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Synthesis of allyl 6-*O*-(3-deoxy-α- and -β-D-*manno*-oct-2-ulopyranosylonic acid)-(1→6)-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-β-D-glucopyranoside 4-phosphate and of the copolymer of the α anomer with acrylamide*

France-Isabelle Auzanneau, Michelle Mondange, Daniel Charon, and Ladislas Szabó‡
Équipe "Endotoxines" du Centre National de la Recherche Scientifique, Université de Paris-Sud (U.R.A. 1116), Centre d'Études Pharmaceutiques, F-92290 Châtenay-Malabry (France)

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ABSTRACT

The title disaccharides were synthesized by mercuric cyanide-catalyzed condensation of methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl bromide)onate and allyl 2-[(3*R*)-3-acetoxytetradecanamido]-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside, followed by phosphorylation of the alcoholic function of the amino sugar. The phosphorylated anomers were separated by chromatography and deprotected by conventional methods. Polymeric material was obtained by copolymerisation, catalyzed by peroxosulphate and *N,N,N',N'*-tetramethylethylenediamine, of the α anomer with acrylamide; it contained *ca.* one disaccharide unit for 18 acrylamide residues.

INTRODUCTION

The hydrophilic and hydrophobic domains of endotoxic lipopolysaccharides are joined by the glycosidic bond of 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo), a characteristic component of these macromolecules. In enterobacterial lipopolysaccharides examined so far, this Kdo unit has the α-D-pyranose configuration[2] and is attached to O-6′ of a disaccharide[3], namely, *O*-(2-amino-2-deoxy-β-D-glucopyranosyl 4-phosphate)-(1→6)-2-amino-2-deoxy-α-D-glucopyranosyl phosphate, the so-called backbone of the hydrophobic domain. The O-4 position of the Kdo molecule is often substituted by a second α-Kdopyranose unit[4,5], as in the tetrasaccharide **1**. The glycose chain present in **1** appears to be a common feature of many endotoxic lipopolysaccharides, and its antigenic properties are of considerable interest to immunologists[6–8]; accordingly numerous efforts were made to reproduce it by synthesis[9–14]. The synthesis of disaccharide **12**, substructure of **1**, which was required for immunological studies is described in this paper. As antigenicity of haptens can be enhanced when they are attached to a "carrier" such as poly(acrylamide)[15], the allyl group was chosen as the

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‡ To whom correspondence should be addressed.

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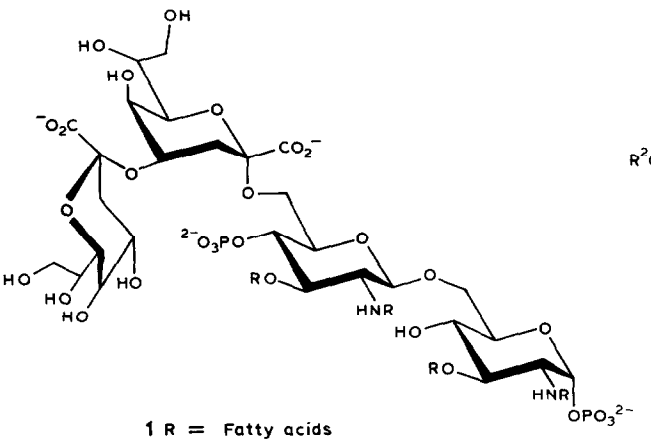
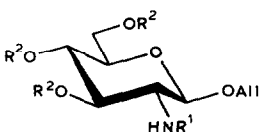
aglycon since it can be polymerized with acrylamide[16] to produce water-soluble polymers that carry the carbohydrate epitope[17].

RESULTS AND DISCUSSION

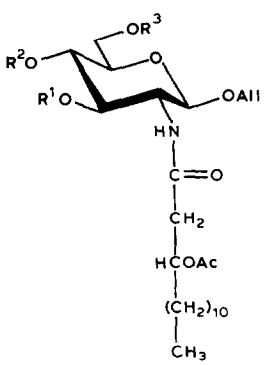
The common starting material for the synthesis of the disaccharides **12** and **16** was allyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside[18] (**2**) that was deacetylated by treatment with aqueous barium hydroxide. Following acylation of the free amino group with (3*R*)-3-acetoxytetradecanoic anhydride[19], amide **4** was transformed into the 4,6-*O*-isopropylidene acetal by treatment with 2-methoxypropene and 4-toluenesulphonic acid, to yield alcohol **5**. This was benzoylated with benzoic anhydride in pyridine with 4-dimethylaminopyridine as catalyst. Selective removal of the isopro-



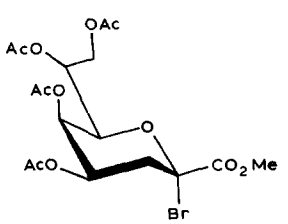
1 R = Fatty acids



2 $R^1 = R^2 =$ Ac
3 $R^1 = R^2 = H$



4 $R^1 = R^2 = R^3 = H$
5 $R^1 = H; R^2, R^3 =$ CMe_2
6 $R^1 =$ Bz; $R^2, R^3 =$ CMe_2
7 $R^1 =$ Bz, $R^2 = R^3 = H$



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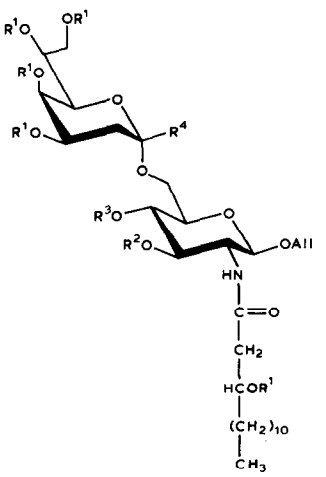
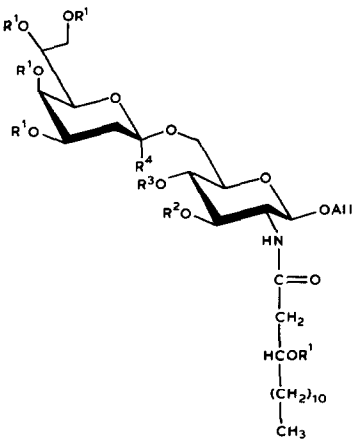
pylidene group with trifluoroacetic acid-on-silica gel[20] in the solid phase afforded diol **7**. The overall yield for the 5 steps was 54%.

Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl bromide) onate[21] (**8**) was used to glycosylate selectively the primary alcohol group of diol **7** with mercuric cyanide as the catalyst. The ratio of α to β glycosides formed under these conditions is known to depend on both the solvent used[22–24] and the nature of the aglycon (or sugar unit being glycosylated)[22,23]. When the glycosylation was performed in acetonitrile solution, the isolated and purified mixture of glycosides consisted of the α and β anomers (**9** and **13**; 65%) in the ratio 3:5, as calculated from the realive intensities of the CO_2CH_3 signals of the ^{1}H-n.m.r. spectrum. In nitromethane solution, a solvent that favors the formation of α anomers[22], the α to β anomer ratio was 4:1, but the yield only 45%; however, 48% of **7** was recovered.

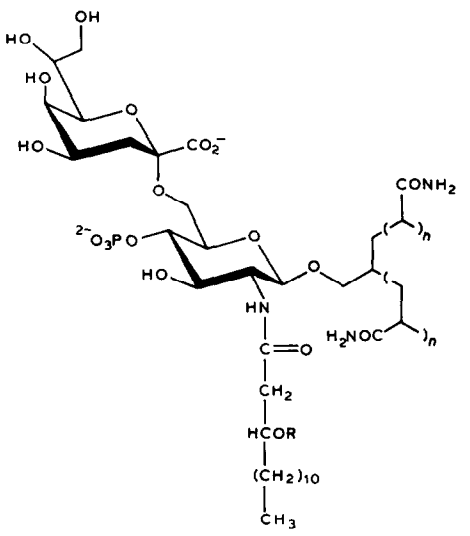
As the mixture of anomers thus formed could not be resolved at this stage, the only alcoholic function (O-4 of the 2-amino-2-deoxy-D-glucose unit) was phosphorylated with bis(trichloroethyl) phosphorochloridate[25] in oxolane and in the presence of *N*-methylimidazole. The phosphorylated anomers **10** and **14** thus produced were separated by chromatography. The β-D configuration (**14**) was assigned to the anomer for which, in the ^{1}H-n.m.r. spectrum, $\Delta\delta$ of H-3*a* and H3-*e* equalled 0.25 Hz, since this value is 0.2–0.4 Hz for β, but only 0.04–0.1 Hz for α anomers of Kdopyranosides[9,22,26]. This conclusion was confirmed by the chemical shifts of H-4', the signals being found at δ 5.38 for the α anomer (**10**) and at δ 4.9 for the β anomer (**14**), in close agreement with values observed[23,27] for α and β anomers of peracetylated glycosides of Kdo. Treatment of the separated phosphoric triesters with "activated" zinc[25] and silver carbonate[28] in





9 R^1 = Ac, R^2 = Bz, R^3 = H, R^4 = CO_2Me
10 R^1 = Ac, R^2 = Bz, R^3 = $PO(OCH_2CCl_3)_2$, R^4 = CO_2Me
11 R^1 = Ac, R^2 = Bz, R^3 = PO_3^{2-}, R^4 = CO_2Me
12 R^1 = R^2 = H, R^3 = PO_3^{2-}, R^4 = CO_2^-

13 R^1 = Ac, R^2 = Bz, R^3 = H, R^4 = CO_2Me
14 R^1 = Ac, R^2 = Bz, R^3 = $PO(OCH_2CCl_3)_2$, R^4 = CO_2Me
15 R^1 = Ac, R^2 = Bz, R^3 = PO_3^{2-}, R^4 = CO_2Me
16 R^1 = R^2 = H, R^3 = PO_3^{2-}, R^4 = CO_2^-



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pyridine containing 10% (v/v) acetic acid removed the trichloroethyl groups and afforded the phosphoric monoesters **11** and **15**, respectively. These were treated first with 0.2M sodium methoxide, and then with 0.5M sodium hydroxide to remove all ester groups. The unprotected α- and β-linked disaccharides (**12** and **16**) were then isolated as their calcium salts. Copolymerisation[16] of the α anomer with acrylamide, followed by chromatography on Sephadex G-50 to remove unpolymerized material, afforded polymer **17** which was desalted on Bio-Gel P-2, and recovered by lyophilization as an amorphous solid. The amount of glycose incorporated into the polymer was estimated by determining the ratio of glycose protons and CH + CH_2 protons in the ^{1}H-n.m.r. spectrum of the acrylamide polymer; it was found to be 1:18 ± 2. The molecular mass (M_r) of acrylamide copolymers is dependent on the concentrations of peroxosulphate and tetramethylethylenediamine in the reaction medium[16]. Under the conditions used, it can be expected to be superior to 100 kDa.

EXPERIMENTAL

General methods. — These were described earlier[1]. Optical rotations were measured at 20–22°. ^{1}H-N.m.r. spectra were obtained at 200 MHz. Column dimensions (diameter by height) are given in cm.

*Allyl 2-[(3R)-3-acetoxytetradecanamido]-2-deoxy-4,6-*O*-isopropylidene-β-*D*-glucopyranoside* (**5**). — *(a) Deacetylation of allyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside.* The acetylated derivative **2** (6 g, 15 mmol) was treated with $Ba(OH)_2 \cdot 8H_2O$ (19.5 g) in water (82.5 mL) contained in a Teflon vessel, and the stirred mixture was kept in the closed vessel at 110° overnight, when deacetylation was complete (t.l.c. in 13:7:2

$CHCl_3$–MeOH–conc.NH_4OH). The cooled mixture was diluted with 1:1 EtOH–water, (~ 150 mL), saturated with CO_2, the volume was diminished to ~ 10 mL, EtOH (60 mL) was added, and the solids were sedimented by centrifugation. The supernatant was decanted, the residue was re-extracted with EtOH, and the supernatants were pooled. The residue remaining after removal of the solvents was taken up in 13:7:2 $CHCl_3$–MeOH–conc.NH_4OH, and passed through a layer (8 × 4) of silica gel wet with the same solvent. Pooled fractions (20 mL) containing the product (t.l.c. as described above) were concentrated (3–4 mL) and loaded onto a column (2 × 13) of AG 50W-X8 (H^+) resin. The column was washed with water until neutrality of the effluent, the product was eluted with M NH_4OH, appropriate fractions were pooled, and the solvent was removed. The ^{1}H-n.m.r. spectrum of the remaining syrup (3.3 g; 100%) indicated that it was the pure, deacetylated allyl glycoside **3**. It was used without further treatment for the acylation step.

(b) Acylation. (3*R*)-3-Acetoxytetradecanoic anhydride (2 g, 3.6 mmol) was added to a solution of allyl 2-amino-2-deoxy-β-D-glucopyranoside (**3**; 790 mg, 3.6 mmol) in anhydr. MeOH (25 mL), and the mixture was stirred 48 h at room temperature; progress of the reaction was monitored by t.l.c. (10:1 EtOAc–EtOH). Crystalline inorganic material was filtered off and rinsed with EtOAc, and solvents were removed from the pooled filtrate and washings. Column (3 × 20) chromatography (solvent as for t.l.c.) of the residue, followed by removal of the solvents from pooled fractions, and thorough drying *in vacuo* over P_2O_5 and paraffin chips afforded **4** as a solid (1.53 g; 87%); ^{1}H-n.m.r. (CD_3OD–$CDCl_3$–D_2O): δ 0.8–1.5 [m, 23 H, $(CH_2)_{10}CH_3$], 2.05 (s, 3 H, CH_3CO), 2.5 [dd, 2 H, –(CH_2)–], 3.2–3.6 [m, 4 H, H-5,4,3,2], 3.7–3.9 (m, 2 H, H-6a,6b), 4.0–4.4 (2 dd, 2 H, OCH_2), 4.5 (s, 1 H, $J_{1,2}$ 9 Hz, H-1), 5.1–5.4 (m, 3 H, *H*COAc and CH_2=), and 5.85 (m, 1 H, CH=). The material was used without any further purification.

(c) Isopropylidenation. Anhydrous toluene (2 × 20 mL) was added to and distilled off compound **4** (1.53 g, 3.14 mmol) under water-pump vacuum. The dry residue was suspended in dry 1:1 oxolane–acetonitrile (50 mL), and dry 4-toluenesulphonic acid (120 mg) was added. The vessel was closed with a septum, cooled to −78°, and 2-methoxypropene (360 μL, 3.8 mmol) was added by injection to the stirred mixture, which was then allowed to reach ambient temperature. One h later, the reaction appeared (t.l.c., EtOAc) to be complete. Carbon tetracloride saturated with aq. NH_4OH was added to neutralize the acid, the mixture was soaked into a dry layer (4 × 9) of silica gel on the top of which solid $NaHCO_3$ had been dispersed, and the product was eluted with EtOAc. Solvents were removed from appropriately pooled fractions (50 mL) to obtain **5** (1.3 g, 81%) as a waxy solid, m.p. 63–64°, $[\alpha]_D$ −42° (*c* 1.4, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 0.8–1.8 [m, 29 H, $(CH_2)_{10}CH_3$, $C(CH_3)_2$], 2.05 (s, 3 H, CH_3CO), 2.5 (dd, 2 H, –OCH_2–), 3.30 (m, 1 H, H-5), 3.45 (dq, 1 H, $J_{2,NH}$ 7, $J_{1,2}$ 9, $J_{2,3}$ 10 Hz, H-2), 3.60 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ 9 Hz, H-4), 3.70–4.40 (m, 5 H, H-6a,6b,3, CH_2–C=), 4.73 (d, 1 H, H-1), 5.05–5.35 (m, 3 H, *H*COAc, CH_2=), 5.90 (m, 1 H, HC=), and 6.08 (d, 1 H, NH).

Anal. Calc. for $C_{28}H_{49}NO_8$: C, 63.8; H, 9.3; N, 2.7. Found: C, 63.8; H, 9.3; N, 2.7.

Allyl 2-[(3R)-3-acetoxytetradecanamido]-3-O-benzoyl-2-deoxy-4,6-O-isopropy-

lidene-β-D-glucopyranoside (**6**). — Benzoic anhydride (1 g, 4.4 mmol) and 4-dimethylaminopyridine (123.5 mg, 1 mmol) were added to a solution of **5** (1.3 g, 2.46 mmol) in dry pyridine (30 mL), and the stirred mixture was kept at 30° overnight. T.l.c. (3:7 EtOAc–cyclohexane) then showed the reaction to be complete. After addition of a few drops of MeOH, and some standing, the solvents were removed. Column (3 × 20) chromatography (7:3 cyclohexane–EtOAc) of the residue afforded benzoate **6** which crystallized from 1:1 Et_2O–cyclohexane (1.25 g, 80%), m.p. 139–140°, $[\alpha]_D$ −15.8° (*c* 1.2, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 0.8–1.75 [m, 29 H, $-(CH_2)_{10}CH_3$, CMe_2], 2.00 (s, 3 H, CH_3CO), 2.35 (m, 2 H, CH_2), 3.45 (m, 1 H, H-5), 3.8–4.4 (m, 6 H, H-2,4,6a,6b, $CH_2C=$), 4.65 (d, 1 H, $J_{1,2}$ 9 Hz, H-1), 5.00 (m, 1 H, *H*COAc), 5.10–5.45 (m, 3 H, H-3, $CH_2=$), 5.7–6.1 (m, 2 H, HC=, NH), and 7.35–8.10 (2 m, 3 and 2 H, arom.).

Anal. Calc. for $C_{35}H_{53}NO_9$: C, 66.56; H, 8.40; N, 2.22. Found: C, 66.49; H, 8.47; N, 2.12.

Allyl 2-[(3R)-3-acetoxytetradecanamido]-3-O-benzoyl-2-deoxy-β-D-glucopyranoside (**7**). — 1.5M Trifluoroacetic acid in CH_2Cl_2 (300 μL) and silica gel (3 g) were thoroughly mixed by shaking. A solution of benzoate **6** (600 mg, 0.95 mmol) in CH_2Cl_2 (500 μL) was added, and the mixture was stirred for 4 h at room temperature. Progress of the reaction was monitored by t.l.c. (EtOAc) of samples eluted with EtOAc. The solid reaction medium was deposited on a pad (2.5 × 4) of dry silica gel and eluted with EtOAc. Concentration of the appropriate fractions (20 mL) afforded diol **7** (528 mg, 94%), m.p. 94°, $[\alpha]_D$ +4.4° (*c* 1.65, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 0.8–1.5 [m, 23 H, $(CH_2)_{10}CH_3$], 2.00 (s, 3 H, CH_3CO), 2.35 (m, 2 H, CH_2), 3.52 (m, 1 H, $J_{5,4}$ 10, $J_{5,6a} = J_{5,6b}$ 4 Hz, H-5), 3.8–4 (m, 3 H, H-4,6a,6b), 4–4.4 (m, 3 H, H-2, CH_2–C=), 4.7 (d, 1 H, $J_{1,2}$ 9 Hz, H-1), 5.00 (m, 1 H, *H*COAc), 5.1–5.4 (m, 3 H, H-3, $=CH_2$), 5.90 (m, 1 H, HC=), 6.35 (d, 1 H, $J_{NH,2}$ 9 Hz, NH), and 7.35–8.1 (2 m, 3 and 2 H, arom.).

Anal. Calc. for $C_{32}H_{49}NO_9$: C, 64.97; H, 8.29; N, 2.37. Found: C, 64.73; H, 8.23; N, 2.33.

Allyl O-*[methyl (4,5,7,8-tetra*-O-*acetyl-α-* (**9**) and *-β-D-manno-oct-2-ulopyranosidonate)]-(1→6)-2-[(3R)-3-acetoxytetradecanamido]-3-*O-*benzoyl-2-deoxy-β-D-glucopyranoside* (**13**). — (*a*) *In acetonitrile solution.* A solution of methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl bromide)onate[21] (**8**, 478 mg, 1.5 mol.equiv) in acetonitrile (1.5 mL) was added, portionwise, within 3 h, to a stirred mixture of the diol **7** (391 mg, 0.66 mmol), $Hg(CN)_2$ (350 mg, 2 molar equivs.), and molecular sieves 4A in acetonitrile (5 mL). It was kept under Ar and, after 15 min, chloroform (15 mL) was added, solids were filtered off and rinsed, and the solvents were removed from the pooled filtrate and washings. The residue was taken up in CH_2Cl_2 (50 mL), washed with a saturated $NaHCO_3$ solution (30 mL) and with a saturated NaCl solution (2 × 20 mL), and dried (Na_2SO_4). Column (2.5 × 20) chromatography (2:3 EtOAc–toluene) of the residue remaining after removal of the solvent gave a mixture of **9** and **13** (430 mg, 65%) as an oil (single spot, R_F 0.2, solvent as described above).

(*b*) *In nitromethane solution.* A solution of **8** (558 mg, 1.3 mol.equiv) in nitromethane (1.5 mL) was added under Ar at room temperature to a stirred mixture of the diol **7** (528 mg, 0.89 mmol), $Hg(CN)_2$ (450 mg, 2 molar equivs.), and molecular sieves 4A

(1 g) in nitromethane (5 mL). Stirring was continued for 20 h under Ar at room temperature. More glycosyl bromide **8** (107 mg, 0.25 molar equiv.) in nitromethane (0.3 mL) was added, the addition was repeated 1 h later, and the reaction allowed to proceed overnight. The mixture was diluted with $CHCl_3$ (15 mL), the solids were filtered off, rinsed ($CHCl_3$, 60 mL), and the organic phase was washed first with saturated aq. $NaHCO_3$ (40 mL), and then with saturated aq. NaCl (2 × 50 mL). The aqueous washings were re-extracted with $CHCl_3$ (3 × 50 mL), and all organic phases were pooled and dried. Upon column (4.5 × 17) chromatography (3:7 EtOAc–toluene, 600 mL; then EtOAc) of the residue remaining after removal of the solvents were eluted sequentially: *methyl (4,5,7 8-tetra-*O*-acetyl-2,6-anhydro-2,3-dideoxy-*α-D-manno*-oct-2-ulopyranos)onate*[29] (150 mg, 17%), the mixture of **9** and **13** (400 mg, 45%), and unreacted diol **7** (250 mg, 48%).

Allyl O*-[methyl (4,5,7,8-tetra-*O*-acetyl-*α-D-manno*-oct-2-ulopyranos)onic acid]-(1→6)-{2-[(3*R*)-3-acetoxytetradecanamido]-3-*O*-benzoyl-2-deoxy-β-D-glucopyranoside 4-[bis(trichloroethyl) phosphate]}* (**10**) *and allyl* O*-[methyl (4,5,7,8-tetra-*O*-acetyl-β-D-manno-oct-2-ulopyranosyl)onic acid]-(1→6)-{2-[(3*R*)-3-acetoxytetradecanamido]-3-*O*-benzoyl-2-deoxy-β-D-glucopyranoside 4-[bis(trichloroethyl) phosphate]}* (**14**). — (*a*) *From the anomeric mixture of disaccharides produced in acetonitrile solution.* *N*-Methylimidazole (150 μL, 4 molar equivs.) and bis(trichloroethyl) phosphorochloridate (308 mg, 2 molar equivs.) were added to a solution of the mixed anomers α (**9**) and β (**13**) (400 mg, 0.40 mmol) in anhydrous oxolane (5 mL). After stirring at 60° for 3 h, more *N*-methylimidazole (90 μL, 2.8 molar equiv.) and bis(trichloroethyl) phosphorochloridate (210 mg, 1.4 molar equivs.) were added, and the temperature and stirring were maintained overnight. The cooled mixture was then diluted with diethyl ether and deposited on a bed (4 × 2) of silica gel wetted with 2:3 EtOAc–toluene, and then eluted with the same solvent (100 mL). Fractions (30 mL) containing the phosphorylated isomers (t.l.c., 2:3 EtOAc–toluene; R_F 0.55 and 0.49) were pooled and concentrated. The anomers were then separated by flash column (3.5 × 27) chromatography (500 kPa; SiO_2, Merck 60; 230–400 mesh, 3:7 EtOAc–toluene) to afford the α anomer **10** (98.5 mg, 18%), a mixture (70 mg, 13%) of the anomers **10** and **14**, and the β anomer **14** (167 mg, 31%).

(*b*) Phosphorylation of the mixture of anomers produced by condensation in nitromethane afforded the α anomer **10** (220 mg, 41%), a mixture (54.5 mg, 10%) of the anomers **10** and **14**, and the β anomer **14** (55 mg, 10%).

Compound **10**. Oil, $[\alpha]_D$ +27.4° (*c* 1.1, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$, 250 MHz): δ 0.8–1.5 [m, 23 H, $(CH_2)_{10}CH_3$], 1.9–2.4 [m, 19 H, 5 CH_3CO, $-COCH_2-$, H-3′*a*,3′*e*), 3.6–3.85 (m and s, 5 H, CO_2CH_3, H-6a or 6b,5), 4.05–4.23 (m, 4 H, H-2,6′ or 8′a, $CH_2C=$), 4.26–4.53 (m, 4 H, H-5 or 6b, 6′ or 8′b, 2 $CHCCl_3$), 4.56 (d, 2 H, 2 $CHCCl_3$), 4.60–4.76 (m, 3 H, H-1,4,8′b), 4.96 [m, 1 H, CH(OAc)], 5.18–5.27 (m, 2 H, H-7′, CH=), 5.29–5.43 (m, 3 H, H-4′,5′, CH=), 5.60 (dd, 1 H, H-3), 5.89 (m, 1 H, CH=), 6.15 (d, 1 H, NH), 7.40–7.62 and 8.07–8.15 (2m, 3 and 2 H, arom.).

Anal. Calc. for $C_{53}H_{74}Cl_6NO_2P$: C, 47.6; H, 5.5; N, 1.0. Found: C, 47.7; H, 5.6; N, 1.1.

Compound **14**. Oil, $[\alpha]_D$ +28.7° (*c* 1.5, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$, 250 MHz): δ 0.8–1.5 [m, 23 H, $(CH_2)_{10}CH_3$], 1.9–2.15 [m, 15 H, 5 CH_3CO), 2.21 (t, 1 H, $J_{3'a,3'e} = J_{3'a,4'}$ 13 Hz, H-3'a), 2.29 and 2.44 (2 dd, 2 × 1 H, $-COCH_2-$), 2.46 (dd, 1 H, $J_{3'e,4'}$ 5.5 Hz, H-3'*e*), 3.85 (s, 3 H, CO_2CH_3), 3.77–3.92 (m, 2 H, H-6a,5), 4.05–4.6 (m, 11 H, H-2,6b,6',8'a,8'b, $CH_2C=$, 2 CH_2CCl_3), 4.6–4.78 (m, 2 H, H-1,4), 4.92 (ddd, 1 H, $J_{4',5'}$ 3 Hz, H-4'), 4.98 [m, 1 H, –CH(OAc)–], 5.17–5.4 (m, 4 H, H-5',7', $CH_2=$), 5.61 (dd, 1 H, $J_{3,2 \text{ or } 3,4}$ 11, $J_{3,4 \text{ or } 3,2}$ 9 Hz, H-3), 5.89 (m, 1 H, CH=), 6.15 (d, 1 H, NH), 7.40–7.62 and 8.07–8.15 (2 m, 3 and 2 H, arom.).

Anal. Calc. for $C_{53}H_{74}Cl_6NO_2P$: C, 47.6; H, 5.5; N, 1.0. Found: C, 47.6; H, 5.6; N, 1.1.

Allyl O-*(α*-D-manno-*oct-2-ulopyranosylonic acid)-(1→6)-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-β*-D-*glucopyranoside 4-phosphate* (**12**). — "Activated" Zn (150 mg) and Ag_2CO_3 (20 mg) were added to a stirred solution of the phosphoric triester **10** (316 mg; 0.23 mmol) in 9:1 pyridine–acetic acid (3 mL) at room temperature; 18 h later, solids were filtered off and rinsed with MeOH (3 mL). Column (2.5 × 100) chromatography (130:60:40:2:1 CH_2Cl_2–MeOH–EtOAc–conc.NH_4OH) on Sephadex LH-20 gel of the residue, which remained after removal of the solvents from the pooled filtrate and washings, gave an oil (230 mg) that was taken up in 0.3M NaOMe (7 mL). The mixture was stirred for 24 h at room temperature, and its volume was reduced to ~ 3.5 mL. After extraction with cyclohexane (2 × 10 mL), water (2 mL) and 0.5M NaOH (2 mL) were added and the mixture was stirred for 2 h. Cations were removed with Dowex AG 50W–X8 (H^+) cation-exchange resin from the cooled (0°) solution, and the resin was filtered off, and rinsed with water (3 mL). The pH of the filtered solution was adjusted to 7.5 with 0.02M $Ca(OH)_2$, whereupon the Ca salt of **12** precipitated; it was collected by centrifugation, washed with acetone (4 × 3 mL), and dried (room temp., P_2O_5; yield, 130 mg, 61%). The ammonium salt, for which the ^{1}H-n.m.r. data are reported below, was obtained by ion-exchange [AG 50W–X8 (H^+)] and neutralization with dilute (~ 20%) aq. NH_4OH, $[\alpha]_D$ +20° (*c* 0.75, water; for the free acid); ^{1}H-n.m.r. (D_2O, 250 MHz): δ 0.8–1.55 (m, 23 H, $(CH_2)_{10}CH_3$), 1.8 (t, 1 H, $J_{3'a,3'e} = J_{3'a,4'}$ 12.5 Hz, H-3'*a*), 2.07 (dd, 1 H; $J_{3'e,4'}$ 5 Hz, H-3'*e*), 2.45 [m, 2 H, $-CH_2CH(OH)-$], 3.5–4.45 (m, 15 H, H-2,3,4,5,6a,6b,4',5',6',7',8'a,8'b, *H*C(OH), $CH_2C=$), 4.58 (m, 1 H, H-1), 5.18–5.41 (m, 2 H, $CH_2=$), and 5.9 (m, 1 H, HC=).

Anal. Calc. for $C_{31}H_{53}Ca_{1.5}NO_{17}P \cdot 7H_2O$: C, 40.1; H, 7.2; N, 1.5. Found: C, 40.1; H, 6.9; N, 1.6.

Allyl O-*(β*-D-manno-*oct-2-ulopyranosylonic acid-(1→6)-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-β*-D-*glucopyranoside 4-phosphate* (**16**). — Deprotection of the phosphoric triester **14** (160 mg, 0.12 mmol), as described for the α anomer, gave **16** isolated as its diammonium salt (50 mg, 50%). $[\alpha]_D$ +23° (*c* 0.9, water); ^{1}H-n.m.r. (D_2O, 250 MHz): δ 0.8–1.60 [m, 23 H, $(CH_2)_{10}CH_3$], 1.82 (t, 1 H, $J_{3'a,3'e} = J_{3'a,4}$ 12.5 Hz, H-3'*a*), 2.42 (m, 3 H, H-3'*e*, $CH_2C(OH)$, 3.5–4.4 (m, 15 H, H-2,3,4,5,6a,6b,4',5',6',7',8'a,8'b, HCOH, $CH_2C=$), 4.56 (m, 1 H, H-1), 5.16–5.38 (m, 2 H, $CH_2=$), and 5.87 (m, 1 H, HC=).

Anal. Calc. for $C_{31}H_{62}N_3O_{17}P \cdot 3H_2O$: C, 44.6; H, 8.2; N, 5.0. Found: C, 44.4; H, 7.7; N, 4.7.

Copolymerisation. — The Ca salt of **12** (19 mg, 0.025 mmol) was suspended in water (200 μL) and decationized by addition of a small amount of AG-50W-X8 (H^+) cation-exchange resin. The resin was filtered off and washed with water (300 μL). 2.5M aqueous ammonia (25 μL, 2.5 mol.equiv) was added to the pooled filtrate and washings, followed by acrylamide (9 mg, 5 molar equivs.) and *N,N,N',N'*-tetramethylethylenediamine (2 μL), and the mixture was kept under water-pump vacuum to remove dissolved gases (30 min). Ammonium peroxosulfate (1 mg) was added and the mixture was kept at room temperature for 1 h, and then at 4° overnight. The solution was then passed through a column (1.6 × 47) of Sephadex G-50 gel in 0.01M $NaHCO_3$ and eluted with the same solvent. Fractions containing the copolymer (monitored by the thiobarbituric acid assay[30]) were pooled, concentrated, and desalted by passage through a column (1.6 × 70) of Bio-Gel P-2 in water. Appropriate fractions were pooled and lyophilized to afford the copolymer **17** (20 mg) as an amorphous powder.

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