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SYNTHESIS OF α-Kdo-(2→4)-Kdo DISACCHARIDE DERIVATIVES AND THEIR CONJUGATION WITH A PROTECTED FORM OF GLA-60, A BIOLOGICALLY ACTIVE ANALOG OF A LIPID A SUBUNIT*

MAKOTO KISO, MINORU FUJITA, YUJI OGAWA, HIDEHARU ISHIDA, AND AKIRA HASEGAWA
Department of Applied Bioorganic Chemistry, Gifu University, Yanagido, Gifu 501-11 (Japan)
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ABSTRACT

A variety of the protected *O*-[(3-deoxy-α-D-*manno*-2-octulopyranosyl)onic acid]-(2→4)-3-deoxy-D-*manno*-2-octulosonic acid [α-Kdo-(2→4)-Kdo] derivatives have been synthesized starting from methyl [2-(trimethylsilyl)ethyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosid]onate. Some of these were conjugated with a protected form of a bacterial lipid A subunit-analog (GLA-60) having beneficial immunopharmacological activity, namely benzyl 2-[(3*R*)-3-(benzyloxymethoxy)tetradecanamido]-2-deoxy-4-*O*-(diphenoxyphosphinyl)-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside.

INTRODUCTION

The inner-core region of bacterial lipopolysaccharide[1,2] (LPS) consists in part of an α-(2→4)-linked disaccharide 3-deoxy-D-manno-octulosonic acid (Kdo), which is attached to O-6′ of the *O*-(β-D-glucosaminyl)-(1→6)-D-glucosamine backbone of lipid A.

In previous papers[3–5] we have described the synthesis of a variety of novel disaccharides combining one Kdo molecule with biologically active lipid A subunit analogs of the GLA series[6], in order to investigate the importance of Kdo for the expression of immunopharmacological activities[7].

We now describe the synthesis of α-Kdo-(2→4)-Kdo disaccharide derivatives[8] and their conversion into conjugates with a protected form of GLA-60 (ref. 9). Among the synthetic 4-*O*-phosphono-D-glucosamine derivatives related to bacterial lipid A, this compound had shown the most marked beneficial biological activities[7d,10,11].

RESULTS AND DISCUSSION

As previously described[5], the 2-*O*-(tetrahydro-2-pyranyl) (2-*O*-THP) deriva-

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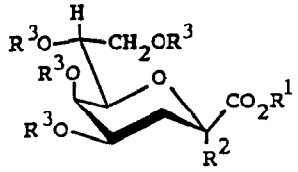
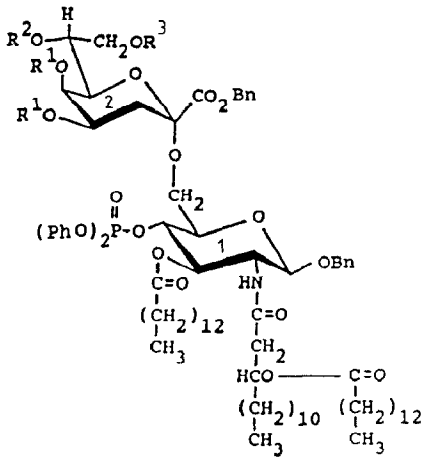
tives of Kdo have been used for the preparation of Kdo glycosyl donors, because of their facile deprotection under mildly acidic conditions. The 2-*O*-THP derivatives, however, are usually obtained as diastereomeric mixtures reflecting the asymmetry of C-2 of the THP group. In the present study, the anomeric OH of Kdo was more conveniently protected by the 2-(trimethylsilyl)ethyl (SE) group[12–14], which is stable over a wide range of pH values and easily removable under certain specific but mild conditions.



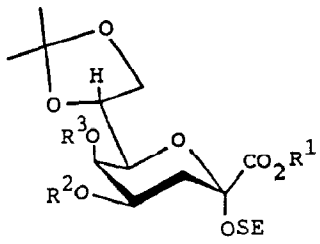
	R^1	R^2	R^3
1	Me	Br	Ac
2	Me	OSE	Ac
3	Me	OSE	H
4	Bn	OSE	H
5	Bn	OSE	ClAc
6	Bn	OAc	ClAc
7	Bn	Br	ClAc

OSE = $OCH_2CH_2Si(CH_3)_3$
Bn = $PhCH_2$
ClAc = $ClCH_2CO$



	R^1	R^2	R^3
8	H	H	H
9	H	—Ipd—	

Ipd = isopropylidene



	R^1	R^2	R^3
10	Me	H	H
11	Bn	H	H
12	Me	Bn	H
13	Me	Bn	Me
14	Me	H	Me

Treatment of **1** (ref. 15) with 2-(trimethylsilyl)ethanol in the presence of silver carbonate, silver perchlorate, and molecular sieves 4A in dichloromethane gave **2** in 86% yield. Saponification of **2**, and benzyl esterification of the carboxyl group afforded **4**, which was per(chloroacetyl)ated as described previously[5]. The resulting **5** was treated with acetic anhydride and boron trifluoride etherate in dichloromethane, to give **6**, and this was converted by treatment with titanium tetrabromide into the corresponding bromide **7**.

For the synthesis of the desired α-Kdo-(2→4)-α-Kdo-(2→6)-GLA-60 derivatives, earlier[5] we attempted to couple **7** with the protected α-Kdo-(2→6)-GLA-27 derivative **9**, prepared from **8**. However, no glycosylation product was obtained, probably because of strong steric hindrance by the lipid A moiety. Accordingly, the alternative of coupling **32** with the previously prepared α-Kdo-(2→4)-Kdo unit was now tested.

The glycosyl acceptors **10** and **11** were respectively prepared by the treatment of **3** and **4** at 0° with 2-methoxypropene and a catalytic amount of *p*-toluenesulfonic acid in *N,N*-dimethylformamide (DMF)[16]. Since the coupling of **7** with **11** had not yielded the desired disaccharide, compound **1** was chosen as the glycosyl donor for preparing the α-Kdo-(2→4)-Kdo unit.

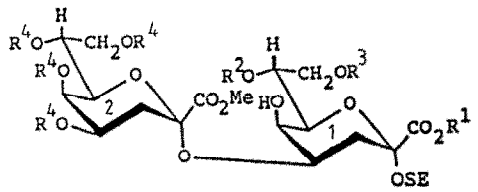
Condensation of the acceptors **10** and **11** with **1** was performed with mercuric cyanide and mercuric bromide as the catalysts, to give the desired α-glycosides **15** (51%) and **17** (52%), respectively. These were converted, by treatment with methanolic sodium methoxide, into a single product **19** in quantitative yield. Hydrolytic cleavage of the methyl ester functions of **19**, and benzyl esterification of the liberated carboxyl groups, afforded **20**, which was per(chloroacetyl)ated to give **22** (70% from **19**). The structure of **20** was confirmed by analyzing the ^{1}H-n.m.r. spectrum of the corresponding per-*O*-acetyl derivative **21**. Compound **22** was then converted into the bromide **24** as described for **6**.

The selective 4-*O*-benzylation of **10**, and the subsequent methylation of the OH at C-5, a position normally occupied by heptose in LPS, gave **13**, which was then hydrogenolyzed to afford **14** in high yield (80% from **10**). Coupling of **14** with **1** was performed as described for the synthesis of **15** and **17**, to give **25** in 75% yield. Saponification of **25**, benzyl esterification of the released carboxyl groups, and deisopropylidenation afforded **28**, which was then per(chloroacetyl)ated. The resulting **29** was acetolyzed to **30** by the method of Jansson *et al.*[13], and finally converted into **31**.

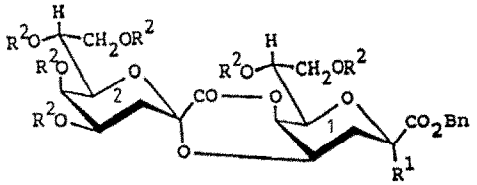
The glycosyl donors **24** and **31** were each coupled with the acceptor **32**, to give the corresponding protected trisaccharides **33** (28%) and **35** (37%), respectively. Selective removal of the chloroacetyl groups of **33** and **35** was achieved with hydrazine dithiocarbonate[17] to afford **34** and **36**, which should be convertible[5], by hydrogenolytic removal of the benzyl and phenyl groups, into the respective α-Kdo-(2→4)-α-Kdo-(2→6)-GLA-60 derivatives. Very recently, the chemical synthesis of the 1-dephospho derivative of *E. coli* Re lipopolysaccharide has been achieved[18] by use of benzyl (3-deoxy-4,5;7,8-di-*O*-isopropylidene-α-D-*manno*-2-octulopyranosyl

1 + 10

1 + 11

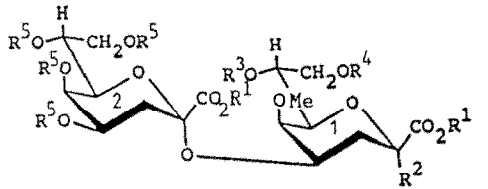


	R^1	R^2	R^3	R^4
15	Me	—Ipd—		Ac
16	Me	H	H	Ac
17	Bn	—Ipd—		Ac
18	Bn	H	H	Ac
19	Me	H	H	H

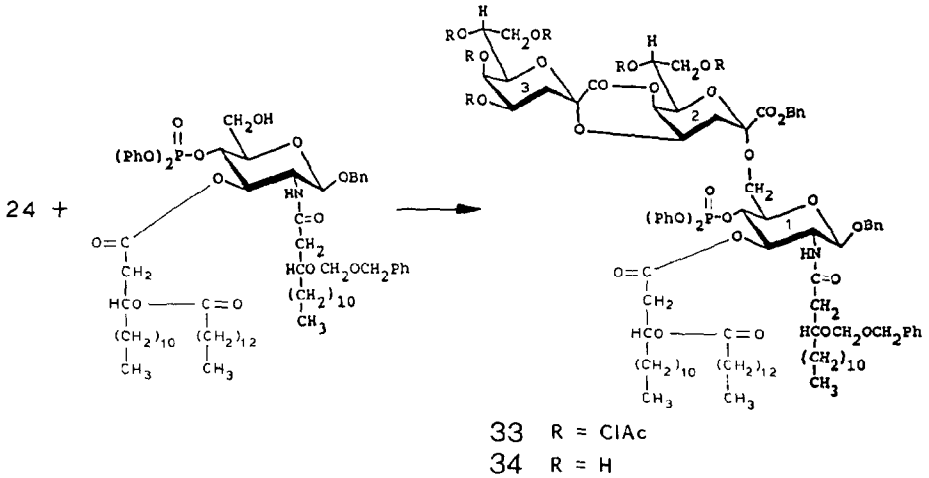


	R^1	R^2
20	OSE	H
21	OSE	Ac
22	OSE	ClAc
23	OAc	ClAc
24	Br	ClAc

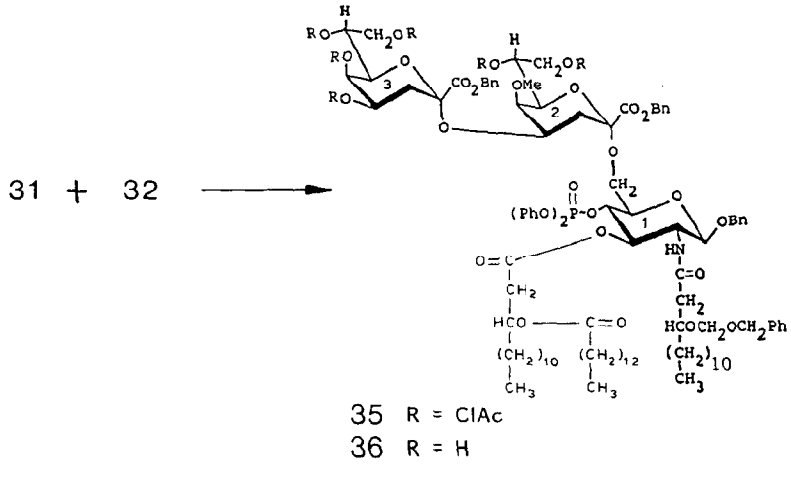
1 + 14



	R^1	R^2	R^3	R^4	R^5
25	Me	OSE	—Ipd—		Ac
26	Me	OSE	—Ipd—		H
27	Bn	OSE	—Ipd—		H
28	Bn	OSE	H	H	H
29	Bn	OSE	ClAc	ClAc	ClAc
30	Bn	OAc	ClAc	ClAc	ClAc
31	Bn	Br	ClAc	ClAc	ClAc



33 R = ClAc
34 R = H



35 R = ClAc
36 R = H

fluoride)onate as a glycosyl donor. The present study may also contribute toward the elucidation of the biological significance of Kdo in bacterial lipopolysaccharides.

EXPERIMENTAL

General methods. — These are as described in the previous paper in this series[5]. ^{1}H-N.m.r. data for the ring protons of compounds **2–5** and **10–14** (selected chemical shifts and coupling constants) are given in tabular form.

*Methyl [2-(trimethylsilyl)ethyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D*-manno-2-octulopyranosid]onate* (**2**). — A mixture of Ag_2CO_3 (1.19 g), $AgClO_4$ (20 mg), 2-(trimethylsilyl)ethanol (0.51 g), and molecular sieves 4A (0.2 g) in dichloromethane (5 mL) was stirred for 5 h at room temperature in the dark. Methyl

TABLE I

^{1}H-N.M.R. DATA FOR THE RING PROTONS OF SOME KdO DERIVATIVES

Compound	*Chemical shifts in p.p.m. (coupling constants in Hz)*						
	H-3ax(t)	*H-3eq(dd)*	*H-4(ddd)*	*H-5(~s)*	*H-6(dd)*	*H-7(ddd)*	*H-8(dd)*
2		2.34 (13.2, 4.8)	4.86 (11.4, 2.9)	5.25	4.13 (9.5, 1.5)	5.15 (9.5, 4.0, 2.6)	4.30, 4.40 (12.5)
3	2.00 (12.5)	2.36 (12.5, 4.4)	3.59 (4.4, 2.9)	3.94			
4	2.06 (12.5)	2.49 (12.5, 4.4)	3.63 (12.5, 4.4, 2.9)		3.51 (8.8)		3.79 (12.5, 5.5)
5	2.18 (12.8)	2.52 (12.8, 4.8)	4.98 (13.2, 2.9)	5.33	4.33 (11.7, 2.6)	5.28 (4.8, 2.2)	4.44, 4.61 (12.8, 4.8, 2.2)
10	1.96 (12.8)	2.42 (4.8)			3.51 (8.4)		
11	2.01 (12.5)	2.51 (4.4)	3.67 (2.9)	3.95	3.53 (8.4)		
12	2.07 (12.5)	2.47 (4.4)	3.49 (3.0)	4.09	3.45 (8.4)	4.41 (6.2, 5.5)	4.08, 4.17 (8.4, 6.2, 5.5)
13	2.20 (12.5)	2.47 (3.3)		3.68	3.44 (8.4, 1.1)	4.32 (6.2, 5.9)	4.06, 4.15 (8.4, 6.2, 5.9)
14	1.89 (12.5)	2.44 (4.4)		3.54	3.52 (8.8, 1.1)	4.29 (6.2, 5.5)	4.09, 4.18 (8.4, 6.2, 5.5)

(4,5,7,8-tetra-*O*-acetyl-3-deoxy-D-*manno*-2-octulopyranosyl bromide)onate[15] (**1**; 1.25 g) was added, and stirring was continued overnight at room temperature. The mixture was filtered through Celite, and the solid washed with dichloromethane. The filtrate and washings were combined and evaporated. The residue was chromatographed on a column of silica gel with 500:1 dichloromethane–methanol as eluent to give **2** (0.97 g, 86%); $[\alpha]_D$ +43° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 0.87 (m, 2 H, CH_2SiMe_3), 1.94, 1.96, 2.05, 2.07 (4 s, 12 H, CH_3CO), 3.39, and 3.87 (2 m, 2 H, $OCH_2CH_2SiMe_3$).

Anal. Calc. for $C_{22}H_{36}O_{12}Si$ (520.60): C, 50.76; H, 6.97. Found: C, 51.07; H, 7.19.

*Benzyl [2-(trimethylsilyl)ethyl 3-deoxy-α-*D-manno*-2-octulopyranosid]onate* (**4**). — Compound **2** (0.4 g) was treated with methanolic sodium methoxide to give **3** (quantitative), $[\alpha]_D$ +45° (*c* 1, 1:1 chloroform–methanol); ^{1}H-n.m.r. ($CDCl_3$–CD_3OD): δ 0.87 (m, 2 H, CH_2SiMe_3) and 3.81 (s, 3 H, CO_2CH_3).

To a solution of **3** (0.2 g) in dry 1,4-dioxane (5 mL) was added 0.2M aqueous KOH (5.8 mL), and the mixture was stirred for 1 h at room temperature. The solvent was removed by evaporation, and the residue was treated with K_2CO_3 (80

mg) and benzyl bromide (0.2 g) in DMF (1.5 mL) for 12 h at room temperature. Ice-water was added, and the mixture was concentrated to dryness. The residue was chromatographed on a column of silica gel with 30:1 dichloromethane–methanol as eluent to give **4** (0.19 g): $[\alpha]_D$ +54° (*c* 1.9, 1:1 chloroform–methanol); ^{1}H-n.m.r. (CD_3OD): δ 5.27, 5.32 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), and 7.37–7.45 (m, 5 H, Ph-H).

Anal. Calc. for $C_{20}H_{32}O_8Si$ (428.55): C, 56.05; H, 7.53. Found: C, 55.76; H, 7.41.

*Benzyl [2-(trimethylsilyl)ethyl 4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-α-*D*-manno-2-octulopyranosid]onate* (**5**). — To a solution of **4** (0.15 g) in dichloromethane (5 mL) were added dry 2,6-dimethylpyridine (1 mL) and triethylamine (0.39 mL), and the mixture was stirred at 0°. Chloroacetic anhydride (0.48 g) was added, and stirring was continued overnight at room temperature. Methanol was added at 0°, and the mixture was concentrated. The residue was taken up in dichloromethane, successively washed with ice-cold 2M hydrochloric acid, water, and dilute sodium hydrogencarbonate, dried, and evaporated. The product was purified by chromatography on a column of silica gel (elution with dichloromethane) to give syrupy **5** (0.2 g); $[\alpha]_D$ +31° (*c* 0.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 4.02, 4.05, 4.14, 4.15 (4 s, 8 H, $COCH_2Cl$), 5.22, 5.33 (2 d, 2 H, CH_2Ph), and 7.41 (m, 5 H, Ph-H).

Anal. Calc. for $C_{28}H_{36}Cl_4O_{12}Si$ (734.50): C, 45.79; H, 4.94. Found: C, 46.05; H, 5.11.

*Benzyl 2-*O*-acetyl-4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-α-*D*-manno-2-octulopyranosonate* (**6**). — To a solution of **5** (0.2 g) in 4:1 acetic anhydride–dichloromethane (5 mL) was added boron trifluoride etherate (0.06 mL) at 0°, and the mixture was stirred for 2 h at 20°. After completion of the reaction (t.l.c., 15:1 dichloromethane–ether). The mixture was poured into ice-cold, aqueous sodium hydrogencarbonate, the product was extracted with chloroform, and the extract was washed with water, dried, and evaporated. The residue was chromatographed on a column of silica gel with dichloromethane as eluent to give amorphous **6** (80%). The $[\alpha]_D$ value (+47°), and the i.r. and ^{1}H-n.m.r. spectra were identical with those of the title compound[5] obtained by treatment of benzyl 4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-D-*manno*-2-octulopyranosonate with acetyl chloride.

Benzyl O-*[(benzyl 3-deoxy-7,8-*O*-isopropylidene-α-*D*-manno-2-octulopyranosyl)onate]-(2→6)-2-deoxy-4-*O*-(diphenoxyphosphinyl)-3-*O*-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**9**). — To a solution of **8** (ref. 5) (0.132 g) in DMF (2 mL) was added 2-methoxypropene (0.04 mL) and a catalytic amount of *p*-toluenesulfonic acid, and the mixture was stirred at 0°. After completion of the reaction (t.l.c., 10:1 dichloromethane–ethanol), sodium hydrogencarbonate was added to neutralize the acid, and the suspension was filtered. The solid was washed with dichloromethane, and the filtrate and washings were combined and evaporated. The residue was chromatographed on a column of silica gel with 150:1 dichloromethane–methanol as eluent to give the title compound **9**

(82%); m.p. 42–43.5°, $[\alpha]_D$ +3° (*c* 1.1, dichloromethane); ν_{max} 3700–3150 (OH, NH), 1740 (ester), 1650, 1550 (amide), and 960 cm^{-1} (P-O-Ph); ^{1}H-n.m.r. ($CDCl_3$)*: δ 0.88 (t, 9 H, terminal CH_3), 0.95–1.7 (m, 64 H, alkyl CH_2), 1.32, 1.34 [2 s, 6 H, $C(CH_3)_2$], 1.75–2.95 (m, 8 H, $COCH_2$, H-3^2*ax*,3^2*eq*), 4.20 (dd, 1 H, $J_{6,7}$ 7.7, $J_{5,6}$ 1.5 Hz, H-6^2), 4.31 (m, 1 H, H-7^2), 4.50 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^1), 4.59, 4.87 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph at O-1^1), 4.62 (q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P}$ 9–10 Hz, H-4^1), 5.0, 5.1 (2 d, 2 H, J_{gem} 12 Hz, CO_2CH_2Ph), 5.77 (d, 1 H, *J* 8.8 Hz, N*H*), and 7.0–7.4 (m, 20 H, Ph-H).

Anal. Calc. for $C_{85}H_{128}NO_{19}P$ (1498.86): C, 68.11; H, 8.61; N, 0.93. Found: C, 68.35; H, 8.54; N, 0.90.

*Methyl [2-(trimethylsilyl)ethyl 3-deoxy-7,8-*O*-isopropylidene-α-*D*-manno-2-octulopyranosid]onate* (**10**). — To a solution of **3** (0.52 g) in DMF (5 mL) were added 2-methoxypropene (0.2 mL) and a catalytic amount of *p*-toluenesulfonic acid, and the mixture was stirred at 0°. After completion of the reaction (t.l.c., 3:1 dichloromethane–methanol), the mixture was treated as described for **9**. The product was purified by chromatography on a column of silica gel (elution with 250:1 dichloromethane–methanol) to give **10** (0.52 g, 90%); $[\alpha]_D$ +35° (*c* 0.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (m, 2 H, CH_2SiMe_3), 1.38, 1.41 [2 s, 6 H, $C(CH_3)_2$], 3.39, 3.85 (2 m, 2 H, OCH_2CH_2SiMe), and 3.80 (s, 3 H, CO_2CH_3).

Anal. Calc. for $C_{17}H_{32}O_8Si$ (392.52): C, 52.02; H, 8.22. Found: C, 52.28; H, 8.20.

*Benzyl [2-(trimethylsilyl)ethyl 3-deoxy-7,8-*O*-isopropylidene-α-*D*-manno-2-octulopyranosid]onate* (**11**). — Compound **11** was prepared in 91% yield by the treatment of **4** with 2-methoxypropene as described for **10**, $[\alpha]_D$ +48° (*c* 1.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 1.41 [s, 6 H, $C(CH_3)_2$], 5.22, 5.33 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), and 7.35–7.44 (m, 5 H, Ph-H).

Anal. Calc. for $C_{23}H_{36}O_8Si$ (468.62): C, 58.95; H, 7.74. Found: C, 58.73; H, 7.68.

*Methyl [2-(trimethylsilyl)ethyl 4-*O*-benzyl-3-deoxy-7,8-*O*-isopropylidene-α-*D*-manno-2-octulopyranosid]onate* (**12**). — A mixture of **10** (0.19 g), dibutyltin oxide (0.2 g), and molecular sieves 4A (0.5 g) in benzene (7 mL) was heated for 5 h at reflux temperature. Benzyl bromide (0.5 mL) and tetrabutylammonium bromide (0.1 g) were added, and the mixture was heated for another 5 h. The solid was filtered off, and the filtrate was evaporated. The residue was chromatographed on a column of silica gel with 400:1 dichloromethane–methanol as eluent to give **12** (0.2 g, 86%); $[\alpha]_D$ +37° (*c* 0.64, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 1.40, 1.42 [2 s, 6 H, $C(CH_3)_2$], 2.28 (br. s, 1 H, O*H*), 3.76 (s, 3 H, CO_2CH_3), 4.58, 4.64 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), and 7.3–7.38 (m, 5 H, Ph-H).

Anal. Calc. for $C_{24}H_{38}O_8Si$ (482.65): C, 59.73; H, 7.94. Found: C, 59.90; H, 7.75.

*A superscript on the locant of an H atom indicates the glycose residue to which the atom belongs. The reducing residue is numbered 1.

*Methyl [2-(trimethylsilyl)ethyl 4-O-benzyl-3-deoxy-7,8-O-isopropylidene-5-O-methyl-α-*D-manno-*2-octulopyranosid]onate* (**13**). — A mixture of **12** (0.49 g), powdered KOH (0.27 g), and methyl iodide (0.2 mL) in dry dimethyl sulfoxide (6 mL) was stirred for 40 min at room temperature. After completion of the reaction (t.l.c., 40:1 dichloromethane–methanol), the mixture was poured into ice–water. The product was extracted with chloroform, and the extract was dried, and evaporated. The residue was chromatographed on a column of silica gel with 500:1 dichloromethane–methanol as eluent to give **13** (0.49 g, 97%); $[\alpha]_D$ +40° (*c* 0.6, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 3.65 (s, 3 H, OCH_3), 3.73 (s, 3 H, CO_2CH_3), 4.63 (br. s, 2 H, CH_2Ph), and 7.28–7.37 (m, 5 H, Ph-H).

Anal. Calc. for $C_{25}H_{40}O_8Si$ (496.67): C, 60.46; H, 8.12. Found: C, 60.25; H, 8.08.

*Methyl [2-(trimethylsilyl)ethyl 3-deoxy-7,8-O-isopropylidene-5-O-methyl-α-*D-manno-*2-octulopyranosid]onate* (**14**). — Compound **13** (0.55 g) in methanol (50 mL) was hydrogenolyzed in the presence of 10% Pd–C catalyst (0.3 g) to give **14** (0.43 g, 96%); $[\alpha]_D$ +3.8° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 1.39, 1.42 [2 s, 6 H, $C(CH_3)_2$], 3.65 (s, 3 H, OCH_3), and 3.80 (s, 3 H, CO_2CH_3), with complete disappearance of signals for the benzyl group.

Anal. Calc. for $C_{18}H_{34}O_8Si$ (406.55): C, 53.18; H, 8.43. Found: C, 53.42; H, 8.36.

Methyl O-[*methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D-manno-*2-octulopyranosyl)onate*]-*(2→4)*-[*2-(trimethylsilyl)ethyl 3-deoxy-7,8-*O*-isopropylidene-α-*D-manno-*2-octulopyranosid]onate* (**15**). — To a solution of **10** (0.56 g) in dry nitromethane (3 mL) were added $Hg(CN)_2$ (1.01 g), $HgBr_2$ (0.72 g), and molecular sieves 4A (1 g), and the mixture was stirred for 5 h at room temperature. The glycosyl donor **1** (2 mol equiv.) was added, and the stirring was continued overnight at room temperature. The mixture was filtered, and the solid washed with chloroform. The filtrate and washings were combined, successively washed with aqueous KI and water, dried, and evaporated. The residue was chromatographed on a column of silica gel (elution with 400:1 dichloromethane–methanol) to give the desired α-glycoside **15** (0.58 g, 51%) and the β isomer (30 mg, 2.6%).

Compound **15** had $[\alpha]_D$ +60° (*c* 1.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 0.87 (m, 2 H, CH_2SiMe_3), 1.36, 1.39 [2 s, 6 H, $C(CH_3)_2$], 1.98, 2.00, 2.08, 2.09 (4 s, 12 H, $COCH_3$), 2.02–2.20 (m, 2 H, H-3^1*ax*,3^2*ax*), 2.24, 2.36 (2 dd, 2 H, $J_{3ax,3eq}$ 12.8, $J_{3eq,4}$ 4.4 Hz, H-3^1*eq*,3^2*eq*), 3.31 (d, 1 H, $J_{6,7}$ 8.4 Hz, H-6^1), 3.45, 3.84 (2 m, 2 H, $OCH_2CH_2SiMe_2$), 3.78, 3.81 (2 s, 6 H, CO_2CH_3), 4.04 (dd, 1 H, $J_{8a,8b}$ 12, $J_{7,8}$ 5.1 Hz, H-8^{2}a), 4.35 (m, 1 H, $J_{7,8a} = J_{7,8b}$ 5.9, $J_{6,7}$ 8.4 Hz, H-7^1), 4.72 (dd, 1 H, $J_{7,8b}$ 2.9 Hz, H-8^{2}b), 5.23–5.36 (m, 2 H, H-4^2,7^2), and 5.39 (br. s, 1 H, H-5^2).

Anal. Calc. for $C_{34}H_{54}O_{19}Si$ (794.87): C, 51.38; H, 6.85. Found: C, 51.60; H, 6.77.

The β isomer of **15** had $[\alpha]_D$ +48° (*c* 0.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 0.89 (m, 2 H, CH_2SiMe_3), 1.40 [s, 6 H, $C(CH_3)_2$], 1.98, 1.99, 2.08, 2.09 (4 s, 12 H, $COCH_3$), 2.34 (dd, 1 H, $J_{3ax,3eq}$ 13.6, $J_{3eq,4}$ 4.4 Hz, H-3^2*eq*), 3.40 (m, 1 H,

$OCH_2CH_2SiMe_3$), 3.55 (d, 1 H, $J_{6,7}$ 8.4 Hz, H-6[1]), 3.78, 3.81 (2 s, 6 H, CO_2CH_3), 4.08 (dd, 1 H, $J_{7,8a}$ 5.9, $J_{8a,8b}$ 8.4 Hz, H-8[1]a), 4.14 (dd, 1 H, $J_{7,8a}$ 4.8, $J_{8a,8b}$ 12.5 Hz, H-8[2]a), 4.17 (dd, 1 H, $J_{7,8b}$ 5.9 Hz, H-8[1]b), 4.27 (ddd, 1 H, H-7), 4.57 (d, 1 H, $J_{6,7}$ 9.2 Hz, H-6[2]), 4.59 (dd, 1 H, $J_{7,8b}$ 2.6 Hz, H-8[2]b), 5.15 (ddd, 1 H, H-7[2]), 5.37 (br. s, 1 H, H-5[2]), and 5.48 (ddd, 1 H, $J_{3ax,4}$ 12.5, $J_{3eq,4}$ 4.4, $J_{4,5}$ 3.3 Hz, H-4[2]).

Anal. Found: C, 51.59; H, 6.82.

Methyl O-[*methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D-manno*-2-octulopyranosyl)onate*]-*(2→4)*-[*2-(trimethylsilyl)ethyl 3-deoxy-α-*D-manno*-2-octulopyranosid*]-*onate* (**16**). — A solution of **15** (0.15 g) in 80% aq. acetic acid (10 mL) was heated for 4 h at 45°. The solvent was evaporated and the residue was chromatographed on a column of silica gel with 50:1 dichloromethane–methanol as eluent to give **16** (0.129 g, 90%); $[\alpha]_D$ −73° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): complete loss of the peaks for the isopropylidene group.

Anal. Calc. for $C_{31}H_{50}O_{19}Si$ (754.81): C, 49.33; H, 6.68. Found: C, 49.07; H, 6.74.

Benzyl O-[*methyl(4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D-manno*-2-octulopyranosyl)onate*]-*(2→4)*-[*2-(trimethylsilyl)ethyl 3-deoxy 7,8-*O*-isopropylidene-α-*D-manno*-2-octulopyranosid*]*onate* (**17**). — Compound **11** (0.38 g) was coupled with **1** (1.6 mol equiv.) as described for the synthesis of **15**. The title compound **17** (52%) was obtained by chromatography on a column of silica gel with 3:1 hexane–ethyl acetate as eluent; $[\alpha]_D$ +77° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 0.86 (m, 2 H, CH_2SiMe_3), 1.39, 1.40 [2 s, 6 H, $C(CH_3)_2$], 2.04, 2.05, 2.10, 2.14 (4 s, 12 H, $COCH_3$), 2.49 (dd, 1 H, $J_{3ax,3eq}$ 12.8, $J_{3eq,4}$ 4.8 Hz, H-3[3]*eq*), 3.33 (near d, 1 H, $J_{6,7}$ 7.7 Hz, H-6[2]), 3.45 (m, 1 H, $OCH_2CH_2SiMe_3$), 3.83 (s, 3 H, CO_2CH_3), 4.38 (m, 1 H, $J_{7,8a} = J_{7,8b}$ 5.9, $J_{6,7}$ 8 Hz, H-7[1]), 4.74 (dd, 1 H, $J_{7,8b}$ 2.9, $J_{8a,8b}$ 12.5 Hz, H-8[2]b), 5.21–5.34 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 5.44 (s, 1 H, H-5[2]), and 7.32–7.44 (m, 5 H, Ph-H).

Anal. Calc. for $C_{40}H_{58}O_{19}Si$ (870.97): C, 55.16; H, 6.71. Found: C, 55.45; H, 6.83.

Benzyl O-[*methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D-manno*-2-octulopyranosyl)onate*]-*(2→4)*-[*2-(trimethylsilyl)ethyl 3-deoxy-α-*D-manno*-2-octulopyranosid*]*onate* (**18**). — Compound **17** (0.27 g) was treated with aqueous 80% acetic acid as described for the preparation of **16**, to give **18** (93%); $[\alpha]_D$ +74° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 0.84 (m, 2 H, CH_2SiMe_3), 2.05 (6 H), 2.11, 2.15 (3 s, 12 H, $COCH_3$), 2.56 (dd, 1 H, $J_{3ax,3eq}$ 12.8, $J_{3eq,4}$ 4.8 Hz, H-3[3]*eq*), 3.37 (d, 1 H, $J_{6,7}$ 8.8 Hz, H-6[1]), 3.85 (s, 3 H, CO_2CH_3), 4.21 (dd, 1 H, $J_{6,7}$ 9.5, $J_{5,6}$ 1.5 Hz, H-6[2]), 4.69 (dd, 1 H, $J_{8a,8b}$ 12, $J_{7,8b}$ 2.9 Hz, H-8[2]b), 5.44 (br. s, 1 H, H-5[2]), and 7.43 (br. s, 5 H, Ph-H).

Anal. Calc. for $C_{37}H_{54}O_{19}Si$ (830.91): C, 53.48; H, 6.55. Found: C, 53.22; H, 6.57.

Methyl O-[*methyl (3-deoxy-α-*D-manno*-2-octulopyranosyl)onate*]-*(2→4)*-[*2-(trimethylsilyl)ethyl 3-deoxy-α-*D-manno*-2-octulopyranosid*]*onate* (**19**). — Compound **16** or **18** was treated with methanolic sodium methoxide at 0° to give **19**

(quantitative); $[\alpha]_D$ +60°, (*c* 1.0, 1:1 chloroform–methanol); ^{1}H-n.m.r. (CD_3OD): δ 1.92, 2.09 (2 t, 2 H, *J* 12.5–12.8 Hz, H-3^1*ax*,3^2*ax*), 2.17, 2.27 (2 dd, 2 H, $J_{3eq,4}$ 4.8 Hz, H-3^1*eq*,3^2*eq*), 3.77, and 3.84 (2 s, 6 H, CO_2CH_3).

Anal. Calc. for $C_{23}H_{42}O_{15}Si$ (586.66): C, 47.09; H, 7.22. Found: C, 46.83; H, 7.12.

*Benzyl {2-(trimethylsilyl)ethyl 7,8-bis-*O*-(chloroacetyl)-3-deoxy-4-*O*-[4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-α-*D*-manno-2-octulopyranosylono-1′,5-lactone]-α-*D*-manno-2-octulopyranosid}onate* (**22**). — Compound **19** (0.3 g) was saponified with 0.2M KOH (8 mL) in 1,4-dioxane (6 mL), and the product was treated with benzyl bromide (0.97 g) as described for the preparation of **4**. Compound **20** (0.56 g) thus obtained was dissolved in dichloromethane (3 mL), and treated with chloroacetic anhydride (1.1 g) in the presence of 2,6-dimethylpyridine (2.2 mL) and triethylamine (0.89 mL) as described for the synthesis of **5**. The title compound **22** (70% from **19**) was obtained by chromatography on a column of silica gel (500:1 dichloromethane–methanol as eluent; $[\alpha]_D$ +32° (*c* 2.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 4–4.4 (m, 12 H, $COCH_2Cl$), and 7.43 (s, 5 H, Ph-H).

Anal. Calc. for $C_{40}H_{48}Cl_6O_{20}Si$ (1089.63): C, 44.09; H, 4.44. Found: C, 44.31; H, 4.55.

To confirm the lactone structure, **20** was acetylated with acetic anhydride in pyridine to give **21**; ^{1}H-n.m.r. ($CDCl_3$): δ 0.86 (m, 2 H, CH_2SiMe_3), 2.04 (6 H), 2.07, 2.08, 2.11, 2.13 (5 s, 18 H, $COCH_3$), 1.86 (t, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ 13 Hz, H-3^1*ax*), 1.93 (dd, 1 H, $J_{3eq,4}$ 4.4 Hz, H-3^2*eq*), 2.68 (dd, 1 H, $J_{3ax,3eq} = J_{3ax,4}$ 13 Hz, H-3^2*ax*), 3.39, 3.85 (2 m, 2 H, $OCH_2CH_2SiMe_3$), 4.06 (ddd, 1 H, $J_{3eq,4}$ 5, $J_{4,5}$ 3.7 Hz, H-4^1), 4.15 (dd, 1 H, $J_{7,8a}$ 3.3, $J_{8a,8b}$ 12.5 Hz, H-8^{2}a), 4.17 (dd, 1 H, $J_{5,6}$ 1.5, $J_{6,7}$ 9.2 Hz, H-6^2), 4.32 (dd, 1 H, $J_{5,6}$ 1.1, $J_{6,7}$ 9.9 Hz, H-6^1), 4.39 (dd, 1 H, $J_{7,8a}$ 2.2, $J_{8a,8b}$ 12.5 Hz, H-8^{1}a), 4.50 (dd, 1 H, $J_{7,8b}$ 2.9 Hz, H-8^{2}b), 5.04 (m, 1 H, H-5^1), 5.15 (m, 1 H, $J_{7,8a} = J_{7,8b}$ 2.2 Hz, H-7^1), 5.23, 5.28 (2 d, 2 H, J_{gem} 12 Hz, CH_2Ph), 5.3–5.42 (m, 2 H, H-4^2,7^2), 5.39 (br. s, 1 H, H-5^2), and 7.43 (s, 5 H, Ph-H).

*Benzyl 2-*O*-acetyl 7,8-bis-*O*-(chloroacetyl)-3-deoxy-4-*O*-[4,5,7,8-tetrakis-O-(chloroacetyl)-3-deoxy-α-*D*-manno-2-octulopyranosylono-1′,5-lactone]-*D*-manno-2-octulopyranosonate* (**23**). — Compound **22** (0.253 g) was treated with acetic anhydride and boron trifluoride etherate, as described for the synthesis of **6**, to give **23** (95%); m.p. 59–61°, $[\alpha]_D$ +42° (*c* 0.75, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 2.35 (s, 3 H, $COCH_3$), and complete loss of the signals for $OCH_2CH_2SiMe_3$.

Anal. Calc. for $C_{37}H_{38}Cl_6O_{21}$ (1031.41): C, 43.08; H, 3.71. Found: C, 43.29; H, 3.82.

Methyl O-*[methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D*-manno-2-octulopyranosyl)onate]-(2→4)-[2-(trimethylsilyl)ethyl-3-deoxy-7,8-*O*-isopropylidene-5-*O*-methyl-α-*D*-manno-2-octulopyranosid]onate* (**25**). — Compound **14** (0.447 g) was coupled with **1** (1.6 mol equiv.), as described for the preparation of **15**, to give **25** (0.67 g, 75%); $[\alpha]_D$ +61° (*c* 0.56, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 0.87 (m, 2 H, CH_2SiMe_3), 1.37, 1.40 [2 s, 6 H, $C(CH_3)_2$], 2.00, 2.02, 2.09, 2.11 (4 s, 12 H, $COCH_3$), 2.23 (t, 1 H, *J* 12.5 Hz, H-3^1*ax* or 3^2*ax*), 2.27 (dd, 1 H, $J_{3eq,4}$ 5 Hz, H-

3^1eq), 2.38 (dd, 1 H, $J_{3ax,3eq}$ 12.5, $J_{3eq,4}$ 4.6 Hz, H-3^2eq), 3.29 (near d, 1 H, $J_{6,7}$ 8.8 Hz, H-6^1), 3.39 (m, 1 H, $OCH_2CH_2SiMe_3$), 3.46 (br. s, 1 H, H-5^1), 3.60 (s, 3 H, OCH_3), 3.77, 3.84 (2 s, 6 H, CO_2CH_3), 4.0–4.15 (m, 3 H, H-8^1a,8^1b,8^2a), 4.24 (near d, 1 H, $J_{6,7}$ 9.5 Hz, H-6^2), 4.2–4.3 (m, 1 H, H-7^1), 4.74 (dd, 1 H, $J_{7,8b}$ 2.9, $J_{8a,8b}$ 12 Hz, H-8^2b), 5.26 (ddd, 1 H, $J_{7,8a}$ 4.8 Hz, H-7^2), 5.37 (m, 1 H, H-4^2), and 5.41 (br. s, 1 H, H-5^2).

Anal. Calc. for $C_{35}H_{56}O_{19}Si$ (808.90): C, 51.97; H, 6.98. Found: C, 52.29; H, 7.03.

Methyl O-[*methyl (3-deoxy-α-*D-manno-*2-octulopyranosyl)onate*]-*(2→4)*-[*2-(trimethylsilyl)ethyl-3-deoxy-7,8-*O*-isopropylidene-5-*O*-methyl-α-*D-manno-*2-octulopyranosid*]*onate* (**26**). — *O*-Deacetylation of **25** was achieved by Zemplén's method, and the product was purified by chromatography on a column of silica gel with 30:1 dichloromethane–methanol as eluent to give **26** (95%); $[\alpha]_D$ +61° (*c* 2.4, 1:1 chloroform–methanol); ^{1}H-n.m.r. ($CDCl_3$–CD_3OD): δ 1.36, 1.40 [2 s, 6 H, $C(CH_3)_2$], 1.99, 2.11 (2 t, 2 H, *J* 12.5–12.8 Hz, H-3^1ax,3^2ax), 2.17, 2.29, (2 d, 2 H, *J* 12–13 and 4.4 Hz, H-3^1eq,3^2eq), 3.38 (s, 3 H, OCH_3), 3.81, and 3.84 (2 s, 6 H, CO_2CH_3).

Anal. Calc. for $C_{27}H_{48}O_{15}Si$ (640.75): C, 50.61; H, 7.55. Found: C, 50.50; H, 7.61.

Benzyl O-[*benzyl (3-deoxy-α-*D-manno-*2-octulopyranosyl)onate*]-*(2→4)*-[*2-(trimethylsilyl)ethyl-3-deoxy-7,8-*O*-isopropylidene-5-*O*-methyl-α-*D-manno-*2-octulopyranosid*]*onate* (**27**). — Compound **26** (0.116 g) was saponified and then esterified with benzyl bromide as described for the preparation of **4**. The product was purified by chromatography on a column of silica gel with 30:1 dichloromethane–methanol as eluent to give **27** (0.125 g, 87%); $[\alpha]_D$ +57° (*c* 1.1, 1:1 chloroform–methanol); ^{1}H-n.m.r. ($CDCl_3$–CD_3OD): δ 1.43, 1.46 [2 s, 6 H, $C(CH_3)_2$], 2.10, 2.17 (2 t, 2 H, *J* 12–13 Hz, H-3^1ax,3^2ax), 2.26, 2.43 (2 dd, 2 H, $J_{3eq,4}$ 4.8 resp. 4.4 Hz, H-3^1eq,3^2eq), 3.55 (s, 3 H, OCH_3), 5.20, 5.23, 5.33, 5.45 (4 d, 4 H, J_{gem} 12 Hz, CH_2Ph), and 7.44–7.50 (m, 10 H, Ph-H).

Anal. Calc. for $C_{39}H_{56}O_{15}Si$ (792.95): C, 59.07; H, 7.12. Found: C, 59.31; H, 7.19.

A portion of **27** was acetylated to afford the corresponding 4^2,5^2,7^2,8^2-tetra-*O*-acetyl derivative of **27**; ^{1}H-n.m.r. ($CDCl_3$): δ 2.06 (6 H), 2.13, 2.15 (3 s, 12 H, $COCH_3$), 3.54 (br. s, 1 H, H-5^1), 3.59 (s, 3 H, OCH_3), 7.43, and 7.48 (2 s, 10 H, Ph-H).

Benzyl O-{*benzyl* [*4,5,7,8-tetrakis-*O*-(chloroacetyl)-3-deoxy-α-*D-manno-*2-octulopyranosyl*]*onate*}-*(2→4)*-[*2-(trimethylsilyl)ethyl-7,8-bis-*O*-(chloroacetyl)-3-deoxy-5-*O*-methyl-α-*D-manno-*2-octulopyranosid*]*onate* (**29**). — Compound **27** (0.2 g) was treated with aqueous 80% acetic acid for 5 h at 45° to give 97% of benzyl-*O*-[benzyl (3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]-(2→4)-[2-(trimethylsilyl)-ethyl-3-deoxy-5-*O*-methyl-α-D-*manno*-2-octulopyranosid]onate (**28**); $[\alpha]_D$ +63° (*c* 1, 1:1 chloroform–methanol). Chloroacetylation of **28** (0.15 g) was performed as described for the preparation of **22**, to afford **29** (92%); $[\alpha]_D$ +41° (*c* 1, chloro-

form); ^{1}H-n.m.r. ($CDCl_3$): δ 3.53 (s, 3 H, OCH_3), 4.0–4.3 (m, 12 H, $COCH_2Cl$), 5.0–5.5 (m, 8 H, H-4^2,5^2,7^1,7^2, and 2 × CH_2Ph), and 7.3–7.5 (m, 10 H, Ph-H).

Anal. Calc. for $C_{48}H_{58}Cl_6O_{21}Si$ (1211.80): C, 47.58; H, 4.82. Found: C, 47.23; H, 4.60.

Benzyl O-{*benzyl* [*4,5,7,8-tetrakis*-O-*(chloroacetyl)-3-deoxy*-α-D-manno-*2-octulopyranosyl]onate*}-*(2→4)*-*2*-O-*acetyl-7,8-bis*-O-*(chloroacetyl)-3-deoxy-5*-O-*methyl*-α-D-manno-*2-octulopyranosonate* (**30**). — The title compound **30** (95%) was synthesized by the treatment of **29** (0.156 g) with acetic anhydride and boron trifluoride etherate in dichloromethane as described for the preparation of **6** and **23**, m.p. 49–51°; $[\alpha]_D$ +53° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 2.35 (s, 3 H, $COCH_3$), and complete loss of the signals for $OCH_2CH_2SiMe_3$.

Anal. Calc. for $C_{45}H_{48}Cl_6O_{22}$ (1153.60): C, 46.85; H, 4.19. Found: C, 46.66; H, 4.25.

Benzyl O-{*benzyl* [*3-deoxy-4*-O-*(3-deoxy*-α-D-manno-*2-octulopyranosylono-1′,5-lactone)*-α-D-manno-*2-octulopyranosyl]onate*}-*(2→6)*-*2*-[*(3*R)-*3-(benzyloxymethoxy)-tetradecanamido*]-*2-deoxy-4*-O-*(diphenoxyphosphinyl)-3*-O-[*(3*R)-*3-tetradecanoyloxytetradecanoyl*]-β-D-*glucopyranoside* (**34**). — To a solution of **23** (0.227 g) in dry dichloromethane (4 mL) was added, at 0°, a solution of titanium tetrabromide (0.22 g) in dry 10:1 dichloromethane–ethyl acetate (1.25 mL), and the mixture was stirred at room temperature. After completion of the reaction (t.l.c., 100:1 dichloromethane–methanol), dry dichloromethane (10 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° to give the crude bromide **24**.

A mixture of **32** (0.16 g, 0.15 mmol), molecular sieves 4A (1 g), $Hg(CN)_2$ (0.202 g), and $HgBr_2$ (0.144 g) in dichloromethane (3 mL) was stirred for 5 h. Bromide **24** (0.2 mmol) was added, and the mixture was stirred for 2 days at room temperature. After usual work-up (see **15**, above), the product was purified by chromatography on a column of silica gel (elution with 300:1 dichloromethane–methanol) to give **33** (28%). About 50% of unreacted acceptor **32** was recovered.

Compound **33** had m.p. 56–58°, $[\alpha]_D$ +23° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 9 H, CH_3), 1.1–1.8 (m, 62 H, alkyl CH_2), 1.8–2.8 (m, 10 H, $COCH_2$, H-3^2, H-3^3), and 7.1–7.5 (m, 25 H, Ph-H).

The chloroacetyl groups of **33** were selectively removed by treatment with hydrazine dithiocarbonate, as described in a previous paper[5], to yield **34**; m.p. 54–56°, $[\alpha]_D$ +14° (*c* 0.5, 3:1 ethanol–chloroform).

Anal. Calc. for $C_{98}H_{142}NO_{27}P$ (1797.17): C, 65.50; H, 7.96; N, 0.78. Found: C, 65.18; H, 8.22; N, 0.69.

Benzyl O-[*benzyl (3-deoxy*-α-D-manno-*2-octulopyranosyl)onate*]-*(2→4)*-O-[*benzyl (3-deoxy-5*-O-*methyl*-α-D-manno-*2-octulopyranosyl)onate*]-*(2→6)*-*2*-[*(3*R)-*3-(benzyloxymethoxy)-tetradecanamido*]-*2-deoxy-4*-O-*(diphenoxyphosphinyl)-3*-O-[*(3*R)-*3-tetradecanoyloxytetradecanoyl)*-β-D-*glucopyranoside* (**36**). — Compound **31** (0.123 mmol), prepared by treatment of **30** with titanium tetrabromide as described

for **24**, was coupled to **32** (0.106 g, 0.1 mmol) in dichloromethane (2 mL) in the presence of $Hg(CN)_2$ (62 mg), $HgBr_2$ (44 mg), and molecular sieves 4A (0.5 g). The reaction mixture was stirred for 3 days at room temperature, and worked up. The main product was purified by chromatography on a column of silica gel with 300:1 dichloromethane–methanol as eluent to give **35** (37%); $[\alpha]_D$ +19° (*c* 1.1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 0.88 (t, 9 H, CH_3), 1.1–1.8 (m, 62 H, alkyl CH_2), 2.0–2.5 (m, 10 H, $COCH_2$, H-3^2,3^3), 3.46 (s, 3 H, OCH_3), 3.84, 3.94, 3.98, 4.04, 4.07 (5 s, 10 H, $COCH_2Cl$), 3.92 (d, 2 H, $COCH_2Cl$), 6.17 (d, 1 H, NH), and 7.0–7.5 (m, 30 H, Ph-H).

De(chloroacetyl)ation of **35** was achieved as described for **34**, and the product was purified by chromatography on a column of silica gel (elution with 20:1 dichloromethane–methanol), to give **36** (91%); m.p. 55–56°, $[\alpha]_D$ +15° (*c* 1.4, 3:1 ethanol–chloroform).

Anal. Calc. for $C_{106}H_{152}NO_{28}P$ (1919.34): C, 66.33; H, 7.98; N, 0.73. Found: C, 66.58; H, 7.76; N, 0.64.

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