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Synthesis of structures corresponding to the capsular polysaccharide of *Neisseria meningitidis* group A

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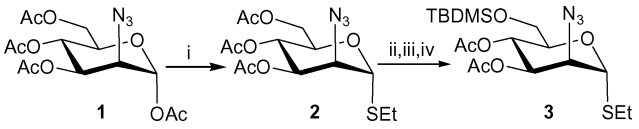
Four differently substituted trimers of the CPS repeating unit have been synthesised in order to investigate the dependence on oligosaccharide size, acetylation and mode of phosphorylation of glycoconjugate vaccines against *Neisseria meningitidis* group A. A spacer-containing starting monomer, a H-phosphonate elongating monomer and a 6-*O*-phosphorylated H-phosphonate cap monomer have been synthesised and coupled together to afford, after deprotection, the target trimer structures differing in their acetylation and phosphorylation substitution pattern.

Introduction

Meningitis is caused both by viruses and bacteria. The two major types of bacteria causing meningitis are *Haemophilus influenzae* and *Neisseria meningitidis*. In the case of *H. influenzae* only one serotype, type b, is important, whereas with *N. meningitidis*, five groups, *i.e.*, A, B, C, W and Y-135, are associated with meningococcal meningitis. The various groups have different geographical prevalence, *e.g.*, group B (MenB) and C (MenC) in Europe and North America and group A (MenA) in Africa and South America.[1,2] The serotyping is based on the structure and antigenicity of the capsular polysaccharide (CPS) surrounding the bacteria, and the purified polysaccharide itself can be used as a vaccine. Especially efficient vaccines (glycoconjugate vaccines) can be made by attaching the saccharide to a carrier protein, which induces a T-cell dependent immune response with memory and is also effective in small children.[3] Such vaccines against *H. influenzae* type b (Hib) have been commercially available for more than ten years and have more or less eradicated disease caused by this bacterium in countries that have implemented mass vaccination. Recently a Hib glycoconjugate vaccine based on synthetic oligosaccharides was licensed.[4] For MenC, three monovalent glycoconjugate vaccines are commercially available,[5] and development of vaccines against the other groups are under way,[6] MenB being a severe problem since its CPS structure is identical to a human carbohydrate structure. Another issue is the poor stability of MenA vaccines due to the inherent instability of the anomeric phosphate diester linkages of the CPS.[7] The repeating unit of MenA is a monosaccharide, 2-acetamido-2-deoxy-α-D-mannopyranose linked 1 → 6 *via* a phosphodiester bridge.[8] In the native polysaccharide the 3-OH is acetylated to an extent of about 90%.[9] The immunological importance of this acetylation has not been completely investigated, but there are indications that it is not of major significance. To investigate the various characteristics of an optimal glycoconjugate vaccine and to develop routes to more stable analogues of the native structure we now report on the synthesis of a number of trimers, **13**, **14**, **21** and **23**, of the repeating unit of the MenA CPS. These will be conjugated to carrier proteins and used in immunological experiments to evaluate the importance of oligosaccharide chain length, acetates, and terminal phosphates on the immune response.

Results and discussion

In order to circumvent the problems associated with the acid lability of the native anomeric phosphodiester linkage during the synthesis, an approach based on 2-azido-2-deoxy-glucose derivatives has been developed and used.[10,11] The properties of the azido group (electron-withdrawing, non-participating) strongly stabilize the anomeric linkage as further proven by Pozsgay and coworkers in their synthesis of trimer **14**.[12] A common precursor for both the starting monomer and the elongating monomer was synthesized by transformation of the known tetraacetate **1** into the corresponding ethyl thioglycoside (→**2**, 68%),[13,14] followed by deacetylation, regioselective silylation and acetylation to give **3** (99% over three steps) (Scheme 1).



Scheme 1 Synthesis of monosaccharide precursor. (i) EtSH, BF_3OEt, CH_2Cl_2; (ii) NaOMe, MeOH; (iii) TBDMSCl, pyridine; (iv) Ac_2O.

NIS-promoted coupling (NIS = *N*-iodosuccinimide) of donor **3** with a spacer, Z-protected ethanolamine (Z = benzyloxycarbonyl), gave the α-linked glycoside **4** in 86% yield (Scheme 2). Interestingly, if the corresponding donor with 3,4-di-*O*-benzyl protection is used in the coupling instead, a high amount of β-linked glycoside is also formed.[12] Removal of the silyl protecting group with TREAT–HF (triethylamine tris(hydrogen fluoride))[15] then afforded the starting acceptor **5** (97%) without any indication of acetyl migration. Compound **3** was also processed to give the elongating monomer by hydrolysis of the thioglycoside (→**6**, 82%) and then phosphitylation using PCl_3–imidazole–Et_3N[16] to give the α-anomeric H-phosphonate **7** (97%). Earlier, with 3-*O*-acetyl-4-*O*-benzyl-protected derivatives, the exclusive formation of the α-H-phosphonate was an issue,[10] problems also encountered in the first synthesis of MenA structures.[17] However, here with 3,4-di-*O*-acetyl-protection, this was not a problem. When the hydrolysis reaction was performed at −20 °C exclusively, the α-anomer was formed. Formation of the first phosphate diester bridge then proceeded smoothly by activation of the H-phosphonate **7** with pivaloyl chloride in the

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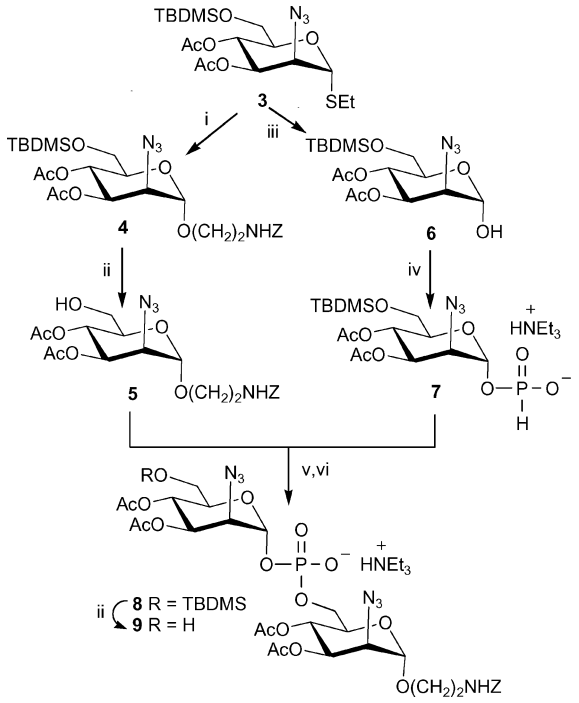


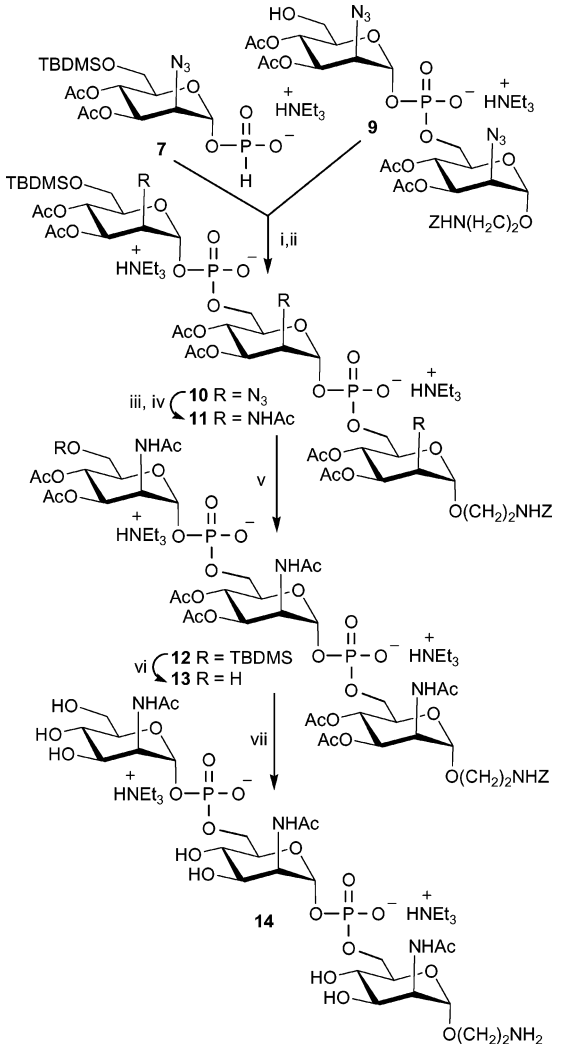
Scheme 2 Synthesis of a repeating unit dimer. (i) $HO(CH_2)_2NHZ$, NIS–AgOTf, CH_2Cl_2; (ii) TREAT–HF, THF; (iii) NIS–AgOTf, wet CH_2Cl_2; (iv) imidazole, PCl_3, Et_3N, MeCN; (v) PivCl, pyridine; (vi) I_2, pyridine–H_2O.

presence of acceptor **5** followed by oxidation using iodine in pyridine–water to yield the dimer **8** in 96% yield (Scheme 2).[16]

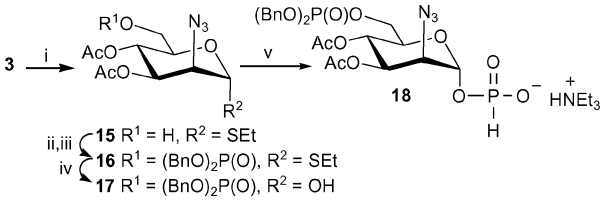
This procedure could then be iterated to give oligomers. Even with these stabilized anomeric phosphodiester linkages, there is still a large difference in lability as compared to non-anomeric phosphates. Thus, the yields decrease as the number of phosphates present in the molecules to be coupled increases,[11,12,18] and cannot compete with the ones obtained in nucleotide chemistry. Even so, an acceptable yield was obtained in the next coupling. Removal of the silyl group from compound **8** gave derivative **9** (91%). Compound **9** can be processed to yield an analogue of the dimer present in the native MenA CPS, but is also a new acceptor and was elongated once more with H-phosphonate **7** to afford the trimer **10** (62%, Scheme 3). The stabilizing azido functions were now transformed into the native acetamido group through reduction followed by acetylation (→**11**, 89%). Catalytical hydrogenolysis of the Z-group (→**12**, 83%) and subsequent removal of the silyl group (TREAT–HF) then gave the first target structure **13** (85%). Treatment of **13** with sodium methoxide afforded the deacetylated target structure **14** (94%), having NMR-data identical to those published by Pozsgay and co-workers.[12]

In the first attempt at synthesis of the target structures with terminal phosphates at the non-reducing end, derivative **10** was desilylated and tried as an acceptor. However, the yield after phosphorylation using dibenzyl *N*,*N*-diisopropyl phosphoramidite[19] was quite low and an alternative pathway involving an already 6-*O*-phosphorylated "cap" H-phosphonate donor was investigated. Precursor **3** was thus desilylated (→**15**) and the obtained 6-OH derivative phosphorylated to yield compound **16** (Scheme 4), which was then transformed as discussed for derivative **3** into the H-phosphonate **18** (37% overall yield from **3**). Once more only the α-anomer was obtained.

Elongation of acceptor **9** with **18** then proceeded in good yield (59%) to give compound **19** (Scheme 5), which could be converted into target structures as discussed above. Reduction of the azido groups followed by acetylation yielded derivative **20** (64%), which was either directly hydrogenolysed to give the acetylated target structure **21** (85%), or first deacetylated



Scheme 3 Synthesis of target trimers **13** and **14**. (i) PivCl, pyridine; (ii) I_2, pyridine–H_2O; (iii) $NaBH_4$, $NiCl_2$, MeOH; (iv) Ac_2O; (v) H_2, Pd/C, MeOH; (vi) TREAT–HF, THF; (vii) NaOMe, MeOH.



Scheme 4 Synthesis of phosphorylated elongating monomer. (i) TREAT–HF, THF; (ii) $(BnO)_2PN(CH(CH_3)_2)_2$, tetrazole, CH_2Cl_2; (iii) *m*CPBA; (iv) NIS–AgOTf, wet CH_2Cl_2; (v) imidazole, PCl_3, Et_3N, MeCN.

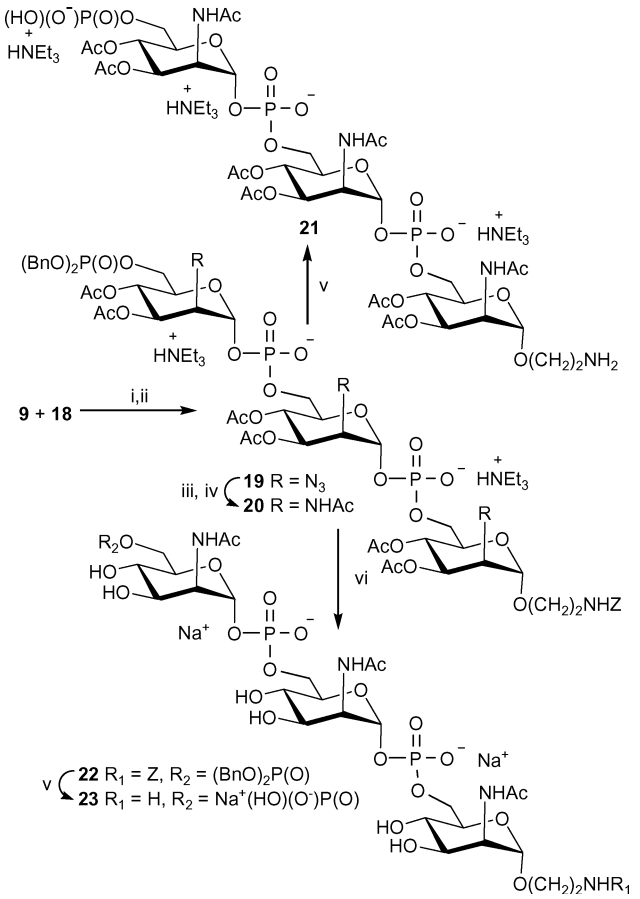
(→**22**, 80%) and then hydrogenolysed to give the deacetylated target structure **23** (64%). Attempts to deacetylate compound **21** resulted in low yields of **23**.

In conclusion, a straightforward synthesis of oligomers of the MenA CPS has been developed. The pathway allows for synthesis of both acetylated and non-acetylated structures and also introduction of a terminal phosphate. Furthermore, inherent in the design is the possibility of synthesising analogues differing in the nature of the 2-amino group.

Experimental

General methods

TLC was carried out on Merck precoated 60 F_{254} plates using AMC (ammonium molybdate–cerium(IV) sulfate–10% sulfuric acid; 100 g : 2 g : 2 L) or 8% H_2SO_4 for visualization. Column



Scheme 5 Synthesis of target trimers **21** and **23**. (i) PivCl, pyridine; (ii) I_2, pyridine–H_2O; (iii) $NaBH_4$, $NiCl_2$, MeOH; (iv) Ac_2O; (v) H_2, Pd/C, MeOH; (vi) NaOMe, MeOH.

chromatography was performed on silica gel (0.040–0.063 mm, Amicon) or reversed phase gel (C18 60A 40–63 μm). NMR spectra were recorded in $CDCl_3$ (Me_4Si, $\delta = 0.00$) or D_2O (acetone $^{13}C\,\delta = 30.89$, $^1H = 2.22$) at 25 °C on a Varian 300 MHz or 400 MHz instrument. For ^{31}P NMR spectra, H_3PO_4 ($\delta = 0.00$) was used as a reference. Organic solutions were concentrated at 30 °C under reduced pressure.

Ethyl 3,4-di-*O*-acetyl-2-azido-6-*O*-(*tert*butyldimethylsilyl)-2-deoxy-1-thio-α-D-mannopyranoside (3). To a solution of **1** (5.04 g, 13.5 mmol) in CH_2Cl_2 (60 mL) was added EtSH (1.6 mL, 21.6 mmol) and MS (4 Å). The mixture was stirred under argon at rt for 30 min. BF_3-etherate (4.8 mL, 38.1 mmol) dissolved in CH_2Cl_2 (10 mL) was then added during 1 h. After another 7 h the mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$, filtered through silica and concentrated. Silica gel chromatography (1 : 0 → 1 : 1 toluene–EtOAc) of the residue gave ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α-D-mannopyranoside[14] (**2**, 3.46 g, 9.22 mmol, 68%); NMR: ^{13}C, δ 14.8 (SCH_2CH_3), 20.6, 20.7, 20.8 (CH_3CO), 25.6 (SCH_2CH_3), 62.3, 62.9, 66.2, 69.0, 71.4 (C-2–6), 82.5 (C-1), 169.6, 170.0, 170.7 (CH_3CO). To a solution of **2** (3.71 g, 9.88 mmol) in MeOH (30 mL), NaOMe (1 M) was added. After 1 h the mixture was neutralized with AcOH and concentrated. The dry residue was dissolved in pyridine (15 mL) and *tert*butyldimethylsilyl chloride (1.94 g, 12.87 mmol) was added. The reaction mixture was stirred at rt overnight. Acetylation with acetic anhydride followed by dilution with toluene, filtration through silica, concentration and purification by silica gel chromatography (1 : 0 → 1 : 1 toluene–EtOAc) gave **3** (4.357 g, 9.73 mmol, 99%); $[\alpha]_D$ +109° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ −5.33, −5.28 (CH_3Si), 14.7 (SCH_2CH_3), 18.4 ($(CH_3)_3CSi$), 20.7, 20.9 (CH_3CO), 25.0 (SCH_2CH_3), 25.9 ($(CH_3)_3CSi$), 62.6, 62.9, 67.0, 71.7, 71.9 (C-2–6), 81.5 (C-1), 169.7, 170.1 (CH_3CO); ^1H, δ 0.05 (s, 3H), 0.05 (s, 3H), 0.90 (s, 9H), 1.30 (t, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.55–2.75 (m, 2H), 3.60–3.75 (m, 2H), 4.10 (m, 1H), 4.15–4.20 (m, 1H), 5.20–5.25 (m, 1H), 5.30–5.35 (m, 1H); HRMS calcd for $C_{18}H_{33}N_3NaO_6SSi$ $[M + Na]^+$ 470.1757, found 470.1750.

2-(Benzyloxycarbonyl)aminoethyl 3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside (5). Compound **3** (527 g, 1.18 mmol) was dissolved in CH_2Cl_2 (10 mL) containing MS (4 Å). Benzyl *N*-(2-hydroxyethyl) carbamate (300 mg, 1.54 mmol) was added and the mixture was stirred under argon at −20 °C for 30 min before NIS (345 mg, 1.53 mmol) and AgOTf (catalytic amount) were added. After 30 min, the reaction mixture was neutralized with Et_3N, diluted with CH_2Cl_2, washed with $Na_2S_2O_3$ (10%), filtered (silica) and concentrated. Chromatography (1 : 0 → 1 : 1 toluene–EtOAc) gave 2-(benzyloxycarbonyl) aminoethyl 3,4-di-*O*-acetyl-2-azido-6-*O*-(*tert*butyldimethylsilyl)-2-deoxy-α-D-mannopyranoside (**4**, 595 mg, 1.02 mmol, 86%); $[\alpha]_D$ +46° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ −5.38, −5.34 (CH_3Si), 18.4 ($(CH_3)_3CSi$), 20.6, 20.8 (CH_3CO), 25.9 ($(CH_3)_3CSi$), 40.7 ($HOCH_2CH_2NH$), 61.6, 62.6, 66.6, 66.9, 67.6, 71.3, 71.8 (C-2–6, $PhCH_2O$, OCH_2CH_2N), 98.0 (C-1), 128.2, 128.3, 128.6, 136.5 (aromatic C), 156.4 ($NHCOOCH_2$), 169.6, 170.1 (CH_3CO); ^1H, δ 0.03 (s, 3H), 0.04 (s, 3H), 0.89 (s, 9H), 2.03 (s, 3H), 2.09 (s, 3H), 2.35 (s, 1H), 3.32–3.40 (m, 1H), 3.44–3.52 (m, 1H), 3.58–3.66 (m, 3H), 3.72–3.80 (m, 2H), 3.98–4.00 (m, 1H), 5.11 (s, 2H), 5.18–5.23 (m, 1H), 5.32–5.36 (m, 1H), 7.16–7.36 (m, 5H). To a solution of compound **4** (575 mg, 0.99 mmol) in THF (5 mL), TREAT–HF (0.81 mL, 4.97 mmol) was added. The mixture was stirred under argon at rt overnight. Concentration and silica gel chromatography (2 : 1 → 0 : 1 toluene–EtOAc) gave **5** (448 mg, 0.96 mmol, 97%); $[\alpha]_D$ +60° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ 20.7, 20.8 (CH_3CO), 40.7 ($HOCH_2CH_2NH$), 61.4, 61.6, 66.4, 67.0, 67.5, 70.9, 71.0 (C-2–6, $PhCH_2O$, OCH_2CH_2N), 98.2 (C-1), 128.3, 128.3, 128.7, 136.5 (aromatic C), 156.5 ($NHCOOBn$), 170.1, 170.5 (CH_3CO); ^1H, δ 2.04 (s, 3H), 2.1 (s, 3H), 3.32–3.46 (m, 2H), 3.54–3.60 (m, 2H), 3.62–3.78 (m, 3H), 4.02–4.04 (m, 1H), 5.06–5.18 (m, 3H), 5.22–5.28 (m, 1H), 5.36–5.40 (m, 1H), 7.28–7.38 (m, 5H); HRMS calcd for $C_{20}H_{26}N_4NaO_9$ $[M + Na]^+$ 489.1597, found 489.1574.

3,4-Di-*O*-acetyl-2-azido-6-*O*-(*tert*butyldimethylsilyl)-2-deoxy-α-D-mannopyranosyl hydrogen-phosphonate, triethylammonium salt (7). NIS (394 mg, 1.75 mmol) and AgOTf (catalytic amount) were added to a solution of **3** (653 mg, 1.46 mmol) in wet CH_2Cl_2 (10 mL). The reaction mixture was stirred at −20 °C for 30 min. CH_2Cl_2 was added and the mixture was washed with $Na_2S_2O_3$ (10%), filtered (silica) and concentrated. The residue was purified by chromatography (1 : 0 → 1 : 1 toluene–EtOAc) to give 3,4-di-*O*-acetyl-2-azido-6-*O*-(*tert*butyldimethylsilyl)-2-deoxy-α-D-mannopyranoside (**6**, 487 mg, 1.21 mmol, 82%); NMR: ^{13}C, δ −5.29, −5.22 (CH_3Si), 18.6 ($(CH_3)_3CSi$), 20.7, 20.9 (CH_3CO), 26.0 ($(CH_3)_3CSi$), 62.1, 63.2, 66.9 71.0, 71.6 (C-2–6), 92.7 (C-1), 169.8, 170.3 (CH_3CO); ^1H, δ 0.05 (s, 3H), 0.06 (s, 3H), 0.89 (s, 9H), 2.03 (s, 3H), 2.08 (s, 3H), 3.65–3.70 (m, 2H), 4.00–4.02 (m, 2H), 5.19–5.23 (m, 2H), 5.42–5.47 (m, 1H). A mixture of imidazole (915 mg, 13.45 mmol), PCl_3 (335 μL, 3.84 mmol) and Et_3N (2.0 mL, 14.35 mmol) in MeCN (25 mL) was stirred at 0 °C for 30 min. A solution of compound **6** (388 mg, 0.96 mmol) in MeCN (25 mL) was added during 30 min at 0 °C. The reaction mixture was then stirred at rt for 10 min, quenched with TEAB (triethylammonium bicarbonate) (0.5 M) and concentrated. The residue was diluted ($CHCl_3$), washed with TEAB (0.5 M), filtered (cotton) and concentrated. Chromatography (1 : 0 → 10 : 1 $CHCl_3$–MeOH + 1.0% Et_3N) of the residue gave **7** (499 mg, 0.93 mmol, 97%); $[\alpha]_D$ +67° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ −5.43, (CH_3Si), 8.98, 18.3 ($(CH_3)_3CSi$), 20.6, 20.8 (CH_3CO), 25.8 ($(CH_3)_3CSi$), 45.7, 62.4, 62.5, 66.5, 71.1, 72.1 (C-2–6), 93.1 (C-1), 169.4, 170.0 (CH_3CO); ^1H, δ −0.06 (s, 3H), −0.05 (s, 3H), 0.79 (s, 9H), 1.94 (s, 3H), 1.99 (s, 3H), 3.59–3.63 (m, 2H), 3.93–4.00 (m, 2H), 5.21–5.28 (m, 1H),

5.37–5.41 (m, 1H), 5.51–5.50 (m, 1H); ^{31}P, δ 0.28; HRMS calcd for $C_{16}H_{29}N_3O_9PSi$ [M + H]$^-$ 466.1416, found 466.1400.

2-(Benzyloxycarbonyl)aminoethyl (3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside) triethylammonium salt (9). A mixture of **5** (217 mg, 0.47 mmol) and **7** (325 mg, 0.60 mmol) was dissolved in pyridine (3 mL). Pivaloyl chloride (144 μL, 1.18 mmol) was added and the mixture was stirred under argon at rt for 1 h. The reaction mixture was cooled to −40 °C and a solution of I_2 (143 mg, 0.56 mmol) in pyridine–H_2O (3 mL 49 : 1) was added. The oxidation was completed at 0 °C and the mixture was diluted with $CHCl_3$, washed with $Na_2S_2O_3$ (10%) and cold TEAB (0.5 M). Filtration (cotton), concentration and chromatography (1 : 0 → 10 : 1 $CHCl_3$–MeOH + 0.5% Et_3N) gave 2-(benzyloxycarbonyl)aminoethyl (3,4-di-*O*-acetyl-2-azido-6-*O*-(*tert*butyldimethylsilyl)-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside) triethylammonium salt (**8**, 469 mg, 0.45 mmol, 96%); $[\alpha]_D$ +52° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ −5.48, −5.38 (*C*H_3Si), 9.27, 18.4 ((CH$_3$)$_3$*C*Si), 20.6, 20.7, 20.8, 20.8 (*C*H_3CO), 25.9 ((*C*H$_3$)$_3$CSi), 40.7 (HOCH$_2$*C*H$_2$NH), 45.8, 61.7, 62.2, 64.5, 66.3, 66.6, 66.7, 67.3, 69.8, 69.9, 71.2, 71.3, 71.8 (C-2–6, 2′–6′, Ph*C*H$_2$O, O*C*H$_2$CH$_2$N), 94.1, 97.9 (C-1, 1′), 128.1, 128.2, 128.6, 136.8 (aromatic C), 156.7 (NH*C*OOBn), 169.3, 169.7, 170.0, 170.1 (CH$_3$*C*O); ^{1}H, δ 0.00 (s, 3H), 0.02 (s, 3H), 0.88 (s, 9H), 1.98 (s, 3H), 2.00 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 3.30–3.38 (m, 1H), 3.42–3.52 (m, 1H), 3.56–3.64 (m, 1H), 3.66–3.68 (m, 2H), 3.74–3.80 (m, 1H), 3.90–4.04 (m, 5H), 4.12–4.14 (m, 1H), 5.1 (s, 2H), 2.16–2.22 (m, 1H), 5.29 (m, 1H), 5.32–5.47 (m, 3H), 5.51–5.54 (m, 1H), 5.63–5.68 (m, 1H), 7.28–7.38 (m, 5H); ^{31}P, δ −3.61. Compound **8** (472 mg, 0.46 mmol) was dissolved in THF (10 mL) and TREAT–HF (372 μL, 2.28 mmol) was added. The mixture was stirred at rt for 24 h followed by concentration and purification on silica gel (1 : 0 → 5 : 1 $CHCl_3$–MeOH + 0.5% Et_3N) to give **9** (390 mg, 0.42 mmol, 91%); $[\alpha]_D$ +34° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ 10.6, 20.6, 20.8, 20.8, (*C*H_3CO), 40.7 (HOCH$_2$*C*H$_2$NH), 46.1, 61.5, 61.7, 62.2, 62.3, 64.6, 66.6, 66.6, 66.8, 67.4, 69.9, 70.0, 70.9, 71.2, 71.9, (C-2–6, 2′–6′, Ph*C*H$_2$O, O*C*H$_2$CH$_2$N), 94.0, 98.0 (C-1, 1′), 128.1, 128.2, 128.6, 136.6 (aromatic C), 156.6 (NH*C*OOBn), 169.9, 170.3 (CH$_3$*C*O); ^{31}P, δ −3.51; HRMS calcd for $C_{30}H_{39}N_7O_{18}P$ [M]$^-$ 816.2089, found 816.2078.

2-(Benzyloxycarbonyl)aminoethyl (3,4-di-*O*-acetyl-2-azido-6-*O*-(*tert*butyldimethylsilyl)-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside) bis-triethylammonium salt (10). To a mixture of **5** (273 mg, 0.51 mmol) and **9** (357 mg, 0.39 mmol) in pyridine (3 mL) was added pivaloyl chloride (119 μL, 0.97 mmol). After 2 h the mixture was cooled to −40 °C and a solution of I_2 (119 mg, 0.47 mmol) in pyridine–H_2O (3 mL 49 : 1) was added. The mixture was diluted with $CHCl_3$ when the temperature reached −10 °C. Extraction with $Na_2S_2O_3$ (10%), cold TEAB (0.5 M), filtration (cotton) and chromatography (1 : 0 → 5 : 1 $CHCl_3$–MeOH + 0.5% Et_3N) gave **10** (351 mg, 0.24 mmol, 62%); $[\alpha]_D$ +68° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ −5.53, −5.42 (*C*H_3Si), 10.1, 18.3 ((CH$_3$)$_3$*C*Si), 20.5, 20.6, 20.6, 20.8 (*C*H_3CO), 25.9 ((*C*H$_3$)$_3$CSi), 40.6 (HOCH$_2$*C*H$_2$NH), 45.9, 61.7, 62.1, 62.3, 64.2, 66.2, 66.4, 66.5, 67.1, 69.7, 70.4, 71.0, 71.2, 71.4, 71.6, 77.4 (C-2–6, 2′–6′, 2″–6″, Ph*C*H$_2$O, O*C*H$_2$CH$_2$N), 93.8, 94.0, 97.8 (C-1, 1′, 1″), 128.0, 128.2, 128.5, 136.9 (aromatic C), 156.8 (NH*C*OOBn), 169.3, 169.6, 169.7, 169.8, 169.9, 170.0 (CH$_3$*C*O); ^{1}H, δ −0.05 (s, 3H), −0.03 (s, 3H), 0.80 (s, 9H), 1.90–2.00 (m, 18H), 3.23–3.31 (m, 1H), 3.36–3.45 (m, 1H), 3.50–3.64 (m, 4H), 3.69–3.76 (m, 1H), 3.79–3.97 (m, 7H), 4.02–4.15 (m, 3H), 5.00–5.06 (m, 2H), 5.12–5.21 (m, 2H), 5.24–5.31 (m, 1H), 5.33–5.37 (m, 2H), 5.38–5.45 (m, 3H), 6.04–6.10 (m, 1H), 7.18–7.30 (m, 5H); ^{31}P, δ −3.89, −3.56; HRMS calcd for $C_{46}H_{66}N_{10}NaO_{27}P_2Si$ [M + Na]$^-$ 1303.3241, found 1303.3197.

2-(Benzyloxycarbonyl)aminoethyl (2-acetamido-3,4-di-*O*-acetyl-6-*O*-(*tert*butyldimethylsilyl)-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-mannopyranoside) bis-triethylammonium salt (11). Compound **10** (83 mg, 0.056 mmol) was dissolved in MeOH (3 mL) and $NiCl_2(H_2O)_6$ was added (catalytic amount). Reduction was performed by adding $NaBH_4$ in small amounts over a period of 1 h at 0 °C. The mixture was then subjected to acetic anhydride followed by dilution (MeOH) and concentration. The residue was purified on silica gel (1 : 0 → 5 : 1 $CHCl_3$–MeOH + 0.5% Et_3N) and on LH-20 gel (MeOH + 1.5% Et_3N), to give compound **11** (76 mg, 0.050 mmol, 89%); $[\alpha]_D$ +63° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ −5.44, −5.38 (*C*H_3Si), 8.46, 18.4 ((CH$_3$)$_3$*C*Si), 20.9, 21.0, 21.0, 21.1, 23.0, 23.0, 23.0, 23.3, 23.9 (*C*H_3CO, *C*H_3CONH), 26.0 ((*C*H$_3$)$_3$CSi), 40.8 (HOCH$_2$*C*H$_2$NH), 46.1, 50.1, 50.4, 50.7 (C-2, 2′, 2″), 59.1, 61.6, 64.5, 65.0, 65.7, 65.9, 66.7, 67.1, 70.0, 70.2, 70.3, 71.3, 77.4 (C-3–6, 3′–6′, 3″–6″, Ph*C*H$_2$O, O*C*H$_2$CH$_2$N), 94.9, 95.1, 99.1 (C-1, 1′, 1″), 128.0, 128.1, 128.6, 136.8 (aromatic C), 156.6 (NH*C*OOBn), 169.5, 169.6, 170.0, 170.5, 170.6, 170.8, 171.3 (CH$_3$*C*O, CH$_3$*C*ONH); ^{31}P, δ −3.75, −3.39; HRMS calcd for $C_{52}H_{79}N_4O_{30}P_2Si$ [M + H]$^-$ 1329.3956, found 1329.3984.

2-Aminoethyl (2-acetamido-3,4-di-*O*-acetyl-6-*O*-(*tert*butyldimethylsilyl)-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-mannopyranoside) bis-triethylammonium salt (12). To a solution of compound **11** (37 mg, 0.024 mmol) in MeOH (1.5 mL) was added amberlite IR-45(OH$^-$) resin (40 mg) and palladium on activated carbon. The mixture was hydrogenolysed at 100 psi overnight, diluted (MeOH), centrifuged and concentrated. Purification on reversed phase gel (1 : 0 → 0 : 1 H_2O–MeOH) gave **12** (28 mg, 0.020 mmol, 83%); $[\alpha]_D$ +60° (*c* 1.0, MeOH); NMR (D_2O): ^{13}C, δ −5.9, −5.4 (*C*H_3Si), 8.89, 18.7 ((CH$_3$)$_3$*C*Si), 20.9, 21.0, 22.4, 22.5 (*C*H_3CO, *C*H_3CONH), 26.0 ((*C*H$_3$)$_3$CSi), 39.7 (HOCH$_2$*C*H$_2$NH), 47.3, 50.6, 51.4 (C-2, 2′, 2″), 62.4, 64.4, 64.7, 64.9, 65.9, 66.2, 66.6, 70.1, 70.8, 71.0, 71.2, 71.5 (C-3–6, 3′–6′, 3″–6″, O*C*H$_2$CH$_2$N), 95.4, 95.5, 99.1 (C-1, 1′, 1″), 173.1, 173.3, 173.5, 173.6, 173.8, 174.9, 175.1, 175.2 (CH$_3$*C*O, CH$_3$*C*ONH); ^{31}P, δ −3.04, −2.87; HRMS calcd for $C_{44}H_{72}N_4O_{28}P_2Si$ [M + H]$^-$ 1195.3589, found 1195.3567.

2-Aminoethyl (2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-mannopyranoside) bis-triethylammonium salt (13). A solution of TREAT–HF (17 μL, 0.10 mmol) in THF (1.5 mL) was treated with Et_3N (17 μL, 0.12 mmol). This solution was added to compound **12** (28 mg, 0.020 mmol). After 30 minutes of stirring at rt the mixture was concentrated and purified on reversed phase gel (1 : 0 → 0 : 1 H_2O–MeOH) which gave **13** (22 mg, 0.017 mmol, 85%); $[\alpha]_D$ +45° (*c* 1.0, MeOH); NMR (D_2O): ^{13}C, δ 8.87, 20.9, 20.9, 22.3, 22.4 (*C*H_3CO, *C*H_3CONH), 39.6 (HOCH$_2$*C*H$_2$NH), 47.3, 50.6, 51.3, 51.4 (C-2, 2′, 2″), 60.2, 64.3, 64.5, 64.9, 66.2, 66.4, 69.9, 70.0, 70.5, 70.5, 70.7, 70.8, 71.0, 71.6 (C-3–6, 3′–6′, 3″–6″, O*C*H$_2$CH$_2$N), 95.3, 95.3, 99.5 (C-1, 1′, 1″), 173.3, 173.3, 173.6, 173.7, 173.8, 175.0, 175.1, 175.3 (CH$_3$*C*O, CH$_3$*C*ONH); ^{1}H, δ 2.03 (s, 3H), 2.08 (s,3H), 2.09 (s, 3H), 2.14 (s, 3H), 2.14 (s, 3H), 2.18 (s, 3H), 2.24 (s, 3H), 2.24 (s, 3H), 2.24 (s, 3H), 3.28–3.36 (m, 2H), 3.68–3.84 (m, 3H), 4.00–4.18 (m, 5H), 4.27–4.33 (m, 1H), 4.57–4.64 (m, 2H), 5.22–5.30 (m, 1H), 5.31–5.37 (m, 2H), 5.44–5.49 (m, 1H); ^{31}P, δ −3.02, −2.95; HRMS calcd for $C_{38}H_{59}N_4O_{28}P_2$ [M + H]$^-$ 1081.2729, found 1081.2687.

2-Aminoethyl (2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-2-deoxy-α-D-mannopyranoside) bis-triethylammonium salt (14). Compound **13** (22 mg,

0.017 mmol) was dissolved in MeOH (1 mL) and NaOMe (1 M) was added. The mixture was concentrated after 30 min and the residue was purified on reversed phase gel ($H_2O \rightarrow$ MeOH). The fractions containing the product were freeze dried to give **14** (14 mg, 0.016 mmol, 94%); $[a]_D$ +12.4° (*c* 0.5, MeOH); NMR (D_2O): ^{13}C, δ 22.6 (CH_3CONH), 40.1 ($HOCH_2CH_2NH$), 53.1, 53.8, 53.9 (C-2, 2′, 2″), 60.8, 65.3, 66.6, 67.0, 69.1, 69.3, 69.5, 72.2, 73.0, 74.1 (C-3–6, 3′–6′, 3″–6″, OCH_2CH_2N), 95.8, 95.8, 99.6 (C-1, 1′, 1″), 175.5, 175, 5, 175.6 (CH_3CONH); ^{31}P, δ −2.36, −2.22; HRMS calcd for $C_{26}H_{47}N_4O_{22}P_2$ $[M + H]^-$ 829.2090, found 829.2064.

Ethyl 3,4-di-*O*-acetyl-2-azido-2-deoxy-6-*O*-dibenzyloxyphosphoryl-1-thio-α-D-mannopyranoside (16). To compound **3** (305 mg, 0.68 mmol) dissolved in THF (6 mL), TREAT–HF (0.55 ml, 3.38 mmol) was added. The mixture was stirred at rt overnight. Concentration and chromatography (10 : 1 → 0 : 1 toluene–EtOAc) gave ethyl 3,4-di-*O*-acetyl-2-azido-2-deoxy-1-thio-α-D-mannopyranoside (**15**, 171 mg, 0.51 mmol, 75%); NMR: ^{13}C, δ 14.7 (SCH_2CH_3), 20.6, 20.8 (CH_3CO), 25.4 (SCH_2CH_3), 61.3, 63.0, 66.6, 71.1, 71.3 (C-2–6), 82.2 (C-1), 170.0, 170.6 (CH_3CO); ^1H, δ 1.26–1.30 (t, 3H), 2.05 (s, 3H), 2.08 (s, 3H), 2.32 (s, 1H), 2.56–2.68 (m, 2H), 3.58–3.67 (m, 2H), 4.09–4.14 (m, 2H), 5.24–5.35 (m, 3H). To compound **15** (171 mg, 0.51 mmol) dissolved in CH_2Cl_2 (10 mL), tetrazole (125 mg, 1.78 mmol) and dibenzyl *N*,*N*-diisopropyl phosphoramidite[19] (264 μL, 0.76 mmol) were added. The reaction mixture was stirred for 30 min at rt. *m*CPBA (176 mg, 1.02 mmol) was added at 0 °C and stirring was continued for 30 min. The mixture was diluted (CH_2Cl_2), washed with $Na_2S_2O_3$ (10%) and $NaHCO_3$. Filtration (Na_2SO_4), concentration and chromatography (10 : 1 → 2 : 1 toluene–EtOAc) gave **16** (200 mg, 0.34 mmol, 67%); $[a]_D$ +92° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ 14.6 (SCH_2CH_3), 20.5, 20.6 (CH_3CO), 25.3 (SCH_2CH_3), 62.9, 65.7, 66.1, 69.4, 69.4, 69.5, 71.4, (C-2–6, PhCH_2O), 82.0 (C-1), 127.9, 128.0, 128.5, 128.5, 128.6, 128.6, 128.7, 135.8, 135.9 (aromatic C), 169.5, 169.9 (CH_3CO); ^{31}P, δ −1.36; HRMS calcd for $C_{26}H_{32}N_3NaO_9PS$ $[M + Na]^+$ 616.1495, found 616.1487.

3,4-Di-*O*-acetyl-2-azido-2-deoxy-6-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl hydrogenphosphonate, triethylammonium salt (18). Compound **16** (281 mg, 0.47 mmol) was dissolved in wet CH_2Cl_2 (10 mL) and cooled to −20 °C. NIS (137 mg, 0.61 mmol) and AgOTf (catalytic amount) were added and the mixture was stirred for 30 min at −20 °C. The mixture was diluted (CH_2Cl_2), washed with $Na_2S_2O_3$ (10%), filtered (Na_2SO_4) and concentrated. Chromatography (2 : 1 → 0 : 1 toluene–EtOAc) gave 3,4-di-*O*-acetyl-2-azido-2-deoxy-6-*O*-dibenzyloxyphosphoryl-α-D-mannopyranoside (**17**, 201 mg, 0.37 mmol, 79%); NMR: ^{13}C, δ 20.7, 20.7, 62.5, 66.2, 66.3, 66.4, 68.6, 68.7, 69.6, 69.7, 69.8, 69.8, 71.0 (C-2–6, PhCH_2O), 92.5 (C-1), 128.0, 128.1, 128.3, 128.6, 128.7, 128.7, 135.6, 135.7 (aromatic C), 169.8, 170.1 (CH_3CO); ^{31}P, δ −1.87. A mixture of imidazole (349 mg, 5.13 mmol), PCl_3 (128 μL, 1.47 mmol) and Et_3N (765 μL, 5.49 mmol) in MeCN (10 mL) was stirred at 0 °C for 30 min. A solution of compound **17** (201 mg, 0.37 mmol) in MeCN (10 mL) was added during 30 min at 0 °C. The reaction mixture was then stirred at rt for 10 min, quenched with TEAB (0.5 M) and concentrated. The residue was diluted ($CHCl_3$), washed with TEAB (0.5 M), filtered (cotton) and concentrated. Chromatography (1 : 0 → 10 : 1 $CHCl_3$–MeOH + 1.0% Et_3N) of the residue gave **18** (243 mg, 0.34 mmol, 92%); $[a]_D$ +55° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ 9.34, 20.6, 20.7 (CH_3CO), 45.9, 62.4, 62.5, 65.6, 65.7, 69.4, 69.5, 69.9, 70.0, 70.9 (C-2–6, PhCH_2O), 93.0 (C-1), 128.0, 128.0, 128.1, 128.5, 128.5, 128.6, 135.8 (aromatic C), 169.5, 169.9 (CH_3CO); ^{31}P, δ −1.32, 0.29; HRMS calcd for $C_{24}H_{28}N_3O_{12}P_2$ $[M]^-$ 612.1148, found 612.1129.

2-(Benzyloxycarbonyl)aminoethyl (3,4-di-*O*-acetyl-2-azido-2-deoxy-6-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl phosphate)-(1 → 6)-(3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside) bis-triethylammonium salt (19). A mixture of **18** (96 mg, 0.13 mmol) and **9** (103 mg, 0.11 mmol) was dissolved in pyridine (3 mL). Pivaloyl chloride (34 μL, 0.28 mmol) was added and the mixture was stirred under argon at rt for 2 h. The mixture was cooled to −40 °C and a solution of I_2 (34 mg, 0.13 mmol) in pyridine–H_2O (3 mL 49 : 1) was added. The oxidation was completed at −10 °C and the mixture was diluted with $CHCl_3$, washed with $Na_2S_2O_3$ (10%) and cold TEAB (0.5 M). Filtration (cotton), concentration and chromatography (1 : 0 → 5 : 1 $CHCl_3$–MeOH + 0.5% Et_3N) gave **19** (106 mg, 0.065 mmol, 59%); $[a]_D$ +80° (*c* 1.0, $CHCl_3$); NMR: ^{13}C, δ 10.2, 20.6, 20.7, 20.8 (CH_3CO), 40.6 ($HOCH_2CH_2NH$), 45.9, 57.9, 61.7, 62.3, 64.4, 65.5, 65.7, 66.1, 66.5, 66.6, 67.2, 69.4, 69.5, 70.3, 71.0, 71.4 (C-2–6, 2′–6′, 2″–6″, PhCH_2O, OCH_2CH_2N), 93.9, 94.0, 97.8 (C-1, 1′, 1″), 128.0, 128.1, 128.2, 128.5, 128.6, 136.0, 136.9 (aromatic C), 156.8 (NH*C*OOBn), 169.6, 169.7, 169.8, 169.9 (CH_3CO); ^{31}P, δ −4.13, −3.58, −1.52.

2-Aminoethyl (2-acetamido-3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl 1,6-bisphosphate)-(1 → 6)-(2-acetamido-3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside) tris-triethylammonium salt (21). Compound **19** (76 mg, 0.047 mmol) was dissolved in MeOH (3 mL) and $NiCl_2(H_2O)_6$ was added (catalytic amount). Reduction was performed by adding $NaBH_4$ in small amounts over a period of 30 min at 0 °C. The mixture was then subjected to acetic anhydride followed by dilution (MeOH) and concentration. The residue was purified on silica gel (1 : 0 → 5 : 1 $CHCl_3$–MeOH + 0.5% Et_3N) and on LH-20 gel (MeOH + 1.5% Et_3N), to give compound 2-(benzyloxycarbonyl) aminoethyl (2-acetamido-3,4-di-*O*-acetyl-2-azido-2-deoxy-6-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-3,4-di-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranoside) bis-triethylammonium salt (**20**, 50 mg, 0.030 mmol, 64%); NMR (D_2O): ^{13}C, δ 8.89, 20.7, 20.8, 22.4 (CH_3CO, CH_3CONH), 40.7 ($HOCH_2CH_2NH$), 47.3, 50.7, 51.4 (C-2, 2′, 2″), 64.5, 65.9, 66.1, 66.5, 66.6, 67.2, 67.3, 69.7, 69.8, 70.5, 70.8, 70.9, 71.1 (C-3–6, 3′–6′, 3″–6″, PhCH_2O, OCH_2CH_2N), 95.2, 95.3, 98.8 (C-1, 1′, 1″), 128.2, 128.9, 129.0, 129.3, 129.5, 129.7, 135.7, 135.8, 137.1 (aromatic C), 158.7 (NH*C*OOBn), 172.8, 172.9, 173.2, 173.3, 173.4, 173.4, 174.8, 174.9, 175.0 (CH_3CO, CH_3CONH); ^{31}P, δ −3.05, −2.75, −1.20. To a solution of compound **20** (46 mg, 0.027 mmol) in MeOH (2 mL) was added Amberlite IR-45(OH^-) resin (46 mg) and palladium on activated carbon. The mixture was hydrogenolysed at 100 psi overnight, diluted (MeOH), centrifuged and concentrated. Purification on a reversed phase silica gel column (1 : 0 → 0 : 1 H_2O–MeOH) gave **21** (34 mg, 0.023 mmol, 85%); $[a]_D$ +43° (*c* 1.0, MeOH); NMR (D_2O): ^{13}C, δ 8.89, 20.9, 20.9, 22.4, 22.4 (CH_3CO, CH_3CONH), 39.6, ($HOCH_2CH_2NH$), 47.3, 50.6, 51.3, 51.4 (C-2, 2′, 2″), 63.6, 64.4, 64.8, 66.1, 66.2, 66.4, 69.9, 70.0, 70.6, 70.7, 71.0 (C-3–6, 3′–6′, 3″–6″, OCH_2CH_2N), 95.3, 95.4, 99.1 (C-1, 1′, 1″), 173.2, 173.3, 173.4, 173.6, 173.7, 173.7, 175.0, 175.1, 175.2 (CH_3CO, CH_3CONH); ^{31}P, δ −3.08, −2.95, 0.05; HRMS calcd for $C_{38}H_{59}N_4O_{31}P_3$ $[M + H]^{2-}$ 580.1157, found 580.1142.

2-Aminoethyl (2-acetamido-2-azido-2-deoxy-6-*O*-phosphoryl-α-D-mannopyranosyl 1,6-bisphosphate)-(1 → 6)-(2-acetamido-2-azido-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-2-azido-2-deoxy-α-D-mannopyranoside) tris-sodium salt (23). Compound **20** (50 mg, 0.030 mmol) was dissolved in MeOH (3 mL) and NaOMe (1 M) was added. The reaction mixture was stirred for 1 h at rt. Concentration and purification on reversed phase gel (1 : 0 → 0 : 1 H_2O–MeOH) gave 2-(benzyloxycarbonyl)aminoethyl (2-acetamido-2-azido-2-deoxy-6-*O*-dibenzyloxyphosphoryl-α-D-mannopyranosyl

phosphate)-(1 → 6)-(2-acetamido-2-azido-2-deoxy-α-D-mannopyranosyl phosphate)-(1 → 6)-(2-acetamido-2-azido-2-deoxy-α-D-mannopyranoside) bis-sodium salt (**22**, 30 mg, 0.024 mmol, 80%); NMR (D_2O): ^{13}C, δ 22.6 (CH_3CONH), 40.7 ($HOCH_2CH_2NH$), 53.1, 53.8, 54.0 (C-2, 2′, 2″), 65.1, 66.4, 66.5, 66.8, 67.5, 68.9, 69.1, 69.6, 71.0, 71.1, 71.9, 72.0, 72.3 73.1 (C-3–6, 3′–6′, 3″–6″, $PhCH_2O$, OCH_2CH_2N), 95.6, 95.8, 99.4 (C-1, 1′, 1″), 128.1, 128.4, 129.0, 129.0, 129.4, 129.5, 129.8, 135.7, 135.8, 137.1 (aromatic C), 158.8 ($NHCOOBn$), 175.3 (CH_3CONH); ^{31}P, δ −2.35, −2.23, −0.96. Compound **22** (30 mg, 0.024 mmol) was dissolved in MeOH (2 mL). Amberlite IR-45(OH^-) resin (30 mg) and palladium on activated carbon were added. The mixture was hydrogenolysed at 100 psi overnight, diluted (MeOH), centrifuged and concentrated. Purification on reversed phase gel (1 : 0 → 0 : 1 H_2O–MeOH) gave **23** (15 mg, 0.015 mmol, 64%); $[\alpha]_D$ +13.6° (*c* 0.5, MeOH); NMR (D_2O): ^{13}C, δ 22.6, 22.6, 22.6 (CH_3CONH), 39.7 ($HOCH_2CH_2NH$), 49.5, 53.0, 53.7, 53.8 (C-2, 2′, 2″), 63.8, 64.2, 65.1, 65.2, 65.4, 66.5, 66.7, 67.0, 68.9, 69.2, 69.5, 72.2, 72.3, 72.9, 73.1, 73.2, 73.4, 73.4 (C-3–6, 3′–6′, 3″–6″, OCH_2CH_2N), 95.9, 99.6 (C-1, 1′, 1″), 175.4, 175.5, 175.5 (CH_3CONH); ^{31}P, δ −2.35, −2.30, 2.2; HRMS calcd for $C_{26}H_{46}N_4Na_2O_{25}P_3$ $[M + 2Na]^-$ 953.1459, found 953.1440.

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