Abstract—The arabinogalactan of mycobacteria contains both monosaccharides in the furanose ring form, which are absent in mammals. We report here the first synthesis of the tetrasaccharide fragment α-DD-Araf-(1\(^\rightarrow\)5)-β-DD-Galf-(1\(^\rightarrow\)5)-β-DD-Galf-(1\(^\rightarrow\)6)-DD-Gal, conveniently derivatized for further elongation. The strategy relied on the use of suitably substituted DD-galactono-1,4-lactones as precursors for the galactofuranose units. Reduction of lactone tetrasaccharide 9 with disiamylborane afforded the tetrasaccharide synthon 1. The tetrasaccharide contains the linker unit of the arabinan to the galactan. © 2008 Elsevier Ltd. All rights reserved.

Keywords: Mycobacteria; Arabinogalactan; Galactofuranose; Arabinofuranose; Galactonolactone
The synthesis began with disaccharide imidate 2, which was recently described \(^{10}\) (Scheme 1). For the introduction of the terminal nonreducing arabinofuranose in 2, tin(IV) chloride-promoted glycosylation has been conveniently used.\(^{10}\) Elongation of the chain with galactofuranoses was achieved using the milder trichloroacetimidate method.\(^{8,17}\) DD-Galactono-1,4-lactone, besides being a stable precursor for the reducing sugar, can be selectively substituted by acylation reactions.\(^{18}\) Thus, 2,6-di-O-pivaloyl-d-galactono-1,4-lactone (3),\(^{8}\) obtained in one step from d-galactono-1,4-lactone, was used for the introduction of the two internal galactose residues in 1 (Scheme 1). For the incorporation of the galactose in the reducing end, the easily obtained 2,3,5-tri-O-benzoyl-d-galactono-1,4-lactone (8)\(^{16}\) was used as the precursor.

Selective glycosylation of the exocyclic OH-5 of lactone derivative 3 with 2 gave trisaccharide lactone 4 in 74% yield after purification by column chromatography. The same regioselectivity was found on glycosylation of the lactone with other imidates\(^ {8}\) or by using the perbenzoyl sugar and tin(IV) chloride as promoter.\(^ {10}\) The structure of 4 was confirmed by one- and two-dimensional NMR spectroscopy. The \(^{13}\)C NMR spectrum
showed C-1″ and C-1′ at 106.1 and 105.6 ppm, characteristic of α-D-arabinofuranosyl and β-D-galactofuranosyl linkages. In the 1H NMR spectrum, H-1″ and H-1′ appeared as broad singlets at 5.64 and 5.21 ppm and H-2″ and H-2′ as doublets, with J_{2,3} < 1.5 Hz, at 5.38 and 5.13 ppm. Acetylation of compound 4 at OH-3 was performed before reduction of the lactone to sugar 6. In the 1H NMR spectrum of 5, the signal of H-3 was shifted ≈1 ppm downfield with respect to 4. Reduction of the trisaccharide lactone derivative 5 with DSB yielded the furanosidic derivative 6 as a 4.6:5.4 α:β anemic mixture as indicated by the 1H NMR spectrum. The anomeric protons appeared at 5.34 (H-1β) as a doublet (J = 3.6 Hz) and at 5.44 ppm as a doublet of doublets (H-1α, J = 4.8, 9.4 Hz). After D$_2$O exchange, the signal for H-1b appeared as a broad singlet and the signal for H-1a was simplified to a doublet (J = 4.8 Hz). The 13C NMR spectrum showed all the anomeric carbons for the reducing unit appeared at 106.1 and 105.6 ppm, characteristic of 2,3,5-tri-O-acetyl-2,6-di-pivaloyl-D-galactono-1,4-lactone (3)

2,3,5-Tri-O-benzoyl-α-D-arabinofuranosyl-(1→5)-O-acetyl-2,6-di-O-pivaloyl-β-D-galactofuranosyl-(1→5)-2,6-di-O-pivaloyl-D-galactono-1,4-lactone (4)

A vigorously stirred solution of dried trichloroacetimidate 2$^{10}$ (1.07 g, 1.09 mmol), 2,6-di-O-pivaloyl-D-galactono-1,4-lactone (3, 848 mg, 1.40 mmol), and activated 4A powdered molecular sieves (0.7 g) in anhyd CH$_2$Cl$_2$ (60 mL) was cooled to −10 °C, and TMSOTf (79 µL, 0.44 mmol) was slowly added. After 1 h of stirring, the mixture was filtered into satd aq NaHCO$_3$ (150 mL) and then extracted with CH$_2$Cl$_2$ (2 × 200 mL). The organic layer was washed with water (3 × 150 mL), dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (15:1 toluene-EtOAc) affording 2,3,5-tri-O-benzoyl-α-D-arabinofuranosyl-(1→5)-3-O-acetyl-2,6-di-O-pivaloyl-β-D-galactofuranosyl-(1→5)-2,6-di-O-pivaloyl-D-galactono-1,4-lactone (4, 933 mg, 73%) as a syrup: [α]$_D$ +29.7 (c 1.0, CHCl$_3$); 1H NMR (CDCl$_3$, 500 MHz): δ 8.08–8.04 (m, 15H, aromatic), 5.65 (dd, 1H, J = 1.2, 5.0 Hz, H-3′), 5.64 (br s, 1H, H-1″), 5.56 (dd, 1H, J = 8.7 Hz, H-2), 5.38 (dd, 1H, J = 1.2 Hz, H-2″), 5.21 (br s, 1H, H-1′), 5.18 (dd, 1H, J = 1.3, 4.2 Hz, H-3′), 5.13 (dd, 1H, J = 1.3 Hz, H-2′), 4.83 (dt, 1H, J = 4.2, 8.2 Hz, H-3), 4.80 (dd, 1H, J = 3.5, 11.4 Hz, H-5′a), 4.77 (dd, 1H, J = 4.2 Hz, OH), 4.75 (m, 1H, H-4″), 4.69 (dd, 1H, J = 4.7, 11.4 Hz, H-5″b), 4.49 (dd, 1H, J = 5.6, 14.0 Hz, H-6′a), 4.42 (dd, 1H, J = 4.2, 7.1 Hz, H-4′), 4.33 (dd, 1H, J = 4.3, 7.7 Hz, H-4), 4.32–4.30 (m, 2H, H-6a, H-6b), 4.19–4.13 (m, 3H, H-5′, H-5′′, H-6b), 2.07 (s, 3H, CH$_3$), 1.19, 1.18, 1.17, 1.16 (4s, 36H, (CH$_3$)$_3$CCO); 13C NMR (CDCl$_3$, 125.8 MHz): δ 178.2, 177.8, 177.4, 176.7 ((CH$_3$)$_3$CCO), 170.0 (C-1), 168.9–165.7 (CH$_3$CO and COPh), 134.4–128.2 (aromatic), 106.1 (C-1″), 105.6 (C-1′), 84.9 (C-4′), 83.2 (C-2″), 80.9 (C-4′′), 80.2 (C-2′), 79.2 (C-4), 77.4 (C-3′), 76.2 (C-3′′), 75.5 (C-5′or C-5), 75.0 (C-2), 72.2 (C-5 or C-5′), 71.4 (C-3), 64.2 (C-6′), 63.5 (C-5′′), 63.0 (C-6), 38.7

1. Experimental

1.1. General methods

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 343 polarimeter at 25 °C. TLC was performed on 0.2 mm Silica Gel 60 F254 (Merck) aluminum-supported plates. Detection was effected by exposure to UV light or by spraying with 5% (v/v) sulfuric acid in EtOH and charring. Column chromatography was performed on Silica Gel 60 (230–400 mesh, Merck). NMR spectra were recorded with a Bruker AVANCE II 500 spectrometer at 500 MHz (1H) and 125.8 MHz (13C), or with a Bruker AC 200 at 200 MHz (1H) and 50.3 MHz (13C). Chemical shifts are given relative to the signal of internal acetone standard at 2.16 ppm and 30.8 ppm for 1H NMR and 13C NMR spectra when recorded in D$_2$O. 1H and 13C assignments were supported by DEPT 135, homonuclear COSY and HSQC experiments. High resolution mass spectra (HRMS) were recorded on Agilent LCTOF (2006) equipped with a high resolution TOF analyzer with Windows XP based OS and APCI/ESI ionization.
argon atmosphere was added to a flask containing com-
00
128.2 (aromatic), 106.0 (C-1
O
4.48 (dd, 1H, J = 8.0 Hz, H-2), 5.61 (s, 1H, H-1
J
1.4. 2,3,5-Tri-
J
3.0, 11.2 Hz, H-5a), 4.71 (dd, 1H, J = 3.0, 4.7 Hz, H-4a), 4.68 (dd, 1H, J = 5.0, 11.2 Hz, H-5b), 4.51 (dd, 1H, J = 3.5, 7.7 Hz, H-4), 4.48 (dd, 1H, J = 5.0, 7.0 Hz, H-4a), 4.40 (dd, 1H, J = 3.0, 11.7 Hz, H-6a), 4.36 (dd, 1H, J = 3.7, 10.7 Hz, H-6a), 4.24 (m, 1H, H-5), 4.22 (dd, 1H, J = 6.1, 10.7 Hz, H-6b), 4.18 (ddd, 1H, J = 3.0, 6.2, 7.0 Hz, H-5'), 4.12 (dd, 1H, J = 6.2, 11.7 Hz, H-6'b); 2.10, 2.03 (2s, 6H, CH3); 1.17, 1.16, 1.15, 1.14 (4s, 36H, (CH3)2COC); 13C NMR (CDC13, 125.8 MHz) δ: 178.0, 177.7, 177.0, 176.8 ((CH3)2COC), 170.0 (C-1), 169.4, 168.0 (CH3CO), 166.1–165.2 (COPh), 137.8–128.2 (aromatic), 106.0 (C-1'), 104.4 (C-1'), 83.5 (C-4'), 82.0 (C-2'), 81.2 (C-4'), 80.0 (C-2'), 77.8 (C-3'), 77.0 (C-4'), 76.5 (C-3'), 75.0 (C-5'), 72.2 (C-2'), 72.0 (C-3), 71.2 (C-5), 64.2 (C-6'), 63.6 (C-5'), 62.2 (C-6); 38.7, 38.6, 38.5 ((CH3)2COC); 27.1, 27.0, 26.9, 26.8 ((CH3)2CO); 20.7, 20.4 (CH3); HRMS (ESI/APCI) m/z: [M+Na]+ calcld for C62H76O24Na, 1227.4624; found, 1227.4597.

1.4. 2,3,5-Tri-O-benzoyl-α-D-arabinofuranosyl-(1→5)-3-O-acetyl-2,6-di-O-pivaloyl-β-D-galactofuranosyl-(1→5)-3-O-acetyl-2,6-di-O-pivaloyl-β-D-galactofuranosyl (6)

ture and then processed as previously described.20 The organic layer was washed with water, dried (Na2SO4), and concentrated. Boric acid was eliminated by coevaporation with MeOH (5 × 3 mL) at room temperature. The residue was purified by column chromatography (7:1, toluene–EtOAc) to give 107 mg (73%) of syrup 6 as a 0.46:0.54 α/β anomic mixture; Rf = 0.43 (3:1, toluene–EtOAc); [x]D
0.46H, H-1" α anomer), 5.61 (dd, 0.54H, J = 1.4, 4.8 Hz, H-3"), 5.58 (dd, 0.46H, J = 1.2, 4.8 Hz, H-3"), 5.56 (dd, 0.46H, J = 1.2 Hz, H-2"), 5.55 (dd, 0.56H, J = 1.4 Hz, H-2"), 5.44 (dd, 0.46H, J = 4.8, 9.4 Hz, H-1 α anomer), 5.34 (dd, 0.54H, J = 3.6 Hz, H-1 β anomer), 5.32 (br s, 0.46H, H-1), 5.25 (m, 0.56H, H-3), 5.23 (br s, 0.56H, H-1), 5.21 (dd, 0.46H, J = 2.0, 6.5 Hz, H-3), 3.86 (d, 1H, J = 9.4 Hz, OH), 3.81 (d, 1H, J = 3.6 Hz, OH); 1H NMR (CDCl3, 125.8 MHz): δ 178.2, 177.9, 177.6, 176.9 ((CH3)2COC), 170.0, 169.9, 169.8, 169.7 (CH3CO), 166.1–165.6 (COPh), 133.6–129.0 (aromatic), 106.1 (C-1' α anomer), 106.0 (C-1' β anomer), 105.2 (C-1' β anomer), 105.1 (C-1' α anomer), 100.4 (C-1 β anomer), 95.0 (C-1 α anomer), 82.9, 82.2, 82.3, 81.6, 81.5, 81.4, 81.2, 81.0, 79.7, 77.9, 77.8, 77.7, 76.8, 76.5, 76.0, 75.1, 74.9, 74.8, 73.5, 64.4, 64.2, 63.7, 63.6, 63.5, 63.3, 38.7, 38.6 ((CH3)2COC); 27.1, 27.0, 26.9 ((CH3)2CO); 20.7, 20.6 (CH3); HRMS (ESI/APCI) m/z: [M+Na]+ calcld for C62H76O24Na, 1227.4871; found, 1227.4758.

1.5. 2,3,5-Tri-O-benzoyl-α-D-arabinofuranosyl-(1→5)-3-O-acetyl-2,6-di-O-pivaloyl-β-D-galactofuranosyl-(1→5)-3-O-acetyl-2,6-di-O-pivaloyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-O-benzoyl-β-D-galactono-1,4-lactone (9)

To a stirred solution of 6 (86 mg, 0.071 mmol) and trichloroacetoni trile (0.036 mL, 0.36 mmol) in CH2Cl2 (5 mL), cooled to 0 °C, DBU (5 mL, 0.036 mmol) was slowly added. After 1 h, TLC monitoring showed the consumption of the starting material. The solution was concentrated at room temperature under reduced pressure, and the residue was purified by column chromatography (20:1:0.21, toluene–EtOAc–TEA) to give O-(2,3,5-tri-O-benzoyl-α-D-arabinofuranosyl-(1→5)-3-O-acetyl-2,6-di-O-pivaloyl-β-D-galactofuranosyl-(1→5)-3-O-acetyl-2,6-di-O-pivaloyl-β-D-galactofuranosyl (9) trichloroacetimidate (7, 80.2 mg, 84%) as a syrup. Compound 7 was stable for 1 day at −20 °C: Rf = 0.60 (β-anomer), 0.50 (α-anomer) (5:1:0.06, toluene–EtOAc–TEA); 1H NMR (CDCl3, 200 MHz) δ for the β anomer: 8.72 (s, 0.1H, NH α anomer), 8.59 (s, 0.9H, NH), 8.08–7.15 (m, 15H, aromatic), 6.51 (d, 0.1H, J = 4.0 Hz, H-1 α anomer), 6.25 (s, 0.9H, H-1 β anomer), 5.62 (br s, 0.9H, H-1"), 5.60 (m, 0.9H, H-3"), 5.52 (d, 0.9H, J = 1.2 Hz, H-2"), 5.36–5.31 (m, 1.8H,
H-3', H-1'), 5.26–5.24 (m, 1.8H, H-2, H-3), 5.15 (d, 0.9H, J = 1.6 Hz, H-2'), 4.84–4.64 (m, 3H, H-4', H-5a', H-5b'), 4.50–4.13 (m, 8H, H-4', H-5', H-6a, H-6b, H-6a', H-6b'); 2.06, 2.02 (2s, 6H, CH3); 1.26, 1.23, 1.18, 1.17 (4s, 36H, (CH3)3CCO); 13C NMR (CDCl3, 50.3 MHz) δ for the β anomer: 178.0, 176.9 ((CH3)3CCO); 170.0, 166.9 (CH3CO); 166.1–165.1 (COPh); 160.1 (NHCOCl); 133.5–125.2 (aromatic), 105.6 (C-1' β anomer), 104.6 (C-1' α anomer), 102.7 (C-1 β anomer), 90.8 (C-1 α anomer), 84.8, 83.3, 82.1, 81.5, 81.4, 81.1, 80.4, 77.8, 76.7, 75.5, 74.0, 72.0, 64.2, 63.6, 63.5, 28.7, 38.6, 38.5 ((CH3)3CCO); 27.1, 27.0, 26.9, 26.8 ((CH3)3CCO); 20.7, 20.5 (CH3).

A vigorously stirred suspension of dried trichloroacetimide 7 (55 mg, 0.040 mmol), 2,3,5-tri-O-benzoyl-d-galactono-1,4-lactone (8,140 mg, 0.048 mmol), and dried 4 A powdered molecular sieves (0.2 g) in anhyd CH2Cl2 (4 mL) was cooled to −15 ºC. After 10 min of stirring, TMSOTf (3 µL, 0.016 mmol) was slowly added. After 1 h, TLC monitoring showed the consumption of imidate 7, the mixture was rapidly filtered into sat aq NaHCO3 (25 mL) and then extracted with CH2Cl2 (2 × 25 mL). The organic phase was separated and washed with water (3 × 50 mL), dried (MgSO4), and concentrated. The oily residue was purified by column chromatography (12:1, toluene–EtOAc) to give 44 mg (54%) of syrupy 9. Lactone 9 (26 mg, 0.015 mmol) was reduced with a solution of bis(2-butyl-3-methyl)borane (2.16 mmol) in anhyd THF (0.63 mL) as described for compound 5. Purification by column chromatography (3:1, hexane–EtOAc) gave 14 mg (54%) of syrupy 1 as a 0.1:0.9 α/β anomic mixture: Rf = 0.63 (4:1, toluene–EtOAc); [α]D 30.6 (c 1.0, CHCl3). 1H NMR (CDCl3, 500 MHz) δ for the β anomer: 8.06–7.21 (m, 30H, aromatic), 5.78–5.71 (m, 1H, H-5, H-1 α anomer), 5.72 (d, 0.9H, J = 4.6 Hz, H-1 β anomer), 5.66 (br s, 0.9H, H-1''), 5.59 (dd, 0.9H, J = 1.4, 5.0 Hz, H-3''), 5.54 (d, 0.9H, J = 1.2 Hz, H-2), 5.51 (dd, 0.9H, J = 1.2, 5.5 Hz, H-3), 5.48 (d, 0.9H, J = 1.4 Hz, H-2''), 5.38 (br s, 0.9H, H-1'), 5.25 (dd, 0.9H, J = 1.9, 6.0 Hz, H-3'), 5.23 (dd, 0.9H, J = 1.9, 6.0 Hz, H-3'), 5.18 (d, 0.9H, J = 1.9 Hz, H-2'), 5.04 (d, 0.9H, J = 1.9 Hz, H-2''), 4.98 (s, 0.9H, H-1), 4.91 (d, 0.9H, J = 4.6 Hz, OH), 4.82 (dd, 0.9H, J = 3.4, 11.5 Hz, H-5a''), 4.79 (dd, 0.9H, J = 1.9, 5.5 Hz, H-4), 4.74–4.67 (m, 1.8H, H-4', H-6a' or H-6a''), 4.68 (dd, 0.9H, J = 4.6, 11.5 Hz, H-5b''), 4.46 (t, 0.9H, J = 5.7 Hz, H-4'), 4.41 (dd, 0.9H, J = 3.4, 6.0 Hz, H-4'), 4.33–4.27 (m, 2.7H, H-5', H-5''), 4.21–4.15 (m, 1.8H, H-6b', H-6b''), 4.07 (t, 0.9H, J = 8.4 Hz, H-6a), 4.78 (dd, 0.9H, J = 5.6, 8.3 Hz, H-6b); 2.04, 1.83 (2s, 5.4H, CH3); 1.18, 1.17, 1.13, 1.11 (4s, 32.4H, (CH3)3CCO); 13C NMR (CDCl3, 125.8 MHz) δ for the α anomer: 178.2, 177.0 ((CH3)3CCO), 169.8 (CH3CO × 2), 168.9 (C-1), 166.7–164.1 (COPh), 137.8–128.3 (aromatic), 105.9 (C-1''), 104.0 (C-1', C-1''), 100.5 (C-1), 95.3 (C-1x anomer), 83.4 (C-2''), 82.1 (C-2'), 81.9, 81.8 (C-4', C-4''), 81.6 (C-2', C-2', C-4''), 80.5 (C-4'), 78.0 (C-3''), 77.6 (C-3), 76.9 (C-3'), 75.7 (C-3'), 74.4, 70.2 (C-5', C-5''), 69.5 (C-5); 66.9, 64.0 (C-6', C-6''); 63.6 (C-5''), 62.4 (C-6), 38.7, 38.6 ((CH3)3CCO); 27.1, 27.0, 26.9, 26.8 ((CH3)3CCO); 20.7, 20.5 (CH3); HRMS (ESI/APCI) m/z: [M+Na]+ calec for C39H58O52Na, 1703.6905; found, 1703.6901.

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