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SYNTHETIC ARABINOFURANOSYL OLIGOSACCHARIDES AS MYCOBACTERIAL ARABINOSYLTRANSFERASE SUBSTRATES

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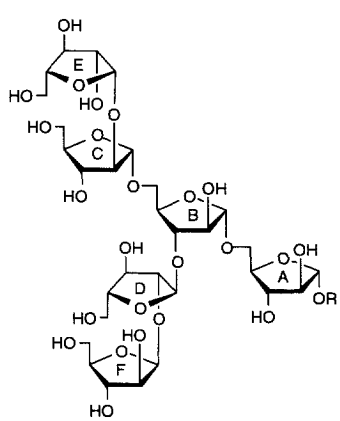
Abstract. A series of arabinofuranosyl oligosaccharides found as constituent parts of the polysaccharide portion of the cell wall of *Mycobacterium tuberculosis* have been chemically synthesized. Screening of these oligosaccharides as substrates for arabinosyltransferases present in mycobacterial membrane preparations suggests that modified oligosaccharide analogs as small as disaccharides may be inhibitors of glycan biosynthesis. Such inhibitors would be of potential utility as lead compounds in the identification of new drugs for the treatment of mycobacterial infections.

Tuberculosis kills nearly three million people worldwide each year making it the single most lethal bacterial disease.[1] It has been estimated that a third of the world's population is infected with *Mycobacterium tuberculosis*, the causative agent of this malady.[2] Recently, mycobacterial infections (e.g., *M. tuberculosis* and AIDS-associated *M. avium*) have attracted renewed attention due to their increasing incidence in both the industrialized and developing world, especially among immunocompromised and homeless populations.[3,4] The resurgence of these diseases has prompted interest in developing new antimycobacterial agents and the great urgency of this task has been underscored by the emergence of *M. tuberculosis* strains resistant to many of the drugs frequently used to treat these infections.[5]

The protective mycobacterial cell wall is an unique and intricate structure of polysaccharides, proteins, and lipids.[6] The two major polysaccharide components of this complex are an arabinogalactan (AG) and a lipoarabinomannan (LAM) in which all of the galactose and arabinose residues are found in the furanosidic form. The distal ends of both polymers are terminated with an arabinofuranosyl hexasaccharide (**1**), which in turn serves as the attachment site for either lipids (in the AG) or mannopyranosyl oligosaccharides (in the LAM); structural moieties that are known to play critical roles in both infection by and survival of the organism in the human host.[6,7] Ethambutol, one of the drugs currently used to treat tuberculosis, has recently been shown to be an arabinosyltransferase inhibitor.[8,9] Thus, new compounds that act, as does ethambutol, in preventing complete arabinan biosynthesis are likely to be potent antimycobacterial agents. Furanose oligo- and polysaccharides are not found in mammalian glycoconjugates and therefore inhibitors of the biosynthetic pathways leading to their formation are particularly attractive drug candidates.



1, R = AG or LAM

Attention is now being focused on understanding mycobacterial cell wall biosynthesis[10] but there is still much to be learned concerning the details of this process, especially the assembly of the arabinan component. Decaprenol arabinofuranosyl phosphate (**2**) has been identified as the source of the arabinose in mycobacteria[11,12]

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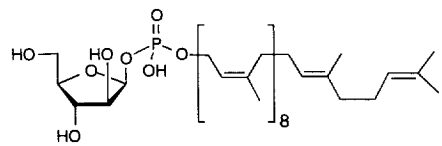
and there is presumably an array of glycosyltransferases that use **2** and various oligosaccharide acceptors to produce the glycan. None of these putative arabinosyltransferases have yet been isolated or purified;[10] however, an assay for their activity using mycobacterial membrane preparations as the enzyme source has been developed.[13] The transfer of arabinose from **2** to an arabinofuranosyl dimer and trimer has been evaluated using this assay; the effect of the aglyconic group (e.g., methyl vs. octyl) was also investigated.[14] However, a major limiting factor in these studies is the lack of availability of discrete oligosaccharide structures that can be used for unraveling the biosynthetic pathways, including the isolation and purification of the enzymes and the development of individual assays for their activity. Such compounds are most easily obtained via chemical synthesis but synthetic studies of oligofuranosides are rare.[15]



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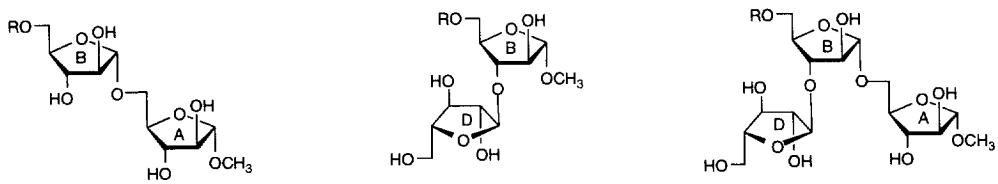
In this communication, we report the synthesis of six fragments of hexasaccharide **1** (Figure 1) and the recognition of these oligosaccharides (**3–8**) by arabinosyltransferases in a mycobacterial membrane extract. In addition to providing important information about the recognition of these oligosaccharides by mycobacterial glycosyltransferases, these glycans will be invaluable in mapping out the substrates specificities of the individual enzymes once they are purified.



3 R = H
6 R = α-D-arabinofuranosyl

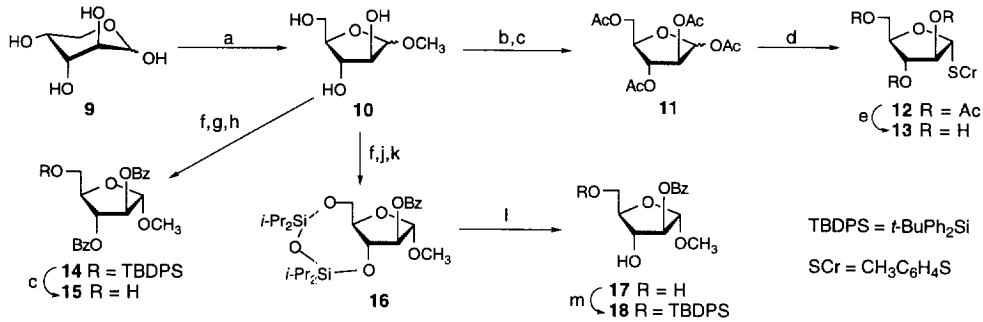
4 R = H
5 R = α-D-arabinofuranosyl

7 R = H
8 R = α-D-arabinofuranosyl

Figure 1. Rings have been lettered to facilitate comparison with **1**

Chemical Synthesis

In designing a synthetic strategy to assemble oligosaccharides **3–8**, we endeavored to develop routes that were readily amenable to the future synthesis of oligosaccharide analogs that would be potential arabinosyltransferase inhibitors.[16] The strategy chosen involved the sequential addition of sugar residues, via thioglycoside donors, to methyl glycoside acceptors.[17] Thus, a series of suitably protected monosaccharide building blocks were required. The arabinofuranoside core was synthesized by treatment of D-arabinose (**9**) with methanol under kinetic Fisher glycosylation conditions leading to the formation a 1:1 α:β mixture of methyl D-arabinofuranosides **10** (Figure 2). This mixture of methyl glycosides was converted, through a two-step acetylation/acetolysis procedure, to the known[18] tetraacetate **11** in 70% yield from D-arabinose. Reaction of **11** with *p*-thiocresol in the presence of boron trifluoride etherate provided thioglycoside **12** in 88% yield which was deacetylated quantitatively to afford triol **13**. Alternatively, chromatographic separation of the anomeric mixture of methyl arabinofuranosides (**10**) yielded the pure α-anomer, which was subsequently converted to methyl glycosides **15**, **17**, and **18**. To this end, **10** was treated with *tert*-butylchlorodiphenylsilane in pyridine and then

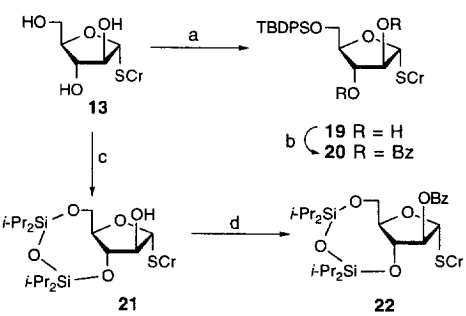


(a) CH_3OH, HCl, 0 °C; (b) Ac_2O, pyridine; (c) Ac_2O, HOAc, H_2SO_4, 3 steps 70%; (d) CrSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 88%, 0 °C; (e) $NaOCH_3$, CH_3OH, quantitative; (f) chromatographic separation of anomers; (g) *t*-$BuPh_2SiCl$, pyridine, 0 °C, 88%; (h) BzCl, pyridine, 82%; (i) *n*-Bu_4NF, THF, 89%; (j) $(Cl(i\text{-}Pr)_2Si)_2O$, pyridine, 68%; (k) BzCl, pyridine, 83%; (l) *n*-Bu_4NF, THF, 76%; (m) *t*-$BuPh_2SiCl$, pyridine, 0 °C, 84%.

Figure 2

benzoylated to provide silyl ether **14** (2 steps, 72%). Desilylation mediated by fluoride ion proceeded in 89% yield affording **15**. Access to diol **17** and alcohol **18** required first the synthesis of siloxane **16**, which could be prepared by the reaction of **10** with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine followed by benzoylation (2 steps, 57%). The siloxane was then removed with fluoride ion yielding **17** (76% yield). Selective protection of **17**, with *tert*-butylchlorodiphenylsilane in pyridine, provided alcohol **18** in 84% yield.

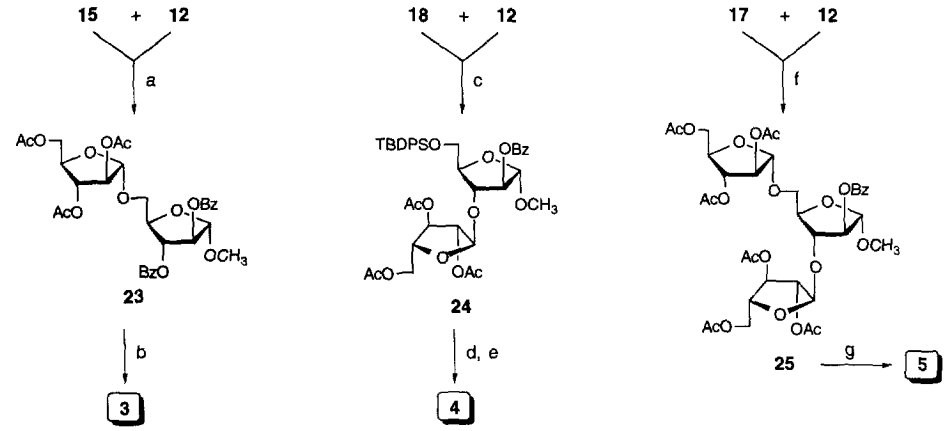
Orthogonally protected glycosyl donors **20** and **22** were synthesized from triol **13** as illustrated in Figure 3. Conversion of **13** to thioglycoside **20** involved selective silylation affording *tert*-butylchlorodiphenylsilyl ether **19** (83%) and then benzoylation, which gave the desired product in 94% yield. Transformation of **13** into **22** was done by way of siloxane **21** in two steps in a combined yield of 76%.



(a) *t*-$BuPh_2SiCl$, pyridine, 0 °C, 83%; (b) BzCl, pyridine 94%; (c) $(Cl(i\text{-}Pr)_2Si)_2O$, pyridine 79%; (d) BzCl, pyridine, 96%.

Figure 3

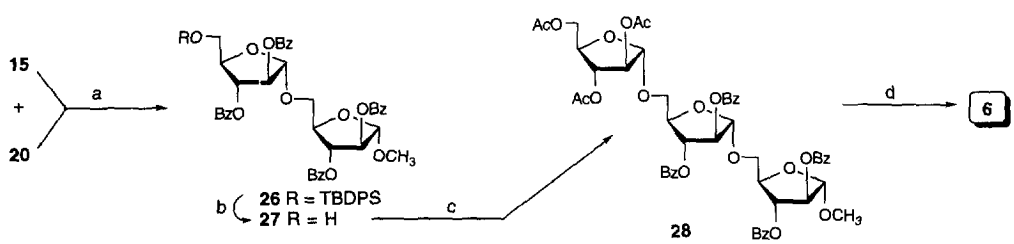
The coupling of the monosaccharide building blocks to yield the oligosaccharides was achieved by the reaction of the appropriate glycosyl acceptor and thioglycoside together with *N*-iodosuccinimide and silver trifluoromethanesulfonate. In all cases this promoter system gave good to excellent yields of the coupled products. Disaccharides **3** and **4** and trisaccharide **5** could each be efficiently synthesized in two steps as shown in Figure 4. Reaction of alcohol **15** with thioglycoside **12** yielded the fully acylated dimer **23** (86%), which was deprotected with sodium methoxide to afford disaccharide **3** (90%). Similarly, disaccharide **4** was prepared by the coupling of **12** with alcohol **18** and deprotection (2 steps, 71% yield). Finally, exhaustive glycosylation of diol **17** with three equivalents of **12** provided trisaccharide **25**; subsequent Zemplén deacylation afforded trisaccharide **5** in 68% yield from **17**.



(a) *N*-Iodosuccinimide, $AgOSO_2CF_3$, CH_2Cl_2, -10 °C, 86%; (b) $NaOCH_3$, CH_3OH, 90%; (c) *N*-Iodosuccinimide, $AgOSO_2CF_3$, CH_2Cl_2, -10 °C, 79%; (d) *n*-Bu_4NF, THF; (e) $NaOCH_3$, CH_3OH, 2 Steps 71%; (f) *N*-Iodosuccinimide, $AgOSO_2CF_3$, CH_2Cl_2, -10 °C, 94%; (g) $NaOCH_3$, CH_3OH, 72%.

Figure 4

The synthesis of trisaccharides **6** and **7** and tetrasaccharide **8** required more extensive transformations, which are illustrated in Figures 5 and 6. Glycosylation of alcohol **15** with thioglycoside **20** provided the protected disaccharide **26** in 82% yield. Removal of the 5'-silyl ether was achieved in 95% yield by reaction with tetra-*n*-butylammonium fluoride to provide **27**. Glycosylation of this disaccharide alcohol with **12** gave an 80% yield of the trisaccharide **28**, which was deprotected to yield **6** (89%). Oligosaccharides **7** and **8** were synthesized from a common intermediate, diol **29**, which was prepared by treatment of alcohol **15** with thioglycoside **22** followed by fluoride-ion mediated desilylation (2 steps, 56% yield). A portion of **29** was treated with *tert*-butylchlorodiphenylsilane in pyridine to afford alcohol **30** in 78% yield, which was converted to trisaccharide **31** upon glycosylation with **12** (76% yield). Desilylation and deacylation provided target oligosaccharide **7** in 73% yield (two steps). The remainder of **29** was glycosylated directly with three equivalents of **12** to give tetrasaccharide **32**, which provided **8** upon Zemplén deacylation (56%, 2 steps).

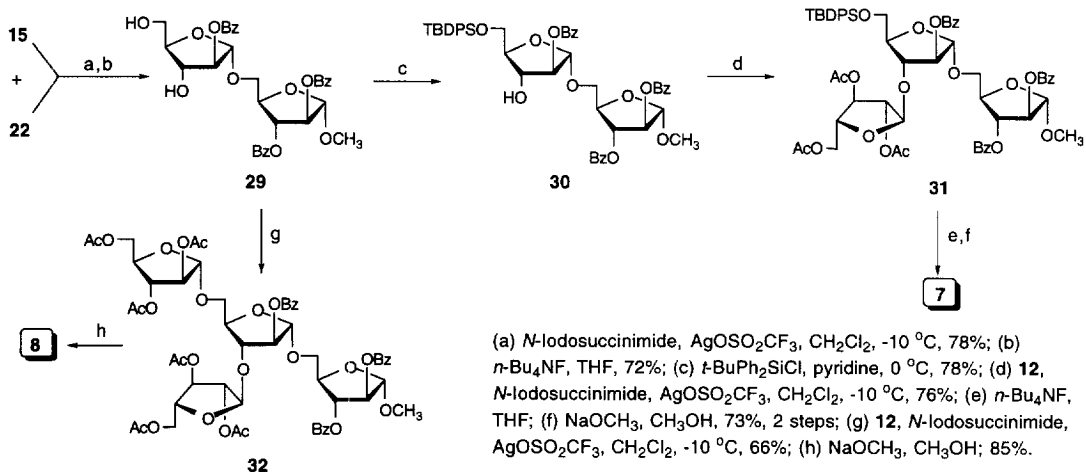


(a) *N*-Iodosuccinimide, $AgOSO_2CF_3$, CH_2Cl_2, -10 °C, 82%; (b) *n*-Bu_4NF, THF, 95%; (c) **12**, *N*-Iodosuccinimide, $AgOSO_2CF_3$, CH_2Cl_2, -10 °C, 80%; (d) $NaOCH_3$, CH_3OH, 89%.

Figure 5

Screening of Oligosaccharides as Arabinosyltransferase Substrates

Recognition of oligosaccharides **3–8** by one or more mycobacterial arabinosyltransferase was carried out as previously reported.[14] Briefly, each oligosaccharide was incubated with $^{14}C_1$-labeled **2** in the presence of membrane extracts from *M. smegmatis*. Residual **2** was removed by passage of the incubation mixture through



(a) *N*-Iodosuccinimide, $AgOSO_2CF_3$, CH_2Cl_2, -10 °C, 78%; (b) *n*-Bu_4NF, THF, 72%; (c) *t*-$BuPh_2SiCl$, pyridine, 0 °C, 78%; (d) **12**, *N*-Iodosuccinimide, $AgOSO_2CF_3$, CH_2Cl_2, -10 °C, 76%; (e) *n*-Bu_4NF, THF; (f) $NaOCH_3$, CH_3OH, 73%, 2 steps; (g) **12**, *N*-Iodosuccinimide, $AgOSO_2CF_3$, CH_2Cl_2, -10 °C, 66%; (h) $NaOCH_3$, CH_3OH; 85%.

Figure 6

an ion-exchange cartridge and the radioactivity in the effluent was measured by scintillation counting. The AG and LAM from *M. tuberculosis* and *M. semgmatis* are identical and thus both organisms possess similar arabinosyltransferase activities; the latter has been used a extensively as a model for *M. tuberculosis* because it is both easier to grow and is not pathogenic to humans. The results of these assays are included in Table 1 and indicate that all six oligosaccharides are converted into radiolabeled products. Since there are a number of arabinosyltransferase activities present in the incubation mixtures, it is not possible to draw definite conclusions about substrate specificities of individual enzymes. It is nevertheless worth noting that all oligosaccharides give similar degrees of incorporation. Oligosaccharides **3** and **6**, synthesized by alternative routes, were included a previous study[14] and our results are in agreement with these earlier findings.

Oligosaccharide	Incorporation of Radiolabel [cpm (%)]			
	Experiment 1	Experiment 2	Experiment 3	Average
3	5148 (12.9)	5148 (12.9)	4990 (12.4)	5095 (12.7)
4	7632 (19.1)	6432 (16.1)	6563 (16.4)	6876 (17.2)
5	7590 (18.9)	7833 (19.6)	7259 (18.1)	7561 (18.9)
6	8732 (21.9)	9011 (22.5)	7271 (18.2)	8338 (20.8)
7	5513 (13.8)	6326 (15.8)	4960 (12.4)	5600 (14.0)
8	6540 (16.4)	6092 (15.2)	-	6433 (16.1)

Table 1. Recognition of oligosaccharides **3-8** by mycobacterial arabinosyltransferases. Assays were carried out using each oligosaccharide at 10 mM concentration with 40,0000 cpm of synthetic, radiolabeled **2**. Each experiment is the average of a set of duplicates and is reported both in terms of cpm and % incorporation.

The most interesting result is that tetrasaccharide **8** is no more efficiently elongated than the smaller fragments, and indeed is less active than **4**, **5**, or **6**. A comparison of **5** with **8** shows that they differ only in the presence of a reducing-end arabinofuranosyl residue. Based on the arabinofuranose-containing structural motifs present in both the AG and LAM (e.g., **1**), it can be anticipated that both **5** and **8** will be substrates for the same enzymes, most notably a 1,2-β-arabinosyltransferase. These results suggest that this reducing end residue is not

critical for recognition by these enzymes. Therefore, it appears that, in common with other previously studied glycosyltransferase/substrate systems,[19] the epitopes for these arabinosyltransferases are di- and trisaccharide structures and that larger glycans are not significantly better substrates. The most important implication of this observation is that analogs of these small oligosaccharides will likely be biosynthetic inhibitors and possible lead compounds for drug development.

In conclusion, we have synthesized six arabinofuranosyl oligosaccharides and have shown that all these glycans can be converted to larger oligosaccharides by arabinosyltransferases present in mycobacterial membrane extracts. The similar degrees to which all are incorporated into labeled products suggests that analogs of these small oligosaccharides (e.g., fluorinated oligosaccharides or *C*-glycosides) are likely to be arabinosyltransferase inhibitors. The synthesis of such analogs is currently in progress.

Acknowledgments

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