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CHEMICAL SYNTHESIS OF THE TRISACCHARIDE UNIT OF THE SPECIES-SPECIFIC PHENOLIC GLYCOLIPID FROM *Mycobacterium leprae*

TSUYOSHI FUJIWARA*,
Laboratory of Chemistry, Institute for Natural Science, Nara University, Horai-cho 1230, Nara (Japan)

GERALD O. ASPINALL,
Department of Chemistry, York University, Downsview, Ontario M3J 1P3 (Canada)

SHIRLEY W. HUNTER, AND PATRICK J. BRENNAN
Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523 (U.S.A.)

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ABSTRACT

O-(3,6-Di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-L-rhamnopyranose, the haptenic trisaccharide of the *Mycobacterium leprae*-specific phenolic glycolipid I (PGL-I) antigen, and related trisaccharides, were synthesized by allylation of O-2 of benzyl 4-*O*-benzyl-α-L-rhamnopyranoside using phase-transfer catalysis, methylation of the product, deallylation, and coupling to *O*-(2,4-di-*O*-acetyl-3,6-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-methyl-L-rhamnopyranosyl bromide or related disaccharides. Anomeric mixtures of the trisaccharide derivatives were separated by preparative t.l.c., deacetylated, and hydrogenolyzed, to give the pure trisaccharides. It had already been demonstrated that only those trisaccharides containing an intact, terminal 3,6-di-*O*-methyl-β-D-glucopyranosyl unit are effective in inhibiting the specific binding between PGL-I and anti-PGL-I immunoglobulin M antibodies in human lepromatous leprosy sera.

INTRODUCTION

Mycobacterium leprae and tissue infected by the bacterium contain three species-specific, glycolipid antigens, called[1–6] phenolic glycolipids I, II, and III. Each is based on a group-specific 29-(4-hydroxyphenyl)-3-methoxy-4-methyl-9,11-nonacosanediol 9,11-dimycocerosate and contains trisaccharide entities unique to *M. leprae*. The haptenic trisaccharide of phenolic glycolipid I (PGL-I) is[1,2] *O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-α-L-rhamnopyranose. We have reported the synthesis of *O*-

*To whom correspondence should be addressed.

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(3,6-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-methyl-L-rhamnopyranose[3,7], the disaccharide unit corresponding to the nonreducing end of the hapten. Others have described alternative synthetic routes[8]. PGL-I itself[9–11] and neoglycoproteins containing the monosaccharide and disaccharide units equivalent to its nonreducing end[3,12–15] have found wide acceptance for the specific serodiagnosis of lepromatous leprosy. The success of these simpler neoglycoproteins in enzyme-linked immunoassays had militated against the difficult process of synthesizing neoglycoproteins containing the entire trisaccharide unit. However, we had briefly described[3] the synthesis of the entire triglycosyl unit of PGL-I in the context of establishing the structure of the native glycolipid and, more recently, had observed[15,16] that antibodies in some lepromatous leprosy sera bind primarily to the reducing-end sugar unit of PGL-I rather than to the nonreducing-end glycosyl group. Accordingly, synthesis of the entire trisaccharide unit of PGL-I and corresponding neoglycoprotins became of considerable importance. In this communication, we describe in full the chemical synthesis of the entire trisaccharide unit of PGL-I.

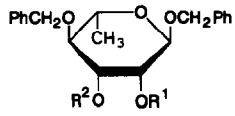
RESULTS AND DISCUSSION

The strategy employed for synthesis of the trisaccharide unit of PGL-I involved condensation of a reducing-end derivative, namely, benzyl 4-*O*-benzyl-3-*O*-methyl-α-L-rhamnopyranoside, and the appropriately derivatized, nonreducing-end disaccharide, described previously[7], *via* the Koenigs–Knorr reaction.

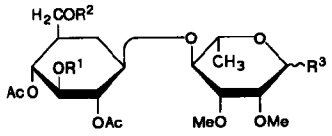
Direct methylation of benzyl 4-*O*-benzyl-α-L-rhamnopyranoside (**1**) with limiting amounts of CH_3I gave mainly the 2-*O*-methylated compound[17]. Therefore, O-2 was temporarily protected by an allyl group, which can be preferentially introduced at this position and then readily removed after methylation of O-3.

The reaction, under phase-transfer conditions[16,18], of **1**, allyl bromide, tetrabutylammonium bromide in dichloromethane, and aqueous sodium hydroxide, yielded the 2,3-di-*O*-allyl derivative (**2**, 5% of the mixture), the 2-*O*-allyl-L-rhamnose derivative (**3**, 70%), the 3-*O*-allyl compound (**4**, 15%), unreacted material (7%), and minor, unidentified materials. Compound **3** was readily purified by chromatography on a column of silica gel. The ^{1}H-n.m.r. spectrum of **3** showed a double triplet at 3.99 p.p.m. ($J_{2,3}$ 3.8, $J_{3,4}$ 9.4, and $J_{3,OH}$ 9.4 Hz), which, by D_2O treatment, was changed to a quartet with one large J value ($J_{3,4}$ 9.4 Hz) and one small J value ($J_{2,3}$ 3.7 Hz), a feature diagnostic of OH-3.

Methylation of **3** with CH_3I in the presence of Ag_2O, and chromatography of the reaction mixture on a column of silica gel resulted in isolation of the 3-*O*-methylated compound **5** as a syrup. Short-term treatment of **5** with potassium *tert*-butoxide in dimethyl sulfoxide caused quantitative rearrangement of the allyl group to the 1-propenyl group to give **6**. Hydrolysis of **6** by action of HCl gave compound **7**. The ^{1}H-n.m.r. spectrum of **7** was in complete accord with the structure of benzyl 4-*O*-benzyl-3-*O*-methyl-α-L-rhamnoside. To confirm the position of the OMe



	R^1	R^2
1	H	H
2	$-CH_2-CH=CH_2$	$-CH_2-CH=CH_2$
3	$-CH_2-CH=CH_2$	H
4	H	$-CH_2-CH=CH_2$
5	$-CH_2-CH=CH_2$	Me
6	$-CH=CH-CH_3$	Me
7	H	Me



	R^1	R^2	R^3
8	Me	Me	OAc
9	Ac	Me	OAc
10	Ac	Ac	OAc
11	Me	Me	Br
12	Ac	Me	Br
13	Ac	Ac	Br

group, **7** was debenzylated, converted into the alditol acetate, and this analyzed by g.l.c.–m.s. in an OV-225 column. The single peak at R_T 1.62 produced fragment ions at *m/z* 203, 189, 143, 129, 101, 87, and 43, namely, those of a 3-*O*-methylated rhamnoside[19].

Acetylated disaccharide **8** (ref. 7) was treated with titanium tetrabromide to give the 1-bromide (**11**); however, t.l.c. indicated that only ~50% of the product had been converted into the bromide. Its i.r. and n.m.r. spectra suggested that the rest consisted of products arising by degradation of the OH-1 disaccharide. Presumably, **11** is unstable because of the presence of the *O*-methyl group adjacent to the anomeric center; attempted purification exacerbated the degradation process. Similar problems arose with **12** and **13**. Accordingly, the coupling reaction was applied to the disaccharide derivatives without attempting their purification.

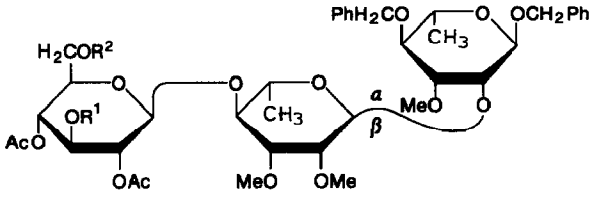
Compound **7** was stirred with bromide **11** in acetonitrile in the presence of mercury cyanide, the products were charged onto a column of silica gel, and the trisaccharide, among other fractions, was eluted with 5:1 benzene–ethyl acetate. T.l.c. of the purified trisaccharide in 4:1 benzene–acetone showed a single spot, and the ^{1}H-n.m.r. spectrum at 60 MHz showed signals for two phenyl, five methoxyl, two acetyl, and two methyl groups. These results indicated that the purified product was the expected trisaccharide. However, the 400-MHz n.m.r. spectrum was unexpected, in that it showed 10 methoxyl and 4 acetyl group signals. In addition, the ^{13}C-n.m.r. spectrum showed four anomeric-carbon signals (96.61, 97.98, 98.30, and 99.61 p.p.m.), in about equal amounts, and an additional anomeric-carbon signal (100.89 p.p.m.) in about twice the amount. The ^{13}C–H coupled spectrum showed coupling constants of ~174 Hz for the signals at 96.61, 97.98, and 98.30 p.p.m.; 158 Hz for that at 99.61 p.p.m.; and 166 Hz for that at 100.89 p.p.m. These results clearly indicated that the purified trisaccharide fraction was a mixture of anomers at the Rha–Rha linkage, in the ratio of ~1:1. Accordingly, preparative t.l.c. in 8:1 dichloromethane–acetone, which, of the

many solvents tested offered the best separation, was used to resolve the α,β mixture. The outcome was that the pure trisaccharide derivatives **14** (R_F 0.72) and **15** (R_F 0.60) were obtained.

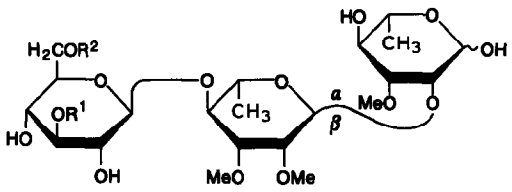
The 360-MHz ^{1}H-n.m.r. spectrum of **14** showed an anomeric signal at 4.39 p.p.m. (singlet) clearly due to a β-L-rhamnoside. The ^{13}C–H coupled n.m.r. spectrum showed three anomeric signals, with coupling constants of 166.3 (100.90 p.p.m., C-1 of 3,6-di-*O*-Me-β-D-Glc*p*), 158.1 (99.61 p.p.m., C-1 of 2,3-di-*O*-Me-β-L-Rha*p*), and 167.1 Hz (96.61 p.p.m., C-1 of 3-*O*-Me-α-L-Rha*p*). These results indicated that **14** was the trisaccharide derivative having the β configuration at the Rha–Rha linkage[20].

The ^{1}H-n.m.r. spectrum of **15** showed an anomeric signal at 5.15 p.p.m. due to 2,3-di-*O*-methyl-α-L-rhamnoside, in addition to a signal at 4.79 p.p.m. attributed to the 3-*O*-methyl-α-L-rhamnoside. The ^{13}C–H coupled n.m.r. spectrum of **15** showed three anomeric signals, at 100.89, 98.30, and 97.50 p.p.m., with respective coupling constants of 164.6 (C-1 of 3,6-di-*O*-Me-β-D-Glc*p*), 168.6 (2,3-di-*O*-Me-α-L-Rha*p*), and 173.8 Hz (3-*O*-Me-α-L-Rha*p*). These results indicated that **15** was the trisaccharide derivative having the desired α configuration at the Rha–Rha linkage. In the ^{1}H-n.m.r. spectrum of **15**, the C–CH_3 signal of 2,3-di-*O*-Me-Rha was observed at a field (1.18 p.p.m.) higher than that of the corresponding signal of **14** (1.27 p.p.m.), which is as expected[21]. The H-5 signal of 2,3-di-*O*-Me-Rha was at 3.18 p.p.m. for **14**, but, for **15**, such a signal was not obvious, and was apparently hidden within the large signals lower than 3.3 p.p.m. All of these data support the proposed structures of **14** and **15**.

The coupling reaction was also applied to **7** and **12**, to yield an anomeric mixture of the corresponding trisaccharide derivatives **16** and **17** in the ratio of ~1:1. These were also separated into the individual anomers by preparative t.l.c.



	R^1	R^2	Rha-Rha link
14	Me	Me	β
15	Me	Me	α
16	Ac	Me	β
17	Ac	Me	α
18	Ac	Ac	β
19	Ac	Ac	α



	R^1	R^2	Rha-Rha link
20	Me	Me	β
21	Me	Me	α
22	H	Me	β
23	H	Me	α
24	H	H	β
25	H	H	α

Likewise, coupling of **7** and **13** yielded a mixture composed of **18** and **19**. These were also successfully resolved.

Each of the trisaccharides (**14–19**) was deacetylated with sodium methoxide, and the product hydrogenolyzed in the presence of Pd–C, to give the corresponding free trisaccharides (**20–25**). These, in turn, were purified by preparative t.l.c. in 60:10:1 $CHCl_3$–CH_3OH–H_2O, and the purity of each was confirmed by analytical t.l.c. The ^{1}H-n.m.r. spectrum of **21** showed three anomeric protons at δ 5.09 (*J* 1.7 Hz, 2,3-di-*O*-Me-α-L-Rha*p*), 5.04 (*J* 1.7 Hz, 3-*O*-Me-α-L-Rha*p*), and 4.53 (*J* 7.8 Hz, 3,6-di-*O*-Me-β-D-Glc*p*), confirming that **21** was *O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-L-rhamnopyranose, the haptenic trisaccharide unit of the most prominent of the family of phenolic glycolipids present in *M. leprae*.

Preparation of the other, related trisaccharides was also accomplished. However, yields from the final steps were poor (~30–40% as the anomeric mixture), due mainly to the instability of the disaccharide bromide; the presence of a methyl group at O-2 of the disaccharide bromide appears to facilitate greatly the hydrolysis to the OH-1 compound, and the lack of neighboring-group assistance probably results in poor stereoselectivity.

The efficacy of the various trisaccharides (**20–25**) described herein in inhibiting binding between PGL-I and anti-PGL-I immunoglobulin M antibodies in lepromatous leprosy sera has already been described[3]. Only those (**20** and **21**) containing the methyl substituent at O-3 and O-6 of the terminal D-glucopyranosyl unit are active. Accordingly, neoglycoproteins containing just the 3,6-di-*O*-methyl-β-D-glucopyranosyl terminal unit[13], a modified nonreducing-end disaccharide, namely, *O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-rhamnopyranose[13], the full nonreducing-end disaccharide[14,15], and the trisaccharide hapten itself[16] are now being used extensively for the serodiagnosis of leprosy.

EXPERIMENTAL

*Benzyl 2,3-di-*O*-, 2-*O*-, and 3-*O*-allyl-4-*O*-benzyl-α-*L*-rhamnopyranoside* (**2**, **3**, *and* **4**). — Compound **1** (3.59 g), allyl bromide (1.68 mL), tetrabutylammonium bromide (0.55 g), 10% aq. sodium hydroxide (10.5 mL), and dichloromethane (105 mL) were shaken at room temperature. Several kinds of ammonium salt (tetrabutylammonium chloride, tetraethylammonium bromide, and lauryltrimethylammonium bromide) were tested in the reaction, but tetrabutylammonium bromide afforded the highest yields of the 2-*O*-allyl derivative. After shaking for 2 d, the organic layer was washed twice with water, and evaporated. The residue consisted of the 2,3-di-*O*-allyl (**2**, 5%), 2-*O*-allyl (**3**, 70%), and 3-*O*-allyl (**4**, 15%) derivatives, and the starting material (**1**). Chromatography on a column of Florisil gave **2** (130 mg), **3** (2.3 mg), **4** (3.82 mg), and unreacted **1** (215 mg). Compound **2**, R_F 0.85 (4:1 benzene–acetone), was not examined further.

Compound **3**, $[\alpha]_D^{20}$ −55.6° (*c* 0.90, chloroform); R_F 0.79 (4:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.40–7.23 (10 H, 2 *Ph*CH_2), 5.92 (m, 1 H, C*H*=CH_2), 5.25 (m, 2 H, OCH_2–CH), 4.93, 4.90, 4.71, 4.68, 4.65 (4 H, 2 CH_2Ph), 4.89 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.20–4.13 (2 H, CH=CH_2), 3.99 (dd, 1 H, $J_{3,4}$ 9.4, $J_{2,3}$ 3.8, $J_{3,OH}$ 9.4 Hz, H-3), 3.73 (dq, 1 H, $J_{5,6}$ 6.3, $J_{4,5}$ 9.5 Hz, H-5), 3.69 (q, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.8 Hz, H-2), 3.30 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 2.36 (broad d, 1 H, $J_{OH,3}$ 9.4 Hz, O*H*), and 1.33 (d, 3 H, $J_{5,6}$ 7.3 Hz, Rha-Me); ^{13}C-n.m.r. ($CDCl_3$, 90 MHz): δ 138.93, 137.58 (2 C, Ph, α-C), 135.11 (–*C*H=), 128.43–127.63 (Ph), 117.53 (=*C*H_2), 97.40 (C-1), 81.87, 80.69, 75.35, 74.60, 72.25, 69.14, 68.17 (7 C), 57.81 (O*C*H_3), and 17.94 (CH_3–C); ν_{max}^{film} 3600–3300 (s, OH), 3100–2850 (C–H), 1970, 1880, 1820, 1730 (monosubstituted benzene), 1655 (m, C=CH_2), 1515, 1472 (m, aromatic C–C), 1405, 1380 (m), 1240 (w), 1230 (m), 1200–1000 (s, broad), 950 (m), 835 (m), 780, 770, and 730 cm^{-1} (s, monosubstituted benzene).

Anal. Calc. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.92; H, 7.46.

Compound **4** had $[\alpha]_D^{20}$ −47.0° (*c* 0.699, chloroform); R_F 0.62 (4:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 60 MHz): δ 7.40–7.20 (10 H, CH_2*Ph*), 6.37–5.53 (m, 1 H, C*H*=CH_2), 5.43–4.90 (m, 2 H, OCH_2–CH), 4.83 (d, 1 H, *J* <2 Hz, H-1), 4.85–4.43 (4 H, 2 CH_2Ph), 4.30–3.90 (3 H, CH=CH_2, H-3), 3.86–3.15 (3 H, H-2,4,5), 2.9 (broad, 1 H, O*H*), and 1.32 (d, 3 H, $J_{5,6}$ 6.3 Hz); ν_{max}^{film} 3600–3200 (s, OH), 3105–2830 (C–H), 1960, 1880, 1825, 1735 (w, monosubstituted benzene), 1655 (m, C=CH_2), 1515, 1473 (aromatic C–C), 1405, 1380 (w), 1235 (m), 1200–975 (s, broad, C–O–C), 950 (m), 835 (m), 773, and 735 cm^{-1} (s, monosubstituted benzene).

Anal. Calc. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.98; H, 7.51.

*Benzyl 2-*O*-allyl-4-*O*-benzyl-3-*O*-methyl-α-*L*-rhamnopyranoside* (**5**). — Compound **3** (4.7 g) and freshly prepared silver oxide (total, 29.8 g), added at four 30-min intervals, were refluxed in CH_3I (40 mL) for 6 h. The mixture was cooled and filtered, and the filtrate evaporated to a syrup. The residue was purified by chromatography on a column of silica gel, giving the 2-*O*-allyl-3-*O*-methylated com-

pound **5** (3.8 g); $[\alpha]_D^{20}$ −50.2° (*c* 1.30, chloroform); R_F 0.59 (49:1 benzene–methanol); *m/z* of the alditol acetate, 43, 87, 101, 129, 189, and 203; ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.50–7.25 (10 H, 2 *Ph*CH_2), 6.0–5.85 (m, 1 H, OCH_2 *CH*=CH_2), 5.30–5.15 (m, 2 H, OCH_2–CH), 4.92, 4.90, 4.70, 4.67, 4.62, 4.59, 4.46, 4.43 (4 H, 2 CH_2Ph), 4.88 (s, 1 H, H-1), 4.15 (m, 2 H, CH=CH_2), 3.80 (t, 1 H, $J_{1,2}$ = $J_{2,3}$ = 2.4 Hz, H-3), 3.73 (o, 1 H, $J_{5,6}$ 6.7, $J_{4,5}$ 9.5 Hz, H-5), 3.63 (q, 1 H, $J_{2,3}$ 2.6, $J_{3,4}$ 9.7 Hz), 3.49 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 3.48 (s, 3 H, OMe-3), and 1.32 (d, 3 H, $J_{5,6}$ 6.7 Hz, Rha-Me); ν_{max}^{film} 3100–2800 (C–H), 1960, 1880, 1820 (monosubstituted benzene), 1740 (m), 1655 (w, CH=CH_2), 1615 (w), 1513, 1470 (m, aromatic C–C), 1400 (m), 1225 (m), 1150–980 (s, broad, C–O–C), 955, 945 (m), 830 (m), 780, 765, and 730 cm^{-1} (s, monosubstituted benzene).

Anal. Calc. for $C_{24}H_{30}O_5$: C, 72.34; H, 7.59. Found: C, 72.51; H, 7.44.

Benzyl 4-O-benzyl-3-O-methyl-2-O-(1-propenyl)-α-L-rhamnopyranoside (**6**). — To a solution of compound **5** (3.5 g) in dry dimethyl sulfoxide (70 mL) was added potassium *tert*-butoxide (1 g), and the mixture was stirred for 1 h at room temperature, diluted with ice–water, extracted four times with ether, and the extracts combined, washed with water four times, dried and evaporated. The residue was purified on a column of silia gel, to give **6** (2.6 g); $[\alpha]_D^{20}$ −54.5° (*c* 1.56, chloroform); R_F 0.75 (20:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.37–7.2 (10 H, 2 CH_2*Ph*), 6.00–5.95 (m, 1 H, OCH=C*H*–), 4.93, 4.90, 4.70, 4.67, 4.64, 4.61, 4.46, 4.43 (4 H, 2 CH_2Ph), 4.87 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.49 (t, 1 H, *J* 6.3 Hz, C*H*–CH_3), 4.00 (dd, 1 H, $J_{1,2}$ 1.78, $J_{2,3}$ 3.0 Hz, H-2), 3.76 (dq, 1 H, $J_{5,6}$ 6.2, $J_{4,5}$ 9.3 Hz, H-5), 3.68 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.3 Hz, H-3), 3.52 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 1.65 (dd, 3 H, *J* 1.7, 6.9 Hz, HC=CH–CH_3), and 1.32 (d, 3 H, $J_{5,6}$ 6.1 Hz, Rha–Me); ν_{max}^{film} 3100–2800 (C–H), 1670 (s, CH=CH–CH_3), 1510, 1460 (m, aromatic C–C), 1400 (m), 1270 (m), 1200–970 (s, broad, C–O–C), 935 (m), 823 (m), 775, 760, and 725 cm^{-1} (s, monosubstituted benzene).

Anal. Calc. for $C_{24}H_{30}O_5$: C, 72.34; H, 7.59. Found: C, 72.39; H, 7.66.

Benzyl 4-O-benzyl-3-O-methyl-α-L-rhamnopyranoside (**7**). — Compound **6** (2.5 g, including a small amount of dimethyl sulfoxide) in 9:1 acetone–1.0M HCl (30 mL) was refluxed for 30 min. The acid was neutralized with saturated sodium hydrogencarbonate, the mixture evaporated, and extracted with chloroform, and the extracts were combined, washed with water, dried, and evaporated. The residue was chromatographed on a column of Florisil, to give **7** (1.92 g); $[\alpha]_D^{24}$ −60.8° (*c* 2.77, chloroform); R_F 0.32 (10:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.36–7.24 (10 H, 2 *Ph*CH_2), 4.90 (d, 1 H, $J_{1,2}$, 1.4 Hz, H-1), 4.86, 4.83, 4.70, 4.67, 4.63, 4.60, 4.48, 4.43 (4 H, 2 CH_2Ph), 4.07 (q, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 3.3 Hz, H-2), 3.78 (dq, 1 H, $J_{4,5}$ 9.6, $J_{5,6}$ 6.3 Hz, H-5), 3.60 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.1 Hz, H-3), 3.47 (s, 3 H, OMe-3), 3.40 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 2.61 (broad s, 1 H, D_2O exchangeable, O*H*), and 1.31 (d, 3 H, $J_{5,6}$ 6.3 Hz, Rha-Me); ^{13}C-n.m.r. ($CDCl_3$, 90 MHz): δ 138.49, 137.26 (2 C, Ph, α-C), 128.36–128.01 (Ph), 98.37 (C-1), 81.80, 81.72, 79.97, 79.94, 75.20, 69.05 (6 C), 57.20 (OCH_3), and 17.95 (C–CH_3); ν_{max}^{film} 3660–3150 (s, OH), 3100–1800 (C–H), 1860, 1875, 1820, 1760 (w, mono-

substituted benzene), 1510, 1470 (m, aromatic C–C), 1400 (m), 1230 (m), 1200–1040 (s, broad, C–O–C), 1008 (m), 940 (m), 830 (m), 780, 770, and 730 cm^{-1} (s, monosubstituted benzene).

Anal. Calc. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.51; H, 7.49.

Benzyl O-(2,4-di-O-acetyl-3,6-di-O-methyl-β-D-glucopyranosyl)-(1→4)-O-(2,3-di-O-methyl-β-L-rhamnopyranosyl)-(1→2)-4-O-benzyl-3-O-methyl-α-L-rhamnopyranoside (**14**) *and benzyl O-(2,4-di-O-acetyl-3,6-di-O-methyl-β-D-glucopyranosyl)-(1→4)-O-(2,3-di-O-methyl-α-L-rhamnopyranosyl)-(1→2)-4-O-benzyl-3-O-methyl-α-L-rhamnopyranoside* (**15**). — To a solution of acetate **8** (516 mg), synthesized by a reported procedure[7], in 10:1 dichloromethane–ethyl acetate (7.7 mL), was added titanium tetrabromide (440 mg), and the mixture was stirred for 1.5 h at room temperature. Acetonitrile (13.6 mL) followed by sodium acetate (1.4 g) was then added. After 10 min, toluene (20 mL) was added, the mixture was filtered through a layer of Celite, and the filtrate evaporated to a syrup (500 mg). T.l.c. showed the syrup to consist of ~50% of bromide **11** and ~50% of the OH-1 compound. Compound **11**: ^{1}H-n.m.r. ($CDCl_3$, 60 MHz): δ 6.53 (s, 1 H, H-1).

To the syrup were added acetonitrile (4 mL), **7** (250 mg), and mercury cyanide (75 mg), and the mixture was stirred overnight at room temperature, evaporated, and the residue extracted with chloroform; the extracts were combined, successively washed with M potassium bromide, saturated sodium hydrogencarbonate, and water, and evaporated to a syrup which was charged onto a column of silica gel. Elution with 5:1 benzene–ethyl acetate yielded the trisaccharide fraction (**14** and **15**) as a syrup (177 mg). Elution with 1:1 benzene–ethyl acetate then gave 192 mg of the hydrolyzed dimer (OH-1 compound). This was acetylated and the acetate processed as before, giving a further 75 mg of the trisaccharide fraction (**14** and **15**).

T.l.c. of the trisaccharide fraction showed only one product (R_1 0.48) in 4:1 benzene–acetone; however, two products were observed in 8:1 dichloromethane–acetone. The bulk of the trisaccharide-containing fraction (177 mg) was separated into two pure compounds, **14** (β anomer, 32.8 mg) and **15** (α anomer, 16 mg), by preparative t.l.c.

Compound **14**: R_F 0.72 (8:1 dichloromethane–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.37–7.28 (10 H, 2 *Ph*CH_2), 4.93 (t, 1 H, *J* 10.5 Hz), 4.90, 4.89, 4.88, 4.86 (2 H), 4.85 (d, 1 H, *J* 1.8 Hz, H-1), 4.76, 4.74, 4.73, 4.70, 4.59, 4.56, 4.50, 4.47 (4 H, 2 C*H*$_2$Ph), 4.39 (s, 1 H, H-1), 4.16 (dd, 1 H, *J* 2.3, *J* 2.4 Hz), 3.80–3.60 (4 H), 3.69 (s, 3 H, OMe-3), 3.55–3.25 (5 H), 3.46 (s, 3 H, OMe-3′), 3.42 (s, 3 H, OMe-2′), 3.38 (s, 3 H, OMe-6″), 3.32 (s, 3 H, OMe-3″), 3.18 (dq, 1 H, *J* 9.15, *J* 6.1 Hz, H-5 or H-5′), 3.08 (dd, 1 H, *J* 3.2, *J* 5.9 Hz), 2.10, 2.08 (2 s, 6 H, 2 AcO), 1.33 (d, 3 H, *J* 6.1 Hz, Rha-Me), and 1.27 (d, 3 H, *J* 6.3 Hz, Rha-Me); ^{13}C-n.m.r. ($CDCl_3$, 90 MHz): δ 100.90 (*J* 166.3 Hz, C-1″, β), 99.61 (*J* 158.1 Hz, C-1′, β), and 96.61 (*J* 167.1 Hz, C-1, α).

Anal. Calc. for $C_{41}H_{58}O_{16}$: C, 61.03; H, 7.24. Found: C, 61.35; H, 7.31.

Compound **15**: R_F 0.60 (8:1 dichloromethane–acetone); ^{1}H-n.m.r. ($CDCl_3$,

360 MHz): δ 7.36–7.26 (10 H, 2 CH_2Ph), 5.15 (d, 1 H, $J_{1',2'}$ 1.2 Hz, H-1'), 4.89 (t, 1 H, *J* 9.4), 4.95–4.85 (2 H), 4.79 (d, 1 H, *J* 1.4 Hz, H-1), 4.77, 4.75 (1 H), 4.70, 4.65, 4.62, 4.49, 4.47 (3 H, CH_2Ph + 1 H), 4.10 (dd, 1 H, *J* 2.3, 2.4 Hz), 3.74 (dq, 1 H, *J* 6.2, 9.26 Hz), 3.63–3.30 (10 H), 3.49, 3.43, 3.42 (3 s, 9 H, OMe-3, OMe-2' and OMe-3'), 3.39 (s, 3 H, OMe-3''), 3.33 (s, 3 H, OMe-6''), 2.14, 2.08 (2 s, 6 H, 2 AcO), 1.32 (d, 3 H, *J* 6.5 Hz, Rha-Me), and 1.18 (d, 3 H, *J* 6.4 Hz, Rha-Me); ^{13}C-n.m.r. ($CDCl_3$, 90 MHz): δ 100.89 (*J* 164.6 Hz, C-1'', β), 98.30 (*J* 168.6 Hz, C-1', α), and 97.50 (*J* 173.8 Hz, C-1, α).

Anal. Calc. for $C_{41}H_{58}H_{16}$: C, 61.03; H, 7.24. Found: C, 61.26; H, 7.14.

Benzyl O-*(2,3,4-tri*-O-*acetyl-6*-O-*methyl-β*-D-*glucopyranosyl)-(1→4)*-O-*(2,3-di*-O-*methyl-β*-L-*rhamnopyranosyl)-(1→2)-4*-O-*benzyl-3*-O-*methyl-α*-L-*rhamnopyranoside* (**16**) *and benzyl* O-*(2,3,4-tri*-O-*acetyl-6*-O-*methyl-β*-D-*glucopyranosyl)-(1→4)*-O-*(2,3-di*-O-*methyl-α*-L-*rhamnopyranosyl)-(1→2)-4*-O-*benzyl-3*-O-*methyl-α*-L-*rhamnopyranoside* (**17**). — Compound **9** (853 mg), which was synthesized by the reported procedure[7], was brominated as for **8**, to give bromide **12** (772 mg). Compounds **12** (772 mg) and **7** (520 mg) were dissolved in acetonitrile (8 mL) and stirred overnight at room temperature in the presence of mercury cyanide (100 mg). The mixture was processed as for (**14, 15**), and the residual syrup purified by chromatography on a column of silica gel to give the anomeric mixture (332 mg). The reaction sequence described under (**14** and **15**) was then applied to the recovered OH-1 disaccharide, to afford a further 53 mg of the anomeric mixture (**16** and **17**). Preparative t.l.c. (230 mg) gave **16** (33 mg) and **17** (28 mg).

Compound **16**: $[\alpha]_D^{20}$ −8.9° (*c* 0.29, chloroform); R_F 0.70 (8:1 dichloromethane–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.37–7.27 (10 H, 2 *Ph*CH_2), 5.15 (t, 1 H, *J* 9.1 Hz), 5.02 (t, 1 H, *J* 9.7 Hz), 4.93, 4.91, 4.90, 4.88, 4.87, 4.85, 4.84 (4 H), 4.73, 4.70, 4.59, 4.54, 4.50, 4.46 (3 H, CH_2Ph + 1 H), 4.39 (s, 1 H, H-1), 4.16 (dd, 1 H, *J* 2.3, *J* 2.9 Hz), 3.80–3.25 (8 H), 3.69 (s, 3 H, OMe-3), 3.46 (s, 3 H, OMe-3'), 3.40 (s, 3 H, OMe-2'), 3.33 (s, 3 H, OMe-6''), 3.18 (dd, 1 H, $J_{4',5'}$ 9.7, $J_{5',6'}$ 6.8 Hz, H-5'), 3.08 (dd, 1 H, *J* 9.7, *J* 3.9 Hz), 2.03, 2.01, 1.99 (3 s, 9 H, 3 AcO), 1.33 (d, 3 H, *J* 6.1 Hz, Rha–Me), and 1.26 (d, 3 H, *J* 6.1 Hz, Rha–Me).

Anal. Calc. for $C_{42}H_{58}O_{17}$: C, 60.42; H, 7.00. Found: C, 60.37; H, 6.96.

Compound **17**: $[\alpha]_D^{20}$ −58.1° (*c* 0.24, chloroform); R_F 0.65 (8:1 dichloromethane–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.37–7.27 (10 H, 2 *Ph*CH_2), 5.22–5.12 (2 H), 5.14 (d, 1 H, $J_{1',2'}$ 1.1 Hz, H-1'), 5.06 (t, 1 H, *J* 10.0 Hz), 4.90–4.79 (2 H), 4.88 (d, 1 H, $J_{1'',2''}$ 1.4 Hz, H-2''), 4.69, 4.66, 4.63, 4.50, 4.46 (3 H, CH_2Ph + 1 H), 4.10 (dd, 1 H, *J* 2.76, *J* 2.4 Hz), 3.75–3.30 (10 H), 3.51, 3.48, 3.46 (3 s, 9 H, OMe-3,-2',-3'), 3.33 (s, 3 H, OMe-6''), 2.08, 2.03, 2.00 (3 s, 9 H, 3 AcO), 1.32 (d, 3 H, *J* 6.1 Hz, Rha–Me), and 1.18 (d, 3 H, *J* 6.8, Rha–Me).

Anal. Calc. for $C_{42}H_{58}O_{17}$: C, 60.42; H, 7.00. Found: C, 60.61; H, 6.97.

Benzyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl)-(1→4)*-O-*(2,3-di*-O-*methyl-β*-L-*rhamnopyranosyl)-(1→2)-4*-O-*benzyl-3*-O-*methyl-α*-L-*rhamnopyranoside* (**18**) *and benzyl* O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl)-(1→4)*-O-*(2,3-di-*

O-methyl-α-L-rhamnopyranosyl)-(1→2)-4-O-benzyl-3-O-methyl-α-L-rhamnopyranoside (**19**). — Compound **10** (320 mg), synthesized by a reported procedure[7], was brominated as for **8**, to give bromide **13** (256 mg). A solution of this and **7** (160 mg) in acetonitrile (2 mL) containing 35 mg of mercury cyanide was stirred overnight at room temperature. The product was chromatographed on a column of silica gel, to give an anomeric mixture of trisaccharide derivatives **18** and **19** (175 mg). Application of the reaction sequence described under (**14** and **15**) to the recovered OH-1 dimer gave a further 26 mg of the mixture. Preparative t.l.c. (450 mg) gave pure **18** (80 mg) and **19** (37 mg).

Compound **18**: $[\alpha]_D^{20}$ −8.7° (*c* 0.24, chloroform); R_F 0.75 (8:1 dichloromethane–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.36–7.28 (10 H, 2 *Ph*CH_2), 5.16 (t, 1 H, *J* 9.2 Hz), 5.00 (t, 1 H, *J* 9.9 Hz), 4.95, 4.93, 4.89, 4.82 (2 H), 4.90 (d, 1 H, *J* 1.4 Hz, H-1′), 4.85 (d, 1 H, *J* 1.8 Hz, H-1), 4.75, 4.70, 4.59, 4.56, 4.50, 4.46 (3 H, C*H*$_2$Ph + 1 H), 4.40 (s, 1 H, H-1′), 4.22–4.04 (3 H), 3.80–3.30 (6 H), 3.69 (s, 3 H, (OMe-3), 3.46, 3.40 (2 s, 6 H, OMe-2′,-3′), 3.19 (dq, 1 H, $J_{5',6'}$ 5.25, $J_{4',5'}$ 10.00 Hz), 3.08 (dd, 1 H, *J* 4.5, *J* 9.3 Hz), 2.08, 2.06, 2.03, 2.00 (4 s, 12 H, 4 AcO), 1.32 (d, 3 H, *J* 6.2 Hz, Rha-Me), and 1.27 (d, 3 H, *J* 6.3 Hz, Rha–Me); ^{13}C-n.m.r. ($CDCl_3$, 90 MHz): δ 100.4 (*J* 166.2 Hz, C-1″, β), 99.6 (*J* 158.1 Hz, C-1′, β), and 97.9 (*J* 173.8 Hz, C-1, α).

Anal. Calc. for $C_{43}H_{58}O_{18}$: C, 59.85; H, 6.74. Found: C, 60.12; H, 6.81.

Compound **19**: $[\alpha]_D^{20}$ −49.8° (*c* 0.238, chloroform); R_F 0.70 (8:1 dichloromethane–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.36–7.29 (10 H, 2 *Ph*CH_2), 5.16 (s, 1 H, H-1′), 5.03 (t, 1 H, *J* 9.8 Hz), 4.96, 4.93, 4.91, 4.90, 4.88, 4.85 (4 H), 4.79 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.70, 4.66, 4.63, 4.49, 4.47 (3 H, C*H*$_2$Ph + 1 H), 4.25–4.05 (3 H), 3.75 (dq, 1 H, $J_{5,6}$ 6.3, $J_{4,5}$ 9.7 Hz, H-5), 3.68–3.42 (6 H), 3.50, 3.48, 3.46 (3 s, 9 H, MeO 3), 3.38 (t, 1 H, *J* 10.2 Hz), 2.08, 2.50, 2.26, 2.00 (4 s, 12 H, 4 AcO), 1.31 (d, 3 H, *J* 6.3 Hz, Rha–Me), and 1.15 (d, 3 H, *J* 6.3 Hz, Rha–Me); ^{13}C-n.m.r. ($CDCl_3$, 90 MHz): δ 100.4 (*J* 166.2 Hz, C-1″, β), 98.3 (*J* 174.1 Hz, C-1′, α), and 9.75 (*J* 173.8 Hz, C-1, α).

Anal. Calc. for $C_{43}H_{58}O_{18}$: C, 59.85; H, 6.74. Found: C, 59.75; H, 6.90.

O-(3,6-Di-O-methyl-β-D-glucopyranosyl)-(1→4)-O-(2,3-di-O-methyl-β-L-rhamnopyranosyl)-(1→2)-3-O-methyl-α-L-rhamnopyranose (**20**) *and O-(3,6-di-O-methyl-β-D-glucopyranosyl)-(1→4)-O-(2,3-di-O-methyl-α-L-rhamnopyranosyl)-(1→2)-3-O-methyl-α-L-rhamnopyranose* (**21**). — Compound **14** (32.8 mg) or **15** (16 mg) was deacetylated with sodium methoxide, and the product debenzylated with H_2 in the presence of Pd–C for 72 h at room temperature. The product was purified by preparative t.l.c. in 60:10:1 dichloromethane–methanol–H_2O, to give free trisaccharide **20** (20.4 mg) or **21** (10 mg).

Compound **20**: $[\alpha]_D^{20}$ +44.4° (*c* 0.036, methanol); R_F 0.30 (60:10:1 dichloromethane–methanol–water).

Compound **21**: $[\alpha]_D^{20}$ −25.8° (*c* 0.093, methanol); R_F 0.35 (60:10:1 dichloromethane–methanol–water); ^{1}H-n.m.r. (CD_3OD, 360 MHz): δ 5.09 (d, 1 H, *J* 1.9 Hz, H-1′), 5.04 (d, 1 H, *J* 1.7 Hz, H-1), 4.53 (d, 1 H, *J* 7.8 Hz, H-1″), 4.00

(q, 1 H, *J* 2.3, *J* 2.4 Hz), 3.81 (dq, 1 H, *J* 6.3, 9.50 Hz), 3.78–3.25 (8 H), 3.62, 3.49, 3.47, 3.45, 3.38 (5 s, 15 H, 5 MeO), 3.20 (t, 1 H, *J* 7.6 Hz), 3.08 (t, 1 H, *J* 6.8 Hz), 1.24 (d, 3 H, *J* 6.1 Hz, Rha–Me), and 1.23 (d, 3 H, *J* 6.2 Hz, Rha–Me).

Anal. Calc. for $C_{23}H_{40}O_{14}$: C, 51.10; H, 7.46. Found: C, 51.26; H, 7.39.

Deacetylation and hydrogenolysis of **16**, **17**, **18**, *and* **19**. — The benzylated and acetylated trisaccharides **16** (33 mg), **17** (28 mg), **18** (80 mg), and **19** (37 mg) were each deacetylated and debenzylated as already described, and finally purified by preparative t.l.c., to give *O*-(6-*O*-methyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-β-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-α-L-rhamnopyranose (**22**, 21.4 mg), *O*-(6-*O*-methyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-α-L-rhamnopyranose (**23**, 16.3 mg), *O*-β-D-glucopyranosyl-(1→4)-*O*-(2,3-di-*O*-methyl-β-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-α-L-rhamnopyranose (**24**, 53.6 mg), and *O*-β-D-glucopyranosyl-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-(1→4)-3-*O*-methyl-α-L-rhamnopyranose (**25**, 21.8 mg). T.l.c. showed that all four free trisaccharides were pure; the R_F values of the free trisaccharides in 60:10:1 dichloromethane–methanol–water were 0.26, 0.31, 0.27, and 0.24, respectively. Further features of the trisaccharides are described in the Results and Discussion section.

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