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SYNTHESIS OF 4-*O*-β-D-MANNOPYRANOSYL-L-RHAMNOPYRANOSE

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ABSTRACT

The title disaccharide (**16**) has been synthesized in 50% overall yield by way of condensation of 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide (**5**) with methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**1**) in chloroform solution, in the presence of silver oxide. The disaccharide was characterized as the crystalline isopropyl alcoholate of methyl 4-*O*-β-D-mannopyranosyl-α-L-rhamnopyranoside (**11**) and as 1,2,3-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranose (**15**). Methyl β-D-mannopyranoside isopropyl alcoholate (**7**) was readily obtained in 85% yield *via* the reaction of bromide **5** with methanol.

Reduction of 2,3-di-*O*-methyl-L-rhamnose with sodium borohydride, followed by acetylation, may result in the formation of an appreciable proportion of a boric ester, namely 1,5-di-*O*-acetyl-4-deoxy-2,3-di-*O*-methyl-L-rhamnitol-4-yl dimethyl borate, depending on the procedure used.

INTRODUCTION

4-*O*-D-Mannopyranosyl-L-rhamnopyranose with the glycosidic linkage in both the α-D and β-D anomeric, glycosidic configurations occurs as a constituent of *Salmonella* cell-wall lipopolysaccharides. The synthesis of each anomer of this disaccharide was undertaken in order to facilitate (*a*) the identification of the isolated 4-*O*-D-mannopyranosyl-L-rhamnopyranoses[1], and (*b*) immunological studies on the immunodominant site of these antigenic lipopolysaccharides[2]. As the α-D anomer is a 1,2-*trans* glycoside, it was synthesized from 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide by a standard Helferich condensation[3].

In order to synthesize the β-D anomer, it was necessary to develop a general method for the synthesis of β-D-mannopyranosides. Although β-D-mannopyranosides have also been identified in *Klebsiella* capsular polysaccharides[4] and are abundant in Nature as plant mannans[5], glucomannans in hemicelluloses[5], and galactomannans in seeds[6,7], methods for their synthesis are very limited. The β-D-mannopyranosides first reported were later shown to have been incorrectly described; they were actually α-D-mannopyranosides[8]. Gorin and Perlin[9] used 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide (**5**) to prepare β-D-mannopyranosides. The convenient, synthetic sequence described here for the preparation of this bromide **5**, and the improved conditions of condensation, make this a feasible method for the preparation

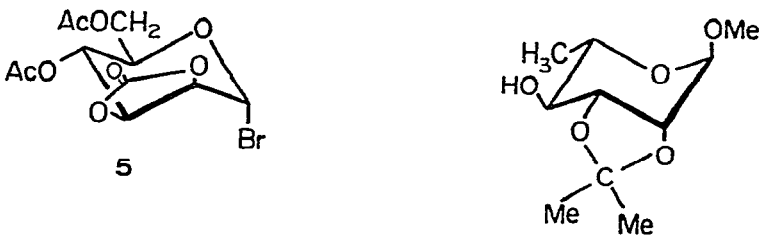
of β-D-mannopyranosides. Lindberg *et al.*[10] have developed an alternative method for the synthesis of β-D-mannopyranosides which has recently been improved by Garegg *et al.*[11]. We now report the synthesis of 4-*O*-β-D-mannopyranosyl-L-rhamnopyranose, a constituent[1] of *Salmonella* types D_2 and E.

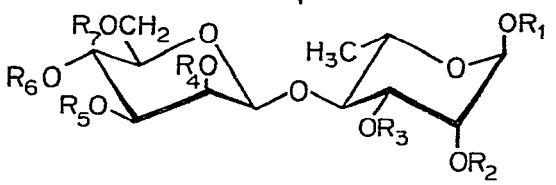
RESULTS AND DISCUSSION

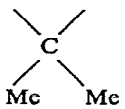
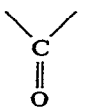
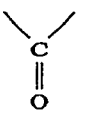
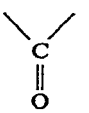
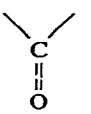
Preparation of methyl 4,6-*O*-benzylidene-2,3-*O*-carbonyl-α-D-mannopyranoside from methyl 4,6-*O*-benzylidene-α-D-mannopyranoside was accomplished by using ethyl chloroformate and triethylamine by a procedure similar to that employed for the D-*gluco* analog[12]. It was found that the addition of just enough triethylamine to make the reaction mixture basic produced the cyclic carbonate. A more complete reaction was achieved by combining with the starting material that quantity of triethylamine required to make the reaction mixture just basic, before adding the ethyl chloroformate dropwise, instead of by the reverse procedure. Acetolysis of the product gave crystalline 1,4,6-tri-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranose (**4**) in 81% yield, m.p. 117.5–118.5°, and subsequent hydrobromination gave 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide (**5**) in 82% yield, m.p. 79–80°.

Condensation of bromide **5** with aglycon hydroxides in acetonitrile, with mercuric cyanide as the condensing agent, gave α-D-mannopyranosides, unless the aglycon hydroxide was present in excess; methanol in excess gave crystalline methyl 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-β-D-mannopyranoside in 87% yield (m.p. 137–138°), and de-esterification thereof afforded methyl β-D-mannopyranoside. However, in the preparation of disaccharides, it is usually not practical to have the aglycon hydroxide present in excessive proportion, although this was the method employed by Gorin and Perlin[9]. If silver oxide in chloroform (without iodine), or mercuric acetate in benzene, is employed as the condensing agent, the β-D-glycoside will be formed without the aglycon hydroxide being present in excess. Thus, **5** was condensed with methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**1**) in chloroform in the presence of silver oxide, to give methyl 4-*O*-(4,6-di-*O*-acetyl-2,3-*O*-carbonyl-β-D-mannopyranosyl)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside as a syrup; the α-D-linked product was formed in less than 5% yield. The subsequent deblocking and derivatization procedures paralleled those for the α-D-*manno*[3] and β-D-*gluco*[13] analogs. Removal of the isopropylidene group followed by acetylation gave crystalline methyl 2,3-di-*O*-acetyl-4-*O*-(4,6-di-*O*-acetyl-2,3-*O*-carbonyl-β-D-mannopyranosyl)-α-L-rhamnopyranoside (**10**), in 73% yield based on **1**, m.p. 204.5–205.5°. Deacylation of **10** afforded methyl 4-*O*-β-D-mannopyranosyl-α-L-rhamnopyranoside (**11**), which crystallized from 2-propanol as an alcoholate containing one molecule of **11** per molecule of 2-propanol, m.p. 108.5–110°.

In contrast to the results obtained for the analogous α-D anomer, acetolysis of either **11** or its peracetate **13** gave a mixture containing the desired 1,2,3-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranose (**15**) and 1,2,3-tri-*O*-acetyl-4-*O*-(1,2,3,4,5,6-hexa-*O*-acetyl-D-*glycero*-D-*galacto*-hexitol-1-yl)-α-L-





	R_1	R_2	R_3	R_4	R_5	R_6	R_7
8	Me					Ac	Ac
9	Me	H	H			Ac	Ac
10	Me	Ac	Ac			Ac	Ac
11	Me	H	H	H	H	H	H
12	Me	Me	Me	Me	Me	Me	Me
13	Me	Ac	Ac	Ac	Ac	Ac	Ac
14	Ac	Ac	Ac			Ac	Ac
15	Ac	Ac	Ac	Ac	Ac	Ac	Ac
16	H	H	H	H	H	H	H

rhamnopyranose in approximately equal amounts. As discussed in greater detail elsewhere[14], acetolysis of **10**, however, gave only one product, namely, 1,2,3-tri-*O*-acetyl-

4-*O*-(4,6-di-*O*-acetyl-2,3-*O*-carbonyl-β-D-mannopyranosyl)-α-L-rhamnopyranose, which was then de-esterified and the product acetylated to the peracetate. As this procedure gave a product containing a considerable proportion of 1,2,3-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-β-L-rhamnopyranose, the mixture was anomerized with zinc chloride in acetic anhydride to give the desired crystalline peracetate **15** in 75% yield (based on **10**), m.p. 164–165°. Deacetylation of **15** gave 4-*O*-β-D-mannopyranosyl-L-rhamnopyranose (**16**) as a syrup. Two further syrupy derivatives, the corresponding alditol **17** and the related alditol acetate **18**, were respectively prepared by reduction of **16**, and subsequent acetylation.

The presence of the (1→4)-linkage in **16** was substantiated by methylation and periodate-oxidation studies on the glycoside **11**. The β-D configuration of the D-mannopyranosyl group was confirmed by the differences between the chromatographic retention-times, the optical rotations, and the proton magnetic resonance (p.m.r.) spectra of this series and those of the α-D series[3]. New crystalline compounds gave satisfactory microanalyses, and the p.m.r. spectra of all of the new compounds described were in agreement with the structures assigned.

When the methylation analysis of disaccharide **16**, and of related compounds[3,13,15], was performed by using a cation-exchange resin to remove sodium ions (prior to removal of the borate and acetylation of the methylated alditols), the gas–liquid chromatograms obtained showed only peaks corresponding to 2,3-di-*O*-methyl-L-rhamnitol triacetate and 2,3,4,6-tetra-*O*-methyl-D-hexitol diacetate (2,3,4-tri-*O*-methyl-L-rhamnitol diacetate in the case of the L-rhamnobiose[15]). If, however, the borohydride was decomposed with acetic acid and the mixture acetylated directly, following the procedure of Albersheim and co-workers[16], gas–liquid chromatography demonstrated the presence of an unexpected, additional peak in the case of each of the four disaccharides.

This unknown material has now been examined and shown to be 1,5-di-*O*-acetyl-4-deoxy-2,3-di-*O*-methyl-L-rhamnitol-4-yl dimethyl borate. This identification was based on the p.m.r. spectrum, which indicated four separate methoxyl groups in addition to two acetate groups, and on the mass spectrum, which showed fragments corresponding to carbon atoms 1, 2, and 3 (*m/e* 161) and to carbon atoms 5 and 6 (*m/e* 87). Confirmation of this structure was obtained by methanolysis and acetylation to yield 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-rhamnitol. The stability of such boric esters, some examples of which have been described[17] in another connection, is indicated by the fact that methanolysis for 4 h was insufficient to achieve complete cleavage, and a reaction time of 16 h was required for this. The difficulty in obtaining correct quantitative data when such borate complexes are formed is obvious.

EXPERIMENTAL

General methods. — Melting points were obtained for samples between glass slides on a Fisher–Johns apparatus and are uncorrected. Optical rotations were

measured with a Perkin–Elmer model 141 polarimeter at 23 ±1°. P.m.r. spectra were recorded on a Varian XL-100 instrument, with tetramethylsilane as the internal standard, except as noted. Gas–liquid partition chromatography (g.l.c.) was conducted with an F and M 720 instrument equipped with dual, thermal-conductivity detectors at a helium flow-rate of 60 ml/min, with the following columns: (*a*) 2 ft × 0.25 in. of 20% of SE-30 (F and M Division, Hewlett Packard, Avondale, Pennsylvania), (*b*) 4 ft × 0.25 in. of 5% of butanediol succinate on Diatoport S (80–100 mesh), and (*c*) 6 ft. × 0.25 in. of 15% of OS-138 on Gas Chrom G (100–120 mesh). Peak areas were determined with an Infotronics CRS-100 electronic integrator. Mass spectra were recorded either with a Micromass 12 gas–liquid chromatography–mass spectrometer, or with an AEI MS 9 instrument. Thin-layer chromatography (t.l.c.) was performed with solvent systems *A* and *B* on silica gel G (from EM Reagents); solvent *A*, 2:1 ethyl ether–toluene; *B*, butanone–water azeotrope. The plates were dried, and components were detected by spraying with 35% ethanolic sulfuric acid and heating for 3–5 min at ~150°. Paper-chromatographic separations were conducted on Whatman No. 1 paper with the upper layer of solvent systems *C* and *D*; solvent *C*, 4:1:1 ethyl acetate–pyridine–water; *D*, 4:1:5 1-butanol–ethanol–water. Zones were made visible by using silver nitrate in acetone[18] for reducing and non-reducing compounds, and *p*-anisidine in trichloroacetic acid[19] for methylated reducing sugars. Solutions were evaporated below 50° under diminished pressure.

Methyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (**1**). — Compound **1** was prepared as described previously[13], except that the Amberlite IR-120 (H^+) resin was presoaked in dry methanol instead of acetone (to avoid formation of acetone polymers).

Methyl 4,6-O-benzylidene-α-D-mannopyranoside (**2**). — Compound **2** was prepared essentially as described by Gibney[20]. Methyl α-D-mannopyranoside (commercial preparation from Calbiochem; finely powdered, 10 g) was dissolved as rapidly as possible in 98–100% formic acid (50 ml), and freshly distilled benzaldehyde (50 ml) was immediately added to the solution. After being allowed to stand for 5 min with occasional shaking, the solution was poured with stirring into a mixture of water (200 ml) and anhydrous potassium carbonate (137 g). The excess of benzaldehyde was immediately removed by steam distillation of the resulting solution, and the resulting, aqueous phase was extracted with chloroform in a continuous extractor. The extract was evaporated to a residue that crystallized from benzene (210 ml); yield 5.5 g (38%). Recrystallization from benzene gave pure **2**, m.p. 146–147°, $[\alpha]_D$ +64.3° (*c* 2.1, chloroform) {lit.[21] m.p. 140–143°, $[\alpha]_D$ +61° (*c* 1.84, chloroform); lit.[22] m.p. 146–147°, $[\alpha]_D$ +71.7° (*c* 1.185, chloroform)}; R_F 0.23 (solvent *A*); p.m.r. ($CDCl_3$): τ 2.44–2.66 (5 protons, aromatic H), 4.44 (1-proton singlet, PhCH), 5.27 (1-proton doublet, $J_{1,2}$ 0.9 Hz, H-1), and 6.62 (3-proton singlet, OCH_3).

Methyl 4,6-O-benzylidene-2,3-O-carbonyl-α-D-mannopyranoside (**3**). — Compound **3** was prepared by minor modifications of the procedure of Doane *et al.*[12] for the corresponding D-glucoside. Compound **2** (3 g, dry) in *p*-dioxane (15 ml) was treated with a solution of triethylamine (7.5 ml) in benzene (45 ml), and the mixture

then cooled in an ice–water bath. While the suspension was being stirred magnetically, ethyl chloroformate (25 ml) was added dropwise so as to keep the effervescence under control (during ~20 min). The mixture (basic to litmus paper) was stirred in the ice–water bath for an additional 20 min, and then diluted with benzene (200 ml) and washed successively with water (2 × 200 ml), 1M hydrochloric acid (200 ml), water (200 ml), saturated sodium hydrogen carbonate solution (200 ml), and water (2 × 200 ml). Evaporation of the solvent gave a solid residue which was recrystallized from ethanol (9 ml); yield 3.1 g (94%). Recrystallization from ethanol gave pure **3**, m.p. 125–127°, $[\alpha]_D$ −21.0° (*c* 2.1, chloroform) {lit.[23] m.p. 125–126°, $[\alpha]_D^{25}$ −19.3° (chloroform)}; R_F 0.75 (solvent *A*); p.m.r. ($CDCl_3$); τ 2.46–2.70 (5 protons, aromatic H), 4.43 (1-proton singlet, PhCH), 4.99 (1-proton singlet, H-1), and 6.62 (3-proton singlet, OCH_3).

1,4,6-Tri-O-acetyl-2,3-O-carbonyl-α-D-mannopyranose (**4**). — A suspension of compound **3** (3 g) in acetic anhydride (15 ml) was shaken with 1% (v/v) concentrated sulfuric acid in acetic anhydride (30 ml) for 2 h at room temperature. The mixture was then diluted with chloroform (200 ml), washed successively with ice–water (2 × 200 ml), saturated sodium hydrogen carbonate solution (2 × 200 ml), and water (2 × 200 ml), and any acetic anhydride or acetic acid remaining was removed by addition and evaporation of ethanol. The resulting syrup crystallized from ethanol (10 ml) to yield 2 g of crystals. A further 0.6 g of syrup containing some of the β anomer was obtained by pressure column-chromatography[24] on silica gel H (from EM Reagents, type 60) and elution with solvent *A* (total yield 81%). Recrystallization from ethanol gave pure **4**, m.p. 117.5–118.5°; $[\alpha]_D$ +15.6° (*c* 2.5, chloroform) {lit.[9] $[\alpha]_D$ +7.5° (*c* 1.0 chloroform)}; R_F 0.21 (solvent *A*); p.m.r. ($CDCl_3$): τ 3.60 (1-proton singlet, H-1), and 7.86, 7.88, and 7.92 (3-proton singlets, 3 OAc).

Anal. Calc. for $C_{13}H_{16}O_{10}$: C, 46.99; H, 4.85. Found: C, 46.90; H, 4.95.

4,6-Di-O-acetyl-2,3-O-carbonyl-α-D-mannopyranosyl bromide (**5**). — Compound **5** was prepared essentially as described by Gorin and Perlin[9]. Compound **4** (2 g) in chloroform (200 ml) was stirred with 30–32% hydrogen bromide in acetic acid (40 ml) for 3 h at 0°. The solution was then quickly washed successively with ice–water (2 × 200 ml), saturated sodium hydrogen carbonate solution (2 × 200 ml), and ice–water (2 × 200 ml), dried, filtered through a layer of calcium oxide and silica gel, and evaporated to a syrup that crystallized from ethyl ether (anhydrous, 25 ml) at −10°. Petroleum ether (b.p. 65–70°, 10 ml) was added in portions to complete the crystallization; yield 1.75 g (82%). Recrystallization from ethyl ether gave pure **5**, m.p. 79–80°; $[\alpha]_D$ +89.8° (*c* 3.3, chloroform) {lit.[9] $[\alpha]_D$ +55° (*c* 1.0, chloroform)}; R_F 0.63 (solvent *A*); p.m.r. ($CDCl_3$): τ 3.33 (1-proton singlet, H-1), 7.86 and 7.92 (3-proton singlets, 2 OAc).

Methyl 4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranoside (**6**). — Compound **5** (1.6 g) was stirred with acetonitrile (10 ml, distilled over calcium hydride), methanol (10 ml, anhydrous), and mercuric cyanide (1.6 g) for 4 h. The solvents were evaporated, the resulting syrup was dissolved in chloroform (125 ml), and the solution was washed successively with 1M potassium bromide (2 × 100 ml), water (100 ml),

saturated sodium hydrogen carbonate solution (100 ml), and water (2 × 100 ml), and evaporated to a syrup that crystallized from ethanol (12 ml); yield 1.2 g (87%). Recrystallization from ethanol gave pure **6**, m.p. 137–138°; $[\alpha]_D$ −85.9° (*c* 2.7, chloroform) {lit.[9] $[\alpha]_D$ −37° (*c* 1.0, chloroform)}; R_F 0.22 (solvent *A*); p.m.r ($CDCl_3$): τ 5.17 (1-proton doublet, $J_{1,2}$ 3 Hz, H-1), 6.43 (3-proton singlet, OCH_3), and 7.89 and 7.91 (3-proton singlets, 2 OAc).

Anal. Calc. for $C_{12}H_{16}O_9$: C, 47.37; H, 5.30. Found: C, 47.49; H, 5.45.

Methyl β-D-mannopyranoside, isopropyl alcoholate (**7**). — Compound **6** (0.50 g) was de-esterified with 0.2M sodium methoxide (15 ml) for 1 h at room temperature. Sodium ions were removed from the chilled solution with Amberlite IR-120 (H^+) resin, and remaining traces of acid were removed with Duolite A-4 (OH^-) resin. The syrup obtained on evaporation crystallized from isopropyl alcohol (5 ml) after addition and evaporation of isopropyl alcohol (2 × 30 ml); yield 0.36 g (86%). Recrystallization from the same solvent gave pure **7**, m.p. 72–73°, $[\alpha]_D$ −52.7° (*c* 2.2, water) {lit.[25] m.p. 74–75°, $[\alpha]_D$ −53.3° (*c* 4, water)}; R_F 0.02 (solvent *B*); R_{Glc} 1.62 (solvent *C*); p.m.r. (D_2O, external tetramethylsilane): τ 5.41 (1-proton doublet, $J_{1,2}$ 0.9 Hz, H-1) and 6.43 (3-proton singlet, OCH_3).

Methyl 4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranosyl)-2,3-O-isopropylidene-α-L-rhamnopyranoside (**8**). — A solution of compound **1** (1.0 g, 4.6 mmoles) in chloroform (10 ml; dry, alcohol-free) was stirred magnetically in the dark for 1 h at room temperature with silver oxide (3 g; freshly prepared, dry) and calcium sulfate (4 g; anhydrous). A solution of **5** (3.0 g) in chloroform (20 ml; dry, alcohol-free) was added dropwise during 3 h, and stirring was continued for an additional 0.5 h. The mixture was filtered, and the filtrate was evaporated to a crude syrup (3.75 g) that showed a major (~80%) component (R_F 0.36) in t.l.c. with solvent *A*. For analytical purposes, a small amount was purified by preparative t.l.c.[26]: $[\alpha]_D$ −65° (*c* 3.1, chloroform); p.m.r. ($CDCl_3$): τ 5.15 (1-proton singlet, H-1), 5.19 (1-proton doublet, $J_{1,2}$ 1.4 Hz, H-1), 6.65 (3-proton singlet, OCH_3), 7.90, 7.92 (3-proton singlets, 2 OAc), 8.49, 8.66 (3-proton singlets, CMe_2), and 8.63 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3).

Methyl 4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranosyl)-α-L-rhamnopyranoside (**9**). — A solution of crude compound **8** (3.75 g) in chloroform (135 ml) was treated for 1 h at room temperature with trifluoroacetic acid containing 1% of water (15 ml). The mixture was then concentrated, and remaining trifluoroacetic acid was removed by addition and evaporation of toluene. The resulting syrup (3.6 g) showed mainly one spot in t.l.c., R_F 0.04 (solvent *A*) and 0.62 (solvent *B*); p.m.r. ($CDCl_3$): τ 6.65 (3-proton singlet, OCH_3), 7.89, 7.92 (3-proton singlets, 2 OAc), and 8.61 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3).

Methyl 2,3-di-O-acetyl-4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranosyl)-α-L-rhamnopyranoside (**10**). — Crude compound **9** (3.6 g) was acetylated with pyridine (20 ml) and acetic anhydride (20 ml) overnight at room temperature. The excess of the reagents was removed by successive evaporation with ethanol and then with water. The resulting syrup crystallized from ethanol (25 ml); yield 1.8 g

(3.37 mmoles; 73%, based on **1**). Recrystallization from ethanol gave pure **10**, m.p. 204.5–205.5°, $[\alpha]_D$ −61.8° (*c* 2.4, chloroform); R_F 0.08 (solvent *A*); p.m.r. ($CDCl_3$): τ 6.65 (3-proton singlet, OCH_3), 7.92, 7.94, 7.96, 8.03 (3-proton singlets, 4 OAc), and 8.61 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3).

Anal. Calc. for $C_{22}H_{30}O_{15}$: C, 49.44; H, 5.66. Found: C, 49.27; H, 5.69.

Methyl 4-O-β-D-mannopyranosyl-α-L-rhamnopyranoside (**11**). — Compound **10** (1.4 g) was de-esterified with sodium methoxide (0.2M, 40 ml) for 1 h at room temperature. Sodium ions were removed from the chilled solution with Amberlite IR-120 (H^+) resin, and remaining traces of acid were removed with Duolite A-4 (OH^-) resin. The syrup (0.85 g, 95%) obtained on evaporation showed one spot in t.l.c., R_F 0.03 (solvent *B*), and crystallized from isopropyl alcohol (50 ml) as an alcoholate after addition and evaporation of this alcohol (2 × 50 ml); yield 0.97 g (97%). Recrystallization from isopropyl alcohol gave pure **11** alcoholate, m.p. 108.5–110°, $[\alpha]_D$ −72.4° (*c* 2.2, water); R_{Glc} 1.44 (solvent *C*); p.m.r. (Me_2SO-d_6): τ 5.08 (1-proton doublet, $J_{1',2'}$ 4 Hz, H-1'), 6.76 (3-proton singlet, OCH_3), 8.77 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3), and 8.92 and 8.98 (3-proton singlets, 2 CH_3 of isopropyl alcohol).

Anal. Calc. for $C_{16}H_{32}O_{11}$: C, 47.99; H, 8.06. Found: C, 47.76; H, 8.20.

When the alcohol of crystallization was lost by exchange with deuterium oxide, the p.m.r. data were as follows (external tetramethylsilane): τ 5.16 (1-proton doublet, $J_{1',2'}$ 0.9 Hz, H-1'), 5.35 (1-proton doublet, $J_{1,2}$ 1.3 Hz, H-1), 6.64 (3-proton singlet, OCH_3), and 8.69 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3).

The methyl glycoside **11** was methylated by a method previously described[13], to give the corresponding heptamethyl ether **12**. A small amount of this was purified by preparative t.l.c.[26] for analytical purposes: R_F 0.17 (solvent *A*); $[\alpha]_D$ −87.5° (*c* 1.4, chloroform); p.m.r. ($CDCl_3$): τ 5.25 (1-proton doublet, $J_{1,2}$ 1.6 Hz, H-1), 5.37 (1-proton doublet, $J_{1',2'}$ 0.8 Hz, H-1'), 6.40–6.63 (21 protons, 7 OCH_3), and 8.63 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3). Hydrolysis of **12** gave 2,3-di-*O*-methylrhamnose and 2,3,4,6-tetra-*O*-methylmannose, identified by paper chromatography (solvent *D*) by using authentic standards. Subsequent reduction and acetylation gave equimolar amounts of 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol, identified by comparative g.l.c. (column *c*, retention times 21.8 and 26.6 min, respectively) and mass-spectrometric analyses with use of authentic standards[27].

Alternatively, the methylated sugars obtained on hydrolysis of **12** were reduced overnight with sodium borohydride in water, followed by acidification with acetic acid, evaporation to dryness, successive additions and evaporations of methanol, and acetylation with acetic anhydride. The product was treated with ethyl acetate, the suspension filtered to remove sodium acetate, and the filtrate injected onto column *c* at 220°. Three peaks in the ratios of 1:3:4 were eluted at 17.8, 21.8, and 26.6 min.

The compound having retention time 17.8 min was collected; it had the following p.m.r. spectrum ($CDCl_3$): τ 6.51–6.56 (12 protons, 4 OCH_3, expansion indicated four separate methoxyl groups), 7.90, 7.94 (3-proton singlets, 2 OAc), and

8.66 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3). The mass spectrum showed *m/e* 43, 45, 58, 71, 87, 101, 117, 130, 131, 143, 149, 161, 177, 191, and 203. The unknown compound was subjected to methanolysis overnight under reflux, followed by neutralization with Duolite A-4 (OH^-) resin, evaporation, and subsequent acetylation; the acetate cochromatographed (column *c*, 220°) with standard 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-rhamnitol.

Periodate oxidation of **11** by a procedure previously described[13] showed a total consumption of 3.0 moles per mole in 50 h. Subsequent reduction, methanolysis, evaporation, and acetylation gave 4-deoxy-L-erythritol (1-deoxy-D-erythritol) triacetate and glycerol triacetate, identified by comparative g.l.c. (column *b*, 130°, retention times 8.4 and 12.8 min, respectively) and by mass-spectrometric analysis with use of authentic standards.

*Methyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranoside* (**13**). — Compound **11** (0.20 g) was acetylated with pyridine (10 ml) and acetic anhydride (10 ml) for 5 h at room temperature. The excess of the reagents was removed by successive evaporation with ethanol and water, yielding the product as a syrup (0.33 g, 95%). For analytical purposes, a small amount was purified by t.l.c.[26]; $[\alpha]_D$ −57° (*c* 2.1, chloroform); R_F 0.36 (solvent *A*); p.m.r. ($CDCl_3$): τ 5.25 (1-proton doublet, $J_{1',2'}$ 0.8 Hz, H-1'), 5.42 (1-proton doublet, $J_{1,2}$ 1.6 Hz, H-1), 6.65 (3-proton singlet, OCH_3), 7.88–8.04 (18 protons, 6 OAc), and 8.64 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3).

*1,2,3-Tri-*O*-acetyl-4-*O*-(4,6-di-*O*-acetyl-2,3-*O*-carbonyl-β-D-mannopyranosyl)-L-rhamnopyranose* (**14**). — Compound **10** (1.5 g) in acetic anhydride (7.5 ml) was shaken with 1% (v/v) concentrated sulfuric acid in acetic anhydride (15 ml) for 2 h at room temperature. The mixture was then diluted with chloroform (200 ml), and washed successively with ice–water (2 × 200 ml), saturated sodium hydrogen carbonate solution (2 × 200 ml), and water (2 × 200 ml); any remaining acetic anhydride or acetic acid was removed by addition and evaporation of ethanol. The resulting syrup showed essentially one spot in t.l.c., R_F 0.07 (solvent *A*); yield 1.5 g (95%). For analytical purposes, a small amount was purified by preparative t.l.c.[26]; $[\alpha]_D$ −64° (*c* 1.7, chloroform); p.m.r. ($CDCl_3$): τ 3.99 (1-proton doublet, $J_{1,2}$ 1.6 Hz, H-1), 7.84, 7.85, 7.88, 7.90, 7.95 (3-proton singlets, 5 OAc), and 8.58 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3).

*1,2,3-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranose* (**15**). — Compound **14** (1.5 g) was de-esterified with sodium methoxide (0.2M, 40 ml) for 1 h at room temperature. Sodium ions were then removed from the chilled solution with Amberlite IR-120 (H^+) resin, and remaining traces of acid were removed with Duolite A-4 (OH^-) resin. The syrup obtained on evaporation was acetylated with pyridine (25 ml) and acetic anhydride (25 ml) overnight at room temperature. The excess of reagents was removed by successive evaporation with ethanol and water, and the resulting syrup was anomerized[28,29] with zinc chloride (0.1 g, freshly fused) in acetic anhydride (10 ml) for 4 h at room temperature. The mixture was then diluted with chloroform (200 ml), washed successively with ice–

water (2 × 200 ml), saturated sodium hydrogen carbonate solution (2 × 200 ml), and water (2 × 200 ml), and the remaining acetic anhydride or acetic acid was removed by addition and evaporation of ethanol. The resulting syrup crystallized from ethanol (9 ml) when nucleated with a crystal obtained from a t.l.c. separation[26]; yield 1.3 g (79%). Recrystallization from ethanol gave pure **15**, m.p. 164–165°, $[\alpha]_D$ −67.8° (*c* 1.3, chloroform); R_F 0.36 (solvent *A*); p.m.r. ($CDCl_3$): τ 4.06 (1-proton doublet, $J_{1,2}$ 1.7 Hz, H-1), 5.27 (1-proton doublet, $J_{1',2'}$ 1.0 Hz, H-1'), 7.89–8.04 (21 protons, 7 OAc), and 8.64 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3).

Anal. Calc. for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.06; H, 5.65.

4-O-β-D-Mannopyranosyl-L-rhamnopyranose (**16**). — The peracetate **15** (0.40 g) was deacetylated with sodium methoxide (0.2M, 15 ml) for 1 h at room temperature. After the usual processing, the resulting syrup (0.20 g, 95%) had $[\alpha]_D$ −46° (*c* 2.5, water); R_{Glc} 0.6 (solvent *C*); p.m.r. (D_2O, external tetramethylsilane): τ 4.97 (0.64 proton doublet, $J_{1,2}$ 1.4 Hz, H-1, α-L form), 5.20 (1-proton doublet, $J_{1',2'}$ 0.9 Hz, H-1'), 5.23 (0.36 proton doublet, $J_{1,2}$ 1.0 Hz, H-1, β-L form), and 8.77 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3).

G.l.c. (column *a* at 240°) of the per-*O*-(trimethylsilyl)ated disaccharide gave one peak (73%) at 8.2 min and a second peak at 10.8 min [in relation to per-*O*-(trimethylsilyl)sucrose, 11.2 min][30].

4-O-β-D-Mannopyranosyl-L-rhamnitol (**17**). — The free disaccharide **16** (0.10 g) was reduced with sodium borohydride (0.05 g) in water (5 ml) overnight. Passage of the solution through Amberlite IR-120 (H^+) resin, concentration, and several successive evaporations with methanol gave **17**; yield 0.1 g (99%), $[\alpha]_D$ −36° (*c* 2.4, water); R_{Glc} 0.5 (solvent *C*); p.m.r. (D_2O, external tetramethylsilane); τ 5.26 (1-proton doublet, $J_{1',2'}$ 0.9 Hz, H-1') and 8.77 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3).

G.l.c. (column *a* at 240°) of the per-*O*-(trimethylsilyl)alditol gave one peak at 14.5 min [in relation to per-*O*-(trimethylsilyl)sucrose, 11.2 min][30].

The alditol **17** (0.1 g) was acetylated with pyridine (4 ml) and acetic anhydride (4 ml) overnight at room temperature, to give the corresponding octaacetate **18** (0.2 g, 98%) as a syrup; $[\alpha]_D$ −67° (*c* 2.6, chloroform); R_F 0.33 (solvent *A*); p.m.r. ($CDCl_3$): τ 5.21 (1-proton doublet, $J_{1',2'}$ 0.9 Hz, H-1'), 7.86–8.02 (24 protons, 8 OAc), and 8.72 (3-proton doublet, $J_{5,6}$ 6 Hz, CH_3).

G.l.c. (column *a* at 270°) of the peracetylated alditol **18** gave one peak at 7.0 min (in relation to sucrose octaacetate, 9.2 min).

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