Synthesis of stable C-phosphonate analogues of *Neisseria meningitidis* group A capsular polysaccharide structures using modified Mitsunobu reaction conditions

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Examples of synthetic C-phosphonate analogues of microbial polysaccharide structures containing inter-residue phosphodiester linkages are most rare. The successful construction of such analogues of the *Neisseria meningitidis* Group A capsular polysaccharide is described. Using a modified Mitsunobu reaction (tris(4-chlorophenyl)phosphine, DIAD, excess of Et_3N) between an anomeric C-phosphonate monoester and a 6-OH ManNAc acceptor a high yield (88%) of a dimer was obtained. Transformation of the dimer into a new 6-OH acceptor through deacetylation and further reaction with the elongating C-phosphonate monomer employing the same conditions afforded the trimer in 92% yield. Iteration of the procedure then afforded the tetramer with a coupling yield of 85%. The di-, tri- and tetramer were deprotected to give target structures ready for conjugation to a carrier protein and subsequent immunological evaluation.

Introduction

Bacterial meningitis is caused mainly by three types of bacteria, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*. With *N. meningitidis* five serogroups, *i.e.*, A, B, C, Y and W-135, are associated with meningococcal meningitis.[1,2] The serotyping is based on the structure and antigenicity of the capsular polysaccharide (CPS) surrounding the bacteria. Efficient bacterial glycoconjugate vaccines can be produced by attaching the polysaccharide to a carrier protein.[3] Against *N. meningitidis* Group C (MenC), there are now three monovalent glycoconjugate vaccines commercially available,[4] and development of vaccines against the other serogroups are under way.[5] However, Group B represents a major issue, since its CPS show molecular mimicry to human carbohydrate structure. Another problem encountered when trying to make multivalent glycoconjugate vaccines against *N. meningitidis* is the poor stability of Group A CPS. The repeating unit of the Group A CPS is a monosaccharide, 2-acetamido-2-deoxy-α-D-mannopyranose, linked 1 → 6 *via* a phosphodiester bridge,[6] and there is an inherent instability of the anomeric phosphate diester linkages of the CPS.[7] Hence, there is an interest in stable analogues of these structures. In a programme directed towards development of stable glycoconjugate vaccine candidates against MenA, we have earlier synthesised a variety of MenA trisaccharide structures[8] and were now interested in exploring C-phosphonate analogues. However, in spite of the obvious applicability of and interest in such derivatives very few have been synthesised, probably because of their difficult preparation. To our knowledge there are only two publications describing synthesis of structures with inter-residue C-phosphonate linked oligosaccharides.[9,10] Herein we describe the efficient synthesis of C-phosphonate analogues of di-, tri- and tetramers of the repeating unit of the *Neisseria meningitidis* Group A capsular polysaccharide, needed for vaccine development. The crucial coupling step is performed using a C-phosphonate monoester and modified Mitsunobu conditions. The synthetic strategy also allows for continued synthesis of larger oligomers and polycondensation. The target structures will be conjugated to a carrier protein and the resulting glycoconjugates evaluated as vaccine candidates in mice.

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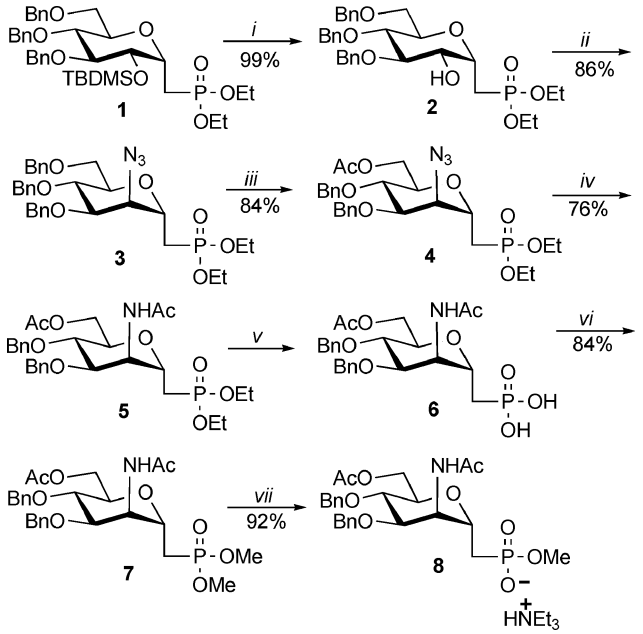
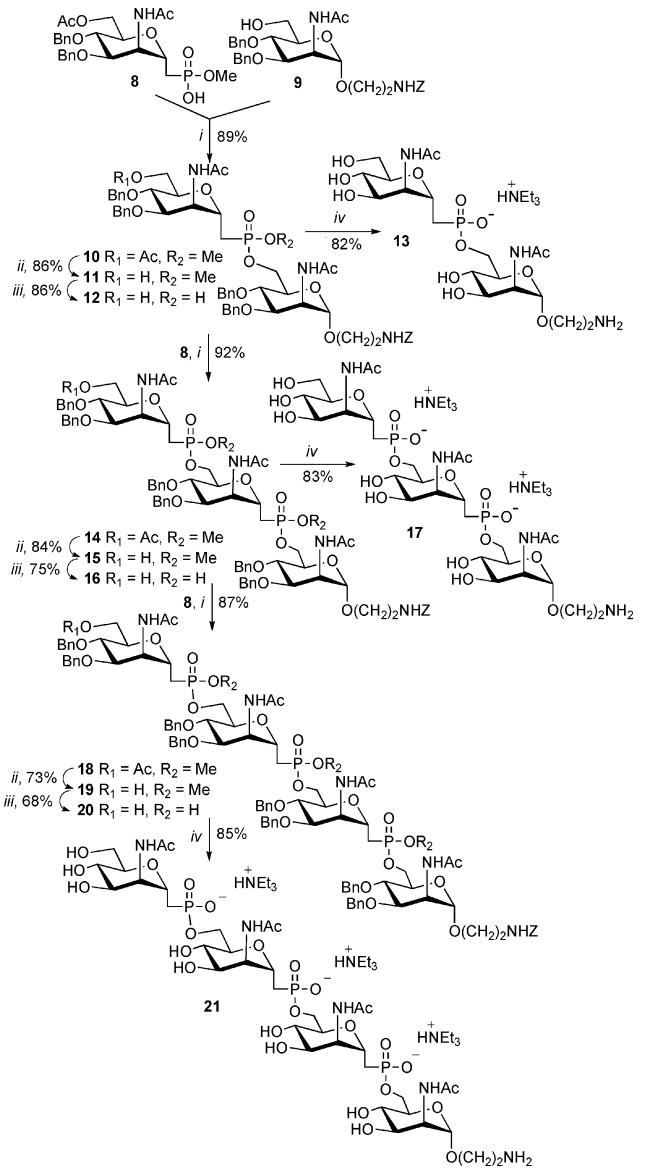
Results and discussion

For the construction of the elongating C-phosphonate monomer a modified version of a published approach was applied (Scheme 1).[11] After desilylation of precursor **1** to afford the 2-OH compound **2**, azide displacements using Mitsunobu conditions gave the 2-azido-2-deoxymannopyranoside **3** (86%). An orthogonal protecting group, to allow later 6-*O* elongation, was introduced by acetolysis to afford the 6-*O*-acetate **4** (84%). Azide reduction followed by acetylation gave **5** (76%), from which the ethyl esters were removed to yield the elongating monomer **6**.

The starting spacer-containing monomer acceptor **9** was already known.[12] In the esterification of anomeric C-phosphonates various examples involving spacer and fatty alcohols have been published,[13] but, as mentioned in the introduction, esterifications using sugar alcohols are most rare. In the publication by Nikolaev and co-workers both DCC and Mitsunobu conditions were employed with yields in the range of 50–70%. However, when esterifications with acceptors already containing phosphonates were performed, the yields dropped to 10–30%.[9] Our first attempt to esterify compound **6** with acceptor alcohol **9** using DCC as condensation reagent gave no product at all. Consequently, the methyl monoester **8** was synthesised (Scheme 1) and tried in the same reaction, which afforded dimer **10** but in a very low yield (around 20%), the pyrophosphonate dimer being the major product according to MALDI-TOF MS. Other condensation



Scheme 1 Synthesis of monosaccharide elongating precursor: (i) TBAF; (ii) Ph_3P, DIAD, DPPA, THF; (iii) HOAc, Ac_2O, H_2SO_4; (iv) (a) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, (b) Ac_2O; (v) Me_3SiBr; (vi) $MeC(OMe)_3$, HOAc; (vii) PhSH, Et_3N.



Scheme 2 Synthesis of a repeating unit di-, tri- and tetramers: (i) (p-$ClC_6H_4)_3P$, DIAD, Et_3N, THF; (ii) KOH, MeOH; (iii) PhSH, DBU, CH_3CN; (iv) H_2, Pd/C, HCl.

reagents (TIPSCl, MSNT) as well as displacements reactions using various leaving groups (triflate, tosylate) at the 6-position of **9** gave even less product. Hence, Mitsunobu conditions were tested using Ph_3P and DIAD, which gave a good yield (47%) of **10**.[14] Under these conditions the pyrophosphate was not a competing side-product since it is the acceptor alcohol that is transformed into a leaving group.

During the course of this work a synthesis of a similar MenA C-phosphonate dimer analogue was published using the same Mitsunobu conditions in the esterification step in an excellent yield of 97%.[10] The structure of both the acceptor (β-linked spacer) and donor (6-*O*-benzylated) differs slightly, but the large difference in yield is still difficult to explain.

In the beginning of the 1990s, Campbell described the use of tris(4-chlorophenyl)phosphine together with a large excess of triethylamine to improve Mitsunobu esterification of phosphonates.[15] The application of these conditions to our reaction proved to be an excellent combination. The desired dimer **10** was obtained in an 89% yield (Scheme 2). Efforts have to be made to exclude external nucleophiles in the reaction mixture to obtain an optimal yield. We found 6-chlorination when acidic chloroform had been used as solvent in a preceding chromatography purification as well as re-acetylation when acetate from the prior deacetylation was still present.

One major problem in the synthesis of anomeric phosphate diester structures is the formation of oligomers since already formed diesters are easily cleaved during the reaction conditions.[16] As mentioned above this might be a problem also for continued C-phosphonate formation.[9] Most pleasingly, using the optimized Mitsunobu conditions, the yield in the trimer formation was found to be comparable and even higher than in the dimer formation. The dimer **10** was transformed into a new acceptor (→**11**) by deacetylation (Scheme 2). Using the modified Mitsunobu reaction between **8** and **11** then afforded the trimer **14** in 92% yield. The procedure could be iterated. Deacetylation of **14**, created a new acceptor **15** and a Mitsunobu reaction between **8** and **15** formed the tetramer **18** in 85% yield. The identity of the products was proven mainly by MS since the NMR spectra were quite complex due to phosphonate diester diastereomers. However, after subsequent removal of the methyl esters using thiophenol and DBU, the NMR data of compounds **12**, **16** and **20** could be interpreted. Final deprotection of these compounds by catalytic hydrogenolysis afforded the unprotected dimer **13**, trimer **17** and tetramer **21** in 79, 62 and 58% overall yield from compounds **11**, **15** and **19**, respectively, all target products ready for conjugation to a carrier protein and immunological studies.

To conclude, an efficient synthesis of C-phosphonate analogues of the *Neisseria meningitidis* Group A capsular polysaccharide has been developed using modified Mitsunobu conditions in the formation of the inter-saccharide phosphonate ester linkages. The building blocks can be prepared in gram quantities and the yields are excellent also for the formation of tri- and tetramers and the approach is suitable also for formation of longer oligomers.

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 chromatography was performed on silica gel (0.040–0.063 mm, Amicon), reversed phase gel (C18 60A 40–63 μm) or liphophilic Sephadex gel (LH20, bead size 20–100 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 reference. Organic solutions were concentrated at 30 °C under reduced pressure.

Diethyl *C*-(3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)methanephosphonate (2). TBAF (2.6 g, 8.24 mmol) was added to a solution of compound **1** (5.36 g, 7.68 mmol) in THF (150 mL). After 20 min the solvent was removed under reduced pressure and the product was purified by silica gel column chromatography (1 : 0 → 0 : 1 toluene–EtOAc) to give **2** (4.44 g, 7.60 mmol, 99%). NMR data were in agreement with those reported earlier.[11]

Diethyl *C*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (3). DIAD (2.4 mL, 12.19 mmol) was added dropwise to a cooled (−5 °C) solution of Ph_3P (3.1 g, 11.82 mmol) in THF (50 mL). After 30 min, a solution of **2** (5.57 g, 9.53 mmol) in THF (20 mL) was added. After an additional 10 min, diphenyl phosphorazidate (DPPA; 2.4 mL, 11.12 mmol) was added and the reaction mixture was allowed to attain rt. After stirring overnight under argon at rt, the solvent was evaporated and the residue was purified by silica gel chromatography (1 : 0 → 0 : 1 toluene–EtOAc) to give **3** (5.0 g, 8.21 mmol, 86%) as a colourless oil. $[\alpha]_D$ +20° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 16.4, 16.5 (CH_3CH_2O), 27.9 (d, $J = 141$ Hz, C-7), 60.9, 61.0, 61.9, 62.0, 62.1, 62.2, 68.8, 69.5, 72.4, 73.5, 73.7, 73.9, 74.3, 78.2 (C-1–6, $PhCH_2O$, CH_3CH_2O), 127.7–128.6, 137.4, 137.9, 138.2 (aromatic C); ^{31}P NMR ($CDCl_3$) δ 27.9; HRMS: calc. for $C_{32}H_{40}N_3NaO_7P$ $[M + Na]^+$: 632.2502, found: 632.2513.

Diethyl *C*-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (4). Compound **3** (3.2 g, 5.25 mmol) was dissolved in HOAc–Ac_2O (1:1, 32 mL). 10 drops of 1% H_2SO_4 in Ac_2O was added. After stirring overnight the mixture was poured into a separation funnel containing ice and CH_2Cl_2. The organic phase was separated, filtered through a plug of silica gel and concentrated. The residue was purified by column chromatography (1 : 0 → 0 : 1 toluene–EtOAc) to give **4** (2.48 g, 4.42 mmol, 84%). $[\alpha]_D$ +70° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.32 (t, $J = 7.2$ Hz, 6H), 2.04 (s, 3H), 2.05–2.16 (m, 2H), 3.72–3.75 (m, 2H), 3.91–3.94 (m, 1H), 3.98 (t, $J = 3.6$ Hz, 1H), 4.06–4.18 (m, 4H), 4.21–4.37 (m, 3H), 4.54–4.64 (m, 2H), 4.72–4.78 (m, 2H), 7.25–7.37 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 16.2, 16.3 (CH_3CH_2O), 20.6 (CH_3CO), 27.6 (d, $J = 141$ Hz, C-7), 60.4, 60.5, 61.7, 61.8, 62.5, 69.3, 71.9, 72.2, 73.4, 74.0, 77.6, 77.8 (C-1–6, $PhCH_2O$, CH_3CH_2O), 127.8–128.3, 137.0, 137.4 (aromatic C), 170.4 (CH_3CO); ^{31}P NMR ($CDCl_3$) δ 27.5; HRMS: calc. for $C_{27}H_{36}N_3NaO_8P$ $[M + Na]^+$: 584.2137, found: 584.2116.

Diethyl *C*-(6-*O*-acetyl-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (5). To a stirred solution of **4** (2.50 g, 4.45 mmol) in MeOH (125 mL) a catalytic amount of $NiCl_2 \cdot 6H_2O$ was added, followed by portions of $NaBH_4$ (in total 0.34 g, 8.99 mmol) every 10 min until no starting material was observed on TLC (2 : 1 toluene–EtOAc). Ac_2O (3 mL, 31.8 mmol) was added and after 50 min the reaction mixture was diluted with toluene, filtered through a plug of silica gel and concentrated. The residue was purified by silica gel chromatography (1 : 0 → 0 : 1 toluene–EtOAc) to give **5** (1.95 g, 3.38 mmol, 76%); $[\alpha]_D$ +37° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 16.1, 16.2, 16.3 (CH_3CH_2O), 20.6 (CH_3CO), 23.0 (CH_3CONH), 28.5 (d, $J = 142$ Hz, C-7), 48.7 (d, C-2), 61.2, 61.3, 61.8, 61.8, 61.9, 67.1, 72.0, 72.1, 72.4, 72.6, 75.3 (C-1, C-3–6, $PhCH_2O$, CH_3CH_2O), 127.4–129.9, 137.1, 137,4 (aromatic C), 170.0, 170.6 (CH_3CO, CH_3CONH; ^{31}P NMR ($CDCl_3$) δ 29.5; HRMS: calc. for $C_{29}H_{40}NNaO_9P$ $[M + Na]^+$: 600.2339, found: 600.2346.

Dimethyl *C*-(6-*O*-acetyl-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (7). To a solution of **5** (250 mg, 0.43 mmol) in dry CH_2Cl_2 (5 mL), bromotrimethylsilane (0.28 mL, 2.16 mmol) was added. After the addition was complete, the mixture was stirred under argon at rt for 1 h. The solution was cooled to 0 °C and Et_3N (1 mL) was added followed by addition of water (1 mL). After 10 min, the solvents were removed under reduced pressure and the residue was desalted by reversed phase chromatography (1 : 0 → 1 : 1 H_2O–MeOH). The product *C*-(6-*O*-acetyl-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonic acid (**6**) was methylated without further purification. To the product from the reaction described above were added AcOH (5.5 mL) and trimethylorthoacetate (11.5 mL). The mixture was heated at reflux for 30 min and then concentrated. (The reaction had to be monitored carefully and stopped exactly when the reaction was completed. Otherwise the result is a mixture of the desired product and the deacetylated product) The residue was purified by silica gel chromatography (1 : 0 → 0 : 1 toluene–EtOAc) to give **7** (200 mg, 0.36 mmol, 84% over two steps); $[\alpha]_D$ +36° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.83 (s, 3H), 2.03 (s, 3H), 2.05–2.09 (m, 2H), 3.51 (t, $J = 3.6$ Hz, 1H), 3.67–3.75 (dd, $J = 23.2$ Hz, $J = 11.2$ Hz, 6H), 4.05–4.19 (m, 4H), 4.28–4.35 (m, 2H), 4.49–4.62 (m, 5H), 5.82 (d, $J = 9.2$ Hz, 1H), 7.22–7.34 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.9 (CH_3CO), 23.3 (CH_3CONH), 28.0 (d, $J = 141$ Hz, C-7), 48.8 (d, $J = 14.8$ Hz, C-2), 52.1 (d, $J = 6.5$ Hz, CH_3O), 52.9 (d, $J = 6.2$ Hz, CH_3O), 61.9, 66.8, 66.9, 72.0, 72.5, 72.6, 73.0, 75.4 (C-1, C-3–6, $PhCH_2O$), 128.0–128.9 137.1, 137.5 (aromatic C), 170.0, 170.7 (CH_3CO, CH_3CONH); ^{31}P NMR ($CDCl_3$) δ 31.6; HRMS: calc. for $C_{27}H_{36}NNaO_9P$ $[M + Na]^+$: 572.2026, found: 572.2000.

Methyl *C*-(6-*O*-acetyl-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate triethylammonium salt (8). To a solution of **7** (200 mg, 0.36 mmol) in THF (0.5 mL)

were added thiophenol (149 μL, 1.45 mmol) and Et_3N (304 μL, 2.18 mmol). After 48 h the reaction mixture was directly put on a silica gel column and eluted (1 : 0 → 5 : 1 $CHCl_3$–MeOH + 1.5% Et_3N) to give **8** (208 mg, 0.33 mmol, 92%); $[\alpha]_D$ +15° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.94 (s, 3H), 1.96 (s, 3H), 3.41 (t, $J = 6.4$ Hz, 1H), 3.53 (d, $J = 10.4$ Hz, 3H), 3.84–3.86 (m, 1H), 4.05 (m, 1H), 4.16–4.20 (m, 2H), 4.34 (dd, $J = 11.6$ Hz, $J = 6$ Hz, 1H), 4.42 (t, $J = 11.2$ Hz, 2H), 4.63–4.72 (m, 3H), 7.21–7.26 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 8.88 [($CH_3CH_2)_3N$], 20.8 (CH_3CO), 23.5 (CH_3CONH), 28.5 (d, $J = 129$ Hz, C-7), 45.3 [($CH_3CH_2)_3N$], 50.3 (C-2), 51.6 (d, $J = 6.1$ Hz, CH_3O), 63.1, 72.2, 73.4, 73.5, 76.3, (C-1, C-3–6, PhCH_2O), 127.7–128.6, 137.9, 138.0 (aromatic C), 169.9, 170.7 (CH_3CO, CH_3CONH); ^{31}P NMR ($CDCl_3$) δ 20.4; HRMS: calc. for $C_{26}H_{33}N_1O_9P_1$ $[M]^-$: 534.1898, found: 534.1902.

2-(Benzyloxycarbonyl)aminoethyl 6-*O*-[methyl *C*-(6-*O*-acetyl-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate] - 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranoside (10). To a solution of compound **8** (100 mg, 0.157 mmol) and compound **9** (70 mg, 0.121 mmol) in THF (0.5 mL), tris(4-chlorophenyl)phosphine (62 mg, 0.17 mmol), Et_3N (84 μL, 0.60 mmol) and DIAD (33 μL, 0.168 mmol) were added. After stirring under argon at rt for 30 min, the residue was directly purified by silica gel chromatography (1 : 0 → 20 : 1 $CHCl_3$–MeOH) followed by further purification on a LH-20 gel column (MeOH) to give the product **10** (118 mg, 0.108 mmol, 89%) as a diastereomeric mixture; HRMS: calc. for $C_{58}H_{70}N_3NaO_{16}P$ $[M + Na]^+$: 1118.4391, found: 1118.4399.

2-(Benzyloxycarbonyl)aminoethyl 6-*O*-[methyl *C*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranoside (11). To a solution of **10** (113 mg, 0.103 mmol) in MeOH (3 mL), KOH (206 μL) from a stock solution of KOH dissolved in MeOH (1 M) was added. After 30 min the reaction mixture was directly purified by silica gel chromatography (1 : 0 → 10 : 1 $CHCl_3$–MeOH) followed by further purification on a LH-20 gel column (MeOH) to give **11** (93 mg, 0.088 mmol, 86%); $[\alpha]_D$ +48° (*c* 1.0, $CHCl_3$); HRMS: calc. for $C_{56}H_{69}N_3O_{15}P_1$ $[M + H]^+$: 1054.4461, found: 1054.4430.

2-Aminoethyl 6-*O*-[*C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-2-deoxy-α-D-mannopyranoside triethylammonium salt (13). To a solution of **11** (44 mg, 0.042 mmol) in CH_3CN (0.5 ml), thiophenol (86 μL, 0.84 mmol) and DBU (125 μL, 0.84 mmol) were added. The reaction mixture was stirred under argon at rt for 2 h and then the product was directly purified by silica gel chromatography (1 : 0 → 5 : 1 $CHCl_3$–MeOH + 1.5% Et_3N). Further purification on a LH-20 gel column (MeOH) gave 2-(benzyloxycarbonyl)aminoethyl 6-*O*-[*C*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranoside (**12**, 41 mg, 0.36 mmol, 86%); $[\alpha]_D$ +32° (*c* 1.0, H_2O); ^{13}C NMR (CD_3OD) δ 8.88 [($CH_3CH_2)_3N$], 22.4 22.6 (CH_3CONH), 29.9 (d, $J = 135$ Hz, C-7′), 41.4 (OCH_2CH_2NH), 45.3 [($CH_3CH_2)_3N$], 50.6, 51.2, 51.3, 54.7, 61.1, 64.6, 67.3, 67.4, 72.0, 72.2, 72.3, 72.9, 74.2, 74.6, 75.2, 75.9, 76.0, 77.8, 78.9 (C-2–6, C-1–6, PhCH_2O, OCH_2CH_2NH), 100.3 (C-1), 125.1–130.4, 138.2, 139.4, 139.6, 139.7, 139.9 (aromatic C), 158.7 ($NHCOOBn$), 172.6, 173.6 (CH_3CONH); ^{31}P NMR ($CDCl_3$) δ 21.6. To a solution of compound **12** (12 mg, 0.011 mmol) in MeOH (4.5 ml), HCl in water (40 μl, 1 M) was added followed by a catalytic amount of palladium on activated carbon. The mixture was hydrogenolysed at 100 psi overnight, diluted (MeOH) and centrifuged to remove the activated carbon. Concentration and purification on a reversed phase silica gel column (H_2O) gave **13** (6 mg, 0.009 mmol, 82%) after freeze-drying; $[\alpha]_D$ +17° (*c* 0.5, H_2O); ^1H NMR (D_2O) δ 2.08 (s, 3H), 2.09 (s, 3H), 2.16 (d, $J = 7.2$ Hz, 1H), 2.21 (d, $J = 7.2$ Hz, 1H), 3.28–3.32 (m, 2H), 3.64–3.66 (m, 2H), 3.71–3.76 (m, 3H), 3.79 (m, 1H), 3.82 (m, 1H), 3.85 (m, 1H), 3.88 (m, 1H), 3.99–4.05 (m, 3H), 4.07–4.11 (m, 1H), 4.14–4.17 (m, 2H), 4.23–4.25 (m, 1H), 4.42 (m, 2H), 4.86 (m, 1H); ^{13}C NMR (D_2O) δ 8.8 [($CH_3CH_2)_3N$], 22.5, 22.6 (CH_3CONH), 27.7 (d, $J = 134$ Hz, C-7′), 39.5 (OCH_2CH_2NH), 47.2 [($CH_3CH_2)_3N$], 52.8 (C-2), 53.2 (d, $J = 10$ Hz, C-2′), 60.0 (C-6′), 63.6, 63.9, 66.8, 67.8, 69.1, 69.6, 72.1, 73.1, 74.5 (C-3–6, C-1′, C-3′–5′, OCH_2CH_2NH), 99.3 (C-1), 174.8, 175.4 (CH_3CONH); ^{31}P NMR (D_2O) δ 23.1; HRMS: calc. for $C_{19}H_{35}N_3O_{13}P$ $[M]^-$: 544.1907, found: 544.1943.

2-(Benzyloxycarbonyl)aminoethyl 6-*O*-[methyl *C*-(6-*O*-[methyl *C*-(6-*O*-acetyl-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranoside (14). To a solution of **11** (38 mg, 0.036 mmol) and **8** (27 mg, 0.042 mmol) in THF (0.5 mL), tris(4-chlorophenyl)phosphine (19 mg, 0.052 mmol), Et_3N (25 μL, 0.18 mmol) and DIAD (10 μL, 0.051 mmol) were added. The mixture was stirred under argon at rt for 40 min followed by purification on silica gel column (1 : 0 → 20 : 1 $CHCl_3$–MeOH). The products were further purified on a LH-20 gel column (MeOH) to give **14** (52 mg, 0.033 mmol, 92%); $[\alpha]_D$ +38° (*c* 1.0, $CHCl_3$). HRMS: calc. for $C_{82}H_{99}N_4NaO_{23}P_2$ $[M + Na]^+$: 1593.6157, found: 1593.6151.

2-(Benzyloxycarbonyl)aminoethyl 6-*O*-[*C*-(6-*O*-[*C*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)-methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranoside bis(triethylammonium) salt (16). To a solution of **14** (59 mg, 0.038 mmol) in MeOH (3 ml), KOH (76 μL) from a stock solution of KOH dissolved in MeOH (1 M) was added. After 30 min the reaction mixture was purified by silica gel chromatography (1 : 0 → 10 : 1 $CHCl_3$–MeOH) followed by further purification on a LH-20 gel column (MeOH) to give 2-(benzyloxycarbonyl)aminoethyl 6-*O*-[methyl *C*-(6-*O*-[methyl *C*-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)-methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranoside **15** (49 mg, 0.032 mmol, 84%); HRMS: calc. for $C_{80}H_{99}N_4O_{22}P_2$ $[M + H]^+$: 1529.6221, found: 1529.6244. To a solution of **15** (36 mg, 0.024 mmol) in CH_3CN (0.5 ml), thiophenol (96 μL, 0.94 mmol) and DBU (70 μL, 0.47 mmol) were added. The reaction mixture was stirred under argon at rt for 2 h and the product was then purified by silica gel chromatography (1 : 0 → 5 : 1 $CHCl_3$–MeOH + 1.5% Et_3N) and further purified on a LH-20 gel column (MeOH) to give **16** (30 mg, 0.018 mmol, 75%); $[\alpha]_D$ +60° (*c* 1.0, MeOH); ^{13}C NMR ($CDCl_3$) δ 9.06 [($CH_3CH_2)_3N$], 23.2, 23.3, 23.5 (CH_3CONH), 29.9 (m, C-7′, C-7″), 40.8

(OCH_2CH_2NH), 48.5, 49.6 (C-2, C-2, C-2″), 59.0, 63.5, 63.7, 66.8, 71.1, 71.5, 71.8, 72.2, 72.6, 72.9, 73.9, 74.2, 74.7, 75.2, 76.1 (C-3–6, C-1′, C-3′–6′, C-1″, C-3″–6″, PhCH_2O, OCH_2CH_2NH), 100.0 (C-1), 127.1–128.8, 136.7, 137.6, 138.1, 138.5, 138.7, 138.9 (aromatic C), 156.6 (NH*C*OOBn), 169.8, 170.8, 171.2 (CH_3CONH); ^{31}P NMR ($CDCl_3$) δ 21.2, 22.4; HRMS: calc. for $C_{78}H_{92}N_4O_{22}P_2$ $[M]^{2-}$: 749.2845, found: 749.2836.

2-Aminoethyl 6-*O*-[*C*-(6-*O*-[*C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-2-deoxy-α-D-mannopyranoside bis(triethylammonium) salt (17). To a solution of **16** (30 mg, 0.018 mmol) in MeOH (4.5 ml), HCl in water (50 μL, 1M) was added followed by a catalytic amount of palladium on activated carbon. The mixture was hydrogenolysed at 100 psi overnight, diluted (MeOH) and centrifuged to remove the activated carbon. Concentration and purification on a reversed phase silica gel column (H_2O) gave **17** (15 mg, 0.015 mmol, 83%) after freeze-drying; $[\alpha]_D$ +70° (*c* 0.5, H_2O);); ^{1}H NMR (D_2O) δ 2.05 (s, 6H), 2.06 (s, 3H), 2.14–2.18 (m, 4H), 2.71 (s, 2H), 3.26–3.27 (m, 2H), 3.61–3.86 (m, 10H), 3.97–4.23 (m, 10H), 4.38–4.40 (m, 2H), 4.44 (m, 1H), 4.84 (m, 1H); ^{13}C NMR (D_2O) δ 8.8 [($CH_3CH_2)_3N$], 22.5, 22.7, 23.9 (CH_3CONH), 27.8 (d, J = 134 Hz, C-7′, C-7″), 39.5 (OCH_2CH_2NH), 47.1 [($CH_3CH_2)_3N$], 52.8 (C-2), 53.2 (d, J = 9.7 Hz, C-2′, C-2″), 60.9 (C-6″), 63.6, 64.0, 66.9, 67.3, 67.8, 69.2, 69.5, 69.7, 72.1, 72.2, 73.2, 74.2, 74.5 (C-3–6, C-1′, C-3′–6′, C-1″, C-3″–5″, O$CH_2CH_2NH_2$), 99.4 (C-1), 174.8, 175.4 (CH_3CONH); ^{31}P NMR (D_2O) δ 22.72, 22.75; HRMS: calc. for $C_{28}H_{51}N_4O_{20}P_2$ $[M\ 2+\ H]^+$: 825.2572, found: 825.2608.

2-(Benzyloxycarbonyl)aminoethyl 6-*O*-[methyl *C*-(6-*O*-[methyl *C*-(6-*O*-[methyl *C*-(6-*O*-acetyl-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranoside (18). Compound **15** (35 mg, 0.023 mmol) and compound **8** (29 mg, 0.046 mmol) were dissolved in THF (0.5 mL). To this solution, tris(4-chlorophenyl)phosphine (17 mg, 0.046 mmol), DIAD (9 μL, 0.046 mmol) and Et_3N (16 μL, 0.115 mmol) were added. The reaction mixture was stirred under argon at rt for 30 min. The mixture was then directly purified by silica gel chromatography (1 : 0 → 20 : 1 $CHCl_3$–MeOH). Concentration of the fractions containing the product and further purification on a LH-20 gel column (MeOH) gave **18** (40 mg, 0.020 mmol, 87%); HRMS: calc. for $C_{106}H_{131}N_5O_{30}P_3$ $[M + H]^+$: 2046.8086, found: 2046.8000.

2-(Benzyloxycarbonyl)aminoethyl 6-*O*-[*C*-(6-*O*-[*C*-(6-*O*-[*C*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranoside tris(triethylammonium) salt (20). To a solution of compound **18** (54 mg, 0.026 mmol) in MeOH (1.5 mL), KOH (32 μL) from a stock solution of KOH, in MeOH (1M) was added. After 20 min the mixture was purified by silica gel chromatography (1 : 0 → 10 : 1 $CHCl_3$–MeOH). The product was then further purified on a LH-20 gel (MeOH) to give 2-(benzyloxycarbonyl)aminoethyl 6-*O*-[methyl *C*-(6-*O*-[methyl *C*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranoside (**19**, 39 mg, 0.019 mmol, 73%). To compound **19** (39 mg, 0.019 mmol) dissolved in CH_3CN (0.5 mL), thiophenol (80 μL, 0.78 mmol) and DBU (116 μL, 0.78 mmol) were added. The mixture was stirred under argon at rt for 2 h and then put on top of a silica gel column and eluted (1 : 0 → 5 : 1 $CHCl_3$–MeOH + 1.5% Et_3N). Additional purification of the product on a LH-20 gel (MeOH) afforded compound **20** (30 mg, 0.013 mmol, 68%); ^{13}C NMR ($CDCl_3$) δ 9.00 [($CH_3CH_2)_3N$], 23.2, 23.3, 23.5 (CH_3CONH), 30.2 (m, C-7, C-7″, C-7‴), 40.8 (OCH_2CH_2NH), 47.0 [($CH_3CH_2)_3N$], 48.8, 49.6 (C-2, C-2′, C-2″, C-2‴), 59.1, 63.9, 66.8, 71.1, 71.5, 72.3, 72.6, 72.9, 73.4, 73.9, 75.2, 76.1 (C-3–6, C-1′ , 3′–6′ , C-1″, C-3″–6″, C-1‴, C-3‴–6‴, PhCH_2O, OCH_2CH_2NH), 100.0 (C-1), 127.3–128.8, 136.7, 137.6, 138.1, 138.5, 138.9 (aromatic C), 156.5 (NH*C*OOBn), 170.6, 170.7, 171.2 (CH_3CONH); ^{31}P NMR ($CDCl_3$) δ 18.7 (2P), 21.4; HRMS: calc. for $C_{101}H_{121}N_5O_{29}P_3$ $[M + 2H]^-$: 1960.7366, found: 1960.7297.

2-Aminoethyl 6-*O*-[*C*-(6-*O*-[*C*-(6-*O*-[*C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate]-2-acetamido-2-deoxy-α-D-mannopyranoside tris(triethylammonium) salt (21). A catalytic amount of palladium on activated carbon and HCl (50 μL, 1 M) was added to a solution of compound **20** (29 mg, 0.013 mmol) in MeOH (4.5 mL). The mixture was hydrogenolysed at 100 psi overnight, diluted (MeOH) and centrifuged to remove the activated carbon. Concentration of the supernatant and purification on a reversed phase silica gel column (H_2O) gave **21** (15 mg, 0.011 mmol, 85%) after freeze-drying; $[\alpha]_D$ +13° (*c* 1.0, H_2O); ^{13}C NMR (D_2O) δ 8.6 [($CH_3CH_2)_3N$], 22.6, 22.7 (CH_3CONH), 27.4, 28.7 (C-7′, C-7″, C-7‴), 39.7 (OCH_2CH_2NH), 47.3 [($CH_3CH_2)_3N$], 53.0 (C-2), 53.6 (m, C-2′, C-2″, C-2‴), 61.1, 63.8, 64.2, 67.2, 67.4, 67.4, 68.0, 69.4, 69.8, 69.8, 69.9, 73.5, 74.4, 74.5, 74.7 (C-3–6, C-1′, C-3′–6′, C-1″, C-3″–6″ , C-1‴, C-3‴–6‴, OCH_2CH_2NH), 99.6 (C-1), 175.1, 175.8, 175.9 (CH_3CONH); ^{31}P NMR (D_2O) δ 22.2 (2P), 22.7; HRMS: calc. for $C_{37}H_{65}N_5O_{27}P_3$ $[M + 2H]^-$: 1106.3242, found: 1106.3206.

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