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Synthesis of a tetrasaccharide of the genus-specific lipopolysaccharide epitope of *Chlamydia*

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ABSTRACT

Allyl 2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-β-D-glucopyranoside was coupled with methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosyl bromide)onate (**1**) to give a good yield of the α-(2→6)-linked disaccharide, isolated after deacetylation and regioselective conversion into the corresponding 7′,8′-*O*-carbonyl or 7′,8′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl) derivatives, respectively. Subsequent glycosylation with **1** gave a high yield of the α- and β-(2″→4′)-linked trisaccharide derivatives **16** and **18**, whereas block synthesis using the α-(2→8)-linked Kdo-disaccharide bromide derivative **19** afforded a low yield of the corresponding α- and β-(2″→4′)-linked tetrasaccharide derivatives **20** and **22**. Removal of the protecting groups furnished the disaccharide allyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-β-D-glucopyranoside, the trisaccharide allyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→4)-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-β-D-glucopyranoside, and the tetrasaccharide allyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→8)-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→4)-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-β-D-glucopyranoside in high yield. Copolymerization of the allyl glycosides with acrylamide gave artificial polyvalent haptens suitable for defining epitope specificities of monoclonal antibodies directed against *Chlamydia* lipopolysaccharides.

INTRODUCTION

Chlamydia species are unique Gram-negative organisms responsible for widespread animal and human diseases[1]. One of the major surface antigens is a glycolipid[2,3], which shares antigenic determinants with lipopolysaccharides from enterobacterial deep rough mutants of the Re chemotype [containing an α-(2→4)-linked Kdo-disaccharide as the core-constituent][3–6]. In addition to this cross-reactive determinant, a *Chlamydia*-specific epitope has been detected serologically with a monoclonal antibody[7].

Further structural investigations on *Chlamydia* lipopolysaccharides using recombinant strains of *Salmonella minnesota* expressing the *Chlamydia*-specific epitope[8,9]

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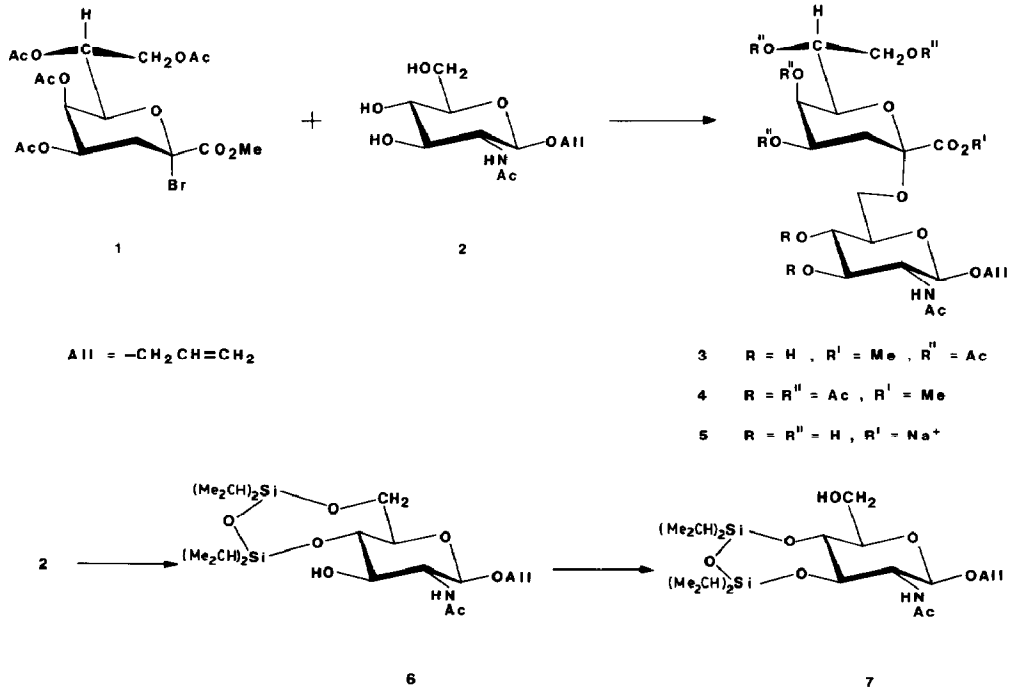
revealed the presence of a Kdo-trisaccharide[10] having the sequence[11] α-Kdo-(2→8)-α-Kdo-(2→4)-α-Kdo.

Within our goal to define epitope specificities of monoclonal antibodies against the inner-core region of lipopolysaccharides[12,13], we report herein the synthesis of allyl glycosides and poly(acrylamide) copolymers[14,15] containing α-Kdo*p*-(2→6)-β-D-GlcNAc, α-Kdo*p*-(2→4)-α-Kdo*p*-(2→6)-β-D-GlcNAc, and α-Kdo*p*-(2→8)-α-Kdo*p*-(2→4)-α-Kdo*p*-(2→6)-β-D-GlcNAc residues.

RESULTS AND DISCUSSION

Reaction of the unprotected allyl glycoside[16] **2** with crystalline methyl (4,5,7,8-tetra-*O*-acetyl-α-D-*manno*-2-octulopyranosyl bromide)onate[17] (**1**) under Helferich conditions [mercury(II) cyanide, *N*,*N*-dimethylformamide, and acetonitrile] gave the α-(2→6)-linked disaccharide derivative **3** with high regio- and stereo-selectivity, and acetylation afforded **4** in 50% overall yield. The α-D-anomeric configuration of the octulopyranosylonate residue was deduced from the downfield signal attributable to H-4′ in a bulk of signals between δ 5.38 and 5.18 in the 250-MHz, ^{1}H-n.m.r. spectrum[18]. Deprotection by Zemplén deacetylation and subsequent hydrolysis of the methyl ester groups in aqueous sodium hydroxide afforded allyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (**5**) in 86% yield.

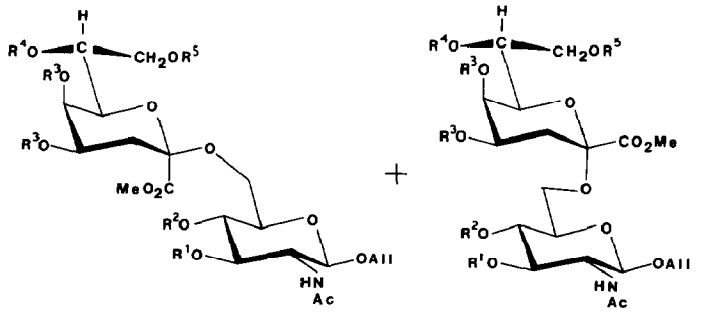
For the preparation of the tri- and tetra-saccharide derivatives **17** and **21**, the glycosyl acceptors **14** and **15** were prepared by regioselective introduction of a 1,1,3,3-



tetraisopropyldisiloxan-1,3-diyl[19–21] or carbonate group, respectively. Reaction of **2** with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine gave a 97% yield of the 4,6-silyl ether **6**, which in turn was isomerized[22,23] into the corresponding 3,4-*O*-substituted compound **7** (51% yield). Glycosylation of **7** with the bromide derivative **1** in acetonitrile in the presence of mercury(II) cyanide and molecular sieves 4A, gave a 4:7 mixture of the β- and α-D-(2→6)-linked disaccharide derivatives **8** and **12** in 64% yield. Promotion of the reaction with 3:1 mercury(II) cyanide–mercury(II) bromide in nitromethane resulted in the favored formation of the α-D isomer **12** (α-to-β ratio 13:1), albeit in lower yield (48%). When 1:1 dichloromethane–toluene was used as solvent, the β-D isomer **8** was obtained as the major component (α-to-β ratio 1:2, 40% yield). Since the isomers **8** and **12** could not be separated by column chromatography on silica gel, the mixture was subjected to Zemplén deacetylation to give a 92% yield of **9** and **13**. Subsequent reaction with 1.1 molar equiv. of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine afforded, in 16 and 45% yield, respectively, the crystalline 7,8-silyl ether derivatives **10** and **14**, which were separated by column chromatography. The anomeric configuration of the isomers was deduced from the ^{1}H-n.m.r. chemical shift difference between H-3*e* and H-3*a* ($\delta_{H\text{-}3e} - \delta_{H\text{-}3a} \sim 0.62$ for **10**, $\delta_{H\text{-}3e} - \delta_{H\text{-}3a} \sim 0.32$ for **14**). Alternatively, conversion of **9** and **13** into the crystalline 7,8-*O*-carbonyl derivatives **11** and **15** was accomplished by the action of trichloromethyl chloroformate[24] in pyridine in 55% yield.

Glycosylation of **14** with the bromide derivative **1** under Helferich conditions [6:1 mercury(II) cyanide–mercury(II) bromide in acetonitrile] afforded the α- and β-D-(2″→4′)-linked trisaccharide derivatives **16** and **18** in 36 and 55% yield, respectively. The α-D-anomeric configuration of **16** was based on the chemical-shift value of the signal attributable to H-4″ (δ 5.22), whereas the corresponding signal for compound **18** occurred at δ 4.82.

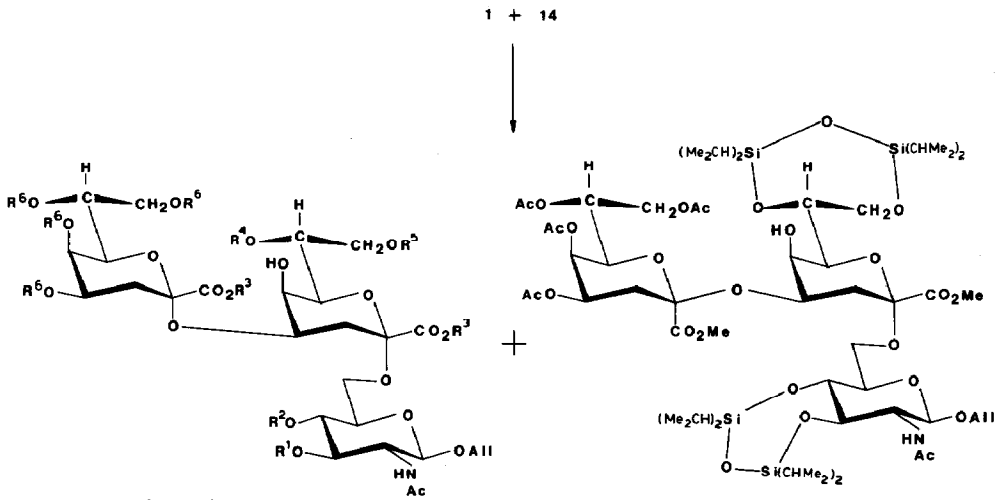
Deprotection of **16** with tetrabutylammonium fluoride[25], followed by deacetyla-



8 R^1, R^2 = TIPDSi; $R^3 = R^4 = R^5$ = Ac
9 R^1, R^2 = TIPDSi; $R^3 = R^4 = R^5$ = H
10 $R^1, R^2 = R^4, R^5$ = TIPDSi; R^3 = H
11 R^1, R^2 = TIPDSi; R^3 = H, R^4, R^5 = =C=O

12 R^1, R^2 = TIPDSi; $R^3 = R^4 = R^5$ = Ac
13 R^1, R^2 = TIPDSi; $R^3 = R^4 = R^5$ = H
14 $R^1, R^2 = R^4, R^5$ = TIPDSi; R^3 = H
15 R^1, R^2 = TIPDSi; R^3 = H, R^4, R^5 = =C=O

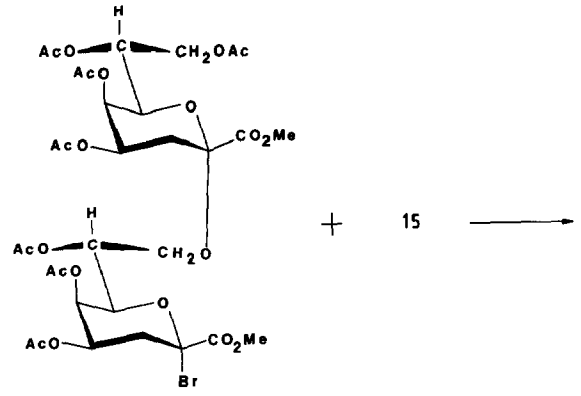
TIPDSi = $-Si(CHMe_2)_2OSi(CHMe_2)_2-$



tion in methanolic sodium methoxide, and deesterification in aqueous sodium hydroxide gave allyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→4)-*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (**17**) in 93% yield. Glycosylation of **14** with the previously described[11] disaccharide bromide derivative **19** in acetonitrile–mercury(II) cyanide gave a mixture of tetrasaccharide derivatives in ~10% yield, which could not be resolved by column chromatography on silica gel. Reaction of the 7′,8′-*O*-carbonyl derivative **15** with **19** using 3:1 mercury(II) cyanide–mercury(II) bromide as catalyst gave an easily separable 3:1 mixture of the α- and β-D-(2″→4′)-linked tetrasaccharide derivatives **20** and **22** in 16% yield. The 360-MHz, ^{1}H-n.m.r. spectra were compatible with the structures assigned; the anomeric configuration of the respective octulopyranosylonate residue was deduced from the upfield shift of the H-4″ signal in the β-D isomer **22** (δ 4.73), whereas the corresponding signal for the α-D isomer **20** occurred in a bulk of signals between δ 5.28 and 5.16.

Deprotection of **20** was accomplished similarly to the preparation of trisaccharide derivative **17** to give allyl *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→8)-*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→4)-*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (**21**) in 89% yield. The purity of **21** was ascertained by ^{13}C-n.m.r. spectroscopy (Fig. 1). The proton-decoupled, ^{13}C-n.m.r. data of **5** and **17** compared favorably with those of reference compounds[26–29], whereas the chemical shift values of **21** are in close agreement with those of a pentasaccharide derivative isolated from *Chlamydia* lipopolysaccharides[30] (Table I).

The allyl glycosides **5**, **17**, and **21** were copolymerized according to the conditions given by Hořejší *et al.*[30], with 4 molar equivs. of acrylamide in the presence of 1,2-bis



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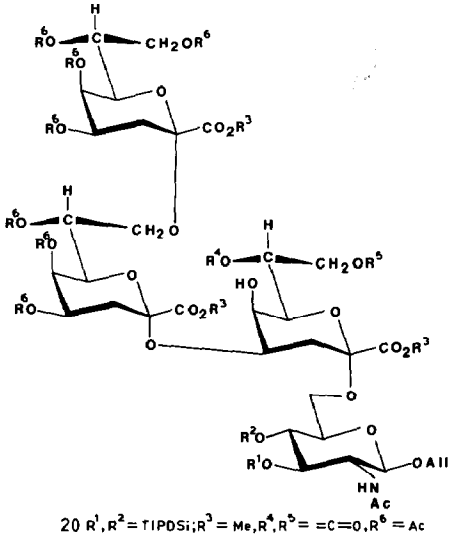
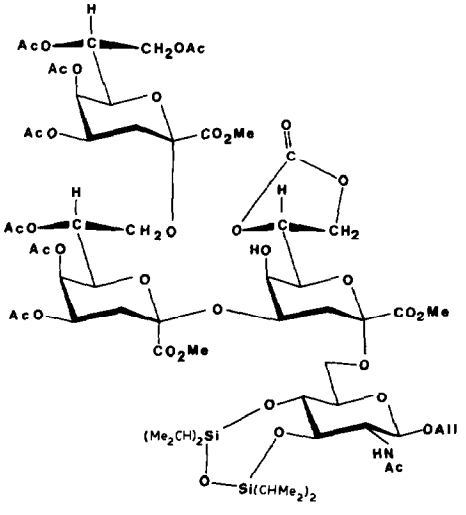


20 R^1, R^2 = TIPDSi; R^3 = Me, R^4, R^5 = =C=O, R^6 = Ac

21 $R^1 = R^2 = R^4 = R^5 = R^6$ = H, R^3 = Na^+



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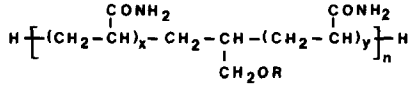

x + y = 19 ± 2

n = 100–150

23 R = α-Kdo-(2→6)-β-D-GlcNAc-(1→

24 R = α-Kdo-(2→4)-α-Kdo-(2→6)-β-D-GlcNAc-(1→

25 R = α-Kdo-(2→8)-α-Kdo-(2→4)-α-Kdo-(2→6)-β-D-GlcNAc-(1→

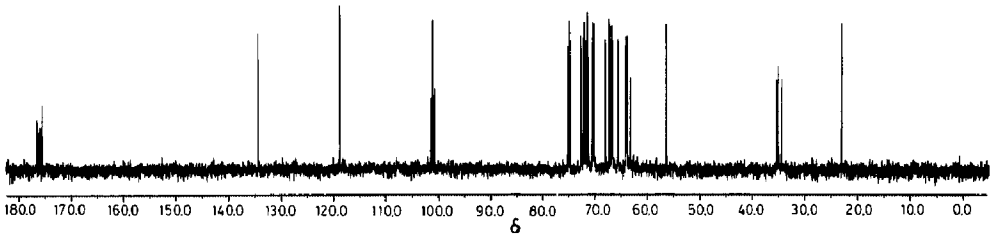

Fig. 1. ^{13}C-N.m.r. spectrum of compound **21**.

TABLE I

Empirical assignment of ^{13}C-n.m.r.-chemical shifts (δ) of compounds **5**, **17**, and **21**

Carbon atom	**5**	**17**	**21**
1	101.15	101.10	101.35
2	56.45	56.39	56.41
3	74.92	75.05[a]	74.82
4	70.46	70.41[b]	70.41[a]
5	75.14	75.10[a]	75.11
6	63.01	62.40	63.21
1′	175.82	175.74	175.77
2′	100.82	100.69	101.00
3′	35.05	34.09	34.30
4′	67.04	69.21	71.73[b]
5′	67.27	65.01	65.60
6′	72.52	72.35	72.13[c]
7′	71.49	71.08	71.36[b]
8′	64.28	64.25[c]	64.03[d]
1″		176.50	176.36[e]
2″		100.09	100.56[f]
3″		35.33	35.04
4″		66.77	66.69[g]
5″		66.99	67.96[h]
6″		73.30	72.62
7″		70.54[b]	70.47[a]
8″		64.21[c]	64.15[d]
1‴			175.99[e]
2‴			100.69[f]
3‴			35.34
4‴			66.79[g]
5‴			67.24[h]
6‴			72.03[c]
7‴			70.14[a]
8‴			63.88[d]
OCH_2	71.49	71.28	71.42
–CH=	134.44	134.18	134.26
CH_2=	119.00	118.77	118.67
CH_3	23.11	22.98	22.97
–NHC=O	175.50	175.40	175.39

[a–h] Assignments within a column may be reversed.

(dimethylamino)ethane and ammonium peroxosulfate to give the linear copolymers **23**, **24**, and **25**, respectively (having a composition of x + y = 19 ±2 and a degree of polymerization of n = 100–150, corresponding to mol. wts. between 60 000 and 100 000). The copolymers were isolated, with recovery of unreacted allyl glycosides by passage through Sephadex G-50 and desalting on Bio-Gel P-2. Immunochemical results obtained with these multivalent haptens will be described elsewhere[31].

EXPERIMENTAL

General methods. — Melting points were determined with a Kofler hot stage and are uncorrected. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. ^{1}H-n.m.r. spectra were recorded with Bruker WM-250 or AM 360 L instruments and tetramethylsilane as the internal standard; coupling constants are first order. ^{13}C-N.m.r. spectra were recorded at 90.6 MHz for solutions in deuterium oxide at 24°; the instrument was operated in the F.t. mode with complete proton-decoupling; chemical shifts (δ) are given from the signal of internal acetonitrile (δ 1.70). T.l.c. was performed on Merck precoated plates (5 × 10 cm, layer thickness 0.25 mm, Silica Gel 60 F_{254}); spots were detected by spraying with an anisaldehyde–H_2SO_4 reagent[32]. Column chromatography was performed on Merck Lichroprep columns (size *A*, 24 × 1; *B*, 31 × 2.5; and *C*, 44 × 3.7 cm; silica gel 40–63 μm) under pressure (0.2 MPa). Elemental analyses were performed by Dr. J. Zak, Mikroanalytisches Laboratorium am Institut für Physikalische Chemie, Universität Wien.

Allyl O-*(methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy*-α-D-manno-*2-octulopyranosylonate)*-*(2→6)*-*2-acetamido-3,4-di*-O-*acetyl-2-deoxy*-β-D-*glucopyranoside* (**4**). — A solution of **1** (500 mg, 1.04 mmol, crystallized from dichloromethane–hexane, m.p. 88–98° with dec.) in dry acetonitrile (5 mL) was added dropwise over a period of 300 min to a suspension of **2** (261 mg, 1.00 mmol), mercury(II) cyanide (300 mg, 1.19 mmol), and molecular sieves 4A (1 g) in 1:1 acetonitrile–*N*,*N*-dimethylformamide (100 mL). After being stirred for 72 h at room temperature, the mixture was diluted with ethyl acetate (100 mL) and filtered over Celite. The organic layer was washed successively with 10% aqueous KI solution, saturated aqueous $NaHCO_3$ solution, dried (Na_2SO_4), and taken to dryness. The residue was purified on a column of silica gel *B* (ethyl acetate → 10:1 ethyl acetate–methanol) to give **3** (330 mg, 50%) as a syrup which was dissolved in pyridine (7 mL) and treated with acetic anhydride (1 mL) and a catalytic amount of 4-dimethylaminopyridine at 0°. The solution was stirred for 12 h at room temperature, the solvent was removed *in vacuo*, and a solution of the residue in dichloromethane (50 mL) was washed with saturated aqueous $NaHCO_3$ solution and dried (Na_2SO_4). Evaporation of the solvent and purification of the residue on a column of silica gel furnished **4** (271 mg, 73%), amorphous solid, m.p. 105–110° (ethyl acetate–hexane), $[\alpha]_D^{20}$ +46° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.90 (m, 1 H, =CH–), 5.56 (d, 1 H, $J_{2,NH}$ ~9.0 Hz, NH), 5.38–5.18 (m, 6 H, including H-3,4′,5′,7′, $=CH_2$), 4.88 (t, 1 H, $J_{3,4} \sim J_{4,5}$ ~9.5 Hz, H-4), 4.65 (dd, 1 H, $J_{8'a,8'b}$ ~ −12.5, $J_{8'a,7'}$ ~2.5 Hz, H-7′), 4.64 (d, 1 H, $J_{1,2}$ ~8.5 Hz, H-1), 4.38 (m, 1 H, OCH_2), 4.27 (d, 1 H, $J_{6',5'}$ ~1.0, $J_{6',7'}$ ~10.0 Hz, H-6′),

4.13 (m, 1 H, OCH_2), 4.10 (dd, 1 H, $J_{8'b,7'}$ ~4.5 Hz, H-8'b), 3.93 (dt, 1 H, $J_{2,3}$ ~10.5 Hz, H-2), 3.81 (s, 3 H, CO_2CH_3), 3.71 (ddd, 1 H, $J_{5,6a}$ ~2.5, $J_{5,6b}$ ~7.5 Hz, H-5), 3.65–3.52 (m, 2 H, H-6a,6b), 2.14 (s, 3 H), 2.10 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 1.99 (s, 3 H), 1.97 (s, 3 H), and 1.96 (s, 3 H, 6 CH_3CO, NHAc), and 2.15–1.99 (m, 2 H, H-3'*e*,3'*a*).

Anal. Calc. for $C_{32}H_{45}NO_{19}$: C, 50.68; H, 6.23; N, 2.11. Found: C, 50.60; H, 5.83; N, 1.73.

Allyl O-*(sodium 3-deoxy-α-*D-manno-*2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-β-*D-*glucopyranoside* (**5**). — A solution of **4** (57 mg) in dry methanol (5 mL) was treated with 0.1M methanolic sodium methoxide (0.15 mL) for 2 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin and filtered. The residue obtained upon evaporation of the filtrate (37 mg) was dissolved in water (5 mL) and stirred with 0.2M aqueous NaOH (1.5 mL) for 3 h at room temperature. The pH of the solution was adjusted to 8.0 by addition of Dowex 50 (H^+) resin. The resin was removed by filtration and the filtrate was lyophilized. Purification on a column of Bio-Gel P-2 afforded **5** (32 mg, 86%), amorphous solid, $[\alpha]_D^{20}$ +14° (*c* 0.5, water); ^{1}H-n.m.r. (D_2O): δ 5.93 (m, 1 H, =CH–), 5.32 dq, 1 H, $=CH_{2trans}$), 5.27 (dq, 1 H, $=CH_{2cis}$), 4.57 (d, 1 H, $J_{1,2}$ ~8.5 Hz, H-1), 4.33 and 4.17 (m, 2 H, OCH_2), 4.15–3.93 (m, 4 H) and 3.77–3.43 (m, 8 H, H-2,3,4,5,6a,6b,4',5',6',7',8'a,8'b), 2.10 (dd, 1 H, $J_{3'e,4'}$ ~5.0, $J_{3'e,3'a}$ ~13.5 Hz, H-3'*e*), 2.05 (s, 3 H, NHAc), and 1.82 (t, 1 H, $J_{3'a,4'}$ ~13.0 Hz, H-3'*a*).

Anal. Calc. for $C_{19}H_{30}NNaO_{13}\cdot 3H_2O$: C, 40.93; H, 6.51; N, 2.51. Found: C, 40.35; H, 6.50; N, 2.40.

*Allyl 2-acetamido-2-deoxy-4,6-*O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-β-*D-*glucopyranoside* (**6**). — 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (1.4 mL, 4.4 mmol) was added dropwise at 0° to a solution of **2** (400 mg, 1.5 mmol) in pyridine (10 mL). The solution was stirred for 4 h, the reaction quenched by addition of 2-propanol (1 mL), and the stirring continued for 30 min. The solution was evaporated, and the residue was dissolved in dichloromethane (50 mL), extracted with saturated aqueous $NaHCO_3$ solution, and dried (Na_2SO_4). Evaporation gave **6** (750 mg, 97%), m.p. 108–110° (hexane), $[\alpha]_D^{20}$ −4.6° (*c* 1.2, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.90 (m, 1 H, =CH–), 5.72 (d, 1 H, $J_{NH,2}$ ~5.0 Hz, N-H), 5.28 (dq, 1 H, $=CH_{2trans}$), 5.22 (dq, 1 H, $=CH_{2cis}$), 4.59 (d, 1 H, $J_{1,2}$ ~8.0 Hz, H-1), 4.40 (m, 1 H, OCH_2), 4.15–3.78 (m, 6 H, including H-3,4,5,6a,6b, OCH_2), 3.43 (dt, 1 H, $J_{2,3}$ ~9.0 Hz, H-2), 3.21 (d, 1 H, $J_{3,OH}$ ~9.0 Hz, OH), 2.04 (s, 3 H, NHAc), and 1.15–0.95 [m, 28 H, 4 $SiCH(CH_3)_2$].

Anal. Calc. for $C_{23}H_{45}NO_7Si_2$: C, 54.84; H, 9.00; N, 2.78. Found: C, 54.35; H, 9.52; N, 2.40.

*Allyl 2-acetamido-2-deoxy-3,4-*O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-β-*D-*glucopyranoside* (**7**). — A solution of **6** (1.63 g, 3.2 mmol) and dry 4-toluenesulfonic acid (50 mg) in dry *N,N*-dimethylformamide was stirred for 20 h at room temperature. The solution was taken to dryness and the residue was dissolved in dichloromethane (50 mL), washed with saturated aqueous $NaHCO_3$ solution, and dried (Na_2SO_4). The residue obtained upon evaporation of the solvent was purified on a column of silica gel (size *C*, 1:3 toluene–ethyl acetate) to give **7** (830 mg, 51%) as the faster-moving isomer,

colorless needles, m.p. 182–184° (ethyl acetate–hexane), $[\alpha]_D^{20}$ +17° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.90 (m, 1 H, =CH–), 5.58 (d, 1 H, $J_{NH,2}$ ~7.5 Hz, NH), 5.28 (dq, 1 H, $=CH_{2trans}$), 5.20 (dq, 1 H, $=CH_{2cis}$), 5.05 (d, 1 H, $J_{1,2}$ ~8.5 Hz, H-1), 4.32 (m, 1 H, OCH_2), 4.17 (dd, 1 H, $J_{2,3}$ ~10.0, $J_{3,4}$ ~9.0 Hz, H-3), 4.12 (m, 1 H, OCH_2), 3.92 (ddd, 1 H, $J_{6a,6b}$ ~12.0, $J_{6a,5}$ ~3.5, $J_{6a,OH}$ ~6.5 Hz, H-6a), 3.75 (ddd, 1 H, $J_{6b,5}$ ~ $J_{6b,OH}$ ~6.5 Hz, H-6b), 3.63 (dd, 1 H, $J_{4,5}$ ~7.5 Hz, H-4), 3.43 (ddd, 1 H, H-5), 3.26 (dt, 1 H, H-2), 2.01 (t, 1 H, OH), 1.97 (s, 3 H, NHAc), and 1.12–0.91 [m, 28 H, 4 $SiCH(CH_3)_2$].

Anal. Calc. for $C_{23}H_{45}NO_7Si_2$: C, 54.84; H, 9.00; N, 2.58. Found: C, 54.15; H, 8.92; N, 2.58.

Glycosylation of **7**. — A solution of **1** (800 mg, 1.66 mmol) in dry acetonitrile (5 mL) was added dropwise at room temperature during 3 h to a suspension of **6** (692 mg, 1.37 mmol), mercury(II) cyanide (500 mg, 1.98 mmol), and molecular sieves 4A (1 g) in acetonitrile (10 mL). The mixture was stirred for 20 h under N_2, and solids were removed by filtration over Celite and thoroughly washed with dichloromethane (100 mL). The organic layer was extracted successively with 10% aqueous KI solution, and saturated aqueous $NaHCO_3$ solution, and dried (Na_2SO_4). Purification of the residue obtained after removal of solvents on a column of silica gel (size *C*, 1:1 toluene–ethyl acetate) afforded a ~3:2 mixture of the α- and β-D-glycosides **12** and **8** (790 mg, 64%), and of unreacted allyl glycoside **6** (200 mg, 29%).

Deacetylation of **8** *and* **12**. — A solution of a mixture of **8** and **12** (1.06 g) in dry methanol (10 mL) was stirred with 0.1M methanolic sodium methoxide (4 mL) for 3 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and evaporated to give a mixture of **9** and **13** (796 mg, 92%).

Allyl O-*[methyl 3-deoxy-7,8*-O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)* -β-D-manno-*2-octulopyranosylonate]-(2→6)* -*2-acetamido-2-deoxy-3,4*-O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)*-β-D-*glucopyranoside* (**10**) and *allyl* O-*[methyl 3-deoxy-7,8*-O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)*-α-D-manno-*2-octulopyranosylonate]-(2→6)* -*2-acetamido-2-deoxy-3,4*-O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)*-β-D-*glucopyranoside* (**14**). — 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (252 mg, 0.8 mmol) was added at −20° to a solution of a mixture of **9** and **13** (518 mg, 0.7 mmol) in dry pyridine (10 mL). The solution was stirred at room temperature for 15 h, methanol (0.5 mL) was added, and stirring was continued for 30 min. The solvents were evaporated and the residue was subjected to column chromatography on silica gel (*C*) using 1:1 toluene–ethyl acetate as eluent, which gave **14** (312 mg, 45%) as the main product, colorless syrup, $[\alpha]_D^{20}$ +22.5° (*c* 2.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.87 (m, 1 H, =CH–), 5.55 (d, 1 H, $J_{2,NH}$ ~9.0 Hz, NH), 5.25 (dq, 1 H, $=CH_{2trans}$), 5.17 (dq, 1 H, $=CH_{2cis}$), 4.93 (d, 1 H, $J_{1,2}$ ~8.0 Hz, H-1), 4.30–4.22 (m, 2 H), 4.15–3.93 (m, 5 H) and 3.84–3.70 (m, 3 H, H-3,6a,4′,5′,6′,7′,8′a, 8′b,OCH_2), 3.73 (s, 3 H, CO_2CH_3), 3.60–3.41 (m, 2 H, H-6b,5), 3.37 (t, 1 H, $J_{4,5}$ ~ $J_{4,3}$ ~8.0 Hz, H-4), 3.25 (dt, 1 H, $J_{2,3}$ ~10.0 Hz, H-2), 2.83 (d, 1 H, $J_{5',OH}$ ~5.0 Hz, OH-5′), 2.34 (d, 1 H, $J_{4',OH}$ ~10.0 Hz, OH-4′), 2.20 (dd, 1 H, $J_{3'e,3'a}$ ~13.5, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 1.96 (s, 3 H, NHAc), 1.88 (t, 1 H, $J_{3'a,4'}$ ~13.5 Hz, H-3′*a*), and 1.15 −0.87 [m, 56 H, 8 $SiCH(CH_3)_2$].

Anal. Calc. for $C_{44}H_{85}NO_{15}Si_4$: C, 53.90; H, 8.74; N, 1.43. Found: C, 54.36; H, 8.80; N, 1.46.

Further elution gave **10** (110 mg, 16%), colorless syrup, $[\alpha]_D^{20}$ +16° (*c* 1.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.90 (m, 1 H, =CH–), 5.55 (d, 1 H, $J_{2,NH}$ ~8.5 Hz, NH), 5.29 (dq, 1 H, $=CH_{2trans}$), 5.19 (dq, 1 H, $=CH_{2cis}$), 4.92 (d, 1 H, $J_{1,2}$ ~8.5 Hz, H-1), 4.36–4.04 (m, 6 H), 3.98 (br., 1 H, H-5′), 3.77 (s, 3 H, CO_2CH_3), 3.74–3.24 (m, 7 H, including H-2,3,4,5,6a,6b,4′,6′,7′,8′a,8′b, OCH_2), 2.84 (br., 1 H, OH-5′), 2.52 (dd, 1 H, $J_{3'e,3'a}$ ~12.5, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 2.43 (d, 1 H, $J_{4',OH}$ ~9.0 Hz, OH-4′), 1.96 (s, 3 H, NHAc), 1.90 (t, 1 H, $J_{3'a,4'}$ ~12.5 Hz, H-3′*a*), and 1.13–0.89 [m, 56 H, 8 $SiCH(CH_3)_2$].

Anal. Calc. for $C_{44}H_{85}NO_{15}Si_4$: C, 53.90; H, 8.74; N, 1.43. Found: C, 54.12; H, 8.85; N, 1.55.

*Allyl O-(methyl 7,8-*O*-carbonyl-3-deoxy-β-*D*-*manno*-2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-β-*D*-glucopyranoside* (**12**) *and allyl* O-*(methyl 7,8-*O*-carbonyl-3-deoxy-α-*D*-*manno*-2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-β-*D*-glucopyranoside* (**15**). — A solution of trichloromethyl chloroformate (75 μL, 0.60 mmol) in dry oxolane (5 mL) was added dropwise under N_2 at −20° to a solution of a mixture of **9** and **13** (466 mg, 0.63 mmol) in pyridine (10 mL). The solution was stirred for 12 h at room temperature, solvents were removed by evaporation, and the residue was purified by column chromatography on silica gel (size *B*, ethyl acetate) to give **12** (255 mg, 20%) as the faster moving isomer, colorless needles, m.p. 144–148° (dec., ethyl acetate–hexane), $[\alpha_D^{20}$ +33° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.89 (m, 1 H, =CH–), 5.63 (d, 1 H, $J_{NH,2}$ ~8.5 Hz, NH), 5.28 (dq, 1 H, $=CH_{2trans}$), 5.18 (dq, 1 H, $=CH_{2cis}$), 4.96 (ddd, 1 H, $J_{7',8'a}$ ~6.5, $J_{7',8'b}$ ~8.5, $J_{7',6'}$ ~5.5 Hz, H-7′), 4.80 (d, 1 H, $J_{1,2}$ ~8.5 Hz, H-1), 4.76 (dd, 1 H, $J_{8'a,8'b}$ ~6.5 Hz, H-8′a), 4.52 (t, 1 H, H-8′b), 4.32 (m, 1 H, OCH_2), 4.09 (m, 1 H, OCH_2), 4.07 (dd, 1 H, $J_{6a,6b}$ ~12.0, $J_{6a,5}$ ~1.5 Hz, H-6a), 3.97 (dd, 1 H, $J_{2,3}$ ~10.0, $J_{3,4}$ ~8.0 Hz, H-3), 3.95 (dd, 1 H, $J_{6',5'}$ ~1.0 Hz, H-6′), 3.89 (br. s, 1 H, H-5′), 3.79 (m, 4 H, CO_2CH_3, H-4′), 3.61 (dd, 1 H, $J_{6b,5}$ ~5.5 Hz, H-6b), 3.60 (t, 1 H, $J_{4,5}$ ~9.0 Hz, H-4), 3.50–3.36 (m, 2 H, H-2,5), 3.03 (d, 1 H, J_{OH} ~6.5 Hz, OH), 2.42 (dd, 1 H, $J_{3'e,3'a}$ ~13.0, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 2.05 (t, 1 H, $J_{3'a,4'}$ ~13.0 Hz, H-3′*a*), 2.05 (br. s, 1 H, OH), 1.96 (s, 3 H, NHAc), and 1.10–0.90 [m, 28 H, 4 $SiCH(CH_3)_2$].

Anal. Calc. for $C_{33}H_{57}NO_{15}Si_2$: C, 51.88; H, 7.40; N, 1.83. Found: C, 51.58; H, 7.42; N, 2.09.

Further elution of the column gave **15** (455 mg, 35%), colorless prisms, m.p. 122–124° (dec., ethyl acetate–hexane), $[\alpha]_D^{20}$ +50° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.87 (m, 1 H, =CH–), 5.82 (d, 1 H, $J_{NH,2}$ ~7.5 Hz, NH), 5.26 (dq, 1 H, $=CH_{2trans}$), 5.19 (dq, 1 H, $=CH_{2cis}$), 4.98 (ddd, 1 H, $J_{7',8'a}$ ~8.0, $J_{7',8'b}$ ~8.5, $J_{7',6'}$ ~6.0 Hz, H-7′), 4.91 (d, 1 H, $J_{1,2}$ ~8.5 Hz, H-1), 4.70 (t, 1 H, $J_{8'a,8'b}$ ~8.0 Hz, H-8′a), 4.59 (t, 1 H, H-8′b), 4.28–4.02 (m, 5 H, H-6a,4′,6′,OCH_2), 3.89 (br. s, 1 H, H-5′), 3.86 (t, 1 H, $J_{3,4}$ ~ $J_{3,2}$ ~9.0 Hz, H-3), 3.77 (s, 3 H, CO_2CH_3), 3.60–3.46 (m, 2 H, H-5,6b), 3.41 (t, 1 H, $J_{4,5}$ ~8.0 Hz, H-4), 3.32 (dt, 1 H, H-2), 3.20 (br. s, 1 H, OH), 3.05 (br. s, 1 H, OH), 2.17 (dd, 1 H, $J_{3'e,4'}$ ~5.0, $J_{3'e,3'a}$ ~13.5 Hz, H-3′*e*), 1.95 (s, 3 H, NHAc), 1.95 (t, 1 H, $J_{3'a,4'}$ ~13.0 Hz, H-3′*a*), and 1.10–0.90 [m, 28 H, 4 $SiCH(CH_3)_2$].

Anal. Calc. for $C_{33}H_{57}NO_{15}Si_2$: C, 51.88; H, 7.40; N, 1.83. Found: C, 51.69; H, 7.38; N, 2.09.

Allyl O-*(methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy*-α-D-manno-*2-octulopyranosylonate)-(2→4)*-O-*[methyl-3-deoxy-7,8*-O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)*-α-D-man-no-*2-octulopyranosylonate]-(2→6)-2-acetamido-2-deoxy-3,4*-O-*(1,1,3,3-tetraisopropyldi-siloxan-1,3-diyl)*-β-D-*glucopyranoside* (**16**) *and allyl* O-*(methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy*-β-D-manno-*2-octulopyranosylonate)-(2→4)*-O-*[methyl 3-deoxy-7,8*-O- *(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)* -α-D-manno-*2-octulopyranosylonate]-(2→6)-2-acetamido-2-deoxy-3,4*-O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)*-β-D-*glucopyranoside* (**18**). A solution of **1** (190 mg, 0.39 mmol) in acetonitrile (2 mL) was added under N_2 to a suspension of **14** (94 mg, 0.10 mmol), mercury(II) cyanide (120 mg, 0.48 mmol), mercury(II) bromide (28 mg, 0.08 mmol), and molecular sieves 4A (500 mg) in acetonitrile (7 mL). The mixture was stirred for 48 h at room temperature, diluted with dichloromethane (100 mL), and filtered over Celite. The organic layer was washed successively with 10% aqueous KI solution and saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated. Purification of the residue on silica gel (size *B*, 3:2 toluene–ethyl acetate) gave **16** and **18** (R_F ~0.53, 1:1 toluene–ethyl acetate), which were further purified by chromatography using 2:1 toluene–ethyl acetate and 3:2 hexane–ethyl acetate as eluents to give **16** (48 mg, 36%) and **18** (73 mg, 55%).

Compound **16**. Colorless syrup, $[\alpha]_D^{20}$ +49° (*c* 1.3, chloroform); ^{1}H-n.m.r. (360 MHz, $CDCl_3$): δ 5.82 (m, 2 H, NH, =CH–), 5.32 (br. s, 1 H, H-5″), 5.23 (dq, 1 H, $=CH_{2trans}$), 5.22 (ddd, $J_{4'',5''}$ ~3.0, $J_{4'',3''e}$ ~5.0, $J_{4'',3''a}$ ~12.5 Hz, H-4″), 5.11 (dq, 1 H, $=CH_{2cis}$), 5.11 (m, 1 H, H-7″), 4.60 (dd, 1 H, $J_{8''a,7''}$ ~2.5, $J_{8''a,8''b}$ ~12.0 Hz, H-8″a), 4.55 (d, 1 H, $J_{1,2}$ ~8.5 Hz, H-1), 4.23–4.09 (m, 5 H), 3.77 (dd, 1 H, $J_{6'',5''}$ ~1.5, $J_{6'',7''}$ ~10.0 Hz, H-6″), 3.95 (dd, 1 H, $J_{8''b,7''}$ ~6.5 Hz, H-8″b), 3.78 (br. s, 1 H, H-5′), 3.75 (m, 1 H), 3.68 (s, 3 H) and 3.65 (s, 3 H, CO_2CH_3), 3.70–3.33 (m, 6 H), 3.28 (dt, 1 H, $J_{2,NH}$ ~ $J_{2,3}$ ~8.5 Hz, H-2), 2.26 (dd, 1 H, $J_{3'e,3'a}$ ~3.0, $J_{3'e,4'}$ ~5.0 Hz, H-3*e*′), 2.13–1.88 (m, 3 H, H-3′*a*,3″*e*,3″*a*), 2.08 (s, 3 H), 2.00 (s, 3 H), 1.93 (s, 3 H), 1.91 (s, 3 H) and 1.89 (s, 3 H, 4 CH_3CO, NHAc), and 1.05–0.80 [m, 56 H, 8 $SiCH(CH_3)_2$].

Anal. Calc. for $C_{61}H_{107}NO_{26}Si_4$: C, 52.98; H, 7.80; N, 1.01. Found: C, 53.15; H, 7.96; N, 1.13.

Compound **18**. Colorless syrup, $[\alpha]_D^{20}$ +41° (*c* 1.0, chloroform); ^{1}H-n.m.r. (360 MHz, $CDCl_3$): δ 5.80 (m, 1 H, =CH–), 5.55 (d, 1 H, $J_{NH,2}$ ~7.8 Hz, H-2), 5.22 (dq, 1 H, $=CH_{2trans}$), 5.21 (br. s, 1 H, H-5″), 5.10 (dq, 1 H, $=CH_{2cis}$), 4.98 (dt, 1 H, $J_{7'',6''}$ ~9.0, $J_{7'',8''a}$ ~3.3 Hz, $J_{7'',8''b}$ ~3.0 Hz, H-7″), 4.87 (d, 1 H, $J_{1,2}$ ~8.4 Hz, H-1), 4.82 (ddd, 1 H, $J_{4'',3e''}$ ~5.0, $J_{4'',3''a}$ ~13.0, $J_{4'',5''}$ ~3.0 Hz, H-4″), 4.26–3.58 (m, 10 H), 3.73 (s, 3 H) and 3.64 (s, 3 H, CO_2CH_3), 3.50–3.37 (m, 2 H, H-5,6b), 3.27 (t, 1 H, $J_{3,4}$ ~ $J_{4,5}$ ~8.5 Hz, H-4), 3.17 (dt, 1 H, H-2), 2.31 (dd, 1 H, $J_{3''e,3''a}$ ~12.5 Hz, H-3″*e*), 2.09 (t, 1 H, H-3″*a*), 2.06 (dd, 1 H, $J_{3'e,3'a}$ ~13.0, $J_{3'e,4'}$ ~4.9 Hz, H-3′*e*), 2.02–1.90 (m, 1 H, H-3′*a*), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.91 (s, 6 H), 1.88 (s, 3 H, 4 CH_3CO, NHAc), and 1.04–0.90 [m, 56 H, 8 $SiCH(CH_3)_2$].

Anal. Calc. for $C_{61}H_{107}NO_{26}Si_4$: C, 52.98; H, 7.80; N, 1.01. Found: C, 52.56; H, 7.54; N, 1.02.

Allyl O-*(sodium 3-deoxy*-α-D-manno-*2-octulopyranosylonate)-(2→4)*-O-*(sodium*

*3-deoxy-α-*D-manno-*2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-β-*D-*glucopyranoside* (**17**). — A solution of **16** (30 mg) in dry oxolane (10 mL) was treated with tetrabutylammonium fluoride (30 mg) for 50 min at room temperature. The residue obtained upon evaporation was dissolved in dry methanol (10 mL) and stirred with 0.1M methanolic sodium methoxide (1 mL) for 2 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and taken to dryness. A solution of the residue in water (15 mL) was stirred with 0.2M aqueous NaOH (1 mL) for 2 h at room temperature. The pH of the solution was adjusted to 8.2 by addition of Dowex 50 (H^+) resin. The resin was removed by filtration, and the filtrate was concentrated and purified on a column of Bio-Gel P-2 to give **17** (14.9 mg, overall yield 93%), amorphous solid, $[\alpha]_D^{20}$ +43° (*c* 0.5, water); ^{1}H-n.m.r. (360 MHz, D_2O): δ 5.80 (m, 1 H, =CH–), 5.20 (dq, 1 H, $=CH_{2trans}$), 5.14 (dq, 1 H, $=CH_{2cis}$), 4.46 (d, 1 H, $J_{1,2}$ ~9.0 Hz, H-1), 4.21 (m, 1 H, OCH_2), 4.06–3.40 (m, 8 H, H-3,4,5,6a,6b,4′,5′,6′,7′,8′a,8′b, 4″,5″,6″,7″,8″a,8″b, OCH_2), 2.03 (dd, 1 H, $J_{3e'',3a''}$ ~13.0, $J_{3''e,4''}$ ~5.0 Hz, H-3*e*″), 1.92 (s, 3 H, NHAc), 1.91 (dd, 1 H, $J_{3'e,4'}$ ~5.0, $J_{3'e,3a'}$ ~12.5 Hz, H-3′*e*), 1.80 (t, 1 H, $J_{3'a,4'}$ ~12.5 Hz, H-3′*a*), and 1.64 (t, 1 H, $J_{3''a,4''}$ ~12.5 Hz, H-3″*a*).

Allyl O-*(methyl 4,5,7,8-tetra-*O-*acetyl-3-deoxy-α-*D-manno-*2-octulopyranosylonate)-(2→8)-*O-*(methyl 4,5,7-tri-*O-*acetyl-3-deoxy-β-*D-manno-*2-octulopyranosylonate)-(2→4)-*O-*(methyl 7,8-*O-*carbonyl-3-deoxy-α-*D-manno-*2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-3,4-*O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-β-*D-*glucopyranoside* (**22**) *and allyl* O-*(methyl 4,5,7,8-tetra-*O-*acetyl-3-deoxy-α-*D-manno-*2-octulopyranosylonate)-(2→8)-*O-*(methyl 4,5,7-tri-*O-*acetyl-α-*D-manno-*2-octulopyranosylonate)-(2→4)-*O-*(methyl 7,8-*O-*carbonyl-3-deoxy-α-*D-manno-*2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-3,4-*O-*(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-β-*D-*glucopyranoside* (**20**). — A solution of **19** (203 mg, 0.24 mmol) in dry acetonitrile (2 mL) was added to a suspension of **15** (140 mg, 0.18 mmol), mercury(II) cyanide (40 mg, 0.16 mmol), mercury(II) bromide (57 mg, 0.16 mmol), and molecular sieves 4A (0.5 g) in acetonitrile (5 mL). The mixture was stirred for 48 h at room temperature, ethyl acetate (100 mL) was added, and the mixture was filtered over Celite. The filtrate was washed sequentially with 5% aqueous KI solution, saturated aqueous $NaHCO_3$ solution, and dried ($MgSO_4$). The residue obtained upon evaporation was subjected to column chromatography on silica gel (size *B*, 1:3 toluene–ethyl acetate) to give **22** (12 mg, 4%) as the faster migrating isomer, $[\alpha]_D^{20}$ +63.5° (*c* 0.5, chloroform); ^{1}H-n.m.r. (360 MHz, $CDCl_3$): δ 5.79 (m, 1 H, =CH–), 5.32–5.04 (m, 8 H, H-7′,5″,7″,4‴,5‴,7‴, $=CH_2$), 4.86 (d, 1 H, $J_{1,2}$ ~8.5 Hz, H-1), 4.77 (t, 1 H, $J_{8'a,8'b}$ ~ $J_{8'a,7'}$ ~8.0 Hz, H-8′a), 4.73 (ddd, 1 H, $J_{3''e,4''}$ ~5.0, $J_{4'',5''}$ ~3.0, $J_{4'',3''a}$ ~13.0 Hz, H-4″), 4.52 (dd, 1 H, $J_{8'''a,8'''b}$ ~12.5, $J_{8'''a,7'''}$ ~2.5 Hz, H-8‴a), 4.50 (t, 1 H, $J_{8'b,7'}$ ~8.5 Hz, H-8′b), 4.25–3.41 (m, 14 H, H-4,5,6a,6b,4′,5′,6′,6″,8″a,8″b,6‴,8‴b, OCH_2), 3.77 (s, 3 H), 3.75 (s, 3 H) and 3.69 (s, 3 H, 3 CO_2CH_3), 3.31 (t, 1 H, $J_{3,4}$ ~8.0 Hz, H-4), 3.13 (t, 1 H, $J_{2,3}$ ~8.5 Hz, H-2), 2.28 (dd, 1 H, $J_{3''e,3''a}$ ~12.5 Hz, H-3″*e*), 2.10 (d, 1 H, $J_{5',OH}$ ~4.0, OH), 2.09 (dd, 1 H, *J* ~5.0, *J* ~12.5, H-3e′ or H-3‴*e*), 2.06–1.87 (m, 4 H, H-3′*e* or H-3‴*e*, 3′*a*,3″*a*,3‴*a*), 2.05 (s, 6 H), 2.03 (s, 3 H), 2.02 (s, 3 H), 1.95 (s, 3 H), 1.92 (s, 3 H) and 1.89 (s, 6 H, 7 CH_3CO, NHAc), and 1.10–0.90 [m, 28 H, 4 $SiCH(CH_3)_2$].

Anal. Calc. for $C_{65}H_{99}NO_{36}Si_2$: C, 51.14; H, 6.54; N, 0.92. Found: C, 51.71; H, 6.45; N, 0.91.

Further elution of the column gave **20** (33 mg, 12%), colorless syrup, $[\alpha]_D^{20}$ +68° (*c* 0.8, chloroform); ^{1}H-n.m.r. (360 MHz, $CDCl_3$): δ 5.96 (d, 1 H, $J_{NH,2}$ ~7.5 Hz, NH), 5.87 (m, 1 H, =CH–), 5.33 (br. s, 1 H, H-5″), 5.28–5.16 (m, 7 H, H-4″,7″,4‴,5‴,7‴, $=CH_2$), 5.09 (d, 1 H, $J_{1,2}$ ~8.5 Hz, H-1), 4.93 (dd, 1 H, $J_{7',8'a}$ ~7.9, $J_{7',6'}$ ~4.6, $J_{7',8'b}$ ~8.0 Hz, H-7′), 4.70 (t, 1 H, $J_{8'a,8'b}$ ~8.4 Hz, H-8′a), 4.55 (t, 1 H, H-8′b), 4.54 (dd, 1 H, $J_{8'''a,7'''}$ ~2.1, $J_{8'''a,8'''b}$ ~12.0 Hz, H-8‴a), 4.32–4.04 (m, 8 H, H-3,6a,4′,6′,6″,8‴b,OCH_2), 3.94 (br. s, 1 H, H-5′), 3.87 (dd, 1 H, $J_{6''',7'''}$ ~9.1, $J_{6''',5'''}$ ~1.0 Hz, H-6‴), 3.80 (m, 1 H, H-6a), 3.80 (s, 6 H), and 3.74 (s, 3 H, 3 CO_2CH_3), 3.68–3.62 (m, 2 H, H-8″a,8″b), 3.54–3.45 (m, 2 H, H-5,6b), 3.30 (t, 1 H, $J_{3,4}$ ~ $J_{4,5}$ ~8.6 Hz, H-4), 3.07 (dd, 1 H, $J_{2,3}$ ~10.0 Hz, H-2), 2.19–1.95 (m, 6 H, H-3′*e*,3′*a*,3″*e*,3″*a*, 3‴*e*, 3‴*a*), 2.09 (s, 3 H), 2.07 (s, 6 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 1.98 (s, 3 H), 1.96 (s, 3 H) and 1.93 (s, 3 H, 7 CH_3CO, NHAc), and 1.04–0.97 [m, 28 H, 4 $SiCH(CH_3)_2$].

Anal. Calc. for $C_{65}H_{99}NO_{36}Si_2$: C, 51.14; H, 6.54; N, 0.92. Found: C, 50.73; H, 6.33; N, 0.74.

Allyl O-*(sodium 3-deoxy-*α-D-manno-*2-octulopyranosylonate)-(2→8)*-O-*(sodium 3-deoxy-*α-D-manno-*2-octulopyranosylonate)-(2→4)*-O-*(sodium 3-deoxy-*α-D-manno-*2-octulopyranosylonate)-(2→6)-2-acetamido-2-deoxy-*β-D-*glucopyranoside* (**21**). — A solution of **20** (21.4 mg) in dry oxolane (5 mL) was stirred with tetrabutylammonium fluoride (25 mg) for 45 min at room temperature. After evaporation of the solvent, methanol (5 mL) and 0.1M methanolic sodium methoxide (0.5 mL) were added and stirring was continued for 2 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and evaporated. A solution of the residue in water (5 mL) was stirred with 0.2M aqueous NaOH (0.5 mL) for 3 h at room temperature. The pH of the solution was adjusted to 8.2 by addition of Dowex 50 (H^+) resin. Filtration and evaporation gave a syrup (**21**), which was desalted on a column of Bio-Gel P-2 (yield 12.2 mg, 89%), amorphous solid, $[\alpha]_D^{20}$ +49° (*c* 0.7, water); ^{1}H-n.m.r. (360 MHz, D_2O): δ 5.81 (m, 1 H, =CH–), 5.20 (dq, 1 H, $=CH_{2trans}$), 5.14 (dq, 1 H, $=CH_{2cis}$), 4.47 (d, 1 H, $J_{1,2}$ ~8.5 Hz, H-1), 4.18 (m, 1 H, OCH_2), 4.08–3.90 (m, 8 H, including H-4′,5′,4″,5″,7″,4‴,5‴, OCH_2), 3.84–3.76 (m, 4 H), 3.66 (dd, 1 H, $J_{5'',6''}$ ~1.0, $J_{6'',7''}$ ~5.0 Hz, H-6″), 3.63–3.35 (m, 12 H), 3.26 (t, 1 H, *J* ~9.2 Hz), 1.99 (dd, 1 H, $J_{3''e,4''}$ ~5.0, $J_{3''e,3''a}$ ~13.0 Hz, H-3″*e*), 1.91 (s, 3 H, NHAc), 1.91 (dd, 2 H, H-3′*e*,3‴*e*), 1.77 (t, 1 H, $J_{3'a,4'}$ ~12.3 Hz, H-3′*a*), 1.68 (t, 1 H, $J_{3''a,4''}$ ~13.0 Hz, H-3″*a*), and 1.66 (t, 1 H, $J_{3'''a,4'''}$ ~12.3 Hz, H-3‴*a*); ^{13}C-n.m.r.: See Table I and Fig. 1.

Copolymerization. — A solution of **5** (18.4 mg), acrylamide (11.4 mg), and *N,N,N′,N′*-tetramethylethylenediamine (2 μL) in water (1 mL) was degassed for 20 min. After addition of $(NH_4)_2S_2O_8$ (1 mg), the solution was kept for 18 h at 4°. The product was isolated by column chromatography on Sephadex G-50 (2.6 × 100 cm; eluent 0.01M aqueous $NaHCO_3$) and desalted on Bio-Gel P-2 (2.6 × 100 cm) to yield **23** (9.9 mg), amorphous powder, $[\alpha]_D^{20}$ +3° (*c* 0.9, water). The copolymers **24** (14.6 mg of **17** and 8 mg of acrylamide) and **25** (4.0 mg of **21** and 1.9 mg of acrylamide) were prepared in a similar manner to yield **24** (4.3 mg), $[\alpha]_D^{20}$ +6° (*c* 0.2, water) and **25** (3.7 mg), $[\alpha]_D^{20}$ +4° (*c* 0.2, water).

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