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CONJUGATES OF BIOLOGICALLY ACTIVE LIPID A-SUBUNIT ANALOGS WITH 3-DEOXY-D-*manno*-2-OCTULOSONIC ACID (KDO) AND ITS METHYL ESTER

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ABSTRACT

The previously described, biologically active 4-*O*-phosphono-D-glucosamine derivatives GLA-27, GLA-47, and GLA-60 (all related to bacterial lipid A) were converted into their respective conjugates with 3-deoxy-D-*manno*-2-octulosonic acid (KDO). The methyl and benzyl esters of [4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-D-*manno*-2-octulopyranosyl fluoride]onic acid and the corresponding glycosyl bromide were prepared, by starting from methyl 2,4,5,7,8-penta-*O*-acetyl-3-deoxy-D-*manno*-2-octulopyranosonate, and coupled to O-6 of suitably protected precursors of the phosphono-D-glucosamines. This gave a series of fully substituted disaccharides that were transformed, by O-de(chloroacetyl)ation and successive hydrogenolytic removal of benzyl, benzyloxymethyl, and phenyl groups, into the target conjugates (KDO methyl ester α- and β-linked to GLA-27, and KDO α-linked to GLA-27, GLA-47, and GLA-60).

INTRODUCTION

Both lipid A and KDO have been noted as the prominent constituents of bacterial lipopolysaccharides (LPS)[1,2]. Recent investigation revealed[3,4] that the α-KDO-(2→4)-KDO disaccharide moiety in the core region of LPS is α-ketosidically linked to O-6′ of the D-glucosamine disaccharide backbone of lipid A, the active center of endotoxin. The biological roles of KDO in LPS are as yet unclear, although its immunological importance has often been suggested[5].

Since the discovery[6,7] that a chemically synthesized lipid A-subunit analog designated GLA-27 can exhibit some of the distinct, beneficial biological activities of endotoxin, many efforts have been focussed on clarifying the relationship between the molecular structure and the biological activity[8,9] of a variety of 4-*O*-phosphono-D-glucosamine derivatives[10,11] related to the nonreducing-sugar subunit of lipid A.

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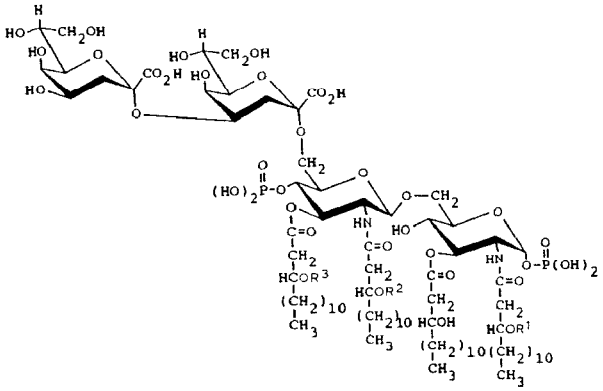
Among the synthetic subunit analogs[10a], GLA-60 showed the most marked immunopharmacological activities[12,13], such as enhancement of nonspecific resistance against infection by *P. aeruginosa* and by some viruses. However, the activity of GLA-47, which corresponds to the nonreducing-sugar subunit of natural lipid A, is much weaker than that of GLA-27, GLA-59, or GLA-60, suggesting the critical importance of the fatty acyl composition.

Earlier[14], 6-*O*-glycosylation of GLA-27 with methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-D-*manno*-2-octulopyranosyl bromide)onate was described, but the KDO part of the resulting disaccharides remained protected by the acetyl groups and as methyl ester. It is clear that acetyl protecting groups could not be removed without cleaving ester groups in the lipid A subunit. We now describe an efficient synthesis of five new disaccharides containing unsubstituted KDO attached to lipid A subunit analogs. The starting materials for these disaccharides were the chloroacetyl-protected KDO halides and the 4-*O*-phosphono-D-glucosamine acceptors.

RESULTS AND DISCUSSION

Treatment of methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-D-*manno*-2-octulopyranosyl bromide)onate[15] with M aqueous sodium hydrogencarbonate at 0° gave compound **1**[16,17] in 85–90% yield. 2-*O*-(Tetrahydropyranyl)ation of **1** was performed in



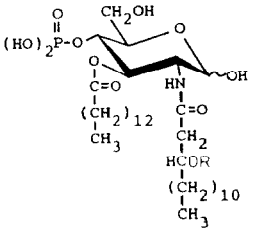
Postulated structure of $(KDO)_2$-lipid A

	R^1	R^2	R^3
E. coli	H	C_{12}	C_{14}
S. minnesota	C_{16}	C_{12}	C_{14}
P. mirabilis	C_{16}	C_{14}	C_{14}

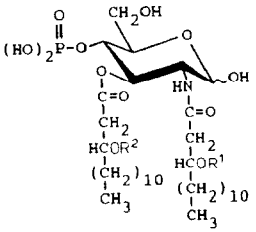
C_{12} = $CH_3(CH_2)_{10}CO$

C_{14} = $CH_3(CH_2)_{12}CO$

C_{16} = $CH_3(CH_2)_{14}CO$



GLA-27 R = C_{14}



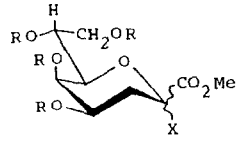
	R^1	R^2
GLA-47	C_{14}	C_{14}
GLA-60	H	C_{14}

the presence of (*a*) *p*-toluenesulfonic acid or (*b*) bis(trimethylsilyl) sulfate[18] as the catalyst, to give **2** in nearly quantitative yield. Whereas method (*a*) gave an ~1:1 mixture of two diastereoisomers (or anomers) of **2**, presumably due to the asymmetric carbon atom (C-2) of the tetrahydropyran-2-yl (THP) group, method (*b*) afforded as the major product (70%) the diastereomer corresponding to the slower-moving compound in t.l.c. Deacetylation of **2** yielded **3**, which is superior to free KDO as a substrate for (chloroacetyl)ation, because it can afford only a single, pyranoid product. Thus, treatment of **3** with chloroacetic anhydride in 2,6-lutidine–triethylamine–2,2-dichloroethane, gave **4** in high yield. Hydrolytic removal of the THP group gave **5**, appearing as a single product in t.l.c. In the ^{1}H-n.m.r. spectrum of **5**, the C-3 methylene protons appear at δ 2.02 (H-3*e*) and 2.47 (H-3*a*) showing a marked, high-field shift of the equatorial proton on C-3. Such a high-field shift of H-3*e* was also observed for **1** [δ 1.91 (H-3*e*) and 2.43 (H-3*a*)], and it is characteristic[19] of 2-OH derivatives of KDO.

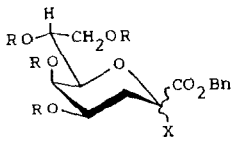
The conversion of **5** into the fluoride **7** was accomplished by use of diethylaminosulfur trifluoride (DAST)[20] as the fluorinating agent. The ^{1}H-n.m.r. data showed that **7** was an ~1:1 mixture of the α- and β-fluoride; a wide multiplet at δ 2.44 having a large H–F coupling[21] ($J_{3a,F}$ 28.6 Hz) was assigned to H-3*a* of the α-fluoride, and a narrower multiplet at δ 2.30 having a smaller H–F coupling ($J_{3a,F}$ 15.8 Hz) was assigned to the β anomer. These assignments are supported by the ^{13}C-n.m.r. data[22]. Saponification of **3**, and benzyl esterification of the carboxyl group afforded **9**, which was per(chloroacetyl)ated as described for **4**. The resulting **10** was treated with aqueous tetrafluoroboric acid in acetonitrile, to give **11**, which was converted, by treatment with DAST, into the corresponding fluoride **13**. 2-*O*-Acetylation of **5** or **11**, and treatment of the product with titanium tetrabromide gave the methyl or benzyl ester of [4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-D-*manno*-2-octulopyranosyl bromide]onate (**8** and **14**), which were used as glycosyl donors for the synthesis of α-disaccharides.

As acceptors, there were used benzyl 2-deoxy-4-*O*-(diphenoxyphosphinyl)-3-*O*-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside[10a] (**15**), benzyl 2-deoxy-4-*O*-(diphenoxyphosphiny)-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-β-D-glucopyranoside[10a] (**15**), benzyl 2-deoxy-4-*O*-(diphenoxyphosphinyl)-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside[10b] (**16**), and benzyl 2-[(3*R*)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4-*O*-(diphenoxyphosphinyl)-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside[10b] (**17**), which had respectively been prepared as the precursors of GLA-27, GLA-47, and GLA-60.

Coupling of the acceptor **15** with **7** was performed by the procedure of Nicolaou *et al.*[23], to give a mixture of α-glycoside (**18α**; 27%) and β-glycoside (**18β**; 41%). The α-disaccharide **18α** was also synthesized in 67% yield by treatment of **15** (1 mol. equiv.) with the bromide **8** (1.3 mol equiv.) in the presence of $Hg(CN)_2$, $HgBr_2$, and molecular sieves 4A in dichloromethane, by a slight modification of the procedure reported by Paulsen *et al.*[15]. The anomeric configurations of the disaccharides were



	R	X
1	Ac	OH
2	Ac	OTHP
3	H	OTHP
4	ClAc	OTHP
5	ClAc	OH
6	ClAc	OAc
7	ClAc	F
8	ClAc	Br

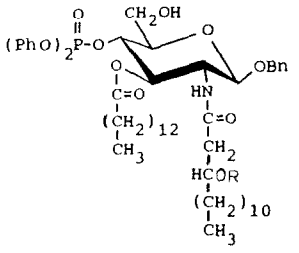


	R	X
9	H	OTHP
10	ClAc	OTHP
11	ClAc	OH
12	ClAc	OAc
13	ClAc	F
14	ClAc	Br

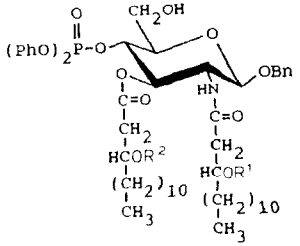
Bn = $PhCH_2$

THP = tetrahydropyran-2-yl

ClAc = $ClCH_2CO$



15 R = C_{14}

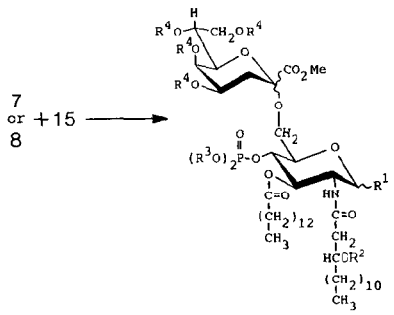


	R^1	R^2
16	C_{14}	C_{14}
17	Bom	C_{14}

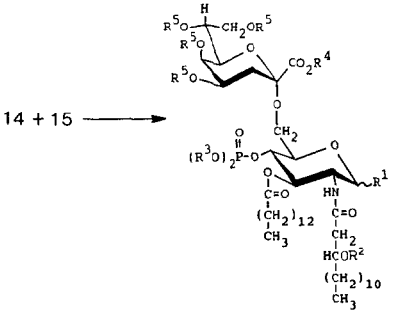
Bom = $PhCH_2OCH_2$

assigned on the basis of glycosylation yields, t.l.c., optical rotations, and n.m.r. spectra[16]. The chloroacetyl groups of **18α** and **18β** were cleaved by treatment with hydrazine dithiocarbonate (HDTC)[24] in 2:1 2,6-lutidine–acetic acid solution at 0°, to afford **19α** and **19β**, respectively, in ~90% yields. Hydrogenolytic removal of the benzyl and phenyl groups was achieved with palladium and Adams' platinum catalysts, respectively, to give **21α** and **21β** in high yields.

When compound **13** was used for the coupling with **15**, in contrast to the results with **7**, the reaction was too slow to permit obtaining disaccharide products in desirable yields. Accordingly, the synthesis of the disaccharides containing the carboxyl-free KDO was accomplished with the bromide **14**, as previously described for the preparation of **21α** with **8**. The three glycosyl acceptors **15**, **16**, and **17** were coupled with **14** to give the corresponding α-disaccharide products **22** (65%), **26** (59%), and **30** (61%), respectively, together with minute proportions of the β-disaccharides. Selective removal of the chloroacetyl groups from **22**, **26**, and **30** was respectively performed with HDTC, as described for **21α**, to afford **23**, **27**, and **31**, which were successively hydrogenolyzed with palladium and platinum catalysts, to yield the desired KDO-containing disaccharides **25**, **29**, and **33**.



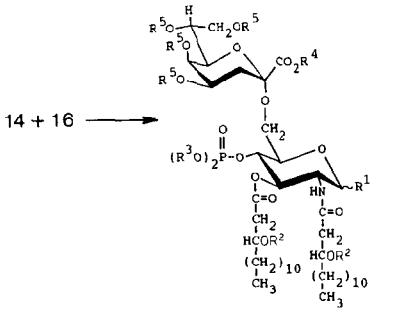
7
or 8 + 15 →



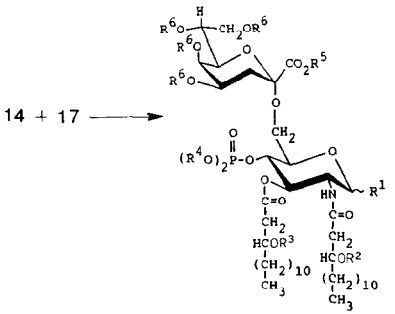
14 + 15 →

	R^1	R^2	R^3	R^4
18α, 18β	OBn(β)	C_{14}	Ph	ClAc
19α, 19β	OBn(β)	C_{14}	Ph	H
20α, 20β	OH	C_{14}	Ph	H
21α, 21β	OH	C_{14}	H	H

	R^1	R^2	R^3	R^4	R^5
22	OBn(β)	C_{14}	Ph	Bn	ClAc
23	OBn(β)	C_{14}	Ph	Bn	H
24	OH	C_{14}	Ph	H	H
25	OH	C_{14}	H	H	H



14 + 16 →



14 + 17 →

	R^1	R^2	R^3	R^4	R^5
26	OBn(β)	C_{14}	Ph	Bn	ClAc
27	OBn(β)	C_{14}	Ph	Bn	H
28	OH	C_{14}	Ph	H	H
29	OH	C_{14}	H	H	H

	R^1	R^2	R^3	R^4	R^5	R^6
30	OBn(β)	Bom	C_{14}	Ph	Bn	ClAc
31	OBn(β)	Bom	C_{14}	Ph	Bn	H
32	OH	H	C_{14}	Ph	H	H
33	OH	H	C_{14}	H	H	H

EXPERIMENTAL

General methods. — T.l.c. was performed on silica gel 60 (Merck, aluminum sheets), and column chromatography on silica gel (Wako Co.; 200 or 300 mesh) was accomplished with the solvent systems (v/v) specified. Evaporations were conducted *in vacuo*. Specific rotations were determined with a Union PM-201 polarimeter, and i.r. spectra were recorded with a Jasco IRA-1 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 270 or 500 MHz with a JEOL JNM-GX270 or JNM-GX500

spectrometer, respectively, and ^{13}C-n.m.r. spectra at 67.5 or 125 MHz with the same instruments.

*Methyl 4,5,7,8-tetra-O-acetyl-3-deoxy-*D-manno-*2-octulosonate* (**1**). — Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-D-*manno*-2-octulopyranosyl bromide)onate[15] (1.19 g) was dissolved in acetone (10 mL), and the solution was cooled to 0°. Aqueous sodium hydrogencarbonate (M, 2.5 mL) was added, and the mixture was stirred for 0.5 h at 0°, the reaction being monitored by t.l.c. (5:1 dichloromethane–ether). The acetone was removed by evaporation, and the residue was extracted with dichloromethane. The organic layer was washed with water, dried, and evaporated to a syrup, which was chromatographed on a column of silica gel (Wakogel C-300) with 2:1 hexane–ethyl acetate, to give **1** in 85% yield; $[\alpha]_D$ +51° (*c* 1, chloroform); ν_{max} 3600–3200 (OH); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.91 (~dd, 1 H, J_{gem} 12.8, $J_{3,4}$ 4.4, $J_{3,5}$ ~1.5 Hz, H-3*e*), 1.99, 2.01, 2.07, 2.11 (4 s, 12 H, CH_3CO), 2.43 (~t, 1 H, $J_{3,4}$ ~12 Hz, H-3*a*), 3.87 (s, 3 H, CO_2CH_3), 4.14 (dd, 1 H, J_{gem} ~12, $J_{7,8}$ ~5 Hz, H-8a), 4.34 (dd, 1 H, $J_{6,7}$ 9.9, $J_{5,6}$ 1.1 Hz, H-6), 4.39 (dd, 1 H, $J_{7,8}$ 2.6 Hz, H-8b), 5.15 (m, 1 H, H-7), 5.35 (m, 1 H, H-4), and 5.37 (m, 1 H, H-5). These n.m.r. data suggest that the α anomer preponderates in the equilibrium mixture in chloroform-*d*.

Anal. Calc. for $C_{17}H_{24}O_{12}$ (420.36): C, 48.57; H, 5.76. Found: C, 48.26; H, 5.58.

When compound **1** thus obtained was treated with acetic anhydride in pyridine for 1 h at 0° and then for 20 h at room temperature, the corresponding pentaacetate was obtained in nearly quantitative yield. This result also supports the preponderance of the α anomer in the solution of **1**.

*Methyl 4,5,7,8-tetra-O-acetyl-3-deoxy-α-*D-manno-*2-O-(tetrahydropyran-2-yl)-2-octulopyranosonate* (**2**). — *(a) With p-toluenesulfonic acid.* To a solution of **1** (0.917 g) in dichloromethane were added dihydropyran (0.99 mL), a catalytic amount of *p*-toluenesulfonic acid monohydrate, and molecular sieves 4A, and the mixture was stirred for 2 h at room temperature. The acid was neutralized by addition of sodium hydrogencarbonate, and the suspension was filtered. The filtrate and washings were combined, and evaporated to a residue that was chromatographed on a column of silica gel (Wakogel C-200) with 3:2 hexane–ethyl acetate, to afford syrupy **2** (1.07 g; 97%) which gave a "near-single" spot in t.l.c. (5:1 dichloromethane–ether); $[\alpha]_D$ +70° (*c* 1.3, chloroform); ν_{max} complete disappearance of the peak at 3600–3200 cm^{-1} (OH); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.45–2.55 (m, 20 H, C-3–C-5 ring methylenes of the THP group, CH_3CO, and H-3), 3.42, 3.56 (2 m, ~0.5 H + 0.5 H, H-6 of THP), 3.75, 3.80 (2 s, 3 H, CO_2CH_3), and 3.75–5.5 (m, 8 H, H-2 and H-6′ of THP, and H-4–H-8 of KDO). These n.m.r. data suggest that **2** is an ~1:1 diastereoisomeric mixture arising from the THP group.

Details of the ^{1}H-n.m.r. data for the slightly slower-moving diastereomer are the following: δ 2.40 (dd, 1 H, J_{gem} 12.8, $J_{3,4}$ 4.8 Hz, H-3*e*), 4.09 (~d, 1 H, $J_{6,7}$ ~10 Hz, H-6), 4.17 (dd, 1 H, J_{gem} 12.5, $J_{7,8}$ 2.9 Hz, H-8a), 4.67 (dd, 1 H, $J_{7,8}$ 2.6 Hz, H-8b), 5.19 (m, 1 H, H-7), and 5.36 (m, 1 H, H-5).

Anal. Calc. for $C_{22}H_{32}O_{13}$ (504.48): C, 52.37; H, 6.39. Found: C, 52.59; H,

6.27.

(b) With bis(trimethylsilyl) sulfate. A stirred mixture of **1** (1.35 g) and dihydropyran (0.76 mL) in dichloromethane (25 mL) was cooled to 0°, and bis(trimethylsilyl) sulfate (0.4 mL of M solution in dichloromethane) was added. The mixture was stirred for 1 h at 0°, the reaction being monitored by t.l.c. (3:1 dichloromethane–ether). Pyridine (0.4 mL) was added, and the mixture was evaporated to a residue which was chromatographed on a column of silica gel with 5:2 hexane–ethyl acetate, to give syrupy **2** (1.16 g); $[\alpha]_D$ +69.5° (*c* 1.3, chloroform). T.l.c. showed that ~70% of the product was the slower-moving diastereomer described in method (*a*).

Methyl 4,5,7,8-tetrakis-O-(chloroacetyl)-3-deoxy-α-D-manno-2-O-(tetrahydropyran-2-yl)-2-octulopyranosonate (**4**). — To a stirred solution of **2** (0.4 g) in dry methanol (3 mL) was added a catalytic amount of sodium metal at 0°. After completion of the reaction (t.l.c., 4:1 dichloromethane–methanol), the alkali was neutralized with Amberlite IR-120 (H^+) ion-exchange resin. The resin was filtered off, and washed with methanol. The filtrate and washings were combined, and evaporated to give a quantitative yield of syrupy **3**, which appeared in t.l.c. (3:1 dichloromethane–methanol) as two spots (~1:1) of closely similar mobility; $[\alpha]_D$ +75° (*c* 1, 10:1 chloroform–methanol); ν_{max} 3600–3100 (OH), 2950 (THP), and 1740 cm^{-1} (CO_2CH_3).

To a solution of **3** (1.14 g) in dichloromethane (5 mL) were added dry 2,6-lutidine (6.5 mL) and triethylamine (2.05 mL). The mixture was stirred at 0°, and chloroacetic anhydride (3.50 g) was added; stirring was continued for 20 h at room temperature, the mixture was cooled, and methanol was added in order to decompose the excess of the reagent; it was then evaporated. The syrupy residue was dissolved in dichloromethane, and the solution was successively washed with ice-cold 2 M hydrochloric acid, water, and dilute sodium hydrogencarbonate, dried, and evaporated. The product was purified by chromatography on a column of silica gel (Wakogel C-300) with 2:1 hexane–ethyl acetate, to give syrupy **4** (2.0 g; 91%). The two diastereomers showed different mobilities in t.l.c. (70:1 dichloromethane–methanol). The chromatographically faster-moving isomer (**4a**; 48%) had $[\alpha]_D$ +77° (*c* 0.5, chloroform); ^{1}H-n.m.r. data: δ 1.45–1.9 (m, 6 H, C-3–C-5 methylene protons of THP), 2.18 (dd, 1 H, J_{gem} 12.8, $J_{3,4}$ ~11 Hz, H-3*a*), 2.25 (dd, 1 H, $J_{3,4}$ ~5 Hz, H-3*e*), 3.82 (s, 3 H, CO_2CH_3), 3.99, 4.04, 4.06, 4.12 (4 s, 8 H, $COCH_2Cl$), 4.28 (dd, 1 H, J_{gem} 12, $J_{7,8}$ 7 Hz, H-8a), 4.57 (~d, 1 H, $J_{6,7}$ ~9, $J_{5,6}$ ~1 Hz, H-6), 4.58 (dd, 1 H, $J_{7,8}$ 2.2 Hz, H-8b), 4.83 (m, 1 H, H-2 of THP), 5.38 (m, 1 H, H-7), 5.42 (m, 1 H, H-5), and 5.45 (m, 1 H, H-4).

Anal. Calc. for $C_{22}H_{28}Cl_4O_{13}$ (642.28): C, 41.14; H, 4.39. Found: C, 41.28; H, 4.46.

The slower-moving isomer (**4b**; 43%) had $[\alpha]_D$ +56° (*c* 0.5, chloroform); ^{1}H-n.m.r. data: δ 2.08 (~t, 1 H, J_{gem} 12.8, $J_{3,4}$ ~12 Hz, H-3*a*), 2.51 (~dd, 1 H, $J_{3,4}$ ~5 Hz, $J_{3,5}$ ~1 Hz, H-3*e*), 3.77 (s, 3 H, CO_2CH_3), 4.00, 4.01, 4.07, 4.09 (4 s, 8 H, $COCH_2Cl$), 4.34 (dd, 1 H, H-8a), and 4.84 (dd, 1 H, H-8b).

Anal. Found: C, 41.32; H, 4.27.

*Methyl 4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-*D*-manno-2-octulosonate* (**5**) *and methyl 2-*O*-acetyl-4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-*D*-manno-2-octulopyranosonate* (**6**). — A solution of **4** (1.90 g) in acetic acid (50 mL) was heated to 45°. Water (5 mL) was added dropwise, and the mixture was kept for 5–6 h at 45°, the reaction being monitored by t.l.c. (50:1 dichloromethane–methanol). Solvents were removed by the combination of evaporation and lyophilization, and the residue was chromatographed on a column of silica gel (Wakogel C-300) with 2:1 hexane–ethyl acetate, to afford **5** (1.4 g; 85%) as a single product; $[\alpha]_D$ +41° (*c* 0.9, chloroform); ν_{max} 3600–3400 (OH); ^{1}H-n.m.r. data: δ 2.20 (~dd, 1 H, J_{gem} 12.5, $J_{3,4}$ 4.8, $J_{3,5}$ ~1 Hz, H-3*e*), 2.47 (~t, 1 H, $J_{3,4}$ ~12 Hz, H-3*a*), 3.88 (s, 3 H, CO_2CH_3), 4.02, 4.06, 4.11, 4.16 (4 s, 8 H, $COCH_2Cl$), 4.27 (dd, 1 H, J_{gem} 12, $J_{7,8}$ 5 Hz, H-8a), 4.45 (dd, 1 H, $J_{6,7}$ 9.5, $J_{5,6}$ 1.1 Hz, H-6), 4.58 (dd, 1 H, $J_{7,8}$ 2.2 Hz, H-8b), 4.87 (broad s, 1 H, OH), 5.27 (m, 1 H, H-7), 5.42 (m, 1 H, H-5), and 5.48 (m, 1 H, $J_{4,5}$ 3.3 Hz, H-4). These n.m.r. data indicate that the α anomer preponderates in the equilibrium mixture in chloroform-*d*, as described for **4**.

Anal. Calc. for $C_{17}H_{20}Cl_4O_{12}$ (558.16): C, 36.58; H, 3.61. Found: C, 36.35; H, 3.49.

Compound **5** was treated with acetyl chloride in dichloromethane containing a small proportion of 2,6-lutidine, to afford **6**, m.p. 44–47°, $[\alpha]_D$ +51° (*c* 0.4, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$): δ 2.17 (s, 3 H, CH_3CO), 2.0–2.4 (m, 2 H, H-3), 3.82 (s, 3 H, CO_2CH_3), 3.98, 4.00, 4.04, 4.11 (4 s, 8 H, $COCH_2Cl$), 4.25 (~d, 1 H, *J* ~10 Hz, H-6), 4.30, 4.61 (2 dd, 2 H, J_{gem} 12.5, $J_{7,8}$ 4 and 2.6 Hz, H-8), and 5.2–5.5 (m, 3 H, H-4,5,7). These data indicate that the α-acetate was preferentially formed.

Anal. Calc. for $C_{19}H_{22}Cl_4O_{13}$ (600.20): C, 38.02; H, 3.69. Found: C, 38.29; H, 3.58.

*Methyl [4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-*D*-manno-2-octulopyranosyl fluoride]onate* (**7**). — A solution of compound **5** (323 mg) in toluene (2 mL) was cooled to −60°, diethylaminosulfur trifluoride (DAST; 0.5 mL) in toluene (2 mL) was added, and the mixture was stirred for 10 min at −60°. Ice water was added, and the mixture was treated with aqueous sodium hydrogencarbonate solution, and extracted with dichloromethane. The extracts were combined, washed with water, dried, and evaporated. The syrupy residue was chromatographed on a column of silica gel (Wakogel C-300) with 2:1 hexane–ethyl acetate, to afford syrupy **7** (0.147 g; 63%) as a single spot in t.l.c. (7:1 dichloromethane–ether); $[\alpha]_D$ +57° (*c* 0.5, chloroform); ν_{max} complete loss of the peak at 3600–3400 cm^{-1} (OH); ^{1}H-n.m.r. data ($CDCl_3$): δ (α fluoride) 2.39 (~dd, 1 H, J_{gem} 12.8, $J_{3,4}$ or $J_{3,F}$ 4.8 Hz, H-3*e*), 2.44 (m, 1 H, $J_{3,F}$ 28.6, $J_{3,4}$ ~12 Hz, H-3*a*), 4.31 (dd, 1 H, J_{gem} 12.5, $J_{7,8}$ ~5 Hz, H-8a), and 4.67 (dd, 1 H, $J_{7,8}$ 2.2 Hz, H-8b); (β fluoride) 2.30 (m, 1 H, $J_{3,F}$ 15.8, J_{gem} = $J_{3,4}$ = 12–13 Hz, H-3*a*), 2.56 (~dd, 1 H, $J_{3,4}$ or $J_{3,F}$ ~5 Hz, H-3*e*), 4.44 (dd, 1 H, J_{gem} 12.5, $J_{7,8}$ 4.4 Hz, H-8a), and 4.61 (dd, 1 H, $J_{7,8}$ 2.2 Hz, H-8b); the other signals observed, both for the α and the β fluoride were the following: δ 3.89, 3.90 (2 s, 3 H,

CO_2CH_3; intensity ratio 15:14, 4.0–4.2 (m, 8 H, $COCH_2Cl$), 4.40, 4.45 (2 dd, 1 H, $J_{6,7}$ 9.5, $J_{5,6}$ 1.1–1.5 Hz, H-6), and 5.25–5.6 (m, 3 H, H-4,5,7) [these n.m.r. data indicated that **7** was a mixture of the α fluoride (~52%) and the β fluoride (~48%)]; ^{13}C-n.m.r. data (125 MHz, $CDCl_3$): δ 30.0 (d, $J_{3,F}$ 26.4 Hz, C-3α), 31.3 (d, $J_{3,F}$ 25.4 Hz, C-3β), 106.6 (d, $J_{2,F}$ 222.0 Hz, C-2β), and 107.7 (d, $J_{2,F}$ 232.9 Hz, C-2α).

Anal. Calc. for $C_{17}H_{19}O_{11}Cl_4F$ (560.15): C, 36.45; H, 3.42. Found: C, 36.67; H, 3.51.

*Benzyl 3-deoxy-2-*O*-(tetrahydropyran-2-yl)-*D-manno*-2-octulopyranosonate* (**9**). — To a solution of **3** (1.14 g) in dry 1,4-dioxane (20 mL) was added 0.2M KOH (26.9 mL), and the mixture was stirred for 1 h at room temperature, the reaction being monitored by t.l.c. (2:1 dichloromethane–methanol). The mixture was treated with Amberlite IR-120 (H^+) ion-exchange resin, to remove the base. The resin was filtered off and washed with 10:1 (v/v) methanol–water, and the filtrate and washings were combined and evaporated to dryness. The residue (1.17 g) was dissolved in *N,N*-dimethylformamide (DMF; 10 mL), and treated with K_2CO_3 (0.373 g) and benzyl bromide (1.4 g) for 12 h at room temperature. Ice–water was added, and the mixture was successively extracted with ethyl acetate and dichloromethane. The extracts were combined, dried (anhydrous sodium sulfate), and evaporated to a syrup which was chromatographed on a column of silica gel with 30:1 dichloromethane–methanol, to give **9** (0.9 g; 81% from **3**).

Because the starting material **3** was a mixture of two diastereomers (or anomers), the product **9** appeared as two spots having closely similar mobilities (t.l.c., 3:1 dichloromethane–methanol). The faster-moving isomer (**9a**) had m.p. <30°, $[\alpha]_D$ +40° (*c* 0.64, dichloromethane); ν_{max} 3600–3100 (OH), 1760 (ester), and 760 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 2.38 (dd, 1 H, J_{gem} 13, $J_{3,4}$ 5 Hz, H-3*e*), 5.03, 5.21 (2 d, 2 H, J_{gem} 12.5 Hz, CH_2Ph), and 7.35 (~s, 5 H, C_6H_5).

Anal. Calc. for $C_{20}H_{28}O_9$ (412.42): C, 58.24; H, 6.84. Found: C, 58.47; H, 6.73.

The slower-moving isomer (**9b**) had m.p. 39–40°, $[\alpha]_D$ +44° (*c* 2.2, dichloromethane); i.r. data similar to those of **9a**; ^{1}H-n.m.r. data ($CDCl_3$): δ 2.31 (m, 1 H, J_{gem} 13, $J_{3,4}$ ~5, $J_{3,5}$ ~1 Hz, H-3*e*), 5.0, 5.17 (2 d, 2 H, J_{gem} 12.5 Hz, CH_2Ph), and 7.33 (s, 5 H, C_6H_5).

Anal. Found: C, 58.45; H, 6.92.

*Benzyl 4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-*D-manno*-2-octulosonate* (**11**) *and benzyl 2-*O*-acetyl-4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-*D-manno*-2-octulopyranosonate* (**12**). — A mixture of **9a** or **9b** (0.3 g), dry dichloromethane (5 mL), 2,6-lutidine (1.9 mL), and triethylamine (0.59 mL) was cooled to 0°, chloroacetic anhydride (1 g) was gradually added, and the mixture was stirred for 18 h at 20°. A small amount of ice was added, and the mixture was evaporated *in vacuo*. The residue was taken up in dichloromethane, and the solution successively washed with ice-cold M hydrochloric acid, water, aq. sodium hydrogencarbonate, and water, dried, and evaporated to a syrup which was chromatographed on a column of silica gel with dichloromethane, to give **10** (0.52 g).

To a solution of **10** (0.5 g) in acetonitrile (20 mL) was added tetrafluoroboric acid (42% in water, 0.1 mL), and the mixture was vigorously stirred for 15 min at room temperature. Triethylamine (0.1 mL) was added, and the solution was evaporated. The residue was chromatographed on a column of silica gel with dichloromethane, to give **11** in 90% yield. Compound **11** had $[\alpha]_D$ +30° (*c* 0.72, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.68 (broad s, 1 H, OH-2), 2.01 (dd, 1 H, J_{gem} 12–13, $J_{3,4}$ ~5 Hz, H-3*e*), 2.52 (~t, 1 H, J_{gem} = $J_{3,4}$ = 12–13 Hz, H-3*a*), 3.99, 4.03, 4.07, 4.11 (4 s, 8 H, $COCH_2Cl$), 4.27 (dd, 1 H, J_{gem} 12–12.5, $J_{7,8}$ 5 Hz, H-8a), 4.44 (dd, 1 H, $J_{6,7}$ ~10, $J_{5,6}$ 1–1.5 Hz, H-6), 4.57 (dd, 1 H, $J_{7,8}$ 2.4 Hz, H-8b), 5.24, 5.34 (2 d, 2 H, J_{gem} 12.5 Hz, CH_2Ph), and 7.39 (~s, 5 H, C_6H_5). These n.m.r. data suggested that the α anomer preponderates in the equilibrium mixture in chloroform-*d*, similarly to **5**.

Anal. Calc. for $C_{23}H_{24}Cl_4O_{12}$ (634.25): C, 43.55; H, 3.81. Found: C, 43.28; H, 3.91.

To a cooled, stirred solution of **11** (0.24 g) in 2:1 dichloromethane–2,6-lutidine (4.5 mL) was added a solution of acetyl chloride (0.1 mL) in dichloromethane (3 mL). The mixture was stirred overnight at 20°, and poured into ice-cold, aq. sodium hydrogencarbonate. The product was extracted with dichloromethane, and the extract was successively washed with ice-cold M hydrochloric acid and water, dried, and evaporated. The residue was chromatographed on a column of silica gel with dichloromethane, to give amorphous **12** (0.22 g; 86%); $[\alpha]_D$ +47° (*c* 1.5, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$): δ 2.14 (s, 3 H, CH_3CO), 2.0–2.4 (m, 2 H, H-3), 3.96, 3.99, 4.03, 4.09 (4 s, 8 H, $COCH_2Cl$), 4.25 (~d, 1 H, *J* ~10 Hz, H-6), 4.29, 4.60 (2 dd, 2 H, J_{gem} 12, $J_{7,8}$ 4, 2.6 Hz, H-8), and 7.2–7.5 (m, 5 H, C_5H_5).

Anal. Calc. for $C_{25}H_{26}Cl_4O_{13}$ (676.30): C, 44.40; H, 3.88. Found: C, 44.31; H, 3.90.

*Benzyl [4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-*D*-manno-2-octulopyranosyl fluoride]onate* (**13**). — Compound **11** was treated with DAST as described for the preparation of **7**, to give **13** (60%) as an ~1:1 mixture of the α and β fluoride, which showed two spots of closely similar mobilities in t.l.c. (5:1 dichloromethane–ether); $[\alpha]_D$ +50° (*c* 0.5, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$): δ (α-fluoride) 2.39 (~dd, 1 H, J_{gem} 12.5, $J_{3,4}$ or $J_{3,F}$ 5 Hz, H-3*e*), 2.43 (m, 1 H, $J_{3,F}$ 28.6, $J_{3,4}$ 11.4 Hz, H-3*a*), 4.00, 4.03, 4.08, 4.11 (4 s, 8 H, $COCH_2Cl$), 4.28, 4.67 (2 dd, 2 H, J_{gem} 12.5, $J_{7,8}$ 5.1, 2.6 Hz, H-8), and 7.39 (~s, 5 H, C_6H_5); (β-fluoride) 2.28 (m, 1 H, $J_{3,F}$ 15.8, J_{gem} = $J_{3,4}$ = 12.5 Hz, H-3*a*), 2.57 (dd, $J_{3,4}$ or $J_{3,F}$ 5 Hz, H-3*e*), 4.0–4.2 (4 s, 8 H, $COCH_2Cl$), 4.31, 4.57 (2 dd, 2 H, J_{gem} 12.5, $J_{7,8}$ 4.8, 2.2 Hz, H-8), and 7.40 (~s, 5 H, C_6H_5).

Anal. Calc. for $C_{23}H_{23}Cl_4FO_{11}$ (636.25): C, 43.42; H, 3.64. Found: C, 43.17; H, 3.76.

Conversion of **12** *into the glycosyl bromide.* — To a solution of **12** (0.1 g) in dry dichloromethane (2 mL) was added, at 0°, a solution of titanium tetrabromide (0.35 g) in 10:1 dry dichloromethane–dry ethyl acetate (2.5 mL), and the mixture was stirred at room temperature. After completion of the reaction (t.l.c., 15:1 di-

chloromethane–ether), dry dichloromethane (15 mL) was added, and the mixture was treated with anhydrous sodium acetate under vigorous stirring. The suspension was filtered through Celite, and the filtrate was evaporated at 20°. The residue of crude *benzyl [4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-*D*-manno-2-octulopyranosyl bromide]onate* (**14**) was lyophilized from 1,4-dioxane solution and used, without further purification, for coupling with the sugar acceptors **15**, **16**, and **17**, respectively.

*Benzyl 2-deoxy-4-*O*-(diphenoxyphosphinyl)-6-*O*-{methyl [4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-α- and -β-*D*-manno-2-octulopyranosyl]onate}-3-*O*-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**18α** and **18β**). — *(a) With* **7**. To a mixture of **7** (98 mg), **15** (0.1 g), molecular sieves 4A (0.2 g), and dichloromethane (2 mL) was added boron trifluoride etherate (3 drops) at 0°, and the mixture was stirred for 24 h at room temperature. After completion of the reaction (t.l.c., 7:1–10:1 dichloromethane–ether), the mixture was filtered, and the solid washed with dichloromethane. The filtrate and washings were combined, washed with aq. sodium hydrogencarbonate, dried, and evaporated. The residue was chromatographed on a column of silica gel with dichloromethane and then 500:1 dichloromethane–methanol, to give **18α** (40 mg; 27%) and **18β** (60 mg; 41%).

Compound **18α** had $[\alpha]_D$ +9.4° (*c* 0.36, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 9 H, CH_3), 1.0–1.8 (m, 64 H, $-CH_2-$), 1.9–2.5 (m, 8 H, $-COCH_2-$ and H-3′), 3.66 (s, 3 H, CO_2CH_3), 3.76(s), 3.98(s), 4.08(s), 4.11(2 d) (8 H, $COCH_2Cl$), 4.36 (~d, 1 H, H-6′), 4.63, 4.92 (2 d, 2 H, J_{gem} 12.5 Hz, CH_2Ph), 4.64 (~q, 1 H, H-4), 4.71 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.73 (dd, 1 H, H-8′), 4.98 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.25 (m, 1 H, H-7′), 5.5 (m, 1 H, H-4′), 5.90 (d, 1 H, N*H*), and 7.05–7.40 (m, 15 H, C_6H_5).

Anal. Calc. for $C_{84}H_{124}Cl_4NO_{23}P$ (1688.70): C, 59.75; H, 7.40; N, 0.83. Found: C, 60.02; H, 7.28; N, 0.97.

Compound **18β** had m.p. 47–50°, $[\alpha]_D$ +4.0° (*c* 0.55, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 9 H, CH_3), 1.0–1.75 (m, 64 H, $-CH_2-$), 3.72 (s, 3 H, CO_2CH_3), 4.38 (dd, 1 H, J_{gem} 12, $J_{7',8'}$ 4.8 Hz, H-8a′), 4.47 (dd, 1 H, $J_{7',8'}$ 2.5 Hz, H-8b′), 4.63, 4.88 (2 d, 2 H, CH_2Ph), 4.62 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.21 (m, 1 H, H-7′), 5.87 (d, 1 H, N*H*), and 7.0–7.45 (m, 15 H, C_6H_5).

Anal. Found: C, 59.87; H, 7.54; N, 0.80.

(b) With **8**. Compound **8** (0.15 g), which was prepared by treatment of **6** with titanium tetrabromide as described for the preparation of **14**, was coupled to **15** (0.2 g) in dichloromethane (2–3 mL) in the presence of $Hg(CN)_2$ (0.11 g), $HgBr_2$ (37 mg), and molecular sieves 4A (0.2 g). The reaction was continued, under stirring, for 24 h at 20°, and the mixture was filtered. The filtrate was successively washed with aq. KI and water, dried, and evaporated. The residue was chromatographed on a column of silica gel with 2:1 hexane–ethyl acetate, to give **18α** (67%). The physical properties and spectral data were identical with those of **18α** obtained by method (*a*).

*Benzyl 2-deoxy-4-*O*-(diphenoxyphosphinyl)-6-*O*-[methyl (3-deoxy-α- and -β-*D*-manno-2-octulopyranosyl)onate]-3-*O*-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxy-*

*tetradecanamido]-β-*D*-glucopyranoside* (**19α** *and* **19β**). — To a stirred solution of **18α** (30 mg) in 2:1 2,6-lutidine-acetic acid (1.8 mL) was added freshly prepared hydrazine dithiocarbonate (HDTC, 0.48 mL) at 0°. The mixture was stirred for 2 h at 0°, and evaporated at 35°. The residue was taken up in chloroform, and successively washed with ice-cold M hydrochloric acid, water, aq. sodium hydrogencarbonate, and water, dried, and evaporated. The syrupy residue was chromatographed on a column of silica gel with (*a*) dichloromethane, (*b*) 100:1 and (*c*) 50:1 dichloromethane–methanol.

Eluant (*c*) gave **19α** (82%); m.p. 54–56°, $[\alpha]_D$ −1.7° (*c* 0.24, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 9 H, CH_3), 3.63 (s, 3 H, CO_2CH_3), 4.56, 4.82 (2 d, 2 H, J_{gem} 12.5 Hz, CH_2Ph), 4.63 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.99 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.31 (~t, 1 H, *J* 9.9, 9.5 Hz, H-3), 6.02 (d, 1 H, N*H*), 7.0–7.4 (m, 15 H, 3 C_6H_5), and complete loss of the peaks of $COCH_2Cl$ at δ 3.7–4.2.

Anal. Calc. for $C_{76}H_{120}NO_{19}P$ (1382.76): C, 66.02: H, 8.75; N, 1.01. Found: C, 66.26; H, 8.67; N, 0.99.

A mixture of **18β** (51 mg) and 2:1 2,6-lutidine–acetic acid (3 mL) was treated with HDTC (0.8 mL) as described for **18α**, to give **19β** (84%); m.p. 62–65°, $[\alpha]_D$ −4.3° (*c* 0.35, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 9 H, CH_3), 3.65 (s, 3 H, CO_2CH_3), 4.68 (q, 1 H, H-4), 5.00 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.29 (~t, *J* ~10 Hz, H-3), 6.12 (d, 1 H, N*H*), 7.05–7.4 (m, 15 H, 3 C_6H_5), and complete loss of the peaks of $COCH_2Cl$.

Anal. Found: C, 66.31; H, 8.63; N, 1.10.

*2-Deoxy-4-*O*-(diphenoxyphosphinyl)-6-*O*-[methyl (3-deoxy-α and -β-*D*-manno-2-octulopyranosyl)onate]-3-*O*-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-*D*-glucose* (**20α** *and* **20β**). — To a solution of **19α** (32 mg) in 3:2 ethanol–methanol (10 mL) was added 10% palladium-on-carbon (Pd–C) catalyst (40 mg) that had been pre-activated, and washed well with ethanol, and the mixture was stirred at room temperature in a hydrogen atmosphere. The catalyst was filtered off, and washed with ethanol–methanol–dichloromethane. The filtrate and washings were combined, and evaporated, to give **20α** (87%); m.p. 75–77°, $[\alpha]_D$ +30° (*c* 0.5, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 0.88 (~t, 9 H, CH_3), 1.0–1.7 (m, 64 H, $-CH_2-$), 1.85–2.55 (m, 8 H, $-COCH_2-$ and H-3′), 3.63 (β anomer), 3.64 (α anomer) (2 s, 3 H, CO_2CH_3; α:β = ~2:1), 5.08 (d, ~2/3 H, $J_{1,2}$ 3.7 Hz, H-1α), 5.15 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.34 (β anomer), 5.50 (α anomer) (~t, 1 H, *J* 10.6, 9.2 Hz, H-3), and 7.05–7.45 (m, 10 H, 2 C_6H_5).

Anal. Calc. for $C_{69}H_{114}NO_{19}P$ (1292.63): C, 64.11; H, 8.89; N, 1.08. Found: C, 64.30; H, 9.01; N, 1.15.

Treatment of **19β** with 10% Pd–C catalyst gave **20β** (93%); m.p. 58–60°, $[\alpha]_D$ +22° (*c* 0.31, chloroform); ^{1}H-n.m.r. data for the α anomer ($CDCl_3$): δ 3.54 (s, 3 H, CO_2CH_3), 4.67 (q, 1 H, *J* ~10 Hz, H-4), 5.12 (m,1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.21 (~s, 1 H, H-1), 5.51 (~t, 1 H, *J* ~10 Hz, H-3), 6.59 (d, 1 H, N*H*), and 7.0–7.4 (m, 10 H, C_6H_5).

Anal. Found: C, 64.32; H, 8.76; N, 1.06.

*2-Deoxy-6-O-[methyl (3-deoxy-α- and -β-*D*-manno-2-octulopyranosyl)onate]-4-O-phosphono-3-O-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamidol]-*D*-glucose* (**21α** *and* **21β**). — To a solution of **20α** or **20β** (35 mg) in ethanol (15 mL) was added pre-reduced Adams' platinum catalyst (30 mg), and the mixture was stirred at room temperature. The catalyst was filtered off with the aid of Celite, and washed with methanol–ethanol–dichloromethane. The filtrate and washings were combined, and evaporated. The product was purified by preparative t.l.c. (2:1 dichloromethane–methanol), to give **21α** (90%) or **21β** (95%), respectively.

Compound **21α** had m.p. 143–145°, $[\alpha]_D$ +29° (*c* 0.42, 3:1 ethanol–dichloromethane).

Anal. Calc. for $C_{57}H_{106}NO_{19}P$ (1140.44): C, 60.03; H, 9.37; N, 1.23. Found: C, 59.81; H, 9.40; N, 1.18.

Compound **21β** had m.p. 138–139°, $[\alpha]_D$ +26° (*c* 0.3, 3:1 ethanol–dichloromethane).

Anal. Found: C, 60.32; H, 9.14; N, 1.02.

*Benzyl 6-O-{benzyl [4,5,7,8-tetrakis-O-(chloroacetyl)-3-deoxy-α-*D*-manno-2-octulopyranosyl]onate}-2-deoxy-4-O-(diphenoxyphosphinyl)-3-O-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**22**). — A mixture of **15** (0.13 g), dry dichloromethane (1 mL), molecular sieves 4A (0.1 g), $Hg(CN)_2$ (55 mg), and $HgBr_2$ (19 mg) was stirred for 3 h at room temperature. A solution of **14** (0.1 mg) in dry dichloromethane was added, and the mixture was stirred for 4 days at 20°. Processing as described for **18α** (method *b*) and column chromatography on silica gel with 2:1 hexane–ethyl acetate gave **22** (0.124 g, 65%); m.p. 32–33°, $[\alpha]_D$ +10° (*c* 1.3, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 9 H, 3 C*H*$_3$), 0.95–1.7 (m, 64 H, -C*H*$_2$-), 1.9–2.5 (m, 8, H, -COC*H*$_2$- and H-3'), 3.72 (s), 3.94 (s), 4.04 (s), 4.09 (2 d) (8 H, COC*H*$_2$Cl), 4.32 (~d, 1 H, $J_{6',7'}$ 9.5 Hz, H-6'), 4.61, 4.88 (2 d, 2 H, J_{gem} ~12 Hz, C*H*$_2$Ph), 4.63 (~q, 1 H, H-4), 4.64 (d, 1 H, H-1), 4.68 (dd, 1 H, H-8b'), 4.90 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.49 (m, 1 H, H-4'), 5.90 (d, 1 H, N*H*), and 7.05–7.50 (m, 20 H, 4 C_6H_5).

Anal. Calc. for $C_{90}H_{128}Cl_4NO_{23}P$ (1764.74): C, 61.25; H, 7.31; N, 0.79. Found: C, 61.49; H, 7.26; N, 0.83.

The β-disaccharide could not be isolated in pure form, because of its difficult separation from the α-disaccharide.

*Benzyl 6-O-[benzyl (3-deoxy-α-*D*-manno-2-octulopyranosyl)onate]-2-deoxy-4-O-(diphenoxyphosphinyl)-3-O-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**23**). — To a stirred solution of **22** (0.13 g) in 2:1 2,6-lutidine–acetic acid (4.5 mL) was added HDTC (2 mL) at 0°. The mixture was stirred for 2 h at 0° as described for the preparation of **19α**. The product was purified by chromatography on a column of silica gel with 50:1 dichloromethane–methanol, to afford **23** (92 mg; 86%); m.p. 56–58°, $[\alpha]_D$ +2° (*c* 1.6, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 0.88 (~t, 9 H, 3 C*H*$_3$), 1.0–1.65 (m, 64 H, -C*H*$_2$-), 1.85–2.55 (m, 8 H, -COC*H*$_2$- and H-3'), 4.59, 4.86 (2 d, 2 H, J_{gem} 12

Hz, CH_2Ph at O-1), 5.00, 5.13 (2 d, 2 H, J_{gem} 12 Hz, CO_2CH_2Ph), 5.35 (~t, 1 H, *J* 9–10 Hz, H-3), 7.05–7.45 (m, 20 H, 4 C_6H_5), and complete loss of the peaks of $COCH_2Cl$ at δ 3.7–4.1.

Anal. Calc. for $C_{82}H_{124}NO_{19}P$ (1458.80): C, 67.51; H, 8.57; N, 0.96. Found: C, 67.34; H, 8.42; N, 1.07.

*2-Deoxy-6-O-[(3-deoxy-α-*D*-manno-2-octulopyranosyl)onic acid]-4-O-(diphenoxyphosphinyl)-3-O-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-*D*-glucose* (**24**). — Compound **23** (78 mg) in 3:2 ethanol–methanol (20 mL) was hydrogenolyzed in the presence of 10% Pd–C catalyst (80 mg) at 40°. After completion of the reduction (t.l.c., 10:1 and 2:1 dichloromethane–methanol), the catalyst was filtered off, and washed with ethanol–methanol–dichloromethane. The filtrate and washings were combined, and evaporated, to give **24** (67 mg; 98%); m.p. 114–115°, $[\alpha]_D$ +13° (*c* 1.3, 3:1 ethanol–dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 0.89 (~t, 9 H, 3 CH_3), 1.0–1.7 (m, 64 H, $-CH_2-$), 1.85–2.55 (m, 8 H, $-COCH_2-$ and H-3′), 5.08 (d, ~1/2 H, $J_{1,2}$ 3.7 Hz, H-1α), 5.14 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.38 (β anomer), 5.51 (α anomer) (2 ~t, 1 H, *J* 9–10 Hz, H-3), 7.05–7.45 (m, 10 H, 2 C_6H_5), and complete loss of the peaks due to benzyl groups.

Anal. Calc. for $C_{68}H_{112}NO_{19}P$ (1278.58): C, 63.87; H, 8.83; N, 1.10. Found: C, 64.18; H, 8.62; N, 0.90.

*2-Deoxy-6-O-[(3-deoxy-α-*D*-manno-2-octulopyranosyl)onic acid]-4-O-phosphono-3-O-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-*D*-glucose* (**25**). — Hydrogenolytic removal of the phenyl groups from **24** (65 mg) in ethanol was performed as described for the preparation of **21α**, to give a colorless, fine powder of **25** (52 mg; 91%); m.p. 168–169°, $[\alpha]_D$ +22° (*c* 1, 1:1 dichloromethane–methanol); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD) showed complete loss of the phenyl protons at δ 7.05–7.45.

Anal. Calc. for $C_{56}H_{104}NO_{19}P$ (1126.38): C, 59.71; H, 9.31; N, 1.24. Found: C, 60.05; H, 9.47; N, 1.10.

*Benzyl 6-O-{benzyl [4,5,7,8-tetrakis-O-6chloroacetyl)-3-deoxy-α-*D*-manno-2-octulopyranosyl]onate}-2-deoxy-4-O-(diphenoxyphosphinyl)-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-3-O-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside* (**26**). — Coupling of **16** with **14** was achieved as described for the preparation of **22**, to give **26** (59%); m.p. <30°, $[\alpha]_D$ +14° (*c* 1.4, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 12 H, CH_3), 1.0–1.7 (m, 84 H, $-CH_2-$), 2.05-2.5 (m, 10 H, $-COCH_2-$ and H-3′), 3.7–4.2 (m, 8 H, $COCH_2Cl$), 4.21 (dd, 1 H, H-8a′), 4.69 (dd, 1 H, H-8b′), 4.63, 4.89 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph at O-1), 5.48 (~t, 1 H, *J* 9–10 Hz, H-3), 6.24 (d, 1 H, N*H*), and 7.05–7.5 (m, 20 H, 4 C_6H_5).

Anal. Calc. for $C_{104}H_{154}Cl_4NO_{25}P$ (1991.09): C, 62.73; H, 7.80; N, 0.70. Found: C, 62.56; H, 7.93; N, 0.81.

*Benzyl 6-O-[benzyl (3-deoxy-α-*D*-manno-2-octulopyranosyl)onate]-2-deoxy-4-O-(diphenoxyphosphinyl)-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-3-O-[(3*R*)-3-tetradecanoyl]-β-*D*-glucopyranoside* (**27**). — The chloroacetyl groups of **26** were

cleaved with HDTC as described for the preparation of **23**, to afford **27** in ~90% yield. Compound **27** had m.p. 51–53°, $[\alpha]_D$ +8° (*c* 0.8, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 0.89 (~t, 12 H, 4 CH_3), 1.0–1.7 (m, 84 H, $-CH_3$), 1.85–2.55 (m, 10 H, $-COCH_2-$ and H-3'), 4.58, 4.84 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph at O-1), 4.68 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.0, 5.13 (2 d, 2 H, J_{gem} 12 Hz, CO_2CH_2Ph), 5.32 (~t, 1 H, *J* 9–10 Hz, H-3), 7.05–7.45 (m, 20 H, 4 C_6H_5), and complete loss of the peaks due to the chloroacetyl groups.

Anal. Calc. for $C_{96}H_{150}NO_{21}P$ (1685.15): C, 68.42; H, 8.97; N, 0.83. Found: C, 68.25; H, 9.18; N, 0.76.

*2-Deoxy-6-*O*-[(3-deoxy-*α*-*D*-*manno*-2-octulopyranosyl)onic acid]-4-*O*-(diphenoxyphosphinyl)-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-*D*-glucose* (**28**) *and 2-deoxy-6-*O*-[(3-deoxy-*α*-*D*-manno-2-octulopyranosyl)onic acid]-4-*O*-phosphono-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-*D*-glucose* (**29**). — Compound **28** was obtained in nearly quantitative yield by hydrogenolytic removal of the benzyl groups from **27**, and had m.p. 105–107°, $[\alpha]_D$ +21° (*c* 0.5, 3:1 ethanol–dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 0.89 (~t, 12 H, 4 CH_3), 1.0–1.8 (m, 84 H, $-CH_2-$), 1.8–2.55 (m, 10 H, $-COCH_2-$ and H-3'), and 7.0–7.5 (m, 10 H, 2 C_6H_5).

Anal. Calc. for $C_{82}H_{138}NO_{21}P$ (1504.91): C, 65.44; H, 9.24; N, 0.93. Found: C, 65.14; H, 9.41; N, 0.82.

Finally, the phenyl groups of **28** were cleaved by hydrogenolysis as described for the preparation of **25**, to give **29** in almost quantitative yield. Compound **29** had m.p. 172–174°, $[\alpha]_D$ +11° (*c* 0.5, 1:1 dichloromethane–methanol); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD) showed complete loss of the phenyl protons at δ 7.0–7.5.

Anal. Calc. for $C_{70}H_{130}NO_{21}P$ (1352.73): C, 62.15; H, 9.69; N, 1.04. Found: C, 62.47; H, 9.43; N, 1.00.

*Benzyl 6-*O*-{benzyl [4,5,7,8-tetrakis-*O*-6chloroacetyl)-3-deoxy-*α*-*D*-manno-2-octulopyranosyl]onate}-2-[(3*R*)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4-*O*-(diphenoxyphosphinyl)-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside* (**30**). — Coupling of **17** with **14** gave the title compound **30** (61%); $[\alpha]_D$ +12° (*c* 1.8, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (~t, 9 H, 3 CH_3), 1.0–1.7 (m, 62 H, $-CH_2-$), 2.0–2.5 (m, 8 H, $-COCH_2-$ and H-3'), 3.74, 3.98, 4.06 (3 s, 6 H, 3 $COCH_2Cl$), 4.06, 4.14 (2 d, 2 H, J_{gem} 15 Hz, $COCH_2Cl$), 4.20 (dd, 1 H, J_{gem} 12.5, $J_{7',8'}$ ~5 Hz, H-8a'), 4.36 (~d, 1 H, $J_{6',7'}$ 9.5 Hz, H-6'), 4.70 (dd, 1 H, H-8b'), 5.50 (m, 1 H, *J* 10.5, 3 Hz, H-4'), 6.39 (d, 1 H, *J* 8.4 Hz, N*H*), and 7.05–7.5 (m, 25 H, 5 C_6H_5).

Anal. Calc for $C_{98}H_{136}Cl_4NO_{25}P$ (1900.89): C, 61.92; H, 7.21; N, 0.74. Found: C, 62.17; H, 7.06; N, 0.77.

*Benzyl 6-*O*-[benzyl (3-deoxy-*α*-*D*-manno-2-octulopyranosyl)onate]-2-[(3*R*)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4-*O*-(diphenoxyphosphinyl)-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside* (**31**). — Selective removal of the chloroacetyl groups from **30**, as described for the preparation of **23**

and **27**, afforded compound **31** (92%); $[\alpha]_D$ +8° (*c* 1.0, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 0.89 (~t, 9 H, 3 CH_3), 1.0–1.75 (m, 62 H, $-CH_2-$), 1.85–2.55 (m, 8 H, $-COCH_2-$ and H-3'), 7.0–7.4 (m, 25 H, 5 C_6H_5), and complete loss of the peaks due to the chloroacetyl groups.

Anal. Calc. for $C_{90}H_{132}NO_{21}P$ (1594.95): C, 67.77; H, 8.34; N, 0.88. Found: C, 68.05; H, 8.21; N, 0.94.

*2-Deoxy-6-O-[(3-deoxy-α-*D*-manno-2-octulopyranosyl)onic acid]-4-O-(diphenoxyphosphinyl)-2-[(3R)-3-hydroxytetradecanamido]-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-β-*D*-glucopyranoside* (**32**) *and 2-deoxy-6-O-[(3-deoxy-α-*D*-manno-2-octulopyranosyl)onic acid]-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-3-O-[(3R)-3-tetradecanoyloxytetradecanoyl]-*D*-glucose* (**33**). — Hydrogenolytic removal of both the benzyl and benzyloxymethyl group from **31**, as described for the preparation of **24** and **28**, gave compound **32** (95%); m.p. 138–140°, $[\alpha]_D$ +23° (*c* 0.6, 3:1 ethanol–dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD): δ 0.89 (~t, 9 H, 3 CH_3), 1.0–1.7 (m, 62 H, $-CH_2-$), 1.8–2.5 (m, 8 H, $-COCH_2-$ and H-3'), 5.39 (~t, 1 H, *J* 9–10 Hz, H-3), and 7.0–7.4 (m, 10 H, 2 C_6H_5).

Anal. Calc. for $C_{68}H_{112}NO_{20}P$ (1294.57): C, 63.09; H, 8.72; N, 1.08. Found: C, 63.38; H, 8.51; N, 0.96.

Compound **33** was obtained by hydrogenolysis of **32** with platinum catalyst as described previously; m.p. 162–164°, $[\alpha]_D$ +26° (*c* 0.5, 1:1 dichloromethane–ethanol); ^{1}H-n.m.r. data ($CDCl_3$ + CD_3OD) showed complete loss of the phenyl protons at δ 7.0–7.4.

Anal. Calc. for $C_{56}H_{104}NO_{20}P$ (1142.38): C, 58.87; H, 9.18; N, 1.23. Found: C, 58.61; H, 9.35; N, 1.04.

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REFERENCES

1 C. Galanos, O. Lüderitz, E. T. Rietschel, and O. Westphal, in T. W. Goodwin (Ed.), *Biochemistry of Lipids, II, Int. Rev. Biochem.*, 14 (1977) 239–335.
2 F. M. Unger, *Adv. Carbohydr. Chem. Biochem.*, 38 (1981) 323–388.
3 R. Christian, G. Schulz, P. Waldstätten, and F. M. Unger, *Tetrahedron Lett.*, (1984) 3433–3436.
4 U. Zähringer, B. Lindner, U. Seydel, E. T. Rietschel, H. Naoki, F. M. Unger, M. Imoto, S. Kusumoto, and T. Shiba, *Tetrahedron Lett.*, (1985) 6321–6324.
5 S. Lebbar, J.-M. Cavaillon, M. Caroff, A. Ledur, H. Brade, R. Sarfati, and N. H.-Cavaillon, *Eur. J. Immunol.*, 16 (1986) 87–91, and references cited therein.
6 (a) M. Kiso, H. Ishida, and A. Hasegawa, *Agric. Biol. Chem.*, 48 (1984) 251–252; (b) M. Matsuura, Y. Kojima, J. Y. Homma, Y. Kubota, A. Yamamoto, M. Kiso, and A. Hasegawa, *FEBS Lett.*, 167 (1984) 226–230.
7 M. Kiso, S. Tanaka, M. Tanahashi, Y. Fujishima, Y. Ogawa, and A. Hasegawa, *Carbohydr. Res.*, 148 (1986) 221–234.

8 (a) M. MATSUURA, A. YAMAMOTO, Y. KOJIMA, J. Y. HOMMA, M. KISO, AND A. HASEGAWA, *J. Biochem. (Tokyo)*, 98 (1985) 1229–1237; (b) M. MATSUURA, Y. KOJIMA, J. Y. HOMMA, Y. KUMAZAWA, A. YAMAMOTO, M. KISO, AND A. HASEGAWA, *ibid.*, 99 (1986) 1377–1384.
9 (a) Y. KUMAZAWA, M. MATSUURA, J. Y. HOMMA, Y. NAKATSURU, M. KISO, AND A. HASEGAWA, *Eur. J. Immunol.*, 15 (1985) 199–201; (b) Y. KUMAZAWA, M. MATSUURA, Y. MARUYAMA, J. Y. HOMMA, M. KISO, AND A. HASEGAWA, *ibid.*, 16 (1986) 1099–1103.
10 (a) M. KISO, S. TANAKA, M. FUJITA, Y. FUJISHIMA, Y. OGAWA, H. ISHIDA, AND A. HASEGAWA, *Carbohydr. Res.*, 162 (1987) 127–140; (b) M. KISO, S. TANAKA, M. FUJITA, Y. FUJISHIMA, Y. OGAWA, AND A. HASEGAWA, *ibid.*, 162 (1987) 247–256.
11 (a) M. KISO, Y. OGAWA, S. TANAKA, H. ISHIDA, AND A. HASEGAWA, *J. Carbohydr. Chem.*, 5 (1986) 621–630; (b) M. KISO, Y. OGAWA, Y. FUJISHIMA, M. FUJITA, S. TANAKA, AND A. HASEGAWA, *ibid.*, 6 (1987) 625–638.
12 Y. KUMAZAWA, M. NAKATSUKA, H. TAKIMOTO, T. FURUYA, T. NAGUMO, J. Y. HOMMA, A. YAMAMOTO, K. INADA, M. YOSHIDA, M. KISO, AND A. HASEGAWA, *Infect. Immun.*, 56 (1988) 149–155.
13 (a) S. IKEDA, Y. KUMAZAWA, C. NISHIMURA, M. NAKATSUKA, J. Y. HOMMA, M. KISO, AND A. HASEGAWA, unpublished work; (b) S. IKEDA, C. NISHIMURA, M. NAKATSUKA, J. Y. HOMMA, M. KISO, AND A. HASEGAWA, unpublished work.
14 M. KISO, M. TANAHASHI, A. HASAGAWA, AND F. M. UNGER, *Carbohydr. Res.*, 163 (1987) 279–284.
15 H. PAULSEN, Y. HAYAUCHI, AND F. M. UNGER, *Justus Liebigs Ann. Chem.*, (1984) 1270–1287.
16 F. M. UNGER, D. STIX, AND G. SCHULZ, *Carbohydr. Res.*, 80 (1980) 191–195.
17 E. C. HEATH AND M. A. GHALAMBOR, *Methods Enzymol.*, 9 (1966) 60–65.
18 Y. MORIZAWA, I. MORI, T. HIYAMA, AND H. NOZAKI, *Synthesis*, (1981) 899–901.
19 W. S. YORK, A. G. DARVILL, M. MCNEIL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 138 (1985) 109–126.
20 W. ROSENBROOK, JR., D. A. RILEY, AND P. A. LARTEY, *Tetrahedron Lett.*, (1985) 3–4.
21 L. D. HALL, R. N. JOHNSON, A. B. FOSTER, AND J. H. WESTWOOD, *Can. J. Chem.*, 49 (1971) 236–240.
22 K. BOCK AND C. PEDERSEN, *Acta. Chem. Scand., Ser. B*, 29 (1975) 682–686.
23 K. C. NICOLAOU, A. CHUCHOLOWSKI, R. E. DOLLE, AND J. L. RANDALL, *J. Chem. Soc., Chem. Commun.*, (1984) 1155–1156.
24 C. A. A. VAN BOECKEL AND T. BEETZ, *Tetrahedron Lett.*, (1983) 3775–3778.