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Synthesis of spacer-containing chlamydial disaccharides as analogues of the α-Kdo*p*-(2→8)-α-Kdo*p*-(2→4)-α-Kdo*p* trisaccharide epitope

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Dedicated to the memory of Professor Nikolay K. Kochetkov

Abstract—On the basis of high-resolution crystal structures of the antigen binding fragment of the chlamydia-specific monoclonal antibody S25-2 in complex with the trisaccharide α-Kdo*p*-(2→8)-α-Kdo*p*-(2→4)-α-Kdo*p* and part structures thereof, seven modified α-Kdo*p*-(2→8)-α-Kdo*p* disaccharide derivatives were synthesized starting from the protected disaccharide allyl ketoside **1**. Hydroboration and subsequent oxidation as well as ozonolysis, respectively, followed by Wittig-reaction for chain elongation were used to install a terminal carboxylic group on spacer entities of various chain lengths. Furthermore, addition of methyl 2-thioacetate to the allyl group furnished the corresponding thioether derivative. Standard deprotection gave the target disaccharides as simplified trisaccharide analogues, which will be used to probe the contribution of the proximal carboxylic group in the binding of chlamydia-specific di- and trisaccharide-reactive monoclonal antibodies.

Keywords: Kdo; Lipopolysaccharide; Chlamydia; Antibody; Spacer

1. Introduction

Chlamydiae are obligate intracellular bacterial parasites, which are responsible for a broad variety of acute and chronic diseases in animals and humans.[1] In addition to acute genital and ocular infections from *Chlamydia trachomatis*, chronic diseases such as atherosclerosis, arthritis, asthma, and neurodegenerative diseases have been associated in the context of chlamydial infections.[2] In the outer membrane of the cell wall, Chlamydiae contain a highly truncated glycolipid, which is composed of Lipid A and 3-deoxy-D-*manno*-octulosonic acid (Kdo) residues only.[3,4] All *Chlamydiaceae* share a common Kdo epitope—formerly called the genus-specific epitope—attached to the Lipid A anchor, which thus resembles the deep rough mutant LPS structures of *Enterobacteriaceae*.[5] In contrast to R*e* mutants, the Kdo region of *Chlamydia*, however, constitutes an immunodominant epitope comprising the sequence α-Kdo-(2→8)-α-Kdo-(2→4)-α-Kdo-(2→6)-Lipid A, wherein the Kdo (2→8)-linkage confers *Chlamydia*-specificity.[6] Additional, species-specific Kdo oligosaccharides such as the linear trisaccharide α-Kdo-(2→4)-α-Kdo-(2→4)-α-Kdo and the branched tetrasaccharide α-Kdo-(2→4)-[α-Kdo-(2→8)]-α-Kdo-(2→4)-α-Kdo are present in the LPS of *Chlamydophila psittaci* and have been isolated from recombinant strains expressing the respective Kdo transferase.[7] Synthetic neoglycoconjugates covering these structural variations have been prepared and used in immunization protocols to generate murine monoclonal antibodies and to characterize their binding epitopes in EIA and EIA-inhibition assays.[8] MAb S25-23—a high affinity antibody with a K_D of 350 nM—requires the complete trisaccharide sequence for binding, whereas mAb S25-2 has a more relaxed binding specificity and binds also the α-(2→8)-linked disaccharide part with reduced affinity.[9] For further definition of the

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epitope specificities of the antibodies, di- and trisaccharide analogues have been prepared containing single carboxyl-reduced Kdo units in order to evaluate the contribution of the acidic substituents to the binding. Whereas reduction of the terminal and proximal carboxylate of the trisaccharide abolished the reactivity completely, mAb S25-23 and S25-2 tolerate carboxyl reduction of the internal Kdo-residue.[10] Recently, crystal structures of two monoclonal antibodies (S25-2 and S45-18) complexed to Kdo ligands have been determined at atomic resolution.[11] The crystal structures of these antibody complexes revealed a conserved and similar binding mode for the distal Kdo unit and the presence of several salt bridges and hydrogen bonds to the carboxylic groups of the Kdo subunits. Moreover, mAb S25-2 displays an induced-fit type of binding, which is mediated by a flexible arginine L30c residue. In particular, this amino acid interacts with the proximal Kdo unit of the trisaccharide, but moves downward to provide a salt bridge with the carboxylic group of the cross-reactive α-Kdo-(2→4)-α-Kdo disaccharide. In order to further evaluate the contribution of this interaction for binding of Kdo analogues, we have set out to prepare spacer-modified α-Kdo-(2→8)-α-Kdo disaccharide derivatives. By replacing the proximal Kdo moiety of the trisaccharide by a carboxylic spacer group, the analogues are designed as simplified trisaccharide mimetics and will be used for ongoing detailed immunochemical and crystallographic studies.

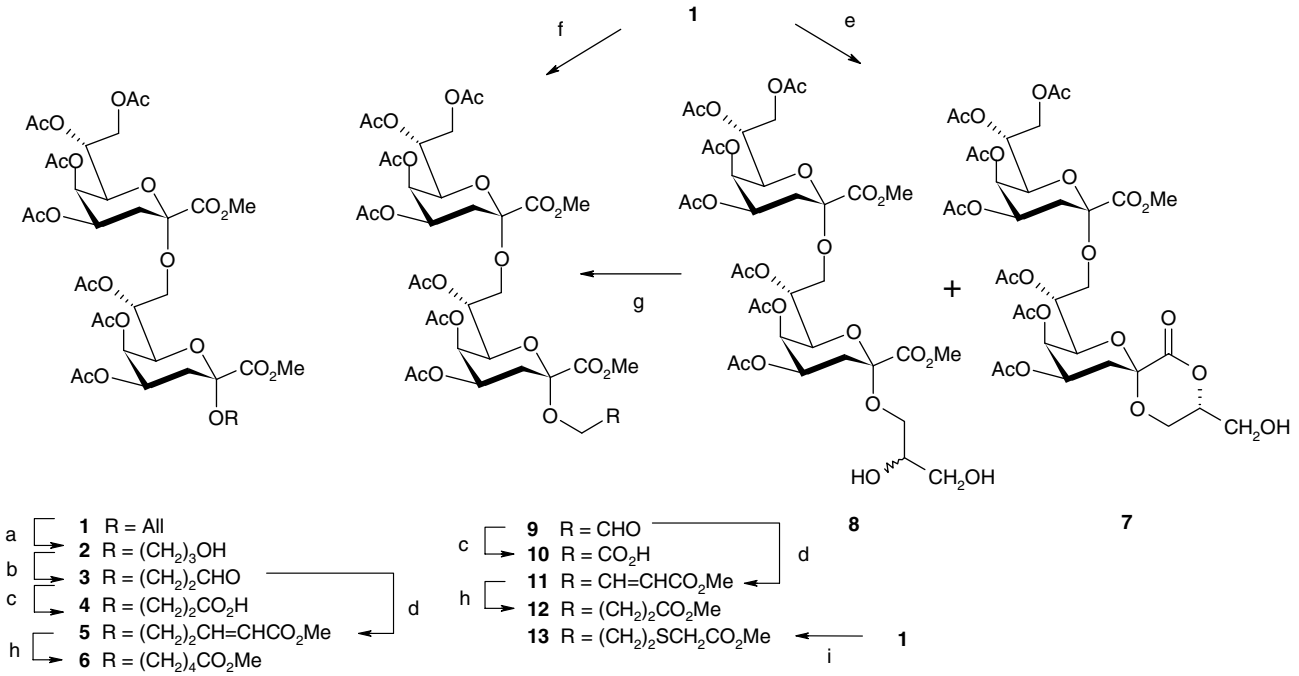
2. Results and discussion

The previously described O-peracetylated α-Kdo-(2 → 8)-α-Kdo allyl disaccharide derivative **1** was used as educt for all homologation and chain cleavage reactions.[12] Hydroboration of the double bond with dimethylsulfide–borane in THF was applied under carefully controlled conditions to avoid hydrolysis of the *O*-acetyl and methyl ester groups.[13] Thus treatment of **1** with the borane reagent at −5 °C for 12 h followed by mild oxidation with H_2O_2 in a phosphate buffer soln at pH 7.5 furnished the propane-1,3-diol glycoside **2** in 53% yield. Whereas many options are available for the oxidation of primary alcohols such as Swern-type,[14] Dess–Martin periodinane,[15] TPAP,[16] SO_3·pyridine complex[17] as well as manganese- or chromium-based protocols, oxidation of the acid-sensitive Kdo moieties requires mild reagents and reaction conditions. Hence, subsequent oxidation was performed with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) in the presence of [bis(acetoxy)iodo]benzene (BAIB) to afford the aldehyde derivative **3** in 80% yield.[18] The formation of the aldehyde was derived from the downfield ^{13}C NMR signal at 200.01 ppm, indicating the presence of the non-hydrated form of the carbonyl function in compound **3**. Further oxidation of **3** with sodium chlorite in a buffered acetonitrile–water solvent system finally gave the 3-substituted propanoic acid derivative **4** in ∼100% yield (Scheme 1). The aldehyde derivative **3** was subjected to a classical Wittig olefination sequence by reaction with methyl(triphenylphosphoranylidene)acetate, which yielded the *E*-configured methyl pent-2-enoate derivative **5** in 89% yield. The *E*-configuration of the alkene was assigned on the basis of the values of the coupling constant $J_{2,3}$ indicating a trans arrangement of the respective protons ($J_{2,3}$ 15.6 Hz). Catalytic hydrogenation of **5** in the presence of 10% Pd/C at atmospheric pressure finally afforded the 5-substituted pentanoic acid glycoside derivative **6** in ∼ quantitative yield.

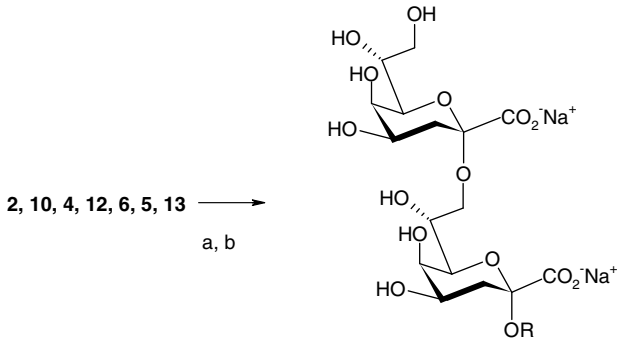
For the synthesis of the second series of analogues, catalytic osmylation of the double bond of **1** in the presence of *N*-methylmorpholine *N*-oxide (NMO) was carried out to afford a ∼1:1 mixture of the diastereoisomeric glycerol derivative **8** (69%) and the lactone **7** in 19% yield. Upon separation of the isomers on silica gel or upon standing in soln, one of the isomers of **8** was prone to intramolecular transesterification to give the corresponding lactone **7**. The formation of the lactone entity was derived from the absence of one methyl ester group, the downfield shift of C-2 of the glycerol aglycon at 80.09 ppm, the upfield shift of the lactone carbonyl signal to 163.76 ppm and the correlation of the lactone carbonyl group to the axial H-3 proton of the proximal Kdo unit in the HMBC measurement. In addition, similar to other Kdo lactones, proton H-3a experienced a distinct downfield shift, whereas the equatorial proton H-3e was shifted upfield. Since NOE experiments were inconclusive, assignments of the new stereogenic center at C-2 is only tentative. Molecular modeling supports the preferred formation of a lactone unit with an equatorially oriented hydroxymethyl substituent, which would thus indicate the *R*-configuration at C-2 of the aglycon.

Sodium periodate promoted cleavage of the diastereoisomeric glycerol-derivative **8** furnished the glycol aldehyde derivative **9** in 61% yield. Better results were obtained by direct ozonolysis of the allyl group of **1** to provide the aldehyde **9** in 89% yield.[19] Subsequent oxidation of **9** as described for **3** afforded the glycolic acid derivative **10** in 96% yield. Similarly to the synthesis of **5**, chain elongation of the aldehyde with the stabilized phosphorane proceeded smoothly to give the α,β-unsaturated ester derivative **11** in 73% yield and an *E*/*Z* ratio of 95:5. Catalytic hydrogenolysis furnished the protected 4-substituted butanoic acid derivative **12** in 85% yield. Finally, in order to place the carboxylic group at a longer distance from the anomeric center, radical thiol addition of methyl 2-mercaptoacetate to the allyl group of educt **1** gave the thioether derivative **13** in fair yield.[20]

Deprotection of compounds **2**, **4**, **5**, **6**, **10**, **12**, and **13** was accomplished via Zemplén O-deacetylation and



Scheme 1. Reagents and conditions: (a) $BH_3{\cdot}Me_2S$ (2 M in THF), 15 h, −5 °C, THF, then H_2O_2 (pH 7.5), 4 h, rt, 53% for **2**; (b) TEMPO, BAIB, CH_2Cl_2, 2 h, rt, 89% for **3**; (c) H_2O_2 (pH 7.5), $NaClO_2$, MeCN, 0 °C, 5 h, 96% for **4**, ~100% for **10**; (d) $Ph_3P{=}CHCO_2Me$, THF, 20 h, rt, 89% for **5**, 73% for **11**; (e) OsO_4, NMO, 2:1:1 dioxane–water–Me_2CO, 5 h, rt, 69% for **8**, 19% for **7**; (f) O_3, 5:1 CH_2Cl_2–MeOH, −6 °C, 20 min, then Me_2S, 10 h, 4 °C, 89% for **9**, (g) $NaIO_4$, 1:1 dioxane–water, 4 h, rt, 61% for **9**, (h) 10% Pd/C, H_2, MeOH, 15 h, rt; 99% for **6**, 85% for **12**, (i) $HSCH_2CO_2Me$, AIBN, dioxane, 75 °C, 5 h, 48% for **13**.



Scheme 2. Reagents and conditions: (a) 1 M NaOMe, MeOH, 1 h, rt; (b) 0.1 M NaOH, 1 h, rt, then BioGel P-2, 99% for **2**, 91% for **15**, 84% for **16**, 85% for **17**, 88% for **18**, 89% for **19**, 88% for **20**.

subsequent alkaline hydrolysis of the methyl ester groups with 0.1 M NaOH (Scheme 2). Purification and desalting on a BioGel P-2 column gave the target disaccharide derivatives **14**–**20** as di- and trisodium salts in high yields.

The ^{13}C NMR data (Table 1) were in full agreement with previous structural assignments of the allyl disaccharide **1**.[21] Immunochemical results obtained with the disaccharide derivatives will be reported elsewhere.

3. Experimental

3.1. General

All solvents were purified and dried by standard procedures. Column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck). Desalting was accomplished on a BioGel P-2 column (water). Analytical TLC was performed using Silica Gel 60 F_{254} HPTLC plates with 2.5 cm concentration zone (E. Merck). Spots were detected by treatment with anisaldehyde–H_2SO_4. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. NMR spectra were recorded at 297 K in D_2O and $CDCl_3$ with a Bruker DPX 300 spectrometer (^1H at 300.13 MHz, and ^{13}C at 75.47 MHz) using standard Bruker NMR software. ^1H NMR spectra were referenced to tetramethylsilane or 2,2-dimethyl-2-silapentane-5-sulfonic acid. ^{13}C NMR spectra were referenced to chloroform for solutions in $CDCl_3$ (δ 77.00) or 1,4-dioxane (δ 67.40) for solutions in D_2O. ESIMS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument.

Table 1. ^{13}C NMR data[a] (ppm) of Kdo disaccharide derivatives **14**–**20**

Atom	Compound number						
	14	**15**	**16**	**17**	**18**	**19**	**20**
α-Kdo-(2→8							
1	176.54[b]	176.70[b]	176.49[b]	16.68[b]	176.68[b]	176.23[b]	176.58[b]
2	101.29	101.40	101.13	101.40	101.43	101.49	101.36
3	34.96[c]	34.67[c]	34.78[c]	35.00[c]	35.00[c]	34.90[c]	34.96[c]
4	66.85[d]	66.51[d]	66.63	66.81	66.83	66.79[d]	66.84
5	67.17[e]	67.04[e]	67.09[d]	67.25[d]	67.21[d]	67.00[e]	67.20[d]
6	72.37	72.18	72.15[e]	72.43[e]	72.49[e]	72.48[f]	72.43[e]
7	70.07	70.02	70.00	70.10	70.12	70.13	70.14
8	63.83	63.59	63.64	63.76	63.85	63.81	63.91
→8)-α-Kdo-(2 →							
1	176.38[b]	175.77[b]	176.07[b]	176.39[b]	176.38[b]	176.23[b]	176.28[b]
2	100.95	100.56	100.64	100.78	100.79	100.95	100.83
3	34.84[c]	34.60[c]	34.65[c]	34.81	34.79[c]	34.74[c]	34.83[c]
4	66.83[d]	66.41[d]	66.63	66.81	66.83	66.86[d]	66.84
5	67.07[e]	66.93[e]	66.92[d]	67.10[d]	67.06[d]	67.18[e]	67.09[d]
6	72.23	71.68	72.02[e]	72.13[e]	72.06[e]	72.16[f]	72.27[e]
7	68.78	68.42	68.76	68.78	68.70	68.61	68.79
8	65.83	65.31	65.60	65.67	65.66	65.62	65.80
Aglycon							
1	59.89	177.32	179.34	183.67	184.33	176.76[b]	178.82
2	32.14	63.04	37.17	35.45	38.37	129.20	37.12
3	61.19		60.45	26.84	23.87	141.97	—
4				63.76	29.74	32.33	29.62
5					63.93	62.60	29.39
6							62.51

[a] ^{13}C NMR data are based on HMQC and HMBC-assignments.
[b–f] Assignments within a column may be reversed.

3.2. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl (3-hydroxyprop-1-yl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (2)

A soln of **1** (175 mg, 0.21 mmol) in dry THF (10 mL) was stirred for 15 h with a 2 M soln of BH_3–SMe_2 in THF (0.35 mL, 0.7 mmol) at −5 °C. One molar of aq sodium phosphate buffer soln (3 mL, pH 7.5) and 50% aq H_2O_2 (0.9 mL) soln were added and stirring was continued for 4 h at room temperature. The reaction mixture was concentrated and CH_2Cl_2 (50 mL) was added. The organic layer was washed with water and dried ($MgSO_4$). The residue obtained upon evaporation was purified by column chromatography on silica gel (1:1 toluene–EtOAc) to give **2** (93.6 mg, 53%) as colorless crystals, mp 195–196 °C (*n*-hexane–EtOAc), $[\alpha]_D^{20}$ +94 (*c* 0.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.30 (br s, 1H, H-5), 5.25 (m, 2H, H-4, 5′), 5.20 (ddd, 1H, $J_{8'a,7'}$ 2.5, $J_{8'b,7'}$ 4.8 Hz, H-7′), 5.07 (ddd, 1H, $J_{8a,7}$ 2.3, $J_{8b,7}$ 5.7 Hz, H-7), 5.07 (ddd, 1H, $J_{3'e,4'}$ 5.1, $J_{5',4'}$ 3.0, $J_{4',3'a}$ 12.4 Hz, H-4′), 4.43 (dd, 1H, $J_{8'a,8'b}$ 12.2 Hz, H-8′a), 4.10 (dd, 1H, H-8′b), 4.07 (dd, 1H, $J_{6,5}$ <1.0, $J_{6,7}$ 9.1 Hz, H-6), 3.93 (dd, 1H, $J_{6',5'}$ 1.1, $J_{6',7'}$ 9.6 Hz, H-6′), 3.75 (dd, 1H, $J_{8a,8b}$ 11.8 Hz, H-8a), 3.73 and 3.73 (s, each 3H, 2 × OMe), 3.68 (t, 2H, OCH_2), 3.59 (dd, 1H, H-8b), 3.60 and 3.38 (dt, 2H, CH_2OH), 2.13 (dd, 1H, $J_{3'a,3'e}$ 13.0 Hz, H-3′e), 2.05 (dd, 1H, $J_{3a,3e}$ 12.0, $J_{3e,4}$ 5.0 Hz, H-3e), 2.02–2.00 (m, 2H, H-3a, 3′a), 2.03 (s, 3H), 2.01 (s, 6H), 2.00 (s, 3H), 1.95 (s, 3H), 1.90 (s, 3H) and 1.89 (s, 3H, 7Ac); ^{13}C NMR ($CDCl_3$): δ 171.01, 170.49, 170.39, 170.08, 169.91, 169.69, and 169.63 (C=O), 168.00 and 167.20 (CO_2Me), 98.89 and 98.85 (C-2, 2′), 68.79 (2C, C-6, 6′), 68.35 (C-7), 67.41 (C-7′), 66.41 and 66.37 (C-4, 4′), 64.42 (C-5), 64.03 (C-5′), 62.82 and 62.77 (C-8, 8′), 60.75 (OCH_2), 58.89 (CH_2OH), 52.76 and 52.71 (OMe), 31.98 (C-3′, CH_2), 31.56 (C-3), 20.78, 20.76, 20.71 and 20.64 (7C, Ac); Anal. Calcd for $C_{35}H_{50}O_{23}$: C, 50.12; H, 6.01. Found: C, 49.98; H, 6.05.

3.3. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl (3-oxoprop-1-yl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (3)

To a cooled and stirred soln of **2** (90 mg, 107 μmol) in dry CH_2Cl_2 (2 mL), TEMPO (2.1 mg, 13 μmol) and [bis(acetoxy)iodo]benzene (70 mg, 0.2 mmol) were added. The ice-bath was removed and after 2 h, aq 10% $Na_2S_2O_3$ soln (2 mL) was added. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and the organic layer was washed with water, satd cold aq $NaHCO_3$, and brine. After drying ($MgSO_4$) and concentration,

the residue was purified on a column of silica gel (1:1 toluene–EtOAc) to give **3** (80 mg, 89%) as a colorless syrup. $[\alpha]_D^{20}$ +81 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 9.77 (s, 1H, CHO), 5.36 (br s, 1H, H-5), 5.30 (br s, 1H, H-5′), 5.24 (ddd, 1H, $J_{3e,4}$ 5.2, $J_{5,4}$ 3.0 Hz, H-4), 5.22 (ddd, 1H, $J_{8'a,7'}$ 2.6, $J_{8'b,7'}$ 4.4 Hz, H-7′), 5.17 (ddd, 1H, $J_{8a,7}$ 2.6, $J_{8b,7}$ 5.0, Hz, H-7), 5.12 (ddd, 1H, $J_{3'e,4'}$ 5.1, $J_{5',4'}$ 3.1, $J_{4',3'a}$ 12.2 Hz, H-4′), 4.56 (dd, 1H, $J_{8'a,8'b}$ 12.3 Hz, H-8′a), 4.26 (dd, 1H, $J_{6,5}$ 1.3, $J_{6,7}$ 9.7 Hz, H-6), 4.14 (dd, 1H, H-8′b), 4.045 (dd, 1H, $J_{6',5'}$ 1.3, $J_{6',7'}$ 9.6 Hz, H-6′), 3.88 and 3.65 (dt, 2H, OCH_2), 3.87 (dd, 1H, $J_{8a,8b}$ 12.6 Hz, H-8a), 3.81 and 3.80 (2s, each 3H, 2 × OMe), 3.79 (dd, 1H, H-8b), 2.92–2.72 (m, 2H, CH_2), 2.215 (dd, 1H, $J_{3'a,3'e}$ 13.4 Hz, H-3′e), 2.14 (dd, 1H, $J_{3a,3e}$ 13.0 Hz, H-3e), 2.13–2.03 (m, 2H, H-3a, 3′a), 2.09 (s, 3H), 2.08 (s, 6H), 2.07, 2.02, 1.96 and 1.95 (5s, each 3H, 7Ac); ^{13}C NMR ($CDCl_3$): δ 200.01 (CHO), 171.56, 170.40, 170.35, 169.84, 169.79, and 169.671 (C=O), 167.48 and 167.28 (CO_2Me), 98.99 and 98.92 (C-2, 2′), 68.85, 68.69, and 68.43 (C-6, C-6′, C-7), 67.59 (C-7′), 66.29 and 66.22 (C-4′, C-4), 64.46 (C-5), 64.14 (C-5′), 62.53 (C-8), 62.19 (C-8′), 57.64 (OCH_2), 52.71 (OMe), 43.03 (CH_2), 31.84 and 31.55 (C-3, 3′), 20.75 and 20.67 (7C, Ac); ESIMS: *m*/*z* calcd for $[C_{35}H_{48}O_{23}+Na]^+$: 859.25. Found: 859.28.

3.4. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl (2-carboxyethyl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (4)

To a stirred soln of **3** (20.0 mg, 24 μmol) in CH_3CN (1.5 mL), an aqueous soln (1.5 mL) containing H_2O_2 (50%, 2.4 μL, ~29 μmol) and $NaH_2PO_4{\cdot}H_2O$ (5.3 mg, 38 μmol) was added. The reaction mixture was cooled to 0 °C and $NaClO_2$ (80% purity, 4.3 mg, ~48 μmol) in water (1.1 mL) was added. After stirring at room temperature overnight, a 0.15 M soln of Na_2SO_3 (0.1 mL) was added and the soln was stirred for 30 min. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with cold aq 1 M $NaHSO_4$. The organic layer was washed with water, dried ($MgSO_4$), and concentrated. Purification of the residue on a column of silica gel (EtOAc) afforded **4** as a syrup (21 mg, 100%). $[\alpha]_D^{20}$ +65 (*c* 0.9, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 5.36 (m, 1H, H-7), 5.33 (m, 3H, H-5, 5′, 7′), 5.22 (m, 2H, H-4, 4′), 4.49 (dd, 1H, $J_{8'a,7'}$ 2.5, $J_{8'a,8'b}$ 12.5 Hz, H-8′a), 4.39 (dd, 1H, $J_{6,5}$ <1.0, $J_{6,7}$ 9.8 Hz, H-6), 4.24 (dd, 1H, $J_{8'b,7'}$ 5.2 Hz, H-8′b), 4.14 (dd, 1H, $J_{6',5'}$ <1.0, $J_{6',7'}$ 9.9 Hz, H-6′), 3.91–3.82 (m, 2H, H-8a, 8b), 3.81 and 3.80 (s, each 3H, 2 × OMe), 3.87 and 3.42 (m, 2H, OCH_2), 2.69 and 2.54 (m, 2H, CH_2), 2.20–2.04 (m, 4H, H-3e, 3a, 3e′, 3′a), 2.12 (s, 3H), 2.11 (s, 3H), 2.07 (s, 6H), 2.02 (s, 3H), and 1.96 (s, 6H, 7Ac); ESIMS: *m*/*z* calcd for $[C_{35}H_{48}O_{24}+Na]^+$: 875.24. Found: 875.22.

3.5. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl ((4-methoxycarbonyl)-3-(*E*)-buten-1-yl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (5)

Methyl (triphenylphosphoranylidene)acetate (34.0 mg, 101.6 μmol) was added to a soln of **3** (65.4 mg, 78.16 μmol) in dry THF (6 mL) and the soln was stirred for 22 h at room temperature. After concentration of the soln, the reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with water, dried ($MgSO_4$), and evaporated. The residue was purified on a column of silica gel (1:1 toluene–EtOAc) to give **5** (61.8 mg, 89%) as a colorless syrup. $[\alpha]_D^{20}$ +59 (*c* 2.1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.95 (dt, 1H, J_{trans} 15.6, $J_{CH=CH_2}$ 6.8 Hz, CH=), 5.93 (dt, 1H, CH=), 5.35 (br s, 1H, H-5), 5.32 (br s, 1H, H-5′), 5.29 (ddd, 1H, $J_{3e,4}$ 4.9, $J_{4,5}$ 2.8, $J_{3a,4}$ 11.9 Hz, H-4), 5.21 (ddd, 1H, $J_{7',8'a}$ 2.4, $J_{7',8'b}$ 3.9 Hz, H-7′), 5.16 (m, 1H, H-7), 5.13 (ddd, 1H, $J_{3'e,4'}$ 5.0, $J_{4',5'}$ 2.9, $J_{3'a,4'}$ 12.1 Hz, H-4′), 4.54 (dd, 1H, $J_{8'a,8'b}$ 12.3 Hz, H-8′a), 4.19 (dd, 1H, H-8′b), 4.08 (dd, 1H, $J_{6,5}$ 1.2, $J_{6,7}$ 9.6 Hz, H-6), 4.02 (dd, 1H, $J_{6',5'}$ 1.2, $J_{6',7'}$ 9.6 Hz, H-6′), 3.86 (dd, 1H, $J_{8a,7}$ 2.5, $J_{8a,8b}$ 11.5 Hz, H-8a), 3.80 (s, 6H) and 3.74 (s, 3H, 3 × OMe), 3.72 (dd, 1H, H-8b), 3.68 and 3.52 (dt, 2H, OCH_2), 2.52 (m, 2H, CH_2), 2.20 (dd, 1H, $J_{3'e,3'a}$ 12.8 Hz, H-3′e), 2.18 (dd, 1H, $J_{3e,3a}$ 12.8 Hz, H-3e), 2.12–2.04 (m, 2H, H-3a, 3′a), 2.10 (s, 3H), 2.08 (s, 3H), 2.06 (s, 6H), 2.01 (s, 3H), and 1.96 (s, each 6H, 7 × Ac); ^{13}C NMR ($CDCl_3$): δ 170.39, 170.35, 170.24, 169.81, 169.77, 169.61, and 169.58 (C=O), 167.41, 167.12, and 166.60 (CO_2Me), 145.20 and 122.90 (CH=), 98.85 and 98.79 (C-2, 2′), 68.74 (2C, C-6, 6′), 68.37 (C-7), 67.59 (C-7′), 66.28 and 66.15 (2C, C-4, 4′), 64.35 (C-5), 64.04 (C-5′), 62.47 (C-8), 62.27 (OCH_2), 61.89 (C-8′), 52.68, 52.62, and 51.40 (3C, OMe), 32.02 (CH_2), 31.73 and 31.44 (C-3, 3′), 20.71, 20.68, 20.61, and 20.55 (7C, Ac); ESIMS: *m*/*z* calcd for $[C_{38}H_{52}O_{24}+Na]^+$: 915.27. Found: 915.30.

3.6. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl ((4-methoxycarbonyl)-but-1-yl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (6)

A soln of **5** (35.2 mg, 39.4 μmol) in dry MeOH (10 mL) was stirred at room temperature in the presence of 10% Pd/C (50 mg) for 15 h under H_2 at atmospheric pressure. After completion of the reaction, the catalyst was removed by filtration through Celite and washed with MeOH. The crude product obtained after filtration and concentration was purified on a column of silica gel (1:2 toluene–EtOAc) to give **6** (35.1 mg, 99%) as a colorless syrup. $[\alpha]_D^{20}$ +66 (*c* 1.2, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 5.35 (br s, 1H, H-5), 5.32 (br s, 1H, H-5′), 5.30 (ddd, 1H, $J_{3e,4}$ 5.3, $J_{4,5}$ 2.8 Hz, H-4), 5.20 (ddd,

1H, $J_{7',8'a}$ 2.5, $J_{7',8'b}$ 4.1, $J_{7',6'}$ 9.4 Hz, H-7′), 5.17 (m, 1H, H-7), 5.13 (ddd, 1H, $J_{3'e,4'}$ 5.3, $J_{4',5'}$ 3.2, $J_{3'a,4'}$ 12.0 Hz, H-4′), 4.57 (dd, 1H, $J_{8'a,8'b}$ 12.5 Hz, H-8′a), 4.16 (dd, 1H, H-8′b), 4.11 (dd, 1H, $J_{6,5}$ 1.0, $J_{6,7}$ 9.6 Hz, H-6), 4.03 (dd, 1H, $J_{6',5'}$ 1.2 Hz, H-6′), 3.86 (dd, 1H, $J_{8a,7}$ 2.6, $J_{8a,8b}$ 11.5 Hz, H-8a), 3.80 (s, 6H) and 3.67 (s, 3H, 3 × OMe), 3.70 (dd, 1H, $J_{8b,7}$ 5.5 Hz, H-8b), 3.51 and 3.36 (dt, 2H, OCH_2), 2.35 (m, 2H, CH_2CO), 2.20 (dd, 1H, $J_{3'e,3'a}$ 13.0 Hz, H-3′e), 2.18 (dd, 1H, H-3e), 2.12–2.04 (m, 2H, H-3a, 3′a), 2.10 (s, 3H), 2.08 (s, 6H), 2.06 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H) and 1.96 (s, 3H, 7 × Ac), 1.76–1.59 (m, 4H, 2 × CH_2); ^{13}C NMR ($CDCl_3$): δ 173.76 (CH_2CO_2Me), 170.44, 170.39, 170.31, 169.90, 169.73, 169.63, and 169.58 (C═O), 167.70 and 167.16 (CO_2Me), 98.78 (2C, C-2, 2′), 68.65 (2C, C-6, 6′), 68.48 (C-7), 67.62 (C-7′), 66.41 and 66.18 (C-4, 4′), 64.43 (C-5), 64.09 (C-5′), 63.66 (OCH_2), 62.59 (C-8), 61.86 (C-8′), 52.69, 52.57, and 51.44 (3C, OMe), 33.55 ($COCH_2$), 31.93 and 31.51 (C-3, 3′), 28.67 and 21.64 (CH_2), 20.72, 20.61 and 20.58 (7C, Ac); ESIMS: *m*/*z* calcd for $[C_{38}H_{54}O_{24}+Na]^+$: 917.29. Found: 917.28.

3.7. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-[2,3-dihydroxyprop-1-yl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid]onic acid 1→2 lactone (7) and methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl [2,3-dihydroxyprop-1-yl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid]onate (8)

A 2% aq soln of OsO_4 (0.5 mL, 40 μmol) was added to a stirred soln of **1** (261 mg, 0.32 mmol) and NMO-monohydrate (86 mg, 0.64 mmol) in 2:1:2 dioxane–water–acetone (2.5 mL) at room temperature. The soln was stirred for 5 h, then diluted with $CHCl_3$ (15 mL). Acetic acid (5 M, 0.7 mL) was added, the organic phase was extracted with aq $Na_2S_2O_5$, water, satd $NaHCO_3$, and water until the pH was neutral. The organic phase was dried ($MgSO_4$), concentrated, and the residue was chromatographed on silica gel to give **7** as the faster migrating compound (50 mg, 19%), which was further chromatographed using 1:2 toluene–EtOAc. $[\alpha]_D^{20}$ +85 (*c* 0.2, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.40 (br s, 1H, H-5), 5.33 (br s, 1H, H-5′), 5.30 (ddd, 1H, $J_{3e,4}$ 4.9, $J_{5,4}$ 3.1 Hz, H-4), 5.26 (ddd, 1H, $J_{3'e,4'}$ 5.1, $J_{5',4'}$ 3.0, $J_{4',3'a}$ 12.2 Hz, H-4′), 5.19 (ddd, 1H, $J_{8'a,7'}$ 2.4, $J_{8'b,7'}$ 3.7 Hz, H-7′), 4.94 (dt, 1H, $J_{8a,7} \sim J_{8b,7}$ 1.9, Hz, H-7), 4.74 (ddd, 1H, CHOH), 4.58 (dd, 1H, $J_{8'a,8'b}$ 12.5 Hz, H-8′a), 4.26 (dd, 1H, $J_{6,5}$ 1.4, $J_{6,7}$ 10.1 Hz, H-6), 4.26 (t, 1H, *J* 12.0 Hz, OCH_a), 4.15–4.08 (m, 3H, H-6′, 8′b, OCH_b), 3.93 and 3.84 (dd, 2H, CH_2OH), 3.80 (m, 2H, H-8a, 8b), 3.77 (s, 3H, OMe), 2.68 (t, 1H, $J_{3a,3e} \sim J_{3a,4}$ 13.4 Hz, H-3a), 2.25 (dd, 1H, $J_{3'a,3'e}$ 12.6 Hz, H-3′e), 2.11 (t, 1H, H-3′a), 2.11 (s, 3H), 2.09 (s, 3H), 2.04 (s, 6H), 2.01 (s, 3H), and 1.99 (s, 6H, 7Ac), 1.93 (dd, 1H, H-3e); ^{13}C NMR ($CDCl_3$): δ 170.69, 170.56, 170.45, 170.43, 169.93, 169.88, and 169.57 (MeC═O), 167.40 (CO_2Me), 163.76 (C═O lactone), 99.46 (C-2′), 96.85 (C-2), 80.09 (CHOH), 69.01 (C-6′), 68.18 (C-7), 67.95 (C-7′), 67.39 (C-6), 66.37 and 65.92 (C-4′, C-4), 64.64 and 64.18 (C-5, 5′), 61.97 and 60.79 (C-8, 8′), 57.64 (CH_2OH), 52.84 (OMe), 43.03 (CH_2), 31.48 (C-3′), 30.75 (C-3), 20.76, 20.72, 20.70, and 20.63 (7C, Ac); ESIMS: *m*/*z* calcd for $[C_{34}H_{46}O_{23}+Na]^+$: 845.23. Found: 845.25.

Further elution of the column afforded diastereoisomeric **8** as colorless syrup (189 mg, 69%), which was immediately used for the preparation of **9**.

3.8. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl (2-oxoethyl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (9)

A soln of **1** (24.1 mg, 29 μmol) in 5:1 dry CH_2Cl_2/dry MeOH (12 mL) was cooled to −6 °C (ice/NH_4Cl) and stirred. Ozonolysis was carried out using an ozone generator (Laborozonisator, Erwin Sander Elektroapparatebau GmbH, Uetze-Eltze) with a flow rate of 80 L/h. Ozone was bubbled through the soln for 20 min. Dimethyl sulfide (0.5 mL) was added and stirring was continued for 30 min. The soln was kept at 4 °C for 10 h to quench the reaction. After removal of the solvent, the residue was purified on silica gel (1:2 toluene–EtOAc) to give **9** (21.5 mg, 89%) as a syrup. $[\alpha]_D^{20}$ +80 (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 9.62 (s, 1H, CHO), 5.34–5.22 (m, 2H, H-4, 5), 5.28 (br s, 1H, H-5′), 5.13 (ddd, 1H, $J_{8'a,7'}$ 2.5, $J_{8'b,7'}$ 3.8, $J_{6',7'}$ 9.5 Hz, H-7′), 5.11 (ddd, 1H, $J_{3e',4'}$ 5.2, $J_{5',4'}$ 3.0, $J_{4',3'a}$ 12.5 Hz, H-4′), 5.07 (ddd, 1H, $J_{8a,7}$ 2.4, $J_{8b,7}$ 4.6, $J_{6,7}$ 9.7 Hz, H-7), 4.48 (dd, 1H, $J_{8'a,8'b}$ 12.3 Hz, H-8′a), 4.21–4.20 (m, 2H, CH_2), 4.12 (dd, 1H, $J_{6,5}$ <1.0 Hz, H-6), 4.11 (dd, 1H, H-8′b), 3.98 (dd, 1H, $J_{6',5'}$ 1.4 Hz, H-6′), 3.77 (dd, 1H, $J_{8a,8b}$ 11.8 Hz, H-8a), 3.73 (s, 6H, 2 × OMe), 3.64 (dd, 1H, H-8b), 2.25–2.05 (m, 4H, H-3e, 3a, 3e′, 3′a), 2.02 (s, 3H), 2.01 (s, 6H), 1.99 (s, 3H), 1.95 (s, 3H), 1.92 (s, 3H) and 1.90 (s, 3H, 7Ac); ^{13}C NMR ($CDCl_3$): δ 198.38 (CHO), 170.48, 170.30, 170.26, 169.93, 169.83, 169.81, and 169.67 (C═O), 167.11 and 166.99 (CO_2Me), 98.90 and 98.83 (C-2, 2′), 69.10 and 69.05 (C-6, 6′, OCH_2), 68.23 (C-7), 67.55 (C-7′), 66.15 (2C, C-4, 4′), 64.31 (C-5), 64.16 (C-5′), 62.30 and 61.97 (C-8, 8′), 52.96 and 52.75 (OMe), 31.38 and 31.23 (C-3, 3′), 20.73, 20.67, and 20.64 (7C, Ac); ESIMS: *m*/*z* calcd for $[C_{34}H_{46}O_{23}+Na]^+$: 845.23. Found: 845.21.

Alternatively, **9** was prepared from **8** as follows: A soln of compound **8** (82.3 mg, 96 μmol) in 1:1 dioxane–water (0.615 mL) was added to a soln of $NaIO_4$ (28.8 mg, 135 μmol) in 1:1 dioxane–water (4 mL) and the soln was stirred at room temperature for 4 h. The

solvent was concentrated and diluted with $CHCl_3$ (50 mL). The organic phase was washed with water, dried ($MgSO_4$), and concentrated. The residue was purified by HPLC (Lichrosorb Si60, 10 μm, 30 cm × 4 cm I.D., 1:2 toluene–EtOAc, flow rate 2 mL min^{-1}) to give **9** as a colorless syrup (59 mg, 61%).

3.9. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl (carboxymethyl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (10)

To a stirred soln of **9** (19.0 mg, 23 μmol) in MeCN (1 mL), an aqueous soln (1.3 mL) containing H_2O_2 (50%, 1.95 μL, ~34 μmol) and $NaH_2PO_4{\cdot}H_2O$ (4.6 mg, 33 μmol) was added (1.3 mL). The reaction mixture was cooled to 0 °C and $NaClO_2$ (80% purity, 7.0 mg, ~62 μmol) in H_2O (1.8 mL) was added. After 5 h at rt, a 0.15 M soln of Na_2SO_3 (0.1 mL) was added and the soln was stirred for 30 min. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with cold aq 1 M $NaHSO_4$. The organic layer was washed with water, dried ($MgSO_4$), and concentrated. Purification of the residue on silica gel (3:1 EtOAc–MeOH) afforded **10** as a syrup (18.6 mg, 96%). $[\alpha]_D^{20}$ +59 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 5.43 (br s, 1H, H-5), 5.42 (ddd, 1H, $J_{3e,4}$ 6.0, $J_{5,4}$ 2.6 Hz, H-4), 5.30 (br s, 1H, H-5′), 5.30 (ddd, 1H, $J_{8'a,7'}$ 2.6, $J_{8'b,7'}$ 5.8 Hz, H-7′), 5.18 (ddd, 1H, $J_{3'e,4'}$ 4.6, $J_{5',4'}$ 2.9, $J_{4',3'a}$ 12.0 Hz, H-4′), 5.15 (ddd, 1H, $J_{8a,7}$ 3.0, $J_{8b,7}$ 5.2, Hz, H-7), 4.57 (dd, 1H, $J_{8'a,8'b}$ 12.1 Hz, H-8′a), 4.57 (dd, 1H, $J_{6,5}$ 0.5, $J_{6,7}$ 10.0 Hz, H-6), 4.28 and 4.16 (AB-system, 2H, J_{AB} 16.4 Hz, CH_2), 4.18 (dd, 1H, H-8′b), 3.95 (dd, 1H, $J_{6',5'}$ 1.2, $J_{6',7'}$ 9.8 Hz, H-6′), 3.81 and 3.79 (2s, each 3H, 2 × OMe), 3.73 (dd, 1H, $J_{8a,8b}$ 11.2 Hz, H-8a), 3.58 (dd, 1H, H-8b), 2.25–2.17 (m, 3H, H-3e, 3e′, 3a), 2.08 (m, 1H, H-3′a), 2.11, 2.09, 2.08, 2.07, 2.03, 1.99, and 1.97 (7s, each 3H, 7Ac); ^{13}C NMR ($CDCl_3$): δ 171.63, 170.99, 170.66, 170.40, 170.34, 169.81, 169.70, and 169.66 (C═O), 167.30 and 167.11 (CO_2Me), 99.29 and 99.03 (C-2, 2′), 69.25 (C-6), 68.93 (C-6′), 67.97 (C-7), 67.17 (C-7′), 66.76 (C-4′), 66.21 (C-4), 64.52 (C-5), 63.93 (C-5′), 63.32 (C-8′), 62.42 (C-8), 61.25 (OCH_2), 52.92 and 52.83 (OMe), 31.55 and 31.28 (C-3, 3′), 20.84, 20.80, 20.74, 20.70, 20.67, and 20.62 (7C, Ac); ESIMS: *m*/*z* calcd for $[C_{34}H_{46}O_{24}+Na]^+$: 861.23. Found: 861.20.

3.10. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl [(3-methoxycarbonyl)-2-(*E*)-propen-1-yl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid]onate (11)

Methyl (triphenylphosphoranylidene)acetate (12.7 mg, 38.1 μmol) was added to a soln of **9** (21.4 mg, 29.3 μmol) in dry THF (3 mL) and the soln was stirred for 20 h at room temperature. After concentration of the soln, the reaction mixture was diluted with CH_2Cl_2 (50 mL) washed with water, dried ($MgSO_4$), and evaporated. The residue was purified on a column of silica gel (1:1 toluene–EtOAc) to give **11** (yield 18.6 mg, 73%) as a colorless syrup. $[\alpha]_D^{20}$ +66 (*c* 1.4, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.93 (dt, 1H, J_{trans} 15.8 Hz, CH═), 6.09 (dt, 1H, CH═), 5.36 (br s, 1H, H-5), 5.34 (m, 1H, H-4), 5.33 (br s, 1H, H-5′), 5.20 (dt, 1H, H-7′), 5.17–5.08 (m, 2H, H-7, H-4′), 4.59 (dd, 1H, $J_{8'a,7'}$ 2.3, $J_{8'a,8'b}$ 12.3 Hz, H-8′a), 4.32 and 4.19 (dddd, 2H, $J_{CH_2,CH=}$ 4.0, $J_{CH_2,CH=}$ 2.5, J_{CH_2,CH_2} 15.8 Hz, OCH_2), 4.15 (dd, 1H, $J_{8'b,7'}$ 4.0 Hz, H-8′b), 4.11 (dd, 1H, $J_{6,5}$ <1.0, $J_{6,7}$ 9.6 Hz, H-6), 4.03 (dd, 1H, $J_{6',5'}$ <1.0, $J_{6',7'}$ 9.8 Hz, H-6′), 3.90 (dd, 1H, $J_{8a,7}$ 2.4, $J_{8a,8b}$ 11.9 Hz, H-8a), 3.80 (s, 6H) and 3.74 (s, 3H, 3 × OMe), 3.75 (dd, 1H, H-8b), 2.25 (dd, 1H, $J_{3e,4}$ 5.3, $J_{3e,3a}$ 12.5 Hz, H-3e), 2.20–2.05 (m, 3H, H-3a, 3e′, 3′a), 2.90, 2.08, 2.07, 2.05, 2.00, 1.98, and 1.97 (7s, each 3H, 7Ac); ^{13}C NMR ($CDCl_3$): δ 170.35, 170.29, 169.95, 169.85, and 169.64 (C═O), 167.13, 167.09, and 166.33 (CO_2Me), 142.72 and 121.08 (CH═), 98.70 and 98.95 (C-2, 2′), 68.84 (2C, C-6, 6′), 68.40 (C-7), 67.60 (C-7′), 66.28 and 66.15 (2C, C-4, 4′), 64.32 (C-5), 64.07 (C-5′), 62.45 (C-8), 62.38 (OCH_2), 61.80 (C-8′), 52.79, 52.69, and 51.61 (OMe), 31.72 and 31.41 (C-3, 3′), 20.74, and 20.64 (7C, Ac); ESIMS: *m*/*z* calcd for $[C_{37}H_{50}O_{24}+Na]^+$: 901.26. Found: 901.28.

3.11. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl [3-methoxycarbonyl-prop-1-yl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid]onate (12)

A soln of **11** (20.4 mg, 23.2 μmol) in dry MeOH (5 mL) was stirred at room temperature in the presence of 10% Pd/C (30 mg) for 15 h under hydrogen at atmospheric pressure. After completion of the reaction, the catalyst was removed by filtration through Celite and washed with MeOH. The crude product obtained after evaporation was purified on a column of silica gel (1:2 toluene–EtOAc) to give **12** (17.3 mg, 85%) as a colorless syrup. $[\alpha]_D^{20}$ +60 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 5.35 and 5.33 (br s, 2H H-5, 5′), 5.29 (ddd, 1H, $J_{3e,4}$ 5.0, $J_{5,4}$ 2.8, $J_{3a,4}$ 12.0 Hz, H-4), 5.21 and 5.20 (m, 2H, H-7, 7′), 5.14 (ddd, 1H, 1H, $J_{3'e,4'}$ 5.0, $J_{5',4'}$ 3.1, $J_{3'a,4'}$ 12.0 Hz, H-4′), 4.58 (dd, 1H, $J_{8'a,7'}$ 2.3, $J_{8'a,8'b}$ 12.2 Hz, H-8′a), 4.165 (dd, 1H, $J_{8'b,7'}$ 4.0 Hz, H-8′b), 4.08 (dd, 1H, $J_{6,5}$ 1.2, $J_{6,7}$ 9.6 Hz, H-6), 4.04 (dd, 1H, $J_{6',5'}$ 1.3, $J_{6',7'}$ 9.7 Hz, H-6′), 3.86 (dd, 1H, $J_{8a,7}$ 2.6, $J_{8a,8b}$ 11.5 Hz, H-8a), 3.80 (s, 6H) and 3.68 (s, 3H, 3 × OMe), 3.71 (dd, 1H, $J_{8b,7}$ 5.2 Hz, H-8b), 3.55 and 3.42 (dt, 2H, OCH_2), 2.44 (t, 2H, *J* 7.2 Hz, CH_2CO), 2.21 (dd, 1H, $J_{3'e,3'a}$ 12.4 Hz, H-3′e), 2.17 (dd, 1H, $J_{3e,3a}$ 12.4 Hz, H-3e), 2.13–1.90 (m, 4H, H-3a, 3′a, CH_2), 2.10 (s, 3H), 2.08 (s, 6H), 2.06 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H) and 1.96 (s, 3H, 7Ac); ^{13}C NMR

($CDCl_3$): δ 173.51, 170.45, 170.34, 169.89, 169.81, and 169.67 (C=O), 167.66 and 167.20 (CO_2Me), 98.84 (2C, C-2, 2′), 68.71 (2C, C-6, 6′), 68.48 (C-7), 67.65 (C-7′), 66.40 and 66.25 (2C, C-4, 4′), 64.44 (C-5), 64.14 (C-5′), 63.085 (OCH_2), 62.60 (C-8), 61.91 (C-8′), 52.71, 52.62, and 51.53 (OMe), 31.91 and 31.54 (C-3, 3′), 30.44 (CH_2CO), 24.58 (CH_2), 20.75 and 20.63 (7C, Ac); ESIMS: *m*/*z* calcd for $[C_{37}H_{52}O_{24}+Na]^+$: 903.27. Found: 903.29.

3.12. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl (5-methoxycarbonyl-4-thia-pent-1-yl 4,5,7-tri-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (13)

A soln of **1** (93 mg, 113 μmol) in dioxane (4 mL) was treated with methyl mercaptoacetate (180 μL, 2 mmol) and azobisisobutyronitrile (4 mg, 24 μmol) for 5 h at 75 °C under Ar. Toluene was added repeatedly and the solvent was removed three times by coevaporation. Purification of the residue on a column of silica gel (1.5:1 toluene–EtOAc) yielded a product, which was purified using HPLC (Lichrosorb Si60, 10 μm, 30 cm × 4 cm I.D., 1.5:1 toluene–EtOAc, flow rate 2 mL min^{-1}) to give **13** (50 mg, 48%) as a syrup. $[\alpha]_D^{20}$ +52 (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.35 and 5.33 (br s, 2H H-5, 5′), 5.31 (m, 1H, $J_{3e,4}$ 5.0, $J_{5,4}$ 3.2 Hz, H-4), 5.22 and 5.19 (m, 2H, H-7′, 7), 5.13 (ddd, 1H, 1H, $J_{3'e,4'}$ 5.2, $J_{5',4'}$ 3.2, $J_{3'a,4'}$ 12.3 Hz, H-4′), 4.57 (dd, 1H, $J_{8'a,7'}$ 2.5, $J_{8'a,8'b}$ 12.3 Hz, H-8′a), 4.17 (dd, 1H, $J_{8'b,7'}$ 4.2 Hz, H-8′b), 4.10 (dd, 1H, $J_{6,5}$ 1.4, $J_{6,7}$ 9.7 Hz, H-6), 4.03 (dd, 1H, $J_{6',5'}$ 1.4, $J_{6',7'}$ 9.9 Hz, H-6′), 3.87 (dd, 1H, $J_{8a,7}$ 2.5, $J_{8a,8b}$ 11.5 Hz, H-8a), 3.81, 3.80, and 3.74 (3s, each 3H, 3 × OMe), 3.72 (dd, 1H, $J_{8b,7}$ 5.3 Hz, H-8b), 3.59 and 3.49 (dt, 2H, OCH_2), 3.25 (s, 2H, SCH_2), 2.74 (dt, 2H, CH_2S), 2.20 (dd, 1H, $J_{3'e,3'a}$ 12.9 Hz, H-3′e), 2.17 (dd, 1H, H-3e), 2.13–2.00 (m, 2H, H-3a, 3′a), 2.11 (s, 3H), 2.08 (s, 6H), 2.06 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H) and 1.96 (s, 3H, 7Ac), 1.90 (m, 2H, CH_2); Anal. Calcd for $C_{38}H_{54}O_{24}S$: C, 49.24; H, 5.87; S, 3.46. Found: C, 49.42; H, 6.03; S, 3.05.

3.13. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)-onate-(2→8)-sodium *O*-(3-hydroxyprop-1-yl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (14)

A soln of 1 M methanolic $NaOCH_3$ (0.1 mL) was added to a soln of **2** (25.0 mg, 30 μmol) in dry MeOH (2 mL). The soln was stirred for 1 h at room temperature and neutralized with Dowex AG-50 WX8 (H^+) ion-exchange resin. The suspension was filtered and the filtrate was concentrated. The residue was dissolved in 0.1 M NaOH (1 mL) and stirred for 60 min at room temperature. The pH of the soln was adjusted to 8 by addition of Dowex 50 (H^+) ion-exchange resin. After filtration and lyophilization, the residue was purified on a Bio-Gel P-2 column (1.3 × 50 cm, water) to give **14** as amorphous powder (16.6 mg, 99%). $[\alpha]_D^{20}$ +67 (*c* 0.1, H_2O); ^1H NMR (D_2O): δ 4.09–3.98 (m, 5H, H-4, 5, 7, 4′, 5′), 3.95–3.87 (m, 2H, H-8′a, 7′), 3.75–3.53 (m, 7H, H-6, 8a, 8b, 6′, 8′b, CH_2OH), 3.46 and 3.35 (dt, 2H, OCH_2), 2.08–1.99 (m, 3H, H-3e, 3′e, CH_2) and 1.85–1.72 (m, 3H, H-3a, 3′a, CH_2); ESIMS: *m*/*z* calcd for $[C_{19}H_{32}O_{16}+Na]^+$: 539.16. Found: 539.15.

3.14. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)-onate-(2→8)-sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyloxy)onate-(2→2)-sodium ethanoate (15)

A soln of 1 M NaOMe in MeOH (100 μL) was added to a soln of **10** (15.6 mg, 18.6 μmol) in dry MeOH (2 mL). The soln was stirred for 1 h at room temperature, neutralized by the addition of Dowex 50 (H^+) exchange resin, filtered and the filtrate was concentrated. A soln of the residue in 0.1 M NaOH (1 mL) was stirred for 1 h at room temperature. The soln was made neutral with Dowex 50 (H^+) resin and filtered. Lyophilization of the filtrate and subsequent purification of the residue on a Bio-Gel P-2 (1.3 × 50 cm, H_2O) gave 9.9 mg (91%) of **15** as amorphous powder. $[\alpha]_D^{20}$ +60 (*c* 1.0); ^1H NMR (D_2O): δ 4.22 (ddd, 1H, $J_{3e,4}$ 5.1, $J_{5,4}$ 2.8, $J_{3a,4}$ 11.9 Hz, H-4), 4.05 (br s, 1H, H-5), 4.04–3.86 (m, 4H, H-7, 8′a, 4′, 5′), 3.77–3.55 (m, 7H, H-6, 8a, 8b, 6′, 8′b, OCH_2), 2.08 (dd, 1H, $J_{3e,3a}$ 13.3 Hz, H-3e), 2.01 (dd, 1H, $J_{3'e,3'a}$ 12.0, $J_{3'e,4'}$ 4.9 Hz, H-3′e), 1.77 (t, 2H, H-3a, 3′a); ESIMS: *m*/*z* calcd for $[C_{18}H_{28}O_{17}+Na]^+$: 539.12. Found: 539.12.

3.15. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)-onate-(2→8)-sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyloxy)onate-(2→3)-sodium propanoate (16)

A soln of 1 M NaOMe (100 μL) was added to a soln of **4** (12 mg, 14 μmol) in MeOH (2 mL) and stirred for 1 h at room temperature. The soln was neutralized by the addition of Dowex 50 (H^+) exchange resin, filtered, and evaporated to dryness. The residue was dissolved in 0.1 M NaOH (1 mL) and processed as described for **14**. Yield: 7 mg (84%) of **16** as amorphous powder. $[\alpha]_D^{20}$ +60 (*c* 0.7, H_2O); ^1H NMR (D_2O): δ 4.10–3.98 (m, 5H, H-4, 5, 7, 4′, 5′), 3.94–3.87 (m, 2H, H-7′, 8′a), 3.70–3.57 (m, 6H, H-6, 8a, 8b, 6′, 8′b, OCH_2), 3.46 (dt, 1H, OCH_2), 2.51 (t, 2H, *J* 7.0 Hz, CH_2), 2.05 and 2.00 (dd, 2H, H-3e, 3′e), 1.79 and 1.75 (2t, 2H, H-3a, 3′a); ESIMS: *m*/*z* calcd for $[C_{19}H_{30}O_{17}+Na]^+$: 553.14. Found: 553.14.

3.16. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)-onate-(2→8)-sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyloxy)onate-(2→4)-sodium butanoate (17)

A soln of 0.1 M NaOMe in MeOH (0.7 mL) was added to a soln of **12** (9.2 mg, 10.4 μmol) in dry MeOH (5 mL).

The soln was stirred for 1 h at room temperature, neutralized by the addition of Dowex 50 (H^+) exchange resin, filtered and the filtrate was concentrated. A soln of the residue in water (10 mL) and 0.1 M NaOH (0.7 mL) was stirred for 1 h at room temperature and processed as described for **14**. Yield: 5.4 mg (85%) of **17** as amorphous solid. $[\alpha]_D^{20}$ +53 (*c* 0.5, H_2O); ^1H NMR (D_2O): δ 4.05 (dd, 1H, $J_{3e,4}$ 5.0, $J_{5,4}$ 2.9, $J_{3a,4}$ 11.9 Hz, H-4), 3.98–3.83 (m, 6H, H-5, 7, 4′, 5′, 7′, 8′a), 3.65–3.55 (m, 5H, H-6, 8a, 8b, 6′, 8′b), 3.28 (m, 2H, OCH_2), 2.21 (dt, 2H, CH_2CO), 2.05–1.95 (m, 2H, H-3e, 3′e), 1.81–1.71 (m, 3H, CH_2, H-3a′), 1.75 (t, 1H, $J_{3e,3a}$ 12.9 Hz, H-3a); ESIMS: *m*/*z* calcd for $[C_{20}H_{32}O_{17}+Na]^+$: 567.15. Found: 567.14.

3.17. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)-onate-(2→8)-sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyloxy)onate-(2→3)-sodium pentanoate (18)

A soln of 0.1 M NaOMe in MeOH (1 mL) was added to a soln of **6** (23 mg, 25.7 μmol) in dry MeOH (5 mL). The soln was stirred for 70 min at room temperature, neutralized by the addition of Dowex 50 (H^+) exchange resin, filtered and the filtrate was concentrated. A soln of the residue in water (10 mL) and 0.1 M NaOH (1.5 mL) was stirred for 1 h at room temperature. The soln was processed as described for **14** to give 14.2 mg (88%) of **18** as amorphous powder. $[\alpha]_D^{20}$ +52 (*c* 0.7, H_2O); ^1H NMR (D_2O): δ 4.02 (ddd, 1H, $J_{3e,4}$ 5.1, $J_{5,4}$ 3.0, $J_{3a,4}$ 12.0 Hz, H-4), 4.00–3.90 (m, 4H, H-5, 7, 4′, 5′), 3.87–3.81 (m, 2H, H-7′, 8′a), 3.60–3.51 (m, 5H, H-6, 8a, 8b, 6′, 8′b), 3.28 (m, 2H, OCH_2), 2.14 (t, 2H, *J* 7.0 Hz, CH_2CO), 2.00 (m, 1H, H-3′e), 1.96 (ddd, 1H, $J_{3e,3a}$ 12.5 Hz, H-3e), 1.74 (dd, 1H, $J_{3'a,4'}$ 11.5, $J_{3'a,3'e}$ 13.1 Hz, H-3′a), 1.96 (dd, 1H, H-3a), 1.61–1.48 (m, 4H, 2 CH_2); ESIMS: *m*/*z* calcd for $[C_{21}H_{34}O_{17}+Na]^+$: 581.17. Found: 581.

3.18. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)-onate-(2→8)-sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyloxy)onate-(2→5)-sodium 2-(*E*)-pentenoate (19)

A soln of 0.1 M NaOMe in MeOH (1 mL) was added to a soln of **5** (18.8 mg, 21.1 μmol) in dry MeOH (5 mL). The soln was stirred for 1 h at room temperature, neutralized by the addition of Dowex 50 (H^+) exchange resin, filtered, and the filtrate was concentrated. A soln of the residue in water (8 mL) and 0.1 M NaOH (1.5 mL) was stirred for 1 h at room temperature. The soln was processed as described to furnish 18.6 mg (89%) of **19** as amorphous powder. $[\alpha]_D^{20}$ +49 (*c* 0.6, H_2O); ^1H NMR (D_2O): δ 6.56 (dt, 1H, *J* 6.9 and 15.7 Hz, CH_2CH=), 5.87 (br d, 1H, =$CHCO_2$), 4.02 (ddd, 1H, $J_{3e,4}$ 5.1, $J_{5,4}$ 3.1, $J_{3a,4}$ 12.0 Hz, H-4), 4.00–3.90 (m, 4H, H-5, 7, 4′, 5′), 3.88–3.82 (m, 2H, H-7′, 8′a), 3.64–3.51 (m, 5H, H-6, 8a, 8b, 6′, 8′b), 3.40 (m, 2H, OCH_2), 2.43 (m, 2H, *J* 7.0 Hz, CH_2CH=), 2.11–2.03 (m, 2H, H-3e, 3′e), 1.83 and 1.80 (2t, 2H, H-3a, 3′a); ESIMS: *m*/*z* calcd for $[C_{21}H_{32}O_{17}+Na]^+$: 579.15. Found: 579.14.

3.19. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)-onate-(2→8)-sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyloxy)onate-(2→6)-sodium 2-thia-hexanoate (20)

A soln of 1 M $NaOCH_3$ (0.2 mL) was added to a soln of **13** (42.0 mg, 45 μmol) in MeOH (4 mL). After 45 min at room temperature, the soln was neutralized with Dowex 50 (H^+) ion-exchange resin, filtered, and concentrated. The residue was dissolved in 0.1 M NaOH (1.5 mL) and stirred for 45 min at room temperature. Processing and work-up as described for **14** afforded **20** as amorphous powder (26.3 mg, 88%). $[\alpha]_D^{20}$ +57 (*c* 0.7, H_2O); ^1H NMR (D_2O): δ 4.11–3.97 (m, 5H, H-4, 5, 7, 4′, 5′), 3.95–3.87 (m, 2H, H-7′, 8′a), 3.68–3.56 (m, 5H, H-6, 8a, 8b, 6′, 8′b), 3.47–3.31 (m, 2H, OCH_2), 3.23 (s, 2H, SCH_2CO), 2.66 (t, 2H, *J* 7.0 Hz, CH_2S), 2.05 and 2.01 (dd, 2H, H-3e, 3′e), 1.92–1.80 (m, 2H, CH_2), 1.79 and 1.75 (2t, 2H, H-3a, 3′a); ESIMS: *m*/*z* calcd for $[C_{21}H_{32}O_{17}+Na]^+$: 579.15. Found: 579.14.

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