H and ^{13}C NMR spectra were assigned with the assistance of COSY, HMQC spectra where necessary. For the assignment of oligosaccharide spectra, the monosaccharride residues were labeled in alphabetical order starting at the reducing end of the compound. Mass spectra (Electrospray) were recorded by the mass spectrometry laboratory at the University of Alberta using a Mariner mass spectrometer.

Methyl 3, 5-*O*-benzyl-α-D-arabinofuranoside (7).



Compound **19** (100 mg, 0.29 mmol) was dissolved in benzyl alcohol (4 mL) and a 1 M solution of sodium benzyloxide (5 mL, 5 mmol) was added. The solution was stirred at 100 °C under an inert atmosphere overnight. The reaction mixture was then cooled to rt and neutralized with acetic acid. Excess benzyl alcohol was removed by vacuum distillation and the residue was purified by chromatograpy (4:1, hexanes:EtOAc) to afford **7** as an oil (117 mg, 80%). R_f 0.36 (2:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.33-7.24 (m, 10 H, Ar), 4.89 (s, 1 H, H-1), 4.67 (d, 1 H, J = 12.3 Hz, OC$\underline{H}_2$Ar), 4.63 (d, 1 H, J = 11.9 Hz, OC$\underline{H}_2$Ar), 4.51 (d, 1H, J = 12.3 Hz, OC$\underline{H}_2$Ar), 4.46 (d, 1 H, J = 11.9 Hz, OC$\underline{H}_2$Ar), 4.25 (dd, 1 H, $J_{3,4}$ = 5.3 Hz, $J_{4,5}$ = 2.5 Hz, H-4), 4.11 (d, 1 H, $J_{2,3}$ = 0.7 Hz, H-2), 3.83 (dd, 1 H, $J_{2,3}$ = 0.7 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 3.65 (dd, 1 H, $J_{4,5}$ = 2.4 Hz, $J_{5,5'}$ = 10.4 Hz, H-5), 3.43 (dd, 1 H, $J_{4,5}$ = 2.5 Hz, $J_{5,5'}$ = 10.0 Hz, H-5′), 3.4 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 137.7 (Ar), 137.0 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 110.5 (C-1), 84.97 (C-3), 83.6 (C-4), 77.9 (C-2), 73.8 (O$\underline{C}H_2$Ar), 72.1 (O$\underline{C}H_2$Ar), 69.7 (C-5), 55.3 (OCH_3). Data matched previously reported one.[80]

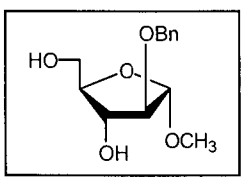
Methyl 2,3-di-*O*-benzyl-α-D-arabinofuranoside (8)



TBAF (14.4 ml, 14.39 mmol) was added to the compound **21** (7 g, 11.99 mmol) in THF (50 mL). The solution was stirred for 8 h at rt.

The mixture was then concentrated and purified by chromatography (2:1, hexanes:EtOAc) to obtain **8** as an oil (2.7 g, 65%). R_f 0.39 (1:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38-7.29 (m, 10 H, Ar), 4.94 (s, 1 H, H-1), 4.62-4.49 (m, 4 H, OC<u>H</u>$_2$Ar), 4.15 (ddd, 1 H, $J_{4,5}$ = 2.9 Hz, $J_{4,5'}$ = 4.1 Hz, $J_{3,4}$ = 6.1 Hz, H-4), 4.01 (dd, 1 H, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 2.6 Hz, H-2), 3.98 (ddd, 1 H, $J_{1,3}$ = 0.6 Hz, $J_{2,3}$ = 2.6 Hz, $J_{3,4}$ = 6.1 Hz, H-3), 3.84 (dd, 1 H, $J_{4,5}$ = 2.9 Hz, $J_{5,5'}$ = 12.0 Hz, H-5), 3.65 (dd, 1 H, $J_{4,5'}$ = 4.1 Hz, $J_{5,5'}$ = 12.0 Hz, H-5′), 3.40 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 137.7 (Ar), 137.4 (Ar), 128.5 (2C, Ar) 128.0 (Ar), 127.9 (3C, Ar), 107.5 (C-1), 87.8 (C-2), 82.6 (C-3), 82.3 (C-4), 72.4 (O<u>C</u>H$_2$Ar), 71.9 (O<u>C</u>H$_2$Ar), 62.3 (C-5), 54.9 (OCH_3). Data matched previously reported one.[81]

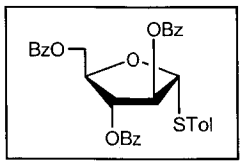
Methyl 2-*O*-benzyl-α-D-arabinofuranoside (9).



TBAF (2 ml, 2.0 mmol) was added to a solution of **23** (1.2 g, 2.4 mmol) in THF (15 mL). The reaction mixture was stirred at rt for 5 h and concentrated. The crude product was purified by chromatography (1:1, hexanes:EtOAc) to obtain **9** as an oil (522 mg, 85%). R_f 0.13 (1:2, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38-7.31 (m, 5 H, Ar), 4.99 (s, 1 H, H-1), 4.65 (d, 1 H, J = 11.7 Hz, OC<u>H</u>$_2$Ar), 4.59 (d, 1 H, J = 11.7 Hz, OC<u>H</u>$_2$Ar), 4.12 (m, 2 H, H-2, H-4), 3.89 (dd, 1 H, $J_{2,3}$ = 0.7 Hz, $J_{3,4}$ = 1.7 Hz, H-3), 3.84 (dd, 1 H, $J_{4,5}$ = 3.0 Hz, $J_{5,5'}$ = 11.9 Hz, H-5), 3.77 (dd, 1 H, $J_{4,5'}$ = 4.1 Hz, $J_{5,5'}$ = 11.9 Hz, H-5′), 3.40 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 128.6 (Ar), 128.1 (Ar), 127.9 (Ar), 106.9 (C-1), 87.3 (C-3),

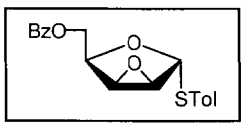
86.3 (C-2), 76.7 (C-4), 71.9 ($O\underline{C}H_2Ar$), 62.6 (C-5), 55.9 (OCH_3). Data matched previously reported one.[3]

_p_-Tolyl 2,3,5-tri-_O_-benzoyl-1-thio-α-D-arabinofuranoside (10).



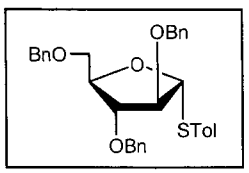
Comound **15** (10 g, 21.0 mmol) was dissolved under an argon atmosphere in CH_2Cl_2 (50 mL). The solution was cooled at 0 °C and $BF_3{\cdot}Et_2O$ (7.9 mL, 63.0 mmol) was added. The mixture was stirred for 10 min, after which time *p*-thiocresol (2.60 g, 21.0 mmol) was added. The reaction mixture was stirred for 5 h, diluted with CH_2Cl_2, washed with a cold saturated $NaHCO_3$ solution and the organic layer dried over anhydrous $MgSO_4$, filtered and concentrated. Upon addition of methanol to the resulting oil, the product crystallized. Recrystallization from methanol afforded **10** as a white solid. (8 g, 67%) R_f 0.72 (2:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 5.76 (s, 1 H, H-1), 5.72 (dd, 1 H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 1.6 Hz, H-2), 5.65 (dd, 1 H, $J_{2,3}$ = 1.6 Hz, $J_{3,4}$ = 4.8 Hz, H-3), 4.86 (ddd, 1 H, $J_{4,5}$ = 3.7 Hz, $J_{3,4}$ = 4.8 Hz, $J_{4,5'}$ = 5.1 Hz, H-4), 4.81 (dd, 1 H, $J_{4,5}$ = 3.7 Hz, $J_{5,5'}$ = 11.9 Hz, H-5), 4.74 (dd, 1 H, $J_{4,5}$ = 5.1 Hz, $J_{5,5'}$ = 11.9 Hz, H-5′), 2.31 (s, 3 H, $ArC\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ_c) 166.0 (C=O), 165.5 (C=O), 165.2 (C=O), 138.2 (Ar), 133.7 (Ar), 133.5 (Ar), 133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.7 (Ar), 129.5 (Ar), 129.0 (Ar), 128.9 (Ar), 128.5 (2C, Ar), 128.3 (Ar), 92.0 (C-1), 82.0 (C-2), 81.2 (C-4), 78.2 (C-3), 64.0 (C-5), 21.0 ($Ar\underline{C}H_3$). Data matched previously reported one.[64]

***p*-Tolyl 2,3-anhydro-5-*O*-benzoyl-1-thio-α-D-lyxofuranoside (11).**



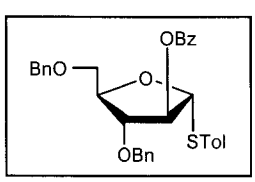
Compound **24** (2 g, 7.8 mmol) was dissolved in THF (50 mL). Triphenylphosphine (5.2 g, 20 mmol) and benzoic acid (1.42 g, 12 mmol) were then added to the solution. After cooling to 0 °C in an ice bath, diisopropylazodicarboxylate (3.86 mL, 19.5 mmol) was added dropwise over 10 min. The reaction mixture was stirred at rt for 45 min, after which time the solution was concentrated. Upon trituration of the residue with cold diethyl ether, triphenylphosphine oxide precipitated. The solid was filtered off and the filtrate concentrated. The resulting oil was purified by chromatography (16:1, hexanes:EtOAc) to obtain **11** as a white crystalline solid (1.3 g, 50%). R_f 0.68 (3:1, hexanes:EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.05-8.02 (m, 2 H, Ar), 7.58-7.53 (m, 1 H, Ar), 7.45-7.39 (m, 4 H, Ar), 7.12-7.09 (m, 2 H, Ar), 5.49 (s, 1 H, H-1), 4.52 (dd, 1 H, $J_{4,5}$ = 5.7 Hz, $J_{5,5'}$ = 11.3 Hz, H-5), 4.47 (dd, 1 H, $J_{4,5}$ = 6.0 Hz, $J_{5,5'}$ = 11.3 Hz, H-5′), 4.26 (dt, 1 H, $J_{3,4}$ = 0.7 Hz, $J_{4,5}$ = 5.7 Hz, $J_{4,5'}$ = 6.0 Hz, H-4), 3.90 (d, 1 H, $J_{2.3}$ = 2.9 Hz, H-2) 3.80 (dd, 1 H, $J_{3,4}$ = 0.7 Hz, $J_{2,3}$ = 2.9 Hz, H-3), 2.32 (s, 3 H, Ar$\underline{C}H_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ_c) 166.0 (C=O), 133.3 (Ar), 133.1 (Ar), 129.9 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 128.6 (Ar), 128.4 (Ar), 87.0 (C-1), 62.5 (C-5), 58.0 (C-2), 56.0 (C-3), 21.0 (Ar$\underline{C}H_3$). Data matched previously reported one.[82]

_p_ –Tolyl 2,3,5-tri-_O_-benzyl-1-thio-α-D-arabinofuranoside (12).



Compound **24** (3 g, 11.8 mmol) was dissolved in THF (50 mL). After cooling to 0 °C in an ice bath, NaH (1.7 g, 70.6 mmol) and benzyl bromide (1.42 g, 70.7 mmol) were added to the solution. The reaction mixture was stirred at rt overnight. The solution was concentrated to yield a crude oil and extracted with CH_2Cl_2 (30 ml X 3). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude oil was purified by chromatography (9:1, hexanes:EtOAc) to obtain **12** as an oil (4.7 g, 77%). R_f 0.55 (5:1, hexanes:EtOAc); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 7.42-7.10 (m, 15 H, Ar), 5.53 (d, 1 H, $J_{1,2}$ = 2.9 Hz, H-1), 4.66-4.48 (m, 6 H, OC<u>H</u>$_2$-Ar), 4.39 (ddd, 1 H, $J_{4,5}$ = 4.0 Hz, $J_{4,5'}$ = 4.6 Hz, $J_{3,4}$ = 6.7 Hz, H-4), 4.12 (dd, 1 H, $J_{1,2}$ = 2.8 Hz, $J_{2,3}$ = 3.4 Hz, H-2), 4.04 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 6.7 Hz, H-3), 3.70 (dd, 1 H, $J_{4,5}$ = 4.0 Hz, $J_{5,5'}$ = 10.9 Hz, H-5), 3.64 (dd, 1 H, $J_{4,5'}$ = 4.9 Hz, $J_{5,5'}$ = 10.9 Hz, H-5′), 2.33 (s, 3 H, Ar<u>C</u>H$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.2 (Ar), 137.8 (Ar), 137.4 (2C, Ar), 132.0 (Ar), 131.0 (Ar), 129.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (2C, Ar), 127.7 (Ar), 127.6 (Ar), 90.6 (C-1), 88.4 (C-2), 83.6 (C-3), 80.5 (C-4), 73.4 (O<u>C</u>H$_2$Ar), 72.3 (O<u>C</u>H$_2$Ar), 72.1 (O<u>C</u>H$_2$Ar), 69.2 (C-5), 21.1 (Ar<u>C</u>H$_3$). Data matched previously reported one.[3]

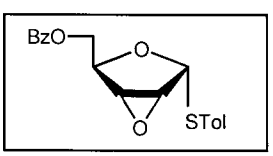
_p_ –Tolyl 2-_O_-benzoyl-3,5-di-_O_-benzyl-1-thio-α-D-arabinofuranoside (13).



Benzoyl chloride (1.4 mL, 11.8 mmol) was added to a solution of **7** (2.7 g, 7.9 mmol) in pyridine (80 mL). The reaction mixture was

stirred at rt overnight, after which time the solution was concentrated. The residue obtained was purified by chromatography (8:1, hexanes:EtOAc) to afford **25** as an oil (3.1 g, 87%). Compound **25** (3.6 g, 8.1 mmol) was dissolved in CH_2Cl_2 (50 mL) under an argon atmosphere and the solution was cooled to 0 °C. $BF_3{\cdot}Et_2O$ (3 mL, 24.4 mmol) was then added. The solution was stirred for 10 min, and then *p*-thiocresol (1.2 g, 9.8 mmol) was added. The reaction mixture was stirred for 8 h, concentrated, and purified by chromatography (8:1, hexanes:EtOAc) to obtain **13** as an oil (2.1 g, 40%). R_f 0.50 (5:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.08-7.11 (m, 15 H, Ar), 5.69 (s, 1 H, H-1), 5.60 (t, 1 H, $J_{2,3}$ = 1.7 Hz, H-2), 4.86-4.52 (m, 5 H, H-4, OC<u>H</u>$_2$Ar), 4.11 (ddd, 1 H, $J_{1,3}$ = 0.8 Hz, $J_{2,3}$ = 1.7 Hz, $J_{3,4}$ = 5.3 Hz, H-3), 3.69 (dd, 1 H, $J_{4,5}$ = 4.1 Hz, $J_{5,5'}$ = 10.8 Hz, H-5), 3.64 (dd, 1H, $J_{4,5'}$ = 4.7 Hz, $J_{5,5'}$ = 10.8 Hz, H-5′) 2.32 (s, 3 H, Ar<u>C</u>H$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 165.4 (C=O), 138.0 (Ar), 137.6 (Ar), 137.5 (Ar), 133.4 (Ar), 132.4 (Ar), 131.8 (Ar), 130.0 (Ar), 129.8 (2C, Ar), 129.7 (Ar), 129.4 (Ar), 128.5 (Ar), 128.4 (3C, Ar), 128.3 (Ar), 127.9 (Ar), 127.8 (2C, Ar), 127.7 (Ar), 127.6 (2C, Ar), 91.6 (C-1), 83.3 (C-3), 82.3 (C-2), 82.0 (C-4), 73.4 (O<u>C</u>H$_2$Ar), 72.3 (O<u>C</u>H$_2$Ar), 69.0 (C-5), 21.1 (Ar<u>C</u>H$_3$). Data matched previously reported one.[3]

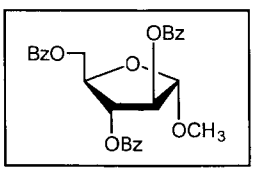
_p_-Tolyl 2,3-anhydro–5-_O_-benzoyl-1-thio-α-D-ribofuranoside (14).



Compound **30** (554.8 mg, 2.3 mmol) was dissolved in pyridine (8 mL), then benzoyl chloride (0.6 mL, 4.2 mmol) was added. The reaction mixture was stirred at rt for 8 h, then concentrated and purified (5:1, hexanes:EtOAc) to obtain **14** as an oil (565 mg, 71%). R_f 0.52 (5:1, hexanes:EtOAc); ^{1}H

NMR (500 MHz, $CDCl_3$, δ_H) 8.12-7.11 (m, 9 H, Ar), 5.44 (s, 1 H, H-1), 4.66 (m, 1 H, H-4), 4.52 (dd, 1 H, $J_{4,5}$ = 3.8 Hz, $J_{5,5'}$ = 12.0 Hz, H-5), 4.39 (dd, 1 H, $J_{4,5'}$ = 4.5 Hz, $J_{5,5'}$ = 12.0 Hz, H-5′), 4.03 (dd, 1 H, $J_{1,2}$ = 0.9 Hz, $J_{2,3}$ = 2.7 Hz, H-2), 4.85 (d, 1 H, $J_{2,3}$ = 2.7 Hz, H-3), 2.33 (s, 3 H, $ArC\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 166.0 (C=O), 137.7 (Ar), 133.4 (Ar) 132.0 (Ar) 130.2 (Ar), 129.8 (Ar), 129.6 (Ar), 129.4 (Ar), 128.6 (Ar), 128.5 (Ar), 87.2 (C-1), 77.7 (C-4), 64.3 (C-5), 59.0 (C-2), 57.3 (C-3), 21.1 ($Ar\underline{C}H_3$). Data matched previously reported one.[66]

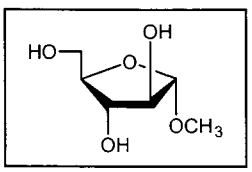
Methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside (15).



D-Arabinose (10 g, 67 mmol) was dissolved in absolute methanol (200 mL). The solution was cooled to 0 °C and 1.06 M acetyl chloride (63 ml) was added slowly. After stirring the mixture at rt for 4 h, the reaction was neutralized by the addition of pyridine (40 mL). The solvent was then evaporated to give a syrup, which was dissolved in pyridine (75 mL). After cooling to 0 °C in an ice bath, benzoyl chloride (31 mL, 2.7 mmol) was added dropwise. The reaction mixture was allowed to warm to rt and was then stirred overnight. Water (5 mL) was added and the solution was diluted with CH_2Cl_2. The organic layer was washed with water, 1M HCl (4 x) and a saturated solution of sodium bicarbonate. After drying the organic extract with $MgSO_4$, the solution was concentrated to provide a syrup. The crude product was recrystallized from absolute ethanol yielding white crystals. Additional product was recovered from the mother liquors by addition of pentane, yielding **15** (15 g, 50%). R_f 0.4 (3:1, hexanes:EtOAc); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 5.56 (ddd, 1 H, $J_{1,2}$ =

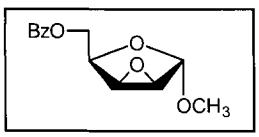
0.7 Hz, $J_{2,3}$ = 1.4 Hz, $J_{3,4}$ = 5.1 Hz, H-3), 5.49 (d, 1 H, $J_{2,3}$ = 1.4 Hz, H-2), 5.16 (d, $J_{1,2}$ = 0.7 Hz, 1 H, H-1), 4.82 (dd, 1 H, $J_{4,5}$ = 3.4 Hz, $J_{5,5'}$ = 12.0 Hz, H-5), 4.67 (dd, 1 H, $J_{4,5'}$ = 4.9 Hz, $J_{5,5'}$ = 12.0 Hz, H-5′), 4.55 (ddd, 1 H, $J_{4,5}$ = 3.4 Hz, $J_{4,5'}$ = 4.9 Hz, $J_{3,4}$ = 5.1 Hz, H-4), 3.48 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ_c) 161.8 (C=O), 165.8 (C=O), 165.4 (C=O), 133.5 (Ar), 133.4 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.1 (Ar), 129.0 (2C, Ar), 128.8 (Ar), 128.5 (2C, Ar), 128.4 (2C, Ar), 128.3 (Ar), 106.9 (C-1), 82.2 (C-2), 80.0 (C-4), 77.9 (C-3), 63.7 (C-5), 55 (OCH_3). Data matched previously reported one.[62]

Methyl α-D-arabinofuranoside (16).



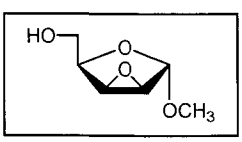
Compound **15** (5 g, 10.5 mmol) was dissolved in an 1:1 mixture of CH_2Cl_2/CH_3OH (100 mL) and treated with a catalytic amount of $NaOCH_3$ in CH_3OH at rt. After stirring for 8 h, the reaction mixture was neutralized with Amberite IR-120 H^+ resin, filtered and concentrated. The crude poduct was purified by column chromatography (3:1, hexanes:EtOAc, then 95% EtOH) to give **16** as an oil (1.7 g, 98%). R_f 0.82 (3:1:1, EtOAc:CH_2Cl_2:CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 4.75 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H-1), 3.93-3.88 (m, 2 H, H-2, H-4), 3.82 (dd, 1 H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 6.2 Hz, H-3), 3.82 (dd, 1 H, $J_{4,5}$ = 3.3 Hz, $J_{5,5'}$ = 11.9 Hz, H-5), 3.62 (dd, 1 H, $J_{4,5}$ = 5.3 Hz, $J_{5,5'}$ = 11.9 Hz, H-5′), 3.37(s, 3 H, OCH_3); ^{13}C NMR (100 MHz, CD_3OD, δ_c) 111.2 (C-1), 86.0 (C-4), 83.8 (C-2), 79.2 (C-3), 63.4 (C-5), 55.6 (OCH_3). Data matched previously reported one.[83]

Methyl 2,3-anhydro-5-*O*-benzoyl-α-D-lyxofuranoside (17).



Compound **16** (2 g, 12.2 mmol) was dissolved in THF (50 mL). Triphenylphosphine (5.2 g, 20 mmol) and benzoic acid (1.42 g, 12 mmol) were then added to the solution. After cooling to 0 °C in an ice bath, diisopropylazodicarboxylate (3.86 mL, 19.5 mmol) was added dropwise over 10 min. The reaction mixture was stirred at rt for 45 min and the solution was concentrated. Trituration of the residue with cold diethyl ether precipitated triphenylphosphine oxide. The solid was filtered off and the filtrate was concentrated. The resulting oil was purified by chromatography (4:1, hexanes:EtOAc) to obtain **17** as a white crystalline solid (1.9 g, 61%). R_f 0.55 (3:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.00 (s, 1 H, H-1), 4.55 (dd, 1 H, $J_{4,5}$ = 5.7 Hz, $J_{5,5'}$ = 11.3 Hz, H-5), 4.51 (dd, 1 H, $J_{4,5'}$ = 5.9 Hz, $J_{5,5'}$ = 11.3 Hz, H-5′), 4.34 (dt, 1 H, $J_{3,4}$ = 0.7 Hz, $J_{4,5}$ = 6.0 Hz, H-4), 3.81 (dd, 1 H, $J_{3,4}$ = 0.7 Hz, $J_{2,3}$ = 2.8 Hz, H-3), 3.69 (d, 1 H, $J_{2,3}$ = 2.8 Hz, H-2), 3.43 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, CD_3OD, δ_c) 166.2 (C=O), 133.2 (Ar), 129.8 (2C, Ar), 128.4 (Ar), 128.2 (Ar), 102.3 (C-1), 74.0 (C-4), 62.9 (C-5), 56.2 (C-2), 55.7 (OCH_3), 54.1 (C-3). Data matched previously reported one.[64]

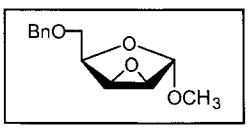
Methyl 2,3-anhydro-α-D-lyxofuranoside (18).



Compound **17** (2 g, 8.1 mmol) was dissolved in an 1:1 mixture of CH_2Cl_2/CH_3OH (50 mL), and treated with a catalytic amount of $NaOCH_3$ in CH_3OH at rt. After stirring for 8 h, the reaction mixture was neutralized with

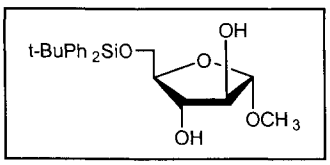
Amberite IR-120 H^+ resin, filtered and concentrated. The crude product was purified by column chromatography (3:1, EtOAc:CH_2Cl_2) to afford **18** as a white solid (1 g, 90%). R_f 0.59 (3:1, EtOAc:CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ_H) 4.97 (s, 1 H, H-1), 4.13 (t, 1 H, $J_{4,5}$ = 5.0 Hz, H-4), 3.89 (d, 2 H, $J_{4,5}$ = 5.0 Hz, H-5, H-5'), 3.74 (d, 1 H, $J_{2,3}$ = 2.8 Hz, H-3), 3.66 (d, 1 H, $J_{2,3}$ = 2.8 Hz, H-2), 3.43 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, CD_3OD, δ_c) 102.2 (C-1), 76.2 (C-4), 61.9 (C-5), 55.8, 55.6 (C-2, OCH_3), 54.1 (C-3). Data matched previously reported one.[64]

Methyl 2,3-anhydro-5-*O*-benzyl-α-D-lyxofuranoside (19).



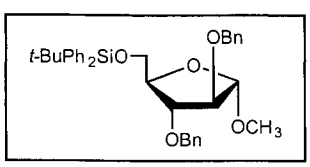
Compound **18** (1 g, 6.9 mmol) was dissolved in THF (50 mL), and NaH (0.2 g, 8.2 mmol) was added. After the solution had stirred for 10 min, benzyl bromide (16.4 g, 9.6 mol) was added. The reaction mixture was allowed to stir at rt for 5 h. The solution was concentrated, and the product obtained was purified by column chromatograpy (4:1, hexanes:EtOAc) to yield **19** as an oil (1.2 g, 74%). R_f 0.46 (3:1, hexanes: EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.37-7.27 (m, 5 H, Ar), 4.96 (s, 1 H, H-1), 4.63 (d, 1 H, J = 12.0 Hz, O$\underline{C}H_2$Ar), 4.56 (d, 1H, J = 12.0 Hz, O$\underline{C}H_2$Ar), 4.21 (dt, 1 H, $J_{3,4}$ = 0.7 Hz, $J_{4,5}$ = 6.5 Hz, H-4), 3.77 (dd, 1 H, $J_{3,4}$ = 0.7 Hz, $J_{2,3}$ = 2.9 Hz, H-3), 3.67-3.65 (m, 3 H, H-2, H-5, H-5'), 3.42 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ_c) 137.9 (Ar), 128.4 (Ar), 127.8 (2C, Ar), 102.3 (C-1), 75.1 (C-4), 73.6 (O$\underline{C}H_2$-Ar), 68.5 (C-5), 56.2 (C-2), 55.6 (OCH_3), 54.3 (C-3). Data matched previously reported one.[85]

Methyl 5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranoside (20).



To a solution of compound **16** (5 g, 30.5 mmol) in pyridine was added *tert*-butylchlorodiphenylsilane (10 mL, 40 mmol). The reaction mixture was stirred at rt overnight. The solution was washed with water (5 mL) and extracted with CH_2Cl_2. The organic layer was washed with water, 1 M HCl (4 X) and a saturated solution of sodium bicarbonate. After drying with $MgSO_4$, the solution was concentrated and purified by chromatography (3:1, hexanes:EtOAc) to obtain **20** as an oil (8.5 g, 70%). R_f 0.38 (1:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.71-7.40 (m, 10 H, Ar), 5.0 (s, 1 H, H-1), 4.15-4.11 (m, 2 H, H-3, H-4), 4.02 (d, 1 H, $J_{2,3}$ = 1.4 Hz, H-2), 3.84 (dd, 1 H, $J_{4,5}$ = 2.3 Hz, $J_{5,5'}$ = 11.4 Hz, H-5), 3.75 (dd, 1 H, $J_{4,5'}$ = 1.7 Hz, $J_{5,5'}$ = 11.4 Hz, H-5′), 3.42 (s, 3 H, OCH_3), 1.06 (s, 9 H, $(C\underline{H}_3)_3CSi$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 136.4 (Ar), 135.7 (Ar), 135.6 (Ar) 131.9 (Ar), 131.8 (Ar), 130.2 (Ar), 130.1 (2C, Ar), 130.0 (Ar), 128.0 (Ar), 127.9 (Ar) 127.8 (Ar), 109.4 (C-1), 87.5 (C-4), 78.4 (C-2), 78.0 (C-3), 64.1 (C-5), 54.9 (OCH_3), 26.7 $(\underline{C}H_3)_3CSi$), 19.1 $(\underline{C}H_3)_3CSi$). Data matched previously reported one.[3]

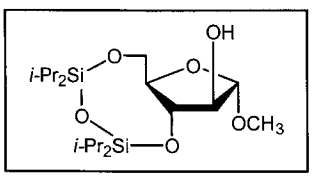
Methyl 2,3-di-*O*-benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranoside (21)



Compound **20** (4 g, 10.0 mmol) was dissolved in DMF (50 mL) and NaH (1.2 g, 50 mmol) was added to the solution at 0 °C. The solution was stirred for 10 min, benzyl bromide (5 mL, 42.0 mmol) was added to the solution, and the reaction mixture was stirred for 6 h at 0 °C. The

solution was diluted with CH_2Cl_2, washed with water (100 ml x 5), dried with $MgSO_4$, and concentrated. The residue was purified by chromatography (9:1, hexanes:EtOAc) to obtain **21** as an oil (3.6 g, 62%). R_f 0.49 (5:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.71-7.25 (m, 20 H, Ar), 4.96 (s, 1 H, H-1), 4.57-4.48 (m, 4 H, O$\underline{C}H_2$Ar), 4.18 (dt, 1 H, $J_{4,5}$ = 4.7 Hz, $J_{3,4}$ = 5.7 Hz, H-4), 4.03 (dd, 1 H, $J_{2,3}$ = 2.7 Hz, $J_{3,4}$ = 5.7 Hz, H-3), 4.01 (dd, 1 H, $J_{1,2}$ = 1.2 Hz, $J_{2,3}$ = 2.7 Hz, H-2), 3.83 (d, 2 H, $J_{4,5}$ = 4.9 Hz, $J_{4,5'}$ = 4.9 Hz, H-5, H-5′), 3.40 (s, 3 H, OCH_3), 1.06 (s, 9H, ($C\underline{H}_3$)$_3$CSi); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.0 (Ar), 137.7 (Ar), 135.7 (2C, Ar) 135.7 (Ar), 133.5 (Ar), 133.5 (Ar), 129.6 (Ar), 129.6 (Ar), 128.4 (Ar), 128.3 (Ar), 127.8 (Ar) 127.7 (2C, Ar), 127.6 (2C, Ar), 107.3 (C-1), 88.2 (C-2), 83.3 (C-3), 82.7 (C-4), 72.1 (O$\underline{C}H_2$Ar), 71.8 (O$\underline{C}H_2$Ar), 64.0 (C-5), 54.9 (OCH_3), 26.9 (($\underline{C}H_3$)$_3$CSi), 19.3 (($\underline{C}H_3$)$_3$CSi). Data matched previously reported one.[3]

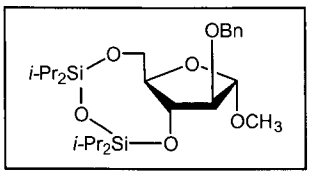
Methyl 3,5-*O*-tetraisopropylsiloxane-1,3-di-yl-α-D-arabinofuranoside (22).



To a compound of **16** (6.57 g, 40.1 mmol) in pyridine (50 mL) was added 1,3-dichloro-1,1,3,3-tetra-isopropyldisiloxane (15 mL, 46.9 mmol). The reaction mixture was stirred overnight at rt and concentrated. The residue was purified by column chromatography (4:1, hexanes: EtOAc) to obtain **22** as an oil (9.7 g, 60%). R_f 0.30 (5:1, hexanes:EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 4.79 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1), 4.17-4.16 (m, 2 H, H-2, H-3), 3.99 (dd, 1 H, $J_{4,5}$ = 3.0 Hz, $J_{5,5'}$ = 12.6 Hz, H-5), 3.95 (dd, 1 H, $J_{4,5'}$ = 3.9 Hz, $J_{5,5'}$ = 12.6 Hz, H-5′), 3.88 (ddd, 1 H, $J_{4,5}$ = 3.0, $J_{4,5'}$ = 3.9 Hz, $J_{3,4}$ = 6.9 Hz, H-4), 3.41 (s, 3 H, OCH_3),

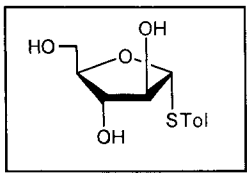
1.12-1.01 (m, 28 H, (i-Pr)$_2$Si); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 107.9 (C-1), 82.6 (C-2), 80.8 (C-4), 77.0 (C-3), 61.5 (C-5), 55.5 (OCH_3), 17.4 ((i-Pr)$_2$Si), 17.3 ((i-Pr)$_2$Si), 17.1 (2C, (i-Pr)$_2$Si), 17.0 (2C, (i-Pr)$_2$Si), 13.5 ((i-Pr)$_2$Si), 13.2 ((i-Pr)$_2$Si), 12.8 ((i-Pr)$_2$Si), 12.6 ((i-Pr)$_2$Si). Data matched previously reported one.[3]

Methyl 2-*O*-benzyl-α-D-arabinofuranoside (23).



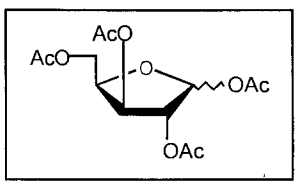
Compound **22** (3.3 g, 8.1 mmol) was dissolved in DMF (35 mL) and the solution was cooled to 0 °C. Benzyl bromide (2 mL, 16.8 mmol) was added, followed by addition of NaH (500 mg, 12.6 mmol). The mixture was stirred for 6 h at 0 °C and concentrated. The crude product was purified by chromatography (16:1, hexanes:EtOAc) to obtain **23** as an oil (3 g, 75%). R_f 0.65 (5:1, hexanes:EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 7.37-7.28 (m, 5 H, Ar), 4.82 (d, 1 H, $J_{1,2}$ = 2.4 Hz, H-1), 4.64 (d, 1 H, J = 11.9 Hz, OCH_2Ar), 4.62 (d, 1 H, J = 11.9 Hz, OCH_2Ar), 4.28 (dd, 1 H, $J_{2,3}$ = 5.9 Hz, $J_{3,4}$ = 8.2 Hz, H-3), 4.00 (dd, 1 H, $J_{4,5}$ = 3.0 Hz, $J_{5,5'}$ = 12.6 Hz, H-5), 3.97-3.93 (m, 2 H, H-2, H-5′), 3.88 (ddd, 1 H, $J_{4,5}$ = 3.0 Hz, $J_{4,5'}$ = 4.2 Hz, $J_{3,4}$ = 8.2 Hz, H-4), 3.36 (s, 3 H, OCH_3), 1.11-1.04 (m, 28 H, (i-Pr)$_2$Si); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 128.3 (Ar), 127.6 (2C, Ar), 106.7 (C-1), 89.7 (C-2), 80.6 (C-4), 76.3 (C-3), 72.5 (OCH_2Ar), 61.7 (C-5), 55.1 (OCH_3), 17.5 ((i-Pr)$_2$Si), 17.3 (2C, (i-Pr)$_2$Si), 17.1 (2C, (i-Pr)$_2$Si), 17.0 ((i-Pr)$_2$Si), 13.5 ((i-Pr)$_2$Si), 13.2 ((i-Pr)$_2$Si), 12.9 ((i-Pr)$_2$Si), 12.6 ((i-Pr)$_2$Si). Data matched previously reported one.[3]

_p_-Tolyl 1-thio-α-D-arabinofuranoside (24)



Compound **10** (5 g, 8.8 mmol) was dissolved in 1:1 mixture of CH_2Cl_2/CH_3OH (100 mL), and treated with a catalytic amount of $NaOCH_3$ in CH_3OH at rt. After stirring for 6 h, the reaction mixture was neutralized with Amberite IR-120 H^+ resin, filtered and concentrated. The crude product was purified by column chromatography (3:1, hexanes:EtOAc, then 95% EtOH) to give **24** as an oil (2.2 g, 98%). R_f 0.89 (2:1, EtOAc:CH_3OH); ^1H NMR (600 MHz, CD_3OD, δ_H) 5.19 (d, 1 H, $J_{1,2}$ = 4.7 Hz, H-1), 3.97 (dd, 1 H, $J_{1,2} = J_{2,3}$ = 4.7 Hz, H-2), 3.95-3.91 (m, 2 H, H-3, H-4), 3.76 (dd, 1 H, $J_{4,5}$ = 2.6 Hz, $J_{5,5'}$ = 12.1 Hz, H-5), 3.64 (dd, 1 H, $J_{4,5}$ = 4.6 Hz, $J_{5,5'}$ = 12.1 Hz, H-5′), 2.29 (s, 3 H, $ArC\underline{H}_3$); ^{13}C NMR (125 MHz, CD_3OD, δ_c) 138.7 (Ar), 133.4 (Ar), 132.4 (Ar), 130.6 (Ar), 93.4 (C-1), 84.3 (C-4), 83.3 (C-2), 77.7 (C-3), 62.5 (C-5), 21.2 ($Ar\underline{C}H_3$). Data matched previously reported one.[84]

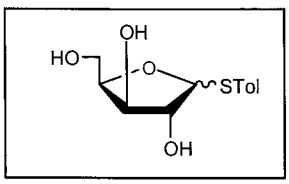
1,2,3,5-tetra-*O*-acetyl-D-xylofuranose (26).



H_3BO_3 (9.1 g, 146.8 mmol) was added to a solution of D-xylose (10.06 g, 67.1 mmol) in acetic acid (150 mL). The reaction mixture was stirred at 50 °C for 1 h. Subsequently, acetic anhydride (150 mL) and H_2SO_4 (0.15 mL) were added. The reaction mixture was continuously stirred at 50 °C for 3 h before adding ice (50 g). The solution was stirred for 1 h at rt, followed by extraction with CH_2Cl_2. The organic layer was washed with a saturated solution of sodium bicarbonate and water. After drying the organic extract with

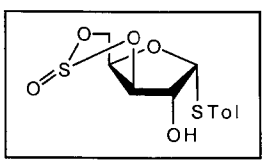
anhydrous Na_2SO_4, the solution was concentrated. The residue was purified by chromatography (3:1, hexanes:EtOAc) to obtain **26** as an oil (1.2 g, 56%). Data matched previously reported one.[66]

_p_-Tolyl 2,3,5-tri-_O_-acetyl-1-thio-D-xylofuranoside (27) and _p_-Tolyl 1-thio-D xylofuranoside (28).



Compound **26** (12 g, 37.7 mmol) was dissolved in CH_2Cl_2 (100 mL). The solution was cooled to 0 °C and $BF_3{\cdot}Et_2O$ (19 mL, 150.5 mmol) was added. The mixture was stirred for 10 min, and then *p*-thiocresol (5 g, 42.0 mmol) was added to the mixture. The solvent was evaporated and the residue was purified by chromatography (4:1, hexanes:EtOAc) to give **27** as an oil (11 g, 76%). To a solution of **27** (11 g, 28.8 mmol) in a 1:1 mixture of CH_2Cl_2/CH_3OH (100 mL) was added a catalytic amount of $NaOCH_3$ in CH_3OH. After stirring for 8 h, the reaction mixture was neutralized with Amberite IR-120 H^+ resin, filtered and concentrated. The crude product was purified by chromatography (2:1, hexanes:EtOAc, then 95% EtOH) to obtain **28** as an oil (7.1 g, 27.7 mmol, 97%). Data matched previously reported one.[66]

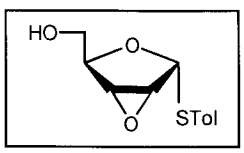
_p_-Tolyl 3,5-_O_-sulfinyl-1-thio-α-D-xylofuranoside (29).



Pyridine (11 mL, 134 mmol) and thionyl chloride (10.1 mL, 136 mmol) were added to a solution of compound **28** (6.2 g, 24.2 mmol)

in CH_3CN (120 mL) at 0 °C. After stirring for 5 h, water (20 mL) was added and the mixture was stirred for 10 additional min. The solution was then extracted with ethyl acetate (100 mL x 3), and the organic layer was washed with water and brine. After drying with anhydrous Na_2SO_4, the solution was filtered, and concentrated. The crude product was purified by chromatography (1:1, hexanes:EtOAc) to obtain **29** as a white solid (0.9 g, 12%). R_f 0.43 (1:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.44 (m, 2 H, Ar), 7.16 (m, 2 H, Ar), 5.75 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.98 (d, 1 H, $J_{3,4}$ = 2.0 Hz, H-3), 4.93 (dd, 1 H, $J_{4,5}$ = 2.1 Hz, $J_{5,5'}$ = 13.0 Hz, H-5), 4.43 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-2), 4.33 (dd, 1 H, $J_{3,4}$ = 2.0 Hz, H-4), 4.15 (dd, 1 H, $J_{4,5'}$ = 0.6 Hz, $J_{5,5'}$ = 13.0 Hz, H-5′) 2.35 (s, 3 H, ArC<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.3 (Ar), 132.6 (Ar) 130.1 (Ar) 129.0 (Ar), 93.7 (C-1), 76.6 (C-2), 72.0 (C-4), 71.1 (C-3), 55.9 (C-5), 21.1 (Ar<u>C</u>H$_3$). Data matched previously reported one.[66]

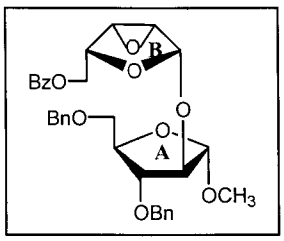
_p_-Tolyl 2,3-anhydro-1-thio-α-D-ribofuranoside (30).



Compound **29** (489.5 mg, 1.6 mmol) was dissolved in DMF (10 mL), then triethylamine (1.2 mL, 7.9 mmol) and $NaHCO_3$ (673 mg, 7.9 mmol) were added. The reaction mixture was heated at 90 °C and stirred overnight. The solution was concentrated and the crude product was purified by chromatography (1:2, hexanes:EtOAc) to obtain **30** as an oil (208 mg, 54%). R_f 0.21 (1:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.45 (m, 2 H, Ar), 7.14 (m, 2 H, Ar), 5.42 (d, 1 H, $J_{1,2}$ = 1.0 Hz, H-1), 4.42 (dd, 1 H, $J_{4,5}$ = $J_{4,5'}$ = 4.2 Hz, H-4), 4.02 (dd, 1 H, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 2.8 Hz, H-2), 3.81-3.67 (m, 3 H, H-3, H-5, H-5′), 2.34 (s, 3 H,

Ar$\underline{C}H_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 137.8 (Ar), 132.5 (Ar), 130.3 (Ar) 129.8 (Ar), 87.7 (C-1), 80.4 (C-4), 62.9 (C-5), 59.5 (C-2), 58.0 (C-3), 21.1 (Ar$\underline{C}H_3$). Data matched previously reported one.[66]

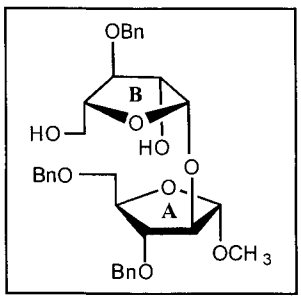
Methyl 2-*O*-(2,3-anhydro-5-*O*-benzoyl-β-D-lyxofuranosyl)-3,5-di-*O*-benzyl-α-D-arabinofuranoside (31).



The donor **11** (205 mg, 0.6 mmol) and the acceptor **7** (172 mg, 0.5 mmol) were combined and vacuum dried overnight over P_2O_5. Molecular sieves (4 Å, 0.3 g) were added followed by CH_2Cl_2 (10 mL). The mixture was cooled to –40 °C and *N*-iodosuccinimide (135 mg, 0.6 mmol) followed by silver triflate (38.5 mg, 0.15 mmol) were added. After stirring for 15-30 min at this temperature, the reaction mixture turned dark red/brown, it was then neutralized by addition of triethylamine. The solution mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was concentrated and the residue was purified by chromatography to obtain **31** as an oil (228 mg, 81%). R_f 0.32 (2:1, hexanes:EtOAc); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 8.05-8.02 (m, 2 H, Ar_B), 7.57-7.53 (m, 2 H, Ar_B), 7.43-7.22 (m, 10 H, Ar_A), 5.24 (d, 1 H, $J_{1,2}$ = 0.6 Hz, H_B-1), 4.89 (s, 1 H, H_A-1), 4.72 (d, 1 H, J = 12.0 Hz, $OC\underline{H}_2Ar_A$), 4.59-4.50 (m, 5 H, $OC\underline{H}_2Ar_A$, H_B-5, H_B-5′), 4.38 (dd, 1 H, $J_{1,2}$ = 1.3 Hz, $J_{2,3}$ = 3.1 Hz, H_A-2), 4.25 (dt, 1 H, $J_{3,4}$ = 1.0 Hz, $J_{4,5}$ = 6.0 Hz, H_B-4), 4.19 (ddd, 1 H, $J_{4,5}$ = 3.7, $J_{4,5'}$ = 5.6 Hz, $J_{3,4}$ = 6.6 Hz, H_A-4), 4.00 (dd, 1 H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 6.6 Hz, H_A-3), 3.80 (dd, 1 H, $J_{3,4}$ = 1.0 Hz, $J_{2,3}$ = 2.9 Hz, H_B-3), 3.76 (dd, 1 H, $J_{1,2}$ = 0.6 Hz, $J_{2,3}$ = 2.9 Hz, H_B-2), 3.62 (dd, 1 H, $J_{4,5}$ = 3.7 Hz, $J_{5,5'}$ = 10.7 Hz , H_A-5),

3.57 (dd, 1H, $J_{4,5'}$ = 5.6 Hz, $J_{5,5'}$ = 10.8 Hz, H_A-5'), 3.4(s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 166.2 (C=O), 138.1 (Ar), 137.9 (Ar), 133.2 (Ar), 129.7 (Ar), 128.4(2 C, Ar), 128.3 (2 C, Ar), 128.0 (Ar), 127.6 (Ar), 107.8 (C_A-1), 101.2 (C-$_B$1), 87.2 (C_A-2), 83.3 (C_A-3), 80.9 (C_A-4), 74.6 (C_B-4), 73.4 ($O\underline{C}_BH_2Ar$), 72.1 ($O\underline{C}_BH_2Ar$), 69.8 (C_A-5), 63.1 (C_B-5), 56.1 (C_B-2), 55.1 (OCH_3), 55.0 (C_B-3). Data matched previously reported one.[65]

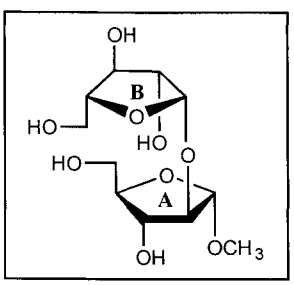
Methyl 2-*O*-(3-*O*-benzyl-β-D-arabinofuranosyl)-3,5-di-*O*-benzyl-α-D-arabinofuranoside (32).



Lithium metal (2.14 mmol) was added to benzyl alcohol (1 mL) and the solution was stirred at 65 °C for 15 min before (-)-sparteine (2.14 mmol) was added. After cooling, the mixture was added to a solution of the epoxide **31** (227.6 mg, 0.40 mmol) in benzyl alcohol (0.30 mL). The mixture was subsequently warmed to 70 °C and allowed to stir until the reaction was completed as observed by TLC. After cooling to rt, the solution was neutralized with acetic acid and diluted with CH_2Cl_2. The organic layer was washed with water, dried, filtered, and concentrated. The crude product was purified by chromatography (4:1, hexanes:EtOAc). The product obtained (190 mg, 0.34 mol) was dissolved in pyridine (15 mL) and acetic anhydride (0.30 mL, 3.1 mmol) was added to the mixture. The residue was purified by chromatography (9:1, toluene:EtOAc) to give **33** as an oil. To a solution of **32** (200 mg, 0.31 mmol) in 1:1 mixture of CH_2Cl_2/CH_3OH (100 mL) was added a catalytic amount of $NaOCH_3$ in CH_3OH and the crude product was

purified to afford **32** as an oil (146.6 mg, 64%). R_f 0.24 (1:1, hexanes:EtOAc); $[\alpha]_D$ +11.64 (*c* 0.5, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.37-7.27 (m, 15 H, Ar), 5.12 (d, 1 H, $J_{1,2}$ = 4.9 Hz, H_B-1), 4.93 (s, 1 H, H_A-1), 4.81 (d, 1 H, J= 11.8 Hz, $OCH_{2B}Ar$), 4.66 (d, 1 H, J = 12.1 Hz, OCH_2Ar), 4.61-4.50 (m, 4 H, $OCH_{2B}Ar$), 4.32 (d, 1 H, $J_{2,3}$ = 2.7 Hz, H_A-2), 4.25 (dd, 1 H, $J_{3,4}$ = 4.9 Hz, $J_{2,3}$ = 6.2 Hz, H_B-2), 4.16 (dt, 1 H, $J_{4,5}$ = 3.8 Hz, $J_{3,4}$ = 6.1 Hz, H_A-4), 4.11 (dd, 1 H, $J_{2,3}$ = 2.7 Hz, $J_{3,4}$ = 6.1 Hz, H_A-3), 4.01 (ddd, 1 H, $J_{4,5}$ = 3.1 Hz, $J_{4,5}$ = 4.4 Hz, $J_{3,4}$ = 6.2 Hz, H_B-4), 3.95 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 6.2 Hz, H_B-3), 3.64-3.61 (m, 2 H, H_A-5, H_B-5), 3.53-3.50 (m, 2 H, H_A-5′, H_B-5′), 3.4 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 137.8 (Ar), 137.7 (Ar), 137.6 (Ar), 129.0 (Ar), 128.9 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 127.8 (Ar), 127.8 (2 C, Ar), 127.7 (2 C, Ar), 127.6 (Ar), 125.2 (Ar), 106.9 (C_A-1), 101.5 (C-$_B$1), 86.6 (C_A-2), 82.6 (3 C, C_A-3, C_B-3, C_B-4), 81.3 (C_A-4), 77.8 (C_B-2), 73.4 (OCH_2Ar), 72.2 (OCH_2Ar), 72.1 (OC_BH_2Ar), 69.0 (C_A-5), 62.9 (C_B-5), 54.9 (OCH_3); HRMS calcd for $C_{32}H_{38}O_9Na$ ($M+Na^+$) 589.24087, found 589.24080.

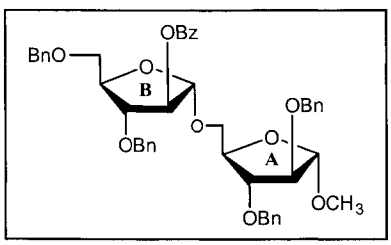
Methyl 2-*O*-(β-D-arabinofuranosyl)-α-D-arabinofuranoside (1).



Compound **32** (48.9 mg, 0.086 mmol) was dissolved in a 4:1 mixture of CH_3OH/EtOAc (2.5 mL). The catalyst, 20% $Pd(OH_2)$ (60 mg) was added and the reaction mixture was stirred at rt under a hydrogen atmosphere until completion as observed by TLC. The mixture was filtered through Celite, and concentrated to give **1** as an oil (25.2 mg, 98%). R_f 0.36 (3:1, CH_2Cl_2:CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 4.93 (d, 1 H,

$J_{1,2}$ = 4.3 Hz, H_B-1), 4.85 (d, 1 H, $J_{1,2}$ = 2.1 Hz, H_A-1), 4.04-3.94 (m, 4 H, H_A-2, H_B-2, H_A-3, H_B-3), 3.85 (ddd, 1 H, J = 2.8, J = 5.0, J = 7.6 Hz, H_A-4), 3.77-3.74 (m, 2 H, H_B-4, H_A-5), 3.71 (dd, 1 H, $J_{4,5}$ = 3.3 Hz, $J_{5,5'}$ = 11.9 Hz, H_B-5), 3.63-3.59 (m, 2 H, H_A-5′, H_B-5′), 3.35 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_c) 108.4 (C_A-1), 102.5 (C-$_B$1), 89.5 (C_A-2), 84.4 (C_B-4), 83.8 (C_A-4), 78.8 (C_B-2), 76.4, 75.8 (2 C, C_A-3, C_B-3), 64.4 (C_B-5), 62.5 (C_A-5), 55.4 (OCH_3); HRMS calcd for $C_{11}H_{20}O_9Na$ ($M+Na^+$) 319.10018, found 319.09995. Data matched previously reported one.[3]

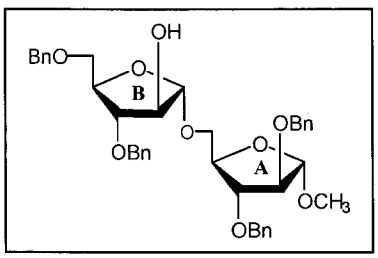
Methyl 5-*O*-(2-*O*–benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (34).



The donor **13** (109 mg, 0.32 mmol) and the acceptor **8** (202 mg, 0.38 mmol) were combined and dried under vaccum overnight over P_2O_5. Molecular sieves (4 Å, 0.2 g) was added followed by CH_2Cl_2 (10 mL). The mixture was cooled to –40 °C, and *N*-iodosuccinimide (85.5 mg, 0.379 mmol) and silver triflate (25.7 mg, 0.10 mmol) were added. After stirring for 30-60 min at this temperature, the reaction mixture turned dark red/brown, then it was neutralized by the addition of triethylamine. The solution was then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was concentrated and the crude residue was purified by chromatography (5:1, hexanes:EtOAc) to obtain **34** as an oil (178 mg, 74%). R_f 0.36 (3:1, hexanes:EtOAc); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.99-7.21 (m, 25 H, Ar), 5.46 (d, 1 H, $J_{2,3}$ = 1.3 Hz, H_B-2), 5.28 (s, 1 H, H_B-1), 4.93 (s, 1 H, H_A-1), 4.82-4.46 (m, 1 H, OCH_2Ar), 4.32 (dd, 1 H, $J_{4,5'}$

= 5.1 Hz, $J_{4,5}$ = 3.8 Hz, H_B-4), 4.22 (m, 1H, H_A-4), 4.06 (dd, 1 H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 6.4 Hz, H_A-3), 4.00 (Broad d, 1 H, H_B-3), 3.98 (dd, 1H, $J_{1,2}$ = 1.3 Hz, $J_{2,3}$ = 3.1 Hz, H_A-2), 3.94 (dd, 1H, $J_{4,5}$ = 4.5 Hz, $J_{5,5'}$ = 11.2 Hz, H_A-5), 3.70 (dd, 1 H, $J_{4,5'}$ = 4.3 Hz, $J_{5,5'}$ = 11.2 Hz, H_A-5′), 3.65 (dd, 1 H, $J_{4,5}$ = 3.8 Hz, $J_{5,5'}$ = 10.8 Hz , H_B-5), 3.59 (dd, 1 H, $J_{4,5'}$ = 5.1 Hz, $J_{5,5'}$ = 10.8 Hz, H_B-5′), 3.37 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 165.3 (C=O), 138.1 (Ar), 138.0 (Ar), 137.8 (Ar), 137.7 (Ar), 133.3 (Ar), 129.8 (Ar), 129.6 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (2 C, Ar), 127.9 (Ar), 127.9 (2 C, Ar), 127.8 (Ar), 127.7 (2 C, Ar), 127.6 (2 C, Ar), 107.3 (C_A-1), 106.3 (C_B-1), 88.2 (C_B-3), 83.6 (C_A-2), 83.3 (C_A-3), 82.4 (C_B-4), 81.7 (C_B-2), 80.6 (C_A-4), 73.4 ($CH_2O\underline{C}H_2Ar$), 72.3 ($CH_2O\underline{C}H_2Ar$), 72.2 ($CH_2O\underline{C}_BH_2Ar$), 71.9 ($CH_2O\underline{C}_BH_2Ar$), 69.4 ($C_A$-5), 66.3 ($C_B$-5), 55.0 ($OCH_3$). Data matched previously reported one.[3]

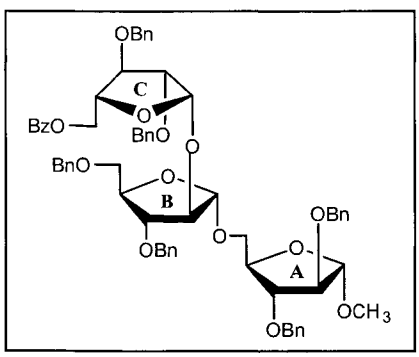
Methyl 5-*O*-(3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (35).



Compound **34** (174.3 mg, 0.23 mmol) was dissolved in 1:1 mixture of CH_2Cl_2/CH_3OH (5 ml). The solution was treated with a catalytic amount of $NaOCH_3$ in CH_3OH at rt. After stirring for 3 h, the reaction mixture was neutralized with Amberite IR-120 H^+ resin, filtered and concentrated. The crude product was purified by column chromatography (4:1, hexanes:EtOAc) to obtain **35** as an oil (142 mg, 94%). R_f 0.47 (1:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.35-7.23 (m, 20 H, Ar), 5.10 (s, 1 H, H_B-1), 4.93 (s, 1 H, H_A-1), 4.64-4.44 (m, 8 H, $OC\underline{H}_2Ar$), 4.22 (m,

1 H, H_B-4), 4.20 (br-s, 1 H, H_B-2), 4.18 (m, 1 H, H_A-4), 4.08 (dd, 1 H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 6.5 Hz, H_A-3), 3.99 (d, 1 H, $J_{2,3}$ = 3.1 Hz, H_A-2), 3.91 (dd, 1 H, $J_{4,5}$ = 3.9 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5), 3.88 (dd, 1 H, $J_{2,3}$ = 1.7 Hz, $J_{3,4}$ = 3.8 Hz, H_B-3), 3.71 (dd, 1 H, $J_{4,5'}$ = 3.6 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5′), 3.65 (dd, 1 H, $J_{4,5}$ = 2.4 Hz, $J_{5,5'}$ = 10.5 Hz, H_B-5), 3.51 (dd, 1 H, $J_{4,5'}$ = 2.8 Hz, $J_{5,5'}$ = 10.5 Hz, H_B-5′), 3.38 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 128.5 (3 C, Ar), 128.4 (2 C, Ar), 128.3 (2 C, Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (2 C, Ar), 127.7 (Ar), 127.6 (Ar), 109.3 (C_B-1), 107.2 (C_A-1), 88.3 (C_A-2), 84.9 (C_B-3), 83.1 (C_B-4), 83.0 (C_A-3), 80.9 (C_A-4), 78.1 (C_B-2), 73.7 ($O\underline{C}H_2Ar$), 72.3 ($O\underline{C}H_2Ar$), 72.0 ($O\underline{C}_BH_2Ar$), 71.9 ($O\underline{C}_BH_2Ar$), 69.8 (C_B-5), 66.2 (C_A-5), 54.9 (OCH_3). Data matched previously reported one.[3]

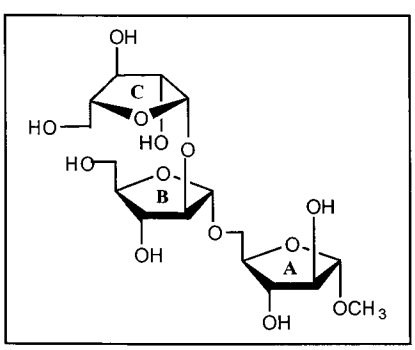
Methyl 2,3-di-*O*-(3,5-di-*O*-benzyl-2-*O*-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-α-D-arabinofuranosyl)-benzyl-5-*O*-α-D-arabinofuranoside (36).



The donor **12** (137.2 mg, 0.263 mmol) and the acceptor **35** (140 mg, 0.219 mmol) were combined and dried under vaccum overnight over P_2O_5. Molecular sieves (4 Å, 0.2 g) were added followed by CH_2Cl_2 (5 ml). The mixture was cooled to –40 °C, and *N*-iodosuccinimide (59.3 mg, 0.26 mmol) and silver triflate (20 mg, 0.078 mmol) were added. After stirring for 30-60 min at this temperature, the reaction mixture turned dark red/brown, and was neutralized by addition of triethylamine. The solution was then diluted with CH_2Cl_2 (10 mL) and filtered through Celite. The filtrate was concentrated

and the crude residue was purified by chromatography (3:1, hexanes:EtOAc) to obtain **36** as an oil (181 mg, 65%). R_f 0.32 (hexanes:EtOAc, 0.32); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 7.36-7.20 (m, 35 H, Ar), 5.13 (s, 1 H, H_B-1), 5.12 (d, 1 H, $J_{1,2}$ = 4.4 Hz, H_C-1), 4.91 (s, 1 H, H_A-1), 4.69-4.35 (m, 14 H, OC<u>H</u>$_2$-Ar, H_B-2), 4.25 (m, 1 H, H_B-4), 4.18 (m, 1 H, H_A-4), 4.12-4.09 (m, 2 H, H_C-3, H_C-4), 4.07 (dd, 1 H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 6.7 Hz, H_A-3), 4.04-4.02 (m, 2 H, H_B-3, H_C-2), 3.99 (d, 1 H, $J_{2,3}$ = 3.1 Hz, H_A-2), 3.91 (dd, 1 H, $J_{4,5}$ = 4.2 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5), 3.73 (dd, 1 H, $J_{4,5'}$ = 3.4 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5′) 3.62-3.52 (m, 4 H, H_B-5, H_B-5′, H_C-5, H_C-5′), 3.35 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.2 (2C, Ar), 138.1 (Ar), 138.0 (Ar), 137.7 (Ar), 138.7 (Ar), 128.5 (Ar), 128.4 (3 C, Ar), 128.3 (2 C, Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (3 C, Ar), 127.7 (2C, Ar), 127.6 (Ar), 127.5 (2 C, Ar), 107.2 (C_A-1), 104.4 (C_B-1), 100.4 (C_C-1), 88.3 (C_A-2), 85.8 (C_B-2), 84.3 (C_B-3), 84.0 (C_C-3), 83.3 (C_A-3), 83.1 (C_C-2), 81.5 (C_B-4), 80.7 (C_A-4), 80.0 (C_C-4), 73.3 (O<u>C</u>H$_2$Ar), 73.1 (O<u>C</u>H$_2$Ar), 72.4 (C_C-5), 72.4 (O<u>C</u>$_B$H$_2$Ar), 72.3 (O<u>C</u>$_B$H$_2$Ar), 72.2 (O<u>C</u>$_B$H$_2$Ar), 72.2 (O<u>C</u>$_B$H$_2$Ar), 71.9 (O<u>C</u>$_B$H$_2$Ar), 70.1 (C_B-5), 66.0 (C_A-5), 54.9 (OCH_3). Data matched previously reported one.[3]

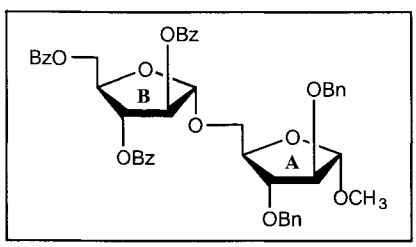
Methyl 5-*O*-((2-*O*-β-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (2).



Compound **36** (70 mg, 0.066 mmol) was dissolved in a 4:1 mixture of CH_3OH/EtOAc (2.5 ml). The catalyst, 20% Pd(OH_2) (50 mg) was added and the reaction mixture was stirred under a hydrogen atmosphere until

completion of the reaction. The mixture was filtered through Celite, and concentrated. The crude product was purified (3:1, CH_2Cl_2:CH_3OH) on Iatrobeads to give **2** as an oil (25.1 mg, 89%). R_f 0.28 (3:1, CH_2Cl_2:CH_3OH); ^{1}H NMR (500 MHz, D_2O, δ_H) 5.18 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H_B-1), 5.15 (d, 1 H, $J_{1,2}$ = 4.7 Hz, H_C-1), 4.94 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H_A-1), 4.21 (dd, 1 H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.9 Hz, H_B-2), 4.18-4.13 (m, 3 H, H_A-4, H_C-2, H_B-3), 4.10-4.04 (m, 3 H, H_B-4, H_A-2, H_C-3), 4.00 (dd, 1 H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 5.9 Hz, H_A-3), 3.93-3.67 (m, 7 H, H_C-4, H_A-5, H_A-5′, H_B-5, H_B-5′, H_C-5, H_C-5′), 3.42 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, D_2O , δ_c) 109.3 (C_A-1), 106.6 (C_B-1), 101.5 (C_C-1), 87.7 (C_B-2), 83.8 (C_B-3), 83.1 (C_A-4), 82.9 (C_C-4), 81.5 (C_B-4), 77.4 (C_A-3), 77.1 (C_C-2), 75.7 (C_A-2), 75.0 (C_C-3), 67.7 (C_A-5), 63.9 (C_C-5), 61.5 (C_B-5), 55.9 (OCH_3). Data matched previously reported one.[3]

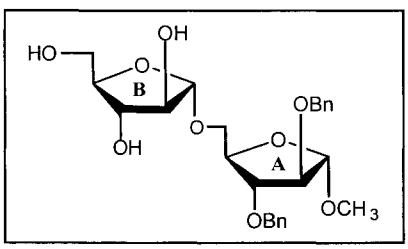
Methyl 5-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (37).



The donor **10** (693 mg, 1.22 mmol) and the acceptor **8** (350 mg, 1.017 mmol) were combined and dried under vaccum overnight over P_2O_5. Molecular sieves (4 Å, 0.5 g) were added followed by CH_2Cl_2 (10 mL). The mixture was cooled to –40 °C, and *N*-iodosuccinimide (275 mg, 1.22 mmol) and silver triflate (87.36 mg, 0.314 mmol) were added. After stirring for 30-60 min at this temperature, the solution turned dark red/brown, and was neutralized by addition of triethylamine. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered through

Celite. The filtrate was concentrated and the residue was purified by chromatography (3:1, hexanes:EtOAc) to obtain **37** as an oil (739 mg, 91%). R_f 0.28 (3:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.07-7.21 (m, 25 H, Ar), 5.59 (s, 1 H, H_B-2), 5.55 (d, 1 H, $J_{3,4}$ = 5.3 Hz, H_B-3), 5.36 (s, 1 H, H_B-1), 4.95 (d, 1 H, $J_{1,2}$ = 0.6 Hz, H_A-1), 4.80 (dd, 1 H, $J_{4,5}$ = 3.3 Hz, $J_{5,5'}$ = 12.0 Hz, H_B-5), 4.63 (dd, 1 H, $J_{4,5'}$ = 4.9 Hz, $J_{5,5'}$ = 12.0 Hz, H_B-5′), 4.58-4.43 (m, 5 H, $OC\underline{H}_2Ar$, H_B-4), 4.23 (m, 1 H, H_A-4), 4.03-4.00 (m, 2 H, H_A-2, H_A-3), 3.95 (dd, 1 H, $J_{4,5}$ = 4.8 Hz, $J_{5,5'}$ = 11.0 Hz, H_A-5), 3.74 (dd, 1 H, $J_{4,5'}$ = 3.8 Hz, $J_{5,5'}$ = 11.0 Hz, H_A-5′), 3.37 (s, 3 H, OCH_3); ^{13}C NMR (125MHz, $CDCl_3$, δ_c) 166.2 (C=O), 165.8 (C=O), 165.3 (C=O), 137.8 (Ar), 137.5 (Ar), 133.4 (Ar), 133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 129.2 (Ar), 129.1 (Ar), 128.5 (Ar), 128.4 (2 C, Ar), 128.3 (Ar), 127.9 (2 C, Ar), 127.7 (Ar), 107.2 (C_B-1), 105.8 (C_A-1), 88.3 (C_A-2), 83.3 (C_A-3), 81.9 (C_B-2), 81.4 (C_B-4), 80.2 (C_A-4), 77.9 (C_B-3), 72.2 ($O\underline{C}H_2Ar$), 72.1 ($O\underline{C}H_2Ar$), 66.5 (C_A-5), 63.7 (C_B-5), 54.9 (OCH_3). Data matched previously reported one.[3]

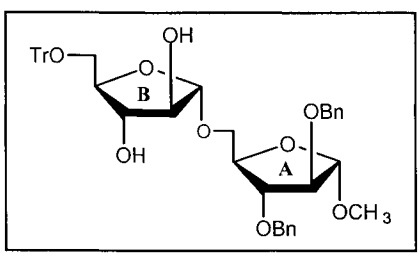
Methyl 5-*O*-(α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (38).



Compound **37** (600 mg, 0.76 mmol) was dissolved in an 1:1 mixture of CH_2Cl_2/ CH_3OH (15 mL) and the solution was treated with a catalytic amount of $NaOCH_3$ in CH_3OH at rt. After stirring for 8 h, the reaction mixture was neutralized with Amberite IR-120 H^+ resin, filtered and concentrated. The crude product was purified by column chromatography (4:1, hexanes:EtOAc) to obtain **38** as an oil (355 mg, 98%). R_f 0.11 (1:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.47-

7.27 (m, 10 H, Ar), 5.02 (s, 1 H, H_B-1), 4.93 (s, 1 H, H_A-1), 4.59-4.38 (m, 4 H, $OC\underline{H}_2Ar$), 4.17 (m, 1 H, H_A-4), 4.02 (s, 1 H, H_B-2), 3.97 (d, 1 H, $J_{2,3}$ = 2.1 Hz, H_A-2), 3.94 (s, 1H, H_B-3), 3.89 (m, 1H, H_B-4), 3.82-3.78 (m, 3 H, H_A-3, H_A-5, H_B-5), 3.74 (dd, 1 H, $J_{4,5'}$ = 1.9 Hz, $J_{5,5'}$ = 11.6 Hz, H_B-5′), 3.65 (dd, 1 H, $J_{4,5'}$ = 3.1 Hz, $J_{5,5'}$ = 10.7 Hz, H_A-5′), 3.36 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 137.5 (Ar), 137.2 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 107.6 (C_B-1), 107.1 (C_A-1), 87.2 (C_B-4), 87.0 (C_A-2), 83.6 (C_A-3), 80.9 (C_A-4), 78.8 (C_B-2), 78.0 (C_B-3), 72.1 ($O\underline{C}H_2Ar$), 72.0 ($O\underline{C}H_2Ar$), 65.9 (C_A-5), 62.0 (C_B-5), 54.9 (OCH_3). Data matched previously reported one.[3]

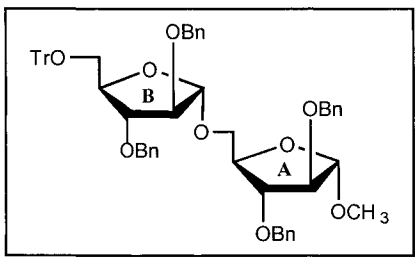
Methyl 5-*O*-(5-*O*-triphenylmethyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D arabinofuranoside (39).



Compound **38** (317.4 mg, 0.667 mmol) was dissolved in pyridine (5 mL) and trityl chloride (279 mg, 1 mmol) was added. The reaction mixture was stirred at 45 °C overnight, concentrated and purified (2:1, hexanes:EtOAc) to obtain **39** as an oil (249 mg, 52%). R_f 0.42 (2:1, hexanes:EtOAc); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.43-7.19 (m, 25 H, Ar), 5.11 (s, 1 H, H_B-1), 4.92 (s, 1 H, H_A-1), 4.58-4.37 (m, 4 H, $OC\underline{H}_2Ar$), 4.17 (m, 1H, H_A-4), 4.01 (s, 1 H, H_B-3), 3.95 (d, 1 H, $J_{2,3}$ = 2.1 Hz, H_A-2), 3.88 (s, 1 H, H_B-2), 3.82 (m, 2 H, H_B-4, H_A-5), 3.77 (dd, 1H, $J_{2,3}$ = 2.1 Hz, $J_{3,4}$ = 5.8 Hz, H_A-3), 3.68 (dd, 1 H, $J_{4,5'}$ = 3.1 Hz, $J_{5,5'}$ = 10.6 Hz , H_A-5′), 3.53 (dd, 1 H, $J_{4,5}$ = 2.8 Hz, $J_{5,5'}$ = 10.5 Hz, H_B-5), 3.34 (s, 3 H, OCH_3), 3.18 (dd, 1 H, $J_{4,5'}$ =

2.2 Hz, $J_{5,5'}$ = 10.5 Hz, H_B-5′); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 142.9 (Ar), 137.5 (Ar), 137.2 (Ar), 128.8 (Ar), 128.5 (Ar), 128.5 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.4 (Ar), 107.4 (C_B-1), 107.1 (C_A-1), 88.3 $(Ph)_3\underline{C}$), 87.0 (C_A-2), 86.3 (C_B-4), 83.6 (C_A-3), 81.0 (C_A-4), 78.9 (C_B-3), 78.1 (C_B-2), 72.0 (2C, $O\underline{C}H_2Ar$), 65.9 (C_A-5), 63.5 (C_B-5), 54.9 (OCH_3). Data matched previously reported one.[3]

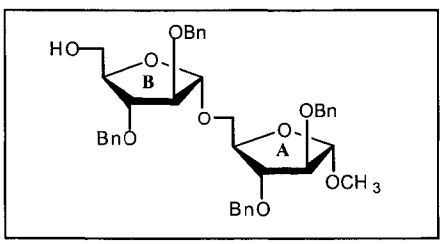
Methyl 5-*O*-(2,3-di-*O*-benzyl-5-*O*-triphenylmethyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D arabinofuranoside (40).



Compound **39** (274.9 mg, 0.382 mmol) was dissolved in THF (10 mL) and NaH (100 mg, 2.51 mmol) was added to the solution at 0 °C. The solution was stirred for 10 min, and benzyl bromide (0.5 ml, 4.1 mmol) was added. The reaction mixture was stirred for 8 h at rt, and diluted with CH_2Cl_2 (20 mL). The organic layer was washed with water, dried over $MgSO_4$ and concentrated. The residue obtained was purified by chromatography (8:1, hexanes:EtOAc) to afford **39** as an oil (226.9 mg, 66%). R_f 0.44 (3:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.46-7.17 (m, 35 H, Ar), 5.20 (s, 1 H, H_B-1), 4.94 (s, 1 H, H_A-1), 4.56-4.42 (m, 8 H, $OC\underline{H}_2Ar$), 4.21-4.17 (m, 2 H, H_A-4, H_B-4), 4.07-4.04 (m, 2 H, H_B-2, H_B-3), 4.01 (dd, 1 H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 6.3 Hz, H_A-3), 3.99 (d, 1 H, $J_{2,3}$ = 3.0 Hz, H_A-2), 3.90 (dd, 1 H, $J_{4,5}$ = 4.4 Hz, $J_{5,5'}$ = 11.5 Hz , H_A-5), 3.72 (dd, 1 H, $J_{4,5'}$ = 3.7 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5′), 3.37 (s, 3 H, OCH_3), 3.28 (dd, 1 H, $J_{4,5}$ = 4.4 Hz, $J_{5,5'}$ = 10.1 Hz, H_B-5), 3.23 (dd, 1 H, $J_{4,5'}$ =

4.7 Hz, $J_{5,5'}$ = 10.1 Hz, H_B-5′); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 144.0 (Ar), 138.0 (Ar), 137.8 (Ar), 137.6 (Ar), 128.8 (Ar), 128.4 (2 C, Ar), 128.3 (3 C, Ar), 128.3 (Ar), 127.9 (Ar), 127.8 (2 C, Ar), 127.7 (2 C, Ar), 127.6 (Ar), 127.5 (Ar), 126.9 (Ar), 107.3 (C_A-1), 106.4 (C_B-1), 88.5 (C_A-2), 88.1 (C_B-2), 86.6 ($(Ph)_3\underline{C}$), 83.8 (C_A-3), 83.3 (C_B-3), 81.1, 80.7 (C_A-4, C_B-4), 72.4 ($O\underline{C}H_2Ar$), 72.0 ($O\underline{C}H_2Ar$), 71.9 ($O\underline{C}H_2Ar$), 71.8 ($O\underline{C}H_2Ar$), 66.1 (C_A-5), 63.6 (C_B-5), 54.9 (OCH_3). Data matched previously reported one.[3]

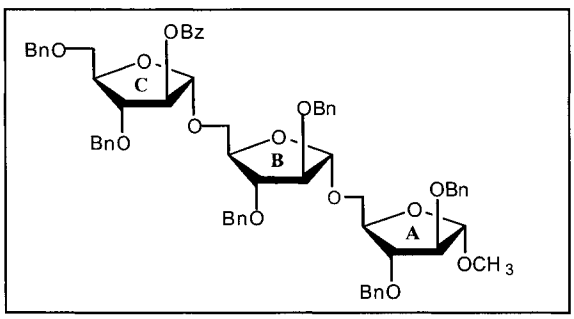
Methyl 5-*O*-(2,3-di-*O*-benzyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (41).



Compound **40** (321 mg, 0.357 mmol) was dissolved in a 4:1 mixture of CH_2Cl_2/CH_3OH (5 mL). *p*-TsOH was added until pH of the solution reached 4. The reaction mixture was stirred at rt overnight. The mixture was then concentrated and purified by chromatography (2:1, hexanes:EtOAc) to obtain **41** as an oil (175.9 mg, 75%). R_f 0.32 (2:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.35-7.26 (m, 20 H, Ar), 5.16 (s, 1 H, H_B-1), 4.94 (s, 1 H, H_A-1), 4.59-4.52 (m, 8 H, $OC\underline{H}_2Ar$), 4.17 (m, 1 H, H_A-4), 4.11 (m, 1 H, H_B-4), 4.07 (dd, 1 H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 6.7 Hz, H_A-3), 4.03 (d, 1 H, $J_{2,3}$ = 3.1 Hz, H_A-2), 3.97 (dd, 1 H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 6.5 Hz, H_B-3) 3.86 (dd, 1H, $J_{4,5}$ = 4.3 Hz, $J_{5,5'}$ = 11.5 Hz , H_A-5), 3.81 (dd, 1 H, $J_{4,5}$ = 2.9 Hz, $J_{5,5'}$ = 12.1 Hz, H_B-5), 3.70 (dd, 1 H, $J_{4,5'}$ = 3.5 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5′), 3.63 (dd, 1 H, $J_{4,5'}$ = 4.0 Hz, $J_{5,5'}$ = 12.1 Hz, H_B-5′), 3.39 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.3 (Ar), 138.1 (Ar), 137.9 (Ar), 137.8 (Ar), 128.7 (Ar), 128.7 (Ar), 128.7

(Ar), 128.7 (Ar), 128.6 (Ar), 128.2 (3 C, Ar), 128.1 (Ar), 128.0 (Ar), 107.3 (C_A-1), 106.5 (C_B-1), 88.4 (C_A-2), 87.8 (C_B-2), 83.3 (C_A-3), 82.8 (C_B-3), 82.1 (C_B-4), 80.6 (C_A-4), 72.3 ($O\underline{C}H_2Ar$), 72.3 ($O\underline{C}H_2Ar$), 72.0 ($O\underline{C}H_2Ar$), 71.9 ($O\underline{C}H_2Ar$), 66.0 (C_A-5), 62.2 (C_B-5), 54.9 (OCH_3). Data matched previously reported one.[3]

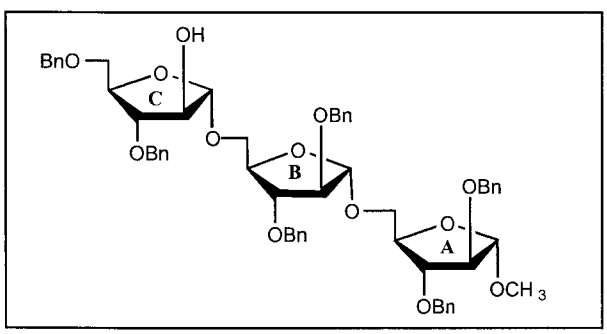
Methyl 5-*O*-(2,3-di-*O*-benzyl-5-*O*-(2-*O*-benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (42).



The donor **13** (153.2 mg, 0.294 mmol) and the acceptor **41** (157.1 mg, 0.239 mmol) were combined and dried under vaccum overnight over P_2O_5. Molecular sieves (4 Å, 0.2 g) were added followed by CH_2Cl_2 (5 mL). The mixture was cooled to –40 °C, and *N*-iodosuccinimide (67 mg, 0.294 mmol) and silver triflate (21.3 mg, 0.083 mmol) were added. After stirring for 30-60 min at this temperature, the reaction mixture turned dark red/brown and was neutralized by addition of triethylamine. The solution was then diluted with CH_2Cl_2, filtered through Celite, and concentrated. The residue obtained was purified by chromatography (5:1, hexanes:EtOAc) to afford **42** as an oil (168 mg, 66%). R_f 0.42 (2:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.00-7.20 (m, 35 H, Ar), 5.45 (d, 1 H, $J_{2,3}$ = 1.3 Hz, H_C-2), 5.28 (s, 1 H, H_C-1), 5.16 (d, 1 H, $J_{1,2}$ = 0.8 Hz, H_B-1), 4.90 (s, 1 H, H_A-1), 4.81-4.41 (m, 12 H, $OC\underline{H}_2Ar$), 4.30 (m, 1 H, H_C-4), 4.20 (m, 1 H, H_B-4), 4.16 (m, 1 H, H_A-4), 4.09 (dd,

1 H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 6.7 Hz, H_B-3), 4.06 (dd, 1 H, $J_{1,2}$ = 1.3 Hz, $J_{2,3}$ = 3.5 Hz, H_B-2), 4.02 (dd, 1 H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 6.6 Hz, H_A-3), 4.00-3.98 (m, 2 H, H_A-2, H_C-3), 3.91 (dd, 1 H, $J_{4,5}$ = 4.3 Hz, $J_{5,5'}$ = 11.4 Hz, H_B-5), 3.86 (dd, 1 H, $J_{4,5}$ = 4.4 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5), 3.70 (dd, 1 H, $J_{4,5'}$ = 3.9 Hz, $J_{5,5'}$ = 11.4 Hz, H_B-5′), 3.68 (dd, 1 H, $J_{4,5}$ = 3.6 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5), 3.62 (dd, 1H, $J_{4,5}$ = 3.7 Hz, $J_{5,5'}$ = 10.8 Hz, H_C-5), 3.6 (dd, 1H, $J_{4,5'}$ = 3.7 Hz, $J_{5,5'}$ = 10.8 Hz, H_C-5′), 3.36 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 165.3 (C=O), 138.2 (Ar), 138.0 (2C, Ar), 137.8 (Ar), 137.8 (Ar), 137.6 (Ar), 133.3 (Ar), 129.8 (Ar), 129.6 (Ar), 128.5 (Ar), 128.4 (4 C, Ar), 127.9 (2 C, Ar), 127.8 (2 C, Ar), 127.7 (3 C, Ar), 127.6 (2C, Ar), 127.5 (Ar), 107.3 (C_A-1), 106.5 (C_B-1), 106.3 (C_C-1), 88.5 (C_A-2), 88.2 (C_B-2), 83.6 (C_C-3), 83.3 (2 C, C_B-3, C_A-3), 82.4 (C_C-4), 81.6 (C_C-2), 80.6 (C_A-4), 80.4 (C_B-4), 73.4 ($O\underline{C}H_2Ar$), 72.3 ($O\underline{C}H_2Ar$), 72.3 ($O\underline{C}H_2Ar$), 72.2 ($O\underline{C}H_2Ar$), 71.9 (2C, $O\underline{C}H_2Ar$), 69.4 (C_C-5), 66.2 (C_A-5), 66.0 (C_B-5), 54.9 (OCH_3). Data matched previously reported one.[3]

Methyl 5-*O*-(2,3-di-*O*-benzyl-5-*O*-(3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (43).

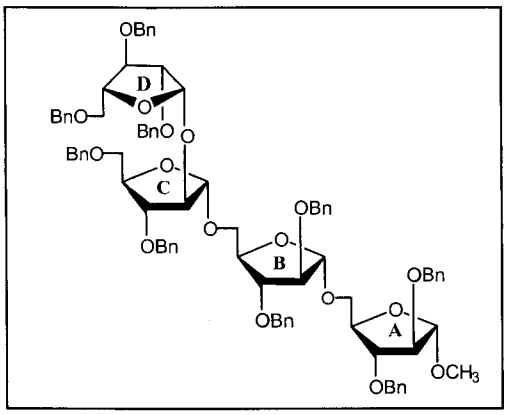


Compound **42** (205 mg, 0.19 mmol) was dissolved in an 1:1 mixture of CH_2Cl_2/CH_3OH (5 mL). The solution was treated with a catalytic amount of $NaOCH_3$ at rt. After stirring for 3 h, the reaction mixture was neutralized with Amberite IR-120 H^+ resin, filtered and

concentrated. The crude product was purified by column chromatography (3:1, hexanes:EtOAc) to obtain **43** as an oil (136 mg, 74%). R_f 0.39 (2:1, hexanes:EtOAc,); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.39-7.21 (m, 30 H, Ar), 5.16 (d, 1 H, $J_{1,2}$ = 1.2 Hz, H_B-1), 5.10 (s, 1 H, H_C-1), 4.93 (s, 1 H, H_A-1), 4.70-4.43 (m, 12 H, OC$\underline{H}_2$Ar), 4.20-4.15 (m, 4 H, H_C-2, H_A-4, H_C-4, H_B-4), 4.09 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 6.9 Hz, H_B-3), 4.06 (dd, 1 H, $J_{1,2}$ = 1.2 Hz, $J_{2,3}$ = 3.3 Hz, H_B-2), 4.02 (dd, 1 H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 6.6 Hz, H_A-3), 3.99 (dd, 1 H, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 3.1 Hz, H_A-2), 3.91-3.86 (m, 3 H, H_C-3, H_B-5, H_A-5), 3.70 (dd, 1 H, $J_{4,5'}$ = 3.3 Hz, $J_{5,5'}$ = 11.6 Hz, H_B-5′), 3.68 (dd, 1 H, $J_{4,5'}$ = 3.7 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5′), 3.63 (dd, 1 H, $J_{4,5}$ = 2.4 Hz, $J_{5,5'}$ = 10.5 Hz, H_C-5), 3.48 (dd, 1 H, $J_{4,5'}$ = 2.8 Hz, $J_{5,5'}$ = 10.5 Hz, H_C-5′), 3.38 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.2 (Ar), 137.9 (Ar), 137.6 (Ar), 137.2 (Ar), 128.5 (3 C, Ar), 128.4 (2 C, Ar), 128.3 (3 C, Ar), 128.0 (Ar), 127.9 (2 C, Ar), 127.8 (3 C, Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 109.2 (C_C-1), 107.2 (C_A-1), 106.4 (C_B-1), 88.4 (C_A-2), 88.3 (C_B-2), 84.8 (C_C-3), 83.3 (C_A-3), 83.1 (C_B-3), 83.0 (C_C-4), 80.6 (2 C, C_A-4, C_B-4), 78.2 (C_C-2), 73.7 (O$\underline{C}H_2$Ar), 72.3 (O$\underline{C}H_2$Ar), 72.2 (O$\underline{C}H_2$Ar), 72.0 (O$\underline{C}H_2$Ar), 71.9 (O$\underline{C}H_2$Ar), 69.7 (C_C-5), 66.2 (C_A-5), 66.0 (C_B-5), 54.9 (OCH_3). Data matched previously reported one.[3]

Methyl 5-*O*-(2,3-di-*O*-benzyl-5-*O*-(3,5-di-*O*-benzyl-2-*O*-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)- α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (44).

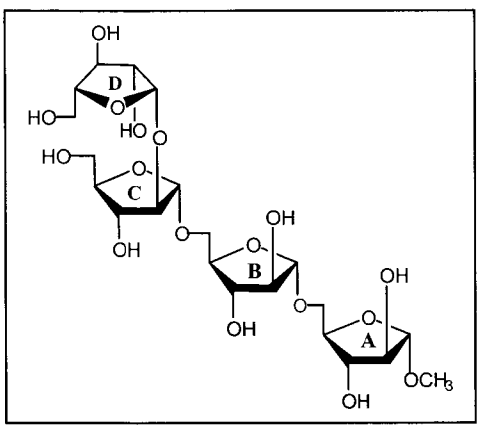
The donor **12** (109.6 mg, 0.21 mmol) and the acceptor **43** (135.4 mg, 0.14 mmol) were combined and dried under vaccum overnight over P_2O_5. Molecular sieves (4 Å, 0.2 g)



were added followed by CH_2Cl_2 (5 mL). The mixture was cooled to –40 °C, and *N*-iodosuccinimide (47.9 mg, 0.21 mmol) and silver triflate (15.2 mg, 0.059 mmol) were added. After stirring for 30-60 min at this temperature, the reaction mixture turned dark red/brown and was neutralized by addition of triethylamine. The solution was then diluted with CH_2Cl_2, filtered through Celite, and concentrated. The residue was purified by chromatography (5:1, hexanes:EtOAc) to obtain **44** as an oil (140 mg, 73%). R_f 0.57 (hexanes:EtOAc, 4:1); (500 MHz, $CDCl_3$, δ_H) 7.34-7.20 (m, 45 H, Ar), 5.11 (s, 2 H, H_B-1, H_C-1), 5.09 (d, 1 H, $J_{1,2}$ = 4.4 Hz, H_D-1), 4.91 (s, 1 H, H_A-1), 4.67-4.33 (m, 19 H, 9OC$\underline{H_2}$Ar, H_C-2), 4.22 (m, 1 H, H_C-4), 4.14-4.12 (m, 2 H, H_A-4, H_B-4), 4.10-4.06 (m, 3 H, H_B-3, H_D-3, H_D-4), 4.04 (dd, 1 H, $J_{1,2}$ = 2.4 Hz, $J_{2,3}$ = 3.4 Hz, H_B-2), 4.00-3.98 (m, 4 H, H_A-2, H_A-3, H_C-3, H_D-2) 3.87 (dd, 1 H, $J_{4,5}$ = 4.0 Hz, $J_{5,5'}$ = 11.7 Hz, H_A-5), 3.82 (dd, 1 H, $J_{4,5}$ = 4.5 Hz, $J_{5,5'}$ = 11.6 Hz, H_B-5), 3.68 (dd, 1 H, $J_{4,5'}$ = 3.1 Hz, $J_{5,5'}$ = 11.7 Hz, H_A-5′), 3.63 (dd, 1 H, $J_{4,5'}$ = 3.5 Hz, $J_{5,5'}$ = 11.6 Hz, H_B-5′), 3.57-3.50 (m, 4 H, H_C-5, H_D-5, H_C-5′, H_D-5′), 3.34 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.2 (2 C, Ar), 138.1 (2 C, Ar), 137.9 (Ar), 137.7 (2 C, Ar), 137.6 (Ar), 128.5 (2 C, Ar), 128.4 (2 C, Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (3 C, Ar), 127.8 (2 C, Ar), 127.7 (Ar), 127.6 (2 C, Ar), 127.5 (3 C, Ar), 107.2 (C_A-1), 106.5, 106.4 (C_B-1, C_C-1), 100.4 (C_D-1), 88.4, 88.3 (C_A-2, C_B-2), 85.7 (C_C-2), 84.3, 84.0, 83.3 , 83.2, 83.1, 81.6, 80.6, 80.5, 80.0 (C_A-3, C_C-3, C_D-2, C_B-3, C_D-3, C_D-4, C_C-4, C_B-4, C_A-4), 73.3, 73.1, 72.4, 72.3, 72.3, 72.2, 72.1,

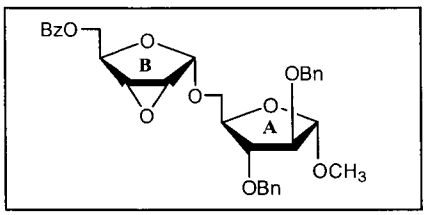
72.0, 71.9 (9 O<u>C</u>H$_2$Ar, C$_D$-5), 70.1 (C$_C$-5), 66.1, 65.6 (C$_A$-5, C$_B$-5), 54.9 (OCH$_3$). Data matched previously reported one.[3]

Methyl 5-*O*-(5-*O*-(2-*O*-(β-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (3).



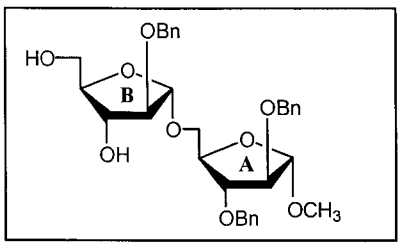
Compound **44** (85 mg, 0.062 mol) was dissolved in a mixture of EtOAc/CH$_3$OH (2.5 ml). The catalyst, 20% Pd(OH$_2$) (70 mg) was added, and the reaction mixture was stirred under a hydrogen atmosphere until completion. The mixture was filtered through Celite, and concentrated to give **3** as an oil (32.3 mg, 93%) R_f 0.26 (3:1, CH$_2$Cl$_2$:CH$_3$OH); ^{1}H NMR (500 MHz, D$_2$O, δ_H) 5.18 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H$_C$-1), 5.14 (d, 1 H, $J_{1,2}$ = 4.7 Hz, H$_D$-1), 5.09 (d, 1 H, $J_{1,2}$ = 1.4 Hz, H$_B$-1), 4.94 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H$_A$-1), 4.22-4.19 (m, 2 H, H$_B$-3, H$_C$-2), 4.18-4.12 (m, 4 H, H$_A$-4, H$_B$-2, H$_C$-3, H$_D$-2), 4.10-4.04 (m, 3 H, H$_A$-2, H$_C$-4, H$_D$-3), 4.02-3.93 (m, 2 H, H$_A$-3, H$_B$-4), 3.93-3.66 (m, 9 H, H$_A$-5, H$_A$-5′, H$_B$-5, H$_B$-5′, H$_C$-5, H$_C$-5′, H$_D$-4, H$_D$-5, H$_D$-5′) 3.42 (s, 3 H, OCH$_3$); ^{13}C NMR (125 MHz, D$_2$O, δ_c) 109.3 (C$_A$-1), 108.4 (C$_B$-1), 106.6 (C$_C$-1), 101.5 (C$_D$-1), 87.6 (C$_C$-2), 83.8, 83.2, 83.1, 82.9, 81.7, 81.5 (C$_C$-3, C$_B$-3, C$_A$-4, C$_D$-4, C$_B$-2, C$_C$-4), 77.6, 77.5, 77.1, 75.7, 75.0 (C$_A$-3, C$_B$-4, C$_D$-2, C$_A$-2, C$_D$-3), 67.9, 67.7 (C$_A$-5, C$_B$-5), 63.9 (C$_D$-5), 61.5 (C$_C$-5), 55.9 (OCH$_3$). Data matched previously reported one.[3]

Methyl 5-*O*-(2,3-anhydro-5-*O*-benzoyl-α-D-ribofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (53).



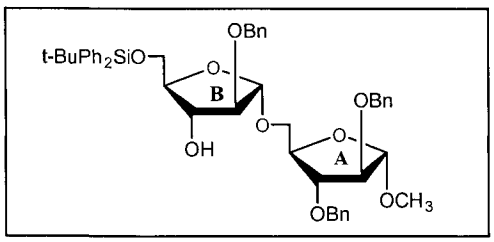
The donor **14** (466.4 mg, 1.364 mmol) and the acceptor **8** (390.9 mg, 1.138 mmol) were combined and dried under vaccum overnight over P_2O_5. Molecular sieves (4 Å, 0.4 g) were added followed by CH_2Cl_2 (10 mL). The mixture was cooled to –40 °C and *N*-iodosuccinimide (308.7 mg, 1.37 mmol) and silver triflate (97.3 mg, 0.38 mmol) were added. After stirring for 30-60 min at this temperature, the reaction mixture turned dark red/brown and was neutralized by addition of triethylamine. The solution was then diluted with CH_2Cl_2, filtered through Celite and concentrated. The residue obtained was purified by chromatography (6:1, hexanes:EtOAc) to afford **52** as an oil (574.9 mg, 75%). R_f 0.39 (2:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.02-7.21 (m, 15 H, Ar), 5.43 (s, 1 H, H_B-1), 4.92 (s, 1 H, H_A-1), 4.59-4.45 (m, 6 H, O$\underline{C}H_2$Ar, H_B-4, H_B-5), 4.38 (dd, 1 H, $J_{4,5'}$ = 4.4 Hz, $J_{5,5'}$ = 11.9 Hz, H_B-5′), 4.19 (m, 1 H, H_A-4), 4.03 (dd, 1 H, $J_{2,3}$ = 2.7 Hz, $J_{3,4}$ = 6.2 Hz, H_A-3), 3.98 (d, 1 H, $J_{2,3}$ = 2.7 Hz, H_A-2), 3.95 (dd, 1 H, $J_{4,5}$ = 4.6 Hz, $J_{5,5'}$ = 11.5 Hz , H_A-5), 3.86 (dd, 1 H, $J_{4,5'}$ = 3.8 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5′), 3.75-3.73 (m, 2 H, H_B-2, H_B-3), 3.37 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 166.0 (C=O), 137.9 (Ar), 137.6 (Ar), 133.4 (Ar), 129.6 (Ar), 129.5 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (2 C, Ar), 128.3 (Ar), 127.9 (2 C, Ar), 127.8 (Ar), 127.7 (Ar), 107.4 (C_A-1), 102.0 (C_B-1), 88.1 (C_A-2), 83.0 (C_A-3), 81.2 (C_A-4), 76.1 (C_B-4), 72.3 (O$\underline{C}H_2$Ar), 71.9 (O$\underline{C}H_2$Ar), 68.0 (C_A-5), 64.7 (C_B-5), 55.9 (2 C, C_B-2, C_B-3), 54.9 (OCH_3). Data matched previously reported one.[65]

Methyl 5-*O*-(2-*O*-benzyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (47).



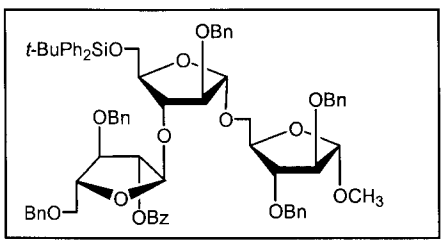
Compound **52** (123.1 mg, 0.219 mmol) was dissolved in benzyl alcohol (5 mL), and the mixture was heated to 100°C. A sodium benzyloxide (5 mL of a 1M solution, 5 mmol) was added. The solution was stirred at 100 °C under an inert atmosphere overnight. The reaction mixture was then cooled to rt and neutralized with acetic acid. Excess benzyl alcohol was removed by vacuum distillation and the crude oil was purified by chromatography.(2:1, hexanes:EtOAc) to yield **47** as an oil (104.7 mg, 85%). R_f 0.44 (2:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.39-7.27 (m, 15 H, Ar), 5.12 (s, 1 H, H_B-1), 4.92 (s, 1 H, H_A-1), 4.61-4.38 (m, 6 H, O$\underline{C}H_2$Ar), 4.16 (m, 1 H, H_A-4), 4.06 (dd, 1 H, $J_{2,3}$ = 1.9 Hz, $J_{3,4}$ = 6.7 Hz, H_B-3), 3.95 (d, 1 H, $J_{2,3}$ = 1.9 Hz, H_A-2), 3.91 (s, 1 H, H_B-2), 3.87 (m, 1 H, H_B-4), 3.82-3.78 (m, 2 H, H_A-3, H_A-5), 3.74-3.67 (m, 2 H, H_B-5, H_B-5′), 3.64 (dd, 1 H, $J_{4,5'}$ = 3.1 Hz, $J_{5,5'}$ = 10.8 Hz, H_A-5′), 3.36 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 137.5 (Ar), 137.2 (Ar), 128.5 (2C, Ar), 129.5 (Ar), 128.2 (Ar), 128.1 (2 C, Ar), 128.0 (Ar), 127.9 (3 C, Ar), 107.2 (C_A-1), 105.1 (C_B-1), 87.2 (C_B-4), 87.0 (C_A-2), 86.8 (C_B-2), 83.5 (C_A-3), 81.0 (C_A-4), 75.2 (C_B-3), 72.1 (O$\underline{C}H_2$Ar), 72.0 (O$\underline{C}H_2$Ar), 71.7 (O$\underline{C}H_2$Ar), 65.7 (C_A-5), 62.7 (C_B-5), 54.9 (OCH_3). Data matched previously reported one.[3]

Methyl 5-*O*-(2-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (48).



To a solution of compound **47** (104.7 mg, 0.185 mmol) in pyridine (5 mL) was added *tert*-Butylchlorodiphenyl silane (0.1 mL, 40 mmol). The reaction mixture was stirred at rt overnight, concentrated and purified by chromatography (4:1, hexanes:EtOAc,) to afford **48** as an oil (72 mg, 59%). R_f 0.24 (4:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.70-7.24 (m, 25 H, Ar), 5.09 (s, 1 H, H_B-1), 4.93 (s, 1 H, H_A-1), 4.57-4.42 (m, 6 H, O$\underline{C}H_2$-Ar), 4.16-4.14 (m, 2 H, H_A-4, H_B-3), 4.03 (ddd, 1 H, J = 3.7 Hz, J = 5.4 Hz, H_B-4), 3.97 (d, 1 H, $J_{2,3}$ = 2.4 Hz, H_A-2), 3.93 (dd, 1 H, H_B-2), 3.85 (dd, 1 H, $J_{2,3}$ = 2.4 Hz, $J_{3,4}$ = 6.1 Hz, H_A-3), 3.83-3.79 (m, 2 H, H_A-5, H_B-5), 3.70 (dd, 1 H, $J_{4,5'}$ = 7.2 Hz, $J_{5,5'}$ = 10.3 Hz, H_B-5'), 3.63 (dd, 1 H, $J_{4,5'}$ = 3.3 Hz, $J_{5,5'}$ = 10.9 Hz, H_A-5'), 3.37 (s, 3 H, OCH_3), 1.09 (s, 9 H, ($C\underline{H}_3$)$_3$CSi); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 137.7 (Ar), 137.6 (Ar), 137.4 (Ar), 135.6 (Ar), 133.4 (2 C, Ar), 129.7 (Ar), 128.5 (Ar), 128.4 (2C, Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (2C, Ar), 127.7 (Ar), 127.6 (Ar), 107.2 (C_A-1), 105.7 (C_B-1), 87.7 (C_B-2), 87.5 (C_A-2), 85.9 (C_B-4), 83.7 (C_A-3), 80.8 (C_A-4), 76.4 (C_B-3), 72.2 (O$\underline{C}H_2$Ar), 72.1 (O$\underline{C}H_2$Ar), 71.6 (O$\underline{C}H_2$Ar), 66.2 (C_A-5), 64.4 (C_B-5), 54.9 (O$\underline{C}H_3$), 26.9 (($\underline{C}H_3$)$_3$CSi), 19.3 ((CH$_3$)$_3\underline{C}$Si). Data matched previously reported one.[3]

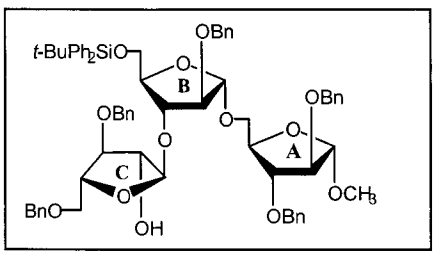
Methyl 5-*O*-(2-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-3-*O*-(2-*O*-benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (49).



Compound **13** (91 mg, 0.16 mmol) and compound **48** (70 mg, 0.106 mmol) were combined and dried under vacuum overnight over P_2O5 before use. Molecular sieves (4 Å, 0.2 g) were added to the mixture followed by CH_2Cl_2 (5 mL). The reaction was cooled to –40 °C and *N*-iodosuccinimide (47.9 mg, 0.21 mmol) was added followed by silver triflate (15.2 mg, 0.059 mmol). After stirring for 30-60 min at this temperature, the reaction mixture turned dark red/brown and was neutralized by addition of triethylamine. The solution was then diluted with CH_2Cl_2, filtered through Celite, and concentrated. The residue obtained was purified by chromatography (5:1, hexanes:EtOAc) to afford **49** as an oil (118 mg, 91%). R_f 0.41 (4:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.02-7.16 (m, 40 H, Ar), 5.35 (d, 1 H, $J_{2,3}$ = 1.5 Hz, H_C-2), 5.29 (s, 1 H, H_C-1), 5.12 (d, 1 H, $J_{1,2}$ = 1.9 Hz, H_B-1), 4.91 (s, 1 H, H_A-1), 4.72-4.38 (m, 10 H, OC$\underline{H}_2$Ar), 4.40 (dd, 1 H, $J_{2,3}$ = 4.0 Hz, $J_{3,4}$ = 6.9 Hz, H_B-3), 4.22-4.16 (m, 4 H, H_A-4, H_C-4, H_B-4, H_B-2), 4.09-4.06 (m, 2 H, H_C-3, H_A-3), 4.00 (d, 1 H, $J_{2,3}$ = 3.3 Hz, H_A-2), 3.89-3.86 (m, 3 H, H_A-5, H_C-5, H_C-5′), 3.67 (dd, 1 H, $J_{4,5'}$ = 3.8 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5′), 3.53 (dd, 1 H, $J_{4,5}$ = 3.7 Hz, $J_{5,5'}$ = 10.9 Hz, H_B-5), 3.50 (dd, 1 H, $J_{4,5'}$ = 4.2 Hz, $J_{5,5'}$ = 10.9 Hz, H_B-5′), 3.37 (s, 3 H, OCH_3), 1.09 (s, 9 H, (C$\underline{H}_3$)$_3$CSi); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 165.33 (C=O), 138.0 (2C, Ar), 137.9 (Ar), 137.8 (Ar), 137.7 (Ar), 135.7 (2 C, Ar), 133.7 (Ar), 133.6 (Ar), 133.3 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6

(Ar), 129.5 (2C, Ar), 128.4 (2C, Ar), 128.3 (3C, Ar), 128.2 (Ar), 127.9 (3C, Ar), 127.8 (2C, Ar), 127.7 (Ar), 127.6 (2C, Ar), 127.5 (Ar), 107.3 (C_A-1), 106.4 (C_B-1), 105.3 (C_C-1), 88.4 (C_A-2), 88.1 (C_B-2), 83.3 (C_A-3), 83.2 (C_C-3), 82.2 (2C, C_B-4, C_C-4), 81.8 (C_C-2), 80.6 (C_A-4), 79.5 (C_B-3), 73.4 ($O\underline{C}H_2Ar$), 72.4 ($O\underline{C}H_2Ar$), 72.2 ($O\underline{C}H_2Ar$), 72.0 ($O\underline{C}H_2Ar$), 71.9 ($O\underline{C}H_2Ar$), 68.8 (C_B-5), 66.4 (C_A-5), 63.3 (C_C-5), 55.0 (OCH_3), 26.8 (($\underline{C}H_3)_3CSi$), 19.4 ($(CH_3)_3\underline{C}Si$). Data matched previously reported one.[3]

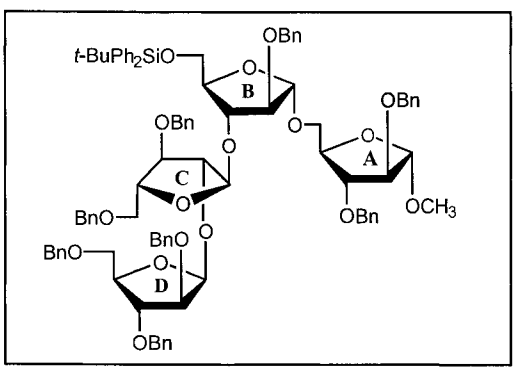
Methyl 5-*O*-(2-O-benzyl-5-*O*-*tert*-butyldiphenylsilyl-3-*O*-(3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (50).



Compound **49** (118 mg, 0.096 mmol) was dissolved in a 1:1 mixture of CH_2Cl_2/CH_3OH (5 mL), and treated with a catalytic amount of $NaOCH_3$ in CH_3OH at rt. After stirring for 3 h, the reaction mixture was neutralized with Amberite IR-120 H^+ resin, filtered and concentrated. The crude product was purified by column chromatography (4:1, hexanes:EtOAc) to obtain **50** as an oil (91.5 mg, 85%). R_f 0.18 (3:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.71-7.22 (m, 35 H, Ar), 5.14 (d, 1 H, $J_{1,2}$ = 1.3 Hz, H_C-1), 5.13 (s, 1 H, H_B-1), 4.92 (s, 1 H, H_A-1), 4.61-4.41 (m, 11 H, $10OC\underline{H}_2Ar$, H_B-3), 4.17 (dt, 1 H, J = 4.0, J = 7.6 Hz, H_A-4), 4.15-4.12 (m, 2 H, H_B-2, H_C-4), 4.10-4.08 (m, 2 H, H_B-4, H_C-2), 4.06 (dd, 1 H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 6.8 Hz, H_A-3), 4.01 (d, 1 H, $J_{2,3}$ = 3.3 Hz, H_A-2), 3.92-3.83 (m, 4 H, H_A-5, H_C-3, H_C-5, H_C-5′), 3.66 (dd, 1 H, $J_{4,5'}$ = 3.5 Hz, $J_{5,5'}$ = 11.4 Hz, H_A-5′), 3.55 (dd, 1 H, $J_{4,5}$ = 2.4

Hz, $J_{5,5'}$ = 10.5 Hz, H_B-5), 3.42 (dd, 1 H, $J_{4,5'}$ = 2.5 Hz, $J_{5,5'}$ = 10.5 Hz, H_B-5'), 3.38 (s, 3 H, OCH_3), 1.06 (s, 9 H, $(C\underline{H}_3)_3$ CSi); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.0 (2 C, Ar), 137.9 (Ar), 137.5 (Ar), 137.3 (Ar), 135.7 (2 C, Ar), 133.8 (Ar), 133.7 (Ar), 129.5 (2 C, Ar), 128.5 (2 C, Ar), 128.4 (2 C, Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (2 C, Ar), 127.7 (3 C, Ar), 127.6 (3 C, Ar), 107.5 (C_B-1), 107.2 (C_A-1), 106.3 (C_C-1), 88.6 (C_A-2), 87.4 (C_C-2), 84.8 (C_C-3), 83.2 (C_A-3), 83.0 (C_B-4), 82.6 (C_C-4), 80.8 (C_A-4), 78.8 (C_B-3), 78.5 (C_B-2), 73.7 ($O\underline{C}H_2Ar$), 72.4 ($O\underline{C}H_2Ar$), 72.1 ($O\underline{C}H_2Ar$), 71.8 (2 C, $O\underline{C}H_2Ar$), 69.5 (C_B-5), 65.8 (C_A-5), 63.4 (C_C-5), 55.0 (OCH_3), 26.8 (($\underline{C}H_3)_3CSi$), 19.4 ($(CH_3)_3\underline{C}Si$). Data matched previously reported one.[3]

Methyl 5-*O*-(2-O-benzyl-5-*O*-*tert*-butyldiphenylsilyl-3-*O*-(3,5-di-*O*-benzyl-2-*O*-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (51).

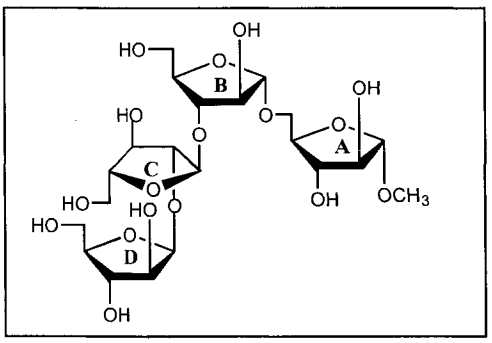


The donor **12** (64.5 mg, 0.12 mmol) and the acceptor **50** (90 mg, 0.08 mmol) were combined in a round bottom flask and dried under vacuum overnight over P_2O_5 before use. Molecular sieves (4 Å, 0.2 g) were added followed by CH_2Cl_2 (5 mL). The mixture was cooled to –40 °C and *N*-iodosuccinimide (47.9 mg, 0.21 mmol) was added followed by silver triflate (15.2 mg, 0.06 mmol). After stirring for 30-60 min at this temperature, the reaction mixture turned dark red/brown, and was neutralized by addition of triethylamine. The

solution was then diluted with CH_2Cl_2, filtered through Celite and concentrated. The crude product was purified by chromatography (5:1, hexanes:EtOAc) to obtain **51** as an oil (89.6 mg, 74%). R_f 0.43 (3:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.73-7.22 (m, 50 H, Ar), 5.17 (d, 1 H, $J_{1,2}$ = 0.6 Hz, H_C-1), 5.13 (d, 1 H, $J_{1,2}$ = 2.1 Hz, H_B-1), 5.03 (d, 1 H, $J_{1,2}$ = 4.4 Hz, H_D-1), 4.89 (s, 1 H, H_A-1), 4.61-4.29 (m, 18 H, 16OC$\underline{H}_2$Ar, H_B-3, H_C-2), 4.19 (dt, 1 H, J = 4.5, J = 6.5 Hz, H_A-4), 4.20-4.06 (m, 7 H, H_A-3, H_B-2, H_B-4, H_C-3, H_C-4, H_D-3, H_D-4), 4.02-3.99 (m, 3 H, H_A-2, H_D-2, H_D-3), 3.89-3.86 (m, 3 H, H_A-5, H_C-5, H_C-5′), 3.69 (dd, 1 H, $J_{4,5'}$ = 3.7 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5′), 3.56-3.48 (m, 4 H, H_B-5, H_B-5′, H_D-5, H_D-5′), 3.37 (s, 3 H, OCH_3), 1.06 (s, 9 H, ($C\underline{H}_3$)$_3$ CSi); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.3 (Ar), 138.2 (2 C, Ar), 138.0 (Ar), 137.9 (Ar), 137.7 (Ar), 137.6 (Ar), 135.7 (2 C, Ar), 133.8 (Ar), 133.7 (Ar), 129.5 (2 C, Ar), 128.4 (2 C, Ar), 128.3 (3 C, Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (2 C, Ar), 127.8 (Ar), 127.7 (2 C, Ar), 127.6 (3 C, Ar), 127.5 (3 C, Ar), 107.3 (C_A-1), 106.2 (C_B-1), 105.4 (C_C-1), 100.1 (C_D-1), 88.4, 84.0 (2C), 83.4, 83.2, 81.9, 81.4, 80.2 (C_A-2, C_A-3, C_B-4, C_C-3, C_C-4, C_D-2, C_D-3, C_D-4), 86.0 (C_C-2), 80.4 (C_A-4), 79.8 (C_B-3), 78.5 (C_B-2), 73.3, 73.1, 72.4, 72.3 (3 C), 72.1, 72.0, 71.8 (O$\underline{C}H_2$Ar, C_D-5), 69.6 (C_B-5), 66.5 (C_A-5), 63.3 (C_C-5), 54.9 (OCH_3), 26.8 (($\underline{C}H_3$)$_3$CSi), 19.4 ((CH_3)$_3\underline{C}$Si). Data matched previously reported one. [3]

Methyl 5-*O*-(3-*O*-(2-*O*-(β-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (4).

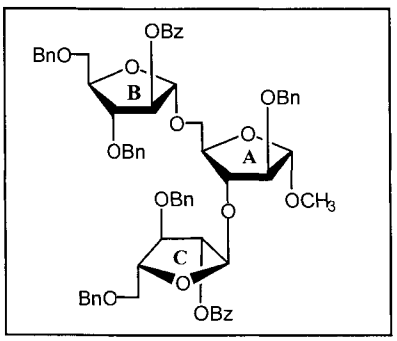
Compound **51** (90 mg, 0.06 mmol) was dissolved in THF (5 mL) and TBAF was added (72 μL, 0.07 mmol). The reaction mixture was stirred for 6h at rt, concentrated and



purified by chromatography (3:1, hexanes:EtOAc) to obtain **52** as an oil. Compound **52** (51 mg, 0.04 mmol) was then dissolved in a 4:1 CH_3OH/EtOAc mixture (2.5 mL). The catalyst, 20% $Pd(OH_2)$ (40 mg) was added, and the reaction mixture was stirred under a hydrogen atmosphere until completion. The mixture was filtered through Celite, and concentrated to afford **4** as an oil (11.6 mg, 52%). R_f 0.32 (3:1, CH_2Cl_2:CH_3OH); ^1H NMR (500 MHz, D_2O, δ_H) 5.23 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H_B-1), 5.12 (d, 1 H, $J_{1,2}$ = 4.5 Hz, H_D-1), 5.08 (s, 1 H, H_C-1), 4.91 (s, 1 H, H_A-1), 4.31 (s, 1 H, H_C-2), 4.19-4.09 (m, 5 H, H_A-3, H_B-2, H_B-3, H_B-4, H_D-2), 4.05-3.99 (m, 4 H, H_A-2, H_A-4, H_C-3, H_D-3), 3.96-3.82 (m, 4 H, H_C-4, H_D-4, 1H_A-5, 1H_B-5), 3.80-3.64 (m, 6 H, H_A-5′, H_B-5′, H_C-5, H_C-5′, H_D-5, H_D-5′), 3.41 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, D_2O, δ_c) 109.3 (C_A-1), 108.3 (C_C-1), 106.2 (C_B-1), 101.6 (C_D-1), 87.9 (C_B-2), 84.2, 83.7, 83.1, 83.0, 82.9, 81.5, 80.0, 77.3, 77.1, 75.6, 75.0 (C_A-4, C_A-2, C_B-3, C_C-2, C_A-3, C_D-2, C_D-3, C_B-4, C_C-4, C_C-3, C_D-4), 67.2 (C_A-5), 63.8, 61.9, 61.5, (C_B-5, C_C-5, C_D-5), 55.9 (OCH_3). Data matched previously reported one.[3]

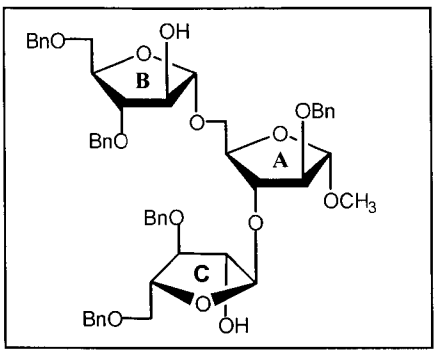
Methyl 3,5-di-*O*-(2-*O*-benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-2-*O*-benzyl-α-D-arabinofuranoside (54).

Compound **13** (309 mg, 0.57 mmol) and compound **9** (acceptor, 76 mg, 0.30 mmol) were combined in a round bottom flask and dried under vacuum overnight over P_2O_5 before use. Molecular sieves (4 Å, 0.3 g) were added to the mixture followed by CH_2Cl_2 (10 mL).



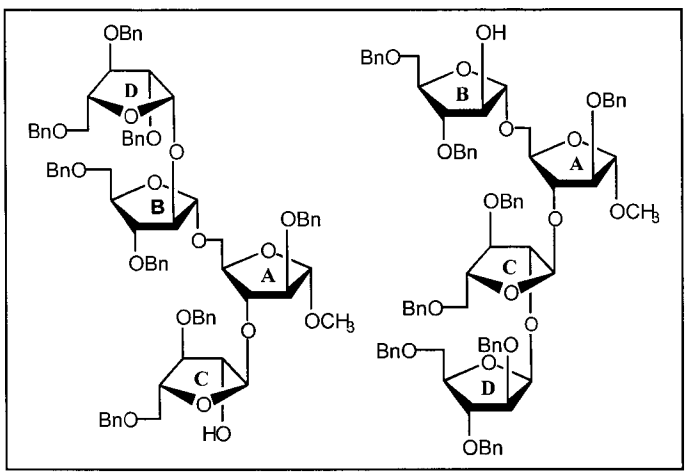
The reaction was cooled to –40 °C, and *N*-iodosuccinimide (129.1 mg, 0.57 mmol) was added followed by silver triflate (47.6 mg, 0.19 mmol). After stirring for 30-60 min at this temperature, the reaction mixture turned dark red/brown and then triethylamine was added to neutralize. The solution mixture was then diluted with CH_2Cl_2, filtered through Celite and concentrated. The residue obtained was purified by chromatography (6:1, hexanes:EtOAc) to afford **53** as an oil (238 mg, 78%). R_f 0.18 (3:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.01-7.12 (m, 35 H, Ar), 5.51 (d, 1 H, $J_{2,3}$ = 1.2 Hz, H_B-2), 5.39 (d, 1 H, $J_{2,3}$ = 1.5 Hz, H_C-2), 5.34 (s, 1 H, H_B-1), 5.30 (s, 1 H, H_C-1), 4.95 (s, 1 H, H_A-1), 4.83-4.44 (m, 11 H, OC$\underline{H}_2$Ar, H_B-4), 4.37-4.35 (m, 2 H, H_A-3, H_C-4), 4.25 (ddd, 1 H, $J_{4,5}$ = 2.6 Hz, $J_{4,5}$ = 4.8 Hz, $J_{3,4}$ = 7.3 Hz, H_A-4), 4.09-4.08 (m, 2 H, H_A-2, H_C-3), 4.06-4.02 (m, 2 H, H_A-5, H_B-3), 3.84 (dd, 1 H, $J_{4,5}$ = 2.6 Hz, $J_{5,5'}$ = 11.4 Hz, H_A-5), 3.69-3.56 (m, 4 H, H_B-5, H_B-5′, H_C-5, H_C-5′), 3.39 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 165.3 (2 C, C=O), 138.2 (Ar), 138.1 (Ar), 138.0 (Ar), 137.8 (2 C, Ar), 135.3 (Ar), 133.2 (Ar), 129.8 (2C, Ar), 129.7 (Ar), 129.6 (Ar), 128.4 (3C, Ar), 128.3 (3 C, Ar), 127.9 (2 C, Ar), 127.7 (Ar), 127.6 (2 C, Ar), 127.5 (2 C, Ar), 107.2 (2 C, C_A-1, C_B-1), 105.7 (C_C-1), 88.5 (C_A-2), 83.7 (C_B-3), 83.3 (C_C-3), 82.4 (C_C-4), 82.3 (C_B-4), 82.2 (C_C-2), 81.7 (C_B-2), 80.7 (C_A-3), 80.1 (C_A-4), 73.4 (2 C, O$\underline{C}H_2$Ar), 72.2 (O$\underline{C}H_2$Ar), 72.1 (O$\underline{C}H_2$Ar), 72.0 (O$\underline{C}H_2$Ar), 69.4 (C_B-5), 69.1 (C_C-5), 66.0 (C_A-5), 55.0 (OCH_3). Data matched previously reported one.[3]

Methyl 3,5-di-*O*-(3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-2-*O*-benzyl-α-D-arabinofuranoside (55).



Compound **53** (193 mg, 0.24 mmol) was dissolved in 1:1 mixture of CH_2Cl_2/CH_3OH (5 mL), treated with a catalytic amount of $NaOCH_3$ at rt. After stirring for 3 h, the reaction mixture was neutralized by Amberite IR-120 H^+ resin, filtered and concentrated. The crude product was purified by column chromatography (4:1, hexanes:EtOAc) to obtain **54** as an oil (150 mg, 76%). R_f 0.35 (1:1, hexanes:EtOAc); ^{1}H NMR(500 MHz, $CDCl_3$, δ_H) 7.35-7.22 (m, 25 H, Ar), 5.09 (s, 1 H, H_C-1), 5.07 (s, 1 H, H_B-1), 4.92 (d, 1 H, $J_{1,2}$ = 1.2 Hz, H_A-1), 4.61-4.41 (m, 10 H, $OC\underline{H}_2Ar$), 4.30-4.27 (m, 2 H, H_A-3, H_B-4), 4.24 (q, J = 2.6 Hz , H_C-4), 4.16 (d, 1 H, $J_{2,3}$ = 1.9 Hz, H_B-2), 4.11-4.07 (m, 2 H, H_C-2, H_A-4), 3.98-3.94 (m, 2 H, H_A-2, H_A-5), 3.84-3.83 (m, 2 H, H_B-3, H_C-3), 3.73 (dd, 1 H, $J_{4,5'}$ = 2.7 Hz, $J_{5,5'}$ = 11.7 Hz, H_A-5'), 3.58 (dd, 2 H, $J_{4,5}$ = 2.3 Hz, $J_{5,5'}$ = 10.6 Hz, H_B-5, H_C-5), 3.47-3.44 (m, 2 H, H_B-5', H_C-5'), 3.34 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.0 (Ar), 137.9 (Ar), 137.5 (Ar), 137.4 (Ar), 137. 2 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (3C, Ar), 128.0 (2C, Ar), 127.9 (2C, Ar), 127.8 (2C, Ar), 127.7 (3C, Ar), 127.6 (Ar), 109.2 (C_B-1), 108.0 (C_C-1), 107.1 (C_A-1), 88.4 (C_A-2), 85.3 (C_B-3), 84.7 (C_C-3), 83.6, 82.5 (C_B-4, C_C-4), 80.8 (C_A-4), 79.5 (C_A-3), 78.5 (C_B-2), 73.7 (2 C, $O\underline{C}H_2Ar$), 72.0 ($O\underline{C}H_2Ar$), 71.9 ($O\underline{C}H_2Ar$), 71.8 ($O\underline{C}H_2Ar$), 69.8 (2C, C_B-5, C_C-5), 65.9 (C_A-5), 54.8 (OCH_3). Data matched previously reported one.[3]

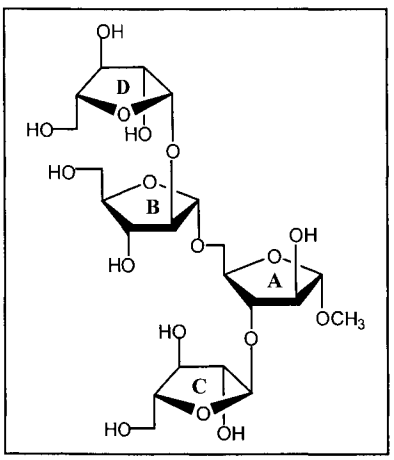
Methyl 3-*O*-(3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-5-*O*-(3,5-di-*O*-benzyl-2-*O*-(2,3,5,-tri-*O*-benzyl-β-D-arabinofuranosyl)-α-D-arabinofuranosyl)-2-*O*-benzyl-α-D-arabinofuranoside and methyl 5-*O*-(3,5-di-*O*-benzyl-α-D-arabinofuranosyl)-3-*O*-(3,5-di-*O*-benzyl-2-*O*-(2,3,5,-tri-*O*-benzyl-β-D-arabinofuranosyl)-α-D-arabinofuranosyl)- 2-*O*-benzyl-α-D-arabinofuranoside (56, 57).



The donor **12** (156.6 mg, 0.30 mmol) and the acceptor **54** (263.4 mg, 0.30 mmol) were combined in a round bottom flask and dried under vacuum overnight over P_2O_5 before use. Molecular sieves (4 Å, 0.3 g) were added followed by CH_2Cl_2 (7 ml). The mixture was cooled to –40 °C, and *N*-iodosuccinimide (68.4 mg, 0.30 mmol) was added followed by silver triflate (25.7 mg, 0.10 mmol). After stirring for 30-60 min at this temperature, the reaction mixture turned dark red/brown and then triethylamine was added to neutralize. The solution was then diluted with CH_2Cl_2, filtered through Celite and concentrated. The residue obtained was purified by chromatography (3:1, hexanes:EtOAc) to afford **56** and **57** as an oil (138.2 mg, 36%) (84.5 mg, 22%). **(56)** R_f 0.54 (3:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.37-7.21 (m, 40 H, Ar), 5.15 (s, 1 H, H_C-1), 5.10 (s, 1 H, H_B-1), 5.09 (d, 1 H, $J_{1,2}$ = 4.7 Hz, H_D-1), 4.88 (s, 1 H, H_A-1), 4.68-4.26 (m, 20 H, $OC\underline{H}_2Ar$, H_A-3, H_B-2, H_C-4, H_B-4), 4.16-4.07 (m, 4 H, H_A-4, H_C-2, H_D-3, H_D-4), 4.02-3.93 (m, 4 H, H_B-3, H_D-2, H_A-2, H_A-5), 3.85 (d, 1 H, J = 2.7

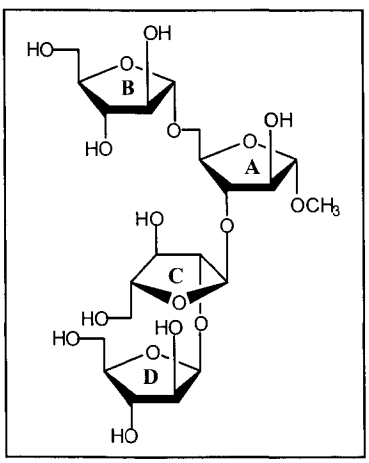
Hz, H_C-3), 3.79 (dd, 1 H, $J_{4,5'}$ = 2.0 Hz, $J_{5,5'}$ = 12.0 Hz, H_A-5′), 3.63-3.37 (m, 6 H, H_B-5, H_B-5′, H_C-5, H_C-5′, H_D-5, H_D-5′), 3.32 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.3 (2C, Ar), 138.2 (Ar), 138.1 (Ar), 138.3 (Ar), 137.7 (2 C, Ar), 137.2 (Ar), 132.5 (Ar), 129.8 (Ar), 129.0 (Ar), 128.6 (6 C, Ar), 128.5 (Ar), 128.4 (4 C, Ar), 128.3 (3 C, Ar), 128.1 (2 C, Ar), 128.0 (2 C, Ar), 127.9 (2 C, Ar), 127.8 (2 C, Ar), 127.7 (3 C, Ar), 127.6 (2 C, Ar), 127.5 (2 C, Ar), 127.4 (Ar), 108.0 (C_C-1), 107.3 (C_A-1), 106.5 (C_B-1), 100.4 (C_D-1), 88.5 (C_A-2), 85.9, 85.2, 84.4, 84.0, 83.8, 83.1, 81.3, 80.7, 79.9, 79.3, 77.7 (C_B-3, C_C-2, C_C-3, C_B-4, C_C-4, C_A-4, C_A-3, C_B-2), 73.7, 73.3, 73.1, 72.3 (3 C), 72.1, 71.9, 71.7, 70.3, 69.8 ($O\underline{C}H_2Ar$, C_D-5, C_B-5, C_C-5), 65.3 (C_A-5), 54.8 (OCH_3). **(57)** R_f 0.36 (3:1, hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.34-7.21 (m, 40 H, Ar), 5.11 (s, 1 H, H_C-1), 5.07 (d, 1 H, $J_{1,2}$ = 0.9 Hz, H_B-1), 4.95 (d, 1 H, $J_{1,2}$ = 4.4 Hz, H_D-1), 4.92 (d, 1 H, $J_{1,2}$ = 1.1 Hz, H_A-1), 4.65-4.32 (m, 18 H, 8$OC\underline{H}_2Ar$, H_A-3, H_C-2), 4.28-4.13 (m, 4 H, H_B-4, H_C-4, H_B-2, H_A-4), 4.11-4.06 (m, 2 H, H_D-3, H_D-4), 4.04-3.95 (m, 4 H, H_A-5, H_C-3, H_A-2, H_D-2), 3.82 (dd, 1 H, J = 2.4 Hz, J = 4.4 Hz, H_C-3), 3.73 (dd, 1 H, $J_{4,5}$ = 3.0 Hz, $J_{5,5'}$ = 11.5 Hz, H_A-5), 3.61-3.36 (m, 6 H, H_B-5, H_B-5′, H_C-5, H_C-5′, H_D-5, H_D-5′), 3.34 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_c) 138.2 (Ar), 138.1 (3 C, Ar), 138.0 (Ar), 137.7 (Ar), 137.5 (Ar), 128.5 (Ar), 128.4 (3 C, Ar), 128.3 (3 C, Ar), 127.9 (2 C, Ar), 127.8 (3 C, Ar), 127.7 (3 C, Ar), 127.6 (4 C, Ar), 127.5 (Ar), 109.1 (C_B-1), 106.9 (C_A-1), 105.3 (C_C-1), 100.0 (C_D-1), 88.4 (C_A-2), 85.5 (C_C-2), 84.5 (C_B-3), 84.2, 84.0, 83.0, 82.3, 81.6, 81.0, 80.5, 80.1, 78.5 (C_A-3, C_A-4, C_B-2, C_B-4, C_C-3, C_C-4, C_D-2, C_D-3, C_D-4), 73.6, 73.3, 73.1, 72.3 (2 C), 72.2, 72.1, 71.9 (2 C), 70.1, 69.7 ($O\underline{C}H_2Ar$, C_D-5, C_B-5, C_C-5), 66.1 (C_A-5), 54.8 (OCH_3). Data matched previously reported one.[3]

Methyl 3-*O*-(α-D-arabinofuranosyl)-5-*O*-(2-*O*-(β-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (5).



Compound **55** (138 mg, 0.11 mmol) was dissolved in a 6:1 mixture CH_3OH/EtOAc (3.5 mL). The catalyst, 20% $Pd(OH_2)$ (60 mg) was added and the reaction mixture was stirred under a hydrogen atmosphere until completion. The mixture was filtered through Celite, and concentrated to give **5** as an oil (53.8 mg, 89%). R_f 0.3 (3:1, CH_2Cl_2:CH_3OH); ^1H NMR (500 MHz, D_2O, δ_H) 5.18 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H_B-1), 5.16 (d, 1 H, $J_{1,2}$ = 4.3 Hz, H_D-1), 5.15 (s, 1 H, H_C-1), 4.97 (s, 1 H, H_A-1), 4.26 (dt, 1 H, J = 2.8, J = 5.4 Hz, H_A-4), 4.23 (dd, 1 H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.9 Hz, H_B-2) 4.21 (d, 1 H, $J_{2,3}$ = 2.1 Hz, H_A-2), 4.18-4.05 (m, 6 H, H_D-2, H_B-3, H_C-2, H_A-3, H_D-3, H_B-4), 4.03 (dt, 1 H, J = 3.2, J = 6.0 Hz, H_C-4), 3.96-3.90 (m, 3 H, H_C-3, 1H_A-5, H_D-4), 3.88-3.67 (m, 7 H, H_A-5, H_B-5, H_B-5′, H_C-5, H_C-5′, H_D-5, H_D-5′), 3.43 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, D_2O, δ_c) 109.4 (C_A-1), 108.1 (C_C-1), 106.6 (C_B-1), 101.5 (C_D-1), 87.7 (C_B-2), 84.9, 83.8, 83.2, 82.9, 82.5, 82.1, 80.0, 77.4, 77.1, 75.7, 75.1 (C_A-4, C_A-2, C_B-3, C_C-2, C_A-3, C_D-2, C_D-3, C_B-4, C_C-4, C_C-3, C_D-4) 67.4 (C_A-5), 63.9, 62.0, 61.5 (C_B-5, C_C-5, C_D-5), 55.9 (OCH_3). Data matched previously reported one.[3]

Methyl 5-*O*-(α-D-arabinofuranosyl)-3-*O*-(2-*O*-(β-D-arabinofuranosyl)-α-D-arabinofuranosyl)-α-D-arabinofuranoside (6).



Compound **56** (85.2 mg, 0.07 mmol) was dissolved in a 6:1 mixture of CH_3OH/EtOAc (3 mL). The catalyst, 20% $Pd(OH_2)$ (50 mg) was added and the reaction mixture was stirred under a hydrogen atmosphere until completion. The mixture was filtered through Celite, and concentrated to afford **6** as an oil (33.8 mg, 90%). R_f 0.27 (3:1, CH_2Cl_2:CH_3OH); ^1H NMR (500 MHz, D_2O, δ_H) 5.24 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H_B-1), 5.14 (d, 1 H, $J_{1,2}$ = 4.6 Hz, H_D-1), 5.09 (s, 1 H, H_C-1), 4.97 (s, 1 H, H_A-1), 4.27 (dt, 1 H, J = 3.1, J = 5.2 Hz, H_A-4), 4.23 (dd, 1 H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 4.7 Hz, H_B-2), 4.20 (d, 1 H, $J_{2,3}$ = 4.7 Hz, H_A-2), 4.19-4.04 (m, 6 H, H_D-2, H_B-3, H_C-2, H_A-3, H_D-4, H_D-3,), 4.03 (dt, 1 H, J = 3.2, J = 6.0 Hz, H_C-4), 3.96-3.90 (m, 3 H, H_C-3, 1H_A-5, H_B-4), 3.74-3.67 (m, 4 H, H_B-5, H_C-5, H_D-5, H_D-5′), 3.42 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, D_2O, δ_c) 109.4 (C_A-1), 108.2 (C_C-1), 106.3 (C_B-1), 101.6 (C_D-1), 87.7 (C_B-2), 84.9, 83.8, 83.4, 82.9, 82.6, 81.8, 79.9, 77.5, 77.1, 75.7, 75.0 (C_A-4, C_A-2, C_B-3, C_C-2, C_A-3, C_D-2, C_D-3, C_B-4, C_C-4, C_C-3, C_D-4) 67.3 (C_A-5), 63.8, 62.1, 61.5 (C_B-5, C_C-5, C_D-5), 55.7 (OCH_3). Data matched previously reported one.[3]

Chapter 3

Screening of compounds by FAC/MS

3.1 Introduction

Frontal affinity chromatography coupled online to electrospray mass spectrometry (FAC/MS) is a high throughput screening method[69,70] that employs a column containing an immobilized biological receptor such as a protein, or a cell fragment and an electrospray mass spectrometer for eluent monitoring. In the FAC-MS system, a mixture of compounds is continuously infused through the column and the compounds are eluted from the column in the order of the affinity to the immobilized receptor, allowing the analysis of the mixture of compounds. (Figure 3.1) In addition, FAC/MS allows the determination of the dissociation constants (K_d's) of an individual compound for the receptor.[71] This technique is rapid and also uses very small amount of protein and ligand. Therefore, we utilized FAC-MS to screen the compounds in hand (**1-6**, **57-65**) to probe their interaction with mAb CS-35 and to determine K_d for compounds that bind.

The determination of K_d's is based on the frontal affinity chromatography theory first introduced by Kasai *et al.*[72] The relationship between the binding capacity (B_t) of the column, the dissociation constant (K_d), the concentration ($[X]_o$) and retention volume (V_x-V_o) of a ligand is given by the equation (1):

$$V_x - V_o = B_t/(K_d + [X]_o) \qquad (1)$$

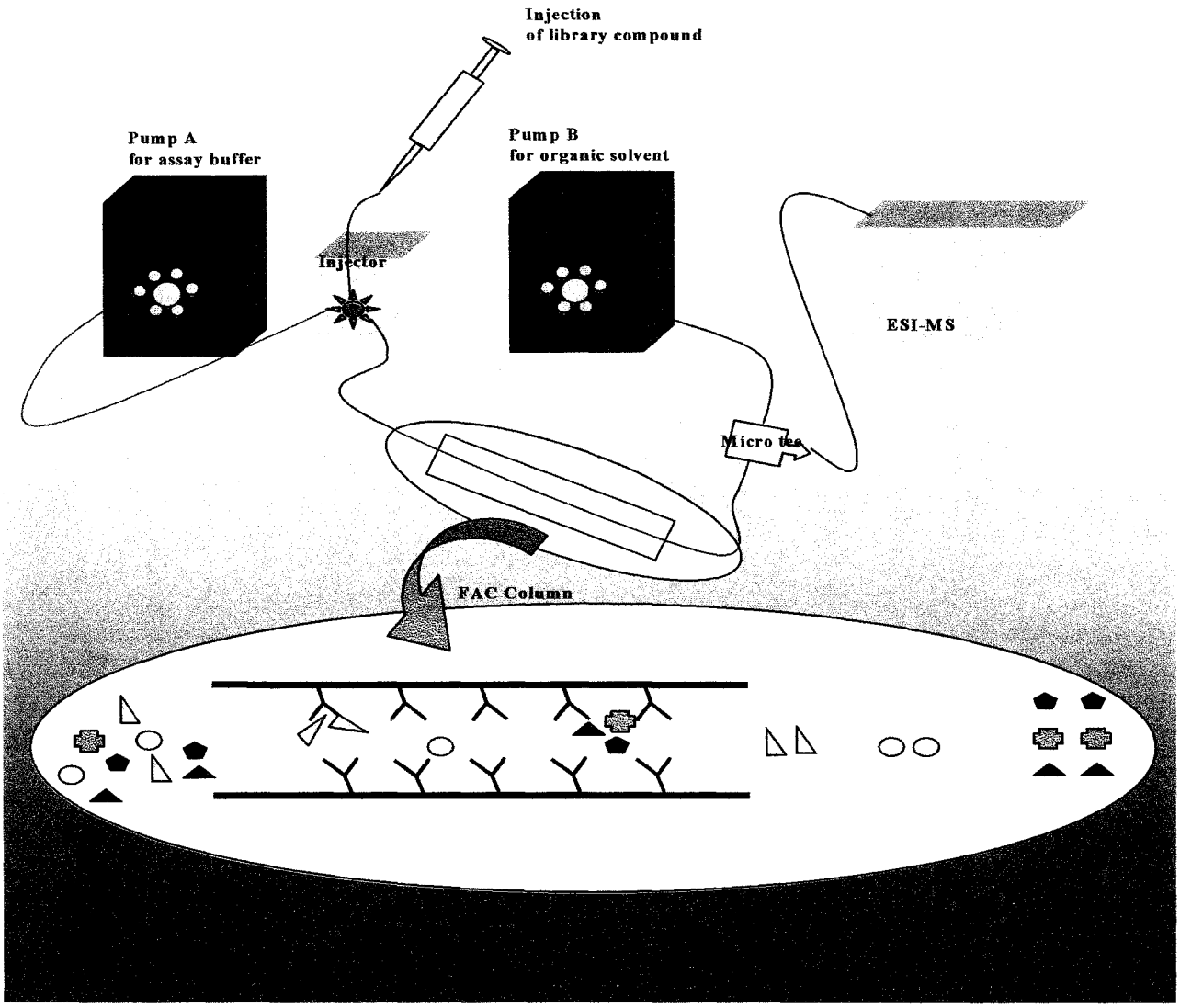


Figure 3.1. Schematic presentation of FAC-MS assay and an overview of FAC/MS concept

V_x is the elution volume of ligand X and V_o is the void volume of the system. As the ligand compound concentration increases, the retention volume (V_x-V_o) decreases, if B_t and K_d remain constant.

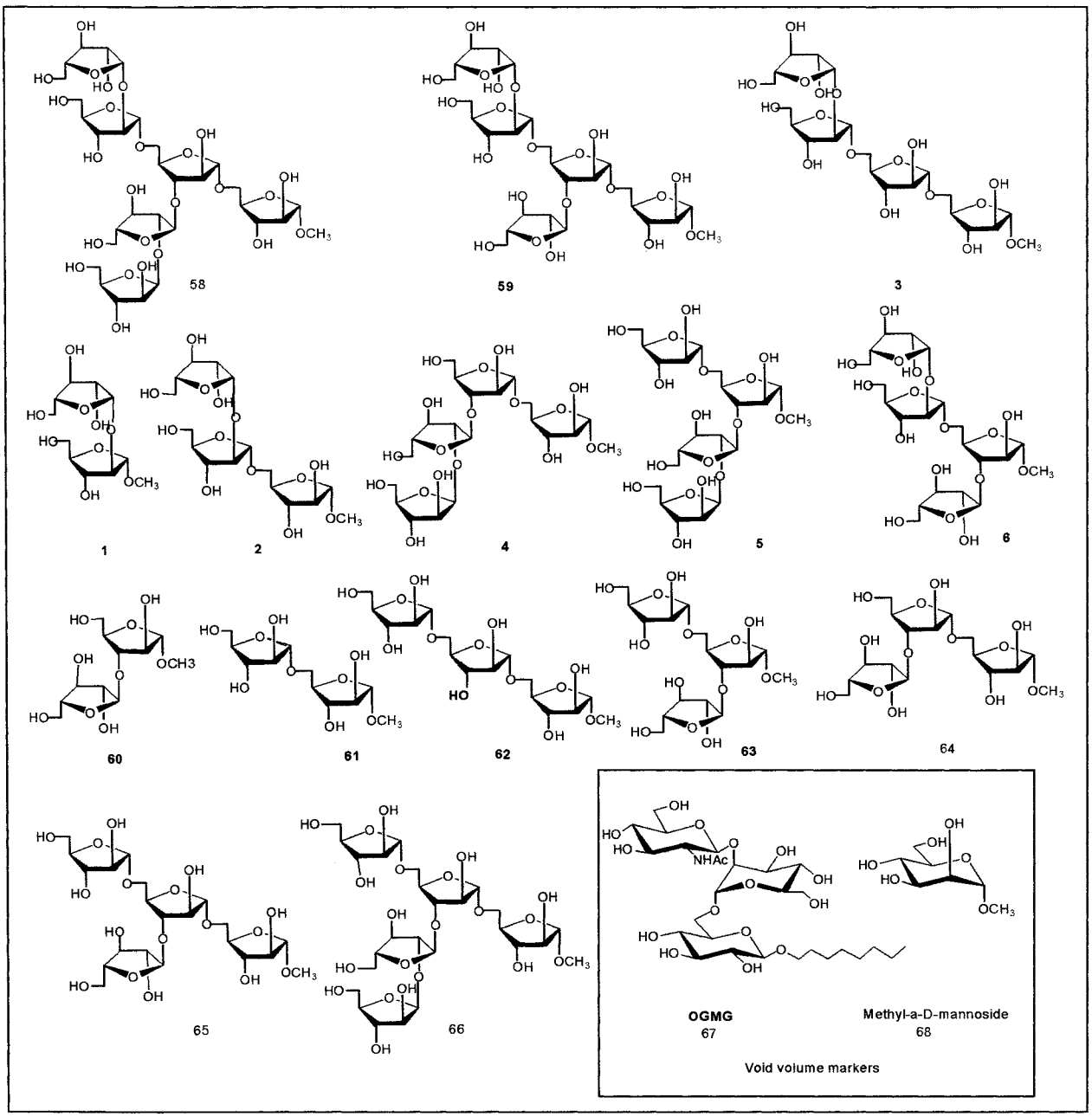


Figure 3.2. Library of compounds screened by FAC-MS

3.2 Binding affinity test of oligosaccharides to CS-35

The oligosaccharides synthesized in chapter 2 (**1-6**) and the previously synthesized compounds[3] were subjected to FAC/MS analysis to determine their binding affinity for mAb CS-35. To test the library of compounds, oligosaccharides were sorted into several groups with different masses. All compounds in the mixture were at the same concentration. Either OGMG (*β*-D-GlcpNAc-(1→2)-*α*-D-Man*p*-(1→6)-*β*-D-Glc*p*-*O*-octyl) or methyl-α-D-mannopyranoside (Figure 3.2), compounds with no affinity to mAb CS-35 were used as a void volume maker. A blank column was prepared by blocking a column with *d*-biotin and the compounds were tested on the column to eliminate the possibility of nonspecific binding.

The oligosaccharides evaluated for binding affinity are shown in Figure 3.2. Among the oligosaccharides, hexasaccharide **58**, pentasaccharide **59** and tetrasaccharide **3** were the only compounds with binding affinity to mAb CS-35. The difference of binding affinity among the oligosaccharides is clearly shown in Figure 3.3. While hexasaccharide **58** and pentasaccharide **59** have similar binding affinity, tetrasaccharide **3** showed much weaker binding affinity, indicating the importance of one of the β-arabinofuranosyl residues and a branched residue at C-3 position for interaction with the mAb CS-35. Because the loss of one branched residue at the C-3 position noticeably decreases binding affinity compared to pentasaccharide **59**, and an isomeric tetrasacharide **5** showed no binding affinity, it is assumed that the linear linkage portion

with β-arabinofuranosyl residue is a fundamental element for binding.

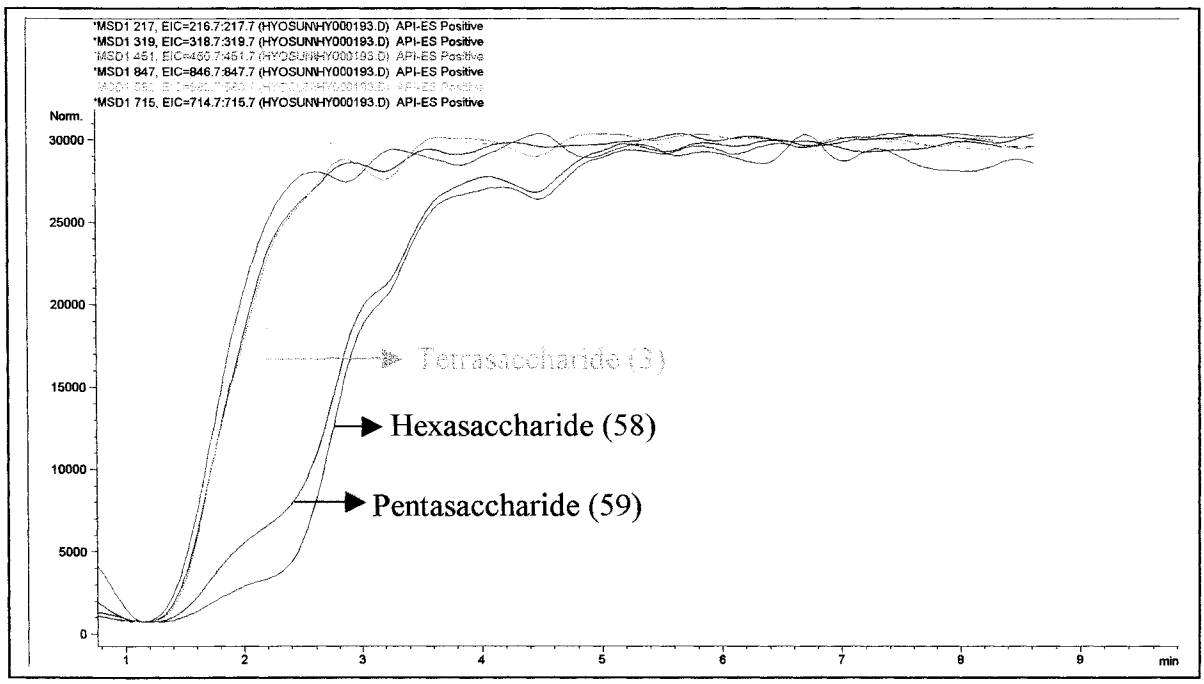


Figure 3.3. Selected ion chromatogram with a mixture (compounds 58, 59, 1, 2, 3)

3.3 Determination of K_d values

Because only three compounds, a hexasaccharide **58**, a pentasaccharide **59**, a tetrasaccharide **3** showed binding affinity with mAb CS-35, we determined their dissociation constants (K_d). Kinetic experiments were performed in triplicate at four different concentrations of each compound. The triplicate data used for the determination of K_d was averaged before processing. The volume ($V-V_o$) was taken as

the midpoints in the extracted ion chromatogram and employed for plotting to obtain B_t and K_d. Plots of $([con]/(V-V_o))^{-1}$ versus $[con]^{-1}$ were then generated. Linear regression analysis of the data using equation (1) gave B_t and K_d values. From the generated linear curve, the y intercept and the x intercept represent B_t and the negative reciprocal of K_d, respectively.

3.3.1 Determination of K_d value for hexasaccharide 58

The K_d value of hexasaccharide **58** was determined with data obtained from the FAC-MS assay. The data are shown in Table 3.1 and were plotted to produce a linear curve. From the linear curve equation, B_t and K_d values were determined to be 18.5 pM and 4.9 μM respectively (Figure 3.4).

[con] μM	(V-Vo) μL	1/[con]	1/[con]/(V-Vo)
2.18	2.61	0.46	0.18
4.34	1.97	0.23	0.12
8.65	1.34	0.12	0.09
17.17	0.86	0.06	0.07

Table 3.1. Obtained data from binding affinity assay with hexasaccharide 58

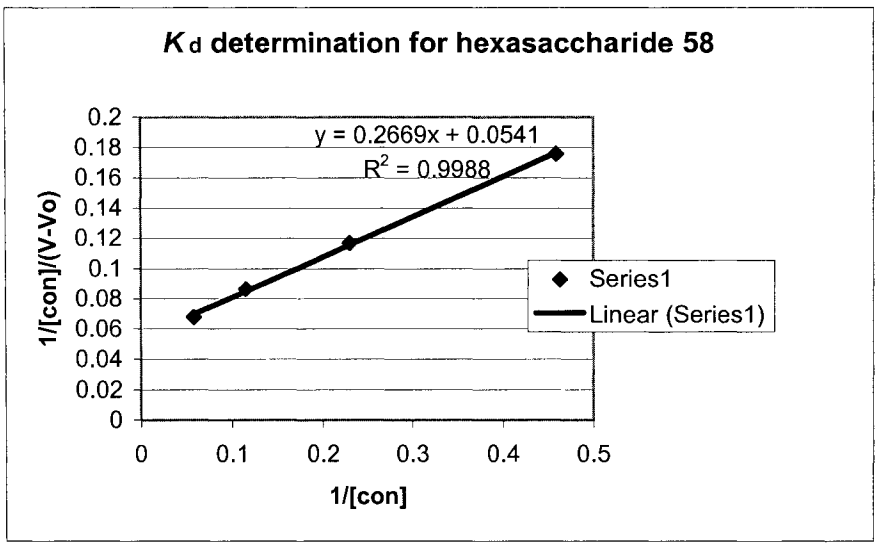


Figure 3.4. Determination of K_d value for hexasaccharide 58

3.3.2 Determination of K_d value for pentasaccharide 59

The K_d value of pentasaccharide **59** was determined with data obtained from FAC-MS assay. The data are shown in Table 3.2 and were plotted to produce a linear curve. From the linear curve equation, B_t and K_d values were obtained as 19.3 pM and 6.2 μM, respectively. (Figure 3.5)

[con] μM	V-Vo μL	1/[con]	1/[con]/(V-Vo)
1.88	2.36	0.53	0.23
3.74	1.99	0.27	0.13
7.47	1.40	0.13	0.10
14.88	0.89	0.07	0.08

Table 3.2. Obtained data from binding affinity assay with pentasaccharide 59

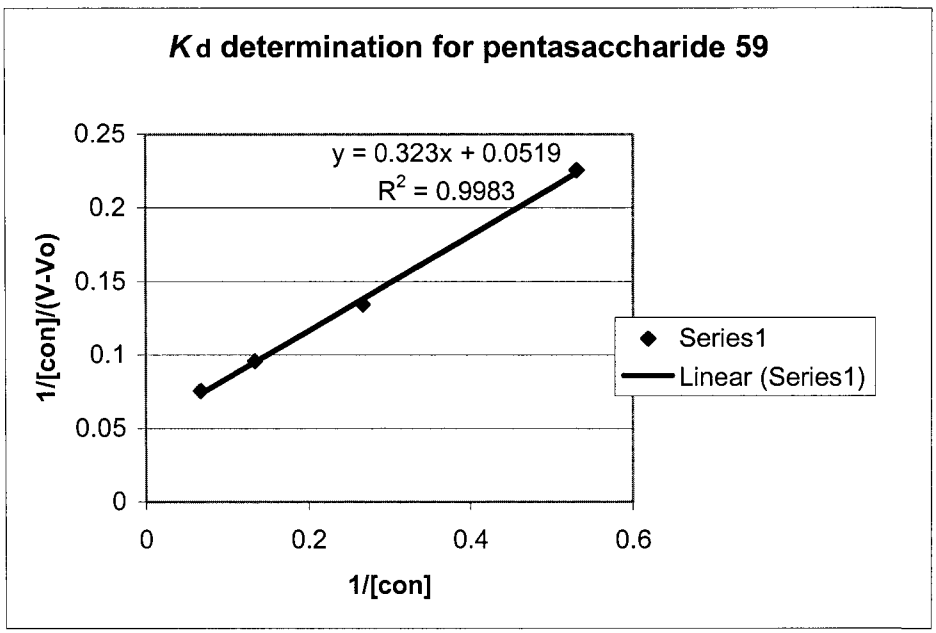


Figure 3.5. Determination of K_d value for pentasaccharide 59

3.3.3 Determination of K_d value for compound 3

The K_d value of compound 3 was determined with data obtained from FAC-MS assay. The data are shown in Table 3.3 and were plotted to produce a linear curve. From the linear curve equation, B_t and K_d values were obtained as 83.3 pM and 32.6 μM respectively. (Figure 3.6)

[con] μM	(V-Vo) μL	1/[con]	1/[con]/(V-Vo)
3.45	2.30	0.29	0.13
6.90	2.15	0.15	0.07
13.80	1.81	0.07	0.04
27.40	1.34	0.04	0.03

Table 3.3. Obtained data from binding affinity assay with compound 3

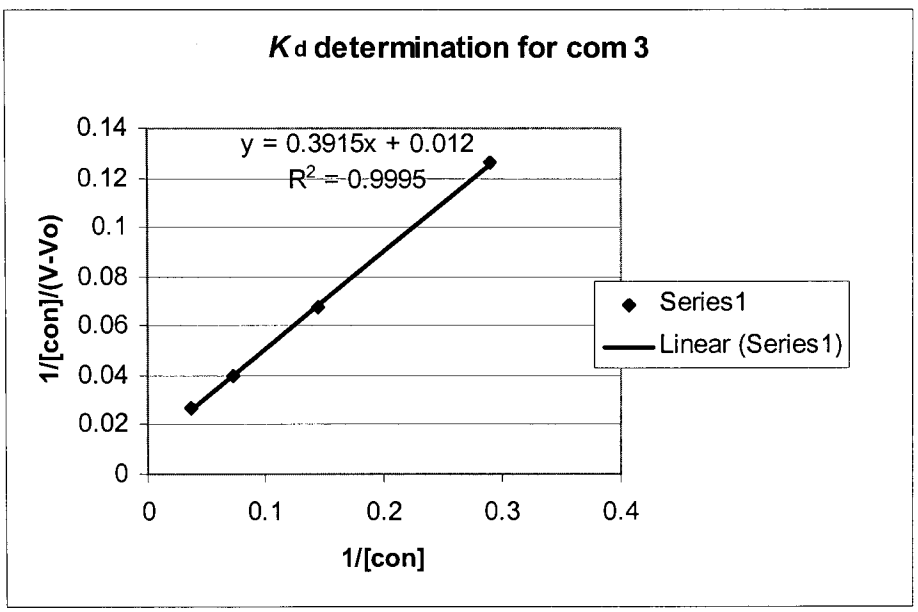


Figure 3.6. Determination of K_d value for compound 3

3.3.4 Comparison of K_d values

A comparison of the K_d values for 3, 58, 59 is presented in table 3.4

Hexasaccharide 58	4.9 μM
Pentasaccharide 59	6.2 μM
Tetrasaccharide 3	32.6 μM

Table 3.4. Comparision of K_d values

Because the pentasaccharide has a very similar K_d value to hexasaccharide, we propose that the minimun structure for binding to mAb CS-35 can be considered to the pentasaccharide. This structure be the starting point for further study as the minimum epitope for a vaccine development.

3.4 Experimental

3.4.1 Preparation of Antibody

3.4.1.1 Tissue culture and mAb CS-35 production

A stock vial for mAb CS-35 was removed from a liquid nitrogen storage tank. After quickly thawing in a water bath the cells were transferred to a 15 mL conical tube and 10 mL of BD media (BD Bioscience) was added slowly. Cells were counted and their viability was determined using 0.1% trypan blue stain in a 1:1 ratio, and a hemocytometer. The cells were resuspended in the appropriate volume of media to obtain the desired cell concentration (500,000-1,000,000 cells/mL) after centrifugation. 10 mL of cells was transferred to a 25 mL flask (T-25) and placed in a humidified CO_2 (8%) incubator for 2 days. Cells were split in 1:10 ratio (established culture:fresh media) until cell viability is greater than 95%. Cells (3×10^7) were resuspended in 15 mL of BD media with 20% FBS and these cells were transferred to a small compartment in an artificial mouse (a CELLLine device, BD company). After 1L of BD media was added to the large compartment, the artificial mouse box was placed in the incubator. One week later, cells were removed from this compartment, and centrifuged. The supernatant containing the Ab was collected and frozen. The cell pellets were resuspended in 15 mL of BD media with 20% FBS and placed back into the cell compartment. These processes were repeated until cell viability dropped below 20%.

3.4.1.2 Purification of mAb CS-35

To the collected supernatant, 1M Tris (pH 8.0) was added to adjust the pH between 7 and 9. Ammonium sulfate was added to the solution to make a final concentration of 50% and the solution was stirred at 4 °C for 2 h, and then centrifuged and the pellets were resuspended in 0.75 M Tris buffer, 0.15 M NaCl, pH 8.0. Additional ammonium sulfate was added to bring the solution to a final concentration of 50% and the solution was stirred at 4 °C for 2 h. After centrifugation, the pellets were resuspended in 0.05 M Tris buffer, 0.15 M NaCl, pH 8.0. This solution was dialyzed against 2 L of the Tris buffer (pH 8.0) overnight at 4 °C. The dialyzed antibody was loaded onto a protein A column with dialysis buffer, and then the antibody was eluted with 0.1 M citric buffer, pH 4.0. The eluate was neutralized and dialyzed to obtain the purified Ab as verified by SDS –PAGE (12.5%). The amount of protein was determined by measuring OD at λ 280 nm with a UV spectrometer. The OD value was divided by the extinction coefficient for a monoclonal antibody (1.3) to determine amount of the antibody produced.

3.4.2 Biotinylation of mAb CS-35

Biotinylated mAb CS-35 was prepared using either a commercially available biotinylation kit (Amersham) or a biotinylating reagent (Pierce). When using the biotinylation kit, 2.5 mg of mAb CS-35 was diluted with 0.04 M sodium bicarbonate (1 mg/mL). The biotinylation reagent was added to the solution and the mixture was

agitated for 1 h at rt. The biotinylated antibody was purified on a Sephadex G25 column. To biotinylate mAb CS-35 using the Biotin-LC-Hydrazide reagent, cold 0.02 M sodium meta-periodate solution 1 mL, was added to a 2 mg/mL solution of mAb CS-35 in 0.1 M NaOAc buffer, pH 5.5, and the oxidation reaction was allowed to proceed for 30 min at 0 °C in the dark. Glycerol was added to reaction mixture up to a final concentration of 15 mM, and the solution was incubated for 5 min at 0 °C. The reaction mixture was dialyzed overnight against 0.1 M NaOAc buffer, pH 5.5. After dialysis, 0.05 M biotin hydrazide in DMSO was added to the solution up to a final concentration of 0.005 M, and then the reaction was let to proceed for 2 h at rt with continuous agitation. The reaction solution was centrifuged to remove unreacted reagent using a Centricon (molecular weight cut off: 10,000) and the resulting solution was stored in PBS buffer. Confirmation of biotinylation with mAb CS-35 was performed by Western Blot.

3.4.3 Preparation of Affinity column

An affinity column was prepared with capillary silica tubing. Either 2.5 or 5 cm capillary tubing (250 ID, 360 OD) was packed with Streptavidin-CPG (controlled-pore glass) beads (Purebiotech, 37-74 μm), and mAb CS-35 was immobilized by infusion in PBS buffer (pH 7.2, 0.5 mL of ~1000 pM) onto the column. To prevent nonspecific binding by substrates, any unoccupied sites on the streptavidin were blocked with *d*-biotin.

3.4.4 FAC/MS Assay

The FAC/MS system used was an integrated module composed of two high flow rate precision nanofluidic delivery system and a six-port valve. (*Scivex Confluent Nano Fluidic Module*, Upchurch) (Figure 3.1) The eluent buffer, 0.01 M NH_4OAc buffer (pH 7.2), was delivered by pump A at a flow rate of 1 μL/min through the six–port injection valve, where library compounds were injected. The FAC column was connected from outlet of the injection valve to a micro tee. Methanol (makeup solution) was introduced through pump B of the FAC system at a flow rate of 3 or 4 μL/min.[79] In online mode, the combined flow was directed into an electrospray mass spectrometer (Hewlett-Packard 1100 MSD). For characterization of the column eluents, the spectrometer scanned from 60-250 m/z in the positive-ion mode. The elution of the compounds was continuously monitored in the selected ion monitoring in positive-ion mode during a run for the screening of mixtures. Breakthrough volumes were measured as midpoints in the extracted ion chromatograms. All data were processed with Microsoft Excel software.

Chapter 4

Conclusion and Future work

We evaluated the binding affinity of all synthesized compounds and identified the binding epitope for the CS-35 antibody. Only three compounds, **3, 58, 59** were shown to bind, all of which had a β-arabinifuranoside residue. Even though we can assume which residues are important for the interaction between the oligosaccharides and CS-35 (Figure 4.1), it is still not possible to draw an exact conclusion. To confirm and identify the scaffold of CS-35, and any conformational changes associated with oligosaccahride binding, X-ray crystallography of the antibody bound to the ligand will be necessary. Such studies are underway.

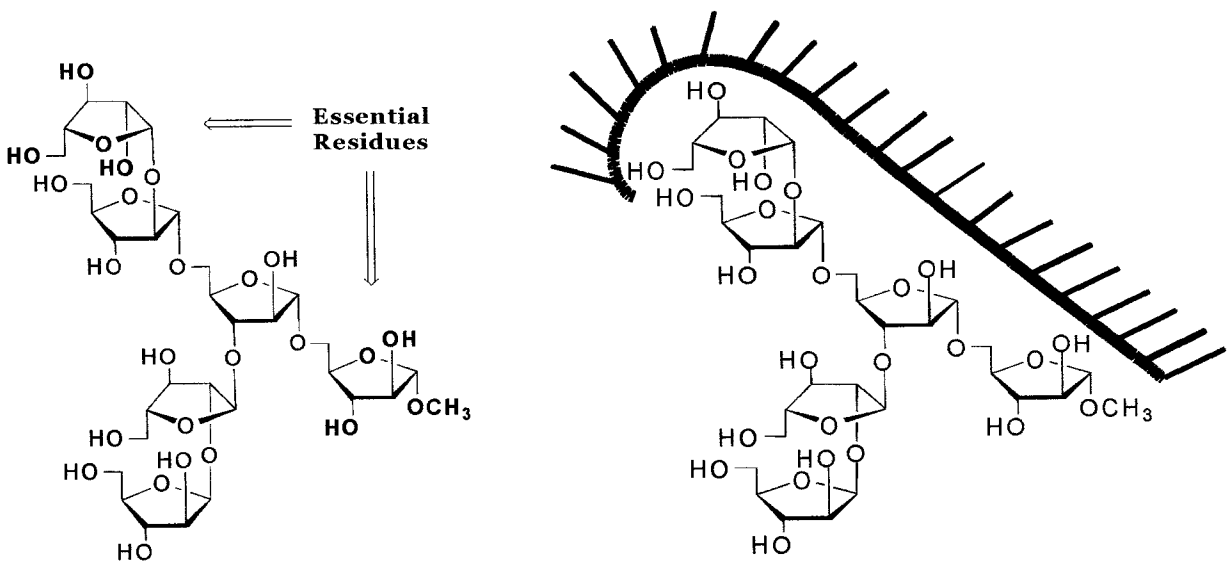


Figure 4.1 Important two residues for affinity to CS-35

Chapter 5

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