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Synthesis and NMR spectroscopic investigation of oligosaccharides containing Kdo and L-*glycero*-D-*manno*-heptopyranosyl residues

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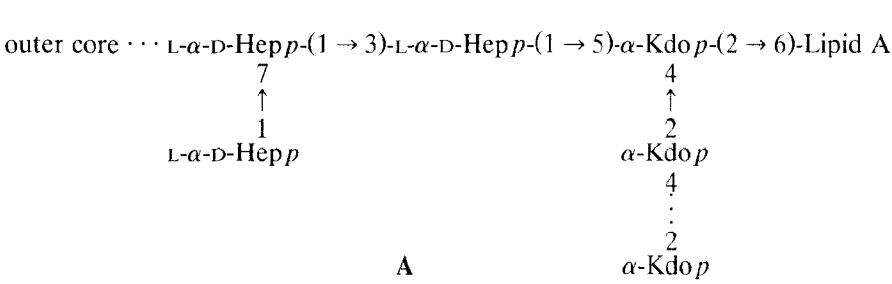
ABSTRACT

The disaccharides *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2 → 8)-sodium (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**8**), *O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1 → 7)-sodium (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**12**), and *O*-α-D-mannopyranosyl-(1 → 7)-sodium (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**21**) and the branched trisaccharides *O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1 → 7)-[*O*-(sodium 3-deoxy-α- and -β-D-*manno*-2-octulopyranosylonate)-(2 → 8)]-sodium (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**15** and **16**) and *O*-α-D-mannopyranosyl-(1 → 7)-[*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2 → 8)]-sodium (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**24**) were prepared. Per-*O*-acetylated mannopyranosyl or Kdo bromide derivatives were employed for the glycosylation steps under Helferich conditions, whereas the imidate derivative **9** was used for the coupling of the L-*glycero*-D-*manno*-heptopyranosyl residues. The oligosaccharides were fully characterized by NMR spectroscopic data. Their structures correspond to an artificial linkage pattern providing a potential cross-reactive epitope for antibodies directed against the inner-core-region of enterobacterial as well as chlamydial lipopolysaccharides.

INTRODUCTION

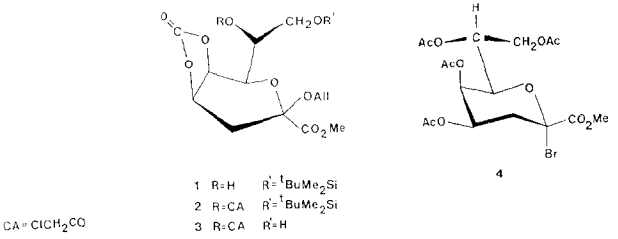
Antibodies that are directed against the core-region of bacterial lipopolysaccharides (LPS) are of significant clinical interest for their potential of neutralizing or enhancing the manifold biological effects of endotoxins[1]. The structure of the *enterobacterial* inner-core region[2,3] comprises the basic sequence **A** and numerous syntheses of the heptose–Kdo region have been performed recently[4–10].

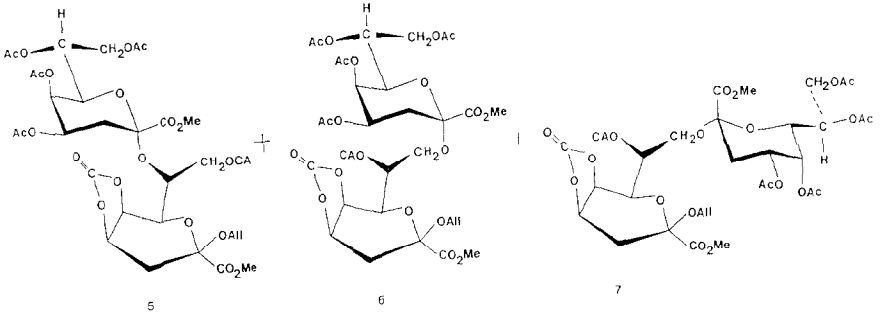


One of the monoclonal antibodies directed against the inner-core region of enterobacterial LPS recognizes the α-(2 → 4)-linked Kdo disaccharide unit[11,12]. In addition, this antibody A 25 cross-reacts with an α-(2 → 8)-linked Kdo–disaccharide, constituting the terminal part of the Kdo region in the LPS of *Chlamydia*[13,14]. In passive hemolysis inhibition assays, an α-(2 → 8)-linked Kdo–disaccharide hapten was an even better inhibitor than the α-(2 → 4)-linked disaccharide. Furthermore, HSEA calculations revealed a close similarity in a part of their topology, which might be further enhanced by the β-anomeric configuration of the reducing Kdo unit[15]. These findings prompted us to investigate whether these related epitopes would also extend into the adjoining heptose region. Herein, we report the synthesis and NMR spectroscopic characterization of the disaccharides α-Kdop-(2 → 8)-β-Kdop, α-Manp-(1 → 7)-β-Kdop, L-α-D-Hepp-(1 → 7)-β-Kdop, and the trisaccharides L-α-D-Hepp-(1 → 7)-[α- and β-Kdop-(2 → 8)-]-β-Kdop, and α-Manp-(1 → 7)-[α-Kdop-(2 → 8)-]-β-Kdop as allyl glucosides, which are suitable for a subsequent conversion into haptens and immunogens[14], to define epitope specificities of monoclonal antibodies against *enterobacterial* and *chlamydial* lipopolysaccharides.

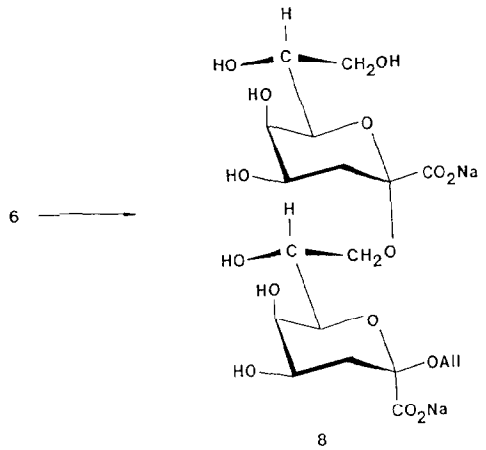
RESULTS AND DISCUSSION

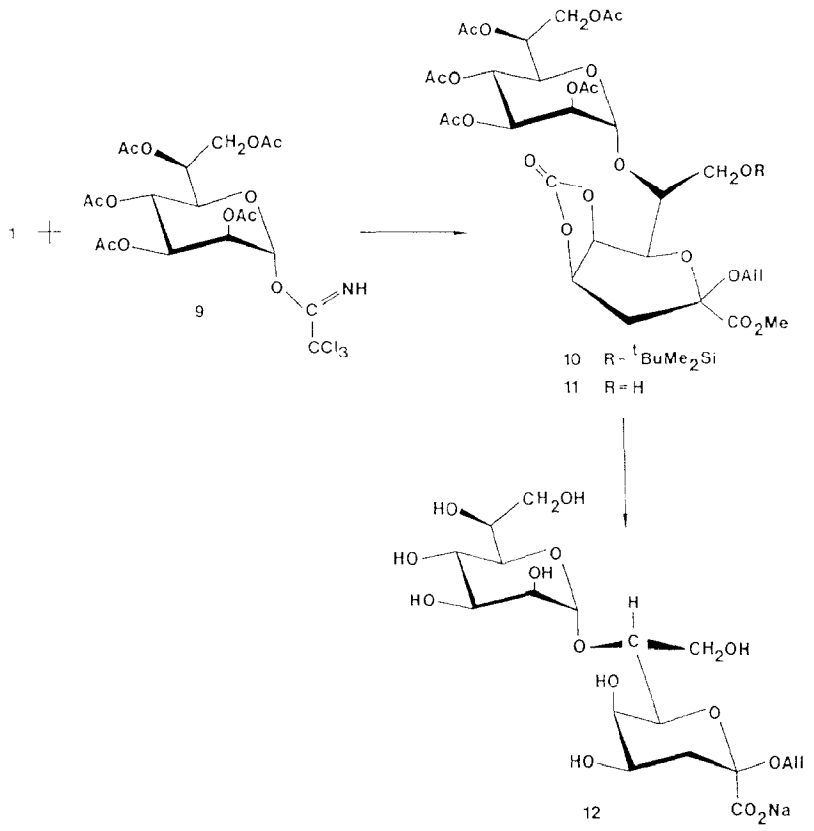
O-Chloroacetylation[16] of the previously described[17] methyl (allyl 8-*O*-*tert*-butyldimethylsilyl-4,5-*O*-carbonyl-3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**1**) gave the crystalline 7-*O*-chloroacetyl derivative **2** in 93% yield, which was treated with 2% HF in acetonitrile[18] to afford the crystalline glycosyl acceptor **3** in 97% yield. Prolonged reaction times, however, led to acyl migration of the 7-*O*-chloroacetyl group. Subsequent glycosylation of **3** with the Kdo-bromide derivative **4** (ref 19) in acetonitrile–$Hg(CN)_2$ gave a separable 3:1 mixture of the α- and β-(2 → 8)-linked disaccharide derivatives **6** and **7** in 30% yield together with the α-(2 → 7)-linked disaccharide derivative **5** (15% yield) arising from chloroacetyl migration under the glycosylation conditions. The assignment of the anomeric configuration was based on the ^1H NMR chemical shift values of the H-4 signals, which showed a downfield shift for the α-linked Kdo residues[20] ($\delta_{H\text{-}4'}$ ~ 5.34 ppm for **5**, ~ 5.41 ppm for **6**, and 4.91 ppm for **7**). Removal of the acyl groups of **6** by methanolic sodium methoxide and hydrolysis of the methyl ester groups by aqueous NaOH afforded *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2 → 8)-sodium (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**8**) in 87% yield.





Glycosylation of **1** with the per-*O*-acetylated heptopyranosyl trichloroacetimidate derivative **9** (ref 6) in CH_2Cl_2–Me_3Si-triflate gave a good yield (82%) of the α-(1 → 7)-linked disaccharide derivative **10**. The α-anomeric configuration of the heptopyranosyl residue was deduced from the value of the heteronuclear coupling constant ($J_{C\text{-}1',H\text{-}1'}$ ~ 172.7 Hz), whereas the (1 → 7) linkage was proved after

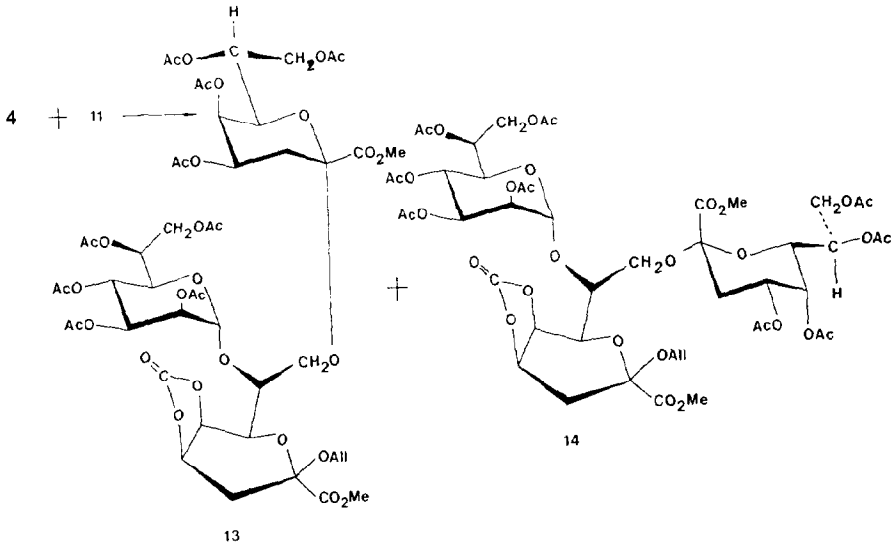




removal of the $^{t}BuMe_2Si$ group, using 2% HF in acetonitrile (81% yield). The OH-group in compound **11** appeared as a doublet of doublets ($J_{8a,OH} \sim 5.5$ and $J_{8b,OH} \sim 8.6$ Hz). Zemplén deacylation and hydrolysis of the methyl ester group furnished *O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1 → 7)-sodium (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**12**) in 98% yield.

Glycosylation of the disaccharide acceptor **11** with the Kdo-bromide derivative **4** in acetonitrile–$Hg(CN)_2$ gave a low yield of the α- and β-(2″ → 8)-linked trisaccharide derivatives **13** and **14** in a ~3:1 ratio. The assignment of the anomeric configuration was based on the downfield shift of the H-4″ signal ($\delta_{H-4''} \sim 5.38$ ppm for **13** and 4.90 ppm for **14**). The isomers were separated by silica gel chromatography, and the deprotection was performed similarly to that for compound **6**, which afforded the trisaccharides *O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1 → 7)-[*O*-(sodium 3-deoxy-α- and -β-D-*manno*-2-octulopyranosylonate)-(2 → 8)-]-sodium (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**15** and **16**) in 98 and 86% yield, respectively.

Glycosylation of the 8-*O*-$^{t}BuMe_2Si$ ether derivative **1** with the per-*O*-acetylated mannopyranosyl bromide derivative **17** (ref 21) in acetonitrile under Helferich

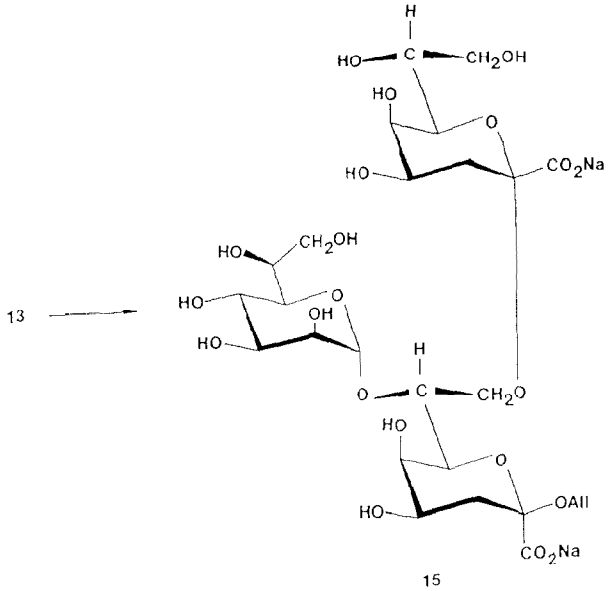


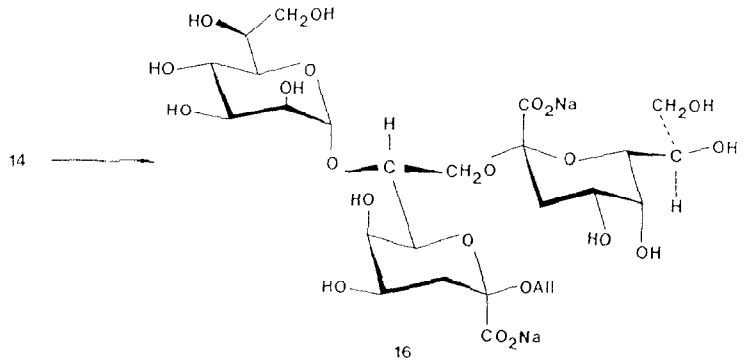
conditions gave a small portion of the *exo*-orthoester derivative **18** and the α-(1 → 7)-linked disaccharide derivative **19** in 36% yield. The assignment of the α-anomeric configuration of compound **19** was in agreement with the value of the heteronuclear coupling constant of C-1′ ($J_{C\text{-}1',H\text{-}1'}$ ~ 170.9 Hz), whereas the assignment of the orthoester derivative **18** was based on the ^{1}H NMR chemical shift of the *endo*-CH_3 group[22] (δ_{CH_3} ~ 1.78 ppm). Better yields (61%) in the glycosylation reaction were obtained using silver triflate in CH_2Cl_2 and 4A molecular sieves. The ^{t}BuMe$_2$Si was then cleaved by 2% HF in acetonitrile in 98% yield, and the ^{1}H NMR signal of the OH group revealed two couplings to the H-8 protons, thus proving the (1 → 7) linkage of the mannopyranosyl residue. Zemplén deacylation of **20** and subsequent hydrolysis of the methyl ester gave *O*-α-D-mannopyranosyl-(1 → 7)-sodium (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**21**) in 92% yield.

Finally, the disaccharide acceptor derivative **20** was coupled with the Kdo bromide derivative **4** in acetonitrile–Hg(CN)$_2$; this reaction afforded a ~ 1:2 mixture of the β- and α-(2 → 8)-linked trisaccharide derivatives **22** and **23** in 34% yield, which were separated by column chromatography. Deblocking of the trisaccharide derivative **23**, as described for compound **6**, gave *O*-α-D-mannopyranosyl-(1 → 7)-[*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2 → 8)]-sodium (allyl 3-deoxy-β-D-*manno*-2-octulopyranosid)onate (**24**) in 99% yield.

The identities of **8, 12, 15, 16, 21**, and **24** were confirmed by the NMR data reported (Tables I–III).

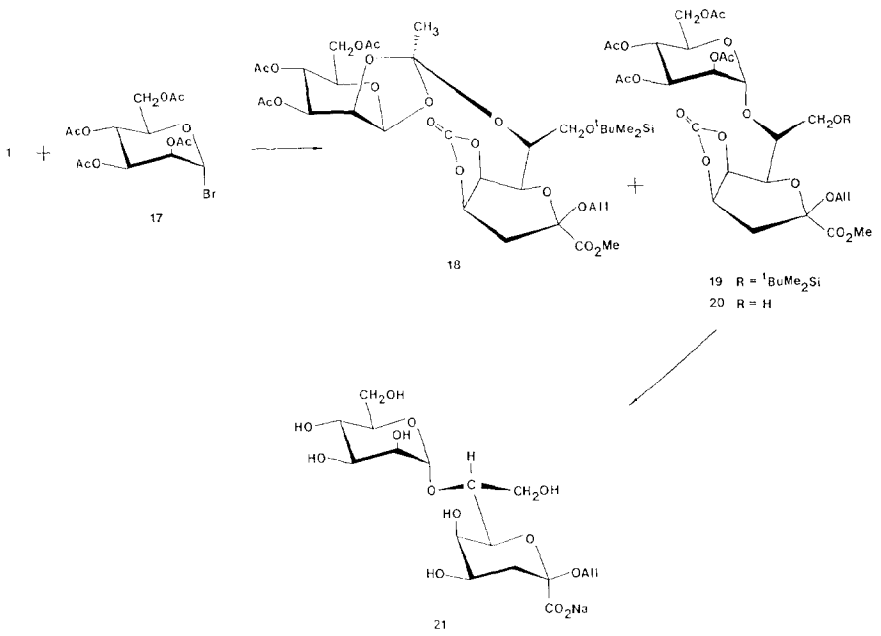
The ^{1} NMR data (500 MHz) for solutions of **8, 12, 15, 16, 21**, and **24** in D_2O at 27°C are given in Tables I and II together with those of the reference monosaccharides, allyl α-Kdo*p* (**25**), methyl α-Man*p* (**26**), allyl L-*glycero*-α-D-*manno*-Hep*p*





(**27**), and allyl β-Kdo*p* (**28**). The assignments were based on COSY[23], relayed COSY, and double-relayed COSY experiments, together with phase-sensitive double-quantum-filtered (DQF) COSY experiments[24]. The ^{13}C NMR data (125.77 MHz) are given in Table III. The assignments were based on heteronuclear correlation spectroscopy[25] with the assigned proton signals and by comparison with data from model compounds[6,26,27]. The *J* values (Table II) indicate that the chair conformations are maintained in the units **a**–**c** for each compound.

Immunochemical results obtained with the allyl glycosides and corresponding multivalent haptens (e.g., via copolymerization with acrylamide) will be reported elsewhere.



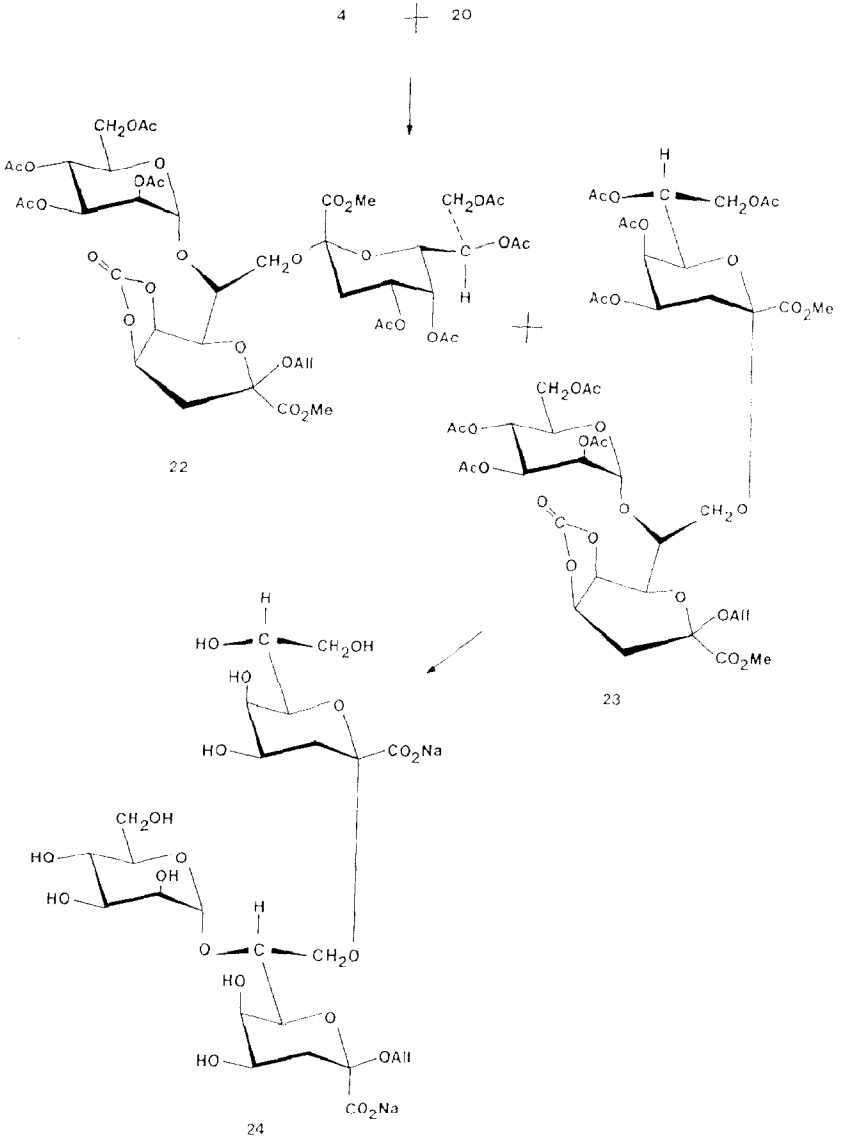
EXPERIMENTAL

General methods.—These were as described previously[17]. Column chromatography was performed on Merck-Lichroprep columns (size *A*, 24 × 1; *B*, 31 × 2.5; *C*, 44 × 3.7; and *D*, 65 × 5.0 cm; silica gel 40–63 μm)

NMR spectroscopy.—Solutions of ~2 mg of **8, 12, 15, 16, 21**, and **24** in 0.5 mL of D_2O were used. Spectra were recorded at 27°C in 5-mm tubes at 500.13 MHz for ^1H and 125.77 MHz for ^{13}C with a Bruker AM-500 spectrometer. The ^1H resonances were measured relative to internal acetone (2.225 ppm, DOH at 4.75 ppm) and coupling constants were determined on a first-order basis. The ^{13}C resonances are relative to internal dioxane (67.4 ppm).

Homonuclear and heteronuclear 2D NMR spectroscopic experiments were performed as published previously[28].

*Methyl (allyl 8-*O*-*tert*-butyldimethylsilyl-4,5-*O*-carbonyl-7-*O*-chloroacetyl-3-deoxy-β-*D*-manno-2-octulopyranosid)onate* (**2**).—A solution of **1** (51 mg, 0.12 mmol), 2,6-dimethylpyridine (100 μL, 0.86 mmol), Et_3N (50 μL, 0.59 mmol), and chloroacetic anhydride (40 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) was stirred for 2.5 h at 4°C. After addition of MeOH (1 mL), the solution was evaporated to dryness. Purification of the residue on a column of silica gel (*A*, 1:1 toluene–EtOAc) gave **2** as colorless needles (55 mg, 93%); mp 124°C (EtOAc–hexane); $[\alpha]_D^{20}$ −27° (*c* 1.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.89 (m, 1 H, =CH–), 5.26 (dq, 1 H, $=CH_{2trans}$), 5.17



(dq, 1 H, =CH_{2cis}), 5.08 (m, 2 H, H-4,7), 4.95 (dd, 1 H, $J_{5,6}$ ~ 1.3, $J_{5,4}$ ~ 9.2 Hz, H-5), 4.20 (dd, 1 H, $J_{6,7}$ ~ 9.0 Hz, H-6), 4.19 (m, 1 H, OCH_2), 4.09 (d, 2 H, CH_2Cl), 4.04 (dd, 1 H, $J_{8a,7}$ ~ 2.7, $J_{8a,8b}$ ~ −11.8 Hz, H-8a), 4.03 (m, 1 H, OCH_2), 3.96 (dd, 1 H, $J_{8b,7}$ ~ 3.6 Hz, H-8b), 3.78 (s, 3 H, CO_2CH_3), 2.57 (dd, 1 H, $J_{3e,4}$ ~ 2.0, $J_{3e,3a}$ ~ −16.0 Hz, H-3*e*), 2.14 (dd, 1 H, $J_{3a,4}$ ~ 4.5 Hz, H-3*a*), 0.91 [s, 9 H, $(CH_3)_3C$], and 0.06 [s, 6 H, $Si(CH_3)_2$]. Anal. Calcd for $C_{21}H_{33}ClO_{10}Si$: C, 49.54; H, 6.53. Found: C, 49.00; H, 6.43.

TABLE I

^{1}H NMR data for Kdo oligosaccharides **8**, **12**, **15**, **16**, **21**, and **24**, and for reference monosaccharides **25**, **26**, **27**, and **28**

Atom	**24**	**16**	**15**	**21**	**12**	**8**	Reference monosaccharides [a]	
Unit **a**							**25**	
α- or β-Kdo-(2 → 8								
H-3*a*	1.75	1.84	1.75			1.80	1.80	
H-3*e*	2.08	2.46	2.07			2.09	2.07	
H-4	4.10	3.76	4.12			4.19	4.10	
H-5	4.01	3.94	4.01			4.04	4.04	
H-6	3.66	3.55	3.65			3.75	3.62	
H-7	3.93	3.92	3.93			3.95	3.96	
H-8a	3.65	3.72	3.63			3.69	3.65	
H-8b	3.94	3.88	3.93			3.94	3.94	
Unit **b**							**26**	**27**
α-Man/Hep-(1 → 7								
H-1	5.17	5.17	5.18	5.00	5.02		4.76	4.90
H-2	4.01	3.98	3.99	3.97	3.95		3.92	3.92
H-3	3.84	3.89	3.84	3.83	3.83		3.75	3.79
H-4	3.69	3.83	3.84	3.64	3.86		3.63	3.84
H-5	3.94	3.93	3.84	3.82	3.72		3.61	3.59
H-6a	3.79	4.04	4.03	3.75	4.04		3.75	4.03
H-6b	3.88			3.89			3.89	
H-7a		3.75	3.72		3.68			3.67
H-7b		3.85	3.77		3.72			3.73
Unit **c**							**28**	
7,8 → β-Kdo-(2 → Allyl								
H-3*a*	1.82	1.83	1.82	1.80	1.80	1.82	1.76	
H-3*e*	2.29	2.35	2.28	2.42	2.42	2.35	2.38	
H-4	3.75	3.72	3.75	3.75	3.74	3.75	3.71	
H-5	3.85	3.84	3.84	3.86	3.84	3.97	3.91	
H-6	4.06	3.95	4.12	3.73	3.72	3.86	3.59	
H-7	4.17	4.15	4.12	3.95	3.95	4.01	3.89	
H-8a	3.58	3.59	3.58	3.84	3.80	3.52	3.71	
H-8b	3.64	4.12	3.61	4.00	4.00	3.66	3.86	
Allyl glycosides							**28**	**25**
H-1a	3.95	3.98	3.95	3.96	3.96	3.96	3.92	3.95
H-1b	4.22	4.25	4.23	4.20	4.20	4.23	4.18	3.85
H-2	5.93	5.95	5.93	5.91	5.91	5.95	5.89	5.97
H-3	5.21	5.23	5.21	5.19	5.20	5.23	5.17	5.24
H-3a	5.33	5.35	5.33	5.30	5.30	5.34	5.28	5.35

[a] **25**, Allyl α-Kdo; **26**, methyl α-D-Man; **27**, allyl α-D-Hep; and **28**, allyl β-Kdo.

*Methyl (allyl 4,5-O-carbonyl-7-O-chloroacetyl-3-deoxy-β-*D-manno*-2-octulopyranosid)onate* (**3**).—A solution of HF (2% in MeCN, 0.6 mL) was added to a solution of **2** (227 mg, 0.46 mmol) in MeCN (10 mL). The solution was stirred for 90 min at room temperature, and $NaHCO_3$ (1 g) was added. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 and the solution was washed with satd aq $NaHCO_3$, dried (Na_2SO_4), and evaporated to give **3** as colorless needles

TABLE II

$J_{H,H}$ values [a] (Hz) for Kdo oligosaccharides **8**, **12**, **15**, **16**, **21**, and **24**, and for reference monosaccharides **25**, **26**, **27**, and **28**

$J_{H,H}$	**24**	**16**	**15**	**21**	**12**	**8**	Reference monosaccharides [b]	
Unit **a**							**25**	
α- or β-Kdo-(2 → 8								
$J_{3a,3e}$	−13.2	−12.1	−13.2			−13.0	−13.0	
$J_{3a,4}$	12.2	12.5	11.9			12.1	12.0	
$J_{3e,4}$	5.1	4.6	5.1			4.4	5.0	
$J_{4,5}$	2.9	3.1	2.8			3.0	3.0	
$J_{5,6}$	1.0	1.0	0.8			1.0	0.8	
$J_{6,7}$	8.5	9.1	8.5			8.5	9.0	
$J_{7,8a}$		4.3	4.6			7.4	7.0	
$J_{7,8b}$		2.0				2.5	3.0	
$J_{8a,8b}$		−12.3				−12.5	−11.5	
Unit **b**							**26**	**27**
α-Man/Hep-(1 → 7								
$J_{1,2}$	1.4	1.7	1.5	1.5	1.5		1.5	1.6
$J_{2,3}$	3.4	3.3	3.1	3.5	3.1		3.5	3.2
$J_{3,4}$	9.8	9.8		9.7	9.5		10.0	9.5
$J_{4,5}$	9.9	9.6		9.7	9.5		10.0	9.5
$J_{5,6a}$	5.3	1.7		6.0			5.8	1.2
$J_{5,6b}$	2.2			1.6			1.9	
$J_{6a,6b}$	−12.4			−11.8			−12.0	
$J_{6,7a}$		3.6	4.3		5.5			5.4
$J_{6,7b}$		8.4	8.3		7.5			7.5
$J_{7a,7b}$		−12.4	−11.6		−11.2			−11.2
Unit **c**						**28**		
→ 7,8)-β-Kdo-(2 → Allyl								
$J_{3a,3e}$	−12.1	−12.1	−13.2	−12.2	−12.2	−12.0	−12.2	
$J_{3a,4}$	12.6	12.7	11.2	12.6	12.6	12.6	12.6	
$J_{3e,4}$	4.6	4.4	4.5	4.7	4.7	5.0	4.7	
$J_{4,5}$	3.0	3.0	2.8	3.0	2.8	3.0	2.8	
$J_{5,6}$	1.0	1.1		0.5	0.8	0.7	0.8	
$J_{6,7}$	6.2	5.8		9.0	9.2	8.7	8.7	
$J_{7,8a}$	6.9	7.3		1.7	1.6	7.6	1.5	
$J_{7,8b}$	2.9	3.0		2.6	2.6	2.8	2.3	
$J_{8a,8b}$	−10.8	−10.3		−12.8	−12.6	−10.5	−12.2	

[a] Observed first-order values, accuracy ±0.3 Hz. [b] See footnote to Table I.

(170 mg, 97%); mp 118–120°C (EtOAc–hexane); $[\alpha]_D^{20}$ −40° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.85 (m, 1 H, =CH–), 5.27 (dq, 1 H, $=CH_{2trans}$), 5.22 (dq, 1 H, $=CH_{2cis}$), 5.14 (dt, 1 H, $J_{6,7}$ ~ 9.4 Hz, H-7), 5.10 (ddd, 1 H, $J_{4,5}$ ~ 9.2, $J_{4,3e}$ ~ 2.2, $J_{4,3a}$ ~ 3.7 Hz, H-4), 4.88 (dd, 1 H, $J_{5,6}$ ~ 1.1 Hz, H-5), 4.21 (m, 1 H, OCH_2), 4.13 (dd, 1 H, H-6), 4.12 (dd, 1 H, $J_{8a,7}$ ~ 2.4, $J_{8a,8b}$ ~ −13.7 Hz, H-8a), 4.11 (d, 2 H, CH_2Cl), 3.94 (dd, 1 H, $J_{8b,7}$ ~ 1.7 Hz, H-8b), 3.90 (m, 1 H, OCH_2), 3.84 (s, 3 H, CO_2CH_3), 2.69 (dd, 1 H, $J_{3e,3a}$ ~ −14.2 Hz, H-3*e*), and 2.02 (dd, 1 H, H-3*a*). Anal. Calcd for $C_{15}H_{19}ClO_{10}$: C, 45.64; H, 4.85. Found: C, 45.34; H, 4.68.

TABLE III

^{13}C NMR data for Kdo oligosaccharides **8**, **12**, **15**, **16**, **21**, and **24**, and for reference monosaccharides **25**, **26**, **27**, and **28**

Atom	**24**	**16**	**15**	**21**	**12**	**8**	Reference monosaccharides [a]	
Unit **a**							**25**	
α- or β-Kdo-(2 → 8								
C-1	176.3	174.2	175.2			176.4	176.1	
C-2	102.0	102.1	100.9			101.2	100.8	
C-3	34.9	35.3	34.9			35.2	35.1	
C-4	66.8	68.4	66.8			67.2	66.8	
C-5	67.2	66.2	67.2			67.5	67.1	
C-6	72.3	74.1	72.3			72.5	72.4	
C-7	70.6	69.9	70.6			70.7	70.3	
C-8	64.1	64.9	64.1			64.4	64.0	
Unit **b**							**26**	**27**
α-Man/Hep-(1 → 7								
C-1	100.8	99.9	100.0	102.7	102.5		101.9	99.9
C-2	70.9	70.9	70.8	71.1	71.1		71.2	70.9
C-3	71.2	71.2	71.3	71.2	71.3		71.8	71.7
C-4	67.3	67.7	68.0 [b]	67.6	66.9		68.0	66.9
C-5	73.7	73.0	72.9	74.1	72.8		73.7	72.2
C-6	61.6	70.4	70.1	61.7	69.7		62.1	69.6
C-7		64.6	64.3		63.7			63.8
Unit **c**							**28**	
β-Kdo-(2 → Allyl								
C-1	174.9	174.6	175.5	174.6	174.5	174.6	174.6	
C-2	101.1	102.1	102.0	101.8	101.8	102.3	101.8	
C-3	35.3	35.4	35.2	35.5	35.5	35.8	35.6	
C-4	68.1	68.1	68.1	68.3	68.3	68.3	68.2	
C-5	66.7	67.1	66.9 [b]	66.1	66.2	67.0	66.1	
C-6	73.8	74.2	73.7 [c]	72.8	72.7	75.0	74.3	
C-7	76.7	75.7	75.5 [c]	78.6	78.1	69.7	69.8	
C-8	64.6	65.4	64.3	63.3	63.4	66.1	64.9	
Allyl glycosides							**27**	**28**
C-1	66.5	66.5	66.4	66.6	66.7	66.7	68.9	66.6
C-2	134.8	134.7	134.8	134.6	134.6	135.2	134.1	134.6
C-3	119.1	119.1	119.0	119.0	119.0	119.1	119.2	119.0

[a] See footnote to Table I. [b,c] Assignments may be reversed.

O-*(Methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy*-α-D-manno-*2-octulopyranosylonate)-(2 → 7)-methyl (allyl 4,5*-O-*carbonyl-8*-O-*chloroacetyl-3-deoxy*-β-D-manno-*2-octulopyranosid)onate* (**5**), O-*(methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy*-α-D-manno-*2-octulopyranosylonate)-(2 → 8)-methyl (allyl 4,5*-O-*carbonyl-7*-O-*chloroacetyl-3-deoxy*-β-D-manno-*2-octulopyranosid)onate* (**6**), *and* O-*(methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy*-β-D-manno-*2-octulopyranosylonate)-(2 → 8)-methyl (allyl 4,5*-O-*carbonyl-7*-O-*chloroacetyl-3-deoxy*-β-D-manno-*2-octulopyranosid)onate* (**7**).—A solution of **4** (300 mg, 0.62 mmol) in MeCN (2 mL) was added during 2 h to a suspension of **3** (160

mg, 0.41 mmol), 4A molecular sieves (1 g), and $Hg(CN)_2$ (532 mg, 2.11 mmol) in MeCN (5 mL) under dry N_2. The suspension was stirred at room temperature for 24 h. Then a solution of **4** (170 mg, 0.35 mmol) in MeCN (1 mL) and $Hg(CN)_2$ (470 mg, 1.85 mmol) were added to the suspension. After 2 days, the mixture was diluted with MeCN (20 mL), filtered over Celite, and evaporated to dryness. The syrup was dissolved in CH_2Cl_2 (100 mL) and washed with satd aq $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated, and the residue was purified on a column of silica gel (*c*, 1 : 1 toluene–EtOAc) to give **5** as a colorless syrup (49 mg, 15%); $[\alpha]_D^{20}$ +41° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.84 (m, 1 H, =CH–), 5.41 (br s, 1 H, H-5′), 5.34 (ddd, 1 H, $J_{4',5'} \sim 2.9$, $J_{4',3'e} \sim 4.6$, $J_{4',3'a} \sim 12.4$ Hz, H-4′), 5.25 (dq, 1 H, $=CH_{2trans}$), 5.20 (ddd, 1 H, H-7′), 5.16 (dq, 1 H, $=CH_{2cis}$), 5.05 (ddd, 1 H, $J_{4,5} \sim 9.1$, $J_{4,3e} \sim 2.2$, $J_{4,3a} \sim 3.9$ Hz, H-4), 4.94 (dd, 1 H, $J_{5,6} \sim 0.9$ Hz, H-5), 4.82 (dd, 1 H, $J_{8a,7} \sim 1.3$, $J_{8a,8b} \sim -12.4$ Hz, H-8a), 4.75 (dd, 1 H, $J_{8'a,7'} \sim 2.7$, $J_{8'a,8'b} \sim -12.4$ Hz, H-8′a), 4.32 (dd, 1 H, $J_{8b,7} \sim 1.9$ Hz, H-8b), 4.30 (dd, 1 H, $J_{6',7'} \sim 8.6$, $J_{6',5'} \sim 1.5$ Hz, H-6′), 4.23 (s, 2 H, CH_2Cl), 4.17 (ddd, 1 H, H-7), 4.16 (m, 1 H, OCH_2), 4.05 (dd, 1 H, $J_{8'b,7'} \sim 4.7$ Hz, H-8′b), 3.95–3.88 (m, 2 H, H-6 and OCH_2), 3.92 and 3.78 (s, 6 H, CO_2CH_3), 2.53 (dd, 1 H, $J_{3e,3a} \sim -16.0$ Hz, H-3*e*), 2.31 (dd, 1 H, $J_{3'e,3'a} \sim -13.9$ Hz, H-3′*e*), 2.11 (dd, 1 H, H-3′*a*), 2.09 (dd, 1 H, H-3*a*), 2.10 (s, 3 H), 2.08 (s, 3 H) and 2.00 (s, 6 H, 4 CH_3CO). Anal. Calcd for $C_{32}H_{41}ClO_{21}$: C, 48.22; H, 5.18. Found: C, 48.51; H, 4.89.

Further elution of the column afforded **6** as colorless needles (71 mg, 22%); mp 176–177°C (EtOAc–hexane); $[\alpha]_D^{20}$ +23° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.86 (m, 1 H, =CH–), 5.41 (ddd, 1 H, $J_{4',5'} \sim 3.1$, $J_{4',3'e} \sim 7.2$, $J_{4',3'a} \sim 13.9$ Hz, H-4′), 5.39 (br s, 1 H, H-5′), 5.26 (dq, 1 H, $=CH_{2trans}$), 5.26 (ddd, 1 H, $J_{7',8'a} \sim 2.3$, $J_{7',6'} \sim 9.7$, $J_{7',8'b} \sim 5.0$ Hz, H-7′), 5.18 (dq, 1 H, $=CH_{2cis}$), 5.085 (ddd, 1 H, $J_{4,3e} \sim 2.2$, $J_{4,3a} \sim 4.2$, $J_{4,5} \sim 9.2$ Hz, H-4), 5.05 (dt, 1 H, $J_{7,8a} \sim J_{7,8b} \sim 2.3$, $J_{7,6} \sim 9.0$ Hz, H-7), 4.96 (dd, 1 H, $J_{5,6} \sim 1.3$ Hz, H-5), 4.60 (dd, 1 H, $J_{8'a,8'b} \sim -12.2$ Hz, H-8′a), 4.39 (dd, 1 H, $J_{6',5'} \sim 1.2$ Hz, H-6′), 4.32 (dd, 1 H, H-6), 4.18 (m, 1 H, OCH_2), 4.17 (s, 2 H, CH_2Cl), 4.11 (dd, 1 H, H-8′b), 3.95–3.77 (m, 3 H, H-8a,8b and OCH_2), 3.85 and 3.80 (s, 6 H, CO_2CH_3), 2.64 (dd, 1 H, $J_{3e,3a} \sim -16.1$ Hz, H-3*e*), 2.14 (dd, 1 H, H-3*a*), 2.12 (s, 3 H, CH_3CO), 2.10 (m, 2 H, H-3′*e*,3′*a*), 2.03 (s, 3 H), 2.00 (s, 3 H) and 1.97 (s, 3 H, 3 CH_3CO). Anal. Calcd for $C_{32}H_{41}ClO_{21}$: C, 48.22; H, 5.18. Found: C, 48.40; H, 5.17.

Final elution of the column afforded **7** as a colorless syrup (23 mg, 7%); $[\alpha]_D^{20}$ +28° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.86 (m, 1 H, =CH–), 5.27 (br s, 1 H, H-5′), 5.20 (dq, 1 H, $=CH_{2trans}$), 5.21-5.12 (m, 3 H, $=CH_{2cis}$ and H-7,7′), 5.06 (ddd, 1 H, $J_{4,5} \sim 9.2$, $J_{4,3e} \sim 2.0$, $J_{4,3a} \sim 4.4$ Hz, H-4), 4.95 (dd, 1 H, $J_{5,6} \sim 1.3$ Hz, H-5), 4.91 (ddd, 1 H, $J_{4',5'} \sim 2.9$, $J_{4',3'e} \sim 4.6$, $J_{4',3'a} \sim 12.9$ Hz, H-4′), 4.30 (dd, 1 H, $J_{8'a,8'b} \sim -11.2$, $J_{8'a,7'} \sim 3.4$ Hz, H-8′a), 4.36–4.27 (m, 2 H, H-8a,8b), 4.17 (m, 1 H, OCH_2), 4.14 (m, 2 H, CH_2Cl), 4.11 (dd, 1 H, $J_{6',7'} \sim 8.0$, $J_{6',5'} \sim 1.2$ Hz, H-6′), 4.06 (dd, 1 H, $J_{6,7} \sim 9.6$ Hz, H-6), 3.99 (m, 1 H, OCH_2), 3.83 and 3.81 (s, 6 H, CO_2CH_3), 3.76 (dd, 1 H, $J_{8'b,7'} \sim 5.0$ Hz, H-8′b), 2.57 (dd, 1 H, $J_{3e,3a} \sim -16.1$ Hz, H-3*e*), 2.38 (dd, 1 H, $J_{3'e,3'a} \sim -12.7$ Hz, H-3′*e*), 2.11 (dd, 1 H, H-3*a*), 2.08 (t, 1 H,

H-3′*a*), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.00 (s, 3 H) and 1.99 (s, 3 H, 4 CH_3CO). Anal. Calcd for $C_{32}H_{41}ClO_{21}$: C, 48.22; H, 5.18. Found: C, 48.36; H, 5.12.

O-*(Sodium 3-deoxy-α-*D*-*manno*-2-octulopyranosylonate)-(2 → 8)-sodium (allyl 3-deoxy-β-*D*-*manno*-2-octulopyranosid)onate* (**8**).—A solution of **6** (27 mg, 0.034 mmol) in dry MeOH (5 mL) and 0.1 M methanolic NaOMe (0.5 mL) was stirred for 3 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 (4 mL) was stirred with 0.2 M aq NaOH (1 mL) for 3 h at room temperature. The pH of the solution was adjusted to 9.0 by addition of Dowex 50 (H^+) resin. The solution was filtered and evaporated, and the residue was desalted on Bio-Gel P-2 to give **8** as an amorphous powder (16 mg, 87%); $[\alpha]_D^{20}$ +39° (*c* 0.3, H_2O). For NMR data, see Tables I–III.

O-*(2,3,4,6,7-Penta-*O*-acetyl-*L*-glycero-α-*D*-manno-heptopyranosyl)-(1 → 7)-methyl (allyl 8-*O*-tert-butyldimethylsilyl-4,5-*O*-carbonyl-3-deoxy-β-*D*-manno-2-octulopyranosid)onate* (**10**).—A suspension of **9** (159 mg, 0.29 mmol), **1** (63 mg, 0.14 mmol), and 4A molecular sieves (1 g) in CH_2Cl_2 (4 mL) was stirred for 2 h at room temperature. A solution of Me_3Si-triflate (0.04 M) in CH_2Cl_2 (0.61 mL) was added and stirring was continued for 20 min. After addition of Et_3N (0.5 mL), the suspension was diluted with CH_2Cl_2 (20 mL), filtered over Celite, washed with satd aq $NaHCO_3$, dried (Na_2SO_4), and evaporated to dryness. Purification of the residue on a column of silica gel (*B*, 1:1 toluene–EtOAc) gave **10** as a colorless syrup (98 mg, 82%); $[\alpha]_D^{20}$ −4° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.88 (m, 1 H, =CH–), 5.36–5.26 (m, 3 H, H-3′,4′,6′), 5.24 (dq, 1 H, $=CH_{2trans}$), 5.21 (dd, 1 H, $J_{2',3'}$ ~ 2.9, $J_{2',1'}$ ~ 1.6 Hz, H-2′), 5.16 (dq, 1 H, $=CH_{2cis}$), 5.11 (d, 1 H, H-1′), 5.10 (ddd, 1 H, $J_{4,5}$ ~ 9.5, $J_{4,3e}$ ~ 1.9, $J_{4,3a}$ ~ 4.4 Hz, H-4), 4.97 (dd, 1 H, $J_{6,5}$ ~ 1.0 Hz, H-5), 4.36 (dd, 1 H, $J_{7'a,6'}$ ~ 3.8, $J_{7'a,7'b}$ ~ −11.8 Hz, H-7′a), 4.30 (dd, 1 H, $J_{7'b,6'}$ ~ 8.3 Hz, H-7′b), 4.17 (m, 1 H) and 4.01 (m, 1 H, OCH_2), 3.93 (dd, 1 H, $J_{6,7}$ ~ 9.5 Hz, H-6), 3.96–3.85 (m, 4 H, H-7,8a,8b,5′), 3.78 (s, 3 H, CO_2CH_3), 2.57 (dd, 1 H, $J_{3e,3a}$ ~ −16.0 Hz, H-3*e*), 2.09 (dd, 1 H, H-3*a*), 2.18 (s, 3 H), 2.14 (s, 3 H), 2.05 (s, 3 H), 2.01 (s, 3 H) and 1.99 (s, 3 H, 5 CH_3CO), 0.90 [s, 9 H, $(CH_3)_3C$], and 0.10 [s, 6 H, $(CH_3)_2Si$]. Anal. Calcd for $C_{36}H_{54}O_{20}Si$: C, 51.79; H, 6.52. Found: C, 51.34; H, 6.18.

O-*(2,3,4,6,7-Penta-*O*-acetyl-*L*-glycero-α-*D*-manno-heptopyranosyl)-(1 → 7)-methyl (allyl 4,5-*O*-carbonyl-3-deoxy-β-*D*-manno-2-octulopyranosid)onate* (**11**).—A solution of HF (2% in MeCN, 0.4 mL) was added to a solution of **10** (153 mg, 0.18 mmol) in MeCN (5 mL) and stirred for 3 h at room temperature. After addition of $NaHCO_3$ (1 g), the mixture was filtered and the filtrate was evaporated to dryness. The residue was purified on a column of silica gel (*B*, EtOAc) to give **11** as a colorless syrup (107 mg, 81%); $[\alpha]_D^{20}$ −7° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.83 (m, 1 H, =CH–), 5.36–5.28 (m, 3 H, H-3′,4′,6′), 5.25 (dq, 1 H, $=CH_{2trans}$), 5.19 (dd, 1 H, $J_{2',1'}$ ~ 1.6, $J_{2',3'}$ ~ 2.1 Hz, H-2′), 5.17 (dq, 1 H, $=CH_{2cis}$), 5.14 (dt, 1 H, H-4), 5.05 (d, 1 H, H-1′), 4.96 (d, 1 H, $J_{5,4}$ ~ 9.2 Hz, H-5), 4.47 (dd, 1 H, $J_{7'a,7'b}$ ~ −11.6, $J_{7'a,6'}$ ~ 4.8 Hz, H-7′a), 4.38 (dt, 1 H, $J_{5',4'}$ ~ 7.9, $J_{5',6'}$ ~ 1.9 Hz, H-5′), 4.25 (dd, 1

H, $J_{7'b,6'}$ ~ 7.9 Hz, H-7'b), 4.18 (m, 1 H, OCH_2), 4.04 (br dd, 1 H, $J_{8a,OH}$ ~ 5.5, $J_{8a,8b}$ ~ −12.8 Hz, H-8a), 3.92-3.81 (m, 4 H, H-6,7,8b and OCH_2), 3.83 (s, 3 H, CO_2CH_3), 3.39 (dd, 1 H, $J_{8b,OH}$ ~ 8.6 Hz, OH), 2.67 (dd, 1 H, $J_{3e,3a}$ ~ −16.4, $J_{3e,4}$ ~ 2.3 Hz, H-3*e*), 2.18 (s, 3 H), 2.14 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), and 1.99 (s, 3 H, 5 CH_3CO), and ~ 2.05 (dd, 1 H, H-3*a*). Anal. Calcd for $C_{30}H_{40}O_{20}$: C, 50.00; H, 5.59. Found: C, 50.00; H, 5.17.

O-L-glycero-α-D-manno-*Heptopyranosyl-(1 → 7)-sodium (allyl 3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**12**).—A solution of **11** (19 mg, 0.027 mmol) in dry MeOH (5 mL) and 0.1 M methanolic NaOMe (0.2 mL) was stirred overnight 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 (3 mL) was stirred with 0.2 M aq NaOH (0.5 mL) for 3 h at room temperature. Workup of the reaction, similarly to the preparation of **8**, gave **12** as an amorphous powder (13 mg, 98%); $[\alpha]_D^{20}$ +18° (*c* 0.8, H_2O). For NMR data, see Tables I–III.

O-*(2,3,4,6,7-Penta-*O-*acetyl-*L-glycero-α-D-manno-*heptopyranosyl)-(1 → 7)-*O-*[methyl 4,5,7,8-tetra-*O-*acetyl-3-deoxy-α-*D-manno-*2-octulopyranosylonate-(2 → 8)]-methyl (allyl 4,5-*O-*carbonyl-3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**13**) *and* O-*(2,3,4,6,7-penta-*O-*acetyl-*L-glycero-α-D-manno-*heptopyranosyl)-(1 → 7)-*O-*[methyl (4,5,7,8-tetra-*O-*acetyl-3-deoxy-β-*D-manno-*2-octulopyranosylonate-(2 → 8)]-methyl (allyl 4,5-*O-*carbonyl-3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**14**).—A solution of **4** (90 mg, 0.19 mmol) in MeCN (1 mL) was added during 2 h to a suspension of **11** (42 mg, 0.059 mmol), $Hg(CN)_2$ (105 mg, 0.42 mmol), and 4A molecular sieves (1 g) in MeCN (5 mL) under dry N_2. A second portion of **4** (90 mg, 0.19 mmol) in MeCN (1 mL) and $Hg(CN)_2$ (105 mg, 0.42 mmol) was added after 15 h and stirring was continued for 48 h. The suspension was diluted with MeCN (20 mL) and filtered over Celite, and the filtrate was evaporated. The residue was dissolved in CH_2Cl_2, washed with satd aq $NaHCO_3$, dried (Na_2SO_4), and evaporated. Purification by chromatography on silica gel (*C*, 1 : 1 2-propanol–hexane) gave **13** as a colorless syrup (11.4 mg, 16%); $[\alpha]_D^{20}$ +35° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.87 (m, 1 H, =CH–), 5.41 (br s, 1 H, H-5″), 5.38 (m, 1 H, H-4″), 5.37–5.26 (m, 4 H, H-3′,4′,6′,7″), 5.26 (dq, 1 H, $=CH_{2trans}$), 5.18 (dd, 1 H, $J_{2',1'}$ ~ 2.0, $J_{2',3'}$ ~ 3.5 Hz, H-2′), 5.16 (dq, 1 H, $=CH_{2cis}$), 5.12 (d, 1 H, H-1′), 5.10 (ddd, 1 H, $J_{4,5}$ ~ 9.2, $J_{4,3e}$ ~ 2.0, $J_{4,3a}$ ~ 4.4 Hz, H-4), 4.97 (dd, 1 H, $J_{5,6}$ ~ 1.0 Hz, H-5), 4.62 (dd, 1 H, $J_{8''a,8''b}$ ~ −12.2, $J_{8''a,7''}$ ~ 2.4 Hz, H-8″a), 4.38 (dd, 1 H, $J_{6'',7''}$ ~ 9.1, $J_{6'',5''}$ ~ 1.4 Hz, H-6″), 4.35 (dd, 1 H, $J_{7'a,7'b}$ ~ −11.5, $J_{7'a,6'}$ ~ 5.1 Hz, H-7′a), 4.26 (br d, 1 H, H-5′), 4.23 (dd, 1 H, $J_{7'b,6'}$ ~ 7.6 Hz, H-7′b), 4.16 (m, 1 H, OCH_2), 4.10 (dd, 2 H, $J_{8''b,7''}$ ~ 3.0 Hz, $J_{6,7}$ ~ 8.9 Hz, H-6,8″b), 4.01 (dt, 1 H, H-7), 3.90 (m, 1 H, OCH_2), 3.86–3.79 (m, 2 H, H-8a,8b), 3.86 and 3.81 (s, 6 H, CO_2CH_3), 2.62 (dd, 1 H, $J_{3e,3a}$ ~ −16.0 Hz, H-3*e*), 2.24 (dd, 1 H, $J_{3''e,3''a}$ ~ −12.5, $J_{3''e,4''}$ ~ 4.5 Hz, H-3″*e*), 2.10 (t, 2 H, H-3*a*,3″*a*), 2.19 (s, 3 H), 2.15 (s, 3 H), 2.11 (s, 3 H), 2.06 (s, 3 H), 2.03 (s, 6 H), 2.01 (s, 3 H), 2.00 (s, 3 H), and 1.97 (s, 3 H, 9 CH_3CO). Anal. Calcd for $C_{47}H_{62}O_{31}$: C, 50.27; H, 5.56. Found: C, 50.72; H, 5.32.

Further elution of the column afforded a mixture of **11** and **14** which was purified on silica gel (1:5 toluene–EtOAc) to give **14** as a colorless syrup (4.0 mg, 6%); $[\alpha]_D^{20}$ +22° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.88 (m, 1 H, =CH–), 5.38–5.23 (m, 6 H, H-2′,3′,4′,6′,5″ and $=CH_{2trans}$), 5.20–5.11 (m, 3 H, H-1′,7″ and $=CH_{2cis}$), 5.08 (ddd, 1 H, $J_{4,5}$ ~ 9.0, $J_{4,3e}$ ~ 2.1, $J_{4,3a}$ ~ 4.1 Hz, H-4), 4.92 (dd, 1 H, $J_{5,6}$ ~ 0.8 Hz, H-5), 4.90 (ddd, 1 H, $J_{4'',3''e}$ ~ 4.7, $J_{4'',5''}$ ~ 2.7, $J_{4'',3''a}$ ~ 13.2 Hz, H-4″), 4.48 (dd, 1 H, $J_{7'a,6'}$ ~ 2.4, $J_{7'a,7'b}$ ~ −9.9 Hz, H-7′a), 4.45 (dd, 1 H, $J_{8''a,8''b}$ ~ −12.1, $J_{8''a,7''}$ ~ 3.0 Hz, H-8″a), 4.36–4.29 (m, 4 H, H-8a,5′,7′b,8″b), 4.25 (dd, 1 H, $J_{6'',5''}$ ~ 1.4, $J_{6'',7''}$ ~ 9.6 Hz, H-6″), 4.22 (m, 1 H, OCH_2), 4.11 (ddd, 1 H, $J_{7,8a}$ ~ 1.8 Hz, H-7), 3.97 (m, 1 H, OCH_2), 3.88 and 3.79 (s, 6 H, CO_2CH_3), 3.72 (dd, 1 H, $J_{6,7}$ ~ 8.7 Hz, H-6), 3.44 (dd, 1 H, $J_{8b,7}$ ~ 7.5, $J_{8b,8a}$ ~ −10.7 Hz, H-8b), 2.58 (dd, 1 H, $J_{3e,3a}$ ~ −16.0 Hz, H-3*e*), 2.41 (dd, 1 H, $J_{3''e,3''a}$ ~ −12.6 Hz, H-3″*e*), 2.19 (t, 1 H, H-3″*a*), 2.19 (s, 3 H), 2.15 (s, 3 H), 2.14 (s, 3 H), 2.10 (s, 3 H), 2.04 (s, 6 H), and 2.00 (s, 9 H, 9 CH_3CO), and 2.00 (t, 1 H, H-3*a*). Anal Calcd for $C_{47}H_{62}O_{31}$: C, 50.27; H, 5.56. Found: C, 50.43; H, 5.27.

O-L-glycero-α-D-manno-*Heptopyranosyl-(1 → 7)-[*O-*(sodium 3-deoxy-*α-D-manno-*2-octulopyranosylonate)-(2 → 8)]-sodium (allyl 3-deoxy-*β-D-manno-*2-octulopyranosid)onate* (**15**).—A solution of **13** (11.4 mg, 0.010 mmol) in dry MeOH (10 mL) and 0.1 M methanolic NaOMe (0.5 mL) was stirred for 3 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 (3 mL) was stirred with 0.2 M aq NaOH (0.5 mL) for 3 h at room temperature. Workup of the reaction, similarly to the preparation of **8**, gave **15** as an amorphous powder (7.3 mg, 98%); $[\alpha]_D^{20}$ +47° (*c* 0.7, H_2O). For NMR data, see Tables I–III.

O-L-glycero-α-D-manno-*Heptopyranosyl-(1 → 7)-[*O-*(sodium 3-deoxy-β-D-*manno-*2-octulopyranosylonate)-(2 → 8)]-sodium (allyl 3-deoxy-β-D-manno-2-octulopyranosid)onate* (**16**).—A solution of **14** (9.6 mg, 0.0085 mmol) in dry MeOH (2.5 mL) and 0.1 M methanolic NaOMe (0.5 mL) was stirred for 1.5 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 (3 mL) was stirred with 0.2 M aq NaOH (0.3 mL) for 3 h at room temperature. Workup of the reaction, similarly to the preparation of **8**, gave **16** as an amorphous powder (5.4 mg, 86%); $[\alpha]_D^{20}$ +87° (*c* 0.2, H_2O). For NMR data, see Tables I–III.

*3,4,6-Tri-*O-*acetyl-β-D-mannopyranose 1,2-{[methyl (allyl 8-*O-tert-*butyldimethylsilyl-4,5-*O-*carbonyl-3,7-dideoxy-β-D-*manno-*2-octulopyranosid)onate]-7-yl orthoacetate}* (**18**) *and* O-*(2,3,4,6-tetra-*O-*acetyl-α-D-mannopyranosyl)-(1 → 7)-methyl (allyl 8-*O-tert-*butyldimethylsilyl-4,5-*O-*carbonyl-3-deoxy-β-D-manno-2-octulopyranosid)onate* (**19**).—A solution of **17** (1.0 g, 2.43 mmol) in MeCN (3 mL) was added during 2 h to a suspension of **1** (434 mg, 0.75 mmol), $Hg(CN)_2$ (44 mg, 3.74 mmol), and 4A molecular sieves in MeCN (10 mL) under dry N_2. The suspension was stirred overnight, diluted with CH_2Cl_2, filtered over Celite, washed with satd aq $NaHCO_3$, dried (Na_2SO_4), and evaporated. Purification of the residue on a column of silica gel (2:1 hexane–EtOAc) gave **18** as colorless needles

(14 mg, 3%); mp 131–135°C (EtOAc–hexane); $[\alpha]_D^{20}$ −7° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.92 (m, 1 H, =CH–), 5.58 (d, 1 H, $J_{1',2'}$ ~ 2.6 Hz, H-1'), 5.26 (dd, 1 H, $J_{4',5'}$ ~ 9.5 Hz, H-4'), 5.26 (dq, 1 H, $=CH_{2trans}$), 5.16 (dq, 1 H, $=CH_{2cis}$), 5.12 (dd, 1 H, $J_{3',4'}$ ~ 9.5, H-3'), 5.07 (dd, 1 H, $J_{5,6}$ ~ 1.0 Hz, H-5), 5.00 (ddd, 1 H, $J_{4,5}$ ~ 9.0 Hz, H-4), 4.66 (dd, 1 H, $J_{2',3'}$ ~ 4.1 Hz, H-2'), 4.24 (dd, 1 H, $J_{6'a,6'b}$ ~ −11.7 Hz, H-6'a), 4.20 (m, 1 H, OCH_2), 4.14 (dd, 1 H, H6'b), 4.05 (m, 1 H, OCH_2), 3.89–3.86 (m, 4 H, H-6,7,8a,8b), 3.76 (s, 3 H, CO_2CH_3), 3.68 (ddd, 1 H, $J_{5',6'a}$ ~ 5.0, $J_{5',6'b}$ ~ 2.7 Hz, H-5'), 2.55 (dd, 1 H, $J_{3e,4}$ ~ 1.9 Hz, H-3*e*), 2.12 (dd, 1 H, $J_{3a,4}$ ~ 4.5, $J_{3a,3e}$ ~ −16.0 Hz, H-3*a*), 2.11 (s, 3 H), 2.08 (s, 3 H) and 2.07 (s, 3 H, 3 CH_3CO), 1.78 (s, 3 H, CH_{3endo}), 0.89 [s, 9 H, $(CH_3)_3C$], and 0.08 [s, 6 H, $Si(CH_3)_2$]. Anal. Calcd for $C_{33}H_{50}O_{18}Si$: C, 51.96; H, 6.61. Found: C, 51.10; H, 6.72.

Further elution of the column afforded **19** as a colorless syrup (186 mg, 36%); $[\alpha]_D^{20}$ +7° (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.88 (m, 1 H, =CH–), 5.36–5.27 (m, 2 H, H-3',4'), 5.23 (dq, 1 H, $=CH_{2trans}$), 5.22 (dd, 1 H, $J_{2',1'}$ ~ 1.9, $J_{2',3'}$ ~ 2.9 Hz, H-2'), 5.15 (dq, 1 H, $=CH_{2cis}$), 5.11 (ddd, 1 H, $J_{4,5}$ ~ 9.3, $J_{4,3e}$ ~ 3.1, $J_{4,3a}$ ~ 4.4 Hz, H-4), 5.06 (d, 1 H, H-1'), 5.02 (dd, 1 H, $J_{5,6}$ ~ 1.3 Hz, H-5), 4.35–4.27 (m, 2 H, H-5',6'a), 4.14 (m, 1 H, OCH_2), 4.10–3.98 (m, 4 H, H-6,8a,6'b and OCH_2), 3.94 (dd, 1 H, $J_{8b,7}$ ~ 2.6, $J_{8b,8a}$ ~ −11.2 Hz, H-8b), 3.85 (dt, 1 H, $J_{7,8a}$ ~ 2.6, $J_{7,6}$ ~ 9.0 Hz, H-7), 3.77 (s, 3 H, CO_2CH_3), 2.57 (dd, 1 H, $J_{3e,3a}$ ~ −15.9 Hz, H-3*e*), 2.16 (s, 3 H), 2.11 (s, 3 H), 2.03 (s, 3 H), and 2.00 (s, 3 H, 4 CH_3CO), 2.09 (dd, 1 H, H-3*a*), 0.95 [s, 9 H, $(CH_3)_3C$], and 0.10 [s, 6 H, $Si(CH_3)_2$]. Anal. Calcd for $C_{33}H_{50}O_{18}Si$: C, 51.96; H, 6.61. Found: C, 52.07; H, 6.50.

Alternatively, **19** was prepared as follows: a solution of **17** (319 mg, 0.78 mmol) in CH_2Cl_2 (5 mL) was added during 2 h to a suspension of **1** (224 mg, 0.39 mmol), silver triflate (200 mg, 0.78 mol), and 4A molecular sieves in CH_2Cl_2 (7 mL) under dry N_2 with exclusion of light. The mixture was stirred overnight at room temperature and then at 40°C for 1.5 h. The suspension was diluted with CH_2Cl_2 (20 mL), filtered over Celite, washed with satd aq $NaHCO_3$, dried (Na_2SO_4), and evaporated. Purification of the residue on a column of silica gel (*D*, 3:1 CH_2Cl_2–Et_2O) gave **19** (170 mg, 61%).

O-(*2,3,4,6-Tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-(*1* → *7*)-*methyl* (*allyl* 4,5-O-*carbonyl*-3-*deoxy*-β-D-manno-*2-octulopyranosid*)*onate* (**20**).—A solution of HF (2% in MeCN, 1 mL) was added to a solution of **19** (160 mg, 0.21 mmol) in MeCN (7 mL). The solution was stirred for 6 h at room temperature. After addition of $NaHCO_3$ (1 g), the mixture was evaporated. The residue was dissolved in CH_2Cl_2, washed with satd aq $NaHCO_3$, dried $(Na_2SO)_4$, and evaporated. Purification by chromatography on a column of silica gel (*C*, 1:1 toluene–EtOAc) gave **20** as colorless needles (143 mg, 99%); mp 118–120°C (EtOAc–hexane); $[\alpha]_D^{20}$ +4° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.84 (m, 1H, =CH–), 5.32–5.25 (m, 2 H, H-3',4'), 5.25 (dq, 1 H, $=CH_{2trans}$), 5.22 (dd, 1 H, $J_{2',3'}$ ~ 2.9, $J_{2',1'}$ ~ 1.8 Hz, H-2'), 5.17 (dq, 1 H, $=CH_{2cis}$), 5.12 (ddd, 1 H, $J_{4,5}$ ~ 9.1, $J_{4,3e}$ ~ 2.5, $J_{4,3a}$ ~ 3.3 Hz, H-4), 5.00 (d, 1 H, H-1'), 4.96 (dd, 1 H, $J_{5,6}$ ~ 0.8 Hz, H-5), 4.33–4.16 (m, 4 H,

H-5′,6′a,6′b and OCH_2), 4.01 (br d, 2 H, H-8a,8b), 3.92–3.83 (m, 3 H, H-6,7 and OCH_2), 3.83 (s, 3 H, CO_2CH_3), 3.34 (t, 1 H, $J_{8a,OH} \sim J_{8b,OH} \sim 7.4$ Hz, OH), 2.67 (dd, 1 H, $J_{3e,3a} \sim -16.4$ Hz, H-3*e*), 2.17 (s, 3 H), 2.13 (s, 3 H), 2.06 (s, 3 H), and 1.99 (s, 3 H, 4 CH_3CO), and 2.01 (dd, 1 H, H-3*a*). Anal. Calcd for $C_{27}H_{36}O_{18}$: C, 50.00; H, 5.59. Found: C, 49.65; H, 5.53.

O-α-D-*Mannoyranosyl-(1 → 7)-sodium (allyl 3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**21**).—A solution of **20** (62 mg, 0.096 mmol) in dry MeOH (21 mL) and 0.1 M methanolic NaOMe (2 mL) was stirred for 6 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 (10 mL) was stirred with 0.2 M aq NaOH (2 mL) for 6 h at room temperature. Workup of the reaction, similarly to the preparation of **8**, gave **21** as an amorphous powder (41.7 mg, 92%); $[\alpha]_D^{20} + 41°$ (*c* 0.7 H_2O). For NMR data, see Tables I–III.

O-*(2,3,4,6-Tetra-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 7)-*O*-[methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-β-*D-manno-*2-octulopyranosylonate-(2 → 8)]-methyl (allyl 4,5-*O*-carbonyl-3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**22**) *and* O-*(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 7)-*O*-[methyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-*D*-manno-2-octulopyranosylonate-(2 → 8)]-methyl (allyl 4,5-*O*-carbonyl-3-deoxy-β-*D-manno-*2-octulopyranosid)onate* (**23**).—A solution of **4** (249 mg, 0.52 mmol) in MeCN (3 mL) was added during 2 h to a suspension of **20** (101 mg, 0.16 mmol), $Hg(CN)_2$ (295 mg, 1.17 mmol), and 4A molecular sieves (1 g) in MeCN (7 mL). The suspension was stirred for 48 h at room temperature, diluted with EtOAc, and filtered over Celite. The filtrate was washed with satd aq $NaHCO_3$, dried (Na_2SO_4), and evaporated. Purification of the residue on a column of silica gel (*C*, 1:5 toluene–EtOAc) gave **22** as a colorless syrup (20 mg, 12%); $[\alpha]_D^{20} + 35°$ (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.88 (m, 1 H, =CH–), 5.41 (t, 1 H, $J_{4',5'} \sim J_{4',3'} \sim 10.3$ Hz, H-4′), 5.30 (dd, 1 H, H-5″), 5.28 (dq, 1 H, $=CH_{2trans}$), 5.27 (dd, 1 H, $J_{3',2'} \sim 3.0$ Hz, H-3′), 5.18 (dq, 1 H, $=CH_{2cis}$), 5.18 (ddd, 1 H, H-7″), 5.16 (dd, 1 H, $J_{2',1'} \sim 1.8$ Hz, H-2′), 5.09 (ddd, 1 H, $J_{4,3e} \sim 2.5$, $J_{4,3a} \sim 4.5$, $J_{4,5} \sim 9.2$ Hz, H-4), 5.08 (d, 1 H, H-1′), 4.94 (dd, 1 H, $J_{5,6} \sim 1.0$ Hz, H-5), 4.91 (ddd, 1 H, $J_{4'',5''} \sim 3.0$, $J_{4'',3''e} \sim 4.5$, $J_{4'',3''a} \sim 13.0$ Hz, H-4″), 4.43–4.30 (m, 5 H, H-5′,6′a,6′b,8″a,8″b), 4.20 (dd, 1 H, $J_{6'',7''} \sim 9.5$, $J_{6'',5''} \sim 1.5$ Hz, H-6″), 4.19 (m, 1 H, OCH_2), 4.14–4.02 (m, 2 H, H-6,7), 3.96 (m, 1 H, OCH_2), 3.88 and 3.80 (s, 6 H, CO_2CH_3), 3.79 (dd, 1 H, $J_{8a,7} \sim 1.5$, $J_{8a,8b} \sim -11.0$ Hz, H-8a), 3.48 (dd, 1 H, $J_{8b,7} \sim 6.5$ Hz, H-8b), 2.58 (dd, 1 H, $J_{3e,3a} \sim -16.5$ Hz, H-3*e*), 2.34 (dd, 1 H, $J_{3''e,3''a} \sim -12.5$ Hz, H-3″*e*), 2.18 (s, 3 H), 2.13 (s, 3 H), 2.12 (s, 3 H), 2.10 (s, 3 H), 2.07 (s, 3 H), 2.02 (s, 6 H), and 2.01 (s, 3 H, 8 CH_3CO), and 2.18–2.00 (m, 2 H, H-3*a*,3″*a*). Anal. Calcd for $C_{44}H_{58}O_{29}$: C, 50.28; H, 5.56. Found: C, 50.58; H, 5.95.

Further elution of the column afforded **23** as a colorless syrup (37 mg, 22%); $[\alpha]_D^{20} + 46°$ (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.86 (m, 1 H, =CH–), 5.41 (br s, 1 H, H-5″), 5.40–5.26 (m, 4 H, H-3′,4′,4″,7″), 5.26 (dq, 1 H, $=CH_{2trans}$), 5.19 (dd, 1 H, $J_{2',3'} \sim 2.8$ Hz, H-2′), 5.18 (dq, 1 H, $=CH_{2cis}$), 5.10 (ddd, 1 H, $J_{4,5} \sim 9.7$, $J_{4,3e} \sim 2.3$, $J_{4,3a} \sim 4.0$ Hz, H-4), 5.06 (d, 1 H, $J_{1',2'} \sim 1.8$ Hz, H-1′), 4.97 (dd, 1 H,

$J_{5,6} \sim 0.8$ Hz, H-5), 4.61 (dd, 1 H, $J_{8''a,7''} \sim 2.5$, $J_{8''a,8''b} \sim -12.5$ Hz, H-8″a), 4.38 (dd, 1 H, $J_{6'',7''} \sim 9.5$, $J_{6'',5''} \sim 1.5$ Hz, H-6″), 4.36–4.10 (m, 5 H, H-5′6′a,6′b,8″b, and OCH_2), 4.05–3.94 (m, 3 H, H-6,7,8a), 3.94 (m, 1 H, OCH_2), 3.83 and 3.80 (s, 6 H, CO_2CH_3), 3.78 (dd, 1 H, $J_{8b,7} \sim 4.0$, $J_{8b,8a} \sim -12.0$ Hz, H-8b), 2.62 (dd, 1 H, $J_{3e,3a} \sim 16.5$ Hz, H-3*e*), 2.18 (s, 3 H), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 2.00 (s, 3 H), and 1.98 (s, 3 H, 8 CH_3CO), and 2.18–2.00 (m, 3 H, H-3″*e*,3*a*,3″*a*). Anal. Calcd for $C_{44}H_{58}O_{29}$: C, 50.28; H, 5.56. Found: C, 51.18; H, 5.71.

O-α-D-*Mannopyranosyl-(1 → 7)-[*O-*(sodium 3-deoxy-*α-D-manno-*2-octulopyranosylonate)-(2 → 8)]-sodium (allyl 3-deoxy-*β-D-manno-*2-octulopyranosid)onate* (**24**). —A solution of **23** (25 mg, 0.024 mmol) in dry MeOH (10 mL) and 0.1 M methanolic NaOMe (0.8 mL) was stirred for 3 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 0.2 M aq NaOH (1 mL) for 6 h at room temperature. Workup of the reaction, similarly to the preparation of **8**, gave **24** as an amorphous powder (16.5 mg, 99%); $[\alpha]_D^{20} + 32°$ (*c* 0.4, H_2O). For NMR data, see Tables I–III.

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