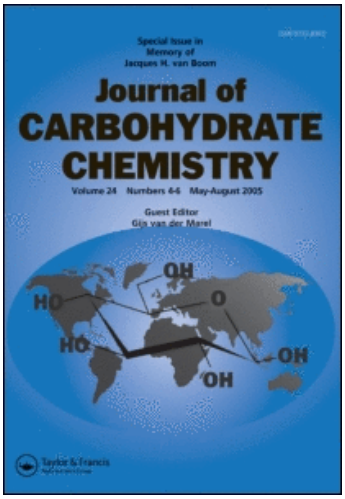

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D-ARABINOFURANOSIDES FROM MYCOBACTERIA: SYNTHESIS AND CONFORMATION*

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I. INTRODUCTION

Unlike the majority of monosaccharides, the pentose arabinose exists in nature in all possible absolute and ring configurations, namely, D-arabinofuranose (D-Ara*f*), L-arabinofuranose (L-Ara*f*), L-arabinopyranose (L-Ara*p*), and D-arabinopyranose (D-Ara*p*). The pyranose forms are more rare and are found primarily either in protozoan parasites[1] or as constituents of plant saponins.[2] Oligo- and polysaccharides comprised of L-arabinofuranose are also widespread in the plant kingdom, where they are present as constituents of arabinoxylans,[3] pectins,[4] and hydroxyproline-rich glycoproteins (HPRGs).[5] This chapter focuses on polysaccharides containing D-arabinofuranose and in particular the most prominent examples of these polymers, which are found as important components of the cell wall of members of the Actinomycetes family including the genera *Mycobacteria*, *Corynebacteria*, *Nocardia*, and *Rhodococcus*.[6]

Although the majority of actinomycetes are benign to humans, among them are two important human pathogens, *Mycobacterium tuberculosis* and *Mycobacterium leprae*.[7] Infection by these organisms causes, respectively, tuberculosis and leprosy. Mycobacterial infections have attracted renewed attention in recent years owing to their increasing incidence in the industrialized world as well as the emergence of drug-resistant strains of these organisms.[8] Additionally, AIDS patients and others with compromised immune systems are susceptible to opportunistic infections caused by ''atypical'' mycobacteria including *M. avium* and *M. kansasii*.[9] The total number of people infected with *M. tuberculosis* has been estimated at a third of the world's population and almost 3 million deaths from tuberculosis occur each year, making it the single most lethal bacterial disease.[10]

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Treating mycobacterial infections is difficult and usually requires a 6–12 month course of antibiotic therapy.[11] The need for antibiotics over an extended period is due largely to the structure of the mycobacterial cell wall, which serves as a formidable barrier to the passage of antibiotics into the organism as well as into the immune system of the human host. Drug regimens that are in current use generally include one or two antibiotics that inhibit cell wall biosynthesis (e.g., ethambutol or isoniazid), in combination with others that have intracellular targets (e.g., rifampicin or streptomycin). When administered together, such combinations destroy the integrity of the cell wall, which in turn allows other antibiotics to pass to the cell more efficiently.[12] Even ignoring the issue of drug resistance, the arsenal of drugs that are active against mycobacteria is relatively small,[8] and thus there is a pressing need for the identification of new antibiotics. When considered along with the emergence of drug-resistant mycobacterial strains, this need becomes even more urgent.

In the search to identify new antibiotics, one area receiving particular attention is the identification of compounds that act by inhibiting mycobacterial cell wall biosynthesis. An increasing number of papers on this topic have appeared in the last few years.[13–17] However, a detailed understanding of the cell wall structure has really become available only in the last decade,[6,13] and an in-depth understanding of its biosynthesis is unavailable.[13] Many more fundamental chemical and biochemical studies need to be completed before the rational design of such antibiotics can be realized.

The mycobacterial cell wall is composed, to a very large degree, of polysaccharides and lipids.[6] The two major polysaccharides are an arabinogalactan (AG) and a lipoarabinomannan (LAM). The predominant lipid species are mycolic acids, branched long-chain lipids characteristic to the actinomycetes. What is particularly unique about both AG and LAM is that all the arabinose and galactose residues are present in the furanose form. Since oligosaccharides containing furanose residues are xenobiotic to mammalian biochemistry, the enzymes that assemble these polysaccharides are ideal targets for drug action.

This chapter focuses on the arabinan portions of the AG and LAM. Sections II–IV outline the structure, biochemical importance, and biosynthesis of these arabinans. Section V discusses the chemical syntheses of oligosaccharides containing D-arabinofuranose residues. Such compounds are important tools in biochemical studies leading to a more detailed understanding of cell wall biosynthesis. Section VI focuses on work that we and others are doing to elucidate the solution conformation of oligo- and polysaccharides containing D-arabinofuranose residues, and Section VII offers some concluding remarks. This review covers work published up to the end of 1998.

II. ARABINAN STRUCTURE

Only recently has a detailed picture of the structure of the mycobacterial cell wall emerged.[6,18,19] Although the constituent species present have been known for many years,[20,21] the assembly of these constituents into a coherent structure has required lengthy investigations and has relied on a number of techniques including, in addition to methylation analysis, both NMR spectroscopy and fast-atom bombardment high-resolution mass spectrometry.[6,18,19] These studies were also greatly facilitated by the discovery[22] of hydrolytic enzymes capable of digesting both AG and LAM into small fragments, which are more amenable to these characterization methods.

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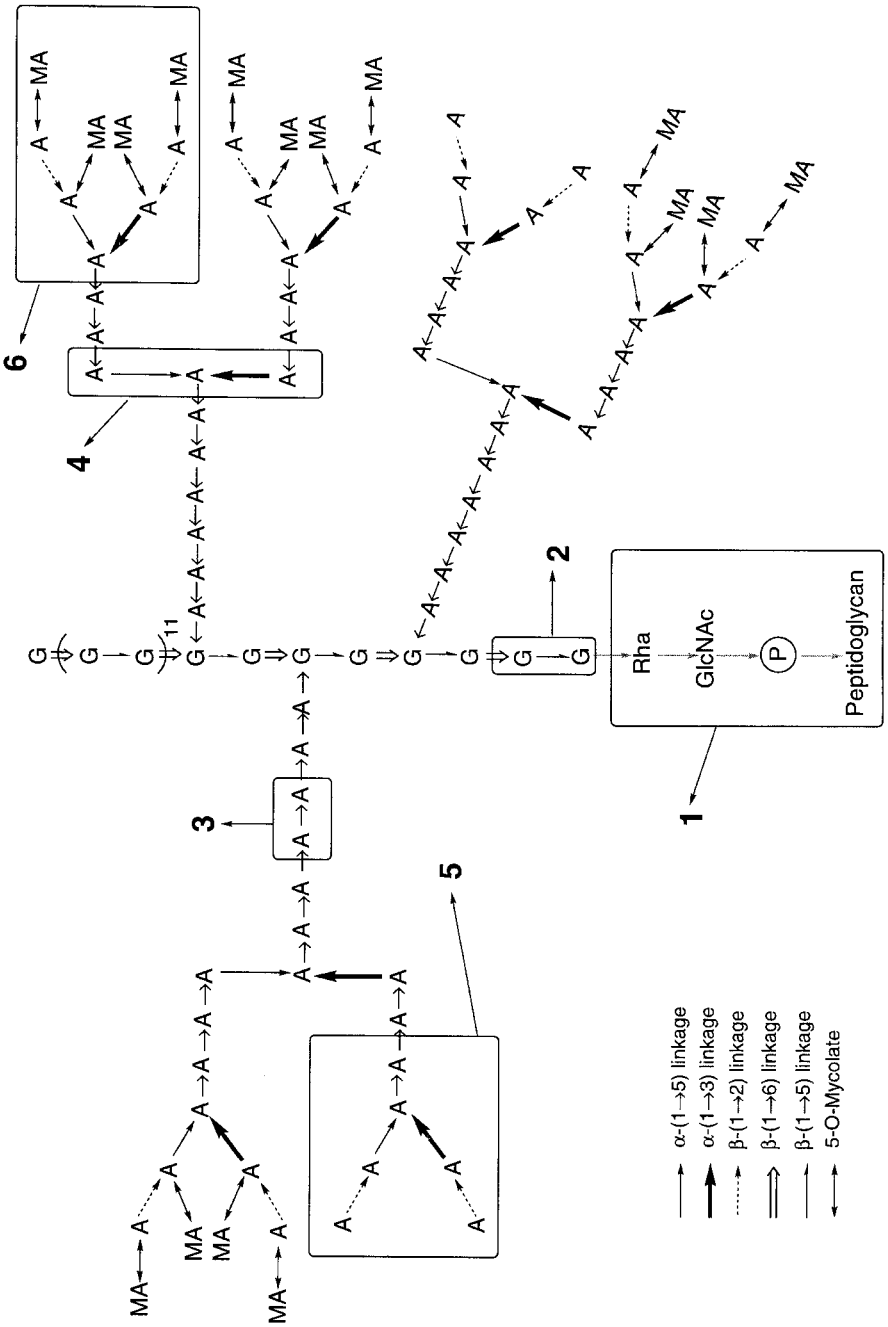


Figure 1. Schematic representation of mycobacterial arabinogalactan (AG).

A. Arabinogalactan

In common with gram-positive bacteria, mycobacteria have a layer of peptidoglycan immediately external to the cytoplasmic membrane. This peptidoglycan serves as the scaffold to which the AG is attached. Figure 1 is a schematic drawing of the overall structure of a single AG molecule.[6] A number of structural motifs are worthy of mention, and these are illustrated in Figure 2. Linkage of the AG to peptidoglycan is by way of phosphodiester through a disaccharide comprised of rhamnose and *N*-acetylglucosamine (**1**). Attached to the 4-hydroxyl group of the rhamnose moiety is a linear chain of approximately 30 D-galactofuranose (D-Gal*f*) residues with alternating β-(1 → 5) and β-(1 → 6) linkages (**2**). Along the galactofuranose chain are branch points at which arabinan chains are attached. This arabinan contains in total approximately 70 D-arabinofuranose residues and consists primarily of linear α-(1 → 5)-linked chains (**3**). However, there are periodic branch points at which another linear arabinan chain is attached via an α-(1 → 3) linkage (**4**). At the distal, nonreducing end of these chains is the branched hexasaccharide (**5**). In a single AG molecule, approximately two-thirds of these hexasaccharide motifs are esterified to mycolic acids at the four primary alcohols to provide (**6**). These lipids, which are unique to mycobacteria and other actinomycetes, are branched fatty acids containing

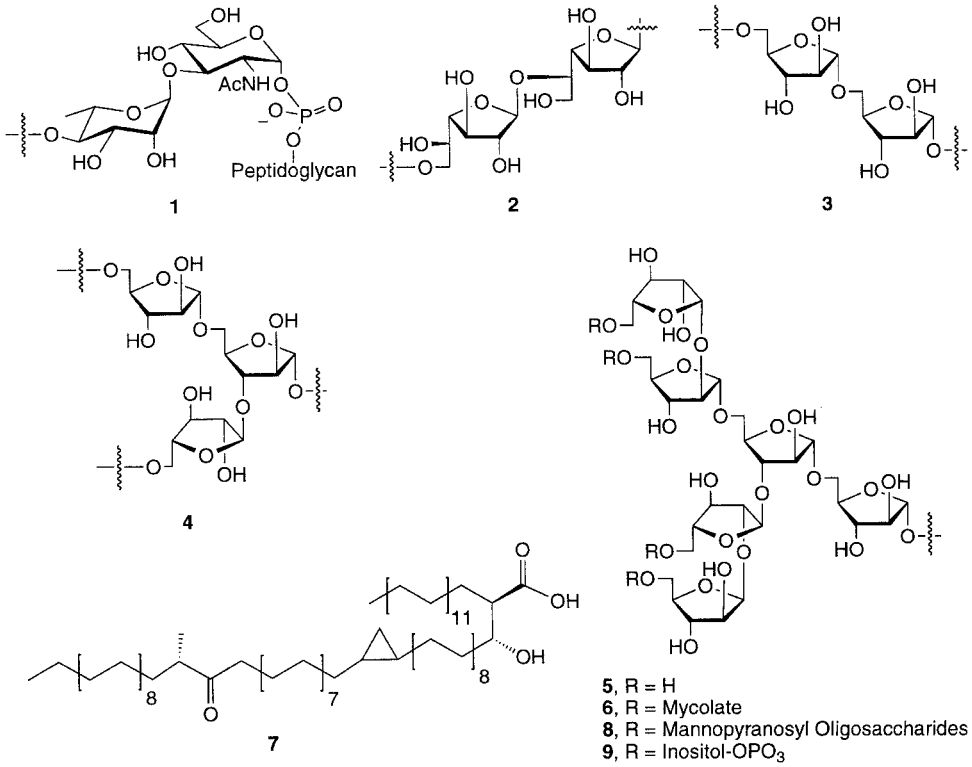


Figure 2. Structural motifs present in mycobacterial AG and LAM.

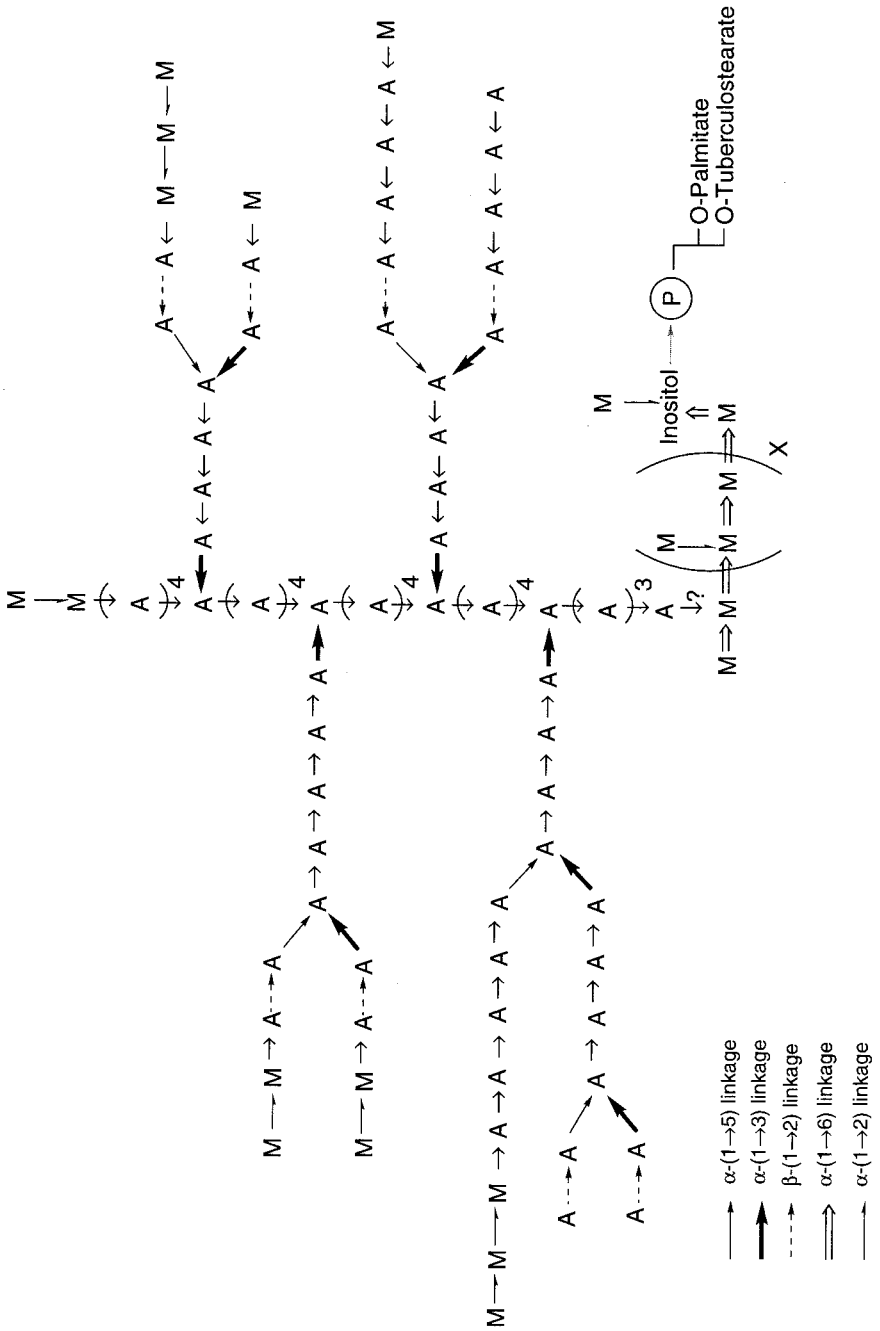


Figure 3. Schematic representation of mycobacterial lipoarabinomannan (LAM).

70–90 carbons as well as cyclopropane, ketone, hydroxyl, and methoxyl functionalities.[23,24] An example of one type of mycolic acid, a ketomycolate, **7**, is shown in Figure 2.

B. Lipoarabinomannan

LAM, which has a less homogeneous structure than the AG, is attached noncovalently to the cytoplasmic membrane through the lipid portion of a phosphatidylinositol (PI) linker. A schematic drawing of LAM is presented in Figure 3.[6] A mannan, comprised of α-(1 → 6) linked D-mannopyranose residues, is covalently bound via a glycosidic linkage to the inositol 06. Approximately half these mannose residues carry an α-(1 → 2) D-mannopyranosyl branch. An arabinan very similar to that found in the AG is attached at the termini of the mannan. In common with the AG arabinan, the major structural motif is an α-(1 → 5)-linked chain with periodic branch points, and hexasaccharide **5** at the nonreducing termini. However, thereare fewer branch points, and thus the ratio of linear to branched arabinofuranose in LAM is greater than in AG. In addition, while the hexasaccharide motifs (**5**) found in the AG are esterified to mycolic acids, in LAM these moieties are found either unsubstituted or, in particularly virulent strains, capped with short mannopyranosyl oligosaccharides (**8**). When capped with the mannose residues, the polymer is referred to as ManLAM. Other structural features have been discovered more recently. For example, hexasaccharide **5** found in LAM of *M. smegmatis* is capped with inositol phosphates (**9**), thus providing a new type of LAM termed PI-GAM (**p**hospho**i**nositol-**g**lycero**a**rabino-**m**annan).[25,26]

III. ROLE OF THE AG AND LAM IN DISEASE PROGRESSION

The AG together with the mycolic acids comprise the major structural component of the mycobacterial cell wall; intercalated within this framework is LAM. These polymers play paramount roles in enabling these organisms to survive in the human host. For example, in contrast to many other microorganisms, mycobacteria can thrive in human macrophages instead of being killed by them.[27] This property is believed to be due, in large part, to the impenetrable nature of the cell wall envelope. In addition to protecting the organism from macrophage destruction, the cell wall is believed to substantially reduce the flow of antibiotics into the organism[12,28] and to be involved in the initial stages of the infection by the binding of cell wall oligosaccharides to host receptors.[29]

A. Arabinogalactan

The mycolic esters at the nonreducing ends of the AG present a formidable hydrophobic barrier that prevents the passage of antibiotics into the organism.[28] The currently accepted structural model of the mycolyl-AG complex was initially proposed by Minnikin in 1982[24] and is illustrated in Figure 4. In this model the mycolic acid chains are packed side by side, perpendicular to the plane of the cytoplasmic mem-

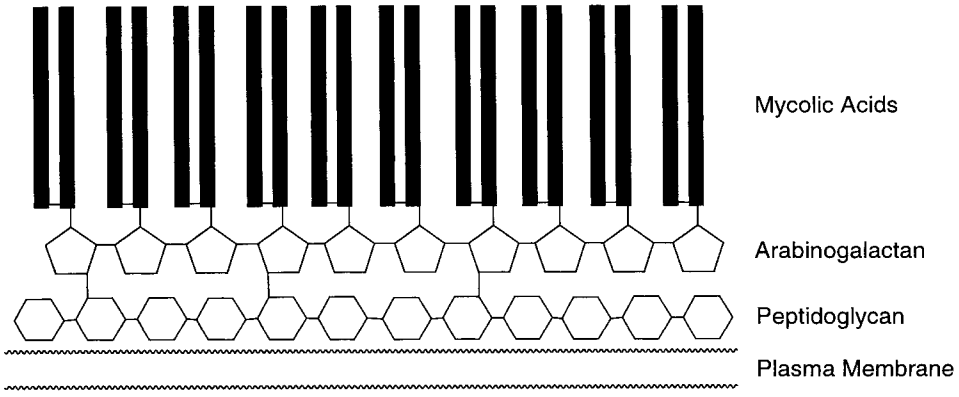


Figure 4. Schematic drawing of the mycolyl–AG complex.

brane. The tight packing of these chains results in very low fluidity of the cell wall, which in some cases is essentially crystalline.[30,31] The fluidity is dependent on the mycolic acids present, which in turn are species specific.[31] However, in all cases a major problem in treating mycobacterial infections is the passage of drugs through this lipid barrier into the host.

This model was initially criticized by some, who suggested that the AG was too large to accommodate the tight packing of the lipid chains.[28] However, a more detailed look at the structure of the AG provides a solution to this problem. A distinguishing feature of mycobacterial AG is the almost exclusive presence of furanosyl (Ara*f* or Gal*f*) residues. Furanoside rings are known to be much less conformationally well defined than their pyranoside counterparts.[32] It has been suggested that this inherent increased flexibility, in addition to the predominant coupling of these residues through linkages that involve primary hydroxyl groups, results in the polysaccharide being a scaffold of high flexibility. This in turn allows the polymer to adopt a conformation that facilitates the orientation of the mycolic acids into tightly packed parallel arrays. Additionally, this proposal provides a reasonable explanation for what, from an evolutionary standpoint, seems odd. Polysaccharides containing furanosyl residues are expected to be less thermodynamically stable than those possessing pyranose rings, and it therefore appears that these organisms have developed a system whereby glycan stability is traded for the protection afforded by the tightly packed mycolic acids.

B. Lipoarabinomannan

The major antigenic component of the mycobacterial cell wall is LAM. It should be pointed out that although not explicitly shown in Figure 4, LAM is interspersed throughout the mycolyl-AG framework, and this polymer is believed to be long enough that any groups found at the nonreducing terminus (e.g., the mannopyranosyl oligosaccharides) extend through the mycolic acid layer and are expressed on the outer

surface of the cell wall.[33] LAM has been suggested to play critical roles in many immunomodulatory events occurring during progression of the disease. Among these are the inhibition of macrophage activation,[34] the neutralization of potentially cytoxic O_2 free radicals,[35] the induction of cytokines,[36–38] the inhibition of protein kinase activities,[39] and the induced expression of collegenases that destroy the extracellular matrix of the lung.[40] LAM in which the primary hydroxyl groups of **5** are capped with mannopyranosyl oligosaccharides (**8**, ManLAM) has been suggested to be involved in the infection process. It is believed that these terminal mannose residues initiate infection by adhering to human cells through their recognition by human mannose binding proteins.[41–43] In contrast to the capping of **5** by mannopyranosyl residues, mycobacterial species (e.g., *M. smegmatis*) in which this motif is capped with inositol phosphate residues (**8**) are avirulent. It has been proposed that the lack of pathogenicity of this species arises from the induction of tumor necrosis factor α (TNF-α) by the inositol phosphate moieties, which in turn results in the killing of the bacteria by macrophages.[26]

Perhaps the most important recent discovery is that T cells recognize LAM via antigen presentation pathways that are independent of the major histocompatibility complex (MHC).[44,45] It was shown that LAM is initially recognized by a mannose receptor and the protein CD14 before it is processed and then complexed with CD1b. This complex is then expressed on the surface of the antigen-presenting cell. Recognition by the T cell requires both the carbohydrate and lipid portion of LAM, and two distinct cell lines have been shown to differentiate between structurally different LAM fragments. Thus it appears that there is a significant amount of specificity in T-cell responses mediated by the LAM-complexed CD1 glycoproteins.

IV. ARABINAN BIOSYNTHESIS

Given the critical importance of AG and LAM to the survival of mycobacteria, there is increasing interest in developing new drugs for the treatment of these diseases, which act by inhibiting the enzymes involved in their biosynthesis. Inhibitors of the glycosyltransferases involved in the assembly of the furanosidic oligosaccharide portions of the glycoconjugates are particularly attractive targets. Glycans of this type are unknown in humans, and therefore blocking these biosynthetic pathways will have little deleterious effect on the host. However, for rational drug design to succeed, more details about the biosynthetic pathways leading to the formation of these polysaccharides are required. Although the overall features of this process are known,[13,18,19] many of the details are not.

The β-(1 $\rightarrow$ 2)- and α-(1 $\rightarrow$ 5)-linked arabinose residues are incorporated into the polymer from the activated polyprenyl sugar phosphate **10**, which is in turn synthesized from glucose via 5-phosphoribose pyrophosphate (pRpp).[46–48] Elongation of the polymer chain is believed to involve a family of arabinosyltranferases (AraT's) that recognize both **10** and arabinofuranoside-based acceptors of differing structures (Figure 5).[18,19,49,50] In AG biosynthesis, the entire polysaccharide appears to be assembled as a polyprenol diphosphate intermediate, which is transferred to peptidoglycan prior to the addition of the mycolate esters.[18] In LAM biogenesis, the

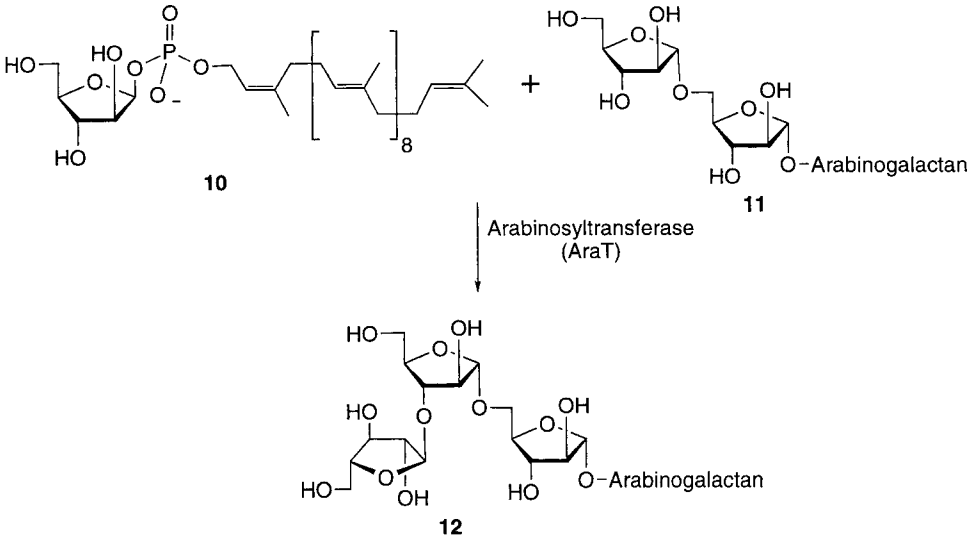


Figure 5. A typical reaction catalyzed by mycobacterial arabinosyltransferases (AraT's).

arabinan portion is believed to be synthesized as a polyprenol phosphate that is transferred to lipomannan.[51]

Incorporation of arabinose into AG has been demonstrated through the use of synthetic, radiolabeled **10** and mycobacterial membrane extracts.[50] These reactions are believed to involve membrane-bound substrates and enzymes and to occur extracellularly. While to date none of these putative glycosyltransferases have been purified to homogeneity, the gene cluster that encodes for one or more of these AraT's has been cloned and expressed.[52–54] In addition, an assay for their activity that uses mycobacterial membrane preparations as the enzyme source has been developed.[49,50] The assay measures the incorporation of radiolabeled arabinose from **10** into arabinan, and it has been used to screen both potential substrates and inhibitors.[46,47,55,56]

Although the natural acceptor substrates for these enzymes are lipid-bound intermediates, these AraT's also recognize small arabinofuranosyl oligosaccharides. A few of the oligoarabinosides that have been shown to be substrates for these enzymes are illustrated in Figure 6.[49,55] Arabinofuranose disaccharides appear to be the minimum epitopes. Furthermore, investigation of the AraT recognition of the disaccharide α-D-Ara*f*-(1 → 5)-α-D-Ara*f*-R (**13**–**15**) and the trisaccharide α-D-Ara*f*-(1 → 5)-α-D-Ara*f*-(1 → 5)-α-D-Ara*f*-R (**16**, **17**) revealed that glycosides with R = octyl (**14**, **17**) are better substrates and those with R = methyl (**13**, **16**). Suprisingly, the dodecyl glycoside **15** was a poorer substrate than **13**. In the investigation of **14** and **17**, the products of the enzymatic reactions were isolated and their structures elucidated.[49] In both cases, mixtures of two products, corresponding to addition of an Ara*f* residue to either the 2- or the 5-position of the terminal residue in the oligosaccharide acceptor, were obtained. From this study it was concluded that **10** was the source of the β-(1 → 2)-, and α-(1 → 5)-linked Ara*f* residues.

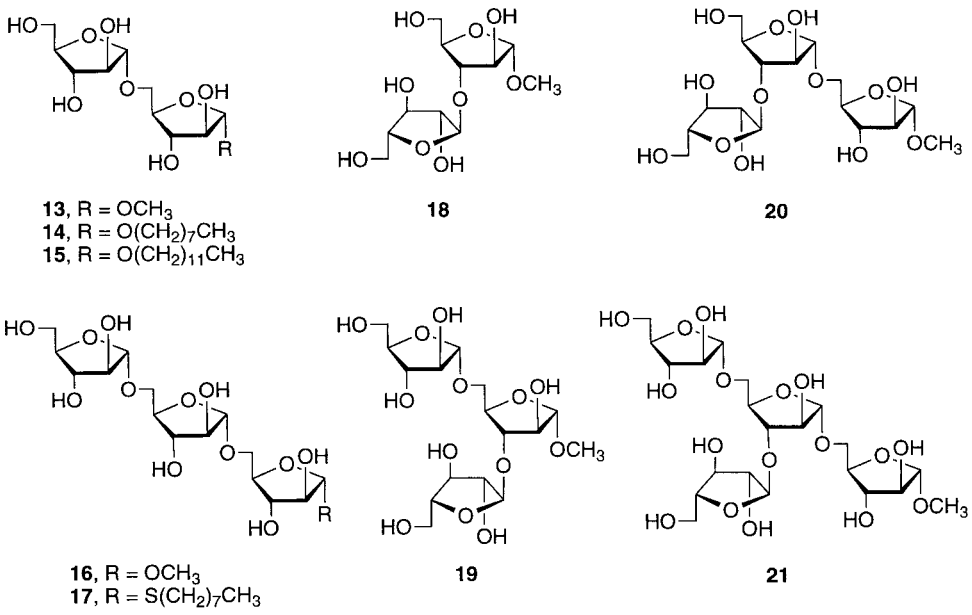
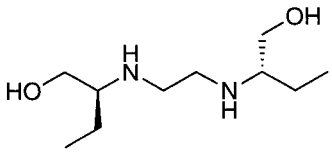


Figure 6. Arabinofuranosyl oligosaccharides tested as substrates for mycobacterial arabinosyltransferases.

Less is known about the formation of the α-(1 → 3) branch points than about the β-(1 → 2) and α-(1 → 5) linkages. Only β-(1 → 2) and α-(1 → 5) linkages are formed by the incubation of small oligosaccharide substrates with **10** in the presence of a mycobacterial membrane preparations.[49] However, additional work[57] demonstrated that when radioactive **10** is incubated with endogenous acceptors and a mycobacterial membrane preparation, a polymer essentially identical to native AG can be obtained. Additionally the radioactivity in the polymer was equally distributed throughout, which suggests that **10** is the major, and possibly sole, source of Ara*f* residues. The lack of α-(1 → 3) linkages formed in the initial study[49] could be attributed to a number of factors: 1) the instability or absence of α-(1 → 3) AraT activity in the membrane preparation, 2) the possibility that this enzyme recognizes oligosaccharide substrates larger than those investigated, or 3) the possibility that another activated donor (e.g., a sugar nucleotide) is used by this AraT. Although the presence of UDP-Ara*f* in mycobacteria has been reported,[58] the incorporation of arabinose from this source into arabinan has not been demonstrated.

The range of donor substrates that are tolerated by these enzymes has also been explored. A series of analogs was prepared, each one differing from **10** only in the identity of the lipid chain, and the set was screened for biological activity.[56] It was discovered that conjugates of C_{50} and C_{55} prenols were substrates, while those compounds with shorter ($<C_{10}$) prenol derivatives (geraniol, nerol, and citronellol) were inactive.



22, Ethambutol

Figure 7. Structure of the antituberculosis drug ethambutol.

A. Action of Ethambutol

From the discussion above is it clear that both AG and LAM play paramount roles in the infection and survival of mycobacteria in the host. Drugs that act by inhibiting the biosynthesis of these polysaccharides are expected to show antimycobacterial action. One of the commonly used antituberculosis agents, ethambutol[(*S,S′*)-2,2′-(ethylenediimino)di-1-butanol, **22**] (Figure 7), has been used for the treatment of tuberculosis since 1961, when it was first reported to have antimycobacterial activity.[59] This drug is active only against mycobacteria,[8] and while it was thought for many years to be involved in inhibiting cell wall biosynthesis,[60,61] its precise target was not elucidated until recently. The structure of the drug is relatively simple and bears little resemblance to any cell wall component, which have complicated the identification of its precise biochemical target. It is now known that the drug acts as an AraT inhibitor and disrupts the biosynthesis of the arabinan portions of both AG and LAM.[43,47,62–64]

V. SYNTHESIS OF D-ARABINOFURANOSIDES

In comparison with the synthesis of oligopyranosides, the preparation of oligosaccharides containing furanose residues has received relatively little attention. However, this will likely change as the biological importance of glycans containing these residues becomes more widely appreciated. The methodologies used to date for assembling these molecules have closely paralleled the methods used for the assembly of pyranosidic linkages. Nevertheless, these interesting structures also open up the possibility for new methodologies to be developed, and these will undoubtedly emerge.

In this section are outlined the chemical synthesis of D-arabinofuranosides that have been reported. As explained in Section IV, although the normal acceptor substrates for mycobacterial arabinosyltransferases are high molecular weight glycolipids, some (and probably most, if not all) of these enzymes will recognize small oligosaccharide fragments of these large glycans.[49,55] In this regard, these enzymes appear to be similar to mammalian glycosyltransferases.[65] Therefore, these small oligosaccharides are invaluable tools not only for mapping out substrate specificities of these enzymes but also as templates for the design of potentially inhibitory analogs.

Route to Arabinofuranose Donors

At equilibrium in solution, most sugars exist predominantly in the pyranose forms. For example, in water, D-arabinose is found in approximately a 95:5 pyranose-to-furanose ratio.[66] Therefore, the initial step in any synthesis of an oligofuranoside is the conversion of this mixture of pyranoses to a furanosyl derivative that can be incorporated into oligosaccharides. This can be done either directly or indirectly as outlined below.

1. Fisher Glycosylation

Furanose glycosides of simple alcohols are conveniently obtained by subjecting a reducing sugar to a controlled Fisher glycosylation reaction. The furanosides are the kinetic products of this reaction, and excellent yields of the product (as a mixture of anomers) are usually obtained if the proper conditions are chosen.[67] The alcohol generally used in this reaction is methanol, which serves as both a reactant and the solvent. The methyl glycosides thus obtained must then be protected and converted to a suitable glycosylation agent. It is also possible to synthesize glycosides of other alcohols by this method; however, since the alcohol is usually employed as the solvent, the choice is somewhat limited. Therefore, from a practical point of view, this method is most useful for the preparation of glycosides of relatively volatile alcohols (e.g., methanol, ethanol, and allyl alcohol).

This method works well for the preparation of methyl D-arabinofuranosides, and an excellent preparation is available[68] that can be used to prepare multigram quantities of the product (Figure 8). Treatment of D-arabinose (**23**) with acidic methanol at room temperature provides a 1:1 α:β mixture of methyl arabinofuranosides (**24**). Benzoylation provides **25**, again as a mixture of anomers; however, the α isomer can be selectively crystallized from ethanol. With either **24** or **25** in hand, it is possible to prepare, in 1–3 steps, the glycosyl donors shown in Figure 9. These include glycosyl acetates **26**[69] and **27**,[70,71] glycosyl halides **28**,[68,72] **29**,[49] **30**,[73] **31**,[73] and thioglycosides **32**–**34**.[55,74–76]

2. Cyclization of Acyclic Dithioacetals and Related Derivatives

An old[67,77] but recently rediscovered[78] method for synthesizing furanose glycosides involves the cyclization of acyclic sugar *S*,*O*-acetals (e.g., **35**) in the

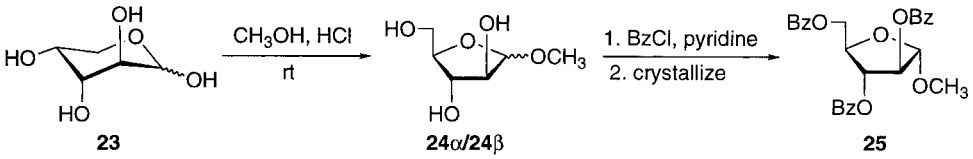


Figure 8. Fisher glycosylation route 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside (**25**).

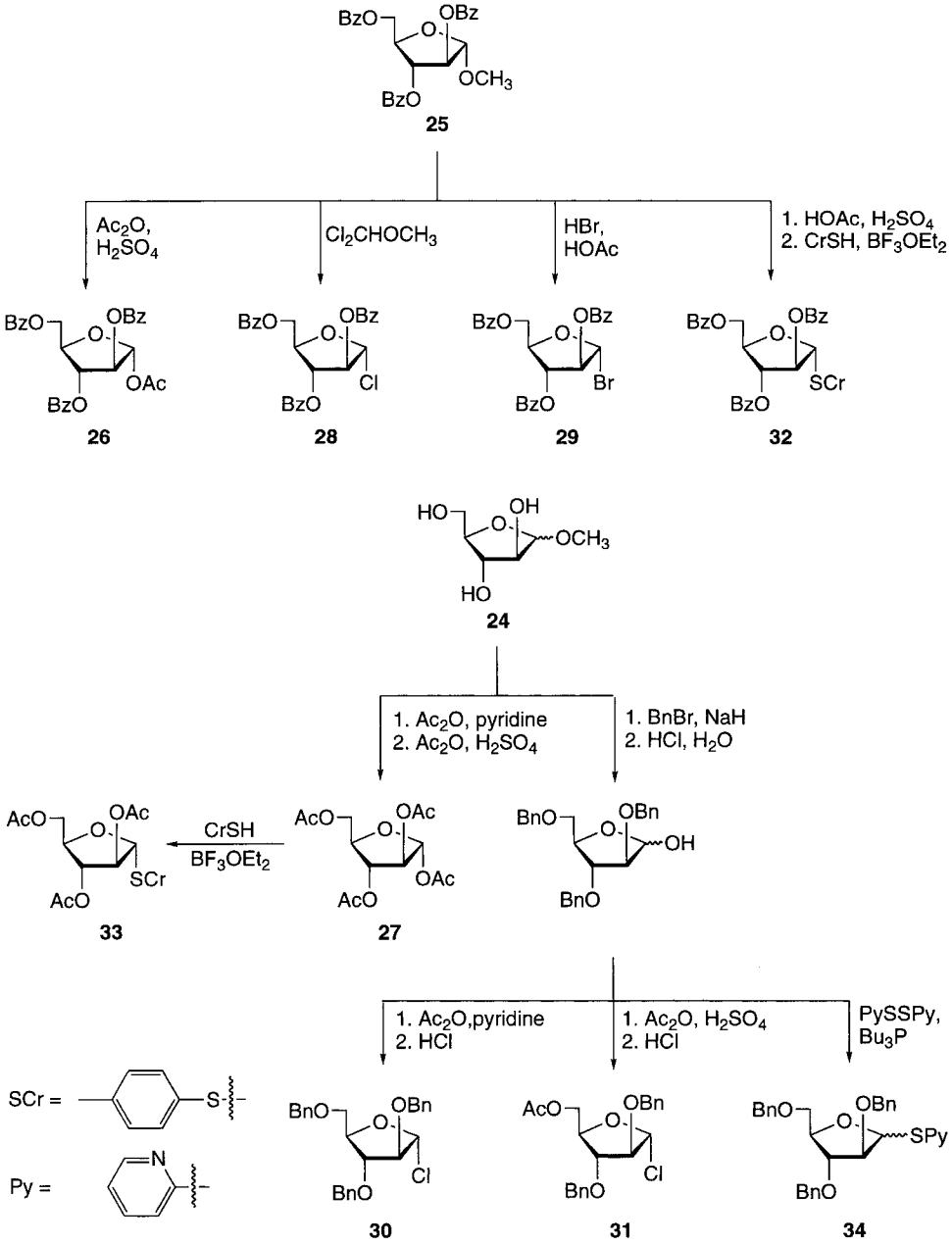


Figure 9. Synthesis of commonly employed D-arabinofuranosyl donors.

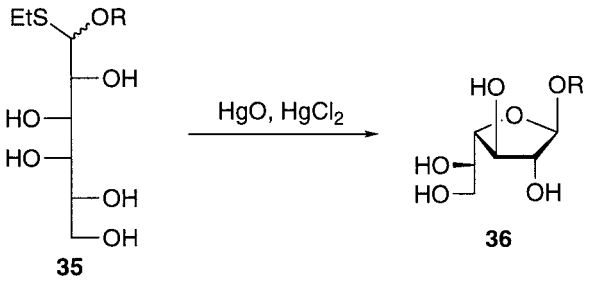


Figure 10. Synthesis of β-galactofuranosides from acylic *S*,*O*-sugar acetals.

presence of mercuric salts (Figure 10). This method is highly selective, producing furanosides in the absence of pyranosides, and is especially useful for the synthesis of galacto-furanosides (e.g., **36**) of both simple and carbohydrate alcohols.[67,78] This approach has not yet been used for the synthesis of D-arabinofuranosides; however, two related syntheses have been reported (Figure 11). Work by Zinner and coworkers[79] demonstrated that the cyclization of the 5-*O*-benzoyl derivative of D-arabinose diethyl dithioacetal (**37**) in mercuric chloride afforded modest yields of thioglycoside **38**. More recently, the isopropylidene derivative **39** has been prepared in good yield from **37** by treatment with mercuric chloride in acetone.[80] Conversion of **37** to any of the derivatives shown in Figure 9 can be achieved in a few steps.

3. Ozonolysis of Glycals

The most recently reported route to the D-arabinofuranose ring nucleus involves an oxidative cleavage of protected glucal derivatives as illustrated in Figure 12.[74] Ozonolysis of 3,4,6-tri-*O*-acetyl D-glucal (**40**), followed by reductive workup with

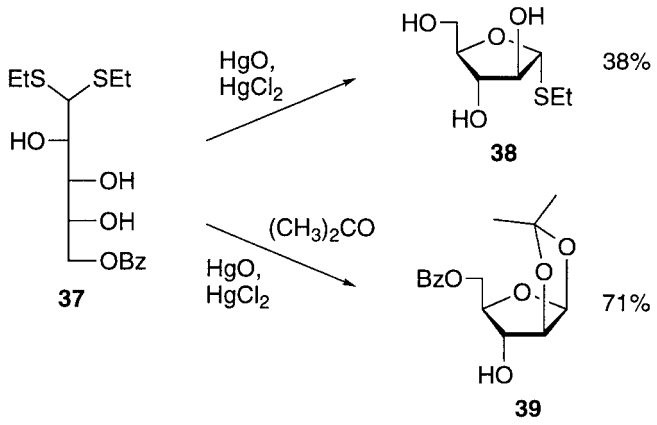


Figure 11. Cyclization of arabinose dithioacetals to give furanose derivatives.

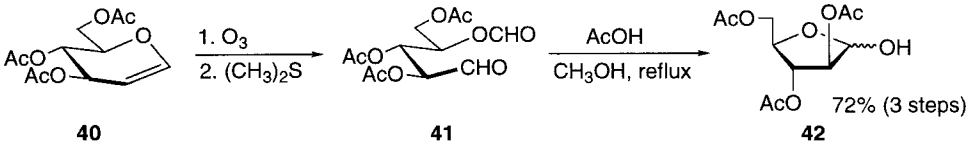


Figure 12. Synthesis of protected arabinofuranose reducing sugars by ozonolysis of acetylated D-glucal.

dimethyl sulfide, affords the formyl aldehyde **41**. The formate ester can be selectively cleaved in the presence of the acetates by refluxing in acidic methanol yielding the reducing sugar **42**. Attempts to use **42** directly in glycosylation reactions via in situ activation of the anomeric hydroxyl group[81,82] failed, but this derivative could be converted in two steps and excellent yield to thioglycoside **33**.[74]

Protected D-glucal derivatives are readily available, and consequently this methodology is an attractive alternative to more conventional routes to arabinofuranosides. In particular, the conversion of fully ^{13}C-labeled glucose to labeled arabinose oligosaccharides by this route has been demonstrated.[79] For the synthesis of ^{13}C-labeled oligoarabinosides, this method is preferable to a strategy involving Fisher glycosylation of labeled D-arabinose because the cost of the starting material (^{13}C-labeled D-glucose) is fourfold cheaper, while the overall yields of the transformations are similar.

B. Glycosylation Reactions with Arabinofuranose Donors

The glycosyl donors illustrated in Figure 9 have been the ones most commonly employed in the preparation of oligosaccharides containing D-arabinofuranosyl residues. The vast majority of glycosidic bonds in mycobacterial arabinan are α linkages, and thus the relationship between the groups at C1 and C2 are trans. Consequently, the synthesis of these linkages can be achieved through the use of glycosyl donors possessing acyl protecting groups on O2. As would be expected, neighboring group participation resulting in the formation of an acyloxonium ion provides, in most cases, good to excellent yields of the 1,2-*trans*-glycoside. Donors **26**–**29**, **32**, and **33** have been used effectively for these glycosylation reactions; however, with some donors the stereocontrol is poorer than in others. For example, it has been demonstrated that while thioglycoside **33** provides good yields of the desired product, peracetate **27** gave significant amounts of the 1,2-*cis* product.[74] Similar observations have been made with other arabinofuranosyl glycosyl donors.[71,83]

In addition to 1,2-*trans*-D-arabinofuranosyl linkages, mycobacterial arabinan also contains β-D-arabinofuranosyl residues (1,2-*cis*-glycosides). These linkages are directly analogous to β-D-mannopyranosides, which are among the most difficult of all glycosidic linkages to prepare.[84] However, in contrast to the β-D-mannoside case, no significant effort has been directed toward the stereocontrolled synthesis of β-D-arabinofuranosides. A minimum prerequisite to the synthesis of any such linkage are donors possessing a nonparticipating group at O2. For this purpose, the 2-*O*-benzyl

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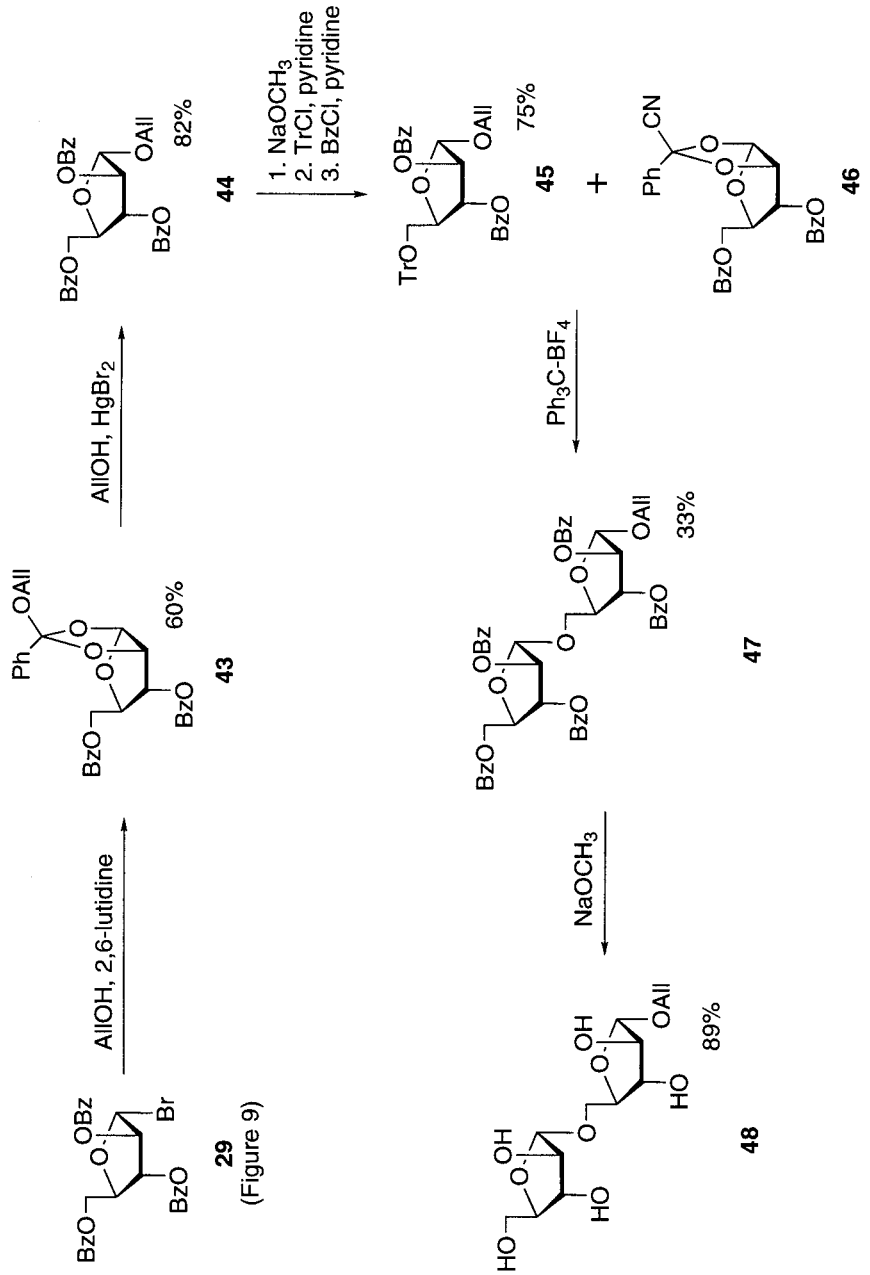


Figure 13. Synthesis of disaccharide **48**: All, allyl; Tr, triphenylmethyl.

derivatives **30**, **31**, and **34**, have been used[73,85,86] to produce, in most cases, mixtures from which the desired compounds could be isolated.

Characterizing the stereochemical course of these glycosylation reactions is easily done by means of standard one-dimensional ^{13}C and ^{1}H NMR experiments.[83] The anomeric carbon of the α anomers resonate between 107 and 110 ppm; the β anomers between 97 and 104 ppm. Furthermore, in the α anomers $^{3}J_{H1,H2}$ is small (0–2 Hz), whereas in the β anomers the magnitude of this coupling is larger (3–5 Hz). It should also be mentioned that although $^{1}J_{C1,H1}$ values are unambiguous determinants of anomeric stereochemistry in pyranosides,[87] they cannot be used to make these assignments in arabinofuranosides as their magnitudes have been shown to be insensitive to anomeric configuration.[83]

1. Syntheses of 1,2-*trans*-Linked Oligosaccharides

The disaccharide α-D-Ara*f*-(1 → 5)-α-D-Ara*f* comprises the core structure of the linear sections of the arabinan. A number of syntheses of this glycan nucleus, as glycosides of a variety of alcohols have been reported. The first synthesis was of the allyl glycoside **48**, which was prepared as illustrated in Figure 13.[72] Orthoester **43** was prepared from the bromide **29** and then rearranged to give the allyl glycoside **44**. A series of protection and deprotection steps afforded the trityl ether **45**, which was subsequently glycosylated with orthoester **46** (also prepared from **29**). Deprotection

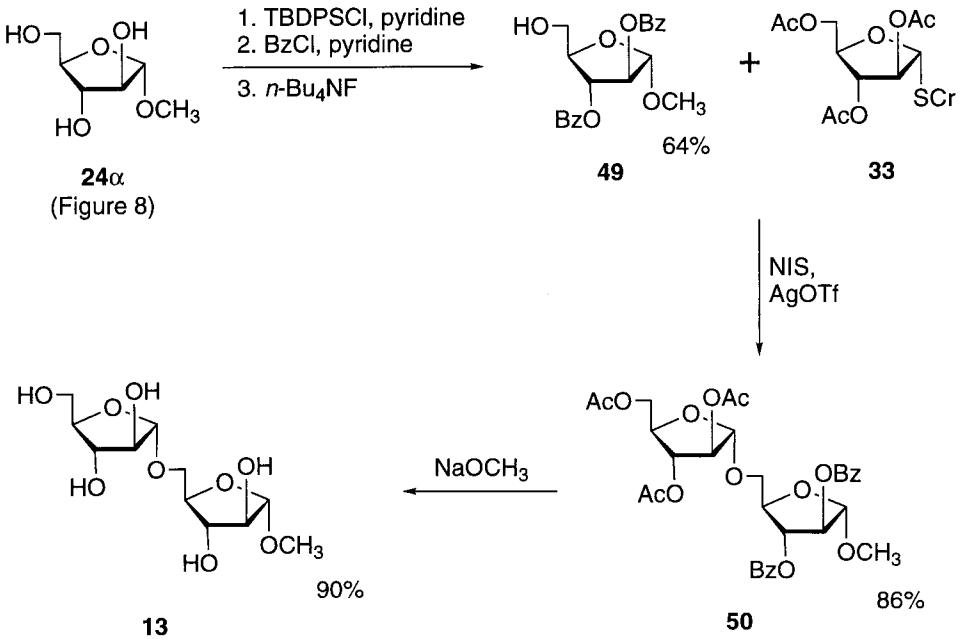


Figure 14. Synthesis of disaccharide **13**: TBDPS, *t*-butyldiphenylsilyl; NIS, *N*-iodosuccinimide; AgOTf, silver trifluoromethanesulfonate.

afforded the tartet, **48**. In the same paper, this route was used to prepare the benzyl glycoside starting from a benzyl orthoester analogous to **43**.

As outlined in Figure 14, a more recent synthesis[55,74] of this disaccharide as the methyl glycoside (**13**, Figure 6) made use of thioglycoside donor **33**. This synthesis began with the preparation of alcohol **49** from **24**α, through a sequence of silylation, benzoylation, and desilylation. Glycosylation promoted by *N*-iodosuccinimide and silver triflate afforded the disaccharide **50**, which could be deprotected with sodium methoxide to provide **13**.

A number of other derivatives of this disaccharide have been synthesized, namely, the 2′-chloroethyl, octyl (**14**), dodecyl (**15**), and hexyl glycosides.[49,71] These syntheses have employed either acetate **27** or chloride **28** as the glycosylating agent.

The trisaccharide α-D-Ara*f*-(1 → 5)-α-D-Ara*f*-(1 → 5)-α-D-Ara*f* is also a core fragment of the linear arabinan, and two synthetic derivatives have been reported. The chloride **28** has been used to prepare the *S*-octyl glycoside analog (**17**), as well as the methyl glycoside (**16**).[49] Another route to **16** is illustrated in Figure 15.[55] Monosaccharide **33** was converted to the differentially protected thioglycoside **51** in three steps. Glycosylation of **49** with **51** provided the protected disaccharide **52**, which upon treatment with tetrabutylammonium fluoride was transformed to alcohol **53**. The trisaccharide was then obtained by glycosylation of **53** with **33** and then deprotection.

Four other oligosaccharides (**18**–**21**, Figure 6) corresponding to fragments of either the linear or the branched hexasaccharide moieties of the arabinan have been

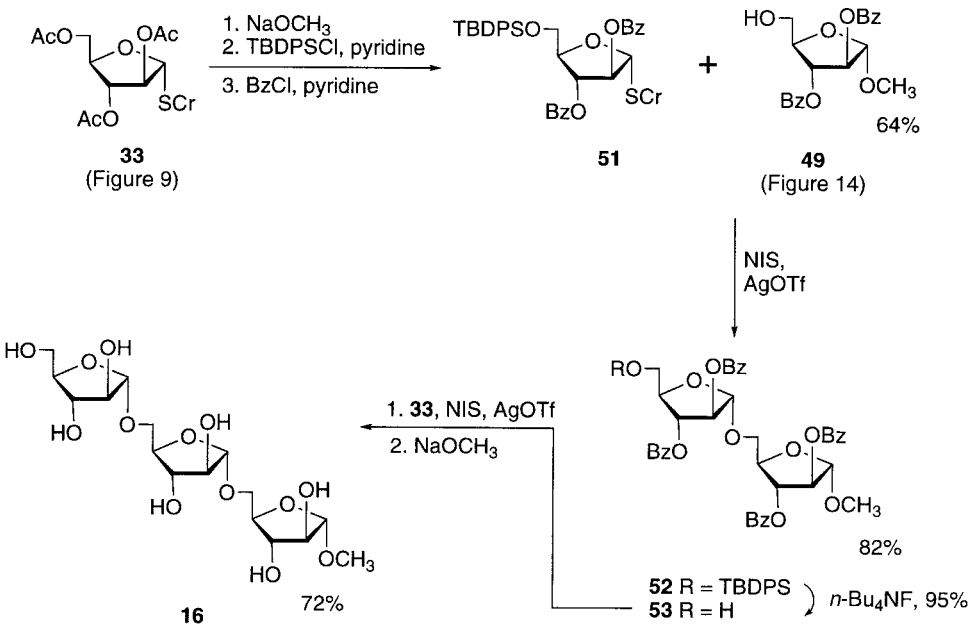


Figure 15. Synthesis of trisaccharide **16**: TBDPS, *t*-butyldiphenylsilyl; NIS, *N*-iodosuccinimide; AgOTf, silver trifluoromethanesulfonate.

synthesized as methyl glycosides.[55,74] These were obtained in a manner similar to the disaccharide and trisaccharide via glycosylation of methyl glycoside acceptors with thioglycoside donors.

2. Syntheses of 1,2-*cis*-Linked Oligosaccharides

The stereocontrolled synthesis of β-arabinofuranosides has received little attention since the reports, over 30 years ago, that solvolyses of 2-*O*-benzylated α-D-arabinofuranosyl halides (e.g., **30** or **31**) with methanol gave mixtures of glycosides in which the β isomer predominates.[86,87] It is of tangential interest to note that kinetic studies were done, and it was proposed that this reaction proceeds through an ion pair S_N1, not S_N2 mechanism.

More recently, this method has been used to synthesize octyl β-D-arabinofuranosides from **30** and **31**.[73] As outlined in Figure 16, reaction of these labile chlorides in the presence of octanol *without a promoter* gave modest yields of β-glycosides **54** and **55**. The reaction of **30** under these conditions gave only the desired product; however, in the case of **31**, traces of the α isomer were also detected. Although the yields are modest, the ease by which the starting material can be prepared (two steps from commercially available 2,3,5-tri-*O*-benzyl arabinofuranose, **56**) makes this an attractive route to these products. Unfortunately, while this method can be used for the synthesis of glycosides of simple primary alcohols, attempts to glycosylate secondary carbohydrate alcohols by this method failed, and thus the method appears to be of limited utility for the synthesis of oligosaccharides.

On the other hand, reaction of the pyridyl thioglycoside **34** with alcohol **57** has been shown[76] to provide good yields of the β-linked disaccharide **58** (Figure 17). No mention was made of the formation of any α-glycoside product in this glycosylation reaction. This disaccharide product was then used as a synthon for the preparation of a pentasaccharide fragment (**62**) of the hexasaccharide motif found at the nonreducing terminus of AG and LAM. Coupling of **58** with alcohol **59** followed by deprotection

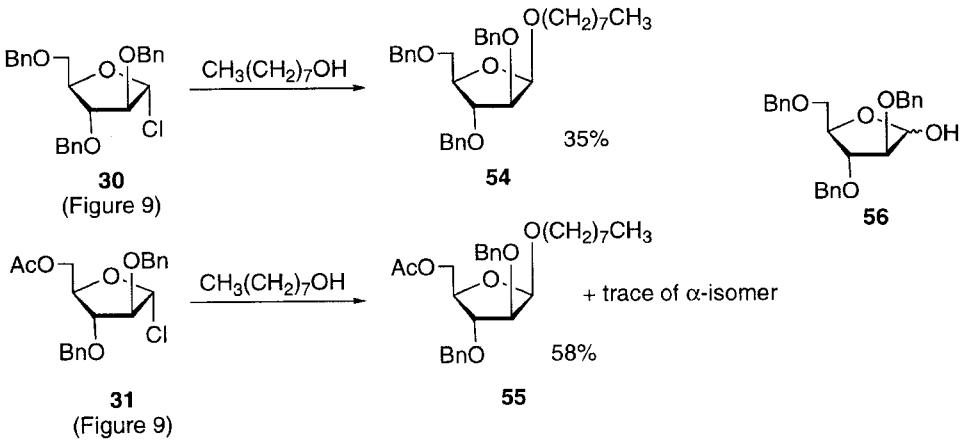


Figure 16. Synthesis of β-D-arabinofuranosides by alcoholysis of glycosyl chlorides.

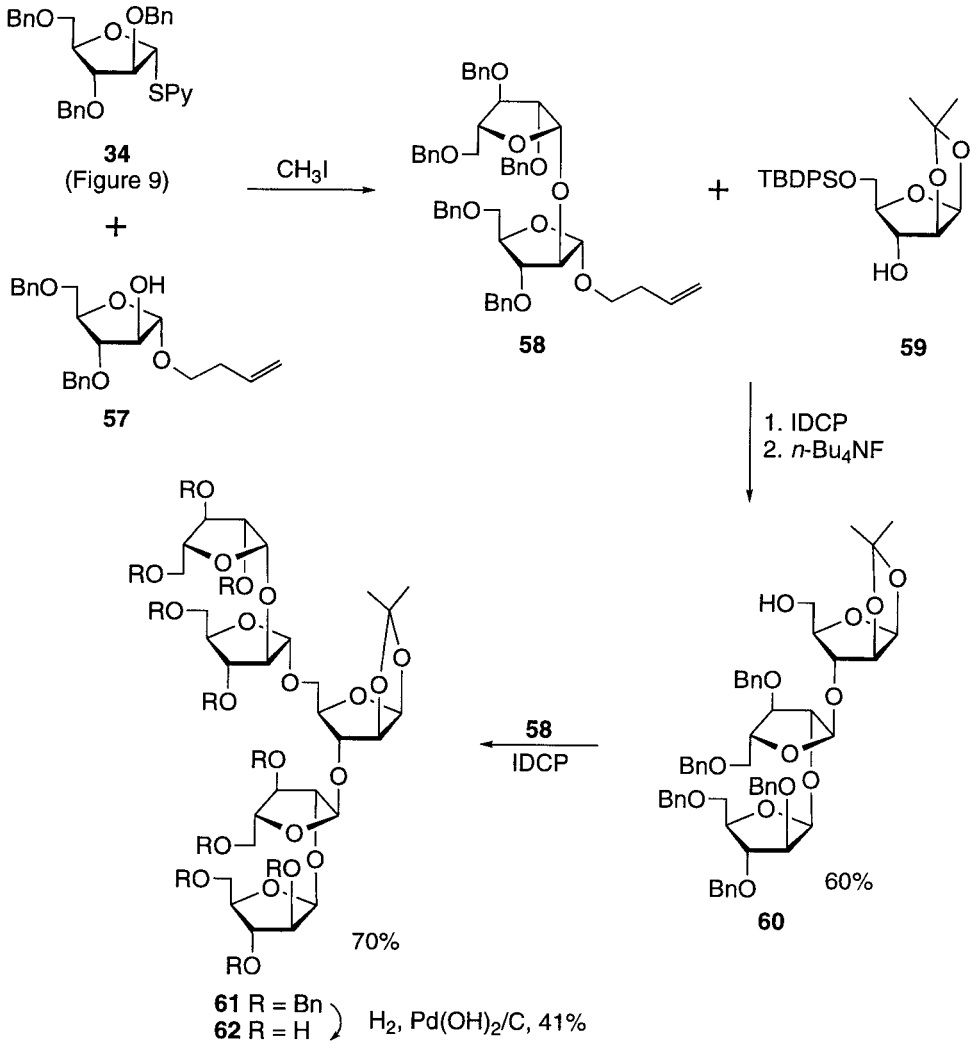


Figure 17. Synthesis of **62**: IDCP, iodine dicollidine perchlorate; TBDPS, *t*-butyldiphenylsilyl.

of the silyl group afforded trisaccharide **60**. Glycosylation of **60**, again with **58**, provided the protected pentasaccharide **61**, which was subsequently hydrogenated to afford the product. In this synthesis the first glycosylation proceeded stereoselectively to afford the α-linked product, but in the second case, a separable 3:2 α:β mixture was produced.

Unquestionably, the stereocontrolled synthesis of β-D-arabinofuranosides is an area ripe for investigation. Indeed, no general methods for their preparation currently exist. Although new strategies will likely appear, it is probable that many of the methodologies used for β-mannoside synthesis can be applied to this problem. Indeed, recent work[88,89] has shown that Ogawa's[90,91] intermolecular aglycone delivery method for β-mannoside synthesis can be used for the stereoselective synthesis of β-D-

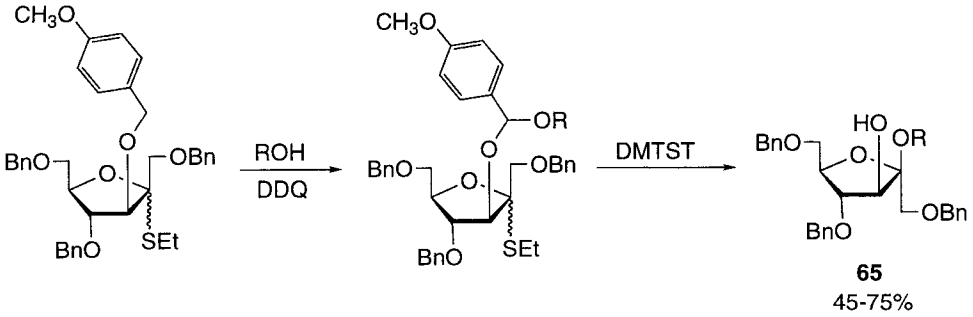


Figure 18. Synthesis of β-D-fructofuranosides by intramolecular aglycone delivery.

fructofuranosides (e.g., **65**) (Figure 18). Given the very close structural similarities between D-arabinofuranose and D-fructofuranose, it is likely that the approachcan be used, but this has yet to be demonstrated.

VI. CONFORMATIONAL STUDIES

A detailed appreciation of the biological function of a molecule requires that its conformation be understood. Therefore, in recent years a great deal of effort has been directed toward elucidating the solution conformation of oligosaccharides.[92] The work, however, has been heavily focused on oligopyranosides. At the same time, a wealth of information detailing the conformation of furanose rings in nucleotides and nucleic acids has accumulated.[93] However, except for sucrose,[94,95] only recently have conformational investigations of oligosaccharides containing furanose residues been reported.[96,97] Outlined in this section are some recent investigations that have explored the solution conformation of methyl α-D-arabinofuranoside and oligosaccharides containing α-D-arabinofuranosyl residues.

A. Background

In oligosaccharides containing pyranosyl residues, most, if not all, of the constituent rings are rigid, existing in one of two possible chair conformations. Thus, the major degrees of freedom available to an oligopyranoside are rotation about glycosidic bonds, which can be described by two torsion angles (Φ, Ψ), and rotation about the exocyclic C—C bond (torsion angle ω), as indicated in Figure 19.[98] Three possible staggered conformations of ω are possible (gg, gt, and tg, Figure 20) and usually a mixture is present in solution.[99] NMR studies focused on elucidating oligosaccharide structure have relied heavily on the measurement, of $^3J_{H,H}$ to determine ring conformations and rotamer populations of exocyclic hydroxymethyl groups of the constituent monosaccharide residues.[92,99] The value of Φ is fixed by the exoanomeric effect, which place one of the lone pairs of the glycosidic oxygen antiperiplanar to the C1-ring oxygen bond, and the value of Ψ is determined largely by steric constraints.

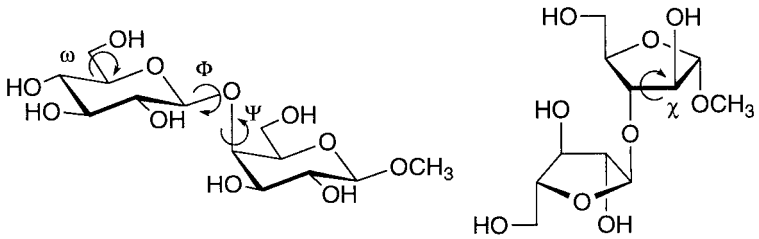


Figure 19. Glycosidic, hydroxymethyl group, and ring torsion angles in pyranosides and furanosides.

The Φ, Ψ angles for a glycosidic linkage cannot be determined by means of $^{3}J_{H,H}$ values. Therefore, to orient the individual residues together in three-dimensional space, most investigations have employed either interresidue nuclear Overhauser effect (NOE) constraints[92] or $^{3}J_{C,H}$ values[92,100–104] measured across glycosidic linkages.

In oligofuranosides, the problem is more complex. In addition to rotation about glycosidic and exocyclic C—C bonds, the rings themselves are flexible. Similar to substituted cyclopentanes, furanose moieties exist in either envelope (E) or twist (T) conformations.[105] At the monosaccharide level, often two of these conformations are of roughly equal energy and are thus present in equilibrium. These puckered conformers generally interconvert through pseudorotation, a process whereby, through a series of small molecular reorganizations, two conformations can equilibrate. An alternate pathway, inversion through the planar ring form, is disfavored because in the planar form, all substituents on the ring are eclipsed, which results in a significant energy penalty due to torsional strain.[106]

A particular ring conformer can be described by two parameters, the puckering amplitude (τ_m) and the pseudorotational phase angle (*P*), which can be illustrated by the pseudorotational wheel shown in Figure 21 for a D-aldofuranose.[106] The radius of the circle is τ_m and *P*, which defines the part of the ring that is most puckered, is indicated along with the associated envelope or twist conformer. A consequence of ring pucker is that in addition to the Φ, Ψ, and ω torsion angles, furanoses possess a ring torsion angle, χ (Figure 19). In solution, furanose rings typically exist as an equilibrating mixture of conformers, one in the northern hemisphere and another in the southern, respectively termed the north (N) and south (S) conformers. Given two low-

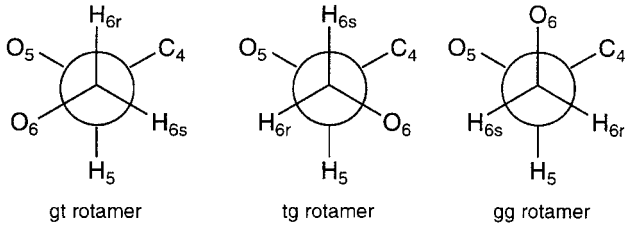


Figure 20. Staggered rotamers about the C5−C6 bond in a pyranose ring (C4−C5 in furanoses).

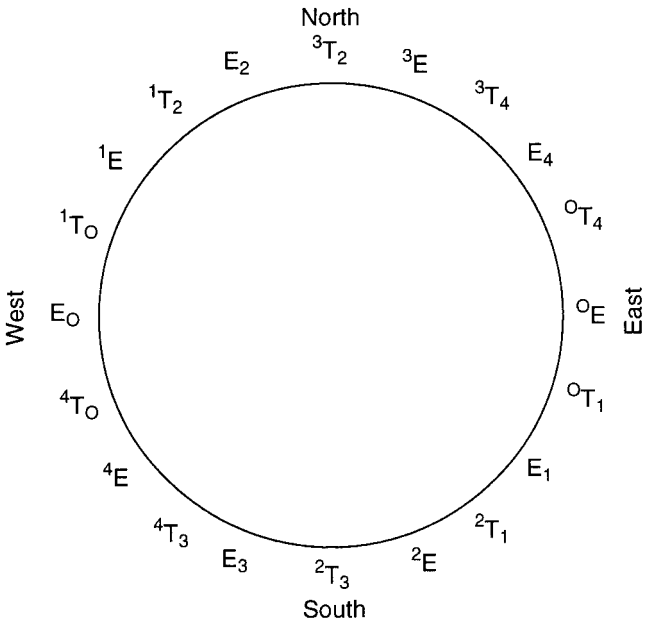


Figure 21. Pseudorotational wheel for a D-aldofuranosyl ring: T, twist; E, envelope; superscript numbers indicate the atoms lying above the plane, while subscript numbers are atoms displaced below the plane.

energy conformations, for example $^3T_2$ and $^2T_3$, equilibration can occur through one of two pathways, either via the east or west. In the east pathway, usually favored for D-pentofuranoses, equilibration proceeds through the following series of conformers: $^3T_2 \leftrightarrow {}^3E \leftrightarrow {}^3T_4 \leftrightarrow E_4 \leftrightarrow {}^oE \leftrightarrow {}^oT_1 \leftrightarrow E_1 \leftrightarrow {}^2T_1 \leftrightarrow E_2 \leftrightarrow {}^2T_3$.[106]

In the case of furanose rings, where a mixture of conformers is equilibrating rapidly on the NMR time scale, conformational analysis becomes complicated because averaging of coupling constants is observed. To facilitate analysis, a commercially available least-squares minimization program (PSEUROT 6.2) can be used, given the observed intracyclic ring $^3J_{H,H}$ and the τ_m for each conformer, tocalculate the N/S ratio and to provide P values for both conformers from which the ring forms (e.g., $^3T_2$) can be determined.[107] Similarly, equations have been derived for calculating populations of rotamers about the exocyclic C—C bond from coupling constant data.[99,108,109]

B. NMR Studies

Mindful of the discussion above, it is clear that when one is probing the conformation of an oligofuranoside, the critical first issue to be addressed is that of the conformational equilibrium of the constituent monosaccharide rings. Once this equilibrium is understood, the three-dimensional orientation of the sugar residues relative to each other can be assessed to provide an overall picture of the shape of the molecule. In recent investigations,[110] the equilibrium populations of ring con-

formers in methyl α-D-arabinofuranoside (**24**α, Figure 8) and a series of related di-, tri-, and tetrasaccharides (**13**, **16**, **18**–**21**, Figure 6) have been studied. These investigations have led to a better understanding of D-arabinofuranose ring conformation and have allowed the determination of the effect of ring substitution on conformer equilibria. High-field (600 and 800 MHz) NMR spectroscopy has been used for these studies. The oligosaccharides of interest contain only α-D-arabinofuranose residues, and consequently the potential for spectral overlap is high. However, in all the compounds studied to date, the spectral resolution at these field strengths has been good enough that standard one- and two-dimensional experiments (e.g., ^{1}H-^{1}H COSY, ^{1}H-^{1}H TOCSY, HMQC) can be used not only in assigning all the ^{1}H and ^{13}C resonances, but also in the measurement of coupling constants. Once the couplings have been obtained, PSEUROT analysis has provided conformer identities and their populations.

As illustrated in Figure 22, this analysis has identified that the ring in **24**α adopts a roughly equimolar distribution of the $^{\circ}T_4$ (N) and $^{2}T_3$ (S) conformations. In both these conformers the anomeric methoxy group is oriented in a pseudoaxial orientation, as would be expected on the basis of the *endo* anomeric effect. Furthermore, in both conformers the hydroxymethyl group lies in a pseudoequatorial fashion, as would be favored on the basis of steric interactions. In the N conformer, both the secondary OH groups adopt sterically favored pseudoequatorial orientations; however, in the S conformer, these groups are pseudoaxial. Although conformers possessing this orientation of substituents might not be expected to be energy minima on the basis of sterics, gauche effects[111] between these hydroxyl oxygens stabilize this conformer. Similar effects have been observed in the furanose rings of nucleic acids.[112] Comparison of the two solution conformers with that of the crystal structure[113] of

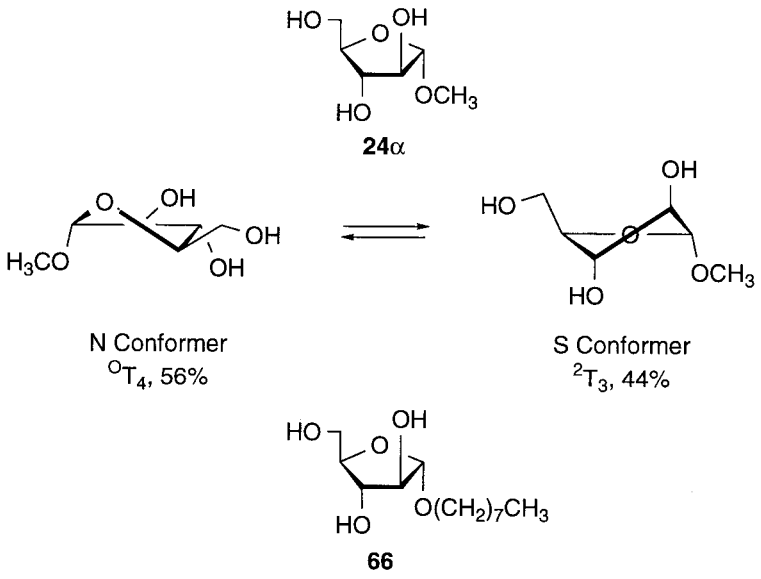
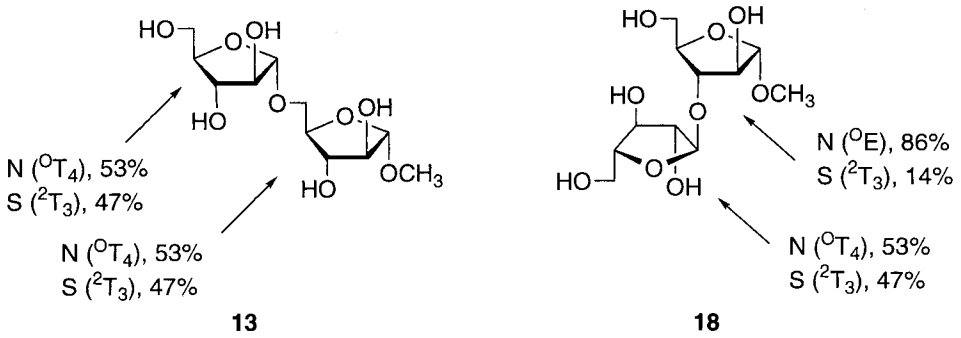


Figure 22. Solution N and S conformers for **24**α as determined by PSEUROT analysis.



13 18

Figure 23. Ring conformers present in disaccharides **13** and **18** and their relative populations.

octyl α-D-arabinofuranoside (**66**) and **24a**[114] reveals that the rings in these crystals adopt a conformation (E_4) very similar to the solution N conformer.

Extension of these investigations to the oligosaccharides showed that both the conformations present and their populations can, in some cases, be affected by glycosylation. Figure 23 illustrates the N and S conformers present for each ring in disaccharides **13** and **18**. In the α-(1 → 5)-linked disaccharide (**13**), each ring adopts the same two conformations as the monosaccharide, and in essentially the same populations. However, in the α-(1 → 3)-linked disaccharide (**18**), while the nonreducing end residue behaves like the monosaccharide, the reducing end residue is profoundly affected by glycosylation. In the 3-O-substituted ring, the S conformer remains at $^{2}T_3$ but the identity of the N conformer changes, slightly, to °E. In addition and more strikingly, the N conformer is favored with an equilibrium population of 86%. Clearly, glycosylation of a D-arabinofuranoside at O3 has a substantial influence upon the conformers available to the ring.

Steric arguments can be used to rationalize these results, and similar trends are observed (Figure 24) when these studies are carried out on the larger oligosaccharides (**16**, **19**–**21**). From the data available to date, some general conclusions can be drawn.

1. Glycosylation, and likely any form of substitution, of a primary hydroxyl group in an arabinofuranose ring does not significantly alter the conformers present or their populations.
2. Similarly, replacement of a methyl group at the anomeric center with a monosaccharide residue does not alter the equilibrium. Hence, all terminal Ara*f* residues behave like the monosaccharide.
3. Glycosylation of O3 on an arabinofuranose ring does alter the conformational equilibria of the ring significantly. The identity of the N conformation changes from °T_4 to °E, and this conformation is favored at equilibrium.
4. In cases in which a ring is glycosylated at both O3 and O5, as in **19** and **21**, favoring of the N conformation becomes even more pronounced.

The populations of rotamers about the C4—C5 bond group are relatively constant and are insensitive to ring conformation. Rotamer populations about this bond have been calculated using $^{3}J_{4,5R}$ and $^{3}J_{4,5S}$ values, and in all the oligosaccharides, the gg

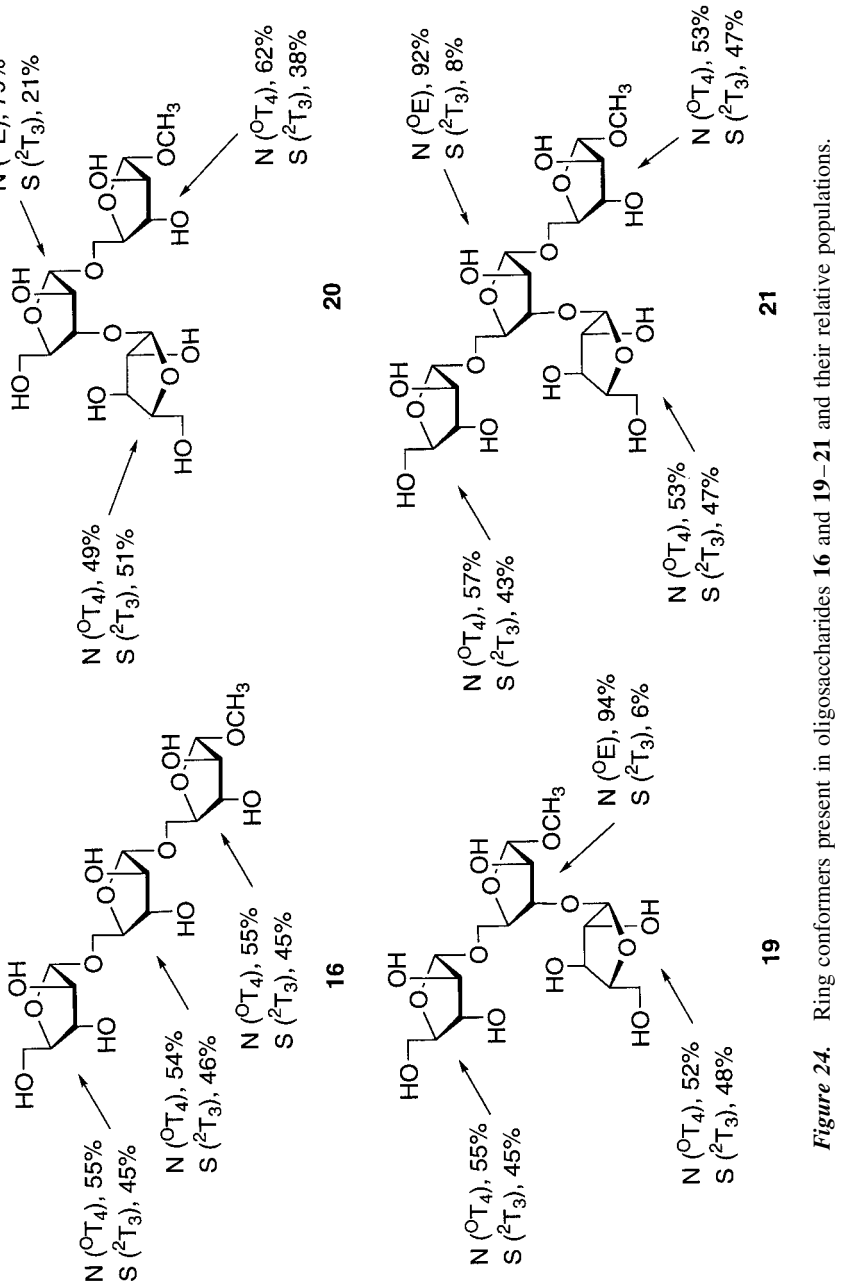


Figure 24. Ring conformers present in oligosaccharides **16** and **19–21** and their relative populations.

orientation is most heavily populated (47–53%). The gt rotamer is also present in significant amounts (34–40%), while the tg rotamer is the least populated (12–15%). In the gg and gt orientations a gauche effect[111] between the ring oxygen and O5 stabilizes these rotamers over the tg conformational isomer, in which O5 is trans to the ring oxygen.

C. Computational Work

The initial computational studies on D-arabinofuranosides were molecular mechanics calculations of methyl β-D-arabinofuranoside done by Pérez and coworkers.[115] Through a process by which the MM3 force field was used to optimize 360 unique conformations, two energy minima, the $^3T_2$ (N) and $^2T_3$ (S) conformations, were identified. The N conformer is about 1.3 kcal/mol more stable than the S, and the favored conformation about the C4—C5 bond was the gt orientation, which was favored over the gg rotamer by less than 2 kcal/mol. Additionally, the most favorable pathway for pseudorotation was shown to be through the east.

More recently, gas phase ab initio (HF/6-31G*, HF/6-31+G**, MP2/6-31+G**, MP3/6-31+G**) and density functional theory (B3LYP/6-31G*, B3LYP/6-31+G**) calculations have been carried out on methyl α-D-arabinofuranose.[116] A protocol developed by Serianni and coworkers,[117,118] was used to construct the 10 possible envelope forms by fixing four atoms in a plane; then, upon minimization, the other parameters (bond lengths, bond angles, etc.) were optimized.

These studies identified ^{3}E and ^{2}E as the N and S minima, respectively, with the latter being the global minima (Figure 25). B3LYP/6-31+G** calculations indicated that in the gas phase, the N conformer was 1.5 kcal/mol less stable than the S conformer. Additionally, comparison of the relative energies of the conformers showed, as expected, that pseudorotation through the east was preferred. The highest energy conformer along this pathway is $^\circ$E (3.2 kcal/mol less stable than the global minima), whereas pseudorotation through the west would require proceeding through the E$_\circ$

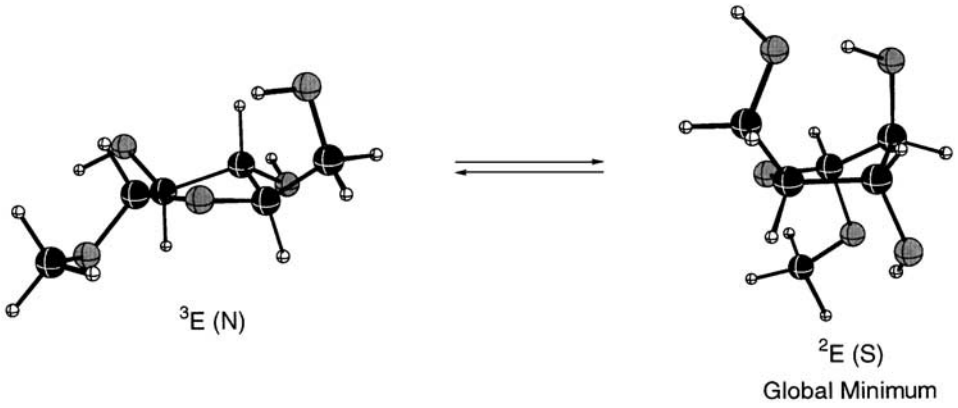


Figure 25. N (^{3}E) and S (^{2}E) conformers for **24**α as determined by gas phase density fashion theory (B3LYP/6-31G*) calculations.

conformer, which is 7.0 kcal/mol less stable than the global minima. Interestingly, these studies also showed that the planar species is approximately equivalent in energy to the E◦ conformer, and thus inversion through the planar ring form is a viable alternative to pseudorotation through the west. However, neither pathway is expected to be important because the east pseudorotation pathway is more favorable.

In 9 of the 10 envelopes, the favored rotamer about the C4—C5 bond is the gg orientation; the gt rotamer is favored in the other. In the gg orientation O5 can form an intramolecular H bond with OH_2 and this capability appears to be of major importance in stabilization of the conformations in the gas phase. Indeed, in the two most stable ring conformers, ^2E and E_1, an intramolecular H bond is formed between these two groups (OH2 $\cdots$ O5) in addition to another such interaction between OH3 and O1 (OH3 $\cdots$ O1). These H bonds undoubtedly stabilize the conformers to a significant degree. This observation is consistent with other recent gas phase calculations on sugars, which revealed that the formation of intramolecular H bonds is critically important in the stabilization of these molecules.[119]

VII. CONCLUDING REMARKS

There is now a thorough understanding of the structure of mycobacterial arabinan, and biosynthetic studies are underway that will lead to the elucidation of the pathways by which it is assembled. These investigations will depend critically on the access to synthetic oligosaccharides and oligosaccharide analogs that can be used as potential substrates or inhibitors of these enzymes. Consequently, the synthesis of arabinofuranosides and more generally oligofuranosides will prove to be an interesting and important research area in the future. Additionally, if inhibitors of the enzymes that biosynthesize these glycans are to be identified, a much better understanding of the conformational preferences of oligofuranosides will be necessary. In this area too, although there have been some fundamental investigations, a great deal more must be accomplished before even a rudimentary understanding of the conformation of these molecules is available.

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