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SYNTHESIS OF ALTERNATE LINEAR AND BRANCHED REPEATING UNITS OF THE *Escherichia coli* LP 1092 CAPSULAR POLYSACCHARIDE CONTAINING 3-DEOXY-α-D-*manno*-2-OCTULOSONIC ACID (KDO) LINKED TO SECONDARY POSITIONS OF D-RIBOSE*

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ABSTRACT

The oligosaccharides, methyl 3-*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-β-D-ribofuranoside, methyl 2-*O*-β-D-ribofuranosyl-3-*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-β-D-ribofuranoside, and methyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→2)-*O*-β-D-ribofuranosyl-(1→2)-β-D-ribofuranoside were prepared in high purity and good over-all yields. The constitutions of the trisaccharide derivatives correspond to the repeating units of the proposed linear and branched structures of the capsular polysaccharide(s) from *Escherichia coli* LP 1092. The α-KDO-(2→3)-β-D-Rib*f* and α-KDO-(2→2)-β-D-Rib*f* units were synthesized by a modification of the Helferich procedure using methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosyl bromide)-onate and appropriate β-D-ribofuranosyl 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

The capsular polysaccharide from *Escherichia coli* strain LP 1092 was the first K antigen reported[1] to contain 3-deoxy-D-*manno*-2-octulosonic acid (KDO), previously discovered as a constituent of the lipopolysaccharide (LPS) of Gram-negative bacteria[2,3]. The first communication[4] dealing with the structure of this K(?) antigen (which had been erroneously designated as the K6 antigen[5,6]) reported data compatible with the branched-chain structure →3)-[β-D-Rib*f*-(1→2)]-β-D-Rib*f*-(1→7)-KDO*p*-(2→3)-[β-D-Rib*f*-(1→2)]-β-D-Rib*f*-(1→. In an independent investigation, Jennings *et al.*[6] concluded that the LP 1092 antigen has the linear

*Dedicated to Professor Otto Hoffmann-Ostenhof on the occasion of his 70th birthday.

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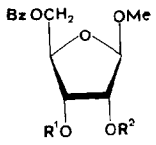
structure β-D-Rib*f*-(1→7)-KDO*p*-(2→2)-β-D-Rib*f*-(1→2)-β-D-Rib*f* . . . . and suggested that the KDO residues had the α anomeric configuration. Furthermore, the latter workers considered the possibility that two closely similar polysaccharides may have been the subject of the studies reported in refs. 4 and 6. This assumption was substantiated by small but distinct differences in the ^{13}C-n.m.r.-spectra of the polysaccharides.

Subsequently, we reported[7] a comparative ^{13}C-n.m.r. study of the LP 1092 (ref. 4) and K 23 (ref. 8) [or *O*-deacetylated K 13 (refs. 9 and 10)] polysaccharides. However, an attempt to interpret the highly complex ^{13}C-n.m.r.-spectrum of the LP 1092 polysaccharide on the basis of empirical rules resulted in only partial assignment. The α-D-anomeric configuration of the KDO residues of the LP 1092 antigen was definitively confirmed by application of c.d.-spectroscopy[11] and by ^{13}C-n.m.r. chemical-shift correlations between the LP 1092 polysaccharide and the two synthetic trisaccharide derivatives β-D-Rib*f*-(1→2)-β-D-Rib*f*-(1→7)-KDO*p*OMe differing only in the anomeric configurations of the respective KDO residues[12,13]. In the course of our studies on the synthesis of partial structures of *E. coli* capsular polysaccharides containing KDO and D-ribose, we report herein the synthesis of trisaccharide derivatives corresponding to the "frame-shifted", alternate repeating units of the LP 1092 polysaccharide, *i.e.*, the sodium salts of the branched trisaccharide β-D-Rib*f*-(1→2)-[α-KDO*p*-(2→3)]-β-D-Rib*f*(OMe) (**14**) and the linear trisaccharide α-KDO*p*-(2→2)-β-D-Rib*f*-(1→2)-β-D-Rib*f*OMe (**25**), which are suitable for spectroscopic and immunological studies of the LP 1092 capsular polysaccharide. The proton-decoupled, ^{13}C-n.m.r.-spectra of these model oligosacharides **14** and **25** have been recorded and presented in a previous communication[13].

RESULTS AND DISCUSSION

For the synthesis of the model oligosaccharides **9, 14**, and **25**, the previously[14] reported methyl 5-*O*-benzoyl-β-D-ribofuranoside (**1**) was employed as the starting material. *O*-Monobenzylation of **1** by way of its 2,3-*O*-dibutylstannylidene derivative[15,16] gave a 2:3 mixture of the 2-*O*- (**2**) and 3-*O*-benzyl (**4**) derivatives in 70% yield. After chromatographic separation of the two isomers, small samples of **2** and **4** were converted into the corresponding 3- and 2-*p*-nitrobenzoates **3** and **5**, respectively, to confirm the assigned structures. Due to the small value of the coupling constant ($J_{1,2}$ ~1.0 Hz), the low field, ^{1}H-n.m.r.-signal due to H-2 in **5** appeared as a doublet (H-2, δ 5.53), whereas the low-field signal due to H-3 in **3** appeared as a dd at δ 5.52.

Glycosylation of **2** with one molar equivalent of methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosyl bromide)onate[17] (**6**) under modified Helferich conditions [mercury (II)cyanide, acetonitrile, and molecular sieve 4A] afforded the (2→3)-α-D-linked disaccharide derivative **7** in 42% yield, together with a small proportion of the glycal ester derivative[17] **10** and unchanged starting material **2** (57%); the components of the mixture were separated by column



1 $R^1 = R^2 = H$

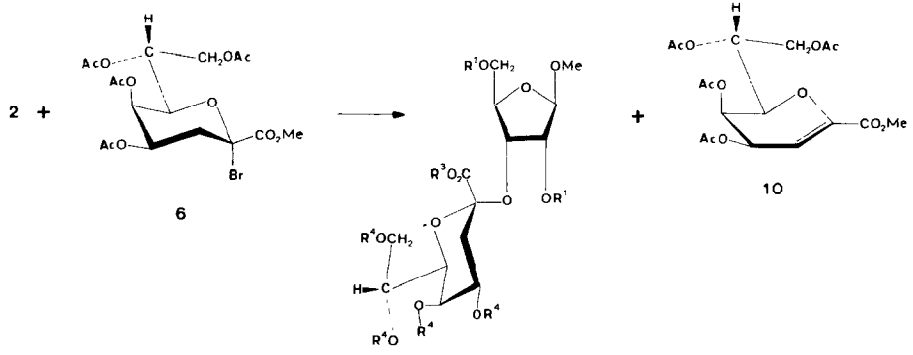
2 $R^1 = H$, $R^2 = Bn$

3 $R^1 = p\text{-}NO_2C_6H_4$, $R^2 = Bn$

4 $R^1 = Bn$, $R^2 = H$

5 $R^1 = Bn$, $R^2 = p\text{-}NO_2C_6H_4$

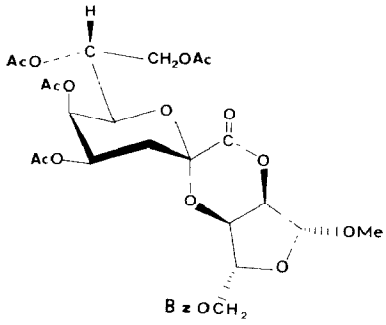
chromatography on silica gel. The α-D anomeric configuration of the KDO residue was ascertained by the chemical shift value (δ 5.35) of the signal attributable to H-4, which is indicative of the α-D anomeric configuration of per-*O*-acetylated KDO derivatives[17]. Hydrogenolysis of **7** in the presence of palladium oxide gave the 2′-*O*-debenzylated compound **8** in quantitative yield. During chromatography on silica gel, however, **8** was converted into the lactone derivative **11** *via* intramolecular transesterification in 10% yield. The structure of **11** was deduced from the ^{1}H-n.m.r.-spectrum; the signal for H-2 of the D-ribofuranosyl residue showed a downfield shift to δ 4.93. In addition, similarly to observations made by Paulsen *et al.*[18,19], the signal at δ 1.97 for the equatorial proton H-3*e* of the KDO residue occurred at a field higher than that of the axial proton H-3*a* (δ 2.66). Deacylation of **8** in methanolic sodium methoxide and subsequent deesterification with aqueous sodium hydroxide afforded the disaccharide methyl 3-*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-β-D-ribofuranoside (**9**) in quantitative yield.



7 $R^1 = Bn$, $R^2 = Bz$, $R^3 = Me$, $R^4 = Ac$

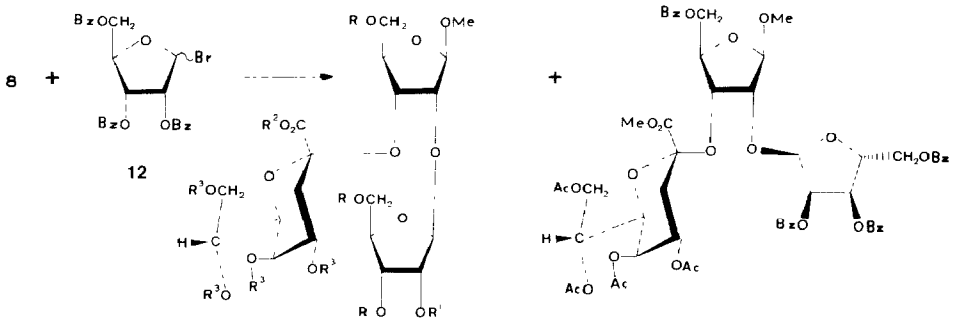
8 $R^1 = H$, $R^2 = Bz$, $R^3 = Me$, $R^4 = Ac$

9 $R^1 = R^2 = R^4 = H$, $R^3 = Na$



11

For the synthesis of the branched trisaccharide **14**, glycosylation of the disaccharide derivative **8** with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide[20] (**12**) was first attempted under catalysis by silver trifluoromethylsulfonate (triflate)[21,22]. This procedure afforded preponderantly the lactone derivative **11** (76% yield) and a small proportion (13%) of a mixture of the (1→2)-β-D- (**13**) and -α-D-linked (**15**) trisaccharide derivatives. Catalysis of the glycosidation reaction by mercury(II) cyanide (dichloromethane, molecular sieve 4A), however, proceeded with pronounced stereoselectivity and gave a mixture of the isomers **13** and **15** in 82% yield in the ratio of **13** to **15** 7:1, as indicated by the relative intensities of the signals due to the methyl ester groups in the 250-MHz, ^{1}H-n.m.r. spectrum. This spectrum for **13** was amenable to first-order analysis, thus allowing unambiguous assignment of all protons. After separation of the isomers by preparative l.c., compound **13** was subjected to Zemplén deacylation and subsequent deesterification with aqueous sodium hydroxide, to give the branched trisaccharide methyl 2-*O*-β-D-ribofuranosyl-3-*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-β-D-ribofuranoside (**14**) in 90% yield.



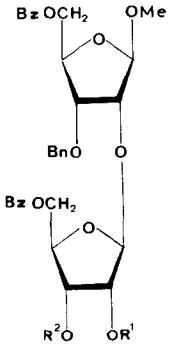
12

15

13 R^1 = Bz , R^2 = Me, R^3 = Ac

14 R^1 = R^3 = H , R^2 = Na

For the synthesis of the linear trisaccharide model compound **25**, methyl 5-*O*-benzoyl-3-*O*-benzyl-β-D-ribofuranoside (**4**) was glycosylated with 2.6 molar equivalents of **12** in the presence of silver triflate and *N,N,N′,N′*-tetramethylurea to give the (1→2)-β-D-linked disaccharide derivative **16** in 96% yield. Selective Zemplén deacylation of **16** furnished a 50% yield of the crystalline 2′,3′-*O*-debenzoylated disaccharide derivative **17** isolated by column chromatography on silica gel. Other, partially deacylated products were conveniently recycled into the starting material **16** by conventional benzoylation (benzoyl chloride–pyridine). The structure of **17** is in accord with the ^{1}H-n.m.r.-spectroscopic data and with the observed reaction of **17** with periodate. The ^{1}H-n.m.r. spectrum indicated the presence of OBz-5 and -5′ and no downfield signals could be attributed to H-2′,3′. *O*-Monobenzylation of **17** by way of its 2′,3′-*O*-dibutylstannylidene derivative[15,16] proceeded with low regioselectivity to give an 11:9 mixture of the 2′-*O*- (**18**) and 3′-*O*-benzyl (**20**) ethers in 80% yield. Separation of the two isomers was facilitated by crystallization of **18**. After removal of the major proportion of **18**, the mother liquor was subjected to preparative l.c. The structural assignments of **18** and **20** were confirmed following their conversion into the corresponding 3′- (**19**) and 2′-*p*-nitrobenzoate (**21**), similarly to the procedure employed for the assignment of the structures of **3** and **5**.



16 $R^1 = R^2 = Bz$

17 $R^1 = R^2 = H$

18 $R^1 = Bn$, $R^2 = H$

19 $R^1 = Bn$, $R^2 = p\text{-}NO_2C_6H_4$

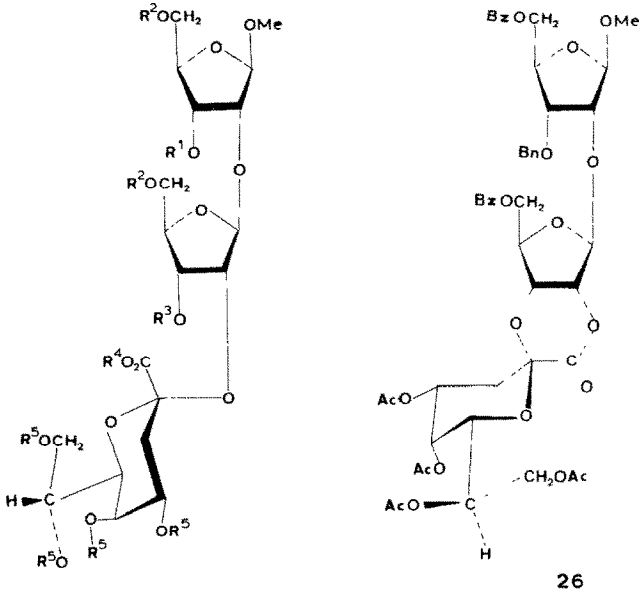
20 $R^2 = H$, $R^1 = Bn$

21 $R^2 = p\text{-}NO_2C_6H_4$, $R^1 = Bn$

Attempts to glycosylate the D-ribofuranosyl disaccharide derivative **20** at OH-2′ with the KDO bromide derivative **6**, under catalysis by silver triflate or mercury(II) cyanide, gave only unchanged **20** in almost quantitative yield. Therefore, the disaccharide derivative **17** having both OH-2 and -3 unprotected was glycosylated with 1.3 molar equivalents of **6** in the presence of mercury(II) cyanide and molecular sieve 4A in acetonitrile, and a 25% yield of the desired α-D-(2→2′)-linked trisaccharide derivative **22** was obtained, together with the α-D-(2→3′)-linked trisaccharide lactone derivative **26** (36% yield) and unreacted **17** (35%). Following separation by chromatography, the structures of **22** and **26** were assigned by interpretation of the 250-MHz, ^{1}H-n.m.r. spectra. Owing to esterification of OH-2′, the signal attributable to H-2′ of the D-ribofuranosyl residue in **26** showed a downfield shift to δ 5.03. As in the case of the α-D-(2→2′)-linked disaccharide lactone derivative **11**, the signal of the equatorial proton H-3*e* of the KDO residue

of the α-D-(2→2′)-linked trisaccharide lactone derivative **26** appeared at a field higher than that of the axial proton H-3*a*.

The structural assignments of **22** were confirmed after acetylation with acetic anhydride–pyridine to give the corresponding 3′-*O*-acetylated trisaccharide derivative **23**. The ^{1}H-n.m.r. signal of H-3′ showed a downfield shift away from the bulk of signals and appeared as a dd (δ 5.64). The α-D configuration of the KDO residue was assigned on the basis of the chemical shift value of H-4 (see ref. 17).



22 R^1 = Bn, R^2 = Bz, R^3 = H, R^4 = Me, R^5 = Ac
23 R^1 = Bn, R^2 = Bz, R^3 = R^5 = Ac, R^4 = Me
24 R^1 = H, R^2 = Bz, R^3 = R^5 = Ac, R^4 = Me
25 R^1 = R^2 = R^3 = R^5 = H, R^4 = Na

Removal of the protecting groups of the trisaccharide derivative **23** was accomplished as follows. Hydrogenolysis in the presence of palladium oxide gave a 96% yield of the *O*-debenzylated derivative **24**, which in turn was converted, in quantitative yield, into the linear trisaccharide model compound methyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→2)-*O*-β-D-ribofuranosyl-(1→2)-β-D-ribofuranoside (**25**) *via* Zemplén deacylation, followed by deesterification in aqueous sodium hydroxide.

EXPERIMENTAL

General methods. — These were as described previously[12]. Column size A, B, and C of Merck prefabricated columns refers to 24 × 1, 31 × 2.5, and 44 × 3.7 cm. L.c. separations were performed at ~20 MPa with a series of three columns (30

× 2.5) containing LiChroSorb Si 60 (Merck, 10 μm) at a flow rate of 15 mL/min using a 1 to 2% gradient (v/v) of 2-propanol in diisopropyl ether.

Methyl 5-O-benzoyl-2-O-benzyl-β-D-ribofuranoside (**2**) *and methyl 5-O-benzoyl-3-O-benzyl-β-D-ribofuranoside* (**4**). — A suspension of **1** (2.57 g) and dibutyltin oxide (2.63 g) in dry benzene (150 mL) was heated at reflux for 2.5 h with continuous removal of water. The resulting solution was evaporated *in vacuo*. The residue was dissolved in dry *N,N*-dimethylformamide (15 mL) and treated with benzyl bromide (1.5 mL) at 85° for 18 h. After evaporation to dryness, the residue was purified on a column of silica gel (80 × 2 cm, Merck Silica gel 60, 230–400 mesh; 5:1 toluene–ethyl acetate) to afford 1.01 g (29%) of the faster-moving compound (**2**) as colorless needles, m.p. 94° (ethyl acetate–pentane), $[\alpha]_D^{20}$ +3.2° (*c* 0.99, chloroform); ^{1}H-n.m.r.: δ 8.10–8.06 (m, 2 H, arom. H), 7.60–7.33 (m, 8 H, arom. H), 4.92 (s, 1 H, H-1), 4.76 and 4.64 (AB, 2 H, $J_{A,B}$ ~11.6 Hz, $C_6H_5CH_2O$), 4.59 (dd, 1 H, $J_{5,4}$ ~3.5, $J_{5,5'}$ ~11.9 Hz, H-5), 4.38 (dd, 1 H, $J_{3,4}$ ~6.8, $J_{3,OH}$ ~9.8, $J_{3,2}$ ~5.2 Hz, H-3), 4.37 (dd, 1 H, $J_{5',4}$ ~5.2 Hz, H-5'), 4.22 (ddd, 1 H, H-4), 3.91 (d, 1 H, H-2), 3.29 (s, 3 H, CH_3O), and 2.64 (d, 1 H, OH).

Anal. Calc. for $C_{20}H_{22}O_6$: C, 67.0; H, 6.2. Found: C, 67.0; H, 6.2.

The combined fractions containing the slower-moving component were evaporated to yield 1.43 g (41%) of **4**, colorless syrup, $[\alpha]_D^{20}$ −23.2° (*c* 0.85, chloroform); ^{1}H-n.m.r.: δ 8.07–8.02 (m, 2 H, arom. H), 7.61–7.28 (m, 8 H, arom. H), 4.90 (s, 1 H, H-1), 4.62 and 4.58 (AB, 2 H, J_{AB} ~11.5 Hz, $C_6H_5CH_2O$), 4.51 (dd, 1 H, $J_{5,4}$ ~3.0, $J_{5,5'}$ ~11.5 Hz, H-5), 4.36 (ddd, 1 H, $J_{4,3}$ ~6.5, $J_{4,5'}$ ~5.0 Hz, H-4), 4.32 (dd, 1 H, H-5'), 4.24 (dd, 1 H, $J_{2,3}$ ~4.3 Hz, H-3), 4.09 (dd, 1 H, $J_{2,OH}$ ~2.5 Hz, H-2), 3.30 (s, 3 H, CH_3O), and 2.67 (d, 1 H, OH).

Anal. Calc. for $C_{20}H_{22}O_6$: C, 67.0; H, 6.2. Found: C, 66.9; H, 6.2.

Methyl 5-O-benzoyl-2-O-benzyl-3-O-(p-nitrobenzoyl)-β-D-ribofuranoside (**3**). — A solution of **2** (36 mg) and *p*-nitrobenzoyl chloride (35 mg) in dry pyridine (2 mL) was stirred for 18 h at room temperature. After addition of water (0.2 mL) stirring was continued for 60 min. The mixture was poured into ice-water, and extracted three times with 10-mL portions of dichloromethane. The organic layer was extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. The residue was purified on a column of silica gel (size A, 4:1 toluene–ethyl acetate) to give syrupy **3** (50 mg, ~100%), $[\alpha]_D^{20}$ +27.5° (*c* 1.04, chloroform); ^{1}H-n.m.r.: δ 8.30–8.15 (m, 4 H, nitroarom. H), 8.07–8.03 (m, 2 H) and 7.60–7.20 (m, 8 H, arom. H), 5.52 (dd, 1 H, $J_{3,2}$ ~5.0, $J_{3,4}$ ~6.5 Hz, H-3), 5.01 (d, 1 H, $J_{1,2}$ ~1.1 Hz, H-1), 4.74–4.52 (m, 4 H, H-4,5, and $C_6H_5CH_2O$), 4.47 (dd, 1 H, $J_{5,5'}$ ~11.8, $J_{5',4}$ ~5.0 Hz, H-5'), 4.30 (dd, 1 H, H-2), and 3.37 (s, 3 H, CH_3O).

Anal. Calc. for $C_{27}H_{25}NO_9$: C, 63.9; H, 5.0; N, 2.8. Found: C, 63.4; H, 5.0; N, 2.9.

Methyl 5-O-benzoyl-3-O-benzyl-2-O-(p-nitrobenzoyl)-β-D-ribofuranoside (**5**). — The preparation of **5** is analogous to that of **3** to give a colorless syrup (49 mg, ~100%), $[\alpha]_D^{20}$ +63.0° (*c* 0.9, chloroform); ^{1}H-n.m.r.: δ 8.33–8.22 (m, 4 H, nitroarom. H), 8.06–8.02 (m, 2 H, arom. H), 7.62–7.17 (m, 8 H, arom. H), 5.53

(d, 1 H, $J_{2,3}$ ~3.7 Hz, H-2), 5.06 (s, 1 H, H-1), 4.68–4.32 (m, 6 H, H-3,4,5,5', and $C_6H_5CH_2O$), and 3.35 (s, 3 H, CH_3O).

Anal. Calc. for $C_{27}H_{25}NO_9$: C, 63.9; H, 5.0; N, 2.8. Found: C, 63.6; H, 4.9; N, 2.8.

Methyl 5-O-benzoyl-2-O-benzyl-3-O-(methyl 4,5,7,8-tetra-O-acetyl-3-deoxy-α-D-manno-2-octulopyranosylonate)-β-D-ribofuranoside (**7**). — A solution of **6** (550 mg, 1.14 mmol) in dry acetonitrile (5 mL) was added dropwise during 5 min to a suspension of **2** (410 mg, 1.14 mmol), $Hg(CN)_2$ (320 mg, 127 mmol), and molecular sieve 4A (400 mg) in acetonitrile (10 mL) under a stream of dry N_2. After being stirred for 20 h at room temperature, the mixture was diluted with dichloromethane (50 mL), filtered, and the filtrate evaporated to dryness. The residue was dissolved in dichloromethane (50 mL), extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated to dryness. The residue was subjected to column chromatography on silica gel (80 × 2 cm; 3:1 toluene–ethyl acetate). Fractions containing the first eluted component were combined and evaporated to give **2** (232 mg, 56%) as colorless needles, m.p. 94° (ethyl acetate–pentane).

The combined fractions containing the later-eluted compound were evaporated to afford **7** (369 mg, 42.5%) as a syrup, $[\alpha]_D^{20}$ +59.2° (*c* 0.94, chloroform); ^{1}H-n.m.r.: δ 8.11–8.06 (m, 2 H, arom. H), 7.62–7.26 (m, 8 H, arom. H), 5.35 (ddd, 1 H, $J_{4,3e}$ ~5.0, $J_{4,5}$ ~3.0, $J_{4,3a}$ ~13.0 Hz, H-4), 5.32 (unresolved signal, 1 H, H-5), 5.18 (ddd, 1 H, $J_{7,8a}$ ~5.0, $J_{7,8b}$ ~2.5, $J_{7,6}$ ~9.0 Hz, H-7), 4.88 (d, 1 H, $J_{1',2'}$ ~1.0 Hz, H-1'), 4.73–4.50 (m, 5 H, including H-8b,3',5'a, and $C_6H_5CH_2O$), 4.49 (ddd, 1 H, $J_{4',5'a}$ ~3.0, $J_{3',4'}$ ~7.0 Hz, H-4'), 4.36 (dd, 1 H, $J_{5'b,5'a}$ ~12.0, $J_{5'b,4'}$ ~5.0 Hz, H-5'b), 4.25 (dd, 1 H, $J_{6,5}$ ~1.4 Hz, H-6), 4.02 (dd, 1 H, $J_{8a,8b}$ ~12.5 Hz, H-8a), 3.93 (dd, 1 H, $J_{2',3'}$ ~4.2 Hz, H-2'), 3.69 (s, 3 H, CH_3OCO), 3.25 (s, 3 H, CH_3O), 2.40 (ddd, 1 H, $J_{3e,3a}$ ~13.5, $^4J_{3e,5}$ ~1.5 Hz, H-3*e*), 2.13 (dd, 1 H, H-3*a*), 2.07, 2.03, 1.99, and 1.94 (s, 12 H, 4 CH_3CO).

Anal. Calc. for $C_{37}H_{44}O_{17}$: C, 58.4; H, 5.8. Found: C, 58.4; H, 5.9.

Further elution of the column afforded **10** (50 mg, 11% based on **6**) as colorless needles, m.p. 132–134° (ethyl acetate–pentane, undepressed on admixture with an authentic sample[17]).

Methyl 5-O-benzoyl-3-O-(methyl 4,5,7,8-tetra-O-acetyl-3-deoxy-α-D-manno-2-octulopyranosylonate)-β-D-ribofuranoside (**8**) *and methyl 5-O-benzoyl-3-O-(4,5,7,8-tetra-O-acetyl-3-deoxy-α-D-manno-2-octulopyranosylono-1',2-lactone)-β-D-ribofuranoside* (**11**). — A suspension of **7** (198 mg) and PdO (86 mg) in 1:1 methanol–ethyl acetate (20 mL) was hydrogenolyzed at atmospheric pressure for 48 h at room temperature. The catalyst was removed by filtration, and the filtrate evaporated and dried to give syrupy **8** (173 mg, 100%), $[\alpha]_D^{20}$ +47.9° (*c* 1.1, chloroform); ^{1}H-n.m.r.: δ 8.13–8.07 (m, 2 H, arom. H), 7.63–7.45 (m, 3 H, arom. H), 5.35 (ddd, 1 H, $J_{4,3e}$ ~5.0, $J_{4,5}$ ~3.0 Hz, H-4), 5.33 (unresolved signal, 1 H, H-5), 5.21 (ddd, 1 H, $J_{7,8a}$ ~2.5, $J_{7,8b}$ ~5.0, $J_{7,6}$ ~9.5 Hz, H-7), 4.89 (s, 1 H, H-1'), 4.71 (dd, 1 H, $J_{8a,8b}$ ~12.5 Hz, H-8a), 4.60 (dd, 1 H, $J_{5'a,5'b}$ ~11.0, $J_{5'a,4'}$ ~3.0 Hz, H-5'a), 4.51–4.38 (m, 3 H, H-3',4',5'b), 4.12 (dd, 1 H, $J_{6,5}$ ~1.5 Hz, H-6), 3.93

(dd, 1 H, H-8b), 3.87 (s, 3 H, CH_3OCO), 3.86 (dd, 1 H, $J_{2',3'}$ ~4.0, $J_{2',OH}$ ~2.5 Hz, H-2'), 3.30 (s, 3 H, CH_3O), 3.09 (d, 1 H, OH), 2.30 (ddd, 1 H, $J_{3e,3a}$ ~13.0, $^4J_{3e,5}$ ~1.0 Hz, H-3*e*), 2.08 (t, 1 H, H-3*a*), 2.08, 2.04, 2.00, and 1.89 (s, 12 H, 4 CH_3CO).

Anal. Calc. for $C_{30}H_{38}O_{17}$: C, 53.7; H, 5.7. Found: C, 54.2; H, 5.7.

In a second experiment (136 mg of **7**, 70 mg of PdO), the mixture was filtered, evaporated, and subjected to column chromatography on silica gel (size B, 1:1 toluene–ethyl acetate); evaporation of the combined fractions containing the first-eluted component afforded **11** (10 mg, 9%) as a colorless syrup, $[\alpha]_D^{20}$ +36.5° (*c* 1.6, chloroform); ^{1}H-n.m.r.: δ 8.11–8.06 (m, 2 H, arom. H), 7.65–7.46 (m, 3 H, arom. H), 5.38 (ddd, 1 H, $J_{5,6}$ ~1.5, $J_{5,4}$ ~3.0, $^4J_{5,3e}$ ~1.0 Hz, H-5), 5.32 (ddd, 1 H, $J_{4,3a}$ ~12.5, $J_{4,3e}$ ~5.0 Hz, H-4), 5.14 (ddd, 1 H, $J_{7,6}$ ~9.5, $J_{7,8a}$ ~2.0, $J_{7,8b}$ ~2.5 Hz, H-7), 5.11 (s, 1 H, H-1'), 4.94 (d, 1 H, $J_{2',3'}$ ~5.0 Hz, H-2'), 4.61 (dd, 1 H, $J_{8a,8b}$ ~12.5 Hz, H-8a), 4.58 (dd, 1 H, $J_{3',4'}$ ~5.5 Hz, H-3'), 4.55–4.43 (m, 3 H, H-4',5'a,5'b), 4.32 (dd, 1 H, H-6), 4.05 (dd, 1 H, H-8b), 3.37 (s, 3 H, CH_3O), 2.66 (t, 1 H, $J_{3a,3e}$ ~12.5 Hz, H-3*a*), 2.11 (s, 3 H), 2.01 (s, 6 H) and 1.99 (s, 3 H, CH_3CO), and 1.97 (ddd, 1 H, H-3*e*).

Anal. Calc. for $C_{29}H_{34}O_{16}$: C, 54.6; H, 5.4. Found: C, 55.0; H, 5.4.

Further elution of the column afforded **8** (103 mg, 86%) as a colorless syrup.

*Methyl 3-*O*-(sodium 3-deoxy-α-*D*-manno-2-*O*-octulopyranosylonate)-β-*D*-ribofuranoside* (**9**). — A solution of **8** (63 mg) and 0.1M methanolic sodium methoxide (2 mL) in dry methanol (5 mL) was stirred for 5 h at room temperature. The mixture was de-ionized using Dowex 50 (H^+) cation-exchange resin, filtered, and evaporated to dryness. The resulting glass was extracted three times with 10-mL portions of diethyl ether and dried. The residue (37 mg) was dissolved in water (4 mL) and treated with 0.2M aqueous NaOH (0.5 mL) for 90 min at room temperature. The pH of the reaction mixture was adjusted to 7.8 by careful addition of Dowex 50 (H^+) cation-exchange resin. Filtration of the mixture followed by lyophilization of the filtrate gave **9** (37 mg, ~100%, based on **8**) as a colorless glass, $[\alpha]_D^{20}$ +35.8° (*c* 1.7, water); ^{1}H-n.m.r. (D_2O): δ 4.97 (s, 1 H, H-1'), 4.19–3.58 (m, 11 H, H-4,5,6,7,8a,8b,2',3',4',5',5'a, and 5'b), 3.39 (s, 3 H, CH_3O), 2.13 (dd, 1 H, $J_{3e,4}$ ~5.0, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), and 1.83 (t, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*).

*Methyl 5-*O*-benzoyl-3-*O*-(methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D*-manno-2-octulopyranosylonate)-2-*O*-(2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranosyl)-β-*D*-ribofuranoside* (**13**) *and methyl 5-*O*-benzoyl-3-*O*-(methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D*-manno-2-octulopyranosylonate)-2-*O*-(2,3,5-tri-*O*-benzoyl-α-*D*-ribofuranosyl)-β-*D*-ribofuranoside* (**15**). — A solution of **12** (476 mg, 0.9 mmol) in dichloromethane (5 mL) was added to a suspension of **8** (176 mg, 0.26 mmol), $Hg(CN)_2$ (230 mg, 0.91 mmol), and molecular sieve 4A (400 mg) in dichloromethane (5 mL) and stirred under a steady stream of dry N_2 at room temperature for 5 h. The mixture was diluted with dichloromethane (50 mL), filtered, extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated to dryness. The residue was purified on a column of silica gel (80 × 2 cm, 3:1

toluene–ethyl acetate) which afforded a mixture of **13** and **15** (238 mg, 82%) as a syrup. Subsequent l.c. separation of an aliquot (200 mg) afforded **15** (13 mg) as the slightly faster-moving component, colorless syrup, $[\alpha]_D^{20}$ +91.1° (*c* 1.3, chloroform); ^{1}H-n.m.r.: δ 8.20-8.01 (m, 6 H, arom. H), 7.78–7.73 (m, 2 H, arom. H), 7.63–7.17 (m, 12 H, arom. H), 5.82 (dd, 1 H, $J_{3'',2''}$ ~7.0, $J_{3'',4''}$ ~3.0 Hz, H-3″), 5.66 (d, 1 H, $J_{1'',2''}$ ~4.5, H-1″), 5.33 (unresolved signal, 1 H, H-5), 5.28 (dd, 1 H, H-2″), 5.20 (ddd, 1 H, $J_{4,3e}$ ~5.5, $J_{4,3a}$ ~12.5, $J_{4,5}$ ~2.5 Hz, H-4), 5.15 (ddd, 1 H, $J_{7,6}$ ~8.0, $J_{7,8a}$ ~2.5, $J_{7,8b}$ ~5.5 Hz, H-7), 4.84 (ddd, 1 H, $J_{4'',5''a}$ ~3.5, $J_{4'',5''b}$ ~3.0 Hz, H-4″), 4.75 (dd, 1 H, $J_{5''a,5''b}$ ~13.0 Hz, H-5″a), 4.71 (s, 1 H, H-1′), 4.66 (dd, 1 H, H-5″b), 4.65–4.57 (m, 3 H, H-8a,3′,5′a), 4.30–4.22 (m, 2 H, H-4′,5′b), 4.17 (dd, 1 H, $J_{6,5}$ ~1.5 Hz, H-6), 4.14 (d, 1 H, $J_{2',3'}$ ~4.5 Hz, H-2′), 3.93 (dd, 1 H, $J_{8b,8a}$ ~13.0 Hz, H-8b), 3.87 (s, 3 H, CH_3OCO), 3.18 (s, 3 H, CH_3O), 2.17 (dd, 1 H, $J_{3e,3a}$ ~13.5 Hz, H-3*e*), 2.05 (t, 1 H, H-3*a*), 2.04, 1.99, 1.90, and 1.88 (s, 12 H, 4 CH_3CO).

Further elution of the column gave pure **13** (155 mg), colorless syrup, $[\alpha]_D^{20}$ +46.0° (*c* 0.7, chloroform); ^{1}H-n.m.r.: δ 8.11–8.00 (m, 6 H, arom. H), 7.97–7.89 (m, 2 H, arom. H), 7.61–7.30 (m, 12 H, arom. H), 5.88 (dd, 1 H, $J_{3'',2''}$ ~5.0, $J_{3'',4''}$ ~6.5 Hz, H-3″), 5.79 (d, 1 H, H-2″), 5.57 (s, 1 H, H-1″), 5.46 (unresolved signal, 1 H, H-5), 5.35 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.5 Hz, H-4), 5.19 (ddd, 1 H, $J_{7,6}$ ~9.5, $J_{7,8a}$ ~3.0, $J_{7,8b}$ ~5.0 Hz, H-7), 5.03 (s, 1 H, H-1′), 4.78 (ddd, 1 H, $J_{4'',5''a}$ ~4.5, $J_{4'',5''b}$ ~6.0 Hz, H-4″), 4.72 (dd, 1 H, $J_{5''a,5''b}$ ~11.5 Hz, H-5″a), 4.71 (dd, 1 H, $J_{5'a,5'b}$ ~12.5, $J_{5'a,4'}$ ~2.5 Hz, H-5′a), 4.64 (dd, 1 H, $J_{8a,8b}$ ~13.0 Hz, H-8a), 4.58 (dd, 1 H, H-5″b), 4.55 (dd, 1 H, $J_{3',2'}$ ~4.0, $J_{3',4'}$ ~7.0 Hz, H-3′), 4.40 (ddd, 1 H, $J_{4',5'b}$ ~5.0 Hz, H-4′), 4.32 (dd, 1 H, $J_{6,5}$ ~1.5 Hz, H-6), 4.30 (dd, 1 H, H-5′b), 4.13 (d, 1 H, H-2′), 4.00 (dd, 1 H, H-8b), 3.79 (s, 3 H, CH_3OCO), 3.13 (s, 3 H, CH_3O), 2.40 (dd, 1 H, $J_{3e,3a}$ ~13.5 Hz, H-3*e*), 2.11 (t, 1 H, H-3*a*), 2.07, 2.04, 1.94, and 1.87 (s, 12 H, 4 CH_3CO).

Anal. Calc. for $C_{56}H_{58}O_{24}$: C, 60.3; H, 5.2. Found: C, 60.0; H, 5.4.

*Methyl 3-O-(sodium 3-deoxy-α-*D-manno*-2-octulopyranosylonate)-2-O-β-*D*-ribofuranosyl-β-*D*-ribofuranoside* (**14**). — A solution of **13** (91 mg) and 0.1M methanolic sodium methoxide (2 mL) in dry methanol (25 mL) was stirred for 24 h at room temperature. The mixture was de-ionized by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and evaporated to dryness. The residue (41 mg) was extracted three times with 10-mL portions of diethyl ether, dissolved in water (5 mL), and stirred with 0.2M aqueous NaOH (0.4 mL) for 60 min at room temperature. The pH of the mixture was adjusted to 7.5 by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and lyophilized to give **14** (39 mg, 90%) as a glass, $[\alpha]_D^{20}$ +27.5° (*c* 0.99, water); ^{1}H-n.m.r.: δ 5.21 (s, 1 H, H-1″), 5.11 (s, 1 H, H-1′), 4.32–3.53 (m, 16 H, H-4,5,6,7,8a,8b,2′,3′,4′,5′a,5′b,2″,3″,4″,5″a,5″b), 3.40 (s, 3 H, CH_3O), 2.23 (ddd, 1 H, $J_{3e,3a}$ ~13.5, $J_{3e,4}$ ~5.0, $^4J_{3e,5}$ ~1.0 Hz, H-3*e*), and 1.85 (dd, 1 H, $J_{3a,4}$ ~13.5 Hz, H-3*a*).

*Methyl 5-O-benzoyl-3-O-benzyl-2-O-(2,3,5-tri-O-benzoyl-β-*D*-ribofuranosyl)-β-*D*-ribofuranoside* (**16**). — A solution of **12** (2.52 g, 5 mmol) in dichloromethane

(10 mL) was added dropwise during 10 min to a suspension of **4** (678 mg, 1.9 mmol), *N,N,N',N'*-tetramethylurea (0.72 mL), silver triflate (1.41 g, 5.5 mmol), and molecular sieve 4A (2 g) at room temperature under a steady stream of dry N_2 and with exclusion of light. After being stirred for 3 h, the mixture was diluted with dichloromethane (50 mL), filtered, and extracted sequentially with saturated aqueous $NaHCO_3$, 5% aqueous $Na_2S_2O_3$, and water. The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified on a column of silica gel (80 × 2 cm, 20:1 toluene–ethyl acetate). Evaporation of the combined fractions containing the major product yielded **16** (1.46 g, 96%) as a colorless syrup, $[\alpha]_D^{20}$ +24.8° (*c* 2.3, chloroform); ^{1}H-n.m.r.: δ 8.10–7.98 (m, 6 H, arom. H), 7.90–7.86 (m, 2 H, arom. H), 7.63–7.09 (m, 17 H, arom. H), 5.90 (dd, 1 H, $J_{3',2'}$ ~5.0, $J_{3',4'}$ ~6.5 Hz, H-3'), 5.80 (dd, 1 H, $J_{2',1'}$ ~0.7 Hz, H-2'), 5.41 (d, 1 H, H-1'), 4.97 (s, 1 H, H-1), 4.78–4.70 (m, 2 H, H-4',5'a), 4.64 and 4.57 (AB, 2 H, J_{AB} ~12.5 Hz, $C_6H_5CH_2O$), 4.56 (dd, 1 H, $J_{5'b,4'}$ ~7.0, $J_{5'a,5'b}$ ~12.0 Hz, H-5'b), 4.52 (dd, 1 H, $J_{5a,5b}$ ~11.5, $J_{5a,4}$ ~3.0 Hz, H-5a), 4.38 (ddd, 1 H, $J_{4,5b}$ ~4.5, $J_{4,3}$ ~8.0 Hz, H-4), 4.30 (dd, 1 H, H-5b), 4.25 (dd, 1 H, $J_{3,2}$ ~4.0 Hz, H-3), 4.15 (d, 1 H, H-2), and 3.16 (s, 3 H, CH_3O).

Anal. Calc. for $C_{46}H_{42}O_{13}$: C, 68.8; H, 5.3. Found: C, 69.2; H, 5.5.

Methyl 5-O-benzoyl-2-O-(5-O-benzoyl-β-D-ribofuranosyl)-3-O-benzyl-β-D-ribofuranoside (**17**). — A solution of **16** (1.53 g) and 0.2M methanolic sodium methoxide (10 mL) in dry methanol (50 mL) was stirred for 65 min at room temperature. Progress of the reaction was monitored by t.l.c. (1:1 toluene–ethyl acetate) at 5-min intervals. The reaction was stopped by addition of Dowex 50 (H^+) cation-exchange resin. After filtration and evaporation of the filtrate to dryness, the resulting syrupy residue was subjected to column chromatography on silica gel (80 × 2 cm, 1:1 toluene–ethyl acetate). Evaporation of the combined fractions containing the major product afforded **17** (568 mg, 50%) as colorless needles, m.p. 99–100° (ethyl acetate–hexane), $[\alpha]_D^{20}$ −12.2° (*c* 1.0, chloroform); ^{1}H-n.m.r.: δ 8.10–7.99 (m, 4 H, arom. H), 7.60–7.16 (m, 11 H, arom. H), 5.10 (s, 1 H, H-1'), 4.93 (s, 1 H, H-1), 4.62–4.14 (m, 11 H, H-3,4,5a,5b,2',3',4',5'a,5'b, and $C_6H_5CH_2O$), 4.07 (d, 1 H, $J_{2,3}$ ~4.0 Hz, H-2), 3.14 (s, 3 H, CH_3O), and 2.98–2.91 (m, 2 H, 2 OH).

Anal. Calc. for $C_{32}H_{34}O_{11}$: C, 64.6; H, 5.8. Found: C, 64.6; H, 5.9.

After further elution of the column with ethyl acetate, the fractions containing some product were evaporated. The residue was dissolved in pyridine (5 mL) and treated with benzoyl chloride (1 mL) at 0° for 50 min. The mixture was poured into ice–water and extracted with dichloromethane (50 mL). The organic layer was extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated to dryness to give **16** (745 mg, 48%) as a syrup.

Methyl 5-O-benzoyl-2-O-(5-O-benzoyl-2-O-benzyl-β-D-ribofuranosyl)-3-O-benzyl-β-D-ribofuranoside (**18**) *and methyl 5-O-benzoyl-2-O-(5-O-benzoyl-3-O-benzyl-β-D-ribofuranosyl)-3-O-benzyl-β-D-ribofuranoside* (**20**). — A suspension of **17** (472 mg) and dibutyltin oxide (200 mg) in dry benzene (150 mL) was heated

under reflux with continuous separation of water for 5 h. After removal of the solvent *in vacuo,* the residue was dissolved in dry *N,N*-dimethylformamide (5 mL), and benzyl bromide (0.12 mL) and molecular sieve 4A (400 mg) were added. The mixture was stirred at 80–85° for 48 h. After evaporation to dryness, the residue was dissolved in dichloromethane (30 mL), extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Subsequent purification of the residue by column chromatography on silica gel (80 × 2 cm, 3:1 toluene–ethyl acetate) afforded a mixture of **18** and **20** (433 mg, 80%) as a syrup. Upon addition of 1:1 ethyl acetate–hexane, crystallization was induced to afford 175 mg (32%) of **18** as colorless needles, m.p. 145–146° (ethyl acetate–hexane), $[\alpha]_D^{20}$ +5.5° (*c* 1.0, chloroform); ^{1}H-n.m.r.: δ 8.10–7.99 (m, 4 H, arom. H), 7.59–7.19 (m, 16 H, arom. H), 5.32 (d, 1 H, $J_{1',2'}$ ~1.0 Hz, H-1'), 4.84 (s, 1 H, H-1), 4.63–4.21 (m, 12 H, H-3,4,5a,5b,3',4',5'a,5'b, and 2 $C_6H_5CH_2O$), 4.15 (d, 1 H, $J_{2,3}$ ~3.7 Hz, H-2), 4.03 (dd, 1 H, $J_{2',3'}$ ~5.0 Hz, H-2'), 3.12 (s, 3 H, CH_3O), 2.65 (d, 1 H, $J_{OH,3'}$ ~8.6 Hz, OH); ^{13}C-n.m.r. ($CDCl_3$, 62.9 MHz, Δ: deuterium induced shift difference[23]): 106.94 (C-1), 105.62 (C-1'), 82.40 (C-4', Δ −0.03), 82.04 (C-4), 78.43 and 77.89 (C-2,2',3), 71.38 (C-3', Δ −0.11), 73.04 and 72.61 ($OCH_2C_6H_5$), 64.87 and 64.73 (C-5,5'), and 54.74 (OCH_3).

Anal. Calc. for $C_{39}H_{40}O_{11}$: C, 68.4; H, 5.9. Found: C, 68.6; H, 6.0.

After removal of **18** by filtration and evaporation of the filtrate to dryness, the residue was subjected to l.c. separation, the purity of the fractions being checked with the aid of 90-MHz ^{1}H-n.m.r. spectrometry, to yield syrupy **20** (150 mg, 28%), $[\alpha]_D^{20}$ +3.9° (*c* 0.97, chloroform); ^{1}H-n.m.r.: δ 8.05–7.98 (m, 4 H, arom. H), 7.60–7.21 (m, 16 H, arom. H), 5.16 (s, 1 H, H-1'), 4.93 (s, 1 H, H-1), 4.66–4.17 (m, 13 H, H-3,4,5a,5b,2',3',4',5'a,5'b, and 2 $C_6H_5CH_2O$), 4.03 (d, 1 H, $J_{2,3}$ ~4.0 Hz, H-2), 3.11 (s, 3 H, CH_3O), and 2.65 (br. s, 1 H, OH).

Anal. Calc. for $C_{39}H_{40}O_{11}$: C, 68.4; H, 5.9. Found: C, 68.1; H, 5.8.

*Methyl 5-*O*-benzoyl-2-*O*-[5-*O*-benzoyl-2-*O*-benzyl-3-*O*-(*p*-nitrobenzoyl)-β-*D*-ribofuranosyl]-3-*O*-benzyl-β-*D*-ribofuranoside* (**19**). — A solution of **18** (34 mg) and *p*-nitrobenzoyl chloride (20 mg) in dry pyridine (2 mL) was stirred for 20 h at room temperature. After addition of water (0.1 mL), stirring was continued for 30 min. The mixture was poured into ice–water, extracted with dichloromethane (50 mL), washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Purification on a column of silica gel (size A, 5:1 toluene–ethyl acetate) gave **19** (38 mg, 92%) as a syrup, $[\alpha]_D^{20}$ +14.8° (*c* 1.1, chloroform); ^{1}H-n.m.r.: δ 8.28–8.14 (m, 4 H, nitroarom. H), 8.06–8.00 (m, 4 H, arom. H), 7.60–7.07 (m, 16 H, arom. H), 5.54 (dd, 1 H, $J_{2',3'}$ ~5.0, $J_{3',4'}$ ~6.0 Hz, H-3'), 5.40 (d, 1 H, $J_{1',2'}$ ~2.0 Hz, H-1'), 4.90 (s, 1 H, H-1), 4.72 (ddd, 1 H, $J_{4',5'a}$ ~4.5, $J_{4',5'b}$ ~5.0 Hz, H-4'), 4.63 and 4.55 (AB, 2 H, J_{AB} ~11.0 Hz, $C_6H_5CH_2O$), 4.61 (dd, 1 H, $J_{5'a,5'b}$ ~11.5 Hz, H-5'a), 4.51 and 4.43 (AB, 2 H, J_{AB} ~11.0 Hz, $C_6H_5CH_2O$), 4.50 (dd, 1 H, H-5'b), 4.40 (dd, 1 H, H-2'), 4.37–4.24 (m, 4 H, H-3,4,5a,5b), 4.19 (d, 1 H, $J_{2,3}$ ~4.0 Hz, H-2), and 3.16 (s, 3 H, CH_3O).

Anal. Calc. for $C_{46}H_{43}NO_{14}$: C, 66.2; H, 5.2; N, 1.7. Found: C, 65.7; H, 5.3; N, 1.5.

Methyl 5-O-benzoyl-2-O-[5-O-benzoyl-3-O-benzyl-2-O-(p-nitrobenzoyl)-β-D-ribofuranosyl]-3-O-benzyl-β-D-ribofuranoside (**21**). — The procedure was analogous to that for the preparation of **19** to give syrupy **21** (36 mg, 87%), $[\alpha]_D^{20}$ +27.4° (*c* 1.8, chloroform); ^{1}H-n.m.r.: δ 8.32–8.18 (m, 4 H, nitroarom. H), 8.04–7.98 (m, 4 H, arom. H), 7.60–7.08 (m, 16 H, arom. H), 5.65 (d, 1 H, $J_{2',3'}$ ~3.0 Hz, H-2'), 5.28 (s, 1 H, H-1'), 4.86 (s, 1 H, H-1), 4.62 and 4.51 (AB, 2 H, J_{AB} ~12.0 Hz, $C_6H_5CH_2O$), 4.58 (s, 2 H, $C_6H_5CH_2O$), 4.54–4.22 (m, 8 H, H-3,4,5a,5b,3',4',5'a,5'b), 4.10 (d, 1 H, $J_{2,3}$ ~4.0 Hz, H-2), and 3.10 (s, 3 H, CH_3O).

Anal. Calc. for $C_{46}H_{43}NO_{14}$: C, 66.2; H, 5.2; N, 1.7. Found: C, 66.3; H, 5.2; N, 1.9.

Methyl O-(methyl 4,5,7,8-tetra-O-acetyl-3-deoxy-α-D-manno-2-octulopyranosylonate)-(2→2)-O-(5-O-benzoyl-β-D-ribofuranosyl)-(1→2)-5-O-benzoyl-3-O-benzyl-β-D-ribofuranoside (**22**) *and methyl O-[3-O-(4,5,7,8-tetra-O-acetyl-3-deoxy-α-D-manno-2-octulopyranosylono-1',2-lactone)-5-O-benzoyl-β-D-ribofuranosyl]-(1→2)-5-O-benzoyl-3-O-benzyl-β-D-ribofuranoside* (**26**). — A solution of **6** (431 mg, 0.9 mmol) in acetonitrile (1 mL) was added to a suspension of **17** (407 mg, 0.68 mmol), $Hg(CN)_2$ (252 mg, 1.0 mmol), and molecular sieve 4A (500 mg) in acetonitrile (2 mL) under a stream of dry N_2. After being stirred for 6 h at room temperature, the mixture was diluted with dichloromethane (50 mL), filtered, and evaporated. The residue was dissolved in dichloromethane (100 mL), and the solution extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Chromatography of the residue on a column of silica gel (80 × 2 cm, 3:1 toluene–ethyl acetate) gave the faster-moving compound **26** (239 mg, 36%) as a colorless syrup, $[\alpha]_D^{20}$ +26.4° (*c* 1.1, chloroform); ^{1}H-n.m.r.: δ 8.11–7.99 (m, 4 H, arom. H), 7.63–7.17 (m, 11 H, arom. H), 5.38 (unresolved signal, 1 H, H-5), 5.32 (s, 1 H, H-1'), 5.30 (ddd, 1 H, $J_{4,3a}$ ~12.5, $J_{4,3e}$ ~5.0, $J_{4,5}$ ~3.0 Hz, H-4), 5.08 (ddd, 1 H, $J_{7,6}$ ~10.5, $J_{7,8a}$ ~2.0, $J_{7,8b}$ ~2.5 Hz, H-7), 5.03 (d, 1 H, $J_{2',3'}$ ~5.5 Hz, H-2'), 4.82 (s, 1 H, H-1''), 4.63 (dd, 1 H, $J_{8a,8b}$ ~12.5 Hz, H-8a), 4.62–4.44 (m, 7 H), 4.30 (dd, 1 H, $J_{6,5}$ ~1.5 Hz, H-6), 4.30–4.22 (m, 3 H), 4.07 (m, 1 H), 3.94 (dd, 1 H, H-8b), 3.14 (s, 3 H, CH_3O), 2.67 (t, 1 H, $J_{3a,3e}$ ~13.5 Hz, H-3*a*), 2.17 (s, 3 H), 2.04 (s, 3 H) and 2.01 (s, 6 H, 4 CH_3CO), and 1.97 (dd, 1 H, H-3*e*).

Anal. Calc. for $C_{48}H_{52}O_{21}$: C, 59.7; H, 5.4. Found: C, 59.2; H, 5.4.

Further elution of the column gave **22** (168 mg, 25%) as a colorless syrup, $[\alpha]_D^{20}$ +92.7° (*c* 0.85, chloroform); ^{1}H-n.m.r.: δ 8.12–7.96 (m, 4 H, arom. H), 7.60–7.16 (m, 11 H, arom. H), 5.39–5.32 (m, 2 H, H-5,4), 5.29 (s, 1 H, H-1'), 5.17 (ddd, 1 H, $J_{7,8a}$ ~2.5, $J_{7,8b}$ ~4.5, $J_{7,6}$ ~10.0 Hz, H-7), 4.91 (s, 1 H, H-1''), 4.76 (dd, 1 H, $J_{6,5}$ ~1.0 Hz, H-6), 4.67 (dd, 1 H, $J_{8a,8b}$ ~12.5 Hz, H-8a), 4.73 (d, 1 H, *J* ~12.0 Hz) and 4.60–4.15 (m, 11 H, H-2',3',4',5'a,5'b,2'',3'',4'',5''a,5''b, and $C_6H_5CH_2O$), 4.09 (dd, 1 H, H-8b), 3.66 (s, 3 H, CH_3OCO), 3.14 (s, 3 H, CH_3O), 2.67 (d, 1 H, $J_{3',OH}$ ~6.5 Hz, OH), 2.30 (dd, 1 H, $J_{3e,3a}$ ~12.5, $J_{3e,4}$ ~5.0 Hz, H-3*e*), 2.17 (t, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*), 2.08 (s, 3 H), 2.04 (s, 3 H), and 1.98 (s, 6 H, 4 CH_3CO).

Anal. Calc. for $C_{49}H_{56}O_{22}$: C, 59.0; H, 5.7. Found: C, 58.8; H, 5.7.

Upon further elution of the column with 1:1 toluene–ethyl acetate, **17** (144

mg, 35%) was recovered as a crystalline solid, m.p. 99–100° (ethyl acetate–hexane).

Methyl O-*(methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→2)*-O-*(3*-O-*aeetyl-5*-O-*benzoyl-β*-D-*ribofuranosyl)-(1→2)-5*-O-*benzoyl-3*-O-*benzyl-β*-D-*ribofuranoside* (**23**). — A solution of **22** (168 mg) and acetic anhydride (0.5 mL) in pyridine (5 mL) was stirred for 10 h at room temperature. After removal of the solvent *in vacuo,* the residue was dissolved in dichloromethane (50 mL), extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Purification of the residue on a column of silica gel (size B, 1:1 toluene–ethyl acetate) yielded syrupy **23** (164 mg, 94%), $[\alpha]_D^{20}$ +47.0° (*c* 1.0, chloroform); ^{1}H-n.m.r.: δ 8.11–7.96 (m, 4 H, arom. H), 7.59–7.16 (m, 11 H, arom. H), 5.64 (dd, 1 H, $J_{3',2'}$ ~$J_{3',4'}$ ~5.5 Hz, H-3'), 5.42 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.5, $J_{4,3a}$ ~12.0 Hz, H-4), 5.36 (s, 1 H, H-1'), 5.36 (unresolved signal, 1 H, H-5), 5.13 (ddd, 1 H, $J_{7,8a}$ ~2.5, $J_{7,8b}$ ~3.5, $J_{7,6}$ ~9.5 Hz, H-7), 4.90 (s, 1 H, H-1''), 4.76–4.60 (m, 4 H), 4.51–4.37 (m, 4 H) and 4.33–4.11 (m, 5 H, H-6,8a,2',4',5'a,5'b,2'',3'',4''5''a,5''b, and $C_6H_5CH_2O$), 3.93 (dd, 1 H, $J_{8b,8a}$ ~12.5 Hz, H-8b), 3.70 (s, 3 H, CH_3OCO), 3.08 (s, 3 H, CH_3O), 2.23, 2.09, 2.07, 2.00, and 1.98 (s, 15 H, 5 CH_3CO), and 2.27–2.04 (m, 2 H, H-3*e*,3*a*).

Anal. Calc. for $C_{51}H_{58}O_{23}$: C, 59.0; H, 5.6. Found: C, 59.0; H, 5.7.

Methyl O-*(methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→2)*-O-*(3*-O-*acetyl-5*-O-*benzoyl-β*-D-*ribofuranosyl)-(1→2)-5*-O-*benzoyl-β*-D-*ribofuranoside* (**24**). — A suspension of **23** (126 mg) and PdO (120 mg) in dry methanol (30 mL) was hydrogenolyzed at atmospheric pressure and room temperature for 15 h. The catalyst was removed by filtration and the filtrate evaporated to dryness. Purification of the residue on a column of silica gel (size B, 1:1 toluene–ethyl acetate) gave syrupy **24** (110 mg, 96%), $[\alpha]_D^{20}$ +14.3° (*c* 0.8, chloroform); ^{1}H-n.m.r.: δ 8.10–8.06 (m, 4 H, arom. H), 7.60–7.42 (m, 6 H, arom. H), 5.52 (dd, 1 H, $J_{3',2'}$ ~5.5, $J_{3',4'}$ ~3.5 Hz, H-3'), 5.33 (unresolved signal, 1 H, H-5), 5.30 (ddd, 1 H, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.5, $J_{4,5}$ ~3.0 Hz, H-4), 5.23 (d, 1 H, $J_{1',2'}$ ~3.0 Hz, H-1'), 5.07 (ddd, 1 H, $J_{7,6}$ ~9.5, $J_{7,8a}$ ~2.0, $J_{7,8b}$ ~3.0 Hz, H-7), 4.96 (s, 1 H, H-1''), 4.64 (dd, 1 H, H-2'), 4.60–4.44 (m, 6 H), 4.41 (ddd, 1 H, $J_{3'',2''}$ ~4.7, $J_{3'',OH}$ ~11.0, $J_{3'',4''}$ ~7.0 Hz, H-3''), 4.35 (dd, 1 H, *J* ~5.0 and 12.0 Hz, not assigned), 4.19 (dd, 1 H, $J_{6,5}$ ~1.0 Hz, H-6), 4.19–4.08 (m, 1 H), 4.00 (d, 1 H, H-2''), 3.90 (dd, 1 H, $J_{8b,8a}$ ~12.0 Hz, H-8b), 3.83 (s, 3 H, CH_3OCO), 3.25 (d, 1 H, OH), 3.19 (s, 3 H, CH_3O), 2.29 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.25 (s, 3 H, CH_3CO), 2.14 (t, 1 H, H-3*a*), 2.09, 2.06, 1.99, and 1.98 (s, 12 H, 4 CH_3CO).

Anal. Calc. for $C_{44}H_{52}O_{23}$: C, 55.7; H, 5.5. Found: C, 56.1; H, 5.5.

Methyl O-*(sodium 3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→2)*-O-*β*-D-*ribofuranosyl-(1→2)-β*-D-*ribofuranoside* (**25**). — A solution of **24** (80 mg) in dry methanol (25 mL) and 25mM methanolic sodium methoxide (5 mL) was stirred for 20 h at room temperature. The reaction mixture was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and the filtrate evaporated to dryness. The syrupy residue was extracted three times with 10-mL portions of diethyl ether and dried. The residue was dissolved in water (5 mL) and 0.2M aqueous

NaOH (0.5 mL), and stirred for 60 min at room temperature. The mixture was adjusted to pH 8.0 by addition of Dowex 50 (H^+) cation-exchange resin and filtered. Lyophilization of the filtrate gave 45 mg (100%) of **25** as a colorless glass, $[\alpha]_D^{20}$ +16.1° (*c* 1.08, water); ^{1}H-n.m.r. (D_2O): δ 5.28 (d, 1 H, $J_{1',2'}$ ~1.5 Hz, H-1′), 5.08 (d, 1 H, $J_{1'',2''}$ ~1.5 Hz, H-1″), 4.30–3.56 (m, 16 H, H-4,5,6,7,8a,8b,2′,3′,4′,5′a,5′b,2″,3″,4″,5″a,5″b), 3.41 (s, 3 H, CH_3O), 2.22 (dd, 1 H, $J_{3e,4}$ ~5.0, $J_{3e,3a}$ ~13.5 Hz, H-3*e*), and 1.79 (t, 1 H, $J_{3a,4}$ ~13.5 Hz, H-3*a*).

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