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SYNTHESIS OF TRISACCHARIDES CONTAINING 3-DEOXY-D-*manno*-2-OCTULOSONIC ACID RESIDUES RELATED TO THE KDO-REGION OF ENTEROBACTERIAL LIPOPOLYSACCHARIDES

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ABSTRACT

Glycosylation of methyl (allyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate with an α-(2→4) linked per-*O*-acetylated KDO-disaccharide bromide derivative under Helferich conditions afforded a 2:1 mixture of the α- and β-linked trisaccharide derivatives in 50% yield. Removal of the protecting groups gave sodium *O*-[sodium (3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]-(2→4)-*O*-[sodium (3-deoxy-α- and -β-D-*manno*-2-octulopyranosyl)onate]-(2→4)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate. Radical copolymerization of the allyl glycosides afforded artificial antigens, suitable for defining antibody specificities directed against the KDO-region of enterobacterial lipopolysaccharides.

INTRODUCTION

3-Deoxy-D-*manno*-2-octulosonic acid (KDO) has been originally discovered[1] as a characteristic component of enterobacterial lipopolysaccharides (LPS), constituting the link between Lipid A and the heptose-containing inner-core oligosaccharide[2,3]. Recent structural and synthetic investigations on the inner-core-region of a number of rough-mutant lipopolysaccharides showed the presence of an α-(2→4)-linked KDO-disaccharide, which may additionally be substituted by a third α-(2→4)-linked KDO-residue in various proportions[4–11]. Previously, we reported[12–14] the synthesis of polyacrylamide copolymers[15,16] substituted by α-KDO*p*, β-KDO*p*, α- and β-KDO*p*-(2→4)-α-KDO*p*, α- and β-KDO*p*-(2→4)-β-KDO*p*, α- and β-KDO*p*-(2→8)-α-KDO*p*, and α-KDO*p*-(2→8)-α-KDO*p*-(2→4)-α-KDO*p* residues. By use of these antigens, the epitope specificities of two monoclonal antibodies directed against the KDO-region of enterobacterial LPS could be deter-

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mined[17,18]. One of them (clone 25) recognizes, in enterobacterial R-lipopolysaccharides, an α-(2→4)-linked KDO-disaccharide, whereas the other (clone 20) reacts with a terminal KDO-pyranosyl group in the α-anomeric configuration. Extending the series of these antigens, we report the synthesis of poly(acrylamide) copolymers containing α-KDO*p*-(2→4)-α- and -β-KDO*p*-(2→4)-α-KDO*p* residues.

RESULTS AND DISCUSSION

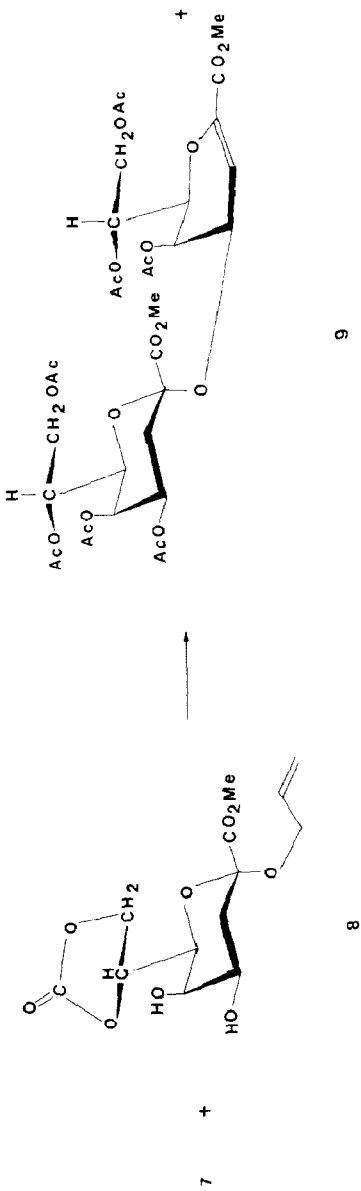
For the synthesis of the trisaccharide derivatives **12** and **13**, the previously described[15] allyl glycoside **1** was converted into the per-*O*-acetylated disaccharide derivative **2**, in 90% yield, with acetic anhydride–pyridine–4-(dimethylamino)-pyridine. Removal of the allyl group was accomplished *via* isomerization into the propenyl glycoside **3** with bis(methyldiphenylphosphine)cycloocta-1,5-diene iridium(I) hexafluorophosphate[19,20] and subsequent hydrolysis by the action of iodine in aqueous oxolane[21] in 67% overall yield. Acetylation of **4** gave a 1:5 mixture of the 2-*O*-acetyl-βα and -αβ derivatives **5** and **6** in 96% yield, which were separated by column chromatography on silica gel. The assignment of the anomeric configuration of the isomers was based on the chemical shift of the signal of the equatorial H-3 of the β anomer **5**, which is shifted downfield to δ 2.53, as compared to the corresponding signal of the α anomer **6**. Conversely, H-4 of **6** experiences a downfield shift relative to H-4 in the β anomer (δ 4.68 *vs.* 4.47) (ref. 22).

Reaction of the crystalline α anomer **6** with titanium tetrabromide in dichloromethane[23] gave a quantitative yield of the disaccharide bromide derivative **7**, which was immediately used for the glycosylation of 3 molar equivalents of the 7,8-*O*-carbonyl derivative[14] **8**. Promotion of the reaction with mercury(II) cyanide in acetonitrile gave a 50% yield of the disaccharide glycal ester derivative **9** and a 1:2 mixture of the β-(2′→4)- and α-(2′→4)-linked trisaccharide derivatives **11** and **10** in 50% yield, which were separated by column chromatography on silica gel. The assignment of the anomeric configuration of the KDO residues was confirmed by the 250-MHz ^{1}H-n.m.r. chemical shift values of H-4′ (δ 4.52 for **10**, 4.07 for **11**) and H-3′*e* (δ 2.25 for **10**, 2.47 for **11**), respectively. Zemplén deacylation and hydrolysis of the methyl ester groups afforded *O*-[sodium (3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]-(2→4)-*O*-[sodium (3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]-(2→4)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**12**) and *O*-[sodium (3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]-(2→4)-*O*-[sodium (3-deoxy-β-D-*manno*-2-octulopyranosyl)onate]-(2→4)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**13**) in quantitative yield.

The 250-MHz ^{1}H-n.m.r. spectra of **12** and **13** confirmed the structures assigned. Thus, the signal of the equatorial H-3*e* of the internal octulopyranosylonate residue of **13** occurred at δ 2.37 which is indicative of the β-anomeric configuration; this could also be deduced from the upfield shift of the corresponding ^{13}C-n.m.r. signal of the carboxylate carbon atom (δ 174.42). The assignment of the complex ^{13}C-n.m.r. spectra will be published elsewhere[24].

1 R = H
2 R = Ac

3 R = OCH=CHCH$_3$, R′ = CO_2Me
4 R = CO_2Me , R′ = OH
5 R = OAc , R′ = CO_2Me
6 R = CO_2Me , R′ = OAc
7 R = CO_2Me , R′ = Br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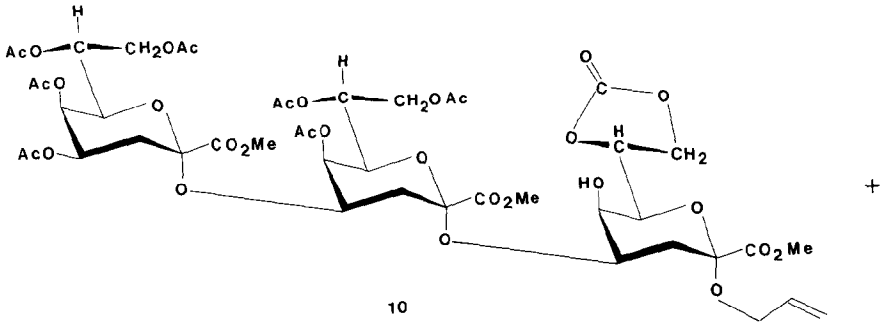
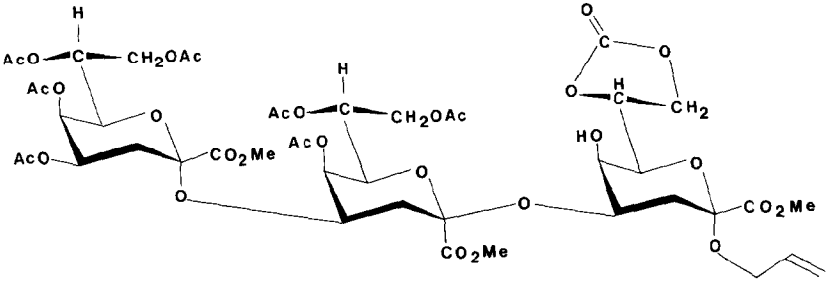
CH2OAc
AcO
AcO
AcO
AcO
CO2Me
CH2OAc
AcO
AcO
AcO
CO2Me
CH2
HO
HO
CO2Me

9 + 8 + 7



10



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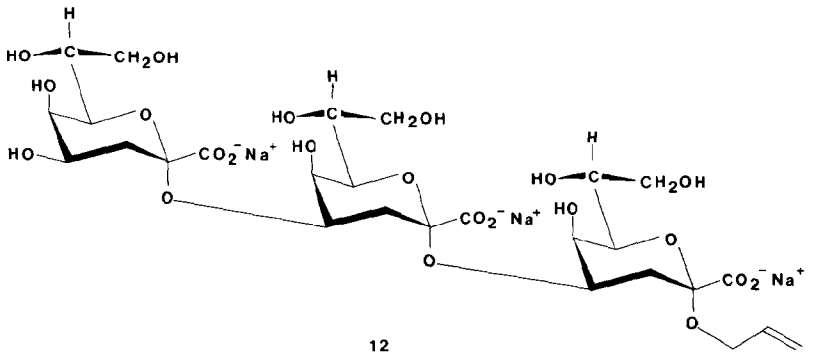

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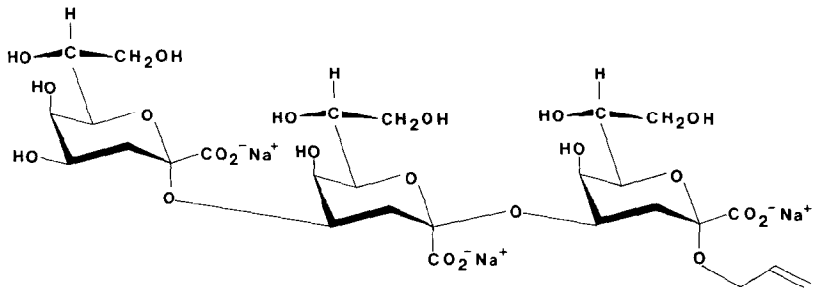

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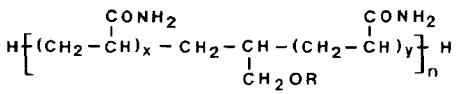

$x + y = 18 \pm 2$

$n = 100 - 150$

14 R = α-KDOp-(2→4)-α-KDOp-(2→4)-α-KDOp-(2→

15 R = α-KDOp-(2→4)-β-KDOp-(2→4)-α-KDOp-(2→

The allyl glycosides **12** and **13** were copolymerized with acrylamide according to the conditions given by Hořejší *et al.*[25]. Thus, reaction of **12** and **13** with 4 molar equivalents of acrylamide in the presence of 1,2-bis(dimethylamino)ethane and ammonium peroxosulfate gave the linear copolymers **14** and **15** ($x + y = 18 \pm 2$, $n = 100 - 150$, corresponding to molecular weights between 60 000 and 100 000) which were isolated, with recovery of unreacted allyl glycoside, by passage through Sephadex G-50 and desalting on Bio-Gel P-2. Immunochemical results obtained with these artificial antigens will be published in an accompanying paper[18].

EXPERIMENTAL

General methods. — These were as described previously[14]. The ^{13}C-n.m.r. spectra were recorded with a Bruker WM 250 instrument at 62.9 MHz, for solutions in D_2O at 24°, by use of 32 K of memory and a spectral width of 12 kHz. Chemical shifts are given from the signal of tetramethylsilane (shift set at δ 67.40 relative to the signal of 1,4-dioxane in D_2O). 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).

O-*(Methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→4)-methyl (allyl 5,7,8-tri*-O-*acetyl-3-deoxy-β*-D-manno-*2-octulopyranosid)onate* (**2**). — To a solution of *O*-(methyl 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→4)-methyl (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate[15] (**1**; 185 mg, 0.26 mmol) in pyridine (10 mL) were added acetic anhydride (0.6 mL, 6.4 mmol), and 4-(dimethylamino)pyridine (5 mg) at 0°. After being stirred overnight at room temperature, the mixture was taken to dryness. The residue was dissolved in dichloromethane (50 mL), and the resulting solution washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated. Purification of the residue on a column of silica gel (B, 1:1 toluene–ethyl acetate) afforded **2** as a colorless syrup (yield 197.2 mg, 90%), $[\alpha]_D^{20}$ +89° (*c* 0.75, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.85 (m, 1 H, –CH=), 5.35 (br. s, 1 H, H-5′), 5.25 (dq, 1 H, $=CH_2$-*trans*), 5.25–5.18 (m, 3 H, H-5,4′,7′), 5.16 (dq, 1 H, $=CH_2$-*cis*), 5.10 (dt, 1 H, $J_{7,8a}$ ~$J_{7,8b}$ ~3.0, $J_{7,6}$ ~9.5 Hz, H-7), 4.70 (dd, 1 H, $J_{7',8'a}$ ~2.5, $J_{8'a,8'b}$ ~12.0 Hz, H-8′a), 4.41–4.30 (m, 2 H, H-8a,8b), 4.30–4.22 (m, 1 H, OCH_2–CH=), 4.16 (dd, 1 H, $J_{6,5}$ ~1.5 Hz, H-6), 4.09 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.5 Hz, H-4), 4.02 (dd, 1 H, $J_{7',8'b}$ ~5.0

Hz, H-8'b), 3.99 (dd, 1 H, $J_{6',5'}$ ~1.0, $J_{6',7'}$ ~9.5 Hz, H-6'), 3.98–3.90 (m, 1 H, OCH_2–CH=), 3.85 (s, 3 H) and 3.78 (s, 3 H, 2 MeO), 2.48 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.12 (t, 1 H, H-3*a*), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.07 (s, 3 H), 2.06 (s, 3 H), 2.02 (s, 3 H), 1.99 (s, 3 H), and 1.97 (s, 3 H) (7 Ac), and 2.12–1.97 (m, 2 H, H-3'*e*,3'*a*).

Anal. Calc. for $C_{35}H_{48}O_{22}$: C, 51.22; H, 5.89. Found: C, 51.45; H, 5.86.

O-*(Methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→4)-methyl 4,5,7-tri*-O-*acetyl-3-deoxy-β*-D-manno-*2-octulopyranosidonate* (**4**). — A solution of $[Ir(cod)(PMePh_2)_2]PF_6$ (8 mg; Alfa Products, Denvers, MA 01923, U.S.A.) in dry oxolane (10 mL) was degassed, placed under O_2-free N_2, and degassed once more. The catalyst was activated in an H_2 atmosphere for 5 min. The solution was degassed and added to a solution of **2** (180 mg, 0.22 mmol) in dry oxolane (10 mL). After being stirred for 4 h at room temperature, the solution was evaporated. The residue was dissolved in dichloromethane (50 mL), extracted with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated. A solution of the residue in 4:1 oxolane–water (5 mL) was treated with I_2 (80 mg, 0.31 mmol) for 15 min at room temperature. The mixture was diluted with dichloromethane (50 mL) and the resulting solution washed sequentially with 20% aqueous $NaHSO_3$, saturated aqueous $NaHCO_3$, and water. The organic layer was dried (Na_2SO_4) and evaporated. Column chromatography of the residue on silica gel (B, 1:1 toluene–ethyl acetate) afforded **4** as a syrup (yield 115 mg, 67%), $[\alpha]_D^{20}$ +98° (*c* 1.2, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.36 (br. s, 1 H, H-5'), 5.22 (ddd, 1 H, $J_{4',5'}$ ~2.5, $J_{4',3'e}$ ~5.0, $J_{4',3'a}$ ~12.5 Hz, H-4'), 5.19 (br. s, 1 H, H-5), 5.18 (ddd, 1 H, $J_{7',6'}$ ~9.5, $J_{7',8'a}$ ~2.5, $J_{7',8'b}$ ~4.0 Hz, H-7'), 5.03 (ddd, 1 H, $J_{7,6}$ ~9.5, $J_{7,8a}$ ~2.5, $J_{7,8b}$ ~4.0 Hz, H-7), 4.90 (dd, 1 H, $J_{8'a,8'b}$ ~12.5 Hz, H-8'a), 4.85 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.5 Hz, H-4), 4.38 (dd, 1 H, $J_{8a,8b}$ ~12.0 Hz, H-8a), 4.23 (dd, 1 H, $J_{6',5'}$ ~1.0 Hz, H-6'), 4.13 (dd, 1 H, H-8b), 4.12 (dd, 1 H, $J_{6,5}$ ~1.0 Hz, H-6), 4.10 (dd, 1 H, H-8'b), 3.94 (d, 1 H, *J* ~2.5 Hz, OH), 3.87 (s, 6 H, 2 MeO), 2.43 (dt, 1 H, $J_{3a,3e}$ ~12.5 Hz, H-3*a*), 2.17 (t, 1 H, $J_{3'a,3'e}$ ~12.5 Hz, H-3'*a*), 2.10 (s, 3 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 2.02 (s, 6 H) and 1.98 (s, 6 H), (7 Ac), 2.10–1.98 (1 H, H-3'*e*), and 1.91 (dd, 1 H, H-3*e*).

Anal. Calc. for $C_{32}H_{44}O_{22}$: C, 49.23; H, 5.68. Found: C, 49.75; H, 5.72.

O-*(Methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→4)-methyl 2,5,7,8-tetra*-O-*acetyl-3-deoxy-β*-D-manno-*2-octulopyranosonate* (**5**) *and* O-*(methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→4)-methyl 2,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosonate* (**6**). — A solution of **4** (105 mg, 0.13 mmol), acetic anhydride (0.25 mL, 2.7 mmol), and 4-(dimethylamino)pyridine (2 mg) in dry pyridine (5 mL) was stirred at room temperature for 15 h. The solution was evaporated and the residue dissolved in dichloromethane (50 mL). The organic layer was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and taken to dryness. Purification of the residue on a column of silica gel (B, 2:1 toluene–ethyl acetate) afforded 18 mg (16%) of **5** as the faster moving compound, colorless syrup, $[\alpha]_D^{20}$ +86° (*c* 1.1, chloroform); ^{1}H-

n.m.r. ($CDCl_3$): δ 5.34 (br. s, 1 H, H-5′), 5.27 (br. s, 1 H, H-5), 5.23 (ddd, 1 H, $J_{7',6'}$ ~9.5, $J_{7',8'a}$ ~3.0, $J_{7',8'b}$ ~5.5 Hz, H-7′), 5.20 (ddd, 1 H, $J_{4',5'}$ ~3.0, $J_{4',3'e}$ ~6.0, $J_{4',3'a}$ ~12.0 Hz, H-4′), 5.07 (ddd, 1 H, $J_{7,6}$ ~9.5, $J_{7,8a}$ ~2.5, $J_{7,8b}$ ~4.5 Hz, H-7), 4.73 (dd, 1 H, $J_{8'a,8'b}$ ~12.0 Hz, H-8′a), 4.47 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.5 Hz, H-4), 4.43 (dd, 1 H, $J_{8a,8b}$ ~12.5 Hz, H-8a), 4.34 (dd, 1 H, $J_{6,5}$ ~1.5 Hz, H-6), 4.17 (dd, 1 H, H-8b), 4.08 (dd, 1 H, $J_{6',5'}$ ~1.5 Hz, H-6′), 4.00 (dd, 1 H, H-8′b), 3.86 (s, 3 H) and 3.77 (s, 3 H, 2 MeO), 2.53 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.16 (dd, 1 H, $J_{3'e,3'a}$ ~12.5 Hz, H-3′*e*), 2.13 (t, 1 H, H-3*a*), 2.13 (s, 3 H), 2.12 (s, 3 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 2.02 (s, 6 H), 1.99 (s, 3 H) and 1.97 (s, 3 H) (8 Ac), and 2.05 (t, 1 H, H-3′*a*).

Further elution of the column gave **6** (87 mg, 80%) as colorless needles, m.p. 178–179° (hexane–ethyl acetate), $[\alpha]_D^{20}$ +136° (*c* 0.6, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.36 (br. s, 1 H, H-5′), 5.20 (br. s, 1 H, H-5), 5.26–5.15 (m, 2 H, H-4′,7′), 5.10 (ddd, 1 H, $J_{7,8a}$ ~2.5, $J_{7,8b}$ ~3.0, $J_{7,6}$ ~9.5 Hz, H-7), 4.78 (dd, 1 H, $J_{8'a,8'b}$ ~12.0, $J_{8'a,7'}$ ~3.0 Hz, H-8′a), 4.68 (ddd, 1 H, $J_{4,5}$ ~2.5, $J_{4,3e}$ ~5.5, $J_{4,3a}$ ~13.0 Hz, H-4), 4.50 (dd, 1 H, $J_{8a,8b}$ ~12.5 Hz, H-8a), 4.13–4.02 (m, 4 H, H-6,6′,8b,8′b), 3.88 (s, 3 H) and 3.83 (s, 3 H, 2 MeO), 2.15 (s, 3 H), 2.13 (s, 3 H), 2.11 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.00 (s, 3 H) and 1.98 (s, 3 H) (8 Ac), and 2.24–1.98 (m, 4 H, H-3*e*,3*a*,3′*e*,3′*a*).

Anal. Calc. for $C_{34}H_{46}O_{23}$: C, 49.63; H, 5.64. Found: C, 49.87; H, 5.61.

O-*(Methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→4)-methyl (5,7,8-tri*-O-*acetyl*-D-manno-*2-octulopyranosyl bromide)onate* (**7**). — To a solution of **6** (79 mg, 0.1 mmol) in dry dichloromethane (25 mL) was added $TiBr_4$ (300 mg, 0.82 mmol) at −10°. The solution was kept at +4° for 48 h, diluted with chloroform (100 mL), and the resulting solution washed with saturated ice-cold aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and evaporated to give 81 mg (100%) of **7** as a slightly yellow syrup which was immediately used in the glycosylation step.

O-*(Methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→4)-methyl 5,7,8-tri*-O-*acetyl-2,6-anhydro-2,3-dideoxy*-D-manno-*2-octenonate* (**9**), O-*(methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→4)*-O-*(methyl 5,7,8-tri*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosyl)-onate-(2→4)-methyl (allyl 7,8*-O-*carbonyl-3-deoxy-α*-D-manno-*2-octulopyranosid)*-onate (**10**), *and* O-*(methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosylonate)-(2→4)*-O-*(methyl 5,7,8-tri*-O-*acetyl-3-deoxy-β*-D-manno-*2-octulopyranosylonate)-(2→4)-methyl (allyl 7,8*-O-*carbonyl-3-deoxy-α*-D-manno-*2-octulopyranosid)onate* (**11**). — A solution of **7** (81 mg, 0.096 mmol) in dry acetonitrile (2 mL) was added to a suspension of **8** (92 mg, 0.29 mmol), $Hg(CN_2)$ (100 mg, 0.4 mmol), and molecular sieve 4A (300 mg) in acetonitrile (5 mL) under dry N_2. After being stirred for 48 h at room temperature, the mixture was diluted with dichloromethane (100 mL) and filtered over Celite. The filtrate was washed sequentially with saturated aqueous $NaHCO_3$, 10% aqueous KI, and water. The organic layer was dried (Na_2SO_4) and evaporated. Purification of the residue on silica gel (B, 1:1

toluene–ethyl acetate) afforded **9** (R_F 0.60, 1:2 toluene–ethyl acetate) as a syrup (yield 36 mg, 50%), $[\alpha]_D^{20}$ +49° (*c* 1.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.84 (dd, 1 H, $J_{3,4}$ ~2.0, $J_{3,5}$ ~2.0 Hz, H-3), 5.42 (br. s, 1 H, H-5′), 5.34 (br. s, 1 H, H-5), 5.28 (ddd, 1 H, $J_{4',5'}$ ~3.0, $J_{4',3'e}$ ~5.0, $J_{4',3'a}$ ~12.5 Hz, H-4′), 5.17 (ddd, 1 H, $J_{7,6}$ ~9.5, $J_{7,8a}$ ~2.5, $J_{7,8b}$ ~3.0 Hz, H-7), 5.16 (ddd, 1 H, $J_{7',6'}$ ~9.5, $J_{7',8'a}$ ~2.5, $J_{7',8'b}$ ~4.0 Hz, H-7′), 5.08 (ddd, 1 H, $J_{4,5}$ ~4.5, $J_{4,6}$ ~1.3 Hz, H-4), 4.64 (dd, 1 H, $J_{8a,8b}$ ~12.5 Hz, H-8a), 4.63 (dd, 1 H, $J_{8'a,8'b}$ ~12.5 Hz, H-8′a), 4.28 (dd, 1 H, $J_{6,5}$ ~1.3 Hz, H-6), 4.22 (dd, 1 H, H-8′b), 4.20 (dd, 1 H, $J_{6',5'}$ ~1.5 Hz, H-6′), 4.18 (dd, 1 H, H-8b), 3.87 (s, 3 H), and 3.84 (s, 3 H, 2 MeO), 2.21 (dd, 1 H, $J_{3'a,3'e}$ ~13.0 Hz, H-3′*a*), 2.00 (dd, 1 H, H-3′*e*), and 2.11 (s, 3 H), 2.08 (s, 3 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 1.99 (s, 3 H) (7 Ac).

Anal. Calc. for $C_{32}H_{42}O_{21}$: C, 50.39; H, 5.55. Found: C, 50.65; H, 5.52.

Further elution of the column with 1:1 toluene–ethyl acetate gave **11** as a syrup (yield 17 mg, 16%), $[\alpha]_D^{20}$ +80° (*c* 0.6, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.90 (m, 1 H, =CH–), 5.35 (br. s, 1 H, H-5″), 5.28 (dq, 1 H, $=CH_2$-*trans*), 5.25 (br. s, 1 H, H-5′), 5.25–5.14 (m, 2 H, H-7″,4″), 5.17 (dq, 1 H, $=CH_2$-*cis*), 5.07 (ddd, 1 H, $J_{7',8'a}$ ~2.5, $J_{7',8'b}$ ~4.5, $J_{7',6'}$ ~9.5 Hz, H-7′), 5.05 (ddd, 1 H, $J_{7,8a}$ ~7.0, $J_{7,8b}$ ~9.0, $J_{7,6}$ ~2.5 Hz, H-7), 4.84 (dd, 1 H, $J_{8a,8b}$ ~9.0 Hz, H-8a), 4.60 (dd, 1 H, $J_{8''a,7''}$ ~2.5, $J_{8''a,8''b}$ ~12.0 Hz, H-8″a), 4.56 (t, 1 H, H-8b), 4.36–4.25 (m, 2 H, H-8′a,8′b), 4.20 (ddd, 1 H, $J_{4,5}$ ~2.5, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.0 Hz, H-4), 4.16–3.96 (m, 9 H, H-5,6,4′,6′,6″,8″b, OCH_2–CH=, OH), 3.86 (s, 6 H) and 3.79 (s, 3 H, 3 Me), 2.47 (dd, 1 H, $J_{3'e,4'}$ ~5.0, $J_{3'e,3'a}$ ~12.5 Hz, H-3′*e*), 2.17–1.95 (m, 4 H, H-3*e*,3*e*″,3′*a*,3″*a*), 2.12 (s, 3 H), 2.10 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 2.00 (s, 3 H), and 1.96 (s, 3 H) (7 Ac), and 1.91 (t, 1 H, H-3*a*); ^{13}C-n.m.r. ($CDCl_3$): δ 170.6, 170.5, 170.2, 170.0, 169.8, 169.7 and 169.5 (7 CH_3CO), 167.9, 167.7, 166.9 (3 CO_2CH_3), 155.4 (OCO), 133.6 (–CH=), 116.7 ($=CH_2$), 100.5 (C-2′), 98.7 (C-2,2″), 76.8 (C-7), 71.3 (C-6), 71.0, 70.6, and 69.9 (C-4,6′,6″), 68.3 and 68.1 (C-4′,7′), 67.6 (C-7″), 67.1, 66.0, and 65.9 (C-8,4″, $-OCH_2CH=$), 64.6 and 64.3 (C-5,5′,5″), 62.5 and 62.4 (C-8′,8″), 53.2, 52.8, and 52.6 (3 OCH_3), 34.7, 32.5, and 32.4 (C-3,3′,3″), 20.8, 20.7, and 20.6 (7 CH_3CO).

Anal. Calc. for $C_{45}H_{60}O_{30}$: C, 50.00; H, 5.59. Found: C, 50.16; H, 5.63.

Further elution of the column afforded 35 mg (34%) of **10**, colorless needles, m.p. 197–199° (hexane–ethyl acetate), $[\alpha]_D^{20}$ +122° (*c* 0.6, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.84 (m, 1 H, =CH–), 5.34 (br. s, 1 H, H-5″), 5.24 (dq, 1 H, $=CH_2$-*trans*), 5.23 (br. s, 1 H, H-5′), 5.16 (dq, 1 H, $=CH_2$-*cis*), 5.28–5.13 (m, 2 H, H-7″,4″), 5.12 (ddd, 1 H, $J_{7',8'a}$ ~3.0, $J_{7',8'b}$ ~4.5, $J_{7',6'}$ ~9.5 Hz, H-7′), 4.82 (ddd, 1 H, $J_{7,8a}$ ~7.0, $J_{7,8b}$ ~9.0, $J_{7,6}$ ~4.0 Hz, H-7), 4.75 (dd, 1 H, $J_{8a,8b}$ ~9.0, H-8a), 4.75 (dd, 1 H, $J_{8''a,8''b}$ ~12.5 Hz, H-8″a), 4.57 (dd, 1 H, $J_{8'a,8'b}$ ~11.5 Hz, H-8′a), 4.55 (t, 1 H, H-8b), 4.52 (ddd, 1 H, $J_{4',5'}$ ~3.0 Hz, H-4′), 4.26 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~6.0, $J_{4,3a}$ ~11.5 Hz, H-4), 4.08 (dd, 1 H, $J_{8''b,7''}$ ~5.0 Hz, H-8″b), 4.04–3.95 (m, 6 H, H-4′,6′8′b,6″, OCH_2), 3.89 (dd, 1 H, $J_{6,5}$ ~2.0 Hz, H-6), 3.88 (s, 3 H), 3.86 (s, 3 H), and 3.80 (s, 3 H) (3 Me), 3.74 (br. s, 1 H, H-5), 3.34 (dd, 1 H, *J* ~1.0, *J* ~4.0 Hz, OH), 2.19 (dd, 1 H, $J_{3e,3a}$ ~12.0 Hz, H-3*e*), 2.19–1.95 (m, 5 H, H-

3*a*,3′*e*,3′*a*,3″*e*,3″*a*), and 2.11 (s, 6 H), 2.07 (s, 3 H), 2.04 (s, 3 H), 1.99 (s, 6 H), 1.97 (s, 3 H) (7 Ac); ^{13}C-n.m.r. ($CDCl_3$): δ 170.2, 170.1, 170.0, and 169.6 (7 CH_3CO), 168.7, 167.9, and 167.0 (C-1,1′,1″), 155.4 (OCO), 133.3 (-*C*H=), 117.3 (=CH_2), 98.9, 98.7, and 98.1 (C-2,2′,2″), 76.2 (C-7), 71.3 (C-6), 70.2 and 70.1 (C-6′,6″), 68.5 and 68.1 (C-4,4′), 67.4 (C-7′), 66.4 (C-7″), 66.1 (C-8,4″), 65.3, 65.0, and 64.9 (C-5,5′, OCH_2CH=), 64.1 (*C*-5″), 63.0 and 61.6 (C-8′,8″), 53.3, 52.9, and 52.7 (3 CO_2CH_3), 34.6 (C-3′), 32.9 and 31.7 (*C*-3,3″), and 20.8, 20.7, 20.6 (7 *C*H_3CO).

Anal. Calc. for $C_{45}H_{60}O_{30}$: C, 50.00; H, 5.59. Found: C, 49.90; H, 5.51.

O-*(Sodium 3-deoxy-α-*D-manno-*2-octulopyranosylonate)-(2→4)-*O-*(sodium 3-deoxy-α-*D-manno-*2-octulopyranosylonate)-(2→4)-sodium (allyl 3-deoxy-α-*D-manno-*2-octulopyranosid)onate* (**12**). — A solution of **12** (17.9 mg) in dry methanol (10 mL) was treated with 0.1M methanolic sodium methoxide (0.5 mL) for 2 h at room temperature. The solution was de-ionized 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 (1 mL) for 2.5 h at room temperature. The pH of the solution was adjusted to 8.0 by addition of Dowex 50 (H^+) resin and filtered. The filtrate was lyophilized and the product was desalted on a column of Bio-Gel P-2 to give **12** (13 mg, 100%) an amorphous powder, $[\alpha]_D^{20}$ +68° (*c* 0.7, water); ^{1}H-n.m.r. (D_2O): δ 5.97 (m, 1 H, =CH–), 5.34 (dq, 1 H, =CH_2-*trans*), 5.23 (dq, 1 H, =CH_2-*cis*), 4.19–3.33 (m, 20 H, H-4,5,6,7,8a,8b,4′,5′,6′,7′,8′a,8′b,4″, 5″,6″,7″,8″a,8″b, OCH_2), 2.14 (dd, 1 H, $J_{3''e,4''}$ ~5.0, $J_{3''e,3''a}$ ~12.5 Hz, H-3″*e*), 2.08 (dd, 1 H, $J_{3'e,3'a}$ ~12.5, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 2.03 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 1.95 (t, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*), 1.90 (t, 1 H, $J_{3'a,4a'}$ ~12.5 Hz, H-3′*a*), and 1.78 (t, 1 H, $J_{3''a,4''}$ ~12.5 Hz, H-3″*a*); ^{13}C-n.m.r. (D_2O): δ 176.80, 176.76, and 176.03 (C-1,1′,1″), 135.11 (=*C*H–), 118.10 (=CH_2), 101.11 (C-2), 100.23 and 99.99 (C-2′,2″), 73.35, 73.26, and 72.41 (C-6,6′,6″), 70.92, 70.71, and 70.52 (C-7,7′,7″), 69.35 and 69.18 (C-4,4′), 67.17 and 66.88 (C-4″,5″), 65.14 and 65.02 (OCH_2–CH=, C-5,5′), 64.10 (C-8,8′,8″), 35.45 (C-3″), 34.51, and 34.19 (C-3,3′).

O-*(Sodium 3-deoxy-α-*D-manno-*2-octulopyranosylonate)-(2→4)-*O-*(sodium 3-deoxy-β-*D-manno-*2-octulopyranosylonate)-(2→4)-sodium (allyl 3-deoxy-α-*D-manno-*2-octulopyranosid)onate* (**13**). — A solution of **11** (12.3 mg) in dry methanol (5 mL) was treated with 0.1M methanolic sodium methoxide (0.5 mL) for 2 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) resin, the suspension filtered, and the solution evaporated. The residue was treated in the same manner as described for **12** to give **13** (9.0 mg, 100%), amorphous powder, $[\alpha]_D^{20}$ +65° (*c* 0.55, water): ^{1}H-n.m.r. (D_2O): δ 5.96 (m, 1 H, =CH–), 5.34 (dq, 1 H, =CH_2-*trans*), 5.23 (dq, 1 H, =CH_2-*cis*), 4.27–3.54 (m, 20 H, H-4,5,6,7,8a,8b, 4′,5′,6′,7′,8′a,8′b,4″,5″,6″,7″,8″a,8″b, OCH_2CH=), 2.37 (dd, 1 H, $J_{3'e,3'a}$ ~12.5, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 2.15 (dd, 1 H, $J_{3''e,3''a}$ ~12.5, $J_{3''e,4''}$ ~5.0 Hz, H-3″*e*), 1.98 (dd, 1 H, $J_{3e,3a}$ ~12.5, $J_{3e,4}$ ~5.0 Hz, H-3*e*), 1.95 (t, 1 H, $J_{3'a,4'}$ ~12.5 Hz, H-3′*a*), 1.85 (t, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*), and 1.78 (t, 1 H, $J_{3''a,4''}$ ~12.5 Hz, H-3″*a*); ^{13}C-n.m.r. (D_2O): δ 176.85 and 176.02 (C-1,1″), 174.42 (C-1′), 134.96 (=CH–), 118.29 (=CH_2), 103.17 (C-2′), 101.07 and 100.12 (C-2,2″), 74.20 (C-6′), 73.32 (C-6), 72.21

(C-6″), 71.82 (C-4′), 70.82, 70.44, and 70.01 (C-7,7′,7″,4), 67.31, 67.24, and 66.80 (C-5′,4″,5″), 65.17 (C-5, $OCH_2CH=$), 64.23, 64.03, and 63.92 (C-8,8′,8″), 35.42 (C-3″), 34.99 and 34.01 (C-3,3′).

Copolymerization. — A solution of **12** (13.2 mg), acrylamide (6.4 mg) and 1,2-bis(dimethylamino)ethane (1 μL) in water (1.0 mL) was degassed for 30 min. After addition of $(NH_4)_2S_2O_8$ (1 mg), the solution was kept at +4° for 18 h. 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 give **14** (yield 7.38 mg), amorphous powder, $[\alpha]_D^{20}$ +11.3° (*c* 0.7, water). Unreacted **12** was recovered in 68% yield (9.0 mg). The copolymer **15** (9.0 mg of **13** and 5.5 mg of acrylamide) was prepared in a similar manner (yield 6.9 mg), amorphous powder, $[\alpha]_D^{20}$ +8.1° (*c* 0.6, water).

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