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SYNTHESES OF REPEATING UNITS OF *Escherichia coli* CAPSULAR POLYSACCHARIDES CONTAINING D-RIBOSE AND 3-DEOXY-D-*manno*-2-OCTULOSONIC ACID (KDO)*

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ABSTRACT

The oligosaccharides, sodium (methyl 3-deoxy-7-*O*-β-D-ribofuranosyl-β-D-*manno*-2-octulopyranosid)onate, methyl 2-*O*-β-D-ribofuranosyl-β-D-ribofuranoside, and the anomeric sodium [methyl 3-deoxy-7-*O*-(2-*O*-β-D-ribofuranosyl-β-D-ribofuranosyl)-α- and -β-D-*manno*-2-octulopyranosid]onate were prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose and the anomeric methyl (methyl 8-*O*-benzyl-4,5-*O*-carbonyl-3-deoxy-α- and -β-D-*manno*-2-octulopyranosid)onate in high purity and in acceptable over-all yields. They constitute a first series of model compounds for spectroscopic and immunochemical studies of the capsular polysaccharides from *Escherichia coli* strains LP 1092 and K 23. The essential, interglycosidic linkages [β-D-Rib*f*-(1→7)-α- or -β-D-dOclA, and β-D-Rib*f*-(1→2)-β-D-Rib*f*] were formed by a modification of the silver triflate procedure using appropriate D-ribofuranosyl bromide derivatives. The constitutional and configurational assignments were based on the 250-MHz ^{1}H-n.m.r.-spectra of protected derivatives of the oligosaccharides.

INTRODUCTION

An increasing number of capsular polysaccharides (K-antigens) from Gram-negative bacteria have been reported[2–6] to contain 3-deoxy-D-*manno*-2-octulosonic acid (KDO), initially discovered[7,8] as a constituent of the lipopolysaccharide (LPS) of these organisms. Of these capsular materials, we have previously studied, and proposed[9] a structure for, the K-antigen from *Escherichia coli* LP 1092. Subsequently, we have reported[10] on a comparative ^{13}C-n.m.r.-study of the LP 1092 (ref. 2) and K 23 (ref. 5) [or *O*-deacetylated K 13 (ref. 4)] polysaccharides. The structure of the K 23 antigen was readily deduced from its ^{13}C-n.m.r.-spec-

*A preliminary account of this work has appeared[1].

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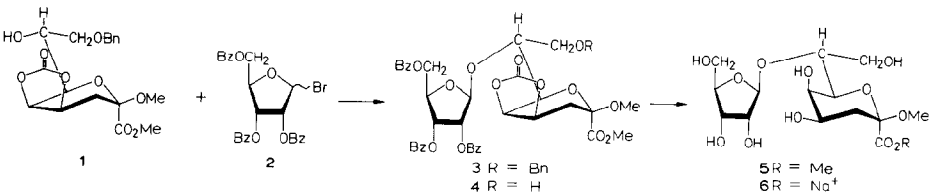
trum[10]. However, in the case of the LP 1092 polysaccharide, an attempt to interpret the ^{13}C-n.m.r.-spectrum on the basis of empirical rules has resulted in only partial assignment[11]. Measurement of the longitudinal relaxation times (T_1) by the inversion-recovery technique has afforded evidence for the presence of one D-ribofuranosyl group per repeating unit as a side branch[10], but controversy persists regarding the constitution of the LP 1092 polysaccharide[10,11]. Furthermore, the evidence[11] for the α-D anomeric configuration of the KDO residues in the LP 1092 polysaccharide is not entirely convincing. Finally, the serological reactivity of the LP 1092 polysaccharide is unclear: in one instance, it cross-reacted with a K 6-antiserum, so that it was erroneously designated as the K 6-antigen[12]. In the light of these spectroscopic and immunochemical ambiguities, the need was felt for additional data, possibly to be obtained with the aid of synthetic model compounds corresponding to "repeating units" of the polysaccharides. We report herein the syntheses of a first series of such model oligosaccharides.

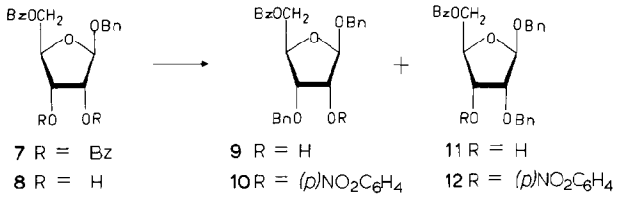
RESULTS AND DISCUSSION

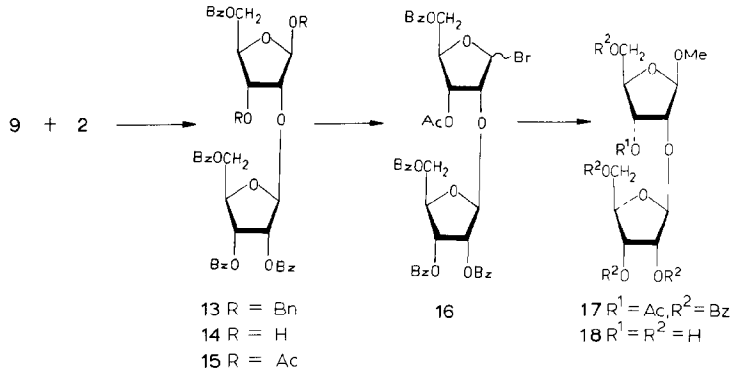
For the synthesis of the β-D-(1→7)-linked disaccharide, sodium (methyl 3-deoxy-7-*O*-β-D-ribofuranosyl-β-D-*manno*-2-octulopyranosid)onate (**6**), glycosylation of methyl (methyl 8-*O*-benzyl-4,5-*O*-carbonyl-3-deoxy-β-D-*manno*-2-octulopyranosid)onate[13] (**1**) with highly reactive 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide[14] (**2**) was first attempted under modified Koenigs–Knorr conditions[15,16] (silver carbonate, Drierite, and molecular sieve 3A). These conditions afforded only unchanged **1** and materials presumably resulting from degradation of the halide **2**. Under catalysis by silver trifluoromethylsulfonate (triflate)[17,18], the glycosidation reaction (15 min, −25°) gave the β-D-(1→7)-linked disaccharide **3** in near quantitative yield. The reaction was stopped by the addition of pyridine to prevent decomposition of the acid-sensitive glycosidation product. Addition of *N,N,N',N'*-tetramethylurea[17] to the glycosidation mixture did not influence the outcome of the reaction. The glycosidation proceeded with high stereospecificity, t.l.c. indicating the absence of a major proportion of a second isomer. N.m.r.-spectroscopic evidence confirmed the β-D configuration of the ribosyl residue ($J_{1',2'}$ ~1.5 Hz; H-7, δ 4.16). The protective groups of **3** were removed by catalytic hydrogenation in the presence of palladium oxide to afford **4**, followed by Zemplén deacylation giving **5**. The structures of the disaccharide derivatives **3–5** were confirmed by interpretation of the 250 MHz ^{1}H-n.m.r.-spectra. In the case of **3**, the 2-D homo-*J*-resolved n.m.r. spectrum allowed unambiguous assignment of all signals. Saponification of



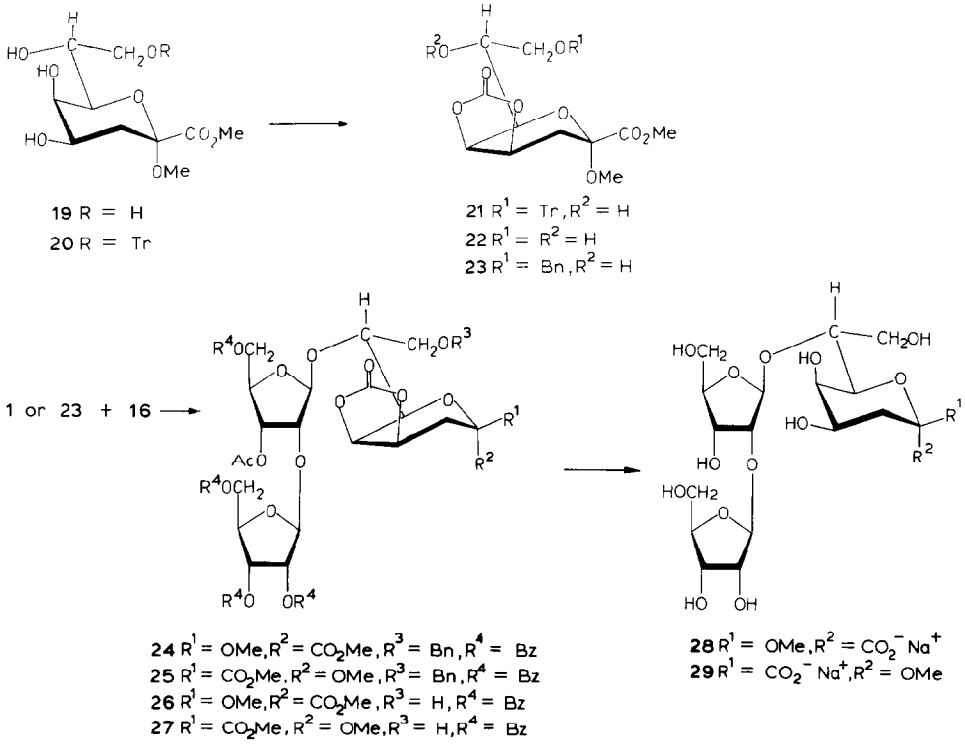
the disaccharide ester **5** afforded the model disaccharide **6** in an overall yield of 54% (based on **1**).

For the synthesis of the model compounds **18, 28,** and **29**, crystalline benzyl 5-*O*-benzoyl-β-D-ribofuranoside (**8**) was the starting material. It was conveniently prepared in 65% yield by controlled Zemplén deacylation of known[19] benzyl 2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside (**7**). The structure of **8** was in accord with the n.m.r.-spectroscopic data and with the observed reactivity of **8** towards periodate. Mono-*O*-benzylation of **8** by way of its 2,3-*O*-dibutylstannylidene derivative[20,21] proceeded with low regioselectivity to give an 11:9 mixture (isolated yields; total yield, 60%) of the 3- and 2-benzyl ethers **9** and **11**. After chromatographic separation of the two isomers, small samples of compounds **9** and **11** were converted into the corresponding 2- and 3-*p*-nitrobenzoate (**10** and **12**) to facilitate their n.m.r.-spectroscopic identification. Depending on the position of *O*-benzylation, H-2 or H-3 experienced characteristic deshielding due to the *p*-nitrobenzoyl group.





Glycosylation of **9** with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**2**) under catalysis by silver triflate afforded the disaccharide derivative **13** in yields >80%. The reaction proceeded to completion within 15 min with high stereoselectivity due to the neighboring-group participation[22] of the 2-*O*-benzoyl group. Hydrogenation of **13** in the presence of palladium oxide gave the partially protected, reducing disaccharide **14** (65%). The ^{1}H-n.m.r.-spectrum of **14** indicated a 3:4 ratio of α- and β-D anomers. Acetylation with acetic anhydride–pyridine of **14** yielded crystalline 1,3-di-*O*-acetyl-5-*O*-benzoyl-2-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranose (**15**). The ^{1}H-n.m.r. spectrum of **15** was amenable to first-order



analysis and confirmed the structure and anomeric configurations assigned. Compound **15** was converted into the disaccharide halide **16** by the action of titanium tetrabromide in dry dichloromethane. The rather unstable **16** was employed for glycosidation immediately. Glycosylation of methanol under Koenigs–Knorr conditions afforded a ~7:1 mixture of the methyl β- and α-D-ribofuranosyl disaccharides in a total yield of 55%. The anomers were separated by column chromatography on silica gel. Zemplén deacylation of the β-D anomer **17** afforded the desired disaccharide model compound **18**.

Glycosylation of the KDO derivative[13] **1** with the disaccharide halide **16** under catalysis by silver triflate gave the β-D-(1″→2′)-β-D-(1′→7)-linked trisaccharide derivative **24** in 43% yield. The β-D configuration at C-1′ was ascertained by the low value of the coupling constant ($J_{1',2'}$ ~2 Hz). Catalytic hydrogenation of **24** in the presence of palladium catalyst, followed by Zemplén deacylation and saponification with aqueous sodium hydroxide afforded the trisaccharide, sodium [methyl3-deoxy-7-*O*-(2-*O*-β-D-ribofuranosyl-β-D-ribofuranosyl)-β-D-*manno*-2-octulopyranosid]onate **28**. For the synthesis of **29** (the model compound analogous to **28** but containing the octulopyranoside residue in the α-D anomeric configuration), methyl (methyl 8-*O*-benzyl-4,5-*O*-carbonyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**23**) was prepared as follows. Tritylation of methyl (methyl 3-

deoxy-α-D-*manno*-2-octulopyranosid)onate[23,24] (**19**) according to the procedure of Hanessian and Staub[25] afforded the 8-*O*-trityl derivative **20**. Carbonylation[26] of **20** and subsequent hydrogenation of the carbonate-trityl ether **21** in the presence of palladium oxide[27] afforded, in good yield, methyl (methyl 4,5-*O*-carbonyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**22**) which was converted into the 8-*O*-benzyl derivative **23** by regioselective benzylation of its 7,8-*O*-dibutylstannylidene derivative[20,21]. Compound **23** was glycosylated with bromide **16** under catalysis by silver triflate, which gave the trisaccharide derivative **25** in 22% yield. The markedly reduced yield of **25**, as compared to that of **24**, may be explained by the increased steric hindrance at OH-7 of the KDO-derivative **23**, possibly due to conformational changes caused by the anomeric effect of the methoxyl group in α-D configuration. Removal of the protecting groups was accomplished by catalytic hydrogenation of **25** in the presence of palladium oxide, which afforded **27** as a crystalline solid. Zemplén deacylation of **27**, followed by saponification with aqueous sodium hydroxide gave the trisaccharide, sodium [methyl 3-deoxy-7-*O*-(2-*O*-β-D-ribofuranosyl-β-D-ribofuranosyl)-α-D-*manno*-2-octulopyranosid]onate (**29**).

The ^{13}C-n.m.r.-spectra of the model oligosaccharides **6**, **18**, **28**, and **29** were recorded and compared[28] to those of the *O*-deacetylated K 13 (K 23) and LP 1092 polysaccharides[10].

EXPERIMENTAL

General methods. — Melting points were determined with a Kofler hot-stage and are uncorrected. Optical rotations were determined with a Perkin–Elmer 141 polarimeter. ^{1}H-N.m.r. spectra were recorded with Bruker WH-90 and WH-250 (at 90 and 250 MHz) instruments using tetramethylsilane as the internal standard. Coupling constants (in Hz) are first order. Unless indicated otherwise, spectra were recorded at 250 MHz for solutions in chloroform-*d*. Thin-layer chromatography (t.l.c.) was performed on Merck precoated plates (5 × 10 cm, layer thickness 0.25 mm, Silica gel 60 F_{254}). Spots were detected by u.v. light and by spraying with an anisaldehyde–H_2SO_4 reagent[29]. Column chromatography was performed on Merck-Lichroprep columns, size A, B, and C (silica gel, 40–63 μm), under pressure (~0.2 MPa). Glycosidation reactions were performed under rigorously anhydrous conditions and with exclusion of light. Dichloromethane (purified through a column of Al_2O_3 followed by distillation in the presence of P_2O_5) was distilled in the presence of CaH_2 prior to use. Elemental analyses were performed by Dr. J. Zak, Mikroanalytisches Laboratorium am Institut für physikalische Chemie, Universität Wien.

Methyl [methyl 8-O-benzyl-4,5-O-carbonyl-3-deoxy-7-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-manno-2-octulopyranosid]onate (**3**). — A solution of **2** (126 mg) in dichloromethane (2 mL) was added dropwise at −25° during 5 min to a suspension of **1** (62 mg), silver triflate (57 mg), and Drierite (400 mg) in dichloromethane (5 mL). After 15 min, pyridine (0.1 mL) was added, the mixture

was filtered through Celite, and the residue washed twice with dichloromethane (10 mL). The filtrate was extracted with water, 5% aqueous $Na_2S_2O_3$, and water. The organic layer was dried ($MgSO_4$) and evaporated. Chromatography of the residue on a silica gel column (size C; 6:1 toluene–ethyl acetate) afforded **3** (131 mg, 98%) as a syrup, $[\alpha]_D^{20}$ +7.9° (*c* 0.58, chloroform); ^{1}H-n.m.r.: δ 1.85 (dd, 1 H, $J_{3a,3e}$ ~16.0, $J_{3a,4}$ ~4.5, H-3*a*), 2.41 (dd, 1 H, $J_{3e,4}$ ~2.0, H-3*e*), 3.29 (s, 3 H, CH_3O), 3.63 (dd, 1 H, $J_{6,7}$ ~9.0, H-6), 3.63 (dd, 1 H, $J_{7,8b}$ ~6.2, H-8b), 3.76 (s, 3 H, CH_3OCO), 3.93 (dd, 1 H, $J_{7,8a}$ ~2.3, $J_{8a,8b}$ ~10.8, H-8a), 4.16 (ddd, 1 H, H-7), 4.54 (dd, 1 H, $J_{4',5'b}$ ~4.9, H-5'b), 4.58 (s, 2 H, CH_2O), 4.69 (ddd, 1 H, $J_{4',5'a}$ ~3.6, H-4'), 4.73 (ddd, 1 H, $J_{4,5}$ ~9.1, H-4), 4.82 (dd, 1 H, $J_{5'a,5'b}$ ~11.7, H-5'a), 5.08 (dd, 1 H, $J_{5,6}$ ~1.4, H-5), 5.55 (d, 1 H, $J_{1',2'}$ ~1.5, H-1'), 5.76 (dd, 1 H, $J_{2',3'}$ ~4.8, H-2'), 5.87 (dd, 1 H, $J_{3',4'}$ ~7.1, H-3'), 7.15–7.65 (m, 14 H) and 7.85–8.08 (m, 6 H, arom. H).

Anal. Calc. for $C_{44}H_{42}O_{16}$: C, 63.9; H, 5.1. Found: C, 63.6; H, 5.1.

Methyl [*methyl 4,5-*O*-carbonyl-3-deoxy-7-*O*-(2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranosyl)-β-*D*-manno-2-octulopyranosid*]*onate* (**4**). — A solution of **3** (100 mg) in 1:1 methanol–ethyl acetate (10 mL) was hydrogenolyzed in the presence of PdO (25 mg) at room temperature and atmospheric pressure. After 18 h, the mixture was filtered and the filtrate evaporated. The residue was purified on a column of silica gel (size B; 1:1 toluene–ethyl acetate) and afforded **4** (82 mg, 92%) as a colorless syrup, $[\alpha]_D^{20}$ −2.5° (*c* 0.68, chloroform); ^{1}H-n.m.r.: δ 1.77 (dd, $J_{3a,3e}$ ~16.2, $J_{3a,4}$ ~3.8, H-3*a*), 2.46 (dd, 1 H, $J_{3e,4}$ ~2.1, H-3*e*), 3.23 (t, 1 H, $J_{8,OH}$ ~7.7, OH), 3.26 (s, 3 H, CH_3O), 3.70 (dd, 1 H, $J_{6,7}$ ~9.1, H-6), 3.81 (s, 3 H, CH_3OCO), 3.95 (m, 2 H, $J_{7,8a}$ ~2.6, $J_{7,8b}$ ~5.5, H-8a, H-8b), 4.05 (ddd, 1 H, H-7), 4.54 (dd, 1 H, $J_{5'a,5'b}$ ~11.9, $J_{4',5'b}$ ~4.75, H-5'b), 4.72 (ddd, 1 H, $J_{4',5'a}$ ~3.56, H-4'), 4.76 (ddd, 1 H, $J_{4,5}$ ~9.3, H-4), 4.84 (ddd, 1 H, H-5'a), 5.14 (dd, 1 H, $J_{5,6}$ ~1.2, H-5), 5.50 (d, 1 H, $J_{1',2'}$ ~1.15, H-1'), 5.69 (dd, 1 H, $J_{2',3'}$ ~4.9, H-2'), 5.87 (dd, 1 H, $J_{3',4'}$ ~6.9, H-3'), 7.15–7.64 (m, 9 H) and 7.90–8.10 (m, 6 H, arom. H).

Anal. Calc. for $C_{37}H_{36}O_{16} \cdot 0.5\ H_2O$: C, 59.6; H, 5.0. Found: C, 59.7; H, 5.0.

*Methyl (methyl 3-deoxy-7-*O*-β-*D*-ribofuranosyl-β-*D*-manno-2-octulopyranosid)onate* (**5**). A solution of **4** (67 mg) in dry methanol (5 mL) was treated with 0.2M methanolic sodium methoxide (2 mL) for 18 h at room temperature. The mixture was made neutral with Dowex 50 (H^+) resin, filtered, and the filtrate evaporated to dryness. The residue was dissolved in water (5 mL) and extracted twice with ether (5 mL). Lyophilization of the aqueous layer afforded **5** (26 mg, 72%) as a syrup, $[\alpha]_D^{20}$ −3.5° (*c* 0.87, methanol); ^{1}H-n.m.r. (90 MHz, D_2O): δ 1.94 (dd, 1 H, $J_{3a,3e}$ ~13, $J_{3a,4}$ ~13, H-3*a*), 2.39 (dd, 1 H, $J_{3e,4}$ ~4.5, H-3*e*), 3.38 (s, 3 H, CH_3O), 3.87 (s, 3 H, CH_3OCO), 3.60–4.30 (m, 11 H, including H-4,5,6,7,8a,8b,2',3',4',5'a,5'b), 5.27 (d, 1 H, $J_{1',2'}$ ~1.5, H-1').

Anal. Calc. for $C_{15}H_{26}O_{12} \cdot 1.5\ H_2O$: C, 42.4; H, 6.9. Found: C, 42.5; H, 6.8.

*Sodium (methyl 3-deoxy-7-*O*-β-*D*-ribofuranosyl-β-*D*-manno-2-octulopyra-*

nosid)onate (**6**). — A solution of **5** (16.4 mg) in water (1 mL) was treated with 0.2M NaOH (0.3 mL) for 45 min at room temperature. The solution was de-ionized with Dowex 50 (H^+) resin, and the product converted into the sodium salt by titration of the acidic solution to pH 7.0 with 20mM NaOH. Lyophilization and subsequent purification of the residue on Sephadex G-10 afforded **6** (14.0 mg, 84%) as a colorless glass, $[\alpha]_D^{20}$ −22.6° (*c* 1.1, water); ^{1}H-n.m.r. (90 MHz, D_2O): δ 1.77 (dd, 1 H, $J_{3a,3e}$ ~13, $J_{3a,4}$ ~13, H-3*a*), 2.41 (dd, 1 H, $J_{3e,4}$ ~4.5, H-3*e*), 3.31 (s, 3 H, CH_3O), 3.56–4.30 (m, 11 H, including H-4,5,6,7,8a,8b,2′,3′,4′,5′a,5′b), 5.23 (d, 1 H, $J_{1',2'}$ ~1, H-1′).

Anal. Calc. for $C_{14}H_{23}O_{12}Na \cdot H_2O$: C, 39.6; H, 6.0. Found: C, 39.2; H, 6.3.

Benzyl 5-O-*benzoyl*-β-D-*ribofuranoside* (**8**). — A suspension of **7** (13.3 g) in dry methanol (150 mL) was stirred with 0.1M methanolic sodium methoxide (30 mL). After 2.5 h, t.l.c. indicated the absence of starting material (2:1 toluene–ethyl acetate). The mixture was made neutral with Dowex 50 (H^+) resin, filtered, and the filtrate evaporated to dryness. The residue crystallized from ether–pentane, and was washed thoroughly with water (3 × 25 mL), and dried (5.4 g, 65%), colorless needles, m.p. 108–109° (ethyl acetate–pentane), $[\alpha]_D^{20}$ −57° (*c* 1.0, chloroform); ^{1}H-n.m.r. (90 MHz, dimethyl sulfoxide-d_6): δ 3.88 (d, 1 H, $J_{2,3}$ ~3.6, H-2), 4.10–4.61 (m, 4 H, including H-3,4,5a,5b), 4.40 and 4.58 (AB, 2 H, J_{AB} ~11.0, CH_2O), 4.90 (s, 1 H, H-1), 5.03 (d, 1 H, *J* ~5.4, OH), 5.13 (d, 1 H, *J* ~3.6, OH), 7.14–7.70 (m, 8 H) and 7.90–8.05 (m, 2 H, arom. H).

Anal. Calc. for $C_{19}H_{20}O_6$: C, 66.3; H, 5.9. Found: C, 66.1; H, 5.9.

Benzyl 5-O-*benzoyl*-3-O-*benzyl*-β-D-*ribofuranoside* (**9**) *and benzyl* 5-O-*benzoyl*-2-O-*benzyl*-β-D-*ribofuranoside* (**11**). — A mixture of **8** (4.53 g) and dibutyltin oxide (3.3 g) in dry toluene (100 mL) was heated under reflux for 3 h with continuous separation of water. After evaporation, the residue was dissolved in dry *N,N*-dimethylformamide (10 mL) and treated with benzyl bromide (1.5 mL) for 48 h at 70°. After removal of the solvent *in vacuo*, the residue was partitioned between dichloromethane and aqueous $NaHCO_3$, and the dichloromethane layer filtered through Celite. The solid residue was washed thoroughly with dichloromethane (200 mL), and the combined organic layers were extracted with saturated aqueous $NaHCO_3$ and water, dried ($MgSO_4$), and evaporated. Repeated chromatography of the residue on a column of silica gel (80 × 4 cm; Merck silica gel 60, 230–400 mesh; 10:1 toluene–ethyl acetate) afforded 1.50 g (27%) of the faster-moving compound (**11**) as colorless needles, m.p. 78–79° (ethyl acetate–pentane), $[\alpha]_D^{20}$ −30.4° (*c* 1.0, chloroform); ^{1}H-n.m.r. (90 MHz): δ 2.36 (s, 1 H, OH), 4.02 (d, 1 H, $J_{2,3}$ ~4.5, H-2), 4.20–4.82 (m, 8 H, including H-3,4,5a,5b, CH_2O), 5.13 (s, 1 H, H-1), 7.20–7.70 (m, 13 H) and 8.02–8.16 (m, 2 H, arom. H).

Anal. Calc. for $C_{26}H_{26}O_6$: C, 71.9; H, 6.0. Found: C, 71.8; H, 6.1.

The combined fractions containing the slower-moving component were evaporated and yielded 1.84 g (33%) of **9**, colorless needles, m.p. 76–77° (ethyl acetate–pentane), $[\alpha]_D^{20}$ −26.6° (*c* 1.0, chloroform); ^{1}H-n.m.r. (90 MHz): δ 2.48 (s, 1 H, OH), 4.18–4.80 (m, 9 H, including H-2,3,4,5a,5b, CH_2O), 5.12 (s, 1 H, H-1), 7.20–7.70 (m, 13 H) and 7.99–8.10 (m, 2 H, arom. H).

Anal. Calc. for $C_{26}H_{26}O_6$: C, 71.9; H, 6.0. Found: C, 71.7; H, 6.2.

Benzyl 5-O-benzoyl-3-O-benzyl-2-O-(p-nitrobenzoyl)-β-D-ribofuranoside (**10**). — A solution of **9** (22 mg) in pyridine (2 mL) was treated with *p*-nitrobenzoyl chloride (25 mg). After 4 h at room temperature, water (0.1 mL) was added and stirring was continued for 30 min. The reaction mixture was poured into ice–water and extracted three times with 10-mL portions of dichloromethane. The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified on a column of silica gel (size A; 10:1, toluene–ethyl acetate); yield 29 mg (98%) of syrupy **10**, $[\alpha]_D^{20}$ +12.2° (*c* 1.77, chloroform); ^{1}H-n.m.r.: δ 4.43–4.52 (m, 4 H, including H-3,4,5a,5b), 4.50 and 4.66 (AB, 2 H, J_{AB} ~11, CH_2O), 4.52 and 4.73 (AB, 2 H, J_{AB} ~12, CH_2O), 5.25 (d, 1 H, $J_{1,2}$ ~1, H-1), 5.63 (dd, 1 H, $J_{2,3}$ ~3.3, H-2), 7.15–7.60 (m, 13 H), 7.96–8.04 (m, 2 H, arom. H), and 8.20–8.32 (m, 4 H, nitroarom. H).

Anal. Calc. for $C_{33}H_{29}NO_9$: C, 67.9; H, 5.0; N, 2.4. Found: C, 68.2; H, 5.0; N, 2.3.

Benzyl 5-O-benzoyl-2-O-benzyl-3-O-(p-nitrobenzoyl)-β-D-ribofuranoside (**12**). — The procedure was analogous to that for the preparation of **10**; yield 26.5 mg (90%); slightly yellow crystals, m.p. 92–93° (ethyl acetate–pentane); $[\alpha]_D^{20}$ −3.1° (*c* 1.0, chloroform); ^{1}H-n.m.r.: δ 4.39 (dd, 1 H, $J_{2,3}$ ~5.0, H-2), 4.50 (dd, 1 H, $J_{5a,5b}$ ~12.0, H-5b), 4.52 and 4.60 (AB, 2 H, J_{AB} ~12.0, CH_2O), 4.51 and 4.77 (AB, 2 H, J_{AB} ~12.0, CH_2O) 4.62 (dd, 1 H, $J_{5a,4}$ ~4.5, H-5a), 4.74 (ddd, 1 H, $J_{4,5b}$ ~4.5, H-4), 5.20 (d, 1 H, $J_{1,2}$ ~1.0, H-1), 5.56 (dd, 1 H, $J_{3,4}$ ~6.5, H-3) 7.15–7.55 (m, 13 H) and 7.95–8.05 (m, 2 H, arom. H), 8.15–8.30 (m, 4 H, nitroarom. H).

Anal. Calc. for $C_{33}H_{29}NO_9$: C, 67.9; H, 5.0; N, 2.4. Found: C, 68.1; H, 5.1; N, 2.3.

Benzyl 5-O-benzoyl-3-O-benzyl-2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranoside (**13**). — A solution of **2** (1.3 g) in dichloromethane (15 mL) was added dropwise to a suspension of **9** (0.94 g), silver triflate (0.58 g), and Drierite (1 g) in dichloromethane (50 mL) during 10 min at −25°. After an additional 10 min, pyridine (1 mL) was added. The mixture was filtered through Celite, and the filtrate extracted sequentially with 10% aqueous $Na_2S_2O_3$ and water. The organic layer was dried ($MgSO_4$) and evaporated. The residue was subjected to column chromatography on silica gel (60 × 2 cm, Merck, 230–400 mesh; 10:1 toluene–ethyl acetate). The combined fractions containing the major product were evaporated to afford **13** (1.54 g, 81%) as a syrup, $[\alpha]_D^{20}$ +8.6° (*c* 0.37, chloroform); ^{1}H-n.m.r.: δ 4.24–4.75 (m, 12 H, including H-2,3,4,5a,5b,4′,5′a,5′b, CH_2O), 5.23 (s, 1 H, H-1′), 5.39 (s, 1 H, H-1), 5.79 (d, 1 H, $J_{2',3'}$ ~5.0, H-2′), 5.88 (dd, 1 H, $J_{3',4'}$ ~7.0, H-3′) 7.10–7.63 (m, 24 H) and 7.85–8.10 (m, 8 H, arom. H).

Anal. Calc. for $C_{52}H_{46}O_{13}$: C, 71.1; H, 5.3. Found: C, 71.2; H, 5.4.

5-O-Benzoyl-2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-D-ribofuranose (**14**). — A solution of **13** (1.54 g) in 2:1 methanol–ethyl acetate (150 mL) was hydrogenolyzed in the presence of palladium oxide (0.17 g) for 18 h, at room temperature and atmospheric pressure. After filtration and evaporation of the filtrate, the resulting residue was purified on a column of silica gel (size B, 2:1 toluene–ethyl

acetate) to give syrupy **17** (0.79 g, 65%); ^{1}H-n.m.r. (ratio of α to β anomer 3:4; α anomer): δ 3.13 (d, 1 H, $J_{3,OH}$ ~6.5, OH), 3.95 (d, 1 H, $J_{1,OH}$ ~8.5, OH), 4.19 (dd, 1 H, $J_{2,3}$ ~4.0, H-2), 5.37 (dd, 1 H, $J_{1,2}$ ~4.0, H-1), 5.49 (d, 1 H, $J_{1',2'}$ ~1.0, H-1'), 5.77 (dd, 1 H, $J_{2',3'}$ ~5.0, H-2'), 5.86 (dd, 1 H, $J_{3',4'}$ ~6.5, H-3'); (β anomer): 2.69 (d, 1 H, $J_{3,OH}$ ~6.5, OH), 3.02 (d, 1 H, $J_{1',OH}$ ~3.5, OH), 4.14 (dd, 1 H, $J_{2,3}$ ~5.0, H-2), 5.51 (d, 1 H, $J_{1',2'}$ ~1.0, H-1'), 5.52 (dd, 1 H, $J_{1,2}$ ~1.0, H-1), 5.81 (dd, 1 H, $J_{2',3'}$ ~4.5, H-2'), 5.84 (dd, 1 H, $J_{3',4'}$ ~6.5, H-3'); (α and β anomers): 4.25–4.85 (m, 7 H, including H-3,4,5a,5b,4',5'a,5'b), 7.15–7.62 (m, 12 H) and 7.92–8.12 (m, 8 H, arom. H).

Anal. Calc. for $C_{38}H_{34}O_{13} \cdot 0.5\ H_2O$: C, 64.5; H, 4.9. Found: C, 64.9; H, 4.9.

*1,3-Di-*O*-acetyl-5-*O*-benzoyl-2-*O*-(2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranosyl)-β-*D*-ribofuranose* (**15**). — A solution of **14** (0.61 g) in pyridine (15 mL) was treated with acetic anhydride (1 mL) for 3 h at room temperature. The mixture was evaporated to dryness and the residual solvent coevaporated three times with toluene (10 mL); yield 659 mg (97%), colorless crystals, m.p. 75–78° (ethyl acetate–pentane), $[\alpha]_D^{20}$ +38° (*c* 0.39, chloroform); ^{1}H-n.m.r.: δ 1.93 (s, 3 H, OAc), 2.13 (s, 3 H, OAc), 4.38 (dd, 1 H, $J_{5a,5b}$ ~11.5, H-5a), 4.50 (ddd, 1 H, $J_{4,5a}$ ~4.0, $J_{4,5b}$ ~3.5, H-4), 4.53 (d, 1 H, $J_{2,3}$ ~4.5, H-2), 4.60 (dd, 1 H, H-5b), 4.60 (dd, 1 H, $J_{5'a,5'b}$ ~11.5, H-5'a), 4.68 (dd, 1 H, $J_{4',5'b}$ ~4.0, H-5'b), 4.78 (ddd, 1 H, $J_{4',5'}$ ~5.5, H-4'), 5.22 (dd, 1 H, $J_{3',4'}$ ~7.0, H-3), 5.34 (d, 1 H, $J_{1',2'}$ ~1.0, H-1'), 5.70 (dd, 1 H, $J_{2',3'}$ ~5.0, H-2'), 5.80 (dd, 1 H, $J_{3',4'}$ ~6.5, H-3'), 6.38 (s, 1 H, H-1), 7.15–7.60 (m, 12 H) and 7.87–8.10 (m, 8 H, arom. H).

Anal. Calc. for $C_{42}H_{38}O_{15}$: C, 64.4; H, 4.9. Found: C, 64.7; H, 5.0.

*3-*O*-Acetyl-5-*O*-benzoyl-2-*O*-(2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranosyl)-*D*-ribofuranosyl bromide* (**16**) *and methyl 3-*O*-acetyl-5-*O*-benzoyl-2-*O*-(2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranosyl)-β-*D*-ribofuranoside* (**17**). — To a solution of **15** (156 mg) in dry dichloromethane (15 mL) was added $TiBr_4$ (700 mg) at −10°. The mixture was kept at +4° for 18 h, and was then extracted with ice-cold, saturated aqueous $NaHCO_3$ as rapidly as possible. The organic layer was dried ($MgSO_4$) and evaporated to dryness at room temperature; yield 162 mg of **16**, slightly yellow syrup.

A suspension of freshly prepared **16** (162 mg), Ag_2CO_3 (200 mg), and Drierite (1 g) in dry methanol (25 mL) was stirred for 3 h under exclusion of light and moisture. The mixture was filtered and the filtrate evaporated to dryness. The residue was purified on a column of silica gel (size C; 5:1 toluene–ethyl acetate), and the fractions containing the major product were combined and evaporated; yield 72 mg (48%) of syrupy **17**, $[\alpha]_D^{20}$ +37.8° (*c* 1.38, chloroform); ^{1}H-n.m.r.: δ 2.24 (s, 3 H, CH_3O), 4.36 (dd, 1 H, $J_{5a,5b}$ ~11.0, H-5a), 4.41 (dd, 1 H, $J_{2,3}$ ~4.5, H-2), 4.46 (ddd, 1 H, $J_{4,5a}$ ~4.5, $J_{4,5b}$ ~3.5, H-4), 4.54 (dd, 1 H, H-5b), 4.58 (dd, 1 H, $J_{5'a,5'b}$ ~12.0, H-5'a), 4.70 (dd, 1 H, $J_{4',5'b}$ ~4.0, H-5'b), 4.76 (ddd, 1 H, $J_{4',5'a}$ ~5.5, H-4'), 5.06 (d, 1 H, $J_{1,2}$ ~1.0, H-1), 5.21 (dd, 1 H, $J_{3,4}$ ~6.5, H-3), 5.32 (d, 1 H, $J_{1',2'}$ ~1.0, H-1'), 5.70 (dd, 1 H, $J_{2',3'}$ ~5.0, H-2'), 5.83 (dd, 1 H, $J_{3',4'}$ ~6.5, H-3'), 7.15–7.63 (m, 12 H) and 7.90–8.10 (m, 8 H, arom. H).

Anal. Calc. for $C_{41}H_{38}O_{14}$: C, 65.3; H, 5.1. Found: C, 65.4; H, 5.0.

Methyl 2-O-β-D-ribofuranosyl-β-D-ribofuranoside (**18**). — A solution of **17** (41 mg) in 0.1M methanolic sodium methoxide (20 mL) was stirred at room temperature for 15 h. The solution was neutralized with Dowex 50 (H^+) resin, filtered, and evaporated to dryness. The syrupy residue was purified on Sephadex G-10 and lyophilized; yield 15.3 mg (95%), $[\alpha]_D^{20}$ −59° (*c* 0.85, water); ^{1}H-n.m.r. (90 MHz, D_2O): δ 3.43 (s, 3 H, CH_3O), 3.50–4.53 (m, 10 H), 5.11 (d, 1 H, $J_{1',2'}$ ~1 Hz, H-1'), 5.20 (s, 1 H, H-1).

Anal. Calc. for $C_{11}H_{20}O_9 \cdot 0.5\ H_2O$: C, 43.3; H, 6.9. Found: C, 43.3; H, 6.6.

Methyl {methyl 7-O-[3-O-acetyl-5-O-benzoyl-2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]-8-O-benzyl-4,5-O-carbonyl-3-deoxy-β-D-manno-2-octulopyranosid}onate (**24**). — A solution of freshly prepared **16** (200 mg) in dichloromethane (5 mL) was added to a suspension of **1** (80 mg), silver triflate (260 mg), and Drierite (500 mg) in dry dichlormethane (15 mL) at −15° under a steady stream of dry nitrogen. After 20 min, pyridine (0.1 mL) was added and the mixture was processed as described for **3**. Final purification on a column of silica gel (size C; 4:1 toluene–ethyl acetate) afforded **24** (100 mg, 43%) as a colorless syrup, $[\alpha]_D^{20}$ +7.1° (*c* 0.91, chloroform); ^{1}H-n.m.r.: δ 1.91 (dd, 1 H, $J_{3a,3e}$ ~16.0, $J_{3a,4}$ ~4.5, H-3*a*), 2.36 (dd, 1 H, $J_{3e,4}$ ~2.0, H-3*e*), 3.26 (s, 3 H, CH_3O), 3.71 (s, 3 H, CH_3OCO), 3.63 (dd, 1 H, $J_{8a,8b}$ ~11.0, $J_{8a,7}$ ~5.0, H-8a), 3.77 (dd, 1 H, $J_{6,7}$ ~9.5, $J_{6,5}$ ~1.5, H-6), 3.86 (dd, 1 H, $J_{8b,7}$ ~2.0, H-8b), 4.01 (ddd, 1 H, H-7), 4.30–4.76 (m, 10 H including H-4,2',4',5'a,5'b,4'',5''a,5''b, CH_2O), 4.87 (dd, 1 H, $J_{4,5}$ ~9.0, H-5), 5.23 (d, 1 H, $J_{1'',2''}$ ~1.0, H-1''), 5.26 (dd, 1 H, $J_{3',4'}$ ~6.0, $J_{2',3'}$ ~5.0, H-3'), 5.47 (d, 1 H, $J_{1',2'}$ ~2.0, H-1'), 5.65 (dd, 1 H, $J_{2'',3''}$ ~5.0, H-2''), 5.71 (dd, 1 H, $J_{3'',4''}$ ~7.0, H-3''), 7.10–7.60 (m, 17 H) and 7.86–8.10 (m, 8 H, arom. H).

Anal. Calc. for $C_{58}H_{56}O_{22}$: C, 63.0; H, 5.1. Found: C, 63.6; H, 5.1.

Upon further elution of the column with ethyl acetate, **1** (45 mg, 56%), m.p. 101–102°, was recovered (confirmed by comparison with an authentic sample).

Methyl {methyl 7-O-[3-O-acetyl-5-O-benzoyl-2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]-4,5-O-carbonyl-3-deoxy-β-D-manno-2-octulopyranosid}onate (**26**). — A solution of **24** (85 mg) in 1:1 methanol–ethyl acetate (30 mL) was hydrogenolyzed in the presence of PdO (80 mg) at room temperature and atmospheric pressure for 48 h. The reaction mixture was filtered and the filtrate evaporated to dryness. Purification of the syrupy residue on a column of silica gel (size C; 1:1 toluene–ethyl acetate) afforded **26** (75 mg, 96%) as a colorless syryp, $[\alpha]_D^{20}$ −0.6° (*c* 1.4, chloroform); ^{1}H-n.m.r.: δ 1.83 (dd, 1 H, $J_{3a,3e}$ ~11.5, $J_{3a,4}$ ~4.0, H-3*a*), 2.41 (dd, 1 H, $J_{3e,4}$ ~2.0, H-3*e*), 3.25 (s, 3 H, CH_3O), 3.77 (s, 3 H, CH_3OCO), 3.74–3.93 (m, 4 H, including H-6,7,8a,8b), 4.34–4.82 (m, 8 H, including H-4,2',4',5'a,5'b,4'',5''a,5''b), 4.92 (dd, 1 H, $J_{5,6}$ ~1.0, $J_{4,5}$ ~9.5, H-5), 5.27 (dd, 1 H, $J_{2',3'}$ ~5.5, $J_{3',4'}$ ~5.5, H-3'), 5.34 (d, 1 H, $J_{1'',2''}$ ~1.0, H-1''), 5.41 (d, 1 H, $J_{1',2'}$ ~2.0, H-1'), 5.69 (dd, 1 H, $J_{2'',3''}$ ~5.0, H-2''), 5.84 (dd, 1 H, $J_{3'',4''}$ ~7.5, H-3''), 7.15–7.63 (m. 12 H) and 7.90–8.07 (m, 8 H, arom. H).

Anal. Calc. for $C_{51}H_{50}O_{22}$: C, 60.3; H, 5.0. Found: C, 60.2; H, 5.1.

*Sodium [methyl 3-deoxy-7-O-(2-O-β-*D*-ribofuranosyl-β-*D*-ribofuranosyl)-β-*D*-manno-2-octulopyranosid]onate* (**28**). — A solution of **26** (25.4 mg) in dry methanol was stirred with 0.13M methanolic sodium methoxide for 20 h. After addition of 10mM HCl (10 mL), the solution was lyophilized and purified on a column of Sephadex G-10. The product (12 mg) was stirred with 0.2M NaOH (5 mL) for 90 min. The solution was de-ionized with Dowex 50 (H^+) resin, and the product converted into the sodium salt by titration of the acidic solution to pH 7.5 with 20mM NaOH. Lyophilization and subsequent purification of the residue on Sephadex G-10 afforded **28** (10.7 mg, 80%) as a colorless glass, $[\alpha]_D^{20}$ −16.5° (*c* 0.4, water); ^{1}H-n.m.r. (90 MHz, D_2O): δ 1.76 (t, 1 H, $J_{3a,3e}$ ~$J_{3a,4}$ ~12.5, H-3*a*), 2.40 (dd, 1 H, $J_{3e,4}$ ~4.5, H-3*e*), 3.34 (s, 3 H, OCH_3), 3.60–4.40 (m, 16 H, including H-4,5,6,7,8a,8b,2′,3′4′,5′a,5′b, 2″,3″,4″,5″a,5″b), 5.22 (s, 1 H, H-1″), 5.44 (s, 1 H, H-1′).

*Methyl (methyl 3-deoxy-8-*O*-triphenylmethyl-α-*D*-manno-2-octulopyranosid)onate* (**20**). — A solution of **19** (1.45 g) and tritylpyridinium tetrafluoroborate (2.5 g) in dry acetonitrile (75 mL) was stirred for 2 h at room temperature. The solution was evaporated *in vacuo* and the residual solvent coevaporated twice with addition of toluene (10 mL). The residue was dissolved in chloroform and extracted twice with saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and evaporated to dryness. The residue was purified on a column of silica gel (size C; ethyl acetate). Evaporation of the combined fractions containing the main product afforded **20** (2.25 g, 82%); colorless crystals, m.p. 170–172° (dec.; ethyl acetate–pentane), $[\alpha]_D^{20}$ +39.5° (*c* 1.0, chloroform), ^{1}H-n.m.r.: δ 1.87 (dd, 1 H, $J_{3a,3e}$ ~12.5, $J_{3a,4}$ ~11.0, H-3*a*), 2.12 (dd, 1 H, $J_{3e,4}$ ~5.0, H-3*e*), 2.26 (d, 1 H, *J* ~10.0, OH), 3.06 (s, 3 H, CH_3O), 3.07 (d, 1 H, OH), 3.15 (d, 1 H, *J* ~3.0, OH), 3.41 (ddd, 2 H, $J_{8,7}$ ~5.0, H-8a,8b), 3.63 (d, 1 H, *J* ~5.5, H-6), 3.77 (s, 3 H, CH_3OCO), 3.95–4.04 (m, 2 H, H-5,7), 4.17 (m, 1 H, H-4), 7.20–7.55 (m, 15 H, arom. H).

Anal. Calc. for $C_{29}H_{32}O_8$: C, 68.5; H, 6.3. Found: C, 68.3; H, 6.4.

*Methyl (methyl 4,5-*O*-carbonyl-3-deoxy-8-*O*-triphenylmethyl-α-*D*-manno-2-octulopyranosid)onate* (**21**). — A solution of **20** (2.1 g) and *p*-nitrophenyl chloroformate (1.0 g) in dry pyridine (75 mL) was stirred for 24 h at room temperature. After dropwise addition of water (1 mL), the solvent was removed *in vacuo*. The residue was dissolved in dichloromethane and extracted seven times with saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified on a column of silica gel (size C; 2:1 toluene–ethyl acetate). Pooling and evaporation of the fractions containing the main product afforded **21** (1.7 g, 77%), colorless crystals, m.p. 193–194° (ethyl acetate–pentane), $[\alpha]_D^{20}$ +11.9° (*c* 1.08, chloroform); ^{1}H-n.m.r.: δ 1.99 (dd, 1 H, $J_{3a,3e}$ ~16.1, $J_{3a,4}$ ~3.0, H-3*a*), 2.54 (d, 1 H, $J_{7,OH}$ ~5.5, OH), 2.71 (dd, 1 H, $J_{3e,4}$ ~3.3, H-3*e*), 2.87 (s, 3 H, CH_3O), 3.30 (dd, 1 H, $J_{8a,8b}$ ~9.8, $J_{8a,7}$ ~5.6, H-8a), 3.54 (dd, 1 H, $J_{8b,7}$ ~2.8, H-8b), 3.77 (s, 3 H, CH_3OCO), 3.81 (dd, 1 H, $J_{6,7}$ ~9.3, $J_{6,5}$ ~1.4, H-6), 4.16 (ddd, 1 H, H-7), 4.95 (ddd, 1 H, $J_{4,5}$ ~8.8, H-4), 5.02 (dd, 1 H, H-5), 7.06–7.28 (m, 15 H, arom. H).

Anal. Calc. for $C_{30}H_{30}O_9$: C, 67.4; H, 5.7. Found: C, 67.3; H, 5.7.

*Methyl (methyl 4,5-O-carbonyl-3-deoxy-α-*D-manno*-2-octulopyranosid)onate* (**22**). — A suspension of **21** (1.57 g) and PdO (700 mg) in 1:1 methanol–ethyl acetate (80 mL) was hydrogenolyzed for 75 h at 4.5 MPa. The catalyst was removed by filtration and the filtrate evaporated to dryness. The crystalline residue was purified on a column of silica gel (size C; ethyl acetate) to afford **22** (850 mg, 100%), colorless crystals, m.p. 130–134° (dec.; ethyl acetate–pentane), $[\alpha]_D^{20}$ +57.1° (*c* 1.0, chloroform); ^{1}H-n.m.r.: δ 2.10 (dd, 1 H, $J_{3a,3e}$ ~16.0, $J_{3a,4}$ ~2.8, H-3*a*), 2.19 (dd, 1 H, $J_{8b,OH}$ ~5.0, $J_{8a,OH}$ ~6.5, 8-OH), 2.75 (dd, 1 H, $J_{3e,4}$ ~3.2, H-3*e*), 2.92 (d, 1 H, $J_{7,OH}$ ~6.0, 7-OH), 3.27 (s, 3 H, CH_3O), 3.80 (ddd, 1 H, $J_{8a,8b}$ ~11.0, $J_{8a,7}$ ~4.5, H-8a), 3.83 (s, 3 H, CH_3OCO), 3.90 (dd, 1 H, $J_{6,7}$ ~9.0, $J_{6,5}$ ~1.0, H-6), 3.94 (ddd, 1 H, $J_{8b,7}$ ~3.0, H-8b), 4.10 (ddd, 1 H, H-7), 5.02 (dd, 1 H, $J_{5,4}$ ~9.0, H-5), 5.05 (ddd, 1 H, H-4).

Anal. Calc. for $C_{11}H_{16}O_9$: C, 45.2; H, 5.5. Found: C, 45.0; H, 5.5.

*Methyl (methyl 8-O-benzyl-4,5-O-carbonyl-3-deoxy-α-*D-manno*-2-octulopyranosid)onate* (**23**). — A solution of **22** (770 mg) and dibutyltin oxide (700 mg) in dry toluene (200 mL) was heated for 3 h under reflux with continuous separation of water. After evaporation to dryness, the residue was dissolved in dry *N,N*-dimethylformamide (10 mL), benzyl bromide (0.6 mL) was added, and the mixture was stirred for 18 h at 90–95°. After evaporation *in vacuo*, the residue was purified on a column of silica gel (size C; 1:2 toluene–ethyl acetate). Pooling and evaporation of the fractions containing the main product afforded **22** (719 mg, 72%) as a syrup, $[\alpha]_D^{20}$ +40.4° (*c* 0.73, chloroform); ^{1}H-n.m.r.: δ 2.05 (dd, 1 H, $J_{3a,3e}$ ~16.0, $J_{3a,4}$ ~2.5, H-3*a*), 2.64 (d, 1 H, $J_{7,OH}$ ~7.0, 7-OH), 2.74 (dd, 1 H, $J_{3e,4}$ ~3.0, H-3*e*), 3.16 (s, 3 H, CH_3O), 3.75 (m, 2 H, $J_{8a,7}$ ~$J_{8b,7}$ ~3.5, H-8a, H-8b), 3.82 (s, 3 H, CH_3OCO), 3.88 (dd, 1 H, $J_{6,7}$ ~9.0, $J_{6,5}$ ~1.0, H-6), 4.13 (ddd, 1 H, H-7), 4.58 (AB, 2 H, J_{AB} ~12.0, $OCH_2C_6H_5$), 5.00 (m, 2 H, H-4,5), 7.25–7.40 (m, 5 H, arom. H).

Anal. Calc. for $C_{18}H_{22}O_9$: C, 56.5; H, 5.8. Found: C, 56.8; H, 5.9.

*Methyl {methyl 7-O-[3-O-acetyl-5-O-benzoyl-2-O-(2,3,5-tri-O-benzoyl-β-*D*-ribofuranosyl)-β-*D*-ribofuranosyl]-8-O-benzyl-4,5-O-carbonyl-3-deoxy-α-*D*-manno-2-octulopyranosid}onate* (**25**). — A solution of freshly prepared **16** (370 mg) in dichloromethane (5 mL) was added to a suspension of **23** (350 mg), silver triflate (130 mg), and Drierite (500 mg) in dichloromethane (15 mL) at −20° under dry nitrogen. After 10 min, pyridine (0.2 mL) was added, and the mixture was processed as described for **3**. Final purification on a column of silica gel (size C; 4:1 toluene–ethyl acetate) afforded **25** (117 mg, 22%) as a colorless syrup, $[\alpha]_D^{20}$ +13.4° (*c* 0.23, chloroform); R_F 0.48, 2:1 toluene-ethyl acetate, ^{1}H-n.m.r.: δ 1.88 (dd, 1 H, $J_{3a,4}$ ~3.5, $J_{3a,3e}$ ~16.0, H-3*a*), 2.13 (s, 3 H, CH_3CO), 2.64 (dd, 1 H, $J_{3e,4}$ ~3.5, H-3*e*), 3.10 (s, 3 H, CH_3O), 3.60 (dd, 1 H, $J_{8a,8b}$ ~11.0, $J_{8a,7}$ ~4.0, H-8a), 3.77 (dd, 1 H, $J_{8b,7}$ ~2.0, H-8b), 3.79 (s, 3 H, CH_3OCO), 3.89 (dd, 1 H, $J_{6,7}$ ~9.0, $J_{6,5}$ ~1.5, H-6), 4.10 (ddd, 1 H, H-7), 4.32–4.74 (m, 9 H, including H-4,4′,4″,5′a,5′b,5″a,5″b, $C_6H_5CH_2O$), 4.81 (dd, 1 H, $J_{5,4}$ ~9.0, H-5), 5.22 (d, 1 H, $J_{1'',2''}$ ~0.5, H-1″), 5.24

(dd, 1 H, $J_{3',2'}$ ~6.0, $J_{3',4'}$~6.0, H-3'), 5.45 (d, 1 H, $J_{1',2'}$~2.0, H-1'), 5.65 (dd, 1 H, $J_{2'',3''}$ ~5.0, H-2''), 5.72 (dd, 1 H, $J_{3'',4''}$ ~7.0, H-3'), 7.10–7.60 (m, 17 H) and 7.85–8.10 (m, 8 H, arom. H).

Anal. Calc. for $C_{58}H_{56}O_{22}$: C, 63.0; H, 5.1. Found: C, 62.8; H, 5.1.

After elution of the column with ethyl acetate, 300 mg of **23** was recovered.

Methyl {methyl 7-O-[3-O-acetyl-5-O-benzoyl-2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]-4,5-O-carbonyl-3-deoxy-α-D-manno-2-octulopyranosid}onate (**27**). — A solution of **25** (107 mg) in 1:1 dry methanol–ethyl acetate (30 mL) and PdO (60 mg) was hydrogenolyzed at atmospheric pressure and room temperature for 18 h. The mixture was filtered, and the filtrate evaporated to dryness. Purification of the residue on a column of silica gel (size C, 2:1 toluene–ethyl acetate) afforded **27** (88 mg, 90%), colorless needles, m.p. 183–184° (from ethyl acetate–hexane), $[\alpha]_D^{20}$ +17.4° (*c* 0.59, chloroform); ^{1}H-n.m.r.: δ 1.92 (dd, 1 H, $J_{3a,3e}$ ~16.1, $J_{3a,4}$ ~3.3, H-3*a*), 2.10 (s, 3 H, CH_3CO), 2.68 (dd, 1 H, $J_{3e,4}$ ~3.6, H-3*e*), 2.73 (dd, 1 H, $J_{8a,OH}$ ~5.0, $J_{8b,OH}$ ~8.5, OH), 3.24 (s, 3 H, OCH_3), 3.70 (ddd, 1 H, $J_{8b,8a}$ ~12.5, $J_{8b,7}$ ~3.5, H-8b), 3.80 (s, 3 H, CH_3OCO), 3.90 (dd, 1 H, $J_{6,7}$ ~9.1, $J_{5,6}$ ~1.4, H-6), 3.98 (ddd, 1 H, $J_{8a,7}$ ~2.5, H-8a), 4.07 (ddd, 1 H, H-7), 4.44 (dd, 1 H, $J_{1',2'}$ ~2.3, $J_{2',3'}$ ~5.2, H-2'), 4.67 (ddd, 1 H, $J_{4,5}$ ~8.8, H-4), 4.34–4.47 and 4.60–4.81 (m, 6 H, including H-4',5'a,5'b,4'',5''a,5''b), 4.87 (dd, 1 H, H-5), 5.27 (t, 1 H, $J_{3',4'}$ ~5.2, H-3'), 5.34 (d, 1 H, $J_{1'',2''}$ ~1.1, H-1''), 5.43 (d, 1 H, $J_{1',2'}$ ~2.2, H-1'), 5.67 (dd, 1 H, $J_{2'',3''}$ ~5.0, H-2''), 5.80 (dd, 1 H, $J_{3'',4''}$ ~6.5, H-3''), 7.10–7.60 (m, 17 H) and 7.80–8.15 (m, 8 H, arom. H).

Anal. Calc. for $C_{51}H_{50}O_{22}$: C, 60.4; H, 5.0. Found: C, 60.4; H, 5.0.

Sodium [methyl 3-deoxy-7-O-(2-O-β-D-ribofuranosyl-β-D-ribofuranosyl)-α-D-manno-2-octulopyranosid]onate (**29**). — A suspension of **27** (35 mg) in dry methanol (10 mL) and 0.2M methanolic sodium methoxide (2 mL) was stirred for 2.5 h at room temperature. The solution was de-ionized with Dowex 50 (H^+) resin and evaporated to dryness. The residue was dissolved in water (2 mL) and extracted three times with ether (5 mL). The aqueous layer was lyophilized. The resulting syrupy residue (18 mg) was dissolved in water (2 mL) and treated with 0.2M NaOH (0.3 mL) for 45 min. The solution was de-ionized with Dowex 50 (H^+) resin and the product converted into the sodium salt by titration of the acidic solution to pH 7.5 with 20mM NaOH. Lyophilization and subsequent purification of the residue on Sephadex G-10 afforded **29** (15 mg, 80%) as a colorless glass, $[\alpha]_D^{20}$ −10.4° (*c* 0.52, water); ^{1}H-n.m.r. (250 MHz, D_2O): δ 1.78 (dd, 1 H, $J_{3a,4}$ ~$J_{3a,3e}$ ~12.5, H-3*a*), 2.03 (dd, 1 H, $J_{3e,4}$ ~5.0, H-3*e*), 3.17 (s, 3 H, CH_3O), 3.66–4.37 (m, 16 H, including H-4,5,6,7,8a,8b,2',3',4',5'a,5'b,2'',3'',4'',5''a,5''b), 5.22 (s, 1 H, H-1''), 5.51 (s, 1 H, H-1').

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