VOLUME 122, NUMBER 7
FEBRUARY 23, 2000


JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

# Arabinofuranosyl Oligosaccharides from Mycobacteria: Synthesis and Effect of Glycosylation on Ring Conformation and Hydroxymethyl Group Rotamer Populations

**Francis W. D'Souza, Joseph D. Ayers, Patrick R. McCarren, and Todd L. Lowary***

*Contribution from the Department of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, Ohio 43210*

*Received October 4, 1999*

**Abstract:** A series of α-D-arabinofuranosyl oligosaccharides (**2**−**8**) that are fragments of the arabinan portions of two polysaccharides present in the cell wall of *Mycobacterium tuberculosis* have been synthesized. Preparation of the oligosaccharides involved the sequential addition of arabinofuranosyl residues from thioglycoside donors to methyl glycoside acceptors. High-resolution NMR studies on the final products provided all $^3J_{H,H}$ values, which were in turn used in PSEUROT 6.2 calculations to determine both the identity and equilibrium populations of preferred conformers for each furanose ring in these glycans. Comparison of the ring conformers present in **2**−**8** with those available in the parent monosaccharide, methyl α-D-arabinofuranose (**16**), allowed the determination of the effect of glycosylation upon ring conformation. At equilibrium, **16** exists as an approximately equimolar mixture of $^OT_4$ (North, N) and $^2T_3$ (South, S) conformers. These studies showed that glycosylation of **16** at $OH_5$ resulted in no significant change in conformer identity or population relative to **16**. However, glycosylation of $OH_3$ resulted in a change in the identity of the N species (to $^OE$) and a significant favoring of this conformer at equilibrium. These trends were seen in all of the oligosaccharides. The populations of the three possible staggered rotamers (gg, gt, tg) about the C4−C5 bond were essentially the same for all residues in **2**−**8**, and thus this equilibrium does not appear to be sensitive to glycosylation.

## Introduction

Although unknown in human biology, polysaccharides containing furanosidic residues are important constituents of glycoconjugates from many lower organisms including bacteria,[1] parasites,[2] and fungi.[3] Glycans containing furanose moieties are generally found in the extracellular glycocalyx (cell wall complex) and consequently play critical roles in the survival and pathogenicity of these microorganisms. A well-established method for the treatment of bacterial disease is the use of antibiotics that act by inhibiting cell wall biosynthesis.[4] Given the xenobiotic nature of furanose polysaccharides to humans, the biosynthetic pathways leading to their formation are particularly attractive targets for drug action. However, the processes by which these polysaccharides are assembled in nature is not well understood, and much additional research in this area is needed before this goal can be achieved.

While a number of microorganisms produce polysaccharides

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10.1021/ja993543l CCC: $19.00 
Published on Web 02/02/2000

containing furanose residues,[1−3] among the most important are the mycobacteria.[1] This genus of bacteria contains a number of species, including the well-known human pathogens *Mycobacterium tuberculosis* and *Mycobacterium leprae*, respectively the causes of tuberculosis and leprosy. In recent years infection by other members of this genus have become health threats. Most notable among these are *Mycobacterium avium* infections, which are now commonplace in AIDS patients.[5] Although mycobacterial diseases have attracted renewed attention in recent years because of their increasing incidence in the western world[6] and the emergence of drug-resistant strains,[7] these diseases have been a constant health threat worldwide for decades. For example, over a third of the world's population is estimated to be infected with *M. tuberculosis*, and tuberculosis remains the single most lethal bacterial disease, resulting in over 3 million deaths each year.[8]

The treatment of mycobacterial diseases is difficult, requiring adherence to a several-month regimen of antibiotics.[7,9,10] Such long treatments are necessary, in large part, as a result of the incredible thickness and impermeability of the cell wall complex, which prevents the effective passage of drugs into the organism.[1,7,11] The morphology of the cell wall is unique to *Mycobacteria* and other members of the *Actinomycetes* family, and the two major polysaccharide components are an arabinogalactan (AG) and a lipoarabinomannan (LAM) in which all of the galactose and arabinose residues are present in the furanose form.[1] The organism's ability to make these polymers is critical to its survival, and it has been shown that one of the drugs often used to treat tuberculosis (ethambutol) acts by inhibiting the biosynthesis of the arabinan portions of the AG and LAM.[12]

A detailed structural model of the mycobacterial cell wall is now available.[1,13,14] The terminal ends of both AG and LAM are capped with the hexasaccharide motif **1** (Figure 1), which is linked to the remainder of the polymer via an α-(1→5)-linked linear chain of arabinofuranose residues. Hexasaccharide **1** in turn serves as the attachment site for other functionalities present in the cell wall. These groups are located at the periphery of the cell wall complex and are therefore the interface between the microorganism and its environment. In LAM, the primary hydroxyl groups in **1** are often substituted with mannopyranosyl oligosaccharides, which have been implicated in the initial stages of infection through their interaction with human mannose-binding proteins.[15] In the AG, these same hydroxyl groups are esterified with mycolic acids, branched, long-chain fatty acids.[1,11,14] Through the tight packing of the alkyl chains, the mycolic acids form a protective hydrophobic facade that in some cases is nearly crystalline.[11,16]

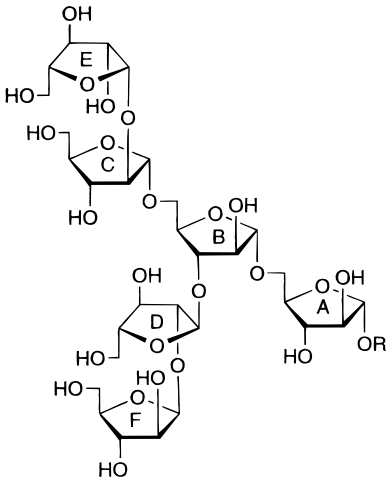


**Figure 1.** Hexaarabinoside motif (**1**) found at the nonreducing termini of mycobacterial arabinogalactan (AG) and lipoarabinomannan (LAM).

It is unknown why mycobacteria synthesize cell wall polysaccharides containing predominantly furanose residues. However, it has been suggested that the protection afforded to the organism by the tightly packed mycolic acids in the AG is one of the reasons why mycobacteria have evolved to produce polyfuranosides and not polypyranosides.[11] Polysaccharides containing furanose rings are expected to be more conformationally flexible than their counterparts composed of pyranose residues,[17] and thus the former would better allow the mycolic acids to align in the proper orientation for side-by-side packing. This proposal is, however, without any experimental support.

Identifying new antibiotics for the treatment of mycobacterial diseases is an area of current interest,[10,18] and inhibitors of mycobacterial arabinosyltransferases are ideal synthetic targets.[19] However, progress in this field has been hampered by the limited amount of detailed information that is available concerning the biosynthesis of mycobacterial arabinan.[20] To some degree, this is due to the lack of synthetic substrates that can be used for fundamental biochemical studies leading to the isolation and

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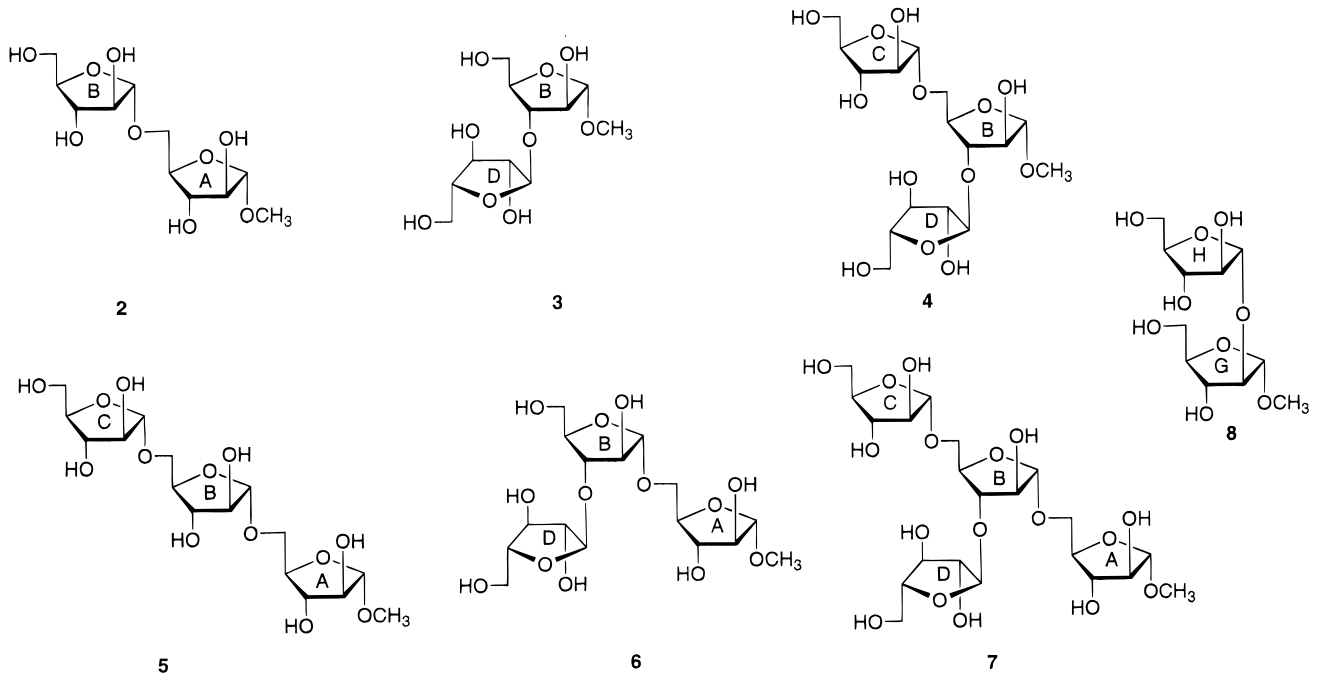


**Figure 2.** Synthetic targets; rings have been lettered to facilitate comparison with **1**.

purification of the appropriate biosynthetic enzymes. Furthermore, the rational design of inhibitors would be facilitated by a greater appreciation of the conformational preferences of these polysaccharides, which is currently unavailable. Such conformational investigations could also provide experimental support for the aforementioned hypothesis that the flexibility of these molecules is a key factor as to why mycobacteria have evolved to synthesize polysaccharides containing furanose, not pyranose, rings.

In a previous communication,[21] we have described preliminary synthetic details on the preparation of six oligosaccharide fragments (**2**−**7**, Figure 2) of hexasaccharide **1** and showed that these glycans are substrates for the arabinosyltransferases that are involved in the biosynthesis of the mycobacterial cell wall. In this paper we report a full account of the synthesis of oligosaccharides **2**−**7** and the related disaccharide, **8**. These oligosaccharides are composed of 1,2-*trans*-arabinofuranosyl residues; the preparation of glycans containing 1,2-*cis*-arabinofuranosyl linkages, which are also found in these polysaccharides, is underway and will be reported in the future. Additionally, to probe the conformation of these molecules, we describe the effect that glycosylation has upon not only the conformers available to each ring in these oligosaccharides but also the rotamer populations about the exocyclic C−C bonds. Although a number of reports have described the conformation of furanose monosaccharides and nucleotides,[22] conformational studies of oligosaccharides containing furanose rings have been more limited, with the majority of studies being carried out on an α-L-arabinofuranosyl disaccharide,[23] sucrose and related fructofuranosyl oligomers,[24] or glycans containing *β*-D-galactofuranosyl residues.[25] This report represents the first study of the effect of glycosylation on ring conformer populations in oligosaccharides containing solely furanose rings.

## Results and Discussion

**Synthesis of Oligosaccharides.** Although syntheses of pyranosidic oligosaccharides are now common, similar studies of furanosidic oligosaccharides are far less so. A relatively small number of reports have described syntheses of glycoconjugates containing D-galactofuranosyl,[26] L-arabinofuranosyl,[27] D-ribofuranosyl,[28] D-arabinofuranosyl[21,29,34] and D-fructofuranosyl[30] residues. A range of glycosylating agents have been used in

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these investigations, including thioglycosides, glycosyl imidates, selenoglycosides, and glycosyl halides.

In designing the synthesis of oligosaccharides **2**−**8** we endeavored to develop a route that would (1) be amenable to the future preparation of analogues (potential arabinosyltransferase inhibitors) and (2) employ glycosyl donors that could be stored for extended periods. We thus chose a linear approach by which the glycans would be synthesized by the sequential addition of thioglycoside donors to methyl glycoside acceptors. We envisioned that the oligosaccharide targets could be prepared from the seven building blocks (**9**−**15**) shown in Figure 3. The linear approach would allow the easy synthesis of analogues by substitution of the appropriately modified (e.g., fluorinated) building blocks for one of those shown in Figure 3. Thioglycosides were chosen as the glycosyl donors because of their hydrolytic stability and range of activation methods.[31]

The preparation of **9**−**15** from methyl α-D-arabinofuranoside **16**[32] and tetraacetate **17**[33] is described in the Supporting Information. With these building blocks in hand, all glycosylation reactions were carried out by reacting the appropriate combination of thioglycoside donor and methyl glycoside acceptor in the presence of *N*-iodosuccinimide and silver triflate.[31] In all cases, this promoter system provided good to excellent yields of the oligosaccharide products, with excellent stereoselectivities. The stereochemical outcome of the glycosylations was straightforwardly determined by standard one-dimensional $^{13}C$ and $^{1}H$ NMR experiments. The anomeric carbons of α-arabinofuranosides resonate in the range of 107−110 ppm, and the $\beta$-anomers appear between 97 and 104 ppm. In addition, $^{3}J_{H1,H2}$ is small (0−2 Hz) for the α-anomers and larger (4−5 Hz) for the $\beta$-anomers.[27c,34]

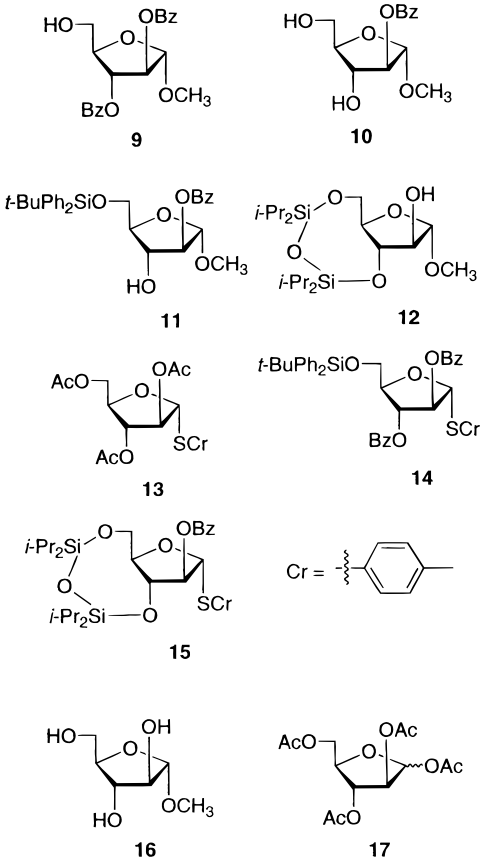


**Figure 3.** Building blocks required for the synthesis of **2**−**8**.

Disaccharides **2**, **3**, and **8** and trisaccharide **4** were readily assembled in 2−3 steps as illustrated in Scheme 1. The α-(1→5) linked disaccharide **2** and its α-(1→3) linked isomer **3** were prepared, respectively, in 77% and 56% overall yields as described previously[34] via the protected derivatives **18** and **19**. Glycosylation of methyl glycoside **12** with thioglycoside **13** provided disaccharide **20** in 86% yield. The α-(1→2) linked disaccharide **8** was obtained in 79% yield upon treatment of **20** with tetra-*n*-butylammonium fluoride (TBAF) followed by Zemplén deacetylation. Finally, reaction of diol **10** with 2.5 equiv of **13** provided trisaccharide **21** in 95% yield. Reaction of **21** with sodium methoxide afforded a 72% yield of **4**.

The assembly of the other three targets (**5**−**7**), although requiring more extensive transformations, proceeded without incident. The linear trisaccharide **5** was prepared as outlined in Scheme 2. Reaction of alcohol **9** with thioglycoside **14** provided the protected disaccharide **22** in 82% yield. The silyl protecting group was cleaved (95% yield), and the resulting disaccharide product **23** was glycosylated with **13** to provide trisaccharide **24** in 80% yield. Zemplén deacetylation proceeded in 89% yield to give **5**. The remaining glycans, trisaccharide **6** and tetrasaccharide **7**, were synthesized from a common intermediate, diol **26**, as illustrated in Schemes 3 and 4. Glycosylation of **9** with thioglycoside **15** afforded, in 78% yield, disaccharide **25** (Scheme 3). Subsequent treatment with TBAF provided a 72% yield of diol **26**. A portion of **26** was reacted with *tert*-butyldiphenylsilyl chloride in pyridine to give **27** in 78% yield. Glycosylation of **27** with **13** afforded the protected trisaccharide **28** (76%), which was deprotected in two steps and 73% yield to give **6**. Alternatively (Scheme 4), glycosylation of **26** with an excess of **13** provided tetrasaccharide **29** in 65% yield. Treatment with sodium methoxide afforded an 85% yield of **7**.

**NMR Studies.** Although NMR-based conformational inves-

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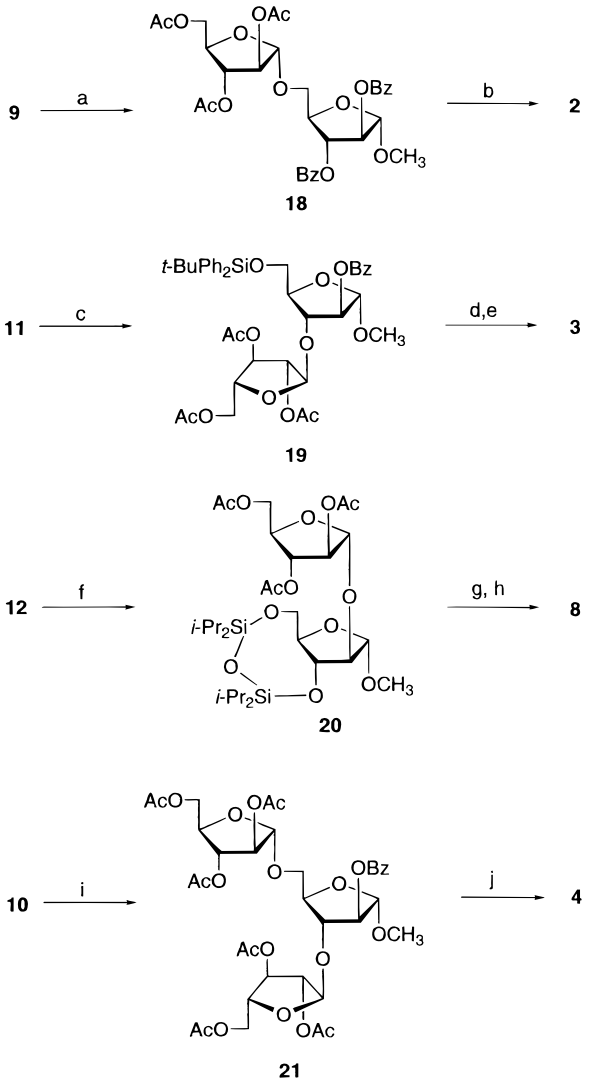
(34) D'Souza, F. W.; Cheshev, P. E.; Ayers, J. D.; Lowary, T. L. *J. Org. Chem.* **1998**, *63*, 9037.

**Scheme 1**[a]

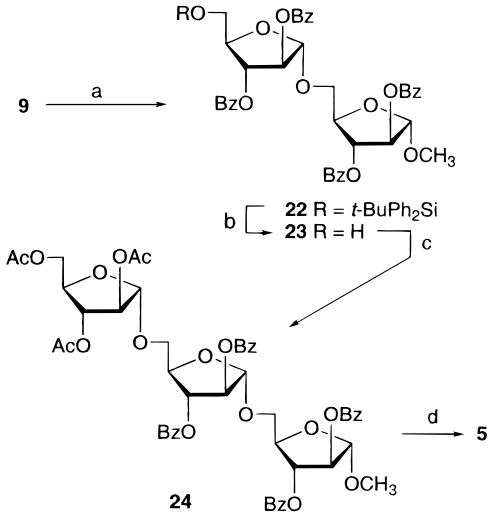



[a] (a) **13**, *N*-iodosuccinimide, $AgOSO_2CF_3$, $CH_2Cl_2$, 0 °C, 86%; (b) $NaOCH_3$, $CH_3OH$, rt, 90%; (c) **13**, *N*-iodosuccinimide, $AgOSO_2CF_3$, $CH_2Cl_2$, 0 °C, 79%; (d) *n*-$Bu_4NF$, THF, rt; (e) $NaOCH_3$, $CH_3OH$, rt, two steps, 71%; (f) **13**, *N*-iodosuccinimide, $AgOSO_2CF_3$, $CH_2Cl_2$, 0 °C, 86%; (g) *n*-$Bu_4NF$, THF, rt; (h) $NaOCH_3$, $CH_3OH$, rt, two steps, 79%; (i) **13**, *N*-iodosuccinimide, $AgOSO_2CF_3$, $CH_2Cl_2$, 0 °C, 94%; (j) $NaOCH_3$, $CH_3OH$, rt, 72%.

tigations of oligosaccharides are increasingly routine,[35] the vast majority of these studies have dealt with oligopyranosides, which, in comparison with their furanosidic counterparts, are relatively inflexible species. Most pyranose rings are rigid, existing in one of two possible chair conformations. Therefore, the major degrees of freedom available to an oligopyranoside are rotation about glycosidic bonds (torsion angles $\Phi,\Psi$), and the exocyclic C−C bond (torsion angle $\omega$).[35a] Understanding the conformational properties of oligofuranosides is a more complicated problem, because in addition to rotation about glycosidic and exocyclic C−C bonds, the rings themselves are flexible.[36] Consequently, in addition to $\Phi$, $\Psi$, and $\omega$ torsion angles, furanoses also possess a ring torsion angle.

Furanose rings, like substituted cyclopentanes, exist in either envelope (E) or twist (T) conformations. A particular ring conformer can be described by two parameters, the puckering amplitude ($\tau_m$) and the pseudorotational phase angle ($P$), which can be illustrated by the pseudorotational wheel[36] shown in Figure 4 for a D-furanose ring. The radius of the circle is $\tau_m$

**Scheme 2**[a]




[a] (a) **14**, *N*-iodosuccinimide, $AgOSO_2CF_3$, $CH_2Cl_2$, 0 °C, 82%; (b) *n*-$Bu_4NF$, THF, rt, 95%; (c) **13**, *N*-iodosuccinimide, $AgOSO_2CF_3$, $CH_2Cl_2$, 0 °C, 80%; (d) $NaOCH_3$, $CH_3OH$, rt, 89%.

and $P$, which defines the part of the ring that is most puckered, is indicated along with the associated envelope or twist conformer. For a given furanose ring, the standard model used for assessing conformation in solution assumes that there are two conformers existing in equilibrium. Typically, one of these species is present in the northern hemisphere of the pseudorotational wheel and the other in the southern hemisphere, respectively termed the North (N) and South (S) conformers (Figure 4).[36] Interconversion between these puckered conformers generally occurs through pseudorotation rather than inversion via the planar ring form, in which the ring substituents are eclipsed.[37] The pathway by which the two interconvert (via the West or East) depends on the identity and orientation of the substituents on the ring.

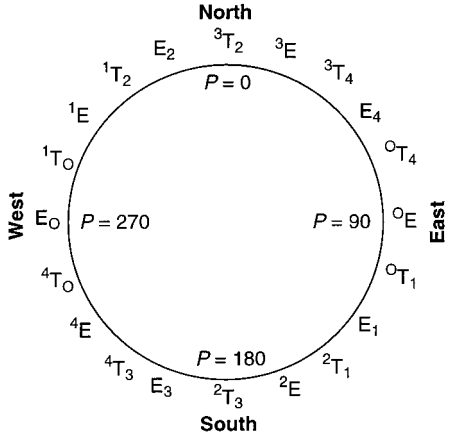
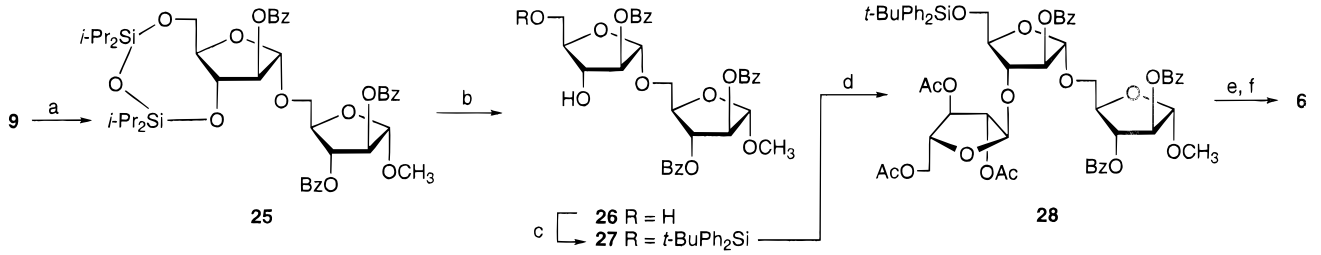



**Figure 4.** Pseudorotational itinerary for a D-aldofuranose ring.

In cases such as these where two conformers are equilibrating rapidly on the NMR time scale, conformational analysis becomes complicated because averaging of coupling constants occurs. Fortunately, for furanose rings, a least-squares minimization program (PSEUROT 6.2)[38] is available that, given the observed intracyclic ring $^3J_{H,H}$ and the $\tau_m$ for each conformer, will calculate the N/S ratio and provide $P$ values for both conformers from which the ring forms (e.g., $^3T_2$) can be determined.

Prior to the development of the PSEUROT method, Angyal reported a more qualitative study on the conformations adopted

**Scheme 3**[a]



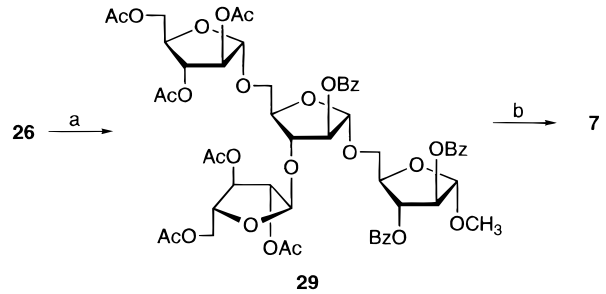

[a] (a)**15**, *N*-iodosuccinimide, $AgOSO_2CF_3$, $CH_2Cl_2$, 0 °C, 78%; (b) *n*-$Bu_4NF$, THF, rt, 72%; (c) *t*-$BuPh_2SiCl$, pyridine, 0 °C, 78%; (d) **13**, *N*-iodosuccinimide, $AgOSO_2CF_3$, $CH_2Cl_2$, 0 °C, 76%; (e) *n*-$Bu_4NF$, THF, rt; (f) $NaOCH_3$, $CH_3OH$, rt, two steps, 73%.

**Table 1.** PSEUROT Analysis of **2**−**8** and **16**[a]

| | compound | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **16** | **2** | | **3** | | **8**[e] | **4** | | | **5** | | | **6** | | | **7** | | | |
| | A[b] | A | B | B | D | H | B | C | D | A | B | C | A | B | D | A | B | C | D |
| $P_N$ (deg)[c] | 72 | 77 | 75 | 94 | 70 | 78 | 95 | 73 | 71 | 74 | 74 | 73 | 74 | 93 | 70 | 75 | 97 | 74 | 73 |
| N conformer[d] | $^OT_4$ | $^OT_4$ | $^OT_4$ | $^OE$ | $^OT_4$ | $^OT_4$ | $^OE$ | $^OT_4$ | $^OT_4$ | $^OT_4$ | $^OT_4$ | $^OT_4$ | $^OT_4$ | $^OE$ | $^OT_4$ | $^OT_4$ | $^OE$ | $^OT_4$ | $^OT_4$ |
| $X_N$ (%) | 56 | 59 | 53 | 86 | 49 | 73 | 94 | 55 | 52 | 55 | 54 | 55 | 62 | 79 | 49 | 53 | 92 | 57 | 53 |
| $P_S$ (deg)[c] | 183 | 184 | 183 | 184 | 183 | 178 | 185 | 183 | 183 | 183 | 183 | 183 | 183 | 184 | 183 | 183 | 185 | 183 | 183 |
| S conformer[d] | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ | $^2T_3$ |
| $X_S$ (%) | 44 | 41 | 47 | 14 | 51 | 27 | 6 | 45 | 48 | 45 | 46 | 45 | 38 | 21 | 51 | 47 | 8 | 43 | 47 |
| RMS (Hz) | 0.02 | 0.01 | 0.02 | 0.02 | 0.11 | 0.14 | 0.12 | 0.6 | 0.10 | 0.00 | 0.04 | 0.06 | 0.02 | 0.07 | 0.11 | 0.02 | 0.06 | 0.06 | 0.07 |

[a] Calculated using a constant $\tau_m = 39°$. [b] See Figure 2 for assignment of ring letters. [c] $P$ = pseudorotational phase angle.[36] [d] See Figures 4, 6, and 8 for conformer definitions. [e] Analysis could not be done for ring G because of non-first-order coupling between ring protons.

**Scheme 4**[a]




[a] (a) **13**, *N*-iodosuccinimide, $AgOSO_2CF_3$, $CH_2Cl_2$, 0 °C, 65%; (b) $NaOCH_3$, $CH_3OH$, rt, 85%.

by methyl furanosides in solution.[39] Using $^3J_{H,H}$ data, three criteria were developed for predicting the region of the pseudorotational itinerary in which the favored conformers of a furanose ring would be found. It was proposed that the preferred conformations will be ones in which (1) the anomeric methoxy group is placed pseudoaxial, (2) the hydroxymethyl group at $C_4$ is oriented in a pseudoequatorial fashion, and (3) the ring substituents are staggered as much as possible. The first of these criteria is a consequence of the *endo* anomeric effect, which necessitates an approximately antiperiplanar orientation between one of the lone pairs on the ring oxygen and the $C_1-O_1$ bond.[40] The second and third criteria would arise from steric effects.

From this discussion, it is clear that the first step in assessing the conformation of an oligosaccharide containing furanose residues is necessarily the determination of the ring conformers that are present for each constituent monosaccharide. Consequently, we have measured all $^3J_{H,H}$ in monosaccharide **16** and oligosaccharides **2**−**8** and then used these couplings in PSEUROT 6.2 calculations to determine the conformers that each ring adopts. Table S1 in the Supporting Information contains the couplings used in these calculations. Through these investigations we have been able to more fully appreciate the effect of substitution (glycosylation) on the equilibrium populations of ring conformers. This work represents the first steps in probing

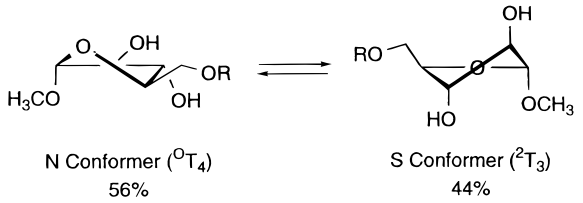



**Figure 5.** Conformational equilibrium of **16**.

the conformations of the molecules as a whole and the results, which are presented in Table 1, are discussed in greater detail below.

**Ring Conformers Present in Monosaccharide 16.** The parent monosaccharide, methyl α-D-arabinofuranoside, **16**, was investigated first and used as a basis of comparison with the oligosaccharides. PSEUROT analysis of **16** identifies the $^OT_4$ (N) and $^2T_3$ (S) conformers as those that are in equilibrium in solution. Both conformations are present in roughly equimolar amounts with the N form being favored slightly. In these two conformers, the methoxy group occupies a pseudoaxial position, and the $C_4$ hydroxymethyl group is pseudoequatorial (Figure 5). However, in the S conformer, the orientation of this hydroxymethyl group is oriented slightly less equatorially than in its N counterpart. These results compare favorably with those

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(36) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205.
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(38) (a) *PSEUROT 6.2*; Gorlaeus Laboratories, University of Leiden. (b) de Leeuw, F. A. A. M.; Altona, C. *J. Comput. Chem.* **1983**, *4*, 428. (c) Altona, C. *Recl. Trav. Chem. Pays-Bas* **1982**, *101*, 413.
(39) Angyal, S. J. *Carbohydr. Res.* **1979**, *77*, 37.
(40) (a) Juaristi, E.; Cuevas, G. *Tetrahedron* **1992**, *48*, 5019. (b) Thatcher, G. R. J. *The Anomeric Effect and Associated Stereoelectronic Effects*; American Chemical Society: Washington, D.C., 1993. For papers specifically addressing the anomeric effect in furanose rings, see: (c) Ellervik, U.; Magnusson, G. *J. Am. Chem. Soc.* **1994**, *116*, 2340. (d) Cossé-Barbi, A.; Dubois, J. E. *J. Am. Chem. Soc.* **1987**, *109*, 1503. (e) Cossé-Barbi, A.; Watson, D. G.; Dubois, J. E. *Tetrahedron Lett.* **1989**, *30*, 163. (f) O'Leary, D. J.; Kishi, Y. *J. Org. Chem.* **1994**, *59*, 6629.

of Angyal[39] and Serianni and Barker[22j] which predicted, without the use of PSEUROT analysis, that the most favored solution conformation of **16** would be in the $^{O}E/E_1$ region of the pseudorotational itinerary.

In a previous investigation, we have carried out gas-phase ab initio (Hartree−Fock, HF) and density functional theory (DFT) calculations on **16**.[41] In that study, the gas-phase energies of each of the 10 envelope conformers were calculated by first fixing four atoms in a plane and then allowing the rest of the molecule to minimize at HF and B3LYP levels with the 6-31G* basis set. In the gas phase, the N and S conformers were determined to be the $^{3}E$ and $^{2}E$ conformers, respectively, with the latter being the global minima. In addition to the previously mentioned steric and stereoelectronic factors, intramolecular hydrogen bonding, particularly in the $^{2}E$ conformer, was a key stabilization factor. The $^{O}T_4$ and $^{2}T_3$ solution conformers are in reasonable agreement with the computationally derived geometries, especially when considering that in solution solvation will, without question, profoundly affect the energies of each conformer. The agreement between S conformers is the best, with the predicted solution structure ($^{2}T_3$) being immediately adjacent to the gas-phase minimum ($^{2}E$).

The two solution conformers presumably equilibrate via pseudorotation through the east ($^{O}T_4 \leftrightarrow {}^{O}E \leftrightarrow {}^{O}T_1 \leftrightarrow E_1 \leftrightarrow {}^{2}T_1 \leftrightarrow {}^{2}E \leftrightarrow {}^{2}T_3$) as there is a favorable orientation of both the $C_1$ and $C_4$ substituents in all of these conformers. Pseudorotation via the west would require proceeding through the $E_O$ conformer in which the group at $C_4$ is pseudoaxial and the anomeric methoxy group is pseudoequatorial. Both of these factors would significantly destabilize this conformer, in turn making pseudorotation through the west a higher energy pathway than the one involving the southeast quadrant. Our computational studies support this proposal as the gas-phase barrier for pseudorotation through the west (via $E_O$) is approximately 4 kcal/mol higher in energy than through the east (via $^{O}E$).[41]

To further corroborate the structures of solution conformers of **16** proposed to be in equilibrium, comparison was made with two available crystal structures. Both X-ray and neutron diffraction structures of **16** have recently been reported.[42] In the crystal, the unit cell is composed of two distinct structures that are almost identical; the ring in both adopts an $E_4$ conformation with a $\tau_m$ of 39°. In addition to having crystal data for **16**, we have also recently obtained an X-ray structure of octyl α-D-arabinofuranoside (**30**, Figure 6).[43] Similar to the methyl glycoside, the unit cell has two distinct forms of the molecule, with each furanose ring existing in an $E_4$ conformation with a $\tau_m$ of 39°. Thus, the structure of the N conformer of **16** observed in solution ($^{O}T_4$) is very similar to the conformation of the rings present in two different α-D-arabinofuranosyl glycosides in the solid state.

In addition to providing us with a potential energy surface across the pseudorotational itinerary, our previous computational studies[41] allow the determination of all bond lengths, bond angles, and dihedral angles of each envelope conformer in **16**. For a given parameter, taking an average of the values from two adjacent envelopes provides an estimate of this parameter in the intermediate twist conformer. Although clearly the values obtained from these gas-phase calculations must be interpreted with some caution when applied to experimental data obtained in aqueous solution, they nevertheless provide a basis for rationalizing our results.

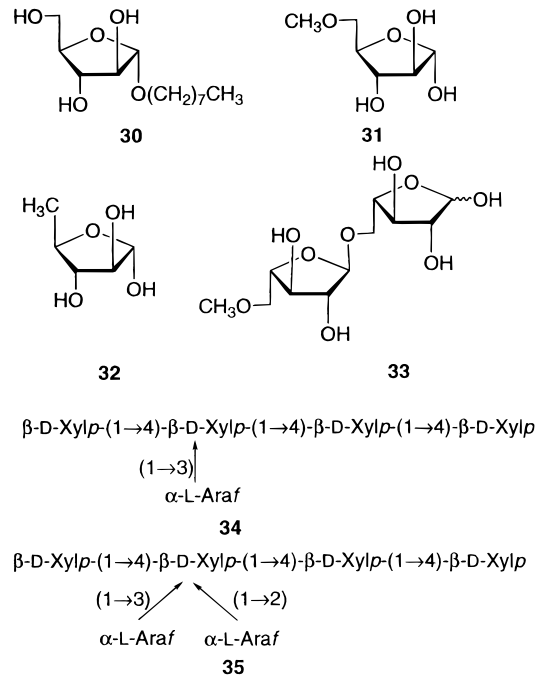


**Figure 6.** Arabinofuranose derivatives that have been the subject of previous conformational investigations.

In the gas phase, the global minima of **16** is the $^{2}E$ conformer, which is located in the southeast quadrant of the itinerary and between the two conformations observed in solution. Given that in the $^{O}T_4$ (N) conformation all of the ring substituents, except the anomeric methoxyl group, are pseudoequatorial, it is not surprising that this is one of the low-energy conformations in solution. What is perhaps more surprising is the identity of the S species, because in this conformer ($^{2}T_3$) both secondary OH groups are pseudoaxial. Inspection of the gas-phase interatomic distances[41] show that the distance between $O_3$ and $O_1$ moves from 4.41 Å in the $^{O}T_4$ conformer to 3.01 Å in the $^{2}T_3$ form. A similar diminution in the interatomic distance between $O_2$ and $C_5$ is observed: 4.68 Å ($^{O}T_4$) to 3.48 Å ($^{2}T_3$).[41] However, concomitant with these changes is the formation of two intramolecular hydrogen bonds, one between $OH_2\cdots O_5$ and the other between $OH_3\cdots O_1$. Although the formation of these intramolecular hydrogen bonds will certainly be diminished in solution, the ability of these groups to interact in this manner may be a factor contributing to the stabilization of the S conformer.

A more compelling explanation is that this conformer is stabilized by the gauche interactions[44] that both $OH_2$ and $OH_3$ make with the ring oxygen. From earlier structural work done in nucleic acids (Figure 7),[22m−q,45] it is known that in double stranded RNA, the ribofuranose rings exist in the $^{3}T_2$ ($C_3'$-*endo*) conformation. This ring form is stabilized both by the anomeric effect of the base and by the gauche relationship of the 2′ hydroxy group with the ring oxygen. In contrast, the furanose rings in double stranded DNA, which lack this hydroxyl group, can adopt either $^{3}T_2$ ($C_3'$-*endo*) or $^{2}T_3$ ($C_2'$-*endo*) conformations. The former is stabilized by the anomeric effect of the base, and the latter by the gauche effect between $OH_3$ and the ring oxygen. In **16**, the inherent stereochemistry of the ring substituents is such that in the $^{2}T_3$ conformer stabilization is achieved via both the anomeric effect of the aglycone and the gauche effects between the two secondary hydroxyl group and the ring oxygen.

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(44) Wolfe, S. *Acc. Chem. Res.* **1972**, *5*, 102.

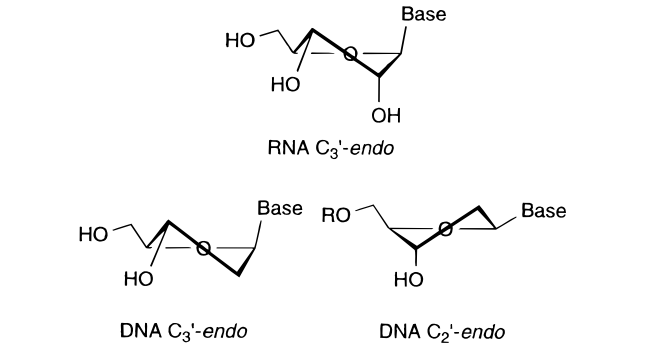


**Figure 7.**

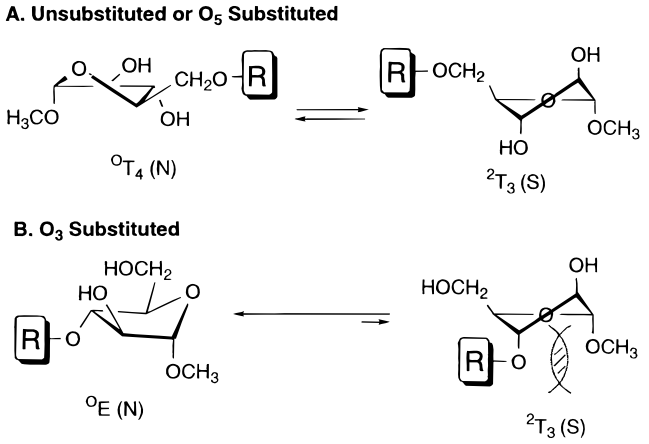


**Figure 8.** (A) Conformational equilibrium in an unsubstituted or $O_5$ substituted α-D-arabinofuranosyl ring. (B) Conformational equilibrium in an $O_3$ substituted α-D-arabinofuranosyl ring.

**Ring Conformers Present in the Disaccharides.** Armed with an understanding of the conformation of the monosaccharide, we next investigated disaccharides **2**, **3**, and **8** to determine the effect of glycosylation on the conformer populations of each ring. These results are presented in Table 1 and discussed in detail below.

**α-(1→5)-Linked Disaccharide.** Both rings in the α-(1→5)-linked disaccharide **2** adopt conformations identical to those of the monosaccharide, namely the $^{O}T_4$ (N) and $^{2}T_3$ (S) twist forms. Clearly, glycosylation of an arabinofuranose ring at the primary position does not alter the conformation of the ring. Similarly, the replacement of a methyl group at the anomeric center with another sugar does not change the conformer equilibrium either.

That the conformation of the reducing-end residue (ring A) of **2** does not differ from that of **16** is perhaps not surprising. As mentioned previously, the N/S conformers of **16** both place the $C_4$ hydroxymethyl group in approximately the same, pseudoequatorial position (Figure 8A). Given this sterically favorable orientation, it could be expected that the glycosylation of the primary hydroxyl group in **16** to give **2** would not dramatically alter the identity of the conformers or their equilibrium populations. When considering the conformations available to the nonreducing arabinofuranose residue (ring B), it might be expected that the replacement of the methoxy group at the anomeric center with a furanose moiety would result in a significant change in the conformation of the ring, due to increased steric congestion between $OH_3$ and the aglycone, especially in the S conformer. However, it should be appreciated that as a result of the *exo*-anomeric effect,[46] the methyl group in **16** would be expected to be oriented gauche to the ring oxygen and trans to the $C_1-C_2$ bond and hence on the opposite side of the molecule from $OH_3$. Therefore, the effective steric bulk that is presented to $OH_3$ by the aglycone is minimal, and the identity of this group should make little difference to the conformation of the ring.

Our results for the reducing-end residue of **2** are in agreement with previous studies on 5-*O*-methyl α-D-arabinofuranose (**31**),[47] 5-deoxy α-D-arabinofuranose (**32**),[47] and the L-arabinofuranosyl disaccharide **33**[22k,23] (Figure 6). The $^{3}J_{H,H}$ values for the $C_5$ modified monosaccharides **31** and **32** are very similar to those of **16**, thus indicating that the identity of the group at $C_5$ appears to not dramatically affect conformation.[47] In the case of disaccharide **33**,[22k,23] each ring was shown to adopt conformations similar to those of the monosaccharide parent, methyl α-L-arabinofuranoside, the enantiomer of **16**. Molecular mechanics calculations on **33** suggested that although the conformations of the rings were unchanged, the energy barrier to pseudorotation for each ring was higher than in the monosaccharide.[22k,23]

The data presented here for the nonreducing residue in **2** also compare favorably with previous conformational investigations of L-arabinofuranosyl residues present in oligosaccharide fragments of wheat arabinoxylans.[22i] In this earlier investigation, two oligosaccharides, **34** and **35** (Figure 6), containing a total of three different L-arabinofuranosyl residues were studied. Although there were small differences between the three furanose residues, all were shown to adopt approximately 1:1 mixtures of $E_4/^{O}T_4$ (N) and $^{2}T_1/^{2}E$ (S) conformers in solution. The best correlation with the results reported here is for the L-arabinofuranosyl residue in **34**, which exists as a 54:46 mixture of $E_4/^{O}T_4$ (N):$^{2}E$ (S) conformers. For the furanose residues in **35**, poorer agreement is observed. This may arise from the attachment of two arabinofuranose moieties on a single xylopyranosyl residue, which may result in conformational changes not observed in less sterically congested systems.

**α-(1→3)-Linked Disaccharide.** Although the identity of the conformers and their equilibrium populations of the individual rings in **2** do not differ appreciably from **16**, the situation in the α-(1→3) linked disaccharide **3** is rather different. The nonreducing end residue (ring D) behaves as does the monosaccharide, adopting the same two twist conformations in roughly equal proportions. However, the conformational equilibria of the reducing-end ring (ring B), which is glycosylated at $OH_3$, is significantly different from the monosaccharide. The N conformer changes slightly from $^{O}T_4$ to $^{O}E$ and predominates (86%) over the S form, $^{2}T_3$ (14%). Clearly, glycosylation of this secondary alcohol profoundly changes the conformation of the ring.

To understand the origin of these changes two issues must be addressed: one is the favoring of the N conformation over the S; the other is the change in the identity of the N conformer. The equilibrium preponderance of the $^{O}E$ (N) form can be rationalized on the basis that in this conformer, the large glycosyl group at $O_3$ is in a more pseudoequatorial orientation than in the $^{2}T_3$ (S) conformer in which this group is pseudoaxial. Our previous gas-phase calculations[41] show that the $O_1/O_3$ distance changes from 4.22 ($^{O}E$) to 3.01 Å ($^{2}T_3$). Although in the

(45) (a) Saenger, W. *Principles of Nucleic Acid Structure*; Spring-Verlag: Berlin, 1988. (b) Birnbaum, G. I.; Shugar, D. Biologically Active Nucleosides and Nucleotides: Conformational Features and Interaction with Enzymes. In *Topics In Nucleic Acid Structure*; Neidle, S., Ed.; MacMillan Press: London, 1987; p 1.

(46) Lemieux, R. U.; Koto, S. *Tetrahedron* **1974**, *30*, 1933.

(47) Snyder, J. R.; Serianni, A. S. *Carbohydr. Res.* **1987**, *163*, 169.

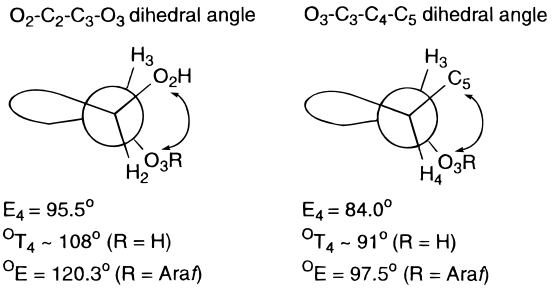


**Figure 9.** $O_2-C_2-C_3-O_3$ and $O_3-C_3-C_4-C_5$ dihedral angles in $^OT_4$ and $^OE$ conformers of an α-D-arabinofuranosyl ring.

monosaccharide the S conformer can potentially be stabilized by the formation of an $OH_3\cdots O_1$ hydrogen bond, in the disaccharide this is not possible. More importantly, because of unfavorable steric interactions in the S conformer, it would be expected that its population will be reduced (Figure 8B). Clearly these steric effects are enough to override, at least in part, the stabilization provided by the gauche relationship between the ring oxygen and both $OH_2$ and $O_3$.[22m−q]

Our previous computational studies[41] on **16** are again useful in explaining why the identity of the N conformer of an α-D-arabinofuranosyl ring changes upon substitution of $O_3$. An analysis of the bond dihedral angles in **16** reveals that the $O_2-C_2-C_3-O_3$ torsion angle increases from 108° in $^OT_4$ to 120° in $^OE$ (Figure 9). A similar, though less dramatic, trend is observed for the $O_3-C_3-C_4-C_5$ dihedral angle, which increases from 91° ($^OT_4$) to 98° ($^OE$). When taken together, these results suggest that when **16** is glycosylated at $O_3$, the ring adopts a conformation that places the substituent group not only as far away as possible from those adjacent to it but also pseudoequatorially.

**α-(1→2)-Linked Disaccharide.** For the nonreducing end residue in the α-(1→2)-linked disaccharide **8** (ring H), the same two conformers present in **16**, $^OT_4$ and $^2T_3$, are found. This behavior is therefore similar to the two other disaccharides. However, in contrast to **2** and **3**, there is a distinct favoring of the N conformer (73%) in this residue in **8**. Unfortunately, the coupling between $H_1$, $H_2$, and $H_3$ on the reducing-end residue in **8** (ring G) is not first-order, and this makes the measurement of accurate $^3J_{H,H}$ and subsequent PSEUROT analysis impossible. The coupling between $H_3$ and $H_4$ on ring G ($J_{3,4} = 5.8$ Hz) is similar to the magnitude of this value in ring H ($J_{3,4} = 6.1$ Hz). Although this observation may suggest that the two rings adopt similar conformations, in the absence of additional data this proposal must be regarded as highly speculative. Indeed, one might expect to see changes similar to those observed for ring B in **3**.

**Ring Conformers Present in the Tri- and Tetrasaccharides.** We next investigated the conformer populations present in each ring of oligosaccharides **4**−**7**. The trends observed in the disaccharides are also seen in the larger oligomers (Table 1).

In all cases, the residues at the nonreducing termini of the oligosaccharides (rings C and D) behave as does **16**, existing as a nearly equimolar mixture of $^2T_3$ and $^OT_4$ conformers. Similar to **3**, in the larger oligosaccharides when a residue is glycosylated at $OH_3$ (ring B in **4**, **6**, and **7**), the N conformer changes to $^OE$ and becomes favored at equilibrium. When this residue is also glycosylated at $OH_5$, as in **4** and **7**, the population of the N conformer exceeds 90%, indicating significant rigidity in this ring relative to the monosaccharide parent, **16**. In all cases, the attachment of a single glycosyl residue to $O_5$ (ring A in **5**−**7** and ring B in **5**) does not alter the identity of the conformers relative to the monosaccharide. Furthermore, in most cases the conformer populations of the $O_5$ substituted rings are essentially the same as the monosaccharide. Only in ring A of **6** is there a noticeable, albeit moderate, favoring (62%) of the N conformer. Also unique to **6** is that, in comparison to **4** and **7**, the N conformer of the $O_3$ substituted ring (ring B) has a lower population. In **6**, the N:S ratio for ring B is 79:21, whereas for **4** and **7**, the ratios are, respectively, 94:6 and 92:8. The reasons why **6** behaves slightly differently than the other oligosaccharides are currently unknown.

These results suggest the conformers available to a particular α-D-arabinofuranosyl ring can be predicted on the basis of their substitution pattern and that, in general, these populations are independent of the size of the molecule. That substitution of either $O_5$ or $O_1$ with a variety of groups appears to not significantly alter ring conformer populations is of particular importance. Linear polymers of α-(1→5)-linked L-arabinofuranose are found in plants and have been suggested to adopt helical conformations both in solution[23] and in the solid state.[48] The arabinan core to which **1** is attached (Figure 1) is a similar α-(1→5)-linked polymer of D-arabinofuranose. It would therefore be expected that these regions of the polymer would also form helices and that this type of structural organization would undoubtedly be of importance in the overall organization of the cell wall complex. This could be probed through fluorescence energy transfer experiments,[49] through the synthesis of α-(1→5)-linked oligosaccharides appropriately modified with fluorescent groups. Our studies suggest that the attachment of the (typically large) groups used for this purpose will not dramatically change the overall conformation of the molecule.

**$C_4-C_5$ Rotamer Populations.** Rotamer populations about the $C_4-C_5$ bond in each ring of **16** and **2**−**8** were calculated as described previously[50] using coupling constants measured from 1D $^1H$ NMR spectra. The populations of each rotamer (Table 2) are essentially the same in all of the compounds investigated; the major one is gg (Figure 10) with a population of approximately 50%. The next most populated rotamer is gt (∼38%) followed by tg (∼14%). In both the gg and gt rotamers $O_5$ is gauche to the ring oxygen and would be stabilized by the gauche effect.[44] Hence, it would be expected that these two conformations would predominate over the tg rotamer, in which there is no gauche effect stabilization. In the crystal structures of both **16**[42] and **30**,[43] the $C_4-C_5$ bond adopts a gg orientation. Furthermore, it is well-known that in nucleosides both in the solid state[51a] and in solution,[51b] the preferred conformation about this bond is gg.

## Conclusions

In this paper we report the synthesis of a series of arabinofuranosyl oligosaccharides that are fragments of two mycobacterial cell wall polysaccharides. These compounds have previously been shown to be substrates for the enzymes involved in the biosynthesis of these polysaccharides in mycobacteria.[21] In addition to the utility of these compounds in biochemical investigations, the synthetic routes developed will be invaluable in the synthesis of additional analogues. We have also, for the

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**Table 2.** $C_4-C_5$ Rotamer Populations in **2**−**8** and **16**[a]

| | compound | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **16** | **2** | | **3** | | **8** | | **4** | | | **5** | | | **6** | | | **7** | | | |
| | A[b] | A | B | B | D | G | H | B | C | D | A | B | C | A | B | D | A | B | C | D |
| $X_{tg}$ (%)[c] | 14 | 15 | 16 | 15 | 15 | 14 | 15 | 12 | 15 | 14 | 13 | 13 | 14 | 13 | 15 | 12 | 13 | 12 | 13 | 13 |
| $X_{gg}$ (%) | 48 | 47 | 50 | 47 | 47 | 48 | 47 | 54 | 47 | 46 | 48 | 50 | 48 | 52 | 48 | 48 | 52 | 53 | 50 | 50 |
| $X_{gt}$ (%) | 38 | 38 | 34 | 38 | 38 | 38 | 38 | 34 | 38 | 40 | 39 | 37 | 38 | 35 | 37 | 40 | 35 | 35 | 37 | 37 |

[a] Calculated using the following equations:[50a] $1.3X_{gg} + 2.7X_{gt} + 11.7X_{tg} = {}^3J_{H4,H5S}$; $1.3X_{gg} + 11.5X_{gt} + 5.8X_{tg} = {}^3J_{H4,H5R}$; $X_{gg} + X_{gt} + X_{tg} = 1$ [b] See Figure 2 for assignment of ring letters. [c] See Figure 10 for rotamer definitions.

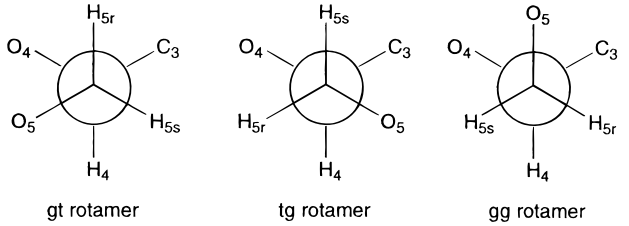


**Figure 10.**

first time, investigated the role that glycosylation has on conformer populations in furanose rings. This has led to a greater understanding of substituent effects in these molecules and to the identification of some underlying principles that appear to dictate the conformers available to both unsubstituted and substituted α-D-arabinofuranosyl residues. Notable conclusions from these conformational investigations are as follows:

(1) The monosaccharide parent, **16**, exists in solution as an approximately equimolar mixture of two twist conformers, ${}^{O}T_4$ and ${}^{2}T_3$. These conformers are similar to those observed in both the solid state[42] and the gas phase.[41]

(2) Glycosylation and likely any form of substitution of a primary hydroxyl group on an arabinofuranose ring does not significantly alter the conformers present or their populations.

(3) Replacement of a methyl group at the anomeric center with a monosaccharide residue does not alter the identity of the conformers present. Hence, all terminal Ara*f* residues exist as equilibrium mixtures of the same two conformers present in **16**. Furthermore, although in the terminal Ara*f* residue in **8** there is a favoring of the N (${}^{O}T_4$) conformer, for the other oligosaccharides the conformer populations for these residues are essentially the same as in the monosaccharide.

(4) Glycosylation of $OH_3$ on an arabinofuranose ring does alter the conformational equilibria of the ring significantly. The identity of the N conformation changes from ${}^{O}T_4$ to ${}^{O}E$, and this conformation is favored at equilibrium. In cases in which a ring is glycosylated at both $OH_3$ and $OH_5$, as in **4** and **7**, the favoring of the N conformation becomes even more pronounced.

(5) Rotamer populations about the $C_4-C_5$ bond are essentially the same for each ring, regardless of the substitution pattern, indicating that this parameter is insensitive to glycosylation.

The studies reported here will serve as the foundation for additional studies on both the synthesis of furanose oligosaccharides and their solution conformations. The synthesis of additional analogues, including oligosaccharides containing 1,2-*cis*-linked residues as found in the native polysaccharides, is currently in progress. We are also carrying out further conformational studies including the measurement of interresidue NOEs, which will lead to an even greater appreciation of the solution conformation of molecules of this type.

## Experimental Section

See Supporting Information.

**Acknowledgment.** This research has been supported by The Ohio State University and the National Science Foundation (CHE-9875163). P.R.M. is the recipient of a NSF-REU Fellowship. We thank Dr. Charles Cottrell for the high field NMR spectra and Dr. Christopher M. Hadad for helpful discussions.

**Supporting Information Available:** Full Experimental Section and analytical data for all new compounds, discussion of the preparation of **9**−**15**, NMR conditions used for the conformational studies, and table containing all ${}^3J_{H,H}$ values used in the PSEUROT calculations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA993543L