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Synthesis of 5-O-oligoglucosyl extended α -(2→4)-Kdo disaccharides corresponding to inner core fragments of Moraxellaceae lipopolysaccharides

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Abstract

The heptose-deficient inner core of the lipopolysaccharide of several pathogenic strains of the Moraxellaceae family (*Moraxella*, *Acinetobacter*) and of *Bartonella henselae*, respectively, comprises an α -D-glucopyranose attached to position 5 of Kdo. In continuation of the synthesis of fragments of *Acinetobacter haemolyticus* LPS, the branched α -Glc_p-(1→5)[α -Kdo-(2→4)]- α -Kdo trisaccharide motif was elaborated. The glycosylation of a suitably protected, α -(2→4)-interlinked Kdo-disaccharide was achieved in high yield and fair anomeric selectivity using a 4,6-O-benzylidene *N*-phenyltrifluoroacetimidate glucosyl donor. Subsequent regioselective reductive benzylidene opening afforded a trisaccharide acceptor, which was extended with β -D-glucopyranosyl and isomaltosyl residues. Global deprotection provided tri- to pentasaccharide structures corresponding to the inner core region of *A. haemolyticus* lipopolysaccharide.

Keywords

oligosaccharide synthesis; *Acinetobacter*; glycosylation; Kdo; lipopolysaccharide

1. Introduction

The Gram-negative bacterial cell wall is covered by densely packed lipopolysaccharide (LPS) molecules which trigger major immune responses of the respective host during pathogenesis.^{1,2} In structural terms, these glycolipids comprise the endotoxically active lipid anchor (lipid A) and an extended oligosaccharide fraction, which harbors a core region of limited structural variability and the strain-specific, highly diverse O-antigenic polysaccharides.^{3,4} The core domain may be further divided into an outer and an inner core region, wherein a 3-deoxy-D-manno-2-octulosonic acid (Kdo) usually forms the link to the lipid A part. In many bacterial genera, this Kdo unit is extended by a lateral α -(2→4)-linked Kdo moiety.⁵ In a few cases - such as in some *Acinetobacter* strains - Kdo is non-stoichiometrically replaced by its 3-oxy analogue D-glycero-D-talo-2-octulosonic acid (Ko).⁶ The α -(2→4)-Kdo disaccharide is frequently substituted at position 5 by a L-glycero-D-manno-heptose residue, and D-mannose, D-galactosamine and Kdo itself were also

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occasionally identified as the branching sugar.⁷ Specifically, an α -(1 \rightarrow 5)-linked β -glucopyranosyl residue has been found in LPS of the Moraxellaceae family and was shown to elicit cross-reactive antibodies recognizing different serotypes.⁸ In addition, *Bartonella henselae* – the causative agent of the cat-scratch disease – expresses a truncated core region being composed of α -GlcP-(1 \rightarrow 5)[α -Kdo-(2 \rightarrow 4)]- α -Kdo linked to lipid A.⁹ Furthermore, in *A. haemolyticus* this basic trisaccharide pattern is elongated at the glucose unit by a β -(1 \rightarrow 4)-linked isomaltotriose.¹⁰ The observation of a high binding affinity of isolated inner core fragments from *A. haemolyticus* towards different mannose binding lectins¹¹ prompted us to synthesize part structures thereof in order to identify the relevant binding epitopes.

Previously, stereoselective α -(1 \rightarrow 5)-coupling of glucosyl donors to Kdo monosaccharide acceptors was successfully performed by Oscarson using a thioglycoside donor in the presence of DMTST.¹² In a previous contribution we have capitalized on the use of an NPTFA-glucosyl donor and an orthogonal blocking group at position-4 of Kdo to allow for the introduction of the lateral Kdo at a later stage.¹³ A limited number of papers has been published addressing the synthesis of 5-*O*-glycosylated α -Kdo-(2 \rightarrow 4)- α -Kdo disaccharides.¹⁴ Ichiyangi *et al.* recently reported on the convenient preparation of 5-*O*-substituted Kdo disaccharides applying a Kdo disaccharide acceptor for coupling with heptose, β -mannose and 2-azido-2-deoxy- β -galactose (as a precursor for β -galactosamine) donors.¹⁵ Extending these studies, we present herein a high-yielding method to access the branched α -GlcP-(1(5)[α -Kdo-(2 \rightarrow 4)]- α -Kdo trisaccharide with fair stereoselectivity and its subsequent elongation into tetra- and pentasaccharide derivatives.

2. Results and discussion

2.1. Approach A: Using an α -Glc-(1 \rightarrow 5)-Kdo acceptor

Previously, α -Glc-(1 \rightarrow 5)-Kdo disaccharide **1** has been prepared with excellent stereoselectivity and in a high yield.¹³ The orthogonal protecting group pattern allowed for selective cleavage of the *p*-methoxybenzyl group (PMB) affording the 4-OH Kdo acceptor **2**. Starting from **2** we envisaged to introduce the lateral Kdo moiety using the Kdo fluoride donors **3** and **4**, which were recently reported to yield only α -anomeric products without significant elimination side reaction.¹⁶ Specifically, the per-*O*-acetyl protected Kdo donor **3** proved to be an efficient glycosyl donor for regioselective coupling to a 4,5-diol Kdo acceptor yielding the α -(2 \rightarrow 4)-interconnected Kdo disaccharide as a single isomer in high yield.^{16a} The donor, however, was unreactive in the presence of base – such as triethylamine or *sym*-collidine – which were added to capture hydrogen fluoride. Since the 7,8-*O*-disiloxane-1,3-diyl (TIPDS) and the 4,6-*O*-acetal group in acceptor **2** were not compatible with glycosylation conditions lacking a HF-scavenger, these protecting groups were replaced by acetyl groups. Accordingly, acid hydrolysis of the benzylidene group, followed by cleavage of the silyl ether, *O*-acetylation and DDQ-oxidation of the PMB group furnished the disaccharide acceptor **5** in 40% overall yield (Scheme 1). Attempted coupling of **5** with acetyl-protected donor **3** under BF₃·Et₂O promotion (2 eq.) in dichloromethane in the presence of 3 Å molecular sieves at room temperature, however, failed and trisaccharide formation was not detected.

Next, the armed benzylated donor **4** was tried, which had been shown to react with 2-propanol under $\text{BF}_3 \cdot \text{Et}_2\text{O}$ promotion also in the presence of triethylamine, although higher temperatures were necessary.^{16b} First, when using donor **4** in the absence of base and under conditions as used for donor **3**, degradation of acceptor **2** was observed. In the presence of triethylamine, acceptor **2** was unaffected, but glycosylation did not take place. In a last attempt we intended to exploit the high reactivity of **4** and the compatibility of glycosyl acceptor **5** towards base-free conditions. Again, however, glycoside formation could not be achieved. NMR analysis of **5** revealed a through-space interaction between the axial hydrogen at position-3 of Kdo with H-5' of the glucosyl residue (Fig. 1). This would indicate that the glucose unit is in close proximity to the Kdo ring and thus sterically shields the 4-OH group towards the incoming electrophile.

In addition to the Kdo-fluoride based glycosylation attempts, coupling of acceptor **2** with peracetylated Kdo bromide under Helferich conditions was also tested but did not lead to significant product formation. Previously, this approach had successfully been performed for the introduction of a lateral Kdo-unit onto a *L-glycero-D-manno*-heptosyl-(1→5)-Kdo glycosyl acceptor in 65% yield.¹⁷

2.2. Approach B: Using an α -Kdo-(2→4)-Kdo acceptor

As an alternative approach, the sequence of glycosylation steps was inverted by attaching a suitably protected glucose donor to a preassembled α -Kdo-(2→4)-Kdo disaccharide acceptor. Thus, we could capitalize on the previously elaborated route towards Kdo₂ **7**, which was obtained in high yields and without any formation of β -products.^{16a} Recently, Ichiyanagi *et al.* successfully coupled a Kdo₂ 5-OH acceptor with heptose and mannose trichloroacetimidate donors exploiting the *trans*-directing effect of C-2 ester groups.¹⁵ First, the 5-OH acceptor **8** was obtained from iodo-precursor **7** by hydrogen atom transfer¹⁸ in cyclohexane catalyzed by lauroyl peroxide. Previously, dehalogenation depended on fully blocked compounds to guarantee sufficient solubility in cyclohexane for smooth conversion.^{16a} Notably, using a mixture of cyclohexane and 1,2-dichloroethane¹⁸ allowed for direct and neat dehalogenation of disaccharide **7** which provided acceptor **8** in high yield (92%).

In previous experiments, *N*-phenyltrifluoroacetimidate glucose donor **6**¹³ afforded excellent α -selectivity in the glycosylation of a 5-OH Kdo monosaccharide due to the directing effect of the 4,6-*O*-benzylidene group.¹⁹ Coupling of 5-OH acceptor **8** with donor **6** in CH_2Cl_2 under TMSOTf catalysis in the presence of ground 4 Å molecular sieves provided traces of both α - and β -trisaccharides **9 α** and **9 β** . By exploiting the known²⁰ 1,2-*cis* directing effect of ether solvents, the α/β -ratio could be increased significantly (from $\alpha/\beta = 1:1.2$ in CH_2Cl_2 to 2.7:1 in Et_2O in preliminary experiments). The observation that glycoside formation ceased after a short initial phase led to the assumption that TfOH formed by partial hydrolysis of TMSOTf was the active promotor, which would then be rapidly scavenged by 4 Å molecular sieves. Indeed, applying TfOH as activator in the presence of 5 Å molecular sieves improved the total yield to 31%. This still disappointing outcome was attributed to donor degradation that proceeded faster than glycoside formation. Eventually, using inverse glycosylation conditions²¹ in diethyl ether containing molecular sieves (5 Å) afforded a

mixture of anomers **9a** and **9b** in a total yield of 91% ($\alpha/\beta = 4:1$) which could be conveniently separated on an HPLC column (isolated yield of α -product **9a**: 67%). The α -anomeric configuration was readily assigned on the basis of the coupling constant $J_{1'',2''}$ (3.8 Hz). Lactone formation between C-1' of the lateral Kdo unit and the free 5-hydroxyl group was also observed but to a low degree only. Sequential removal of benzyl (Pd/C, H₂) and acetyl (NaOMe) groups from trisaccharide **9a** and ensuing methyl ester saponification under alkaline conditions (NaOH) gave trisaccharide **10** in 94% yield (Scheme 2).

2.3. Synthesis of oligoglucosyl fragments

Proceeding towards the oligoglucosyl core structures, regioselective opening of the benzylidene group in compound **9a** to provide the 6-OBn protected derivative **11** was foreseen, to serve as an acceptor for the extended glucosyl core structure of *A. haemolyticus*.

Hence, treatment of trisaccharide **9a** with triethylsilane/BF₃·Et₂O²² in dry dichloromethane afforded the desired 4-OH acceptor derivative **11** as the major product (72%) together with its 6-OH regioisomer **12** (5%) and traces of diol **13** (2%). By using the peracetylated glucopyranosyl NPTFA donor **14**²³ under TfOH promotion (5 mol% for **15**, 12.5 mol% for **18**), tetrasaccharide **15** was obtained (Scheme 3). The isolated yield for the β -tetrasaccharide **14**, however, did not exceed 46%, since several purification steps by HPLC were needed to produce a pure tetrasaccharide. Using the same strategy with the isomaltosyl donor **17**, the pentasaccharide **18** was readily accessible in a 3+2 approach in a good yield (71%). Global deprotection afforded the oligosaccharide ligands **16** (99%) and **19** (90%) in excellent yields. The ¹³C NMR data of the oligosaccharide (Table 1) are in good agreement with the LPS-oligosaccharides isolated from *B. henselae* ATCC 49882^{T*} and *A. haemolyticus* respectively.^{9,10} Deviating assignments were only noted for C-7 of both Kdo units.⁹

2.4. Conclusions and outlook

In summary, a straightforward method to prepare the α -Glc_p-(1→5)[α -Kdo-(2→4)]- α -Kdo trisaccharide capitalizing on a Kdo disaccharide acceptor was established. Good α -selectivity relied on diethyl ether as a solvent and high yields were obtained by slowly adding the donor to a pre-stirred mixture of the Kdo₂ acceptor and TfOH as a promotor. The 4,6-*O*-benzylidene blocking group of the donor eventually provided access to extended oligosaccharides after regioselective reductive opening. Subsequent elongation with 2-*O*-acetyl protected *N*-phenyl trifluoroacetimidate glucosyl donors allowed for the introduction of β -Glc_p-(1→4)- as well as α -Glc_p-(1→6)- β -Glc_p-(1→4)- units. Thus, three fragments of the *A. haemolyticus* inner core were obtained which serve as ligands in binding studies with C-type collectins.

3. Experimental

3.1. General

All purchased chemicals were used without further purification unless stated otherwise. The promotor BF₃·Et₂O was used as a solution in diethyl ether (46% according to the manufacturer). Solvents were dried over activated 3 Å (acetone, Et₂O) or 4 Å (CH₂Cl₂, ClCH₂CH₂Cl, cyclohexane, *N,N*-dimethylformamide, pyridine) molecular sieves. Dry

MeOH (Merck) and dry THF (Sigma-Aldrich) were purchased. Cation exchange resin DOWEX 50 H⁺ was regenerated by consecutive washing with HCl (3 M), water and dry MeOH. Aqueous solutions of salts were saturated unless stated otherwise. Concentration of organic solutions was performed under reduced pressure < 40 °C. Optical rotations were measured with a Perkin-Elmer 243 B Polarimeter. $[\alpha]_D^{20}$ values are given in units of 10⁻¹deg cm²g⁻¹. Thin layer chromatography was performed on Merck precoated plates: generally on 5 × 10 cm, layer thickness 0.25 mm, Silica Gel 60F₂₅₄; alternatively on HPTLC plates with 2.5 cm concentration zone (Merck). Spots were detected by dipping reagent (anisaldehyde-H₂SO₄). For column chromatography silica gel (0.040 – 0.063 mm) was used. HP-column chromatography was performed on pre-packed columns (YMC-Pack SIL-06, 0.005 mm, 250×10 mm and 250×20 mm). Desalting after ester saponification was performed on pre-packed PD-10 columns (GE Healthcare, Sephadex™ G-25 M). NMR spectra were recorded with a Bruker Avance III 600 instrument (600.22 MHz for ¹H, 150.93 MHz for ¹³C) using standard Bruker NMR software. ¹H spectra were referenced to 7.26 (CDCl₃) and 0.00 (D₂O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) ppm unless stated otherwise. ¹³C spectra were referenced to 77.00 (CDCl₃) and 67.40 (D₂O, external calibration to 1,4-dioxane) ppm. ESI-MS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument.

3.2. 4,6-Di-O-acetyl-2,3-di-O-benzyl- α -D-glucopyranosyl-(1→5)-methyl [methyl 7,8-di-O-acetyl-3-deoxy- α -D-manno-oct-2-ulopyranosid]onate (5)

A solution of disaccharide **1**¹³ (100 mg, 0.094 mmol) in dry MeOH (4.0 mL) containing *p*-toluenesulfonic acid-monohydrate (1.6 mg, 0.008 mmol) was kept at 40 °C for 2 h under an atmosphere of argon. The cooled solution was treated with solid NaHCO₃ (ca. 50 mg) for 5 min, the solvent was removed under reduced pressure and the residue was partitioned between EtOAc and aq. NaHCO₃. The organic phase was washed with brine, dried (MgSO₄), filtered and concentrated. The residue was dissolved in dry THF (6.0 mL) and treated with tetrabutylammonium fluoride (1 M in THF, 0.14 mL, 0.142 mmol) at 0 °C for 20 min. After addition of dry MeOH (1.0 mL) the solvent was removed and EtOAc was added. The residue was filtered over a short pad of silica and rinsed with EtOAc. Concentration of the filtrate afforded a crude product, which was acetylated using acetic anhydride (0.8 mL), 4-(*N,N*-dimethylamino)pyridine (5 mg) and dry pyridine (4.0 mL). After 14 h at ambient temperature excessive reagent was destroyed with dry MeOH (1.0 mL) at 0 °C (10 min) followed by removal of solvent by codistillation with toluene (3×). Chromatography of the crude product provided the PMB-protected intermediate (52 mg, 61%) as a colorless oil.

This intermediate (41 mg, 0.046 mmol) was dissolved in dry CH₂Cl₂ (2.1 mL) and dry MeOH (0.7 mL) under Ar and treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (164 mg, 0.672 mmol) in eight portions within 2.5 h. After complete addition the mixture was stirred for 1 h, diluted with CHCl₃ and washed with aq. NaHCO₃. The aqueous phase was once more extracted with CHCl₃ and the combined organic phases were washed with aq. NaHCO₃ (2×), dried (MgSO₄) and filtered. Concentration of the filtrate and purification of the residue by chromatography (toluene/EtOAc 9:1 to remove excessive reagent, then 2:1 to elute product) afforded 4-OH acceptor **5** (23 mg, 65%, total yield: 40%) as a colorless oil:

$[\alpha]_D^{20} + 41.9$ (c 0.56, CHCl_3); R_f 0.18 (n -hexane/EtOAc 1:1); $^1\text{H NMR}$ (CDCl_3): δ 7.36 - 7.27 (m, 10H, Ar), 5.43 (app dt, 1H, $J_{7,6} \sim 5.7$, $J_{7,8a}$ 2.1 Hz, H-7), 4.87 (app t, 1H, $J_{4',3'} \sim J_{4',5'}$ 9.5 Hz, H-4'), 4.86 (d, 1H, $J_{1',2'}$ 3.7 Hz, H-1'), 4.85 (d, 1H, J 11.1 Hz, CHHPH), 4.82 (dd, 1H, $J_{8a,8b}$ 12.5 Hz, H-8a), 4.74 (d, 1H, J 12.2 Hz, CHHPH), 4.70 (d, 1H, J 12.4 Hz, CHHPH), 4.68 (d, 1H, J 11.6 Hz, CHHPH), 4.29 (dd, 1H, $J_{8b,7}$ 5.3 Hz, H-8b), 4.10 (dt, 1H, $J_{5',6'}$ 4.6 Hz, H5'), 4.07 - 4.00 (m, 3H, H-4, H-6'a, H-6'b), 3.98 - 3.96 (br s, 1H, H-5), 3.95 (dd, $J_{6,5}$ 1.0 Hz, H-6), 3.93 (app t, 1H, H-3'), 3.83 (s, 3H, CO_2CH_3), 3.56 (dd, 1H, $J_{2',3'}$ 9.5, $J_{2',1'}$ 3.5 Hz, H-2'), 3.37 (d, 1H, $J_{\text{OH},4}$ 11.5 Hz, OH), 3.27 (s, 3H, OCH_3), 2.20 - 2.16 (m, 1H, H-3eq), 2.03, 2.01, 1.95 and 1.94 (4 s, each 3H, COCH_3), 1.80 (app t, 1H, $J_{3ax,3eq} \sim J_{3ax,4}$ 12.5 Hz, H-3ax); $^{13}\text{C NMR}$ (CDCl_3): δ 170.53, 170.43, 169.95 and 169.76 (4 s, 4C, COCH_3), 168.02 (s, C-1), 138.39 and 137.85 (2 s, 2C, Ar), 128.56, 128.39, 128.00, 127.95, 127.67 and 127.63 (6 d, 10C, Ar), 99.73 (d, C-1'), 99.04 (s, C-2), 79.42 (d, C-5), 78.79 (d, C-3'), 78.30 (d, C-2'), 75.17 (t, CH_2Ph), 73.73 (t, CH_2Ph), 71.54 (d, C-6), 71.13 (d, C-7), 69.90 and 69.51 (2 d, 2C, C-4', C-5'), 66.19 (d, C-4), 62.84 (t, C-6'), 62.25 (t, C-8), 52.62 (q, CO_2CH_3), 51.04 (q, OCH_3), 36.08 (t, C-3'), 21.02, 20.76, 20.70 and 20.63 (4 q, 4C, COCH_3); ESI-TOF HRMS: $m/z = 799.2784$; calcd for $\text{C}_{38}\text{H}_{48}\text{O}_{17}\text{Na}^+$: 799.2784.

3.3. Methyl (4,5,7,8-tetra-O-acetyl-3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2 \rightarrow 4)-methyl (methyl 7,8-O-carbonyl-3-deoxy- α -D-manno-oct-2-ulopyranosid)onate (8)

A suspension of disaccharide **7**^{16a} (0.71 g, 0.87 mmol) in a mixture of dry $\text{ClCH}_2\text{CH}_2\text{Cl}$ (9.0 mL) and dry cyclohexane (65.0 mL) was degassed with argon and heated to reflux for 15 min. Lauroyl peroxide (0.12 g, 0.30 mmol) was added to the warm solution and the refluxed for 2 h. The solvent was removed under reduced pressure and the residue was subjected to chromatography (n -hexane/EtOAc 1:2) to provide dehalogenated compound **8** (0.56 g, 92%) as a colorless amorphous solid: $[\alpha]_D^{20} + 73.1$ (c 0.63, CHCl_3); R_f 0.31 (toluene/EtOAc 2:3); $^1\text{H NMR}$ (CDCl_3): δ 5.40 - 5.38 (m, 1H, H-5'), 5.26 - 5.19 (m, 2H, H-4', H-7'), 4.91 (ddd, 1H, $J_{7,8b}$ 8.2, $J_{7,8a}$ 6.9 Hz, $J_{7,6}$ 3.7, H-7), 4.75 (dd, 1H, $J_{8a,8b}$ 8.9, H-8a), 4.65 (dd, 1H, $J_{8'a,8'b}$ 12.5, $J_{8'a,7'}$ 2.7 Hz, H-8'a), 4.54 (app t, 1H, H-8b), 4.25 (ddd, 1H, $J_{4,3ax}$ 11.2, $J_{4,3eq}$ 5.7, $J_{4,5}$ 3.2 Hz, H-4), 4.11 (dd, 1H, $J_{6',7'}$ 9.6, $J_{6',5'}$ 1.3 Hz, H-6'), 4.08 (dd, 1H, $J_{8'b,7'}$ 3.5 Hz, H-8'b), 3.89 - 3.87 (m, 1H, H-6), 3.84 (s, 3H, CO_2CH_3), 3.81 (s, 3H, CO_2CH_3), 3.69 - 3.67 (m, 1H, H-5), 3.22 (s, 3H, OCH_3), 2.46 (bs, 1H, OH), 2.20 (dd, 1H, $J_{3'eq,3'ax} \sim J_{3'eq,4'}$ 12.7, H-3'eq), 2.13 (s, 3H, COCH_3), 2.12 - 2.03 (m, 6H, H-3ax, H-3'ax, H-3eq, COCH_3), 2.00 and 1.98 (2 s, each 3H, COCH_3); $^{13}\text{C NMR}$ (CDCl_3): δ 170.98, 170.20, 169.88 and 169.61 (4 s, 4C, COCH_3), 168.87 and 167.58 (2 s, 2C, C-1, C-1'), 154.66 [s, $\text{OC}(=\text{O})\text{O}$], 99.10 and 98.08 (2 s, 2C, C-2, C-2'), 76.03 (d, C-7), 70.38 (d, C-6), 69.61 (d, C-6'), 67.64 and 67.45 (2 d, 2C, C-4, C-7'), 66.10 (t, C-8), 65.88 (d, C-4'), 64.73 (d, C-5), 64.18 (d, C-5'), 61.58 (t, C-8'), 53.43 and 52.71 (2 q, 2C, CO_2CH_3), 51.14 (q, OCH_3), 33.00 and 32.29 (2 t, 2C, C-3, C-3'), 20.70, 20.60, 20.58 and 20.56 (4 q, 4C, COCH_3); ESI-TOF HRMS: $m/z = 712.2298$; calcd for $\text{C}_{28}\text{H}_{38}\text{O}_{20}\text{NH}_4^+$: 712.2295.

3.4. Methyl (4,5,7,8-tetra-O-acetyl-3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2 \rightarrow 4)-[2,3-di-O-benzyl-4,6-O-benzylidene- α -D-glucopyranosyl-(1 \rightarrow 5)]-methyl (methyl 7,8-O-carbonyl-3-deoxy- α -D-manno-oct-2-ulopyranosid)onate (9a) and methyl (4,5,7,8-tetra-O-acetyl-3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2 \rightarrow 4)-[2,3-di-O-benzyl-4,6-O-benzylidene- β -D-

glucopyranosyl-(1→5)]-methyl (methyl 7,8-O-carbonyl-3-deoxy- α -D-manno-oct-2-uloopyranosid)onate (9 β)

A solution of disaccharide acceptor **8** (77 mg, 0.111 mmol) in dry Et₂O (1.5 mL) containing ground 5 Å molecular sieves (75 mg) was stirred at ambient temperature for 2 h under Ar. Diluted triflic acid (0.49 μ L, 5.55 μ mol) in dry CH₂Cl₂ (0.1 mL) was added and after 5 min a separately prepared solution of donor **6**¹³ (344 mg, 0.555 mmol) in dry CH₂Cl₂ (3.0 mL) was added to this mixture using a syringe pump (1 mL/h). After complete addition the promotor was neutralized by adding triethylamine (32 μ L, 0.222 mmol) and the suspension was filtered over Celite. The filtrate was concentrated and the residual solid was purified by chromatography (*n*-hexane/EtOAc 1:1) affording a mixture of the anomers **9 α** and **9 β** (114 mg, 91%, α : β = 4:1 according to ¹H NMR). The compounds were separated on an HPLC column (toluene/EtOAc 3:1 → 2:1) providing the desired α -anomer **9 α** (83 mg, 67%) and the slower migrating β -isomer **9 β** (20 mg, 16%) as colorless oils. Data for **9 α** : [α]_D²⁰ + 111.4 (*c* 0.82, CHCl₃); R_f 0.44 (toluene/EtOAc 2:1, HP-TLC); ¹H NMR (CDCl₃): δ 7.51 - 7.28 (m, 15H, Ar), 5.58 (s, 1H, PhCH), 5.35 - 5.33 (m, 1H, H-5'), 5.27 (ddd, 1H, *J*_{4',3'ax} 12.5, *J*_{4',3'eq} 4.8, *J*_{4',5'} 3.0 Hz, H-4'), 5.19 (ddd, 1H, *J*_{7',6'} 9.6, *J*_{7',8'b} 3.9, *J*_{7',8'a} 2.8 Hz, H-7'), 5.07 (d, 1H, *J*_{1'',2''} 3.8 Hz, H-1''), 5.00 (d, 1H, *J* 11.5 Hz, CHHPh), 4.92 (d, 1H, *J* 10.6 Hz, CHHPh), 4.85 (d, 1H, *J* 11.5 Hz, CHHPh), 4.67 (dd, 1H, *J*_{8'a,8'b} 12.4 Hz, H-8'a), 4.66 - 4.62 (m, 1H, H-7), 4.59 (d, 1H, *J* 10.9 Hz, CHHPh), 4.44 (dd, 1H, *J*_{8a,8b} 8.6, *J*_{8a,7} 5.8 Hz, H-8a), 4.30 (dd, 1H, *J*_{6''a,6''b} 10.4, *J*_{6''a,5''} 4.8 Hz, H-6''a), 4.20 (dd, 1H, *J*_{6',5'} 1.5 Hz, H-6'), 4.16 (ddd, 1H, *J*_{4,3ax} 11.8, *J*_{4,3eq} 4.7, *J*_{4,5} 2.4 Hz, H-4), 4.06 (app t, 1H, *J*_{8b,7} ~ 8.4 Hz, H-8b), 4.01 (dd, 1H, H-8'b), 3.93 - 3.88 (m, 2H, H-3'', H-5''), 3.84 (s, 3H, CO₂CH₃), 3.83 - 3.82 (m, 1H, H-5), 3.79 (s, 3H, CO₂CH₃), 3.75 - 3.69 (m, 2H, H-6, H-6''b), 3.66 (app t, 1H, *J*_{4'',3''} ~ *J*_{4'',5''} 9.3 Hz, H-4''), 3.59 (dd, 1H, H-2''), 3.16 (s, 3H, OCH₃), 2.21 - 2.15 (m, 2H, H-3^{eq}, H-3'^{eq}), 2.10 (app. t, 1H, *J*_{3ax,3eq} ~ 12.1 Hz, H-3^{ax}), 2.09 and 2.07 (2 s, each 3H, COCH₃), 2.07 (app t, 1H, *J*_{3'ax,3'eq} 12.7 Hz, H-3'^{ax}), 1.99 and 1.95 (2 s, each 3H, COCH₃); ¹³C NMR (CDCl₃): δ 170.85, 170.27, 169.69 and 169.60 (4 s, 4C, COCH₃), 168.06 and 167.35 (2 s, 2C, C-1, C-1'), 154.57 [s, OC(=O)O], 138.24, 137.70 and 137.28 (3 s, 3C, Ar), 128.95, 128.64, 128.46, 128.33, 128.25, 128.23, 128.20, 127.93 and 125.99 (9 d, 15C, Ar), 101.28 (d, PhCH), 100.56 (d, C-1''), 99.34 and 98.92 (2 s, C-2, C-2'), 82.63 (d, C-4''), 79.86 (d, C-2''), 77.86 (d, C-3''), 76.00 (d, C-5), 75.16 (d, C-7), 75.01 (t, CH₂Ph), 74.81 (t, CH₂Ph), 72.03 (d, C-6), 69.50 (d, C-4), 69.44 (d, C-6'), 68.95 (t, C-6''), 67.72 (d, C-7'), 65.95 (t, C-8), 65.92 (d, C-4'), 64.37 (d, C-5'), 63.59 (d, C-5''), 61.58 (t, C-8'), 53.11 and 52.69 (2 q, 2C, CO₂CH₃), 51.23 (q, OCH₃), 33.85 (t, C-3), 32.40 (t, C-3'), 20.70, 20.63 and 20.58 (3 q, 4C, 4 COCH₃); ESI-TOF HRMS: *m/z* = 1147.3625; calcd for C₅₅H₆₄O₂₅Na⁺: 1147.3629.

9 β : [α]_D²⁰ + 49.8 (*c* 0.82, CHCl₃); R_f 0.23 (toluene/EtOAc 2:1, HP-TLC); ¹H NMR (CDCl₃): δ 7.49 - 7.24 (m, 15H, Ar), 5.56 (s, 1H, PhCH), 5.35 - 5.33 (m, 1H, H-5'), 5.23 (ddd, 1H, *J*_{4',3'ax} 12.3, *J*_{4',3'eq} 4.9, *J*_{4',5'} 3.0 Hz, H-4'), 5.18 (ddd, 1H, *J*_{7',6'} 9.2, *J*_{7',8'b} 4.3, *J*_{7',8'a} 2.8 Hz, H-7'), 4.94 (d, 1H, *J*_{1'',2''} 7.7 Hz, H-1''), 4.90 (d, 1H, *J* 11.5 Hz, CHHPh), 4.89 (d, 1H, *J* 11.3 Hz, CHHPh), 4.83 - 4.74 (m, 4H, 2 × CHHPh, H-7, H-8a), 4.62 (dd, 1H, *J*_{8'a,8'b} 12.5 Hz, H-8'a), 4.44 (app t, 1H, *J*_{8b,8a} ~ *J*_{8b,7} 8.1 Hz, H-8b), 4.37 (dd, 1H, *J*_{6''a,6''b} 10.4, *J*_{6''a,5''} 5.0 Hz, H-6''a), 4.29 (ddd, 1H, *J*_{4,3ax} 11.7, *J*_{4,3eq} 5.2, *J*_{4,5} 2.7 Hz, H-4), 4.11 - 4.09 (m, 1H, H-5), 4.04 - 4.00 (m, 2H, H-6', H-8'b), 3.96 - 3.94 (m, 1H, H-6), 3.85 (s, 3H, CO₂CH₃), 3.81 (app t, 1H, *J*_{3'',2''} ~ *J*_{3'',4''} 8.9 Hz, H-3''), 3.74 (s, 3H, CO₂CH₃), 3.74 (app t,

1H, $J_{6''b,5''}$ 10.4 Hz, H-6''b), 3.60 (app t, 1H, $J_{4'',5''}$ 9.2 Hz, H-4''), 3.46 (app dt, 1H, H-5''), 3.31 (app t, 1H, H-2''), 3.24 (s, 3H, OCH₃), 2.23 - 2.19 (m, 1H, H-3'eq), 2.16 (app t, 1H, $J_{3ax,3eq}$ ~ 12.2 Hz, H-3ax), 2.10 - 2.04 (m, 8H, 2 × COCH₃, H-3eq, H-3'ax), 2.00 and 1.98 (2 s, each 3H, COCH₃); ¹³C NMR (CDCl₃): δ 170.93, 170.23, 169.64 and 169.57 (4 s, 4C, COCH₃), 168.18 and 167.52 (2 s, 2C, C-1, C-1'), 154.64 [s, OC(=O)O], 138.47, 138.03 and 137.13 (3 s, 3C, Ar), 129.02, 128.36, 128.25, 127.91, 127.76, 127.69, 127.58 and 125.96 (8 d, 15C, Ar), 102.92 (d, C-1''), 101.22 (d, PhCH), 99.16 and 99.04 (2 s, 2C, C-2, C-2'), 81.67 (d, C-4''), 81.32 (d, C-2''), 80.72 (d, C-3''), 76.72 (d, C-7), 75.14 (t, CH₂Ph), 74.50 (t, CH₂Ph), 71.06 (d, C-6), 69.97 (d, C-5), 69.78 (d, C-6'), 68.88 (d, C-4), 68.67 (t, C-6''), 67.76 (d, C-7'), 66.11 (d, C-4'), 65.95 (d, C-5''), 65.51 (t, C-8), 64.23 (d, C-5'), 61.86 (t, C-8'), 53.16 and 52.68 (2 q, 2C, CO₂CH₃), 51.32 (q, OCH₃), 34.12 (t, C-3), 32.40 (t, C-3'), 20.78, 20.64 and 20.62 (3 q, 4C, COCH₃); ESI-TOF HRMS: m/z = 1142.4088; calcd for C₅₅H₆₄O₂₅NH₄⁺: 1142.4075.

3.5. Sodium (3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2→4)-[α -D-glucopyranosyl-(1→5)]-sodium (methyl α -D-manno-oct-2-ulopyranosid)onate (10)

Trisaccharide **9a** (11.3 mg, 0.010 mmol) was dissolved in dry MeOH (1.0 mL), the atmosphere was exchanged to argon and 10% Pd-C (1 mg) was added. The atmosphere was exchanged successively to argon and hydrogen (1 atm). After 4 h the catalyst was filtered off, rinsed with MeOH and the filtrate was concentrated. The residue was dried and then dissolved in anhydrous MeOH (1.0 mL) and treated with sodium methoxide (0.1 M in MeOH, 60 μ L, 0.006 mmol) for 17 h at rt. Ion exchange resin DOWEX 50 (H⁺ form) was added until the solution reacted neutral. The resin was filtered off immediately, rinsed with MeOH and the filtrate was concentrated. A solution of the residue in H₂O (1.0 mL) was treated with aq NaOH (0.1 M, 1.0 mL) at 0 °C for 4 h at ambient temperature. The mixture was made neutral by adding DOWEX 50 resin (H⁺-form). Filtration of the resin and freeze-drying of the filtrate afforded a salt-containing product, which was desalted on a PD10 SEC column (H₂O). Lyophilisation of the pooled fractions yielded trisaccharide **10** (6.4 mg, 94%) as a colorless amorphous solid: [α]_D²⁰ + 114.9 (*c* 0.59, H₂O); ¹H NMR (D₂O, pD ~ 7.4): δ 5.24 (d, 1H, $J_{1'',2''}$ 3.6 Hz, H-1''), 4.19 - 4.17 (m, 1H, H-5), 4.08 - 4.03 (m, 2H, H-5'', H-7), 3.97 - 3.91 (m, 3H, H-4, H-5', H-7'), 3.91 (ddd, 1H, $J_{4',5'}$ 3.0 Hz, H-4'), 3.89 - 3.86 (m, 2H, H-8a, H-8'a), 3.85 (dd, 1H, $J_{6''a,6''b}$ 12.2, $J_{6''a,5''}$ 2.6 Hz, H-6''), 3.78 (app t, 1H, $J_{3'',4''}$ 9.2 Hz, H-3''), 3.665 (br d, 1H, $J_{6',7'}$ 7.5 Hz, H-6'), 3.66 (dd, 1H, $J_{8'a,8'b}$ 11.8, $J_{7',8'b}$ 7.2 Hz, H-8'b), 3.58 (dd, 1H, $J_{8b,8a}$ 11.8, $J_{8b,7}$ 6.5 Hz, H-8b), 3.51 (app t, 1H, $J_{5'',4''}$ 9.7 Hz, H-4''), 3.50 (br d, 1H, $J_{6,7}$ 9.5 Hz, H-6), 3.46 (dd, 1H, $J_{2'',3''}$ 9.9 Hz, H-2''), 3.07 (s, 3H, OCH₃), 2.05 (dd, 1H, $J_{3'eq,3'ax}$ 13.2, $J_{3'eq,4'}$ 4.7 Hz, H-3'eq), 2.01 (dd, 1H, $J_{3eq,3ax}$ 12.6, $J_{3eq,4}$ 4.8 Hz, H-3eq), 1.94 (app t, 1H, $J_{3ax,4}$ 12.3 Hz, H-3ax), 1.75 (app t, 1H, $J_{3'ax,4'}$ 12.7 Hz, H-3'ax); ¹³C NMR (D₂O, pD ~ 7.4): see Table 1; ESI-TOF HRMS: m/z = 657.1838; calcd for C₂₃H₃₈O₂₀Na⁺: 657.1849.

3.6. Methyl (4,5,7,8-tetra-O-acetyl-3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2→4)-[2,3,6-tri-O-benzyl- α -D-glucopyranosyl-(1→5)]-methyl (methyl 7,8-O-carbonyl-3-deoxy- α -D-manno-oct-2-ulopyranosid)onate (11), methyl (4,5,7,8-tetra-O-acetyl-3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2→4)-[2,3,4-tri-O-benzyl- α -D-glucopyranosyl-(1→5)]-methyl (methyl 7,8-O-carbonyl-3-deoxy- α -D-manno-oct-2-ulopyranosid)onate (12) and methyl

(4,5,7,8-tetra-O-acetyl-3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2 \rightarrow 4)-[2,3-di-O-benzyl- α -D-glucopyranosyl-(1 \rightarrow 5)]-methyl (methyl 7,8-O-carbonyl-3-deoxy- α -D-manno-oct-2-ulopyranosid)onate (13)

A cooled (0 °C) solution of compound **9a** (188 mg, 0.167 mmol) in dry CH₂Cl₂ (6.0 mL) was treated with triethylsilane (400 μ L, 2.506 mmol) and BF₃·Et₂O (41 μ L, 0.334 mmol) successively. After 1 h at 0 °C the mixture was diluted with CH₂Cl₂ and washed with aq. NaHCO₃. The aqueous phase was once again extracted with CH₂Cl₂ and the combined organic phases were washed with water, dried (MgSO₄) and concentrated. The residue was purified on an HPLC column (*n*-hexane/EtOAc 3:2 \rightarrow 1:2) providing some recovered starting material **9a** (7 mg, 3%), the desired 6-OBn compound **11** (135 mg, 72%), the 4-OBn isomer **12** (10 mg, 5%) and the diol **13** (3 mg, 2%) successively. Data for **11**: Colorless oil; $[\alpha]_D^{20} + 102.6$ (*c* 1.12, CHCl₃); R_f 0.38 (*n*-hexane/EtOAc 1:1, HP-TLC); ¹H NMR (CDCl₃): δ 7.41 - 7.27 (m, 15H, Ar), 5.34 - 5.33 (m, 1H, H-5'), 5.23 (ddd, 1H, $J_{4',3'ax}$ 12.6, $J_{4',3'eq}$ 5.0, $J_{4',5'}$ 2.8 Hz, H-4'), 5.20 (ddd, 1H, $J_{7',6'}$ 9.4, $J_{7',8'b}$ 4.4, $J_{7',8'a}$ 2.8 Hz, H-7'), 5.10 (d, 1H, $J_{1'',2''}$ 3.6 Hz, H-1''), 4.97 (d, 1H, J 11.4 Hz, CHHPh), 4.87 (d, 1H, J 11.4 Hz, CHHPh), 4.82 (d, 1H, J 10.8 Hz, CHHPh), 4.77 - 4.73 (m, 1H, H-7), 4.62 (dd, 1H, $J_{8'a,8'b}$ 12.1, $J_{8'a,7'}$ 2.6 Hz, H-8'a), 4.59 (d, 1H, J 10.8 Hz, CHHPh), 4.55 (d, 1H, J 11.5 Hz, CHHPh), 4.48 (d, 1H, J 11.5 Hz, CHHPh), 4.49 - 4.46 (m, 1H, H-8a), 4.22 - 4.18 (m, 2H, H-4, H-8b), 4.10 (dd, 1H, $J_{6',5'}$ 1.7 Hz, H-6'), 4.06 (dd, 1H, H-8'b), 3.86 - 3.85 (m, 1H, H-5), 3.84 - 3.81 (m, 4H, H-5'', CO₂CH₃), 3.80 (s, 3H, CO₂CH₃), 3.76 (dd, 1H, $J_{6''a,6''b}$ 10.1, $J_{6''a,5''}$ 3.8 Hz, H-6''a), 3.74 - 3.67 (m, 3H, H-3'', H-4'', H-6), 3.58 (dd, 1H, $J_{6''b,5''}$ 4.5 Hz, H-6''b), 3.53 (dd, 1H, $J_{2'',3''}$ 9.2 Hz, H-2''), 3.18 (s, 3H, OCH₃), 2.62 (d, 1H, $J_{OH,4''}$ 2.2 Hz, OH), 2.24 - 2.20 (m, 1H, H-3'eq), 2.12 (app t, 1H, $J_{3ax,3eq} \sim J_{3ax,4}$ 12.2 Hz, H-3ax), 2.09 and 2.08 (2 s, each 3H, COCH₃), 2.09 - 2.01 (m, 2H, H-3'ax, H-3eq), 1.99 and 1.91 (2 s, each 3H, COCH₃); ¹³C NMR (CDCl₃): δ 170.93, 170.30, 169.71 and 169.63 (4 s, 4C, COCH₃), 168.36 and 167.46 (2 s, 2C, C-1, C-1'), 154.49 [s, O(C=O)O], 138.50, 137.90 and 137.74 (3 s, 3C, Ar), 128.65, 128.58, 128.41, 128.24, 128.00, 127.95, 127.92, 127.76 and 127.62 (9 d, 15C, Ar), 99.76 (d, C-1''), 99.12 and 99.04 (2 s, 2C, C-2, C-2'), 80.95 (d, C-3''), 80.12 (d, C-2''), 75.79 (d, C-5), 75.14 (t, CH₂Ph), 74.97 (d, C-7), 74.15 (t, CH₂Ph), 73.45 (t, CH₂Ph), 72.22 (d, 2C, C-4'', C-6), 70.70 (d, C-5''), 70.10 (t, C-6''), 69.62 (d, C-6'), 68.70 (d, C-4), 67.82 (d, C-7'), 66.21 (t, C-8), 66.06 (d, C-4'), 64.29 (d, C-5'), 61.94 (t, C-8'), 53.12 (q, CO₂CH₃), 52.64 (q, CO₂CH₃), 51.20 (q, OCH₃), 34.18 (t, C-3), 31.96 (t, C-3'), 20.69, 20.62 and 20.59 (3 q, 4C, COCH₃); ESI-TOF HRMS: $m/z = 1144.4239$; calcd for C₅₅H₆₆O₂₅NH₄⁺: 1144.4231.

12: Colorless oil; $[\alpha]_D^{20} + 110.8$ (*c* 0.89, CHCl₃); R_f 0.30 (*n*-hexane/EtOAc 1:1, HP-TLC); ¹H NMR (CDCl₃): δ 7.41 - 7.27 (m, 15H, Ar), 5.36 - 5.32 (m, 2H, H-4', H-5'), 5.19 (ddd, 1H, $J_{7',6'}$ 9.4, $J_{7',8'b}$ 4.1, $J_{7',8'a}$ 2.8 Hz, H-7'), 5.09 (d, 1H, $J_{1'',2''}$ 3.6 Hz, H-1''), 4.97 (d, 1H, J 11.2 Hz, CHHPh), 4.94 (d, 1H, J 11.2 Hz, CHHPh), 4.88 (d, 1H, J 10.7 Hz, CHHPh), 4.83 (d, 1H, J 10.8 Hz, CHHPh), 4.72 (d, 1H, J 10.6 Hz, CHHPh), 4.66 (dd, 1H, $J_{8'a,8'b}$ 12.3 Hz, H-8'a), 4.61 - 4.54 (m, 3H, H-7, H-8a, CHHPh), 4.32 (app t, 1H, $J_{8b,8a} \sim J_{8b,7}$ 7.6 Hz, H-8b), 4.18 (dd, 1H, $J_{6',5'}$ 1.3 Hz, H-6'), 4.12 (ddd, 1H, $J_{4,3ax}$ 12.0, $J_{4,3eq}$ 4.6, $J_{4,5}$ 2.5 Hz, H-4), 4.02 (dd, 1H, H-8'b), 3.86 (s, 3H, CO₂CH₃), 3.86 - 3.84 (m, 1H, H-5), 3.83 - 3.79 (m, 3H, H-3'', H-6''a, H-6''b), 3.78 (s, 3H, CO₂CH₃), 3.75 - 3.72 (m, 2H, H-5'', H-6), 3.67 (dd, 1H, $J_{9,8}$, $J_{9,1}$ Hz, H-4''), 3.54 (dd, 1H, $J_{2'',3''}$ 9.6 Hz, H-2''), 3.16 (s, 3H, OCH₃), 2.90 (app

t, 1H, J 6.6 Hz, OH), 2.28 - 2.24 (m, 1H, H-3'*eq*), 2.16 - 2.12 (m, 1H, H-3'*eq*), 2.08 and 2.06 (2 s, each 3H, COCH₃), 2.07 - 2.01 (m, 2H, H-3*ax*, H-3'*ax*), 2.00 and 1.99 (2 s, each 3H, COCH₃); ¹³C NMR (CDCl₃): δ 170.79, 170.52, 170.16 and 169.68 (4 s, 4C, COCH₃), 168.27 and 167.44 (2 s, 2C, C-1, C-1'), 154.65 [s, O(C=O)O], 138.40, 137.91 and 137.70 (3 s, 3C, Ar), 128.70, 128.56, 128.51, 128.38, 128.19, 128.13, 127.99 and 127.83 (8 d, 15C, Ar), 99.89 (d, C-1''), 99.67 and 98.93 (2 s, 2C, C-2, C-2'), 81.50 (d, C-3''), 80.56 (d, C-2''), 77.72 (d, C-4''), 76.15 (d, C-5), 75.93 (d, C-7), 75.38 (t, CH₂Ph), 75.35 (t, CH₂Ph), 74.47 (t, CH₂Ph), 72.57 (d, C-5''), 71.84 (d, C-6), 70.05 (d, C-4), 69.65 (d, C-6'), 67.80 (d, C-7'), 66.41 (d, C-4'), 65.88 (t, C-8), 64.36 (d, C-5'), 61.63 (t, C-8'), 61.07 (t, C-6''), 53.17 (q, CO₂CH₃), 52.67 (q, CO₂CH₃), 51.20 (q, OCH₃), 33.81 and 32.53 (2 t, 2C, C-3, C-3'), 20.86, 20.63 and 20.59 (3 q, 4C, COCH₃); ESI-TOF HRMS: m/z = 1149.3771; calcd for C₅₅H₆₆O₂₅Na⁺: 1149.3785.

13: Colorless oil; R_f 0.06 (*n*-hexane/EtOAc 1:1); ¹H NMR (CDCl₃): δ 7.40 - 7.31 (m, 10H, Ar), 5.35 - 5.33 (m, 1H, H-5'), 5.31 (ddd, 1H, $J_{4',3'ax}$ 12.1, $J_{4',3'eq}$ 4.7, $J_{4',5'}$ 3.0 Hz, H-4'), 5.20 (ddd, 1H, $J_{7',6'}$ 9.4, $J_{7',8'b}$ 4.2, $J_{7',8'a}$ 2.8 Hz, H-7'), 5.13 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1''), 5.02 (d, 1H, J 11.5 Hz, CHHPh), 4.83 (d, 2H, J 11.3 Hz, CHHPh, CHHPh), 4.68 (ddd, 1H, $J_{7,8b}$ 8.3, $J_{7,8a}$ 5.7, $J_{7,6}$ 3.9 Hz, H-7), 4.65 (dd, 1H, $J_{8'a,8'b}$ 12.4 Hz, H-8'a), 4.60 (d, 1H, J 10.9 Hz, CHHPh), 4.57 (dd, 1H, $J_{8a,8b}$ 8.4 Hz, H-8a), 4.32 (app t, 1H, H-8b), 4.18 - 4.14 (m, 2H, H-4, H-6'), 4.04 (dd, 1H, H-8'b), 3.88 - 3.87 (m, 1H, H-5), 3.86 (s, 3H, CO₂CH₃), 3.80 (s, 3H, CO₂CH₃), 3.79 - 3.76 (m, 2H, H-6''a, H-6''b), 3.75 - 3.66 (m, 4H, H-3'', H-4'', H-5'', H-6), 3.56 - 3.51 (m, 1H, H-2''), 3.18 (s, 3H, OCH₃), 2.85 (b s, 1H, OH), 2.44 (b s, 1H, OH), 2.27 (dd, 1H, $J_{3'eq,3'ax}$ 12.7 Hz, H-3'*eq*), 2.14 - 2.10 (m, 2H, H-3*ax*, H-3'*eq*), 2.083 and 2.077 (2 s, each 3H, COCH₃), 2.05 (app t, 1H, H-3'*ax*), 2.00 and 1.98 (2 s, each 3H, COCH₃); ¹³C NMR (CDCl₃): δ 170.83, 170.53, 170.15 and 169.68 (4 s, 4C, COCH₃), 168.30 and 167.46 (2s, 2C, C-1, C-1'), 154.54 [s, O(C=O)O], 138.41 and 137.62 (2s, 2C, Ar), 128.73, 128.69, 128.39, 128.06, 128.03 and 127.81 (6 d, 10C, Ar), 99.71 (d, C-1''), 99.54 and 99.02 (2 s, 2C, C-2, C-2'), 81.21 (d, C-3''), 80.43 (d, C-2''), 75.85 (d, C-5), 75.41 (d, C-7), 75.12 (t, CH₂Ph), 74.17 (t, CH₂Ph), 72.41 (d, C-5''), 72.03 (d, C-6), 70.50 (d, C-4''), 69.76 and 69.66 (2 d, 2C, C-4, C-6'), 67.77 (d, C-7'), 66.40 (d, C-4'), 66.09 (t, C-8), 64.34 (d, C-5'), 61.88 (t, C-6''), 61.74 (t, C-8'), 53.20 (q, CO₂CH₃), 52.69 (q, CO₂CH₃), 51.24 (q, OCH₃), 33.94 (t, C-3), 32.40 (t, C-3'), 20.86, 20.64 and 20.59 (3 q, 4C, COCH₃); ESI-TOF HRMS: m/z = 1059.3303; calcd for C₄₈H₆₀O₂₅Na⁺: 1059.3316.

3.7. Methyl (4,5,7,8-tetra-O-acetyl-3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2→4)-[2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl-(1→4)-2,3,6-tri-O-benzyl- α -D-glucopyranosyl-(1→5)]-methyl (methyl 7,8-O-carbonyl-3-deoxy- α -D-manno-oct-2-ulopyranosid)onate (15)

A solution of acceptor **11** (24.8 mg, 0.022 mmol) and donor **14**²¹ (22.9 mg, 0.044 mmol) in dry CH₂Cl₂ (1.0 mL) containing 5 Å molecular sieves (50 mg) was stirred at ambient temperature for 1 h. The cooled (0 °C) mixture was treated with TfOH (0.10 μ L, 1.1 μ mol) in dry CH₂Cl₂ (20 μ L) and stirred for 15 min at 0 °C. The promotor was neutralized with triethylamine (15 μ L, 0.110 mmol), the suspension was filtered over Celite[®], rinsed with CH₂Cl₂ and the filtrate was concentrated. The residue was subjected to HP-chromatography (toluene/EtOAc 2:1 → 1:2) affording impure product fractions which were further purified by HPLC (*n*-hexane/EtOAc 2:1 → 1:2) to obtain pure **15** (14.7 mg, 46%) as a colorless oil:

$[\alpha]_D^{20} + 93.5$ (c 0.50, CHCl_3); R_f 0.30 (toluene/EtOAc 1:1, HP-TLC); $^1\text{H NMR}$ (CDCl_3): δ 7.44 - 7.18 (m, 15H, Ar), 5.36 - 5.34 (m, 1H, H-5'), 5.26 (ddd, 1H, $J_{4',3'ax}$ 12.5, $J_{4',3'eq}$ 5.0, $J_{4',5'}$ 2.9 Hz, H-4'), 5.21 (ddd, 1H, $J_{7',6'}$ 9.2, $J_{7',8'b}$ 4.1, $J_{7',8'a}$ 2.7 Hz, H-7'), 5.07 (d, 1H, $J_{1'',2''}$ 3.8 Hz, H-1''), 5.05 (d, 1H, J 11.5 Hz, *CHHP*), 5.01 (app t, 1H, $J_{4''',3''}$ ~ $J_{4''',5''}$ 9.5 Hz, H-4'''), 4.94 (app. t, 1H, $J_{3''',2''}$ 9.2 Hz, H-3'''), 4.92 - 4.89 (m, 1H, H-2'''), 4.75 - 4.71 (m, 3H, $2 \times$ *CHHP*, *CHHP*), 4.68 (app td, 1H, $J_{7,8b}$ 8.1, $J_{7,6}$ ~ $J_{7,8a}$ 5.0 Hz, H-7), 4.61 (dd, 1H, $J_{8'a,8'b}$ 12.4 Hz, H-8'a), 4.50 (d, 1H, J 10.9 Hz, *CHHP*), 4.48 (d, 1H, $J_{1''',2''}$ 7.7 Hz, H-1'''), 4.45 (dd, 1H, $J_{8a,8b}$ 8.5, $J_{8a,7}$ 5.5 Hz, H-8a), 4.31 (d, 1H, J 11.6 Hz, *CHHP*), 4.20 (ddd, 1H, J 9.7, J 7.0, $J_{4,5}$ 2.5 Hz, H-4), 4.14 (dd, 1H, $J_{6''a,5''}$ 4.3, $J_{6''a,6''b}$ 12.5 Hz, H-6''a), 4.12 (br, d, 1H, $J_{6',7'}$ 9.2 Hz, H-6'), 4.11 (app t, 1H, H-8b), 4.07 (dd, 1H, H-8'b), 3.94 (app t, 1H, $J_{4'',3''}$ ~ $J_{4'',5''}$ 9.5 Hz, H-4''), 3.90 (dd, 1H, $J_{6''b,6''a}$ 12.2, $J_{6''b,5''}$ 2.3 Hz, H-6''b), 3.85 - 3.84 (m, 1H, H-5), 3.82 (s, 3H, CO_2CH_3), 3.81 (dd, 1H, $J_{6''a,6''b}$ 10.5, $J_{6''a,5''}$ 2.5 Hz, H-6''a), 3.81 (s, 3H, CO_2CH_3), 3.68 (app t, 1H, H-3''), 3.67 - 3.65 (m, 2H, H-5'', H-6), 3.53 (dd, 1H, $J_{6''b,5''}$ 1.1 Hz, H-6''b), 3.48 (dd, 1H, $J_{2'',3''}$ 9.8 Hz, H-2''), 3.29 (ddd, 1H, $J_{5''',6''a}$ 4.2 Hz, H-5'''), 3.19 (s, 3H, OCH_3), 2.25 (dd, 1H, $J_{3'eq,3'ax}$ 13.0 Hz, H-3'eq), 2.10 (app t, 1H, H-3'ax), 2.095 and 2.08 (2 s, each 3H, COCH_3), 2.04 - 2.01 (m, 2 H, H-3ax, H-3eq), 2.01, 1.99, 1.98, 1.97, 1.94 and 1.90 (6 s, each 3H, COCH_3); $^{13}\text{C NMR}$ (CDCl_3): δ 170.92, 170.59, 170.33, 170.16, 169.91, 169.62, 169.34 and 169.10 (8 s, 8C, COCH_3), 168.36 and 167.56 (2 s, 2C, C-1, C-1'), 154.57 [s, $\text{O}(\text{C}=\text{O})\text{O}$], 139.00, 137.81 and 137.76 (3 s, 3C, Ar), 128.73, 128.54, 128.31, 128.23, 128.18, 127.56 and 127.48 (7 d, 15C, Ar), 100.10 (d, C-1''), 99.97 (d, C-1'''), 99.07 and 98.97 (2 s, 2C, C-2, C-2'), 79.68 (d, C-2''), 79.62 (d, C-3''), 77.0 (d, C-4'', overlapped by solvent peak), 76.16 (d, C-5), 75.06 (d, C-7), 75.03 (t, CH_2Ph), 74.52 (t, CH_2Ph), 73.49 (t, CH_2Ph), 73.19 (d, C-3'''), 72.26 (d, C-6), 71.90 (d, C-2'''), 71.46 (d, C-5'''), 71.35 (d, C-5''), 69.66 (d, C-6'), 68.55 (d, C-4), 68.21 (d, C-4'''), 67.85 (d, C-7'), 67.75 (t, C-6''), 66.06 (t, C-8), 66.03 (d, C-4'), 64.25 (d, C-5'), 61.92 (t, C-8'), 61.66 (t, C-6'''), 53.17 and 52.65 (2 q, 2C, CO_2CH_3), 51.22 (q, OCH_3), 34.28 (t, C-3), 31.91 (t, C-3'), 20.72, 20.63, 20.59 and 20.56 (4 q, 8C, COCH_3); ESI-TOF HRMS: m/z = 1479.4740; calcd for $\text{C}_{69}\text{H}_{84}\text{O}_{34}\text{Na}^+$: 1479.4736.

3.8. Sodium (3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2 \rightarrow 4)-[β -D-glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl-(1 \rightarrow 5)]-sodium (methyl α -D-manno-oct-2-ulopyranosid)onate (16)

According to the procedure for compound **10**, tetrasaccharide **15** (14.0 mg, 0.010 mmol) was debenzylated in dry MeOH (1.0 mL) over 10% Pd-C (1 mg) under hydrogen at atmospheric pressure for 3 h at rt, and deacetylated by treatment with sodium methoxide (0.1 M in MeOH, 24 μL , 2.4 μmol) in dry MeOH (1.0 mL) for 14 h at rt. A solution of the deblocked intermediate in H_2O (2 mL) and aq NaOH (0.1 M, 0.5 mL) was stirred for 2 h at 0 $^\circ\text{C}$ and for 1 h at ambient temperature followed by neutralization with Dowex resin and processing as described for **10**. The filtrate was lyophilized and the residue was desalted on a PD10 SEC column (H_2O) to give **16** (8.0 mg, 99%) as a colorless amorphous solid: $[\alpha]_D^{20} + 91.4$ (c 0.56, H_2O); $^1\text{H NMR}$ (D_2O , pD ~ 7.4): δ 5.25 (d, 1H, $J_{1'',2''}$ 3.5 Hz, H-1''), 4.49 (d, 1H, $J_{1''',2''}$ 7.7 Hz, H-1'''), 4.25 - 4.20 (m, 2H, H-5, H-5''), 4.03 (ddd, 1H, $J_{7,6}$ 9.4, $J_{7,8b}$ 6.4, $J_{7,8a}$ 2.9 Hz, H-7), 3.97 - 3.84 (m, 10H, H-3'', H-4, H-4', H-5', H-6''a, H-6''b, H-6''a, H-7', H-8a, H-8'a), 3.71 (app t, 1H, $J_{4'',3''}$ ~ $J_{4'',5''}$ 9.7 Hz, H-4''), 3.70 (dd, 1H, $J_{6''b,6''a}$ 12.1, $J_{6''b,5''}$ 6.1 Hz, H-6''b), 3.67 (d, 1H, $J_{6',7'}$ 8.2 Hz, H-6'), 3.66 (dd, 1H, $J_{8'b,8'a}$ 11.6, $J_{8'b,7'}$ 7.3 Hz, H-8'b), 3.59 (dd, 1H, $J_{8b,8a}$ 11.9, H-8b), 3.52 (dd, 1H, $J_{2'',3''}$ 10.3 Hz, H-2''), 3.51 (d, $J_{6,7}$

10.3 Hz, H-6), 3.49 - 3.44 (m, 2H, H-3''', H-5'''), 3.38 (app t, 1H, $J_{4''',3'''} \sim J_{4''',5'''} 9.5$ Hz, H-4'''), 3.28 (dd, 1H, $J_{2''',3'''} 9.3$ Hz, H-2'''), 3.08 (s, 3H, OCH₃), 2.07 - 1.99 (m, 2H, H-3_{eq}, H-3'_{eq}), 1.95 (app t, 1H, $J_{3ax,3eq} \sim J_{3ax,4}$ 12.3 Hz, H-3_{ax}), 1.76 (app t, $J_{3'ax,3'eq} \sim J_{3'ax,4'}$ 12.5 Hz, H-3'_{ax}); ¹³C NMR (D₂O, pD ~ 7.4): see Table 1; ESI-TOF HRMS: $m/z = 819.2375$; calcd for C₂₉H₄₈O₂₅Na⁺: 819.2377.

3.9. 2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl-(1 \rightarrow 6)-2,3,4-tri-O-acetyl- α , β -D-glucopyranosyl (*N*-phenyl)trifluoroacetimidate (**17**)

A solution of isomaltose (100 mg, 0.292 mmol) in dry pyridine (2.0 mL) was treated with acetic anhydride (0.8 mL) and 4-(*N,N*-dimethylamino)pyridine (5 mg) at 0 °C and after 20 h at ambient temperature, excessive reagent was destroyed by adding dry MeOH (2.0 mL) at 0 °C. After 10 min the solvent was coevaporated with toluene (3 \times) and the residual oil was purified by chromatography (*n*-hexane/EtOAc 1:1 \rightarrow 1:2) affording an α/β -mixture (1:1) of peracetylated isomaltose (198 mg, quantitative yield). The residue (101 mg, 0.149 mmol) was dissolved in dry DMF (2.0 mL) and treated with hydrazine acetate (27 mg, 0.298 mmol) at 40 °C for 2 h under Ar. The mixture was diluted with EtOAc, washed with cold brine and the organic layer was dried (MgSO₄) and filtered. Concentration of the filtrate provided a crude product which was purified by chromatography (*n*-hexane/EtOAc 1:2) to obtain an α/β -mixture (1:0.3) of the hemiacetal (85 mg, 90%): ¹H NMR (CDCl₃): δ 5.53 (dd, 1H, $J_{3\alpha,2\alpha} 10.1$, $J_{3\alpha,4\alpha} 9.4$ Hz, H-3 α), 5.46 (app t, 1H, $J_{3'\beta,2'\beta} \sim J_{3'\beta,4'\beta} 9.8$ Hz, H-3' β), 5.45 (app t, 1H, $J_{3'\alpha,2'\alpha} \sim J_{3'\alpha,4'\alpha} 9.8$ Hz, H-3' α), 5.40 (app t, 1H, $J_{1\alpha,2\alpha} \sim J_{1\alpha,OH} 3.5$ Hz, H-1 α), 5.23 (app t, 1H, $J_{3\beta,2\beta} \sim J_{3\beta,4\beta}$ Hz, H-3 β), 5.14 (d, 1H, $J_{1'\beta,2'\beta} 3.8$ Hz, H-1' β), 5.13 (d, 1H, $J_{1'\alpha,2'\alpha} 3.8$ Hz, H-1' α), 5.02 - 4.97 (m, 3H, H-4' α , H-4 β , H-4 α), 4.94 (dd, 1H, $J_{4\alpha,5\alpha} 10.3$ Hz, H-4 α), 4.85 - 4.78 (m, 4H, H-2 α , H-2' α , H-2 β , H-2' β), 4.73 (app t, 1H, $J 8.0$ Hz, H-1 β), 4.24 (ddd, 1H, $J_{5\alpha,6\alpha\alpha} 6.5$, $J_{5\alpha,6\alpha\beta} 2.5$ Hz, H-5 α), 4.21 - 4.05 (m, 7H, H-5' α , H-5' β , H-6' $\alpha\alpha$, H-6' $\alpha\beta$, H-6' $\beta\alpha$, H-6' $\beta\beta$, OH β), 4.02 - 3.99 (m, 1H, OH α), 3.74 - 3.57 (m, 5H, H-5 β , H-6 $\alpha\alpha$, H-6 $\alpha\beta$, H-6 $\beta\alpha$, H-6 $\beta\beta$), 2.10 to 2.00 (10 s, 14 \times COCH₃); ESI-TOF HRMS: $m/z = 659.1794$; calcd for C₂₆H₃₆O₁₈Na⁺: 659.1798.

The residue (70 mg, 0.110 mmol) was dissolved in dry CH₂Cl₂ (1.8 mL) and dry acetone (1.0 mL). Potassium carbonate (30 mg, 0.220 mmol) and 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (35 μ L, 0.220 mmol) were added successively under Ar. The suspension was stirred for 12 h at rt, then filtered through a pad of Celite, rinsed with CH₂Cl₂ and the filtrate was concentrated. Swift chromatographic purification (*n*-hexane/EtOAc 1:1) provided **17** (80 mg, 90%) which was directly used for the subsequent glycosylation reaction. ¹H NMR spectra of **17** (CDCl₃) displayed broad signals corresponding to a 2.5:1 mixture; ¹³C NMR (125 MHz, CDCl₃, selected data): δ 169.8-168.9 (C=O, Ac), 142.8, 128.9, 128.8 124.7 and 119.4 (arom. C), 96.0 (C-1, C-1'), 66.4 and 66.2 (C-6), 61.7 (C-6'), 20.6-20.4 (CH₃CO).

3.10. Methyl (4,5,7,8-tetra-O-acetyl-3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2 \rightarrow 4)-[2,3,4,6-tetra-O-acetyl- α -D-glucopyranosyl-(1 \rightarrow 6)-2,3,4-tri-O-acetyl- β -D-glucopyranosyl-

(1→4)-2,3,6-tri-O-benzyl- α -D-glucofuranosyl-(1→5)]-methyl (methyl 7,8-O-carbonyl-3-deoxy- α -D-manno-oct-2-ulopyranosid)onate (18)

A suspension of acceptor **11** (20.0 mg, 0.018 mmol) and donor **17** (28.7 mg, 0.035 mmol) in dry CH₂Cl₂ (1.0 mL) containing 5 Å molecular sieves (50 mg) was stirred at ambient temperature for 1 h. The cooled (0 °C) mixture was treated with TfOH (0.04 μ L, 0.44 μ mol) in dry CH₂Cl₂ (10 μ L) and stirred for 30 min at 0 °C. An additional portion of TfOH (0.16 μ L, 1.76 μ mol) was added (0 °C) and after 15 min triethylamine (12.3 μ L, 0.089 mmol) was added. The mixture was filtered over Celite, rinsed with CH₂Cl₂ and the filtrate was concentrated. The residue was subjected to HP-chromatography (toluene/EtOAc 2:1 → 1:2) affording impure product fractions which were further purified by HPLC (*n*-hexane/EtOAc 1:1 → 1:2) to give pure **18** (22.0 mg, 71%) as a colorless oil: $[\alpha]_D^{20} + 105.8$ (*c* 1.10, CHCl₃); R_f 0.16 (*n*-hexane/EtOAc 1:1, HP-TLC); ¹H NMR (CDCl₃): δ 7.44 - 7.25 (m, 15H, Ar), 5.38 (app t, 1H, $J_{3''',2'''} \sim J_{3''',4'''} 9.7$ Hz, H-3'''), 5.34 - 5.33 (m, 1H, H-5'), 5.24 (ddd, 1H, $J_{4',3'ax} 12.6$, $J_{4',3'eq} 4.9$, $J_{4',5'} 2.9$ Hz, H-4'), 5.20 (ddd, 1H, $J_{7',6'} 9.3$, $J_{7',8'b} 4.3$, $J_{7',8'a} 2.8$ Hz, H-7'), 5.03 (d, 1H, $J_{1'',2''} 3.9$ Hz, H-1''), 5.02 (app t, 1H, $J_{4''',5'''} 9.7$ Hz, H-4'''), 5.00 (app t, 1H, $J_{3''',2'''} \sim J_{3''',4'''} 9.3$ Hz, H-3'''), 4.98 (d, 1H, $J_{1''',2'''} 3.4$ Hz, H-1'''), 4.92 (app t, 1H, $J_{4''',5'''} 9.5$ Hz, H-4'''), 4.90 - 4.87 (m, 2H, H-2''', CHHPh), 4.83 - 4.80 (m, 2H, H-2''', CHHPh), 4.79 (d, 1H, $J 11.7$ Hz, CHHPh), 4.69 (d, 1H, $J 11.7$ Hz, CHHPh), 4.67 (ddd, 1H, $J_{7,8b} 8.3$, $J_{7,8a} 5.4$, $J_{7,6} 4.6$ Hz, H-7), 4.60 (dd, 1H, $J_{8'a,8'b} 12.3$ Hz, H-8'a), 4.55 (d, 1H, $J 11.0$ Hz, CHHPh), 4.51 (d, 1H, $J_{1''',2'''} 8.0$ Hz, H-1'''), 4.42 (dd, 1H, $J_{8a,8b} 8.6$ Hz, H-8a), 4.29 (d, 1H, $J 11.8$ Hz, CHHPh), 4.21 (dd, 1H, $J_{6''',a,6''',b} 12.3$, $J_{6''',a,5'''} 4.0$ Hz, H-6''',a), 4.19 (ddd, 1H, $J_{4,3ax} 11.3$, $J_{4,3eq} 5.5$, $J_{4,5} 2.5$ Hz, H-4), 4.11 (dd, 1H, $J_{6',5'} 1.4$ Hz, H-6'), 4.08 - 4.04 (m, 2H, H-8b, H-8'b), 3.96 (dd, 1H, $J_{6''',b,5'''} 2.3$ Hz, H-6''',b), 3.94 (app t, 1H, $J_{4'',3''} \sim J_{4'',5''} 9.5$ Hz, H-4''), 3.88 (ddd, 1H, H-5'''), 3.84 - 3.83 (m, 1H, H-5), 3.82 (s, 3H, CO₂CH₃), 3.80 (s, 3H, CO₂CH₃), 3.78 (dd, 1H, $J_{6''',a,5'''} 10.8$, $J_{6''',a,5'''} 2.8$ Hz, H-6''',a), 3.67 - 3.63 (m, 3H, H-3'', H-5'', H-6), 3.49 (dd, 1H, $J_{6''',b,5'''} 1.5$ Hz, H-6''',b), 3.46 (dd, 1H, $J_{2'',3''} 9.7$, H-2''), 3.42 (dd, 1H, $J_{6''',a,6''',b} 11.6$, $J_{6''',a,5'''} 5.4$ Hz, H-6''',a), 3.35 (dd, 1H, $J_{6''',b,5'''} 4.2$ Hz, H-6''',b), 3.30 - 3.26 (m, 1H, H-5'''), 3.18 (s, 3H, OCH₃), 2.22 - 2.18 (m, 1H, H-3'eq), 2.11 (app t, 1H, $J_{3'ax,3'eq} 13.1$ Hz, H-3'ax), 2.07 - 1.99 (m, 2H, H-3eq, H-3ax), 2.09, 2.08, 2.06, 2.02, 2.01, 2.00, 1.99, 1.98, 1.96 and 1.89 (10 s, 33H, COCH₃); ¹³C NMR (CDCl₃): δ 170.89, 170.48, 170.31, 170.14, 169.94, 169.91, 169.90, 169.60, 169.50, 169.45 and 169.16 (11 s, 11C, COCH₃), 168.38 and 167.55 (2 s, 2C, C-1, C-1'), 154.58 [s, O(C=O)O], 138.81, 137.86 and 137.83 (3 s, 3C, Ar), 128.71, 128.60, 128.33, 128.24, 128.19, 128.14, 127.99 and 127.53 (8 d, 15C, Ar), 100.15 (d, C-1''), 99.76 (d, C-1'''), 99.09 and 98.95 (2 s, 2C, C-2, C-2'), 96.12 (d, C-1'''), 79.71 (d, C-2''), 79.33 (d, C-3''), 76.88 (d, C-4''), 76.07 (d, C-5), 75.09 (d, C-7), 75.01 (t, CH₂Ph), 74.58 (t, CH₂Ph), 73.53 (t, CH₂Ph), 73.24 (d, C-3'''), 72.51 (d, C-5'''), 72.27 (d, C-6), 71.89 (d, C-2'''), 71.36 (d, C-5''), 70.45 (d, C-2'''), 70.01 (d, C-3'''), 69.80 (d, C-4'''), 69.64 (d, C-6'), 68.60 (d, C-4), 68.28 (d, C-4'''), 68.12 (t, C-6'''), 68.04 (t, C-6''), 67.84 (d, C-7'), 67.64 (d, C-5'''), 66.03 (t, C-8), 66.01 (d, C-4'), 64.23 (d, C-5'), 61.92 (t, C-8'), 61.67 (t, C-6'''), 53.14 (q, CO₂CH₃), 52.60 (q, CO₂CH₃), 51.19 (q, OCH₃), 34.23 (t, C-3), 31.90 (t, C-3'), 20.68, 20.64, 20.62, 20.59, 20.56 and 20.55 (6 q, 11C, COCH₃); ESI-TOF HRMS: $m/z = 1762.6029$; calcd for C₈₁H₁₀₀O₄₂NH₄⁺: 1762.6027.

3.11. Sodium (3-deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2 \rightarrow 4)-[α -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl-(1 \rightarrow 5)]-sodium (methyl α -D-manno-oct-2-ulopyranosid)onate (19)

According to the procedure for compound **10**, pentasaccharide **18** (12.0 mg, 0.007 mmol) was debenzylated in dry MeOH (1.0 mL) over 10% Pd-C (1 mg) under an atmosphere of hydrogen (1 atm) for 4 h at rt, and deacetylated by treatment with sodium methoxide (0.1 M in MeOH, 100 μ L, 10.0 μ mol; 3 portions) in dry MeOH (1.0 mL) for 20 h at rt. Workup of the solution as described for **10** afforded a residue which was dissolved in H₂O (2 mL) and treated with aq NaOH (0.1 M, 1.0 mL) for 3 h at 0 °C followed by neutralization and freeze-drying. The residue was desalted on a PD10 column (H₂O) and lyophilisation of the pooled fractions provided compound **19** (6.2 mg, 90%) as a colorless amorphous solid: $[\alpha]_D^{20} + 100.8$ (*c* 0.43, H₂O); ¹H NMR (D₂O, pD ~ 7.4): δ 5.25 (d, 1H, $J_{1'',2''}$ 3.7 Hz, H-1''), 4.94 (d, 1H, $J_{1''',2''''}$ 3.8 Hz, H-1'''), 4.52 (d, 1H, $J_{1''',2''''}$ 8.0 Hz, H-1'''), 4.24 (app td, 1H, $J_{5'',4''}$ 10.1, $J_{5'',6''a} \sim J_{5'',6''b}$ 2.4 Hz, H-5''), 4.21 (d, 1H, $J_{5,6}$ 2.2 Hz, H-5), 4.03 (ddd, 1H, $J_{7,6}$ 9.4, $J_{7,8b}$ 6.3, $J_{7,8a}$ 3.0 Hz, H-7), 3.97 - 3.75 (m, 13H, H-3'', H-3''', H-4, H-4', H-5', H-6''a, H-6''b, H-6'''a, H-6'''b, H-6''''a, H-7', H-8a, H-8'a), 3.73 - 3.63 (m, 6H, H-4'', H-5''', H-5''''a, H-6', H-6''b, H-8'b), 3.58 (dd, 1H, $J_{8'b,8'a}$ 11.9 Hz, H-8b), 3.54 - 3.44 (m, 5H, H-2'', H-2''', H-3''', H-4''', H-6), 3.37 (app t, 1H, $J_{4''',3''''} \sim J_{4''',5''''}$ 9.5 Hz, H-4'''), 3.29 (app t, $J_{2''',3''''} \sim 8.7$ Hz, H-2'''), 3.07 (s, 3H, OCH₃), 2.06 - 1.99 (m, 2H, H-3_{eq}, H-3'_{eq}), 1.94 (app t, 1H, $J_{3ax,3eq} \sim J_{3ax,4}$ 12.1 Hz, H-3_{ax}), 1.76 (app t, 1H, $J_{3'ax,3'eq} \sim J_{3'ax,4'}$ 12.5 Hz, H-3'_{ax}); ¹³C NMR (D₂O, pD ~ 7.4): see Table 1; ESI-TOF HRMS: *m/z* = 981.2906; calcd for C₃₅H₅₈O₃₀Na⁺: 981.2095.

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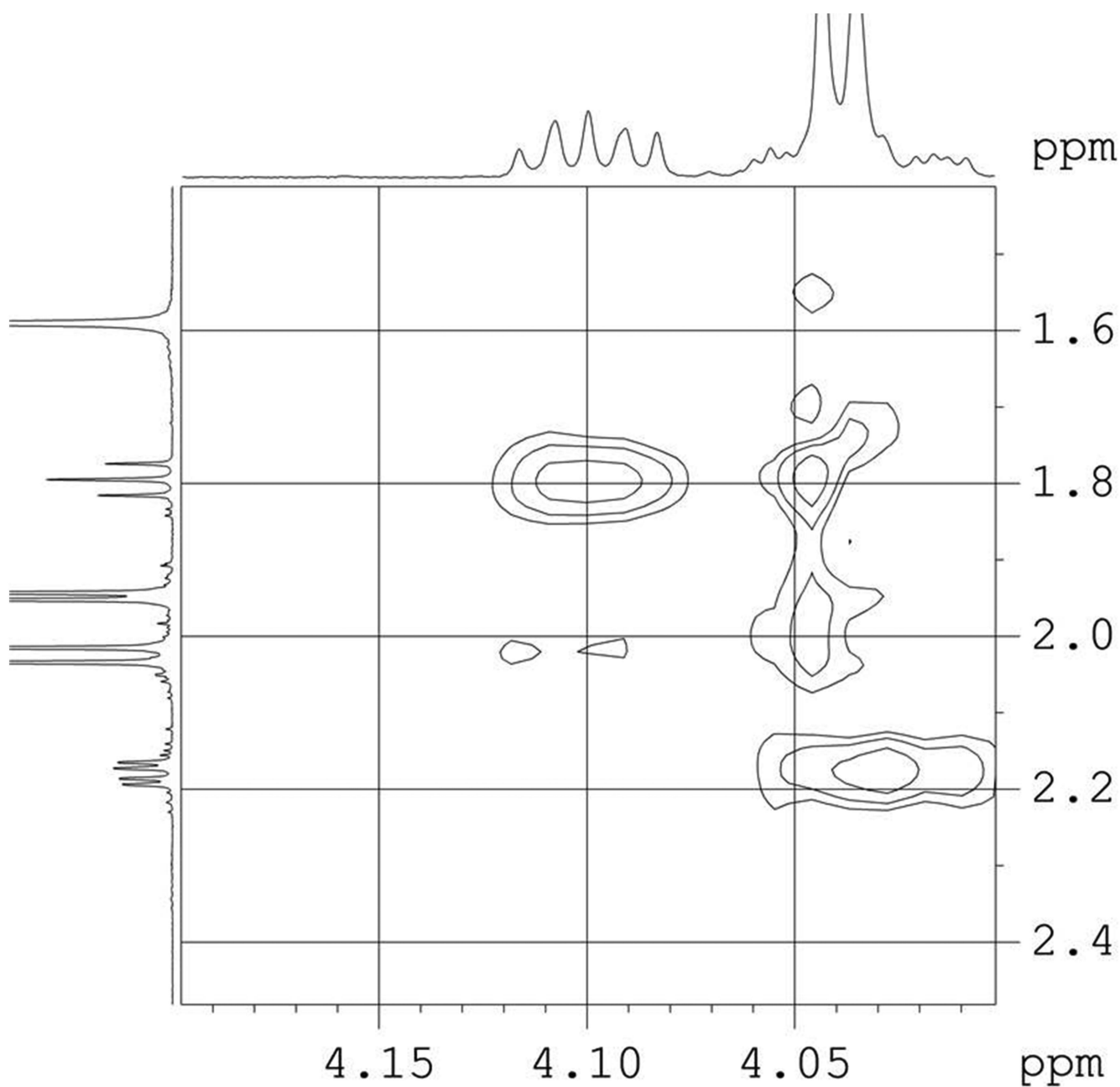
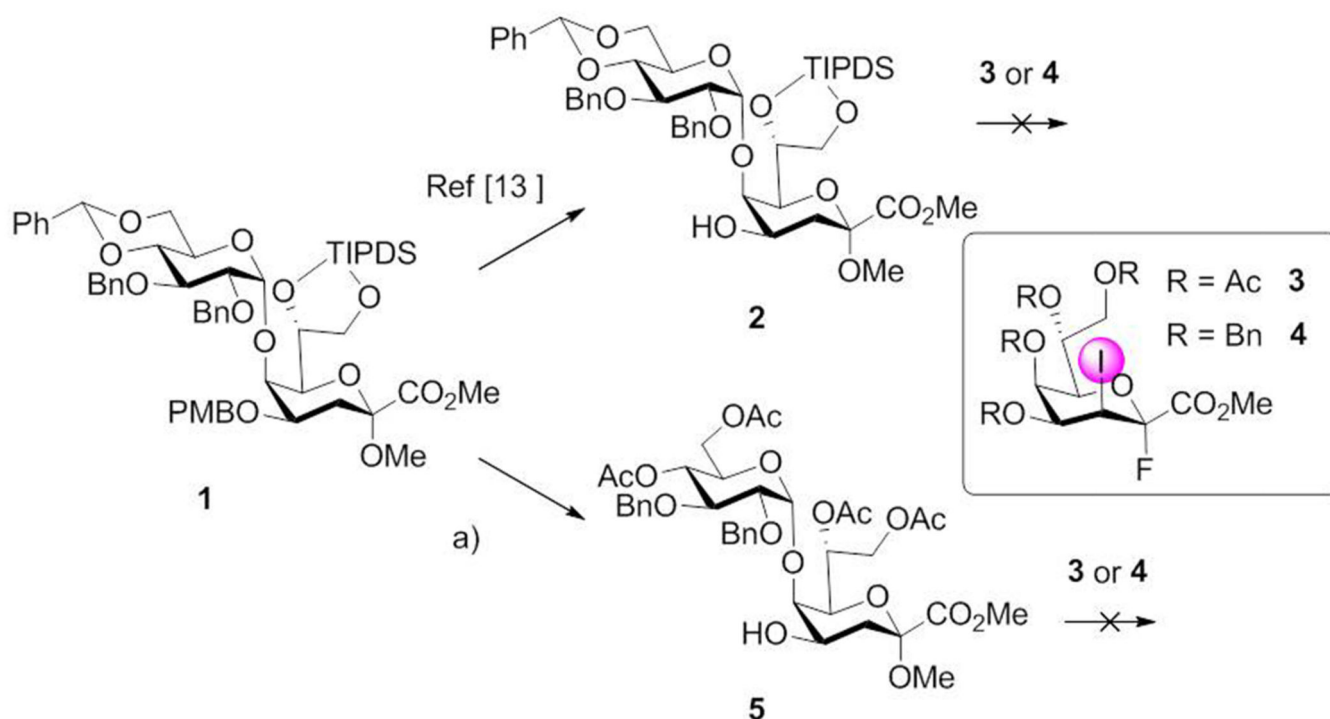
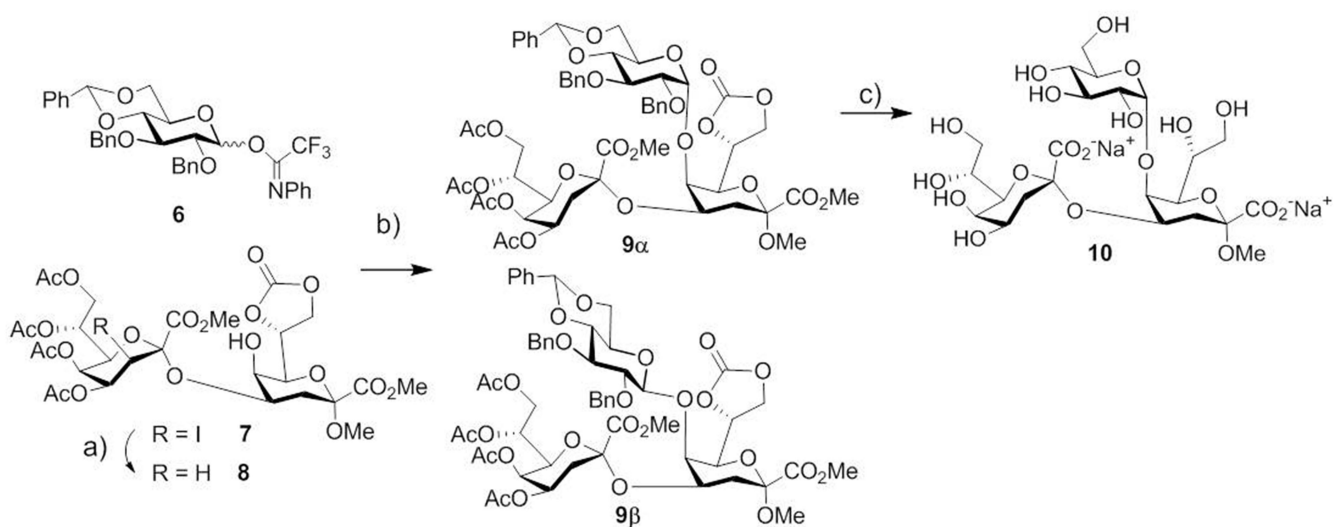


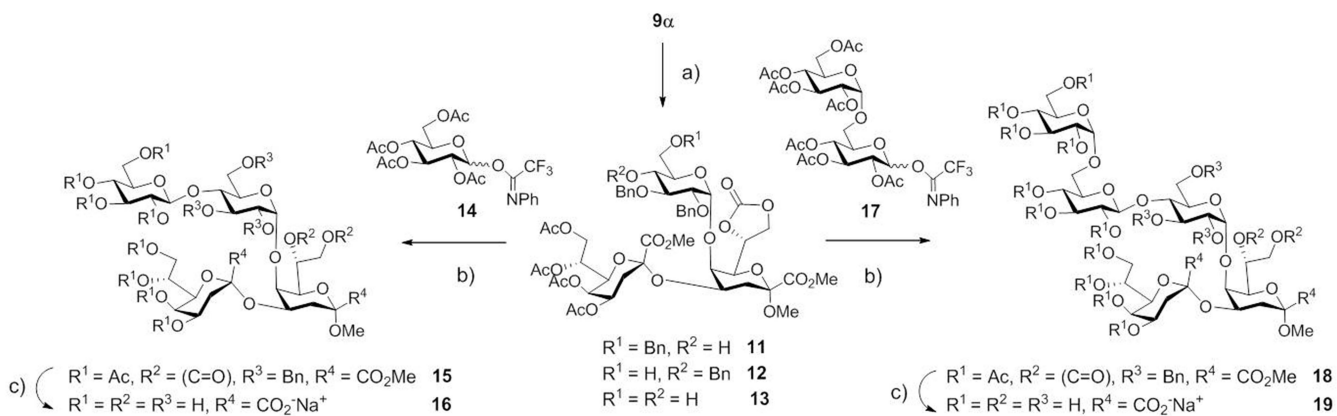
Fig. 1. Section of the NOESY spectrum of compound **5** in CDCl_3 showing a correlation between H-3 α x and H-5'.

**Scheme 1.**

Reagents and conditions: a) *p*TosOH, MeOH, 40 °C, 2 h; then TBAF, THF, 0 °C, 20 min; then Ac₂O, DMAP, pyridine, r.t., 14 h; then DDQ, CH₂Cl₂/MeOH (3:1), r.t., 3.5 h, 40% (4 steps).

**Scheme 2.**

Reagents and conditions: a) Lauroyl peroxide, cyclohexane/ClCH₂CH₂Cl (7.2:1), reflux, 2 h, 92%; b) TfOH, 5 Å molecular sieves, CH₂Cl₂/Et₂O (2:1), r.t., 3 h, 91% (α/β 4:1, isolated **9 α** : 67%, **9 β** : 16%); c) Pd-C (10%), H₂ (1 bar), MeOH, r.t., 4 h; then 0.1 M NaOMe, MeOH, r.t., 17 h; then 0.1 M aq. NaOH, 0 °C → r.t., 4 h, 94%.

**Scheme 3.**

Reagents and conditions: a) Triethylsilane, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 , 0 °C, 1 h, **11**: 72%, **12**: 5%, **13**: 2% ; b) TfOH , 5 Å molecular sieves, CH_2Cl_2 , 0 °C, 15 min (for \rightarrow **15**)/45 min (for \rightarrow **18**), **15**: 46%, **18**: 71%; c) Pd-C (10%), H_2 (1 atm), MeOH, r.t., 3 h (for \rightarrow **16**)/4 h (for \rightarrow **19**); then 0.1 M NaOMe, MeOH, r.t., 14 h (for \rightarrow **16**)/20 h (for \rightarrow **19**) ; then 0.1 M aq. NaOH, 0 °C \rightarrow r.t., 3 h, **16**: 99%, **19**: 90%.

Table 1

 ^{13}C NMR chemical shifts (δ) of compounds **10**, **16** and **19**

Residue	Carbon	10	16	19
<i>→4,5)-α-Kdop</i>				
	1	176.05	175.94	176.05 ^b
	2	101.35	101.35	101.34
	3	35.24	35.21	35.19
	4	72.12	72.04 ^a	72.04 ^c
	5	74.47	73.94	73.78
	6	73.22	73.11	73.10
	7	69.26	69.35	69.29
	8	63.98	64.00	64.08
<i>α-Kdop-(2→4)-</i>				
	1	176.05	176.03	175.92 ^b
	2	102.87	102.82	102.84
	3	35.42	35.48	35.50
	4	66.67	66.73	66.75
	5	67.68	67.66	67.67
	6	72.50	72.55	72.55 ^d
	7	71.52	71.50	71.52
	8	63.58	63.82	63.63
<i>α-GlcP-(1→5)-</i>				
	1	100.31	99.72	99.61
	2	72.88	72.60	72.45
	3	73.29	71.90 ^a	72.03 ^c
	4	69.76	79.07	79.98
	5	72.64	71.25	71.06
	6	60.69	60.29	60.30
<i>β-GlcP-(1→4)-</i>				
	1		103.36	103.69
	2		73.94	73.81
	3		76.33	76.40
	4		70.33	70.49
	5		76.78	74.97
	6		61.46	66.74
<i>α-GlcP-(1→6)-</i>				
	1			98.79
	2			72.32
	3			73.62
	4			70.49

Residue	Carbon	10	16	19
	5			72.71 ^d
	6			61.39
OCH ₃		51.37	51.38	51.37

^{a-d} Assignments within a column may be reversed