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Synthesis and antigenic properties of C-7-modified Kdo mono- and disaccharide ligands and Kdo disaccharide interresidue lactones

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ABSTRACT

In order to define binding interactions of Kdo-specific monoclonal antibodies directed against the chlamydial α-(2→8)-linked Kdo disaccharide epitope on a molecular level, modifications at the 7-position of the proximal and distal Kdo unit were investigated. The synthesis of 7-*O*-methyl and 7-azido-7-deoxy-7-*epi*-Kdo monosaccharide derivatives was achieved via an 8-*O*-TBS protected derivative, whereas methylation of O-7 at the proximal Kdo unit of the α-(2→8)-linked Kdo disaccharide was conveniently accomplished via a 4,5; 4′,5′; 7′,8′-tri-*O*-carbonyl-protected disaccharide intermediate. Attempted epimerization at C-5 of the inner unit of a α-(2→4)-linked Kdo disaccharide, however, resulted in formation of the corresponding 5,6-dehydro derivative, which was fully deprotected. Treatment of unprotected α-(2→8)- as well as α-(2→4)-linked Kdo disaccharides in neat acetic acid furnished the corresponding interresidue lactone derivatives. The lactones displayed limited stability under neutral conditions and were hydrolyzed at pH 7 within 3 days. Access to the lactones, however, provides a means for selective derivatization of the carboxylic group located at the distal Kdo residue, which was demonstrated by methanolysis of the lactone to afford the monomethyl ester of the α-(2→8)-linked Kdo disaccharide. ELISA inhibition experiments of the ligands with two Kdo-specific monoclonal antibodies showed slightly reduced reactivity for the binding of the α-(2→8) Kdo-specific antibody S25-2, whereas the 7-*O*-methyl disaccharide antigen displayed high binding affinity toward the Kdo monosaccharide-specific antibody S67-27.

1. Introduction

Chlamydiae are obligate intracellular bacterial pathogens causing a broad variety of acute and chronic diseases in animals and humans.[1] In addition to acute genital and eye infections caused by *Chlamydia trachomatis* and cases of pneumonia caused by *Chlamydophila pneumoniae* and *Chlamydophila psittaci*, respectively, also chronic diseases such as arteriosclerosis, arthritis, asthma, and neurodegenerative diseases have been linked to chlamydial infections.[2] In the outer leaflet of the cell membrane, Chlamydiae contain a highly truncated glycolipid, which is composed of lipid A and 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) residues only.[3,4] All *Chlamydiaceae* share a common Kdo epitope—formerly called the genus-specific epitope—resembling the deep rough mutant LPS structures of *Enterobacteriaceae*.[5] In contrast to Re mutants, the Kdo region of *Chlamydia*, however, constitutes a prominent antigenic epitope of 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] Recently, the (2→8)-linkage between Kdo residues has also been found in an *Acinetobacter* strain providing structural evidence for the occasionally observed serological cross-reactivity between Chlamydiae and *Acinetobacter* species.[7] 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 *C. psittaci* and have been isolated from recombinant strains expressing the *C. psittaci* Kdo transferase.[8] Synthetic neoglycoconjugates covering these structural variations have been prepared and utilized to generate murine monoclonal antibodies and to characterize their binding epitopes.[9] Whereas the monoclonal antibody S25-23 has a strict specificity for the family-specific epitope, mAb S25-2 has a more relaxed binding specificity and binds in addition to the major epitope α-Kdo-(2→8)-α-Kdo-(2→4)-α-Kdo also the α-(2→8)-linked disaccharide part with reduced affinity.[10] Furthermore, the plasticity of the binding mode of mAb S25-2 was also seen in liganded crystal structures of Kdo disaccharide units modified at the exocyclic chain of Kdo. Similarly, derivatives containing a 3-hydroxy group were still accommodated in the binding site.[11] Notably, the hydroxyl groups at positions C7 and C8 of Kdo being involved in several bridging water interactions do not seem to be essential for Kdo

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recognition. Thus, the α-(2→8)-linked Kdo disaccharide analogue containing a terminal 7-*epi*-Kdo unit was bound by the mAb S25-2 despite having lost the bridging water interactions of both C7 and C8 hydroxyl groups. In order to further evaluate the contribution of major interactions for the binding of Kdo analogues, we now report on the synthesis of 7-O-methylated mono and disaccharide derivatives as well as a 7-azido-7-deoxy-7-*epi*-Kdo analogue.

In the crystal structure of the unliganded α-(2→8)-linked Kdo allyl glycoside, a hydrogen bond is observed between the terminal carboxylic group and the 7-OH group of the inner Kdo unit.[12] The proximity of the carboxylic group of a distal Kdo moiety to the interresidue 7-OH as well as to the 5-OH group in α-(2→8)-linked Kdo disaccharides and α-(2→4)-linked Kdo disaccharides, respectively, should indeed facilitate interresidue lactone formation. While interresidue lactone formation has been studied in several oligomeric neuraminic acid glycosides, the propensity of interglycosidic lactone formation and stability of unprotected Kdo lactones have not been investigated in any detail.[13] Since the Chlamydia-specific α-Kdo-(2→8)-α-Kdo-(2→4)-α-Kdo epitope harbors two potential sites for lactonization, acid-induced lactone formation of the underlying part structures will be described in the present communication for α-Kdo-(2→8)-α-Kdo and α-Kdo-(2→4)-α-Kdo disaccharide allyl glycosides.

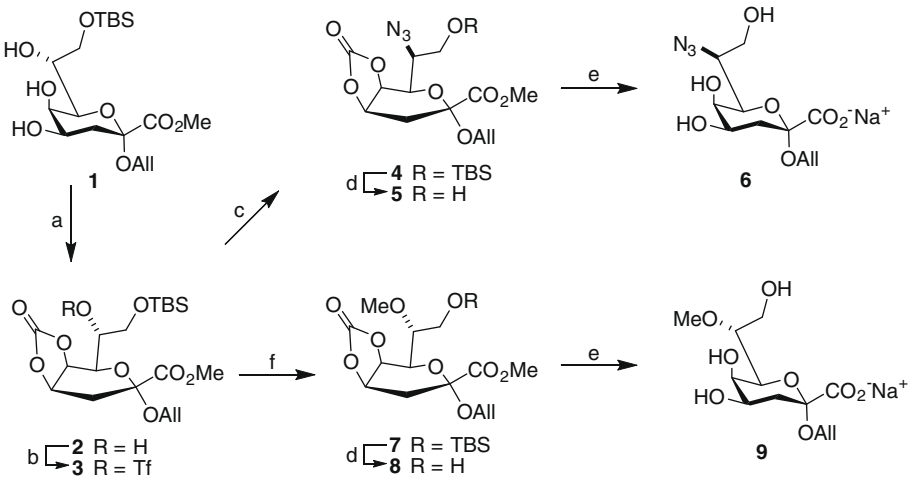
2. Results and discussion

For the synthesis of the 7-azido-7-deoxy analogue **5**, the previously described 8-*O*-TBS protected Kdo allyl glycoside derivative **1** was converted into the 4,5-*O*-carbonyl derivative **2** by reaction with 4-nitrophenyl chloroformate in pyridine in 83% yield.[14] Compound **2** was then converted into the 7-*O*-triflate derivative **3** by reaction with triflic acid anhydride in dichloromethane-pyridine in 76% yield. The triflate derivative **3** reacted smoothly with LiN_3 in DMF with inversion of configuration to furnish the epimeric 7-azido derivative **4** in 71% yield. The formation of the azide was deduced from the high field-shifted ^{1}H NMR signal of H-7 (δ 3.77). Deprotection of 4 was effected by treatment with 2% hydrofluoric acid in MeCN affording compound **5** in 96% yield.[15] Subsequent Zemplén saponification followed by hydrolysis of the methyl ester group with aqueous NaOH furnished the Kdo derivative **6** in 98% yield after purification on a Sephadex G-10 column. The ^{13}C NMR data were consistent with the formation of the 7-azide moiety as seen from the high field-shifted signal of C-7 (δ 64.63).

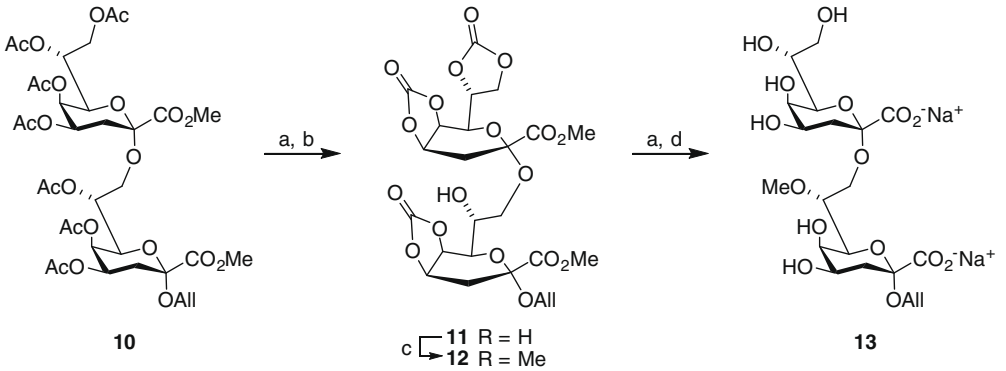
Methylation of **2** was accomplished by treatment of **2** with trimethylsilyl diazomethane in CH_2Cl_2 in the presence of tetrafluoroboric acid, which furnished the 7-*O*-methyl derivative **7** in 54% yield and with recovery of the starting material in 41% yield.[16] The reaction was not performed until full conversion of the educt, since degradation reactions were observed upon prolonged reaction times. Deprotection of the 7-O-methylated Kdo derivative **7** was performed in a similar fashion as for the 7-*epi*-7-azido derivative **6** by cleavage of the silyl protecting group, which afforded the 7-*O*-methyl Kdo allyl glycoside **8** in 82% yield. Removal of the ester groups under alkaline conditions finally gave the 7-O-methylated monosaccharide analogue as the sodium salt **9** in 96% yield (Scheme 1). The structural assignment of compound **9** was confirmed by the low field-shifted ^{13}C NMR signal of C-7 (δ 78.61) and the high field-shifted ^{13}C NMR signals of the neighboring carbons (δ 59.09 for C-8 and 69.95 for C-6, respectively).

Although compound **8** is a suitable precursor for further extension with a Kdo glycosyl donor, the α-(2→8)-linked 7-O-methylated disaccharide was obtained by a different approach. It was envisaged that formation of a tri-*O*-carbonyl derivative derived from the previously described α-(2→8)-linked Kdo disaccharide allyl glycoside **10** should leave the 7-OH group accessible for further modifications.[12] Indeed, reaction of the intermediate disaccharide dimethyl ester derivative—obtained upon Zemplén deacetylation of **10**—with an excess of trichloromethyl chloroformate in THF and *sym* collidine afforded the tris-*O*-carbonate derivative **11** in 84% yield. The ^{1}H NMR spectrum of **11** displayed low field-shifted signals for the protons at positions 4, 5, 4′, 5′, 7′, and 8′, while H-7 was observed in a region of higher field (δ 4.16–4.08). Methylation of **11** with trimethylsilyl diazomethane produced the 7-*O*-methyl derivative **12** in better yield (76%) compared to the monosaccharide **7**, which may be due to the lability of the 8-*O*-TBS group in the latter case. Removal of the carbonate protecting groups using sodium methoxide and subsequent saponification of the ester groups gave the target disaccharide sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-sodium (allyl 7-*O*-methyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate **13** in near quantitative yield (Scheme 2). Similar to compound **9**, the ^{13}C NMR chemical shift of C-7 in compound **13** was observed in the low field region (δ 78.68) confirming the site of methylation at position 7.

The previously reported (2→4)-linked Kdo disaccharide derivative **14** was converted into the 5-*O*-triflate derivative **15** by reac-



Scheme 1. Reagents and conditions: (a) 4-nitrophenyl chloroformate, pyridine, rt, 15 h, 83% for **2**; (b) Tf_2O, CH_2Cl_2–pyridine, −20 °C, 15 h, 76% for **3**; (c) LiN_3, DMF, rt, 12 h, 71% for **4**; (d) 2% HF, MeCN, rt, 2 h, 96% for **5**, 82% for **8**; (e) 0.1 M NaOMe, MeOH, rt, 1 h, then 0.1 M aq NaOH, rt, 2 h, 98% for **6**, 96% for **9**; (f) TMS-N_2, HBF_4, Et_2O, 0 °C, 1 h, 54% for **7**.

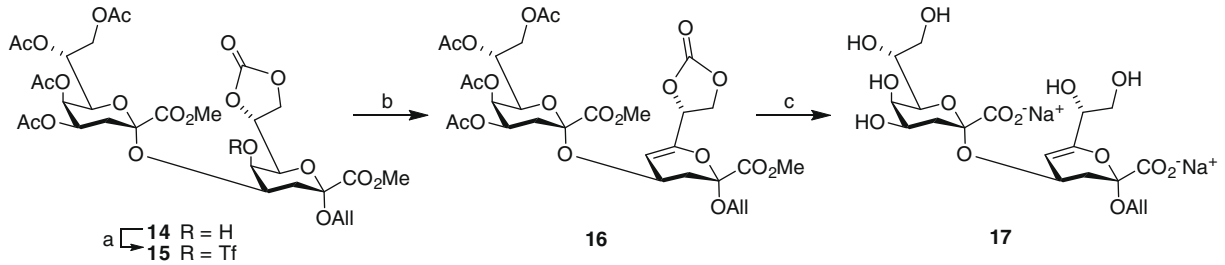


Scheme 2. Reagents and conditions: (a) 0.1 M NaOMe, MeOH, rt, 1 h; (b) $Cl_3COCOCl$, THF, −5 °C, 4 h, 84% for **11**; (c) TMS–N_2, HBF_4, Et_2O, 0 °C, 0.5 h, 76% for **12**; (d) 0.1 M aq NaOH, rt, 1 h, 99% for **13**.

tion with triflic acid anhydride in dry pyridine in 95% yield.[17] Treatment of **15** with cesium trifluoroacetate in butanone did not lead to the 5-epimeric Kdo analogue but resulted in neat elimination of the triflate group to afford compound **16** in 74% yield.[18] Evidence of the formation of the elimination product was derived from the NMR data which revealed a low field-shifted ^{13}C NMR signal attributable to C-5 (δ 103.60), the absence of H-6 and a low field-shifted ^{13}C NMR signal for C-6 (δ 147.10). Deprotection of the 5,6-dehydro derivative **16** was performed via Zemplén deacylation followed by alkaline ester hydrolysis to afford the disaccharide analogue **17** in 60% yield to be used as ligand in cocrystallization experiments with Kdo-(2→4)-Kdo-specific monoclonal antibodies (Scheme 3).

Finally, lactone formation of Kdo disaccharide allyl glycosides was studied by subjecting the crystalline (2→8)-linked Kdo disaccharide **18** to treatment with glacial acetic acid at room temperature.[12] The progress of the reaction was monitored by performing the reaction in deuterated acetic acid and recording NMR spectra at suitable time intervals.[19] The formation of the C-1′→O7 lactone derivative **19** proceeded slowly but in a smooth reaction without hydrolysis of the ketosidic linkages. As shown in Figure 1, an additional low field-shifted signal appeared as a doublet of triplets at δ 4.91 attributable to H-7 of the respective Kdo unit, which was tracked to a low field-shifted carbon signal at δ 74.5 via an HSQC experiment. In addition, a diagnostic reversal of the chemical shifts of the geminal H-3 protons was observed, with the axial proton H-3*ax* occurring at lower field (δ 2.41) than the equatorial proton H-3*eq* (δ 2.12). The low field shift of H-3′*ax* may be rationalized by its 1,3-diaxial interaction with the carbonyl group of the lactone fixed in the spirobicyclic unit and serves as a structural reporter group for lactone formation of α-anomeric Kdo residues (Scheme 4). After standing for 4 weeks at room temperature, the solution was processed by evaporation of the solvent followed by repeated lyophilization of the residue from DMSO to furnish compound **19**, which was not further purified due to inherent lability of the material. The stability of the lactone derivative **19** was studied in situ by NMR spectroscopy for a solution of the lactone in D_2O at pD 7.0. In contrast to glycopeptide lactones of neuraminic acid oligosaccharides, which were stable between 9 and more than 30 days under aqueous conditions, the C-1′→O7 lactone ring was completely hydrolyzed at room temperature within 3 days.[20] Hence the potential application of Kdo lactones as immunogens is rather limited. The easy access to Kdo lactones of Kdo oligosaccharides, however, provides an access to selective activation of the carboxylic group at the distal Kdo unit (Scheme 4).

The reactivity and selective derivatization were tested by treating the crude lactone **19** with methanol which resulted in a smooth conversion into the monomethyl ester derivative **20**, isolated in 91% yield after purification on a BioGel P2 column (calculated on the basis of the crystalline educt **18** subjected to lactonization). Finally, the previously described Kdo-(2→4)-Kdo disaccharide derivative **21** was converted into a C1′→O5 lactone by treatment with glacial acetic acid.[14] Similar to the lactone **19**, NMR data revealed low field-shifted signals of the proton and carbon atoms, respectively, involved in the ring formation. The ^1H NMR signal of H-5 of compound **22** appeared as broad doublet at δ 5.37 and showed a correlation to a ^{13}C NMR cross-peak at δ 68.7. The lactone formation was also evident from the low field-shifted signal of H-3′*ax* observed at δ 2.43. The signal of H-4 was also shifted out of the bulk region and was observed at δ 4.53. In comparison to the generation of the C-1′→O7 lactone derivative **18**, however, the presence of a minor byproduct was seen in the spectrum of the C-1′→O5 lactone **22** (Fig. 2). The second compound being present in ~15% in the mixture presumably corresponds to an intraresidue lactone. The hydrolytic lability of the lactone **22** was comparable to that of compound **19** and the lactone was completely hydrolyzed at pD 7.0 within 3 days at room temperature.



Scheme 3. Reagents and conditions: (a) Tf_2O, CH_2Cl_2–pyridine, 4 °C, 20 h, 95% for **15**; (b) Cs-trifluoroacetate, butanone, pyridine, 40 °C, 24 h, 74% for **16**; (c) 0.1 M NaOMe, MeOH, rt, 2 h, then 0.1 M aq NaOH, rt, 1 h, 60% for **17**.

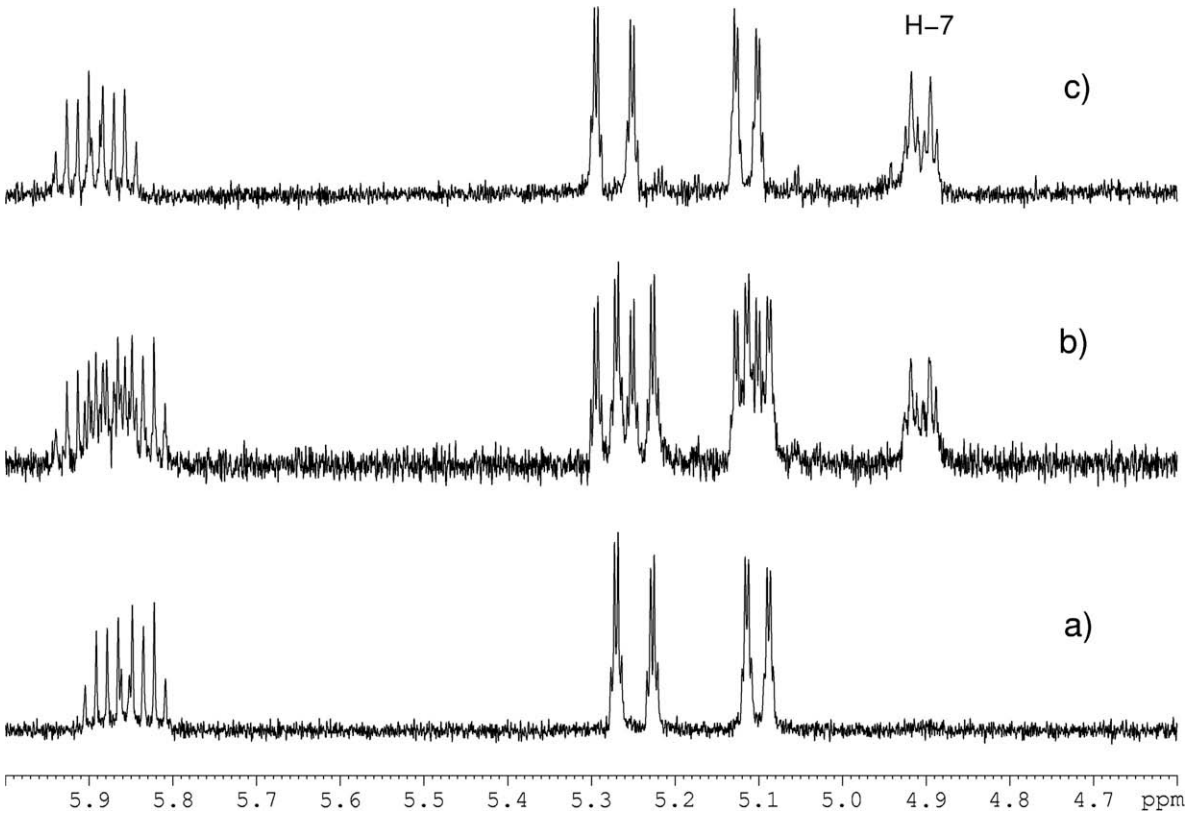
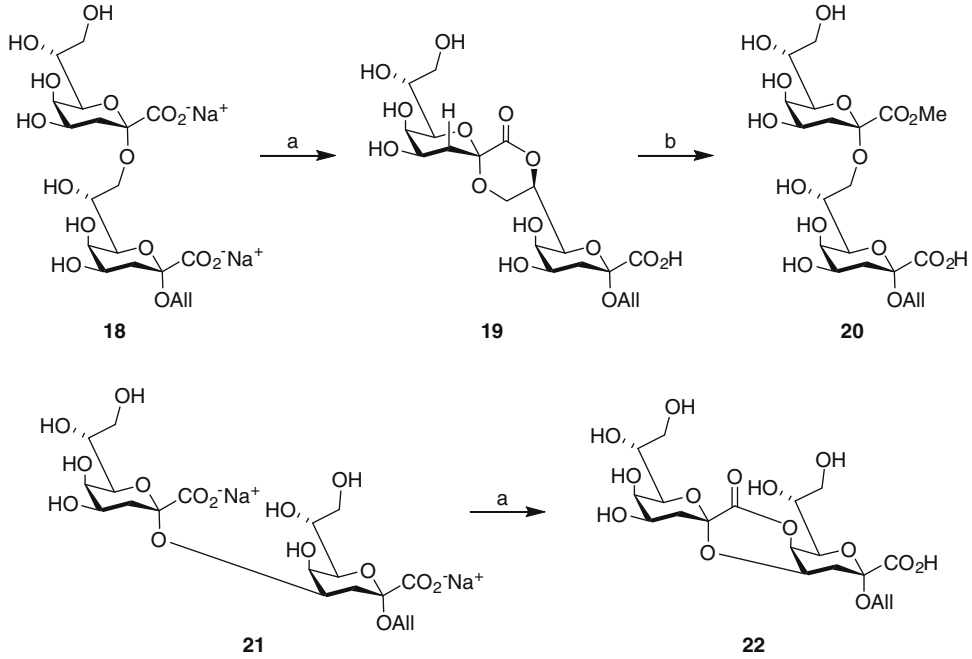


Figure 1. Expansion plots of the low field region recorded for solutions of disaccharide **18** in acetic acid-d_4; (a) start of the reaction; (b) sample recorded after 5 days; (c) sample recorded after 4 weeks.



Scheme 4. Reagents and conditions: (a) acetic acid-d_4, rt, 4 weeks; (b) MeOH, rt, 3 h, then −18 °C, 14 h, 91% for **20**.

The monosaccharide and (2→8)-linked Kdo disaccharide derivatives were then tested as inhibitors for the binding of two monoclonal antibodies, performed by ELISA for their inhibitory capacity for the Kdo-(2→8)-Kdo-specific mAb S25-2 and the Kdo-specific mAb S67-27. In addition, allyl glycosides of Kdo, Kdo-(2→8)-Kdo, and Kdo-(2→8)-Kdo-(2→4)-Kdo were tested as positive controls. As seen in Table 1, mAb S25-2 is inhibited by the homologous antigen Kdo-(2→8)-Kdo disaccharide to a comparable extent as all derivatives tested (IC_{50} 100–200 μM) except the 7-azido-7-deoxy-7-*epi*-Kdo derivative **6** which yielded a fourfold higher inhibitory concentration. MAb 67-27 is inhibited by the homologous antigen Kdo monosaccharide to a comparable extent

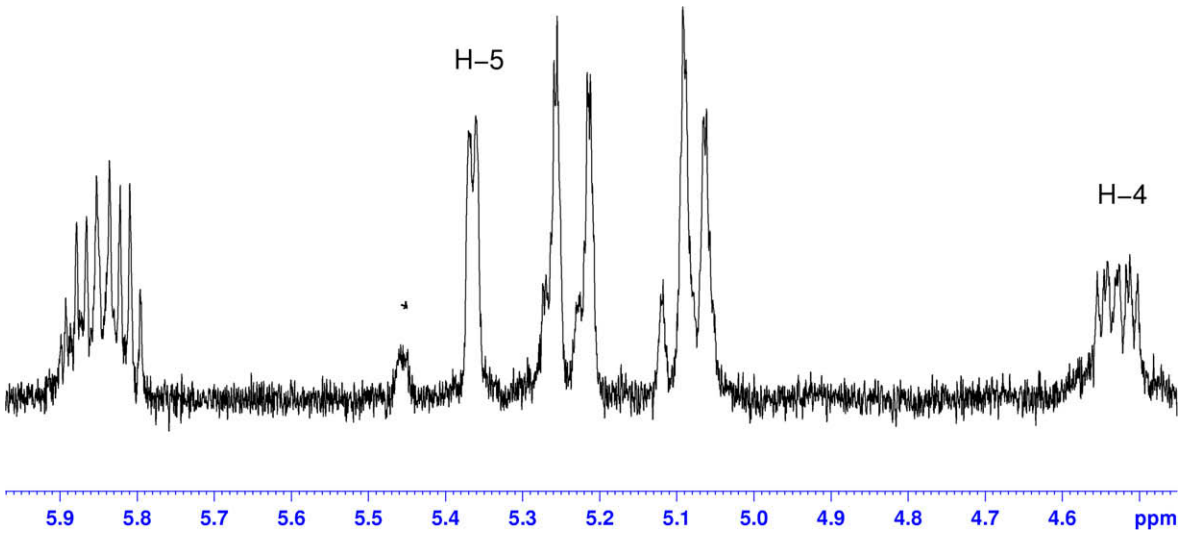


Figure 2. Expansion plot of low field region recorded for a solution of disaccharide **21** in acetic acid-d_4 after a reaction time of 5 days. The * indicates a signal from an additional lactone byproduct.

Table 1
Inhibitory capacity of synthetic Kdo analogues for Kdo-(2→8)-Kdo-specific mAb S25-2 and Kdo-specific mAb S67-27 in ELISA experiments

Inhibitor[b]		Conc. of inhibitor (μM) yielding 50% inhibition of mAb[a]	
		S25-2	S67-27
Kdo		400	25
Kdo-(2→8)-Kdo		100	50
7-Azido-7-*epi*-Kdo	**6**	800	100
7-*O*-Methyl-Kdo	**9**	200	100
Kdo-(2→8)-7-*O*-methyl-Kdo	**13**	100	0.8
Kdo-(2→8)-Kdo-1.7-lactone	**19**	200	50
Kdo-Me-(2→8)-Kdo	**20**	100	25

[a] Mab S25-2 was tested with Kdo-(2→8)-Kdo-BSA and S67-27 with Kdo-BSA as solid phase antigen.
[b] All compounds are α-allyl glycosides.

as 7-azido-7-deoxy-7-*epi*-Kdo (**6**), 7-*O*-methyl-Kdo (**9**), or the disaccharide monomethyl ester derivative **20**. Most surprisingly, the Kdo-(2→8)-7-*O*-methyl-Kdo derivative **13** was 30 times more active (IC_{50} 0.8 μM) than the Kdo monosaccharide. Results from ongoing studies of the ligands by surface plasmon resonance spectroscopy as well as cocrystallization experiments with Kdo-specific monoclonal antibodies will be reported in due course.

3. Conclusions

Modifications at the 7-position of Kdo monosaccharide allyl glycosides have been achieved via selective derivatization of 8-*O*-TBS protected intermediates, whereas per-O-carbonylation of the α-Kdo-(2→8)-α-Kdo disaccharide enabled a selective reaction at OH-7 of the inner Kdo unit. In addition, OH-7 of the α-Kdo-(2→8)-α-Kdo-(2→O-Allyl) and OH-5 of α-Kdo-(2→4)-α-Kdo-(2→O-Allyl) disaccharide, respectively, react smoothly in acetic acid with the carboxylic group of adjacent Kdo units to provide the corresponding interresidue lactones, which may be further utilized for selective derivatization of carboxylic groups at distal Kdo moieties.

3.1. General

Concentration of solutions was performed at reduced pressure at temperatures <40 °C. Dichloromethane and dry pyridine were dried by stirring with CaH_2 (5 g/L) for 16 h, then distilled and stored under argon over molecular sieves 0.4 nm. DMF was stirred with CaH_2 (5 g/L) for 16 h at 20 °C, distilled under reduced pressure, and stored over activated molecular sieves 0.3 nm. Column chromatography was performed on Silica Gel 60 (230–400 mesh, Merck). Analytical TLC was performed using Silica Gel 60 F_{254} HPTLC plates with 2.5 cm concentration zone (Merck). Spots were detected by treatment with anisaldehyde–H_2SO_4. Size-exclusion chromatography was performed either on Bio-Gel® P-2 Gel, Extra fine <45 μm (wet) from Bio-Rad Laboratories or on Sephadex® G-10. Ion exchange chromatography was performed on a Dowex 50 W X 8 resin, H+ form, 50–100 mesh. Melting points were determined on a Kofler hot stage microscope and are uncorrected. 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 or Avance 400 spectrometer (^1H at 300.13 MHz, ^{13}C at 75.47 MHz and ^1H at 400.13 MHz, ^{13}C at 100.61 MHz, respectively) using standard Bruker NMR software. ^1H NMR spectra were referenced to tetramethylsilane for solutions in $CDCl_3$ or 2,2-dimethyl-2-silapentane-5-sulfonic acid for solutions in D_2O and referenced to the solvent peak for solutions in acetic acid-d_4 (δ 1.97). ^{13}C NMR spectra were referenced to chloroform for solutions in $CDCl_3$ (δ 77.00) or dioxane (δ 67.40) for solutions in D_2O. For mass spectrometry analyses, samples were dissolved in the appropriate amount of water to give a solution of approx. 1 nmol/μL. Just before analysis an aliquot of the respective sample was diluted in 50% aq acetonitrile containing 0.1% formic acid to give a final concentration of ~10 pmol/μL. This solution was directly infused into the electrospray ion source of a Waters Micromass Q-TOF Ultima Global. Capillary voltage was adjusted to obtain approx. 200 counts/s. The MS had been previously tuned with [Glu1]-fibrinopeptide B to give highest possible sensitivity and a resolution of approximately 10,000 (FWHM). The TOF analyzer was calibrated using [Glu1]-fibrinopeptide B again to ensure high mass accuracy.

3.2. Inhibition experiments

Kdo-(2→8)-Kdo-BSA and Kdo-BSA in carbonate-buffer (50 mM, pH 9.2) were coated onto MaxiSorp microtiter plates (96-well, U-bottom, NUNC) with 2 pmol of ligand per cup at 4 °C over night. Mab S25-2 and mAb S67-27 were titrated in quadruplicates on Kdo-(2→8)-Kdo-BSA and Kdo-BSA, respectively, and the concen-

tration yielding OD_{405} between 0.9 and 1.1 was determined. An equal volume of this antibody dilution and a serial dilution of the inhibitor in PBS (30 μL each) were mixed in V-shaped microtiter plates (NUNC). After incubation (15 min, 37 °C), 50 μL of the mixture was added to antigen-coated ELISA plates and incubated for 1 h at 37 °C. After two washings, peroxidase-conjugated goat anti-mouse IgG (heavy and light chain-specific; Dianova; diluted 1:1000) was added and incubation was continued for 1 h at 37 °C. After three washings, the plates were washed once in substrate buffer (0.1 M sodium citrate, pH 4.5). Substrate solution was freshly prepared and was composed of azino-di-3-ethylbenzthiazolinesulfonic acid (1 mg) dissolved in substrate buffer (1 mL) with sonication in an ultrasound water bath for 3 min followed by the addition of hydrogen peroxide (25 μL of a 0.1% solution). After 30 min at 37 °C, the reaction was stopped by the addition of aq oxalic acid (2%) and the plates were read by a microplate reader (Dynatech MR 5000) at 405 nm. Further details of the assay have been described already.[21]

3.3. Methyl (allyl 8-*O*-*tert*-butyldimethylsilyl-4,5-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (2)

4-Nitrophenyl chloroformate (403 mg, 2 mmol) was added to a solution of **1** (580 mg, 1.42 mmol) in dry pyridine. The solution was stirred for 12 h at rt and an additional portion of 4-nitrophenyl chloroformate (100 mg, 0.5 mmol) was added and stirring was continued for 3 h at rt. The suspension was concentrated and coevaporated three times with addition of toluene. The residue was dissolved in CH_2Cl_2 (50 mL) and washed three times with satd aq $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated. Purification of the residue on a column of silica gel (3:1 toluene/EtOAc) afforded **2** as a syrup. Yield: 510 mg (83%); $[\alpha]_D^{20} + 36.6$ (*c* 3.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.86–5.73 (m, 1H, =CH–), 5.19 (dq, 1H, ^2J 1.5, ^3J 17.1 Hz, $=CH_{2\ trans}$), 5.11 (dq, 1H, ^2J 1.5, ^3J 10.5 Hz, $=CH_{2\ cis}$), 5.01 (ddd, 1H, $J_{4,3a}$ $J_{4,3e}$ 2.2, $J_{4,3a}$ 3.9 Hz, H-4), 4.88 (dd, 1H, $J_{5,4}$ 9.3, $J_{5,6}$ 1.2 Hz, H-5), 4.14 (dddd, 1H, ^2J 14.5, ^3J 5.0, ^4J 1.5 Hz, OCH_2), 3.94–3.88 (m, 2H, H-7, H-8a), 3.83 (dddd, 1H, OCH_2), 3.76 (dd, 1H, $J_{6,7}$ 9.0 Hz, H-6), 3.75 (s, 3H, CO_2CH_3), 3.64 (m, 1H, H-8b), 2.80 (br s, 1H, OH), 2.56 (dd, 1H $J_{3e,3a}$ 16.1 Hz, H-3*e*), 1.97 (dd, 1H, H-3*a*), 0.85 [s, 9H, $(CH_3)_3C$], 0.11 and 0.09 [2s, 6H, $(CH_3)_2Si$]. Anal Calcd for $C_{19}H_{32}SiO_9$: C, 52.76; H, 7.45. Found: C, 52.17; H, 7.43.

3.4. Methyl (allyl 8-*O*-*tert*-butyldimethylsilyl-4,5-*O*-carbonyl-3-deoxy-7-*O*-trifluoromethanesulfonyl-α-D-*manno*-oct-2-ulopyranosid)onate (3)

To a stirred solution of **2** (34.4 mg, 79.5 μmol) in CH_2Cl_2 (3 mL) pyridine (550 μL) was added. The solution was cooled to −20 °C and Tf_2O (70 μL) was added. TLC (1:1 toluene/EtOAc) showed halfway conversion after 3 h and the reaction was complete after continued stirring overnight. CH_2Cl_2 (20 mL) was added and the reaction mixture was extracted with satd cold aq $NaHCO_3$. The organic phase was washed with water, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified on a column of silica (6:1 toluene/EtOAc) to give **3** (34.3 mg, 76 %) as a colorless syrup; $[\alpha]_D^{20} + 10$ (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.90–5.77 (m, 1H, =CH–), 5.25 (dq, 1H, ^2J 1.5, ^3J 17.2 Hz, $=CH_{2\ trans}$), 5.18 (dq, 1H, ^3J 10.5 Hz, $=CH_{2\ cis}$), 5.16 (m, 1H, H-7), 5.07 (ddd, 1H, $J_{4,5}$ 8.5 Hz, H-4), 4.85 (dd, 1H, $J_{5,6}$ 1.6 Hz, H-5), 4.30 (dd, 1H, $J_{6,7}$ 8.0 Hz, H-6), 4.16 (dd, 1H, $J_{8a,7}$ 2.5, $J_{8a,8b}$ 12.5 Hz, H-8a), 4.12 (dddd, 1H, ^2J 14.5, ^3J 5.5, ^4J 1.5 Hz, OCH_2), 4.02 (dd, 1H, $J_{8b,7}$ 4.0 Hz, H-8b), 3.89 (dddd, 1H, ^2J 12.5, ^3J 5.7 Hz, OCH_2), 3.81 (s, 3H, CO_2CH_3), 2.81 (dd, 1H, $J_{3e,3a}$ 16.2, $J_{3e,4}$ 4.0 Hz, H-3*e*), 2.16 (dd, 1H, $J_{3a,3e}$ 16.2, $J_{3a,4}$ 3.3 Hz, H-3*a*), 0.91 [s, 9H, $(CH_3)_3C$], 0.12 and 0.11 [2s, 6H, $(CH_3)_2Si$]; ^{13}C NMR ($CDCl_3$): δ 168.00 (CO_2CH_3), 152.73 [O–C(=O)–O], 132.84 (=CH–), 118.31 (q, $J_{C,F} \sim 319$ Hz, CF_3SO_3–), 117.49 ($=CH_2$), 97.77 (C-2), 84.32 (C-7), 71.47 (C-5), 71.05 (C-4), 66.46 (C-6), 65.32 (OCH_2), 61.06 (C-8), 53.12 (CO_2CH_3), 32.67 (C-3), 25.73 (SiC*Me*$_3$) and 18.22 (Si–C*Me*$_3$). ESI-TOFMS: *m/z* 565.139; calcd for $[C_{20}H_{31}F_3O_{11}SSi+H]^+$: 565.138.

3.5. Methyl (allyl 7-azido-8-*O*-*tert*-butyldimethylsilyl-4,5-*O*-carbonyl-3,7-dideoxy-β-L-*gulo*-oct-2-ulopyranosid)onate (4)

A stirred solution of **3** (23.6 mg, 41.8 μmol) in dry DMF (~2 mL) was cooled with an ice-bath. A 20% aq LiN_3 solution (23 μL) was added and TLC (3:1 toluene/EtOAc) showed complete conversion after continued stirring at rt overnight. EtOAc (20 mL) was added to the reaction mixture and extracted twice with H_2O. A second 20 mL portion of EtOAc was added to the flask and again extracted with water twice. The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Silica gel chromatography of the residue (5:1 toluene/EtOAc) afforded 13.5 mg (59.6 μmol, 71%) of **4** as a syrup; $[\alpha]_D^{20} + 12$ (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.96–5.86 (m, 1H, =CH–), 5.30 (dq, 1H, ^2J 1.6, ^3J 17.1 Hz, $=CH_{2\ trans}$), 5.20 (dq, 1H, ^3J 10.3 Hz, $=CH_{2\ cis}$), 5.03 (ddd, 1H, $J_{4,5}$ 8.3 Hz, H-4), 4.80 (dd, 1H, $J_{5,6}$ 1.8 Hz, H-5), 4.27 (dddd, 1H, ^2J 12.1, ^3J 5.7 Hz, OCH_2), 4.04 (dd, 1H, $J_{6,5}$ 1.7, $J_{6,7}$ 8.8 Hz, H-6), 3.95 (dddd, 1H, ^3J 5.9, ^4J 1.4 Hz, OCH_2), 3.91–3.88 (m, 2H, H-8a, 8b), 3.83 (s, 3H, CO_2CH_3), 3.77 (ddd, 1H, $J_{7,6}$ 8.7, $J_{7,8a} \sim J_{7,8b}$ 3.2 Hz, H-7), 2.83 (dd, 1H, $J_{3e,3a}$ 16.1, $J_{3e,4}$ 5.0 Hz, H-3*e*), 2.13 (dd, 1H, $J_{3a,4}$ 3.5 Hz, H-3*a*), 0.90 (s, 9H, Me_3C), 0.11 and 0.10 (2s, 6H, Me_2Si); ^{13}C NMR ($CDCl_3$): δ 168.46 (CO_2CH_3), 153.26 [O–C(=O)–O], 133.18 (=CH–), 117.77 ($=CH_2$), 97.44 (C-2), 72.46 (C-5), 71.65 (C-4), 70.14 (C-6), 65.18 (OCH_2), 62.58 (C-8), 62.48 (C-7), 53.06 (CO_2CH_3), 32.65 (C-3), 25.76 (Si–C*Me*$_3$), 18.22 (Si–C*Me*$_3$), 5.46 and 5.62 (Si–Me)$_2$. ESI-TOFMS: *m/z* 475.184; calcd for $[C_{19}H_{31}N_3O_8Si+NH_4]^+$: 475.222.

3.6. Methyl (allyl 7-azido-4,5-*O*-carbonyl-3,7-dideoxy-β-L-*gulo*-oct-2-ulopyranosid)onate (5)

A solution of **4** (12.0 mg, 26.2 μmol) in dry CH_3CN (2 mL) was stirred and cooled to 0 °C with an ice-bath. 300 μL of a solution of HF (2% in CH_3CN) was added and the ice-bath was removed. TLC (1:1 toluene/EtOAc) showed complete conversion after 2 h. A spatula tip of $NaHCO_3$ was added and stirring was continued for 15 min. After concentration of the reaction mixture, CH_2Cl_2 (20 mL) was added and extracted with satd cold aq $NaHCO_3$. The organic phase was dried ($MgSO_4$) and concentrated in vacuo. Column chromatography of the residue on silica gel (5:2 toluene/EtOAc) gave 8.6 mg (25.1 μmol, 96%) **5** as off-white syrup; $[\alpha]_D^{20} + 16$ (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.97–5.84 (m, 1H, =CH–), 5.30 (d, 1H, ^3J 17.1 Hz, $=CH_{2\ trans}$), 5.20 (d, 1H, ^3J 10.5 Hz, $=CH_{2\ cis}$), 5.05–5.00 (m, 1H, H-4), 4.86 (d, 1H, $J_{5,4}$ 8.8 Hz, H-5), 4.26 (dd, 1H, ^2J 12.1, ^3J 5.6, OCH_2), 4.10 (d, 1H, $J_{6,7}$ 9.0 Hz, H-6), 3.99–3.92 (m, 1H, H-8a), 3.96–3.92 (m, 1H, OCH_2), 3.93–3.90 (m, 1H, H-7), 3.83 (s, 3H, CO_2CH_3), 3.83–3.78 (m, 1H, H-8b), 2.86 (dd, 1H, $J_{3e,3a}$ 16.2, $J_{3e,4}$ 4.0 Hz, H-3*e*), 2.13 (dd, 1H, $J_{3a,3e}$ 16.1, $J_{3a,4}$ 3.3 Hz, H-3*a*); ^{13}C NMR ($CDCl_3$): δ 168.45 (CO_2CH_3), 153.25 [O–C(=O)–O], 133.17 (=CH–), 117.67 ($=CH_2$), 97.55 (C-2), 72.31 (C-5), 71.63 (C-4), 70.68 (C-6), 65.27 (OCH_2), 63.07 (C-7), 61.69 (C-8), 53.10 (CO_2CH_3), 32.58 (C-3). ESI-TOFMS: *m/z* 366.077; calcd for $[C_{13}H_{17}N_3O_8+Na]^+$: 366.091.

3.7. Sodium (allyl 7-azido-3,7-dideoxy-β-L-*gulo*-oct-2-ulopyranosid)onate (6)

To a stirred solution of **5** (8.6 mg, 25.1 μmol) in dry MeOH (5 mL) 0.1 M methanolic NaOMe (200 μL) was added at rt. TLC (3:1 EtOAc/MeOH) showed complete conversion after 1 h. Dowex 50 (H^+) cation-exchange resin (prewashed with dry MeOH) was

added until the pH was neutral. The resin was filtered off, washed with dry MeOH, and the filtrate was concentrated in vacuo. The residue was dissolved in H_2O (5 mL) and 0.1 M NaOH (2 mL), and the solution was stirred at rt for 2 h. Dowex 50 (H^+) ion-exchange resin was added until pH was 7.5. After filtration and lyophilization of the filtrate, the residue was purified on a Sephadex G-10 column (1.3 × 50 cm, 95:5 H_2O/EtOH) to give **6** as amorphous powder (8.0 mg, 98%) after lyophilization of appropriate fractions; $[\alpha]_D^{20}$ +48 (*c* 0.5, H_2O); ^{1}H NMR (D_2O, 300 MHz): δ 5.99–5.87 (m, 1H, =CH–), 5.31 (dq, 1H, ^2J 1.7, ^3J 17.1 Hz, $=CH_{2\ trans}$), 5.17 (dq, 1H, ^3J 10.3 Hz, $=CH_{2\ cis}$), 4.00 (ddd, 1H, $J_{4,5}$ 3.0 Hz, H-4), 3.99 (ddd, 1H, ^2J 12.1, ^3J 4.8, ^4J 1.2, OCH_2), 3.87 (ddd, 1H, ^2J 11.1, ^3J 5.9 Hz, OCH_2), 3.87–3.83 (m, 1H, H-7), 3.85–3.80 (m, 1H, H-8a), 3.79 (br d, 1H, H-5), 3.72 (dd, 1H, $J_{6,5}$ 1.1, $J_{6,7}$ 8.4 Hz, H-6), 3.64 (dd, 1H, $J_{7,8b}$ 7.1, $J_{8a,8b}$ 13.0 Hz, H-8b), 2.00 (dd, 1H, $J_{3e,3a}$ 13.3, $J_{3e,4}$ 5.2 Hz, $J_{3e,5}$ < 1 Hz, H-3*e*), 1.75 (dd, 1H, $J_{3a,4}$ 12.0 Hz, H-3*a*); ^{13}C NMR (D_2O): δ 175.93 (CO_2), 134.63 (=CH–), 118.77 ($=CH_2$), 100.93 (C-2), 73.21 (C-6), 67.99 (C-5), 66.56 (C-4), 65.35 (OCH_2), 64.63 (C-7), 61.08 (C-8), 34.93 (C-3). ESI-TOFMS: *m/z* 304.110; calcd for $[C_{11}H_{17}N_3O_7+H]^+$: 304.114.

3.8. Methyl (allyl 8-*O*-*tert*-butyldimethylsilyl-4,5-*O*-carbonyl-3-deoxy-7-*O*-methyl-α-D-*manno*-oct-2-ulopyranosid)onate (7)

A stirred solution of **2** (29.3 mg, 67.7 μmol) in dry CH_2Cl_2 (2 mL) was cooled with an ice-bath (0 °C). A 2-M solution of (trimethylsilyl)diazomethane in Et_2O (35 μL) was added and the solution turned yellow. The color disappeared immediately after a tetrafluoroboric acid solution (9 μL, 48 wt % in H_2O) was added. TLC (2:1 toluene/EtOAc) showed incomplete reaction after 30 min. Addition of TMS-CHN_2 (20 μL) followed by addition of HBF_4 (5 μL) was repeated twice within another 30 min until beginning of decomposition was observed. The solution was diluted with CH_2Cl_2 (20 mL), washed twice with satd aq $NaHCO_3$ (2 × 10 mL) and water (10 mL), and dried ($MgSO_4$). Concentration of the residue and purification of the residue on a column of silica (5:1 toluene/EtOAc) gave **7** (16.2 mg, 54%) as a colorless syrup. Further elution of the column afforded unreacted educt **2** (12.0 mg, 41%); $[\alpha]_D^{20}$ +21 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.91–5.78 (m, 1H, =CH–), 5.24 (dq, 1H, ^2J 1.6, ^3J 17.2 Hz, $=CH_{2\ trans}$), 5.15 (dq, 1H, ^3J 10.5 Hz, $=CH_{2\ cis}$), 5.04–4.99 (m, 1H, H-4), 4.95 (dd, 1H, $J_{5,6}$ 1.8, $J_{5,4}$ 8.8 Hz, H-5), 4.14 (dddd, 1H, ^2J 12.3, ^3J 5.1, ^4J 1.5 Hz, OCH_2), 3.98 (dd, 1H, $J_{8a,7}$ 2.0, $J_{8a,8b}$ 11.8 Hz, H-8a), 3.96 (dd, 1H, $J_{6,5}$ 1.7, $J_{6,7}$ 9.4 Hz, H-6), 3.85 (dddd, 1H, ^2J 12.3, ^3J 5.9, ^4J 1.3 Hz, OCH_2), 3.83 (s, 3H, CO_2CH_3), 3.79 (dd, 1H, $J_{8b,7}$ 3.7 Hz, H-8b), 3.53 (ddd, 1H, H-7), 3.47 (s, 1H, OCH_3), 2.72 (dd, 1H, $J_{3e,3a}$ 16.0, $J_{3e,4}$ 4.0 Hz, H-3*e*), 2.14 (dd, 1H, $J_{3a,4}$ 3.5 Hz, H-3*a*), 0.90 (s, 9H, Me_3C) and 0.06 (s, 6H, $SiMe_2$); ^{13}C NMR ($CDCl_3$): δ 168.71 (CO_2CH_3), 132.32 (=CH–), 117.24 ($=CH_2$), 97.36 (C-2), 78.57 (C-7), 72.89 (C-5), 71.45 (C-4), 67.07 (C-6), 64.91 (OCH_2), 60.57 (C-8), 58.21 (OCH_3), 52.98 (CO_2CH_3), 32.92 (C-3), 25.91 ($SiCMe_3$), 18.38 (Si–CMe_3). ESI-TOFMS: *m/z* 447.212; calcd for $[C_{20}H_{34}O_9Si+H]^+$: 447.203.

3.9. Methyl (allyl 4,5-*O*-carbonyl-3-deoxy-7-*O*-methyl-α-D-*manno*-oct-2-ulopyranosid)-onate (8)

A 2% solution of HF in CH_3CN (50 μL) was added to a stirred and cooled (0 °C) solution of **7** (15.0 mg, 33.6 μmol) in dry CH_3CN (2 mL). TLC (1:1 toluene/EtOAc) showed complete conversion after 2 h. $NaHCO_3$ (~20 mg) was added and stirring was continued for 15 min. The solvent was removed, CH_2Cl_2 (10 mL) was added, and extracted with satd aq $NaHCO_3$. The organic phase was dried ($MgSO_4$) and concentrated in vacuo. Column chromatography of the residue (2:1 toluene/EtOAc) afforded 9.2 mg (27.7 μmol, 82%) of **8** as a colorless syrup; $[\alpha]_D^{20}$ +23 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.94–5.84 (m, 1H, =CH–), 5.29 (dq, 1H, ^2J 1.6, ^3J 17.2 Hz, $=CH_{2\ trans}$), 5.19 (dq, 1H, ^2J 1.4, ^3J 10.5 Hz, $=CH_{2\ cis}$), 5.03 (ddd, 1H, $J_{4,5}$ 8.4 Hz, H-4), 4.93 (dd, 1H, $J_{5,6}$ 1.9 Hz, H-5), 4.15 (dddd, 1H, ^2J 12.2, ^3J 5.4, ^4J 1.5 Hz, OCH_2), 4.02 (dd, 1H, $J_{6,7}$ 9.5 Hz, H-6), 4.00–3.95 (m, 1H, H-8a), 3.87 (dddd, 1H, OCH_2), 3.81 (s, 3H, CO_2CH_3), 3.78–3.71 (m, 1H, H-8b), 3.61 (ddd, 1H, $J_{7,8a} \sim J_{7,8b}$ 3.5 Hz, H-7), 3.49 (s, 1H, OCH_3), 2.75 (dd, 1H, $J_{3e,3a}$ 16.0, $J_{3e,4}$ 4.3 Hz, H-3*e*), 2.16 (dd, 1H, $J_{3a,4}$ 3.8 Hz, H-3*a*); ^{13}C NMR ($CDCl_3$): δ 168.44 (CO_2CH_3), 153.74 [O–C(=O)–O], 133.27 (=CH–), 117.77 ($=CH_2$), 97.41 (C-2), 77.93 (C-7), 72.75 (C-5), 71.51 (C-4), 66.99 (C-6), 65.02 (OCH_2), 58.89 (C-8), 57.97 (OCH_3), 53.05 (CO_2CH_3) and 32.94 (C-3).

3.10. Sodium (allyl 3-deoxy-7-*O*-methyl-α-D-*manno*-2-oct-2-ulopyranosid)onate (9)

A 0.1-M solution of methanolic NaOMe (0.8 mL) was added to a stirred solution of **8** (9.2 mg, 27.7 μmol) in dry MeOH (2 mL). Reaction was complete after 1 h and Dowex 50 (H^+) ion-exchange resin was added until the pH was ~6. After filtration and concentration of the solvent, the residue was dissolved in 0.1 M aq NaOH (1.5 mL) and the solution was stirred for 1 h at rt. Dowex 50 (H^+) was added until the suspension pH was 6. The resin was filtered off and the filtrate was lyophilized. Purification of the residue on a Sephadex G-10 column (1.3 × 50 cm, 95:5 H_2O/EtOH, 10 mg L^{-1} NaCl) gave **9** as white amorphous powder (8.4 mg, 96%); $[\alpha]_D^{20}$ +40 (*c* 0.8, H_2O); ^{1}H NMR (D_2O, 400 MHz): δ 5.93–5.83 (m, 1H, =CH–), 5.27 (dq, 1H, ^2J 1.5, ^3J 17.3 Hz, $=CH_{2\ trans}$), 5.15 (dd, 1H, ^2J 1.3, ^3J 10.7 Hz, $=CH_{2\ cis}$), 4.04–3.99 (m, 1H, H-4), 4.00–3.95 (m, 1H, H-8a), 3.93–3.88 (m, 1H, OCH_2), 3.89–3.88 (m, 1H, H-5), 3.75 (dd, 1H, ^2J 12.1, ^3J 5.9, OCH_2), 3.66–3.61 (m, 1H, H-8b), 3.64–3.61 (m, 1H, H-6), 3.57–3.54 (m, 1H, H-7), 3.45 (s, 1H, OCH_3), 1.99 (dd, 1H, $J_{3e,3a}$ 13.9, $J_{3e,4}$ 5.8 Hz, H-3*e*), 1.72 (dd, 1H, $J_{3a,3e} \sim J_{3a,4}$ 12.7 Hz, H-3*a*); ^{13}C NMR (D_2O): δ 175.25 (CO_2), 133.59 (=CH–), 117.88 ($=CH_2$), 99.96 (C-2), 78.61 (C-7), 69.95 (C-6), 66.28 (C-5), 65.94 (C-4), 64.36 (OCH_2), 59.09 (C-8), 57.70 (OCH_3), 34.08 (C-3). ESI-TOFMS: *m/z* 291.064; calcd for $[C_{12}H_{20}O_8-H]^-$: 291.109.

3.11. Methyl (4,5;7,8-di-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-methyl (allyl 4,5-*O*-carbonyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate (11)

To a stirred solution of **10** (100.8 mg, 122.8 μmol) in dry MeOH (15 mL), 1 M methanolic NaOMe solution (0.6 mL) was added. The reaction was complete after 45 min and Dowex 50 (H+) ion-exchange resin was added to neutralize the solution. The resin was filtered off and the filtrate was concentrated and the residue was dried to give 61.0 mg (115.9 μmol) of the intermediate material. The residue was dissolved in anhydrous THF (10 mL), stirred, and cooled to -5 °C. *Sym*-collidine (200 μL) was added to the reaction mixture. Trichloromethyl chloroformate (50 μL) was dissolved in dry THF (10 mL) and added dropwise within 20 min to the solution. TLC (EtOAc) showed complete reaction after 4 h. The solvent was evaporated and the residue was subjected to column chromatography (1:5 toluene/EtOAc) to give 62.7 mg (103.7 μmol, 84%) of **11** as colorless syrup; $[\alpha]_D^{20}$ +19 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.89–5.75 (m, 1H, =CH–), 5.28 (dq, 1H, ^2J 1.6, ^3J 17.2 Hz, $=CH_{2\ trans}$), 5.19 (dq, 1H, ^2J 1.4, ^3J 10.4 Hz, $=CH_{2\ cis}$), 5.13 (ddd, 1H, $J_{4',3'}$ 3.3, $J_{4',5'}$ 8.7 Hz, H-4′), 5.08–5.05 (m, 1H, H-5), 5.07–5.04 (m, 1H, H-4), 5.06–5.01 (m, 1H, H-7′), 4.96 (dd, 1H, $J_{5',4'}$ 8.8 Hz, $J_{5',6'}$ 1.6, H-5′), 4.78 (dd, 1H, $J_{8a',7'}$ 5.7, $J_{8a',8b'}$ 9.0 Hz, H-8′a), 4.63 (dd, 1H, $J_{8b',7'}$ 8.6, H-8′b), 4.36 (dd, 1H, $J_{6',7'}$ 4.8 Hz, H-6′), 4.16–4.08 (m, 1H, H-7), 4.01 (dddd, 1H, ^2J 12.5, ^3J 5.1, ^4J 1.6 Hz, OCH_2), 3.83 (dddd, 1H, OCH_2), 3.82 (s, 3H, CO_2CH_3), 3.81 (s, 3H, CO_2CH_3), 3.75–3.70 (m, 1H, H-6), 3.71–3.55 (m, 2H, H-8), 2.88 (dd, 1H, $J_{3'e,3'a}$ 16.4, $J_{3'e,4'}$ 3.4 Hz, H-3′*e*), 2.86 (dd, 1H, H-3*e*), 2.26 (dd, 1H, H-3′*a*), 2.09 (dd, 1H, H-3*a*); ^{13}C NMR ($CDCl_3$): δ

168.46 and 168.43 (CO_2CH_3), 154.58 and 153.99 and 152.97 [O–(C=O)–O], 133.08 (=CH–), 117.48 ($=CH_2$), 97.84 and 97.30 (C-2 and C-2′), 74.04 (C-7′), 72.62 (C-5), 72.17 and 71.66 (C-4 and C-5′), 71.42 (C-4′), 69.61 (C-6), 68.86 (C-6′), 67.60 (C-7), 66.26 (C-8), 66.09 (C-8′), 64.74 (OCH_2), 53.42 and 53.14 (CO_2CH_3), 32.45 and 32.39 (C-3 and C-3′). ESI-TOFMS: *m/z* 605.118; calcd for $[C_{24}H_{28}O_{18}+H]^+$: 605.135.

3.12. Methyl (4,5;7,8-di-*O*-carbonyl-3-deoxy-α-D-*manno*-2-octulopyranosyl)onate-(2→8)-methyl (allyl 4,5-*O*-carbonyl-3-deoxy-7-*O*-methyl-α-D-*manno*-2-octulopyranosid)onate (12)

A 48% aq tetrafluoroboric acid solution (25 μL) was added to a stirred and cooled (0 °C) solution of **11** (87 mg, 144 μmol) in dry CH_2Cl_2 (18 mL), followed by addition of a 2-M etheral (trimethylsilyl)diazomethane solution (120 μL). Stirring of the cooled solution was continued and three additional amounts of the $TMSCHN_2$ solution were added portion-wise (120, 120, and 60 μL) within 30 min. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with satd aq $NaHCO_3$ and H_2O. The organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified on a column of silica gel (1:3 toluene/EtOAc) to give **12** (67.8 mg, 76%) as a colorless syrup; $[\alpha]_D^{20}$ +28 (*c* 0.7, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.87–5.77 (m, 1H, =CH–), 5.28 (dq, 1H, ^2J 1.6, ^3J 17.2 Hz, $=CH_{2\ trans}$), 5.19 (dq, 1H, ^2J 1.4, ^3J 10.4 Hz, $=CH_{2\ cis}$), 5.08 (ddd, 1H, $J_{4',5'}$ 8.7 Hz, H-4′), 5.04–4.98 (m, 2H, H-4, H-7′), 4.93 (dd, 1H, $J_{5,4}$ 8.6, $J_{5,6}$ 1.5 Hz, H-5), 4.89 (dd, 1H, $J_{5',6'}$ 1.7 Hz, H-5′), 4.71 (dd, 1H, $J_{8a',7'}$ 5.7, $J_{8a',8b'}$ 9.0 Hz, H-8′a), 4.63 (dd, 1H, $J_{8b',7'}$ 8.1 Hz, H-8′b), 4.13 (dd, 1H, $J_{6',7'}$ 6.1 Hz, H-6′), 4.01 (dddd, 1H, ^2J 12.5, ^3J 5.2, ^4J 1.6 Hz, OCH_2), 3.86 (dddd, 1H, OCH_2), 3.83 (s, 3H, CO_2CH_3), 3.81 (s, 3H, CO_2CH_3), 3.80–3.62 (m, 2H, H-8a, H-8b), 3.80–3.72 (m, 1H, H-6), 3.76–3.68 (m, 1H, H-7), 3.51 (s, 3H, OCH_3), 2.95 (dd, 1H, $J_{3'e,3'a}$ 16.3, $J_{3'e,4'}$ 3.9 Hz, H-3′*e*), 2.82 (dd, 1H, $J_{3e,3a}$ 16.0, $J_{3e,4}$ 4.2 Hz, H-3*e*), 2.11 (dd, 1H, $J_{3'a,4'}$ 3.4 Hz, H-3′*a*), 2.10 (dd, 1H, $J_{3a,3e}$ 16.1, $J_{3a,4}$ 3.7 Hz, H-3*a*); ^{13}C NMR ($CDCl_3$): δ 168.10 and 167.48 (CO_2CH_3), 153.84, 153.45 and 152.58 [O–(C=O)–O], 133.07 (=CH–), 117.56 ($=CH_2$), 97.66 and 97.42 (C-2 and C-2′), 76.92 (C-7), 73.17 and 72.58 (C-7′, C-4), 71.74 (C-5), 71.29 (C-5′), 70.94 (C-4′), 68.93 (C-6′), 68.47 (C-6), 66.15 (C-8′), 64.78 (-OCH_2), 64.55 (C-8), 59.73 (OCH_3), 53.33 and 53.13 (CO_2CH_3), 32.59 and 32.55 (C-3 and C-3′). ESI-TOFMS: *m/z* 641.094; calcd for $[C_{25}H_{30}O_{18}+Na]^+$: 641.132.

3.13. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-sodium (allyl 3-deoxy-7-*O*-methyl-α-D-*manno*-oct-2-ulopyranosid)onate (13)

A solution of **12** (25.8 mg, 41.8 μmol) in dry MeOH (3 mL) and NaOMe in MeOH (0.7 mL, 0.1 M) was stirred for 1 h at rt. The solution was brought to neutral pH by addition of Dowex 50 (H^+) ion-exchange resin. After filtration and removal of the solvent, the residue was dissolved in water (3 mL) and 0.1 M NaOH (2 mL) was added. The solution was stirred for 1 h at rt and the pH of the solution was adjusted to 8 by addition of Dowex 50 (H^+) resin. The resin was removed, and the filtrate was lyophilized. Purification of the residue on a Sephadex G-10 column (1.3 × 50 cm, 95:5 H_2O/EtOH, 10 mg L^{-1} NaCl) furnished **13** as colorless amorphous powder (22.9 mg, 99%); $[\alpha]_D^{20}$ +61 (*c* 0.8, H_2O); ^{1}H NMR (D_2O, 400 MHz): δ 5.93 (m, 1H, =CH–), 5.32 (dq, 1H, ^2J 1.2, ^3J 17.2 Hz, $=CH_{2\ trans}$), 5.22 (dq, 1H, ^2J 1.0, ^3J 10.5 Hz, $=CH_{2\ cis}$), 4.08–3.85 (m, 8H, including H-4, H-5, H-4′, H-5′, H-7′, H-8′a and OCH_2), 3.77 (ddd, 1H, $J_{8a,7}$ 2.4, $J_{7,8b}$ 7.2 Hz, H-7), 3.71 (dd, 1H, $J_{8a,8b}$ 10.4 Hz, H-8a), 3.59 (dd, 1H, $J_{8'b,7'}$ 8.0, $J_{8'a,8'b}$ 12.8 Hz, H-8′b), 3.57 (dd, 1H, $J_{6,7}$ 8.8, $J_{6,5}$ 1.2 Hz H-6), 3.55 (dd, 1H, $J_{6',7'}$ 8.8, $J_{6',5'}$ 1.2 Hz H-6′), 3.54 (s, 3H, OCH_3), 3.43 (dd, 1H, H-8b), 2.11* (dd, 1H, $J_{3e,3a}$ 13.1, $J_{3e,4}$ 4.9 Hz, H-3*e*) and 2.05* (dd, 1H, $J_{3'e,3'a}$ 13.2, $J_{3'e,4'}$ 5.0 Hz, H-3′*e*), 1.81* (dd, 1H, $J_{3a,4}$ 12.8 Hz, H-3*a*) and 1.79* (dd, 1H, $J_{3'a,4'}$ 12.8 Hz, H-3′*a*); ^{13}C NMR (D_2O): δ 175.96 and 175.90 (CO_2), 134.67 (=CH–), 118.33 ($=CH_2$), 101.11 and 101.03 (C-2 and C-2′), 78.68 (C-7), 72.47 (C-6′), 72.05 (C-6), 70.32 (C-7′), 67.26, 67.05, 66.97 and 66.88 (C-5, C-5′, C-4 and C-4′), 65.33 (C-8), 64.72 (OCH_2), 64.23 (C-8′), 60.06 (OCH_3), 34.95 (C-3 and C-3′). ESI-TOFMS: *m/z* 535.132; calcd for $[C_{20}H_{32}O_{15}+Na]^+$: 535.163.

3.14. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-methyl (allyl 7,8-*O*-carbonyl-3-deoxy-5-*O*-trifluoromethanesulfonyl-α-D-*manno*-oct-2-ulopyranosid)onate (15)

Compound **14** (8.2 mg, 11.1 μmol) was dissolved in dry CH_2Cl_2 (3 mL). Dry pyridine (15 μL) and Tf_2O (10 μL) were added under cooling (−20 °C) and the solution was stirred at 4 °C for 20 h. Dry MeOH (100 μL) was added, the solution was stirred for 1 h, and concentrated. Purification of the residue on a column of silica gel (1:2 toluene/EtOAc) gave **15** (9 mg, 95%) as colorless crystals; R_f 0.66 (1:2 toluene/EtOAc); mp 160 °C, $[\alpha]_D^{20}$ +72.8 (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.85 (m, 1H, =CH–), 5.40 (br s, 2H, H-5, H-5′), 5.28–5.19 (m, 3H, H-4′, $=CH_{2\ trans}$, $=CH_{2\ cis}$), 5.15 (ddd, 1H, $J_{8'a,7'}$ 2.8, $J_{6',7'}$ 9.5 Hz, H-7′), 4.84 (m, 2H, H-7, H-4), 4.76 (dd, 1H, $J_{8a',8b'}$ 12.6 Hz, H-8a′), 4.63 (t, 1H, $J_{8a,7}$ 8.4 Hz, H-8a), 4.52 (dd, 1H, $J_{8b,8a}$ 8.8, $J_{8b,7}$ 5.5 Hz, H-8b), 4.16 (dd, 1H, $J_{6',5'}$ 1.1 Hz, H-6′), 4.06 (d, 1H, $J_{6,7}$ 6.2 Hz, H-6), 3.98 (m, 2H, OCH_2), 3.96 (dd, 1H, $J_{8'b,7'}$ 3.6 Hz, H-8b′), 3.86, 3.83 (2s, 6H, 2 × OCH_3), 2.40–2.04 (4H, H-3*e*, H-3*a*, H-3′*e*, H-3′*a*), 2.09 (br s, 6H, 2 × OAc), 1.99 (s, 3H, OAc) and 1.98 (s, 3H, OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.64, 170.24, 169.90 and 169.60 (CH_3CO), 167.34, 166.68 (C-1, C-1′), 132.55 (=CH–), 118.06 ($=CH_2$), 99.07 and 98.71 (C-2, C-2′), 81.08 (C-5), 73.17 (C-7), 70.64 (C-6), 70.08 (C-6′), 67.74 (C-7′), 66.05 (C-8), 65.97 (C-4), 65.82 (C-4′), 65.48 (OCH_2), 64.19 (C-5′), 61.24 (C-8′), 53.36 and 53.01 (2 × OCH_3), 34.06 (C-3′), 30.85 (C-3), 20.75 (OAc), 20.63 (2 × OAc), 20.57 (OAc). ESI-TOFMS: *m/z* 875.19; calcd for $[C_{31}H_{39}F_3O_{22}S+Na]^+$: 875.15.

3.15. Methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-(allyl 7,8-carbonyl-3,5-dideoxy-α-D-*threo*-oct-5-en-2-ulopyranosid)onate (16)

Compound **15** (16 mg, 19 μmol) was dissolved in butanone (10 mL). After warming up to 40 °C, Cs-trifluoroacetate (13 mg, 52 μmol) and four drops of pyridine were added. After 24 h at 40 °C, the solvent was removed. Purification of the residue on a column of silica gel (4:1 toluene/EtOAc→ 2:1 toluene/EtOAc) gave **16** (10 mg, 74%) as a white powder, R_f 0.5 (1:1 toluene/EtOAc); $[\alpha]_D^{20}$ +71.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.84 (m, 1H, =CH–), 5.36 (br s, 1H, H-5′), 5.25 (dq, 1H, $=CH_{2\ trans}$), 5.22–5.14 (m, 4H, H-4′, H-7′, H-5, $=CH_{2\ cis}$), 5.00 (dd, 1H, $J_{7,8a}$ 5.7, $J_{7,8b}$ 8.5 Hz, H-7), 4.74 (dd, 1H, $J_{8'b,8'a}$ 12.3, $J_{7',8'a}$ 4.0 Hz, H-8′a), 4.73 (dd, 1H, $J_{8b,8a}$ 8.5 Hz, H-8a), 4.56 (t, 1H, H-8b), 4.48 (ddd, 1H, $J_{4,3a}$ 5.7, $J_{4,3e}$ 5.3, $J_{4,5}$ 3.8 Hz, H-4), 4.21 (dd, 1H, $J_{6',5'}$ 1.3 Hz, $J_{6',7'}$ 9.3 Hz, H-6′), 4.21–4.16 (m, 1H, OCH_2), 4.08 (dd, 1H, $J_{8'b,7'}$ 2.8, Hz, H-8′b), 3.97 (m, 1H, OCH_2), 3.85 and 3.84 (2s, 6H, 2 × OCH_3), 2.39 (dd, 1H, $J_{3e,3a}$ 13.9 Hz, H-3*e*), 2.31 (dd, 1H, H-3*a*), 2.16–2.01 (m, 2H, H-3′*a*, H-3′*e*), 2.11 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.00 (s, 3H, OAc) and 1.97 (s, 3H, OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.67, 170.26, 169.85 and 169.67 (CH_3CO), 167.90 (C-1, C-1′), 154.50 [O–(C=O)–O], 147.10 (C-6), 132.93 (=CH–), 117.82 ($=CH_2$), 103.60 (C-5), 99.13 (C-2), 98.81 (C-2′), 75.68 (C-7), 69.08 (C-6′), 67.82 (C-7′), 66.61 (C-8), 66.13 (OCH_2), 66.08 (C-4′), 64.31 (C-5′), 63.19 (C-4), 61.19 (C-8′), 53.13 and 53.06 (2 × OCH_3), 36.37 (C-3), 32.19 (C-3′), 20.80 (OAc), 20.74 (OAc) and 20.62 (2 × OAc). ESI-TOFMS: *m/z* 725.22; calcd for $[C_{30}H_{38}O_{19}+Na]^+$: 725.19.

3.16. Sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-sodium (allyl 3,5-dideoxy-α-D-*threo*-oct-5-en-2-ulopyranosid)onate (17)

Compound **16** (10 mg, 14 μmol) was dissolved in dry MeOH (6 mL) and 0.1 M methanolic NaOMe solution (0.5 mL) was added. The solution was stirred for 2 h at rt and made neutral by addition of Dowex H^+ resin. The cation exchange resin was removed by filtration and the filtrate was concentrated. The remaining residue was dissolved in H_2O (2 mL) and 0.1 M aq NaOH solution (0.5 mL) was added. The solution was kept at rt for 1 h and then made neutral with Dowex H^+. The resin was filtered off and the filtrate was lyophilized. Purification of the residue on a Bio-Gel P2 column gave **17** (4.4 mg, 60%) as amorphous powder; $[\alpha]_D^{20}$ +109 (*c* 0.2, MeOH). ^{1}H NMR (400 MHz, D_2O): δ 5.92 (m, 1H, =CH–), 5.29 (dq, 1H, $=CH_{2\ trans}$), 5.18 (dq, 1H, $=CH_{2\ cis}$), 5.16 (d, 1H, $J_{4,5}$ 2.8 Hz, H-5), 4.42 (ddd, 1H, $J_{4,3a}$ 8.0, $J_{4,3e}$ 6.5 Hz, H-4), 4.15 (dd, 1H, H-7), 4.14 – 4.08 (m, 1H, O*CH*$_2$), 4.02 (ddd, 1H, $J_{4',5'}$ 2.1 Hz, H-4′), 4.00 (br s, 1H, H-5′), 3.99–3.94 (m, 3H, O*CH*$_2$, H-8′a, H-7′), 3.79 (dd, 1H, $J_{8a,7}$ 5.2, $J_{8a,8b}$ 11.6 Hz, H-8a), 3.68 (dd, 1H, $J_{6',5'}$ 0.6, $J_{6',7'}$ 8.1 Hz, H-6′), 3.67 (dd, 1H, $J_{8'b,7'}$ 8.1, $J_{8'b,8'a}$ 12.2 Hz, H-8′b), 3.62 (dd, 1H, $J_{8b,7}$ 7.0 Hz, H-8b), 2.30 (ddd, 1H, $J_{3e,3a}$ 13.3, $J_{3e,5}$ 1.1 Hz, H-3*e*), 2.08 (dd, 1H, $J_{3'e,3'a}$ 13.2, $J_{3'e,4'}$ 5.2 Hz, H-3′*e*), 1.96 (dd, 1H, H-3*a*), 1.74 (dd, 1H, $J_{3'a,4'}$ 11.7 Hz, H-3′*a*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.34 (C-1′), 175.08 (C-1), 151.79 (C-6), 134.77 (=CH–), 118.14 ($=CH_2$), 101.79 (C-5), 101.74, 101.45 (C-2, C-2′), 73.03 (C-6′), 72.50 (C-7), 70.99 (C-7′), 67.26 (C-5′), 66.66 (C-4′), 66.16 (OCH_2), 65.14 (C-4), 63.95 (C-8′), 63.76 (C-8), 35.39 (C-3) and 32.83 (C-3′). ESI-TOFMS: *m/z* 503.15; calcd for $[C_{19}H_{26}O_{14}+Na]^+$: 503.13.

3.17. Lactone formation of sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-sodium (allyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate

A solution of **18** (3.35 mg) in CD_3COOD (1 mL) was kept at rt for 4 weeks. The solution was concentrated in vacuo and the residue was lyophilized 3 times from DMSO (2 mL) to give lactone **19** (3.3 mg) as solid material. ^{1}H NMR (400 MHz, CD_3COOD): δ 5.89 (m, 1H, =CH), 5.25 (dq, 1H, $=CH_{2\ trans}$), 5.12 (dq, 1H, $=CH_{2\ cis}$), 4.90 (dt, 1H, $J_{8b,7}$ 3.0, $J_{6,7}$ 8.8 Hz, H-7), 4.21 (ddd, 1H, $J_{3e,4}$ 4.5, $J_{5,4}$ 3.0 Hz, H-4), 4.13–4.00 (m, 6H, including H-5, H-4′, H-5′, H-7′, H-8b and O*CH*$_2$), 3.96 (m, 1H, O*CH*$_2$), 3.90 (dd, 1H, H-6), 3.88 (dd, 1H, H-8′a), 3.85 (dd, 1H, $J_{6',7'}$ 8.8 Hz, H-6′), 3.75 (dd, 1H, $J_{8'b,7'}$ 5.2, $J_{8'a,8'b}$ 11.7 Hz, H-8′b), 2.41 (t, 1H, $J_{3'a,4'} \sim J_{3'a,3'e}$ 12.5 Hz, H-3′*a*), 2.17 (dd, 1H, $J_{3e,3a}$ 12.9 Hz, H-3*e*), 2.01 (t, 1H, H-3*a*), and 1.86 (dd, 1H, $J_{3'e,4'}$ 4.6 Hz, H-3′*e*); ^{13}C-HSQC (100 MHz, CD_3COOD): δ 133.1 (=CH–), 116.2 ($=CH_2$), 74.5 (C-7), 70.9 (C-6′), 69.5 (C-6), 69.0 (C-7′), 66.6 and 66.0 (C-5, C-5′), 65.4 (OCH_2), 65.2 (C-4, C-4′), 62.8 (C-8′), 57.4 (C-8), 33.5 (C-3), 32.6 (C-3′) ESI-TOFMS: *m/z* 521.1329 $[M+H_2O+Na]^+$; calcd for $[C_{19}H_{27}O_{14}+H_2O+Na]^+$: 521.1549.

3.18. Methyl (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→8)-sodium (allyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid) onate (20)

A solution of **19** (3.3 mg) in dry MeOH (2 mL) was stirred at rt for 30 min and then kept at −18 °C for 14 h. The solution was concentrated and the residue was purified on a column of BioGel P-2 (1.3 × 50 cm, 95:5 H_2O/EtOH). Pooling and lyophilization of appropriate fractions furnished **20** as colorless solid. Yield: 2.90 mg (91% based on crystalline **18**); $[\alpha]_D^{20}$ +36 (*c* 0.25, H_2O); ^{1}H NMR (D_2O, 400 MHz): δ 5.96 (m, 1H, =CH–), 5.33 (dq, 1H, $=CH_{2\ trans}$), 5.23 (dq, 1H, $=CH_{2\ cis}$), 4.15–3.85 (m, 8H, H-4, H-4′, H-5, H-5′, H-7, H-7′, H-8′a, O*CH*$_2$), 3.83 (s, 3H, OCH_3), 3.69–3.56 (m, 6H, H-8a, H-8b, H-6, H-6′, H-8′b and O*CH*$_2$), 2.16* (dd, 1H, $J_{3e,3a}$ 13.5, $J_{3e,4}$ 5.2 Hz, H-3*e*) and 2.03* (dd, 1H, $J_{3'e,3'a}$ 13.1, $J_{3'e,4'}$ 5.5 Hz, H-3′*e*), 1.89* (dd, 1H, $J_{3a,3e} \sim J_{3a,4}$ 12.8 Hz, H-3*a*) and 1.79* (t, 1 H, $J_{3'a,3'e} \sim J_{3'a,4'}$ 12.2 Hz, H-3′*a*); selected ^{13}C NMR signals (D_2O): δ 134.4 (=CH–), 118.2 ($=CH_2$), 72.7 (C-6′), 72.0 (C-6), 69.5 (C-7), 64.6 (OCH_2), 54.1 (OCH_3), 34.9 (C-3 and C-3′). ESI-TOFMS: *m/z* 535.1417 $[M+H]^+$; calcd for $[C_{20}H_{32}O_{15}+Na]^+$: 535.1639.

3.19. Lactone formation of sodium (3-deoxy-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→4)-sodium (allyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate

A solution of **21** (0.5 mg) in CD_3COOD (1 mL) was kept at rt for 3 weeks. The solution was concentrated in vacuo and the residue was lyophilized 3 times from DMSO (2 mL) to give **22** (0.5 mg) as solid material. ^{1}H NMR (400 MHz, CD_3COOD): δ 5.84 (m, 1H, =CH), 5.37 (br d, 1H, $J_{5,4}$ 3.8 Hz, H-5), 5.23 (dq, 1H, $=CH_{2\ trans}$), 5.08 (dq, 1H, $=CH_{2\ cis}$), 4.53 (ddd, 1H, H-4), 4.15 – 3.63 (m, 12H, including H-6, H-7, H-8a, H-8b, H-4′, H-5′, H-6′, H-7′, H-8′a, H-8′b, O*CH*$_2$), 2.43 (t, 1H, $J_{3'a,3'e} \sim J_{3'a,4'}$ 13.0 Hz, H-3′*a*), 2.31 (dd, 1H, $J_{3a,3e}$ 13.2, $J_{3e,4}$ 5.2 Hz, H-3*e*) and 1.79 (m, 2H, H-3′*e*, H-3*a*); selected ^{13}C NMR data (CD_3COOD): δ 133.5 (=CH–), 115.9 ($=CH_2$), 68.7 (C-5), 66.1 (C-4), 33.3 (C-3) and 32.8 (C-3′).

3.20. Hydrolysis of lactones

Lactone **19** (0.5 mg) was dissolved in a solution of sodium phosphate buffer (pD 7.0) in D_2O. ^{1}H NMR spectra were recorded in intervals of 5 h. Lactone **22** (0.3 mg) was treated in a similar way.

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