Monatshefte für Chemie 133, 561–570 (2002)

Monatshefte für Chemie
Chemical Monthly

Synthesis of C-8 Deuterated Glycosides of 3-Deoxy-*D*-manno-oct-2-ulosonic Acid (*Kdo*) Related to Chlamydial Lipopolysaccharides

Paul Kosma[1,*], **Martina Strobl**[1], **Andreas Hofinger**[1], **Jens Ø. Duus**[2], **Bent O. Petersen**[2], **Klaus Bock**[2], and **Helmut Brade**[3]

[1] Institut für Chemie, Universität für Bodenkultur, A-1190 Wien, Austria
[2] Department of Chemistry, Carlsberg Laboratory, DK-2500 Valby, Denmark
[3] Forschungszentrum Borstel, Institut für Medizin und Biowissenschaften, D-23845 Borstel, BRD

Summary. Methyl glycosides of *Kdo* and a $(2 \rightarrow 8)$-linked *Kdo* disaccharide were prepared which contain a deuterium label at C-8 of the reducing unit. The label was introduced in fair diastereoselectivity upon reduction of an aldehyde group using a chiral borane complex derived from N-benzyloxycarbonyl-(*S*)-proline which produced the 8-(*S*)-deuterated derivative as the major isomer. Further coupling with a *Kdo* bromide gave the α-$(2 \rightarrow 8)$-linked disaccharide in good yield. The deprotected disaccharide serves as a model for NMR spectroscopic studies on the side chain conformation of a carbohydrate epitope from the bacterial pathogen *Chlamydia*.

Keywords. Carbohydrate; Reduction; *Kdo*; Deuterium labeling; Chlamydia.

Introduction

Members of the order Chlamydiales are intracellular parasites of increasing biomedical importance [1]. In the outer membrane of the cell, the bacteria contain a lipopolysaccharide (LPS) which constitutes a highly immunogenic epitope and induces inflammatory responses in a host organism [2]. The epitope is composed of a trisaccharide of 3-deoxy-*D*-manno-oct-2-ulopyranosylonic acid (*Kdo*) residues of the sequence α-*Kdop*-$(2 \rightarrow 8)$-α-*Kdop*-$(2 \rightarrow 4)$-α-*Kdop* which may be exploited for the serological diagnosis of chlamydial infections [3]. Previously, the terminal disaccharide unit of the epitope has been synthesized as an allyl glycoside, and the crystal structure has been determined [4, 5]. The structural data revealed the presence of an interglycosidic hydrogen bond extending from the terminal carboxyl group to OH-7 of the reducing *Kdo* unit. Although the side chain linkage between the *Kdo* moieties inherently confers additional conformational freedom, it

* Corresponding author. E-mail: pkosma@edv2.boku.ac.at

nonetheless is part of a specific epitope which is recognized by monoclonal antibodies. The binding interaction of the disaccharide with *Kdo*-specific monoclonal antibodies has been thoroughly studied using NOE experiments [6] and immunochemical methods [7]. Meanwhile, preliminary crystal data have been obtained from a complex of the disaccharide ligand with the *Fab* fragment of a $(2 \rightarrow 8)$-linked *Kdo* disaccharide specific monoclonal antibody [8].

The determination of the solution conformation of the disaccharide ligand, however, has been hampered by the fact that the geminal protons at C-8 of the reducing *Kdo* unit occur at identical chemical shifts in the ^{1}H NMR spectrum [9, 10]. Hence we have set out to synthesize *Kdo* glycosides deuterated at C-8 in order to be able to differentiate between H-8-(*R*) and H-8-(*S*) to be used for NOESY and ROESY experiments and conformational studies of the hydroxymethyl groups of *Kdo*-glycosides [11].

Results and Discussion

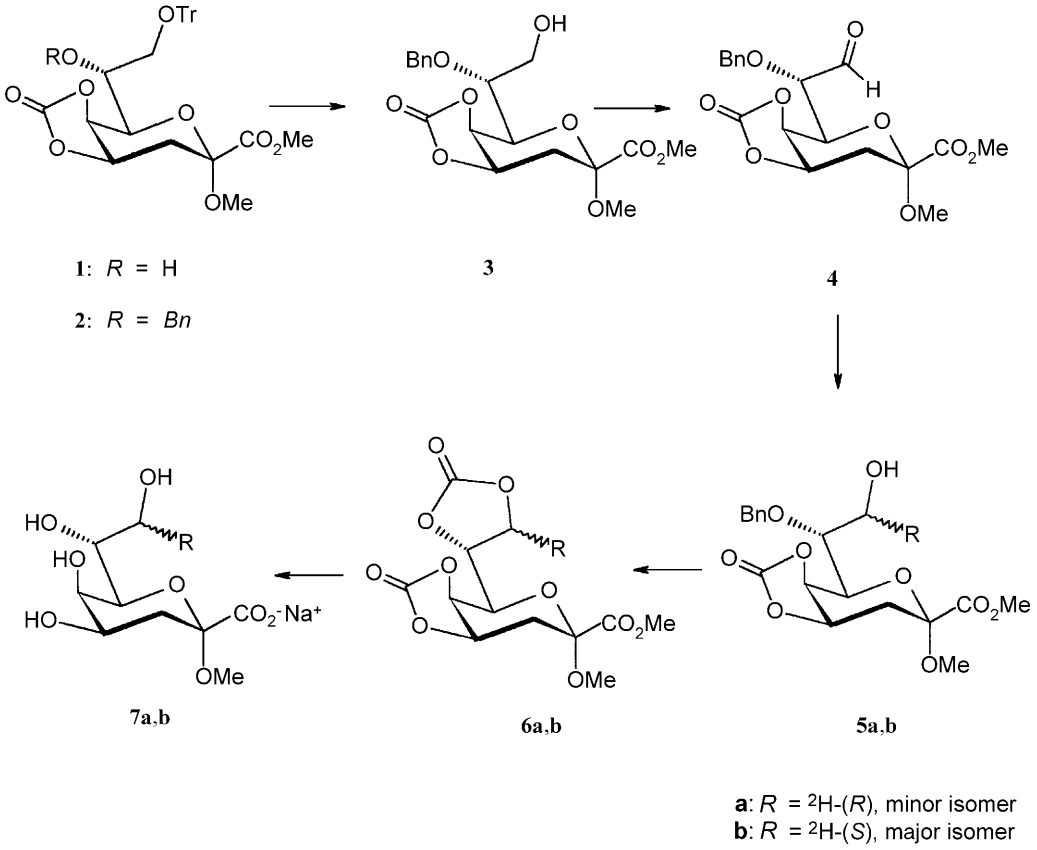
Synthesis of glycosides

For the synthesis of the deuterated *Kdo* glycosides, the previously reported 8-O-trityl-derivative **1** [12] was O-benzylated using O-benzyl trichloroacetimidate/triflic acid [13] to give the 7-O-benzyl derivative **2** in 70% yield. Removal of the trityl group with formic acid in MeCN proceeded smoothly in 84% yield. Subsequent *Swern* oxidation [14] of **3** afforded the aldehyde **4** in 87% yield.

Numerous methods have been developed for the stereoselective reduction of carbonyl groups, which in general are far less selective for aldehydes compared to keto groups [15]. Low diastereoselectivities were observed in the reduction of compound **4** with deuterated boranes (Table 1). The outcome of the reaction was based on the integration values of the H-8-(*R*) and H-8-(*S*) signals in the ^{1}H NMR spectra. The use of deuterated borane complexes prepared from $NaBD_4$ and D-mannitol, (-)-menthol, or (*R*)- and (*S*)-mandelic acid did not result in selective formation of the deuterated hydroxymethyl group. The same held true for other chiral borane complexes derived from phenyl 1,3-dioxaborolidine and an (*R*)-proline complex [16, 17]. By contrast, reduction of **4** at -126°C in *THF* with a complex prepared from $NaBD_4$ and N-benzyloxycarbonyl-(*S*)-proline [17] proceeded in excellent yield (99%) and with fair diastereoselectivity ($\sim 56\%$ *de*)

Table 1. Diastereoselectivities observed upon reduction of **4** with deuterated boranes

T/°C	Solvent	Reagent	Ratio **5a**:**5b**
-70	*THF*	$NaBD_4$, (*S*)-mandelic acid	45:55
-126	*THF*	$NaBD_4$, N-*Fmoc*-(*R*)-proline	50:50
-126	*THF*	$NaBD_4$, N-*Fmoc*-(*S*)-proline	37:63
-126	*THF*	$NaBD_4$, N-*Cbz*-(*R*)-proline	50:50
-126	*THF*	$NaBD_4$, N-*Cbz*-(*S*)-proline	22:78 (*de* = 56)
-126	*THF*	^{2}H-Catecholborane/B-*n*-butyl-oxazaborolidine [15]	60:40

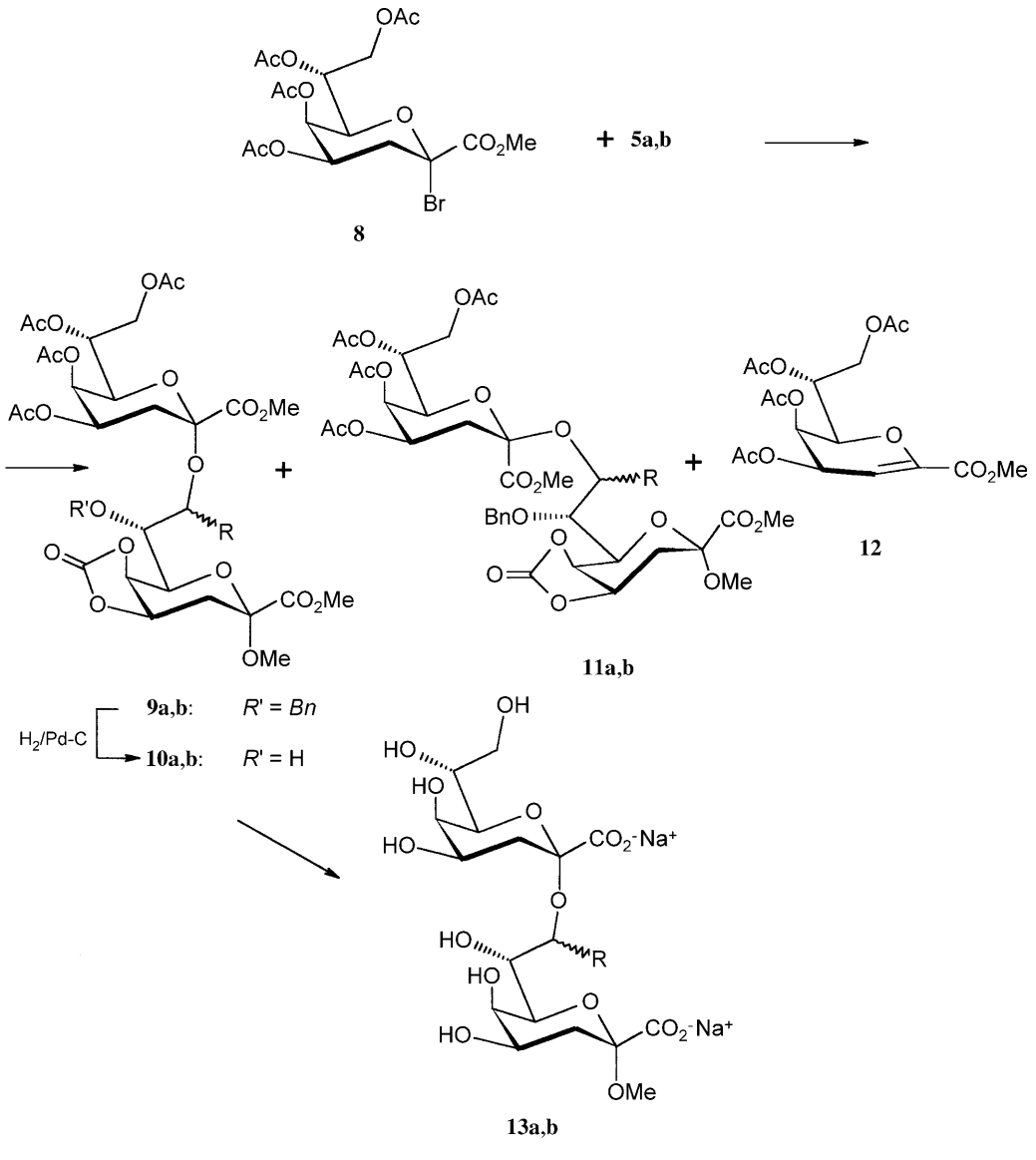


Scheme 1

to give a mixture of **5a** and **5b**. For the assignment of the H-8-(*R*) and H-8-(*S*) protons, the mixture of diastereoisomers **5a** and **5b** was subsequently converted into a cyclic 7,8-O-carbonate ester following quantitative removal of the benzyl group by hydrogenolysis on 5% Pd–C. Reaction of the resulting diol with diphosgene/*sym*-collidine in *THF* at low temperature gave the 4,5;7,8-di-O-carbonyl derivatives **6a** and **6b** in 40% yield (2 steps). Irradiation of H-6 lead to a significant nuclear *Overhauser* enhancement for the H-8 signal observed at 4.62 ppm, whereas the second (minor) H-8 signal at 4.66 ppm remained unaffected. Thus, the major isomer was assigned as the ^{2}H-8-(*S*) substituted isomer **6b**, since only H-8 corresponding to H-8-pro-*R* in the non-labeled compound would be close to H-6. For comparison, compounds **6a** and **6b** were fully deprotected by *Zemplén* de-O-acylation and subsequent hydrolysis of the methyl ester group with aqueous 0.2 *M* NaOH. The ^{1}H NMR data of the methyl ketoside **7b** are in good agreement with reported data for an 8-(*S*) deuterated *Kdo* ammonium salt [18]. In the α-pyranose form of the latter compound, H-8-(*R*) was observed at 3.62 ppm ($J_{7,8(R)} = 5.8$ Hz), which closely matches the corresponding values found for isomer **7b** (3.64 ppm, $J_{7,8(R)} = 6.5$ Hz).

Glycosylation of **5a** and **5b** with 2.5 equivalents of *Kdo* bromide donor **8** using $Hg(CN)_2$ in $MeNO_2$ furnished the glycal ester **12** and the disaccharides **9** and **11** in 85% yield (based on **5**, $\alpha/\beta = 5{:}1$) which were separated by chromatography.

Crystallization of the anomeric disaccharide mixture afforded the crystalline α-(2 → 8)-linked disaccharides **9a** and **9b**. The assignment of the anomeric configuration of the terminal *Kdo* unit was based on the downfield shift of the H-4′ signal (5.33 ppm) and on the upfield shift of the H-3′*e* signal (2.27 ppm). The β-linked isomers **11a** and **11b** displayed the signal of H-3′*e* at 2.42 ppm. Removal of the 7-O-benzyl group by hydrogenolysis with 5% Pd–C afforded **10a** and **10b** in quantitative yield. Subsequent treatment of **10a** and **10b** with sodium methoxide and aqueous 0.2 *M* NaOH gave the target disaccharides **13a** and **13b** in 95% yield after purification on Bio-Gel P2.



Scheme 2

NMR spectroscopic analysis of the glycosides

The assignment of the mixture of **13a** and **13b** was performed from data obtained at 800 MHz using standard one- and two-dimensional experiments. Even at the high field used severe overlap was observed, and some of the important $^{1}H–^{1}H$ coupling constants could only be obtained from slices of two-dimensional spectra acquired with good digital resolution (*e.g.* $^{3}J_{7,8}$ of the ^{2}H-8-(*S*) compound from a slice of an HSQC spectrum, Fig. 1). The identities of the *R* and *S* compounds in the mixtures were assigned based on the relative signal intensity, with the *S* compound as the major component. All chemical shifts except that of H-8 were completely identical for the two compounds; the corresponding data are reported in Table 2.

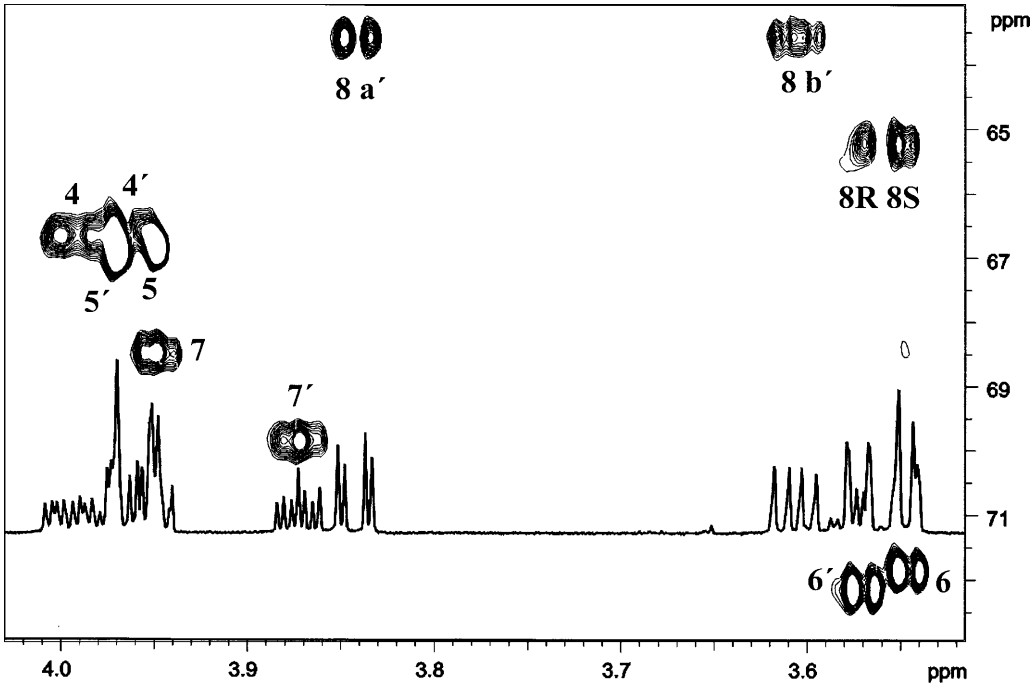


Fig. 1. Two-dimensional plot of the 800 MHz HSQC spectrum of **13a** and **13b** displaying cross-peaks of the side chain protons

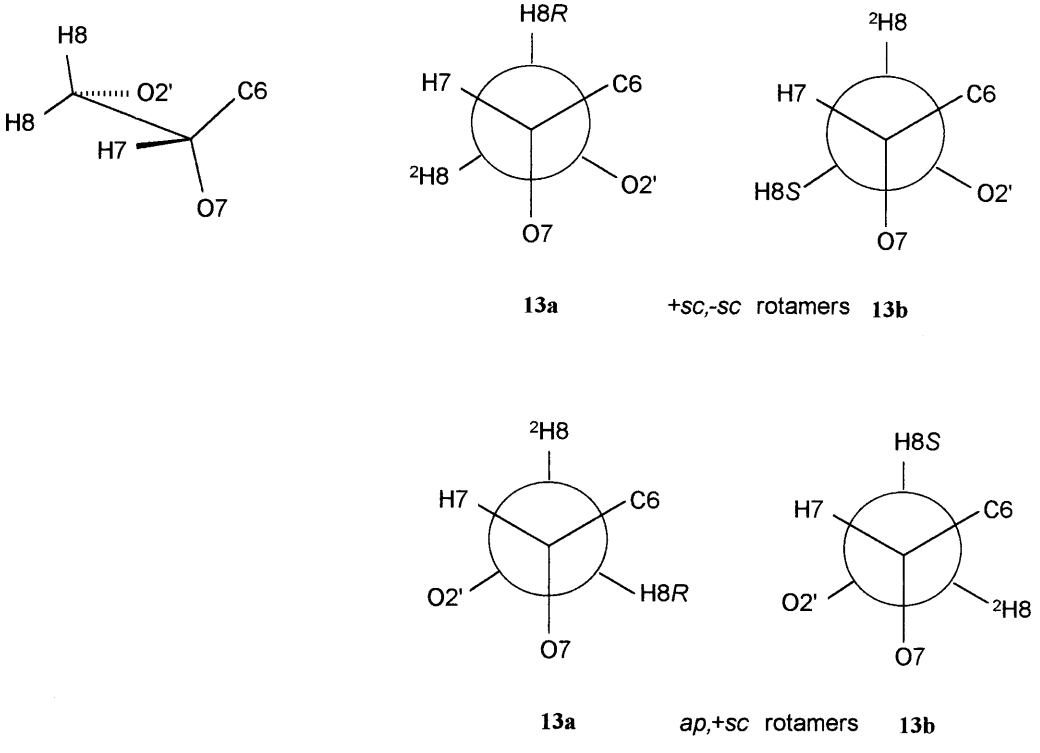
Table 2. NMR parameters for **13a** and **13b** assigned at 800 MHz for ^{1}H and 201 MHz for ^{13}C (the only difference between **13a** and **13b** is observed for H-8)

Position	$\delta(^{1}H)$/ppm	J/Hz	$\delta(^{13}C)$/ppm	Position	$\delta(^{1}H)$/ppm	J/Hz	$\delta(^{13}C)$/ppm
MeO	3.107		51.3				
3*a*	1.725	12.2	34.9	3′a	1.754	12.2	34.9
3*e*	1.952	5.1, 13.0		3′e	2.006	5.1, 12.9	
4	3.965		66.7	4′	3.996	3.0	66.6
5	3.970	<1	66.9	5′	3.950	<1	66.9
6	3.547	7.3	71.9	6′	3.571	8.9	72.2
7	3.949		68.5	7′	3.872		69.9
H-8 of ^{2}H-(*R*)	3.569	<2	65.3	8′a	3.843	2.8	63.6
H-8 of ^{2}H-(*S*)	3.547	5.5		8′b	3.606	6.5, 11.8	

As expected, a difference in the isotope effect between the two compounds in the mixture is too small to be observed.

The assignment of the H-8 protons allows for a more thorough analysis of the conformational preference around the C-7–C-8 linkage of the reducing residue. Previous studies [6] have been hampered by complete overlap of the H-8-pro-*R* and H-8-pro-*S* protons even at 800 MHz. In the present study, the analysis has solely focussed on this problem associated with the side chain conformation, as previous studies have described the overall conformational properties in detail [6, 9].

The major disaccharide **13b** is (*S*)-configured at C-8, as determined in compounds **6a** and **6b**; that is, the proton observed corresponds to H-8-pro-*R* in a nondeuterated disaccharide and *vice versa*. In the published crystal structure of the corresponding allyl disaccharide [5] the rotamer with synclinal orientation between the glycosidic oxygen (O2′) and both O-7 and C-6 of the reducing residue is observed. The $-sc$ arrangement for C-6 and $+sc$ arrangement of O-7 also gives rise to H-7 being placed between the two H-8 protons. Such an arrangement is expected to give two small three-bond coupling constants [11] of approximately 1 and 3 Hz, as H-8 with an antiperiplanar orientation relative to O-7 is expected to give the smallest coupling. The proton in this arrangement is the pro-*R* in the nondeuterated disaccharide and the remaining proton in ^{2}H-8-(*S*), which has a coupling of 5.5 Hz. This indicates that in solution some rotational averaging of the C-7–C-8 linkage takes place, and this is most likely an averaging between the rotamer observed in the crystal and the rotamer which places H-8-pro-*R*



Scheme 3

antiperiplanar to H-7 [11]. Only such an averaging will give a larger value for H-8 in ^{2}H-8-(*S*) and still a small value for H-8 in ^{2}H-8-(*R*). An approximate population of the *sc*,*sc* and *sc*,*ap* rotamers of 1:1 would give a coupling constant close to 5.5 Hz for H-8 in ^{2}H-8-(*S*) (Scheme 3).

Unfortunately, no additional information about the conformation could be obtained from the NOESY spectra, partly due to overlap and partly to very small NOEs observed in general. No attempts were made to obtain ROESY spectra.

Experimental

General

Melting points were determined with a hot stage and are uncorrected. Optical rotations were measured with a Perkin-Elmer 243 B polarimeter. ^{1}H NMR spectra were recorded at 297 K with Bruker AC 300F and DPX instruments operating at 300 MHz for ^{1}H using $CDCl_3$ as solvent and *TMS* as internal standard unless stated otherwise. Coupling constants are given in Hz (first order values). Homo- and heteronuclear 2D NMR spectroscopy was performed with Bruker standard software. High-field NMR was performed in 3 mm tubes at 298 K at 799.96 MHz for ^{1}H and 201.12 MHz for ^{13}C on a Varian UNITY INOVA 800 spectrometer, using acetone (2.225 ppm) and 1,4-dioxane (67.4 ppm) as secondary standards for ^{1}H and ^{13}C, respectively.

TLC was performed on E. Merck precoated plates (5×10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$); detection was effected by spraying with anisaldehyde-H_2SO_4. For column chromatography, silica gel (40–63 μm) was used. Concentration of solutions was performed at reduced pressure and below 40°C. Elemental analyses were provided by Dr. *J. Theiner*, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, University of Vienna; the results were in good agreement with the calculated values.

Methyl (methyl 7-O-benzyl-4,5-O-carbonyl-3-deoxy-8-O-triphenylmethyl-α-D-manno-oct-2-ulopyranosid)-onate (**2**; $C_{37}H_{36}O_9$)

A solution of 880 mg (1.64 mmol) **1** and 1.04 g (4.1 mmol) benzyl 2,2,2-trichloroacetimidate in 10 cm^3 dry CH_2Cl_2 was treated with 40 mm^3 trifluoromethane sulfonic acid for 3 h at room temperature. Solid $NaHCO_3$ (2 g) was added, and stirring was continued for 15 min. The suspension was diluted with 50 cm^3 CH_2Cl_2 and washed with satd. aq. $NaHCO_3$. The organic phase was dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography on silica gel (toluene:EtOAc = 3:1) gave **2** as a colorless syrup.

Yield: 720 mg (70%); $[\alpha]_{20}^{D} = -14°$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 7.48–7.20 (m, 20 H, arom. H), 5.06 (dd, 1 H, $J_{5,4} = 8.8$, $J_{5,6} = 1.5$ Hz. H-5), 4.98 (t, H-4), 4.86 and 4.62 (AB, $J = 10.6$ Hz, OCH_2), 4.12 (dd, $J_{6,7} = 9.5$ Hz, H-6), 4.00 (ddd, $J_{7,8a} = 1.6$, $J_{7,8b} = 4.4$ Hz, H-7), 3.77 (s, OMe), 3.64 (dd, $J_{8a,8b} = 10.3$ Hz, H-8a), 3.29 (dd, H-8b), 2.82 (s, OMe), 2.72 (dd, $J_{3e,4} = 3.7$, $J_{3e,3a} = 16.1$ Hz, H-3*e*), 2.02 (dd, $J_{3a,4} = 3.2$ Hz, H-3*a*) ppm.

Methyl (methyl 7-O-benzyl-4,5-O-carbonyl-3-deoxy-α-D-manno-oct-2-ulopyranosid)-onate (**3**; $C_{18}H_{22}O_9$)

A solution of 52 mg (0.083 mmol) **2** in 5 cm^3 dry CH_3CN was stirred with 2 cm^3 of a mixture of formic acid:Et_2O = 2:1 (v/v), for 15 h at room temperature and for 2 h at 40°C. Solid $NaHCO_3$ (1 g) was added, and the suspension was diluted with 50 cm^3 CH_2Cl_2 and washed with satd. aq. $NaHCO_3$. The organic phase was dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography on silica gel (toluene:EtOAc = 1:1) afforded **3** as colorless crystals.

Yield: 27 mg (84%); m.p.: 171–172°C (EtOAc/hexane); $[\alpha]_{20}^{D} = +11^{\circ}$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 7.40–7.28 (m, 5 arom. H), 5.00 (ddd, $J_{4,5} = 8.5$ Hz, H-4), 4.95 (dd, $J_{5,4} = 8.5$, $J_{5,6} = 1.5$ Hz, H-5), 4.70 and 4.65 (AB, $J = 10.9$ Hz, OCH_2), 4.05 (dd, $J_{6,7} = 9.2$ Hz, H-6), 3.95 (m, H-8a), 3.91 (dt, $J_{7,8a} = 2.5$, $J_{7,8b} = 2.5$ Hz, H-7), 3.83 (s, OMe), 3.77 (m, H-8b), 3.28 (s, OMe), 2.72 (dd, $J_{3e,4} = 3.7$, $J_{3e,3a} = 16.0$ Hz, H-3*e*), 2.09 (dd, $J_{3a,4} = 3.2$ Hz, H-3*a*), 1.82 (dd, $J = 2.9$, $J = 9.6$ Hz, OH) ppm.

Methyl (methyl 2-O-benzyl-4,5-O-carbonyl-6-deoxy-α-D-manno-oct-7-ulo-4,7-pyranosid)-onate (**4**; $C_{18}H_{20}O_9$)

A solution of 112 mm^3 (1.3 mmol) freshly distilled oxalyl chloride in 10 cm^3 dry CH_2Cl_2 was cooled to -40°C. A solution of 222 mm^3 (2.88 mmol) *DMSO* in 1 cm^3 CH_2Cl_2 was added under N_2, followed by addition of 230 mg (0.6 mmol) **3** in 2 cm^3 CH_2Cl_2. After stirring of the solution for 15 min, 0.84 cm^3 (6 mmol) triethylamine in 2 cm^3 CH_2Cl_2 was added, and the temperature was raised to 20°C within 30 min. The suspension was diluted with 50 cm^3 CH_2Cl_2, washed with H_2O, and the organic layer was dried (Na_2SO_4) and concentrated. The residue was chromatographed on silica gel (toluene:EtOAc = 1:1) which furnished **4** as colorless crystals.

Yield: 200 mg (87%); m.p.: 169–172°C (dec., CH_2Cl_2/pentane); $[\alpha]_{20}^{D} = +15^{\circ}$ ($c = 0.6$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 9.80 (d, $J_{1,2} = 1.5$ Hz, H-1), 7.38–7.35 (m, 5 arom. H), 5.00 (ddd, $J_{6e,5} = 3.6$, $J_{6a,5} = 3.1$ Hz, H-5), 4.93 (dd, $J_{5,4} = 8.7$, $J_{4,3} = 1.5$ Hz, H-4), 4.74 and 4.62 (AB, $J =$ 10.9 Hz, OCH_2), 4.30 (d, $J_{1,2} = 1.5$ Hz, H-2), 4.06 (dd, $J_{3,2} = 9.4$ Hz, H-3), 3.83 (s, OMe), 3.19 (s, OMe), 2.81 (dd, $J_{6e,5} = 3.6$, $J_{6e,6a} = 16.2$ Hz, H-6*e*), 2.02 (dd, $J_{6a,5} = 3.1$ Hz, H-6*a*) ppm.

Methyl (methyl 7-O-benzyl-4,5-O-carbonyl-3-deoxy-8-(R)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate (**5a**; $C_{18}H_{21}DO_9$) and *methyl (methyl 7-O-benzyl-4,5-O-carbonyl-3-deoxy-8-(S)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate* (**5b**; $C_{18}H_{21}DO_9$)

N-Benzyloxycarbonyl-(*S*)-proline (1.3 g, 5.23 mmol) was lyophilized twice from 5 cm^3 D_2O and dissolved in 10 cm^3 dry *THF*. $NaBD_4$ (73 mg, 1.74 mmol) was added, and the solution was stirred under Ar for 3 h at room temperature. The reaction vessel was cooled to -126°C (methylcyclohexane and liquid N_2 as coolant), and a solution of 60 mg **4** (0.158 mmol) in 5 cm^3 *THF* was added over 30 min. After additional 30 min at -126°C the reaction was allowed to attain room temperature. The solution was concentrated and purified by column chromatography (toluene: EtOAc = 1:1) which afforded 60 mg of a mixture of **5a** and **5b** (99%) as a syrup.

Selected ^{1}H NMR data ($CDCl_3$, δ, 300 MHz): **5a**: 3.95 (d, $J_{7,8\text{-}(S)\text{-H}} = 3.0$ Hz, H-8-(*S*)); **5b**: 3.76 (d, $J_{7,8\text{-}(R)\text{-H}} = 2.5$ Hz, H-8-(*R*)).

Methyl (methyl 4,5;7,8-di-O-carbonyl-3-deoxy-8-(R)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate (**6a**; $C_{12}H_{13}DO_{10}$) and *methyl (methyl 4,5;7,8-di-O-carbonyl-3-deoxy-8-(S)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate* (**6b**; $C_{12}H_{13}DO_{10}$)

A suspension of 15 mg (0.04 mmol) **5a** and **5b** and 20 mg 5% Pd–C in 5 cm^3 MeOH was hydrogenated at atmospheric pressure for 2 h at room temperature. The suspension was filtered over a pad of Celite, and the filtrate was evaporated to dryness. A solution of the residue in 5 cm^3 pyridine was cooled to -20°C. Diphosgene (15 mm^3, 0.12 mmol) was added, and the mixture was stirred for 30 min at -20°C. MeOH (2 cm^3) was added, and the solution was coevaporated twice with toluene. The residue was chromatographed on silica gel (toluene:EtOAc = 1:3) which afforded a mixture of **6a** and **6b** as colorless crystals.

Yield: 5 mg (40%); m.p.: 154–156°C (EtOAc/pentane); $[\alpha]_{20}^{D} = +57^{\circ}$ ($c = 0.2$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 5.06 (dt, $J_{5,4} = 8.6$, Hz, H-4), 5.00 (m, H-7), 4.85 (dd, $J_{5,6} = 1.7$ Hz, H-5), 4.66 (br d, 0.3 H, H-8-(*S*)), 4.62 (br d, 0.7 H, $J_{7,8} = 6.5$ Hz, H-8-(*R*)), 4.12 (dd, $J_{6,7} = 5.1$ Hz, H-6), 3.85 (s, OMe), 3.29 (s, OMe), 2.84 (dd, $J_{3e,3a} = 16.2$ Hz, H-3*e*), 2.10 (dd, $J_{3a,4} = 3.4$ Hz, H-3*a*) ppm.

Sodium (methyl 3-deoxy-8-(R)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate(**7a**; $C_9H_{14}DO_8Na$) and *sodium (methyl 3-deoxy-8-(S)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate* (**7b**; $C_9H_{14}DO_8Na$)

A solution of 2.5 mg of **6a** and **6b** in 4 cm^3 dry MeOH was stirred with 0.3 cm^3 of an 0.1 *M* methanolic sodium methoxide solution for 4 h at room temperature. The *pH* of the solution was adjusted to 7.5 by adding Dowex-50 H^+ resin. The suspension was filtered, and the filtrate was concentrated. The residue was dissolved in 2 cm^3 H_2O and stirred with 2 cm^3 0.2 *M* NaOH for 12 h at 0°C. The *pH* of the solution was adjusted to 7.5 by adding Dowex-50 H^+ resin, the resin was filtered off, and the filtrate was lyophilized giving 2 mg of **7a** and **7b** (100%) as an amorphous solid.

$[\alpha]_{20}^{D} = +66°$ ($c = 0.2$, H_2O); ^{1}H NMR (D_2O, δ, 300 MHz): 4.02 (ddd, $J_{5,4} = 3.0$, $J_{4,3e} = 5.0$, $J_{4,3a} = 11.5$ Hz, H-4), 4.00 (dd, $J_{4,5} = 3.0$ Hz, H-5), 3.94 (m, H-7), 3.91 (m, 0.3 H, H-8-(*S*)), 3.64 (dd, 0.7 H, $J_{8,7} = 6.5$ Hz, H-8-(*R*)), 3.55 (dd, $J_{7,6} = 9.0$ Hz, H-6), 3.14 (s, OMe), 2.01 (m, H-3*e*), 1.77 (dd, $J_{3e,3a} = 13.2$ Hz, H-3*a*) ppm.

Methyl ((methyl 4,5,7,8-tetra-O-acetyl-α-D-manno-oct-2-ulopyranosyl)-onate)-(2 → 8)-(methyl 7-O-benzyl-4,5-O-carbonyl-3-deoxy-8-(R)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate (**9a**; $C_{35}H_{43}DO_{20}$) and *methyl ((methyl 4,5,7,8-tetra-O-acetyl-α-D-manno-oct-2-ulopyranosyl)-onate)-(2 → 8)-(methyl 7-O-benzyl-4,5-O-carbonyl-3-deoxy-8-(S)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate* (**9b**; $C_{35}H_{43}DO_{20}$)

A suspension of 65 mg (0.17 mmol) **5a** and **5b**, 60 mg (0.24 mmol) $Hg(CN)_2$, and 0.3 g molecular sieve 4 Å in 4 cm^3 dry $MeNO_2$ was stirred for 15 min under N_2. A solution of 320 mg (0.66 mmol) **8** in 2 cm^3 dry $MeNO_2$ was added dropwise during 30 min at room temperature, and stirring was continued for 12 h. The suspension was diluted with 50 cm^3 CH_2Cl_2 and filtered over a pad of Celite. The filtrate was washed sequentially with aq. 5% KI and satd. aq. $NaHCO_3$, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (toluene:EtOAc = 3:2) which furnished first glycal ester **12**, followed by 112 mg of the disaccharides **11a**,**b** and **9a**,**b** as a syrup (85%). Crystallization from EtOAc/ *n*-pentane furnished 72 mg of **9a**,**b** as colorless crystals.

M.p.: 119–126°C; $[\alpha]_{20}^{D} = +51°$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 7.38–7.28 (m, 5 arom. H), 5.33 (ddd, $J_{4',5'} = 3.1$, $J_{4',3'e} = 5.0$, $J_{4',3'a} = 11.8$ Hz, H-4′), 5.30 (br s, H-5′), 5.23 (ddd, $J_{7',6'} = 10.0$, $J_{7',8'a} = 2.6$, $J_{7',8'b} = 4.4$ Hz, H-7′), 5.03–4.93 (m, H-4, H-5), 4.80 and 4.63 (AB, $J = 10.7$ Hz, OCH_2), 4.55 (dd, $J_{8'a,8'b} = 12.3$ Hz, H-8′a), 4.15 (dd, $J_{6',5'} = 1.3$ Hz, H-6′), 4.14 (dd, H-8′b), 4.00 (m, H-7), 3.93 (dd, ~0.3 H, $J_{8(S),7} = 1.4$ Hz, H-8-(*S*)), 3.84 (dd, $J_{6,7} = 9.5$, $J_{6,5} = 1.4$ Hz, H-6), 3.83 (s, CO_2Me), 3.74 (s, CO_2Me), 3.65 (dd, ~0.7 H, $J_{8(R),7} = 6.3$ Hz, H-8-(*R*)), 3.27 (s, OMe), 2.77 (dd, $J_{3e,4} = 3.8$, $J_{3e,3a} = 16.0$ Hz, H-3*e*), 2.27 (dd, $J_{3'e,3'a} = 12.5$ Hz, H-3′*e*), 2.09 (s, Ac), 2.09 (t, $J_{4',3'a} = 11.8$ Hz, H-3′*a*), 2.06 (dd, $J_{3a,4} = 3.3$ Hz, H-3*a*), 1.99 (s, 3×Ac) ppm.

Methyl ((methyl 4,5,7,8-tetra-O-acetyl-α-D-manno-oct-2-ulopyranosyl)-onate)-(2 → 8)-(methyl 4,5-O-carbonyl-3-deoxy-8-(R)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate (**10a**; $C_{28}H_{37}DO_{20}$) and *methyl ((methyl 4,5,7,8-tetra-O-acetyl-α-D-manno-oct-2-ulopyranosyl)-onate)-(2 → 8)-(methyl 4,5-O-carbonyl-3-deoxy-8-(S)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate* (**10b**; $C_{28}H_{37}DO_{20}$)

A solution of 68 mg (0.086 mmol) **9a** and **9b** in 20 cm^3 dry MeOH was stirred with 75 mg 5% Pd–C under H_2 at atmospheric pressure for 2 h at room temperature. The catalyst was filtered off, and the filtrate was taken to dryness to afford **10a** and **10b** as a syrup.

Yield: 58 mg (quant.); $[\alpha]_{20}^{D} = +65°$ ($c = 1.2$, $CHCl_3$); ^{1}H NMR ($CDCl_3$, δ, 300 MHz): 5.35–5.27 (m, 2 H, H-5′, H-4′), 5.23 (ddd, $J_{7',6'} = 10.0$, $J_{7',8'a} = 2.4$, $J_{7',8'b} = 3.7$ Hz, H-7′), 5.03 (m, H-4, H-5), 4.54 (dd, $J_{8'a,8'b} = 12.4$ Hz, H-8′a), 4.17 (dd, $J_{7',8'b} = 3.7$ Hz, H-8′b), 4.16–4.06 (m, H-7, H-6′), 3.90 (dd, $J_{6,7} = 9.4$ Hz, H-6), 3.87–3.72 (m, H-8), 3.87 (s, CO_2Me), 3.81 (s, CO_2Me), 3.49 (br s, OH), 3.33

(s, OMe), 2.73 (dd, $J_{3e,4} = 3.3$, $J_{3e,3a} = 15.7$ Hz, H-3*e*), 2.20–2.02 (m, H-3′*e*, 3′*a*, 3*a*), 2.11, 2.08, 2.05, 2.00, 1.99 (5s, 5×Ac) ppm.

Methyl (sodium (3-deoxy-α-D-manno-oct-2-ulopyranosyl)-onate)-(2 → 8)-(sodium 3-deoxy-8-(R)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate (**13a**) and *methyl (sodium (3-deoxy-α-D-manno-oct-2-ulopyranosyl)-onate)-(2 → 8)-(sodium 3-deoxy-8-(S)-[8-^{2}H]-α-D-manno-oct-2-ulopyranosid)-onate* (**13b**; $C_{17}H_{24}DO_{15}Na_2$)

A solution of 10 mg of (0.013 mmol) **10a** and **10b** in 5 cm^3 dry MeOH was stirred with 0.5 cm^3 0.1 *M* methanolic sodium methoxide solution for 3 h at room temperature. The *pH* of the solution was adjusted to 7.5 by adding Dowex-50 H^+ resin. The suspension was filtered, and the filtrate was concentrated. The residue was dissolved in 2 cm^3 H_2O and stirred with 0.5 cm^3 0.2 *M* NaOH for 15 h at 0°C. The *pH* of the solution was adjusted to 8.5 by adding Dowex-50 H^+ resin, and the resin was filtered off. The filtrate was concentrated and purified on a column of Bio-Gel P2 (100×2.6 cm, EtOH:H_2O = 5:95) which afforded **13a** and **13b** as amorphous solids.

Yield: 5.5 mg (95%); NMR data: see Table 2.

Acknowledgments

Financial support by a grant from FWF (P-13843 CHE) is gratefully acknowledged. The 800 MHz spectra were obtained using the Varian Unity Inova spectrometer of the Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules.

References

[1] Moulder JW (1991) Microbiol Rev **55**: 143
[2] Nurminen M, Leinonen M, Saikku P, Mäkelä PH (1983) Science **220**: 1279
[3] Brade H, Brade L, Nano FE (1987) Proc Natl Acad Sci USA **84**: 2508
[4] Kosma P, Schulz G, Brade H (1988) Carbohydr Res **183**: 183
[5] Mikol V, Kosma P, Brade H (1994) Carbohydr Res **263**: 35
[6] Sokolowski T, Haselhorst T, Scheffler K, Weisemann R, Kosma P, Brade H, Brade L, Peters T (1998) J Biomol NMR **12**: 123
[7] Fu Y, Baumann M, Kosma P, Brade L, Brade H (1992) Infect Immun **60**: 1314
[8] Nguyen H, Evans SL (personal communication)
[9] Bock K, Thomsen U, Kosma P, Christian R, Holst O, Brade H (1992) Carbohydr Res **229**: 213
[10] Maaheimo H, Kosma P, Brade L, Brade H, Peters T (2000) Biochemistry **39**: 12778
[11] Bock K, Duus J (1994) J Carbohydr Chem **13**: 513
[12] Kosma P, Schulz G, Unger FM (1984) Carbohydr Res **132**: 261
[13] Wessel HP, Iversen T, Bundle DR (1985) J Chem Soc Perkin Trans I, 2247
[14] Mancuso AJ, Huang SL, Swern D (1978) J Org Chem **43**: 2480
[15] Nishizawa M, Noyori R (1991) In: Trost BM, Fleming I (eds) Comprehensive Organic Synthesis, vol 8. Pergamon Press, Oxford, New York Seoul Tokyo, p 159
[16] Corey EJ, Link JO (1989) Tetrahedron Lett **30**: 6275
[17] Hayashi M, Terashima S, Koga K (1981) Tetrahedron **37**: 2797
[18] Hori H, Morita M, Ito H, Nishida Y, Ohrui H, Meguro H (1990) XVth Int Carbohydr Symposium, August 12–17, Yokohama, Abstr C030

Received September 17, 2001. Accepted October 17, 2001