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ARTIFICIAL ANTIGENS. SYNTHESIS OF POLYACRYLAMIDE COPOLYMERS CONTAINING 3-DEOXY-D-*manno*-2-OCTULOPYRANOSYLONIC ACID (KDO) RESIDUES*

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ABSTRACT

Starting from an anomeric mixture of the methyl (allyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α- and -β-D-*manno*-2-octulopyranosid)onates, the glycosides sodium (allyl 3-deoxy-α- and -β-D-*manno*-2-octulopyranosid)onate, sodium *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→4)-[allyl 3-deoxy-α-D-*manno*-2-octulopyranosid]onate and sodium (allyl 3-deoxy-7-*O*-β-D-ribofuranosyl-β-D-*manno*-2-octulopyranosid)onate were prepared in several steps. Radical copolymerization of the allyl glycosides with acrylamide afforded linear macromolecular antigens containing mono- and di-saccharide residues corresponding to the KDO-region of *Salmonella minnesota* rough-form lipopolysaccharide and to partial structures of the capsular polysaccharide from *Escherichia coli* K 23, respectively. The copolymers were substituted by KDO-residues in a ratio of 1:18 ±2 (based on acrylamide) and had molecular masses of 60–100 kdaltons.

INTRODUCTION

3-Deoxy-D-*manno*-2-octulosonic acid (KDO) has been originally discovered[1] as a characteristic component of the lipopolysaccharides (LPS) of gram-negative bacteria, constituting the link[2,3] between the core oligosaccharide and Lipid A. In contrast to previous reports[4–6], recent structural investigations on the inner-core region of a number of rough-mutant lipopolysaccharides have revealed an α-(2→4)-linked KDO-disaccharide as a common constituent[7–12]. Furthermore, the chemical synthesis of the tetrasaccharide α-KDO-(2→4)-α-KDO-(2→6)-β-D-GlcN-(1→6)-D-GlcN has been accomplished[13], providing an independent structural proof of a degraded lipopolysaccharide isolated from *Salmonella minnesota* R_e 595. In addition, an increasing number of capsular polysaccharides causing urinary tract in-

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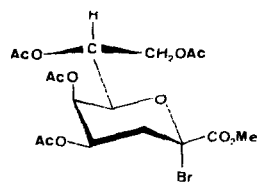

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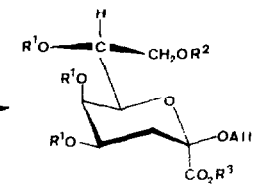
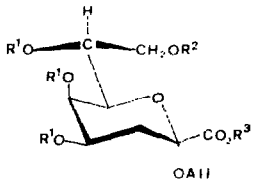
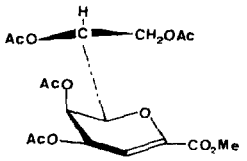


2 $R^1 = R^2 = Ac$, $R^3 = Me$
3 $R^1 = R^2 = H$, $R^3 = Me$
4 $R^1 = R^2 = H$, $R^3 = Na^+$
5 $R^1 = H$, $R^2 = Bu^tMe_2Si$, $R^3 = Me$



6 $R^1 = R^2 = Ac$, $R^3 = Me$
7 $R^1 = R^2 = H$, $R^3 = Me$
8 $R^1 = H$, $R^2 = Bu^tMe_2Si$, $R^3 = Me$
9 $R^1 = Ac$, $R^2 = Bu^tMe_2Si$, $R^3 = Me$
10 $R^1 = R^2 = H$, $R^3 = Na^+$



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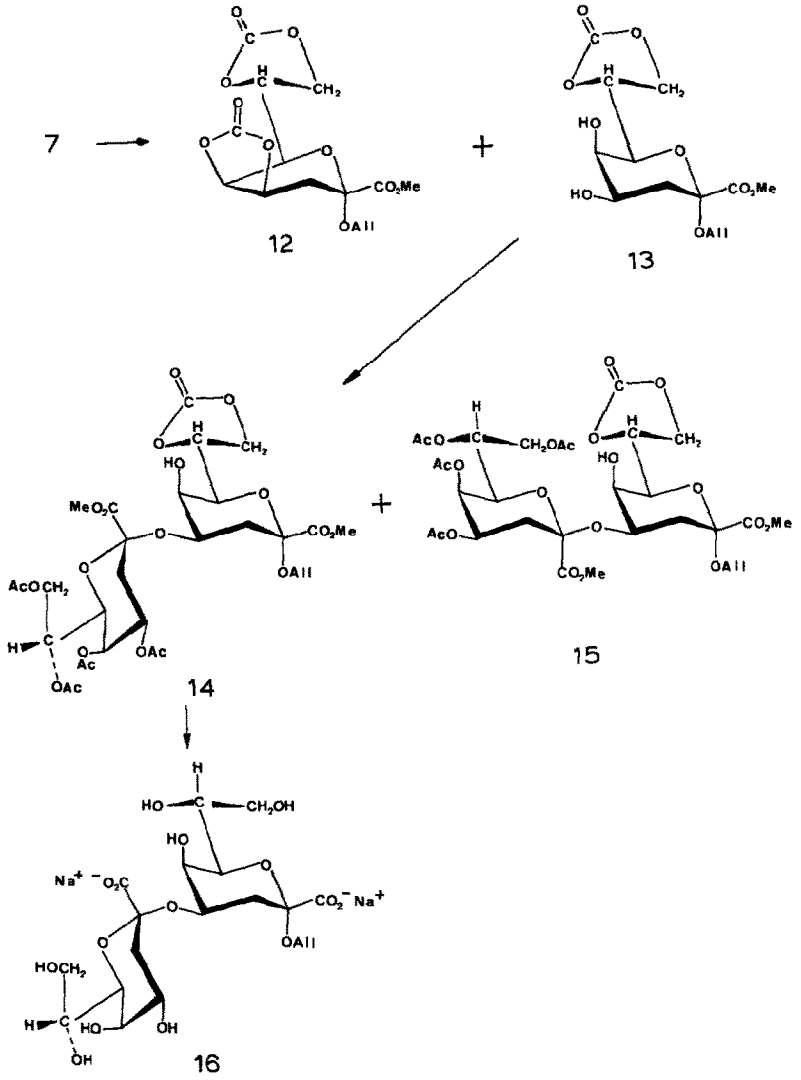
fections have also been reported to contain KDO residues[14]. Thus, artificial antigens containing the corresponding oligosaccharide determinants attached to various carrier molecules would be of value in defining antibody specificities directed against enterobacterial R-lipopolysaccharides, as well as capsular polysaccharides. We report herein the preparation of a first series of polyacrylamide copolymers containing α-KDO*p*-(2→ and α-KDO*p*-(2→4)-α-KDO*p*-(2→ units corresponding to the KDO-region of *Salmonella minnesota* R_c 595 LPS, and β-KDO*p*-(2→, β-D-Rib*f*-(1→7)-β-KDO*p*-(2→ residues corresponding to partial structures of *Escherichia coli* K 13, K 20, and K 23 capsular polysaccharides[15,16]. Polyacrylamide antigens of this type displaying the specificities of O:3 and O:4 factors of *Salmonella* and of the capsular polysaccharide from *Streptococcus pneumoniae* type 3 have first been prepared by Kochetkov *et al.*[17,18].

RESULTS AND DISCUSSION

For the synthesis of the allyl glycosides **4, 10, 16,** and **22,** the allyl α- and β-ketosides of methyl (3-deoxy-D-*manno*-2-octulopyranosid)onate had to be employed as the starting material. Therefore, glycosylation of allyl alcohol with methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosyl bromide)-onate[19] (**1**) was studied under various conditions. Promotion of the glycosylation reaction by insoluble silver salts (silver carbonate or silver zeolith[20] in the presence of molecular sieve 4A in dichloromethane) gave crystalline methyl (allyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**2**) in 72% yield, together with a small proportion (12%) of the glycal ester derivative[21] **11**. Zemplén deacylation of **2** gave **3**, which was converted, in quantitative yield, into sodium

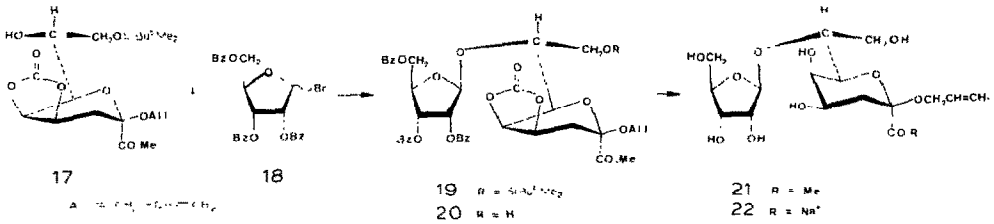
(allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**4**) by the action of aqueous sodium hydroxide. Reaction of allyl alcohol with **1**, catalyzed by mercury(II) cyanide, afforded an homogeneous (by t.l.c.) mixture of the allyl β- and α-glycosides **2** and **6**, which could not be separated by column chromatography on silica gel. The α-to-β ratio of the anomers **2** and **6**, which was calculated from the relative intensities of the 250-MHz, ^{1}H-n.m.r.-signals attributable to H-4 of **2** (δ 4.89) and H-8a of **6** (δ 4.60), was found to depend on the solvent employed in the glycosylation reaction; when dichloromethane was the solvent, an ~1:2 mixture of the α and β anomers was obtained in 96% yield, whereas glycosylation performed in acetonitrile afforded an ~2:3 mixture in 96% yield. In nitromethane, however, the α



anomer **6** was formed as the major isomer (3:1, 81% yield). Separation of the isomers was achieved after deacetylation of the mixture in methanolic sodium methoxide to give **3** and **7**, and subsequent conversion into the crystalline 8-*O*-*tert*-butyldimethylsilyl ethers **5** and **8** with *tert*-butyldimethylchlorosilane and 1,4-diazabicyclo[2.2.2]octane[22] in acetonitrile (72%). After separation of the isomers by column chromatography on silica gel, an aliquot of **8** was acetylated (acetic anhydride–pyridine) to confirm the assignment of the α-anomeric configuration. The 250-MHz, ^{1}H-n.m.r.-chemical shift data of the axial and equatorial H-3 (δ 2.07 and 2.20) and of the signal attributable to H-4 (δ 5.40) of compound **9** are indicative of the α-anomeric configuration[23].

Removal of the Bu^{t}Me$_2$Si group was achieved by the action of 2% hydrofluoric acid in acetonitrile[24], to give **7** in quantitative yield. Compound **7** was further characterized by conversion into the peracetylated derivative **6** in 90% yield. De-esterification of **7** with aqueous sodium hydroxide afforded sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**10**) in 100% yield.

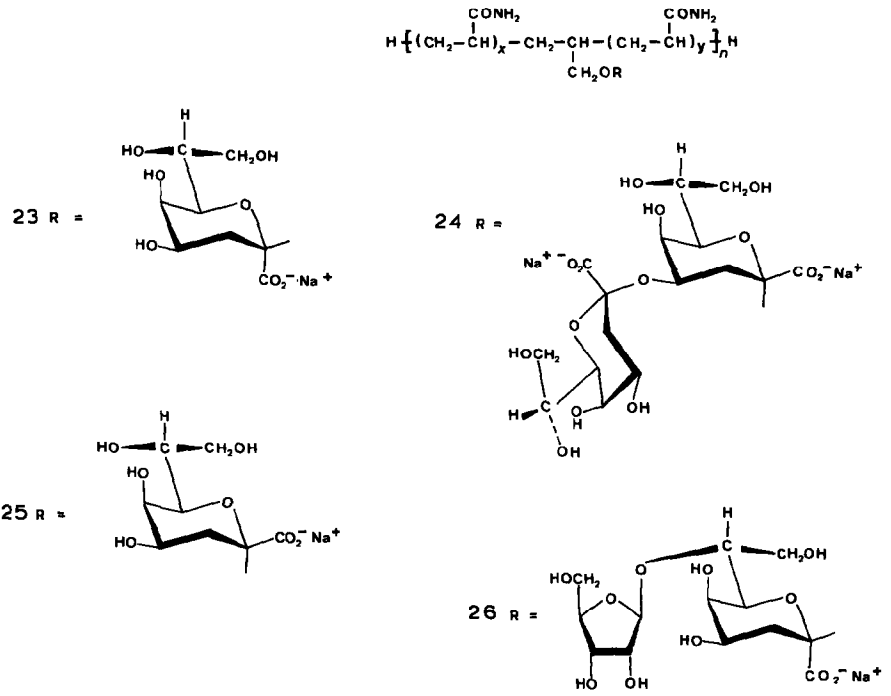
Proceeding towards the synthesis of the disaccharide derivative **16**, compound **7** was treated with 4-nitrophenyl chloroformate–pyridine[25] to give the 4,5:7,8-di-*O*-carbonyl derivative **12** (22%), the crystalline 7,8-*O*-carbonyl-derivative **13** (34%), and unreacted starting material (30%) as the major products, which were separated by column chromatography on silica gel. Glycosylation of **13** with five molar equivalents of **1** under catalysis by mercury(II) cyanide in acetonitrile gave a 1:3 mixture of the β-D-(2→4)-linked **15** and α-D-(2→4)-linked disaccharide derivative **14** in 69% yield. After separation of the isomers by column chromatography on silica gel, **14** was deprotected by sequential deacetylation in methanolic sodium methoxide and subsequent deesterification in aqueous sodium hydroxide to give sodium *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2→4)-(allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**16**) in quantitative yield.



17 18 19 R = Si^{t}Bu^{t}Me$_2$ 20 R = H 21 R = Me 22 R = Na$^+$

For the synthesis of the disaccharide derivative **22**, compound **5** was converted in 88% yield, into the 4,5-*O*-carbonyl derivative **17** by treatment with 4-nitrophenyl chloroformate–pyridine. Glycosylation of **17** with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide[26] (**18**) under catalysis with silver trifluoromethylsulfonate in the presence of *N*,*N*,*N*′,*N*′-tetramethylurea (TMU) gave a low yield (10%) of an anomeric mixture of the α- and β-D-ribofuranosyl derivatives. Promotion of the glycosylation reaction with mercury(II) cyanide in dichloromethane afforded exclu-

sively the β-D-linked disaccharide derivative **19** in 78% yield. The assignment of the β-D-anomeric configuration of the ribofuranosyl residue was based on the value of the coupling constant[27] $J_{1,2}$ (0.8 Hz). Deprotection of **19** was accomplished as follows. Cleavage of Bu^tMe_2Si-3 by treatment with 2% hydrogen fluoride in acetonitrile gave **20** in 100% yield. Zemplén deacylation of **20** afforded **21**, which was deesterified in aqueous sodium hydroxide to give sodium (allyl 3-deoxy-7-*O*-β-D-ribofuranosyl-β-D-*manno*-2-octulopyranosid)onate (**22**) in quantitative yield.



Copolymerization of the allyl glycosides **4, 10, 16,** and **22** with 4 molar equivalents of acrylamide was performed essentially under the conditions given by Horejsi *et al.*[28], *i.e.*, reaction of the allyl glycosides with acrylamide in aqueous solution in the presence of *N,N,N′,N′*-tetramethylethylenediamine and ammonium persulfate. The copolymers **23, 24, 25,** and **26** were purified by gel permeation chromatography on Bio-Gel P-6 (which allowed the recovery of unreacted allyl glycoside in 40–50% yield) and subsequent desalting on Sephadex G-25. Estimation of the molecular masses of the copolymers was performed by analytical fractionation on Sepharose 4B (products were eluted in the void volumes of Sephadex G-25, Bio-Rad P-100, and Bio-Rad P-6–Sephacryl S-200), the main proportion of the copolymers being eluted in a range corresponding to a M_r 60–100 kdalton. The copolymers contained oligosaccharide residues in a ratio of 1:18 ±2 (based on acrylamide), as determined by a thiobarbituric acid assay[5,29] and calculated from

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS (δ) OF THE KDO-REGION OF A TETRASACCHARIDE FROM *Salmonella minnesota* R_c 595 LPS[a], RELATED MONO- AND DI-SACCHARIDE DERIVATIVES, AND POLYACRYLAMIDE COPOLYMERS

Carbon atom	*Compound*						
	α-KDOpOMe[b]	**10**	*α-KDOp-(2→4) α-KDOpOMe*[a]	**16**	*R_c 595 LPS*[a]	**25**	**24**
1	176.1	176.1	176.1	176.0	175.5	176.2	176.0
2	101.3	101.1	101.3	101.0	100.5	101.0	101.0
						100.9	
3	34.9	35.1	34.0	34.2	34.1		
4	66.8	66.9	69.6	69.5	69.3	67.1	69.5
5	67.1	67.2	65.1	65.2	65.2	67.3	65.1
6	72.2	72.5	72.1	72.4	73.2	72.4	72.2
7	70.2	70.4	70.2	70.4	70.4	70.4	70.5
8	63.9	64.1	63.8	64.0	64.2	64.1	63.9
1′			176.7	176.8	176.4		176.4
2′			100.3	100.2	100.2		100.3
3′			35.3	35.4	35.2		
4′			66.7	66.8	66.7		66.7
5′			67.1	67.2	66.9		67.2
6′			73.0	73.3	73.3		73.7
7′			70.7	70.9	70.5		71.1
8′			63.9	64.0	64.2		63.9
OCH_3		65.4		65.0			
–CH=		134.8		135.0			
CH_2=		118.8		118.1			

[a]Ref. 11. [b]Ref. 30.

TABLE II

^{13}C-N.M.R.-CHEMICAL SHIFTS (δ) OF DEACETYLATED K13 POLYSACCHARIDE, RELATED MONO- AND DI-SACCHARIDE DERIVATIVES, AND POLYACRYLAMIDE COPOLYMERS

Carbon atom	*Compound*						
	β-KDOpOMe[a]	**4**	*β-D-Rib-(1→7) β-KDOpOMe*[b]	**22**	*K-13D*[b]	**26**	**23**
1	174.5	174.6	174.4	174.5	174.0	173.4	174.8
2	102.1	101.9	102.1	101.8	102.4	100.9	101.8
3	35.3	35.6	35.3	35.6	35.2		
4	68.2	68.3	68.2	68.2	68.1	67.9	68.4
5	66.2	66.2	66.0	65.9	66.0	66.0	66.3
6	74.3	74.3	72.9	72.9	73.0[c]	73.3	74.2
7	69.9	69.9	75.4[c]	75.3	75.8	75.7	70.0
8	64.8	64.9	60.8	60.7	59.9	60.7	65.1
1′			105.8	105.7	104.3	106.1	
2′			75.7[c]	75.6	73.4[c]	75.7	
3′			71.2	71.2	74.9	71.1	
4′			83.5	83.4	81.9	83.5	
5′			63.2	63.1	63.2	63.1	
OCH_2		66.6		66.6			
–CH=		134.8		134.7			
CH_2=		119.0		118.9			

[a]Ref. 30. [b]Ref. 31. [c]Assignments may be interchanged.

the relative intensities of the bulk of signals attributable to carbohydrate residues *vs.* CH- and CH_2-groups of the polyacrylamide backbone in the 250-MHz, ^{1}H-n.m.r.-spectra.

^{13}C-N.m.r.-chemical shift data compared favorably with data obtained from the corresponding methyl glycosides, *Salmonella minnesota* R_c 595 lipopolysaccharide (Table I), and *Escherichia coli* deacetylated K 13 capsular polysaccharide (Table II). Additional signals were observed for $CONH_2$ (δ 181.1 and 180.3), CH (δ 44.3–42.3), and CH_2 (δ 37.1–34.9) groups. Broad signals corresponding to the CH_2–OR group linked to the carbohydrate residues appeared at δ 67.0–68.0. Immunochemical studies obtained with these copolymers will be published elsewhere.

EXPERIMENTAL

General methods. — Melting points were determined on a Kofler hot-stage and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. ^{1}H-N.m.r.-spectra were recorded with a Bruker WM-250 instrument and tetramethylsilane as the internal standard. Coupling constants are first order. ^{13}C-N.m.r.-spectra were recorded at 62.9 MHz for solutions in deuterium oxide, at 24°, 32 k of memory, and a spectral width of 12 kHz. The instrument was operated in the F.t. mode with complete proton-decoupling. Chemical shifts (δ) are given from the signal of tetramethylsilane whose resonance frequency was set at δ 67.40 upfield from an external signal of 1,4-dioxane in deuterium oxide. Thin-layer chromatography was performed on Merck precoated plates (5 × 10 cm, layer-thickness 0.25 mm, Silica gel 60 F_{254}). Spots were detected by u.v. light and 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 x 3.7 cm; silica gel 40–63 μm) under pressure (0.2 MPa). Acrylamide was twice recrystallized from chloroform. Elemental analyses were performed by Dr. J. Zak, Mikroanalytisches Laboratorium am Institut für Physikalische Chemie, Universität Wien.

*Methyl (allyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-β-*D*-manno-2-octulopyranosid)-onate* (**2**). — A solution of **1** (1.4 g, 2.9 mmol) in dichloromethane (5 mL) was added at −12° to a suspension of silver zeolith (1 g), molecular sieve 4A (1.5 g), and allyl alcohol (400 mg, 6 mmol, dried over CaH_2–molecular sieve 3A) in dichloromethane (5 mL) under dry N_2. After being stirred for 4 h at room temperature, the mixture was diluted with dichloromethane (50 mL), filtered, and extracted with 5% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The organic layer was dried, evaporated, and the residue purified on a column of silica gel (B, 5:1 toluene–ethyl acetate) to give **2** (920 mg, 72%), colorless prisms, m.p. 92° (ethyl acetate–hexane), $[\alpha]_D^{20}$ +54.9° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.88 (m, 1 H, =C*H*–), 5.30 (dd, 1 H, $J_{4,5}$ ~3.0, $J_{5,6}$ ~1.5 Hz, H-5), 5.31–5.16 (m, 2 H, CH_2=), 5.18 (ddd, 1 H, H-7), 4.89 (ddd, 1 H, $J_{3e,4}$ ~5.0, $J_{3a,4}$ ~12.5 Hz, H-4), 4.42 (dd, 1 H, $J_{8a,8b}$ ~12.5, $J_{7,8a}$ ~4.0 Hz, H-8a), 4.33 (dd, 1 H, $J_{8b,7}$ ~2.5, H-8b), 4.28 (m, 1 H, CH_2=CH–C*H*), 4.20 (dd, 1 H, $J_{6,7}$ ~9.5 Hz, H-6), 3.94 (m, 1 H,

CH_2=CH–C*H*), 3.81 (s, 3 H, CH_3OCO), 2.41 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.15 (t, 1 H, H-3*a*), and 2.13, 2.11, 2.02, 1.99 (s, 12 H, 4 CH_3CO); ^{13}C-n.m.r. ($CDCl_3$): 170.4, 170.2, 169.6, 169.5, 168.1 (CO_2), 133.6 (CH=), 117.0 (=CH–), 99.1 (C-2), 70.7 (C-6), 68.0 (C-7), 67.0 (C-4), 65.5 (–CH_2O), 64.0 (C-5), 62.2 (C-8), 52.5 (OCH_3), 32.3 (C-3), and 20.5 (CH_3).

Anal. Calc. for $C_{20}H_{28}O_{12}$: C, 52.17; H, 6.13. Found: C, 52.15; H, 6.09.

*Methyl (allyl 3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**3**). — A solution of **2** (446 mg) and Na (2 mg) in dry methanol (25 mL) was stirred for 2 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and evaporated; yield 278 mg (100%) of **3**, colorless syrup, $[\alpha]_D^{20}$ +58.9° (*c* 1.9, methanol); ^{1}H-n.m.r. (90 MHz, D_2O): δ 6.18–5.72 (m, 1 H, –CH=), 5.53–5.17 (m, 2 H, CH_2=), 4.42–4.06 (m, 2 H, CH_2=CH–C*H*$_2$), 4.05–3.55 (m, 6 H, H-4,5,6,7,8a,8b), 3.88 (s, 3 H, CH_3OCO), 2.47 (dd, 1 H, $J_{3e,3a}$ ~12.5, $J_{3e,4}$ ~5.0 Hz, H-3*e*), and 1.98 (t, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*).

Anal. Calc. for $C_{12}H_{20}O_8$: C, 49.31; H, 6.89. Found: C, 48.89; H, 6.81.

*Sodium (allyl 3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**4**). — A solution of **3** (79 mg) and mM NaOH in water (10 mL) was stirred for 12 h at room temperature. The solution was adjusted to pH 8.5 by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and lyophilized. Purification of the residue on a column (16 × 100 cm) of Bio-Gel P-2 afforded **4** (80 mg, 100%), colorless glass, $[\alpha]_D^{20}$ +37.9° (*c* 1.0, water); ^{1}H-n.m.r. (D_2O): δ 5.94 (m, 1 H, –CH=), 5.32 (dq, 1 H, =CH_{2trans}), 5.22 (dq, 1 H, =CH_{2cis}), 4.27–4.19 (m, 1 H, CH_2=CH–C*H*), 4.01–3.72 (m, 6 H, CH_2=CH–C*H*, H-4,5,7,8a,8b), 3.63 (dd, 1 H, $J_{6,7}$ ~9.5, $J_{6,5}$ ~1.0 Hz, H-6), 2.43 (ddd, $J_{3e,3a}$ ~12.5, $J_{3e,4}$ ~5.0, $^4J_{3e,5}$ ~0.5, Hz, H-3*e*), and 1.81 (t, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*).

*Methyl (allyl 8-*O-tert-*butyldimethylsilyl-3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**5**) *and methyl (allyl 8-*O-tert-*butyldimethylsilyl-3-deoxy-α-*D-manno-*2-octulopyranosid)onate* (**8**). — A solution of **1** (1.5 g, 3.1 mmol) in dry nitromethane (5 mL) was added to a suspension of allyl alcohol (0.67 mL, 10 mmol), mercury(II) cyanide (2.52 g), and molecular sieve 4A (1 g) in nitromethane (10 mL) under dry Ar. After being stirred for 3 h at room temperature, the mixture was diluted with dichloromethane (50 mL), filtered, washed with saturated aqueous $NaHCO_3$, and dried ($MgSO_4$). After removal of the solvents, the residue was subjected to column chromatography on silica gel (C, 5:1 toluene–ethyl acetate) to give 1.16 g (81%) of **2** and **6** as a syrup. The mixture was dissolved in dry methanol (25 mL) containing 10mM methanolic sodium methoxide and stirred 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 709 mg (96%) of **3** and **7**. The products were dissolved in a solution of 1,4-diazabicyclo[2.2.2]octane (409 mg) in dry acetonitrile (10 mL). After addition of *tert*-butyldimethylchlorosilane (439 mg), the mixture was stirred for 2 h at room temperature, filtered, and evaporated. The residue was purified on a column of silica gel (B, 1:5 toluene–ethyl acetate) to give **5** (283 mg, 29%) as the faster-moving component, colorless needles, m.p. 95° (ethyl acetate–

hexane), $[\alpha]_D^{20}$ +44.7° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.98–5.80 (m, 1 H, –C*H*=), 5.30–5.13 (m, 2 H, CH_2=), 4.29–4.22 (m, 1 H, CH_2=CH–C*H*), 4.01 (ddd, 1 H, $J_{5,4}$ ~3.5, $J_{5,6}$ ~1.0 Hz, H-5), 4.00–3.90 (m, 2 H, H-7, CH_2=CH–C*H*), 3.90 (dd, 1 H, $J_{8a,8b}$ ~10.0, $J_{8a,7}$ ~4.5 Hz, H-8a), 3.77 (s, 3 H, CH_3OCO), 3.73 (dd, 1 H, $J_{8b,7}$ ~5.3 Hz, H-8b), 3.66 (dddd, 1 H, $J_{4,3e}$ ~4.5, $J_{4,3a}$ ~12.5 Hz, H-4), 3.61 (dd, 1 H, $J_{7,6}$ ~7.0 Hz, H-6), 3.25 (d, 1 H, $J_{5,OH}$ ~4.0 Hz, 5-OH), 2.91 (d, 1 H, $J_{7,OH}$ ~5.2 Hz, 7-OH), 2.66 (d, 1 H, $J_{4,OH}$ ~9.0 Hz, 4-OH), 2.45 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), 2.01 (t, 1 H, H-3*a*), 0.92 [s, 9 H, C$(CH_3)_3$], and 0.12 [S, 6 H, Si$(CH_3)_2$].

Anal. Calc. for $C_{18}H_{34}O_8Si$: C, 53.18; H, 8.43. Found: C, 53.22; H, 8.40.

Further elution of the column afforded **8**, yield 429 mg (43.5%), colorless needles, m.p. 119–121° (ethyl acetate–hexane), $[\alpha]_D^{20}$ +63.7° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.86 (m, 1 H, =CH), 5.26 (dq, 1 H) and 5.16 (dq, 1 H, CH_2=), 4.12–3.67 (m, 9 H, CH_2=CH–CH_2, H-4,5,6,7,8a,8b,OH), 3.79 (s, 3 H, CH_3OCO), 3.32 (d, 1 H, *J* ~5.5 Hz, OH), 2.93 (1 H, *J* ~9.5 Hz, OH), 2.19 (dd, and d, 2 H, $J_{3e,3a}$ ~12.0, $J_{3e,4}$ ~4.0 Hz, H-3*e*,OH), 1.93 (t, 1 H, $J_{3a,4}$ ~12.0 Hz, H-3*a*), 0.88 [s, 9 H, C$(CH_3)_3$], 0.09 and 0.06 [s, 6 H, Si$(CH_3)_2$].

Anal. Calc. for $C_{18}H_{34}O_8Si$: C, 53.18; H, 8.43. Found: C, 53.25; H, 8.47.

Alternatively, **5** was prepared from **3** as follows. A solution of **3** (600 mg, 1.75 mmol), 1,4-diazabicyclo[2.2.2]octane (337 mg, 3 mmol), and *tert*-butyldimethylchlorosilane (300 mg, 2 mmol) in dry acetonitrile (10 mL) was stirred for 3 h at room temperature. The mixture was evaporated and the residue dissolved in ethyl acetate (50 mL). The organic layer was extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Recrystallization of the residue from ethyl acetate–hexane afforded 497 mg (70%) of **5**.

*Methyl (allyl 4,5,7-tri-*O*-acetyl-8-*O*-tert-butyldimethylsilyl-3-deoxy-α-*D*-manno-2-octulopyranosid)onate* (**9**). — A solution of **8** (70 mg), 4-(dimethylamino)-pyridine (5 mg), and acetic anhydride (0.1 mL) in dry pyridine (5 mL) was stirred for 2.5 h at room temperature. After evaporation to dryness, the residue was purified on a column of silica gel (A, 4:1 toluene–ethyl acetate) which gave **9** (74 mg, 80%), colorless syrup, $[\alpha]_D^{20}$ +58.3° (*c* 2.2, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.87 (m, 1 H, –CH=), 5.40 (ddd, 1 H, $J_{4,5}$ ~3.5, $J_{4,3e}$ ~5.5, $J_{4,3a}$ ~12.0 Hz, H-4), 5.38 (dd, 1 H, H-5), 5.28 (dq, 1 H) and 5.17 (dq, 1 H, CH_2=). 5.04 (dt, 1 H, $J_{7,6}$ ~10.0, $J_{7,8a} \simeq J_{7,8b}$ ~2.5 Hz, H-7), 4.22 (dd, 1 H, $J_{6,5}$ ~1.0 Hz, H-6), 4.20–4.11 (m, 1 H, CH_2=CH–C*H*), 3.96 (dd, 1 H, $J_{8a,8b}$ ~12.0 Hz, H-8a), 3.96–3.89 (m, 1 H, CH_2=CH–C*H*), 3.82 (dd, 1 H, H-8b), 3.80 (s, 3 H, CH_3OCO), 2.20 (dd, 1 H, $J_{3e,3a}$ ~12.0 Hz, H-3*e*), 2.07 (t, 1 H, H-3*a*), 2.07 (s, 3 H), 2.00 (s, 3 H) and 1.96 (s, 3 H, CH_3CO), 0.86 [s, 9 H, $(CH_3)_3C$], 0.05 and 0.01 [s, 6 H, Si$(CH_3)_2$].

Anal. Calc. for $C_{24}H_{40}O_{11}Si$: C, 54.12; H, 7.57. Found: C, 54.32; H, 7.43.

*Methyl (allyl 3-deoxy-α-*D*-manno-2-octulopyranosid)onate* (**7**). — A solution of **8** (276 mg) and 2% HF–acetonitrile (1 mL) in acetonitrile (5 mL) was stirred for 1 h at room temperature. The solution was made neutral by addition of Dowex AG-1 X-8 (HCO_3^-) anion-exchange resin, filtered, and evaporated to dryness to yield **7**, 199 mg (100%), colorless syrup, $[\alpha]_D^{20}$ +86.2° (*c* 1.44, methanol); ^{1}H-n.m.r. (D_2O): δ 5.94 (m, 1 H, –CH=), 5.35 (dq, 1 H) and 5.28 (dq, 1 H, CH_2=), 4.18–3.65

(m, 8 H, CH_2=CH–CH_2, H-4,5,6,7,8a,8b), 2.11 (dd, 1 H, $J_{3a,3e}$ ~12.5, $J_{3e,4}$ ~5.0 Hz, H-3*e*), and 1.94 (dd, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*).

Anal. Calc. for $C_{12}H_{20}O_8$: C, 49.31; H, 6.89. Found: C, 48.95; H, 6.69.

*Methyl (allyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D*-manno-2-octulopyranosid)-onate* (**6**). — A solution of **7** (24 mg) and acetic anhydride (0.1 mL) in pyridine (2 mL) was stirred for 20 h at room temperature. After evaporation of the mixture to dryness, the residue was purified on a column of silica gel (A, 3:1 toluene–ethyl acetate) to give **6**, 29.5 mg (80%), colorless syrup, $[\alpha]_D^{20}$ +86.9° (*c* 1.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.89 (m, 1 H, –CH=), 5.39 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.5 Hz, H-4), 5.39 (dd, 1 H, H-5), 5.31 (dq, 1 H) and 5.21 (dq, 1 H, CH_2=), 5.24 (ddd, 1 H, $J_{7,8a}$ ~2.5, $J_{7,8b}$ ~3.5, $J_{7,6}$ ~9.0 Hz, H-7), 4.60 (dd, 1 H, $J_{8a,8b}$ ~12.5 Hz, H-8a), 4.17 (dd, 1 H, H-8b), 4.14 (dd, 1 H, $J_{6,5}$ ~1.0 Hz, H-6), 4.10–3.88 (m, 2 H, CH_2=CH–CH_2), 3.82 (s, 3 H, CH_3OCO), 2.23 (ddd, 1 H, $J_{3e,3a}$ ~12.5, $^4J_{3e,5}$ ~1.0 Hz, H-3*e*), 2.10 (t, 1 H, H-3*a*), 2.10 (s, 3 H), 2.07 (s, 3 H), 2.00 (s, 3 H), and 1.98 (s, 3 H, CH_3CO); ^{13}C-n.m.r. ($CDCl_3$): δ 170.4, 170.4, 169.9, 169.6 and 167.6 (CO_2), 133.2 (–CH=), 117.1 (CH_2=), 98.6 (C-2), 68.3 (C-6), 67.6 (C-7), 66.4 (C-4), 64.7 (OCH_2–), 64.4 (C-5), 62.0 (C-8), 52.7 (OCH_3), and 32.1 (C-3).

Anal. Calc. for $C_{20}H_{28}O_{12}$: C, 52.17; H, 6.13. Found: C, 52.01; H, 5.93.

*Sodium (allyl 3-deoxy-α-*D*-manno-2-octulopyranosid)onate*. (**10**). — A solution of **7** (60 mg) in water (5 mL) was stirred with 0.2M aqueous NaOH (1.5 mL) at room temperature for 90 min. The pH of the solution was adjusted to 7.8 by addition of Dowex 50 (H^+) cation-exchange resin. The mixture was filtered and lyophilized. Purification of the residue on a column of Bio-Gel P-2 (16 × 100) gave **10**; yield 60 mg (100%), colorless syrup, $[\alpha]_D^{20}$ +73.7° (*c* 1.4, water); ^{1}H-n.m.r. (D_2O): δ 5.97 (m, 1 H, –CH=), 5.35 (dq, 1 H, =CH_{trans}), 5.24 (dq, 1 H, =CH_{cis}), 4.10 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~5.0, $J_{4,3a}$ ~12.0 Hz, H-4), 4.04 (dd, 1 H, $J_{5,6}$ ~1.0 Hz, H-5), 3.96 (ddd, 1 H, $J_{7,6}$ ~9.0, $J_{7,8a}$ ~7.0, $J_{7,8b}$ ~3.0 Hz, H-7), 3.94 (dd, 1 H, $J_{8a,8b}$ ~12.0 Hz, H-8b), 3.97–3.80 (m, 2 H, OCH_2), 3.65 (dd, 1 H, H-8a), 3.62 (dd, 1 H, H-6), 2.07 (dd, 1 H, $J_{3e,3a}$ ~13.0 Hz, H-3*e*), and 1.80 (dd, 1 H, H-3*a*).

*Methyl (allyl 4,5:7,8-di-*O*-carbonyl-3-deoxy-α-*D*-manno-2-octulopyranosid)-onate* (**12**) *and methyl (allyl 7,8-*O*-carbonyl-3-deoxy-α-*D*-manno-2-octulopyranosid)-onate* (**13**). — 4-Nitrophenyl chloroformate (190 mg) was added in three portions during 24 h to a solution of **7** (130 mg) in pyridine (5 mL) at room temperature. The mixture was evaporated to dryness. The residue was subjected to column chromatography on silica gel (B, 1:5 toluene–ethyl acetate). Pooling and evaporation of the fractions containing the faster-moving component afforded **12**, yield 33 mg (22%), colorless syrup, $[\alpha]_D^{20}$ +38.1° (*c* 1.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.87 (m, 1 H, –CH=), 5.30 (dq, 1 H, =CH–$_{trans}$), 5.22 (dq, 1 H, =CH_{cis}), 5.11 (dt, 1 H, $J_{4,5}$ ~8.5, $J_{4,3a} \simeq J_{4,3e}$ ~3.5 Hz, H-4), 5.03 (ddd, 1 H, $J_{7,6}$ ~5.0, $J_{7,8a}$ ~8.5, $J_{7,8b}$ ~6.0 Hz, H-7), 4.93 (dd, 1 H, $J_{5,6}$ ~1.5 Hz, H-5), 4.67 (t, 1 H, $J_{8a,8b}$ ~8.5 Hz, H-8a), 4.58 (dd, 1 H, H-8b), 4.18 (dd, 1 H, H-6), 4.14–4.05 (m, 1 H) and 3.95–3.85 (m, 1 H, OCH_2), 3.82 (s, 3 H, CH_3OCO), 2.89 (dd, 1 H, $J_{3e,3a}$ ~16.5 Hz, H-3*e*), and 2.17 (dd, 1 H, H-3*a*).

Anal. Calc. for $C_{14}H_{16}O_{10}$: C, 48.84; H, 4.68. Found: C, 49.21; H, 4.82.

Further elution of the column afforded **13**, R_F 0.49 (ethyl acetate), yield 48 mg (34%), colorless crystals, m.p. 138–150° (dec.) (ethyl acetate–hexane), $[\alpha]_D^{20}$ +67.3° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.88 (m, 1 H, –CH=), 5.30 (dq, 1 H, $=CH_{trans}$), 5.20 (dq, 1 H, $=CH_{cis}$), 5.00 (ddd, 1 H, $J_{7,8a}$ ~6.5, $J_{7,8b}$ ~9.0, $J_{7,6}$ ~4.5 Hz, H-7), 4.74 (dd, 1 H, $J_{8a,8b}$ ~9.0 Hz, H-8a), 4.59 (t, 1 H, H-8b), 4.20 (br. m, 1 H, H-4), 4.00–3.90 (m, 4 H, H-5,6,OCH_2), 3.81 (s, 3 H, CH_3OCO), 3.05 (br. s, 1 H, OH), 2.80 (br. s, 1 H, OH), 2.80 (dd, 1 H, $J_{3e,4}$ ~5.5, $J_{3e,3a}$ ~13.0 Hz, H-3*e*), and 1.94 (t, 1 H, $J_{3a,4}$ ~12.0 Hz, H-3*a*).

Anal. Calc. for $C_{13}H_{18}O_9$: C, 49.06; H, 5.70. Found: C, 49.37; H, 5.76.

Methyl O-[*methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-*β*-*D-manno*-2-octulopyranosyl)onate*]-*(2→4)*-*(allyl 7,8-*O*-carbonyl-3-deoxy-*α*-*D-manno*-2-octulopyranosid)onate* (**15**) *and methyl* O-[*methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-*α*-*D-manno*-2-octulopyranosyl)onate*]-*(2→4)*-*(allyl 7,8-*O*-carbonyl-3-deoxy-*α*-*D-manno*-2-octulopyranosid)onate* (**14**). — A solution of **1** (320 mg, 0.66 mmol) in dry acetonitrile (2 mL) was added to a suspension of **13** (40 mg, 0.12 mmol), mercury(II) cyanide (252 mg), and molecular sieve 4A (1 g) in acetonitrile (5 mL) under dry Ar. After being stirred for 15 h at room temperature, the mixture was diluted with dichloromethane (50 mL), filtered, and evaporated. The residue was dissolved in dichloromethane (50 mL), and the solution extracted with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated. Purification of the residue on a column of silica gel (B, 1:1 toluene–ethyl acetate) afforded **15**, R_F 0.41 (1:2 toluene–ethyl acetate), yield 15 mg (17%), colorless syrup, $[\alpha]_D^{20}$ +57.7° (*c* 1.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.89 (m, 1 H, –CH=), 5.28 (dq, 1 H, $=CH_{2trans}$), 5.28 (unresolved signal, 1 H, H-5′), 5.18 (dq, 1 H, $=CH_{2cis}$), 5.14 (ddd, 1 H, $J_{7',6'}$ ~9.5, $J_{7',8'a}$ ~4.5, $J_{7',8'b}$ ~3.0 Hz, H-7′), 5.08 (ddd, 1 H, $J_{7,6}$ ~2.5, $J_{7,8a}$ ~7.5, $J_{7,8b}$ ~8.5 Hz, H-7), 4.84 (dd, 1 H, $J_{8a,8b}$ ~8.5 Hz, H-8a), 4.83 (ddd, 1 H, $J_{4',5'}$ ~3.0, $J_{4',3'e}$ ~4.5, $J_{4',3'a}$ ~12.5 Hz, H-4′), 4.55 (t, 1 H, H-8b), 4.34 (dd, 1 H, $J_{8'a,8'b}$ ~12.5 Hz, H-8′a), 4.27 (dd, 1 H, H-8′b), 4.19 (dd, 1 H, $J_{6',5'}$ ~1.5 Hz, H-6′), 4.14 (unresolved signal, 1 H, H-5), 4.12–3.96 (m, 4 H, H-4,6,OCH_2–), 3.86 (s, 3 H, CH_3OCO), 3.78 (s, 3 H, CH_3OCO), 2.39 (dd, 1 H, $J_{3'e,3'a}$ ~12.5 Hz, H-3′*e*), 2.35 (d, 1 H, OH), 2.15–1.95 (m, 3 H, H-3*e*,3′*a*,3*a*), 2.12, 2.10, 2.04, and 1.99 (s, 12 H, CH_3CO).

Anal. Calc. for $C_{30}H_{40}O_{20}$: C, 50.00; H, 5.59. Found: C, 50.78; H, 5.34.

Further elution of the column gave 47 mg (52%) of **14**, R_F 0.35 (1:2 toluene–ethyl acetate), colorless syrup, $[\alpha]_D^{20}$ +95.2° (*c* 0.46, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.85 (m, 1 H, –CH=), 5.39 (unresolved signal, 1 H, H-5′), 5.30–5.14 (m, 4 H, H-4′,7′, $=CH_2$), 4.81 (ddd, 1 H, $J_{7,6}$ ~3.5, $J_{7,8a}$ ~7.0, $J_{7,8b}$ ~7.0 Hz, H-7), 4.79 (dd, 1 H, $J_{8'a,8'b}$ ~12.5, $J_{8'a,7'}$ ~3.0 Hz, H-8′a), 4.75 (dd, 1 H, $J_{8a,8b}$ ~9.0 Hz, H-8a), 4.54 (t, 1 H, H-8b), 4.28 (ddd, 1 H, $J_{4,5}$ ~3.0, $J_{4,3e}$ ~7.0, $J_{4,3a}$ ~10.0 Hz, H-4), 4.11 (dd, 1 H, $J_{6',7'}$ ~9.5, $J_{6',5'}$ ~1.0 Hz, H-6′), 4.00 (dd, 1 H, $J_{8'b,7'}$ ~3.5 Hz, H-8′b), 3.99–3.92 (m, 3 H, H-6, OCH_2–), 3.85 (s, 3 H, CH_3OCO), 3.80 (s, 3 H, CH_3OCO), 3.70 (br. s, 1 H, H-5), 2.54 (br. s, 1 H, OH), 2.21 (dd, 1 H, $J_{3'e,3'a}$ ~13.0, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 2.12, 2.09, 1.99 and 1.98 (s, 12 H, CH_3CO), and

2.15–2.01 (m, 3 H, H-3′*a*,3*e*,3*a*).

Anal. Calc. for $C_{30}H_{40}O_{20}$: C, 50.00; H, 5.59. Found: C, 50.38; H, 5.41.

*O-(Sodium 3-deoxy-α-*D-manno-*2-octulopyranosylonate)-(2→4)-sodium (allyl 3-deoxy-α-*D-manno-*2-octulopyranosid)onate* (**16**). — A solution of **14** (35 mg) and Na (1 mg) in dry methanol (30 mL) was stirred for 2 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and evaporated to dryness. The residue (25 mg) was dissolved in water (10 mL) and 0.2M aqueous NaOH (0.7 mL). After stirring the solution for 2 h at room temperature, the pH was adjusted to 8.0 by addition of Dowex 50 (H^+) resin, the mixture filtered, and the filtrate lyophilized. Subsequent purification of the residue on Bio-Gel P-2 gave **16** (26 mg, 100%), colorless syrup, $[\alpha]_D^{20}$ +76.1° (*c* 0.5, water); ^{1}H-n.m.r. (D_2O): δ 5.99 (m, 1 H, –CH=), 5.35 (dq, 1 H, $=CH_{2trans}$), 5.23 (dq, 1 H, $=CH_{2cis}$), 4.20–3.54 (m, 14 H, H-4,5,6,7,8a,8b,4′,5′,6′,7′,8′a,8′b, OCH_2–), 2.14 (dd, 1 H, $J_{3'e,3'a}$ ~13.0, $J_{3'e,4'}$ ~5.0 Hz, H-3′*e*), 2.02 (dd, 1 H, $J_{3e,3a}$ ~13.0, $J_{3e,4}$ ~5.0 Hz, H-3*e*), 1.82 (dd, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*), and 1.78 (dd, 1 H, $J_{3'a,4'}$ ~12.0 Hz, H-3′*a*).

*Methyl (allyl 8-*O-tert-*butyldimethylsilyl-4,5-*O-*carbonyl-3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**17**). — A solution of **5** (198 mg) and *p*-nitrophenyl chloroformate (181 mg) in pyridine (3 mL) was stirred for 48 h at room temperature. After evaporation to dryness, the residue was dissolved in dichloromethane (50 mL) and washed three times with saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and evaporated. Purification of the residue on a column of silica gel (B, 2:1 toluene–ethyl acetate) gave **17**, yield 184 mg (88%), colorless prisms, m.p. 86–87° (ethyl acetate–hexane), $[\alpha]_D^{20}$ +11.6° (*c* 1.0 chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.91 (m, 1 H, =CH–), 5.25 (dq, 1 H, $=CH_{2trans}$), 5.16 (dq, 1 H, $=CH_{2cis}$), 5.12 (dd, 1 H, $J_{5,6}$ ~1.0, $J_{5,4}$ ~9.0 Hz, H-5), 5.05 (ddd, 1 H, $J_{4,3e}$ ~1.8, $J_{4,3a}$ ~4.5 Hz, H-4), 4.23–4.14 (m, 1 H) and 4.02–3.93 (m, 1 H, OCH_2–), 3.92–3.83 (m, 3 H, H-7,8a,8b), 3.77 (s, 3 H, CH_3OCO), 3.69 (dd, 1 H, $J_{6,7}$ ~8.0 Hz, H-6), 2.56 (dd, 1 H, $J_{3e,3a}$ ~16.0 Hz, H-3*e*), 2.08 (dd, 1 H, H-3*a*), 0.92 [s, 9 H, $(CH_3)_3C$], and 0.12 [s, 6 H, $(CH_3)_2Si$].

Anal. Calc. for $C_{19}H_{32}O_9Si$: C, 52.76; H, 7.45. Found: C, 52.74; H, 7.41.

*Methyl [allyl 8-*O-tert-*butyldimethylsilyl-4,5-*O-*carbonyl-3-deoxy-7-*O-*(2,3,5-tri-*O-*benzoyl-β-*D-*ribofuranosyl)-β-*D-manno-*2-octulopyranosid]onate* (**19**). — A solution of **18** (182 mg, 0.35 mmol) in dichloromethane (3 mL) was added to a suspension of **17** (100 mg, 0.23 mmol), mercury(II) cyanide (116 mg), and molecular sieve 4A (1 g) in dichloromethane (15 mL). After being stirred for 4 h under dry N_2, the mixture was diluted with dichloromethane (50 mL) and filtered. The filtrate was extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Purification of the residue on a column of silica gel (B, 5:1 toluene–ethyl acetate) afforded **19**, yield 165 mg (78%), colorless syrup, $[\alpha]_D^{20}$ +10.4° (*c* 0.91, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.10–7.99 (m, 4 H), 7.90–7.85 (m, 2 H) and 7.65–7.25 (m, 9 H, arom.), 5.89 (dd, 1 H, $J_{2',3'}$ ~5.0, $J_{3',4'}$ ~7.0 Hz, H-3′), 5.88 (m, 1 H, =CH–), 5.76 (dd, 1 H, $J_{2',1'}$ ~0.8 Hz, H-2′), 5.61 (d, 1 H, H-1′), 5.23 (dq, 1

H, $=CH_{2trans}$), 5.14 (dq, 1 H, $=CH_{2cis}$), 5.10 (dd, 1 H, $J_{5,6}$ ~1.0, $J_{5,4}$ ~9.5 Hz, H-5), 4.81 (dd, 1 H, $J_{5'a,5'b}$ ~11.5, $J_{5'a,4'}$ ~3.5 Hz, H-5'a), 4.71 (ddd, 1 H, $J_{4,3e}$ ~2.0, $J_{4,3a}$ ~4.0 Hz, H-4), 4.67 (ddd, 1 H, $J_{4',5'b}$ ~5.0, H-4'), 4.55 (dd, 1 H, H-5'b), 4.19–4.11 (m, 1 H, OCH_2–), 4.08 (dd, 1 H, $J_{8a,8b}$ ~11.0, $J_{8a,7}$ ~2.5 Hz, H-8a), 4.02 (ddd, 1 H, $J_{7,8b}$ ~6.5 Hz, H-7), 4.00–3.91 (m, 1 H, OCH_2–), 3.77 (s, 3 H, CH_3OCO), 3.72 (dd, 1 H, H-8b), 3.57 (dd, 1 H, $J_{6,7}$ ~9.0 Hz, H-6), 2.44 (dd, 1 H, $J_{3e,3a}$ ~16.0 Hz, H-3*e*), 1.83 (dd, 1 H, H-3*a*), 0.85 [s, 9 H, $(CH_3)_3C$], and 0.06 [s, 6 H, $(CH_3)_2Si$].

Anal. Calc. for $C_{45}H_{52}O_{16}Si$: C, 61.63; H, 5.98. Found: C, 61.08; H, 5.89.

Methyl [allyl 4,5-O-carbonyl-3-deoxy-7-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-manno-2-octulopyranosid]onate (**20**). — A solution of HF (2% in acetonitrile, 1 mL) was added to a solution of **19** (330 mg) in acetonitrile (20 mL). This was stirred for 30 min at room temperature, and $NaHCO_3$ (500 mg) was added. After evaporation of the mixture, the residue was dissolved in dichloromethane (60 mL), and the solution extracted with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. Purification of the residue on a column of silica gel (B, 1:1 toluene–ethyl acetate) gave **20**, yield 287 mg (100%), colorless syrup, $[\alpha]_D^{20}$ −2.3° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.10–7.88 (m, 6 H) and 7.62–7.32 (m, 9 H, arom.), 5.88 (dd, 1 H, $J_{3',2'}$ ~5.0, $J_{3',4'}$ ~7.0 Hz, H-3'), 5.83 (m, 1 H, =CH–), 5.69 (dd, 1 H, $J_{1',2'}$ ~1.2 Hz, H-2'), 5.50 (d, 1 H, H-1'), 5.23 (dq, 1 H, $=CH_{2trans}$), 5.15 (dd, 1 H, $=CH_{2cis}$), 4.83 (dd, 1 H, $J_{5'a,5'b}$ ~11.5, $J_{5'a,4'}$ ~4.0 Hz, H-5'a), 4.78 (ddd, 1 H, $J_{4,3e}$ ~2.3, $J_{4,3a}$ ~3.8 Hz, H-4), 4.73 (ddd, 1 H, $J_{4',5'b}$ ~4.5 Hz, H-4'), 4.55 (dd, 1 H, H-5'b), 4.20–4.10 (m, 1 H, OCH_2–), 4.05 (dt, $J_{7,6}$ ~9.0, $J_{7,8a} \approx J_{7,8b}$ ~2.5 Hz, H-7), 3.95 (br. m, 2 H, H-8a,8b), 3.88–3.80 (m, 1 H, OCH_2–), 3.78 (s, 3 H, CH_3OCO), 3.71 (dd, 1 H, H-6), 3.28 (br. s, 1 H, OH), 2.50 (dd, 1 H, $J_{3e,3a}$ ~16.0 Hz, H-3*e*), and 1.78 (dd, 1 H, H-3*a*).

Anal. Calc. for $C_{39}H_{38}O_{16}$: C, 61.41; H, 5.02. Found: C, 61.73; H, 5.44.

Methyl (allyl 3-deoxy-7-O-β-D-ribofuranosyl-β-D-manno-2-octulopyranosid)onate (**21**). — A solution of **20** (248 mg) in dry methanol (20 mL) and 0.2M methanolic sodium methoxide (2 mL) was stirred for 3 h at room temperature. The mixture was de-ionized by addition of Dowex 50 (H^+) resin, filtered, and evaporated. The residue was extracted with four 10-mL portions of diethyl ether and dried, yield 137 mg of **21** (100%), colorless syrup, $[\alpha]_D^{20}$ +3.0° (*c* 1.6, methanol); ^{1}H-n.m.r. (D_2O): δ 5.94 (m, 1 H, =CH–), 5.46–5.16 (m, 3 H, H-1', $=CH_2$), 4.50–3.25 (m, 13 H, H-4,5,6,7,8a,8b,2',3',4',5'a,5'b,OCH_2–), 3.87 (s, 3 H, CH_3OCO), 2.46 (dd, 1 H, $J_{3e,3a}$ ~12.5, $J_{3e,4}$ ~5.5 Hz, H-3*e*), and 1.98 (t, 1 H, $J_{3a,4}$ ~12.5 Hz, H-3*a*).

Sodium (allyl 3-deoxy-7-O-β-D-ribofuranosyl-β-D-manno-2-octulopyranosid)onate (**22**). — A solution of **21** (150 mg) and 0.2M aqueous NaOH (4 mL) was stirred for 4 h at room temperature. The pH of the mixture was adjusted to 4 by addition of Dowex 50 (H^+) resin, which was filtered, and the product was converted into the sodium salt by titration with 10mM NaOH to pH 8.0. Lyophilization and subsequent purification of the residue on Bio-Gel P-2 gave **22**, yield 152.5 mg (100%), colorless syrup, $[\alpha]_D^{20}$ −16.3° (*c* 0.78, water); ^{1}H-n.m.r. (D_2O): δ 5.95 (m,

1 H, =CH–), 5.33 (dq, 1 H, $=CH_{2trans}$), 5.23 (d, 1 H, $J_{1',2'}$ ~1.0 Hz, H-1'), 5.23 (dq, 1 H, $=CH_{2cis}$), 4.24 (m, 1 H, OCH_2–), 4.21 (dd, 1 H, $J_{2',3'}$ ~4.0, $J_{3',4'}$ ~8.0 Hz, H-3'), 4.12 (dd, 1 H H-2'), 4.05 (ddd, 1 H, $J_{7,6}$ ~9.0 Hz, H-7), 4.00–3.90 (m, 4 H, H-5,8a,8b,OCH–), 3.86 (dd, 1 H, $J_{5'a,5'b}$ ~12.0 Hz, H-5'a), 3.74 (ddd, 1 H, $J_{4,5}$ ~3.5, $J_{4,3e}$ ~4.5, $J_{4,3a}$ ~12.5 Hz, H-4), 3.70 (dd, 1 H, H-5'b), 3.68 (dd, 1 H, $J_{6,5}$ ~1.0 Hz, H-6), 2.45 (dd, 1 H, $J_{3e,3a}$ ~12.5 Hz, H-3*e*), and 1.82 (t, 1 H, H-3*a*).

Copolymerization. — A solution of **10** (55 mg), acrylamide (64 mg), and *N,N,N',N'*-tetramethylethylenediamine (2 μL) in water (1 mL) was degassed at aspirator pressure for 30 min. After addition of $(NH_4)_2SO_5$ (1 mg), the mixture was kept at +4° for 18 h. The solution was purified on a column of Sephadex G-50 (16 × 400 mm; eluent, 0.1M aqueous $NaHCO_3$; flow rate 55 mL/h; fractions 2.5 mL). The product-containing fractions (10–17) were pooled, lyophilized, and desalted on a column on Sephadex G-25 to give **25**, yield 73 mg, amorphous powder, $[\alpha]_D^{20}$ +10.2° (*c* 0.9, water).

The copolymers **23** (60 mg of **4** and 57 mg acrylamide), **24** (26 mg of **16** and 14 mg acrylamide), and **26** (21 mg of **22** and 17.8 mg acrylamide) were prepared in a similar manner. Yields: 53 mg of **23**, $[\alpha]_D^{20}$ +8.1° (*c* 10.98, water); 17.6 mg of **24**, $[\alpha]_D^{20}$ +15.5° (*c* 0.37, water); and 27.6 mg of **26**, $[\alpha]_D^{20}$ −2.8° (*c* 0.7, water).

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