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CHEMICAL SYNTHESIS OF DISACCHARIDES OF THE SPECIFIC PHENOLIC GLYCOLIPID ANTIGENS FROM *Mycobacterium leprae* AND OF RELATED SUGARS

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ABSTRACT

O-(3,6-Di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-methyl-L-rhamnopyranose, which is the nonreducing disaccharide of the haptenic trisaccharide of the *Mycobacterium leprae*-specific, phenolic glycolipid I, *O*-(6-*O*-methyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-methyl-L-rhamnopyranose, the nonreducing end of the specific, phenolic glycolipid III, and the nonhaptenic *O*-β-(D-glucopyranosyl)-(1→4)-2,3-di-*O*-methyl-L-rhamnopyranose, were synthesized in relatively good yield from 3-*O*-methyl-D-glucose, or D-glucose, and L-rhamnose *via* Koenigs–Knorr reactions. These disaccharides can be used as precursors in the synthesis of the trisaccharide unit of phenolic glycolipid I and of neoglycoconjugates suitable for the serodiagnosis of leprosy.

INTRODUCTION

Mycobacterium leprae contains[1–6] three species-specific, glycolipid antigens, namely phenolic glycolipids I, II, and III. Each is based on a group-specific 3-methoxy-4-methyl-9,11-dimycocerosoxy-29(4-hydroxyphenyl)nonacosane, and contains trisaccharide entities unique to the leprosy bacillus. The 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. In PGL-II, 2,3-di-*O*-methyl-L-rhamnose is replaced by 3-*O*-methyl-L-rhamnose[3], and, in PGL-III, 3,6-di-*O*-methyl-D-glucose is replaced by 6-*O*-methyl-D-glucose[4]. PGL-I and -II are serologically much more active than PGL-III, pointing to the 3,6-di-*O*-methyl-D-glucose as the primary, antibody-binding moiety[3]. Accordingly, synthesis of the oligosaccharide portion of PGL-I and -III was considered essential (*a*) for further clarification of the relationship between epitope

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and antibody, and (*b*) in order to develop tools for the serodiagnosis of leprosy based on the sugar hapten.

Previously[3,7], we briefly described the synthesis of the terminal disaccharides, of the entire trisaccharide units, and of related oligosaccharides of PGL-I and -III, in the context of their serological activities; this was necessary in order to evolve syntheses of the highly active and leprosy-specific neoglycoproteins[7,8]. Gigg *et al.*[9] have also described, in more detail, the synthesis of 3,6-di-*O*-methyl-D-glucose *via* 5-*O*-allyl-1,2-*O*-isopropylidene-3-*O*-methyl-β-D-glucofuranose, and condensation of the D-glucosyl chloride therefrom with allyl 2,3-*O*-isopropylidene-β-L-rhamnopyranoside to give the corresponding, natural β-D-glycoside derivative, allyl *O*-(2,4-di-*O*-acetyl-3,6-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside, which was then converted into the natural, terminal disaccharide.

Our approach[3] to chemical synthesis of the entire trisaccharides of PGL-I and -III involved synthesis of the nonreducing-end disaccharide, a derivatized *O*-D-glucosyl-rhamnose, and coupling of this product to a reducing-end L-rhamnose[3]. We now describe, in more detail, the synthesis of the D-glucosyl-L-rhamnose-based disaccharides as the first step toward total synthesis of the relevant trisaccharides.

RESULTS AND DISCUSSION

Benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**1**) was prepared by the method of Bebault and Dutton[10]. 3-*O*-Methyl-D-glucose was acetylated, and the tetraacetate treated with HBr in acetic acid, to afford 2,4,6-tri-*O*-acetyl-3-*O*-methyl-D-glucopyranosyl bromide (**2**). However, **2** was unstable and decomposed in part during the preparation, even at 0°. Therefore, **2** was used for the coupling reaction without further purification. Coupling of **1** and **2** was conducted with mercury(II) cyanide in acetonitrile[11]. The resulting disaccharide (**3**) was purified by chromatography on a column of Florisil, and crystallized from ethanol. When 5:3 nitromethane–toluene was used as solvent, the yield was about equal to that when acetonitrile was used. The ^{13}C-n.m.r. spectrum of **3** showed two anomeric signals, at 99.93 (Glc-C-1′) and 95.94 p.p.m. (Rha-C-1), which confirmed the β-D configuration of the D-glucosyl group[12].

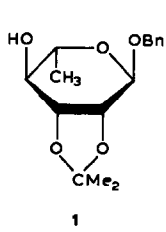
O-Deacetylation of **3**, followed by tritylation with trityl chloride, and acetylation, gave the 6-*O*-tritylated disaccharide **4**. Chromatography on a column of Florisil, resulted in pure **4**. The ^{1}H-n.m.r. spectrum of **4** showed four phenyl group signals, at ~7 p.p.m., indicative of one trityl group, and one benzyl group at the anomeric center of the L-rhamnose residue. One of two acetoxyl signals was observed at very high field (1.76 p.p.m.) due to the magnetic anisotropic effect of the trityl group.

Benzylation[13] of **4** using benzyl chloride–KOH was attempted. However, at the extraction step, the reaction mixture gave an intractable emulsion. Accordingly, the reaction mixture was chromatographed on a column of silica gel, resulting,

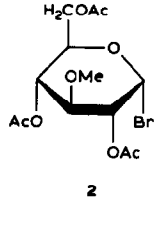
after brief washing and evaporation, in almost pure benzylated compound **5**. A small amount of benzyl chloride remained in the preparation, and, accordingly, preparative t.l.c. was used to prepare benzyl chloride-free **5**. It was on such material that n.m.r.-spectral and other physical analyses were conducted.

Refluxing of **5** in 4:3 60% acetic acid–methanol resulted in two products, separable by t.l.c. on silica gel. The minor spot, **6** (R_F 0.77) in 4:1 benzene–acetone constituted 6% of the mixture. The major spot, **7** (R_F 0.15), was >90% of the mixture. These were obtained in large amounts by chromatography on a column of Florisil. When eluted with 1:99 *tert*-butyl alcohol–benzene, **6** showed i.r. absorption at ~3500 cm^{-1}. The ^1H-n.m.r. spectrum showed two methyl signals (singlet), at 1.47 and 1.31 p.p.m., and only three phenyl signals. One proton signal, a broad doublet at ~1.9 p.p.m. which was exchangeable with D_2O, was evident, indicating the presence of one OH proton in **6**. Therefore, **6** was obviously the 6-*O*-detritylated disaccharide.

Elution of the Florisil column with 1:19 *tert*-butyl alcohol–benzene removed **7**. The ^1H-n.m.r. spectrum of **7** showed no signals attributable either to the isopropylidene or trityl groups. Three OH signals, at 3.88, 2.71 and 1.94 p.p.m. (broad singlets), all D_2O-exchangeable, were evident. The i.r. spectrum of **7** showed broad absorption at 3200 cm^{-1}. These results indicated that **7** consisted of the 6-*O*-de-



1



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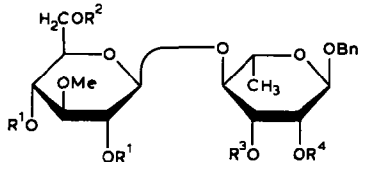
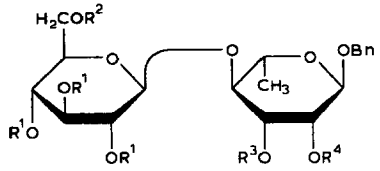


	R^1	R^2	R^3	R^4
3	Ac	Ac	Ip	
4	Ac	Tr	Ip	
5	Bn	Tr	Ip	
6	Bn	H	Ip	
7	Bn	H	H	H
8	Bn	Me	Me	Me

Ip = $>CMe_2$, Ph = C_6H_5, Bn = $PhCH_2$, Tr = CPh_3



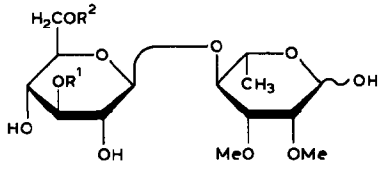
	R^1	R^2	R^3	R^4
9	Bn	Me	Me	Me
10	Bn	Bn	H	H
11	Bn	Bn	Me	H
12	Bn	Bn	Me	Me
13	Ac	Ac	—Ip—	
14	Ac	Tr	—Ip—	
15	Bn	Tr	—Ip—	
16	Bn	H	—Ip—	
17	Bn	H	H	H
18	Bn	Bn	—Ip—	

tritylated, *O*-de-isopropylidenated dimer.

Methylation of **7** with dimethylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide[14] gave the permethylated disaccharide **8** in good yield. Purification by chromatography on a column of silica gel gave pure, syrupy **8**. The ^{1}H-n.m.r. spectrum of **8** showed four methoxyl signals, at 3.63, 3.45, 3.37 and 3.31 p.p.m., which were in accord with a tetra-*O*-methylated disaccharide.

Each methoxyl signal could be assigned by comparison of the spectrum with those of **9** and **12**, both related compounds. Synthesis of **9** was achieved by the same synthetic procedure but using D-glucose instead of 3-*O*-methyl-D-glucose. The results were almost identical, but the yields were higher. Partial methylation of **10** with limiting amounts of methyl iodide gave crystalline 3-*O*-methylated disaccharide **11**, whose structure was confirmed by g.l.c.–m.s. of the alditol acetates. Synthesis of **12** was readily achieved in good yield from D-glucose by using the same procedure, except that the tritylation was omitted. The ^{1}H-n.m.r. spectrum of **9** showed three methoxyl signals, at 3.45 (OMe-3), 3.37 (OMe-6′), and 3.32 p.p.m. (OMe-2). For **12**, only two methoxyl signals were observed, at 3.45 (OMe-3) and 3.33 (OMe-2). The ^{1}H-n.m.r. spectrum of **11** showed one methoxyl signal, at 3.33 p.p.m. Therefore, the four methoxyl signals in the spectrum of **8** were assigned as follows: at 3.31, OMe-2; 3.37, OMe-6′; 3.45, OMe-3; and 3.63 p.p.m., OMe-3′.

Hydrogenolysis of **8** with palladium-on-carbon gave free disaccharide **19**, the nonreducing-end disaccharide of PGL-I. The ^{1}H-n.m.r. spectrum of **19** showed two characteristic, anomeric protons, at 5.14 ($J_{1,2}$ 1.82 Hz; H-1 of 2,3-OMe_2-α-L-Rha*p*) and 4.54 p.p.m. ($J_{1,2}$ 7.76 Hz; H-1 of 3,6-OMe_2-β-D-Glc*p*), four methoxyl signals (3.63, 3.46, 3.38, and 3.38 p.p.m.), and one C-CH_3 signal (1.24 p.p.m.; *J* 6.26 Hz), all strongly supporting the structure proposed for **19**.



	R^1	R^2
19	Me	Me
20	H	Me
21	H	H

To confirm the linkage and positions of the OCH_3 groups, **19** was methylated with CD_3I and the alditol acetates analyzed by g.l.c.–e.i.m.s. in a column of OV-225. Two peaks were present in the ratio of ~1:1 (R_T 0.92 and 1.00). The peak at R_T 0.92 produced fragment ions of *m/z* 203, 161, 143, 117, 101, and 43, and that at R_T 1.00 showed *m/z* 208, 164, 148, 132, 128, 104, and 43. The spectra were in agreement with a 1,4,5-tri-*O*-Ac-2,3-di-*O*-Me-rhamnitol and a 1,5-di-*O*-Ac-3,6-di-*O*-Me-2,4-di-*O*-CD_3-glucitol[1,15]. These results again indicated the correctness of the structure assigned to **19**.

Hydrogenolysis of **9** and **12** by the same procedure as for **8** gave the 6-*O*-methyl-D-glucosyl (**20**) and D-glucosyl (**21**) disaccharides. The overall yields of **20** and **21** were 6.6 and 7.1%, respectively. The proposed structures of **20** and **21** were confirmed by g.l.c.–e.i.m.s. of the perdeuteriomethylated alditol acetates.

EXPERIMENTAL

Melting points were measured with a micro hot-plate apparatus and are uncorrected. Optical rotations were determined with a Horiba SEPA 200 polarimeter, using a 10-cm microtube (2 mL). T.l.c. was conducted on plates of Merck silica gel 60 F-254 (0.25 mm); compounds were detected by charring with 10% sulfuric acid. I.r. spectra were recorded with a Hitachi 210 infrared spectrometer. ^{1}H-N.m.r. spectra were recorded with a Nicolet NT-360 (360 MHz) or Varian EM-360 (60 MHz) nuclear magnetic resonance spectrometer. ^{13}C-N.m.r. spectra were also measured with a Nicolet NT-360 (90 MHz) instrument. G.l.c.–e.i.m.s. was recorded with a Hitachi M-70 mass spectrometer with a column (0.10 m) of OV-225 and an ionization potential of 70 eV.

Benzyl O-*(2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-*D*-glucopyranosyl)-(1→4)-2,3-di-*O*-isopropylidene-α-*L*-rhamnopyranoside* (**3**). — Compound **1** (ref. 16; 12.5 g, 42.5 mmol) and syrupy **2** (21.5 g, 63.2 mmol), prepared from commercial 3-*O*-methyl-D-glucose (20 g, 103 mmol), were stirred in dry acetonitrile (125 mL) in the presence of mercury cyanide (12.5 g, 49.5 mmol) for 5 h at room temperature, the mixture evaporated, the residue extracted with chloroform, and the extracts successively washed with M potassium bromide, water, saturated sodium hydrogencarbonate, and water, dried (sodium sulfate), and evaporated to a syrup. Further purification

was achieved on a column of Florisil. Crystallization and recrystallization from ethanol gave **3** (21.8 g, 36.6 mmol, 86.1%) as white needles, m.p. 136.5–137.0°, $[\alpha]_D^{24}$ −51.9° (*c* 0.93, chloroform); R_F 0.81 (4:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.4–7.28 (5 H, Ph), 5.04 (s, 1 H, H-1), 5.01–4.8 (3 H), 4.70, 4.47 (2 H, PhCH_2), 4.2–4.05 (4 H), 3.69 (o, 1 H, $J_{5,6}$ 6.17, 9.3 Hz, H-5), 3.6–3.5 (2 H), 3.52 (t, 1 H, *J* 4.32 Hz), 3.30 (s, 3 H, OMe-3′), 2.11, 2.09, 2.05 (3 s, 9 H, OAc), 1.52, 1.34 (2 s, 6 H, CMe_2), and 1.27 (d, 3 H, *J* 6.17 Hz, Rha-Me); ^{13}C-n.m.r. ($CDCl_3$, 90 MHz): δ 170.64, 169.45, (3 C, CH_3-*C*OO), 136.92 (Ph, α-C), 128.52–128.03 (Ph), 109.20 [$(CH_3)_2$*C*], 99.93 (C-1′), 95.94 (C-1), 81.33 (C-3′), 79.60 (*C*H_2Ph), 78.25 (C-3), 76.16 (C-2), 72.21 (C-4), 72.06 (C-5′), 69.40 (C-2′), 69.20 (C-4′), 64.24 (C-5), 62.70 (C-6′), 58.63 (OCH_3), 28.00, 26.44 [$(CH_3)_2$*C*], 20.99, 20.81, 20.71 (3 C, *C*H_3-COO), and 17.89 (*C*H_3-C); ν_{max}^{KBr} 1760 (C=O), 1390, 1240 (s, acetyl C–C), 1150–1000 (s, broad, C–O–C), 780, and 728 cm^{-1} (m, monosubstituted benzene).

Anal. Calc. for $C_{29}H_{40}O_{13}$: C, 58.38; H, 6.76. Found: C, 58.16; H, 7.04.

Benzyl O-*(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-(1→4)-2,3-*O*-isopropylidene-α-*L*-rhamnopyranoside* (**13**). — Crystalline **2** (30 g, 78.3 mmol), **1** (10 g, 34.0 mmol), and mercury cyanide (10 g, 40 mmol) were stirred in dry acetonitrile (100 mL), and processed as for **3**. Purification by chromatography on a column of Florisil, and recrystallization from ethanol, gave 18.5 g (29.6 mmol, 87.1%) of crystalline disaccharide **13**, m.p. 102.0–103.1°, $[\alpha]_D^{20}$ −31.9° (*c* 2.023, chloroform); R_F 0.84 (4:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.4–7.28 (5 H, Ph), 5.24 (t, 1 H, *J* 9.15 Hz, H-3′), 5.1–4.9 (4 H), 4.70, 4.67, 4.51, 4.47 (2 H, PhCH_2), 4.25–4.05 (4 H), 3.68–3.20 (2 H), 3.56 (q, 1 H, *J* 6.99, 9.94 Hz), 2.06, 2.05, 2.03, 2.00 (4 s, 12 H, OAc), 1.52, 1.33 (2 s, 6 H, CMe_2), and 1.14 (d, 3 H, *J* 6.18 Hz, Rha-Me); ^{13}C-n.m.r. ($CDCl_3$, 90 MHz): δ 170.53, 170.22, 169.54, 169.44 (4 C, CH_3-*C*OO), 136.91 (Ph, α-C), 128.52–128.03 (Ph), 109.24 [$(CH_3)_2$*C*], 99.60 (C-1′) 95.96 (C-1), 79.79 (*C*H_2Ph), 78.07 (C-3), 76.13 (C-2), 72.90 (C-4), 71.82 (C-5′), 71.52 (C-3′), 69.19 (C-2′), 68.85 (C-4′), 64.13 (C-5), 62.32 (C-6′), 28.00, 26.39 [(*C*H_3)$_2$C], 20.72, 20.60 (4 C, *C*H_3-COO), and 17.46 (*C*H_3-C); ν_{max}^{KBr} 1762 (s, C=O), 1390, 1240 (s, acetyl C–C), 1130–1100 (s, broad, C–O–C), 885 (m), 780, and 730 cm^{-1} (m, monosubstituted benzene).

Anal. Calc. for $C_{30}H_{40}O_{14}$: C, 57.69; H, 6.46. Found: C, 57.58; H, 6.64.

Tritylation of **3**. — Compound **3** (15.2 g, 25.5 mmol) was deacetylated with sodium methoxide, and stirred with trityl chloride (22 g, 78.9 mmol) for 3 h at 80° and then for 2 days at room temperature. Twice the volume of 1:1 pyridine–acetic anhydride was added, and the mixture stirred overnight at room temperature, poured onto ice–water, extracted with ether, and the extracts successively washed with saturated sodium hydrogensulfate, saturated sodium hydrogencarbonate, and water, dried (sodium sulfate), and evaporated. A solution of the residue in 1:1 benzene–hexane was placed on a column of Florisil in benzene–hexane. The column was washed extensively with 1:1 benzene–hexane, the tritylated compound eluted with benzene, and the eluate evaporated, to give benzyl *O*-(2,4-di-*O*-acetyl-

3-*O*-methyl-6-*O*-trityl-β-D-glucopyranosyl)-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**4**) as a syrup (11.5 g, 14.4 mmol, 56.5%); $[\alpha]_D^{20}$ −6.2° (*c* 1.44, chloroform); R_F 0.52 (16:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.5–7.2 (20 H, CPh_3 and Ph), 5.10 (t, 1 H, *J* 9.71 Hz), 5.07 (s, 1 H, H-1), 5.00 (t, 1 H, *J* 8.85 Hz), 4.92, 4.89 (1 H), 4.72, 4.49 (2 H, CH_2Ph), 4.2–4.1 (2 H), 3.8–3.73 (1 H), 3.7–3.62 (1 H), 3.55–3.4 (2 H), 3.37 (s, 3 H, 3'-OMe), 3.10–3.15 (2 H), 2.12, 1.76 (2 s, 6 H, OAc), 1.52, 1.34 (2 s, 6 H, CMe_2), 1.42 (d, 3 H, *J* 6.05 Hz, Rha-Me); i.r. (liquid film): 3150–2800 (s, C–H), 1760 (s, C=O), 1610, 1405, 1460 (m, aromatic C–C), 1390 (m, acetyl C–C), 1260 (s, acetyl C–C), 1200–1000 (s, broad, C–O–C), 926 (m), 890 (m), 780, and 730 cm^{-1} (s, aromatic C–H).

Tritylation of **13**. — Compound **13** (15 g, 24.0 mmol) was treated as for **3**, to give benzyl *O*-(2,3,4-tri-*O*-acetyl-6-*O*-trityl-β-D-glucopyranosyl)-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**14**; 11.2 g, 13.6 mmol, 56.7%); $[\alpha]_D^{20}$ −2.1° (*c* 2.15, chloroform); R_F 0.50 (16:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 60 MHz): δ 7.7–7.1 (20 H, CPh_3 and Ph), 5.5–4.85 (5 H), 4.84, 4.62, 4.57, 4.37 (2 H, CH_2Ph), 4.3–4.1 (2 H), 3.85–3.4 (3 H), 3.35–3.15 (2 H), 2.09, 2.00, 1.71 (3 s, 9 H, OAc), 1.52, 1.38 (2 s, 6 H, CMe_2), and 1.42 (d, 3 H, *J* 7.2 Hz, Rha-Me); $\nu_{max}^{liquid\ film}$ 3200–2800 (s, C–H), 1765 (s, C=O), 1608, 1405 (m, aromatic C–C), 1380 (m, acetyl C–C), 1263 (s, acetyl C–C), 1200–1000 (s, broad), 930 (m), 890 (m), 773, and 725 cm^{-1} (s, aromatic C–H).

Benzylation of **4**. — Compound **4** (8.7 g, 10.9 mmol) in benzyl chloride (110 mL) together with powdered potassium hydroxide (40 g, 336 mmol) was heated for 2 h at 130–140°, diluted with ice–water, extracted with chloroform, and the extract washed with water, dried, and evaporated at <13.3 Pa. The product was placed on a column of silica gel in benzene. Elution with 1:49 ethyl acetate–benzene gave benzyl *O*-(2,4-di-*O*-benzyl-3-*O*-methyl-6-*O*-trityl-β-D-glucopyranosyl)-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**5**; 8.1 g, 9.1 mmol, 83.2%). As this fraction contained a trace of benzyl chloride, a portion was further purified by preparative t.l.c. and used for obtaining physical data for **5**; for the subsequent step, the fraction directly eluted was used without purification; $[\alpha]_D^{20}$ −16.6° (*c* 1.46, chloroform); R_F 0.66 (16:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.55–7.15 (30 H, CPh_3 and Ph), 6.93 (q, 1 H, *J* 5.0, 3.2 Hz), 5.11 (s, 1 H, H-1), 5.0–4.9 (2 H), 4.8–4.7 (2 H), 4.68, 4.64, 4.57, 4.53 (2 H, CH_2Ph), 4.35–4.25 (2 H), 4.17 (d, 1 H, *J* 5.3 Hz), 3.82 (m, 2 H), 3.68 (m, 1 H), 3.64 (s, 3 H, OMe), 3.53 (q, 1 H, *J* 8.3, 1.52 Hz), 3.43–3.32 (2 H), 3.25–3.45 (q, 1 H, *J* 8.3, 2.5 Hz), 1.47 (d, 3 H, *J* 5.60 Hz, Rha-Me), 1.42, 1.45 (2 s, 6 H, CMe_2); $\nu_{max}^{liquid\ film}$ 3110–2800 (s, C–H), 1615, 1470 (aromatic C–C), 1400, 1250 (acetyl C–C), 1200–1000 (s, broad, C–O–C), 895 (m), 790, and 736 cm^{-1} (s, monosubstituted benzene).

Benzylation of **14**. — Compound **14** (7.0 g, 8.5 mmol) was benzylated with the benzyl chloride–KOH system and processed as for **5** to give perbenzylated disaccharide **15**. It was applied to a column of silica gel in 1:1 benzene–hexane and eluted with benzene, to give benzyl *O*-(2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-D-gluco-

pyranosyl)-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**15**; 7.2 g, 7.4 mmol, 87.4%); $[\alpha]_D^{20}$ −20.8° (*c* 0.792, chloroform); R_F 0.68 (16:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 60 MHz): δ 7.7–7.0 (35 H, CPh_3 and Ph), 7.0–6.7 (1 H), 5.1 (s, 1 H, H-1), 5.05–4.5 (8 H), 4.45–4.16 (2 H), 4.15–3.2 (8 H), 1.45 (d, 3 H, *J* 6.0 Hz, Rha-Me), 1.41, and 1.29 (2 s, 6 H, CMe_2); $\nu_{max}^{liquid\ film}$ 3140–2800 (C–H), 1930, 1960, 1840, 1770 (w, monosubstituted benzene), 1605, 1500, 1460 (aromatic), 1390, 1380 (m), 1230 (acetyl C–C), 1200–1000 (s, broad, C–O–C), 873 (m), 760, and 710 cm^{-1} (s, monosubstituted benzene).

Benzylation of **13**. — Compound **13** (10 g, 16.0 mmol) was benzylated with benzyl chloride–KOH, and processed as for **5**, to give the tetrabenzylated disaccharide, which was purified by chromatography on a column of silica gel. After placement of the sample, the column extensively washed with 1:1 benzene–hexane. Elution with pure benzene gave benzyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**18**; 9.5 g, 11.6 mmol, 72.7%). The product contained a small amount of benzyl chloride, but was hydrolyzed without purification. Preparative t.l.c. using 16:1 benzene–acetone was conducted on a small amount in order to isolate pure **18** for measurement of its physical data; $[\alpha]_D^{20}$ −30.7° (*c* 1.52, chloroform); R_F 0.71 (16:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 60 MHz): δ 7.4–7.2 (25 H, Ph), 5.15–4.43 (12 H), 4.46–4.0 (3 H), 3.87–3.18 (7 H), 1.62 (d, 3 H, *J* 6 Hz, Rha-Me), 1.43, and 1.32 (2 s, 6 H, CMe_2); $\nu_{max}^{liquid\ film}$ 3130–2850 (CH), 1600 (w), 1510, 1470 (aromatic C–C), 1395, 1387 (m), 1260, 1240 (m), 1200–950 (s, broad, C–O–C), 890 (m), 765, and 728 cm^{-1} (s, monosubstituted benzene).

Removal of the isopropylidene and trityl groups of **5**. — Compound **5** (3.8 g, 4.25 mmol; containing a small amount of benzyl chloride) was refluxed in methanol (150 mL)–60% acetic acid (200 mL) for 1 h. The solution was cooled and evaporated, the product was repeatedly treated by addition and evaporation of methanol, and the residue was applied to a column of Florisil in benzene. Elution with 1:99 *tert*-butyl alcohol–benzene gave benzyl *O*-(2,4-di-*O*-benzyl-3-*O*-methyl-β-D-glucopyranosyl)-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**6**) as a syrup (120 mg, 0.18 mmol, 4.3%); $[\alpha]_D^{24}$ −73.6° (*c* 0.14, chloroform); R_F 0.77 (4:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.43–7.25 (15 H, Ph), 5.05 (s, 1 H, H-1), 4.93, 4.91, 4.89, 4.88, 4.86, 4.85 (3 H), 4.73, 4.70, 4.53, 4.50 (2 H, CH_2Ph), 4.70, 4.68, 4.66, 4.63 (2 H), 4.23 (t, 1 H, *J* 6.4 Hz), 4.15 (d, 1 H, *J* 5.7 Hz, H-1′), 3.82 (broad d, 1 H, *J* 11.52 Hz, H-6′, after D_2O treatment; 3.82, q, 1 H, *J* 2.85, *J* 12.16 Hz), 3.69–3.60 (3 H), 3.64 (s, 3 H, OMe), 3.49, 3.46, 3.43, 3.42, 3.40 (2 H), 3.35–3.28 (1 H), 3.25 (t, 1 H, *J* 8.28 Hz), 1.88 (broad, 1 H, D_2O-exchangeable, O*H*), 1.47, 1.31 (2 s, 6 H, CMe_2), and 1.29 (d, 3 H, *J* 6.12 Hz, Rha-Me); $\nu_{max}^{liquid\ film}$ 3680–3400 (m, broad, O–H), 3150–2800 (s, C–H), 1515, 1470 (aromatic C–C), 1400 (m), 1245 (acetyl C–C), 1200–1000 (s, broad, C–O–C), 895 (m), 775, and 735 cm^{-1} (s, monosubstituted benzene).

Elution with 5% *tert*-butyl alcohol gave benzyl *O*-(2,4-di-*O*-benzyl-3-*O*-methyl-β-D-glucopyranosyl)-(1→4)-α-L-rhamnopyranoside (**7**) as a syrup (1.57 g,

2.57 mmol, 60.5%); $[\alpha]_D^{24}$ −75.1° (*c* 7.54, chloroform); R_F 0.15 (4:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.43–7.25 (15 H, Ph), 4.90, 4.87, 4.85, 4.82, 4.82, 4.79 (4 H), 4.72, 4.68, 4.66, 4.63, 4.61, 4.59, 4.50, 4.47 (4 H), 3.91 (broad, 1 H, D_2O-exchangeable, O*H*), 3.88 (broad, 1 H; after D_2O treatment, 3.90, q, 1 H, *J* 1.51, 3.25 Hz), 3.90–3.75 (3 H), 3.74–3.60 (2 H), 3.65 (s, 3 H, OMe), 3.55–3.25 (5 H), 2.71 (broad s, 1 H, D_2O-exchangeable, O*H*), 1.94 (broad s, 1 H, D_2O-exchangeable, O*H*), and 1.32 (d, 3 H, *J* 6.2 Hz, Rha-Me); $\nu_{max}^{liquid\ film}$ 3650–3200 (s, O–H), 3150–2800 (C–H), 1930, 1890, 1820, 1740 (monosubstituted benzene), 1515, 1475 (aromatic C–C), 1400 (m), 1240 (s), 1200–1000 (s, broad, C–O–C), 790, and 740 cm^{-1} (m, monosubstituted benzene).

Removal of the isopropylidene and trityl groups of **15**. — Compound **15** (6.5 g, 6.7 mmol) was refluxed in 1:1 methanol–60% acetic acid (200 mL) for 1.5 h. After processing as for **7**, the syrup was applied to a column of silica gel in benzene. Elution with 1:1 benzene–ethyl acetate gave benzyl *O*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-α-L-rhamnopyranoside (**17**; 3.40 g, 4.95 mmol, 73.9%); $[\alpha]_D^{20}$ −31.4° (*c* 2.37, chloroform); R_F 0.22 (2:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 60 MHz): δ 7.45–7.15 (20 H, Ph), 5.0–4.3 (9 H), 4.2–3.2 (14 H), and 1.39 (d, 3 H, *J* 6.0 Hz, Rha-Me); $\nu_{max}^{liquid\ film}$ 3800–3150 (s, O–H), 3130–2800 (C–H), 1960, 1880, 1820, 1740 (monosubstituted benzene), 1508, 1463 (aromatic C–C), 1400, 1380 (m), 1220 (w), 1200–950 (s, C–O–C), 920 (m), 818 (m), 755, and 740 cm^{-1} (s, monosubstituted benzene).

Removal of the isopropylidene group of **18**. — To a suspension of compound **18** (9.0 g, 11.0 mmol) in 0.05M HCl (100 mL) was added ethanol (150 mL), the suspension refluxed for 2 h, and then evaporated with repeated additions of methanol. The residue was applied to a column of Florisil in benzene. Elution with 1% *tert*-butyl alcohol gave benzyl *O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-α-L-rhamnopyranoside (**10**; 3.90 g, 5.0 mmol, 45.6%); $[\alpha]_D^{20}$ −30.7° (*c* 1.52, chloroform); R_F 0.38 (4:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 60 MHz): δ 7.4–7.1 (25 H, Ph), 4.9–4.6 (11 H), 4.0–3.2 (11 H), 2.47 (broad s, 1 H, D_2O-exchangeable, O*H*), 1.62 (broad s, 1 H, D_2O-exchangeable, O*H*), and 1.32 (d, 3 H, *J* 6.1 Hz, Rha-Me); $\nu_{max}^{liquid\ film}$ 3650–3180 (s, O–H), 3150–2800 (C–H), 1970, 1880, 1820, 1760 (w, monosubstituted benzene), 1620, 1600 (w), 1515, 1470 (m, aromatic C–C), 1400, 1380 (m), 1235 (w), 1200–950 (s, broad, C–O–C), 940 (w), 840 (m), 780, 770, and 730 cm^{-1} (s, monosubstituted benzene).

Methylation of **7**. — To a solution of compound **7** (950 mg, 1.56 mmol) in dry dimethyl sulfoxide (5 mL) was added 2M dimethylsulfinyl carbanion[17] (2 mL), and the mixture was stirred for 2 h at 20° under argon gas. Methyl iodide (1.5 mL) was then added, and the mixture was stirred for 2 h at 20°. The reaction was stopped by addition of water. The mixture was extracted with chloroform, and the extract washed, dried, and evaporated to a syrup which was placed on a column of silica gel in benzene. After washing the column with 50:1 benzene–ethyl acetate, elution with 10:1 benzene–ethyl acetate gave benzyl *O*-(2,4-di-*O*-benzyl-3,6-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-methyl-α-L-rhamnopyranoside (**8**; 935 mg,

1.43 mmol, 91.8%); $[\alpha]_D^{20}$ −49.9° (*c* 0.36, chloroform); R_F 0.44 (8:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 360 MHz): δ 7.4–7.2 (15 H, Ph), 4.95 (d, 1 H, *J* 1.26 Hz, H-1), 4.92–4.49 (7 H), 3.72–3.48 (7 H), 3.63 (s, 3 H, OMe-3′), 3.45 (s, 3 H, OMe-3), 3.40–3.29 (2 H), 3.37 (s, 3 H, OMe-6′), 3.31 (s, 3 H, OMe-2), 3.24 (t, 1 H, *J* 4.47 Hz), and 1.34 (d, 3 H, *J* 5.65 Hz, Rha-Me); $\nu_{max}^{liquid\ film}$ 3120–2800 (C–H), 1960, 1885, 1820, 1740 (monosubstituted benzene), 1415 (w), 1470 (aromatic C–C) 1405, 1395 (m), 1200–950 (s, broad, C–O–C), 785, and 732 cm^{-1} (s, monosubstituted benzene).

Benzyl O-*(2,3,4-tri-*O*-benzyl-6-*O*-methyl-β-*D*-glucopyranosyl)-(1→4)-2,3-di-*O*-methyl-α-*L*-rhamnopyranoside* (**9**). — Compound **17** (1.5 g, 2.18 mmol) was methylated with dimethylsulfinyl carbanion and methyl iodide, and the product processed as for **8**. The purified syrup crystallized from ethanol, and was recrystallized from ethanol–ether, to give **9** (1.15 g, 1.58 mmol, 72.3%); m.p. 125.1–126.0°, $[\alpha]_D^{20}$ −39.3° (*c* 0.39, chloroform); R_F 0.53 (8:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 60 MHz): δ 7.4–7.2 (20 H, Ph), 5.15–4.45 (10 H), 3.90–3.55 (8 H), 3.50–3.20 (2 H), 3.45 (s, 3 H, OMe-3), 3.37 (s, 3 H, OMe-6′), 3.32 (s, 3 H, OMe-2), and 1.42 (d, 3 H, *J* 5.9 Hz, Rha-Me); ν_{max}^{KBr} 3120–2800 (C–H), 1960, 1875, 1815, 1760 (w, monosubstituted benzene), 1610, 1595 (w), 1503, 1460 (aromatic C–C), 1390, 1360 (m), 1210, 1200 (m), 1190–950 (s, broad, C–O–C), 755, 740, and 705 cm^{-1} (s, monosubstituted benzene).

Anal. Calc. for $C_{43}H_{52}O_{10}$: C, 70.86; H, 7.19. Found: C, 70.84; H, 7.23.

Benzyl O-*(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-(1→4)-2,3-di-*O*-methyl-α-*L*-rhamnopyranoside* (**12**) *and benzyl* O-*(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-(1→4)-3-*O*-methyl-α-*L*-rhamnopyranoside* (**11**). — Compound **10** (3.5 g, 4.5 mmol) was methylated with dimethylsulfinyl carbanion and methyl iodide[17]. Addition of a large excess of water to the reaction mixture gave crystalline product **12**. Recrystallization from 1:1 ethanol–ether gave pure **12** (1.8 g, 2.24 mmol, 49.7%). Methylation of **10** using a limited amount of dimethylsulfinyl carbanion (1.2 equiv.) gave a mixture of **11** and **12** in the ratio of ~4:3. Purdie methylation[18], using methyl iodide and silver oxide, and refluxing for 5 h gave a mixture of **11** and **12**. In order to confirm the position of the OCH_3 group in **11**, it was hydrolyzed by heating for 2 h in 3M trifluoroacetic acid, and the alditol acetates were prepared[19]. G.l.c.–m.s. in a column of OV 225 showed that the rhamnoside residue was 3-*O*-methylated (*m/z* 43, 87, 101, 129, 189, and 203).

Compound **11** had m.p. 109.1–111.2°, $[\alpha]_D^{20}$ −35.4° (*c* 0.32, chloroform); R_F 0.52 (8:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 60 MHz): δ 7.4–7.2 (20 H, Ph), 5.17–4.15 (12 H), 4.10–3.20 (11 H), 3.33 (s, 3 H, OMe-3), and 1.41 (d, 3 H, *J* 5.95 Hz, Rha-Me); ν_{max}^{KBr} 3750–3300 (O–H), 3150–2800 (C–H), 1960, 1880, 1815, 1735 (monosubstituted benzene), 1610, 1590 (w), 1500, 1460 (m, aromatic C–C), 1380 (m), 1180–950 (s, broad, C–O–C), 755, and 740 cm^{-1} (monosubstituted benzene).

Anal. Calc. for $C_{43}H_{52}O_{10}$: C, 70.86; H, 7.19. Found: C, 70.78; H, 7.07.

Compound **12** had m.p. 106.5–107.4°, $[\alpha]_D^{20}$ −28.1° (*c* 1.12, chloroform); R_F 0.75 (8:1 benzene–acetone); ^{1}H-n.m.r. ($CDCl_3$, 60 MHz): δ 7.4–7.2 (20 H, Ph),

5.17–4.15 (12 H), 4.10–3.20 (11 H), 3.45 (s, 3 H, OMe-3), 3.33 (s, 3 H, OMe-2), and 1.41 (d, 3 H, *J* 6.0 Hz, Rha-Me); ν_{max}^{KBr} 2950–2800 (C–H), 1500, 1455 (aromatic C–C), 1295 (w), 1210 (m) 1200–950 (s, broad, C–O–C), 920 (m), 760, 740, and 703 cm^{-1} (s, monosubstituted benzene).

Anal. Calc. for $C_{49}H_{56}O_{10}$: C, 73.11; H, 7.01. Found: C, 72.40; H, 6.76.

*O-(3,6-Di-O-methyl-β-*D*-glucopyranosyl)-(1→4)-2,3-di-O-methyl-α-*L*-rhamnopyranose* (**19**). To a solution of compound **8** (820 mg, 1.26 mmol) in absolute ethanol (25 mL) containing chloroform (3 mL) was added 5% palladium-on-carbon (350 mg), and the mixture was stirred for 16 h at room temperature under hydrogen, filtered, and the filtrate evaporated, giving free disaccharide **19** (390 mg, 1.02 mmol, 80.9%), which showed a single spot in t.l.c.; $[\alpha]_D^{20}$ −27.1° (*c* 7.8, methanol); lit.[8] $[\alpha]_D^{24}$ −23.2° (*c* 1.18, chloroform); R_F 0.39 (60:10:1 $CHCl_3$–MeOH–H_2O); ^{1}H-n.m.r. (CD_3OD, 360 MHz): δ 5.14 (d, 1 H, *J* 1.82 Hz, H-1), 4.54 (d, 1 H, *J* 7.76 Hz, H-1′), 3.83 (1 H, *J* 6.26, 9.8 Hz, H-5), 3.70–3.53 (5 H), 3.63 (s, 3 H, OMe-3′), 3.46 (s, 6 H, OMe-2 and OMe-3), 3.38 (s, 3 H, OMe-6′), 3.38 (t, 1 H, *J* 9.6 Hz), 3.20 (q, 1 H, *J* 9.1, 7.9 Hz), 3.08 (q, 1 H, *J* 9.0, 9.3 Hz), and 1.24 (d, 3 H, *J* 6.26 Hz, Rha-Me); ^{13}C-n.m.r. (CD_3OD, 90 MHz): δ 104.96 (C-1′), 92.43 (C-1), 87.61 (C-3′), 82.10 (C-4), 79.67 (C-2 or C-3), 78.56 (C-3 or C-2), 76.71 (C-5′), 75.50 (C-2′), 73.00 (C-6′), 71.20 (C-4′), 68.07 (C-5), 60.92, 59.81, 59.07, 57.35 (4 C, O*C*H_3), 18.37 *C*H_3-C); $\nu_{max}^{liquid\ film}$ 3800–3050 (s, O–H), 3040–2800 (C–H), 1460 (m), 1395 (m), 1305 (w), 1200 (m), 1180–990 (s, broad), 945 (m), 918 (w), 883, 840, and 803 cm^{-1} (m).

Hydrogenolysis of **9**. — Compound **9** (1.05 g, 1.44 mmol) was stirred for 16 h at room temperature in absolute ethanol (30 mL) containing 5% palladium-on-carbon (350 mg) under hydrogen. The mixture was processed as for **19**, giving *O*-(6-*O*-methyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-methyl-α-L-rhamnopyranose (**20**) (506 mg, 1.38 mmol, 95.4%); $[\alpha]_D^{20}$ −32.6° (*c* 1.05, methanol); R_F 0.23; (60:10:1 $CHCl_3$–MeOH–H_2O); ^{1}H-n.m.r. (CD_3OD, 360 MHz): δ 5.14 (d, 1 H, *J* 1.66 Hz, H-1), 4.56 (d, 1 H, *J* 7.72 Hz, H-1′), 3.84 (m, 1 H, *J* 6.23, 9.74 Hz, H-5), 3.72–3.65 (2 H), 3.64–3.54 (3 H), 3.47–3.43 (1 H), and 3.45 (s, 6 H, OMe-2 and OMe-3), 3.17 (g, 1 H, *J* 7.86, 8.90 Hz), 1.28 (d, 3 H, *J* 6.24 Hz, Rha-Me); ^{13}C-n.m.r. (CD_3OD, 90 MHz): δ 104.95 (C-1′), 92.45 (C-1), 82.17 (C-4), 79.63 (C-2 or C-3), 78.62 (C-3 or C-2), 77.91 (C-3′), 76.90 (C-5′), 75.63 (C-2′), 73.15 (C-6′), 71.71 (C-4′), 68.09 (C-5), 59.82, 59.10, 57.42 (3 C, O*C*H_3), and 18.40 (*C*H_3=C); $\nu_{max}^{liquid\ film}$ 3800–3060 (s, O–H), 3000–2800 (C–H), 1460 (m), 1400 (w), 1200 (m), 1170–990 (s, broad, C–O–C), 920, 890, 845, 805 (m), and 760 cm^{-1} (s).

Hydrogenolysis of **12**. — Compound **12** (750 mg, 0.93 mmol) was hydrogenolyzed as for **19**, to give *O*-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-methyl-α-L-rhamnopyranose (**21**; 232 mg, 0.65 mmol, 70.4%); $[\alpha]_D^{20}$ −23.9° (*c* 1.10, methanol); R_F 0.11 (60:10:1 $CHCl_3$–MeOH–H_2O); ^{1}H-n.m.r. (CD_3OD, 360 MHz): δ 5.15 (d, 1 H, *J* 1.66 Hz, H-1), 4.56 (d, 1 H, *J* 7.72 Hz, H-1′), 3.89–3.82 (2 H), 3.73–3.60 (5 H), 3.48–3.40 (1 H), 3.45 (s, 6 H, OMe-2 and OMe-3), 3.17 (q, 1 H, *J* 7.86, 8.90 Hz), and 1.28 (d, 3 H, *J* 6.24 Hz, Rha-Me); ^{13}C-n.m.r. (CD_3OD, 90

MHz): δ 105.03 (C-1'), 92.45 (C-1), 82.16 (C-4), 79.71 (C-2 or C-3), 78.61 (C-3 or C-2), 77.92 (C-3'), 77.87 (C-5'), 75.77 (C-2'), 71.80 (C-4'), 68.18 (C-5), 59.10, 57.37 (2 C, OCH_3), and 18.41 (CH_3–C); $\nu_{max}^{liquid\ film}$ 3700–3120 (s, O–H), 2980–2800 (C–H), 1610 (w), 1420 (w), 1380 (w), 1195 (w), 1190–950 (s, broad, C–O–C), 900 (w), 815 (w), and 1795 cm^{-1} (w).

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REFERENCES

1 S. W. HUNTER AND P. J. BRENNAN, *J. Bacteriol.*, 147 (1981) 728–735.
2 S. W. HUNTER, T. FUJIWARA, AND P. J. BRENNAN, *J. Biol. Chem.*, 258 (1983) 7556–7562.
3 T. FUJIWARA, S. W. HUNTER, S.-N. CHO, G. O. ASPINALL, AND P. J. BRENNAN, *Infect. Immun.*, 43 (1984) 245–252.
4 S. W. HUNTER AND P. J. BRENNAN, *J. Biol. Chem.*, 258 (1983) 7556–7562.
5 P. J. BRENNAN, *Int. J. Lepr.*, 51 (1983) 387–396.
6 E. TARELLI, P. DRAPER, AND S. N. PAYNE, *Carbohydr. Res.*, 131 (1984) 346–352.
7 S.-N. CHO, T. FUJIWARA, S. W. HUNTER, T. H. REA, R. H. GELBER, AND P. J. BRENNAN, *J. Infect. Dis.*, 150 (1984) 311–322.
8 D. CHATTERJEE, J. T. DOUGLAS, S.-N. CHO, T. H. REA, R. H. GELBER, G. O. ASPINALL, AND P. J. BRENNAN, *Glycoconj. J.*, 2 (1985) 187–208.
9 R. GIGG, S. PAYNE, AND R. CONANT, *J. Carbohydr. Chem.*, 2 (1983) 207–223.
10 G. M. BEBAULT AND G. G. S. DUTTON, *Can. J. Chem.*, 50 (1972) 3373–3378.
11 H. PAULSEN, *Angew. Chem., Int. Ed. Engl.*, 21 (1982) 155–173.
12 A. S. PERLIN, B. CASU, AND H. J. KOCH, *Can. J. Chem.*, 48 (1970) 2596–2606.
13 H. G. FLETCHER, JR., *Methods Carbohydr. Chem.*, 2 (1963) 166–167.
14 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
15 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem., Int. Ed. Engl.*, 9 (1970) 610–619.
16 V. POZSGAY, P. NÁNÁSI, AND A. NESZMÉLYI, *Carbohydr. Res.*, 90 (1981) 215–231.
17 P. A. SANDFORD AND H. E. CONRAD, *Biochemistry*, 5 (1966) 1508–1516.
18 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021–1037.
19 B. LINDBERG, *Methods Enzymol.*, 28 (1972) 178–195.