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The First Total Synthesis of a Highly Branched Arabinofuranosyl Hexasaccharide Found at the Nonreducing Termini of Mycobacterial Arabinogalactan and Lipoarabinomannan

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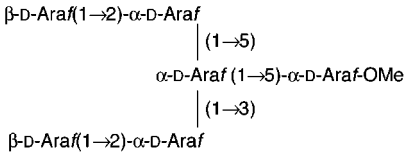
ABSTRACT



The first total synthesis of the arabinofuranosyl hexasaccharide present at the nonreducing termini of mycobacterial arabinogalactan and lipoarabinomannan is reported. The oligosaccharide was prepared as its methyl glycoside via a route that is both highly efficient and convergent. Addition of two *β*-D-arabinofuranosyl residues simultaneously in high yield and with excellent stereocontrol was the key step of the synthesis.

Although most of the greater than 50 known species of the *Mycobacterium* genus are not pathogenic to humans, a few, e.g., *M. tuberculosis*, *M. leprae*, and *M. avium*, pose serious health problems, particularly to individuals with compromised immune systems.[1] Regardless of their pathogenicity, all mycobacteria, in addition to other members of the Actinomycetes family (the *Rhodococcus*, *Corynebacteria*, and *Nocardia* genera), possess a unique cell wall structure comprised predominantly of polysaccharides and lipids (mycolic acids).[2] The two major polysaccharides are an arabinogalactan (AG) and a lipoarabinomannan (LAM), which are unusual in that all of the arabinose and galactose residues exist in the furanose form. At the nonreducing end of these polysaccharides is found the hexasaccharide motif shown in Figure 1, which is either unsubstituted or attached to the mycolic acids (AG) or mannopyranosyl oligosaccharides (LAM).[2]

A number of the immunological events associated with mycobacterial infections have been ascribed to these polysaccharides and in particular LAM.[3] Given its presence at the outermost periphery of the cell wall, it has been suggested that this hexasaccharide motif, when unsubstituted, is a key player in these processes.[3,4] Indeed, removal of the arabinose residues in these polysaccharides upon treatment with arabinofuranosidases has been shown to abolish many of their immunomodulatory properties.[4c]

We have recently initiated a research program directed at the identification of inhibitors of arabinan biosynthesis in mycobacteria.[5] As part of this work, we have focused on

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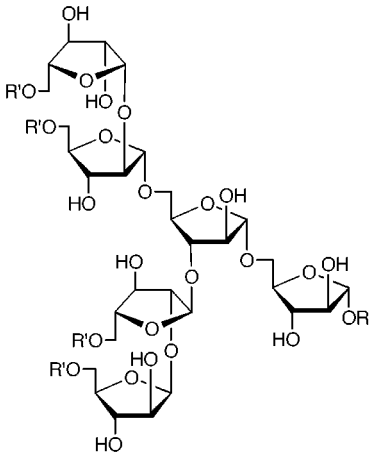

1 AG, R = arabinogalactan; R' = H or mycolic acids
2 LAM, R = arabinomannan; R' = H or Man*p* oligosaccharides
3 R = CH_3; R' = H

Figure 1.

synthesizing fragments of these cell wall polysaccharides, and in this context we have chosen this hexasaccharide as a target.

The chemical synthesis of oligosaccharides containing furanose residues has been relatively unexplored, and this structure presents a particular challenge as a result of the presence of two β-D-arabinofuranosyl residues. These moieties are analogous to the notoriously difficult to synthesize β-D-mannopyranosyl linkages found in mammalian glycoconjugates.[6] The *cis* orientation of the groups at C-1 and C-2 in these systems prevents directing the stereoselectivity of the glycosylation reaction via participation of an acyloxy group at C-2 in the glycosyl donor. Furthermore, in the case of mannose and arabinose, recourse cannot be made to donors possessing nonparticipating groups at C-2, because in the absence of neighboring group participation both stereoelectronic (the anomeric effect) and steric effects favor the formation of the α-glycoside. Although there have been a number of investigations on the stereoselective synthesis of β-mannopyranosides,[6] resulting in the development of a number of elegant synthetic methodologies, similar studies on β-arabinofuranosides were virtually nonexistent[7] until recently.[8,9]

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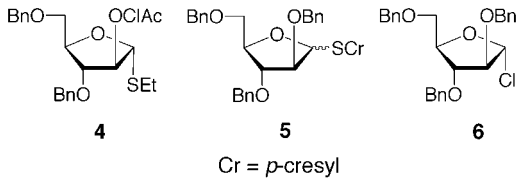


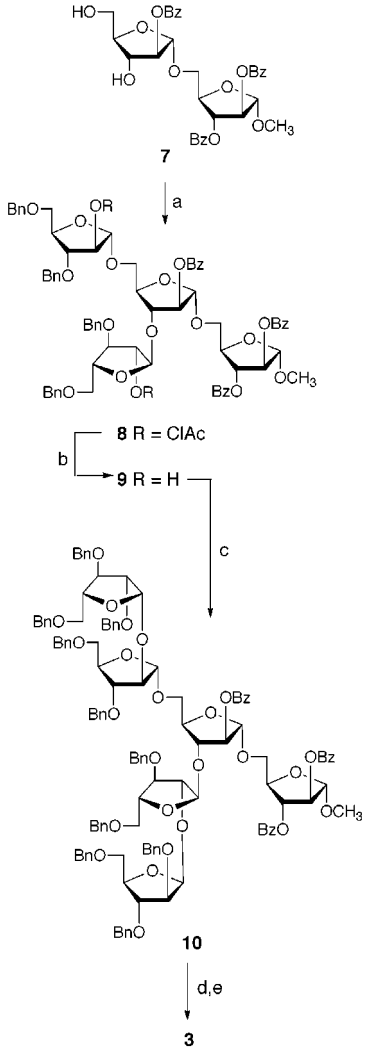
4 **5** **6**

Cr = *p*-cresyl

Figure 2.

In this communication, we report the first total synthesis of this hexasaccharide, as its methyl glycoside, **3**,[10] via a route that is both highly efficient and convergent. The key step is the introduction of two β-D-arabinofuranosyl residues simultaneously in good yield and with excellent stereocontrol. The synthesis of **3** required three building blocks, thioglycosides **4** and **5** (Figure 2) and disaccharide **7** (Scheme 1).

Scheme 1. Synthesis of **3**[a]



[a] Legend: (a) **4**, *N*-iodosuccinimide, AgOTf, CH_2Cl_2, 0 °C, 74%; (b) $H_2NNH_2 \cdot H_2O$, HOAc, CH_2Cl_2, CH_3OH, 40 °C, 91%; (c) **5**, *N*-iodosuccinimide, AgOTf, CH_2Cl_2, −78 °C, 81%; (d) $NaOCH_3$, $CH_3OH-CH_2Cl_2$ (3:1), rt; (e) H_2, Pd/C, $HOAc-H_2O$ (4:1), rt, 86% (two steps).

These were synthesized as described in the Supporting Information (**4**, **5**) or as reported previously (**7**).[5a,11]

As illustrated in Scheme 1, access to these building blocks enabled the synthesis of tetrasaccharide **8** in 74% yield by the reaction of **7** with an excess of thioglycoside **4** using *N*-iodosuccinimide/silver triflate activation at 0 °C. In the subsequent step, the chloroacetate protecting groups were removed (91% yield) by the treatment of **8** with hydrazine acetate. With **9** in hand, the stage was set for the introduction of the β-D-arabinofuranosyl residues.

In previous work, we[8a] and others[7] have demonstrated that the unstable tri-*O*-benzylated arabinofuranosyl chloride **6** can be used for the stereoselective synthesis of β-D-arabinofuranosides of simple alcohols in modest (40−60%) yields. In the case of methanol, this reaction has been shown[7b] to proceed through an S_N1 ion pair mechanism, resulting in net inversion of the anomeric stereochemistry. Unfortunately, in our hands, all attempts to glycosylate either primary or secondary carbohydrate alcohols with **6** failed to give *any* glycosylation product. *We have now found that reaction of diol* ***9*** *with thioglycoside* ***5***[12] *at −78 °C, in the presence of N-iodosuccinimide and silver triflate, affords hexasaccharide* ***10*** *in excellent (81%) yield and with extremely high stereocontrol.* Under these conditions, no α-glycoside products were isolated.[13] The temperature at which the glycosylation was conducted was critical. When the reaction was carried out at either −40 or 0 °C, significant amounts of side products were formed, which were chromatographically indistinguishable from the desired product.[14] Deprotection of **10** was achieved by treatment with catalytic sodium methoxide followed by hydrogenolysis affording hexasaccharide **3** in 86% yield over the two steps.

The anomeric stereochemistry of the glycosyl residues in **3** was determined unequivocally by both ^{1}H and ^{13}C NMR spectroscopy. The ^{13}C NMR spectrum showed six anomeric carbons at 108.83, 107.86, 106.07, 105.90, 101.16, and 101.05 ppm.[15] Similarly, in the ^{1}H NMR spectrum, four of the six anomeric hydrogens appeared as singlets or doublets ($J_{H1,H2} = 0-1.7$ Hz), characteristic of α-arabinofuranosides, and two as doublets ($J_{H1,H2} = 4.6$ Hz), indicative of the β-arabinofuranosyl linkages.[15,16] An HMQC experiment correlated the ^{1}H resonances with $J_{H1,H2} = 4.6$ Hz with the ^{13}C resonances at 101.16 and 101.05 ppm.

We are currently exploring the scope of this reaction to determine if other alcohols are glycosylated by **5** with a similarly high degree of stereocontrol. If so, this should become an extremely attractive method for the synthesis of other oligosaccharide fragments of mycobacterial arabinan. Particularly advantageous is that, rather than resorting to more elaborate methods for the assembly of these linkages (e.g., intermolecular aglycon delivery),[6a−c,8b,9b] excellent stereocontrol appears to be achieved using a readily accessible donor simply by carrying out the glycosylation at low temperature.

In summary, we report here the first chemical synthesis of the hexasaccharide (as its methyl glycoside, **3**) found at the nonreducing termini of mycobacterial LAM and AG. The key step in the synthesis is the stereoselective formation of the two β-D-arabinofuranosyl linkages simultaneously. To the best of our knowledge, there are no previous reports of the stereoselective formation of two 1,2-*cis*-β-glycosyl linkages in a single glycosylation reaction. At this time, we are unsure of the origin of the remarkable stereoselectivity observed in the reaction of **9** with **5**, but it may result via the same ion-pair S_N1 pathway proposed[7b] for glycosylation of methanol with **6**. Routine access to compounds of this type will enable a more detailed investigation of the role that this motif plays in the immunological response arising from mycobacterial infections.

Acknowledgment. We thank Christopher S. Callam and Joseph D. Ayers for technical assistance. This work was supported by the National Institutes of Health (AI44045-01), the National Science Foundation (CHE-9875163), and the Petroleum Research Fund (32026-G1), administered by the American Chemical Society.

Supporting Information Available: Experimental procedures and analytical data for compounds **3**−**5**, **8**−**10**, and the precursors leading to **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Ratio α: β = 8:2.

(13) All other products detected by TLC were isolated and screened by ^{1}H NMR for the presence of the hexasaccharide containing only α-arabinofuranosyl linkages. It was not detected.

(14) These impurities may be α-glycoside products; however, given the identical chromatographic properties of **10** and these byproducts, we were unable to determine their structures.

(15) Anomeric carbons in α-arabinofuranosides resonate between 105 and 110 ppm, and those of β-arabinofuranosides resonate between 100 and 104 ppm: Mizutani, K.; Kasai, R.; Nakamura, M.; Tanaka, O.; Matsuura, H. *Carbohydr. Res.* **1989**, *185*, 27.

(16) Unlike pyranosides, $^1J_{C1,H1}$ values cannot be used reliably in determinations of anomeric stereochemistry in furanosides; see ref 15.