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Bioorganic & Medicinal Chemistry 14 (2006) 3049–3061
Bioorganic & Medicinal Chemistry

Synthesis and TNF-α inducing activities of mycoloyl-arabinan motif of mycobacterial cell wall components

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Received 6 September 2005; revised 8 December 2005; accepted 13 December 2005
Available online 19 January 2006

Abstract—The extract of the cell wall skeleton of Bacillus Calmette–Guérin (BCG-CWS) from *Mycobacterium bovis* is known to be an activator of innate immunity. Synthesis of pentaarabinofuranoside as part of the arabinan moiety of BCG-CWS was achieved by double α-arabinofuranosylation followed by double β-arabinofuranosylation with orthogonally protected donors. Mycolic esters of the arabinan in the terminal lipo-arabinan motif of BCG-CWS were synthesized through alkylation of unprotected mycolic acid with bis- and tetra-tosylates of pentaarabinofuranoside. A series of compounds were subjected to a tumor necrosis factor alpha (TNF-α) secretion-inducing assay, disclosing aspects of the structure–activity relationship which should be useful in finding the site of the activity.

1. Introduction

Mycobacterium tuberculosis, the causative agent of tuberculosis, is rampant across the world and poses a growing threat due to its multi-drug resistance (MDR).[1] For mycobacteria, as well as other bacteria, one of the most important drug targets is cell wall biosynthesis, which is critical for bacterial survival. Clinical use of a variety of anti-mycobacterials has resulted in resistance, although its precise mechanism is yet to be defined.[2] An adjuvant consisting of Bacillus Calmette–Guérin cell wall skeleton (BCG-CWS) from *Mycobacterium bovis* is known to be an activator of innate immunity.[3,4] It has been proposed that macrophages are activated via Toll-like receptors.[5] However, the structural basis of BCG-CWS-initiated immunity is not clear, because of the complexity of BCG-CWS.

BCG-CWS is a supramolecular architecture, composed of a peptidoglycan (PG) linked to mycolic acids (MAs) and arabinogalactan, through linker disaccharide (β-Rha*p*-(1,3)-α-GlcNAc) (Fig. 1).[6] Arabinogalactan consists of furanose forms of D-galactose (Gal*f*) and D-arabinose (Ara*f*), which are potentially immunogenic to mammals. At non-reducing terminal, it has branched pentasaccharides, which are decorated as esters of MA. MAs are a group of long-chain fatty acids, which contain β-hydroxycarboxylic acid moiety and two hydrophobic chains; a linear saturated alkyl (C_{22} or C_{24}) chain and a longer functionalized (e.g., *cis*-cyclopropyl, *cis*-olefin, methyl, and oxo or methoxy) chain called *mero* group.[7] Although the biological roles of MA are largely undefined, it resides at the outer region of the cell wall and therefore is likely to be important for initial contact with receptors.[8] In addition, correlation of MA composition and virulence has been reported.[9]

The aim of this study was to synthesize a series of mono- (**1**), di- (**2**, **3**, **4**), and tetramycolated (**5**) arabinans, which constitute the terminal region of BCG-CWS. In addition, their activities to induce tumor necrosis factor (TNF)-α were evaluated.

2. Results and discussion

2.1. β-Arabinofuranosylation for the synthesis of mycoloyl-arabinan

The terminal pentaarabinofuranoside of BCG-CWS is composed of two β-arabinofuranosides (β-Ara*f*) linked

Keywords: BCG-CWS; Mycoloyl-arabinan; TNF-α secretion-inducer; Stereoselective arabinofuranosylation.
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doi:10.1016/j.bmc.2005.12.037

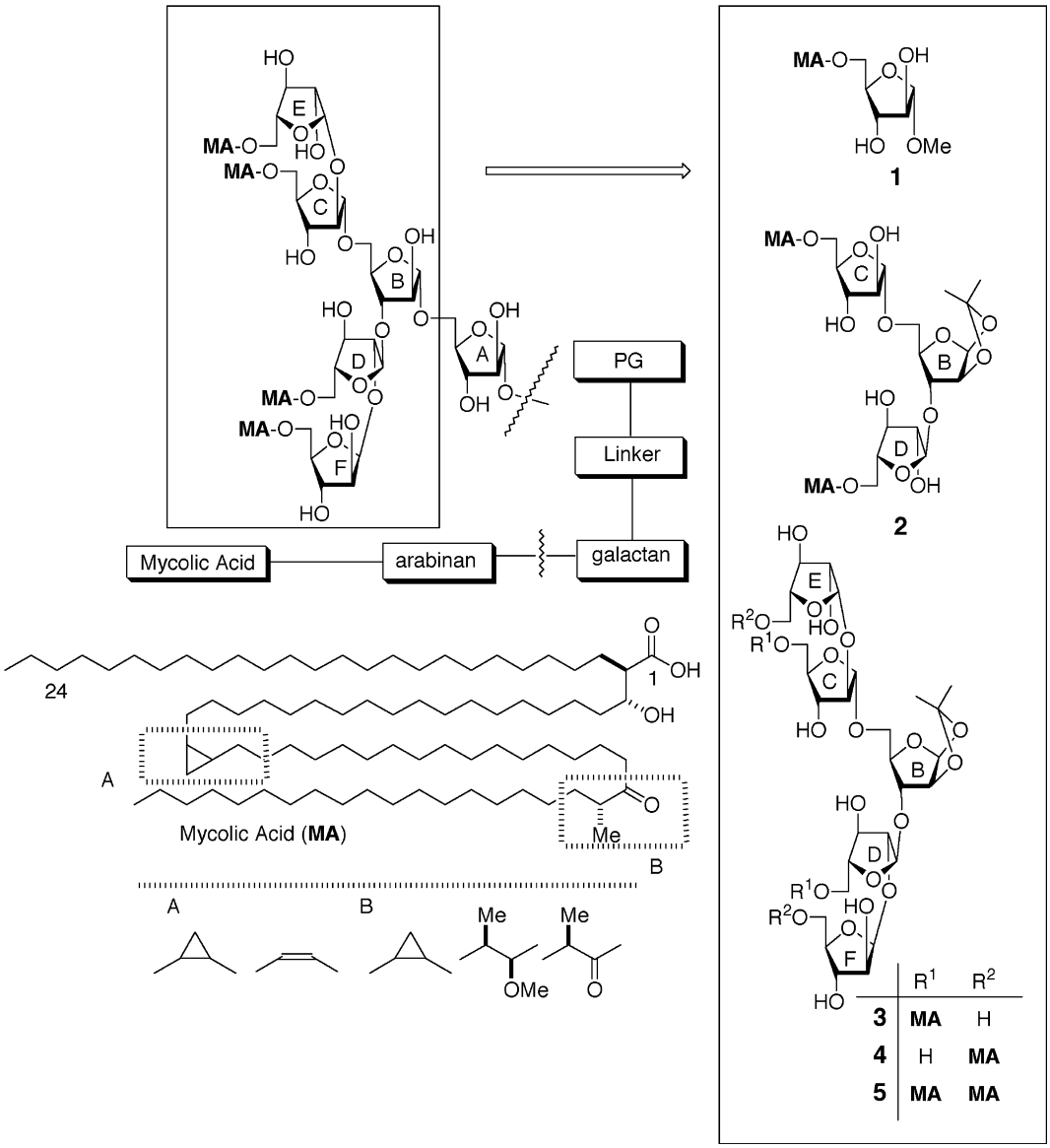


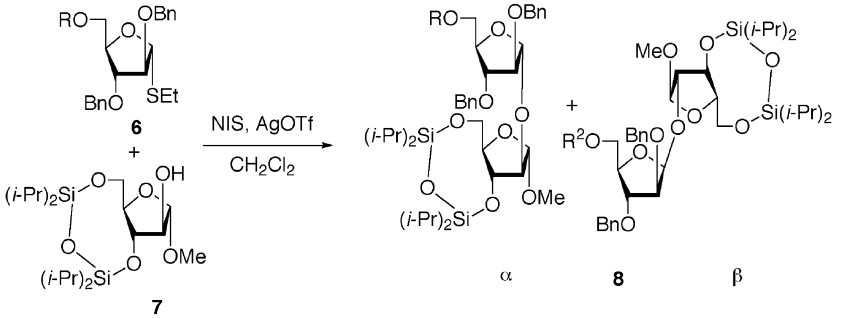
Figure 1. Structure of monomer of BCG-CWS and targets of mycoloyl-arabinans.

to the penultimate α-arabinofuranosides (α-Ara*f*). To approach the branched arabinan portion of BCG-CWS,[10] formation of both the α- (B, C, D) and β-configured (E, F) Ara*f* is required. Basic strategy for the stereoselective synthesis of furanosidic linkage is less explored, compared to that of hexopyranoside. Due to their conformational elasticity, stereoelectronic factors that control the stereochemistry of *O*-furanosides are difficult to be generalized. Formation of β-Ara*f* has been difficult, because of its 1,2-cis orientation.[11] To achieve this, various approaches have been reported.[10a,12–14] We looked into the effect of *O*-5 protecting group, because this position was going to be selectively deprotected later on, in order to be esterified with MA.

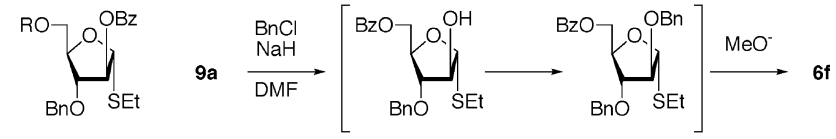
We screened glycosylation of thioglycosides **6a**–**e** with a model acceptor **7**. These glycosyl donors were prepared from known **9a**.[12] Namely, treatment under standard O-benzylation conditions (NaH, $PhCH_2Cl$, and DMF) caused benzoyl migration and provided 2,3-di-O-benzylated product **6f**, which was then converted to **6a**–**e**. It was revealed that the nature of the protecting group introduced to this position gave a significant effect on the stereochemical outcome (Scheme 1); α:β = 1.3:1 (CAc, **6a**), 9.4:1 (TBPS, **6b**), 1.9:1 (2-NBn, **6c**), 1:1.5 (NAP, **6d**), and 1:2.8 (PMB, **6e**).[14] With these results, we decided to use **6e** as the donor for β-Ara*f* synthesis. As an orthogonally protected donor, 5-*O*-TBPS protected donor **9b** was prepared from **9a** and used for the α-arabinofuranosylation.

2.2. Synthesis of arabinan moieties

Syntheses of tri (B/C/D) and pentasaccharide (B/C/E/D/F) were conducted as shown in Scheme 2. Thus, diol **10**[15] was first subjected to glycosylation with 2-*O*-benzoyl protected donor **9b**, which uneventfully provided bis-α-glycosylated product **11**. After deacylation, diol **12** was then glycosylated with 5-*O*-PMB protected donor **6e**. It gave a mixture of pentasaccharides, from which



6	R	Yield (%)	α:β
a	$ClCH_2CO$ (CAc)	65	1.3:1
b	*t*-$BuPh_2Si$ (TBPS)	97	9.4:1
c	*o*-$NO_2C_6H_4CH_2$ (NBn)	88	1.9:1
d	2-Naphthylmethyl (NAP)	93	1:1.5
e	*p*-$MeOC_6H_4CH_2$ (PMB)	88	1:2.8
f	H	–	–



9	R
a	H
b	TBPS

Scheme 1. Examination of β-arabinofuranosylation of donors **6a**–**e**.

the desired pentasaccharide **13** was isolated in 57% yield, together with 35% yield of a mixture of stereoisomers. Its structure was clarified by ^1H NMR, revealing 3 β-Ara*f* [δ_H 5.01 (d, $J = 4.4$ Hz), 5.12 (d, $J = 4.4$ Hz), 5.84 (d, $J = 4.0$ Hz)] and 2 α-Ara*f* [δ_H 5.02 (*s*), 5.09 (d, $J = 0.6$ Hz)] residues. It was then converted to regioisomeric diols **14** and **15** for subsequent esterification.

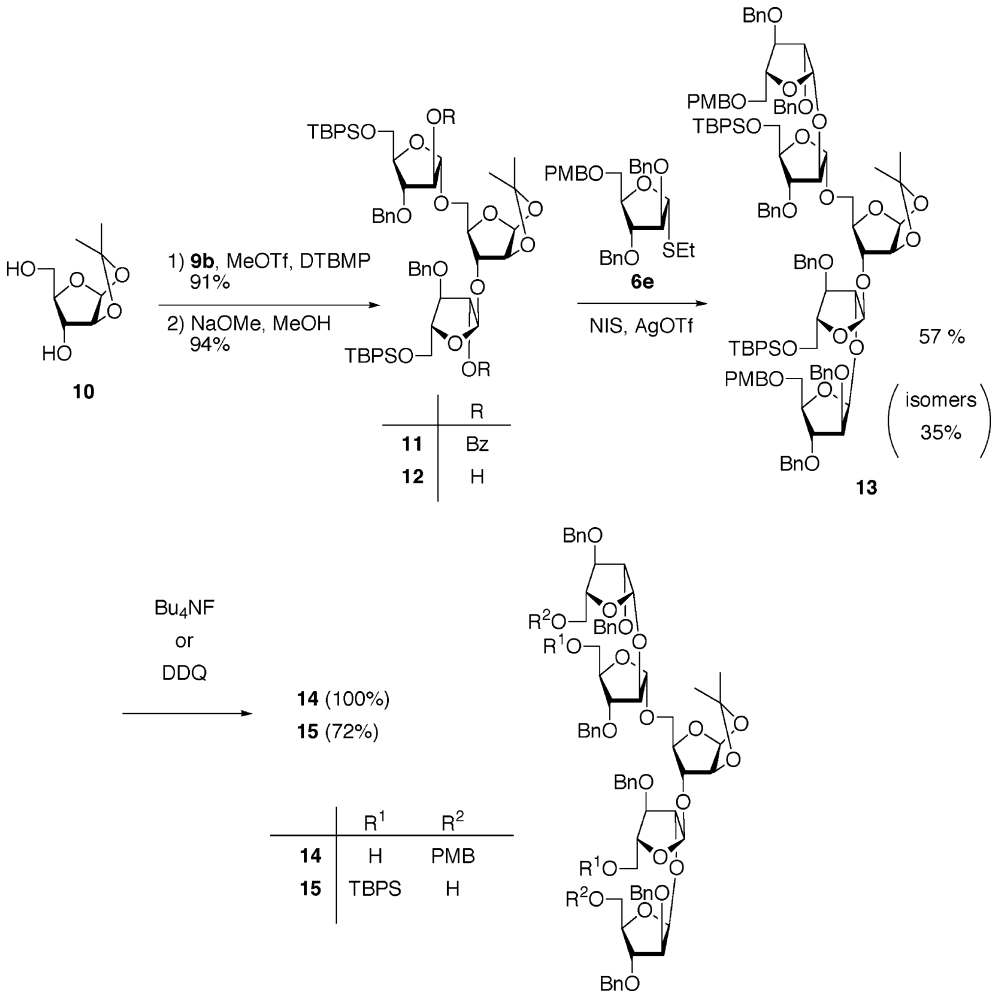
2.3. Synthesis of mycoloyl-arabinans

To introduce MA to arabinans,[16] direct condensation was initially attempted. However, not unexpectedly, this option turned out to be challenging, due to the propensity of hydroxy acid to cause self-condensation. For instance, attempted condensation between monosaccharide **16** and mycolic acid under Yamaguchi's conditions[17] gave only 30% yield of the product **18**. Several attempts to protect the hydroxy group proved to be difficult. We therefore switched to the alkylative esterification strategy,[18] which was examined with monosaccharide tosylate **17** prepared from **16**. When treated with $CsHCO_3$ and mycolic acid, the product **18** was obtained in 79% yield (Scheme 3). It was then subjected to hydrogenolysis to afford $Myc_1Ara f_1$ **1**.

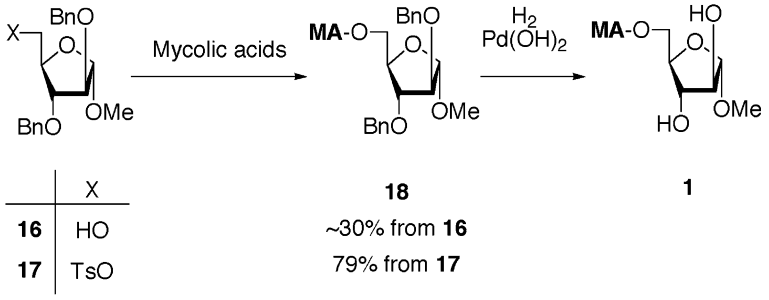
In a similar manner, trisaccharide bis-tosylate (**20**) was prepared from **12** via diol **19** and mycolated to give **24** (Scheme 4). This reaction was conducted either in THF–DMF or toluene; in the latter case, 18-crown-6 was included in the reaction mixture. Subsequently, pentasaccharides **14** and **15** were converted to bis- (**21**, **22**) and tetrakis-(**23**) tosylates, and then to corresponding mycolates **25**, **26**, and **27**. The progress of these reactions was monitored most conveniently with MALDI-TOF mass spectrometer. Because of its heterogeneity, MA and its derivatives share a characteristic peak distribution. After coupling with arabinans, this pattern migrated to the higher MW region, indicating the successful conversion (Fig. 2). Esterificated products were finally deprotected under hydrogenolytic conditions to provide $Myc_2Ara f_3$ **2**, $Myc_{2cd}Ara f_5$ **3**, $Myc_{2ef}Ara f_5$ **4**, and $Myc_4Ara f_5$ **5**.

2.4. TNF-α secretion-inducing assay of mycoloyl-arabinans

To evaluate the activities of mycolated arabinans as immune potentiators, tumor necrosis factor-α (TNF-α) secretion-inducing assay was conducted,[9,19,5a] in comparison with BCG-CWS (Tokyo strain),[20] trehalose dimycolate (TDM)[20] as the positive control, and mycolic acid (MA). Namely, RAW 264.7 mouse macrophage was used for this assay in vitro. All of the synthesized mycolates ($Myc_1Ara f_1$ **1**, $Myc_2Ara f_3$ **2**, $Myc_{2cd}Ara f_5$ **3**, $Myc_{2ef}Ara f_5$ **4**, and $Myc_4Ara f_5$ **5**) induced TNF-α in vitro, with activities similar to that of BCG-CWS,



Scheme 2. Synthesis of tri- and pentaarabinfuranosides.

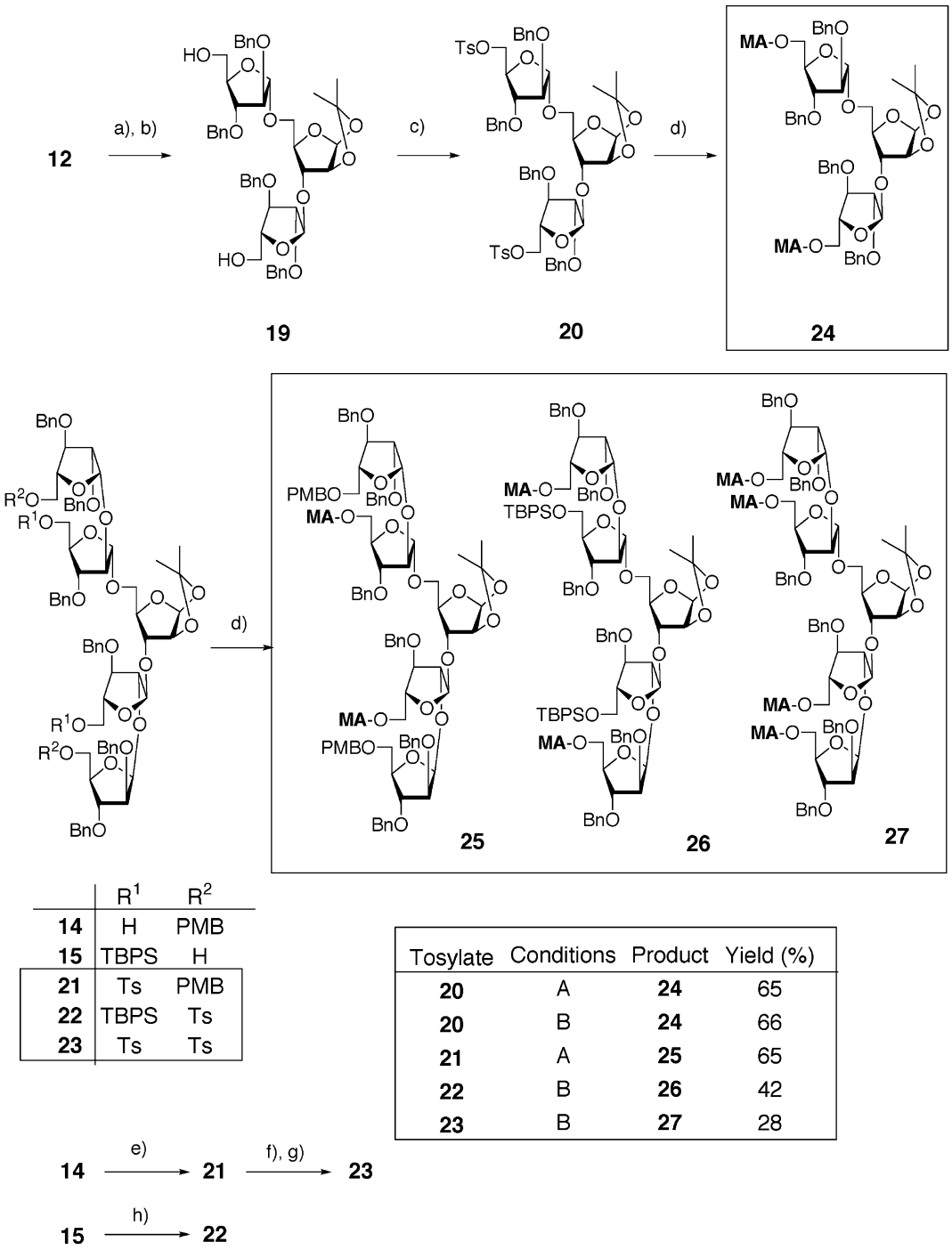


Scheme 3. Examination of esterification of arabinofuranoside with mycolic acid.

although mycolic acid and arabinan[21] by themselves induced TNF-α secretion to a minimum extent (Fig. 3). To induce TNF-α, both hydrophilic arabinan and lipophilic mycolic acid were required.

3. Conclusion

We have demonstrated the first synthesis of mycobacterial lipo-pentaarabinofuranoside as a terminal section of BCG-CWS from *M. bovis*. Synthesis of the non-reducing terminal arabinan of BCG-CWS was achieved via double α-arabinofuranosylation followed by double β-arabinofuranosylation with orthogonal protections. Alkylative esterification via an arabinan tosylate is effective for the synthesis of mycolate without protection of the β-hydroxyl group in mycolic acid moiety. A series of compounds were subjected to a TNF-α secretion-inducing assay and it was found that all the synthesized mycolates of arabinan showed potent TNF-α inducing activities in vitro in almost the same order as BCG-CWS itself. A detailed mechanism of the action of these compounds is still not clear, although the lipo-arabinan moieties are thought to play an important role in this activity.



	R^1	R^2
14	H	PMB
15	TBPS	H
21	Ts	PMB
22	TBPS	Ts
23	Ts	Ts

Tosylate	Conditions	Product	Yield (%)
20	A	24	65
20	B	24	66
21	A	25	65
22	B	26	42
23	B	27	28

Scheme 4. Completion of synthesis of mycoloyl-arabinans. Reagents and conditions: (a) BnBr, NaH, DMF, 99%; (b) TBAF, THF, 82%; (c) TsCl, pyridine, DMAP, 67%; (d) method A; mycolic acid, $CsHCO_3$, DMF–THF, 70 °C, or method B; mycolic acid, $CsHCO_3$, 18-crown-6, toluene, 70 °C; (e) TsCl, pyridine, 86%; (f) DDQ, 68%; (g) TsCl, pyr, 91%; (h) TsCl, pyridine, 86%.

4. Experimental

4.1. General procedures

All reactions sensitive to air and/or moisture were carried out under nitrogen or argon atmosphere with anhydrous solvents. Column chromatography was performed on silica gel 60 N, 100–210 mesh (Kanto Kagaku Co., Ltd). Preparative TLC was performed on silica gel 60 F_{254}, 0.5 mm (E. Merck). Gel filtration was performed on Sephadex LH-20 (Pharmacia). Melting point was determined with a Büchi 510 melting point apparatus. Optical rotations were measured with a JASCO DIP 370 polarimeter. ^{1}H NMR spectra were recorded at 400 MHz on a JEOL JNM-AL 400 spectrometer and chemical shifts are referred to internal $CDCl_3$ (7.24 ppm). ^{13}C NMR spectra were recorded at 100 MHz on the same instrument and chemical shifts are referred to internal $CDCl_3$ (77 ppm). MALDI-TOF mass spectra were recorded

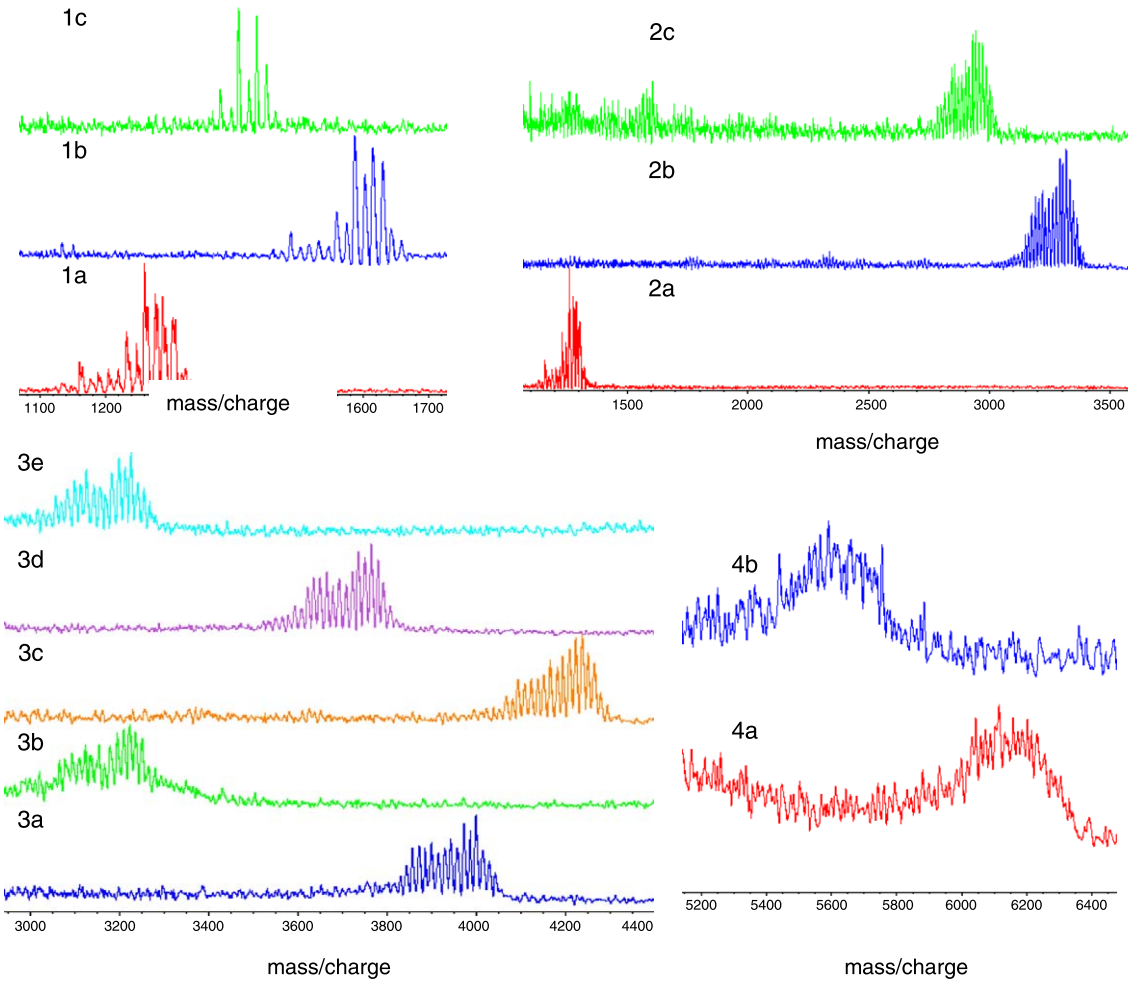


Figure 2. Mass spectra for monitoring of the synthesis of mycolate. (1) Monitoring of the synthesis of Myc_1Araf_1 (**1**): (1a) MA; (1b) **18**; (1c) **1**. (2) Monitoring of the synthesis of Myc_2Araf_3 (**2**): (2a) MA; (2b) **24**; (2c) **2**. (3) Monitoring of the synthesis of Myc_2Araf_5 (**3**, **4**): (3a) **25**; (3b) **3**; (3c) **26**; (3d) desilylated product of **26**; (3e) **4**. (4) Monitoring of the synthesis of Myc_4Araf_5 (**5**): (4a) **27**; (4b) **5**.

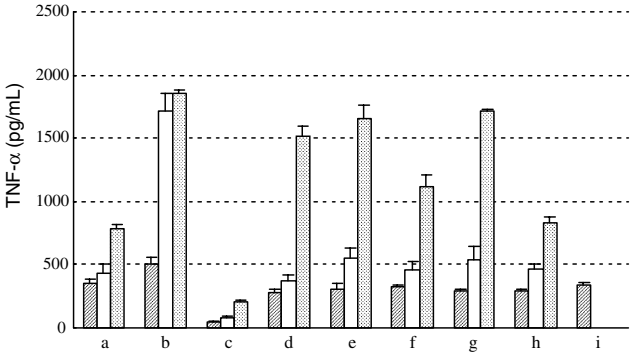


Figure 3. TNF-α secretion-inducing assay; (a) BCG-CWS, (b) TDM, (c) MA, (d) **1** (Myc_1Araf_1), (e) **2** (Myc_2Araf_3), (f) **3** ($Myc_{2cd}Araf_5$), (g) **4** ($Myc_{2ef}Araf_5$), (h) **5** (Myc_4Araf_5), (i) solvent only (control). ▨, 0.01 μg/well; □, 0.1 μg/well; ⊡, 1 μg/well.

on a SHIMADZU Kompact MALDI AXIMA-CFR spectrometer with 2,5-dihydroxybenzoic acid as the matrix. ESI-TOF mass spectra were recorded on a JEOL AccuTOF JMS-T700LCK with CF_3CO_2Na as the internal standard. Elemental analyses were performed with a Fisons EA1108 instrument. Mass units of the mycoloyl esters were referred to those of C_{84} ketomycolic acid for convenience sake.

4.1.1. 3-*O*-Benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1 → 3)-[3-*O*-benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (12). A mixture of **10** (1.00 g, 5.26 mmol), **9b** (7.91 g, 12.6 mmol), 2,6-di-*t*-butyl-4-methylpyridine (5.29 g, 25.2 mmol), and dried powdered molecular sieves 4 Å (5 g) in dry CH_2Cl_2 (75 mL) was stirred at room temperature for 15 min under N_2 and cooled to 0 °C. To the mixture was added dropwise methyl triflate (2.86 mL, 25.3 mmol) and the temperature of the reaction mixture was allowed to rise to room temperature. After stirring overnight, triethylamine (2.5 mL) was added before being filtered. The filtrate was washed successively with satd aq $NaHCO_3$, satd aq NH_4Cl, and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (hexane–EtOAc, 8:1–3:1) to give the trisaccharide **11** (6.29 g, 4.77 mmol, 91%) as a viscous oil.

$[\alpha]_D^{24}$ +61.5 (*c* 0.52, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.94 (s, 9H), 0.97 (s, 9H), 1.30 (s, 3H), 1.50 (s, 3H), 3.71 (dd, *J* = 5.2, 10.8 Hz, 1H), 3.72–3.89 (m, 4H), 3.97 (dd, *J* = 6.4, *J* = 10.8 Hz, 1H), 4.18 (br d, *J* =6.0 Hz, 1H), 4.23–4.30 (m, 4H), 4.38 (br d, *J* = 3.2 Hz, 1H), 4.51 (d, *J* = 12.4 Hz, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 4.68 (br d, *J* = 4.2 Hz, 1H), 4.70 (d, *J* = 12.4 Hz, 1H), 4.72 (d, *J* = 12.0 Hz, 1H), 5.21 (br s, 1H), 5.32 (br s, 1H), 5.36 (br d, *J* = 0.8 Hz, 1H), 5.40 (br d, *J* = 12.0 Hz, 1H), 5.91 (d, *J* = 4.2 Hz, 1H), 7.15–7.40 (m, 26H), 7.52–7.63 (m, 10H), 7.91–7.96 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 19.3, 19.3, 26.5, 26.8, 26.8, 27.2, 62.6, 62.6, 66.6, 72.0, 72.2, 80.0, 82.2, 82.4, 82.5, 82.6, 83.2, 83.4, 83.7, 85.1, 105.1, 105.5, 106.1, 113.0, 127.4, 127.5, 127.6, 127.7, 127.8, 128.2, 128.3, 129.2, 129.4, 129.5, 129.7, 133.0, 133.2, 133.3, 135.4, 135.5, 137.5, 137.7, 165.1, 165.4. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{78}H_{86}O_{15}Si_2Na$, 1341.5. Found 1341.8. Anal. Calcd for $C_{78}H_{86}O_{15}Si_2$: C, 70.99; H, 6.57. Found: C, 70.81; H, 6.55.

The dibenzoate **11** (6.39 g, 4.84 mmol) was dissolved in CH_2Cl_2 (20 mL) and NaOMe (390 mg, 7.3 mmol) in MeOH (20 mL) was added at 0 °C. After stirring at 0 °C for 2 h, the reaction mixture was neutralized with dil HCl and extracted with EtOAc. The organic layer was washed successively with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (hexane–EtOAc, 5:1–2:1) to give **12** (5.03 g, 4.53 mmol, 94%) as a viscous oil.

$[\alpha]_D^{26}$ +74.6 (*c* 0.54, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.96 (s, 18H), 1.31 (s, 3H), 1.51 (s, 3H), 3.20 (d, *J* = 10.0 Hz, 1H), 3.40 (d, *J* = 10.8 Hz, 1H), 3.41–3.44 (m, 2H), 3.62–3.70 (m, 3H), 3.90–3.93 (m, 3H), 4.10–4.16 (m, 5H), 4.39 (br d, *J* = 4.0 Hz, 1H), 4.41 (d, *J* = 12.0 Hz, 1H), 4.43 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.59 (d, *J* = 12.0 Hz, 1H), 4.62 (br d, *J* = 4.0 Hz, 1H), 5.05 (br s, 1H), 5.18 (br s, 1H), 5.82 (d, *J* = 4.0 Hz, 1H), 7.18–7.39 (m, 22H), 7.51–7.60 (m, 8H); ^{13}C NMR ($CDCl_3$): δ 19.0, 19.1, 26.7, 26.7, 26.8, 27.3, 63.8, 63.9, 66.4, 71.7, 71.8, 77.2, 77.9, 79.3, 83.0, 83.8, 84.1, 84.5, 84.7, 85.7, 105.1, 107.5, 108.8, 113.1, 127.5, 127.6, 127.7, 127.8, 128.3, 129.7, 129.8, 129.9, 131.9, 132.1, 132.2, 132.4, 135.4, 135.5, 137.6, 137.7. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{64}H_{78}O_{13}Si_2Na$, 1133.5. Found 1133.4. Anal. Calcd for $C_{64}H_{78}O_{13}Si_2$: C, 69.16; H, 7.07. Found: C, 69.14; H, 7.05.

4.1.2. Ethyl 2,3-di-*O*-benzyl-5-*O*-*p*-methoxybenzyl-1-thio-α-D-arabinofuranoside (6e). To compound **9a**[13] (5.08 g, 13.1 mmol) in dry DMF (40 mL) was added sodium hydride (60% dispersion in oil, 1.10 g, 27.5 mmol) at 0 °C. After stirring at 0 °C for 20 min, benzyl chloride (1.58 mL, 13.7 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for additional 2 h and then methanol (25 mL) was added before being diluted with CH_2Cl_2. The organic layer was washed successively with H_2O, satd aq NH_4Cl, and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (hexane–EtOAc, 6:1–3:1) to give dibenzylated primary alcohol **6f** (3.43 g, 9.17 mmol, 70%) as a colorless solid.

$[\alpha]_D^{27}$ +153.0 (*c* 0.34, $CHCl_3$); m.p. 35–36 °C; ^1H NMR ($CDCl_3$): δ 1.29 (t, *J* = 7.4 Hz, 3H), 2.07 (br s, 1H), 2.57–2.75 (m, 2H), 3.67 (dd, *J* = 4.0, 12.0 Hz, 1H), 3.83 (dd, *J* = 2.8, 12.0 Hz, 1H), 3.98–4.02 (m, 2H), 4.23 (m, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.59 (d, *J* = 12.0 Hz, 1H), 5.35 (d, *J* = 2.0 Hz, 1H), 7.25–7.37 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 14.8, 25.3, 61.7, 71.9, 72.3, 81.3, 82.8, 87.4, 88.5, 127.6, 127.7, 127.8, 128.3, 128.3, 137.1, 137.5. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{21}H_{26}O_4SNa$, 397.1. Found 396.8. Anal. Calcd for $C_{21}H_{26}O_4S$: C, 67.35; H, 7.00. Found: C, 67.35; H, 6.86.

To the alcohol **6f** (3.15 g, 8.41 mmol) in dry DMF (25 mL) was added sodium hydride (60% dispersion in oil, 640 mg, 16.0 mmol) at 0 °C and the suspension was stirred for 10 min. Then *p*-methoxybenzyl chloride (1.28 mL, 9.25 mmol) was added dropwise. After the temperature of the solution was allowed to rise to room temperature overnight, methanol (3.5 mL) was added at 0 °C. The reaction mixture was diluted with CH_2Cl_2. The organic layer was washed successively with H_2O, satd aq NH_4Cl, and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (hexane–EtOAc, 20:1–15:1) to give **6e** (3.89 g, 7.86 mmol, 94%) as a colorless oil.

$[\alpha]_D^{28}$ +107.8 (*c* 1.40, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.30 (t, *J* = 7.6 Hz, 3H), 2.68 (m, 2H), 3.59 (dd, *J* = 4.8, 10.8 Hz, 1H), 3.64 (dd, *J* = 3.6, 10.8 Hz, 1H), 3.79 (s, 3H), 3.95–3.97 (m, 2H), 4.28 (m, 1H), 4.46–4.62 (m, 6H), 5.37 (d, *J* = 2.0 Hz, 1H), 6.84 (br d, *J* = 8.8 Hz, 2H), 7.21–7.37 (m, 12H); ^{13}C NMR ($CDCl_3$): δ 14.9, 25.3, 55.3, 68.7, 72.0, 72.2, 73.0, 79.9, 83.6, 87.1, 88.8, 113.6, 127.7, 127.8, 127.9, 128.3, 129.3, 130.1, 137.4, 137.7, 159.0. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{29}H_{34}O_5SNa$, 517.2. Found 517.4. Anal. Calcd for $C_{29}H_{34}O_5S$: C, 70.42; H, 6.93. Found: C, 70.57; H, 6.88.

4.1.3. 2,3-Di-*O*-benzyl-5-*O*-*p*-methoxybenzyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-5-*O*-*p*-methoxybenzyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (13). A mixture of **12** (31.9 mg, 0.0287 mmol), **6e** (45.0 mg, 0.0906 mmol), *N*-iodosuccinimide (26 mg), 4 Å (0.12 mmol), and dried powdered molecular sieves (5 g) in dry CH_2Cl_2 (1.5 mL) was cooled to −78 °C and stirred for 15 min under N_2. To this mixture was dropwise added silver triflate (7.4 mg, 0.029 mmol) in dry toluene (0.08 mL) and the suspension was stirred for 10 min. Then the temperature of the solution was allowed to rise to −40 °C. After stirring for additional 1 h, triethylamine (1 mL) was added before being filtered. The filtrate was washed successively with 20% aq sodium thiosulfate, satd aq NH_4Cl, and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by LH-20 column chromatography ($CHCl_3$–MeOH, 1:1) and preparative TLC (developed with hexane–EtOAc, 2:1 and eluted with $CHCl_3$–MeOH, 4:1) to give **13** (32.2 mg, 0.0163 mmol, 57%) as a colorless foam and also a mix-

ture of diastereomers (19.8 mg, 0.0100 mmol, 35%) as a colorless foam.

For desired ββ diastereomer of **13**: $[\alpha]_D^{23}$ −6.14 (*c* 0.96, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.04 (s, 9H), 1.04 (s, 9H), 1.32 (s, 3H), 1.52 (s, 3H), 3.48–3.67 (m, 4H), 3.60 (dd, *J* = 4.0, 10.4 Hz, 1H), 3.75 (s, 3H), 3.75 (s, 3H), 3.73–3.83 (m, 4H), 3.96 (dd, *J* = 6.8, 10.4 Hz, 1H), 4.02–4.38 (m, 18H), 4.56 (br d, *J* = 4.0 Hz, 1H), 4.45–4.69 (m, 12H), 5.01 (d, *J* = 4.4 Hz, 1H), 5.02 (br s, 1H), 5.09 (d, *J* = 4.4 Hz, 1H), 5.12 (d, *J* = 0.6 Hz, 1H), 5.84 (d, *J* = 4.0 Hz, 1H), 6.78 (d, *J* = 8.8 Hz, 1H), 6.79 (d, *J* = 8.8 Hz, 1H), 7.11 (d, *J* = 8.8 Hz, 1H), 7.13 (d, *J* = 8.8 Hz, 1H), 7.22–7.38 (m, 42H), 7.64–7.69 (m, 8H); ^{13}C NMR ($CDCl_3$): δ 19.3, 26.6, 26.9, 27.3, 55.2, 63.5, 66.8, 71.9, 72.0, 72.1, 72.2, 72.3, 72.5, 72.6, 72.7, 77.2, 79.9, 80.0, 82.1, 82.9, 83.0, 83.2, 83.3, 83.5, 83.8, 83.9, 85.0, 85.7, 85.9, 100.0, 100.1, 104.3, 105.4, 105.8, 112.9, 113.5, 113.6, 127.2, 127.4, 127.5, 127.6, 127.8, 128.1, 128.2, 128.3, 129.0, 129.2, 129.5, 129.9, 130.0, 133.1, 133.2, 133.4, 135.5, 137.6, 137.9, 138.0, 138.1, 158.9, 158.9. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{118}H_{134}O_{23}Si_2Na$, 1997.9. Found 1997.4. Anal. Calcd for $C_{118}H_{134}O_{23}Si_2$: C, 71.71; H, 6.83. Found: C, 71.35; H, 6.84.

4.1.4. 2,3-Di-*O*-benzyl-5-*O*-*p*-methoxybenzyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-5-*O*-*p*-methoxybenzyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (14). To compound **13** (29 mg, 0.015 mmol) in THF (0.1 mL) was added 1 M THF solution of TBAF (0.03 mL, 0.03 mmol) at 0 °C. After stirring for 2 h at ambient temperature, the reaction mixture was diluted with EtOAc. The organic layer was washed successively with satd aq NH_4Cl and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by preparative TLC (developed with hexane–EtOAc, 1:1 and eluted with $CHCl_3$–MeOH, 5:1) to give **14** (22 mg, 0.015 mmol, 100%) as a colorless oil.

$[\alpha]_D^{24}$ −8.30 (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.25 (s, 3H), 1.43 (s, 3H), 3.42–3.75 (m, 9H), 3.71 (s, 6H), 3.85–4.07 (m, 11H), 4.15–4.63 (m, 21H), 4.92 (d, *J* = 4.4 Hz, 1H), 4.95 (br s, 1H), 4.98 (d, *J* = 3.2 Hz, 1H), 5.03 (br s, 1H), 5.76 (d, *J* = 4.0 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 4H), 7.11 (d, *J* = 8.4 Hz, 4H), 7.20–7.30 (m, 30H); ^{13}C NMR ($CDCl_3$): δ 27.0, 27.5, 55.2, 62.8, 62.8, 65.2, 71.5, 72.1, 72.3, 72.4, 72.4, 72.6, 72.6, 72.7, 72.7, 79.9, 80.0, 82.2, 82.6, 82.7, 83.3, 83.3, 83.4, 83.7, 83.8, 83.9, 85.4, 85.5, 86.3, 100.0, 100.5, 104.8, 105.2, 105.7, 113.5, 113.6, 127.5, 127.6, 128.0, 128.2, 128.4, 129.2, 129.8, 129.9, 137.4, 137.4, 137.7, 137.8, 137.9, 138.0, 159.0, 159.0. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{86}H_{98}O_{23}Na$, 1521.6. Found 1521.9. Anal. Calcd for $C_{86}H_{98}O_{23}$: C, 68.88; H, 6.59. Found: C, 68.64; H, 6.57.

4.1.5. 2,3-Di-*O*-benzyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (15). To a solution of compound **13** (82 mg, 0.041 mmol) in CH_2Cl_2–H_2O (18:1, 1.3 mL) was added DDQ (29 mg, 0.13 mL) and stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc. The organic layer was washed successively with ascorbic acid/citric acid buffer* and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (hexane–EtOAc, 1:1) and LH-20 column chromatography ($CHCl_3$–MeOH, 1:1) to give **15** (51 mg, 0.030 mmol, 72%) as a colorless foam. *L-Ascorbic acid (0.7 g), citric acid monohydrate (1.2 g) and NaOH (0.92 g) in H_2O (100 mL).

$[\alpha]_D^{27}$ +104.0 (*c* 0.05, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.03 (s, 18H), 1.30 (s, 3H), 1.49 (s, 3H), 3.48–3.81 (m, 9H), 3.90–4.16 (m, 7H), 4.20–4.26 (m, 6H), 4.32 (m, 1H), 4.37 (m, 1H), 4.47–4.73 (m, 13H), 5.00 (br s, 1H), 5.04 (d, *J* = 4.4 Hz, 1H), 5.10 (d, *J* = 4.4 Hz, 1H), 5.14 (br s, 1H), 5.82 (d, *J* = 4.0 Hz, 1H), 7.24–7.39 (m, 42H), 7.62–7.66 (m, 8H); ^{13}C NMR ($CDCl_3$): δ 19.3, 26.5, 26.9, 27.2, 63.4, 63.4, 63.5, 63.5, 67.2, 72.0, 72.2, 72.2, 72.4, 72.5, 72.6, 79.9, 80.6, 80.7, 81.5, 81.8, 81.9, 82.4, 82.5, 82.6, 83.3, 83.8, 84.0, 85.0, 85.4, 85.5, 99.6, 99.8, 104.3, 105.4, 105.5, 112.9, 127.4, 127.6, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 129.5, 129.6, 133.0, 133.1, 133.2, 135.5, 137.4, 137.5, 137.6, 137.7, 137.9, 138.0. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{102}H_{118}O_{21}Si_2Na$, 1757.8. Found 1757.8. Anal. Calcd for $C_{102}H_{118}O_{21}Si_2$: C, 70.56; H, 6.85. Found: C, 69.99; H, 6.74.

4.1.6. Methyl 2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside (17). A mixture of **16** (118 mg, 0.343 mmol), pyridine (230 mg, 2.9 mmol) and 4-dimethylaminopyridine (catalytic amount) in dry CH_2Cl_2 (1 mL) was cooled to 0 °C and *p*-toluenesulfonyl chloride (140 mg, 0.734 mmol) was added. After the temperature of the solution was allowed to rise to room temperature overnight, the reaction mixture was diluted with EtOAc. The organic layer was washed successively with dil HCl, satd aq $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (hexane–EtOAc, 4:1) to give **17** (155 mg, 0.311 mmol, 91%) as a colorless oil.

$[\alpha]_D^{27}$ +57.0 (*c* 0.40, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H), 3.30 (s, 3H), 3.78 (dd, *J* = 2.8, 5.6 Hz, 1H), 3.92 (br d, *J* = 2.8 Hz, 1H), 4.10 (br d, *J* = 5.2 Hz, 2H), 4.15 (m, 1H), 4.37–4.52 (m, 4H), 4.82 (s, 1H), 7.22–7.33 (m, 12H), 7.73 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 21.6, 55.0, 68.8, 71.8, 72.2, 79.1, 82.7, 87.4, 107.3, 127.7, 127.8, 127.9, 128.3, 129.6, 132.6, 137.1, 137.2, 144.6. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{27}H_{30}O_7SNa$, 521.2. Found 521.4. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{27}H_{30}O_7SNa$, 521.1610. Found 521.1617. Anal. Calcd for $C_{27}H_{30}O_7S$: C, 65.04; H, 6.06. Found: C, 65.03; H, 5.92.

4.1.7. Preparation of mycolic acid. Mycolic acid was prepared from the extract of BCG-CWS following a Patent Application No. 2005-135840. BCG-CWS,[20] shown in Figure 1, was hydrolyzed with KOH in ethanol–toluene–H_2O at 65 °C for 3 h. After addition of 2 M HCl

aq hydrophobic residues were extracted with hexane. Combined organic phase was filtered, washed with water and concentrated.

MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{84}H_{164}O_4Na$, 1260.3. Found 1260.4.

4.1.8. Methyl 2,3-di-*O*-benzyl-5-*O*-mycoloyl-α-D-arabinofuranoside (18). [*General procedure for the synthesis of mycolate: Method A*]. To a mixture of **17** (6.0 mg, 0.012 mmol) and MA (12 mg, 0.0097 mmol) in DMF–THF (1:5, 1.2 mL) was added $CsHCO_3$ (10 mg, 0.052 mmol). After stirring at 70 °C for 2 days, the suspension was diluted with EtOAc. The organic layer was washed successively with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by LH-20 column chromatography ($CHCl_3$–MeOH, 1:1) and silica gel column chromatography (hexane–EtOAc, 4:1) to give **18** (11.9 mg, 0.00761 mmol, 79%) as a colorless oil.

^{1}H NMR (sugar moiety) ($CDCl_3$): δ 3.35 (s, 3H), 3.81 (dd, J = 2.8, 6.4 Hz, 1H), 3.96 (br d, J = 2.8 Hz, 1H), 4.19 (m, 1H), 4.27–4,28 (m, 2H), 4.45 (d, J = 12.0 Hz, 1H), 4.48 (d, J = 12.0 Hz, 1H), 4.54 (d, J = 12.0 Hz, 1H), 4.56 (d, J = 12.0 Hz, 1H), 7.24–7.33 (m, 10H); ^{13}C NMR (sugar moiety) ($CDCl_3$): δ 54.9, 63.5, 72.2, 72.4, 79.4, 83.7, 87.8, 107.1, 126.2, 127.8, 127.9, 128.4, 133.0, 137.2. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{104}H_{186}O_8Na$, 1586.4. Found 1586.2.

4.1.9. Methyl 5-*O*-mycoloyl-α-D-arabinofuranoside (1). Compound **18** (4.0 mg, 0.0026 mmol) was dissolved in hexane–EtOAc (1:1, 0.5 mL) and 20% $Pd(OH)_2$/C (2.5 mg) was added. After the reaction mixture was stirred under hydrogen atmosphere at room temperature for 5 days, it was filtered through Celite and washed with EtOAc and the combined filtrate was concentrated in vacuo. The resulting residue was purified by LH-20 column chromatography ($CHCl_3$–MeOH, 1:1) to give **1** (2.9 mg, 0.0021 mmol, 82%) as a colorless powder.

^{1}H NMR (sugar moiety) ($CDCl_3$): δ 3.38 (s, 3H), 3.96 (m, 1H), 4.05 (br s, 1H), 4.16 (m, 1H), 4.30 (dd, J = 4.0, 12.0 Hz, 1H), 4.48 (dd, J = 4.4, 12.0 Hz, 1H), 4.87 (s, 1H); ^{13}C NMR (sugar moiety) ($CDCl_3$): δ 55.0, 63.2, 78.4, 80.4, 83.8, 108.7. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{90}H_{174}O_8Na$, 1406.3. Found 1406.2.

4.1.10. 2,3-Di-*O*-benzyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (19). To a mixture of **12** (87 mg, 0.078 mmol) and benzyl bromide (43 mg, 0.25 mmol) in dry DMF (0.5 mL) was added sodium hydride (60% dispersion in oil, 10 mg, 0.25 mmol) at 0 °C. After stirring at 0 °C for 5 h, the reaction was quenched with satd aq NH_4Cl. The reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (hexane–EtOAc, 7:1–5:1) to give the tetrabenzylated compound (101 mg, 0.0778 mmol, 99%) as a colorless syrup.

$[\alpha]_D^{24}$ +47.1 (c 1.46, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.99 (s, 9H), 1.00 (s, 9H), 1.29 (s, 3H), 1.47 (s, 3H), 3.56 (dd, J = 4.8, 10.8 Hz, 1H), 3.70–3.78 (m, 4H), 3.92 (dd, J = 6.4, 10.8 Hz, 1H), 4.02–4.22 (m, 8H), 4.39–4.52 (m, 9H), 5.02 (s, 1H), 5.14 (s, 1H), 5.82 (d, J = 4.0 Hz, 1H), 7.20–7.35 (m, 32H), 7.59–7.63 (m, 8H); ^{13}C NMR ($CDCl_3$): δ 19.3, 26.6, 26.9, 27.2, 63.3, 67.0, 71.8, 71.9, 72.1, 72.1, 80.1, 81.9, 82.7, 82.8, 83.0, 83.7, 85.0, 88.5, 105.2, 105.5, 106.1, 112.9, 127.4, 127.5, 127.6, 127.7, 127.8, 128.2, 128.3, 128.4, 129.4, 129.5, 129.6, 133.2, 133.3, 135.5, 135.6, 137.5, 137.7, 137.8. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{78}H_{90}O_{13}Si_2Na$, 1313.6. Found 1313.6. Anal. Calcd for $C_{78}H_{90}O_{13}Si_2$: C, 72.53; H, 7.02. Found: C, 72.67; H, 7.07.

To this tetrabenzylated compound (70 mg, 0.054 mmol) in THF (0.6 mL) was added 1 M THF solution of TBAF (0.36 mL, 0.36 mmol) at 0 °C. After stirring for 4 h at ambient temperature, the reaction mixture was diluted with EtOAc. The organic layer was washed successively with satd aq NH_4Cl and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (hexane–EtOAc, 2:1–1:2) to give **19** (36 mg, 0.044 mmol, 82%) as a colorless oil.

$[\alpha]_D^{25}$ +78.6 (c 1.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.30 (s, 3H), 1.47 (s, 3H), 3.50 (dd, J = 6.1, 12.2 Hz, 1H), 3.59 (dd, J = 5.2, 11.8 Hz, 1H), 3.64–3.68 (m, 3H), 3.73 (dd, J = 3.2, 11.8 Hz, 1H), 3.80 (dd, J = 3.2, 7.2 Hz, 1H), 3.82–3.86 (m, 2H), 3.95–4.04 (m, 4H), 4.17 (m, 1H), 4.28 (dd, J = 1.2, 4.8 Hz, 1H), 4.40–4.51 (m, 9 H), 5.02 (s, 1H), 5.09 (s, 1H), 5.76 (d, J = 4.0 Hz, 1H), 7.19–7.31 (m, 20 H); ^{13}C NMR ($CDCl_3$): δ 27.0, 27.5, 62.6, 62.7, 65.3, 72.0, 72.2, 72.2, 72.3, 80.2, 82.3, 83.0, 83.2, 86.3, 87.7, 88.3, 104.8, 105.7, 105.8, 113.5, 127.6, 127.7, 127.8, 127.9, 128.3, 128.4, 128.5, 137.1, 137.2, 137.5, 137.5. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{46}H_{54}O_{13}Na$, 837.4. Found 837.4. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{46}H_{54}O_{13}Na$, 837.3462. Found 837.3480. Anal. Calcd for $C_{46}H_{54}O_{13}$: C, 67.80; H, 6.68. Found: C, 67.65; H, 6.70.

4.1.11. 2,3-Di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (20). Compound **20** was synthesized from **19** according to the procedure for the synthesis of **17** except that twofold equivalent of TsCl and pyridine was used and that the mixture was stirred for 4 h (67% as a colorless oil).

$[\alpha]_D^{26}$ +62.2 (c 0.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.31 (s, 3H), 1.48 (s, 3H), 2.37 (s, 3H), 2.38 (s, 3H), 3.50 (dd, J = 5.2, 10.4 Hz, 1H), 3.79 (dd, J = 5.2, 10.4 Hz, 1H), 3.81–3.84 (m, 2H), 3.94 (dd, J = 0.8, 3.2 Hz, 1H), 4.00 (dd, J = 0.8, 3.2 Hz, 1H), 4.02–4.15 (m, 7H), 4.19–4.23 (m, 1H), 4.38–4.51 (m, 9H), 4.93 (s, 1H), 5.01 (s, 1H), 5.78 (d, J = 4.0 Hz, 1H), 7.22–7.35 (m, 24H), 7.71 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 8.4 Hz, 2H); ^{13}C NMR

($CDCl_3$): δ 21.6, 26.6, 27.2, 66.5, 68.6, 68.7, 72.0, 72.1, 72.3, 78.8, 79.4, 80.0, 82.9, 82.9, 83.3, 85.0, 87.8, 87.8, 105.3, 106.1, 113.0, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 129.7, 132.6, 132.7, 137.1, 137.2, 137.3, 137.5, 144.6, 144.7. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{60}H_{66}O_{17}S_2Na$, 1145.4. Found 1145.4. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{60}H_{66}O_{17}S_2Na$, 1145.3639. Found 1145.3601.

4.1.12. 2,3-Di-*O*-benzyl-5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (24). [*Method A*] Compound **24** was synthesized from **20** according to a general procedure (Method A) for the synthesis of **18** except that twofold equivalent of MA and $CsHCO_3$ was used and that the mixture was stirred for 4 days (65% as a colorless foam).

[*General procedure for the synthesis of mycolate: Method B*] A mixture of **20** (6.5 mg, 0.0058 mmol), MA (18 mg, 0.015 mmol), $CsHCO_3$ (7.2 mg, 0.037 mmol), and 18-crown-6 (13 mg, 0.049 mmol) was dissolved in dry toluene (2 mL). After stirring at 70 °C for 4 days, the reaction mixture was diluted with EtOAc. The organic layer was washed successively with satd aq $NaHCO_3$, satd aq NH_4Cl, and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by preparative TLC (developed with hexane–EtOAc, 3:1 and eluted with $CHCl_3$–MeOH, 5:1) to give **24** (12.4 mg, 0.0038 mmol, 66%) as a colorless foam.

^1H NMR (sugar moiety) ($CDCl_3$): δ 1.31 (s, 3H), 1.49 (s, 3H), 3.56–3.60 (m, 1H), 3.85–3.90 (m, 3H), 3.97 (br d, J = 2.8 Hz, 1H), 4.01 (br d, J = 2.8 Hz, 1H), 4.09 (m, 1H), 4.15 (m, 1H), 4.20–4.29 (m, 6H), 4.40 (br d, J = 4.0 Hz, 1H), 4.43–4.54 (m, 8H), 5.02 (br s, 1H), 5.09 (br s, 1H), 5.79 (d, J = 4.0 Hz, 1H); ^{13}C NMR (sugar moiety) ($CDCl_3$): δ 57.7, 62.7, 62.9, 72.1, 72.1, 72.3, 72.4, 79.2, 79.7, 79.9, 83.2, 83.7, 85.2, 85.4, 88.1, 88.1, 105.2, 105.2, 105.9, 113.1, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5, 137.1, 137.3, 137.4, 137.5. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{214}H_{379}O_{19}Na$, 3275.9. Found 3275.6.

4.1.13. 5-*O*-Mycoloyl-α-D-arabinofuranosyl-(1 → 3)-[5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (2). Compound **24** (15.9 mg, 0.00488 mmol) was dissolved in hexane–EtOAc (1:1, 1 mL) and 20% $Pd(OH)_2$/C (6 mg) was added. Hydrogen was bubbled through the suspension at room temperature for 5 days. The solution was filtered over Celite and the filtrate was concentrated in vacuo. The resulting residue was purified by LH-20 column chromatography ($CHCl_3$–MeOH, 1:1) and preparative TLC (developed with $CHCl_3$–MeOH, 10:1 and eluted with $CHCl_3$–MeOH, 5:1) to give **2** (10.2 mg, 0.00352 mmol, 72%) as a colorless foam.

^1H NMR (sugar moiety) ($CDCl_3$): δ 1.30 (s, 3H), 1.51 (s, 3H), 3.58 (dd, J = 7.6, 9.2 Hz, 1H), 3.92–3.95 (m, 2H), 4.00 (m, 1H), 4.08–4.15 (m, 4H), 4.19 (m, 1H), 4.25–4.30 (m, 3H), 4.45 (dd, J = 3.6, 10.8 Hz, 2H), 4.58 (d, J = 4.0 Hz, 1H), 4.99 (s, 1H), 5.11 (s, 1H), 5.83 (d, J = 4.0 Hz, 1H); ^{13}C NMR (sugar moiety) ($CDCl_3$): δ 26.4, 27.2, 63.3, 63.5, 66.9, 78.8, 80.6, 81.0, 81.2, 83.0, 83.1, 83.4, 84.9, 85.4, 105.4, 106.9, 107.5, 113.1. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{186}H_{354}O_{19}Na$, 2915.7. Found 2916.1.

4.1.14. 2,3-Di-*O*-benzyl-5-*O*-*p*-methoxybenzyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-5-*O*-*p*-methoxybenzyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (21). Compound **21** was synthesized from **14** according to the procedure for the synthesis of **17** except that twofold equivalent of TsCl and pyridine was used (86% as a colorless oil).

$[\alpha]_D^{23}$ −4.07 (c 2.80, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.29 (s, 3H), 1.47 (s, 3H), 2.31 (s, 3H), 2.33 (s, 3H), 3.46–3.54 (m, 5H), 3.74 (s, 6H), 3.69–3.77 (m, 1H), 3.93–4.23 (m, 18H), 4.31–4.37 (m, 4H), 4.44 (br d, J = 4.0 Hz, 1H), 4.47–4.66 (m, 12H), 4.86 (br s, 1H), 4.88 (d, J = 4.4 Hz, 1H), 4.93 (br s, 1H), 5.02 (d, J = 4.4 Hz, 1H), 5.75 (d, J = 4.0 Hz, 1H), 6.78 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.8 Hz, 2H), 7.13–7.31 (m, 38H), 7.69 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 21.6, 26.7, 27.2, 55.2, 66.2, 68.8, 69.0, 71.4, 71.6, 72.1, 72.2, 72.4, 72.4, 72.6, 72.7, 72.7, 79.6, 79.8, 79.9, 80.0, 80.2, 82.5, 82.7, 83.0, 83.4, 83.5, 83.8, 83.9, 85.1, 85.2, 85.7, 100.1, 100.5, 104.8, 105.2, 106.1, 113.1, 113.6, 113.7, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.9, 129.2, 129.6, 129.7, 129.9, 130.0, 132.6, 132.7, 137.5, 137.6, 137.7, 137.9, 138.1, 144.5, 144.6, 159.0, 159.0. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{100}H_{110}O_{27}S_2Na$, 1829.7. Found 1830.0. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{100}H_{110}O_{27}S_2Na$, 1829.6574. Found 1829.6600.

4.1.15. 2,3-Di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (22). Compound **22** was synthesized from **15** according to the procedure for the synthesis of **17**, except that twofold equivalent of TsCl and pyridine was used (86% as a colorless oil).

$[\alpha]_D^{27}$ −26.0 (c 0.06, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.00 (s, 18H), 1.29 (s, 3H), 1.47 (s, 3H), 2.31 (s, 3H), 2.32 (s, 3H), 3.55 (dd, J = 4.4, 5.6 Hz, 1H), 3.66–3.78 (m, 4H), 3.89–4.26 (m, 19H), 4.39–4.64 (m, 13H), 4.94 (br s, 1H), 4.96 (d, J = 4.0 Hz, 1H), 5.04 (d, J = 4.4 Hz, 1H), 5.05 (br s, 1H), 5.79 (d, J = 4.0 Hz, 1H), 7.12 (br d, J = 8.8 Hz, 2H), 7.14 (br d, J = 8.8 Hz, 2H), 7.23–7.37 (m, 42H), 7.61–7.65 (m, 12H); ^{13}C NMR ($CDCl_3$): δ 19.3, 26.6, 26.9, 27.3, 63.5, 63.5, 66.8, 70.1, 70.2, 72.1, 72.2, 72.3, 72.4, 72.5, 78.3, 78.4, 79.9, 82.1, 82.1, 82.3, 83.1, 83.2, 83.3, 83.4, 85.0, 85.9, 86.1, 100.3, 100.4, 104.0, 105.4, 105.6, 113.0, 127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.1, 128.2, 128.3, 128.4, 129.5, 129.6, 129.7, 132.7 133.1, 133.2, 133.4, 135.5, 135.7, 137.3, 137.4, 137.5, 137.6, 137.9, 138.1. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{116}H_{130}O_{25}S_2Si_2Na$, 2065.8.

Found 2066.1. Anal. Calcd for $C_{116}H_{130}O_{25}S_2Si_2$: C, 68.14; H, 6.41. Found: C, 68.18; H, 6.66.

4.1.16. 2,3-Di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (23). To a solution of **21** (64 mg, 0.035 mmol) in CH_2Cl_2–H_2O (18:1, 1.9 mL) was added DDQ (27 mg, 0.12 mmol) and stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc. The organic layer was washed successively with ascorbic acid/citric acid buffer and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (toluene–acetone, 15:1–10:1) and LH-20 column chromatography ($CHCl_3$–MeOH, 1:1) to give the corresponding diol (38 mg, 0.024 mmol, 68%) as a colorless oil.

$[\alpha]_D^{23}$ +6.17 (*c* 0.79, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.29 (s, 3H), 1.47 (s, 3H), 2.35 (s, 3H), 2.36 (s, 3H), 3.50 (dd, *J* = 5.6, 10.4 Hz, 1H), 3.49–3.59 (m, 2H), 3.60–3.67 (m, 2H), 3.77 (dd, *J* = 5.6, 10.4 Hz, 1H), 3.93–4.13 (m, 11H), 4.15–4.20 (m, 6H), 4.24 (dd, *J* = 0.9, 3.2 Hz, 1H), 4.45 (dd, *J* = 0.6, 4.0 Hz, 1H), 4.37–4.71 (m, 12H), 4.87 (d, *J* = 0.9 Hz, 1H), 4.91 (d, *J* = 4.4 Hz, 1H), 4.98 (d, *J* = 0.9 Hz, 1H), 5.02 (d, *J* = 4.4 Hz, 1H), 5.77 (d, *J* = 4.0 Hz, 1H), 7.20–7.33 (m, 34H), 7.71 (br d, *J* = 8.4 Hz, 2H), 7.73 (br d, *J* = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 21.6, 26.6, 27.2, 63.5, 63.6, 66.6, 68.6, 68.8, 72.2, 72.3, 72.5, 72.6, 72.7, 79.1, 79.9, 79.9, 80.7, 80.8, 81.8, 81.9, 82.8, 82.8, 83.2, 83.9, 84.0, 85.1, 85.7, 100.1, 100.2, 104.9, 105.3, 105.8, 113.1, 127.6, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5, 129.7, 129.8, 132.5, 137.4, 137.9, 144.7, 144.8. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{84}H_{94}O_{25}S_2Na$, 1589.5. Found 1590.3. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{84}H_{94}O_{25}S_2Na$, 1589.5423. Found 1589.5411. Anal. Calcd for $C_{84}H_{94}O_{25}S_2$: C, 64.35; H, 6.04. Found: C, 64.20; H, 6.07.

Compound **23** was synthesized from the diol according to the procedure for the synthesis of **17** except that twofold equivalent of TsCl and pyridine was used (91% as a viscous oil).

$[\alpha]_D^{23}$ +4.18 (*c* 0.49, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.23 (s, 3H), 1.39 (s, 3H), 2.26 (s, 3H), 2.27 (s, 3H), 2.29 (s, 3H), 2.30 (s, 3H), 3.39 (dd, *J* = 4.8, 10.4 Hz, 1H), 3.68 (dd, *J* = 6.0, 10.4 Hz, 1H), 3.87–4.10 (m, 22H), 4.40 (d, *J* = 4.0 Hz, 1H), 4.27–4.59 (m, 12H), 4.77 (br s, 1H), 4.81 (d, *J* = 4.0 Hz, 1H), 4.87 (br s, 1H), 4.97 (d, *J* = 4.0 Hz, 1H), 5.68 (d, *J* = 4.0 Hz, 1H), 7.10–7.25 (m, 38H), 7.62–7.69 (m, 8H); ^{13}C NMR ($CDCl_3$): δ 21.6, 26.7, 27.3, 66.1, 69.0, 69.1, 70.0, 70.3, 71.6, 72.3, 72.3, 72.5, 72.6, 72.6, 78.2, 78.3, 79.8, 79.9, 80.5, 81.5, 81.7, 83.0, 83.3, 83.4, 83.6, 83.6, 85.3, 85.5, 86.1, 100.6, 101.0, 104.7, 105.2, 106.0, 113.2, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.9, 129.6, 129.7, 129.8, 130.8, 130.9, 132.5, 132.6, 137.1, 137.3, 137.4, 137.5, 137.6, 137.7, 144.5, 144.7, 144.8, 144.9. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{98}H_{106}O_{29}S_4Na$, 1897.6. Found 1898.4. HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{98}H_{106}O_{29}S_4Na$, 1897.5600. Found 1897.5637.

4.1.17. 2,3-Di-*O*-benzyl-5-*O*-*p*-methoxybenzyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-5-*O*-*p*-methoxybenzyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (25). Compound **25** was synthesized from **21** according to the general procedure (Method A) for the synthesis of **18** except that twofold equivalent of MA and $CsHCO_3$ was used and that the mixture was stirred for 5 days (65% as a colorless powder).

^{1}H NMR (sugar moiety) ($CDCl_3$): δ 1.29 (s, 3H), 1.49 (s, 3H), 3.47–3.52 (m, 4H), 3.57–3.62 (m, 1H), 3.73 (s, 3H), 3.73 (s, 3H), 3.88 (dd, *J* = 5.6, 10.4 Hz, 1H), 3.88–4.11 (m, 9H), 4.17–4.36 (m, 13H), 4.42–4.65 (m, 13H), 4.90 (d, *J* = 4.4 Hz, 1H), 4.96 (br s, 1H), 5.02 (d, *J* = 4.4 Hz, 1H), 5.03 (br s, 1H), 5.78 (d, *J* = 3.6 Hz, 1H), 6.76 (d, *J* = 8.8 Hz, 2H), 6.77 (d, *J* = 8.8 Hz, 2H), 7.11 (d, *J* = 8.8 Hz, 2H), 7.11 (d, *J* = 8.8 Hz, 2H), 7.21–7.32 (m, 30H); ^{13}C NMR (sugar moiety) ($CDCl_3$): δ 55.2, 63.1, 68.5, 71.6, 72.0, 72.1, 72.2, 72.3, 72.4, 72.5, 72.6, 72.7, 72.8, 79.9, 79.9, 80.5, 82.7, 82.9, 83.8, 83.9, 84.0, 84.2, 85.1, 85.4, 99.8, 100.2, 104.6, 104.7, 105.2, 113.2, 113.6, 113.7, 127.5, 127.6, 127.7, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 129.2, 129.8, 129.9, 137.4, 137.7, 137.8, 138.0, 138.5, 159.0, 159.0. MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{254}H_{422}O_{29}Na$, 3960.1. Found 3960.3.

4.1.18. 2,3-Di-*O*-benzyl-5-*O*-mycoloyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-5-*O*-mycoloyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-*t*-butyldiphenylsilyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (26). Compound **26** was synthesized from **22** according to the general procedure (Method B) for the synthesis of **24** except that coevaporated substrates and 18-crown-6 with toluene were used and that the mixture was stirred for 7 days (42% as a colorless oil).

^{1}H NMR (sugar moiety) ($CDCl_3$): δ 1.2–1.3 (s, 3H), 1.46 (s, 3H), 3.52–3.58 (m, 1H), 3.63–3.77 (m, 4H), 3.90 (dd, *J* = 6.8, 10.8 Hz, 1H), 3.95–4.31 (m, 18H), 4.38–4.68 (m, 13H), 4.96 (br s, 1H), 4.96 (d, *J* = 3.6 Hz, 1H), 5.05 (br s, 1H), 5.06 (d, *J* = 4.0 Hz, 1H), 5.77 (d, *J* = 4.0 Hz, 1H), 7.22–7.30 (m, 42H), 7.60–7.62 (m, 8H). MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{270}H_{442}O_{27}Si_2Na$, 4196.3. Found 4196.2.

4.1.19. 2,3-Di-*O*-benzyl-5-*O*-mycoloyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 3)-[2,3-di-*O*-benzyl-5-*O*-mycoloyl-β-D-arabinofuranosyl-(1 → 2)-3-*O*-benzyl-5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (27). Compound **27** was synthesized from **23** according to the general procedure (Method B) for the synthesis of **24** except that twofold equivalent of

MA and $CsHCO_3$ were used. Furthermore, coevaporated substrates and 18-crown-6 with toluene were used and the mixture was stirred for 5 days (28% as a colorless powder).

^{1}H NMR (sugar moiety) ($CDCl_3$): δ 1.30 (s, 3H), 1.50 (s, 3H), 3.55–3.65 (m, 1H), 3.85–3.92 (m, 1H), 3.99–4.30 (m, 22H), 4.47–4.70 (m, 13H), 4.90 (d, J = 3.6 Hz, 1H), 4.97 (br s, 1H), 5.03 (br s, 1H), 5.03 (d, J = 3.6 Hz, 1H), 5.77 (d, J = 3.6 Hz, 1H), 7.22–7.31 (m, 30H). MALDI-TOF MS: $[M+Na]^+$ calcd. for $C_{406}H_{730}O_{33}Na$, 6158.5. Found 6159.2.

4.1.20. β-D-Arabinofuranosyl-(1 → 2)-5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 3)-[β-D-arabinofuranosyl-(1 → 2)-5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (3). Compound **25** (4.2 mg, 0.0011 mmol) and 20% $Pd(OH)_2$/C (3 mg) were suspended in hexane–EtOAc (1:1, 1 mL) and hydrogen was bubbled at room temperature. After 2 days, EtOAc–MeOH (1:1, 2 mL) was added to the suspension and stirred for additional 5 h under hydrogen atmosphere. The reaction mixture was filtered over Celite and washed with $CHCl_3$, MeOH, and EtOAc, successively. The filtrate was combined and concentrated in vacuo. The resulting residue was purified by LH-20 column chromatography ($CHCl_3$–MeOH, 1:1) to give **3** (3.0 mg, 0.00095 mmol, 89%) as a colorless powder.

^{1}H NMR (sugar moiety) ($CDCl_3$): δ 1.2–1.3 (s, 3H), 1.51 (s, 3H), 3.6–4.6 (br m, 25H), 5.0–5.2 (br m, 4H), 5.8–5.9 (br m, 1H). MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{196}H_{370}O_{27}Na$, 3179.8 Found 3179.8.

4.1.21. 5-*O*-Mycoloyl-β-D-arabinofuranosyl-(1 → 2)-α-D-arabinofuranosyl-(1 → 3)-[5-*O*-mycoloyl-β-D-arabinofuranosyl-(1 → 2)-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (4). To compound **26** (2.4 mg, 0.00058 mmol) in THF (0.1 mL) were added AcOH (0.47 μL, 0.0082 mmol) and TBAF (0.01 M THF solution, 1.2 mL, 0.012 mmol) at 0 °C. After stirring for 8 days at ambient temperature, the solution was concentrated in vacuo. The resulting residue was purified by LH-20 column chromatography ($CHCl_3$–MeOH, 1:1) to give the desilylated diol (1.5 mg, 0.00041 mmol, 71%) as a colorless powder.

^{1}H NMR (sugar moiety) ($CDCl_3$): δ 1.32 (s, 3H), 1.50 (s, 3H), 3.47–3.73 (m, 5H), 3.90 (m, 1H), 3.94–4.12 (m, 9H), 4.18–4.28 (m, 8H), 4.34 (br d, J = 4.4 Hz, 1H), 4.46–4.69 (m, 13H), 4.93 (d, J = 4.0 Hz, 1H), 4.97 (br s, 1H), 4.99 (d, J = 3.6 Hz, 1H), 5.05 (br s, 1H), 5.77 (d, J = 4.4 Hz, 1H), 7.25–7.31 (m, 30H). MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{238}H_{406}O_{27}Na$, 3720.0. Found 3720.4.

This diol (5.0 mg, 0.0014 mmol) and 20% $Pd(OH)_2$/C (6 mg) were suspended in hexane–EtOAc (1:1, 2 mL) and hydrogen was bubbled at room temperature overnight. The reaction mixture was filtered over Celite and washed with $CHCl_3$–MeOH (4:1), $CHCl_3$, and EtOAc, successively. The filtrate was combined and concentrated in vacuo. The resulting residue was purified by LH-20 column chromatography ($CHCl_3$–MeOH, 1:1) to give **4** (4.1 mg, 0.0013 mmol, 96%) as a colorless powder.

^{1}H NMR (sugar moiety) ($CDCl_3$): δ 1.2–1.3 (s, 3H), 1.52 (s, 3H), 3.6–4.4 (br m, 24H), 4.61 (br s, 1H), 4.99 (br s, 1H), 5.02 (br s, 1H), 5.09 (br s, 1H), 5.15 (br s, 1H), 5.83 (br s, 1H). MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{196}H_{370}O_{27}Na$, 3179.8. Found 3180.4.

4.1.22. 5-*O*-Mycoloyl-β-D-arabinofuranosyl-(1 → 2)-5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 3)-[5-*O*-mycoloyl-β-D-arabinofuranosyl-(1 → 2)-5-*O*-mycoloyl-α-D-arabinofuranosyl-(1 → 5)]-1,2-*O*-isopropylidene-β-D-arabinofuranose (5). Compound **27** (8.8 mg, 0.0014 mmol) and 20% $Pd(OH)_2$/C (5.3 mg) were suspended in hexane–EtOAc (1:1, 4 mL) and hydrogen was bubbled at room temperature for 3 days. The reaction mixture was filtered over Celite and washed with $CHCl_3$–MeOH (4:1). The filtrate was combined and concentrated in vacuo. The resulting residue was purified by LH-20 column chromatography ($CHCl_3$–MeOH, 3:1) to give **5** (7.5 mg, 0.0013 mmol, 94%) as a colorless powder.

^{1}H NMR (sugar moiety) ($CDCl_3$): δ 1.2–1.3 (s, 3H), 1.51 (s, 3H), 3.6–4.4 (br m, 24H), 4.58 (br d, J = 4.0 Hz, 1H), 5.00–5.02 (br m, 2H), 5.02 (br d, J = 4.0 Hz, 1H), 5.09 (br s, 1H), 5.83 (br d, J = 4.0 Hz, 1H). MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{364}H_{694}O_{33}Na$, 5618.3. Found 5618.8.

4.2. Methods and materials for TNF-α secretion-inducing assay

4.2.1. Cells and reagents. A murine macrophage cell line RAW264.7 was purchased from American Type Culture Collection (Manassas, VA). The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin. BCG-CWS, and trehalose-dimycolate (TDM) were prepared from *M. bovis* BCG Tokyo strain.[20]

4.2.2. In vitro macrophage stimulation. Arabinomycolates (**1**–**5**), BCG-CWS and TDM were dissolved or suspended in chloroform and diluted with hexane–EtOH (9:1 v/v). After the samples were dispensed into 96-well polypropylene microplates, the solvent was evaporated in the clean bench. RAW264.7 cell suspension was added in the plates at 5×10^5 cells/well and incubated overnight.

4.2.3. ELISA. TNF-α in the culture supernatant was measured using a commercial kit (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol.

Acknowledgments

We thank Dr. M. Murata for in vitro assay, Ms. Y. Uenishi for a preparation of mycolic acid (Sumitomo Pharmaceuticals Co.), and Ms. A. Takahashi for her technical assistance (RIKEN).

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