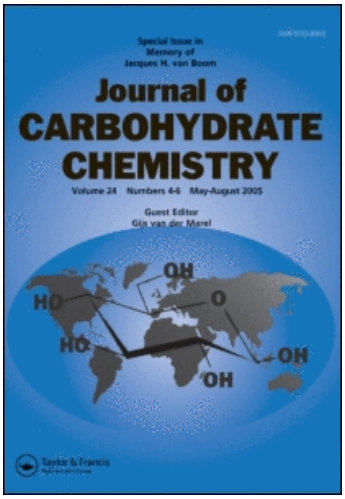

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Eva Segerstedt[a]; Karin Mannerstedt[a]; Mikael Johansson[a]; Stefan Oscarson[a]
[a] Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden

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Eva Segerstedt, Karin Mannerstedt, Mikael Johansson, and Stefan Oscarson*

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden

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*Correspondence: Stefan Oscarson, Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Floor 6, S-106 91 Stockholm, Sweden; Fax: +46 8 15 49 08; E-mail: s.oscarson@organ.su.se.

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ABSTRACT

An efficient synthesis of the protected branched trisaccharide (2′*S*,3′*S*)-(7-*O*-benzyl-6-*O*-chloroacetyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-2-*O*-*p*-methoxybenzyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1 → 3)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 4)]-7-*O*-acetyl-1,6-anhydro-2-*O*-benzyl-L-*glycero*-β-D-*manno*-heptopyranose, which is a key intermediate in the synthesis of inner core structures of *Haemophilus* and *Neisseria* LPSs, is described. The heptoses were formed by Grignard reactions using a benzyloxymethyl chloride or a commercial vinyl reagent. The anhydro bridge was formed by treatment of a 6-OH methyl α-heptoside precursor with $FeCl_3$. The protecting group pattern allows modifications at the 2-, 3-, 4-, and 6-positions of the second heptose moiety and also, after acetolysis of the anhydro bridge, elongation at the reducing end, all known alterations found in the bacterial LPSs.

Key Words: Oligosaccharide synthesis; 1,6-Anhydro acceptors; Glycoconjugate vaccines.

INTRODUCTION

The lipopolysaccharide (LPS) of the gram-negative bacteria *Haemophilus influenzae* is extremely heterogeneous, which has made the analysis of its structure most complex. The LPS is truncated and lacks the polymeric O-antigen. Through the phase-variable expression of several transferases and kinases, the bacteria can build up a large number of structures, possibly to be able to evade the human immune system. With the elucidation of the bacterial genome, subsequent use of bacterial mutants, and new analysis techniques, it has become possible during the last decade to determine many of these structures and the genetics behind them.[1,2] These results strongly indicated that the bacterium makes a conservative core pentasaccharide (Fig. 1), which is linked to Lipid A and is found in all bacteria so far investigated. This core is then modified in numerous ways by glycans, various phosphate groups, amino acids, and acetates.

Glycoconjugate vaccines based on capsular polysaccharide structures have been most successful.[3,4] Due to the heterogeneity of the *H. influenzae* LPS it is not possible to use these carbohydrate structures for the construction of a conjugate vaccine. One conceivable solution instead would be to use well-defined synthetic structures corresponding to the conservative part of the LPS. Another advantage of this approach would be that detoxification of the Lipid A part is not needed. As part of a program directed toward LPS-based vaccines against *H. influenzae*, we are trying to synthesize part structures of the LPS inner core and evaluate these as vaccine candidates after conjugation to a carrier protein.

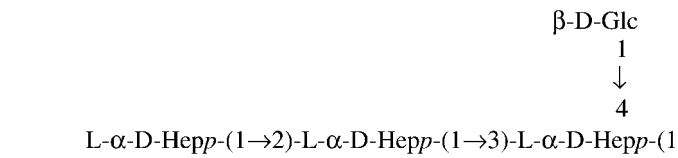


Figure 1. Structure of the inner core of dephosphorylated *H. influenzae* LPS.

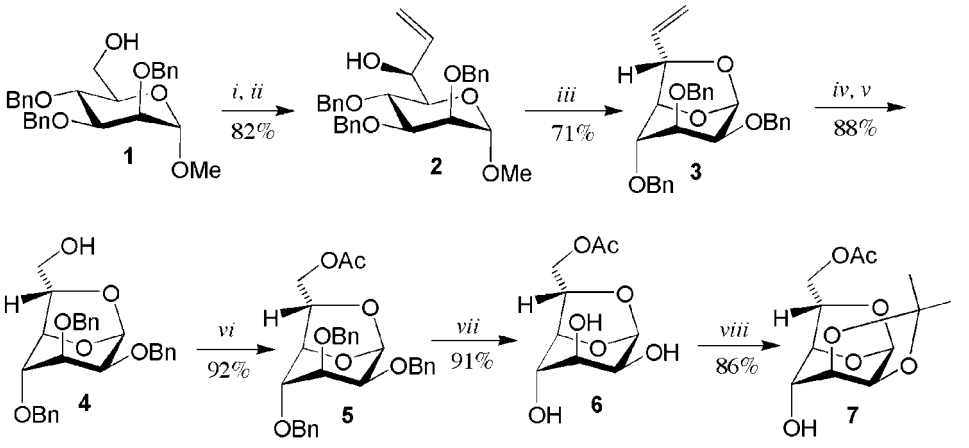
A key intermediate in these syntheses is the branched trisaccharide L-*glycero*-α-D-*manno*-heptopyranosyl-(1 $\rightarrow$ 3)-[β-D-glucopyranosyl-(1 $\rightarrow$ 4)]-L-*glycero*-β-D-*manno*-heptopyranose.

In an earlier publication we showed that the use of a 1,6-anhydro-heptose acceptor facilitated the formation of the 3,4-branched trisaccharide.[5] Obviously, the conformational change, from $^4C_1$ to $^1C_4$, in the acceptor released the steric strain in the transition state to the target trisaccharide and allows for an efficient α-glycosylation of the free 3-position in derivative **8**. Herein, a more efficient route to the 1,6-anhydro-heptose acceptor is presented, starting from commercial methyl α-D-mannopyranoside and using a commercial vinyl Grignard reagent in the carbon elongation step. Furthermore, the synthesis and introduction of a highly differentially protected heptose donor is reported, which permits subsequent selective glycosylations or other modifications, for example phosporylation, in positions 2, 3, 4, and 6 in the second heptose residue, variations frequently found in the native LPS of *Neisseria* and *Haemophilus* strains.

RESULTS AND DISCUSSION

Our earlier published synthesis of the 1,6-anhydro heptose acceptor **7**, although efficient, involved features that make a scale-up difficult. Ethyl 1-thio-α-D-mannopyranoside (prepared in four steps from commercial D-mannose) was used as precursor and the labile benzyloxymethyl chloride Grignard reagent was utilized in the carbon elongation, which is not completely stereoselective (L/D 9 : 1). Thus, modifications were investigated that would allow an efficacious large scale synthesis of donor **7** (Sch. 1).

Åberg and Ernst have described the formation of 1,6-anhydrohexoses in good yields from the corresponding methyl glycosides by simple treatment with $FeCl_3$ in acetonitrile,[6] which should make it possible to use commercially available methyl α-D-mannopyranoside as heptose precursor. Regarding the carbon elongation reaction,



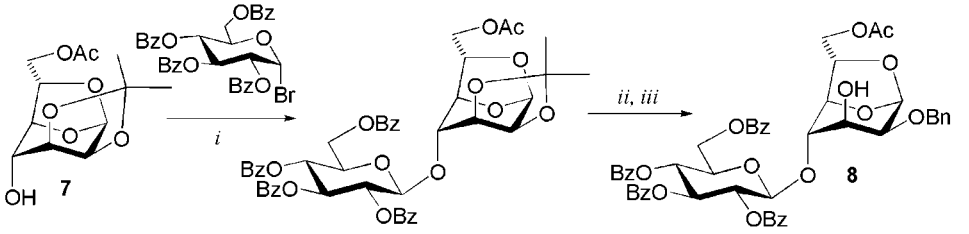
Scheme 1. *i*) a. $(COCl)_2$, DMSO, THF, -60°C; b. DIPEA, rt; *ii*) $CH_2CHMgBr$, THF, -60°C; *iii*) $FeCl_3$, CH_3CN, reflux; *iv*) OsO_4, $NaIO_4$, acetone-H_2O 5 : 1; *v*) $NaBH_4$, EtOH; *vi*) Ac_2O, pyridine; *vii*) H_2 $Pd(OH)_2$/C, EtOH; *viii*) $Me_2C(OMe)_2$ CSA, DMF.

Dasser et al. reported already in 1990 the use of a vinyl Grignard reagent for this purpose.[7] Although this approach involves additional steps (i.a. dihydroxylation and periodate cleavage, excluding thioglycoside precursors), the advantages are several: a stable (commercial) reagent and very high stereoselectivity. Recently, a large scale synthesis of methyl L-*glycero*-D-*manno*-heptopyranoside employing this methodology on a BDA-protected methyl mannoside was published.[8]

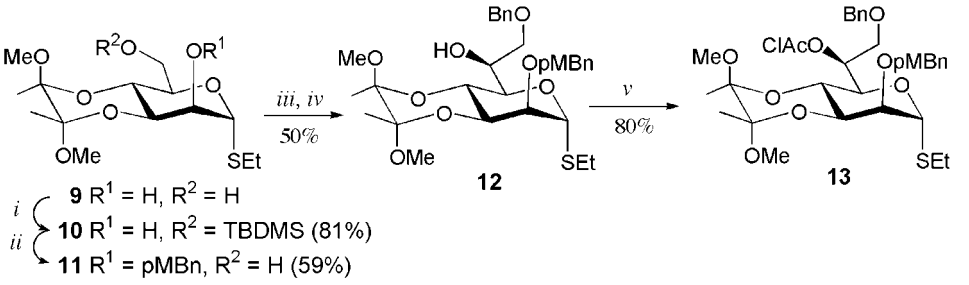
Methyl mannoside derivative **1** was prepared by known procedures.[9] One-pot Swern oxidation and consecutive Grignard vinylation afforded the vinyl derivative **2** in 82% yield and with complete stereoselectivity for the L-*glycero* form (Sch. 1). Treatment of **2** with $FeCl_3$ in acetonitrile at reflux then smoothly afforded the 1,6-anhydro derivative **3** (71%). The alkene was dihydroxylated, the obtained diol was cleaved through periodate oxidation, and the resulting aldehyde was reduced with $NaBH_4$ to give **4** in an 88% overall yield. Straightforward acetylation (→**5**, 92%), debenzylation (→**6**, 91%), and isopropylidenation then afforded the desired heptose acceptor **7** (86%). As compared to the earlier synthesis of **7**, the yield is only slightly better (35% vs. 30% calculated from the 6-OH mannose precursors), but the real advantage is the possibility to start from the methyl mannoside in combination with the reproducibility of the Grignard reaction.

Compound **7** was then transformed as previously described into the 3-OH disaccharide donor **8** (Sch. 2).[5]

As mentioned in the introduction, the second heptose residue is frequently heavily substituted both in *Neisseria* and in *Haemophilus* LPS. In *Neisseria meningitidis* there are glycosylations found in the 2- and 3-positions and phosphorylations at the 3-, 6-, and 7-hydroxyl groups.[10] In *H. influenzae* there are glycan substituents found in the 2- and 3-positions, whereas phosphate groups are found mainly at the 6-hydroxyl group.[1,2] To make later introduction of these substituents feasible, a heptosyl thioglycoside donor with orthogonal temporary protecting groups in the 2-, 3-, 4-, and 6-positions was designed and synthesised (Sch. 3). Starting from ethyl 1-thio-α-D-mannopyranoside, the known 3,4-BDA-acetal **9**[11] was prepared. Attempted regioselective *p*-methoxybenzylation at the 2-position, in accordance with benzylation results obtained by Yamasaki et al.,[8] afforded instead mainly the 6-*O*-benzylated derivative. Interestingly, the same selectivity was observed also for the corresponding *O*-methyl glycoside, which is the substrate used by Yamasaki et al. Thus, regioselective benzylation of the 2,6-diol produced 2-*O*-selectivity, whereas *p*-methoxybenzylation gave 6-*O*-selectivity. Hence, the 2,6-diol **9** was first silylated selectively at the 6-position (→**10**) and then *p*-methoxybenzylated and desilylated to yield **11** in 48% overall yield. Swern oxidation to the aldehyde



Scheme 2. *i*) AgOTf, CH_2Cl_2, 0°C, 77%; *ii*) HOAc (80% aq.), 80°C, 85%; *iii*) a. Bu_2SnO, benzene, reflux, b. BnBr, Bu_4NBr, reflux, 80%.

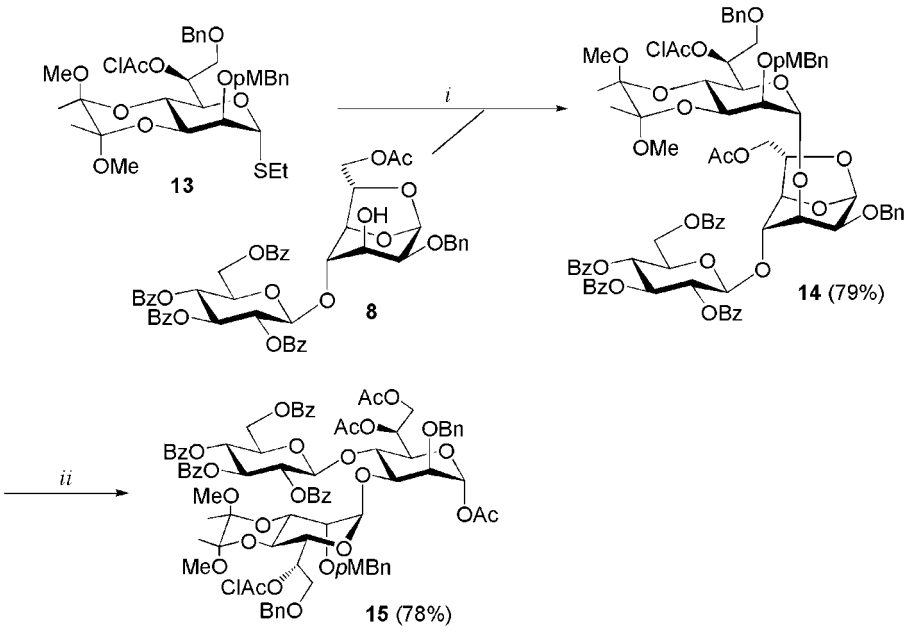


Scheme 3. *i*) TBDMSCl, DMF, imidazole; *ii*) a. *p*-MBnBr, NaH, DMF; b. TBAF, THF; *iii*) a. $(COCl)_2$, DMSO, THF, $-60°C$; b. DIPEA, rt; *iv*) $BnOCH_2Cl$, Mg, THF; *v*) ClAcCl, pyridine/CH_2Cl_2.

followed by a Barbier reaction using benzyloxymethyl chloride afforded in 50% yield the heptoside derivative **12**, chloroacetylation of which afforded the desired thioglycoside donor **13**.

Dimethyl(methylthio)sulfonium triflate (DMTST)-promoted coupling of donor **13** and acceptor **8** yielded the trisaccharide **14** in 79% yield with complete α-selectivity ($J_{C,H}$ 169 Hz), proving once more **8** to be an excellent acceptor for the construction of these 3,4-branched trisaccharide structures (Sch. 4).

Trisaccharide **14** comprises the possibilities for the syntheses of a plethora of *Neisseria* and *Haemophilus* LPS inner core structures. Removal of the *p*-methoxybenzyl



Scheme 4. *i*) DMST, Et_2O; *ii*) $Sc(OTf)_3$, Ac_2O.

group allows for the introduction in the 2′-position of a 2-acetamido-2-deoxy-α-D-glucopyranosyl (*N. meningitidis*) or a third heptosyl (*H. influenzae*) moiety, whereas removal of the chloroacetyl-protecting group permits formation of a 6′-ethanolamine-phosphate group (*N. meningitidis* and *H. influenzae*). Removal of the BDA-acetal make 3′-substitution possible,[12] and acetolysis of the 1,6-anhydro bridge [→ **15** (78%), Sch. 4] and subsequent transformation into a donor would enable elongation at the reducing end as shown previously.[5]

EXPERIMENTAL

General methods. TLC was carried out on Merck precoated 60 F_{254} plates using UV-light and/or 8% sulfuric acid for visualization. Column chromatography was performed on silica gel (0.040–0.063 mm, Amicon). NMR spectra were recorded in $CDCl_3$ (internal Me_4Si, $\delta = 0.00$) at 25°C on a Varian 300 MHz or 400 MHz instrument. Organic phases were dried over $MgSO_4$ before evaporation, which was performed under reduced pressure. Benzyloxymethyl chloride was synthesized according to the literature,[13] dried over $CaCl_2$, and stored without drying agent in a sealed container at −18°C.

Methyl 2,3,4-tri-*O*-benzyl-7,8-dideoxy-α-D-*manno*-oct-7-enopyranoside (2). Oxalyl chloride (2.52 mL, 28.9 mmol) and dry THF (75 mL) were added to a 1000 mL flask fitted with a dropping funnel and a stirrer. The solution was cooled to −70°C, DMSO (4.1 mL, 57.8 mmol) in dry THF (10 mL) was added, and the mixture was stirred for 15 min. Methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside[9] (**1**, 12.2 g, 26.3 mmol) in dry THF (50 mL) was added drop-wise. The reaction mixture was stirred for 1 hr at −60°C and then for 1 hr at −40°C. *N*-Ethyldiisopropylamine (22.9 mL, 131 mmol) was added, and the solution was slowly (overnight) brought to rt. The mixture was recooled down to −60°C. Vinyl magnesium bromide (1 M in THF, 131 mmol) was added drop-wise, and the reaction mixture was stirred for 2 hr at −60°C. The reaction was quenched by the addition of ethanol (10 mL), and after adding saturated aqueous NH_4Cl (25 mL), the whole reaction mixture was warmed to rt. The mixture was extracted with EtOAc (50 mL × 3), and the combined extracts were sequentially washed with 5% sodium hypochlorite in H_2O (50 mL × 4) and brine (50 mL × 2). After drying ($MgSO_4$), the organic solution was concentrated to a syrup, which was purified by silica gel chromatography (toluene/EtOAc 9 : 1) to give compound **2** (10.6 g, 21.6 mmol, 82%); $[\alpha]_D$ +9 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ: 7.37–7.32 (m, 15H, Ar-H), 6.02 (m, 1H, H-7), 5.39 (dt, 1H, $J_{7,8a} = 17.3$ Hz, $J_{8a,8b} = 1.4$ Hz, H-8a), 5.22 (dt, 1H, $J_{7,8b} = 10.4$ Hz, H-8b), 5.00 (d, 1H, J = 11 Hz, C*H*Ph), 4.81–4.65 (m, 6H, 5 × C*H*Ph, H-1), 4.44 (m, 1H, $J_{6,7} = 4.9$ Hz, H-6), 4.17 (t, 1H, $J_{4,5} = 9.6$ Hz, H-4), 3.92 (dd, 1H, $J_{3,4} = 9.3$ Hz, H-3), 3.80 (dd, 1H, $J_{2,3} = 3$Hz, $J_{1,2} = 1.9$, H-2), 3.59 (dd, $J_{5,6} = 1.7$ Hz, H-5), 3.28 (s, 3H, OC*H*$_3$); ^{13}C NMR: δ 54.7, 70.7, 73.2, 72.9, 73.8, 74.7, 74.9, 75.3, 80.3, 99.4, 115.4, 127.6–128.5, 138.4, 138.5, 138.6, 138.7.

Anal. Calcd for $C_{30}H_{34}O_6$: C, 73.45; H, 6.99; Found: C, 73.20; H, 7.06.

7-*O*-Acetyl-1,6-anhydro-2,3,4-tri-*O*-benzyl-L-*glycero*-β-D-*manno*-heptopyranose (5). Compound **2** (450 mg, 0.91 mmol) was dissolved in dry CH_3CN (10 mL), and $FeCl_3$ (104 mg, 0.64 mmol) was added. The reaction mixture was refluxed for 1.5 hr and then cooled to room temperature. Concentration followed by silica gel chromatography

(toluene/EtOAc 6:1) yielded 1,6-anhydro-2,3,4-tri-*O*-benzyl-7,8-dideoxy-β-D-*manno*-oct-7-enopyranose (**3**, 300 mg, 0.65 mmol, 71%); $[\alpha]_D$ −37 (*c* 1.0, $CHCl_3$); ^{13}C NMR: δ 71.4, 71.5, 73.5, 74.4, 74.4, 76.3, 76.5, 78.7, 100.9, 116.8, 127.8–128.6, 137.3, 137.7, 138.0. Compound **3** (403 mg, 0.88 mmol) in acetone/H_2O (5 : 1, 6 mL) was cooled to 0°C and treated with $NaIO_4$ (958 mg, 4.48 mmol) and 1% aqueous OsO_4 (1.6 mL). After stirring the reaction mixture for 24 hr at room temperature, H_2O (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (10 mL × 2). The combined extracts were washed with H_2O (10 mL), dried ($MgSO_4$), and concentrated to a syrup, which was then treated with $NaBH_4$ in EtOH (7 mL) for 15 hr at room temperature. The solution was concentrated, and the residue was dissolved in CH_2Cl_2 (10 mL) and washed with H_2O (5 mL × 2). The organic phase was separated, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel chromatography (toluene/EtOAc 4 : 1) to yield 1,6-anhydro-2,3,4-tri-*O*-benzyl-L-*glycero*-β-D-*manno*-heptopyranose (**4**, 359 mg, 0.78 mmol, 88%); ^{13}C NMR: δ 63.8, 71.2, 71.3, 73.1, 73.9, 74.0, 75.4, 75.5, 76.0, 100.6, 127.6–128.4, 137.5, 137,6, 137.7. Compound **4** (235 mg, 0.51 mmol) was dissolved in Ac_2O/pyridine (2 : 1, 3 mL), and the reaction mixture was stirred at room temperature for 1.5 hr. MeOH was added and the mixture was concentrated. Silica gel chromatography (toluene/EtOAc 6 : 1) of the residue afforded **5** (238 mg, 0.47 mmol, 92%); $[\alpha]_D$ −32 (*c* 1.0, $CHCl_3$); ^1H NMR δ: 7.37–7.23 (m, 15H, Ar-H), 5.50 (s, 1H, H-1), 4.65–4.53 (m, 7H, 6 × C*H*Ph, H-6), 4.26 (s, 1H, H-5), 4.02 (m, 2H, $J_{6,7a} = 6.3$ Hz, $J_{6,7b} = 6.6$ Hz, $J_{7a,7b} = 11.0$ Hz, H-7a, H-7b), 3.83 (m, 1H, $J_{3,4} = 1.6$ Hz, H-3), 3.56 (dd, 1H, $J_{2,3} = 5.2$ Hz, $J_{1,2} = 1.6$ Hz, H-2), 3.50 (s, 1H, H-4), 2.06 (s, 3H, CH_3CO); ^{13}C NMR: δ 21.1, 65.2, 71.6, 71.7, 73.3, 73.6, 74.3, 74.3, 76.2, 76.4, 101.1, 128.0–128.128.8, 137.8, 138.0, 138.1, 170.9.

Anal. Calcd for $C_{30}H_{34}O_7$: C, 71.41; H, 6.39; Found: C, 71.17; H, 6.37.

7-*O*-Acetyl-1,6-anhydro-2,3-*O*-isopropylidene-L-*glycero*-β-D-*manno*-heptopyranose (7). Compound **5** (178 mg, 0.35 mmol) was dissolved in EtOH (4 mL). $Pd(OH)_2$ (20%, 50 mg), 5 drops of H_2O and 5 drops of EtOAc were added to the solution. The mixture was hydrogenolyzed at 110 psi for 72 hr. The mixture was filtered through Celite, concentrated, and purified on a silica gel column (CH_2Cl_2/MeOH 7 : 1) to give 7-*O*-acetyl-1,6-anhydro-L-*glycero*-β-D-*manno*-heptopyranose (**6**, 75 mg, 0.32 mmol, 91%); $[\alpha]_D$ −97 (*c* 1.0, $CHCl_3$); ^{13}C NMR: δ 20.8, 65.1, 65.7, 70.6, 71.5, 72.8, 78.0, 102.6, 171.3. Compound **6** (33 mg, 0.14 mmol) was dissolved in DMF (2 mL). 2,2-Dimethoxypropane (69 μL, 0.56 mmol) was added and the solution was adjusted to pH 2 by addition of (+/−)-10-camphorsulfonic acid. The mixture was stirred for 3 hr at rt and then concentrated. Toluene was added and the solution was neutralized by addition of triethylamine. The solution was concentrated and co-evaporated three times with toluene. Silica gel chromatography (toluene/EtOAc 3 : 1→1 : 1) yielded **7** (33 mg, 0.12 mmol, 86%), which NMR data were identical to the earlier prepared sample.[5]

(2′S,3′S)-Ethyl 3,4-*O*-(2′,3′-Dimethoxybutane-2′,3′-diyl)-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (11). Compound **9**[11] (4.3 g, 12.7 mmol) was treated with tert-butyldiphenylsilyl chloride (2.3 g, 15.2 mmol) in dry DMF (50 mL) in the presence of imidazole (2.16 g, 31.8 mmol) for 20 hr at rt. After quenching the reaction by adding H_2O (30 mL), the product was extracted with EtOAc (100 mL × 3). The combined organic extracts were dried ($MgSO_4$), concentrated, and purified on a silica gel column (toluene/EtOAc 6 : 1 → 1 : 1) to yield ethyl 6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-α-D-mannopyranoside (**10**, 4.68 g, 10.3 mmol, 81%);

^{13}C NMR: δ −5.1, −5.4, 14.8, 17.6, 17.8, 18.2, 24.7, 25.9, 47.8, 48.0, 61.7, 63.3, 69.1, 71.1, 71.8, 83.8, 99.8, 100.3. Compound **10** (3.96 g, 8.76 mmol) was dissolved in dry DMF (17 mL) and added drop-wise to a chilled (0°C) mixture of p-methoxybenzyl bromide (2.9 mL, 17.5 mmol) and sodium hydride (660 mg, 95% in oil) in dry DMF (5 mL). The ice bath was removed, and the reaction was allowed to continue until TLC showed complete reaction. MeOH (2 mL) was added slowly and the mixture was stirred overnight. Toluene was added, and the organic phase was washed with H_2O, dried ($MgSO_4$), and concentrated. Silica gel chromatography (toluene/EtOAc) gave ethyl 6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (4.92 g, 8.59 mmol, 98%). This compound (5.58 g, 9.74 mmol) was treated with tetrabutylammonium fluoride (1 M in THF, 26 mL) in dry THF (26 mL) for 24 hr at rt. The reaction mixture was diluted with H_2O (100 mL) and then extracted with EtOAc (100 mL × 2). The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated. Purification by silica gel chromatography yielded **11** (2.68 g, 5.84 mmol, 60%); $[\alpha]_D$ +226 (*c* 1.0, $CHCl_3$); ^{13}C NMR: δ 15.0, 17.9, 17.9, 25.5, 48.0, 48.0, 55.4, 61.8, 64.2, 69.7, 71.3, 72.9, 77.6, 84.2, 99.7, 100.0, 113.8, 129.7–130.8, 159.3.

HRMS: Calcd for $C_{22}H_{33}O_8S$: 457.1896; Found: 457.1895.

(2′S,3′S)-Ethyl 7-*O*-Benzyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-2-*O*-*p*-methoxybenzyl-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside (12). Compound **11** (2.4 g, 5.23 mmol) was submitted to Swern oxidation according to the procedure described above for compound **2** to give the corresponding aldehyde. Freshly activated magnesium turnings (725 mg, 29.8 mmol), 50 mg of sublimed $HgCl_2$ and dry THF (5 mL) were added (under argon atmosphere) to a flame-dried flask equipped with an internal thermometer, a stirrer, and a dropping funnel. The solution was stirred for 10 min. Benzyloxymethyl chloride (2.17 mL, 15.7 mmol) was added to the dropping funnel, and a few drops were added to the magnesium at rt. Once the exothermic reaction had started (about +30°C, as monitored by the thermometer, which must extend into the reaction slurry), the flask was partially immersed into an ice bath (0°C). The aldehyde (approx. 5.23 mmol) dissolved in freshly distilled THF (10 mL) was added into the dropping funnel, and this aldehyde/alkyl halide mixture was added drop-wise while the inner flask temperature was kept between 20–30°C. The mixture was stirred overnight and then diluted by diethyl ether (200 mL), whereafter freshly prepared saturated aqueous NH_4Cl (400 mL) was added. The organic phase was separated and washed with saturated aqueous NH_4Cl, dried ($MgSO_4$), filtered, and concentrated to give ethyl 7-*O*-benzyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-2-*O*-*p*-methoxybenzyl-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside (**12**, 1.6 g, 2.61 mmol, 50%) after silica gel column chromatography (toluene/EtOAc 12 : 1 → 8 : 1); $[\alpha]_D$ +211 (*c* 1.0, $CHCl_3$); ^{13}C NMR: δ 14.8, 17.9, 25.2, 48.0, 48.0, 55.4, 63.1, 67.6, 69.9, 70.4, 71.8, 72.7, 73.4, 76.7, 84.0, 99.7, 100.0, 113.7, 127.7, −130.8, 138.2, 159.2.

HRMS: Calcd for $C_{30}H_{41}O_9S$: 577.2471; Found, 577.2462.

[(2′S,3′S)-7-*O*-Benzyl-6-*O*-chloroacetyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-2-*O*-*p*-methoxybenzyl-L-*glycero*-α-D-*manno*-heptopyranosyl]-(1 → 3)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 4)]-7-*O*-acetyl-1,6-anhydro-2-*O*-benzyl-L-*glycero*-β-D-*manno*-heptopyranose (14). Chloroacetyl chloride (448 μL, 5.62 mmol) was added to a chilled (0°C) solution of compound **12** (1.3 g, 2.25 mmol) in CH_2Cl_2/pyridine (15 : 1, 16 mL). The reaction was stirred at rt for 45 min. H_2O (10 mL) was added, and the organic

phase was separated, dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography gave ethyl 7-*O*-benzyl-6-*O*-chloroacetyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-2-*O*-*p*-methoxybenzyl-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside (**13**, 1.18 g, 1.80 mmol, 80%); ^{1}H NMR δ: 7.37–7.30 (m, 7H, Ar-H), 6.86 (d, 2H, Ar-H), 5.63, (m, 1H, $J_{6,7a} = 7.5$ Hz, $J_{6,7b} = 6.0$ Hz, H-6), 5.30 (s, 1H, H-1), 4.82 (d, 1H, C*H*Ph), 4.62–4.45 (m, 3H, J = 11.5 Hz, 3 × C*H*Ph), 4.29 (dd, 1H, $J_{5,6} = 1.6$ Hz, H-5), 4.19 (t, 1H, $J_{4,5} = 10.1$ Hz, H-4), 4.05 (s, 2H, Cl*CH2*CO) 4.00 (1H, $J_{3,4} = 10.1$ Hz, H-3), 3.80 (s, 3H, PhOC*H3*), 3.78 (m, $J_{2,3} = 2.6$ Hz, $J_{1,2} = 0.92$ Hz, H-2), 3.65 (m, 2H, $J_{7a,7b} = 10.1$ Hz, H-7a, H-7b), 3.25 (s, 3H, OC*H3*), 3.19 (s, 3H, OC*H3*), 2.52 (m, 2H, SC*H2*), 1.33 (s, 3H, C*H3*), 1.29 (s, 3H, C*H3*), 1.19 (t, 3H, SCH_2CH_3); ^{13}C NMR: δ 14.7, 17.8, 17.9, 25.2, 40.9, 47.9, 48.2, 55.3, 63.0, 67.9, 69.1, 69.8, 70.2, 73.4, 73.0, 76.7, 83.9, 99.9, 100.2, 113.6, 113.7, 127.6–130.7, 137.8 159.2, 167.0. Compound **8** (297 mg, 0.33 mmol) and **13** (347 mg, 0.53 mmol) were dissolved in dry diethyl ether and powdered molecular sieves (4 Å) were added. After stirring at rt for 1 hr, DMTST (408 mg, 1.58 mmol) was added and the reaction was stirred overnight. Triethylamine (300 μL) was added and the mixture was filtered through Celite and concentrated to give **14** after silica gel column chromatography (toluene/EtOAc 5 : 1); Yield: 393 mg (0.26 mmol, 79%); $[\alpha]_D$ +24 (*c* 1.0, $CHCl_3$); ^{13}C NMR: δ 18.0, 18.0, 20.9, 41.0, 47.7, 48.2, 55.5, 62.6, 62.9, 63.9, 65.0, 68.4, 68.9, 69.4, 69.8, 70.2, 71.5, 71.8, 71.9, 72.7, 72.8, 73.1, 73.2, 73.6, 75.5, 75.6, 76.0, 97.4 ($J_{C,H}$ 169 Hz), 99.8, 99.9, 100.4 ($J_{C,H}$ 177 Hz), 100.6 ($J_{C,H}$ 161 Hz), 113.7, 127.2–133.6, 137.9, 138.0, 159.3, 165.1, 165.2, 165.9, 166.2, 167.0, 170.6.

Anal. Calcd for $C_{80}H_{83}ClO_{26}$: C, 64.23; H, 5.59; Found: C, 64.07; H, 5.71.

[(2′S,3′S)-7-*O*-Benzyl-6-*O*-chloroacetyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-2-*O*-*p*-methoxybenzyl-L-*glycero*-α-D-*manno*-heptopyranosyl]-(1 → 3)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 4)]-1,6,7-tri-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranose (15). Compound **14** (72 mg, 0.048 mmol) was dissolved in Ac_2O (2 mL) and cooled to −40°C. $Sc(OTf)_3$ (0.5 mol%) was added and the mixture was stirred at −40°C for 2 hr, whereafter CH_2Cl_2 and sat. aq. $NaHCO_3$ were added. The aqueous layer was extracted twice with CH_2Cl_2, and the combined organic layers were washed with ice water, dried ($MgSO_4$), filtrated, and concentrated. Purification by silica gel chromatography (toluene : EtOAc 5 : 1) yielded **15** (60 mg, 0.038 mmol, 78%); $[\alpha]_D$ +45 (*c* 1.0, $CHCl_3$); ^{13}C NMR: δ 18.0, 20.4, 20.9, 21.0, 21.6, 40.9, 47.7, 48.0, 55.3, 61.7, 62.7, 63.9, 66.8, 67.8, 68.8, 69.1, 69.3, 70.4, 72.1, 72.1, 72.2, 72.4, 72.8, 73.0, 73.4, 74.3, 74.6, 76.0, 91.0 ($J_{C,H}$ 175 Hz), 99.9, 100.1, 101.2, 101.5, 113.8, 125.4, 127.8–130.1, 131.5, 133.3, 133.3, 133.57, 133.6, 137.5, 137.6, 159.1, 164.9, 165.4, 165.6, 166.0, 197.1, 168.5, 169.9, 170.4.

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