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Stereoselective Synthesis of a Fragment of Mycobacterial Arabinan

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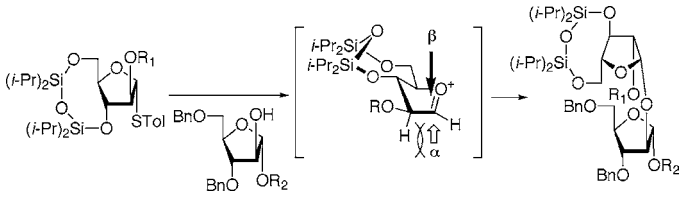
ABSTRACT



Strategies for the stereoselective synthesis of mycobacterial arabinan were explored. Arabinofuranosyl donors with various protective groups were screened in terms of suitability for *β*-(1,2-cis)-selective glycosylation. The protective group was found to affect the stereoselectivity of arabinofuranosylation. *β*-Selectivity was drastically enhanced by using donors protected with 3,5-TIDPS, possibly due to conformational constraints on the furanose ring. Synthesis of heptaarabinofuranoside was then performed to demonstrate the practicality of this methodology.

Glycans that consist of furanosides are widespread constituents of glycoconjugates and cell-surface polysaccharides in bacteria,[1] fungi,[2] and parasites.[3] They play key roles in the infectivity and pathogenicity of these microbes. Among them, mycobacterial cell wall arabinans are attracting particular attention. *Mycobacterium tuberculosis*, the causative agent of *tuberculosis*, remains rampant and is a growing threat worldwide due to the emergence of strains that have multidrug resistance (MDR).[4] Arabinan biosynthesis by mycobacterial arabinofuranosyl transferases (Ara*f*Ts)[5] is a novel therapeutic target,[6] although the precise mechanism has yet to be elucidated. On the other hand, adjuvants derived from the cell wall skeleton of mycobacteria, such as Bacillus Calmette-Guérin (BCG-CWS) from *M. bovis*, are known to be activators of innate immunity.[7,8] Although the mechanism of BCG-CWS-initiated immunity has been proposed to involve the activation of macrophages via a Toll-like receptor (TLR)2,[9] its structural basis is difficult to define due to the extreme complexity of BCG-CWS. The structure of the CWS is unique, being composed of mycolic acid (MA), D-arabinan, D-galactan, a linker disaccharide (α-Rha*p*-(1,3)-α-GlcNAc), and peptidoglycan (PG) (Figure 1).[10] Mycobacterial arabinans consist of an [α-Ara*f*(1→5)α-Ara*f*]$_n$ repeat, which is linked to the inner complex and is capped by a branched motif. The latter contains terminal *β*-Ara*f*, linked to the 2-position

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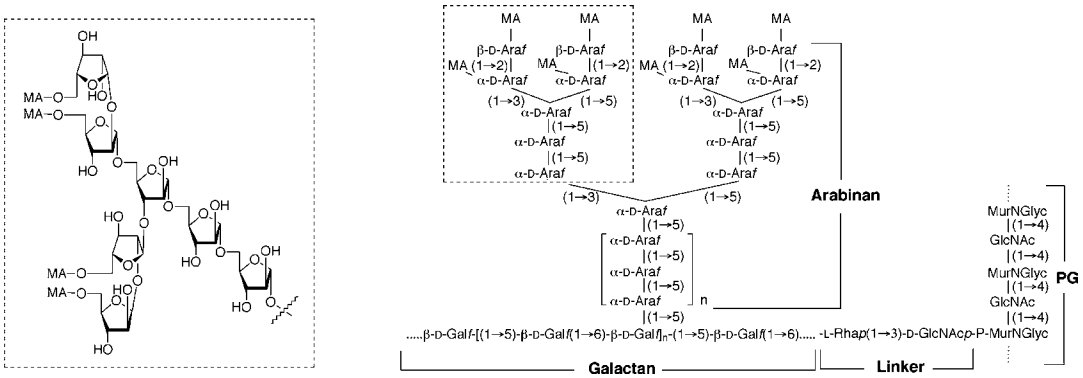


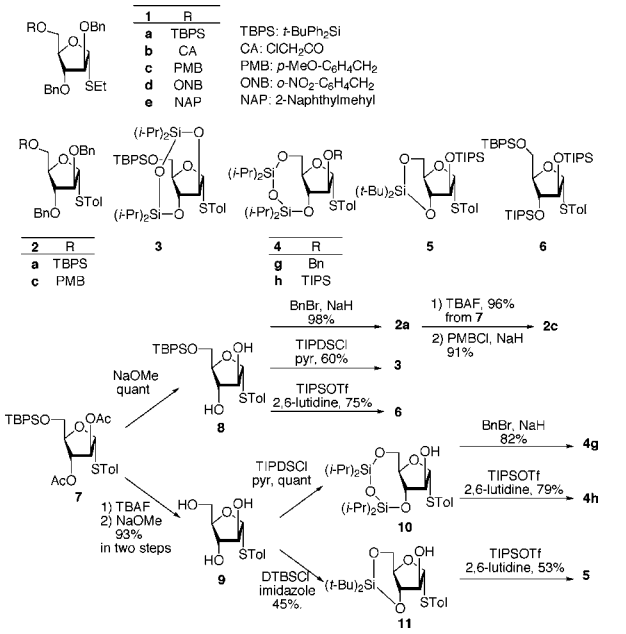
Figure 1. Structure of a mycobacterial arabinan terminus.

of the penultimate α-Ara*f*. These Ara*f* residues are O-acylated at their C-5 positions by mycolic acid.

To achieve the synthesis of mycobacterial arabinan, introduction of the terminal *β*-Ara*f* residue is potentially problematic. Whereas α-Ara*f* linkage is easily accessible with a 2-O-acylated donor, formation of the *β*-Ara*f* linkage is not straightforward. The difficulty of the latter derives from its 1,2-cis relative stereochemistry, which prohibits the use of neighboring group participation for stereochemical control. Because the conformation of the furanoside ring is more flexible than that of pyranoside, factors that affect the stereochemistry of O-furanosylation are difficult to generalize. For the construction of *β*-linked Ara*f* glycoside, several innovative methods have been reported.[11] Of particular note were approaches through S_N2 displacement via α-triflate (a 2,3-anhydro-type donor and a carboxybenzyl (CB) donor) developed by Lowary[11a] and by Kim,[11b] respectively. As an alternative to these, we explored the possibility of achieving *β*-selective furanosylation by tuning the protective group patterns. We report herein the stereoselective synthesis of *β*-Ara*f* using 3,5-*O*-tetraisopropyldisiloxanylidene (TIPDS)-protected thioglycoside as a donor. This method was then successfully applied to the synthesis of heptaarabinofuranoside.[12]

Because terminal *β*-Ara*f* residues of AG are O-5 mycolated, this position must be distinguishable from others to approach mycolated arabinan. Therefore, we began by screening the effects of O-5 protective groups on the stereoselectivity of glycosylation using 2,3-*O*-benzyl-protected donors **1** and **2** (Scheme 1), through activation by NIS-AgOTf.[13] We previously reported that selectivity was particularly sensitive to the nature of R (Scheme 2 and Table 1, entries 1∼5).[14] Specifically, the *p*-methoxybenzyl (PMB)-

Scheme 1. Preparation of Arabinofuranosyl Donors



protected donor **1c** gave a favorable result, although the selectivity was modest. On the other hand, *o*-nitrobenzyl- (**1d**) or acyl-substituted (**1b**) donors gave the α-glycoside as the major product, suggesting that the electron density of O-5 is important. The *tert*-butyldiphenylsilyl (TBDPS) group at O-5 strongly disfavored the formation of the *β*-glycoside, possibly because its bulkiness hindered the nucleophilic attack of **12**[12c] from the upper face. Corresponding tolylthio glycosides **2a** and **2c**, prepared from known compound **8**,[15]

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Table 1. Effect of Protection of the Glycosyl Donor with Various Protections at the 5 Position on Arabinofuranosylation

entry	donor	acceptor	solvent[b]	temp	time/h	product	yield (α/β)
1[a]	**1a**	**12**	D[c]	−78 °C	1	**14a**	97% (9.4:1)
2[a]	**1b**	**12**	D[c]	−40 °C	3.5	**14b**	65% (1.3:1)
3[a]	**1c**	**12**	D[c]	−78 °C	2.5	**14c**	93% (1:2.6)
4[a]	**1d**	**12**	D[c]	−40 °C	1	**14d**	88% (1.9:1)
5[a]	**1e**	**12**	D[c]	−78 °C	1	**14e**	93% (1:1.5)
6	**2a**	**12**	D[c]	−78 °C	1	**14a**	89% (7.6:1)
7	**2c**	**12**	D[c]	−78 °C	1	**14c**	81% (1:2.7)
8	**1c**	**12**	D[d]	rt	24	**14c**	82% (1.3:1)
9	**1c**	**12**	T[d]	0 °C	8	**14c**	45% (2.0:1)
10	**1c**	**12**	X[d]	0 °C	1	**14c**	75% (1:1.1)
11	**2c**	**13**	D[c]	−40 °C	3	**15c**	94% (1:4.3)
12	**2c**	**13**	D[c]	−60 °C	3	**15c**	85% (1:8.6)

[a] From ref 13. [b] D: CH_2Cl_2. T: toluene. X: xylene. [c] NIS (1.2∼2.0 equiv), AgOTf (0.3∼1.0 equiv). [d] MeOTf (2.4 equiv), DTBMP (2.4 equiv).

gave similar results (Table 1, entries 6 and 7). A weaker activating agent, MeOTf,[16] gave low selectivity (entries 8 and 9); however, as we previously reported, substantial rate enhancement[17] was observed in frozen solvent (entry 10). The stereochemistry of the anomeric center of glycosylated products was confirmed by δ (C-1) and $^3J_{H1-H2}$ values:[18] β-isomer, δ (C-1) 97∼103 ppm, $^3J_{H1-H2}$ = 4∼5 Hz; α-isomer, δ (C-1) 104∼111 ppm, $^3J_{H1-H2}$ = 1∼3 Hz. Further validation for the stereochemsitry of the β-isomer was obtained by differential NOE experiments, which revealed the NOE between H-1 and H-2.

Subsequent investigation revealed that the selectivity was also sensitive to the structure of the acceptor. When **2c** was reacted with 3,5-*O*-benzyl-protected acceptor **13**,[15] significant enhancement of β-selectivity (α/β = 1:4.6) was observed. Selectivity was improved to 1:8.6, when the reaction was conducted at −60 °C. To achieve higher selectivity, we turned our attention to the effects of cyclic protective groups (Scheme 1). Our hope was that the conformational perturbation associated with the formation of fused rings would cause favorable stereoelectronic effects.[19] To that end, donors having 2,3-O- (**3**) or 3,5-O- (**4g**, **4h**, **5**) cyclic protection were prepared. When glycosylation with acceptor **13** was performed, it was found that the use of 3,5-*O*-TIPDS-protected[20] donor **4g** or **4h** resulted in the formation of the desired β-isomer in a markedly selective manner (Table 2). That tri-

Scheme 2. Various Arabinofuranosyl Donors

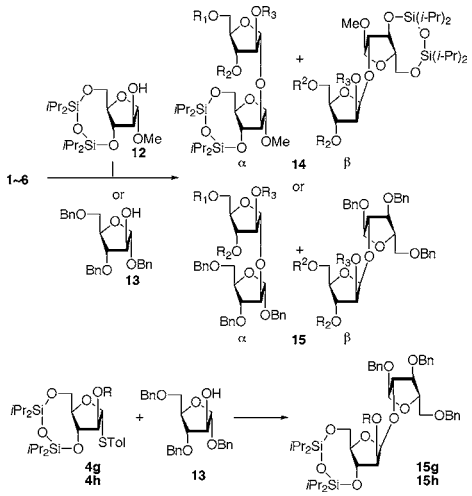

Table 2. Effect of Protection of the Glycosyl Donor on Arabinofuranosylation

entry	donor	acceptor	product	yield (α/β)
1	**3**	**13**	**15f**	96% (1:2.45)
2	**4g**	**13**	**15g**	94% (1:12.5)
3	**4h**	**13**	**15h**	93% (1:20.0)
4	**5**	**13**	**15i**	70% (1:5.36)
5	**6**	**13**	**15j**	99% (5.26:1)

O-silyl protection itself was not the dominant factor for this selectivity was evident from the results with regioisomerically protected (**3**), 3,5-*O*-di-*tert*-butylsilylene[21,22] masked (**5**), and noncyclically protected (**6**) donors. Comparison of these results suggested that the conformational restraint introduced by the eight-membered ring 3,5-O-protection was responsible for the enhanced β-selectivity.

The marked differences in selectivity between **4h** (α/β = 1:20) and **5** (α/β = 1:5.36) are reminiscent of the work of Woerpel et al.,[23] who investigated the allylation of bicyclic lactol acetates **16** and **17** (Figure 2). They found that eight-five bicyclic acetate **16** gave higher selectivity in favor of the β-isomer than the six-five counterpart **17**. In light of Woerpel's hypothesis, we surmise that the nucleophilic attack from the α-face is disfavored for both **5** and **4h** due to the 1,2-gauche interaction between the entering acceptor and the pseudoaxially oriented C-2 hydrogen. In the case of six-five bicyclic **5**, however, the β-attack should lead to an initial conformer with a 3,5-silylene group possessing a distorted nonchairlike conformation, thereby reducing the preference of the pathway toward the β-isomer. Molecular modeling studies[15] of glycosylated products provided an alternative interpretation of the selectivity; the total energy of the β-linked product was 3.7 kcal/mol lower than that of the α-isomer, suggesting that the formation of the β-isomer was the thermodynamically favored process, which may rational-

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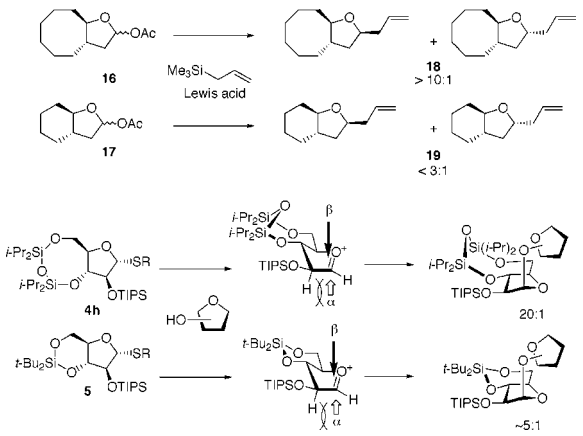


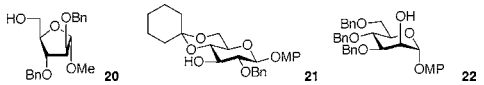
Figure 2. Plausible explanation for the β-selective addition to the activated donor. The black arrow shows the direction for β-attack to the anomeric carbon, and the white arrow shows the direction for α-attack. In the case of α-attack, there seems to be a large steric repulsion from the α-hydrogen atom at C2.

ize the selectivity based on Hammond's postulate.[24] In the global minimum structure, the β-product had pseudoaxially oriented glycoside linkages, which may be favorable in light of the anomeric effect (see Supporting Information).

Inspection of other acceptors shows that, to achieve the β-selective glycosylation, the acceptor should be moderately bulky. For instance, a reaction of **4g** with less-hindered acceptor **20**[25] displayed no selectivity (Table 3). We speculate

Table 3. Results of Glycosylation of **4g**/**4h** with Other Acceptors

entry	donor	acceptor	yield/%	α/β
1	**4g**	**13**	94	1:12.5
2	**4h**	**13**	93	1:20.0
3	**4g**	**20**	97	1:1.15
4	**4g**	**21**	100	1:7.35
5	**4h**	**21**	61	1.80:1
6	**4g**	**22**	77	1:2.66
7	**4h**	**22**	47	1:2.80



that, when the acceptor is not sufficiently hindered, steric repulsion with the C-2 hydrogen would be inconsequential. In addition, the results with hindered acceptor **21** were drastically different between **4g** and **4h**, thus suggesting that the steric factor plays a major role in controlling the stereochemical outcome, and there seems to be a matched/mismatched effect in the donor−acceptor combination.

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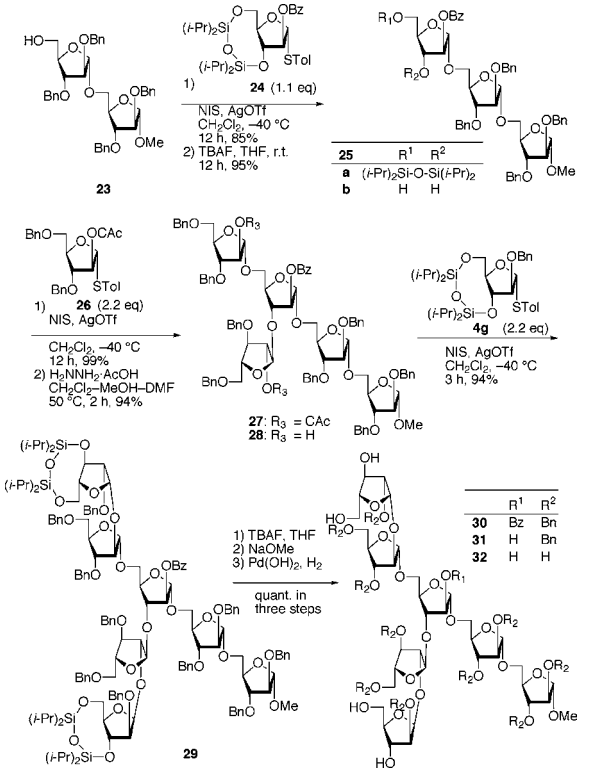
With an arabinosyl donor suitable for the stereoselective formation of the β-Ara*f*1→2Ara*f* linkage at our disposal, we conducted the synthesis of heptasaccharide **32** (Scheme 3),

Scheme 3. Synthesis of Heptaarabinofuranoside



which corresponds to the branched terminal structure of mycobacterial arabinogalactan (Figure 3). Thus, disaccharide **23**[12e] was first glycosylated with 2-*O*-Bz-protected donor **24**[12c] to give **25a**. Removal of the TIPDS group gave **25b**, which was further glycosylated with 2-*O*-CAc-protected **26**.[12b] The resultant pentasaccharide **27** was converted to diol **28**, which was subjected to a reaction with the β-selective donor **4g** to give **29** in high yield and selectivity (**29**/other isomers = 10.8:1). Subsequent deprotection was conducted in a stepwise manner to give **32**.

In conclusion, the stereoselective synthesis of mycobacterium arabinan was achieved. This β-selective arabinofuranosylation was applied to the synthesis of nonreducing terminal heptaarabinofuranoside in the mycobacterial cell wall.

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Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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