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Synthesis and immunoreactivity of poly(acrylamide) copolymers containing C-3- and C-7-modified, carboxyl-reduced, 4-*O*- and 5-*O*-phosphorylated Kdo residues

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ABSTRACT

Sodium (allyl 3-deoxy-α-D-*lyxo*-2-heptulopyranosid)onate (**6**), allyl 3-deoxy-β- and -α-D-*manno*-2-octulopyranoside, sodium (allyl 3-deoxy-β-L-*gulo*-2-octulopyranosid)onate, sodium (allyl α-D-*glycero*-D-*talo*-2-octulopyranosid)onate, sodium (allyl α-D-*glycero*-D-*galacto*-2-octulopyranosidonate, ammonium (allyl 3-deoxy-4-*O*- and -5-*O*-phosphono-α-D-*manno*-2-octulopyranosid)onate, and ammonium (allyl 3-deoxy-4-*O*- and -5-*O*-phosphono-β-D-*manno*-2-octulopyranosid)onate were prepared and copolymerized with acrylamide to give multivalent haptens, which were used in immune inhibition assays. The monoclonal antibody A 20, directed against α-pyranoside Kdo residues, did not react with these compounds, except with heptulosonic acid derivative **6**, thus proving that the terminal hydroxymethyl group of Kdo is not required for binding.

INTRODUCTION

Lipopolysaccharides (LPS) are common constituents of the outer membrane of Gram-negative bacteria. Potentially, antibodies directed against the core- and lipid A-region of LPS would be of clinical interest to counteract the deleterious effects of endotoxins[1]. Previously, monoclonal antibodies have been raised[2] against the rough mutant *Salmonella minnesota* R 595 and shown to recognize part structures of the 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) region[3], as well as the Kdo–lipid A domain[4]. Among the Kdo-specific clones, one (A 20) was found to react with α-linked Kdo*p* monosaccharide residues and a second one (A 25) to be directed against the disaccharide α-Kdo*p*-(2 → 4)-α-Kdo*p*, a common constituent of enterobacterial LPS. Furthermore, A 20, a murine monoclonal antibody of the IgM

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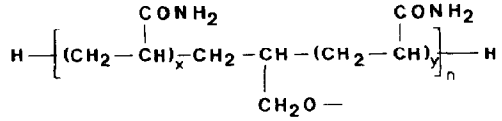
isotype, has been reported to confer cross-protection against lethal sepsis in an *Escherichia coli* model[5]. Within our study of immunoreactivity of LPS-structures[6], we report herein on the further characterization of the epitope specificity of monoclonal antibody, A 20, by use of poly(acrylamide) copolymers **1** (ref. 7) containing carboxyl-reduced-, 4-*O*- and 5-*O*-phosphorylated-, as well as C-3- and C-7-modified Kdo-groups.



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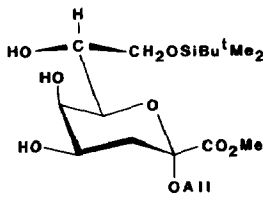
RESULTS AND DISCUSSION

For the synthesis of the 3-deoxy-α-D-*lyxo*-2-octulosonic acid derivative **6**, the previously described[8] 8-*O*-*tert*-butyldimethylsilyl ether derivative **2** was converted into the 4,5-*O*-carbonyl derivative **3** by treatment with 4-nitrophenyl chloroformate–pyridine[9] in 65% yield. Removal of the Bu^tMe_2Si group by 2% HF in acetonitrile[10] afforded the diol **4** in 97% yield. Periodate oxidation and subsequent reduction of the aldehyde with BH_3–NH_3 complex[11] gave an 88% yield of the heptulosonic acid derivative **5**. Zemplén *O*-deacylation and hydrolysis of the methyl ester group in aqueous NaOH gave sodium (allyl 3-deoxy-α-D-*lyxo*-2-heptulopyranosid)onate (**6**) in 91% yield. 3-Deoxy-2-heptulosaric acid of the D-*lyxo* configuration has previously been isolated from plant cell-wall polysaccharides[12] and from the cell-wall glycan of green algae[13]. The configuration of a similar heptulosaric acid found in the LPS of *Acinetobacter calcoaceticus*[14] has not yet been assigned.

For the synthesis of the carboxyl-reduced derivatives **9** and **11**, the previously reported[8] allyl glycosides **8** and **10** were reduced with $NaBH_4$ in EtOH in 62 and 65% yields, respectively. Whereas **9** could be purified by recrystallization, the corresponding α anomer **11** had to be subjected to *O*-acetylation, purification by column chromatography on silica gel, and subsequent Zemplén *O*-deacetylation. Protected derivatives of 3-deoxy-D-*manno*-2-octulopyranoside have been described[15,16].

For the synthesis of the Kdo-derivatives with inverted configuration at C-7 (7-*epi*-Kdo), the Cornforth reaction of L-xylose with oxalacetic acid and $NiCl_2$-catalyzed decarboxylation[17] of the intermediate was employed to give 3-deoxy-L-*gulo*-2-octulosonic acid[18] as a syrup in 32% yield. Subsequent per-*O*-acetylation (acetic anhydride, 4-dimethylaminopyridine, and pyridine) and esterification of the Cs salt with CH_3I[19] afforded the methyl ester derivative **16** (30%), which was separated from furanosidic byproducts by silica gel chromatography. Reaction of



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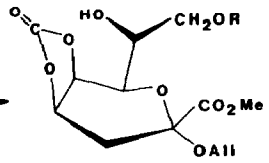
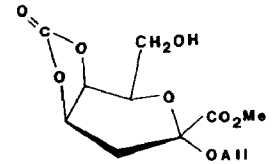


3 R = $SiBu^tMe_2$

4 R = H



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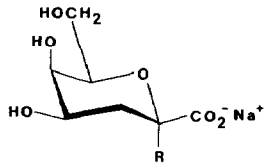
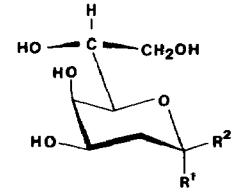


6 R = OAll

7 R = 1



8 $R^1 = CO_2Me$, R^2 = OAll

9 $R^1 = CH_2OH$, R^2 = OAll

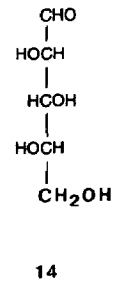
10 R^1 = OAll, $R^2 = CO_2Me$

11 R^1 = OAll, $R^2 = CH_2OH$

12 $R^1 = CH_2OH$, R^2 = 1

13 R^1 = 1, $R^2 = CH_2OH$

16 with $TiBr_4$ in dichloromethane gave the corresponding bromide derivative **17** in 94% yield. Subsequent glycoside formation with allyl alcohol, $Hg(CN)_2$, and 4A molecular sieves in nitromethane furnished a 1:3 mixture of the allyl α- and β-L-glycosides **18** and **19** in 77% yield, which could not be separated by column



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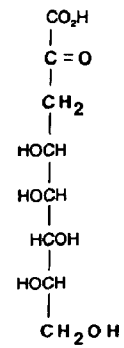

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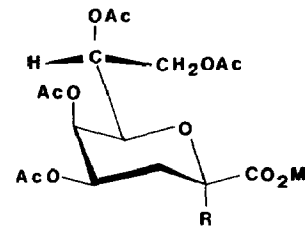
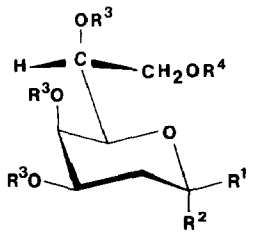


16 R = OAc

17 R = Br

17 →



18 R^1 = OAll, R^2 = CO_2Me, R^3 = R^4 = Ac

19 R^1 = CO_2Me, R^2 = OAll, R^3 = R^4 = Ac

20 R^1 = OAll, R^2 = CO_2Me, R^3 = H, R^4 = $SiBu^tMe_2$

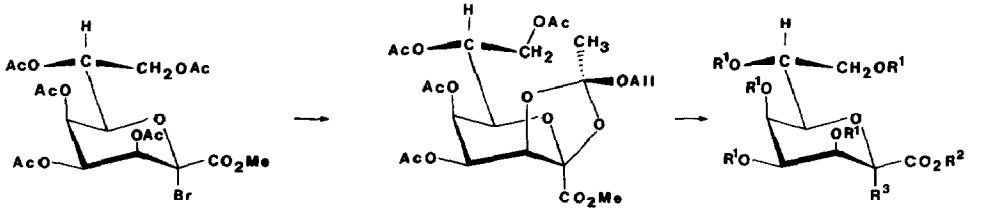
21 R^1 = CO_2Me, R^2 = OAll, R^3 = H, R^4 = $SiBu^tMe_2$

22 R^1 = $CO_2^-Na^+$, R^2 = OAll, R^3 = R^4 = H

23 R^1 = $CO_2^-Na^+$, R^2 = 1, R^3 = R^4 = H

chromatography on silica gel. Hence, the mixture was *O*-deacetylated with methanolic NaOMe and subsequently converted into a separable mixture of the 8-Bu^tMe_2Si ethers **20** and **21** with 1,4-diazabicyclo[2.2.2]octane[8] and Bu^tMe_2SiCl in acetonitrile. The assignment of the anomeric configuration[20] was based on the chemical shift differences between H-3*a* and H-3*e* ($\delta_{H\text{-}3e} - \delta_{H\text{-}3a} \sim 0.40$ ppm for **20**, $\delta_{H\text{-}3e} - \delta_{H\text{-}3a} \sim 0.28$ ppm for **21**). Removal of the Bu^tMe_2Si group and hydrolysis of the methyl ester group in aqueous NaOH afforded sodium (allyl 3-deoxy-β-L-*gulo*-2-octulopyranosid)onate (**22**) in 95% yield.

For the synthesis of the α-D-*glycero*-D-*talo* derivative **27**, previously reported[21] per-*O*-acetyl methyl (α-D-*glycero*-D-*talo*-2-octulopyranosyl bromide)onate derivative **24** was converted into the *exo*-orthoester **25** by reaction with allyl alcohol and $Hg(CN)_2$ in acetonitrile in 75% yield. The assignment of the orthoester configuration was based on the ^1H NMR chemical shift value of the *endo*-CH_3 group (δ 1.81). Trimethylsilyl triflate-catalyzed orthoester rearrangement[22] gave the allyl α-glycoside **26** in 46% yield. Zemplén *O*-deacetylation and hydrolysis of the methyl ester group in aqueous NaOH afforded sodium (allyl α-D-*glycero*-D-*talo*-2-octulopyranosid)onate (**27**) in 93% yield. Glycoside formation of **29** with allyl alcohol[21] in dichloromethane in the presence of trimethylsilyl triflate gave a 1:1 mixture of the anomeric allyl glycosides of D-*glycero*-D-*galacto* configuration, which were *O*-acetylated prior to the chromatography (52% overall yield). The mixture of anomers could not be resolved by column chromatography on silica gel; however, the allyl α-glycoside **30** could be isolated by crystallization in 19% yield. The assignment of the anomeric configuration was based on the ^{13}C NMR chemical shift value of C-6 by comparison with published values[21] of the corresponding



24 25

26 R^1 = Ac , R^2 = Me , R^3 = OAll

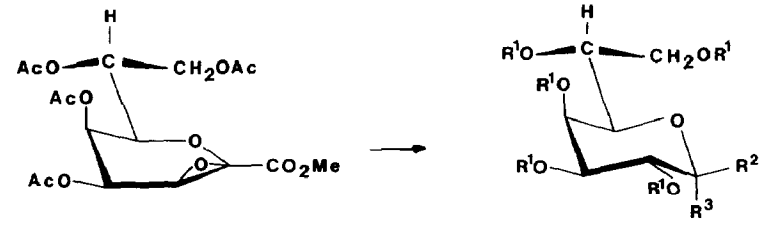
27 R^1 = H , R^2 = Na^+, R^3 = OAll

28 R^1 = H , R^2 = Na^+, R^3 = 1

methyl glycosides, which showed a downfield shift ($\delta_{C\text{-}6}$ 70.9) for the β-linked glycoside. Furthermore, the chemical shift differences between the geminal H-8 protons were significantly higher for the α anomers ($\delta_{H\text{-}8a} - \delta_{H\text{-}8b} \sim 0.5$ ppm) than for the β-glycosides ($\delta_{H\text{-}8a} - \delta_{H\text{-}8b} \sim 0.1$ ppm). Zemplén *O*-deacetylation of **30** and hydrolysis of the methyl ester group gave sodium (allyl α-D-*glycero*-D-*galacto*-2-octulopyranosid)onate (**31**) in 84% yield. The axial orientation of H-3 was confirmed from the large value of the coupling constant ($J_{3,4} \sim 10.0$ Hz). ^{13}C NMR data of **6**, **9**, **11**, **22**, **27**, and **31** are in agreement with the structural assignments (Table I).

The synthesis of the anomeric 4-*O*- and 5-*O*-phosphono derivatives **33**, **35**, **37**, and **39** has been described[23].

Copolymerisation of the allyl glycosides **6**, **9**, **11**, **22**, **27**, **31**, **33**, **35**, **37**, and **39** with 4 molar equivalents of acrylamide was performed essentially under the conditions given by Hořejši et al.[24]. The copolymers were purified by gel permeation chromatography on Sephadex G-25 and subsequently desalted on Bio-Gel P-2. The degree of ligand-incorporation (see Table II) was determined by the thiobarbiturate assay[25] for **7** and **23**, or by phosphate determination[26] for **34**, **36**, **38**, and **40**. The carbohydrate content of **12**, **13**, **28**, and **32** was estimated on the



29

30 R^1 = Ac , R^2 = CO_2Me , R^3 = OAll

31 R^1 = H , R^2 = $CO_2^-Na^+$, R^3 = OAll

32 R^1 = H , R^2 = $CO_2^-Na^+$, R^3 = 1

TABLE I

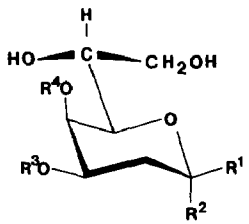
Assignments of ^{13}C NMR chemical shifts (δ) of compounds **6**, **9**, **11**, **22**, **27**, and **31**

C atom	**6**	**9**	**11**	**22**	**27**	**31**
1	176.26	60.86	63.85	176.29	174.41	174.78
2	100.76	101.70	101.40	100.68	102.83	100.82
3	35.04	31.35	33.65	34.96	72.56[b]	71.31
4	66.78	66.49	66.79	66.82	67.32	70.07
5	68.26	67.95	67.01	68.37	69.10	69.25
6	73.66	73.99	72.06	73.64	72.45[b]	71.81
7	62.52	70.20	70.08	72.32	70.38	71.39
8		63.85[a]	63.85	62.40	63.92	63.81
–CH=	134.66	135.43	134.88	134.59	134.41	134.88
CH_2=	118.96	118.43	118.26	118.95	118.77	118.10
OCH_2	65.44	63.99[a]	62.68	65.35	65.44	65.10

[a,b] Assignments may be reversed.

basis of the relative intensities of the ^1H NMR signals between δ 2.5 and 4.5 in relation to the $-CH_2-$ and CH signals of the poly(acrylamide) backbone, respectively.

Characterization of monoclonal antibody A 20 by the passive hemolysis–inhibition assay.—The reactivity of the monoclonal antibody A 20 towards various Kdo-derivatives was tested in the passive-hemolysis inhibition assay using the synthetic copolymerization products listed in Table II. In agreement with the previous



33 $R^1 = CO_2^-NH_4^+$, $R^2 = OAll$, $R^3 = HPO_3^-NH_4^+$, $R^4 = H$

34 $R^1 = CO_2^-NH_4^+$, $R^2 = 1$, $R^3 = HPO_3^-NH_4^+$, $R^4 = H$

35 $R^1 = OAll$, $R^2 = CO_2^-NH_4^+$, $R^3 = HPO_3^-NH_4^+$, $R^4 = H$

36 $R^1 = 1$, $R^2 = CO_2^-NH_4^+$, $R^3 = HPO_3^-NH_4^+$, $R^4 = H$

37 $R^1 = CO_2^-NH_4^+$, $R^2 = OAll$, $R^3 = H$, $R^4 = HPO_3^-NH_4^+$

38 $R^1 = CO_2^-NH_4^+$, $R^2 = 1$, $R^3 = H$, $R^4 = HPO_3^-NH_4^+$

39 $R^1 = OAll$, $R^2 = CO_2^-NH_4^+$, $R^3 = H$, $R^4 = HPO_3^-NH_4^+$

40 $R^1 = 1$, $R^2 = CO_2^-NH_4^+$, $R^3 = H$, $R^4 = HPO_3^-NH_4^+$

TABLE II

Synthetic copolymers and their inhibition values in the passive-hemolysis assay with monoclonal antibody A 20

Compound	Structure [a]	Ligand [b] (nmol/mg)	Inhibition value [c] (pmol/mL)
	[α-Kdo]$_n$-PA [d]	323	0.6
7	[α-Kdh]$_n$-PA	346	18
12	[β-Kdol]$_n$-PA	675	> 500
13	[α-Kdol]$_n$-PA	628	> 500
23	[α-(7*epi*)Kdo]$_n$-PA	179	448
28	[α-D*g*D*t*-Ko]$_n$-PA	502	> 500
32	[α-D*g*D*g*-Ko]$_n$-PA	478	> 500
34	[α-Kdo-4-P]$_n$-PA	148	71
36	[β-Kdo-4-P]$_n$-PA	37	> 500
38	[α-Kdo-5-P]$_n$-PA	79	> 500
40	[β-Kdo-5-P]$_n$-PA	135	133

[a] Abbreviations: PA, poly(acrylamide); Kdo, 3-deoxy-D-*manno*-2-octulosonic acid; Kdh, 3-deoxy-D-*lyxo*-2-heptulosonic acid; Kdol, 3-deoxy-D-*manno*-octulose; (7*epi*)Kdo, 3-deoxy-L-*gulo*-2-octulosonic acid; D*g*D*t*-Ko, D-*glycero*-D-*talo*-2-octulosonic acid; D*g*D*g*-Ko, D-*glycero*-D-*galacto*-2-octulosonic acid; P, OPO_3H^-. [b] Error bounds of assays for the hapten incorporation are ±5%. [c] Based on the effective carbohydrate ligand concentration. [d] Ref. 6.

study[6], only the heptulosonic acid derivative **7** was an effective inhibitor; however, the inhibition value was significantly higher (18 pmol/ml) compared to those compounds containing α-Kdo*p* residues. The reduced activity of **7** may, in part, be attributable to the rotamer distribution of the hydroxymethyl group, since the 7-*epi*-Kdo derivative **23** was not active. Phosphorylation at either O-4 or O-5 of Kdo abolished reactivity with this antibody. The octulosonic acid derivatives **28** and **32** having OH-3 in the axial or equatorial orientation, as well as the carboxyl-reduced derivatives **12** and **13**, were found to be inactive, too. In conclusion, the main epitope recognized by A 20 is composed of the α-pyranoside ring of Kdo and the carboxyl group, whereas the hydroxymethyl group of Kdo is not involved in the binding of this antibody.

EXPERIMENTAL

General methods.—Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. ^{1}H NMR spectra were recorded with a Bruker, AC 300F instrument with Me_4Si as the internal standard; coupling constants are first order. ^{13}C NMR spectra were recorded at 75.47 MHz for solutions in D_2O at 24°C; the instrument was operated in the FT mode with complete proton-decoupling; chemical shifts (δ) are given from the signal of Me_4Si (shift set at δ 67.40 relative to the signal of 1,4-dioxane in D_2O). TLC was performed on Merck precoated plates (5 × 10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$); spots were detected by spraying with an anisaldehyde–H_2SO_4 reagent[27]. Column chromatography was performed on Merck Lichroprep columns (size *A*, 24 × 1; *B*, 31 × 2.5; and *C*,

44 × 3.7 cm; silica gel 40–63 μm) under pressure (0.2 MPa). Elemental analyses were performed by Mag. J. Theiner, Mikoranalytisches Laboratorium am Institut für Physikalische Chemie, Universität Wien.

Serology.—Sheep erythrocytes (SRBCs) were washed three times in phosphate-buffered saline solution (PBS). Packed cells (200 μL) were suspended in PBS (5 mL) and passively coated with *O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)-(2 → 6)-*O*-{2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-β-D-glucopyranosyl}-(1 → 6)-2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-D-glucose (30 μg) at 37°C (ref. 28) for 30 min with occasional shaking. After being washed in PBS, the cells were suspended at a final concentration of 0.5% in veronal-buffered saline solution (VBS). A dilution (25 μL) of monoclonal antibody A 20 (ref. 2) in VBS containing 3–4 hemolytic units was preincubated with serial two-fold dilutions (25 μL) of inhibitor in VBS at 37°C for 15 min in 96-well microtiter plates. Sensitized SRBCs (50 μL) and guinea pig complement (25 μL, diluted 1:20 in VBS) were added, followed by incubation at 37°C for 1 h. The plates were centrifuged (500*g*, 5 min) and 50% inhibition values were read.

*Methyl (allyl 8-*O*-tert-butyldimethylsilyl-4,5-*O*-carbonyl-3-deoxy-α-*D*-manno-2-octulopyranosid)onate* (**3**).—4-Nitrophenyl chloroformate (200 mg, 1.0 mmol) was added to a solution of methyl (allyl 8-*O*-*tert*-butyldimethylsilyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate (**2**; 200 mg, 0.49 mmol) in dry pyridine (10 mL). The solution was stirred for 24 h at room temperature and the solvent evaporated. The residue was applied to a column of silica gel (*B*, 3:1 toluene–EtOAc). Elution of the main component afforded **3** as a syrup (yield, 140 mg, 65%); $[\alpha]_D^{20}$ +34° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.84 (m, 1 H, =CH–), 5.26 (dq, 1 H, $=CH_{2trans}$), 5.18 (dq, 1 H, $=CH_{2cis}$), 5.03 (br s, 2 H, H-4,5), 4.08 (m, 1 H, OCH_2), 4.01 (dddd, 1 H, H-7), 3.88 (dd, 1 H, $J_{8a,7} \sim 3.6$, $J_{8a,8b} \sim -10.4$ Hz, H-8a), 3.87 (m, 1 H, OCH_2), 3.81 (s, 3 H, CO_2CH_3), 3.80 (d, 1 H, $J_{6,7} \sim 8.0$ Hz, H-6), 3.73 (dd, 1 H, $J_{8b,7} \sim 4.5$ Hz, H-8b), 2.77 (dd, 1 H, $J_{3e,4} \sim 2.5$, $J_{3a,3e} \sim 14.6$ Hz, H-3*e*), 2.58 (d, 1 H, $J_{7,OH} \sim 6.3$ Hz, OH), 2.14 (dd, 1 H, $J_{3a,4} \sim 2.0$ Hz, H-3*a*), 0.91 [s, 9 H, $(CH_3)_3C$], 0.10 and 0.09 [s, 6 H, $(CH_3)_2Si$]. Anal. Calcd for $C_{19}H_{32}O_9Si$: C, 52.76; H, 7.45. Found: C, 52.69; H, 7.28.

*Methyl (allyl 4,5-*O*-carbonyl-3-deoxy-α-*D*-manno-2-octulopyranosid)onate* (**4**).—A solution of **3** (140 mg, 0.33 mmol) in acetonitrile (5 mL) was treated with 2% HF in acetonitrile (1 mL) for 1 h at room temperature. The pH of the solution was made neutral by addition of Dowex AG-1X8 (HCO_3^-) anion-exchange resin. The mixture was filtered and the filtrate was concentrated. Column chromatography of the residue on silica gel (*B*, EtOAc) gave 102 mg (97%) of **4** as a syrup; $[\alpha]_D^{20}$ +46° (*c* 0.7, 1:1 $CHCl_3$–MeOH); ^1H NMR ($CDCl_3$–CD_3OD): δ 5.88 (m, 1 H, =CH–), 5.28 (dq, 1 H, $=CH_{2trans}$), 5.17 (dq, 1 H, $=CH_{2cis}$), 5.13–5.06 (m, 2 H, H-4,5), 4.16 (m, 1 H, OCH_2), 3.91–3.81 (m, 4 H, OCH_2, H-6,7,8a), 3.81 (s, 3 H, CO_2CH_3), 3.65 (dd, 1 H, $J_{8b,7} \sim 3.3$, $J_{8b,8a} \sim -11.0$ Hz, H-8b), 2.83 (dd, 1 H, $J_{3e,4} \sim 2.5$, $J_{3e,3a} \sim 16.0$ Hz, H-3*e*), and 2.16 (dd, 1 H, $J_{3a,4} \sim 2.5$ Hz, H-3*a*). Anal. Calcd for $C_{13}H_{18}O_9$: C, 49.06; H, 5.70. Found: C, 48.78; H, 5.47.

*Methyl (allyl 4,5-O-carbonyl-3-deoxy-α-*D*-lyxo-2-heptulopyranosid)onate* (**5**).—A solution of **4** (30 mg) in dry MeOH (5 mL) was treated at −20°C with 5 portions of $NaIO_4$ (400 mg) during 2 h; BH_3–NH_3 (15 mg) was added and stirring was continued for 15 min at −20°C. The solvent was evaporated and the residue was purified by column chromatography on silica gel (*A*, EtOAc), which gave **5** as a syrup (yield, 24 mg, 88%); $[\alpha]_D^{20}$ +41° (*c* 1.0, MeOH); ^{1}H NMR (CD_3OD): δ 5.88 (m, 1 H, =CH–), 5.27 (dq, 1 H, $=CH_{2trans}$), 5.17 (dq, 1 H, $=CH_{2cis}$), 5.10 (dt, 1 H, $J_{4,5} \sim 8.7$, $J_{4,3a} \sim 3.6$, $J_{4,3e} \sim 3.2$ Hz, H-4), 4.92 (dd, 1 H, $J_{5,6} \sim 1.5$ Hz, H-5), 4.21 (m, 1 H, OCH_2), 3,99 (dt, 1H, $J_{6,7a} \sim J_{6,7b} \sim 6.5$ Hz, H-6), 3.85 (m, 1 H, OCH_2), 3.83 (s, 3 H, CO_2CH_3), 3.81 (m, 2 H, H-7a,7b), 2.82 (dd, 1 H, $J_{3a,3e} \sim 16.2$ Hz, H-3*e*), and 2.18 (dd, 1 H, H-3*a*). Anal. Calcd for $C_{12}H_{16}O_8$: C, 50.02; H, 5.59. Found: C, 49.74; H, 5.48.

*Sodium (allyl 3-deoxy-α-*D*-lyxo-2-heptulopyranosid)onate* (**6**).—A solution of **5** in dry MeOH (5 mL) was treated with 0.1 M methanolic NaOMe (0.5 mL) for 3 h at room temperature. The pH of the solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, and the mixture was filtered and taken to dryness. A solution of the residue in water (5 mL) was stirred with 0.2 M NaOH (8 mL) for 3 h at room temperature. The pH of the solution was adjusted to 8.5 by addition of Dowex 50 (H^+) cation-exchange resin. The resin was removed by filtration, the filtrate was lyophilized, and the residue was desalted on Bio-Gel P-2 to give **6** (16 mg, 91%), amorphous solid; $[\alpha]_D^{20}$ +37° (*c* 0.5, H_2O); ^{1}H NMR (D_2O); δ 5.94 (m, 1 H, $=CH_2$–), 5.32 (dq, 1 H, $=CH_{2trans}$), 5.20 (dq, 1 H, $=CH_{2cis}$), 4.08 (ddd, 1 H, $J_{4,5} \sim 3.0$, $J_{4,3e} \sim 5.1$, $J_{4,3a} \sim 12.7$ Hz, H-4), 3.97 (m, 1 H, OCH_2), 3.84 (dd, 1 H, $J_{7a,6} \sim 6.9$ Hz, H-7a), 3.81 (br s, 1 H, H-5), 3.84–3.78 (m, 1 H, OCH_2), 3.77 (ddd, 1 H, $J_{6,5} \sim 3.2$ Hz, H-6), 3.72 (dd, 1 H, $J_{7b,6} \sim 3.3$, $J_{7b,7a} \sim -12.3$ Hz, H-7b), 2.07 (dd, 1 H, $J_{3e,3a} \sim 13.2$ Hz, H-3*e*), and 1.75 (t, 1 H, H-3*a*). Anal. Calcd for $C_{10}H_{15}NaO_7$: C, 44.45; H, 5.59. Found: C, 44.15; H, 5.70.

*Allyl 3-deoxy-β-*D*-manno-2-octulopyranoside* (**9**).—A solution of $NaBH_4$ (0.78 g, 20.6 mmol) in dry EtOH (50 mL) was added dropwise at 0°C during 15 min to a solution of **8** (950 mg, 3.26 mmol) in EtOH (50 mL). The solution was stirred for 3 h at room temperature and made neutral by addition of Dowex 50 (H^+) cation-exchange resin. The suspension was filtered and the filtrate taken to dryness. The solid residue crystallized from EtOH to give **9** (0.534 g, 62%) as colorless needles; mp 159–160°C; $[\alpha]_D^{20}$ +51° (*c* 1.0, H_2O); ^{1}H NMR (250 MHz, D_2O): δ 5.95 (m, 1 H, –CH=), 5.34 (dq, 1 H, $=CH_{2trans}$), 5.22 (dq, 1 H, $=CH_{2cis}$), 4.20 (m, 2 H, OCH_2), 3.98 (br s, 1 H, H-5), 3.91 (ddd, 1 H, $J_{4,5} \sim 3.0$, $J_{4,3e} \sim 5.4$, $J_{4,3a} \sim 12.0$ Hz, H-4), 3.87–3.60 (m, 5 H, H-7,8a,8b, CH_2), 3.48 (dd, 1 H, $J_{6,7} \sim 8.0$, $J_{6,5} < 1.0$ Hz, H-6), 1.97 (dd, 1 H, $J_{3e,3a} \sim 12.0$ Hz, H-3*e*), and 1.91 (t, 1 H, H-3*a*). Anal. Calcd for $C_{11}H_{20}O_7$: C, 50.00; H, 7.63. Found: C, 49.59; H, 7.60.

*Allyl 3-deoxy-α-*D*-manno-2-octulopyranoside* (**11**).—A solution of **10** (67 mg) was treated with $NaBH_4$ (70 mg) for 18 h at room temperature. Workup as described for **9** gave a syrup, which was dissolved in dry pyridine (6 mL) and stirred with Ac_2O (0.2 mL) and 4-dimethylaminopyridine (2 mg) for 15 h at room temperature.

The solvent was evaporated and a solution of the residue in CH_2Cl_2 (50 mL) was washed with satd aq $NaHCO_3$, dried (Na_2SO_4), and evaporated. The residue was purified by chromatography on silica gel (*A*, 2:1 toluene–EtOAc). Evaporation of the fractions containing the main product left a syrup (35 mg), which was dissolved in dry MeOH (5 mL) and stirred with 0.1 M methanolic NaOMe (0.1 mL) for 2 h at room temperature. The solution was de-ionized by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and evaporated to give **11** (yield, 39 mg, 65%), syrup; $[\alpha]_D^{20}$ +84° (*c* 0.6, H_2O); ^1H NMR (D_2O): δ 5.95 (m, 1 H, =CH–), 5.33 (dq, 1 H, $=CH_{2trans}$), 5.21 (dq, 1 H, $=CH_{2cis}$), 4.06 (ddd, 1 H, $J_{4,5} \sim 3.0$, $J_{4,3e} \sim 5.0$, $J_{4,3a} \sim 12.0$ Hz, H-4), 4.02 (m, 3 H, H-5, OCH_2), 3.85 (m, 2 H, H-7,8a), 3.70 and 3.49 (AB-system, $J_{A,B} \sim 12.2$ Hz, CH_2), 3.59 (dd, 1 H, $J_{6,5} < 1.0$, $J_{6,7} \sim 9.0$ Hz, H-6), 3.58 (dd, 1 H, $J_{8a,8b} \sim -12.5$, $J_{8b,7} \sim 6.5$ Hz, H-8b), 1.96 (dd, 1 H, $J_{3e,3a} \sim 13.1$ Hz, H-3*e*), and 1.78 (t, 1 H, H-3*a*). Anal. Calcd for $C_{11}H_{20}O_7$: C, 50.00; H, 7.63. Found: C, 49.78; H, 7.55.

*Methyl 2,4,5,7,8-penta-*O*-acetyl-3-deoxy-β-*L*-gulo-2-octulopyranosylonate* (**16**).— Oxalacetic acid (8 g, 60 mmol) was added in portions during 30 min at 0°C to a solution of $NaHCO_3$ (500 mg) in water (400 mL), while maintaining pH 10 by simultaneous addition of 10 M NaOH. A solution of L-xylose (25 g, 166 mmol) in water (100 mL) was added and stirring was continued for 2 h at room temperature. Dowex 50 (H^+) cation-exchange resin was added at 50°C and the pH was adjusted to 5.7. $NiCl_2 \cdot 6H_2O$ (141 mg) in water (5 mL) was added, and the mixture was kept at 50°C for 90 min and filtered. The filtrate was applied to a column of Dowex AG-1X8 (HCO_3^-), eluted first with water to remove residual L-xylose, then with NH_4HCO_3 (0.1 → 0.25 M final concentration). Fractions containing TBA-reactive material were pooled and lyophilized to give **15** (4.9 g, 32%) as a syrup. A portion (1.0 g) was dissolved in pyridine (30 mL) and 4-dimethylaminopyridine (47 mg). A solution of Ac_2O (4 mL) in pyridine (4 mL) was added dropwise at −20°C during 15 min. The solution was stirred for 3 h at room temperature, MeOH (5 mL) was added, and stirring was continued for 5 min. The solution was diluted with $CHCl_3$ (100 mL) and washed with satd aq $NaHCO_3$ solution. The aqueous layer was acidified with 4 M $NaHSO_4$ and extracted with two 50-mL portions of $CHCl_3$. The organic layer was dried ($MgSO_4$) and concentrated. The residue was dissolved in dry *N*,*N*-dimethylformamide, and Cs_2CO_3 (845 mg) and MeI (0.13 mL) were added. The mixture was stirred for 15 h at room temperature, diluted with $CHCl_3$ (50 mL), and washed with water. The organic layer was dried ($MgSO_4$) and taken to dryness. Purification of the residue on a column of silica gel (*B*, 1:1 butanol–hexane) afforded **16** (540 mg, 30%) as the faster moving component, colorless syrup; $[\alpha]_D^{20}$ +59° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.40 (dd, 1 H, $J_{5,4} \sim 2.9$, $J_{5,6} \sim 1.3$ Hz, H-5), 5.28 (ddd, 1 H, H-7), 5.27 (ddd, 1 H, $J_{4,3e} \sim 4.4$, $J_{4,3a} \sim 12.5$ Hz, H-4), 4.38 (dd, 1 H, $J_{8a,7} \sim 3.4$, $J_{8a,8b} \sim 12.3$ Hz, H-8a), 4.15 (dd, 1 H, $J_{6,7} \sim 7.6$ Hz, H-6), 4.00 (dd, $J_{8b,7} \sim 6.2$ Hz, H-8b), 3.80 (s, 3 H, CO_2CH_3), 2.25 (m, 2 H, H-3*e*,3*a*), 2.19 (s, 3 H), 2.15 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), and 2.00 (s, 3 H, 5 CH_3CO). Anal. Calcd for $C_{19}H_{26}O_{13}$: C, 49.35; H, 5.67. Found: C, 49.39; H, 5.75.

*Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-β-*L*-gulo-2-octulopyranosyl bromide)onate* (**17**).—$TiBr_4$ (300 mg) was added at 0°C to a solution of **16** (120 mg, 0.26 mmol) in CH_2Cl_2 (20 mL). The solution was kept at 4°C for 18 h, diluted with $CHCl_3$ (50 mL), and washed with ice-cold aq $NaHCO_3$ solution. The organic layer was dried ($MgSO_4$) and concentrated to dryness (yield, 117 mg, 94%), slightly yellow syrup; ^{1}H NMR ($CDCl_3$): δ 5.48 (br s, 1 H, H-5), 5.48 (ddd, 1 H, H-4), 5.36 (ddd, 1 H, $J_{7,6} \sim 9.2$, $J_{7,8a} \sim 3.6$, $J_{7,8b} \sim 5.7$ Hz, H-7), 4.39 (dd, 1 H, $J_{8a,8b} \sim -12.4$ Hz, H-8a), 4.35 (dd, 1 H, $J_{6,5} \sim 1.1$ Hz, H-6), 3.97 (dd, 1 H, H-8b), 3.90 (s, 3 H, CO_2CH_3), 2.66 (ddd, 1 H, $J_{3e,4} \sim 4.6$, $J_{3e,3a} \sim 14.0$, $J_{3e,5} \sim 1.1$ Hz, H-3*e*), 2.40 (dt, 1 H, $J_{3a,4} \sim 12.7$, $J_{3a,5} \sim 1.03$ Hz, H-3*a*), 2.16 (s, 3 H), 2.10 (s, 3 H), 2.08 (s, 3 H), and 2.01 (s, 3 H, 4 CH_3CO).

*Methyl (allyl 8-*O*-tert-butyldimethylsilyl-3-deoxy-α-*L*-gulo-2-octulopyranosid)onate* (**20**) *and methyl (allyl 8-*O*-tert-butyldimethylsilyl-3-deoxy-β-*L*-gulo-2-octulopyranosid)onate* (**21**).—A solution of **17** (75 mg, 0.15 mmol) in dry nitromethane (2 mL) was added to a suspension of allyl alcohol (20 μL), $Hg(CN)_2$ (50 mg, 0.2 mmol), and 4A molecular sieves (200 mg) in nitromethane (5 mL). The mixture was stirred for 15 h at room temperature, diluted with CH_2Cl_2 (50 mL) and filtered over Celite. The filtrate was washed with 5% aq KI solution, satd aq $NaHCO_3$ solution, and dried ($MgSO_4$). The residue obtained upon removal of the solvents was purified on silica gel (*B*, 1:1 toluene–EtOAc) to give **18** and **19** (55 mg, 77%) as a syrup. The mixture was dissolved in dry MeOH (5 mL) and 0.1 M methanolic NaOMe (0.2 mL), and stirred for 3 h at room temperature. The solution was de-ionized by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and taken to dryness. The residue was dissolved in dry acetonitrile (10 mL), 1,4-diazabicyclo-[2.2.2.]octane (22 mg, 0.2 mmol) and *tert*-butylchlorodimethylsilane (23 mg) were added. The mixture was stirred for 15 h at room temperature. The residue obtained upon evaporation was purified by column chromatography on silica gel (*A*, EtOAc), which gave **20** as the faster moving isomer (yield, 9 mg, 19%); $[\alpha]_D^{20}$ +35° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$–CD_3OD): δ 5.89 (m, 1 H, =CH–), 5.26 (dq, 1 H, $=CH_{2trans}$), 5.16 (dq, 1 H, $=CH_{2cis}$), 4.32 (m, 1 H, OCH_2), 4.03 (m, 1 H, OCH_2), 3.99–3.95 (m, 1 H, H-7), 3.86 (br s, 1 H, H-5), 3.82–3.75 (m, 2 H, H-8a,8b), 3.79 (s, 3 H, CO_2CH_3), 3.73 (dd, 1 H, $J_{6,5} \sim 1.2$, $J_{6,7} \sim 4.2$ Hz, H-7), 3.64 (dd, 1 H, $J_{4,5} \sim 2.9$, $J_{4,3e} \sim 4.6$, $J_{4,3a} \sim 12.4$ Hz, H-4), 2.46 (dd, $J_{3e,3a} \sim 12.4$ Hz, H-3*e*), 2.06 (t, 1 H, H-3*a*), 0.91 [s, 9 H, $(CH_3)_3C$], and 0.11 [s, 6 H, $(CH_3)_2Si$]. Anal. Calcd for $C_{18}H_{34}O_8Si$: C, 53.18; H, 8.43. Found: C, 53.06; H, 8.59.

Further elution of the column with EtOAc gave **21** as a colorless syrup (yield, 14 mg, 29%); $[\alpha]_D^{20}$ +64° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$–CD_3OD): δ 5.86 (m, 1 H, =CH–), 5.27 (dq, 1 H, $=CH_{2trans}$), 5.16 (dq, 1 H, $=CH_{2cis}$), 3.96 (m, 1 H, OCH_2), 3.93 (ddd, 1 H, $J_{4,5} \sim 3.1$, $J_{4,3e} \sim 4.9$ Hz, H-4), 3.83 (br d, 1 H, H-5), 3.80–3.57 (m, 5 H, H-6,7,8a,8b, OCH_2), 3.79 (s, 3 H, CO_2CH_3), 2.22 (dd, 1 H, $J_{3e,3a} \sim 12.8$ Hz, H-3*e*), 1.94 (t, 1 H, $J_{3a,4} \sim 12.6$ Hz, H-3*a*), 0.90 [s, 9 H, $(CH_3)_3C$], and 0.08 [s, 6 H, $(CH_3)_2Si$]. Anal. Calcd for $C_{18}H_{34}O_8Si$: C, 53.18; H, 8.43. Found: C, 52.82; H, 8.37.

*Sodium (allyl 3-deoxy-β-*L*-gulo-2-octulopyranosid)onate* (**22**).—A solution of **21** (10 mg) in acetonitrile (2 mL) was treated with 2% HF in acetonitrile (60 μL) for 60 min at room temperature. The solution was made neutral by addition of Dowex AG-1X8 (HCO_3^-) anion-exchange resin, filtered, and concentrated. A solution of the residue in water (2 mL) was stirred with 0.1 M NaOH (4 mL) for 3 h at room temperature. The pH of the solution was adjusted to 8.2 by addition of Dowex 50 (H^+) cation-exchange resin. The resin was removed by filtration and the residue lyophilized. Purification on Bio-Gel P-2 afforded **22** (yield, 7.0 mg, 95%), amorphous solid; $[\alpha]_D^{20}$ +21° (*c* 0.7, H_2O), ^1H NMR (D_2O): δ 5.95 (m, 1 H, =CH–), 5.34 (dq, 1 H, $=CH_{2trans}$), 5.22 (dq, 1 H, $=CH_{2cis}$), 4.08 (ddd, 1 H, $J_{4,5} \sim 3.0$, $J_{4,3e} \sim 5.1$, $J_{4,3a} \sim 12.0$ Hz, H-4), 4.03 (ddd, 1 H, H-7), 4.01 (m, 1 H, OCH_2), 3.87 (br d, 1 H, H-5), 3.82 (m, 1 H, OCH_2), 3.82 (dd, 1 H, $J_{8a,7} \sim 3.2$, $J_{8a,8b} \sim 12.1$ Hz, H-8a), 3.69 (dd, 1 H, $J_{8b,7} \sim 5.7$ Hz, H-8b), 3.68 (dd, 1 H, $J_{6,5} \sim 1.1$, $J_{6,7} \sim 7.0$ Hz, H-6), 2.10 (dd, 1 H, $J_{3e,3a} \sim 12.5$ Hz, H-3*e*), and 1.79 (t, 1 H, H-3*a*). Anal. Calcd for $C_{11}H_{17}NaO_8 \cdot 0.5H_2O$: C, 42.73; H, 5.87. Found: C, 42.91; H, 5.84.

*Methyl {allyl 4,5,7,8-tetra-*O*-acetyl-2,3-*O*[(1-*exo*-allyloxy)ethylidene]-β-*D*-glycero-*D*-talo-2-octulopyranosyl}onate* (**25**).—A solution of **24** (150 mg, 0.28 mmol) in dry acetonitrile (5 mL) was added to a suspension of allyl alcohol (300 μL), $Hg(CN)_2$ (200 mg, 0.79 mmol), and 4A molecular sieves (1 g) in acetonitrile (5 mL) at room temperature. The mixture was stirred for 3 h at 40°C, diluted with EtOAc (50 mL), and filtered over Celite. The filtrate was washed with satd aq $NaHCO_3$, dried ($MgSO_4$), and concentrated. Purification of the residue on a column of silica gel (*B*, 2:1 → 1:1 toluene–EtOAc) afforded **25** as a syrup (yield, 135 mg, 75%); $[\alpha]_D^{20}$ +44° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.92 (m, 1 H, =CH–), 5.35–5.13 (m, 5 H, H-4,5,7, $=CH_{2trans}$, $=CH_{2cis}$), 4.65 (dd, 1 H, $J_{3,4} \sim 4.0$, $^4J_{3,5} \sim 1.0$ Hz, H-3), 4.47 (dd, 1 H, $J_{8a,8b} \sim -12.5$, $J_{8a,7} \sim 2.5$ Hz, H-8a), 4.30 (dd, 1 H, $J_{8b,7} \sim 4.0$ Hz, H-8b), 4.19 (dd, 1 H, $J_{6,7} \sim 9.5$, $J_{5,6} \sim 1.0$ Hz, H-6), 4.19–4.04 (m, 2 H, OCH_2), 3.88 (s, 3 H, CO_2CH_3), 2.11 (s, 3 H), 2.09 (s, 6 H), 2.01 (s, 3 H, 4 CH_3CO), and 1.81 (s, 3 H, *endo*-CH_3). Anal. Calcd for $C_{22}H_{30}O_{14}$: C, 50.96; H, 5.83. Found: C, 50.84; H, 5.74.

*Methyl (allyl 3,4,5,7,8-penta-*O*-acetyl-α-*D*-glycero-*D*-talo-2-octulopyranosid)onate* (**26**).—A suspension of **25** (120 mg, 0.23 mmol), 4A molecular sieves (500 mg), and trimethylsilyl triflate (50 μL) in CH_2Cl_2 (10 mL) was stirred for 2 h at room temperature under N_2. Triethylamine (0.1 mL) was added, and the mixture was diluted with $CHCl_3$ (50 mL) and filtered over Celite. The filtrate was washed with satd aq $NaHCO_3$, dried (Na_2SO_4), and concentrated. Purification of the residue in a column of silica gel (*B*, 1:1 toluene–EtOAc) afforded **26** as a syrup (yield, 55 mg, 46%); $[\alpha]_D^{20}$ +48° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.86 (m, 1 H, =CH–), 5.54 (dd, 1 H, $^4J_{3,5} \sim 1.0$, $J_{3,4} \sim 3.7$ Hz, H-3), 5.42 (t, 1 H, $J_{4,5} \sim 3.7$ Hz, H-4), 5.37 (ddd, 1 H, H-7), 5.36 (ddd, 1 H, H-5), 5.31 (dq, 1 H, $=CH_{2trans}$), 5.23 (dq, 1 H, $=CH_{2cis}$), 4.69 (dd, 1 H, $J_{8a,8b} \sim -12.4$, $J_{8a,7} \sim 2.4$ Hz, H-8a), 4.24 (dd, 1 H, $J_{8b,7} \sim 3.2$ Hz, H-8b), 4.22 (dd, 1 H, $J_{6,5} \sim 1.8$, $J_{6,7} \sim 9.9$ Hz, H-6), 4.11 (m, 1 H, OCH_2), 3.86 (m, 1 H, OCH_2), 3.78 (s, 3 H, CO_2CH_3), 2.07 (s, 3 H), 2.06 (s, 3 H),

2.05 (s, 3 H), 1.99 (s, 3 H), and 1.97 (s, 3 H, 5 CH_3CO). Anal. Calcd for $C_{22}H_{30}O_{14}$: C, 50.96; H, 5.83. Found: C, 50.70; H, 5.74.

*Sodium (allyl α-*D*-glycero-*D*-talo-2-octulopyranosid)onate* (**27**).—A solution of **26** (8 mg, 15 μmol) in dry MeOH (5 mL) was stirred with 0.1 M methanolic NaOMe (0.1 mL) for 12 h at room temperature. The pH of the solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin. The mixture was filtered, and the filtrate was concentrated. A solution of the residue in water (2 mL) was stirred with 0.2 M aq NaOH (2 mL) for 3 h at room temperature. Workup as described for **6** gave **27** as an amorphous powder (yield, 4.1 mg, 93%); $[\alpha]_D^{20}$ +48° (*c* 0.4, H_2O); ^1H NMR (D_2O): δ 5.94 (m, 1 H, =CH–), 5.33 (dq, 1 H, $=CH_{2trans}$), 5.22 (dq, 1 H, $=CH_{2cis}$), 4.08 (ddd, 1 H, H-5), 4.03 (ddd, 1 H, H-7), 4.03–3.94 (m, 4 H, H-3,4,8a, OCH_2), 3.81 (m, 1 H, OCH_2), 3.70 (dd, 1 H, $J_{8b,7} \sim 6.4$, $J_{8b,8a} \sim -11.7$ Hz, H-8b), and 3.65 (dd, 1 H, $J_{6,5} \sim 1.2$, $J_{6,7} \sim 8.6$ Hz, H-6).

*Methyl (allyl 3,4,5,7,8-penta-*O*-acetyl-α-*D*-glycero-*D*-galacto-2-octulopyranosid) onate* (**30**).—Trimethylsilyl triflate (50 μL) was added under N_2 to a suspension of **29** (0.5 g, 1.2 mmol), allyl alcohol (0.13 mL, 1.9 mmol), and 4A molecular sieves in CH_2Cl_2 (7 mL). The mixture was stirred for 30 min at room temperature. Pyridine (3 mL) and acetic anhydride (1.5 mL) were added at 0°C and stirring was continued for 15 h at room temperature. The suspension was diluted with CH_2Cl_2 (50 mL), filtered over Celite, and the filtrate was washed with satd aq $NaHCO_3$, and dried (Na_2SO_4). Evaporation left a syrup which was purified on a column of silica gel (*B*, 1:1 toluene–EtOAc). Pooling and evaporation of the fractions containing the main product gave a syrup (325 mg, 52%) which crystallized upon addition of hexane–EtOAc. Recrystallization afforded **30** as colorless prisms (yield, 120 mg, 19%); mp 136–139°C (dec); $[\alpha]_D^{20}$ +106° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.96 (m, 1 H, =CH–), 5.52 (dd, 1 H, $J_{5,4} \sim 2.9$, $J_{5,6} \sim 1.5$ Hz, H-5), 5.42 (m, 2 H, H-3,4), 5.37 (dq, 1 H, $=CH_{2trans}$), 5.26 (ddd, 1 H, H-7), 5.25 (dq, 1 H, $=CH_{2cis}$), 4.59 (dd, 1 H, $J_{8a,8b} \sim -12.4$, $J_{8a,7} \sim 2.3$ Hz, H-8a), 4.18 (dd, 1 H, $J_{6,7} \sim 9.9$ Hz, H-6), 4.15 (m, 1 H, OCH_2), 4.10 (dd, 1 H, $J_{8b,7} \sim 3.4$ Hz, H-8b), 4.02 (m, 1 H, OCH_2), 3.77 (s, 3 H, CO_2CH_3), 2.13 (s, 3 H), 2.07 (s, 6 H), 1.99 (s, 3 H), and 1.96 (s, 3 H, 5 CH_3CO); ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 170.4, 170.1, 169.9, 169.5, 165.7 (CO), 133.0 (=CH–), 117.3 ($=CH_2$), 98.2 (C-2), 68.1, 67.8, 67.3 (C-3,4,6,7), 66.4 (C-5), 64.9 (OCH_2), 61.8 (C-8), 53.1 (CO_2CH_3), 20.7, and 20.6 (CH_3CO). Anal. Calcd for $C_{22}H_{30}O_{14}$: C, 50.96; H, 5.83. Found: C, 51.18; H, 5.88.

*Sodium (allyl α-*D*-glycero-*D*-galacto-2-octulopyranosid)onate* (**31**).—A solution of **30** (12.2 mg) in dry MeOH (5 mL) was stirred with 0.1 M methanolic NaOMe (2 mL) for 2 h at room temperature. The solution was made neutral by addition of Dowex 50 (H^+) cation-exchange resin, filtered, and taken to dryness. A solution of the residue in water (5 mL) was treated with 0.2 M aq NaOH (2 mL) for 3 h at room temperature. Workup as described for **22** afforded **31** as an amorphous solid (yield, 6.2 mg, 84%); $[\alpha]_D^{20}$ +66° (*c* 0.4, H_2O); ^1H NMR (D_2O): δ 5.99 (m, 1 H, =CH–), 5.35 (dq, 1 H, $=CH_{2trans}$), 5.22 (dq, 1 H, $=CH_{2cis}$), 4.13 (dd, 1 H, $J_{5,6} \sim 1.0$, $J_{5,4} \sim 3.5$ Hz, H-5), 4.00 (m, 2 H, OCH_2), 3.94 (ddd, 1 H, $J_{7,6} \sim 9.9$, $J_{7,8a} \sim 2.6$,

$J_{7,8b} \sim 6.6$ Hz, H-7), 3.91 (dd, 1 H, $J_{8a,8b} \sim -12.1$ Hz, H-8a), 3.90 (dd, 1 H, $J_{4,3} \sim 10.0$ Hz, H-4), 3.77 (d, 1 H, H-3), 3.65 (dd, 1 H, H-6), and 3.63 (dd, 1 H, H-8b). Anal. Calcd for $C_{11}H_{17}NaO_9$: C, 41.78; H 5.42. Found: C, 41.49; H, 5.55.

Copolymerization.—A solution of **6** (17.0 mg), acrylamide (23 mg), and *N,N,N',N'*-tetramethylethylenediamine (2 μL) in water (1 mL) was degassed at 2 kPa for 30 min. After addition of $(NH_4)_2S_2O_8$ (0.5 mg), the mixture was kept for 20 h at 4°C. The solution was purified on a column (2.6 × 100 cm) of Sephadex G-25 with 0.01 M aq $NaHCO_3$ as eluent, at a flow rate of 55 mL/h, and 3.5-mL fractions were collected. Appropriate fractions were pooled and lyophilized. The residue was desalted on a column (2.6 × 100 cm) of Bio-Gel P-2 to give **7** (yield, 19.6 mg), amorphous powder; $[\alpha]_D^{20}$ +9° (*c* 1.0, H_2O). The copolymers **12** (17 mg of **9** and 17.5 mg of acrylamide), **13** (13.8 mg of **11** and 14.9 mg of acrylamide), **23** (13.5 mg of **22** and 14.0 mg of acrylamide), **28** (6.0 mg of **27** and 4.5 mg of acrylamide), **32** (6.2 mg of **31** and 6.1 mg of acrylamide), **34** (5.3 mg of **33** and 4.8 mg of acrylamide), **36** (5.0 mg of **35** and 4.0 mg of acrylamide), **38** (5.0 mg of **37** and 4.9 mg of acrylamide), and **40** (6.5 mg of **39** and 6.0 mg of acrylamide) were prepared in a similar manner. Yields: 11.1 mg of **12**, $[\alpha]_D^{20}$ +7° (*c* 1.0, H_2O); 14.3 mg of **13**, $[\alpha]_D^{20}$ +9° (*c* 1.0, H_2O); 14.0 mg of **23**, $[\alpha]_D^{20}$ +4° (*c* 1.0, H_2O); 6.2 mg of **28**, $[\alpha]_D^{20}$ +5° (*c* 0.5, H_2O); 5.8 mg of **32**, $[\alpha]_D^{20}$ +5° (*c* 0.5, H_2O); 4.1 mg of **34**, $[\alpha]_D^{20}$ +7° (*c* 0.4, H_2O); 4.6 mg of **36**, $[\alpha]_D^{20}$ +8° (*c* 0.4, H_2O); 4.5 mg of **38**, $[\alpha]_D^{20}$ +5° (*c* 0.4, H_2O); and 6.5 mg of **40**, $[\alpha]_D^{20}$ +8° (*c* 0.5, H_2O), respectively.

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