# Arabinofuranosides from Mycobacteria: Synthesis of a Highly Branched Hexasaccharide and Related Fragments Containing β-Arabinofuranosyl Residues

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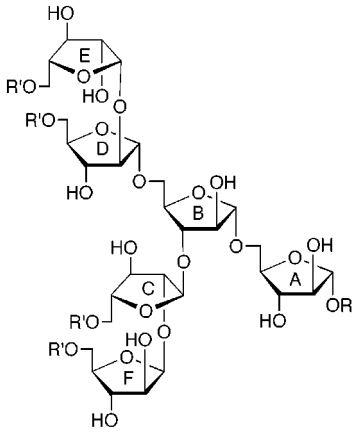
*Received September 10, 2001*

The synthesis of 11 oligosaccharides (**4**−**14**) containing β-arabinofuranosyl residues is reported. The glycans are all fragments of two polysaccharides, arabinogalactan and lipoarabinomannan, which are found in the cell wall complex of mycobacteria. In the preparation of the targets, the key step was a low-temperature glycosylation reaction that installed the β-arabinofuranosyl residues with good to excellent stereocontrol.

## Introduction

Over the past decade, diseases arising from mycobacterial infections have reemerged as global health threats.[1] *Mycobacterium avium* infections are common in HIV-positive individuals,[2] and tuberculosis, which results from infection by *Mycobacterium tuberculosis*, kills 3 million people annually.[1,3] In the future, this number is almost certain to rise given the worldwide increase in drug-resistant strains of this organism. A recent World Health Organization survey of 35 countries (including the United States) revealed that between 2 and 42% of all isolates of *M. tuberculosis* are resistant to one or more antituberculosis agents.[4] Statistics such as these underscore the importance of identifying new therapies for the treatment of these diseases.[5]

The cell wall complex of mycobacteria, like that of their close relatives in the Actinomycetes family (the *Corynebacteria*, *Nocardia*, and *Rhodococcus* genera), is a unique assembly of carbohydrates and lipids.[6] The major carbohydrate components are two polysaccharides, an arabinogalactan (AG) and a lipoarabinomannan (LAM), which are unusual in that all of the galactose and arabinose is present in the furanose ring form. Both polysaccharides have at their nonreducing termini the hexasaccharide motif shown in Figure 1. This moiety can either be unsubstituted (**1**) or covalently attached to other functionalities. In the AG (**2**), a portion of these hexasaccharide motifs is esterified at the four primary hydroxyl groups with long chain branched lipids (mycolic acids). Together these lipids and the polysaccharide comprise the mycolyl-AG complex, which plays an important role in preventing the passage of antibiotics into the organism and in protecting it from its environment.[7] In the LAM (**3**), this hexasaccharide is often capped with short mannopyranosyl oligosaccharides, which are believed to be involved in the infection process through interaction with human mannose binding receptors.[8] In addition to these roles, these polysaccharides (in particular LAM)[9] have been implicated in a number of immunological events that occur upon infection by mycobacteria. It has been postulated that hexasaccharide **1** is an important player in these processes.[10] In support of this hypothesis is the report that removal of the arabinofuranose residues by treatment of the polysac-




**1**, R = AG or LAM, R' = H
**2**, R = AG, R' = mycolic acids
**3**, R = LAM, R' = mannopyranosyl oligosaccharides
**4**, R = $CH_3$, R' = H

**Figure 1.** Hexasaccharide motif found at the nonreducing termini of mycobacterial arabinogalactan (AG) and lipoarabinomannan (LAM).

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10.1021/jo010910e CCC: $22.00 
Published on Web 01/10/2002

charides with arabinofuranosidases abolishes many of their immunomodulatory properties.[10c]

The ability of mycobacteria to synthesize both AG and LAM is critical to their viability. Inhibitors of the enzymes that assemble these polysaccharides should therefore be lethal to these organisms. Indeed, one of the drugs used to treat tuberculosis, ethambutol, has been shown to block the formation of the arabinan portions of both glycans.[11] There has been increasing interest in identifying compounds that act like ethambutol by inhibiting the arabinosyltransferases involved in cell wall assembly.[12] Over the past few years, as part of a program directed at the identification of inhibitors of mycobacterial arabinosyltransferases, we have reported the synthesis of a number of oligosaccharide fragments of AG and LAM.[13] These studies were undertaken in order to provide substrates that could be used not only to elucidate arabinan biosynthesis[13a,14] but also to determine the immunological importance of these glycans.

Among our targets was the hexasaccharide motif shown in Figure 1. We anticipated that the assembly of this oligosaccharide would be complicated by the presence of the two $\beta$-arabinofuranosyl residues (residues E and F). These glycosidic bonds are stereochemically analogous to the $\beta$-D-mannopyranosyl linkages found in mammalian glycoconjugates. The stereoselective formation of these glycosides has been a long-standing problem in oligosaccharide synthesis.[15] The 1,2-*cis* relationship between the substituents at $C_1$ and $C_2$ prohibits the use of glycosyl donors with $C_2$ acyloxy groups because 1,2-*trans* glycosides are produced due to neighboring group participation. The use of donors with nonparticipating groups at $C_2$ also gives predominantly 1,2-*trans* glycosides because, in the absence of neighboring group participation, both stereoelectronic and steric effects favor the $\alpha$-glycoside. Solutions to the $\beta$-mannoside problem have been explored for a number of years, and many elegant synthetic methods have resulted from these investigations.[15] Among the most straightforward and general is a method developed by Crich and co-workers,[16] which has recently been applied to the synthesis of an octasaccharide comprised of eight $\beta$-(1→2)-linked mannopyranose residues.[17]

In contrast to the large number of investigations dedicated to solving the $\beta$-mannoside problem, relatively few investigations have addressed methods for the stereoselective formation of $\beta$-arabinofuranosides, or the stereochemically related $\beta$-fructofuranosides. Early work in this area was done by Fletcher and Glaudemans who, in 1965, reported that methanolysis of 2,3,5-tri-*O*-benzyl-$\alpha$-D-arabinofuranosyl chloride afforded a 92:8 $\beta$: $\alpha$ ratio of methyl glycosides.[18] On the basis of kinetic data, it was proposed that the reaction proceeded through an $S_N1$-ion-pair mechanism. Over the subsequent 35 years, only sporadic syntheses of $\beta$-arabinofuranosides were reported.[19] In most cases only glycosides of simple alcohols were prepared and often as $\alpha$:$\beta$ mixtures. The publication[10a,20] of the structure of **1** has prompted renewed interest in this area, and new methods for the preparation of $\beta$-arabinofuranosides have begun to appear. Among these are the application of the Fletcher and Glaudemans methodology to the preparation of simple $\beta$-arabinofuranosides,[21] the use of intramolecular aglycone delivery (IAD),[22] the glycosylation of 2,3-anhydro-D-lyxofuranosyl thioglycoside and sulfoxides,[23] and the glycosylation of alcohols by thioglycosides at low temperature.[13c,d] These studies have led to the successful synthesis of hexasaccharide **4**[13c] and two related pentasaccharides.[22b,24] New methods for the formation of $\beta$-fructofuranosides have also been described recently. These include the application of the IAD method,[25] the use of glycosyl phosphites,[26] and the development of a new siloxane protected glycosyl donor that provides $\beta$-fructofuranosides with a high degree of stereoselectivity.[27]

In a previous communication,[13c] we reported the synthesis of hexasaccharide **4** via a very efficient and convergent route. We describe here a full account of that synthesis and the application of this methodology to the preparation of all fragments of this glycan that contain a $\beta$-arabinofuranose residue (**5**−**14**, Figure 2). The work reported here complements our earlier synthetic investigations, in which we synthesized all fragments of **4** that contained only $\alpha$-arabinofuranosyl residues.[28] These oligosaccharides will be used in investigations focused on

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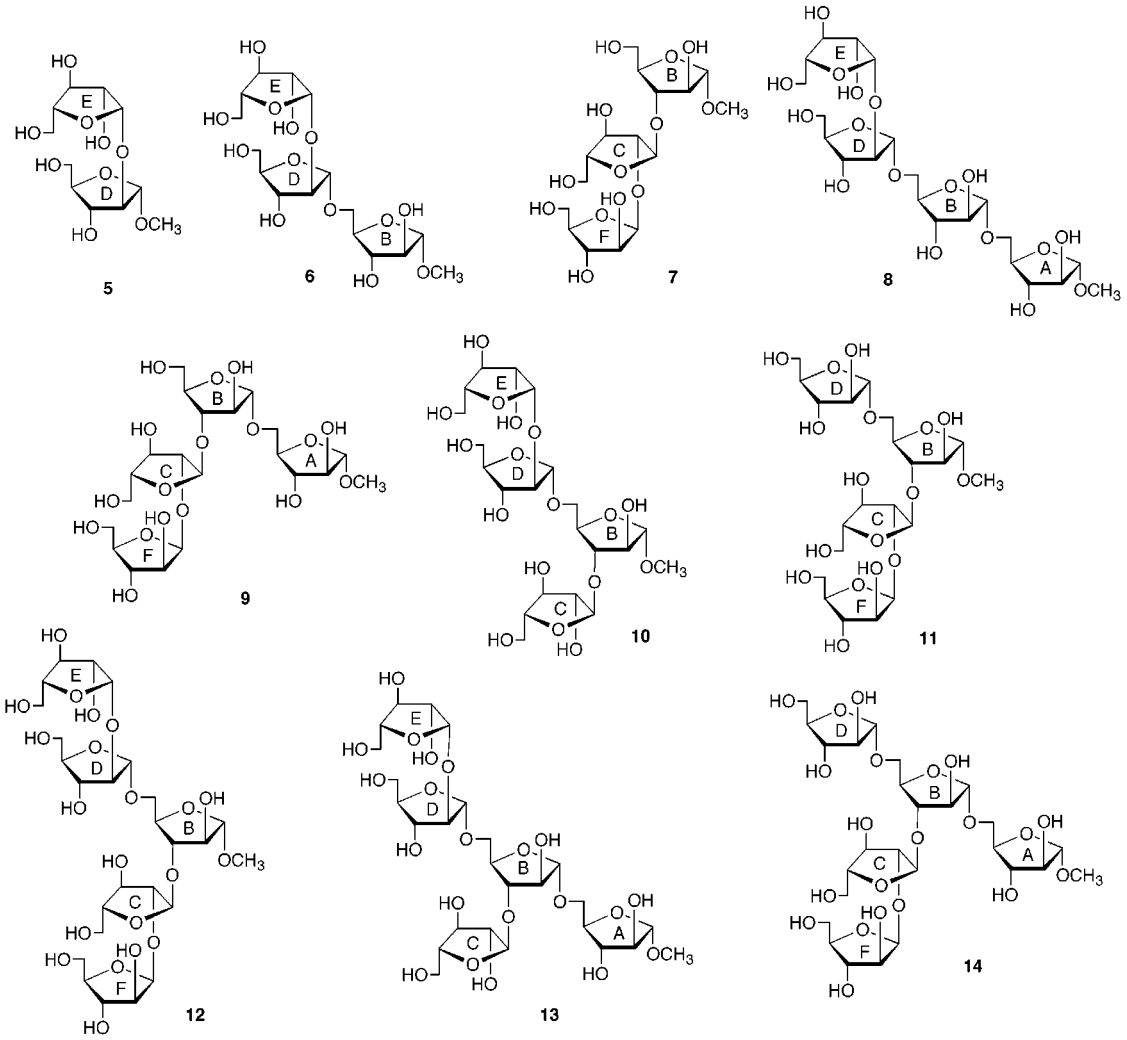


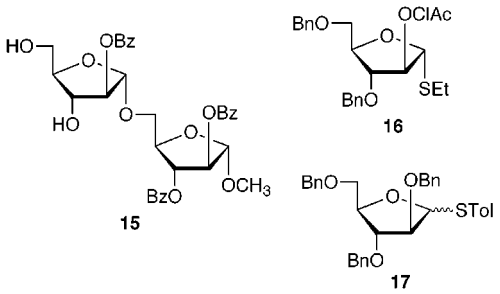

**Figure 2.** Synthetic targets; rings have been lettered to facilitate comparison with **1**.

elucidating the biosynthetic pathway by which mycobacterial AG and LAM are assembled and in the characterization of antibodies generated against these polysaccharides.

## Results and Discussion

**Synthesis of Hexasaccharide 4. A. Strategy.** In designing a synthetic route to **4** we endeavored to develop an approach that could be readily applied to the synthesis of all of the oligosaccharides in Figure 2. Such a requirement ruled out the possibility of a block synthesis, e.g., the simultaneous attachment of both Ara*f*-*β*-(1→2)-Ara*f* disaccharide fragments (residues DE and CF) to the AB disaccharide. We also wanted to avoid the somewhat cumbersome IAD method. Instead, we proposed to rapidly assemble this molecule from the AB disaccharide fragment (**15**, Chart 1) via the stepwise addition of the arabinofuranose residues in pairs. Thioglycoside **16** was to serve as the precursor to the C and D residues; rings E and F were to be added via thioglycoside **17**. Although very efficient, this approach requires that both *β*-arabinofuranosyl residues be introduced simultaneously. At the time we started this work, there were no examples of two 1,2-*cis*-*β*-glycosides being installed in a single step.[29] We were, however, hopeful that conditions could be found under which this transformation could be successfully carried out.

**Chart 1. Building Blocks Used for the Preparation of 4**




**B. Synthesis of Building Blocks.** The synthesis of **4** began with the preparation of building blocks **15**−**17**. Disaccharide **15** is known.[28] The preparation of **16** and **17** is illustrated in Scheme 1.

To access **16** (Scheme 1A), we started with the diacetate **18**.[30] Conversion to the thioglycoside was carried

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(29) A later synthesis of pentasaccharide **26** (Figure 3, ref 22b) did install both *β*-linked residues in a single step through the use of the IAD protocol.

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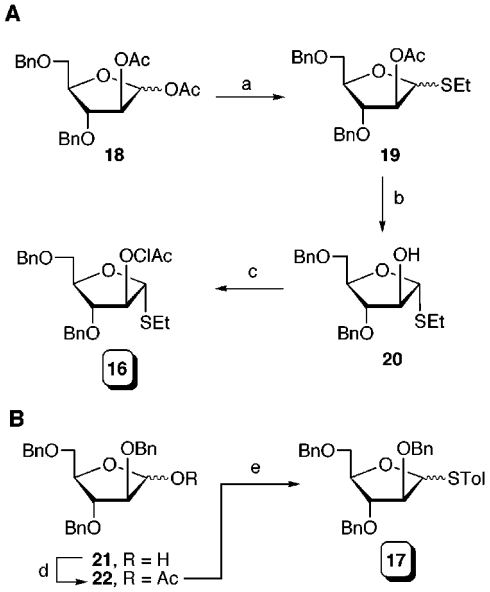
**Scheme 1**[a]




[a] (a) EtSH, $BF_3-OEt_2$, $CH_2Cl_2$, 0 °C, 80%; (b) $NaOCH_3$, $CH_3OH$, rt, 97%; (c) $(ClAc)_2O$, $NaHCO_3$, DMF, rt, 83%; (d) $Ac_2O$, pyridine, DMAP, 0 °C, quantitative; (e) *p*-TolSH, $BF_3-OEt_2$, $CH_2Cl_2$, 0 °C, 92%.

out by reaction with ethanethiol and boron trifluoride etherate, which provided **19** as a 4:1 α:*β* mixture in 80% yield. The products were separated by chromatography and the α-isomer then deprotected (in 97% yield) upon treatment with sodium methoxide. The resulting alcohol, **20**, was converted to **16** in 83% yield upon reaction with chloroacetic anhydride and sodium bicarbonate.

Thioglycoside **17** was synthesized (Scheme 1B) in two steps from the commercially available hemiacetal **21**. First, treatment of **21** with acetic anhydride and pyridine provided **22** in quantitative yield. Without further purification, **22**[30] was then reacted with *p*-thiocresol and boron trifluoride etherate, which afforded **17** as a 4:1 α:*β* mixture in 92% yield.

**C. Glycosylations and Deprotection.** After all of the building blocks were in hand, conditions for the assembly of the hexasaccharide were explored (Scheme 2). Coupling of disaccharide **15** with 2.4 equiv of thioglycoside **16** proceeded without incident, upon activation with *N*-iodosuccinimide and silver triflate. The desired tetrasaccharide, **23**, was obtained in 74% yield. A number of conditions were explored for the selective removal of the chloroacetate group in the presence of the benzoate esters. These included the use of thiourea,[31] hydrazinedithiocarbonate,[32] tetrabutylammonium fluoride, and hydrazine acetate.[33] After considerable optimization, it was found that the best results were obtained with hydrazine acetate in a mixture of dichloromethane and methanol at 40 °C. Under these conditions, deprotection of tetrasaccharide **23** afforded a 91% yield of diol **24**.

With an efficient route to tetrasaccharide **24** in place, the stage was set for the key reaction, the addition of both *β*-arabinofuranosyl residues. Using *N*-iodosuccinimide and silver triflate activation, a series of reaction conditions were investigated for the coupling of **24** with an excess of thioglycoside **17**. Through these studies it was determined that the temperature was critical to the stereoselectivity. When the glycosylation was carried out at either −40 °C, 0 °C, or room temperature, a number of glycoside products were formed. However, when the reaction was carried out at −78 °C, the conversion proceeded cleanly, affording the desired hexasaccharide **25** in 81% yield. In an effort to determine if any hexasaccharide possessing solely α-arabinofuranosyl linkages had been formed, all of the fractions obtained from the chromatography column during purification were screened by $^1H$ NMR spectroscopy. None of this product was detected. We have since found that the same degree of stereocontrol can be achieved by initiating the reaction at −78 °C and then allowing the reaction to warm to 0 °C. Under these conditions the reaction proceeds more quickly, with the characteristic orange color of these reactions developing as the solution reaches −60 °C.

The stereochemistry of the newly formed glycosidic linkages could be easily determined by NMR spectroscopy.[34] The $^{13}C$ chemical shift of *β*-arabinofuranosyl residues is upfield relative to the α-isomers (100−104 ppm vs 105−109 ppm). In the $^{13}C$ NMR spectrum of **25**, six anomeric carbons were apparent (106.75, 106.63, 105.95, 105.84, 100.39, and 100.01 ppm). Further support for the structure came from the $^1H$ NMR spectrum. Four of the anomeric hydrogens appeared as doublets or singlets with $^3J_{H1,H2} = 0-1.7$ Hz; the remaining two were split into doublets with $^3J_{H1,H2} = 4.6$ Hz. For α-arabinofuranosides, $^3J_{H1,H2}$ values of 0−2 Hz are typical, while this coupling constant is significantly larger for the *β*-anomer (3−5 Hz).[35] Furthermore, an HMQC spectrum[36] of **25**, correlated the $^1H$ resonances with $J_{H1,H2} = 4.6$ Hz with the $^{13}C$ resonances at 100.39 and 100.01 ppm.

The stereoselectivity of this glycosylation reaction is impressive, especially when considering the relative ease with which the reaction can be carried out. For example, the key step in the previous synthesis of pentasaccharide **26** (Figure 3)[22b] was the simultaneous introduction of both *β*-arabinofuranosyl residues via the highly stereoselective IAD method. The product of this reaction was obtained in 23% yield. The method described here is procedurally more simple than the IAD protocol and the yield of the desired product is significantly higher. Moreover, the glycosyl donor used in the glycosylation is more straightforwardly accessed.

Through the use of this method it was possible to synthesize **25** in multi-milligram quantities. Conversion to the deprotected oligosaccharide was achieved in two steps by treatment of **25** with sodium methoxide in methanol followed by hydrogenolysis of the benzyl ethers. Hexasaccharide **4** was obtained 86% yield from **25**.

**Synthesis of Oligosaccharides 5−14. A. Strategy and Preparation of Building Blocks.** Once the synthesis of **4** was complete, we turned our attention to the preparation of smaller fragments of this glycan. However, before proceeding with the synthesis of these oligosac-

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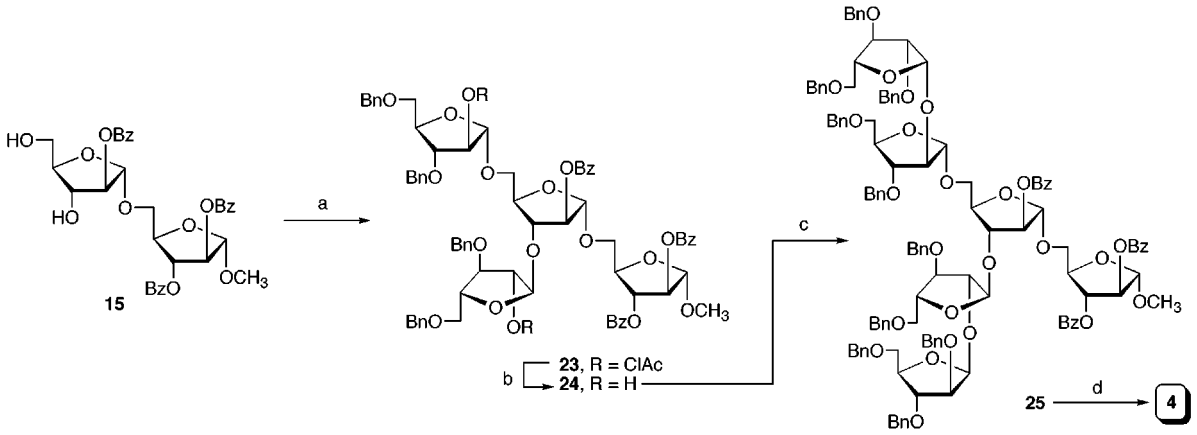
(35) Although $^1J_{C,H}$ values are unambiguous determinants of 1,2-*cis*-*β*-glycosides in pyranosides, in conformationally unrestricted furanose rings this parameter is not a reliable indicator of anomeric stereochemistry (see ref 34).
(36) Müller, L. *J. Am. Chem. Soc.* **1979**, *101*, 4481.

**Scheme 2**[a]




[a] (a) **16**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, 0 °C, 74%; (b) $NH_2NH_2-H_2O$, HOAc, $CH_3OH$, $CH_2Cl_2$, 40 °C, 91%; (c) **17**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, −78 °C, 81%; (d) $NaOCH_3$, $CH_3OH$, rt, 97%; then $H_2$, Pd/C, HOAc, $H_2O$, 86%.

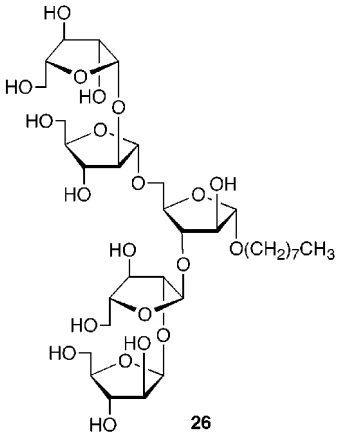
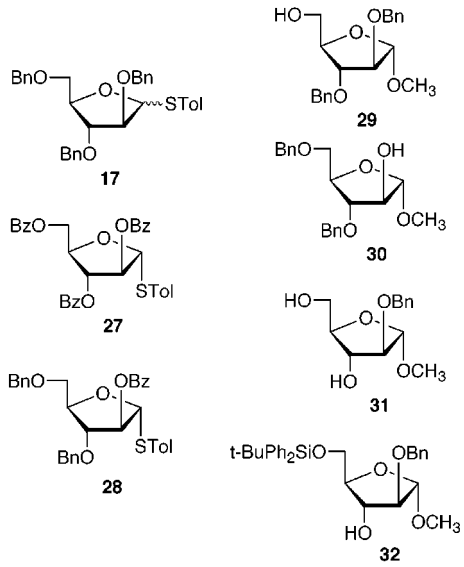



**Figure 3.** Pentasaccharide **26** synthesized by Prandi and coworkers (ref 22b).

charides, we decided to modify the strategy. Identical to the method developed for the preparation of **4**, the key reaction in our routes to **5**−**14** remained the introduction of the $\beta$-arabinofuranosyl residues via low-temperature glycosylation of the appropriate acceptor species with thioglycoside **17**. The most significant difference in strategy was the replacement of the benzoate ester protecting groups on rings A and B with benzyl ethers. This substitution not only eliminated the need for the selective cleavage of the chloroacetate groups in the presence of the benzoate esters but it also simplified the final deprotection for most of the targets (see below). With these goals in mind, we chose the building blocks shown in Chart 2 as precursors to all of the oligosaccharides shown in Figure 2. The synthesis of **17** was described above (Scheme 1B) and **27**,[37] **29**,[38] and **30**[39] were prepared as previously reported. The preparation of **28**, **31**, and **32** is discussed below.

Access to **28** was achieved from methyl glycoside **30**[39] in two steps (Scheme 3A). First, the hydroxyl group in **30** was benzoylated to provide **33** (in 90% yield). This product was subsequently converted, in 72% yield, to thioglycoside **28** under standard conditions. As illustrated

**Chart 2. Building Blocks Used for the Preparation of 5−14**



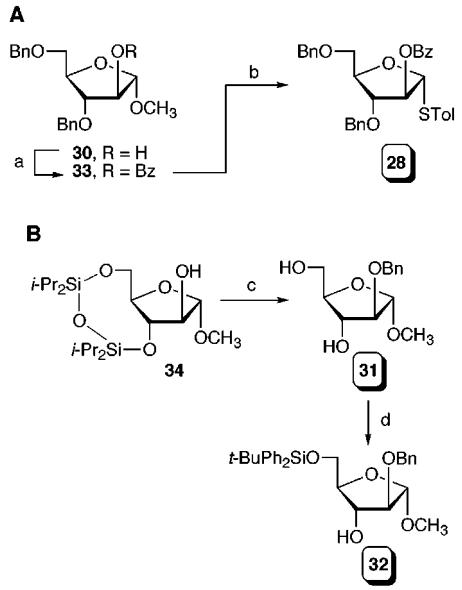

in Scheme 3B, both **31** and **32** were synthesized from alcohol **34**,[28] in two and three steps, respectively. Benzylation of alcohol **34** followed by cleavage of the siloxane protecting group afforded **31** in 89% overall yield. The primary alcohol in **31** was subsequently silylated using *tert*-butylchlorodiphenylsilane and pyridine, providing a 76% yield of **32**.

With the necessary building blocks in hand, we could then assemble oligosaccharides **5**−**14**. As discussed below, although all of the key glycosylation reactions involving thioglycoside **17** proceeded to give the $\beta$-arabinofuranoside as the major product, the stereoselectivities were generally not as high as those observed in the synthesis of **25**. In many cases, the corresponding α-glycoside was also produced in 10−25% yield. These undesired stereoisomers could be separated by chromatography and were not characterized.

**B. Synthesis of Disaccharide 5.** The synthesis of disaccharide **5** (Scheme 4) was achieved in two steps from alcohol **30** and thioglycoside **17**. Coupling of these two species under the promotion of *N*-iodosuccinimide and silver triflate afforded **35** in 73% yield. In this and following glycosylation reactions, the stereochemistry of

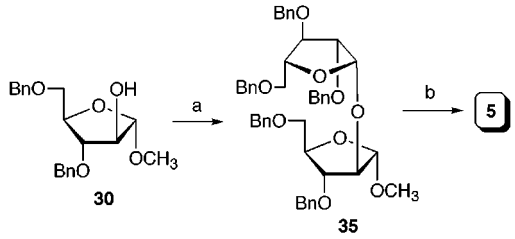
(37) Callam, C. S.; Gadikota, R. R.; Lowary, T. L. *J. Org. Chem.* **2001**, *66*, 4549.
(38) Montgomery, J. A.; Shortnacy, A. T.; Thomas, H. J. *J. Med. Chem.* **1974**, *17*, 1197.
(39) Ning, J.; Kong, F. *Carbohydr. Res.* **2001**, *330*, 165.

**Scheme 3**[a]




[a] (a) BzCl, pyridine, rt, 90%; (b) *p*-TolSH, $BF_3{-}OEt_2$, $CH_2Cl_2$, 0 °C, 72%; (c) NaH, BnBr, DMF, 0 °C; then *n*-$Bu_4NF$, THF, rt, 89%; (d) *t*-$BuPh_2SiCl$, pyridine, rt, 76%.

**Scheme 4**[a]




[a] (a) **17**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, −78 → 0 °C, 73%; (b) $H_2$, Pd/C, $CH_3OH$, 79%.

the newly formed glycosidic linkage was established via $^1H$ and $^{13}C$ NMR spectroscopy as described previously. Disaccharide **35** was subsequently deprotected by reaction with hydrogen and palladium on carbon to afford **5** in 79% yield.

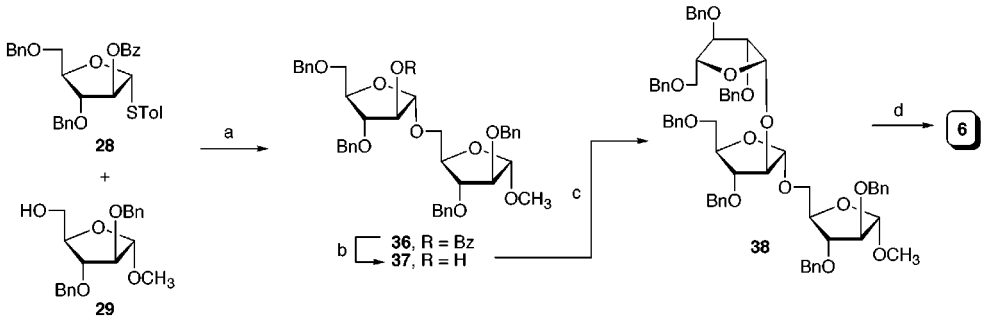
**C. Synthesis of Trisaccharides 6 and 7.** As outlined in Scheme 5, the preparation of trisaccharide **6** began with the glycosylation of methyl glycoside **29** with thioglycoside **28**. The reaction afforded, in 91% yield, disaccharide **36**, which was next subjected to Zemplén deacylation. Alcohol **37** was obtained in 91% yield. Introduction of the β-arabinofuranosyl residue was carried out through the use of **17** as was done for the synthesis of **35**. A 54% yield of trisaccharide **38** was obtained after chromatography. The product was hydrogenated, affording **6** in 76% yield.

The preparation of trisaccharide **7**, which is illustrated in Scheme 6, proceeded along lines similar to the synthesis of **6**. Coupling of thioglycoside **28** and alcohol **32** provided disaccharide **39** in 60% yield. The benzoate ester was removed, giving **40** in 68% yield, and the resulting alcohol glycosylated with **17**. This reaction produced trisaccharide **41** in 63% yield. Reaction of the protected glycan with tetrabutylammonium fluoride and then hydrogen and palladium on carbon provided **7** in 62% yield over the two steps.

**D. Synthesis of Tetrasaccharides 8 and 9.** The preparation of tetrasaccharide **8** is shown in Scheme 7. Glycosylation of **29** with **27** provided **42** in 84% yield. This disaccharide was the converted to triol **43** upon reaction with sodium methoxide in methanol. The product was formed in 95% yield and the primary hydroxyl group was then protected as a trityl ether. Benzylation of the remaining secondary hydroxyl groups afforded **45** and the trityl ether was then removed, affording **46** in 48% yield over the three steps from **43**. The remaining α-arabinofuranosyl linkage was installed by reaction of **46** with thioglycoside **28**. Produced in 88% yield was trisaccharide **47**, which was then converted to alcohol **48** in 79% yield under standard conditions. The final glycosyl residue was then added via the reaction of **17** and **48** to provide tetrasaccharide **49** in 52% yield. Conversion of **49** into **8** was achieved in one step and 79% yield by hydrogenolysis of the benzyl ethers.

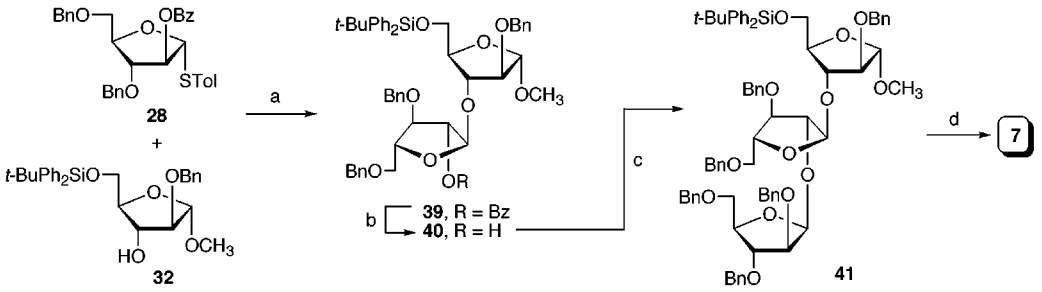
The synthesis of tetrasaccharide **9** was carried out as illustrated in Scheme 8. Disaccharide **43** was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane and pyridine to provide **50** in 68% yield. This product was then converted to benzyl ether **51** and the siloxane protecting group cleaved upon treatment with tetrabutylammonium fluoride. The product was isolated in 53% overall yield from **50**. The primary alcohol was then protected as a *tert*-butyldiphenylsilyl ether and the resulting alcohol was glycosylated with thioglycoside **28**. The resulting product, trisaccharide **54**, was obtained in 75% yield. Cleavage of the benzoyl group afforded, in 80% yield, alcohol **55**, which was in turn reacted with **17** under the standard conditions for the formation of β-arabinofuranosides. Tetrasaccharide **56** was isolated in 60% yield. Deprotection of **56** to give **9** was achieved in two steps and 65% overall yield.

**Scheme 5**[a]



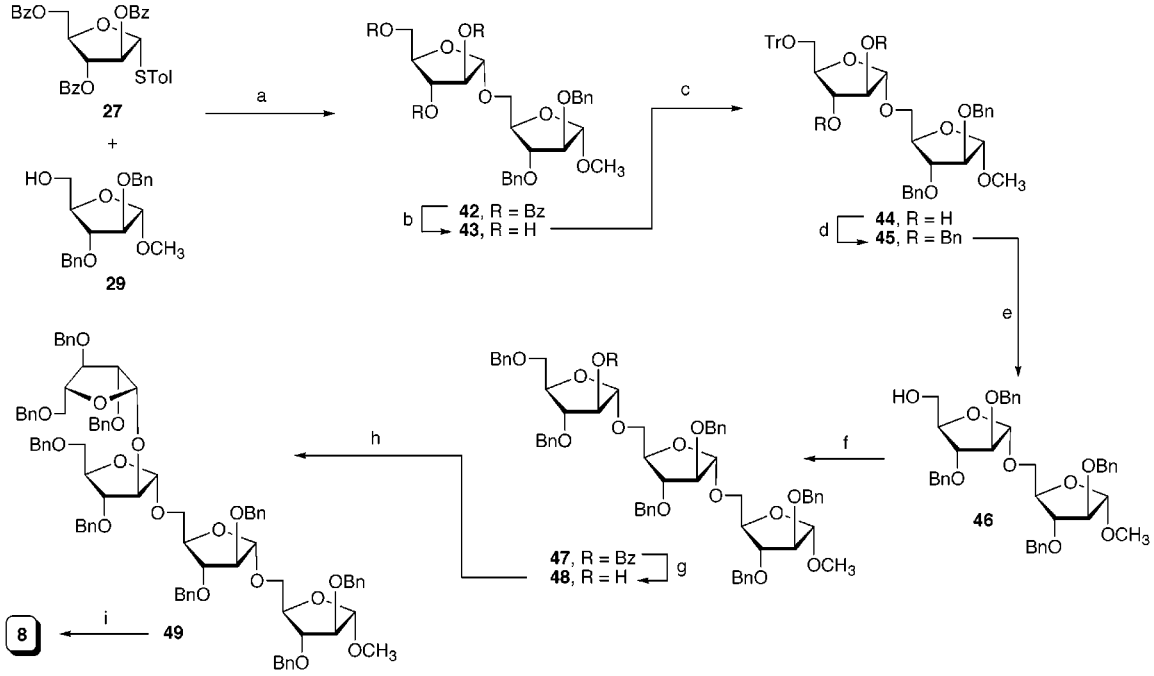

[a] (a) *N*-Iodosuccinimide, silver triflate, $CH_2Cl_2$, 0 °C, 91%; (b) $NaOCH_3$, $CH_3OH$, rt, 91%; (c) **17**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, −78 → 0 °C, 54%; (d) $H_2$, Pd/C, $CH_3OH$, 76%.

**Scheme 6**[a]




[a] (a) *N*-Iodosuccinimide, silver triflate, $CH_2Cl_2$, 0 °C, 60%; (b) $NaOCH_3$, $CH_3OH$, rt, 68%; (c) **17**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, −78 → 0 °C, 63%; (d) *n*-$Bu_4NF$, THF, rt, then $H_2$, Pd/C, $CH_3OH$, 62%.

**Scheme 7**[a]



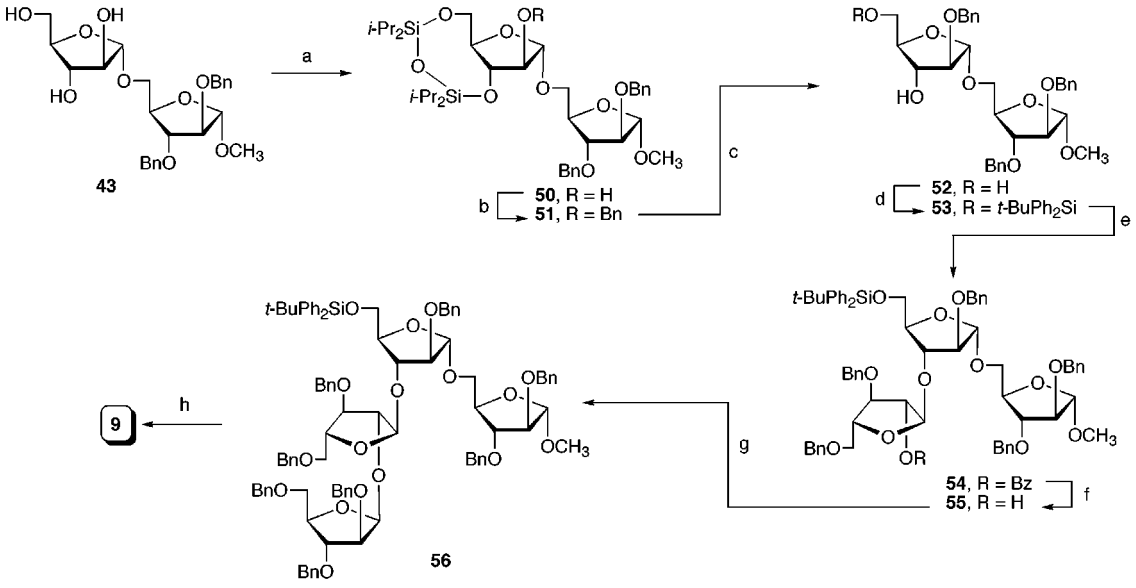

[a] (a) *N*-Iodosuccinimide, silver triflate, $CH_2Cl_2$, 0 °C, 84%; (b) $NaOCH_3$, $CH_3OH$, rt, 95%; (c) TrCl, pyridine, rt; (d) BnBr, NaH, DMF, 0 °C; (e) *n*-$Bu_4NF$, THF, rt, 48%, three steps from **43**; (f) **28**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, 0 °C, 88%; (g) $NaOCH_3$, $CH_3OH$, rt, 79%; (h) **17**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, −78 → 0 °C, 52%; (i) $H_2$, Pd/C, $CH_3OH$, 79%.

**E. Synthesis of Tetrasaccharides 10 and 11 and Pentasaccharide 12.** The most efficient route to **10** and **11** involved a common intermediate, **58** (Scheme 9). Alcohol **31** was glycosylated with an excess of thioglycoside **28**, which afforded trisaccharide **57** in 82% yield. The benzoyl groups were removed under basic conditions, resulting in a 78% yield of **58**. This diol was then glycosylated with 1 equiv of thioglycoside **17**. This reaction produced a mixture of tetrasaccharides, **59** and **60**, which were separated by chromatography. The isolated yields of the products were 41% (**59**) and 26% (**60**). Both products were then deprotected by hydrogenolysis of the benzyl ethers under standard conditions, in 83% and 86% yields, respectively.

The structures of the products produced from the glycosylation of diol **58** with a limiting amount of **17** were determined through the use of $^{13}C$ NMR spectroscopy, by inspection of the chemical shifts of the anomeric carbons on residues C and D (Figure 4). Access to a panel of protected oligosaccharide derivatives (e.g., **38**−**41**, **47**−**49**, **56**) allowed us to determine markers for the attachment of a *β*-arabinofuranosyl moiety to residue C vs residue D. When residue C possesses a free hydroxyl group at $C_2$, the chemical shift of the anomeric carbon ($C_{1C}$) is between 108.0 and 108.5 ppm. Benzoylation or glycosylation of this functionality results in a 2.5 ppm upfield shift of the $C_{1C}$ resonance. A similar trend is seen for residue D. In oligosaccharides in which this ring has a 2-hydroxyl group, $C_{1D}$ resonates between 109.0 and 109.5 ppm. If this position is glycosylated or benzoylated the chemical shift of $C_{1D}$ is shifted upfield (to 106.6−107.0 ppm). The 1 ppm difference between the chemical shift $C_{1D}$ and $C_{1C}$ when these rings possess a $C_2$-hydoxyl group is consistent for all compounds investigated and allowed us to determine the structures of **59** and **60** (Figure 4). Additional data supporting these chemical shifts as markers for determining the regioselectivity of this glycosylation can be found in the Supporting Information (Figure S1).

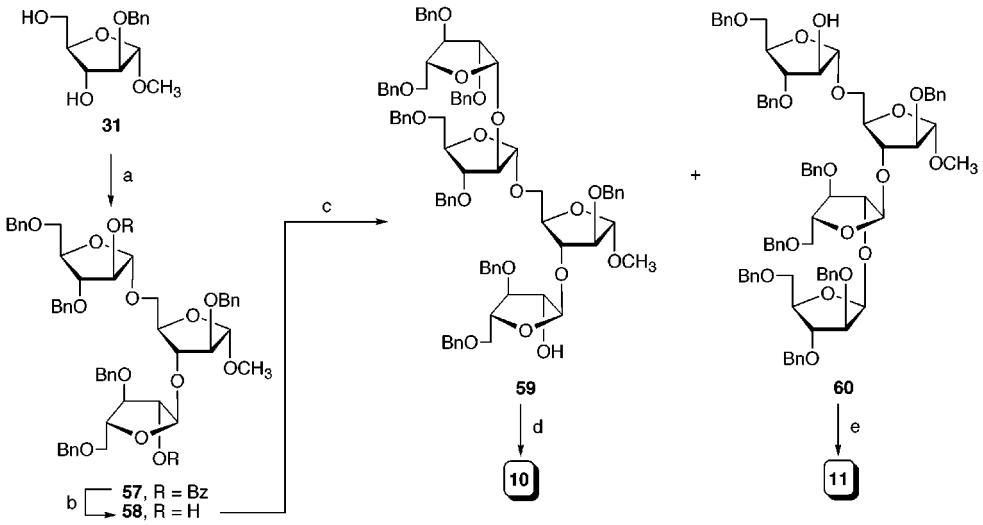
The synthesis of pentasaccharide **12** also involved intermediate **58** (Scheme 10). Glycosylation of this trisaccharide diol with 2 equiv of **17** provided **61** in 63% yield. The benzyl groups were then removed by hydrogenolysis, affording **12** in 86% yield.

**Scheme 8**[a]




[a] (a) 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine, rt, 0 °C, 68%; (b) NaH, BnBr, DMF, 0 °C; (c) *n*-$Bu_4NF$, THF, rt, 53% from **50**; (d) *t*-$BuPh_2SiCl$, pyridine, rt, 68%; (e) **28**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, 0 °C, 75%; (f) $NaOCH_3$, $CH_3OH$, rt, 80%; (g) **17**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, −78 → 0 °C, 60%; (h) *n*-$Bu_4NF$, THF, rt, then $H_2$, Pd/C, $CH_3OH$, 65%.

**Scheme 9**[a]




[a] (a) **28**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, 0 °C, 82%; (b) $NaOCH_3$, $CH_3OH$, rt, 78%; (c) **17**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, −78 → 0 °C, 41% **59** and 26% **60**; (d) $H_2$, Pd/C, $CH_3OH$, 83%; (e) $H_2$, Pd/C, $CH_3OH$, 86%.

**F. Synthesis of Pentasaccharides 13 and 14 and Hexasaccharide 4.** The route used for the preparation of **13** and **14** (Scheme 11) was analogous to that used for the preparation of **10** and **11**. First, disaccharide **52**, prepared as outlined in Scheme 8, was glycosylated with an excess of thioglycoside **28**. The resulting product, tetrasaccharide **62**, was produced in 89% yield. The benzoyl groups in **62** were then cleaved (84%) and the resulting diol was glycosylated with a limiting amount of **17**. This reaction afforded three chromatographically separable products, hexasaccharide **64** and pentasaccharides **65** and **66** in 21%, 28%, and 10% yield, respectively. Differentiation of the structures of **65** and **66** was carried out as described above for **59** and **60**. All three products were deprotected upon reaction with hydrogen and palladium on carbon, to afford oligosaccharides **4**, **13**, and **14**.

In conclusion, we report here the synthesis of a panel of oligosaccharides containing *β*-arabinofuranosyl residues. The key reaction in the formation of the targets is a low-temperature glycosylation of alcohols with a readily available thioglycoside (**17**) promoted by *N*-iodosuccinimide and silver triflate. Under these conditions, the *β*-arabinofuranosides are produced with good to excellent stereoselectivity. The method has been used to synthesize a hexasaccharide motif found at the nonreducing end of two mycobacterial cell wall polysaccharides. In addition, all fragments of this oligosaccharide containing a *β*-arabinofuranosyl residues have been synthesized.

Although in the preparation of **5**−**14** the stereocontrol of the key glycosylation reactions is not as high as in the synthesis of **4**, the desired products are nevertheless produced as the major products. The sensitivity of the stereocontrol to the coupling partners employed in the

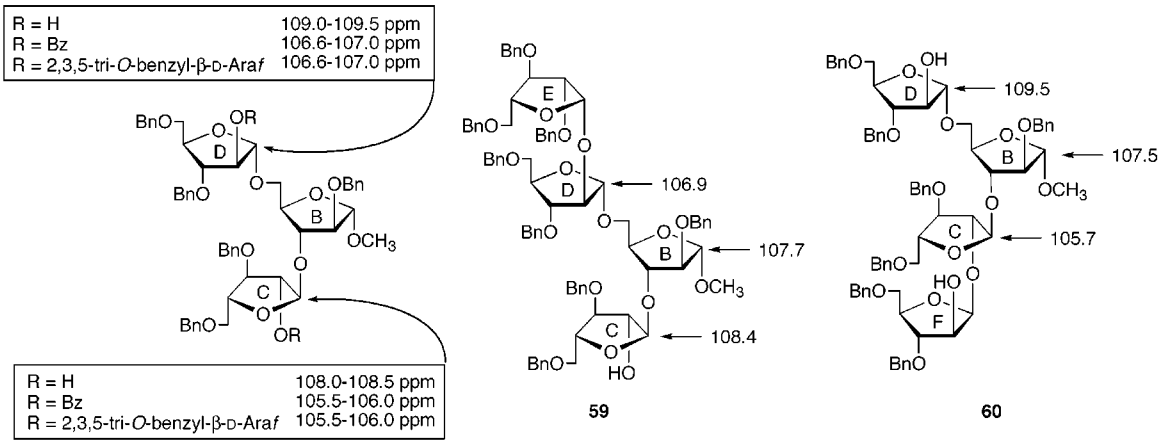
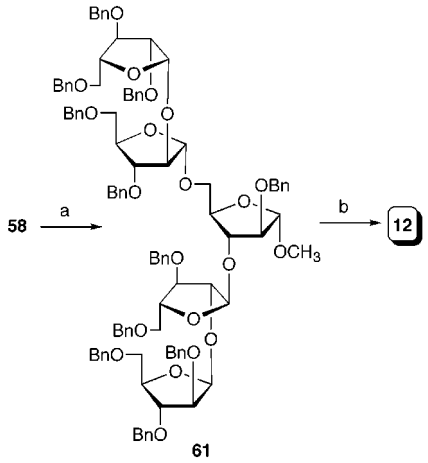



**Figure 4.** $^{13}C$ NMR spectroscopic markers used to determine structures of oligosaccharides **59**, **60**, **65**, and **66**.

**Scheme 10**[a]




[a] (a) **17**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, −78 → 0 °C, 63%; (b) $H_2$, Pd/C, $CH_3OH$, 86%.

glycosylations is reminiscent of many syntheses of $\beta$-mannosides, prior to the development of the Crich method described in the Introduction.[16] Often, seemingly small changes in the structure of the donor and acceptor result in significant alterations in $\beta$:$\alpha$ ratios.[40] Clearly additional work is needed to provide a general and highly stereoselective $\beta$-arabinofuranoside synthesis.

Glycans **4**−**14** will be of much use in biosynthetic studies of hexasaccharide **1** as well as in investigations of the specificity of various antibodies that have been raised against mycobacterial AG and LAM. It is also likely that many of these oligosaccharides will serve as substrates for the enzymes that further elaborate hexasaccharide **1**. These include the mycolyltransferases, which add the mycolic acids to this hexasaccharide in AG and the mannosyltransferases that glycosylate this motif in LAM. The use of these glycans in a range of biochemical studies is underway.

## Experimental Section

**General Methods.** Solvents were distilled from the appropriate drying agents before use. Unless stated otherwise all reactions were carried out at room temperature and under a positive pressure of argon and were monitored by TLC on silica gel 60 $F_{254}$ (0.25 mm, E. Merck). Spots were detected under UV light or by charring with 10% $H_2SO_4$ in EtOH. Solvents were evaporated under reduced pressure and below 40 °C (bath). Column chromatography was performed on silica gel 60 (40−60 $\mu$M). Iatrobeads refers to a beaded silica gel 6RS-8060, which is manufactured by Iatron Laboratories (Tokyo). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured at 22 ± 2 °C. $^1H$ NMR spectra were recorded at 400, 500, or 800 MHz, and chemical shifts are referenced to either to TMS (0.0, $CDCl_3$) or HOD (4.78, $D_2O$). $^{13}C$ NMR spectra were recorded at 100 or 125 MHz, and $^{13}C$ chemical shifts are referenced to internal $CDCl_3$ (77.00, $CDCl_3$) or external dioxane (67.40, $D_2O$). Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. Electrospray mass spectra were recorded on samples suspended in mixtures of THF with $CH_3OH$ and added trifluoroacetic acid or NaCl. Analytical data for all new compounds ($^1H$ NMR, $^{13}C$ NMR, elemental analysis, HRMS, $[\alpha]_D$) is provided in the Supporting Information.

**Ethyl 2-*O*-Acetyl-3,5-di-*O*-benzyl-1-thio-D-arabinofuranoside (19).** To a solution of **18** (3.80 g, 9.17 mmol) in dry $CH_2Cl_2$ (10 mL) at 0 °C was added dropwise ethanethiol (0.75 mL, 10.13 mmol). After stirring for 10 min, boron trifluoride etherate (1.39 mL, 10.97 mmol) was added dropwise. After 2 h, $Et_3N$ (3 mL) was added and the reaction mixture was concentrated. The residue was then purified by chromatography (hexanes/EtOAc, 5:1) to provide the product **19** (3.07 g, 80%) as two separable anomers in a 4:1 $\alpha$:$\beta$ ratio as oils.

**Ethyl 3,5-Di-*O*-benzyl-1-thio-α-D-arabinofuranoside (20).** To a solution of **19** ($\alpha$-isomer) (1.85 g, 4.44 mmol) in dry $CH_3OH$ (10 mL) was added dropwise 0.1 M methanolic sodium methoxide (4 mL). After 2 h, the reaction mixture was neutralized with prewashed Amberlite IR-120 ($H^+$) resin, filtered, and concentrated. The residue was purified by chromatography (hexanes/EtOAc, 4:1) to give **20** (1.61 g, 97%) as an oil.

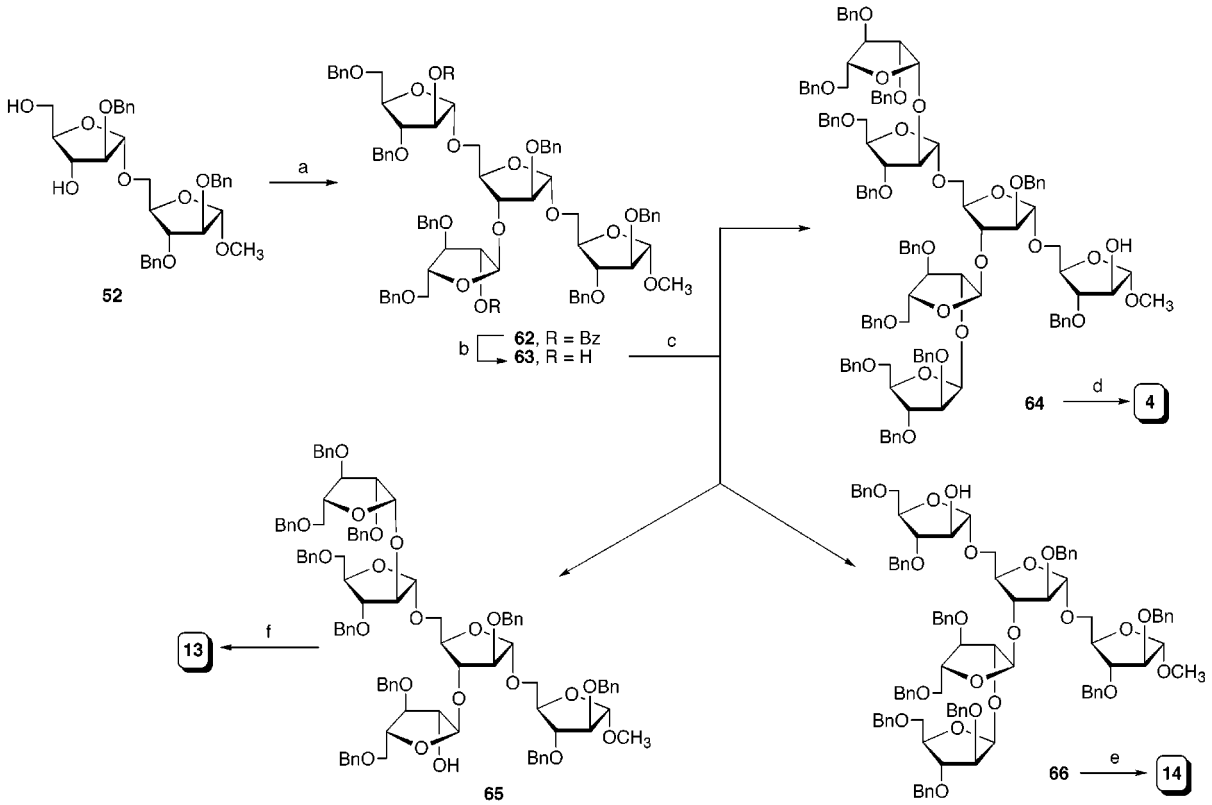
**Ethyl 3,5-Di-*O*-benzyl-2-*O*-chloroacetyl-1-thio-α-D-arabinofuranoside (16).** Alcohol **20** (150 mg, 0.40 mmol) was dissolved in dry DMF (5 mL), and then $NaHCO_3$ (50 mg, 0.60 mmol) and chloroacetic anhydride (103 mg, 0.60 mmol) were added. The reaction mixture was stirred for 2 h and was then diluted with $CH_2Cl_2$. The organic layer was washed with water, dried ($Na_2SO_4$), ad concentrated and the residue chromatographed (hexanes/EtOAc, 6:1) to give **16** (150 mg, 83%) as an oil.

**1-*O*-Acetyl-2,3,5-tri-*O*-benzyl-1-D-arabinofuranose (22).** To a solution of 2,3,5-tri-*O*-benzyl-D-arabinofuranose[41] (**21**, 5.0 g, 11.9 mmol) in dry pyridine (10 mL) at 0 °C was added acetic anhydride (1.35 mL, 14.31 mmol) dropwise. A catalytic amount of DMAP was added, and the solution was stirred for 4 h at 0 °C. The reaction mixture was then diluted with $CH_2Cl_2$ and then washed with 5% HCl, a saturated solution of $NaHCO_3$, and then water. The organic layer was dried ($Na_2SO_4$), filtered,

(40) Paulsen, H. *Angew. Chem., Intl Ed. Engl.* **1982**, *21*, 155.

(41) Commercially available from Pfanstiel Laboratories.

**Scheme 11**[a]




[a] (a) **28**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, 0 °C, 89%; (b) $NaOCH_3$, $CH_3OH$, rt, 84%; (c) **17**, *N*-iodosuccinimide, silver triflate, $CH_2Cl_2$, −78 → 0 °C, 21% **64**, 28% **65**, and 10% **66**; (d) $H_2$, Pd/C, HOAc, $H_2O$, 67%; (e) $H_2$, Pd/C, $CH_3OH$, 75%; (f) $H_2$, Pd/C, $CH_3OH$, 71%.

and concentrated to give the product **22**[30] in quantitative yield as an oil. The product was used in the next step without further purification.

***p*-Cresyl 2,3,5-Tri-*O*-benzyl-1-thio-D-arabinofuranoside (17).** Acetate **22** (1.64 g, 3.55 mmol) was dissolved in dry $CH_2Cl_2$ (10 mL), and the solution was cooled to 0 °C. *p*-Thiocresol (485 mg, 3.90 mmol) was added, and after stirring for 10 min, boron trifluoride etherate (540 μL, 4.26 mmol) was added dropwise. The solution was stirred for 1 h at 0 °C before $Et_3N$ (590 μL, 4.23 mmol) was added, and then the reaction mixture was diluted with $CH_2Cl_2$. After washing with a saturated solution of $NaHCO_3$, the organic layer was dried ($Na_2SO_4$) and concentrated to give a residue that was chromatographed (hexanes/EtOAc, 8:1) to give **17** (1.73 g, 4:1 α:β mixture, 92%) as an oil.

**Tetrasaccharide 23.** To a solution of disaccharide alcohol **15** (533 mg, 0.88 mmol) and donor **16** (948 mg, 2.10 mmol) in dry $CH_2Cl_2$ (10 mL) was added powdered molecular sieves (4 Å, 1.0 g). The reaction mixture was stirred for 20 min at 0 °C, and then *N*-iodosuccinimide (568 mg, 2.52 mmol) and silver triflate (162 mg, 0.63 mmol) were added. After stirring for 2 h at 0 °C, $Et_3N$ was added. The reaction mixture was then diluted with $CH_2Cl_2$ and filtered through Celite. The filtrate was washed successively with a saturated solution of $Na_2S_2O_3$, water, and brine. The organic phase was dried ($Na_2SO_4$), filtered, concentrated, and purified by chromatography (hexanes/EtOAc, 5:1 → 2:1) to give **23** (894 mg, 74%) as an oil.

**Tetrasaccharide 24.** To a stirred solution of tetrasaccharide **23** (414 mg, 0.30 mmol) in dry $CH_2Cl_2$ (5 mL) and dry $CH_3OH$ (2 mL) were added acetic acid (171 μL, 2.99 mmol) and hydrazine monohydrate (145 μL, 2.99 mmol). The solution was stirred at 40 °C for 3 h and then cooled to room temperature before being concentrated. The residue was taken up in $CH_2Cl_2$ and washed with water. The organic phase was then dried ($Na_2SO_4$), filtered, concentrated, and purified by chromatography (hexanes/EtOAc, 3:1 → 1:1) to give **24** (335 mg, 91%) as an oil.

**Hexasaccharide 25.** Tetrasaccharide diol **24** (255 mg, 0.21 mmol) and thioglycoside **17** (436 mg, 0.83 mmol) were dissolved in dry $CH_2Cl_2$ (6 mL). The solution was stirred under $N_2$ for 30 min with activated, powdered molecular sieves (4 Å, 2.0 g) at −78 °C, and then *N*-iodosuccinimide (186 mg, 0.83 mmol) and silver triflate (64 mg, 0.25 mmol) were added. After being stirred for 90 min at this temperature, $Et_3N$ was added. The reaction mixture was diluted with $CH_2Cl_2$ and filtered through Celite. The filtrate was washed successively with a saturated aqueous solution of $Na_2S_2O_3$, water, brine, and dried ($Na_2SO_4$). After evaporation of the solvent, chromatography of the residue (hexanes/EtOAc, 3:1 → 2:1) gave **25** (343 mg, 81%) as an oil.

**Hexasaccharide 4.** A solution of **25** (184 mg, 0.09 mmol) in dry $CH_2Cl_2$ (2 mL) and dry $CH_3OH$ (6 mL) was treated with a catalytic amount of 0.1 M methanolic sodium methoxide. After stirring for 4 h, the reaction mixture was neutralized with prewashed Amberlite IR-120 ($H^+$) resin, filtered, and concentrated. The resulting residue was purified by chromatography (hexanes/EtOAc, 2:1 → 1:2). The residue was dissolved in AcOH/$H_2O$ (4:1, 5 mL) and hydrogenolyzed over 10% Pd/C (60 mg) for 3 h. The reaction mixture was filtered through Celite and concentrated, and the residue was purified by chromatography on Iatrobeads ($CH_2Cl_2$:$CH_3OH$:$H_2O$, 60:35:5) to give **4** (64 mg, 86%) as a white solid.

**Methyl 2-*O*-Benzoyl-3,5-di-*O*-benzyl-α-D-arabinofuranoside (33).** To a solution of **30** (400 mg, 1.16 mmol) in dry pyridine (5 mL) at room temperature was added benzoyl chloride (150 μL, 1.25 mmol). The reaction mixture was stirred for 2 h and then concentrated. The residue was dissolved in $CH_2Cl_2$, and the resulting organic solution was washed with brine and concentrated. Purification by chromatography (hexanes/EtOAc, 4:1) afforded **33** (490 mg, 90%) as an oil.

***p*-Cresyl 2-*O*-Benzoyl-3,5-di-*O*-benzyl-1-thio-α-D-arabinofuranoside (28).** To a solution of **34** (2.2 g, 4.91 mmol) in dry $CH_2Cl_2$ (80 mL) was added thiocresol (620 mg, 5 mmol). The solution was stirred for 10 min at 0 °C, and boron trifluoride etherate (1.2 mL, 10 mmol) was added dropwise.

After 2 h at 0 °C, $Et_3N$ (2 mL) was added and the solution was concentrated. The residue was purified by column chromatography (hexanes/EtOAc, 6:1) to provide product **28** (1.96 g, 72%) as an oil.

**Methyl 2-*O*-Benzyl-α-D-arabinofuranoside (31).** To a solution of **35** (1.50 g, 3.0 mmol) in dry DMF (5 mL) at 0 °C were added sodium hydride (60% dispersion in oil, 240 mg, 6 mmol) and benzyl bromide (370 μL, 3.1 mmol). After stirring at 0 °C for 2 h, $CH_3OH$ (1 mL) was added and the solvent was evaporated. The resulting residue was dissolved in EtOAc, and this organic solution was washed with water and brine and then dried ($Na_2SO_4$). The solvent was evaporated, the residue was dissolved in THF (10 mL), and *n*-$Bu_4NF$ (200 mg) was added. After stirring overnight, the solvent was evaporated and the product purified by chromatography (hexanes/EtOAc, 1:1) to give **31** (580 mg, 89%) as an oil.

**Methyl 2-*O*-Benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranoside (32).** To compound **31** (600 mg, 2.36 mmol) in pyridine (10 mL) was added, dropwise, *tert*-butylchlorodiphenylsilane (730 μL, 2.8 mmol). The solution was stirred 24 h and the solvent evaporated. Purification by chromatography (hexanes/EtOAc, 4:1) yielded **32** (890 mg, 76%) as an oil.

**Disaccharide 35.** Alcohol **30** (700 mg, 2.0 mmol) and thioglycoside **17** (1.1 g, 2.1 mmol) were dissolved in $CH_2Cl_2$ (50 mL) and stirred with 4 Å molecular sieves at room temperature for 15 min. The reaction mixture was then cooled to −78 °C, and NIS (520 mg, 2.3 mmol) and AgOTf (130 mg, 0.51 mmol) were added in succession. The temperature of the reaction was allowed to rise to 0 °C over 3 h, and then $Et_3N$ (2 mL) and Celite were added. The suspension was stirred for 15 min and then filtered. The filtrate was concentrated and the resulting residue purified by chromatography (hexanes/EtOAc, 4:1) to afford **35** (1.12 g, 73%) as an oil.

**Disaccharide 5.** Oligosaccharide **35** (400 mg, 0.52 mmol) was dissolved in $CH_3OH$ (20 mL), and 10% Pd/C (150 mg) was added. Hydrogen was bubbled through the suspension at room temperature, and the reaction was monitored by TLC. After the hydrogenation was complete, the solution was filtered through Celite and the filtrate was concentrated. The residue was purified by chromatography on Iatrobeads ($CH_2Cl_2/CH_3OH$, 8:1) to give **5** (122 mg, 79%) as an oil.

**Disaccharide 36.** Alcohol **29** (550 mg, 1.60 mmol) and thioglycoside **28** (970 mg, 1.8 mmol) were dissolved in $CH_2Cl_2$ (30 mL), and 4 Å molecular sieves (1 g) were added. The solution was stirred for 15 min and cooled to 0 °C. After stirring for an additional 15 min, NIS (450 mg, 2 mmol) and AgOTf (130 mg, 0.5 mmol) were successively added to the reaction mixture. The progress of the reaction was followed by TLC, and after the disappearance of the alcohol, $Et_3N$ (2 mL) was added followed by Celite. This suspension was stirred for another 15 min, before being filtered. The filtrate was concentrated, and the resulting residue was purified by chromatography (hexanes/EtOAc, 6:1), yielding **36** (1.1 g, 91%) as an oil.

**Disaccharide 37.** Disaccharide **36** (820 mg, 0.68 mmol) was dissolved in a 0.1 M solution of $NaOCH_3$ in $CH_3OH$ (10 mL) and stirred for 2 h at room temperature. The reaction mixture was then neutralized with prewashed Amberlite IR-120 (H+) resin and filtered. The filtrate was concentrated, and the residue was purified by chromatography (hexanes/EtOAc, 6:1) to provide **37** (600 mg, 91%) as an oil.

**Trisaccharide 38.** Alcohol **37** (600 mg, 0.91 mmol) and thioglycoside **17** (500 mg, 0.95 mmol) were coupled using NIS (230 mg, 1 mmol) and AgOTf (50 mg, 0.2 mmol) in $CH_2Cl_2$ (20 mL) as described for the preparation of **35**. Purification by chromatography (hexanes/EtOAc, 6:1) yielded **38** (520 mg, 54%) as an oil.

**Trisaccharide 6.** Oligosaccharide **38** (470 mg, 0.44 mmol) was debenzylated via hydrogenation over 10% Pd/C (150 mg) in $CH_3OH$ (20 mL) as described for the preparation of **5**. Purification by chromatography ($CH_2Cl_2/CH_3OH$, 8:1) on Iatrobeads yielded **6** (145 mg, 76%) as an oil.

**Disaccharide 39.** Alcohol **32** (0.74 g, 1.5 mmol) and thioglycoside **28** (700 mg, 1.3 mmol) were coupled using NIS (380 mg, 1.70 mmol) and AgOTf (100 mg, 0.39 mmol) in $CH_2Cl_2$ (30 mL) as described for the preparation of **36**. Purification by chromatography (hexanes/EtOAc, 4:1) yielded **39** (840 mg, 60%) as an oil.

**Disaccharide 40.** Compound **39** (560 mg, 0.62 mmol) was debenzoylated in a 0.1 M solution of $NaOCH_3$ in $CH_3OH$ (20 mL) as described for the preparation of **37**. Purification by chromatography (hexanes/EtOAc, 4:1) yielded **40** (340 mg, 68%) as an oil.

**Trisaccharide 41.** Alcohol **40** (340 mg, 0.42 mmol) and thioglycoside **17** (260 mg, 0.50 mmol) were coupled using NIS (120 mg, 0.55 mmol) and AgOTf (50 mg, 0.19 mmol) in $CH_2Cl_2$ (30 mL) as described for the preparation of **35**. Purification by chromatography (hexanes/EtOAc, 4:1) yielded **41** (320 mg, 63%) as an oil.

**Trisaccharide 7.** Oligosaccharide **41** (320 mg, 0.26 mmol) was dissolved in THF (20 mL), and *n*-$Bu_4NF$ (100 mg, 0.40 mmol) was added. The solution was stirred at room temperature for 6 h and then concentrated. The crude product was purified by chromatography (hexanes/EtOAc, 4:1) to give the desilylated compound, which was immediately debenzylated via hydrogenation over 10% Pd/C (100 mg) in $CH_3OH$ (10 mL) as described for the preparation of **5**. Purification by chromatography ($CH_2Cl_2/CH_3OH$, 8:1) on Iatrobeads yielded **7** (68 mg, 62%) as an oil.

**Disaccharide 42.** Alcohol **29** (2.60 g, 7.56 mmol) and thioglycoside **27** (4.50 g, 7.92 mmol) were coupled using NIS (1.85 g, 8.2 mmol) and AgOTf (200 mg, 0.8 mmol) in $CH_2Cl_2$ (30 mL) as described for the preparation of **36**. Purification by chromatography (hexanes/EtOAc, 2:1) yielded **42** (5.01 g, 84%) as an oil.

**Disaccharide 43.** Disaccharide **42** (3.20 g, 4.06 mmol) was debenzoylated in a 0.1 M solution of $NaOCH_3$ in $CH_3OH$ (20 mL) as described for the preparation of **37**. Purification by chromatography (hexanes/EtOAc, 2:1) yielded **43** (1.84 g, 95%) as an oil.

**Disaccharide 46.** Compound **43** (1.25 g, 2.93 mmol) and trityl chloride (1.18 g, 4 mmol) were dissolved in pyridine (60 mL) and stirred overnight. The reaction mixture was concentrated, and the residue was extracted with EtOAc (2 × 30 mL). The organic extracts were then washed with brine (2 × 20 mL) and concentrated to provide crude **44**. The residue was dissolved in DMF/THF (3 mL/20 mL), and the solution was cooled to 0 °C before sodium hydride (60% dispersion in oil, 0.8 g, 20 mmol) and benzyl bromide (1 mL, 8.1 mmol) were added. The suspension was stirred at 0 °C for 2 h, and then $CH_3OH$ was added slowly. The reaction mixture was concentrated, and the resulting residue was extracted with EtOAc (100 mL). The organic extract was washed with water and brine and then evaporated to dryness to provide crude **45**. The residue was dissolved in $CH_3OH/CH_2Cl_2$ (10 mL/40 mL) and *p*-TsOH (0.4 g, 2.3 mmol) was added. After stirring for 3 h at room temperature, the solution was concentrated and extracted with $CH_2Cl_2$. The organic extract was washed with brine and water and then dried over $Na_2SO_4$ before being concentrated. The resulting crude product was purified by chromatography (hexanes/EtOAc, 4:1) to yield **46** (910 mg, 48%) as an oil.

**Trisaccharide 47.** Alcohol **46** (200 mg, 0.32 mmol) and thioglycoside **28** (180 mg, 0.33 mmol) were coupled using NIS (77 mg, 0.34 mmol) and AgOTf (8 mg, 0.07 mmol) in $CH_2Cl_2$ (20 mL) as described for the preparation of **36**. Purification by chromatography (hexanes/EtOAc, 6:1) yielded **47** (210 mg, 88%) as an oil.

**Trisaccharide 48.** Compound **47** (210 mg, 0.21 mmol) was debenzoylated in a 0.1 M solution of $NaOCH_3$ in $CH_3OH$ (10 mL) as described for the preparation of **37**. Purification by chromatography (hexanes/EtOAc, 4:1) yielded **48** (150 mg, 79%) as an oil.

**Tetrasaccharide 49.** Alcohol **48** (120 mg, 0.12 mmol) and thioglycoside **17** (75 mg, 0.14 mmol) were coupled using NIS (35 mg, 0.15 mmol) and AgOTf (10 mg, 0.04 mmol) in $CH_2Cl_2$ (20 mL) as described for the preparation of **35**. Purification by chromatography (hexanes/EtOAc, 4:1) yielded **49** (88 mg, 52%) as an oil.

**Tetrasaccharide 8.** Oligosaccharide **49** (88 mg, 0.06 mmol) was debenzylated via hydrogenation over 10% Pd/C (32 mg) in $CH_3OH$ (20 mL) as described for the preparation of **5**. Purification by chromatography ($CH_2Cl_2/CH_3OH$, 8:1) on Iatrobeads yielded **8**, (27 mg, 79%) as an oil.

**Disaccharide 50.** Compound **43** (1.80 g, 3.78 mmol) was dissolved in pyridine (20 mL) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.40 g, 4.44 mmol) was added dropwise. The reaction mixture was stirred for 8 h at room temperature and then concentrated. Purification by chromatography (hexanes/EtOAc, 3:1) yielded **50** (1.84 g, 68%) as an oil.

**Disaccharide 52.** Compound **50** (1.84 g, 2.56 mmol) was dissolved in dry DMF (10 mL) and cooled to 0 °C. To this solution were added sodium hydride (60% dispersion in oil, 200 mg, 5 mmol) and benzyl bromide (355 $\mu$L, 3 mmol). After stirring at 0 °C for 2 h, $CH_3OH$ (1 mL) was added. The solvent was evaporated and the resulting residue was diluted with EtOAc. This organic solution was washed with water and brine and then dried ($Na_2SO_4$) and concentrated. The resulting oil was dissolved in THF (10 mL), *n*-$Bu_4NF$ (200 mg) was added, and the solution was stirred overnight at room temperature. The solvent was then evaporated, and the product was purified by chromatography (hexanes/EtOAc, 1:3) to afford **52** (770 mg, 53%) as an oil.

**Disaccharide 53.** To a solution of **52** (500 mg, 0.88 mmol) in pyridine (20 mL) was added, dropwise, *tert*-butylchlorodiphenylsilane (300 mg, 1.09 mmol). The solution was stirred for 36 h and the solvent evaporated. The product was purified by chromatography (hexanes/EtOAc, 4:1) to yield **53** (480 mg, 68%) as an oil.

**Trisaccharide 54.** Compound **53** (430 mg, 0.53 mmol) and thioglycoside **28** (350 mg, 0.65 mmol) were coupled using NIS (150 mg, 0.65 mmol) and AgOTf (40 mg, 0.16 mmol) in $CH_2Cl_2$ (10 mL) as described for the preparation of **36**. Purification by chromatography (hexanes/EtOAc, 6:1) yielded **54** (490 mg, 75%) as an oil.

**Trisaccharide 55.** Compound **54** (470 mg, 0.39 mmol) was debenzoylated in a 0.1 M solution of $NaOCH_3$ in $CH_3OH$ (20 mL) as described for the preparation of **37**. Purification by chromatography (hexanes/EtOAc, 2:1) yielded **55** (350 mg, 80%) as an oil.

**Tetrasaccharide 56.** Alcohol **55** (350 mg, 0.31 mmol) and thioglycoside **17** (190 mg, 0.35 mmol) were coupled using NIS (84 mg, 0.37 mmol) and AgOTf (20 mg, 0.08 mmol) in $CH_2Cl_2$ (20 mL) as described for the preparation of **35**. Purification by chromatography (hexanes/EtOAc, 4:1) afforded **56** (280 mg, 60%) as an oil.

**Tetrasaccharide 9.** Compound **56** (280 mg, 0.18 mmol) was dissolved in THF (10 mL), and *n*-$Bu_4NF$ (58 mg, 0.22 mmol) was added. The solution was stirred at room temperature for 6 h and then concentrated. The crude product was purified by chromatography (hexanes/EtOAc, 6:1) to give the desilylated compound, which was debenzylated via hydrogenation over 10% Pd/C (100 mg) in $CH_3OH$ (10 mL) as described for the preparation of **5**. Purification by chromatography ($CH_2Cl_2/CH_3OH$, 8:1) on Iatrobeads yielded **9** (66 mg, 65%) as an oil.

**Trisaccharide 57.** Diol **31** (280 mg, 1.1 mmol) and thioglycoside **28** (1.30 g, 2.4 mmol) were coupled using NIS (590 mg, 2.6 mmol) and AgOTf (100 mg, 0.4 mmol) in $CH_2Cl_2$ (40 mL) as described for the preparation of **36**. Purification by chromatography (hexanes/EtOAc, 4:1) yielded **57** (970 mg, 82%) as an oil.

**Trisaccharide 58.** Compound **57** (820 mg, 0.76 mmol) was debenzoylated in a 0.1 M solution of $NaOCH_3$ in $CH_3OH$ (20 mL) as described for the preparation of **37**. Purification by chromatography (hexanes/EtOAc, 4:1) yielded **58** (520 mg, 78%) as an oil.

**Tetrasaccharide 59 and Tetrasaccharide 60.** Diol **58** (550 mg, 0.63 mmol) and thioglycoside **17** (330 mg, 0.63 mmol) were coupled using NIS (160 mg, 0.71 mmol) and AgOTf (32 mg, 0.13 mmol) in $CH_2Cl_2$ (20 mL) as described for the preparation of **35**. Purification by chromatography (hexanes/EtOAc, 4:1) yielded **59** (330 mg, 41%) and **60** (210 mg, 26%) as oils.

**Tetrasaccharide 10.** Oligosaccharide **59** (280 mg, 0.22 mmol) was debenzylated via hydrogenation over 10% Pd/C (90 mg) in $CH_3OH$ (10 mL) as described for the preparation of **5**. Purification by chromatography ($CH_2Cl_2/CH_3OH$, 8:1) on Iatrobeads yielded **10** (98 mg, 83%) as an oil.

**Tetrasaccharide 11.** Oligosaccharide **60** (190 mg, 0.14 mmol) was debenzylated via hydrogenation over 10% Pd/C (60 mg) in $CH_3OH$ (20 mL) as described for the preparation of **5**. Purification by chromatography ($CH_2Cl_2/CH_3OH$, 8:1) on Iatrobeads yielded **11** (67 mg, 86%) as an oil.

**Pentasaccharide 61.** Diol **58** (510 mg, 0.59 mmol) and thioglycoside **17** (62 mg, 1.18 mmol) were coupled using NIS (340 mg, 1.5 mmol) and AgOTf (90 mg, 0.35 mmol) in $CH_2Cl_2$ (40 mL) as described for the preparation of **35**. The product was purified by chromatography (hexanes/EtOAc, 4:1) to afford **61** (610 mg, 63%) as an oil.

**Pentasaccharide 12.** Oligosaccharide **61** (590 mg, 0.35 mmol) was debenzylated via hydrogenation over 10% Pd/C (200 mg) in $CH_3OH$ (20 mL) as described for the preparation of **5**. Purification by chromatography ($CH_2Cl_2/CH_3OH$, 8:1) on Iatrobeads yielded **12** (208 mg, 86%) as an oil.

**Tetrasaccharide 62.** Compound **52** (1.10 g, 1.94 mmol) and thioglycoside **28** (2.16 g, 4 mmol) were coupled using NIS (900 mg, 4.0 mmol) and AgOTf (200 mg, 0.8 mmol) in $CH_2Cl_2$ (30 mL) as described for the preparation of **36**. Purification by chromatography (hexanes/EtOAc, 5:1) yielded **62** (2.40 g, 89%) as an oil.

**Tetrasaccharide 63.** Compound **62** (1.4 g, 1 mmol) was debenzoylated in a 0.1 M solution of $NaOCH_3$ in $CH_3OH$ (20 mL) as described for the preparation of **37**. Purification by chromatography (hexanes/EtOAc, 5:1) yielded **63** (990 mg, 84%) as an oil.

**Hexasaccharide 64**, **Pentasaccharide 65, and Pentasaccharide 66.** Diol **63** (750 mg, 0.63 mmol) and thioglycoside **17** (470 mg, 0.89 mmol) were coupled using NIS (250 mg, 1.1 mmol) and AgOTf (70 mg, 0.27 mmol) in $CH_2Cl_2$ (20 mL) as described for the preparation of **35**. The products were purified by chromatography (hexanes/EtOAc, 6:1) to afford **64** (260 mg, 21%), **65** (280 mg, 28%), and **66** (100 mg, 10%) as oils.

**Hexasaccharide 4.** Oligosaccharide **64** (220 mg, 0.11 mmol) was debenzylated via hydrogenation over 10% Pd/C (70 mg) in a 4:1 mixture of acetic acid and water (20 mL) as described for the preparation of **5**. Purification by chromatography ($CH_2Cl_2/CH_3OH$, 8:1) on Iatrobeads yielded **4** (61 mg, 67%) as a white solid. The NMR data for this compound were identical to those of the compound obtained by deprotection of **25**.

**Pentasaccharide 13.** Oligosaccharide **65** (250 mg, 0.16 mmol) was debenzylated via hydrogenation over 10% Pd/C (80 mg) in $CH_3OH$ (20 mL) as described for the preparation of **5**. Purification by chromatography ($CH_2Cl_2/CH_3OH$, 8:1) on Iatrobeads yielded **13** (83 mg, 75%) as an oil.

**Pentasaccharide 14.** Oligosaccharide **66** (100 mg, 0.06 mmol) was debenzylated via hydrogenation over 10% Pd/C (30 mg) in $CH_3OH$ (20 mL) as described for the preparation of **5**. Purification by chromatography ($CH_2Cl_2/CH_3OH$, 8:1) on Iatrobeads yielded **14** (29 mg, 71%) as an oil.

**Acknowledgment.** This work was supported by the National Institutes of Health (AI44045-01) and the National Science Foundation (CHE-9875163).

**Supporting Information Available:** Analytical data for all new compounds, NMR spectra of selected compounds, figure comparing anomeric chemical shifts in protected oligosaccharides. This material is available free of charge via the Internet at http://pubs.acs.org.

JO010910E