ALTERNATIVE SYNTHESIS OF THE END DISACCHARIDE OF THE SPECIFIC PHENOLIC GLYCOLIPID I ANTIGEN FROM MYCOBACTERIUM LEPRAE AND OTHER PARTIALLY MODIFIED DISACCHARIDES

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ABSTRACT

A simpler, more productive variation on the previously described [*Infect. Immun.*, *43*, 245 (1984)] synthesis of the haptenic non-reducing-end disaccharide of phenolic glycolipid I of *Mycobacterium leprae* is described. Benzyl 2, 3-*O*-isopropylidene-4-*O*-(3-*O*-methyl-2, 4-di-*O*-acetyl-6-*O*-trityl-β-D-glucopyranosyl)-α-L-rhamnopyranoside, synthesized as described previously, was de-tritylated and de-isopropylidenated, and methylated with diazomethane/BF_3-etherate to give mostly the benzyl 2, 3-di-*O*-methyl-4-*O*-(2, 4-di-*O*-acetyl-3, 6-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside, but also some benzyl 2, 3-di-*O*-methyl-4-*O*-(2, 6-di-*O*-acetyl-3, 4-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside, due to acetyl migration. Likewise, benzyl 2, 3-*O*-isopropylidene-4-*O*-(2, 4, 6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside upon de-isopropylidenation and methylation yielded benzyl 2, 3-di-*O*-methyl-4-*O*-(2, 4, 6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranosyl)-α-L-rhammopyranoside. Deacetylation and hydrogenolysis of these disaccharide derivatives yielded unblocked disaccharides which are suitable precursors of neoglycoconjugates to be used in the serodiagnosis of leprosy and probing the molecular requirements for antibody binding and immunogenesity.

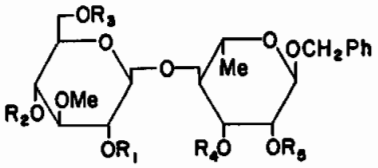
I. INTRODUCTION

In previous papers,[1,2,3] we have reported on the synthesis of the di- and trisaccharide units of the *Mycobacterium leprae* specific phenolic glycolipid I (PGL-I) antigen. Serological analysis of the synthetic compounds showed that only di- and trisaccharides containing the full 3, 6-di-*O*-methyl-β-D-glucopyranosyl terminus were active against human lepromatous leprosy sera.[1] It was also previously demonstrated that the non-reducing end disaccharide, 2, 3-di-*O*-methyl-4-*O*-(3, 6-di-*O*-methyl-β-D-glucopyranosyl)-L-rhamnopyranose, was as active as the entire trisaccharide unit in antibody inhibition[1] and that a neoglycoprotein prepared by reductive amination of the end disaccharide was serologically highly active in direct assays.[4]

The reported synthetic procedures were complicated, and yields of the final products were less than desirable. One of the reasons for inadequate yields was a benzylation step resulting in an intractable emulsion. In order to bypass this step and to simplify the synthetic pathway, methylation of partially acetylated disaccharides using diazomethane/BF_3-etherate, which does not cause acetyl migration or deacetylation,[5] was used. In order to ultimately study the relationship between structure and serological activities, other compounds related to the natural disaccharide were also synthesized by this modified route. Gigg *et al* [6] have also described a highly satisfactory route for synthesis of the non-reducing-end disaccharide.

II. RESULTS AND DISCUSSION

A. Synthesis of 2, 3-di-*O*-methyl-4-*O*-(3, 6-di-*O*-methyl-β-D-glucopyranosyl)-L-rhamnopyranose Using Diazomethane/BF_3-etherate.



1 $R_1 = R_2 = Ac$, $R_3 = Tr$, R_4-$R_5 = I_p$

2 $R_1 = R_2 = Ac$, $R_3 = R_4 = R_5 = H$

3 $R_1 = Ac$, $R_2 = Me$, $R_5 = Ac$, $R_4 = R_5 = Me$

4 $R_1 = R_2 = Ac$, $R_3 = R_4 = R_5 = Me$

5 $R_1 = Ac$, $R_2 = H$, $R_3 = R_4 = R_5 = Me$

6 $R_1 = R_2 = H$, $R_3 = R_4 = R_5 = Me$

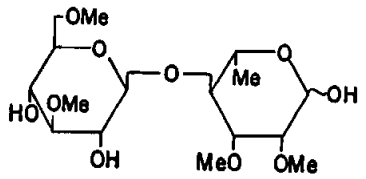
7 $R_1 = H$, $R_2 = Me$, $R_3 = H$, $R_4 = R_5 = Me$

Ac = $COCH_3$, I_p = $>C(CH_3)_2$, Me = CH_3, Ph = $CH_2C_6H_5$, Tr = $C(C_6H_5)_3$

Compound **1**, which was synthesized by the reported procedure,[2] was treated with 60% acetic acid to give the de-tritylated, de-isopropylidenated disaccharide **2** in about 56% yield after purification by silica gel column chromatography. Methylation of **2** with diazomethane using catalytic amounts of BF_3-etherate gave complete methylation but resulted in two products: **3**, R_f 0.64 in benzene-acetone (4:1), 25% yield; **4**, R_f 0.56, 75% yield. These two were separated by silicagel column chromatography. They were hydrolyzed and converted to the alditol acetates and analyzed by GLC/MS using an OV225 S. C. O. T. column. Compound **3** gave two peaks with R_t 0.92 and 4.37 in a ratio of about 1:1. The fragmentation pattern of the peak of R_t 0.92 (m/z 43, 101, 117, 143, 203) was that of a 1, 4, 5-tri-*O*-acetyl-2, 3-di-*O*-methyl-6-deoxyhexitol and that of the R_t 4.37 peak (m/z 43, 87, 129, 189) was of a 1, 2, 5, 6-tetra-*O*-acetyl-3, 4-di-*O*-methylhexitol. Accordingly, **3** was a 3, 4-di-*O*-methylglucosyl-2, 3-di-*O*-methylrhamnoside derivative.[8] It has been reported that acetyl groups do not migrate under the conditions used.[5] However, the results suggest that the acetyl group on the 4-OH migrated to 6-OH. NMR of **3** indicated benzyl 2, 3-di-*O*-methyl-4-*O*-(2, 6-di-*O*-acetyl-3, 4-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside. Compound **4** also gave two alditol acetate peaks with R_t 0.92 and 3.60 in a ratio of about 1:1. The fragmentation pattern of the peak of R_t 0.92 (m/z 43, 117, 143, 203) was that of a 1, 4, 5-tri-*O*-acetyl-2, 3-di-*O*-methyl-6-deoxyhexitol and that of R_t 3.60 (m/z 43, 87, 113, 129, 189, 233) was of a 1, 2, 4, 5-tetra-*O*-acetyl-3, 6-di-*O*-methylhexitol.[7] Thus, **4** was a 3, 6-di-*O*-methylglucosyl-2, 3-di-*O*-methylrhamnoside derivative. NMR of **4** indicated benzyl 2, 3-di-*O*-methyl-4-*O*-(2, 4-di-*O*-acetyl-3, 6-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside.

Treatment of **4** with the usual concentration of sodium methoxide (0.01 M, see EXPERIMENTAL) resulted in incomplete deacetylation, to give **5**. NMR of **5** showed one acetoxyl signal. In order to determine the position of the *O*-acetyl group, **5** was methylated with diazomethane/BF_3-etherate and the alditol acetates prepared. GLC of these showed two peaks, R_t 0.92 and R_t 2.57 in a ratio of about 1:1. The mass fragmentation patterns of the peaks of R_t 0.92 (m/z 43, 101, 117, 143, 203) and R_t 2.57 (m/z 43, 129, 161, 189; hence, 1, 2, 5-tri-*O*-acetyl-3, 4, 6-tri-*O*-methylhexitol) indicated that **5** was the 2-*O*-acetyl derivative of **6**. Treatment with relatively concentrated sodium methoxide (0.1 M) gave the completely deacdtylated compound **6**. Hydrogenolysis of **6** with H_2 in the presence of Pd-C gave **8**, quantitatively.

This synthetic pathway is much simpler and resulted in much better yields than that described previously. Accordingly, this procedure was used for further synthesis.



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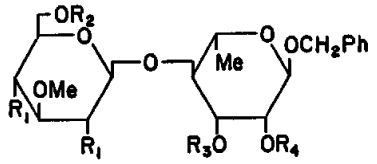
B. Synthesis of the 3-*O*-Methylglucosyl Disaccharide. Previously, we had shown a near-absolute requirement for the 3-*O*-methyl group on the terminal 3, 6-di-*O*-methylglucopyranosyl unit for binding to anti-glycolipid antibodies.[14] In order to eventually examine the role of the 6-*O*-methyl group in antibody recognition, **12** was synthesized.



9 $R_1 = R_2 = Ac$, R_3-$R_4 = I_p$

10 $R_1 = R_2 = Ac$, $R_3 = R_4 = H$

11 $R_1 = R_2 = Ac$, $R_3 = R_4 = Me$

12 $R_1 = R_2 = H$, $R_3 = R_4 = Me$

Mild acid treatment of **9** with 60% acetic acid gave de-isopropylidenated compound **10**, but the yield was less than 25%. However, heating in diluted HCl resulted in preferential loss of the isopropylidene group and better yields. Methylation of **9** with diazomethane/BF_3-etherate gave **11**, and deacetylation with sodium methoxide gave **12**. The NMR spectrum of **12**, showing three OCH_3 signals (3.52 ppm, $1xOCH_3$; 3.44 ppm, $2xOCH_3$), supported the proposed structure. To confirm the positions of the OCH_3 group, **12** was hydrolyzed with 3 M trifluoroacetic acid, the alditol acetates prepared and analyzed by GLC/MS. Two products only were present, R_t 0.92 and R_t 5.96, which were completely coincident with the derivatives of authentic 2, 3-di-*O*-methylrhamnose and 3-*O*-methylglucose, respectively. MS of the peaks of R_t 0.92 and R_t 5.96 showed m/z 43, 101, 117, 143, 203 and m/z 43, 87, 99, 129, 189, 203, respectively. These results were in complete accord with the proposed strtcture of **12**.

Ⅲ. EXPERIMENTAL

A. Benzyl 4-*O*-(2, 4-di-*O*-acetyl-3-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (2).

Compound **1** (33.8 g, including small amounts of trityl alcohol) was dissolved in 500 ml of 60% acetic acid and refluxed for 1.5 hr. The mixture was chilled

and the precipitate removed, poured onto ice-water, extracted with chloroform twice, washed with saturated sodium hydrogen carbonate, and water. It was dried with sodium sulfate, evaporated, and purified by silicagel colum chromatography, to give 12.3 g of **2**. $[\alpha]_D$ -65.70 (c 1.12, chloroform), Rf 0.33 (benzene-acetone, 2:1). Proton NMR ($CDCl_3$, 60 MHz) : δ, 7.45 (s, 5H, $CH_2\phi$), 5.25-4.85 (4 H), 4.84-4.3 (3H), 4.2-3.0 (10H), 3.41 (s, 3H, OMe), 2.1 (6H, 2xOAc), 1.28 (d, 3H, *J* 7.2 Hz, Rha-Me). IR (liquid film) : cm^{-1}, 3750-3200 (s, O-H), 3200-2800 (s, C-H), 1760-1720 (s, C=O), 1500 (w), 1380 (m), 1230 (s, acetyl C-O), 1200-950 (s, broad, C-O-C), 913 (m), 815 (m), 740, 700 (s, monosubstituted benzene).

B. Benzyl 2, 3-di-*O*-methyl-4-*O*-(2, 6-di-*O*-acetyl-3, 4-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (3) and benzyl 2, 3-di-*O*-methyl-4-*O*-(2, 4-di-*O*-acetyl-3, 6-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (4).

Compound **2** (7 g) was dissolved in 120 ml of dry dichloromethane. Boron trifloride-ether complex (0.4 ml) was added at 0 ℃. Dichloromethane-diazomethane was added dropwise at 0 ℃ untill the faint yellow color persisted. After 1.5 hr at 0 ℃, the white precipitate was removed. The filtrate was washed with saturated sodium hydrogen chrbonate, water, then dried and evaporated to a syrup. The syrup was chromatographed on a silicagel colum, to give **3** (1.3 g) and **4** (2.18 g).

Compound **3**, $[\alpha]_D$ - 49.91 (c 1.23, chloroform), Rf 0.64 (benzene-acetone, 4:1). Proton NMR ($CDCl_3$, 60MHz) : δ, 7.35 (s. 5H), 4.96-4.45 (5H), 4.40-4.10 (2H), 3.8-3.1 (7H), 3.52 (s, 6H, 2xOMe), 3.41 (s, 3H, OMe), 3.39 (s, 3H, OMe), 2.11, 2.06 (2s, 6H, 2xOMe), 1.25 (d, 3H, *J* 7.2 Hz, Rha-Me). IR (liquid film) : cm^{-1}, 3150-2750 (s, C-H), 1738 (s, C=O), 1440 (m, aromatic C-C), 1380 (s, aromatic C-C), 1225 (s, acetyl C-O), 1150-1000 (s, broad, C-O-C), 900, 838, 798 (w), 740, (s, monosubstituted benzene).

Compond **4**, $[\alpha]_D$ -52.15 (c 1.185, chloroform), Rf 0.56 (benzene-acetone, 4:1). Proton NMR ($CDCl_3$, 60 MHz) : δ, 7.37 (5H; $CH_2\phi$), 5.2-4.3 (7H), 3.8-3.3 (7H), 3.47, 3.43, 3.39, 3.32 (4s, 12H, 4xOMe), 2.11, 2.08 (2s, 6H, 2xOAc), 1.31 (d, 3H, *J* 7.2 Hz, Rha-Me). IR (liquid film) : cm^{-1}, 3100-2800 (m, C-H), 1750 (s, C=O), 1450 (m), 1375 (m), 1220 (s, acetyl C-O-C), 1150-1000 (s, broad, C-O-C), 905 (w), 840, 800 (w), 735, 700 (m, monosubstituted benzene).

C. Benzyl 2, 3-di-*O*-methyl-4-*O*-(2-*O*-acetyl-3, 6-di-*O*-methyl-β-D-glucopyranosyl-α-L-rhamnopyranoside (5).

Compound **4** (970 mg) was dissolved in 20 ml of dry methanol, and 0.4 M sodium methoxide was added to a final concentration of 0.01 M. The mixture was boiled for 2 min, stood for 40 min at room temperature, neutralized with Amberlite IR 120 (H^+), and evaporated to a syrup. The syrup was purified by silicagel colum chromagraphy, giving **5** (420 mg). $[\alpha]_D$ -58.11 (c 1.25, chloroform), Rf 0.51 (benzene-acetone, 2:1). Proton NMR ($CDCl_3$, 60 MHz) : δ, 7.32 (5H, $CH_2\phi$), 5.0-4.31 (5H). 3.8-3.20 (9H), 3.09 (broad, 1H, OH), 3.49, 3.43,

3.41, 3.28, (4s, 12H, 4xOMe), 2.09 (s, 3H, OAc), 1.28 (d, 3H, *J* 7.2 Hz, Rha-Me). IR (liquid film) : cm^{-1}, 3700 - 3200 (m, O-H), 3100, 2800 (s, C-H), 1745 (s, C=O), 1450 (m), 1370 (m), 1235 (s, acetyl C-O), 1150-1000 (s, broad, 910 (w), 800 (w), 750, 700 (m, monosubstituted benzene).

D. Benzyl 2, 3-di-*O*-methyl-4-*O*-(3, 6-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (6).

Compound **4** (600 mg) was treated as above except that the sodium methoxide concentration was 0.1 M. Silicagel column chromatography gave **6** (470 mg). $[\alpha]_D$ - 64.13 (c 1.01, chloroform). Rf 0.36 (benzene-acetone, 2:1). Rroton NMR ($CDCl_3$, 60 MHz) : δ. 7.35 (5H, $CH_2\phi$), 4.96 (1H), 4.7-4.3 (3H), 3.9-3.3 (12H), 3.60 (s, 6H, 2xOMe), 3.49 (s, 6H, 2xOMe), 1.38 (d, 3H, *J* 7.2 Hz, Rha-Me). IR (liquid film) : cm^{-1}, 3800-3100 (s, O-H), 3100-2800 (s. C-H), 1450 (m), 1380 (w), 1290 (w), 1200 (w), 1270-980 (s, broad, C-O-C), 940, 905, 880. 840. 800 (w), 750, 700 (m, monosubstituted benzene).

E. Preparation of 8 from 6 by hydrogenolysis.

6 (480 mg) was dissoloved in 20 ml of dry ethanol. Palladium-carbon chtalyst (10 %, 100 mg) was added and stirred overnight at 35℃ under hydrogen gas. Filtration and evaporation gave **8** (340 mg). The physical data have already been reported.

F. Benzyl 2, 3-di-*O*-methyl-4-*O*-(3, 4-di-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (7).

Compound **5** (190 mg) was deacetylated as for **6** with 0.1 M sodium methoxide. Silicagel column chromatography gave pure **7** (78.2 mg). $[\alpha]_D$ -42.28 (c 2.60, chloroform), Rf 0.42 (benzene-acetone, 2:1). Proton NMR ($CDCl_3$, 60 MHz) : δ, 7.35 (5H, $CH_2\phi$), 4.95 (1H), 4.7-4.3 (3H), 3.85-320 (12H), 3.68 (s, 6H, 2xOMe), 3.48 (s, 6H, 2xOMe), 1.35 (d, 3H, *J* 7.2 Hz, Rha-Me). IR (liquid film) : cm^{-1}, 3650-3150 (s, O-H), 3100-2800 (s, C-H), 1450 (m), 1380 (w), 1280 (w), 1195 (w), 1270-980 (s, broad. C-O-C), 945. 905, 880, 840, 795 (w), 740, 695 (m, monosubstituted benzene).

G. Benzyl 4-*O*-(2, 4, 6-tri-*O*-acetyl-3-*O*-metbyl-β-D-glucopyranosyl)-α-L-rhamnpyranoside (10).

Compound **9** (220 mg) was refluxed for 45 min in 8 ml of 0.05 M HCl-ethanol (1:1). The mixture was chilled and evaporated with repeating addition of methanol to a syrup. The syrup was purified by silicagel column chromatography, giving **10** (179 mg). $[\alpha]_D$ -22.54 (c 5.98, chloroform), Rf 0.51 (benzene-acetone, 2:1). Proton NMR ($CDCl_3$, 60 MHz) : δ, 7.36 (5H, $CH_2\phi$), 5.3-4.5 (5H), 4.4-4.0 (2H), 4.0-3.2 (7H), 3.41 (s, 3H, OMe), 2.65 (broad, 2H, OH), 2.18, 2.12, 2.09 (3s, 9H, 3xOAc), 1.32 (d, 3H, *J* 7.3 Hz, Rha-Me). IR (liquid film) : cm^{-1}, 3700-3150 (m, O-H), 3100-2800 (m, C-H), 1745 (s, C=O), 1450 (w), 1370 (s), 1230 (s, acetyl C-O), 1120 (w), 1100-1000 (s, broad, C-O-C), 980, 905, 810, 750, 700 (w).

H. Benzyl 2, 3-di-*O*-methyl-4-*O*-(2, 4, 6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (11).

Methylation of **10** (179 mg) with diazomethane-BF_3 was carried out as stated before, the product was purified by silica gel column chromatography, to give **11** (99 mg). $[\alpha]_D$ - 48.60 (c 1.557, chloroform), R_f 0.68 (benzene-acetone, 4:1). Proton NMR ($CDCl_3$, 60 MHz) : δ, 7.35 (5H), 5.2-4.5 (5H), 4.4-3.8 (2H), 3.8-3.3 (7H), 3.45 (s, 6H, 2xOMe), 3.38 (s, 3H, OMe), 2.10 (s, 6H, 2xOAc), 2.06 (s, 3H, OAc), 1.29 (d, 3H, J 7.1 Hz, Rha-Me). IR (liquid film) ; cm^{-1}, 3100-2800 (m. C-H), 1743 (s, C=0), 1450 (w), 1370 (m), 1230 (s, acetyl C-O), 1120 (m), 1100-1000 (s, broad, C-O-C). 975, 905, 895, 840, 800. 745, 700 (w).

1. Benzyl 2, 3-di-*O*-methyl-4-*O*-(3-*O*-methyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (12).

Treatment of **11** (80 mg) with 0.1 M sodium methoxide was carried out as for **6**. Purification was carried out by preparative TLC using silicagel plate, to give **12** (35 mg). $[\alpha]_D$ - 54.99 (c 1.033, methanol), R_f 0.20 (benzene-acetone, 2:1). Proton NMR (CD_3OD. 60 MHz) : δ, 7.32 (5H, $CH_2\phi$), 4.95 (s, 1H), 4.7-4.4 (4H), 3.8-3.4 (7H), 3.62 (s, 3H, OMe), 1.30 (d, 3H, J 7.0 Hz, Rha-Me). IR (liquid film) : cm^{-1}, 3700-3100 (s, O-H), 3030-2800 (m, C-H), 1450 (m), 1390 (w), 1300-950 (s, broad, C-O-C), 910, 880, 840, 800, 750, 700 (w).

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らい菌 *Mycobacterium leprae* に特異的なフェノール性糖脂質Iの末端二糖および類縁体の改良法による合成

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