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Note

Synthesis of the terminal trisaccharide unit of the lipo-oligosaccharide from *Mycobacterium linda* *

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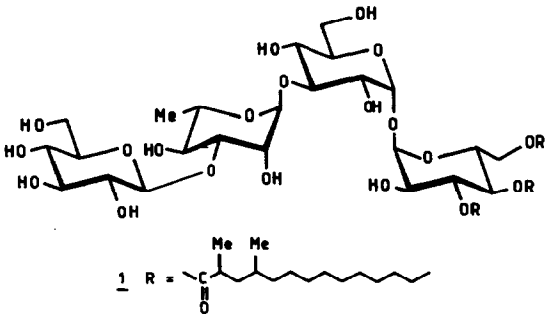
Mycobacterium (M) linda was first isolated from patients suffering from Crohn's disease[1]. There are doubts about the implication of this bacterium in Crohn's disease because later studies[2] revealed the presence of mycobacteria in healthy patients. However, literature evidence[1,3] did suggest that *M. linda* has a definite role to play in the pathogenesis of Crohn's disease. The identification of the species-specific antigen therefore becomes important, particularly for serodiagnosis and for the identification of the epidiology and epidemiology of the disease. The surface glycolipid (**1**), classified[4] as a trehalose-containing lipo-oligosaccharide, contained the oligosaccharide: β-D-Glc*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-D-Glc*p*-(1 ↔ 1)-α-D-Glc*p*. The antigenic activity[5] in lipo-oligosaccharides resides in those sugars units which are placed distant from the trehalose end. This report describes the synthesis of the terminal trisaccharide (**11**), as a methyl glycoside, of *M. linda*. The *O*-glycosylation reactions were performed using Schmidt's trichloroacetimidate approach[6].



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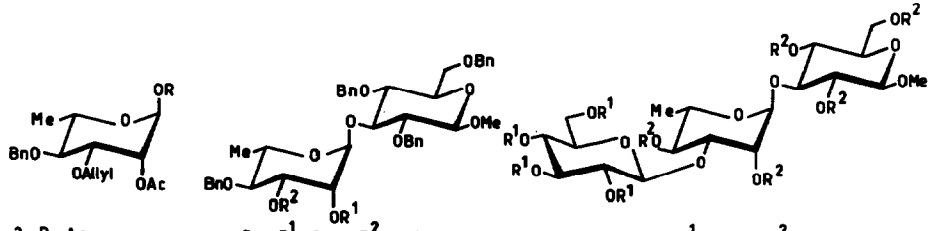
The requisite trichloroacetimidate derivative (**4**) was prepared from the known[7] 1,2-di-*O*-acetyl-3-*O*-allyl-4-*O*-benzyl-α-L-rhamnopyranose (**2**) by selective *O*-deacetylation[8] at C-1 with tributyltin ethoxide in refluxing dichloroethane to give **3**, which was then treated with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene in dichloromethane. Subsequent condensation of **4** with methyl 2,4,6-tri-*O*-benzyl-β-D-glucopyranoside[9] was effected with boron trifluoride etherate as catalyst in dichloromethane at −20°C to give **5**, the acetyl group of which was removed by Zemplén methanolysis to provide **6**. In the ^{1}H NMR spectrum of **6**, the anomeric proton signals were located as doublets at δ 4.32 ($J_{1,2}$ 8.0 Hz) and 5.50 ($J_{1',2'}$ 1.0 Hz). The characteristic coupling constants (δ 104.5, $J_{C\text{-}1,H\text{-}1}$ 158.3 Hz and δ 99.4, $J_{C\text{-}1',H\text{-}1'}$ 171.1 Hz) observed for anomeric carbons of **6** in the ^{13}C NMR spectrum confirmed the structure.



The free OH group in **6** was benzylated by the conventional procedure, to give **7**, the allyl group of which was removed by using tris(triphenylphosphine)rhodium(I) chloride for isomerisation[10] and mercuric chloride–mercuric oxide for hydrolysis[10], to afford **8**. The coupling reaction of **8** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate[11], as described earlier, provided **9**, whose *O*-acetyl groups were removed by Zemplén methanolysis to give **10**. Its ^{13}C NMR spectrum revealed anomeric carbon signals at δ 98.09 (C-1', $J_{C\text{-}1',H\text{-}1'}$ 171.1 Hz), 103.66 (C-1'', $J_{C\text{-}1'',H\text{-}1''}$ 160.0 Hz), and 104.51 (C-1, $J_{C\text{-}1,H\text{-}1}$ 161.5 Hz), which confirmed the structure. Upon hydrogenolytic debenzylation of **10** over 10% palladium-on-charcoal, the trisaccharide **11** was isolated, in whose ^{1}H NMR spectrum the anomeric proton signals appeared at δ 4.26 (H-1, $J_{1,2}$ 8.5 Hz), 4.66 (H-1'', $J_{1'',2''}$ 8.0 Hz), and 5.28 (H-1', $J_{1',2'}$ 1.0 Hz). In addition, the ^{13}C NMR spectrum revealed anomeric carbon signals at δ 98.1 (C-1', $J_{C\text{-}1',H\text{-}1'}$ 171.7 Hz), 103.6 (C-1'', $J_{C\text{-}1'',H\text{-}1''}$ 160.0 Hz), and 104.5 (C-1, $J_{C\text{-}1,H\text{-}1}$ 161.5 Hz)[12]. Compound **11** was further converted into the nona-acetate **12** by conventional acetylation. The ^{1}H NMR spectrum of **12** showed characteristic doublets for CH_3-5' at 1.02 ppm. In addition, acetyl methyl signals appeared between 1.9 and 2.2 ppm. The anomeric proton signals were located at δ 4.22 (d, *J* 8.3 Hz, H-1), 4.50 (d, *J* 7.9 Hz, H-1''), and 4.70 (d, *J* 1.0 Hz, H-1').

EXPERIMENTAL

General methods.—See ref. 12.

Methyl 3-O-(3-O-allyl-4-O-benzyl-α-L-rhamnopyranosyl)-2,4,6-tri-O-benzyl-β-D-glucopyranoside (**6**).—A solution of 1,2-di-*O*-acetyl-3-*O*-allyl-4-*O*-benzyl-α-L-rhamnopyranose[7] (**2**; 5.0 g, 13.2 mmol) and tributyltin ethoxide (4.43 g, 13.2 mmol) in dichloroethane was boiled under reflux for 3 h, cooled, washed with water, dried, and concentrated. Column chromatography of the residue, by eluting with 1:9 EtOAc–light petroleum, gave **3** (3.1 g, 70%).

A solution of **3** (2.4 g, 7.14 mmol), trichloroacetonitrile (6.6 mL), and 1,8-diazobicyclo[5.4.0]undec-7-ene (0.2 mL) in CH_2Cl_2 was stirred at room temperature for 10 min and then poured onto a silica gel column. Elution with CH_2Cl_2 provided **4** (2.49 g, 73%), which was used as such for the next reaction.

To a mixture of **4** (2.4 g, 5.0 mmol), methyl 2,4,6-tri-*O*-benzyl-β-D-glucopyranoside[9] (2.7 g, 5.8 mmol), and 4A molecular sieves (5 g) in dry CH_2Cl_2 at −20°C was added boron trifluoride etherate (25 μL). After 30 min, the reaction mixture was decomposed by the addition of a few drops of pyridine and then filtered over a bed of Celite which was washed with CH_2Cl_2. The combined filtrate was washed with water, dried, and concentrated. The residue was chromatographed on silica gel by using 1:9 EtOAc–light petroleum, to give **5** (2.73 g, 70%), which was further stirred with MeOH (50 mL) containing sodium (75 mg) for 3 h, then deionised by adding Amberlite IR-120 (H^+) resin, and filtered. The filtrate was concentrated to give **6** (2.32 g, 90%), isolated as a syrup, $[\alpha]_D$ −31° (*c* 1.6, $CHCl_3$); NMR data ($CDCl_3$): ^1H, δ 1.06 (d, 3 H, *J* 6.5 Hz, H-6,6,6), 3.60 (s, 3 H, OMe), 4.32 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.25 (dd, 1 H, *J* 1.0, 10.0 Hz, 1/2 CH_2=), 5.35 (dd, 1 H, *J* 1.0, 17.0 Hz, 1/2 CH_2=), 5.50 (d, 1 H, $J_{1',2'}$ 1.0 Hz, H-1'), 5.9 (m, 1 H, CH=), 7.3 (m, 20 H, 4 Ph); ^{13}C, δ 99.4 (*J* 171.1 Hz, C-1'), 104.5 (*J* 158.3 Hz, C-1). Anal. Calcd for $C_{44}H_{52}O_{10}$: C, 71.3; H, 7.0. Found: C, 71.4; H, 7.1.

Methyl 2,4,6-tri-O-benzyl-3-O-(2,4-di-O-benzyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**8**).—To a solution of **6** (0.42 g, 0.57 mmol) in dry THF was added sodium hydride (0.2 g, 50% dispersion in oil), and the mixture was stirred for 1 h. Benzyl bromide (0.2 mL) was introduced, and after 3 h, the reaction was worked up in the usual fashion. Column chromatography of the product with 1:10 EtOAc–light petroleum gave **7** (0.40 g, 85%), which was heated with tris(triphenylphosphine)rhodium(I) chloride (40 mg) and 1,4-diazobicyclo[2.2.2]octane (0.1 g) in 7:3:1 ethanol–benzene–water (15 mL) for 12 h. The solvents were evaporated, and a solution of the residue in aq 50% acetone (15 mL) was stirred with mercuric chloride (0.3 g) and mercuric oxide (75 mg) for 1 h. The reaction mixture was filtered over a bed of Celite which was then washed with acetone. The filtrate and washings were combined and concentrated to afford a residue which was subjected to column chromatography with 1:10 EtOAc–light petroleum, to give **8** (0.29 g, 76%), isolated as a syrup, $[\alpha]_D$ −3.3° (*c* 1.0, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 1.06 (d, 3 H, *J* 6.4 Hz, H-6',6',6'), 3.26 (t, 1 H, $J_{3',4'} = J_{4',5'} = 10.6$ Hz, H-4'), 3.57 (s,

3 H, OMe), 4.31 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.51 (s, 1 H, H-1′), 7.3 (m, 25 H, 5 Ph). Anal. Calcd for $C_{48}H_{54}O_{10}$: C, 72.9; H, 6.8. Found: C, 72.7; H, 6.9.

Methyl 3-O-(3-O-β-D-glucopyranosyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**11**).—To a mixture of **8** (0.23 g, 0.29 mmol), 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate[11] (0.15 g, 0.30 mmol), and 4A molecular sieves (0.5 g) in CH_2Cl_2 (15 mL) at −20°C was added boron trifluoride etherate (10 μL). After 14 h, the reaction mixture was worked up followed by column chromatography with 1:10 EtOAc–light petroleum to give **9** (0.165 g, 50%), which was *O*-deacetylated with MeOH (10 mL) and sodium (20 mg) for 3 h. After workup, column chromatography with 10:1 $CHCl_3$–MeOH afforded **10** (0.10 g, 71%); ^{13}C NMR data ($CDCl_3$): δ 98.09 (171.1 Hz, C-1′), 103.66 (160.0 Hz, C-1″), 104.51 (161.5 Hz, C-1). Compound **10** was hydrogenated over 10% palladium-on-charcoal (20 mg) in MeOH (5 mL) at normal temperature and pressure for 36 h. The catalyst was filtered off on a bed of Celite, and the filtrate was concentrated. The residue was purified by column chromatography with 3:1 $CHCl_3$–MeOH to give **11** (33 mg, 62%), isolated as a syrup (R_f 0.4, 1:1 $CHCl_3$–MeOH), $[\alpha]_D$ −34.5° (*c* 1.7, $CHCl_3$); NMR data (CD_3OD): ^1H, δ 1.36 (d, 3 H, *J* 6.5 Hz, H-6′,6′,6′), 3.60 (s, 3 H, OMe), 4.26 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.66 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), 5.28 (d, 1 H, $J_{1',2'}$ 1.0 Hz, H-1′); ^{13}C, δ 98.1 (171.7 Hz, C-1′), 103.6 (160.0 Hz, C-1″), 104.5 (161.5 Hz, C-1). Anal. Calcd for $C_{19}H_{34}O_{15}$: C, 45.4; H, 6.8 Found: C, 45.4; H, 6.6.

Methyl 2,4,6-tri-O-acetyl-3-O-[2,4-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**12**).—To a solution of **11** (20 mg, 0.04 mmol) in pyridine (2 mL) were added acetic anhydride (2 mL) and a catalytic amount of 4-(dimethylamino)pyridine, and the mixture was stirred for 24 h. Ice-cold water (2 mL) was added and, after 20 min, the solution was extracted with EtOAc which was washed with aq 5% $NaHCO_3$ and water, dried, and concentrated. The residue, on column chromatography with 1:1 EtOAc–light petroleum, afforded **12** (22 mg, 62.8%), $[\alpha]_D$ −30.2° (*c* 0.715, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 1.02 (d, 3 H, *J* 6.0 Hz, H-6′,6′,6′) 1.92, 1.94, 1.96, 2.08 (4 s, 12 H, 4 CH_3CO), 2.02 (bs, 15 H, 5 CH_3CO), 3.42 (s, 3 H, OMe), 4.22 (d, *J* 8.3 Hz, H-1), 4.50 (d, *J* 7.9 Hz, H-1″), 4.70 (d, *J* 1.0 Hz, H-1′). Anal. Calcd for $C_{37}H_{52}O_{24}$: C, 50.45; H, 5.9. Found: C, 50.5; H, 6.0.

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