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Synthesis of the trisaccharide segment of phenolic glycolipid from *Mycobacterium tuberculosis* (strain Canetti)

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Abstract

The trisaccharide, 2,3,4-$(OMe)_3$-α-L-Fucp-(1→3)-α-L-Rhap-(1→3)-2-O-Me-α-L-Rhap (**1**, R=lipid), was the phenolic glycolipid fraction of *Mycobacterium tuberculosis* (strain Canetti). The synthesis of this trisaccharide (**2**) as methyl ether has been described. The critical O-glycosylation reaction has been carried out with 1-O-acetylhexoses, promoted by borontrifluoride–etherate to provide a high degree of α-selectivity.

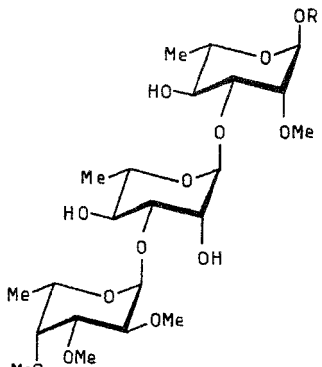
Key words: Trisaccharide, phenolic glycolipid, *Mycobacterium tuberculosis*, synthesis.

1. Introduction

The current level of interest in pathogenic mycobacteria is indicated by the number of publications appearing in this area[1]. Tuberculosis is caused by *Mycobacterium* (M.) *tuberculosis*. Deaths due to tuberculosis are as high as 3 million per year out of 16 million active cases. The increasing incidents of tuberculosis throughout the world including developed countries are now linked to the AIDS disease becoming epidemic[2]. In fact, *M. avium* serotype 4 is observed in majority of patients suffering from AIDS infection[3]. The precise serological identification of mycobacterium species, therefore, becomes tremendously important to detect the infection at an early stage for proper chemotherapy. The phenolic glycolipid of *M. tuberculosis* (strain Canetti) contains a novel trisaccharide unit, the structure of which was established by Daffe *et al*[4] as 2,3,4-tri-O-Me-α-L-Fucp-(1→3)-α-L-Rhap-(1→3)-2-O-Me-α-L-Rhap-(1→lipid) (**1**, R=lipid) based on spectral and chemical studies.

2. Results and discussion

To commence the synthesis of **2**, our first concern was to prepare the disccharide (**10**) containing a free hydroxyl group at 3′ position. Methyl 3-O-allyl-4-O-benzyl-α-L-rhamnopyranoside (**4**) was prepared from methyl α-L-rhamnopyranoside (**3**) in five high-yielding steps[5]. Subsequent hydrolysis of **4** with 3N H_2SO_4 in dioxan at 100°



1 R = Lipid

2 R = Me

gave the hemiacetal which was transformed into the diacetate derivative **5** by using acetic anhydride-pyridine. Compound **5** represents the glycosylating agent.

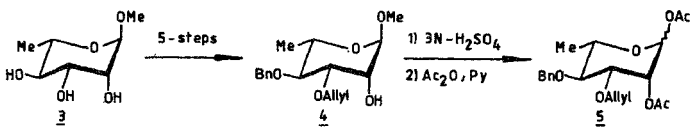
The preparation of methyl 3-O-allyl-4-O-benzyl-2-O-methyl-α-L-rhamnopyranoside (**6**) was earlier described[6], starting from **3**, in this laboratory. In order to remove the allyl group at C-3 the isomerisation of the double bond by using Wilkinson's catalyst and DABCO in refluxing mixture of benzene–ethanol–water was first conducted followed by hydrolysis with mercuric chloride–mercuric oxide in aqueous acetone to give **7**[7]. The condensation of **5** and **7** was promoted by borontrifluoride–etherate[8] in methylene chloride at 0° to give **8**. Deacetylation of **8** under Zemplen condition followed by benzylation with NaH–BnBr in tetrahydrofuran gave **9** whose allyl group at C-3′ position was deprotected by the procedure, described earlier, to furnish **10**.

Finally, the disaccharide **10** and 1-O-acetyl-2,3,4-trio-O-methyl-L-fucopyranose[9] were coupled in the presence of borontrifluoride–etherate to furnish the trisaccharide (**11**). Removal of benzyl groups by hydrogenolysis over 10% palladium-on-charcoal in ethyl acetate at normal temperature and pressure gave the trisaccharide (**2**, R=Me). In the ^{1}H NMR spectrum of **2**, the anomeric protons resonated at δ 4.72 (singlet, **H-1**), 5.04 (singlet, H-1′) and 5.12 (doublet, J=2.9 Hz, H-1″). The anomeric carbons of **2** in a proton decoupled ^{13}C NMR spectrum appeared at δ 97.45 (C-1), 102.06 (C-1′) and 100.72 (C-1′).

3. Experimental

1,2-Di-O-acetyl-3-O-allyl-4-O-benzyl-L-rhamnopyranose (**5**)

A solution of **4** (2.0 g, 6.5 mmol) and 3N H_2SO_4 (20 ml) in dioxane (20 ml) was



SCHEME 1.

heated at 100° for 8 h and then neutralised with solid barium carbonate. The reaction mixture was filtered through celite, washed with dioxane and the combine filtrate concentrated to afford a residue which was stirred with acetic anhydride (5 ml) and pyridine (10 ml) for 18 h. After workup, the residue was purified by column chromatography on silica gel by using ethyl acetate–light petroleum (1:3) to give **5** (2.1 g, 85%); ^{1}H NMR ($CDCl_3$) data: δ 1.28 (d, 3H, J=6.5 Hz, H-6,6′,6″), 2.11, 2.22 (2s, 6H, 2×AcO), 4.72 (ABq, 2H, PhCH_2), 5.20 (m, 2H, CH_2=), 5.72 (m, 1H, CH=), 5.89 (d, 1H, J=1.5 Hz, H-1), 7.30 (s, 5H, Ph).

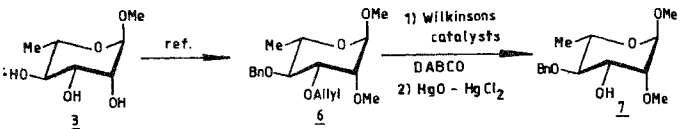
Methyl 4-O-benzyl-2-O-methyl-3-O-(2-O-acetyl-3-O-allyl-4-O-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**8**)

Methyl 3-O-allyl-4-O-benzyl-2-O-methyl-α-L-rhamnopyranoside (**6**) (1.4 g, 4.3 mmol), DABCO (0.38 g), Wilkinson's catalyst (75 mg) in ethanol–benzene–water (7:3:1, 30 ml) were heated under reflux for 10 h, filtered and concentrated. The residue was dissolved in ethyl acetate and successively washed with 1N HCl, $NaHCO_3$ solution, water, dried and concentrated. The residue, mercuric chloride (0.4 g) and mercuric oxide (0.4 g) in 1:1 aqueous acetone (10 ml) were stirred at room temperature for 1 h and filtered. The residue was washed with acetone, the combined filtrate concentrated and then purified on silica gel by using ethyl acetate–light petroleum (1:20) to give **7** (0.9 g, 73%), $[\alpha]_D$ −39° (c 0.5, $CHCl_3$), ^{1}H NMR ($CDCl_3$) data: δ 1.25 (d, 3H J=6.5 Hz, H-6,6′,6″), 3.25 (s, 3H, OMe), 3.45 (s, 3H, OMe), 4.81 (ABq, 2H, PhCH_2), 7.23 (s, 5H, Ph).

To the solution of **7** (0.8 g, 2.8 mmol) and **5** (1.4 g, 3.7 mmol) in methylene chloride (20 ml) under nitrogen at 0° was added freshly distilled borontrifluoride–etherate (100 μl). After 45 min, the reaction was quenched with pyridine (0.5 ml) and then washed with water, dried and concentrated. The residue was chromatographed on silica gel by using ethyl acetate–light petroleum (3:7) to give **8** (0.75 g, 44%), ^{1}H NMR ($CDCl_3$) data: δ 1.31, 1.43 (2s, 6H, 5-CH_3, 5′-CH_3), 2.09 (s, 3H, OAc), 3.39, 3.48 (2s, 6H, 2×OMe), 4.55 (s, 1H, H-1), 4.65 (ABq, 4H, 2×PhCH_2), 5.31 (m, 2H, CH_2=), 5.81 (m, 1H, CH=), 7.31 (s, 10H, 2-Ph).

Methyl 2-O-methyl-4-O-benzyl-3-O-(3-O-allyl-2,4-di-O-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**9**)

To a solution of **8** (0.75 g, 12.5 mmol) in methanol (5 ml), sodium metal (20 mg) was added. After 3 h, the reaction was deionized by Amberlite IR 120 (H) resin,



SCHEME 2.

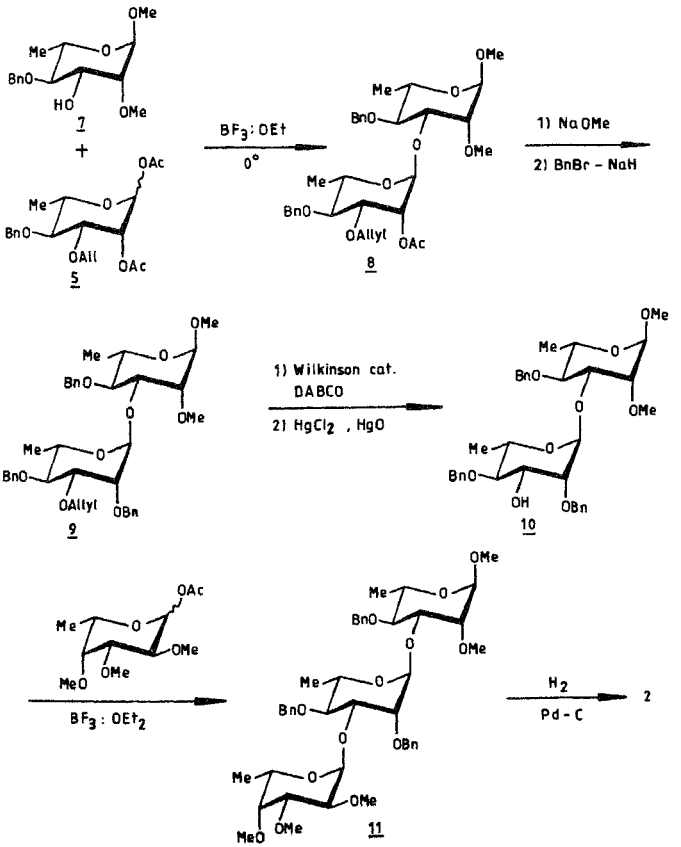
filtered and concentrated. The residue (0.4 g) was dissolved in dry tetrahydrofuran (5 ml) and sodium hydride (0.3 g, 50% dispersion in oil) was added. After 3 h, benzyl bromide (0.5 ml) was added and then the reaction mixture stirred overnight. After workup, the residue was purified by column chromatography on silica gel by using ethyl acetate–light petroleum (1:4) to give **9** (0.4 g, 50%), $[\alpha]_D$ −31.4° (c 0.3, $CHCl_3$), ^1H NMR ($CDCl_3$) data: δ 1.25 (d, 3H, J=6.5 Hz, 5-CH_3), 1.37 (d, 3H, J=6.5 Hz, 5′-CH_3), 3.37, 3.50 (2s, 6H, 2×OMe), 4.81 (s, 1H, H-1), 5.12 (m, 2H, CH_2=), 5.81 (m, 1H, C*H*=), 7.30 (m, 15H, 3×Ph). Anal. Calcd for $C_{38}H_{48}O_9$: C, 70.3; H, 7.4. Found: C, 70.1; H, 7.65.

Methyl 4-O-benzyl-2-O-methyl-3-O-(2,4-di-O-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**10**)

Compound **9** (0.4 g, 0.62 mmol) was deallylated, according to the procedure described for **7**, to give **10** (0.26 g, 70%), $[\alpha]_D$ −28.2 (c 1, $CHCl_3$); ^1H NMR ($CDCl_3$) data: δ 1.25 (d, 3H, J=6.0 Hz, 5-CH_3), 1.37 (d, 3H, J=6.5 Hz, 5′-CH_3), 3.37, 3.43 (2s, 6H, 2×OMe), 5.18 (s, 1H, H-1), 7.2 (m, 15H, 3×Ph).

Methyl 2-O-methyl-3-O-[3-O-(2,3,4-tri-O-methyl-α-L-fucopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (**2**)

To a solution of **10** (0.24 g, 0.4 mmol) and 1-O-acetyl-2,3,4-tri-O-methyl-L-fucopyranose (0.2 g, 0.8 mmol) in methylene chloride (10 ml) at 0°C, borontrifluoride–etherate (12 μl in 0.3 ml of CH_2Cl_2) was added. After 1 h, the reaction mixture was worked up to give a residue which was chromatographed on silica gel by using ethyl acetate–light petroleum (2:3) to give **11** (0.16 g, 50%). It was dissolved in ethyl acetate (10 ml) and 10% palladium-on-charcoal (50 mg) was added. The reaction was stirred under hydrogen atmosphere at normal pressure and temperature for 24 h. The catalyst was filtered and the filtrate concentrated. The residue was purified on silica gel by using chloroform–methanol (19:1) to give **2** (75 mg, 75%), $[\alpha]_D$ −136° (c 0.2, MeOH), ^1H NMR ($CDCl_3$) data: δ 1.28 (d, 3H, J=6.5 Hz, 5-CH_3), 1.32 (d, 3H, J = 6.5 Hz, 5′-CH_3), 1.35 (d, 3H, J = 6.5 Hz, 5″-CH_3), 3.37, 3.48, 3.52, 3.58, 3.61 (5s, 15H, 5×OMe), 4.72 (s, 1H, H-11), 5.03 (s, 1H, H-1′), 5.12 (d, 1H, J=2.9 Hz, H-1″); ^{13}C NMR ($CDCl_3$) data: δ 97.45 (C-1), 102.06 (C-1′), 100.72 (C-1″), CI-Mass spectrum (*m/z*): 526 (M^+). Anal. Calcd for $C_{23}H_{42}O_{13}$: C, 52.5; H, 8.0. Found: C, 52.5; H, 7.9.


OMe
Me
O
BnO
HO
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7
+
OAc
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O
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OAll
OAc
5
BF3 : OEt
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OMe
Me
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BnO
O
OMe
Me
O
BnO
OAllyl
OAc
8
1) NaOMe
2) BnBr - NaH
OMe
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OAllyl
OBn
9
1) Wilkinson cat.
DABCO
2) HgCl2 , HgO
OMe
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O
BnO
O
OMe
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OAc
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OMe
MeO
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11
H2
Pd - C
2

SCHEME 3.

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