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# SYNTHESIS OF POLYACRYLAMIDE COPOLYMERS CONTAINING α-(2→4)-β-, β-(2→4)-β-, AND β-(2→4)-α-LINKED *O*-(3-DEOXY-D-*manno*-2-OCTULOPYRANOSYLONATE)-(3-DEOXY-D-*manno*-2-OCTULOPYRANOSYLONO) (KDO) RESIDUES*

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ABSTRACT

Glycosylation of methyl (allyl 7,8-di-*O*-*tert*-butyldimethylsilyl-3-deoxy-β-D-*manno*-2-octulopyranosid)onate with methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosyl bromide)onate under Helferich conditions gave a 3:1 mixture of the corresponding α- and β-(2→4)-linked disaccharide derivatives in 58% yield. Separation and subsequent deprotection of the isomers afforded sodium *O*-[sodium (3-deoxy-α- and -β-D-*manno*-2-octulopyranosyl)onate]-(2→4)-(allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate. Similarly, the glycoside sodium *O*-[sodium (3-deoxy-β-D-*manno*-2-octulopyranosyl)onate]-(2→4)-(allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate was obtained by glycosylation of methyl (allyl 7,8-*O*-carbonyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate, followed by removal of the protecting groups. Radical copolymerization of the allyl glycosides with acrylamide afforded linear macromolecular antigens suitable for the determination of epitope-specificities of monoclonal antibodies directed against the KDO-region of enterobacterial lipopolysaccharides.

## INTRODUCTION

3-Deoxy-D-*manno*-2-octulosonic acid (KDO) was originally discovered[1] as a characteristic component of the enterobacterial lipopolysaccharides (LPS), constituting the link between the core oligosaccharide and lipid A[2,3]. Based upon recent structural investigations on the inner-core region of a number of rough-mutant lipopolysaccharides[4–10] showing the presence of an α-(2→4)-linked KDO-disaccharide, we reported[11] the synthesis of polyacrylamide copolymers[12,13] substituted by α-KDO*p*, β-KDO*p*, α-KDO*p*-(2→4)-α-KDO*p*, and β-D-Rib*f*-(1→7)-β-KDO*p* residues. By use of these antigens, the epitope-specificities of two mono-

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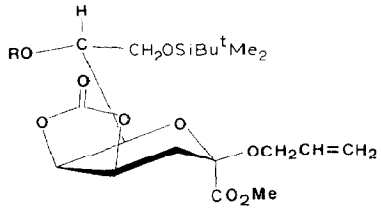
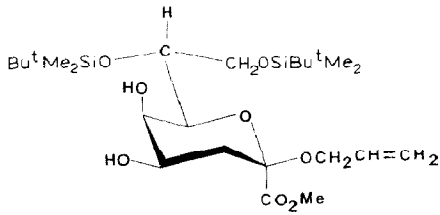
clonal antibodies directed against the KDO region of enterobacterial LPS could be determined[14]. Extending the series of these artificial antigens, we report herein the synthesis of polyacrylamide copolymers containing α-(2→4)-β-, β-(2→4)-β-, and β-(2→4)-α-linked KDO-disaccharide as substituents which are suitable for defining the antibody specificities in relation to different anomeric linkages of the KDO residues.

## RESULTS AND DISCUSSION

For the synthesis of the disaccharide derivatives **8** and **11**, the previously described[11] methyl (allyl 8-*O*-*tert*-butyldimethylsilyl-4,5-*O*-carbonyl-3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**1**) was converted into the crystalline 7,8-di-*O*-*tert*-butyldimethylsilyl ether derivative **2** by treatment with *tert*-butylchlorodimethyl-




1 R = H
2 R = $Bu^tMe_2Si$

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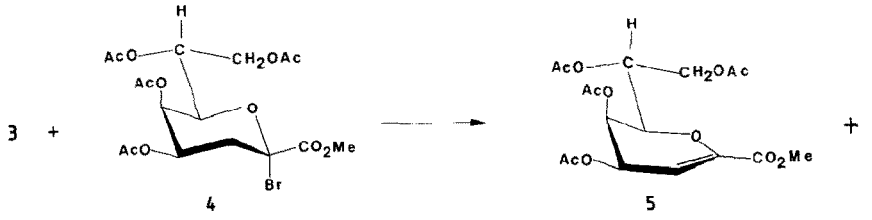
silane in the presence of imidazole[15] in *N*,*N*-dimethylformamide in 93% yield. Zemplén deacylation of **2** gave a quantitative yield of the diol **3**, which was subjected to glycosylation with 1.8 equivalents of methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosyl bromide)onate[16] (**4**) in the presence of mercury(II) cyanide and molecular sieves 4A in acetonitrile. Thus, a ~3:1 mixture of the α- and β-(2→4)-linked disaccharide derivatives **6** and **9** was obtained in 58% yield, together with the glycal ester derivative[17] **5**. The mixture was separated by column chromatography on silica gel. The assignment of the anomeric configuration of the KDO-residues in **6** and **9** was based on the different chemical shifts attributable to the signal[18] of H-4. Thus, in the 250-MHz $^1$H-n.m.r. spectrum, this signal for **6** was observed at δ 5.33, whereas the corresponding signal for **9** appeared at δ 4.86.

Removal of the $Bu^tMe_2Si$ groups was achieved by the action of 2% hydrofluoric in acetonitrile[19], giving **7** and **10** in 98 and 93% yield, respectively. Deprotection of **7** and **10** by deacetylation in methanolic sodium methoxide and subsequent deesterification in aqueous sodium hydroxide gave **8** and **11** in 95 and 96% yield, respectively.

The disaccharide *O*-(sodium 3-deoxy-β-D-*manno*-2-octulopyranosyl)onate-(2→4)-[sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate] (**13**) was pre-



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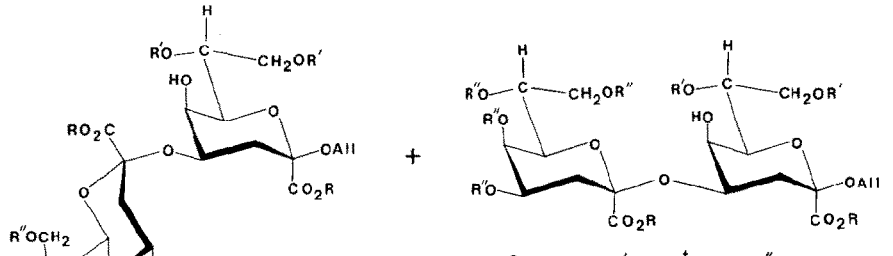


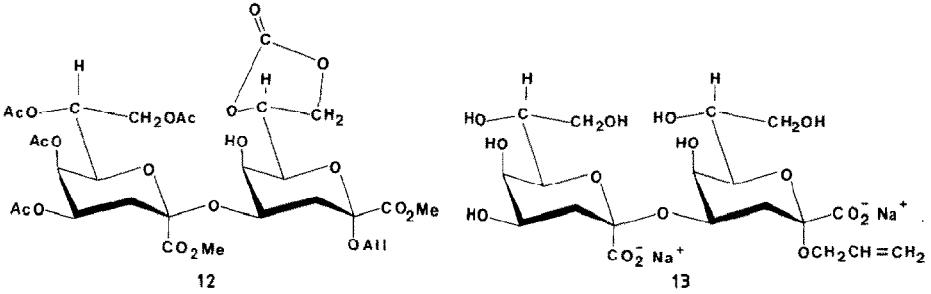
6 R = Me, R′ = Bu$^t$Me$_2$Si, R″ = Ac
7 R = Me, R′ = H, R″ = Ac
8 R = Na$^+$, R′ = R″ = H

9 R = Me, R′ = Bu$^t$Me$_2$Si, R″ = Ac
10 R = Me, R′ = H, R″ = Ac
11 R = Na$^+$, R′ = R″ = H

pared by deprotection of the previously described[11] disaccharide derivative **12**. Deacylation of **12** in methanolic sodium methoxide and subsequent saponification of the methyl ester groups in aqueous sodium hydroxide gave **13** in 94% yield.

Copolymerization of the allyl glycosides **8, 11,** and **13** with acrylamide was performed under the conditions of Kochetkov and assoc.[12,13], *i.e.*, reaction of the allyl glycosides with acrylamide in aqueous solution in the presence of *N,N,N′,N′*-tetramethylethylenediamine and ammonium persulfate[20]. The copolymers **14, 15,** and **16** were isolated by column chromatography on Sephadex G-50 and subsequent desalting on Bio-Gel P-2. The distribution of the molecular masses as well as their carbohydrate content were similar to the previously reported data[11].

$^{13}$C-N.m.r. chemical-shifts (Table I) of the allyl glycosides **8, 11,** and **13**, and



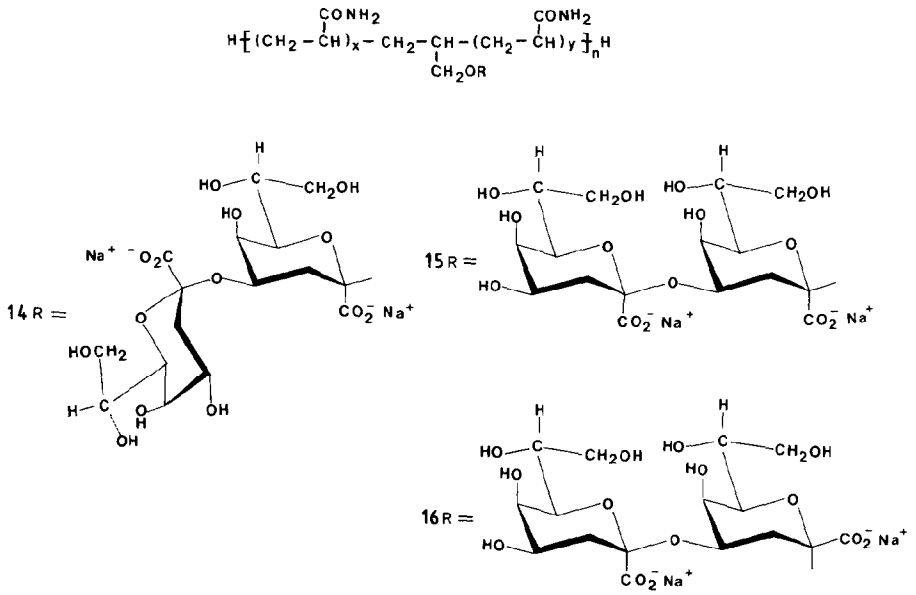
12 13

TABLE I

EMPIRICAL ASSIGNMENT[a] OF $^{13}$C-N.M.R. CHEMICAL SHIFTS ($\delta$) OF KDO-DISACCHARIDE–POLYACRYLAMIDE COPOLYMERS AND RELATED MONO- AND DI-SACCHARIDE DERIVATIVES[b]

| *Carbon atom* | *α-KDOpOMe*[b] | *β-KDOpOMe*[b] | **8** | **14**[c] | **11** | *β-KDOp-(2→4)-* β-KDOp*OMe*[b] | **15**[c] | **13** | **16**[c] |
|---|---|---|---|---|---|---|---|---|---|
| 1′ | | | 176.8 | [d] | 174.5 | 174.4 | 174.5 | 174.7 | 174.5 |
| 2′ | | | 100.2 | 100.2 | 103.1 | 103.0[e] | 103.2 | 103.1 | 103.0 |
| 3′ | | | 35.4 | [d] | 36.1 | 35.9 | [d] | 36.1 | [d] |
| 4′ | | | 66.8 | 66.8 | 68.2 | 68.1 | 68.2 | 68.3 | 68.3 |
| 5′ | | | 67.2 | 67.2 | 66.3 | 66.2 | 66.3 | 66.3 | 66.3 |
| 6′ | | | 73.2 | 73.2 | 74.4 | 74.3 | 74.4 | 74.4 | 74.3 |
| 7′ | | | 70.7 | 70.8 | 70.0 | 69.8[f] | 69.9 | 70.0 | 70.0 |
| 8′ | | | 63.8 | 63.9 | 65.2[g] | 65.1[h] | 65.2 | 65.2 | 65.2 |
| 1 | 176.1 | 174.5 | 174.4 | [d] | 174.5 | 174.4 | 174.5 | 176.0 | 176.1 |
| 2 | 101.3 | 102.1 | 101.9 | 102.1 | 101.9 | 102.0[e] | 102.0 | 101.0 | 100.9 |
| 3 | 34.9 | 35.3 | 34.5 | [d] | 34.5 | 34.1 | [d] | 34.0 | 34.0 |
| 4 | 66.8 | 68.2 | 70.6 | 70.8 | 72.9 | 72.8 | 73.1 | 71.7 | 71.8 |
| 5 | 67.1 | 66.2 | 64.0 | 63.9 | 66.3 | 66.2 | 66.3 | 67.4 | 67.3 |
| 6 | 72.2 | 74.3 | 74.2 | 74.1 | 73.9 | 73.8 | 73.7 | 72.2 | 71.8 |
| 7 | 70.2 | 69.9 | 70.0 | 70.0 | 70.0 | 69.9[f] | 69.9 | 70.4 | 70.3 |
| 8 | 63.9 | 64.8 | 64.9 | 65.0 | 65.0[g] | 64.8[h] | 65.2 | 64.2 | 64.0 |
| $OCH_2$– | | | 66.6 | [d] | 66.5 | | [d] | 65.1 | [d] |
| –CH= | | | 134.9 | | 134.8 | | | 134.9 | |
| $CH_2$= | | | 119.0 | | 118.9 | | | 118.2 | |

[a]Ref. 21. [b]Ref. 22. [c]The copolymers had additional signals for $CONH_2$ ($\delta$ 181.1–180.3), CH– ($\delta$ 44.3–42.3), and $CH_2$– ($\delta$ 37.1–34.9), groups. [d]Signal not resolved. [e–h]Assignments may be interchanged.



the corresponding copolymers **14, 15,** and **16** are in agreement with the assigned structures and compare favorably with reported values of related methyl glycosides[22]. Immunochemical results obtained with these copolymers will be published elsewhere.

## EXPERIMENTAL

*General methods.* — These were as described previously[11]. 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 32k of memory and a spectral width of 12 kHz. Chemical shifts are given from the signal of tetramethylsilane (shift set at $\delta$ 67.40 relative to the signal of 1,4-dioxane in $D_2O$).

*Methyl (allyl 7,8-di-*O*-tert-butyldimethylsilyl-4,5-*O*-carbonyl-3-deoxy-β-*D*-manno-2-octulopyranosid)onate* (**2**). — A solution of **1** (1.2 g, 2.77 mmol), imidazole (340 mg, 5 mmol) and *tert*-butyldimethylchlorosilane (452 mg, 3 mmol) in dry *N,N*-dimethylformamide (10 mL) was stirred for 15 h at room temperature. The solvent was removed *in vacuo*, and the residue dissolved in dichloromethane (50 mL). The organic layer was extracted with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated. Purification of the residue on a column of silica gel (size B, 1:1 toluene–ethyl acetate) afforded **2** (1.42 g, 93%), colorless crystals, m.p. 128° (ethyl acetate–hexane), $[\alpha]_D^{20}$ −11.7° (*c* 1.0, chloroform); $^1$H-n.m.r.: $\delta$ 5.90 (m, 1 H, –CH=), 5.24 (dq, 1 H, =C$H_2$-*trans*), 5.15 (dq, 1 H, =C$H_2$-*cis*), 5.04 (m, 2 H, H-4,5), 4.18 and 4.00 (m, 2 H, OC$H_2$–CH=), 3.92 (dd, 1 H, $J_{7,8a}$ ~2.0, $J_{7,8b}$ ~3.5 Hz, H-7), 3.79–3.72 (m, 2 H, H-8a,8b), 3.77 (s, 3 H, $CO_2CH_3$),

2.54 (d, 1 H, $J_{3e,3a}$ ~14.0 Hz, H-3*e*), 2.07 (dd, 1 H, $J_{3a,4}$ ~$J_{3a,5}$ ~2.5 Hz, H-3*a*), 0.91 and 0.89 [s, 18 H, $(CH_3)_3C$], 0.14 and 0.07 [s, 12 H, $(CH_3)_2Si$].

*Anal.* Calc. for $C_{25}H_{46}O_9Si_2$: C, 54.91; H, 8.48. Found: C, 55.02; H, 8.37.

*Methyl (allyl 7,8-di-*O-tert-*butyldimethylsilyl-3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**3**). — A solution of **2** (1.08 g) in dry methanol (20 mL) was stirred with 0.1M methanolic sodium methoxide (10 mL) for 10 h at room temperature. The mixture was made neutral by addition of Dowex 50 ($H^+$) cation-exchange resin, filtered, and evaporated. Purification of the residue on a column of silica gel (B, 2:1 toluene–ethyl acetate) gave **3** (1.02 g, 100%), colorless syrup, $[\alpha]_D^{20}$ +18.2° (*c* 1.2, chloroform); $^1$H-n.m.r.: δ 5.87 (m, 1 H, –C*H*=), 5.26 (dq, 1 H, =C$H_2$-*trans*), 5.15 (dq, 1 H, =C$H_2$-*cis*), 4.34 (m, 1 H, OC$H_2$–CH=), 4.29 (br. s, 1 H, OH-5), 4.19 (ddd, 1 H, $J_{7,6}$ ~10.0, $J_{7,8a}$ ~3.0, $J_{7,8b}$ ~6.0 Hz, H-7), 4.04 (br. s, 1 H, H-5), 3.98 (m, 1 H, OC$H_2$–CH=), 3.76 (s, 3 H, $CH_3OCO$), 3.75 (dd, 1 H, $J_{8a,8b}$ ~10.5 Hz, H-8a), 3.71 (dd, 1 H, $J_{6,5}$ ~2.0 Hz, H-6), 3.66 (dd, 1 H, H-8b), 3.56 (ddd, 1 H, H-4), 2.44 (dd, 1 H, $J_{3e,3a}$ ~12.5, $J_{3e,4}$ ~5.0 Hz, H-3*e*), 2.38 (d, 1 H, $J_{4,OH}$ ~10.0 Hz, OH-4), 2.07 (t, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*), 0.92 and 0.88 [s, 18 H, $(CH_3)_3C$], 0.17, 0.13, and 0.07 [s, 12 H, $(CH_3)_2Si$].

*Anal.* Calc. for $C_{24}H_{48}O_8Si_2$: C, 55.35; H, 9.29. Found: C, 55.51; H, 9.26.

*Methyl* O-[*methyl (4,5,7,8-tetra-*O-*acetyl-3-deoxy-α-*D-manno-*2-octulopyranosyl)onate*]-(2→4)-*(allyl 7,8-di-*O-tert-*butyldimethylsilyl-3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**6**) *and methyl* O-[*methyl (4,5,7,8-tetra-*O-*acetyl-3-deoxy-β-*D-manno-*2-octulopyranosyl)onate*]-(2→4)-*(allyl 7,8-di-*O-tert-*butyldimethylsilyl-3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**9**). — A solution of **4** (710 mg, 1.47 mmol) in dry acetonitrile (7 mL) was added dropwise during 4 h at room temperature to a suspension of **3** (420 mg, 0.8 mmol), mercury(II) cyanide (540 mg, 2.14 mmol), and molecular sieves 4A (1 g) in acetonitrile (5 mL) under a stream of dry $N_2$. After stirring for 15 h, the mixture was diluted with dichloromethane (50 mL), filtered, extracted with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated. Purification of the residue on a column of silica gel (C, 4:1 toluene–ethyl acetate) gave **6** and **7** (429 mg, 58%; $R_F$ 0.45, 2:1 toluene–ethyl acetate) as a colorless syrup. The mixture was further purified by column chromatography on silica gel (0.015–0.040 mm) using 5:2 hexane–ethyl acetate as the eluent. Pooling and evaporation of the fractions containing the faster moving component afforded **6** (310 mg, 42%), colorless syrup, $[\alpha]_D^{20}$ +73° (*c* 0.74, chloroform); $^1$H-n.m.r.: δ 5.84 (m, 1 H, –C*H*=), 5.41 (br. s, 1 H, H-5′), 5.33 (ddd, 1 H, $J_{4',5'}$ ~2.7, $J_{4',3'e}$ ~4.5, $J_{4',3'a}$ ~12.5 Hz, H-4′), 5.24 (ddd, 1 H, $J_{7',6'}$ ~9.5, $J_{7',8'a}$ ~2.7, $J_{7',8'b}$ ~4.5, Hz, H-7′), 5.23 (dq, 1 H, =$CH_2$-*trans*), 5.13 (dq, 1 H, =$CH_2$-*cis*), 4.70 (dd, 1 H, $J_{8'a,8'b}$ ~ −12.0 Hz, H-8′a), 4.30 (m, 1 H, OC$H_2$CH=), 4.24 (dd, $J_{6',5'}$ ~1.3 Hz, H-6′), 4.10 (ddd, 1 H, $J_{6,7}$ ~5.0, $J_{7,8a}$ ~5.0, $J_{7,8b}$ ~6.0 Hz, H-7), 4.05 (dd, 1 H, H-8′b), 4.00 (m, 1 H, OC$H_2$CH=), 3.99 (unresolved signal, 1 H, H-5), 3.79 (dd, 1 H, $J_{8a,8b}$ ~10.5 Hz, H-8a), 3.79 and 3.75 (s, 6 H, $CH_3OCO$), 3.71 (ddd, 1 H, $J_{4,5}$ ~2.5, $J_{4,3e}$ ~5.2, $J_{4,3a}$ ~12.0 Hz, H-4), 3.61 (dd, 1 H, H-8b), 3.53 (d, 1 H, $J_{5,OH}$ ~1.2 Hz, OH-5), 3.47 (dd, 1 H, $J_{6,5}$ ~ 1.0 Hz, H-6), 2.41 (dd, 1 H, $J_{3e,3a}$ ~12.0 Hz,

H-3*e*), 2.32 (t, 1 H, H-3*a*), 2.30 (dd, 1 H, $J_{3'e,3'a}$ ~12.5 Hz, H-3'*e*), 2.06 (t, 1 H, H-3'*a*), 2.07, 2.06, 1.99 and 1.96 (s, 12 H, $CH_3CO$), 0.89 [s, 18 H, $(CH_3)_3C$], 0.14, 0.12, and 0.06 [s, 12 H, $(CH_3)_2Si$].

*Anal.* Calc. for $C_{41}H_{70}O_{19}Si_2$: C, 53.34; H, 7.64. Found: C, 53.50; H, 7.65.

Further elution of the column with 2:1 hexane–ethyl acetate afforded **9** (109 mg, 15%), colorless syrup, $[\alpha]_D^{20}$ +39° (*c* 0.56, chloroform); $^1$H-n.m.r.: δ 5.85 (m, 1 H, –CH=), 5.26 (unresolved signal, 1 H, H-5'), 5.24 (dq, 1 H, =C*H*$_2$-*trans*), 5.14 (dq, 1 H, =C*H*$_2$-*cis*), 5.13 (ddd, 1 H, $J_{7',8'b}$ ~2.0, $J_{7',8'a}$ ~3.5, $J_{7',6'}$ ~9.5 Hz, H-7'), 4.86 (ddd, 1 H, $J_{4',5'}$ ~3.0, $J_{4',3'e}$ ~5.0, $J_{4',3'a}$ ~13.0 Hz, H-4'), 4.60 (dd, 1 H, $J_{8'a,8'b}$ ~12.0 Hz, H-8'a), 4.31 (m, 1 H, OC*H*$_2$–CH=), 4.22 (dd, 1 H, $J_{6',5'}$ ~1.5 Hz, H-6'), 4.13 (ddd, 1 H, $J_{7,6}$ ~6.0, $J_{7,8a}$ ~5.0, $J_{7,8b}$ ~6.0 Hz, H-7), 4.11 (unresolved signal, 1 H, H-5), 4.00 (m, 1 H, OC*H*$_2$–CH=), 3.99 (dd, 1 H, H-8'b), 3.92 (dd, 1 H, H-8a), 3.83 and 3.81 (s, 6 H, $CH_3OCO$), 3.80 (ddd, 1 H, $J_{4,5}$ ~2.5, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.5 Hz, H-4), 3.75 (d, 1 H, $J_{5,OH}$ ~1.0 Hz, OH-5), 3.65 (dd, 1 H, H-8b), 3.63 (dd, 1 H, $J_{6,5}$ ~1.0 Hz, H-6), 2.39 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.27 (t, 1 H, $J_{3e,3a}$ ~12.0 Hz, H-3*a*), 2.17 (t, 1 H, $J_{3'e,3'a}$ ~13.0 Hz, H-3'*a*), 2.12 (dd, 1 H, H-3'*e*), 2.11, 2.09, 2.01, and 1.98 (s, 12 H, $CH_3CO$), 0.91 and 0.90 [s, 18 H, $(CH_3)_3C$], 0.17, 0.13, and 0.07 [s, 12 H, $(CH_3)_2Si$].

*Anal.* Calc. for $C_{41}H_{70}O_{19}Si_2$: C, 53.34; H, 7.64. Found: C, 53.21; H, 7.58.

*Methyl* O-[*methyl (4,5,7,8-tetra*-O-*acetyl-3-deoxy-α-*D-manno-*2-octulopyranosyl)onate*]-*(2→4)-(allyl 3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**7**). — A solution of **6** (177 mg) in dry acetonitrile (10 mL) was stirred with 2% HF in acetonitrile (0.6 mL) for 2 h at room temperature. After addition of $NaHCO_3$ (1 g), the mixture was taken to dryness. The residue was dissolved in ethyl acetate (50 mL), extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Purification of the residue on a column of silica gel (B, ethyl acetate) gave **7** (130 mg, 98%), colorless syrup, $[\alpha]_D^{20}$ +86° (*c* 1.4, chloroform); $^1$H-n.m.r.: δ 5.83 (m, 1 H, –CH=), 5.40 (unresolved signal, 1 H, H-5'), 5.32 (ddd, 1 H, $J_{4',5'}$ ~3.0, $J_{4',3'e}$ ~5.0, $J_{4',3'a}$ ~12.5 Hz, H-4'), 5.26 (ddd, 1 H, $J_{7',6'}$ ~9.5, $J_{7',8'a}$ ~3.0, $J_{7',8'b}$ ~5.0 Hz, H-7'), 5.23 (dq, 1 H, =C*H*$_2$-*trans*), 5.14 (dq, 1 H, =C*H*$_2$-*cis*), 4.75 (unresolved signal, OH), 4.70 (dd, 1 H, $J_{8'a,8'b}$ ~12.0 Hz, H-8'a), 4.26 (m, 1 H, OC*H*$_2$CH=), 4.20 (dd, 1 H, $J_{6',5'}$ ~1.5 Hz, H-6'), 4.02 (dd, 1 H, H-8'b), 4.02–3.88 (m, 5 H, H-5,7,8a,8b, OC*H*$_2$–CH=), 3.83 and 3.80 (s, 6 H, $CH_3OCO$), 3.73 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.5 Hz, H-4), 3.25 (dd, 1 H, $J_{6,5}$ ~1.0, $J_{6,7}$ ~9.0 Hz, H-6), 2.48 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.28 (dd, 1 H, $J_{3'e,3'a}$ ~12.5 Hz, H-3'*e*), 2.20 (t, 1 H, H-3*a*), 2.06 (t, 1 H, H-3'*a*), 2.09, 2.06, 2.01, and 1.99 (s, 12 H, $CH_3CO$).

*Anal.* Calc. for $C_{29}H_{42}O_{19}$: C, 50.14; H, 6.09. Found: C, 49.71; H, 6.01.

*Sodium* O-[*sodium (3-deoxy-α-*D-manno-*2-octulopyranosyl)onate*]-*(2→4)-(allyl 3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**8**). — A solution of **7** (30 mg) in dry methanol was stirred with 0.1M methanolic sodium methoxide (0.45 mL) for 2 h at room temperature. The mixture was made neutral by addition of Dowex 50 ($H^+$) cation-exchange resin, filtered, and evaporated. The residue (20 mg) was dissolved in water (5 mL) and treated with 0.2M NaOH (1.0 mL) for 3 h at room

temperature. The pH of the mixture was adjusted to 8.0 by addition of Dowex 50 ($H^+$) resin. After filtration and lyophilization, the product in water was purified on a column of Bio-Gel P-2 and lyophilized, (yield, 21.9 mg, 95%), $[\alpha]_D^{20}$ +52° (*c* 0.4, water); $^1$H-n.m.r.: δ 5.95 (m, 1 H, –CH=), 5.33 (dq, 1 H, =C*H*$_2$-*trans*), 5.23 (dq, 1 H, =C*H*$_2$-*cis*), 4.23 (m, 1 H, OC*H*$_2$–CH=), 4.15–3.58 (m, 13 H, H-4,5,6,7,8a,8b,-4′,5′,6′,7′,8′a,8′b, OC*H*$_2$–CH=), 2.38 (dd, 1 H, $J_{3e,3a}$ ~12.5, $J_{3e,4}$ ~5.0 Hz, H-3*e*), 2.14 (dd, 1 H, $J_{3'e,3'a}$ ~12.5, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 1.91 (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*).

*Methyl* O-[*methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-β-*D-manno-*2-octulopyranosyl)onate*]-*(2→4)-(allyl 3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**10**). — A solution of **9** (60 mg) in dry acetonitrile (5 mL) was treated with 2% HF in acetonitrile (0.35 mL) for 45 min at room temperature. After addition of $NaHCO_3$ (1 g), the mixture was evaporated. The residue was dissolved in ethyl acetate (50 mL), extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Purification of the residue on a column of silica gel (A, ethyl acetate) afforded **10** (42 mg, 93%), colorless syrup, $[\alpha]_D^{20}$ +41.5° (*c* 0.6, chloroform); $^1$H-n.m.r.: δ 5.85 (m, 1 H, –CH=), 5.28 (unresolved signal, 1 H, H-5′), 5.24 (dq, 1 H, =C*H*$_2$-*trans*), 5.15 (ddd, 1 H, H-7′), 5.15 (dq, 1 H, =C*H*$_2$-*cis*), 4.89 (ddd, 1 H, $J_{4',5'}$ ~3.0, $J_{4',3'e}$ ~4.5, $J_{4',3'a}$ ~13.0 Hz, H-4′), 4.41 (dd, 1 H, $J_{8'a,8'b}$ ~12.0, $J_{8'a,7'}$ ~5.0 Hz, H-8′a), 4.28 (m, 1 H, OC*H*$_2$–CH=), 4.25 (dd, $J_{8'b,7'}$ ~2.0 Hz, H-8′b), 4.21 (unresolved signal, 1 H, H-5), 4.20 (dd, 1 H, $J_{6',7'}$ ~9.5, $J_{6',5'}$ ~1.5 Hz, H-6′), 4.00 (ddd, 1 H, $J_{8a,8b}$ ~12.0 Hz, H-8a), 3.85 and 3.83 (s, 6 H, $CH_3O$), 3.79 (dd, 1 H, H-8b), 3.74 (ddd, 1 H, $J_{4,5}$ ~2.5, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.0 Hz, H-4), 3.49 (dd, 1 H, $J_{6,5}$ ~1.0 Hz, H-6), 2.40 (dd, 1 H, $J_{3'e,3'a}$ ~12.5 Hz, H-3′*e*), 2.25 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.15 (t, 2 H, H-3*a*), 2.12, 2.11, 2.03, and 1.99 (s, 12 H, 4 $CH_3CO$).

*Anal.* Calc. for $C_{29}H_{42}O_{19}$: C, 50.14; H, 6.09. Found: C, 50.30; H, 6.22.

*Sodium* O-[*sodium (3-deoxy-β-*D-manno-*2-octulopyranosyl)onate*]-*(2→4)-(allyl 3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**11**). — A solution of **10** (47 mg) in dry methanol (5 mL) was stirred with 0.1M methanolic sodium methoxide (1.0 mL) for 1 h at room temperature. The mixture was made neutral by addition of Dowex 50 ($H^+$) cation-exchange resin, filtered and evaporated. The residue (33 mg) was dissolved in water (10 mL) and treated with 0.2M NaOH (2.0 mL) for 4 h at room temperature. The pH of the reaction mixture was adjusted to 8.0 by addition of Dowex 50 ($H^+$) resin. The mixture was filtered and lyophilized. Purification on Bio-Gel P-2 afforded **11** (35.3 mg, 96%), amorphous powder, $[\alpha]_D^{20}$ +41.5° (*c* 0.44, water); $^1$H-n.m.r.: δ 5.94 (m, 1 H, –CH=), 5.33 (dq, 1 H, =C*H*$_2$-*trans*), 5.22 (dq, 1 H, =C*H*$_2$-*cis*), 4.26–3.60 (m, 14 H, H-4,5,6,7,8a,8b,4′,5′,6′,7′,8′a,8′b, OC*H*$_2$–CH=), 2.46 (dd, 1 H, $J_{3'e,3'a}$ ~12.5, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 2.37 (dd, 1 H, $J_{3e,3a}$ ~12.5, $J_{3e,4}$ ~5.0 Hz, H-3*e*), 1.87 (t, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*), and 1.85 (t, 1 H, $J_{3'a,4'}$ ~12.5 Hz, H-3′a).

*Anal.* Calc. for $C_{19}H_{28}Na_2O_{15}$: C, 42.07; H, 5.20. Found: C, 41.61; H, 5.15.

*Sodium* O-[*sodium (3-deoxy-β-*D-manno-*2-octulopyranosyl)onate*]-*(2→4)-(allyl 3-deoxy-α-*D-manno-*2-octulopyranosid)onate* (**13**). — A solution of **12** (80

mg) in dry methanol (5 mL) was stirred with 0.1M methanolic sodium methoxide (1 mL) for 1 h at room temperature. The reaction mixture was made neutral by addition of Dowex 50 ($H^+$) resin, filtered, and evaporated. A solution of the residue (50 mg) in water (5 mL) was treated with 0.2M NaOH (2.5 mL) for 90 min at room temperature. Processing of the mixture as described for **11** afforded **13** (56.4 mg, 94%), amorphous powder, $[\alpha]_D^{20}$ +73° (*c* 0.4, water); $^1$H-n.m.r.: $\delta$ 5.97 (m, 1 H, –CH=), 5.35 (dq, 1 H, =C$H_2$-*trans*), 5.23 (dq, 1 H, =C$H_2$-*cis*), 4.28–4.18 (m, 2 H) and 3.98–3.55 (m, 12 H, H-4,5,6,7,8a,8b,4′,5′,6′,7′,8′a,8′b, OC$H_2$–CH=), 2.46 (ddd, 1 H, $J_{3'e,3'a}$ ~12.5, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 1.90 (t, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*), and 1.86 (t, 1 H, $J_{3'a,4'}$ ~12.5 Hz, H-3′*a*).

*Anal.* Calc. for $C_{19}H_{28}Na_2O_{15}$: C, 42.07; H, 5.20. Found: C, 41.73; H, 5.31.

*Copolymerization.* — A solution of **8** (15.2 mg), acrylamide (8.0 mg), and *N,N,N′N′*-tetramethylethylenediamine (1 μL) in water (1 mL) was degassed at aspirator pressure for 25 min. After addition of $(NH_4)_2S_2O_8$ (1 mg), the mixture was kept for 20 h at +4°. The solution was purified on a column (2.6 × 100 cm) of Sephadex G-50 with 0.01M aqueous $NaHCO_3$ as eluent, at a flow rate of 55 mL/h, and 3.5-mL fractions were collected. Product-containing fractions 38–45 were pooled and lyophilized. The residue was desalted on a column (2.6 × 100 cm) of Bio-Gel P-2 to give **14** (yield 12.2 mg), amorphous powder, $[\alpha]_D^{20}$ +11° (*c* 0.4, water). The copolymers **15** (29.4 mg of **11** and 15.4 mg of acrylamide) and **16** (32 mg of **13** and 17 mg of acrylamide) were prepared in a similar manner to yield **15** (20.2 mg), $[\alpha]_D^{20}$ +14° (*c* 0.56, water); and **16** (17.6 mg), $[\alpha]_D^{20}$ +16° (*c* 0.67, water), respectively.

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