Synthesis of a common tetrasaccharide motif of *Haemophilus influenzae* LPS inner core structures†

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Received 13th November 2007, Accepted 23rd January 2008
First published as an Advance Article on the web 14th February 2008
DOI: 10.1039/b717564g

A conserved tetrasaccharide structure, L-*glycero*-α-D-*manno*-heptopyranosyl-(1→2)-(6-*O*-aminoethylphosphono-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-[β-D-glucopyranosyl-(1→4)]-L-*glycero*-α-D-*manno*-heptopyranose, from the LPS inner core of *Haemophilus influenzae* has been synthesised as its ethylamino glycosides to allow later conjugations. Starting from a previously synthesised suitably protected trisaccharide intermediate, the third heptose and subsequently the spacer were introduced using thioglycoside donor chemistry. The phosphoethanolamine was formed employing a Boc-protected phosphoamidite. Final deprotection and conjugation to biotin gave conjugates that will be used to study the specificity of MAbs raised against native LPS structures.

Introduction

The lipopolysaccharide (LPS) produced by the Gram-negative bacteria *Haemophilus influenzae* is extremely heterogeneous, which makes its structural analysis most difficult. However, with the sequencing of the bacterial genome, subsequent use of bacterial mutants and new analytical techniques, many structures, as well as the genetics behind them, have recently been elucidated.[1–3] The results strongly indicates that the bacterium makes a conservative core pentasaccharide (Fig. 1) linked to Lipid A *via* the Kdo moiety. This conserved core is then modified in numerous ways by glycans, various phosphate groups, amino acids and acetates.

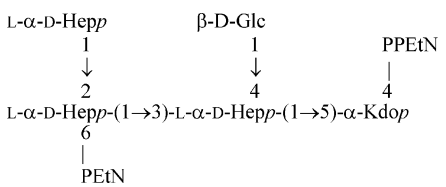

Fig. 1 Structure of the conserved inner core of *H. influenzae* LPS.

Several glycoconjugate vaccines based on capsular polysaccharide structures (CPS) are now commercial and have been most successful, *inter alia*, against *H. influenzae* type b.[4–6] However, *H. influenzae* bacteria are frequently found as non-capsulated (also referred to as non-typeable) *H.influenzae* (NTHi). Due to the heterogeneity of the native LPS it is not possible to use these carbohydrate structures for the construction of a conjugate vaccine. One conceivable solution is to use LPS from bacterial mutants expressing less variation.[3] Still, there are several problems connected with this approach, such as identification of protective motifs, detoxification of the lipid A part and conjugation of the LPS structures to a carrier protein. Another possibility would be to use synthetic oligosaccharide structures. Apart from being well-defined vaccine candidates, these synthetic derivatives can also be of assistance in establishing immunodominant motifs, *inter alia*, by aiding in the determination of the specificity of MAbs. As part of a programme directed towards LPS-based vaccines against *H. influenzae*, we are involved in synthesising partial structures of the LPS inner core and evaluating these as vaccine candidates. Obvious primary target structures are motifs from the conserved inner core. Herein, we describe the synthesis of the outer tetrasaccharide part of the conserved inner core including the phosphoethanolamino group on the middle heptose, an inner core feature, which in molecular models appears to be exposed and accessible, and thus, might be immunologically important.[7–9]

Results and discussion

The synthesis starts from a published trisaccharide precursor **1**[10] containing features allowing all the transformation necessary to reach the target structures, namely orthogonal protecting groups in the 2′- and 6′-positions to permit the introduction of a heptose residue and a phosphoethanolamine group, respectively, and a 1,6-anhydro bridge, both protecting the reducing end and making elongation possible after acetolysis and transformation into a thioglycoside donor.

Removal of the *p*-methoxybenzyl group from **1** by DDQ-oxidation afforded the 2′-OH acceptor **2** in 77% yield (Scheme 1). Coupling with the perbenzoylated thioglycoside **3** using NIS/AgOTf as promoter gave a high yield of the exclusively α-linked tetrasaccharide **4**, as proven by $J_{C,H}$-couplings (>170 Hz) of the anomeric carbon.[11] Hydrolysis using TFA (90% aqueous) removed the BDA-acetal without touching the anhydro linkage to produce the 3′,4′-diol **5** (89%), leaving open the possibility for substitution in these positions.[12] Since this time we only were interested in 6′-phosphorylation, a scandium triflate-mediated acetolysis was performed to accomplish cleavage of the anhydro linkage and (concurrently) acetylation of all free hydroxyl groups.

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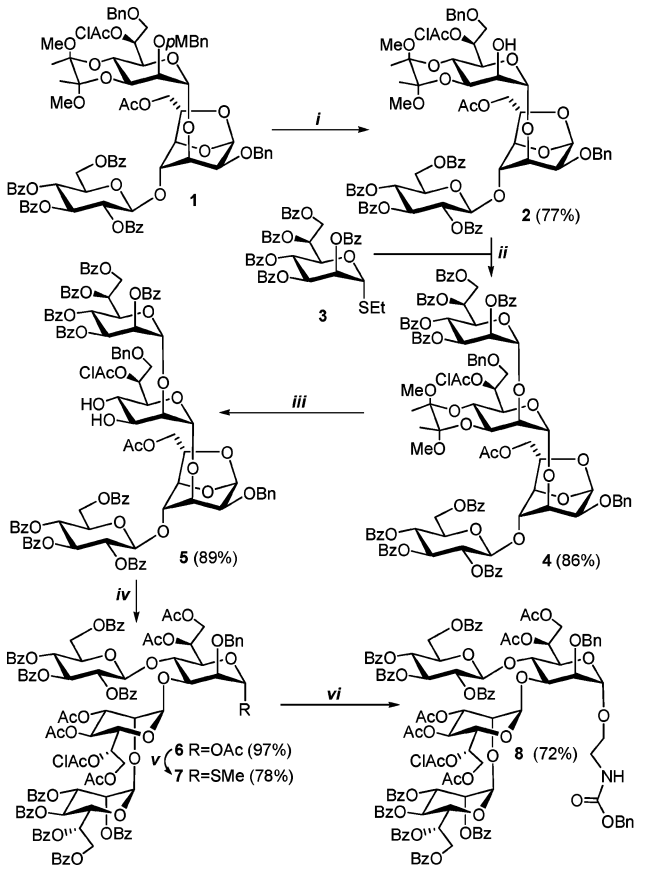
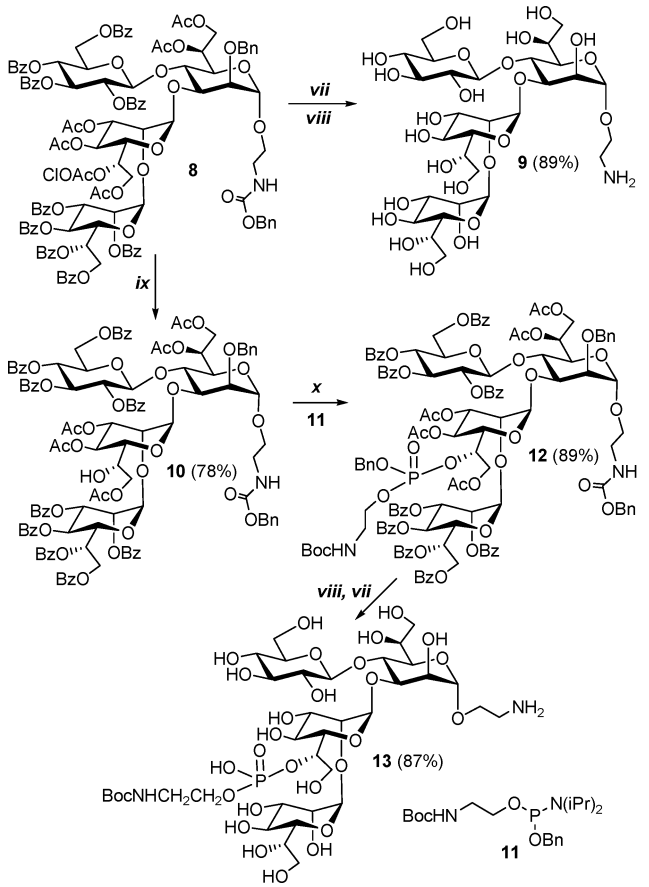
† Electronic supplementary information (ESI) available: Copies of ^{1}H NMR spectra of compounds **9** and **13**. See DOI: 10.1039/b717564g



Scheme 1 Synthesis of the protected tetrasaccahride spacer glycoside. *Reagents and conditions*: (i) DDQ; (ii) NIS, AgOTf; (iii) 90% TFA; (iv) $Sc(OTf)_3$, Ac_2O; (v) TMSSMe, TMSOTf; (vi) *N*-(benzyloxycarbonyl)-aminoethanol, NIS, TfOH.



Scheme 2 Synthesis of target structures **9** and **13**. *Reagents and conditions*: (vii) NaOMe; (viii) Pd/C, HCl; (ix) hydrazine dithiocarbonate; (x) a. **11**, tetrazole. b. mCPBA.

Concomitant acetolysis of the primary benzyl group was also observed to different extent. To get a single product the acetolysis was allowed to go to completion to produce derivative **6** in almost quantitative yield

Efforts to convert this compound into the corresponding ethyl thioglycoside were difficult to monitor both by TLC (no difference in R_f value) and also by MALDI-TOF MS (almost the same MW). Hence, the methyl thioglycoside, the formation of which could easily be followed by MALDI-TOF MS, was synthesised by treatment with TMSSMe/TMSOTf, affording **7** (78%). As proven before with a similar trisaccharide donor, coupling with a spacer acceptor yielded exclusively the α-linked spacer glycoside **8** (72%), although a non-participating benzyl group was present in the 2-position.[13] These results are different from the ones obtained with monosaccharide donors, where often a substantial amount of β-linked product is produced.[14–16]

Compound **8** was deprotected in two effective steps, Zemplén deacylation followed by catalytic hydrogenolysis, to give the non-phosphorylated target structure **9** in high yield (Scheme 2). Selective removal of the chloroacetyl group from **8** was first tried with a reagent, DABCO, reported in the literature to prevent acetyl migration.[17] However, in this case, this reagent caused substantial acetyl migration and removal, whereas the reagent we normally use, hydrazine dithiocarbonate,[18] removed the chloroacetyl group smoothly without any indication of acetyl migration to yield the 6′-OH derivative **10** (78%). When introducing the phosphoethanolamine substituent, a Boc-protection of the amino group was chosen, so as to be able to differentiate this from the spacer amino group in later conjugations. Preliminary attempts using H-phosphonate chemistry were not successful, and so a phosphoamidite, **11**, was synthesised and tested. This time the phosphotriester **12** was produced in 89% yield as a diastereomeric mixture. Again, deprotection in two steps was effective to afford the Boc-protected aminoethanolphosphorylated target structure **13** (87%).

Biotin conjugates were of interest for ELISA-screening of MAbs produced against NTHi bacteria with a known specificity for inner core structures. Treatment of compounds **9** and **13** with the commercial NHS-ester of biotin, afforded derivatives **14** and **15**, respectively, in almost quantitative yield according to MALDI-TOF MS (Scheme 3). Mild TFA-hydrolysis of the Boc-group in **15** then yielded the last target structure **16**.

In conclusion, an effective synthesis of *Haemophilus* inner core structures from the versatile precursor **1** has been performed. In four steps and 45% overall yield a tetrasaccharide thioglycoside intermediate **7** was prepared. This was then successfully converted to the two target structures **9** and **13**, in 30 and 20% overall yield (from **1**) respectively. The target structures are designed

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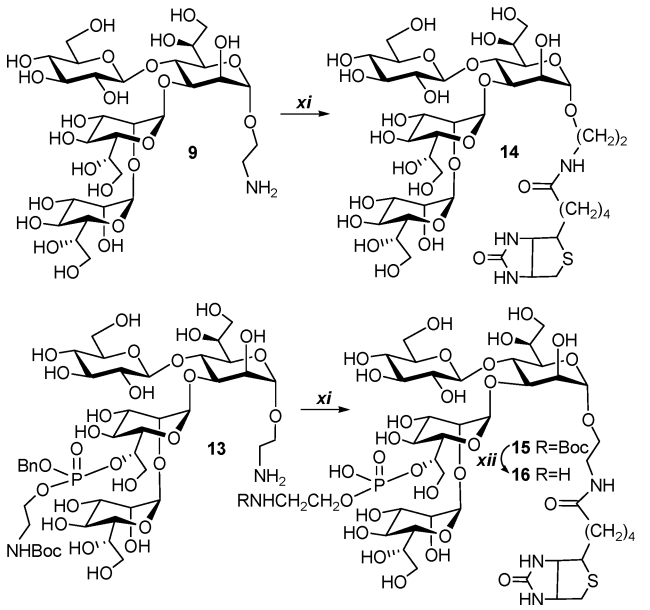

Scheme 3 Synthesis of biotin conjugates. *Reagents and conditions*: (xi) (+)-biotin *N*-hydroxysuccinimide ester, Et_3N, 0.1 M phosphate buffer pH 7.0; xii: $TFA_{(aq)}$.

to allow selective activation and conjugation through the spacer amino group. Biotin conjugates have been constructed and protein conjugates are under way. The former will be used to investigate the epitope specificity of MAbs, whereas the latter will be used in immunological experiments and evaluated as vaccine candidates.

Experimental

General methods

Organic solvents were dried over $MgSO_4$ before concentration, which was performed under reduced pressure at <40 °C (bath temperature). TLC was carried out on Merck precoated 60 F_{254} plates with detection by UV-light and/or 8% sulfuric acid or ammonium molybdate (100 g) : Ce(IV) sulfate (2 g) : sulfuric acid (10%, 2 L). Column chromatography was performed on silica gel (35–70 µm, Millipore), and reverse-phase chromatography was performed on silica gel (C18 60A 40–60 µm). NMR spectra were recorded in $CDCl_3$ at 25 °C on a Varian 300 MHz or 400 MHz instrument, unless otherwise stated. MALDI-TOF spectra were recorded on a Bruker Biflex III instrument using 2′,4′,6′-trihydroxyacetophenone trihydrate (THAP) as matrix.

(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*-β-D-*manno*-heptopyranose (2). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 45 mg, 0.197 mmol) and H_2O (0.5 mL) were added to a solution of **1**[10] (197 mg, 0.131 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 4 h, then diluted with CH_2Cl_2, washed with $Na_2S_2O_3$ (5% aq), dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (toluene–EtOAc 1 : 1) to give **2** (138 mg, 0.100 mmol, 76%): $[\alpha]_D$ +33 (*c* 1.0, $CHCl_3$); ^{13}C NMR δ 17.9 (CH_3 BDA), 20.8 (CH_3CO), 41.0 ($ClCH_2CO$), 47.9, 48.2 (CH_3O BDA), 62.1, 62.8, 64.9, 68.1, 68.2, 69.1, 69.4, 70.0, 70.1, 71.5, 71.8, 72.1, 72.7, 72.8, 72.8, 73.4, 75.6, 75.7 (C-2-7, C-2′-7′, C-2″-6″, PhCH_2O), 98.1, 100.0, 100.3, 100.3, 100.4 (C-1, C-1′, C-1″, *C* BDA), 127.4-137.9 (aromatic C), 165.1, 165.2, 165.9, 166.2, 167.0, 170.6 (CH_3CO, Ph*C*O, $ClCH_2CO$).

(2′*S*,3′*S*)-(2,3,4,6,7-Penta-*O*-benzoyl-L-*glycero*-α-D-*manno*-heptopyransyl)-(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*-β-D-*manno*-heptopyranose (4). A solution of **2** (43 mg, 31.3 µmol) and **3**[13] (48 mg, 62.5 µmol) in CH_2Cl_2 containing powdered molecular sieves (4 Å) was stirred at room temperature under an argon atmosphere for 1 h. The mixture was cooled to −30 °C, NIS (14 mg, 62.5 µmol) and AgOTf (cat.) were added, and the reaction was slowly brought to 5 °C. After neutralisation with Et_3N, the mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with $Na_2S_2O_3$ (10% aq) and water, dried and concentrated. Purification by silica gel chromatography (toluene–EtOAc 10:1) gave **4** (56 mg, 26.8 µmol, 86%): $[\alpha]_D$ −16 (*c* 1.0, $CHCl_3$); ^{13}C NMR δ 17.7, 17.8 (CH_3 BDA), 20.8 (CH_3CO), 41.5 ($ClCH_2CO$), 47.8, 48.2 (CH_3O BDA), 61.7, 62.3, 65.2, 65.5, 68.7, 68.8, 69.4, 69.8, 70.2, 70.3, 70.7, 71.5, 71.6, 72.2, 72.8, 73.4, 73.7, 74.1, 74.9, 76.3 (C-2-6, C-2′-6′, C-2″-6″, PhCH_2O), 97.1 ($J_{C,H}$ 171 Hz), 98.8 ($J_{C,H}$ 176 Hz), 99.0 ($J_{C,H}$ 163 Hz), 99.9, 100.2, 100.4 ($J_{C,H}$ 176 Hz) (C-1, C-1′, C-1″, *C* BDA), 127.5–138.2 (aromatic C), 164.7, 165.0, 165.2, 165.5, 165.5, 165.7, 165.8, 166.1, 166.9, 167.6, 170.8 (CH_3CO, Ph*C*O, $ClCH_2CO$). HRMS calcd for $C_{114}H_{107}ClO_{36}$ [M + Na]$^+$ 2091.6129, found 2091.6204.

(2,3,4,6,7-Penta-*O*-benzoyl-L-*glycero*-α-D-*manno*-heptopyransyl)-(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*-β-D-*manno*-heptopyranose (5). Compound **4** (148 mg, 70.9 µmol) was dissolved in 90% TFA (aq, 5 mL) and the reaction mixture was stirred at room temperature for 1 h, concentrated and co-concentrated with toluene. Purification by silica gel chromatography (toluene–EtOAc 1 : 1) gave **5** (124 mg, 62.8 µmol, 89%): $[\alpha]_D$ −45° (*c* 1.0, $CHCl_3$); ^{13}C NMR δ 20.9 (CH_3CO), 41.1 ($ClCH_2CO$), 60.7, 65.2, 65.4, 65.8, 67.2, 68.6, 68.8, 69.0, 70.2, 70.6, 70.7, 70.9, 71.3, 71.4, 71.8, 72.3, 72.7, 72.7, 72.9, 73.2, 73.5, 74.1, 74.7, 76.7 (C-2-7, C-2′-7′, C-2″-6″, C-2‴-7‴, PhCH_2O), 96.2, 98.2, 99.3, 100.2 (C-1, C-1′, C-1″, C-1‴), 127.8–138.0 (aromatic C), 164.9, 165.1, 165.2, 165.6, 165.7, 165.7, 165.7, 166.1, 166.9, 169.3, 170.9 (CH_3CO, Ph*C*O, $ClCH_2CO$).

(2,3,4,6,7-Penta-*O*-benzoyl-L-*glycero*-α-D-*manno*-heptopyransyl)-(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 (6). $Sc(OTf)_3$ (0.5 mol%) was added to a solution of **5** (51 mg, 25.8 µmol) in Ac_2O and the mixture was stirred at room temperature over night., The reaction was quenched by the addition of MeOH whereafter the mixture was concentrated. Purification by silica gel chromatography (toluene–EtOAc 2 : 1) gave **6** (53 mg, 25.1 µmol, 97%): $[\alpha]_D$ −15 (*c* 1.0, $CHCl_3$); ^{13}C NMR δ 20.4, 20.6, 20.7, 20.9, 21.0 (CH_3CO), 41.1 ($ClCH_2CO$), 61.7, 62.0, 63.8, 64.7, 65.5, 66.0, 66.3, 67.7, 68.9, 69.1, 69.2, 69.5, 69.8, 70.8, 71.2, 71.7, 71.9, 72.0, 73.1, 73.4, 73.8, 74.8, 75.4, 78.7

(C-2-7, C-2′-7′, C-2″-6″, C-2‴-7‴, Ph*C*H$_2$O), 90.4 ($J_{C,H}$ 174 Hz), 99.8 ($J_{C,H}$ 173 Hz, 2C), 101.9 ($J_{C,H}$ 163 Hz) (C-1, C-1′, C-1″, C-1‴), 128.2–137.1 (aromatic C), 164.2, 165.1, 165.2, 165.5, 165.6, 165.7, 165.9, 166.0, 166.3, 167.4, 169.0, 169.5, 169.9, 170.1, 170.5, 170.9 (CH$_3$*C*O, Ph*C*O, ClCH$_2$*C*O). MALDI-TOF MS calcd for $C_{111}H_{103}ClO_{40}$ [M + Na]$^+$ 2134,56, found 2134.52.

Methyl (2,3,4,6,7-penta-*O*-benzoyl-L-*glycero*-α-D-*manno*-heptopyransyl)-(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 (7). TMSSMe (158 μL, 1.11 mmol) and TMSOTf (536 μL, 2.96 mmol) were added to a stirred solution of **6** (277 mg, 0.131 mmol) in CH_2Cl_2, containing molecular sieves (AW-300). The reaction mixture was stirred at room temperature for 72 h (monitored by MALDI-TOF MS), filtered through Celite and concentrated. Purification by silica gel chromatography (toluene–EtOAc 3 : 1) gave **7** (214 mg, 0.102 mmol, 78%): $[\alpha]_D$ −20 (*c* 1.0, $CHCl_3$); ^{13}C NMR δ 13.9, 20.4, 20.7, 20.9, 20.9 (*C*H$_3$CO), 41.3 (Cl*C*H$_2$CO), 61.3, 62.4, 64.1, 64.1, 65.6, 66.1, 67.9, 69.4, 69.7, 69.8, 69.9, 70.0, 70.1, 70.4, 70.9, 71.9, 72.0, 73.3, 73.3, 75.6, 76.0, 78.0 (C-2-7, C-2′-7′, C-2″-6″, C-2‴-7‴, Ph*C*H$_2$O), 83.5, 99.6, 99.6, 102.0 (C-1, C-1′, C-1″, C-1‴), 127.9–137.6 (aromatic C), 164.5, 165.2, 165.2, 165.5, 165.6, 165.9, 166.0, 166.1, 167.7, 169.5, 170.0, 170.4, 170.5, 170.7 (CH$_3$*C*O, Ph*C*O, ClCH$_2$*C*O). HRMS calcd for $C_{110}H_{103}ClO_{38}S$ [M + Na]$^+$ 2121.5435, found 2121.5435.

2-(*N*-Benzyloxycarbonyl)aminoethyl (2,3,4,6,7-penta-*O*-benzoyl-L-*glycero*-α-D-*manno*-heptopyransyl)-(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 (8). A solution of **7** (35 mg, 16.7 μmol) and *N*-(benzyloxycarbonyl)aminoethanol (13 mg, 66.7 μmol) in Et$_2$O containing powered molecular sieves (4 Å) was stirred at room temperature for 1 h. The reaction mixture was cooled to 10 °C, NIS (7.5 mg, 33.4 μmol) and TfOH (cat.) were added, and the reaction mixture was stirred for 3 h. After dilution with Et$_2$O and filtration through Celite, the organic phase was washed with $Na_2S_2O_3$ (10% aq), dried and concentrated. Purification by silica gel chromatography (toluene–EtOAc 2 : 1) gave **8** (26 mg, 12.0 μmol, 72%): $[\alpha]_D$ −22 (*c* 1.0, $CHCl_3$); ^{13}C NMR δ 20.4, 20.7, 21.0, 21.1 (*C*H$_3$CO), 40.8, 41.2 (OCH$_2$*C*H$_2$N, Cl*C*H$_2$CO), 61.5, 62.2, 63.9, 64.8, 65.6, 66.0, 66.5, 67.0, 67.9, 68.3, 68.9, 69.4, 69.6, 69.9, 70.1, 70.8, 71.3, 71.9, 73.2, 73.5, 74.9, 75.9, 78.7 (C-2-7, C-2′-7′, C-2″-6″, C-2‴-7‴, Ph*C*H$_2$O, O*C*H$_2$CH$_2$N), 97.9 ($J_{C,H}$ 176 Hz), 99.5 ($J_{C,H}$ 179 Hz), 99.8 ($J_{C,H}$ 171 Hz), 102.1 ($J_{C,H}$ 164 Hz) (C-1, C-1′, C-1″, C-1‴), 128.0–137.7 (aromatic C), 156.7, 164.3, 165.2, 165.3, 165.5, 165.7, 165.8, 165.9, 166.4, 166.5, 167.4, 169.5, 169.9, 170.5, 170.8 (CH$_3$*C*O, Ph*C*O, ClCH$_2$*C*O). HRMS calcd for $C_{119}H_{112}ClNO_{41}$ [M + 2Na]$^{2+}$ 1145.8097, found 1145.8025.

2-Aminoethyl (L-*glycero*-α-D-*manno*-heptopyransyl)-(1→2)-(L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-[(β-D-glucopyranosyl)-(1→4)]-L-*glycero*-α-D-*manno*-heptopyranoside (9). Compound **8** (22 mg, 9.79 μmol) was dissolved in MeOH and the pH was adjusted to 11 by treatment with 1 M NaOMe (in MeOH). The mixture was stirred for 2 h, neutralised with Dowex 50 (H$^+$) ion exchange resin, filtered and concentrated. The residue was dissolved in EtOH, HCl (0.1 M, 98 μL) palladium on activated carbon powder was added, and the mixture was hydrogenolyzed at 110 psi over night, followed by filtration through Celite and concentration. Purification by Biogel P2-column (1% BuOH in H$_2$O) gave **9** (7 mg, 8.75 μmol, 89%): $[\alpha]_D$ +174 (*c* 0.5, H$_2$O); ^{1}H NMR (D$_2$O, 30 ° C) δ 3.20–3.41 (7H), 3.57–4.04 (22 H), 4.11 (bs, 1H), 4.21 (t, *J* 10 Hz, 1H), 4.56 (d, $J_{1,2}$ 7.5 Hz, 1H), 4.88 (s, 1H), 5.13 (s, 1H), 5.68 (s, 1H). HRMS calcd for $C_{36}H_{59}NO_{24}$ [M + H]$^+$ 800.2958, found 800.2991.

2-(*N*-Benzyloxycarbonyl)aminoethyl (2,3,4,6,7-penta-*O*-benzoyl-L-*glycero*-α-D-*manno*-heptopyransyl)-(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 (10). Hydrazine dithiocarbonate[18] (71 μL, 26.7 μmol) was added to a stirred solution of **8** (20 mg, 8.90 μmol) in DMF. After 1 h, the solution was diluted with CH_2Cl_2, washed with 1M H_2SO_4, $NaHCO_3$ (sat., aq), water, dried and concentrated. Purification by silica gel chromatography (toluene–EtOAc 2 : 1) gave **10** (15 mg, 6.91 μmol, 78%): $[\alpha]_D$ −18 (*c* 1.0, $CHCl_3$); ^{13}C NMR δ 20.4, 21.0, 21.1, 21.2 (*C*H$_3$CO), 40.8 (OCH$_2$*C*H$_2$N), 61.4, 64.0, 64.7, 65.8, 66.5, 66.7, 67.0, 67.5, 67.9, 68.3, 69.3, 69.9, 70.3, 70.5, 70.7, 71.0, 71.9, 72.1, 73.3, 73.5, 74.8, 75.1, 76.0, 78.4 (C-2-7, C-2′-7′, C-2″-6″, C-2‴-7‴, Ph*C*H$_2$O, O*C*H$_2$CH$_2$N), 98.1, 99.1, 99.1, 102.1 (C-1, C-1′, C-1″, C-1‴), 127.9–138.0 (aromatic C), 156.7, 164.3, 165.2, 165.4, 165.5, 165.6, 165.8, 166.0, 166.4, 166.4, 170.0, 170.6, 170.8, 171.1, 171.3 (CH$_3$*C*O, Ph*C*O, ClCH$_2$*C*O).

Benzyloxy-[2-(*tert*-butyloxycarbonylamino)ethoxy]-(*N*,*N*-diisopropylamino)phosphine (11). 2-(*tert*-Butyloxycarbonylamino)-ethanol (0.343 g, 2.13 mmol) in CH_2Cl_2 was added to a stirred solution of benzyloxybis(*N*,*N*-diisopropylamino)phosphine[19] (1.08 g, 3.19 mmol) and tetrazole (75 mg, 1.07 mmol) in CH_2Cl_2. The mixture was stirred at room temperature for 3 h and concentrated. Purification by silica gel chromatography (pentane–EtOAc–Et$_3$N 90 : 10 : 5) gave **11** (0.611 g, 1.53 mmol, 72%): ^{1}H NMR δ 1.19 (dd, 12H), 1.42 (s, 9H), 3.31 (d, 2H), 3.67 (m, 4H), 4.71 (m, 2H), 4.95 (bs, 1H), 7.30 (m, 5H); ^{13}C NMR δ 24.7 (t, *J* 6.9 Hz), 28.5, 41.9 (d, *J* 5.94 Hz), 43.0 (d, *J* 12.2 Hz), 62.8 (d, *J* 16.8 Hz), 65.5 (d, *J* 18.3 Hz), 79.1, 127.1, 127.4, 128.4, 139.3 (d, *J* 7.63 Hz), 156.0; ^{31}P NMR δ 147.9.

2-(*N*-Benzyloxycarbonyl)aminoethyl (2,3,4,6,7-penta-*O*-benzoyl-L-*glycero*-α-D-*manno*-heptopyransyl)-(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 (12). Tetrazole (8 mg, 111 μmol) was added to a solution of **10** (48 mg, 22.1 μmol) and **11** (44 mg, 111 μmol) in dry CH_2Cl_2. The reaction mixture was stirred at room temperature for 4 h, and after cooling to 0 °C, *m*-chloroperbenzoic acid (17 mg, 66.3 μmol) was added. The mixture was stirred for another hour at room temperature and the solution was diluted with CH_2Cl_2, washed with $NaHCO_3$ (sat., aq), dried ($MgSO_4$), filtered and concentrated. Purification by silica gel chromatography (toluene–EtOAc 2 : 1) gave **12** (49 mg, 19.7 μmol, 89%): ^{31}P NMR δ 8.08; MALDI-TOF MS calcd for $C_{131}H_{131}N_2O_{45}P$ [M + Na]$^+$ 2506.78, found 2507.33.

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2-Aminoethyl (L-*glycero*-α-D-*manno*-heptopyransyl)-(1→2)-(6-*O*-(benzyl-2-((*tert*-butyloxycarbonyl)aminoethyl)phosphono)-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-[(β-D-glucopyranosyl)-(1→4)]-L-*glycero*-α-D-*manno*-heptopyranoside (13). Compound **12** (10 mg, 4.03 μmol) was dissolved in THF–EtOH 3 : 1, HCl (0.1 M, 40 μL), palladium on activated carbon powder was added and the mixture was hydrogenolyzed at 110 psi over night, followed by filtration through Celite and concentration. The residue was dissolved in MeOH and the pH was adjusted to 11 by treatment with 1 M NaOMe (in MeOH). The mixture was stirred for 2 h, neutralised with Dowex 50 (H^+) ion exchange resin, filtered and concentrated. Purification by reverse-phase chromatography (H_2O–MeOH 1 : 0 → 1 : 1) gave **13** (3.6 mg, 3.52 μmol, 87%): $[\alpha]_D$ +110 (*c* 0.1, H_2O); ^{1}H NMR (D_2O, 30 °C) δ 1.38 (s, 9H, $(CH_3)_3$), 3.20–3.41 (9H), 3.59–4.05 (23 H), 4.10 (bs, 1H), 4.23 (t, *J* 10 Hz, 1H), 4.42 (dd, 1H, H-6′), 4.56 (d, $J_{1,2}$ 7.5 Hz, 1H, H-1″), 4.88 (s, 1H), 5.12 (s, 1H), 5.77 (s, 1H) (H-1,1′,1‴); ^{31}P (D_2O) δ 0.56. HRMS calcd for $C_{131}H_{131}N_2O_{45}P$ $[M + H]^+$ 1023.3567, found 1023.3627.

2-[(+)-Biotinylamino]ethyl (L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→2)-(L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-[(β-D-glucopyranosyl)-(1→4)]-L-*glycero*-α-D-*manno*-heptopyranoside (14). (+)-Biotin *N*-hydroxysuccinimide ester (0.8 mg, 2.50 μmol) and Et_3N (50 μL) were added to **9** (1 mg, 1.25 μmol) in 0.1 M phosphate buffer pH 7.0 (0.5 mL). After 30 min the reaction was completed (monitored by MALDI-TOF MS) and the residue was purified on a Biogel P2 column eluted with H_2O (1% *n*-BuOH), followed by lyophilisation to give **14**: HRMS calcd for $C_{39}H_{67}N_3O_{26}S$ $[M + Na]^+$ 1048.3626, found 1048.3613.

2-[(+)-Biotinylamino]ethyl (L-*glycero*-α-D-*manno*-heptopyransyl)-(1→2)-(6-*O*-aminoetylphosphono-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-[(β-D-glucopyranosyl)-(1→4)]-L-*glycero*-α-D-*manno*-heptopyranoside (16). Compound **13** (1 mg, 0.777 μmol) was treated with biotin *N*-hydroxysuccinimide (0.7 mg, 1.96 μmol) and Et_3N (50 μL) in 0.1 M phosphate buffer pH 7.0 (0.5 mL) as described for compound **14** to give compound **15**. The Boc-protecting group was subsequently removed by treatment of **15** with TFA (100 μL) in H_2O (500 μL) for 2 h. Purification by size-exclusion chromatography using a Biogel P2 column eluted with H_2O (1% *n*-BuOH) and lyophilisation gave **16**: MALDI-TOF MS calcd for $C_{41}H_{73}N_4O_{29}PS$ $[M + Na]^+$ 1171.28, found 1172.28.

Acknowledgements

We are thankful to the Swedish Research Council for financial support. Dr Petter Tollbäck, Department of Analytical Chemistry, SU, is acknowledged for recording the HRMS spectra.

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