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Synthesis of phosphorylated *Neisseria meningitidis* inner core lipopolysaccharide structures

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This paper is dedicated to Professor George Fleet, on the occasion of his 65th birthday

ABSTRACT

A phosphoethanolamine-substituted tetrasaccharide structure, 2-aminoethyl 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→2)-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, corresponding to the non-reducing part of the conserved part of *Neisseria meningitidis* lipopolysaccharides has been synthesized. Orthogonal protection of the phosphoethanolamino group in combination with the presence of a free amino-containing anomeric spacer allows conjugation to proteins to construct conjugate vaccine candidates. The tetrasaccharide is built up using a linear strategy, where the introduction of the terminal α-GlcNAc moiety is performed using a 2-azido-thioglucoside as a donor and NIS/AgOTf as a promoter. The synthetic pathway includes tetrasaccharide intermediates appropriately designed to permit other phosphorylation patterns as well as elongation at the reducing end.

1. Introduction

Efficient glycoconjugate vaccines against *Neisseria meningitidis* based on the surface capsular polysaccharide (CPS) are already commercial or under development for four, A, C, W-135 and Y, of the five serotypes that are the main causes of meningococcal meningitis.[1] Type B, however, constitutes a problem, since the structure of the CPS mimics a human structure.[2] An alternative, which has been pursued for some time, is to try to base a vaccine on lipopolysaccharide (LPS) structures instead.[3] These structures conjugated to a carrier protein should be good vaccine candidates since they are genetically stable and have proven to be accessible to host immune mechanisms and to induce protective antibodies.[4] An advantage with the LPS-structures is that they are conserved in all clinically relevant strains across the species; LPS-based vaccine protection should not be dependent on the capsular serotype of the infecting bacteria. However, there are other issues with LPS-based vaccines against *Neisseria meningitidis*, for example, the toxicity of the Lipid A part and a heterogeneity in the outer parts of the LPS-molecule. Furthermore, the activation and subsequent protein conjugation are not trivial. To try to identify protecting epitopes, well-defined synthetic part structures would be an asset. Herein, a synthesis of a tetrasaccharide **20**, corresponding to the non-reducing part of the conserved inner core structure of *N. meningitidis* LPS (Fig. 1), is presented. In addition phosphorylated structures **18** and **19** are reported, containing a phosphoethanolamine moiety at the 6-position of the outer heptose residue, a determinant believed to be of immunological importance.[4] The synthetic strategy also allows for the introduction of a phosphoethanolamine in the 3-position at the same heptose residue, which is the other important phosphorylation position found. The synthesis proceeds via a tetrasaccharide thioglycoside donor **14**. Glycosylation of this with an aminospacer alcohol was performed to facilitate efficient conjugation of the target structures, but this intermediate is also designed to be a vital building donor block in the formation of larger structures containing the Kdo and LipidA parts.

2. Results and discussion

Starting from intermediate **7**,[5] *Haemophilus influenzae* structures similar to the target *N. meningitidis* structures **18–20**, differing only in the non-reducing end moiety, being an α-GlcNAc residue in *Neisseria* (Fig. 1) and an α-LD-Hep residue in *Haemophilus*, have earlier been efficiently synthesized in our group.[6] However, initial experiments showed that the coupling of glucosamine donors to the intermediate **7** was not trivial (see below). Hence, a different pathway, a 2+2-block approach, was attempted to reach the desired tetrasaccharide **9** (Scheme 1). Using the well-known trichloroacetimidate donor **3**[7] and TMSOTf-activation in a coupling to the easily accessible acceptor **2**, a good yield and an excellent α-stereoselectivity were obtained to give disaccharide **4** (63%, only α). But when glycosylation reactions between disaccharide donor **4** and acceptor **6**[5] were attempted, no tetrasaccharide product was formed at all in spite of the use of a variety of promoters and coupling conditions. It has been observed earlier that heptose donors are generally less

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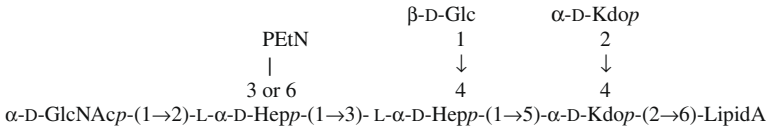
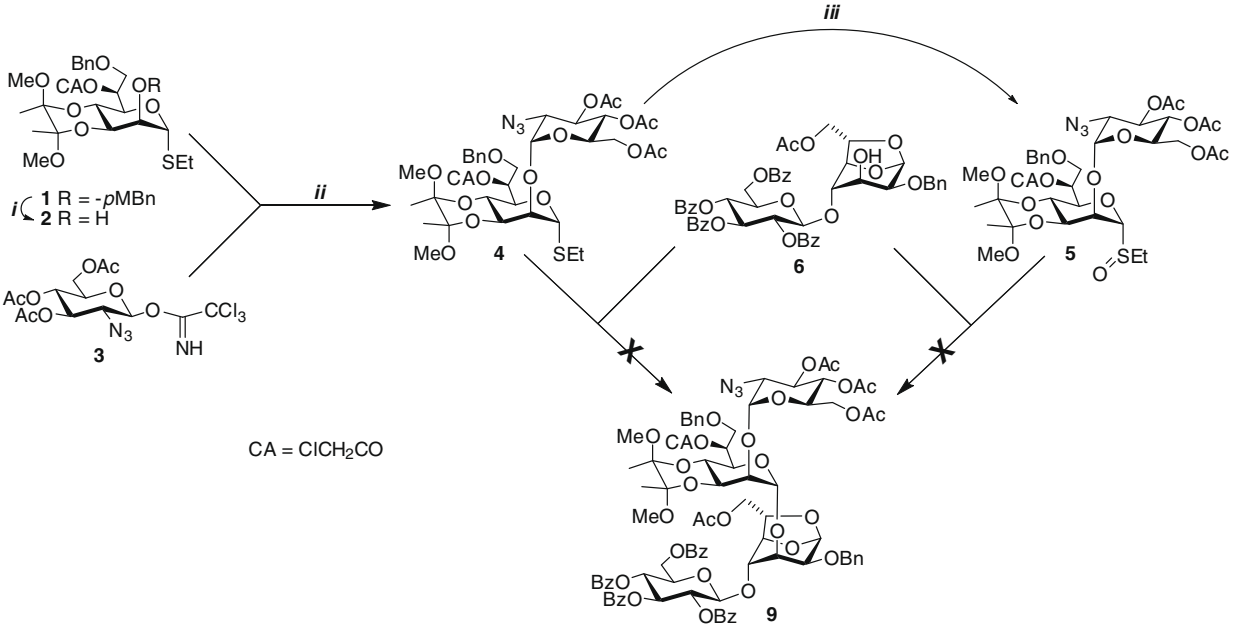


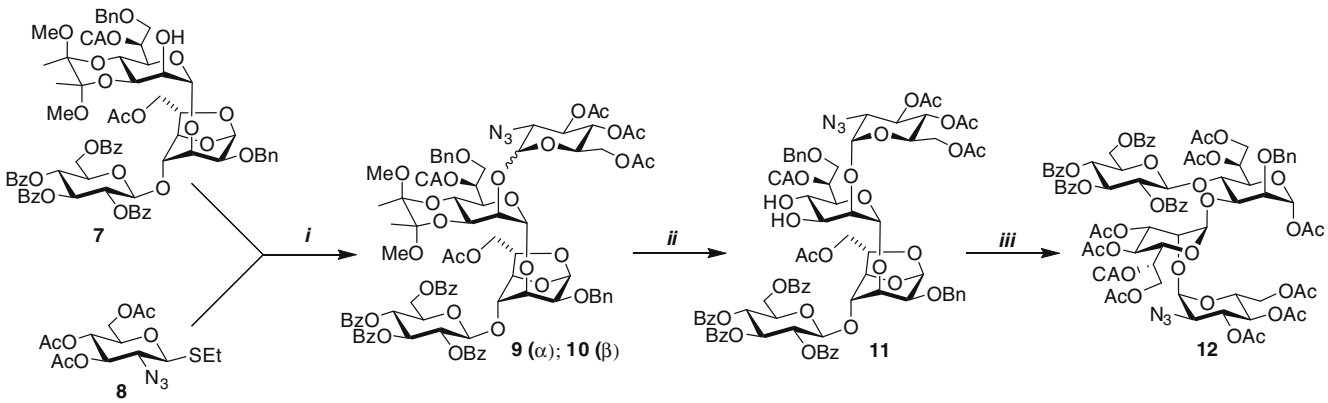
Figure 1. Conserved inner core structure of *N. meningitidis* LPS.



Scheme 1. Attempted block approach towards tetrasaccharide intermediate **9**. Reagents and conditions: (i) DDQ, CH_2Cl_2/H_2O (15:1), 94%; (ii) TMSOTf, 4 A MS, Et_2O, 0 °C 63%; (iii) *m*-CPBA, CH_2Cl_2, −60 °C, 61%.

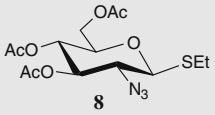
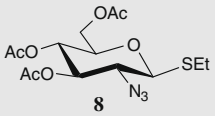
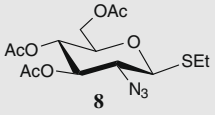
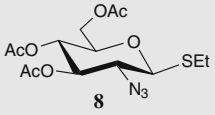
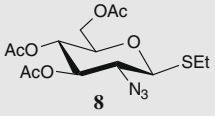
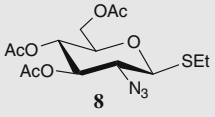
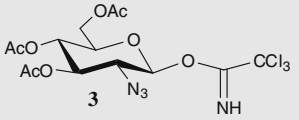
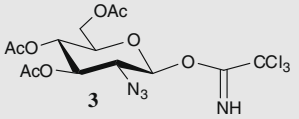
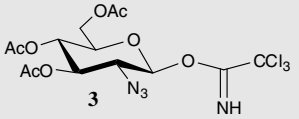
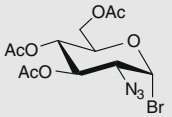
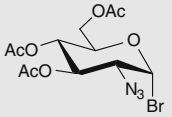
reactive than hexose donors,[8] but this disaccharide donor was unusually difficult to be activated even by thiophilic promoters that are considered to be very effective (Me_2S_2/Tf_2O[9] and Ph_2SO/Tf_2O[10]). To try to increase the reactivity, the corresponding sulfoxide donor was constructed, donors that have been advocated by Kahne et al. to be most reactive.[11] Using this donor, small amounts of tetrasaccharide were obtained, but the results were difficult to be reproduced and even the best yields were quite disappointing (<15%).

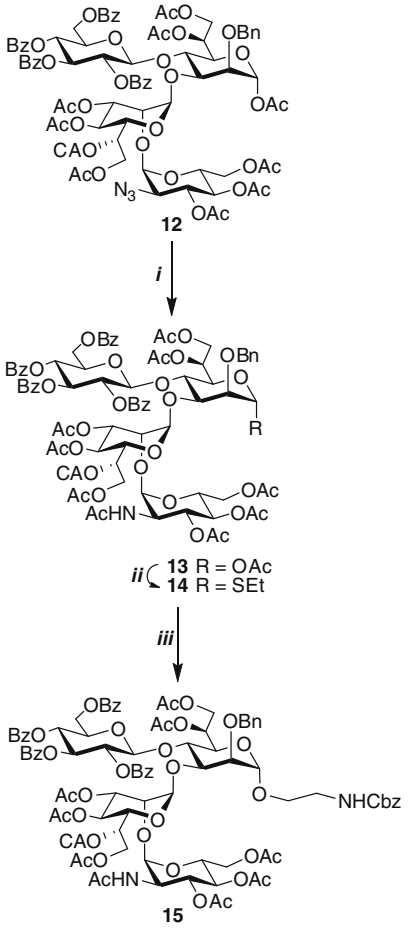
We were therefore forced back to the earlier used linear (1+3)-approach (Scheme 2 and Table 1). Glycosylations of acceptor **7** with the above mentioned donor **3** gave, with TMSOTf as promoter, again no tetrasaccharide product at all, and with BF_3-etherate low yields and low stereoselectivity were obtained (22%, α/β 1:1.6, entries vii–ix). With the corresponding bromide donor activated by AgOTf/DTBMP, only slightly better yield and α-selectivity (34%, α/β 1:1, entries x–xi) were observed. The thioglycoside donor **8**[12] activated by DMTST or Me_2S_2/Tf_2O also gave very poor results (entries i–ii), however, surprisingly, activation by NIS/AgOTf in CH_2Cl_2 gave excellent yields of the tetrasaccharide **9**/**10,** though once more with low stereoselectivity (entries iv–vi). The α-selectivity was found to increase with increase in temperature, but, again surprisingly, a change of solvent from CH_2Cl_2 to Et_2O (generally considered α-directing[13]) afforded complete β-selectivity (entry iii). We have earlier obtained interesting results in glycosylation with



Scheme 2. Synthesis of tetrasaccharide intermediate **12** using a linear approach. Reagents and conditions: (i) NIS, AgOTf, 4 A MS, CH_2Cl_2, 10 °C, 95% (α/β 1.5:1); (ii) 90% TFA (aq), 86%; (iii) $Sc(OTf)_3$, Ac_2O, 96%.

Table 1
Glycosylation investigation to optimize formation of tetrasaccharide **9**

Entry	Donor	Acceptor	Promoter	Solvent	Yield
i	 **8**	7	DMTST	Et_2O (0 °C)	Traces
ii	 **8**	7	Me_2S_2/Tf_2O	Et_2O (0 °C)	Decomposition
iii	 **8**	7	NIS/AgOTf	Et_2O (0 °C)	82% (α/β 0/1)
iv	 **8**	7	NIS/AgOTf	CH_2Cl_2 (−20 °C)	95% (α/β 1/1.4)
v	 **8**	7	NIS/AgOTf	CH_2Cl_2 (0 °C)	93% (α/β 1/1)
vi	 **8**	7	NIS/AgOTf	CH_2Cl_2 (rt)	95% (α/β 1.5/1)
vii	 **3**	7	BF_3–OEt_2	CH_2Cl_2 (−30 °C)	22% (α/β 1/1.6)
viii	 **3**	7	TMSOTf	Et_2O/CH_2Cl_2 (0 °C)	Traces
ix	 **3**	7	TMSOTf	Et_2O (0 °C)	Decomposition
x		7	AgOTf	CH_2Cl_2 (−60 °C)	Decomposition
xi		7	AgOTf/DTBMP	CH_2Cl_2 (−35 °C)	34% (α/β 1/1)

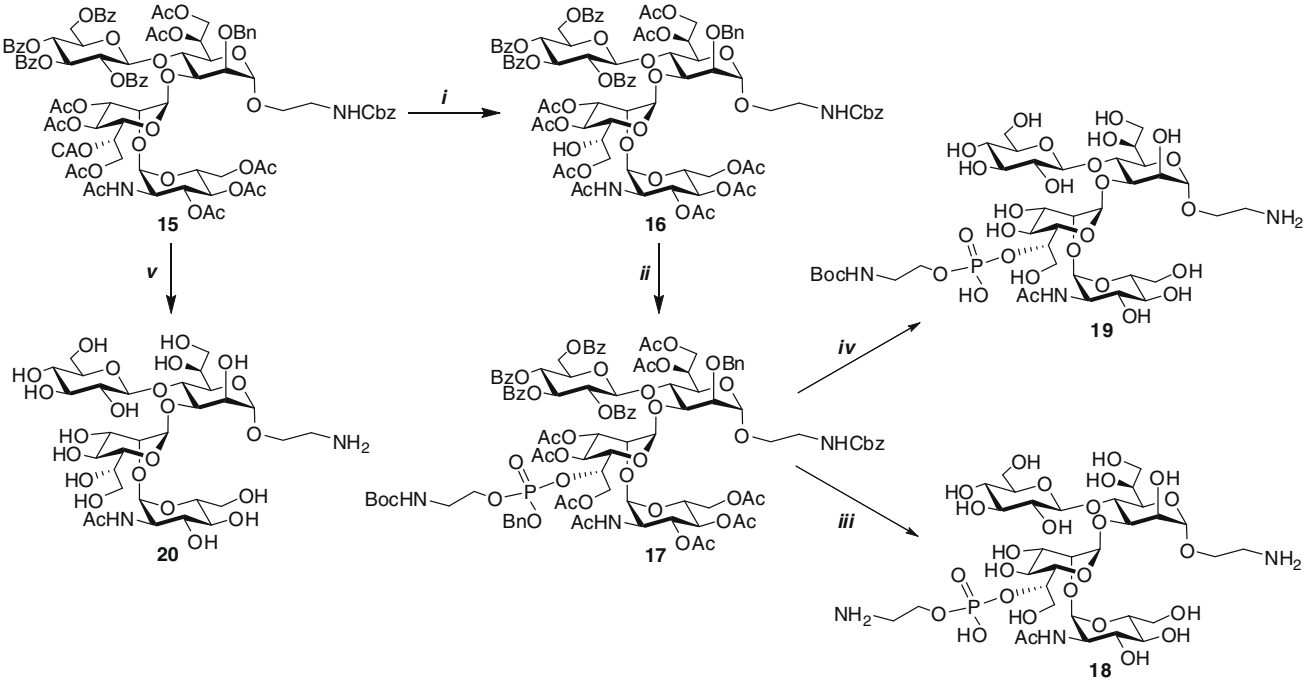


Scheme 3. Synthesis of tetrasaccharide intermediate **15**. Reagents and conditions: (i) (a) PPh_3, THF, H_2O, reflux; (b) Ac_2O/pyridine (1:1), 83%; (ii) EtSH, BF_3–OEt_2, CH_2Cl_2, 68%; (iii) *N*-Cbz-2-aminoethanol, NIS, AgOTf, CH_2Cl_2/Et_2O (2:1), 4 Å MS, 0 °C, 85%.

2-*N*-acetyl-2*N*,3*O*-oxazolidinone-protected glucosamine donors activated by NIS/AgOTf, where the stereoselective outcome is determined by the amount of AgOTf added, catalytic amounts afforded β-linked products whereas 0.5 equiv gave α-linked products.[14] This methodology was applied to the present glycosylation reaction and excellent yield (94%) and complete α-selectivity were obtained.[15] Unfortunately, the carbamate-protecting group is not orthogonal to the chloroacetyl temporary-protecting group in the molecule. To proceed, the best conditions found were applied to the coupling reaction between donor **3** and acceptor **7** on a preparative scale to yield an α/β-mixture, which was separated on a silica gel column affording the desired tetrasaccharide **9** (57%) and also **10** (38%).

Tetrasaccharide **9** was then converted to the target structures **18–20,** partly using methodologies worked out for the synthesis of the *Haemophilus* structures.[6] Hydrolysis of the BDA-acetal (→**11**, 86%) followed by acetolysis of the 1,6-anhydro-linkage using $Sc(OTf)_3$ as a catalyst very efficiently afforded the anomeric acetate **12** (96%). At this stage, chemoselective reduction of the azido group was performed using Staudinger conditions. Acetylation of the obtained amine yielded **13** (83%), which was converted into the corresponding thioglycoside donor **14** (68%) by EtSH/BF_3-etherate treatment (Scheme 3). This donor will be a key intermediate in the synthesis of larger *Neisseria* LPS structures including the Kdo- and lipid A parts of the native molecule. To test its donor properties glycosylation with a spacer, *N*-Cbz-protected ethanolamine, using again NIS/AgOTf as promoter was performed which afforded an 85% yield of spacer tetrasaccharide **15**.

From this intermediate, the non-phosphorylated deprotected target structure **20** as well as the phosphorylated structures **18** and **19** was produced (Scheme 4). A two-step deprotection scheme comprising Zemplen deacylation followed by catalytic hydrogenolysis yielded tetrasaccharide **20** in 92% overall yield. To introduce the phosphoethanolamine regioselectively in the O-6″ position, the chloroacetyl group was removed by treatment with ammonia in MeOH/CH_2Cl_2 (→**16**, 75%). The phosphoethanolamine was introduced by phosphoramidite chemistry using a Boc-protected amino



Scheme 4. Synthesis of target structures **18–20**. Reagents and conditions: (i) NH_3 (satd) MeOH/CH_2Cl_2 (2:1), 75%; (ii) a) (2-*tert*-butyloxycarbonyl aminoethyl)benzyl *N*,*N*-diisopropyl phosphoramidite, tetrazole, CH_2Cl_2; (b) *m*-CPBA, CH_2Cl_2, 0 °C, 76%; (iii) (a) Pd/C (10%), H_2, 0.1 M HCl, EtOAc/MeOH (1:1); (b) NaOMe, MeOH (c) Dowex-H^+, MeOH, 40 °C, 68% over three steps; (iv) a) Pd/C (10%), H_2, 0.1 M HCl, EtOAc/MeOH (1:1); (b) NaOMe, MeOH, 68% over two steps; (v) (a) NaOMe, MeOH; (b) Pd/C (10%), H_2, 0.1 M HCl, MeOH, 92% over two steps.

group to attain orthogonality with the protection of the spacer amino group. Treatment of alcohol **16** with (*N*-Boc-aminoethyl)benzyl *N*,*N*-diisopropyl phosphoramidite[6] and tetrazole followed by *m*-CPBA oxidation gave phosphotriester **17** in 76% yield. Deprotection of **17** was performed using the two-step procedure discussed above but this time in reversed order to give target structure **19** (68%) with a free spacer amino group which can be selectively activated and conjugated in the presence of the Boc-protected phosphoethanolamine group. Acidic workup after the deacylation step afforded target structure **18** with two free amino groups.

3. Conclusion

In conclusion, an effective synthesis of a tetrasaccharide thioglycoside donor, corresponding to the outer part of the conserved inner core LPS structure of *N. meningitidis* and allowing elongation through glycosylation at the reducing end and regioselective phosphorylation at the 6″-position, is reported. Spacer-equipped target structures were produced, with or without phosphoethanolamine substituents, ready for conjugation and following immunological evaluation.

4. Experimental

4.1. General methods

Normal workup means drying the organic phase with $MgSO_4$ (s) or Na_2SO_4 (s), filtering and evaporation of the solvent in vacuo at ~35 °C. CH_2Cl_2 was distilled over calcium hydride and collected onto 4 Å predried MS. Thin Layer Chromatography (TLC) was carried out on 0.25 mm precoated 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 ppm 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% H_3PO_4 (δ = 0.00) was used as an external reference.

4.2. (2′*S*,3′*S*)-Ethyl 7-*O*-benzyl-6-*O*-chloroacetyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside 2

(2′*S*,3′*S*)-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[5] **1** (381 mg, 0.58 mmol) was dissolved in CH_2Cl_2/H_2O (15:1, 8 mL) and DDQ (152 mg, 0.67 mmol) was added. After 3 h, the mixture was diluted with CH_2Cl_2 (10 mL), washed with $Na_2S_2O_3$ (5% aq, 10 mL) and subjected to normal workup. FC (toluene/EtOAc 6:1 → 3:1) gave **2** (260 mg, 0.55 mmol, 94%); R_f 0.46 (toluene/EtOAc 2:1); $[\alpha]_D$ = +17 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.6, 17.7, 17.8, 24.9, 41.0, 48.1, 48.2, 62.6, 67.8, 68.7, 69.2, 70.1, 71.0, 73.1, 84.6, 100.2, 100.6, 127.7 (2C), 127.8, 128.4 (2C), 137.7, 167.0; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (t, 3H, *J* = 7.6 Hz), 1.27 (s, 3H), 1.29 (s, 3H), 2.55 (m, 2H), 3.13 (s, 3H), 3.24 (s, 3H), 3.62 (dd, 1H, *J* = 6.4, 10.0 Hz), 3.68 (dd, 1H, *J* = 7.2, 10.0 Hz), 3.95 (dd, 1H, *J* = 2.8, 10.0 Hz), 4.00–4.16 (m, 4H), 4.35 (dd, 1H, *J* = 0.8, 10.0 Hz), 4.47 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.55 (benzylic d, 1H, J_{gem} = 11.6 Hz), 5.34 (s, 1H), 5.60 (ddd, *J* = 0.8, 1.6, 3.6 Hz), 7.22–7.34 (m, 5H); HRMS: Calcd for $C_{24}H_{35}ClO_9S$: $[M+Na]^+$ 557.1583; Found 557.1611.

4.3. (2′*S*,3′*S*)-Ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl-(1→2)-7-*O*-benzyl-6-*O*-chloroacetyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside 4

2-Azido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl trichloroacetimidate[7] **3** (128 mg, 0.269 mmol) and **2** (80 mg, 0.149 mmol) were dissolved in dry Et_2O (3 mL) and 4 A MS was added. After stirring for 30 min under an Ar-atmosphere, the mixture was cooled to 0 °C and TMSOTf (0.015 mmol, from Et_2O stock solution) was added. After 2.5 h, Et_3N (150 μL) was added and the mixture was diluted with CH_2Cl_2 (6 mL), filtered through Celite and subjected to normal workup. FC (toluene/EtOAc 9:1 → 6:1) gave **4** (80 mg, 0.094 mmol, 63%); R_f 0.68 (toluene/EtOAc 2:1); $[\alpha]_D$ = +16 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.7, 17.7, 17.9, 20.7, 20.8, 20.8, 25.2, 41.2, 48.1, 48.3, 61.2, 62.3, 62.7, 67.8, 68.1, 69.0, 69.1, 69.5 (2C), 69.6, 73.2, 75.2, 83.9, 98.4 ($J_{C,H}$ = 177 Hz), 100.1, 100.4, 127.7 (2C), 127.8, 128.5 (2C), 137.8, 167.3, 169.3, 170.1, 170.7; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (t, 3H, *J* = 7.6 Hz), 1.26 (s, 6H), 2.05 (s, 3H), 2.09 (s, 3H), 2.09 (s, 3H), 2.57 (m, 2H), 3.09 (s, 3H), 3.23 (s, 3H), 3.22 (dd, 1H, *J* = 3.6, 10.8 Hz), 3.60 (dd, 1H, *J* = 6.8, 9.6 Hz), 3.67 (dd, 1H, *J* = 6.8, 9.6 Hz), 4.04 (dd, 1H, *J* = 3.2, 9.6 Hz), 4.04–4.35 (m, 10H), 4.48 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.55 (benzylic d, 1H, J_{gem} = 11.6 Hz), 5.01 (dd, 1H, *J* = 9.6, 9.6 Hz), 5.32 (s, 1H), 5.47 (d, 1H, *J* = 3.6 Hz), 5.51 (dd, 1H, *J* = 9.2, 10.8 Hz), 5.61 (dd, 1H, *J* = 6.8, 6.8 Hz), 7.22–7.35 (m, 5H); HRMS: Calcd for $C_{36}H_{50}ClN_3O_{16}S$: $[M+Na]^+$ 870.2493; Found 870.2467.

4.4. (2′*S*,3′*S*)-Ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl-(1→2)-7-*O*-benzyl-6-*O*-chloroacetyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside S-oxide 5

Compound **4** (67 mg, 0.079 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and the solution was cooled to −60 °C followed by addition of *m*-CPBA (14 mg, 0.083 mmol). After 30 min (−60 °C → −20 °C), the reaction was diluted with CH_2Cl_2 (5 mL), washed with $NaHCO_3$ (satd, 10 mL) and subjected to normal workup followed by FC (toluene/EtOAc 1:1) to give **5** (42 mg, 0.048 mmol, 61%); R_f 0.26 (toluene/EtOAc 1:1); $[\alpha]_D$ = +22 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 5.8, 17.7, 17.9, 20.7, 20.8, 20.9, 41.0, 43.6, 48.4, 48.5, 61.2, 61.5, 62.0, 66.4, 68.4, 68.9 (2C), 69.3, 69.5, 73.6, 74.1, 93.1, 98.7, 100.3, 100.5, 127.8 (2C), 128.1, 128.7 (2C), 137.4, 167.2, 169.7, 170.3, 171.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (t, 3H, *J* = 7.2 Hz), 1.28 (s, 3H), 1.30 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.11 (s, 3H), 2.65 (m, 1H), 2.88 (m, 1H), 3.11 (s, 3H), 3.27 (s, 3H), 3.27 (dd, 1H, *J* = 4.0, 10.4 Hz), 3.57–3.66 (m, 2H), 3.98–4.10 (m, 5H), 4.23–4.28 (m, 2H), 4.39 (dd, 1H, *J* = 10.0, 10.0 Hz), 4.49 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.53 (benzylic d, 1H, J_{gem} = 11.6 Hz), 5.55 (s, 1H), 4.70 (dd, 1H, *J* = 1.2, 2.8 Hz), 5.02 (dd, 1H, *J* = 10.0, 10.0 Hz), 5.45 (dd, 1H, *J* = 7.6, 7.6 Hz), 5.50 (dd, 1H, *J* = 9.2, 10.8 Hz), 5.64 (d, 1H, *J* = 3.6 Hz), 7.25–7.38 (m, 5H); HRMS: Calcd for $C_{36}H_{50}ClN_3O_{17}S$: $[M+Na]^+$ 886.2442; Found 886.2384.

4.5. (2′*S*,3′*S*)-3,4,6-tri-*O*-Acetyl-2-azido-2-deoxy-α-D-glucopyranosyl-(1→2)-7-*O*-benzyl-6-*O*-chloroacetyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-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 9 and (2′*S*,3′*S*) 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranosyl-(1→2)-7-*O*-benzyl-6-*O*-chloroacetyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-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

(2′*S*,3′*S*) 7-*O*-Benzyl-6-*O*-chloroacetyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-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[5] **7** (61 mg, 0.044 mmol) and ethyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside[12] **8** (65 mg, 0.173 mmol) were dissolved in dry CH_2Cl_2 (5 mL) and 4 A MS was added. After stirring for 30 min under an Ar-atmosphere, the mixture was cooled to 10 °C, followed by addition of NIS (41 mg, 0.180 mmol) and AgOTf (cat.). After 30 min, Et_3N (150 μL) was added and the mixture was diluted with CH_2Cl_2 (10 mL), filtered through Celite, washed with $Na_2S_2O_3$ (10% aq, 15 mL) and subjected to normal workup. FC (pentane/EtOAc 2:1 → 3:2 → 1:1) eluted the α-anomer **9** (42 mg, 0.025 mmol, 57%) followed by the β-anomer **10** (28 mg, 0.017 mmol, 38%). For **9** (α-anomer): R_f 0.46 (toluene/EtOAc 2:1); $[\alpha]_D$ = +58 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100.0 MHz, $CDCl_3$): δ 17.8, 17.9, 20.6, 20.8 (2C), 21.0, 41.3, 47.7, 48.1, 60.5, 61.4, 62.1, 62.4, 65.1, 67.2, 68.1, 68.2, 69.3, 69.6, 69.7, 71.7 (2C), 72.1, 72.6, 72.8, 72.9, 73.3, 73.7, 74.8, 74.9, 75.3, 96.9 (J_{CH} = 171 Hz), 98.3 (J_{CH} = 178 Hz), 99.9, 100.0 (J_{CH} = 168 Hz), 100.2, 100.5 (J_{CH} = 173 Hz), 127.5–129.9 (Ar-C), 133.5, 133.6, 137.8, 138.0, 165.1, 165.2, 165.9, 166.1, 167.3, 169.8, 170.1, 170.6, 170.8; ^1H NMR (400 MHz, $CDCl_3$): δ 1.26 (s, 6H), 1.98 (s, 3H), 2.04 (s, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 3.09 (s, 3H), 3.16 (s, 3H), 3.31 (dd, 1H, *J* = 3.6, 10.8 Hz), 3.38–3.42 (m, 2H), 3.50 (dd, 1H, *J* = 8.0, 10.0 Hz), 3.78 (dd, 1H, *J* = 6.4, 11.2 Hz), 3.91 (dd, 1H, *J* = 6.0, 11.2 Hz), 3.94 (m, 1H), 4.08–4.38 (m, 16H), 4.47 (dd, 1H, *J* = 3.6, 12.4 Hz), 4.79 (dd, 1H, *J* = 3.2, 12.0 Hz), 5.07 (dd, 1H, *J* = 9.6, 9.6 Hz), 5.12 (s, 1H), 5.15 (d, 1H, *J* = 8.0 Hz), 5.22 (s, 1H), 5.35 (dd, 1H, *J* = 6.4, 6.8 Hz), 5.45 (d, 1H, *J* = 3.6 Hz), 5.50 (dd, 1H, *J* = 9.6, 9.6 Hz), 5.58 (dd, 1H, *J* = 9.6, 10. Hz), 5.82 (dd, 1H, *J* = 9.6, 9.6 Hz), 5.96 (dd, 1H, *J* = 9.6, 9.6 Hz), 7.17–7.57 (m, 22 H), 7.84 (d, 2H, *J* = 8.0 Hz), 7.89 (d, 2H, *J* = 8.0 Hz), 7.94 (d, 2H, *J* = 8.0 Hz), 8.03 (d, 2H, *J* = 8.0 Hz); HRMS: Calcd for $C_{84}H_{90}ClN_3O_{32}$: $[M+Na]^+$ 1710.5094; Found 1710.5138. For **10** (β-anomer): R_f 0.64 (toluene/EtOAc 1:1); $[\alpha]_D$ = −25 (*c* 1.0, $CHCl_3$); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 17.9, 18.0, 20.7, 20.8 (2C), 20.8, 41.0, 47.7, 48.0, 61.3, 62.2, 62.9, 63.9, 65.0, 68.0 (2C), 68.5, 69.4, 70.1, 70.1, 71.5, 71.6, 72.1, 72.4, 72.9, 72.8 (2C), 73.1, 73.3, 73.5, 75.4, 75.6, 77.0, 96.9 (J_{CH} = 168 Hz), 99.9, 100.1, 100.3 (J_{CH} = 178 Hz), 100.5 (J_{CH} = 161 Hz), 101.2 (J_{CH} = 166 Hz), 127.4–129.9 (Ar-C), 133.3, 133.4 (2C), 133.6, 137.8, 138.0, 165.2, 165.2, 165.9, 166.2, 167.0, 169.6, 170.2, 170.7, 170.8; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.23 (s, 3H), 1.25 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.08 (s, 3H), 2.09 (s, 3H), 3.07 (s, 3H), 3.20 (s, 3H), 3.37–3.60 (m, 4H), 3.76 (dd, 1H, *J* = 6.6, 11.1 Hz), 3.89–4.38 (m, 19H), 4.44 (dd, 1H, *J* = 4.2, 12.0 Hz), 4.69 (dd, 1H, *J* = 2.7, 12.0 Hz), 4.78 (d, 1H, *J* = 8.1 Hz), 4.92–5.10 (m, 3H), 5.18 (s, 1H), 5.27 (s, 1H), 5.36 (m, 1H), 5.56 (dd, 1H, *J* = 7.5, 9.3 Hz), 5.72 (dd, 1H, *J* = 9.9, 9.9 Hz), 5.90 (dd, 1H, *J* = 9.9, 9.9 Hz), 7.19–7.55 (m, 22H), 7.82 (dd, 2H, *J* = 1.5, 8.4 Hz), 7.88 (d, 2H, *J* = 1.5, 8.4 Hz), 7.93 (d, 2H, *J* = 1.2, 8.4 Hz), 8.03 (d, 2H, *J* = 1.5, 8.4 Hz); HRMS: Calcd for $C_{84}H_{90}ClN_3O_{32}$: $[M+Na]^+$ 1710.5094; Found 1710.5161.

4.6. 3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl-(1→2)-7-*O*-benzyl-6-*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 11

Compound **9** (91 mg, 0.054 mmol) was dissolved in 90% TFA (aq, 4 mL). After stirring for 20 min, the mixture was evaporated followed by co-evaporation with toluene (2 × 10 mL). FC (toluene/EtOAc 2:1 → 1:1) gave **11** (73 mg, 0.046 mmol, 86%); R_f 0.33 (toluene/EtOAc 1:1); $[\alpha]_D$ = +12 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6, 20.8, 21.0, 21.0, 41.1, 61.8, 62.1, 62.6, 65.1, 67.6, 68.0, 68.5, 68.8, 69.4, 70.8, 71.0, 71.1, 71.5, 72.1, 72.6, 72.7, 72.8, 73.1, 73.5, 73.8, 75.0, 75.3, 79.9, 96.5, 99.2, 99.6, 100.2, 127.9–130.0 (Ar-C), 133.5, 133.6, 133.7, 137.5, 138.0, 165.2, 165.3, 166.1, 168.4, 169.8, 170.1, 170.7, 170.8; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.97 (s, 3H), 2.04 (s, 3H), 2.08 (s, 3H), 2.08 (s, 3H), 3.36 (d, 1H, *J* = 4.4 Hz), 3.37 (dd, 1H, *J* = 4.8, 10.4 Hz), 3.54 (dd, 1H, *J* = 3.6, 10.4 Hz), 3.55–3.61 (m, 2H), 3.84–3.88 (m, 2H), 3.92–3.96 (m, 2H), 4.00–4.04 (m, 2H), 4.10–4.34 (m, 13 H), 4.46 (dd, 1H, *J* = 3.6, 12.0 Hz), 4.72 (dd, 1H, *J* = 3.6, 12.0 Hz), 5.04 (dd, 1H, *J* = 9.6 Hz), 5.13–5.15 (m, 3H), 5.21–5.24 (m, 2H), 5.38 (dd, 1H, *J* = 9.6, 9.6 Hz), 5.57 (dd, 1H, *J* = 4.0, 10.0 Hz), 5.77 (dd, 1H, *J* = 10.0, 10.0 Hz), 5.95 (dd, 1H, *J* = 10.0, 10.0 Hz), 7.15–7.57 (m, 22H), 7.84 (dd, 2H, *J* = 1.2, 8.4 Hz), 7.89 (dd, 2H, *J* = 0.8, 8.0 Hz), 7.95 (dd, 2H, *J* = 1.2, 8.4 Hz), 8.03 (dd, 2H, *J* = 0.8, 8.0 Hz); HRMS: Calcd for $C_{78}H_{80}ClN_3O_{30}$: $[M+Na]^+$ 1596.4413; Found 1596.4430.

4.7. 3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl-(1→2)-3,4,7-tri-*O*-acetyl-6-*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*-heptopyranose 12

Compound **11** (59 mg, 0.037 mmol) was dissolved in Ac_2O (2 mL) and $Sc(OTf)_3$ (1.0 mg, 0.002 mmol) was added. After 2 h, the mixture was cooled to 0 °C and MeOH (3 mL) was added. The solvent was evaporated, and the residue was co-evaporated with toluene (2 × 5 mL). FC (toluene/EtOAc 3:1 → 2:1) gave **12** (61 mg, 0.35 mmol, 96%); R_f 0.57 (toluene/EtOAc 1:1); $[\alpha]_D$ = +40 (*c* 1.0, $CHCl_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.1, 20.2, 20.5, 20.6, 20.7 (4C), 20.8, 40.9, 61.1, 61.2, 61.3, 62.2, 63.4, 65.1, 67.5, 68.6, 68.7, 69.0 (2C), 69.1, 70.2, 70.3, 71.6, 71.9, 72.1, 72.8, 73.1, 73.3, 74.2, 74.5, 78.5, 90.4 (J_{CH} = 175 Hz), 99.5 (J_{CH} = 178 Hz), 100.0 (J_{CH} = 171 Hz), 101.3 (J_{CH} = 165 Hz), 128.3–130.1 (Ar-C), 133.4, 133.4, 133.7, 133.8, 137.1, 164.5, 165.4, 165.6, 165.7, 167.2, 168.6, 169.1, 169.6, 169.7, 169.7, 170.0, 170.3, 170.7, 170.8; ^1H NMR (500 MHz, $CDCl_3$): δ 1.62 (s, 3H), 1.75 (s, 3H), 1.94 (s, 3H), 2.01 (s, 3H), 2.08 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.13 (s, 3H), 2.18 (s, 3H), 3.51 (m, 1H), 3.56–3.59 (m, 2H), 3.67 (dd, 1H, *J* = 3.5, 11.0 Hz), 3.76 (dd, 1H, *J* = 7.0, 11.0 Hz), 3.92–3.98 (m, 2H), 4.05 (dd, 1H, *J* = 6.5, 11.0 Hz), 4.11–4.19 (m, 3H), 4.24 (benzylic d, 1H, J_{gem} = 15.0 Hz), 4.33 (m, 1H), 4.34 (benzylic d, 1H, J_{gem} = 15.0 Hz), 4.43–4.55 (m, 5H), 4.64–4.72 (m, 2H), 4.92–4.97 (m, 2H), 5.25 (dd, 1H, *J* = 10.0, 10.0 Hz), 5.36–5.39 (m, 2H), 5.46–5.56 (m, 5H), 5.69 (s, 1H), 5.86 (dd, 1H, *J* = 10.0, 1.0 Hz), 6.14 (d, 1H, *J* = 1.5 Hz), 7.23–7.52 (m, 17H), 7.80 (d, 2H, *J* = 7.0 Hz), 7.91 (d, 2H, *J* = 7.5 Hz), 7.96 (d, 2H, *J* = 7.5 Hz), 8.05 (d, 2H, *J* = 7.0 Hz). HRMS: Calcd for $C_{81}H_{86}ClN_3O_{36}$: $[M+Na]^+$ 1734.4572; Found 1734.4499.

4.8. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl-(1→2)-3,4,7-tri-*O*-acetyl-6-*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*-heptopyranose 13

Derivative **12** (101 mg, 0.059 mmol) was dissolved in THF (5 mL), and PPh_3 (31 mg, 0.118 mmol) was added. The mixture was refluxed for 6 h before H_2O (1.5 mL) was added. The mixture was refluxed for an additional 90 min and then evaporated. The residue was dissolved in pyridine (2 mL) and cooled to 0 °C followed by dropwise addition of Ac_2O (2 mL). After 12 h, the mixture was concentrated and the residue was co-evaporated with toluene (2 × 4 mL). FC (toluene/EtOAc 2:1 → 1:1 → 1:2) gave **13** (85 mg, 0.49 mmol, 83%); R_f 0.55 (toluene/EtOAc 1:2); $[\alpha]_D$ = +20 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.3, 20.5, 20.7, 20.8 (2C), 20.8 (3C), 21.1, 23.3, 40.7, 52.3, 61.4, 61.8, 61.9, 63.7, 65.4, 67.6, 68.1, 68.9 (2C), 69.2, 70.1, 70.5, 71.4, 71.5, 72.0 (2C), 73.1, 73.2, 73.5, 74.6, 74.9, 77.9, 90.3, 100.2 (2C), 101.7, 128.3–130.1 (Ar-C), 133.2, 133.3, 133.5, 133.7, 136.9, 164.8, 165.2, 165.7, 165.9,

167.3, 168.8, 169.3, 169.8, 169.9, 170.1, 170.2 (2C), 170.8, 171.0, 171.3; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.64 (s, 3H), 1.81 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.10 (s, 3H), 2.23 (s, 3H), 3.48 (m, 2H), 3.55 (d, 1H, J = 9.6 Hz), 3.67 (dd, 1H, J = 6.8, 11.2 Hz), 3.79 (dd, 1H, J = 5.2, 11.2 Hz), 3.94 (dd, 1H, J = 2.0, 8.8 Hz), 4.02 (dd, 1H, J = 6.8, 11.2 Hz), 4.05–4.17 (m, 4H), 4.21–4.36 (m, 5H), 4.39 (dd, 1H, J = 3.6, 12.0 Hz), 4.45 (benzylic d, 1H, J_{gem} = 12.8 Hz), 4.50 (dd, 1H, J = 3.6, 9.6 Hz), 4.56 (dd, 1H, J = 0.8, 12.4 Hz), 4.65 (benzylic d, 1H, J_{gem} = 12.8 Hz), 4.69 (dd, 1H, J = 7.2, 12.4 Hz), 4.92 (d, 1H, J = 8.0 Hz), 5.04 (m, 1H), 5.16 (d, 1H, J = 3.2 Hz), 5.26–5.54 (m, 7H), 5.89 (dd, 1H, J = 9.6, 9.6 Hz), 6.14 (d, 1H, J = 9.6 Hz), 6.16 (d, 1H, J = 2.0 Hz), 7.22–7.56 (m, 17H), 7.81 (d, 2H, J = 7.2 Hz), 7.89 (d, 2H, J = 8.0 Hz), 7.91 (d, 2H, J = 8.0 Hz), 8.05 (d, 2H, J = 7.6 Hz); HRMS: Calcd for $C_{83}H_{90}ClNO_{37}$: $[M+Na]^+$ 1750.4772; Found 1750.4688.

4.9. Ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl-(1→2)-3,4,7-tri-*O*-acetyl-6-*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-1-thio-L-*glycero*-α-D-*manno*-heptopyranoside 14

Compound **13** (83 mg, 0.048 mmol) was dissolved in dry CH_2Cl_2 (4 mL), EtSH (35 μL, 0.48 mmol) and $BF_3{\cdot}OEt_2$ (157 μL, 1.25 mmol) were added. After 30 h (reaction monitored by HRMS) under an Ar-atmosphere, the mixture was diluted with CH_2Cl_2 (5 mL), washed with $NaHCO_3$ (satd, 10 mL) and subjected to normal workup followed by FC (toluene/EtOAc 2:1 → 1:1 → 1:2) to give **14** (57 mg, 0.033 mmol, 68%); R_f 0.28 (toluene/EtOAc 1:1); $[\alpha]_D$ = +28 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.8, 20.4, 20.8, 20.9, 20.9 (2C), 21.0 (2C), 21.2, 23.4, 25.4, 40.9, 52.4, 61.0, 62.1, 63.9, 65.5, 67.8, 68.2, 69.2, 69.3, 69.4, 69.9, 70.2, 70.6, 71.4, 71.5, 72.1, 73.2, 73.2, 75.6, 75.8, 76.8, 77.4, 77.9, 81.7, 100.2 (2C), 101.9, 128.3–130.3 (Ar-C), 133.4, 133.5, 133.6, 133.6, 137.4, 164.7, 165.3, 165.8, 166.0, 167.5, 169.4, 169.9, 170.0, 170.2 (2C), 170.4, 170.8, 171.0, 171.3; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (t, 3H, J = 7.2 Hz), 1.59 (s, 3H), 1.88 (s, 3H), 2.00 (s, 3H), 2.00 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H), 2.24 (s, 3H), 2.35–2.50 (m, 2H), 3.57 (m, 1H), 3.65 (m, 1H), 3.70 (dd, 1H, J = 7.2, 10.8 Hz), 3.85–3.88 (m, 2H), 3.98–4.50 (m, 13H), 4.53 (dd, 1H, J = 4.0, 12.4 Hz), 4.65–4.70 (m, 2H), 4.89 (d, 1H, J = 8.0 Hz), 5.07 (m, 1H), 5.14 (d, 1H, J = 2.8 Hz), 5.27–5.40 (m, 4H), 5.46 (dd, 1H, J = 2.8, 10.4 Hz), 5.50–5.55 (m, 4H), 5.88 (dd, 1H, J = 9.6, 9.6 Hz), 6.09 (d, 1H, J = 9.6 Hz), 7.24–7.51 (m, 17H), 7.81 (dd, 2H, J = 1.6, 8.4 Hz), 7.88–7.92 (m, 4H), 8.08 (dd, 2H, J = 1.2, 8.8 Hz); HRMS: Calcd for $C_{83}H_{92}ClNO_{35}S$: $[M+Na]^+$ 1752.4751; Found 1752.4808.

4.10. 2-(*N*-Benzyloxycarbonyl)aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl-(1→2)-3,4,7-tri-*O*-acetyl-6-*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

Derivative **14** (33 mg, 0.019 mmol) and *N*-Cbz-2-aminoethanol (11 mg, 0.057 mmol) were dissolved in dry CH_2Cl_2/Et_2O (2:1, 3 mL) followed by addition of 4 A MS. After stirring for 30 min under an Ar-atmosphere, the mixture was cooled to 0 °C followed by addition of NIS (9 mg, 0.038 mmol) and AgOTf (cat.). After stirring for 45 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) and subjected to normal workup. FC (toluene/EtOAc 2:1 → 1:1 → 1:2) gave **15** (30 mg, 0.016 mmol, 85%); R_f 0.50 (toluene/EtOAc 1:3); $[\alpha]_D$ = +18 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 20.5, 20.9 (3C), 21.0 (2C), 21.2, 23.4, 40.8 (2C), 52.4, 60.8, 62.1, 63.8, 65.6, 67.1, 67.2, 67.7, 68.3, 69.1 (2C), 69.3, 69.8, 70.2, 70.6, 71.5, 71.8, 72.1, 73.2, 73.3, 74.7, 75.0, 75.4, 77.5, 78.1, 97.6 (J_{CH} = 170 Hz), 99.9 (J_{CH} = 178 Hz), 100.2 (J_{CH} = 174 Hz), 101.8 (J_{CH} = 165 Hz), 128.2–130.2 (Ar-C), 133.4, 133.5, 133.6, 133.7, 136.7, 137.5, 156.8, 164.7, 165.3, 165.8, 166.0, 167.4, 169.4, 169.9, 169.9 (2C), 170.3 (2C), 170.8, 171.1, 171.4; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.56 (s, 3H), 1.71 (s, 3H), 2.00 (s, 3H), 2.05 (s, 6H), 2.05 (s, 3H), 2.06 (s, 3H), 2.10 (s, 3H), 2.24 (s, 3H), 3.09 (m, 1H), 3.35–3.39 (m, 2H), 3.49–3.51 (m, 3H), 3.60 (m, 1H), 3.73 (m, 1H), 3.93–3.99 (m, 2H), 4.03–4.14 (m, 5H), 4. 19 (benzylic d, 1H, J_{gem} = 15.0 Hz), 4.26–4.42 (m, 6H), 4.47–4.56 (m, 2H), 4.62 (benzylic d, 1H, J_{gem} = 15.0 Hz), 4.71 (m, 1H), 4.85–4.88 (m, 2H), 5.02 (d, 1H, J = 3.5 Hz), 5.06–5.11 (m, 2H), 5.17 (d, 1H, J = 3.5 Hz), 5.29–5.31 (m, 2H), 5.35 (dd, 1H, J = 10.5, 10.5 Hz), 5.42–5.47 (m, 3H), 5.51 (dd, 1H, J = 8.0, 9.5 Hz), 5.87 (dd, 1H, J = 9.5, 9.5 Hz), 6.18 (d, 1H, J = 9.5 Hz), 7.24–7.50 (m, 22H), 7.80 (dd, 2H, J = 1.5, 8.0 Hz), 7.89–7.92 (m, 4H), 8.05 (dd, 2H, J = 1.5, 8.5 Hz); HRMS: Calcd for $C_{91}H_{99}ClN_2O_{38}$: $[M+Na]^+$ 1885.5457; Found 1885.5474.

4.11. 2-(*N*-Benzyloxycarbonyl)aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl-(1→2)-3,4,7-tri-*O*-acetyl-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-(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

Compound **15** (14 mg, 7.5 μmol) was dissolved in CH_2Cl_2 (1 mL), the solution was cooled to −10 °C and MeOH saturated with NH_3 (2 mL) was added dropwise. After stirring for 30 min (−10 °C → 0 °C), the solvents were evaporated followed by FC (toluene/EtOAc 1:3 → EtOAc) of the residue to give **16** (10 mg, 5.5 μmol, 75%); R_f 0.44 (EtOAc); $[\alpha]_D$ = +17 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 20.9, 20.9, 21.0 (2C), 21.0 (2C), 21.2, 23.4, 40.7, 52.5, 60.9, 62.4, 63.8, 65.2, 66.8, 67.0 (2C), 67.3, 67.8, 68.6, 69.8, 70.4, 70.5, 70.6 (2C), 71.3, 72.1 (2C), 73.3 (2C), 75.1 (2C), 76.6, 77.5, 98.0, 99.7 (2C), 101.9, 128.0–130.2 (Ar-C), 133.4, 133.4, 133.6, 133.7, 136.8, 137.9, 156.8, 164.8, 165.3, 165.8, 166.1, 169.7, 169.9, 170.1, 170.5, 170.6, 170.8, 171.3, 171.4, 171.5; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.57 (s, 3H), 1.99 (s, 3H), 2.03 (s, 6H), 2.04 (s, 6H), 2.08 (s, 3H), 2.12 (s, 3H), 2.22 (s, 3H), 3.10 (m, 1H), 3.35 (m, 3H), 3.49–3.65 (m, 6H), 3.75–3.83 (m, 2H), 4.02–4.14 (m, 6H), 4.27–4.52 (m, 6H), 4.63 (benzylic d, 1H, J_{gem} = 13.2 Hz), 4.71 (m, 1H), 4.81 (s, 1H), 4.93 (d, 1H, J = 8.0 Hz), 5.01 (d, 1H, J = 3.2 Hz), 5.23 (d, 1H, J = 3.6 Hz), 5.33 (dd, 1H, J = 10.0, 10.0 Hz), 5.40–5.57 (m, 7H), 5.87 (dd, 1H, J = 9.6, 9.6 Hz), 6.36 (1H, bs), 7.21–7.50 (m, 22H), 7.81 (dd, 2H, J = 1.2, 8.4 Hz), 7.89–7.92 (m, 4H), 8.06 7.81 (dd, 2H, J = 1.2, 8.4 Hz); HRMS: Calcd for $C_{89}H_{98}N_2O_{37}$: $[M+Na]^+$ 1809.5741; Found 1809.5720.

4.12. 2-(*N*-Benzyloxycarbonyl)aminoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl-(1→2)-3,4,7-tri-*O*-acetyl-6-*O*-[benzyl-2-(*tert*-butyloxycarbonylaminoethyl)-phosphono]-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

(2-*tert*-Butyloxycarbonyl aminoethyl)benzyl *N*,*N*-diisopropyl phosphoramidite[5] (11 mg, 28 μmol) and **16** (10 mg, 5.6 μmol) were dissolved in dry CH_2Cl_2 (1 mL), followed by addition of tetrazole (2 mg, 28 μmol). After stirring for 2 h, the solution was cooled to 0 °C and *m*-CPBA (4 mg, 17 μmol) was added. After an additional 15 min (0 °C → rt), the solution was diluted with CH_2Cl_2 (4 mL), washed with $NaHCO_3$ (satd, 5 mL) and subjected to normal workup. FC (toluene/EtOAc 1:4) gave **17** (9 mg, 4.3 μmol, 76%) as an

inseparable diastereomeric mixture; R_f 0.60 (EtOAc); ^{13}C NMR (100 MHz, $CDCl_3$, diastereomeric mixture): δ 20.2–21.1 (several carbons), 23.1, 28.4 (3C), 28.5 (3C), 29.7, 40.5 (2C), 40.7, 40.9, 52.1, 55.2, 57.3, 60.4, 61.7, 63.5 (2C), 66.5 (2C), 67.0, 67.1, 67.4 (2C), 68.1, 68.3, 69.3 (3C), 69.9, 70.3, 71.3, 71.6, 71.8, 73.0 (2C), 73.1, 74.4, 74.6, 75.1, 77.4, 80.0, 97.6, 99.7, 100.0, 101.5, 127.9–130.3, 133.2, 133.4, 133.5, 133.6, 135.9, 136.5, 137.3, 156.6, 164.5, 165.0, 165.6, 165.8, 169.7–171.2 (several carbons); ^{31}P NMR (decoupled, 162 Hz, $CDCl_3$): δ −1.15, −0.62; HRMS: Calcd for $C_{103}H_{118}N_3O_{42}P$: $[M+Na]^+$ 2122.6820; Found 2122.6731.

4.13. 2-Aminoethyl 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→2)-6-*O*-(2-aminoethyl-phosphono)-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-L-*glycero*-α-D-*manno*-heptopyranoside 18

Derivative **17** (11 mg, 4.7 μmol) was dissolved in MeOH/EtOAc (1:1, 2 mL) followed by addition of 0.1 M HCl (104 μL, 10.0 μmol) and Pd/C (10%, cat.). The mixture was stirred under H_2 pressure (110 psi) for 14 h, then filtered through Celite and concentrated. The residue was dissolved in MeOH (1 mL) and NaOMe (50 μl, 1 M solution) was added. After stirring for 7 h, the temperature was increased to 40 °C and the stirring was continued for an additional 45 min. Dowex-H^+ ion-exchange resins were added (excess) and the stirring was continued for additional 15 min at 40 °C. The mixture was then filtered and concentrated. Reversed phase chromatography (H_2O) of the residue and freeze-drying of the product gave **18** (3 mg, 3.2 μmol, 68%); ^{13}C NMR (125 MHz, D_2O): δ 21.9, 39.0, 40.0 (d, J = 6 Hz), 54.3, 60.3, 60.6, 60.9, 62.5 (d, J = 5 Hz), 62.4, 63.3, 67.8, 69.7, 69.8 (2C), 70.2, 70.7, 70.8 (d, J = 5 Hz), 72.2, 73.0, 73.5, 73.6 (d, J = 5 Hz) 74.2, 75.8, 76.5 (2C), 80.0, 99.2, 99.7, 99.8, 102.4, 174.3; ^{1}H NMR (500 MHz, D_2O): δ 2.05 (s, 3H), 3.14 (m, 1H), 3.26–3.30 (m, 5H), 3.35–3.38 (m, 2H), 3.45–3.53 (m, 4H), 3.61–4.01 (m, 15H), 4.07–4.17 (m, 6H), 4.26 (dd, 1H, J = 10.0, 10.0 Hz), 4.54 (d, 1H, J = 8.0 Hz), 4.57 (m, 1H), 4.87 (s, 1H), 5.03 (d, 1H, J = 3.5 Hz), 5.62 (s, 1H); ^{31}P NMR (decoupled, 162 Hz, D_2O): δ −0.98; HRMS: Calcd for $C_{32}H_{60}N_3O_{26}P$: $[M + H]^+$ 934.3286; Found 934.3285.

4.14. 2-Aminoethyl 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→2)-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** (6 mg, 2.8 μmol) was dissolved in MeOH/EtOAc (1:1, 2 mL) followed by addition of 0.1 M HCl (57 μL, 5.7 μmol) and Pd/C (10%, cat.). The mixture was stirred under H_2 pressure (110 psi) for 14 h, filtered through Celite, concentrated and purified by FC (CH_2Cl_2/MeOH 4:1). The resulting amine was dissolved in MeOH (1 mL) and NaOMe (20 μl, 1 M solution) was added. After stirring for 10 h, the solution was cooled to 0 °C and neutralized with Dowex-H^+ ion exchange resins, filtered and concentrated. Reversed phase chromatography ($H_2O \rightarrow H_2O$/MeOH 1:1) of the residue and freeze-drying of the product gave **19** (2 mg, 1.9 μmol, 68%); ^{13}C NMR (125 MHz, D_2O): δ 21.9, 27.7 (3C), 39.4, 40.7 (d, J = 10 Hz), 54.3, 60.2, 60.7, 61.3, 62.5, 64.9, 65.3, 66.2, 67.8, 69.8, 69.8, 69.9, 70.2, 70.7, 70.8, 71.3 (d, J = 5 Hz), 72.0, 73.0, 73.4 (d, J = 5 Hz), 73.5, 74.1, 75.8, 76.5, 79.7, 99.1, 99.6, 99.9, 102.4, 158.2, 174.3. (Note: C_q from—NBoc not detected); ^{1}H NMR (500 MHz, D_2O): δ 1.46 (s, 9H), 2.07 (s, 3H), 3.11 (m, 2H), 3.27–3.39 (m, 4H), 3.46–3.52 (m, 3H), 3.62–4.13 (m, 23 H), 4.26 (dd, 1H, J = 10.0, 10.0 Hz), 4.53 (m, 1H), 4.54 (d, 1H, J = 8.0 Hz), 4.86 (s, 1H), 5.03 (d, 1H, J = 3.0 Hz), 5.64 (s, 1H); ^{31}P NMR (decoupled, 162 Hz, D_2O): δ = -0.57; HRMS: Calcd for $C_{37}H_{68}N_3O_{28}P$: $[M+Na]^+$ 1056.3619; Found 1056.3596.

4.15. 2-Aminoethyl 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→2)-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-L-*glycero*-α-D-*manno*-heptopyranoside 20

Compound **15** (10 mg, 5.4 μmol) was dissolved in MeOH (1 mL) and NaOMe (50 μl, 1 M solution) was added. After stirring for 3 h, the solution was neutralized with Dowex-H^+ ion exchange resins, filtered and evaporated. The resulting compound was dissolved in MeOH (2 mL) and 0.1 M HCl (107 μl, 11 μmol) and Pd/C (10% cat.) were added. The mixture was stirred under H_2 pressure (110 psi) for 18 h, then filtered through Celite and concentrated. Reversed phase chromatography (H_2O)of the residue and freeze-drying of the product gave **20** (4 mg, 4.9 μmol, 92%); ^{13}C NMR (100 MHz, D_2O): δ = 22.0, 39.3, 54.4, 60.7, 61.5, 62.6, 62.8, 63.7, 66.5, 68.0, 69.0, 69.9, 70.1, 70.4, 70.5, 70.9, 71.0, 71.7, 72.4, 73.1, 73.7, 74.6, 76.0, 76.6, 80.1, 99.7, 100.0, 100.2, 102.6, 174.5; ^{1}H NMR (400 MHz, D_2O): δ = 2.07 (s, 3H), 3.25–3.31 (m, 2H), 3.39 (m, 1H), 3.46–3.56 (m, 3H), 3.62–3.85 (m, 10H), 3,89–4.00 (m, 6H), 4.06–4.14 (m, 5H), 4.26 (dd, 1H, J = 9.6, 9.6 Hz), 4.55 (d, 1H, J = 8.0 Hz), 4.88 (s, 1H), 5.04 (d, 1H, J = 3.2 Hz), 5.53 (s, 1H); HRMS: Calcd for $C_{30}H_{54}N_2O_{23}$: $[M+Na]^+$ 833.3010; Found 833.2994.

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References

1. See for example, Canada Communicable Disease Report, Vol. 33. ACS-3 (http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/07vol33/acs-03/index-eng.php) and references cited therein.
2. Finne, J.; Leinonen, M.; Makela, P. H. *Lancet* **1983**, *ii*, 355–357.
3. Jennings, H. J.; Lugowski, C.; Ashton, F. E. *Infect. Immun.* **1984**, *43*, 407–412.
4. Gidney, M. A. J.; Pledstad, J. S.; Lacelle, S.; Coull, P. A.; Wright, J. C.; Makepeace, K.; Brisson, J.-R.; Cox, A. D.; Moxon, E. R.; Richards, J. C. *Infect. Immun.* **2004**, *72*, 559–569.
5. Segerstedt, E.; Mannerstedt, K.; Johansson, M.; Oscarson, S. *J. Carbohydr. Chem.* **2004**, *23*, 443–452.
6. Mannerstedt, K.; Segerstedt, E.; Olsson, J. D. M.; Oscarson, S. *Org. Biomol. Chem.* **2008**, *6*, 1087–1091.
7. Grundler, G.; Schmidt, R. R. *Liebigs Ann. Chem.* **1984**, 1826–1847.
8. Paulsen, H.; Heitmann, A. C. *Liebigs Ann. Chem.* **1989**, 655–663.
9. Tatai, J.; Fugedi, P. *Org. Lett.* **2007**, *9*, 4647–4650.
10. Codee, J. D. C.; Litjens, R. E. J. N.; den Heeten, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1519–1522.
11. Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881.
12. Ekelöf, K.; Oscarson, S. *J. Org. Chem.* **1996**, *61*, 7711–7718.
13. Lönn, H. *J. Carbohydr. Chem.* **1987**, *6*, 301–306.
14. Boysen, B.; Gemma, E.; Lahmann, M.; Oscarson, S. *Chem. Commun.* **2005**, *16*, 3044–3046.
15. Olsson, J. D. M.; Lahmann, M.; Oscarson, S. *J. Org. Chem.* **2008**, *73*, 7181–7188.