Carbohydrate Research 345 (2010) 1331–1338

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Synthesis of phosphorylated 3,4-branched trisaccharides corresponding to LPS inner core structures of *Neisseria meningitidis* and *Haemophilus influenzae*

Johan D. M. Olsson [a], Stefan Oscarson [b,*]

[a] *Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*
[b] *Centre for Synthesis and Chemical Biology, UCD School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland*

ARTICLE INFO

Article history:
Received 15 January 2010
Received in revised form 1 March 2010
Accepted 4 March 2010
Available online 7 March 2010

Keywords:
Oligosaccharide synthesis
Thioglycosides
Lipopolysaccharides
Phosphoethanolamine

ABSTRACT

2-(*N*-Benzyloxycarbonyl)aminoethyl 7-*O*-acetyl-6-*O*-allyl-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-6,7-di-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside, a spacer-equipped protected derivative of the common 3,4-branched diheptoside trisaccharide structure of the lipopolysaccharide core of *Neisseria meningitidis* and *Haemophilus influenzae* has been synthesized. The protecting group pattern installed allows regioselective introduction of phosphoethanolamine residues in the 3- and 6-position of the second heptose unit in accordance with native structures. From this intermediate the 3-and 6-monophosphoethanolamine as well as the non-phosphorylated deprotected trisaccharides have been synthesized to be used in evaluation of antibody binding specificity and in investigation of the substrate specificity of glycosyl transferases involved in the biosynthesis of LPS core structures.

1. Introduction

The structures of the conserved parts of the lipopolysaccharides from *Neisseria meningitidis* (Fig. 1) and *Haemophilus influenzae* (Fig. 2) show significant similarity, the main differences being the substitution in the 4-position of Kdo (*N. meningitidis*: α-D-Kdo*p*; *H. influenzae*: PPEtN*)* and in the 2-position of the second heptose unit (*N. meningitidis*: α-D-GlcNAc*p*; *H. influenzae*: L-α-D-Hep*p*).[1–5] Another difference is the phosphoethanolamine substitution of the second heptose unit, which in *H. influenzae* is almost exclusively in the 6-position, whereas in *N. meningitidis* it is mainly in the 3-position, although 6- and 3,6-disubstitution have also been identified.

To in detail evaluate the specificity of antibodies raised against these native structures, well-defined synthetic structures are almost a necessity. As part of a programme involving synthesis of core LPS structures for the development of LPS-based glycoconjugate vaccines,[6,7] we now report on the synthesis of regioselectively phosphorylated structures of the common 3,4-branched trisaccharide L-α-D-Hep*p*-(1→3)-[β-D-Glc-(1→4)]-L-α-D-Hep*p* found in *N. meningitidis* and *H. influenzae* LPS, the 3-*O*-phosphoethanolamine derivative **18** and the 6-*O*-phosphoethanolamine derivative **19** as well as the non-phosphorylated trisaccharide **20**. These synthetic derivatives will also be useful in establishing the substrate specificity of various glycosyltransferases involved in the biosynthetic elongation of this trisaccharide unit, for example, the transferase introducing the (1→2)-linked α-D-GlcNAc*p* unit in *N. meningitidis*. The gene coding for this transferase has been identified and cloned, but it is still not known if large substrates are needed to get high activity or if phosphorylated or non-phosphorylated substrates are preferred by the enzyme. Investigations such as these will be greatly facilitated with the help of the synthesized derivatives.[8]

2. Results and discussion

In our earlier syntheses of tetrasaccharide structures from *N. menigitidis* and *H. influenzae* we have utilized a 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*-heptapyranoside donor for the introduction of the second heptose unit.[6,7] This protection group pattern allowed for continued substitution with glycans in the 2-position, after selective removal of the *p*-methoxybenzyl group, and also introduction of a phosphoethanolamine moiety in the 6-position after removal of the chloroacetyl group. However, since the target molecules this time only contain 3- and 6-substitutions and it is not possible to remove the BDA-acetal prior to the removal of the *p*-methoxybenzyl group a new protecting group pattern was developed for the heptosyl donor (Scheme 1).

The known ethyl 4-*O*-benzyl-2,3-*O*-isoproylidene-1-thio-α-D-mannopyranoside[9] (**1**) was prepared and its transformation into a heptose derivative was accomplished as before, first a Swern oxidation to give the 6-aldehyde followed by a Barbier reaction with benzyloxymethyl chloride.[10] This afforded the thioheptosides **2**

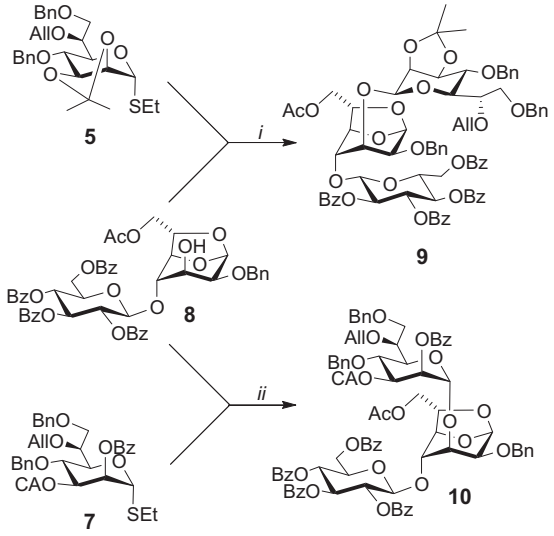
* Corresponding author. Tel.: +353 17162318; fax: +353 17162501.
E-mail address: stefan.oscarson@ucd.ie (S. Oscarson).

0008-6215/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2010.03.006

and **3** in good yield but with low stereoselectivity. A total yield of 56% with a D/L-ratio of 4:5 was obtained. To utilize also the undesired 6-D-stereoisomer **2**, this compound was transformed into the 6-L-isomer **3** via a Mitsunobu reaction with *p*-nitro-benzoic acid and subsequent deacylation in an overall yield of 57%. For temporary protection of the 6-position an allyl group was selected and introduced (→**5**, 94%). In spite of the double bond function, allyl groups are normally orthogonal to thiophilic promoters. Two routes to the target structures were now investigated, one involving the use of thioglycoside **5** directly as a donor and a subsequent selective protection release of the 3-position at the trisaccharide level, and another one comprising the latter protecting group manipulation performed already at the monosaccharide stage to give a thioglycoside donor **7** protected in the 3- and 6-position with orthogonal temporary protecting groups.

Thus, donor **5** was coupled using DMTST as promoter to the disaccharide acceptor **8,** earlier developed by us as an efficient acceptor to obtain these quite elusive 3,4-branched structures (Scheme 2).[10,11] Diethyl ether was used as solvent to improve α-selectivity and a single stereoisomer **9** was obtained in a 52% yield. Because of the *manno*-configuration of the donor we expected this to be the α-product, but no conclusive evidence could be obtained from NMR data. The shifts of the anomeric carbon (δ 101.2 ppm) and proton (δ 4.66 ppm) rather suggested a β-linkage, and the $^1J_{C1-H1}$ of 167 Hz was not diagnostic without the value of the other anomer for purposes of comparison. Finally, the configuration was established by transforming the trisaccharide **9** (hydrolysis of the isopropylidene acetal followed by selective chloroacetylation of the 3-OH) into an already synthesized derivative where we had both anomers for comparison. This proved that exclusively the β-linked product (C-1 δ 102.2 ($^1J_{C1-H1}$ 161 Hz), H-1 δ 4.43) had been formed in the glycosylation; therefore, this approach was abandoned. The complete stereoselectivity was a surprise, but our experience when coupling various heptosyl donors with a non-participating group in the 2-position to acceptor **8** is that a large ratio of β-anomer is always formed and to obtain α-selectivity the α-directing 3,4-BDA acetal has to be present in the donor.[6,7,12,13] Interestingly, the 1,2-isopropylidene acetal has in a recent publication been found also to be α-directing, but this investigation involved mannuronic acid donors and other acceptors.[14]

To introduce an orthogonal temporary protecting group in the 3-position, the isopropylidene acetal was removed by acid hydrolysis from compound **5** to yield the 2,3-diol **6** (96%, Scheme 1). As a temporary protecting group, a chloroacetyl group was selected and for the protection of the 2-position we hypothesized that a participating group was required, considering the stereoselectivity problems discussed with donor **5**. Hence, the 3-*O*-CA-2-*O*-Bz derivative **7** was prepared through tin activation of **6** followed by chloroacetylation and subsequent benzoylation in a 72% overall yield.



Scheme 2. Synthesis of trisaccharides **9** and **10**. Reagents: (i) DMTST, Et_2O, 52%; (ii) Me_2S_2, Tf_2O, CH_2Cl_2, 70%.

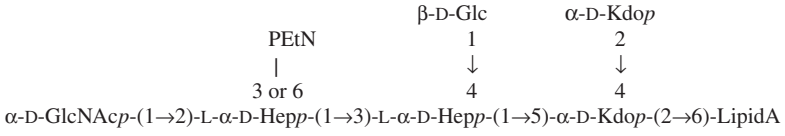


Figure 1. Conserved structure of *N. meningitidis* LPS.

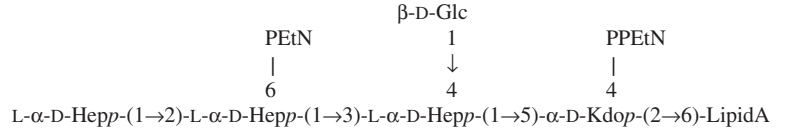
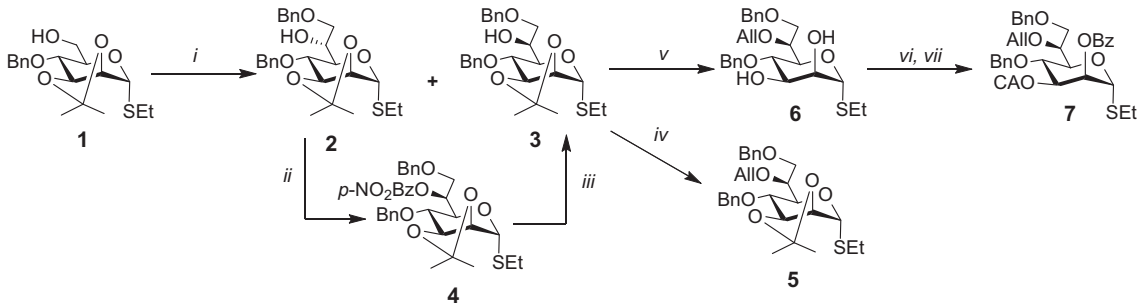


Figure 2. Conserved structure of *H. influenzae* LPS.



Scheme 1. Synthesis of heptosyl thioglycosides **5** and **7**. Reagents: (i) (a) $(COCl)_2$, DMSO, DIPEA, CH_2Cl_2; (b) $BnOCH_2Cl$, Mg, $HgBr_2$, I_2, THF, 56% (**2**:**3** 4:5) over two steps; (ii) *p*-nitrobenzoic acid, PPh_3, DIAD, THF, 77%; (iii) NaOMe, MeOH–CH_2Cl_2 (2:1), 74%; (iv) AllBr, NaH, DMF, 94%; (v) (a) AllBr, NaH, DMF; (b) 80% HOAc (aq), 90% over two steps; (vi) (a) Bu_2SnO, toluene; (b) chloroacetyl chloride, toluene, 82%; (vii) BzCl, pyridine, CH_2Cl_2, 88%.

Coupling of donor **7** with acceptor **8**, using the stronger promoter system Me_2S_2–Tf_2O[15] and CH_2Cl_2 as solvents, now efficiently afforded the trisaccharide **10** in 70% yield and with complete α-selectivity (J_{C1-H1} = 172 Hz; ^{13}C 96.9 ppm; ^{1}H 5.19 ppm) (Scheme 2).

The spacer moiety and the phosphoethanolamine residues could now be introduced using methodology already developed during synthesis of the tetrasaccharide structures.[6,7] $ScOTf_3$-promoted acetolysis of the 1,6-anhydro bridge afforded the anomeric acetate **11** (75%), which was converted to the ethyl thioglycoside by treatment with ethanethiol and BF_3·diethyl etherate (→**12**, 69%) (Scheme 3). NIS–AgOTf-promoted glycosylation between donor **12** and CBz-protected ethanolamine gave the spacer-equipped trisaccharide **13** in 77% yield and with complete α-selectivity, although a non-participating benzyl group is present in the 2-position of the donor.

From derivative **13** now various phosphorylation patterns can be easily accomplished, 3-mono, 6-mono and 3,6-diphosphorylation, and obviously also the non-phosphorylated trisaccharide can be obtained. Removal of the monochloroacetate by NH_3–MeOH treatment yielded the 3′-OH derivative **14** (90%), whereas deallylation through Ir-catalyzed rearrangement followed by NIS-promoted hydrolysis of the obtained enol ether afforded the 6′-OH compound **15** (73%) (Scheme 4). The phosphoethanolamino groups were introduced using phosphoramidite chemistry. Treatment of derivatives **14** and **15** with (2-*tert*-butyloxycarbonyl aminoethyl)benzyl *N*,*N*-diisopropyl phosphoramidite[6] and tetrazole followed by *m*-CPBA oxidation gave the phosphotriesters **16** and **17** in 80% and 73% yields, respectively. The Boc-protection of the ethanolamino group provides orthogonality to the spacer amino group protection to allow selective activation and conjugation of the latter.

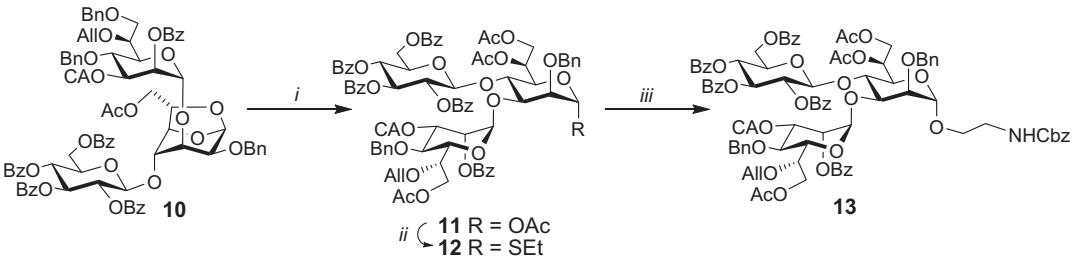
Deprotection of derivative **16** in three steps, deallylation followed by catalytic hydrogenolysis and Zemplen deacylation, and of derivative **17** in two steps, deacylation and catalytic hydrogenolysis, gave the two target structures **18** and **19** in 49% and 76% yield, respectively. Deprotection of **14** as described for compound **16** gave the nonposphorylated trisaccharide **20** in 75% yield.

In conclusion, an effective synthesis of a regioselectively protected spacer equipped trisaccharide intermediate **13** has been developed and its transformation into phosphoethanolamine containing structures corresponding to *N. meningitidis* and *H. influenzae* LPS core structures has been performed.

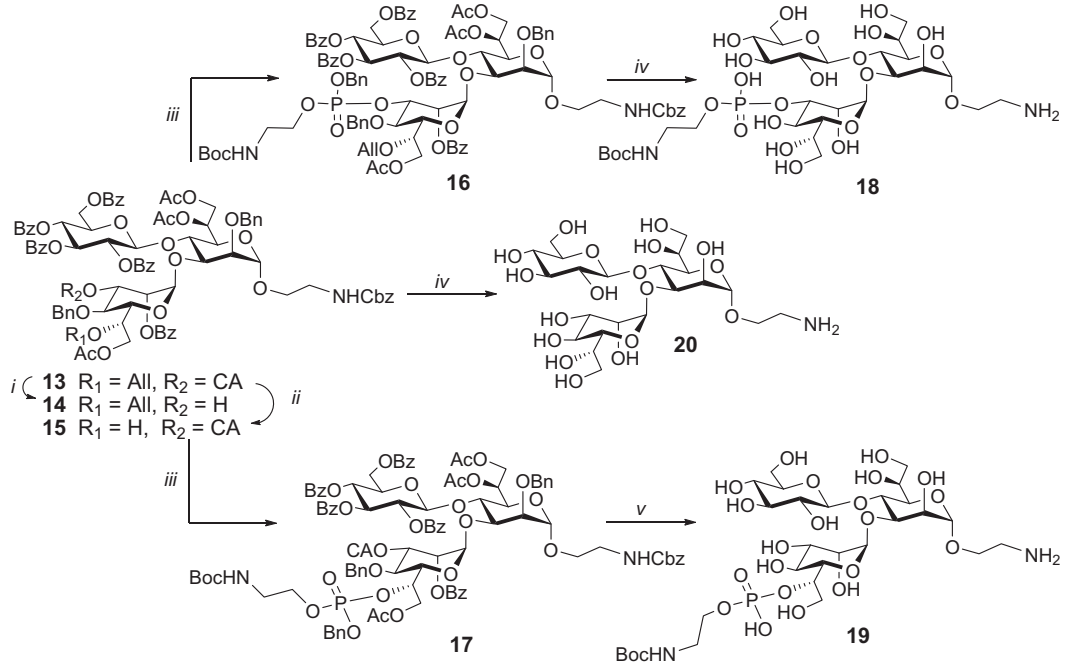
3. Experimental

3.1. General methods

Normal work-up means drying the organic phase with $MgSO_4$ (s) or Na_2SO_4 (s), filtering and evaporation of the solvent in vacuo



Scheme 3. Synthesis of spacer trisaccharide **13**. Reagents: (i) $Sc(OTf)_3$, Ac_2O, 75%; (ii) EtSH, BF_3·OEt_2, CH_2Cl_2, 69%; (iii) *N*-(benzyloxycarbonyl) aminoethanol, NIS, AgOTf, CH_2Cl_2–Et_2O (2:1), 77%.



Scheme 4. Synthesis of target structures **18–20**. Reagents: (i) NH_3 (satd) MeOH–CH_2Cl_2 (2:1), 90%; (ii) (a) H_2 (g), [bis(methyldiphenylphosphine)](1,5-cyclooctadine) $Ir(I)PF_6$, THF; (b) NIS, H_2O, 73%; (iii) (a) (2-*tert*-butyloxycarbonyl aminoethyl)benzyl *N*,*N*-diisopropyl phosphoramidite, tetrazole, CH_2Cl_2; (b) *m*-CPBA, CH_2Cl_2, 0 °C (for **16** 80% and for **17** 73%); (iv) (a) H_2 (g), [bis(methyldiphenylphosphine)](1,5-cyclooctadine) $Ir(I)PF_6$, THF; (b) NIS, H_2O; (c) Pd–C (10%), H_2 (g), 0.1 M HCl (aq), EtOAc–MeOH (1:1); (d) NaOMe, MeOH (for **18** 49% and for **20** 74% over four steps); (v) (a) Pd–C (10%), H_2 (g), 0.1 M HCl (aq), EtOAc–MeOH (1:1); (b) NaOMe, MeOH, 76% over two steps.

at ~35 °C. CH_2Cl_2 was distilled over calcium hydride and collected onto 4 A pre-dried MS. Thin Layer Chromatography (TLC) was carried out on 0.25 mm pre-coated silica-gel plates (Merck Silica-Gel 60 F_{254}); detected with UV-abs (254 nm) and/or by charring with 8% sulfuric acid or AMC (ammonium molybdate (10 g) and cerium sulfate (2 g) dissolved in 10% H_2SO_4 (200 mL)) followed by heating to ~250 °C. FC means Flash Column chromatography using silica gel (Amicon, (0.040–0.063 mm)). ^{1}H NMR, ^{31}P NMR and ^{13}C NMR spectra were performed on a Varian or Bruker instrument (300, 400 or 500 MHz) at 25 °C unless otherwise stated. Chemical shifts are given in parts per million relative to solvent peaks in $CDCl_3$ (δ 77.17 for ^{13}C and δ 7.26 for ^{1}H) and in D_2O (δ 4.79 for ^{1}H) or TMS (δ 0.00 for ^{13}C NMR and ^{1}H NMR. For ^{31}P NMR 85% aq H_3PO_4 (δ 0.00 was used as an external reference.

3.2. Ethyl 4,7-di-*O*-benzyl-2,3-*O*-isopropylidene-1-thio-D-*glycero*-α-D-*manno*-heptopyranoside (2) and ethyl 4,7-di-*O*-benzyl-2,3-*O*-isopropylidene-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside (3)

Oxalyl chloride (0.230 mL, 2.64 mmol) was dissolved in dry CH_2Cl_2 (3 mL) and cooled to −60 °C. DMSO (0.375 mL, 5.28 mmol) in dry CH_2Cl_2 (3 mL) was added dropwise. After stirring for 10 min, ethyl 4-*O*-benzyl-2,3-*O*-isopropylidene-1-thio-α-D-mannopyranoside[9] (**1**, 0.851 g, 2.40 mmol) dissolved in dry CH_2Cl_2 (5 mL) was added dropwise and the stirring was continued for an additional 60 min at −60 °C followed by the addition of DIPEA (2.06 mL, 12.0 mmol). The mixture was slowly brought to rt, diluted with CH_2Cl_2 (15 mL), washed with H_2O (30 mL) and subjected to normal work-up to yield the corresponding aldehyde, which was used without further purification. Magnesium (0.408 g, 16.8 mmol, activated), $HgBr_2$ (cat.) and I_2 (cat.) were dissolved in dry THF (4 mL). Benzyloxymethyl chloride (1.67 mL, 7.20 mmol, 60%) was measured in a dropping funnel and a small amount (~0.200 mL) was added dropwise to the magnesium mixture until the exothermic reaction started and the purple-brown colour disappeared. After the initial increase in temperature the reaction temperature was kept at 25 °C by immediately cooling of the mixture. The crude aldehyde dissolved in dry THF (10 mL) was added to the dropping funnel containing the benzyloxymethyl chloride. This aldehyde reagent solution was carefully added dropwise to the magnesium mixture over 45 min, strictly keeping the reaction temperature at 25–28 °C. After completed addition, the mixture was stirred for 14 h at rt, then diluted with Et_2O (30 mL), washed with NH_4Cl (satd, 100 mL) and subjected to normal work-up. FC (toluene–EtOAc 19:1→14:1→9:1) afforded first the D-D-heptoside **2** (0.283 g, 0.60 mmol, 25%) followed by the L-D-heptoside **3** (0.354 g, 0.75 mmol, 31%).

Compound **2** (D-D-heptoside): R_f = 0.44 (toluene–EtOAc 6:1); $[\alpha]_D$ +106 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3, 24.1, 26.4, 28.0, 68.6, 71.0, 72.2, 72.8, 73.5, 76.5, 77.9, 78.6, 79.6, 109.5, 127.6, 127.8 (2C), 127.9, 128.2 (2C), 128.4 (2C), 128.5 (2C), 137.7, 138.2; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, 3H, *J* = 7.6 Hz), 1.39 (s, 3H), 1.56 (s, 3H), 2.49–2.72 (m, 2H), 3.56–3.63 (m, 2H), 3.73 (dd, 1H, *J* = 7.6, 8.4 Hz), 4.10–4.14 (m, 2H), 4.21 (d, 1H, *J* = 5.2 Hz), 4.34 (dd, 1H, *J* = 5.2, 6.0 Hz), 4.52 (benzylic d, 1H, J_{gem} = 12.5 Hz), 4.52 (benzylic d, 1H, J_{gem} = 12.5 Hz), 4.62 (benzylic d, 1H, J_{gem} = 11.2 Hz), 4.95 (benzylic d, 1H, J_{gem} = 11.2 Hz), 5.55 (s, 1H), 7.28–7.40 (m, 10H); HRMS calcd for $C_{26}H_{35}O_6S$ $[M+H]^+$ 475.2154; found 475.2169.

Compound **3** (L-D-heptoside): R_f = 0.36 (toluene–EtOAc 6:1); $[\alpha]_D$ +108 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.4, 24.3, 26.6, 28.1, 68.0, 68.7, 71.8, 73.4, 73.5, 75.7, 76.6, 78.8, 80.1, 109.5, 127.8, 127.8, 127.9 (2C), 128.1 (2C), 128.4 (2C), 128.5 (2C), 138.0, 138.4; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24 (t, 3H, *J* = 7.6 Hz), 1.37 (s, 3H), 1.53 (s, 3H), 2.17 (br s, 1H), 2.47 (dq, 1H, *J* = 7.6, 13.2 Hz), 2.59 (dq, 1H, *J* = 7.6, 13.2 Hz), 3.52 (dd, 1H, *J* = 5.2, 9.2 Hz), 3.62 (dd, 1H, *J* = 7.6, 9.2 Hz), 3.85 (dd, 1H, *J* = 7.2, 10.0 Hz), 3.99 (dd, 1H, *J* = 1.6, 10.0 Hz), 4.18–4.23 (m, 2H), 4.31 (dd, 1H, *J* = 6.0, 7.2 Hz), 4.53 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.70 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.67 (benzylic d, 1H, J_{gem} = 11.2 Hz), 4.93 (benzylic d, 1H, J_{gem} = 11.2 Hz), 5.57 (s, 1H), 7.28–7.39 (10H, Ar-H); HRMS calcd for $C_{26}H_{35}O_6S$ $[M+H]^+$ 475.2154; found 475.2140.

3.3. Ethyl 4,7-di-*O*-benzyl-2,3-*O*-isopropylidene-6-*O*-*p*-nitrobenzoyl-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside (4)

PPh_3 (0.324 g, 1.23 mmol) and *para*-nitrobenzoic acid (0.206 g, 1.23 mmol) were added to a solution of **2** (0.293 g, 0.62 mmol) in THF (5 mL). DIAD (0.242 mL, 1.23 mmol) was subsequently added dropwise. The resulting reaction mixture was stirred for 3 h whereafter the solvent was evaporated. FC (toluene→toluene–EtOAc 39:1→19:1) gave **4** (0.298 g, 0.48 mmol, 77%). R_f = 0.68 (toluene–EtOAc 9:1); $[\alpha]_D$ +88 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.4, 24.3, 26.6, 28.2, 67.4, 67.9, 71.0, 72.6, 73.3, 74.9, 76.5, 78.8, 80.0, 109.6, 123.6 (2C), 127.7 (2C), 127.8, 127.8, 128.4 (2C), 128.5 (2C), 128.7 (2C), 129.1, 131.0 (2C), 137.6, 137.9, 150.7, 163.9; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (t, 3H, *J* = 7.2 Hz), 1.39 (s, 3H), 1.45 (s, 3H), 2.51 (dq, 1H, *J* = 7.2, 12.8 Hz), 2.66 (dq, 1H, *J* = 7.2, 12.8 Hz), 3.58 (dd, 1H, *J* = 5.4, 10.0 Hz), 3.75 (dd, 1H, *J* = 6.4, 9.6 Hz), 3.83 (dd, 1H, *J* = 6.4, 9.6 Hz), 4.25 (dd, 1H, *J* = 0.8, 5.6 Hz), 4.35–4.39 (m, 2H), 4.45 (benzylic d, 1H, J_{gem} = 11.2 Hz), 4.54 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.61 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.82 (benzylic d, 1H, J_{gem} = 11.2 Hz), 5.65 (s, 1H), 5.83 (ddd, 1H, *J* = 1.6, 6.4, 6.4 Hz), 7.10–7.36 (m, 10H), 8.18 (d, 2H, *J* = 8.8 Hz), 8.26 (d, 2H, *J* = 8.8 Hz); HRMS calcd for $C_{33}H_{37}NO_9S$: $[M+H]^+$ 624.2267; found 624.2257.

*Synthesis of **3** from **4***: NaOMe (cat.) was added to a stirred solution of **4** (0.204 g, 0.33 mmol) in MeOH–CH_2Cl_2 (2:1, 6 mL). After 8 h the reaction mixture was neutralized with Dowex-H^+ ion exchange resins, filtered and concentrated. FC (toluene–EtOAc 19:1→9:1) of the residue gave **2** (0.115 g, 0.24 mmol, 74%).

3.4. Ethyl 6-*O*-allyl-4,7-di-*O*-benzyl-2,3-*O*-isopropylidene-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside (5)

Compound **3** (0.289 g, 0.61 mmol) was dissolved in dry DMF (6 mL) and allyl bromide (0.105 mL, 1.21 mmol) was added. This solution was added dropwise at 0 °C to a slurry of NaH (0.048 g, 1.21 mmol, 60%) in DMF (2 mL). After 45 min, MeOH (2 mL) was added and the solution was diluted with toluene (50 mL). The organic phase was washed with H_2O (40 mL) and subjected to normal work-up followed by FC (toluene→toluene–EtOAc→toluene–EtOAc 1:0→19:1→9:1) to give **5** (0.293 g, 0.570 mmol, 94%). R_f = 0.68 (toluene–EtOAc 9:1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.5, 24.3, 26.6, 28.2, 69.4, 70.7, 72.7, 72.9, 73.5, 75.5, 75.7, 76.8, 79.1, 80.2, 109.6, 116.7, 127.7 (2C), 127.7 (2C), 128.0 (2C), 128.4 (2C), 128.5 (2C), 135.2, 138.3, 138.6; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (t, 3H, *J* = 7.2 Hz), 1.37 (s, 3H), 1.56 (s, 3H), 2.41–2.63 (m, 2H), 3.61 (dd, 1H, *J* = 6.4, 9.6 Hz), 3.72 (dd, 1H, *J* = 6.4, 9.6 Hz), 3.87 (dd, 1H, *J* = 7.2, 10.0 Hz), 3.93 (dd, 1H, *J* = 6.0, 12.4 Hz), 4.00 (ddd, 1H, *J* = 1.5, 6.4, 6.4 Hz), 4.07 (dd, 1H, *J* = 1.6, 10.0 Hz), 4.19 (dd, 1H, *J* = 1.5, 5.6 Hz), 4.26 (dd, 1H, *J* = 6.0, 12.4 Hz), 4.43 (dd, 1H, *J* = 6.4, 6.4 Hz), 4 52 (s, 2H), 4.56 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.97 (benzylic d, 1H, J_{gem} = 11.6 Hz), 5.09 (dd, 1H, *J* = 1.5, 10.4 Hz), 5.21 (dd, 1H, *J* = 1.5, 17.2 Hz), 5.59 (s, 1H), 5.87 (m, 1H), 7.25–7.37 (m, 10H); HRMS calcd for $C_{29}H_{38}O_6S$: $[M+Na]^+$ 537.2281; found 537.2298.

3.5. Ethyl 6-*O*-allyl-4,7-di-*O*-benzyl-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside (6)

Compound **3** (0.184 g, 0.387 mmol) was treated as described above to give compound **5**. The resulting oil was dissolved in 80%

AcOH (aq, 8 mL) and the mixture was heated to 80 °C. After 2 h the solvent was evaporated and toluene (2 × 10 mL) was co-evaporated twice from the residue. FC (toluene–EtOAc→toluene–EtOAc 2:1→1:1) gave diol **6** (0.166 g, 0.348 mmol, 90%) as a colourless oil. R_f = 0.39 (toluene–EtOAc 1:1); $[\alpha]_D$ +130 (*c* 1.0, $CHCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.6, 24.7, 70.5, 71.3, 72.4, 72.7, 72.8, 73.4, 74.4, 75.5, 75.8, 84.1, 117.3, 127.6 (2C), 127.7, 127.7 (2C), 127.8, 128.4 (2C), 128.5 (2C), 134.9, 138.0, 138.6; ^1H NMR (500 MHz, $CDCl_3$): δ 1.20 (t, 3H, *J* = 7.5 Hz), 2.44–2.59 (m, 2H), 3.63 (dd, 1H, *J* = 6.0, 10.0 Hz), 3.74 (dd, 1H, *J* = 6.0, 10.0 Hz), 3.85–3.92 (m, 2H), 3.95–4.03 (m, 3H), 4.13 (d, 1H, *J* = 8.0 Hz), 4.30 (m, 1H), 4.51 (s, 2H), 4.67 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.90 (benzylic d, 1H, J_{gem} = 12.0 Hz), 5.14 (dd, 1H, *J* = 1.5, 10.5 Hz), 5.25 (dd, 1H, *J* = 1.5, 17.5 Hz), 5.31 (s, 1H), 5.93 (m, 1H), 7.27–7.36 (m, 10H); HRMS calcd for $C_{26}H_{34}O_6S$: $[M+H]^+$ 475.2154; found 475.2133.

3.6. Ethyl 6-*O*-allyl-2-*O*-benzoyl-4,7-di-*O*-benzyl-3-*O*-chloroacetyl-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside (7)

A mixture of Bu_2SnO (0.177 g, 0.713 mmol) and **6** (0.282 g, 0.594 mmol) in dry toluene (5 mL) was refluxed for 60 min and then cooled to −35 °C at which temperature ClAcCl (0.057 mL, 0.713 mmol) was added. After stirring for 15 min, the mixture was diluted with toluene (10 mL), washed with brine (10 mL) and subjected to normal work-up. FC (toluene–EtOAc→toluene–EtOAc 3:1→2:1) gave the corresponding 3-*O*-chloroacetyl derivative (0.269 g, 0.488 mmol, 82%). This was dissolved in dry CH_2Cl_2 (7 mL) and pyridine (0.807 mL, 10.0 mmol) was added. The mixture was cooled to 0 °C and BzCl (0.581 mL, 5.0 mmol) was added. After 90 min, additional CH_2Cl_2 (10 mL) was added and the solution was washed with 1 M HCl (aq, 25 mL) and subjected to normal work-up. FC (toluene→toluene–EtOAc→toluene–EtOAc→toluene–EtOAc 1:0→39:1→19:1→9:1) afforded **7** (0.274 g, 0.429 mmol, 88%). R_f = 0.65 (toluene–EtOAc 6:1); $[\alpha]_D$ +19 (*c* 1.0, $CHCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.6, 25.0, 40.5, 70.1, 71.8, 71.9, 72.1, 73.1, 73.5, 74.8, 74.9, 75.3, 82.0, 116.4, 127.5–130.0 (Ar-C), 133.5, 135.0, 138.0, 138.1, 165.6, 166.3; ^1H NMR (500 MHz, $CDCl_3$): δ 1.23 (t, 3H, *J* = 7.5 Hz), 2.52–2.64 (m, 2H), 3.69 (dd, 1H, *J* = 6.5, 9.0 Hz), 3.75 (benzylic d, 1H, J_{gem} = 14.5 Hz), 3.82 (dd, 1H, *J* = 6.5, 9.0 Hz), 3.83 (benzylic d, 1H, J_{gem} = 14.5 Hz), 4.00 (dd, 1H, *J* = 5.5, 13.0 Hz), 4.09 (dd, 1H, *J* = 6.5, 6.5 Hz), 4.29–4.33 (m, 2H), 4.42 (dd, 1H, *J* = 5.5, 13.0 Hz), 4.54 (s, 2H), 4.66 (benzylic d, 1H, J_{gem} = 11.0 Hz), 4.72 (benzylic d, 1H, J_{gem} = 11.0 Hz), 5.21 (dd, 1H, *J* = 1.5, 10.0 Hz), 5.34 (dd, 1H, *J* = 1.5, 18.0 Hz), 5.42–5.44 (m, 2H), 5.62 (dd, 1H, *J* = 1.5, 3.5 Hz), 6.00 (m, 1H), 7.26–7.34 (m, 10H), 7.46 (dd, 2H, *J* = 7.5, 7.5 Hz), 7.60 (t, 1H, *J* = 7.5 Hz), 8.09 (d, 2H, *J* = 7.5 Hz); HRMS calcd for $C_{35}H_{39}ClO_8S$ $[M+Na]^+$ 677.1946; found 677.1912.

3.7. 6-*O*-Allyl-4,7-di-*O*-benzyl-2,3-*O*-isopropylidene-L-*glycero*-β-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*-α-D-*manno*-heptopyranose (9)

Compounds **5** (0.034 g, 0.066 mmol) and **8** (0.037 g, 0.041 mmol) were dissolved in dry Et_2O (3 mL) followed by the addition of 4 A MS. After stirring under an Ar-atmosphere for 30 min, the mixture was cooled to 0 °C and DMTST (0.032 g, 0.123 mmol) was added. After 2 h (0 °C→rt), Et_3N (0.150 mL) was added and the mixture was filtered through Celite and concentrated. FC (toluene–EtOAc 6:1→3:1) of the residue gave **9** (0.029 g, 0.021 mmol, 52%). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.0, 26.4, 27.3, 63.2, 65.2, 69.7, 71.6, 72.1, 72.3, 72.5, 72.7, 72.9, 72.9, 73.6, 73.8, 74.0, 74.1, 74.9, 75.5, 75.9, 76.1, 78.8, 79.7, 100.8 (J_{CH} = 161 Hz), 101.1 (J_{CH} = 176 Hz), 101.2 (J_{CH} = 167 Hz), 111.3, 117.8, 127.8–130.0, 133.4, 133.5, 133.7, 135.3, 138.3, 138.5, 165.2, 165.4, 166.0, 166.3, 170.7; ^1H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 3H), 1.56 (s, 3H), 1.96 (s, 3H), 3.46 (m, 1H), 3.53 (dd, 1H, *J* = 3.6, 9.2 Hz), 3.74–3.84 (m, 4H), 3.89–3.93 (m, 3H), 3.98 (dd, 1H, *J* = 6.0, 12.4 Hz), 4.02–4.05 (m, 2H), 4.09–4.25 (m, 5H), 4.31–4.41 (m, 3H), 4.52–4.70 (m, 5H), 4.87 (benzylic d, 1H, J_{gem} = 11.2 Hz), 4.86 (benzylic d, 1H, J_{gem} = 10.4 Hz), 4.99 (dd, 1H, *J* = 1.2, 10.4 Hz), 5.15 (dd, 1H, *J* = 1.2, 17.6 Hz), 5.19 (d, 1H, *J* = 8.0 Hz), 5.27 (s, 1H), 5.54 (dd, 1H, *J* = 8.0, 9.6 Hz), 5.71 (dd, 1H, *J* = 10.0, 10.0 Hz), 5.84–5.95 (m, 2H), 7.19–7.53 (m, 27H), 7.83–7.87 (m, 4H), 7.92 (dd, 2H, *J* = 1.6, 8.8 Hz), 8.01 (d, 2H, *J* = 7.6 Hz).

3.8. 6-*O*-Allyl-2-*O*-benzoyl-4,7-di-*O*-benzyl-3-*O*-chloroacetyl-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*-α-D-*manno*-heptopyranose (10)

Tf_2O (51 μL, 0.302 mmol) was added at 0 °C to a solution of Me_2S_2 (30 μL, 0.330 mmol) in dry CH_2Cl_2 (0.5 mL). After 30 min the Tf_2O–Me_2S_2 mixture was added dropwise at −60 °C and under Ar to a stirred (10 min) solution of **7** (0.052 g, 0.079 mmol) and **8** (0.050 g, 0.055 mmol) in Et_2O (4 mL). After 60 min (−60 °C→rt), Et_3N (300 μL) was added and the mixture was diluted with CH_2Cl_2 (5 mL), washed with H_2O (10 mL) and subjected to normal work-up. FC (toluene–EtOAc 9:1→6:1) gave **10** (0.058 g, 0.039 mmol, 70%). R_f = 0.63 (toluene–EtOAc 2:1); $[\alpha]_D$ −22 (*c* 1.0, $CHCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.0, 40.8, 62.3, 64.9, 69.2, 70.3, 71.6, 71.7, 72.1, 72.5, 72.6, 72.7, 72.7, 72.9, 73.3, 73.4, 73.6, 74.6, 74.7, 74.9, 75.5, 75.7, 76.8, 96.9 (J_{CH} = 172 Hz), 100.1 (J_{CH} = 175 Hz), 100.5 (J_{CH} = 161 Hz), 115.8, 127.6–130.2 (Ar-C), 133.2, 133.4, 133.4, 133.5, 133.6, 135.5, 137.6, 138.3, 138.4, 165.1, 165.2, 165.8, 166.0, 166.1, 166.5, 171.0; ^1H NMR (500 MHz, $CDCl_3$): δ 2.07 (s, 3H), 3.44–3.48 (m, 2H), 3.79–4.02 (m, 8H), 4.18–4.47 (m, 11 H), 4.53 (dd, 1H, *J* = 6.0, 6.0 Hz), 4.60 (benzylic d, 1H, J_{gem} = 11.0 Hz), 4.69 (benzylic d, 1H, J_{gem} = 11.0 Hz), 4.71 (dd, 1H, *J* = 3.0, 12.0 Hz), 5.10 (d, 1H, *J* = 8.5 Hz), 5.15 (dd, 1H, *J* = 1.5, 10.5 Hz), 5.19 (s, 1H), 5.32 (s, 1H), 5.33 (dd, 1H, *J* = 1.5, 18.0 Hz), 5.50–5.51 (m, 2H), 5.53 (dd, 1H, *J* = 8.5, 10.0 Hz), 5.76 (dd, 1H, *J* = 9.5, 9.5 Hz), 5.92 (dd, 1H, *J* = 9.5, 9.5 Hz), 5.96 (m, 1H), 7.14–7.58 (m, 30H), 7.82 (dd, 2H, *J* = 1.5, 8.5 Hz), 7.86 (dd, 2H, *J* = 1.5, 9.0 Hz), 7.92–7.96 (m, 4H), 8.07 (dd, 2H, *J* = 1.5, 8.5 Hz); HRMS calcd for $C_{83}H_{79}ClO_{24}$ $[M+Na]^+$ 1517.4542; found 1517.4460.

3.9. 7-*O*-Acetyl-6-*O*-allyl-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-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*-heptopyranoside (11)

$Sc(OTf)_3$ (3 mg, 0.006 mmol) was added to a stirred solution of **10** (63 mg, 0.042 mmol) in Ac_2O (5 mL). After 2.5 h, the mixture was cooled to 0 °C and MeOH (2 mL) was added dropwise. The mixture was concentrated, and the residue was co-evaporated with toluene (2 × 5 mL), whereafter FC (toluene–EtOAc 6:1) gave **11** (48 mg, 0.032 mmol, 75%). R_f = 0.61 (toluene–EtOAc 2:1); $[\alpha]_D$ +10 (*c* 1.0, $CHCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.4, 20.5, 20.8, 20.9, 40.8, 61.6 (2C), 61.9, 63.8, 67.8, 70.3, 70.4, 71.6, 71.6, 72.2 (3C), 72.9, 73.0, 73.7, 73.9, 74.1, 74.3, 74.7, 75.2, 91.0, 99.5, 100.5, 116.8, 127.7–130.5 (Ar-C), 132.9, 133.1, 133.3, 133.4, 133.5, 134.6, 137.3, 138.1, 164.8, 165.4, 165.6, 165.7, 166.1, 166.6, 168.7, 170.0, 170.4, 170.8; ^1H NMR (500 MHz, $CDCl_3$): δ 1.67 (s, 3H), 1.71 (s, 3H), 2.00 (s, 3H), 2.15 (s, 3H), 3.60–3.67 (m, 3H), 3.84–4.17 (m, 10H), 4.33–4.44 (m, 4H), 4.63 (benzylic d, 1H, J_{gem} = 13.0 Hz), 4.65 (benzylic d, 1H, J_{gem} = 11.0 Hz), 4.70 (dd, 1H, *J* = 3.5, 12.0 Hz), 4.77 (benzylic d, 1H, J_{gem} = 13.0 Hz), 4.84 (benzylic d, 1H, J_{gem} = 11.0 Hz), 4.91 (d, 1H *J* = 8.0 Hz), 5.26 (dd, 1H, *J* = 1.5, 9.5 Hz), 5.34 (dd, 1H, *J* = 6.5, 6.5 Hz), 5.43 (dd, 1H, *J* = 1.5, 17.5 Hz), 5.50 (dd, 1H, *J* = 9.0, 9.0 Hz), 5.53 (d, 1H, *J* = 1.0 Hz), 5.71 (dd, 1H, *J* = 3.0, 9.5 Hz), 5.83–5.90 (m, 2H), 5.98

(s, 1H), 5.98–6.04 (m, 2H), 6.15 (d, 1H, J = 1.5 Hz), 7.24–7.55 (m, 24 H), 7.68 (t, 1H, J = 7.5 Hz), 7.78 (dd, 2H, J = 1.5, 7.5 Hz), 7.95 (dd, 2H, J = 1.0, 8.0 Hz), 8.00 (d, 2H, J = 8.0 Hz), 8.05 (dd, 1H, J = 1.0, 8.0 Hz), 8.28 (d, 1H, J = 7.5 Hz); HRMS calcd for $C_{82}H_{81}ClO_{28}$ $[M+Na]^+$ 1571.4495; found 1571.4488.

3.10. Ethyl 7-*O*-acetyl-6-*O*-allyl-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-6,7-tri-*O*-acetyl-2-*O*-benzyl-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside (12)

EtSH (20 μL, 0.27 mmol) and $BF_3{\cdot}OEt_2$ (68 μL, 0.54 mmol) were added to a solution of **11** (42 mg, 0.027 mmol) in dry CH_2Cl_2 (3 mL). After stirring for 3 h (reaction monitored by MS) at 27 °C under Ar, the mixture was diluted with CH_2Cl_2 (5 mL), washed with $NaHCO_3$ (satd) (10 mL) and subjected to normal work-up followed by FC (toluene–EtOAc 6:1→9:2) to give **12** (29 mg, 0.019 mmol, 69%). R_f 0.55 (toluene–EtOAc 3:1); $[\alpha]_D$ +21 (c 1.0, $CHCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.7, 20.3, 20.9, 20.9, 25.0, 40.8, 61.0, 61.5, 63.8, 68.0, 69.8, 70.2, 70.4, 71.3 (2C), 71.6, 72.0, 72.2, 72.9, 72.9, 73.7, 74.0, 74.1, 75.1 (2C), 75.5, 82.6, 99.3, 100.5, 116.6, 127.7–128.8 (Ar-C), 129.2, 129.2, 129.8–130.4 (Ar-C), 132.8, 133.0, 133.2, 133.2, 133.4, 134.7, 137.7, 138.2, 164.6, 165.3, 165.5, 165.6, 166.0, 166.5, 170.0, 170.4, 170.7; ^1H NMR (500 MHz, $CDCl_3$): δ 1.15 (t, 3H, J = 7.5 Hz), 1.66 (s, 3H), 1.75 (s, 3H), 2.15 (s, 3H), 2.37–2. 49 (m, 2H), 3.74–4.01 (m, 10H), 4.08 (dd, 1H, J = 6.5, 11.0 Hz), 4.13 (m, 1H), 4.31–4.40 (m, 3H), 4.54 (dd, 1H, J = 6.5, 11.0 Hz), 4.62 (benzylic d, 1H, J_{gem} = 13.0 Hz), 4.66 (benzylic d, 1H, J_{gem} = 11.5 Hz), 4.71 (dd, 1H, J = 3.5, 12.0 Hz), 4. 78 (benzylic d, 1H, J_{gem} = 13.0 Hz), 4.82 (benzylic d, 1H, J_{gem} = 11.5 Hz), 4.87–4.91 (m, 2H), 5.26 (d, 1H, J = 10.5 Hz), 5.31 (s, 1H), 5.41–5.50 (m, 3H), 5.53 (s, 1H), 5.72 (dd, 1H, J = 3.0, 10.5 Hz), 5.84–5.87 (m, 2H), 5.96–6.05 (m, 2H), 7.13–7.43 (m, 22H), 7.49 (t, 1H, J = 7.5 Hz), 7.54 (t, 1H, J = 7.5 Hz), 7.67 (t, 1H, J = 7.5 Hz), 7.77 (dd, 2H, J = 1.0, 7.5 Hz), 7.94 (dd, 2H, J = 1.5, 8.0 Hz), 7.99 (d, 2H, J = 8.0 Hz), 8.08 (dd, 2H, J = 8.0 Hz), 8.28 (d, 2H, J = 8.0 Hz); HRMS calcd for $C_{82}H_{83}ClO_{26}S$ $[M+Na]^+$ 1573.4474; found 1571.4487.

3.11. 2-(*N*-Benzyloxycarbonyl) aminoethyl 7-*O*-acetyl-6-*O*-allyl-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-6,7-di-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (13)

Compound **12** (16 mg, 0.010 mmol) and *N*-Cbz-2-aminoethanol (5 mg, 0.026 mmol) were dissolved in dry CH_2Cl_2–Et_2O (2:1, 1.5 mL) followed by the addition of 4 A MS. After stirring for 30 min under an Ar-atmosphere the mixture was cooled to 0 °C and NIS (4 mg, 0.018 mmol) and AgOTf (cat.) were added. After stirring for 30 min (0 °C→rt), Et_3N (70 μL) was added and the mixture was diluted with CH_2Cl_2 (5 mL), filtered through Celite, washed with $Na_2S_2O_3$ (10% aq, 8 mL) and subjected to normal work-up. FC (toluene–EtOAc 6:1→3:1) gave **13** (13 mg, 0.0077 mmol, 77%). R_f = 0.69 (toluene–EtOAc 1:1); $[\alpha]_D$ −7 (c 1.0, $CHCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.2, 20.3, 20.9, 40.6, 40.8, 60.7, 60.9, 63.2, 63.8, 66.6, 67.0, 67.8, 69.7, 70.3, 70.4, 71.3, 71.8, 72.2, 72.3, 72.9, 73.0, 73.7, 73.8, 74.1, 75.0, 75.1 (2C), 98.4. 99.4, 100.5, 116.6, 127.7–128.7 (Ar-C), 129.1, 129.2, 129.2, 129.7–130.4 (Ar-C), 132.9, 133.0, 133.3, 133.3, 133.4, 134.6, 136.7, 137.7, 138.2, 156.6, 164.7, 165.4, 165.5, 165.6, 166.0, 166.6, 169.8, 170.7, 171.1; ^1H NMR (400 MHz, $CDCl_3$): δ 1.59 (s, 3H), 1.61 (s, 3H), 2.13 (s, 3H), 3.08 (m, 1H), 3.32 (m, 2H), 3.51–3.66 (m, 3H), 3.83–4.37 (m, 12 H), 4.58 (benzylic d, 1H, J_{gem} = 13.2 Hz), 4.63 (benzylic d, 1H, J_{gem} = 11.2 Hz), 4.67 (dd, 1H, J = 3.6, 12.0 Hz), 4.74 (benzylic d, 1H, J_{gem} = 13.2 Hz), 4.81 (benzylic d, 1H, J_{gem} = 11.2 Hz), 4.82 (d, 1H, J = 8.0 Hz), 4.89 (m, 1H), 5.01 (s, 2H), 5.25 (dd, 1H, J = 1.6, 10.8 Hz), 5.34 (dd, 1H, J = 6.8, 6.8 Hz), 5.41 (dd, 1H, J = 1.6, 17.2 Hz), 5.45–5.49 (m, 2H), 5.68 (dd, 1H, J = 3.2, 10.0 Hz), 5.81–6.03 (m, 5H), 7.10–7.54 (m, 24H), 7.63 (m, 1H), 7.75 (dd, 2H, J = 1.2, 8.4 Hz), 7.92 (dd, 2H, J = 1.2, 8.0 Hz), 7.97 (dd, 2H, J = 1.2, 8.4 Hz), 8.03 (dd, 2H, J = 1.6, 8.4 Hz), 8.26 (dd, 2H, J = 1.2, 8.4 Hz); HRMS calcd for $C_{90}H_{90}ClNO_{29}$ $[M+Na]^+$ 1706.5179; found 1706.5152.

3.12. 2-(*N*-Benzyloxycarbonyl) aminoethyl 7-*O*-acetyl-6-*O*-allyl-2-*O*-benzoyl-4-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-6,7-di-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (14)

MeOH saturated with NH_3 (2 mL) was added dropwise to a cooled (0 °C) solution of **13** (15 mg, 0.009 mmol) in CH_2Cl_2 (1 mL). After stirring for 90 min at 0 °C, the solvent was evaporated and FC (toluene–EtOAc 2:1) of the residue gave **14** (13 mg, 0.008 mmol, 90%). R_f = 0.66 (toluene–EtOAc 1:1); $[\alpha]_D$ +3 (c 1.0, $CHCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.2 (2C), 20.0, 39.6, 60.0, 62.1, 62.3, 65.4, 66.0, 66.9, 68.7, 69.0 (2C), 70.2, 70.4, 70.7, 70.9, 71.4, 71.9 (2C), 72.6, 72.8, 73.7, 73.8 (2C), 74.2, 97.5, 98.7, 99.5, 115.5, 126.5–129.4 (Ar-C), 132.0, 132.2, 132.3, 133.6, 133.7, 135.7, 136.7, 138.1, 155.6, 163.5, 164.3, 164.5, 165.0, 165.5, 168.8, 169.5, 170.0; ^1H NMR (500 MHz, $CDCl_3$): δ 1.61 (s, 6H), 2.28 (s, 3H), 2.76 (m, 1H), 3.05 (m, 1H), 3.27–3.67 (m, 8H), 3.85–4.44 (m, 11H), 4.58–4.86 (m, 4H), 5.00–5.07 (m, 3H), 5.19–5.46 (m, 5H), 5.57 (dd, 1H, J = 9.0 Hz), 5.81–6.01 (m, 5H), 7.16–7.55 (m, 30H), 7.83 (d, 2H, J = 7.5 Hz), 8.00 (d, 2H, J = 7.5 Hz), 8.03–8.07 (m, 4H), 8.26 (d, 2H, J = 7.5 Hz); HRMS calcd for $C_{88}H_{89}NO_{28}$ $[M+Na]^+$ 1630.5463; found 1630.5512.

3.13. 2-(*N*-Benzyloxycarbonyl) aminoethyl 7-*O*-acetyl-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-6,7-di-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (15)

A solution of **13** (14 mg, 8.2 μmol) in dry THF (2.5 mL) was degassed and placed under an argon atmosphere. [Bis(methyldiphenylphosphine)](1,5-cyclooctadine) $Ir(I)PF_6$ (0.7 mg, 0.82 μmol) was added and the solution was again degassed but now placed under a H_2-atmosphere. After stirring for 5 min, the H_2 was replaced by Ar and the stirring was continued for 15 min whereafter NIS (18 mg, 82 μmol) and H_2O (0.400 mL) were added. After an additional 10 min, the solution was diluted with CH_2Cl_2 (6 mL), washed with $Na_2S_2O_3$ (10% aq, 10 mL) and subjected to normal work-up followed by FC (toluene–EtOAc 2:1) to afford **15** (10 mg, 6.0 μmol, 73%). R_f = 0.68 (toluene–EtOAc 1:1); $[\alpha]_D$ +2 (c 1.0, $CHCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.2, 20.3, 20.8, 40.5, 40.7, 60.7, 63.6, 65.1, 66.5, 66.9, 67.7, 69.6, 70.2, 71.3, 72.2, 72.4, 72.8, 73.3, 73.5, 74.9 (2C), 75.3, 79.2, 98.2, 99.2, 100.4, 127.9–130.1 (Ar-C), 132.8, 133.0, 133.2, 133.3, 133.5, 136.6, 137.4, 137.9, 156.6, 164.9, 165.2, 165.4, 166.0, 166.4, 169.8, 170.6, 171.3; ^1H NMR (500 MHz, $CDCl_3$): δ 1.59 (s, 6H), 2.15 (s, 3H), 3.09 (m, 1H), 3.35 (m, 2H), 3.51–3.54 (m, 4H), 3.78–4.11 (m, 11H), 4.28 (dd, 1H, J = 10.0, 10.0 Hz), 4.54 (benzylic d, 1H, J_{gem} = 13.0 Hz), 4.63 (dd, 1H, J = 3.5, 12.0 Hz), 4.70 (benzylic d, 1H, J_{gem} = 13.0 Hz), 4.73 (benzylic d, 1H, J_{gem} = 11.5 Hz), 4.78–4.85 (m, 3H), 5.01 (s, 2H), 5.32 (m, 1H), 5.49–5.52 (m, 2H), 5.67 (d, 1H, J = 10.0 Hz), 5.81–5.91 (m, 2H), 7.17–7.54 (m, 30H), 7.76 (d, 2H, J = 7.5 Hz), 7.92 (d, 2H, J = 7.5 Hz), 7.96 (d, 2H, J = 6.0 Hz), 8.04 (d, 2H, J = 8.5 Hz),

8.22 (d, 2H, J = 8.0 Hz); HRMS calcd for $C_{87}H_{86}ClNO_{29}$ $[M+Na]^+$ 1660.4866; found 1660.4929.

3.14. 14 2-(*N*-Benzyloxycarbonyl) aminoethyl 7-*O*-acetyl-6-*O*-allyl-3-*O*-[benzyl-2-(*tert*-butyloxycarbonylaminoethyl)-phosphono]-2-*O*-benzoyl-4-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-6,7-di-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (16)

Tetrazole (3 mg, 0.0043 mmol) was added to a solution of (2-*tert*-butyloxycarbonyl aminoethyl)benzyl *N*,*N*-diisopropyl phosphoramidite[6] (17 mg, 0.043 mmol) and **14** (14 mg, 0.009 mmol) in dry CH_2Cl_2 (1.5 mL). After stirring for 90 min, the solution was cooled to 0 °C and *m*-CPBA (6 mg, 0.026 mmol) was added. After an additional 10 min at 0 °C, the solution was diluted with CH_2Cl_2 (4 mL), washed with $NaHCO_3$ (satd, 10 mL) and subjected to normal work-up. FC (toluene–EtOAc 3:1→2:1) yielded **16** (14 mg, 0.0072 mmol, 80%) as an inseparable diastereomeric mixture. R_f = 0.25 (toluene–EtOAc 2:1); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.2, 20.3, 20.6, 28.3, 28.3, 40.5, 40.7, 60.7, 63.3, 63.4, 64.4, 66.5, 66.8, 66.8, 67.3, 67.8, 69.2, 69.4, 69.5, 69.6, 69.7, 70.8, 71.0, 71.3, 71.6, 72.1–72.4 (several carbons), 72.8, 72.9, 73.6, 73.8, 74.9, 75.0, 78.9, 79.0, 79.2, 98.3, 98.4, 99.3, 99.4, 100.0, 116.5, 127.5–130.4 (Ar-C), 132.8, 132.9, 133.0, 133.1, 133.2, 133.3, 133.4, 134.5, 135.6, 135.8, 136.6, 137.5, 137.7, 138.2, 155.7, 156.0, 156.6, 164.6, 164.4, 165.4, 165.5, 165.6, 166.0, 166.1, 169.8, 170.6, 170.6, 171.0; ^{31}P NMR (decoupled, 162 Hz, $CDCl_3$): δ −1.64, −1.02; HRMS calcd for $C_{102}H_{109}N_2O_{33}P$ $[M+Na]^+$ 1943.6542; found 1943.6540.

3.15. 2-(*N*-Benzyloxycarbonyl) aminoethyl 7-*O*-acetyl-6-*O*-[benzyl-2-(*tert*-butyloxycarbonylaminoethyl)-phosphono]-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-6,7-di-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (17)

Tetrazole (3 mg, 4.2 μmol) was added to a solution of (2-*tert*-butyloxycarbonyl aminoethyl)benzyl *N*,*N*-diisopropyl phosphoramidite[6] (16 mg, 42 μmol) and **17** (14 mg, 8.4 μmol) in dry CH_2Cl_2 (1 mL). After stirring for 60 min, the solution was cooled to 0 °C and *m*-CPBA (6 mg, 2.6 μmol) was added. After an additional 10 min (0 °C), the solution was diluted with CH_2Cl_2 (6 mL), washed with $NaHCO_3$ (satd, 8 mL) and subjected to normal work-up. FC (toluene–EtOAc 3:1→2:1→1:1) gave **17** (12 mg, 6.2 μmol, 73%) as an inseparable diastereomeric mixture. R_f = 0.55 (toluene–EtOAc 1:1); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.1, 20.8, 21.0, 28.3, 28.4, 29.7, 40.5, 40.6, 40.8 60.4, 60.6, 63.3, 63.6, 65.1, 66.5, 67.1, 67.3, 67.7, 69.4–70.5 (several carbons), 72.1, 72.5, 72.9, 73.6, 74.1, 74.7, 74.9, 79.6, 98.3, 99.0, 100.4, 127.5–128.8 (Ar-C), 129.7, 129.7, 129.9, 130.0, 130.2, 132.8, 133.0, 133.2, 133.3, 133.4, 133.5, 136.6, 137.4, 138.1, 155.7. 155.9, 156.6, 164.8, 165.3, 165.4, 165.6, 166.0, 166.4, 166.5, 169.7, 170.6, 170.8; ^{31}P NMR (decoupled, 162 Hz, $CDCl_3$): δ −0.87, −1.08; HRMS calcd for $C_{101}H_{106}ClN2O_{34}P$ $[M+Na]^+$ 1979.5945; found 1979.5931.

3.16. 2-Aminoethyl 3-*O*-[2-(*tert*-butyloxycarbonylaminoethyl)-phosphono]-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-L-*glycero*-α-D-*manno*-heptopyranoside (18)

A solution of **16** (11 mg, 5.60 μmol) in dry THF (2 mL) was degassed and placed under an argon atmosphere. [Bis(methyldiphenylphosphine)](1,5-cyclooctadine) Ir(I)PF_6 (cat.) was added and the solution was again degassed but now placed under a H_2-atmosphere. After stirring for 5 min, the H_2 was replaced by Ar and the stirring was continued for 15 min whereafter NIS (12 mg, 56.0 μmol) and H_2O (0.400 mL) were added. After an additional 10 min, the solution was diluted with CH_2Cl_2 (4 mL), washed with $Na_2S_2O_3$ (10% aq, 6 mL) and subjected to normal work-up followed by FC (toluene–EtOAc 2:1). The product was directly dissolved in EtOAc–MeOH (1:1) (2 mL) followed by the addition of 0.1 M HCl (aq) (84 μL) and Pd–C (10%) (cat.). The mixture was stirred under H_2 (110 psi) for 14 h, filtered, concentrated and purified by FC (EtOAc–MeOH–H_2O, 7:2:1). The resulting product was dissolved in MeOH (1.5 mL) and the pH was adjusted to 10 by the addition of NaOMe (from a 0.1 M in MeOH stock solution). After stirring for 14 h at 27 °C, the solution was cooled to 0 °C and neutralized with Dowex-H^+ ion exchange resins, filtered and concentrated. Reversed phase silica gel chromatography (H_2O→H_2O–MeOH 1:1) and freeze-drying of the product gave **18** (2.3 mg, 2.7 μmol, 49%); ^{13}C NMR (125 MHz, D_2O): δ 27.7 (3C), 39.5, 40.7 (J = 7.5 Hz), 61.2, 61.5 (J = 5.0 Hz), 62.6, 62.9, 68.0, 68.8, 69.0, 69.6, 69.8, 70.0, 70.2, 70.7, 71.8, 73.0, 73.8 (2C), 75.7, 76.1, 76.3 (J = 5.0 Hz), 99.6, 100.7, 102.6 (Note: Cq and C(O) from –NHBoc not detected); ^1H NMR (500 MHz, D_2O): δ 1.36 (s, 9H), 2.96–2.98 (m, 2H), 3.21 (dd, 1H, J = 8.0, 9.5 Hz), 3.23–3.43 (m, 8H), 3.52–3.76 (m, 8H), 3.85–4.06 (m, 7H), 4.18–4.24 (m, 2H), 4.48 (d, 1H, J = 8.0 Hz), 4.78 (d, 1H, J = 1.5 Hz), 5.28 (s, 1H); ^{31}P NMR (decoupled, 162 Hz, D_2O): δ 0.09; HRMS calcd for $C_{29}H_{55}N_2O_{23}P$ $[M+H]^+$ 831.3006; found 831.3051.

3.17. 2-Aminoethyl 6-*O*-[2-(*tert*-butyloxycarbonylaminoethyl)-phosphono]-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-L-*glycero*-α-D-*manno*-heptopyranoside (19)

Compound **17** (4 mg, 2.5 μmol) was dissolved in EtOAc–MeOH (1:1) (2 mL) followed by the addition of 0.1 M HCl (aq) (50 μL) and Pd–C (10%) (cat.). The mixture was stirred under H_2 (110 psi) for 14 h, filtered, concentrated and purified by FC (EtOAc–MeOH–H_2O, 7:2:1). The resulting product was dissolved in MeOH (1.0 mL) and the pH was adjusted to 10 by the addition of NaOMe (from a 0.1 M in MeOH stock solution). After stirring for 16 h, the solution was cooled to 0 °C and neutralized with Dowex-H^+ ion exchange resins, filtered and concentrated. Reversed phase silica gel chromatography (H_2O→H_2O–MeOH 1:1) and freeze-drying of the product gave **19** (1.6 mg, 1.9 μmol, 76%). ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.7, 39.2, 40.7 (J = 10 Hz), 60.6, 61.5, 62.4, 63.4, 65.0, 66.0, 67.9, 69.6, 69.7, 69.9, 70.4, 70.8, 71.0, 73.4 (J = 6.5 Hz), 73.5, 73.8, 73.9, 75.6, 76.3, 99.8, 101.5, 102.5 (Note: Cq and C(O) from –NHBoc not detected); ^1H NMR (400 MHz, D_2O): δ 1.45 (s, 9H), 3.25–3.37 (m, 6H), 3.44–3.59 (m, 3H), 3.70–3.99 (m, 14 H), 4.11–4.17 (m, 3H), 4.26 (dd, 1H, J = 9.4, 9.4 Hz), 4.51 (m, 1H), 4.56 (d, 1H, J = 8.0 Hz), 4.87 (d, 1H, J = 1.5 Hz), 5.31 (d, 1H, J = 0.8 Hz); ^{31}P NMR (decoupled, 162 Hz, D_2O): δ 0.62; HRMS calcd for $C_{29}H_{55}N_2O_{23}P$ $[M+H]^+$ 831.3006; found 831.3023.

3.18. 2-Aminoethyl L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-L-*glycero*-α-D-*manno*-heptopyranoside (20)

A solution of **14** (7 mg, 4.30 μmol) in dry THF (1.5 mL) was degassed and placed under an argon atmosphere. [Bis(methyldiphenylphosphine)](1,5-cyclooctadine) Ir(I)PF_6 (cat.) was added and the solution was again degassed but now placed under a H_2-atmosphere. After stirring for 5 min, the H_2 was replaced by Ar and the stirring was continued for 15 min whereafter NIS (10 mg, 43.0 μmol) and H_2O (0.300 mL) were added. After an additional 10 min, the solution was diluted with CH_2Cl_2 (5 mL), washed with $Na_2S_2O_3$ (10% aq, 6 mL) and subjected to normal work-up followed

by FC (toluene–EtOAc 2:1). The product was directly dissolved in EtOAc–MeOH (1:1, 2 mL) followed by the addition of 0.1 M HCl (aq, 76 μL) and Pd–C (10%, cat.). The mixture was stirred under H_2 (110 psi) for 10 h, filtered, concentrated and purified by FC (EtOAc–MeOH–H_2O, 7:2:1). The resulting product was dissolved in MeOH (2 mL) and the pH was adjusted to 10 by the addition of NaOMe (from a 0.1 M in MeOH stock solution). After stirring for 16 h, the solution was neutralized with Dowex-H^+ ion exchange resins, filtered and concentrated. Reversed phase silica gel chromatography (H_2O) and freeze-drying of the product gave **20** (2.0 mg, 3.2 μmol, 74%); ^{13}C NMR (125 MHz, D_2O): δ 39.2, 61.5, 62.4, 62.7, 66.1, 67.9, 68.7, 69.7, 69.8, 69.9, 70.7, 70.8, 71.7, 73.5, 73.7, 74.1, 75.6, 76.3 (2C), 99.8, 101.5, 102.5; ^1H NMR (500 MHz, D_2O): δ 3.11–3.14 (m, 2H), 3.20 (dd, 1H, J = 8.0, 9.5 Hz), 3.25 (dd, 1H, J = 9.0, 9.0 Hz), 3.37 (m, 1H), 3.42 (dd, 1H, J = 9.0, 9.0 Hz), 3.51 (dd, 1H, J = 6.0, 12.0 Hz), 3.56–3.71 (m, 9H), 3.77–3.79 (m, 2H), 3.88 (dd, 1H, J = 2.0, 12.0 Hz), 3.95–4.06 (m, 4H), 4.16 (dd, 1H, J = 10.0, 10.0 Hz), 4.47 (d, 1H, J = 8.0 Hz), 4.77 (d, 1H, J = 1.0 Hz), 5.18 (d, 1H, J = 1.5 Hz); HRMS calcd for $C_{22}H_{41}NO_{18}$ $[M+Na]^+$ 630.2216; found 630.2239.

Acknowledgement

We thank the Swedish Research Council and Science Foundation Ireland (Grant Number 08/IN.1/B2067) for financial support.

References

1. Andersen, S. R.; Kolberg, J.; Hoiby, E. A.; Namork, E.; Caugant, D. A.; Froholm, L. O.; Jantzen, E.; Bjune, G. *Microb. Pathog.* **1997**, *23*, 139–155.
2. Cox, A. D.; Li, J. J.; Brisson, J. R.; Moxon, E. R.; Richards, J. C. *Carbohydr. Res.* **2002**, *337*, 1435–1444.
3. Richards, J. C.; Cox, A. D.; Schweda, E. K. H.; Martin, A.; Hood, D. W.; Moxon, E. R. *Adv. Exp. Med. Biol.* **2001**, *491*, 515–524. and references cited therein.
4. Yildirim, H. H.; Hood, D. W.; Moxon, E. R.; Schweda, E. K. H. *Eur. J. Biochem.* **2003**, *270*, 3153–3167. and references cited therein.
5. Moxon, E. R.; Hood, D. W.; Saunders, N. J.; Schweda, E. K. H.; Richards, J. C. *Philos. Trans. R. Soc. London, Ser. B* **2002**, *357*, 109–116.
6. Mannerstedt, K.; Segerstedt, E.; Olsson, J.; Oscarson, S. *Org. Biomol. Chem.* **2008**, *6*, 1087–1091.
7. Olsson, J. D. M.; Oscarson, S. *Tetrahedron: Asymmetry* **2009**, *20*, 879–886.
8. Wakarchuk, W.; Schur, M. J.; St. Michael, F.; Li, J.; Eichler, E.; Whitfield, D. *Glycobiology* **2004**, *14*, 537–546.
9. Boons, G. J. An approach Towards the Development of a Synthetic Vaccine Against *Neisseria meningitides*. Ph.D. Thesis, Leiden University, 1991.
10. Bernlind, C.; Oscarson, S. *J. Org. Chem.* **1998**, *63*, 7780–7788.
11. Segerstedt, E.; Mannerstedt, K.; Johansson, M.; Oscarson, S. *J. Carbohydr. Chem.* **2004**, *23*, 443–452.
12. Olsson, J. D. M. Synthesis of Bacterial Oligosaccharides from *N. meningitidis* and Mechanistic Details of Stereoselective Glycosylations Using a Novel Bicyclic Donor. Ph.D. Thesis, Stockholm University, 2009.
13. Crich, D.; Cai, W.; Dai, Z. *J. Org. Chem.* **2000**, *65*, 1291–1297.
14. Codee, J. D. C.; deJong, A. R.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *Tetrahedron* **2009**, *65*, 3780–3788.
15. Tatai, J.; Fugedi, P. *Org. Lett.* **2007**, *9*, 4647–4650.