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Synthesis and Biological Evaluation of Phosphono Analogues of Capsular Polysaccharide Fragments from *Neisseria meningitidis* A

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Abstract: *Neisseria meningitidis* type A (MenA) is a Gram-negative encapsulated bacterium that may cause explosive epidemics of meningitis, especially in the sub-Saharan region of Africa. The development and manufacture of an efficient glycoconjugate vaccine against *Neisseria meningitidis* A is greatly hampered by the poor hydrolytic stability of its capsular polysaccharide, which is made up of (1→6)-linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate repeating units. Since this chemical lability is a product of the inherent instability of the phosphodiester bridges, here we report the synthesis of phosphonoester-linked oligomers of *N*-acetyl mannosamine as candidates for stabilised analogues of the corresponding phosphate-bridged saccharides. The installation of each interglycosidic phosphonoester linkage was achieved by Mitsunobu coupling of a glycosyl *C*-phosphonate building block with the 6-OH moiety of a mannosaminyl residue. Each of the synthesised compounds contains an *O*-linked aminopropyl spacer at its reducing end (α- or β-oriented) to allow for protein conjugation. The relative affinities of the synthetic molecules were investigated by a competitive ELISA assay and showed that a human polyclonal anti-MenA serum can recognise both the phosphonoester-bridged fragments **1**–**3** and their monomeric subunits, glycosides **20** and **21**. Moreover, the biological results suggest that the abilities of these compounds to inhibit the binding of a specific antibody to MenA polysaccharide are dependent on the chain lengths of the molecules, but independent on the orientations of the anomeric linkers.

Keywords: carbohydrates · phosphonates · immunology · Mitsunobu reaction · *Neisseria meningitidis*

Introduction

Meningitis is a severe bacterial or viral infection of the meninges, still capable of significant impacts on public health. In particular, bacterial forms of meningitis are caused by several kinds of bacteria, but over 80% of the infections recorded worldwide per year are mainly due to three pathogens: *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib).[1] *N. meningitidis* is a Gram-negative bacterium that exclusively inhabits the nasopharynx in about 10% of the human adult population. The bacterium enters the bloodstream, where it multiplies to high density, after which it can cross the blood–brain barrier and cause meningitis. This invasive infection mostly affects infants, children and adolescents, who do not possess specific antibodies. After infection, some of these subjects may develop the disease within a few hours and, of them, about 5–15% die while up to 25% develop permanent damages, such as epilepsy, mental retardation or sensorineural deafness.[2] *N. meningitidis* belongs to the class of encapsulated bacteria: its outer membrane is surrounded by a polysaccharide coat (capsule) that is essential for its pathogenicity, exerting a protective function against the host's immune defence. From the chemical composition of the polysaccharide capsule, 13 capsular serogroups of *N. meningitidis* have so far been defined, although about 90% of infections are caused by the serotypes A, B, C, Y and W135. All these se-

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rotypes can cause epidemics, but their relative incidences are strictly dependent on geographic area. Serogroups B and C (MenB and MenC) are the predominant disease-causing organisms in most of the industrialised countries, while group A strains (MenA) are the main types responsible for epidemics in sub-Saharan Africa (the so-called "meningitis belt"), where the annual disease incidence ranges from 100 to 800 per 100 000 of the population.[1,3]

Literature data clearly demonstrate that resistance to this type of infection is mediated by the production of specific antibodies against the bacterial capsular polysaccharides (CPSs), suggesting that a vaccine composed of purified CPSs as antigenic material may be effective for protection against meningococcal diseases. Tetravalent vaccines of this type, containing the CPSs of serogroups A, C, Y, and W135, have been available for use in adults for three decades.[2] However, these vaccines have limited clinical usefulness. In fact, a) they are T-cell-independent immunogens, b) they induce only short-lasting antibody responses in adults, and c) they are poorly immunogenic in infants and young children.[1] During childhood, particularly, serogroup A vaccines show 90% efficacy over the first year, but almost no protection after 1 year post-vaccination. Moreover, they do not offer long-term immunological memory.[1,4]

Immune response against CPSs can be improved by chemical conjugation of the capsule saccharide components to a protein carrier[5] (typically tetanus toxoid or CRM197, a nontoxic variant of diphtheria toxin), which invokes T-cell recruitment.[6] In this way, boosting of the antibody response occurs on reimmunisation and immunological memory is established.[3a,7] Glycoconjugate vaccines of this kind have been demonstrated to be safe and effective in eliminating the disease; a working glycoconjugate vaccine against MenC has recently been employed in a large-scale vaccination campaign in the United Kingdom,[8] and the development of vaccines against the other groups is under way.[2,9] However, glycoconjugate vaccines, generated from partially hydrolysed size fractionated CPS fragments, are often heterogeneous and difficult to characterise, so the chemical synthesis of highly pure CPS fragments of variable length is essential. Successful examples of synthetic oligosaccharide-protein conjugates have indeed been reported in the literature for *Shigella dysenteriae* type 1[10] and Hib.[11]

Furthermore, the poor stability in water of the CPS fragments employed in the protein conjugation could hamper the development of a fully synthetic glycoconjugate vaccine. This property is mainly a consequence of the nature of the *O*-glycosidic bonds joining the saccharide units, which can be readily hydrolysed in the presence of acids or glycosidases.

The structure of MenA CPS, consisting of (1→6)-linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate residues (Figure 1),[12] offers a typical example of a polysaccharide with poor stability in water.[13]

Chemical lability derives from the inherent instability of the anomeric phosphodiester groups bridging two *N*-acetyl mannosamine units. It should be noted that other research

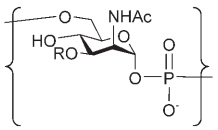


Figure 1. Structure of the repeating unit of the capsular polysaccharide of *N. meningitidis* type A. Structural heterogeneity derives from partial 3-*O*-acetylation (R = H or Ac).

groups have exploited this feature by using anomeric dialkyl phosphates as donors in glycosylation reactions.[14]

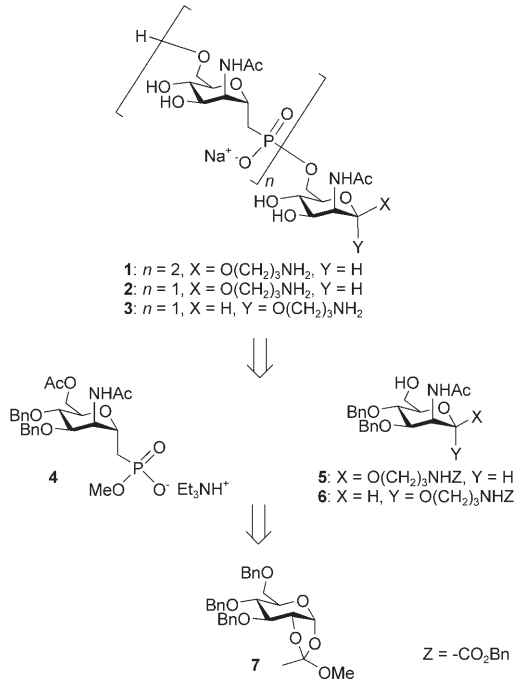
Since glycoconjugate vaccines against *N. meningiditis* A are mainly intended for use in the "meningitis belt" countries, the availability of stable molecules with long shelf-lives is very important. Chemically modified structures (synthetic analogues), endowed both with the immunological properties of the natural compounds (i.e., the ability to induce the production of antibodies that will cross-react with the bacterial capsule) and with an increased stability in water are currently under evaluation.

Sugar 1-*C*-phosphonates have been used as isosteric and nonhydrolysable analogues of glycosyl 1-*O*-phosphates to inhibit a variety of enzymes.[15] In a preliminary communication, we described the synthesis of the phosphono analogue of the dimer of MenA CPS.[16] Here, together with a full account of our previous investigation, we wish to report the synthesis of phosphonoester-bridged fragments **1–3** (Scheme 1) of MenA CPS by an improved and more straightforward synthetic approach. It should be noted that compounds **1–3** each bears an *N*-protected aminopropyl spacer arm suitable for protein conjugation. This linker is β-oriented in fragments **1–2**, while in dimer **3** it is α-linked. In this way we were able to assess the influence of the anomeric configuration at the reducing terminus on the affinity of saccharide for antibody binding.

The relative affinities of oligomers **1–3**, as well as those of their spacer-bearing, monomeric glycosides **20** and **21**, were investigated by a competitive ELISA method. The data we obtained show that the orientation of the anomeric linker does not modify the affinities of the saccharides for antibodies. The chain lengths of the saccharides, however, do seem to influence the efficacies of the compounds.

Results and Discussion

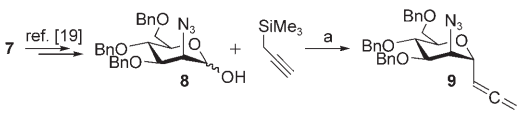
Synthesis: Our approach to the synthesis of mimetic fragments of MenA CPS is outlined in Scheme 1 and is based on Mitsunobu coupling[17] for the creation of the interglycosidic phosphonoester bridges. Oligomers **1–3** were derived from monosaccharide building blocks **4**, **5** (compounds **1–2**) and **6** (compound **3**), which were in turn obtained from the known orthoester **7**. Intermediate **4** contains the α-oriented anomeric phosphonoester unit suitable for Mitsunobu coupling with the 6-*O*-unprotected 2-acetamido-2-deoxy-D-mannopyranosides **5** and **6** bearing the β- and α-oriented anomeric linkers, respectively.



Scheme 1. Retrosynthetic strategy for the synthesis of phosphono analogues of *N. meningitidis* A capsular polysaccharide fragments.

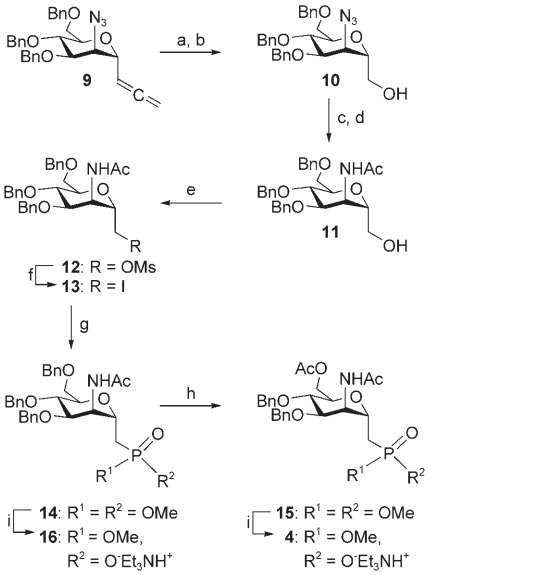
Moreover, the 6-*O*-acetyl group of compound **4** can easily be removed for chain elongation.

Although we had reported the synthesis of the phosphono analogue of *N*-acetyl-α-D-mannosamine 1-phosphate a few years ago,[18] scaling up of that procedure proved unfeasible for the preparation of our key intermediate phosphonate **4**, so we decided to design a different route to such molecules, taking advantage of our procedure that we had used to obtain 2-azido-2-deoxymannopyranosidic building blocks on multigram scales.[19] In this way, the orthoester **7** was converted into the hemiacetal **8** in five steps[19] (Scheme 2). For the attachment of the key α-*C*-glycosidic appendage onto the anomeric carbon of compound **8** we envisaged the allene as a suitable synthetic equivalent of the hydroxymethyl function through ozonolysis with reductive workup. Accordingly, **8** was treated with propargyl trimethylsilane in the presence of various Lewis acids under different reaction conditions. After extensive exploration, we found that the α-*C*-allenyl derivative **9** could be obtained in reasonable yields (58%) and with excellent stereoselectivity (96:4 α/β ratio) through the use of $BF_3{\cdot}Et_2O$ as a Lewis acid in combination with 10 equiv of propargyl trimethylsilane at 50°C (Scheme 2).[20]



Scheme 2. Synthesis of 1-*C*-allene **9**: a) $BF_3{\cdot}OEt_2$, 0.2 M CH_3CN, 0 → 50°C, 5–7 h, 58%.

The introduction of the phosphonate moiety was accomplished as shown in Scheme 3. Allene **9** was subjected to ozonolysis in dichloromethane at −78°C, and reductive workup with sodium borohydride delivered alcohol **10** in 95% yield. Reduction of the azido group under Staudinger's conditions,[21] followed by N-acetylation, gave the acetamido derivative **11** in 96% overall yield.

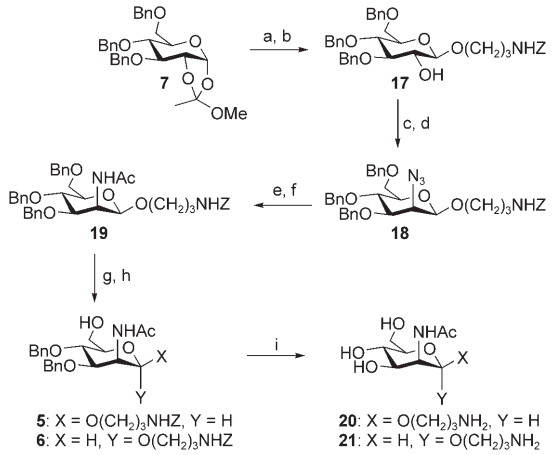


Scheme 3. Synthesis of glycosyl phosphonates **4** and **16**: a) O_3, CH_2Cl_2, −78°C, 10 min; b) $NaBH_4$, THF/H_2O 7:3, −78°C → RT, 95% over two steps; c) PPh_3, THF, RT, 3 h, then H_2O, 14 h; d) Ac_2O, MeOH, RT, 30 min, 96% over two steps; e) CH_3SO_2Cl, Py, CH_2Cl_2, RT, 24 h, 88%; f) NaI, butanone, 100°C, 16 h; g) $P(OMe)_3$, 100°C, vacuum, 42 h, 84% from **12**; h) $ZnCl_2$, Ac_2O/AcOH 2:1, RT, 16 h, 92%; i) Et_3N, PhSH, THF, RT, 24 h: **4** (89%), **16** (93%).

Conversion of the hydroxymethyl group into the iodomethyl function suitable for the Arbuzov reaction was performed in two steps. Firstly, alcohol **11** was transformed into the *O*-mesylated compound **12** (88%), and nucleophilic displacement with sodium iodide in butanone then yielded the labile iodo derivative **13**. It should be noted that the direct conversion of alcohol **11** into **13** by use of iodine and triphenylphosphine (the Garegg reaction)[22] occurred in much lower yield. The synthesis of the glycosyl phosphonate **14** was achieved by a modified Arbuzov procedure:[23] the crude iodo derivative **13** was dissolved in freshly distilled trimethyl phosphite and the reaction mixture was heated at 100°C under vacuum (water pump), in order to remove the volatile methyl iodide. Dimethyl phosphonodiester **14** was obtained in 84% overall yield from **12** (30% without pumping) and was subjected to regioselective acetolysis with $ZnCl_2$ in Ac_2O/AcOH,[24] furnishing the 6-*O*-acetylated derivative **15** in 92% yield. Finally, selective monohydrolysis of **15** with triethylamine and thiophenol in THF[25] gave the monoester **4** as a triethylammonium salt in 89% yield. On the other hand, glycosyl phosphonate **16** was obtained by monodemethylation of **14** as described above in the case of

15 and was employed in our first synthesis[16] of dimer **2** (see below).

The synthesis of the spacer-bearing mannopyranosides **5** and **6** was achieved by the divergent approach illustrated in Scheme 4.



Scheme 4. Synthesis of spacer-bearing mannopyranosides. a) Benzyl *N*-(3-hydroxypropyl)carbamate, TMSOTf (cat.), CH_2Cl_2, 0 °C, 2.5 h; b) NaOMe, MeOH, RT, 2 h, 85% over two steps; c) Tf_2O, Py, CH_2Cl_2, 0 °C, 30 min; d) $Bu_4N^+N_3^-$, toluene, 55–60 °C, 24 h, 64% over two steps; e) PPh_3, THF, RT, 3 h, then H_2O, 14 h; f) Ac_2O, MeOH, RT, 30 min, 93% over two steps; g) $ZnCl_2$, Ac_2O/AcOH 2:1, RT, 2–4 h for **5**, 24 h for **6**; h) NaOMe, MeOH, RT, 1 h: **5** (74% over two steps), **6** (50% over two steps); i) MeOH/H_2O 1:1, 10% Pd/C, H_2: **20** (95%), **21** (96%).

TMSOTf-catalysed opening of orthoester **7** with commercially available benzyl *N*-(3-hydroxypropyl)carbamate and subsequent 2-*O* deacetylation afforded glycoside **17** in 85% yield over two steps. The 2-OH group was then activated as a triflate with trifluoromethanesulfonic anhydride in dichloromethane, followed by nucleophilic displacement with freshly prepared tetrabutylammonium azide, providing β-2-azidomannopyranoside **18** in 64% yield. Conversion of the azide into the acetamide occurred smoothly under Staudinger's conditions to afford compound **19** in 93% yield.

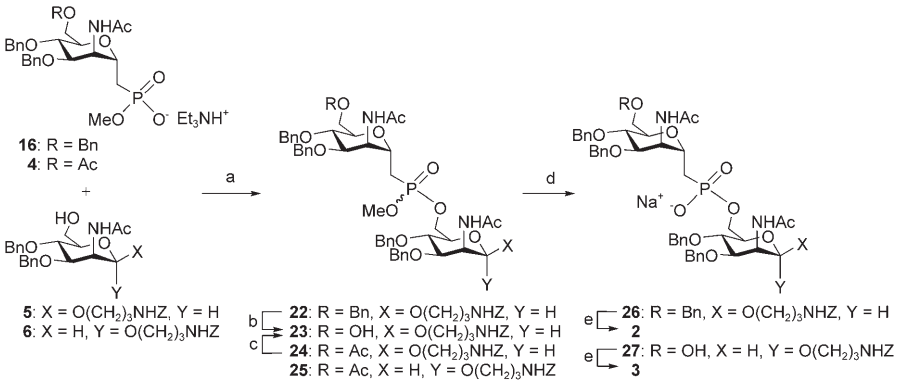
Acetolysis of **19** provided access either to compound **5** or to its α anomer **6**. Treatment of **19** with $ZnCl_2$ in Ac_2O/AcOH resulted in the disappearance of the starting material in 2–4 h and afforded a mixture of 6-*O*-monoacetylated and 6-*O*,3′-*N*-diacetylated derivatives. The crude mixture was directly subjected to Zemplén conditions to provide alcohol **5** in 74% overall yield after chromatographic purification. However, when acetolysis was allowed to proceed for 24 h, two compounds were recovered after deacetylation. Alcohol **5** was obtained in a small amount (20%), while its α-anomer **6** was recovered as the major product (50% from **19**). We conjectured that this reaction outcome is the result of a $ZnCl_2$-assisted, thermodynamically controlled ring-opening/ring-closure sequence affording the more thermodynamically stable α anomer as the main product.

The anomeric configurations of alcohols **5** and **6** were ascertained by 2D-NOESY experiments and measurement of $^1J_{C\text{-}1,H\text{-}1}$ values. The NOESY spectrum of compound **5** showed H-1/H-3 and H-1/H-5 contacts, which are consistent with an axial orientation of H-1 and with an anomeric β configuration. In contrast, these contacts were not observed in the NOESY spectrum of **6**, as would be expected for the α anomer. Moreover, measurement of the one-bond ^{1}H-1–^{13}C-1 coupling constants further confirmed these assignments. Alcohol **5** gave a value of $J = 161$ Hz, while the α configuration of compound **6** was shown by a J value of 173 Hz, consistently with literature data.[26]

For immunological tests, two samples of alcohols **5** and **6** were fully deprotected by hydrogenolysis over Pd on carbon to afford the monomeric glycosides **20** and **21**, respectively.

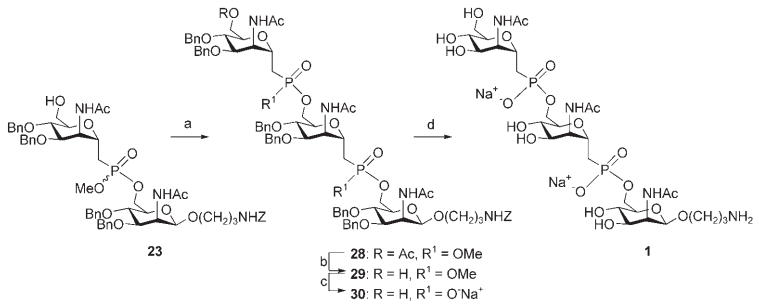
The stage was now set for the synthesis of fragments **1**–**3**. As described above, preliminary experiments to achieve the new phosphonoester linkage were carried out with use of tri-*O*-benzylated phosphonate **16** and alcohol **5** as substrates. Early attempts to accomplish the crucial coupling step by DCC-mediated esterification of **16** or via the phosphonochloridate[27] (derived from **14**) failed. Eventually, phosphonate **16** and alcohol **5** were condensed under Mitsunobu conditions[28] in the presence of triphenylphosphine and diisopropyl azadicarboxylate (DIAD) in dry THF to afford the anomerically pure phosphonoester-linked dimer **22** as a mixture of phosphorus diastereoisomers (97% yield; Scheme 5).

Further chain elongation required selective access to the 6′-OH group of the phosphonodisaccharide. Notably, the 6′-*O*-benzyl group of **22** was removed by regio- and chemose-



Scheme 5. Synthesis of phosphonoester-bridged dimers of MenA CPS. a) PPh_3, DIAD, THF, 0 °C → RT, 24 h: **22** (97%), **24** (90%), **25** (84%); b) $ZnCl_2$, Ac_2O/AcOH 2:1, RT, 36 h, then NaOMe, MeOH, RT, 18 h, 70%; c) NaOMe, MeOH, RT, 14 h, 76%; d) dimer **26** (from **22**): Et_3N, PhSH, toluene, reflux, 36 h, then MeOH, Amberlite IR-120 resin (Na^+ form), 95%; dimer **27** (from **25**): NaOMe, MeOH, RT, 6 h, then DBU, PhSH, THF, RT, 7 h, then MeOH, Amberlite IR-120 resin (Na^+ form), 75%; e) MeOH/H_2O 1:1, 10% Pd/C, H_2, then H_2O, Dowex 50W X8 resin (H^+ form), then Dowex 50W X8 resin (Na^+ form): **2** (96%), **3** (92%). DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DIAD = diisopropyl azadicarboxylate.

lective acetolysis with $ZnCl_2$ in Ac_2O/AcOH followed by Zemplén deacetylation to provide the 6′-*O*-unprotected dimer **23** (mixture of phosphorus diastereoisomers) in 70% overall yield. However, the fairly harsh conditions of the acetolysis reaction prompted us to investigate a more reliable route towards the synthesis of higher oligomers, based on phosphonoester **4** as the key intermediate. Accordingly, Mitsunobu coupling of 6-*O*-acetylated phosphonate **4** with alcohol **5** provided dimers **24** (90%, mixture of phosphorus diastereoisomers) from which the same compound **23** was obtained in a simpler way by Zemplén 6′-*O*-deacetylation (76%). Finally, Mitsunobu coupling of **4** with **6** afforded α,α-dimers **25** in 84% yield (mixture of phosphorus diastereoisomers).



Scheme 6. Synthesis of the phosphonoester-bridged trimer of MenA CPS. a) **4**, PPh_3, DIAD, THF, 0°C → RT, 24 h, 83%; b) NaOMe, MeOH, RT, 6 h; c) DBU, PhSH, THF, RT, 8 h, then MeOH, Amberlite IR-120 resin (Na^+ form), 76% from **28**; d) MeOH/H_2O 1:1, 10% Pd/C, H_2, then H_2O, Dowex 50W X8 resin (H^+ form), then Dowex 50W X8 resin (Na^+ form), 84%.

Full deprotection of dimers **22** and **25** was achieved as follows. Selective monohydrolysis of the phosphonoesters **22** by treatment with thiophenol and triethylamine in refluxing toluene, followed by ion exchange on Amberlite IR-120 resin (Na^+ form), converted the diastereoisomeric mixture into the single compound **26** (sodium salt) in 95% yield. On the other hand, 6′-*O*-deacetylation of **25**, followed by demethylation and ion exchange on Amberlite IR-120 resin (Na^+ form), provided the single compound **27** as a sodium salt (75% from **25**).

It should be noted that the demethylation of **25** was much faster when 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was used instead of triethylamine as a base (7 h at room temperature against 36 h in refluxing toluene). The remaining protecting groups (benzyl and benzyloxycarbonyl) on dimers **26** and **27** were removed by hydrogenolysis over Pd on carbon. Final purification was accomplished by elution of a water solution of the deprotected fragments over a column filled with Dowex 50W X8 resin (H^+ form), followed by a second ion exchange on the same resin in Na^+ form. Lyophilisation of the eluates provided the dimers **2** and **3** as their sodium salts in 96 and 92% yields, respectively.

Next, the panel was completed with the synthesis of trimer **1** (Scheme 6). Satisfyingly, when phosphonate **4** and dimer **23** were subjected to Mitsunobu conditions, trimer **28** was obtained as a mixture of phosphorus diastereoisomers in 83% yield. Zemplén deacetylation of **28** showed that selective access to the primary hydroxy group at the nonreducing terminus for further elongation of **29** by iteration of the Mitsunobu protocol with monomer **4** is still possible. The delicate double selective monohydrolysis of the methyl phosphonoesters then occurred smoothly through the use of thiophenol and DBU in THF at room temperature. Ion exchange on Amberlite IR-120 resin (Na^+ form) furnished the single compound **30** in 76% overall yield (from **28**) as a disodium salt.

Finally, hydrogenolysis of the remaining protecting groups over Pd on carbon, followed by double ion exchange as described above for the synthesis of dimers **2** and **3**, provided **1** in 84% yield.

Biology: The abilities of increasing concentrations (10^{-8}–1 mg mL^{-1}) of the newly synthesised saccharides to inhibit the binding between the MenA, coated onto plates, and anti-MenA human antibodies were evaluated by competitive ELISA assay and compared with the inhibition of either MenA (positive control) or MenY (negative control).

The inhibition curves shown in Figure 2 allowed the calculation of the maximum inhibition that each compound can elicit in the ELISA assay (relative efficacy), as well as the concentration that produces 50% of the maximum possible effect (EC_{50}) of that compound. Moreover, the relative affinity ($1/EC_{50}$) of each compound for binding of anti-polysaccharide antibody may be estimated. Analysis of the results (Table 1) showed that the phosphonoester-bridged fragments **1**–**3** are still recognised by a human polyclonal anti-MenA serum, and that the EC_{50} values for the synthetic compounds (including the monomeric glycosides **20** and **21**) are of the same order of magnitude (10^{-3} mg mL^{-1}), suggesting similar affinities for antibody binding. It is noteworthy that the presence of the phosphonate residue (compare glycosides **20**–**21** with fragments **1**–**3**) and the orientation of the anomeric linker (compare data for compounds **2** and **3**) do

Table 1. Results of competitive ELISA assay.

Substrate	EC_{50} [mg mL^{-1}]	Maximum inhibition [%][a]
MenA	6.6×10^{-6}	100
MenY	–	–
1	2.6×10^{-3}	60
2	4.0×10^{-3}	40
3	2.4×10^{-3}	40
20	5.9×10^{-4}	48
21	4.9×10^{-3}	48

[a] Measured at 10^{-1} mg mL^{-1}.

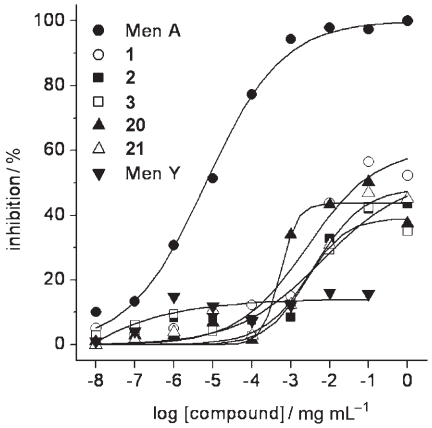


Figure 2. Concentration/response curves of saccharides on the inhibition of the binding between the MenA, coated onto the plates, and the anti-MenA human antibodies were evaluated by a competitive ELISA method (see the Experimental Section). Values are means of at least four experiments run in triplicate.

not appear to affect the affinity of saccharide for antibody binding.

In contrast, the chain lengths of the saccharide molecules seem to be important for their efficacy, and this observation is consistent with previous literature data.[29] It might be that the fragments we synthesised are too short to bind antibody with an affinity similar to that of the native polysaccharide. MenA polysaccharide indeed exhibited both a higher affinity ($EC_{50} = 6.6 \times 10^{-6}$ mg mL^{-1}) and a higher efficacy (100% of inhibition at 10^{-2} mg mL^{-1}) than the other compounds, suggesting that high molecular weight forms of the saccharide may have a conformational specificity (conformational epitopes) more suitable for antibody binding.[30]

Conclusion

In summary, we have described the synthesis of phosphonoester-bridged fragments **1–3** of MenA CPS as stabilised analogues of the corresponding phosphate-bridged oligomers. These compounds have *O*-linked aminopropyl spacer arms at the anomeric positions at the reducing ends to allow their conjugation with a carrier protein. Since the linkers are β-oriented in fragments **1–2** and α-oriented in dimer **3**, we were able to compare the influence of the anomeric configuration on the ability to inhibit antibody binding to the synthetic molecules.

The creation of the key interglycosidic phosphonoester linkages was carried out by Mitsunobu coupling. The synthetic approach was designed in order to allow selective access to the primary hydroxy group at the nonreducing terminus of each fragment. In this way, further elongation and synthesis of higher oligomers is facilitated by iteration of the Mitsunobu procedure with phosphonate **4**, as demonstrated with the synthesis of trimer **1**.[32]

Competitive ELISA assays performed on the newly synthesised saccharides showed that a human polyclonal anti-MenA serum can recognise both the phosphonoester-bridged fragments **1–3** and the spacer-bearing monomeric glycosides **20** and **21**. In addition, our data suggest that the ability of these compounds to inhibit the binding of a specific antibody to MenA polysaccharide is dependent on the chain length of the molecules, but is independent of the anomeric configuration.

Work aimed at the synthesis of higher oligomers and conjugates endowed with improved biological characteristics is in progress in our laboratory.

Experimental Section

General: NMR spectra were recorded on Bruker AC 300 and Bruker Avance 400 spectrometers at 298 K, unless otherwise reported. In ^{13}C NMR spectra, signals corresponding to aromatic carbons are omitted. Chemical shifts are reported on the δ (ppm) scale and in ^{31}P spectra they are relative to H_3PO_4. Peak assignments were based on analysis of 2D spectra (H,H-COSY and HSQC or HMQC spectra). HRMS spectra were recorded in the negative or positive modes on Jeol AX-505 and Bruker Daltonics APEX^{TM} II (FT-ICR) instruments. Optical rotations were measured at room temperature with a Perkin–Elmer 241 polarimeter. TLC and HPTLC were carried out on Merck silica gel 60 F-254 plates (0.25 mm and 0.2 mm thickness, respectively), and spots were viewed by spraying with a solution containing H_2SO_4 (31 mL), ammonium molybdate (21 g) and $Ce(SO_4)_2$ (1 g) in 500 mL water, followed by heating at 110 °C for 5 min. Column chromatography was performed by the flash procedure on Merck silica gel 60 (230–400 mesh). Solvents were dried by standard procedures.

1-*C*-(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)-allene (9): Propargyltrimethylsilane (10 mL, 66.8 mmol), and then $BF_3{\cdot}Et_2O$ (834 μL, 6.68 mmol), were added under argon at 0 °C to a solution of alcohol **8** (3.18 g, 6.68 mmol) in acetonitrile (33 mL). The mixture was heated at 50 °C. After 5–7 h (TLC, hexane/AcOEt 7:3), the solution was neutralised with TEA and concentrated to give a crude residue, which was diluted with CH_2Cl_2, washed with brine and dried over Na_2SO_4. The residue was purified by silica gel chromatography (hexane/AcOEt 9:1) to give allene **9** as a colourless oil (1.93 g, 58%). $R_f = 0.73$ (hexane/AcOEt 7:3); $[\alpha]_D^{25} = +6.0$ ($c = 1.0$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.37$–7.20 (m, 15H; arom.), 5.20 (ddd, $J_{7,9a} = 4.2$, $J_{7,9b} = J_{7,1} = 6.8$ Hz, 1H; H-7), 4.85 (d, $J = 11.0$ Hz, 1H; CH*H*Ph), 4.82–4.67 (m, 3H; H-9a, H-9b, H-1), 4.76 (d, $J = 11.7$ Hz, 1H; CH*H*Ph), 4.71 (d, 1H; CH*H*Ph), 4.65 (d, $J = 12.2$ Hz, 1H; CH*H*Ph), 4.56 (d, 1H; CH*H*Ph), 4.54 (d, 1H; CH*H*Ph), 4.20 (t, 1H; H-2), 3.95 (dd, $J_{2,3} = 3.6$, $J_{4,3} = 8.7$ Hz, 1H; H-3), 3.80 (t, $J_{5,4} = 8.7$ Hz, 1H; H-4), 3.78 (m, 1H; H-5), 3.74 (dd, $J_{6a,6b} = 10.1$, $J_{6a,5} = 5.0$ Hz, 1H; H-6a), 3.72 ppm (dd, $J_{6b,5} = 5.3$ Hz, 1H; H-6b); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 207.50$ (C-8), 89.08 (C-7), 79.09 (C-3), 77.89 (C-9), 75.16, 73.48, 72.36 (3×*C*H$_2$Ph), 75.16 (C-4), 74.02 (C-5), 72.73 (C-1), 69.03 (C-6), 60.82 ppm (C-2); HRMS (MALDI): *m*/*z* (%): 520.2247 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{30}H_{31}N_3O_4$ (497.6): C 72.41, H 6.28, N 8.44; found: C 72.34, H 6.27, N 8.43.

***C*-(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)-methanol (10)**: O_3 was bubbled through a solution of allene **9** (0.32 g, 0.64 mmol) in dry CH_2Cl_2 (24 mL), cooled at −78 °C, until the reaction mixture became grey-blue (10 min). Argon was then bubbled through the mixture for 30 min until the starting material had disappeared (TLC, hexane/AcOEt 7:3). Sodium borohydride (0.076 mg, 2.01 mmol) in THF/H_2O 7:3 (4.40 mL) was then added dropwise at −78 °C and the temperature was allowed to rise to room temperature. The solution was diluted with CH_2Cl_2, washed with brine and dried over Na_2SO_4. The crude product was purified by silica gel chromatography (hexane/AcOEt 3:1→2:1) to give **10** as a colourless oil (0.30 g, 95%). $R_f = 0.32$ (hexane/AcOEt 7:3); $[\alpha]_D^{25} = +4.8$ ($c = 1.0$ in chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 7.37–7.10 (m, 15H; arom.), 4.62 (d, $J = 11.6$ Hz, 1H; CH*H*Ph), 4.59 (s, 2H; CH_2Ph), 4.58 (d, 1H; CH*H*Ph), 4.48 (s, 2H; CH_2Ph), 4.05 (ddd,

$J_{1,7a}=J_{1,7b}=4.7$, $J_{1,2}=8.1$ Hz, 1H; H-1), 3.92 (m, 1H; H-4), 3.90 (t, $J_{3,4}=J_{2,3}=4.6$ Hz, 1H; H-3), 3.78 (dd, $J_{7a,7b}=10.1$ Hz, 1H; H-7a), 3.76–3.72 (m, 3H; H-5, H-6a, H-6b), 3.70 (dd, 1H; H-2), 3.62 ppm (dd, 1H; H-7b); ^{13}C NMR (75.2 MHz, $CDCl_3$): $\delta=77.07$ (C-3), 74.34 (C-4, C-5), 73.37, 73.25, 72.95 (3×CH_2Ph), 71.15 (C-1), 68.11 (C-6), 61.86 (C-7), 56.92 ppm (C-2); HRMS (MALDI): *m/z* (%): 512.2184 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{28}H_{31}N_3O_5$ (489.6): C 68.69, H 6.38, N 8.58; found: C 68.79, H 6.37, N 8.60.

***C*-(2-Acetamido-3,4,6-tri-O-benzyl-2-deoxy-α-D-mannopyranosyl)-methanol (11)**: A solution of compound **10** (0.27 g, 0.54 mmol) and PPh_3 (0.16 g, 0.60 mmol) in dry THF (2.80 mL) was stirred at room temperature for 3 h, H_2O (35 μL, 2.17 mmol) was then added, and the solution was stirred at room temperature for an additional 20 h and finally concentrated. The crude amine was dissolved in MeOH (3 mL), acetic anhydride (887 μL) was added, and the solution was stirred at room temperature for 1 h and then concentrated to dryness. The residue was purified by silica gel chromatography (hexane/AcOEt 1:9) to afford **11** as an oil (0.25 g, 96%). $R_f=0.21$ (hexane/AcOEt 1:9); $[\alpha]_D^{25}=+47.6$ ($c=1.0$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.40$–7.15 (m, 15H; arom.), 5.65 (d, $J=8.9$ Hz, 1H; NH), 4.68 (d, $J=11.8$ Hz, 1H; CH*H*Ph), 4.56 (d, $J=11.2$ Hz, 1H; CH*H*Ph), 4.54 (d, $J=10.6$ Hz, 1H; CH*H*Ph), 4.53 (d, 1H; CH*H*Ph), 4.52 (d, 1H; CH*H*Ph), 4.33 (dt, $J_{2,1}=J_{2,3}=3.5$ Hz, 1H; H-2), 4.27 (dt, $J_{5,6a}=1.8$, $J_{5,6b}=J_{5,4}=5.8$ Hz, 1H; H-5), 4.21 (d, 1H; CH*H*Ph), 3.80 (dd, $J_{6a,6b}=10.8$ Hz, 1H; H-6a), 3.77 (dd, 1H; H-4), 3.75 (m, 1H; H-6b), 3.68 (t, $J_{3,2}=J_{3,4}=3.5$ Hz, 1H; H-3), 3.67–3.60 (m, 2H; H-7a, H-7b), 3.55 (dt, $J_{1,2}=3.5$, $J_{1,7a}=J_{1,7b}=6.5$ Hz, 1H; H-1), 3.38 (s, 1H; OH), 1.85 ppm (s, 3H; $COCH_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta=$ 171.10 (CO), 76.53 (C-3), 73.76 (C-5), 73.56, 72.39, 72.21 (3×CH_2Ph), 71.54 (C-1), 71.31 (C-4), 68.15 (C-6), 62.46 (C-7), 45.12 (C-2), 23.52 ppm (CH_3CO); HRMS (MALDI): *m/z* (%): 528.2387 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{30}H_{35}NO_6$ (505.6): C 71.27, H 6.98, N 2.77; found: C 71.20, H 6.99, N 2.78.

***C*-(2-Acetamido-3,4,6-tri-O-benzyl-2-deoxy-α-D-mannopyranosyl)methyl methanesulfonate (12)**: A solution of compound **11** (0.94 g, 1.86 mmol) in dry CH_2Cl_2 (27 mL) was cooled at 0°C under nitrogen, after which pyridine (0.9 mL, 11.13 mmol) and mesyl chloride (0.43 mL, 5.59 mmol) were added. The reaction mixture was stirred at room temperature overnight and then diluted with CH_2Cl_2, washed with aq. HCl (5%) and satd. aq. $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated to give a crude syrup. The residue was purified by silica gel chromatography (hexane/AcOEt 1:6) to give compound **12** as an oil (0.95 g, 88%). $R_f=0.74$ (hexane/AcOEt 1:9); $[\alpha]_D^{25}=+42.2$ ($c=1.0$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.40$–7.15 (m, 15H; arom.), 5.65 (d, $J=9.1$ Hz, 1H; N–H), 4.60 (d, $J=12.0$ Hz, 1H; CH*H*Ph), 4.56 (d, 1H; CH*H*Ph), 4.53 (d, $J=10.6$ Hz, 1H; CH*H*Ph), 4.52 (d, $J=12.4$ Hz, 1H; CH*H*Ph), 4.51 (d, 1H; CH*H*Ph), 4.34–4.22 (m, 3H; H-2, H-7a, H-7b), 4.24 (d, 1H; CH*H*Ph), 4.15 (dt, $J_{5,4}=3.1$, $J_{5,6a}=J_{5,6b}=6.5$ Hz, 1H; H-5), 3.93 (dt, $J_{1,2}=3.1$, $J_{1,7a}=J_{1,7b}=7.4$ Hz, 1H; H-1), 3.79 (dd, $J_{6a,6b}=10.1$ Hz, 1H; H-6a), 3.72 (t, 1H; H-4), 3.66 (dd, 1H; H-6b), 3.64 (t, $J_{3,2}=J_{3,4}=3.8$ Hz, 1H; H-3), 3.01 (s, 3H; SO_2CH_3), 1.88 ppm (s, 3H; $COCH_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta=169.79$ (CO), 75.74 (C-3), 73.81 (C-5), 73.29, 72.20, 72.12 (3×CH_2Ph), 71.36 (C-4), 70.31 (C-1), 69.90 (C-7), 67.64 (C-6), 45.02 (C-2), 37.66 (SO_2CH_3), 23.23 ppm (CH_3CO); HRMS (MALDI): *m/z* (%): 606.2194 (100) $[M+Na]^+$, 622.1867 (30) $[M+K]^+$; elemental analysis calcd (%) for $C_{31}H_{37}NO_8S$ (583.7): C 63.79, H 6.39, N 2.40; found: C 63.87, H 6.38, N 2.40.

Dimethyl *C*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (14): Sodium iodide (0.42 g, 2.80 mmol) was added under nitrogen to a solution of compound **12** (0.33 g, 0.56 mmol) in butan-2-one (6.5 mL). The reaction mixture was stirred at 100°C for 24 h with monitoring of the course of the reaction by TLC (hexane/AcOEt 1:9). The solvent was removed under reduced pressure, and the crude residue was dissolved in CH_2Cl_2, washed with aq. $NaHSO_3$ (10%) and water, dried over Na_2SO_4 and concentrated to give the iodomethyl derivative **13** as an oil. This was dissolved in freshly distilled trimethylphosphite (6 mL) and the solution was heated to 100°C under vacuum (water pump) for 48 h. After concentration and silica gel chromatography ($CH_3OH/CHCl_3$ 1:100), phosphonate **14** was isolated as a colourless oil (0.28 g, 84%). $R_f=0.66$ ($CH_3OH/CHCl_3$ 1:10); $[\alpha]_D^{25}=+20.7$ ($c=1.0$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.37$–7.19 (m, 15H; arom.), 5.87 (d, $J=9.3$ Hz, 1H; NH), 4.65 (d, $J=11.7$ Hz, 1H; CH*H*Ph), 4.56 (d, 1H; CH*H*Ph), 4.55 (d, $J=11.9$ Hz, 1H; CH*H*Ph), 4.51 (d, 1H; CH*H*Ph), 4.46 (d, $J=11.5$ Hz, 1H; CH*H*Ph), 4.41 (d, 1H; CH*H*Ph), 4.36 (dt, $J_{2,1}=J_{2,3}=3.5$ Hz, 1H; H-2), 4.16 (m, 1H; H-1), 4.00 (dd, $J_{5,6a}=5.1$, $J_{5,4}=J_{5,6b}=9.7$ Hz, 1H; H-5), 3.82 (dd, $J_{6a,6b}=10.0$ Hz, 1H; H-6a), 3.78–3.69 (m, 3H, H-3, H-4, H-6b), 3.74 (d, $J_{Me,P}=11.3$ Hz, 3H; OMe), 3.71 (d, $J_{Me,P}=11.3$ Hz, 3H; OMe), 2.13 (dt, $J_{7a,1}=9.3$, $J_{7a,7b}=J_{7a,P}=15.7$ Hz, 1H; H-7a), 2.04 (dt, $J_{7b,1}=4.0$, $J_{7b,P}=15.7$ Hz, 1H; H-7b), 1.87 ppm (s, 3H; $COCH_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta=170.37$ (CO), 76.49 (C-3), 74.06 (C-5), 73.81, 73.35, 72.31 (3×CH_2Ph), 72.31 (C-4), 68.79 (C-1), 68.69 (C-6), 53.26 (d, $J_{Me,P}=5.9$ Hz; OMe), 52.60 (d, $J_{Me,P}=5.9$ Hz; OMe), 45.28 (d, $J_{2,P}=14.8$ Hz; C-2), 28.06 (d, $J_{7,P}=142.0$ Hz; C-7), 23.71 ppm (CH_3CO); ^{31}P NMR (162 MHz, $CDCl_3$): $\delta=32.48$ ppm; HRMS (MALDI): *m/z* (%): 620.2386 (100) $[M+Na]^+$, 636. 2132 (20) $[M+K]^+$; elemental analysis calcd (%) for $C_{32}H_{40}NO_8P$ (597.6): C 64.31, H 6.75, N 2.34; found: C 64.28, H 6.74, N 2.33.

Dimethyl *C*-(2-acetamido-6-O-acetyl-3,4-di-O-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (15): A solution of freshly fused zinc chloride (6.02 g, 44.20 mmol) in Ac_2O/AcOH 2:1 (10 mL) was added to a solution of compound **14** (2.64 g, 4.42 mmol) in Ac_2O/AcOH 2:1 (4 mL). The reaction mixture was stirred at room temperature for 16 h with monitoring of the course of the reaction by TLC (AcOEt/*i*PrOH 8:2). The reaction mixture was quenched with water, diluted with $CHCl_3$, washed with satd. aq. $NaHCO_3$ until neutralisation and then with water, dried over Na_2SO_4 and concentrated under reduced pressure to give a crude residue, which was purified by silica gel chromatography ($MeOH/CHCl_3$ 1:100) to give compound **15** (2.23 g, 92%). $R_f=0.63$ (AcOEt/*i*PrOH 8:2); $[\alpha]_D^{25}=+38.8$ ($c=0.9$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 7.41–7.25 (m, 10H; arom.), 5.78 (d, $J=9.2$ Hz, 1H; N*H*Ac), 4.64 (d, $J=$ 12.0 Hz, 1H; CH*H*Ph), 4.59 (d, 1H; CH*H*Ph), 4.58 (d, $J=12.0$ Hz, 1H; CH*H*Ph), 4.54 (dd, $J_{6a,6b}=12.0$, $J_{6a,5}=7.5$ Hz, 1H; H-6a), 4.37 (d, 1H; CH*H*Ph), 4.32 (dt, 1H; H-2), 4.22 (dd, $J_{6b,5}=4.3$ Hz 1H; H-6b), 4.17–4.08 (m, 2H; H-1, H-5), 3.78 (d, $J_{Me,P}=11.9$ Hz, 3H; OMe), 3.74 (m, 1H; H-3), 3.73 (d, $J_{Me,P}=11.0$ Hz, 3H; OMe), 3.54 (t, $J_{4,3}=J_{4,5}=3.5$ Hz, 1H; H-4), 2.14–2.02 (m, 2H; H-7a, H-7b), 2.08 (s, 3H; CH_3CO), 1.87 ppm (s, 3H; CH_3CO); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta=169.82$, 169.32 (2× CO), 75.50 (C-3), 72.95 (C-5), 72.51 (2×CH_2Ph), 71.99 (C-4), 66.93 (C-1), 61.89 (C-6), 52.81 (d, $J_{Me,P}=6.0$ Hz, OMe), 52.13 (d, $J_{Me,P}=6.0$ Hz, OMe), 48.77 (d, $J_{2,P}\approx14$ Hz, C-2), 28.04 (d, $J_{7,P}\approx148$ Hz, C-7), 23.35 (CH_3CO), 20.85 ppm (CH_3CO); ^{31}P NMR (162 MHz, $CDCl_3$): $\delta=32.41$ ppm; HRMS (ESI): *m/z* (%): 572.2007 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{27}H_{36}NO_9P$ (549.5): C 59.01, H 6.60, N 2.55; found: C 58.97, H 6.59, N 2.55.

Methyl *C*-(2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-α-D-mannopyranosyl)-methanephosphonate triethylammonium salt (16): TEA (3.0 mL, 21.96 mmol) and thiophenol (1.5 mL, 14.64 mmol) were added under nitrogen to a solution of compound **14** (0.73 g, 1.22 mmol) in dry THF (6.0 mL). The reaction mixture was stirred at room temperature for 24 h with monitoring of the course of the reaction by TLC ($CH_3OH/CHCl_3$ 1:9). The reaction mixture was diluted with TEA and concentrated to give a crude residue, which was purified by silica gel chromatography ($CH_3OH/CHCl_3$ 1:9 with 1% TEA) to provide compound **16** as an oil (0.78 g, 93%). $R_f=0.30$ ($CH_3OH/CHCl_3$ 1:9); $[\alpha]_D^{25}=+8.2$ ($c=2.5$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.35$–7.17 (m, 15H; arom.), 6.56 (d, $J=8.6$ Hz, 1H; NH), 4.89 (ddd, $J_{2,1}=4.5$, $J_{2,3}=3.3$ Hz, 1H; H-2), 4.79 (d, $J=11.1$ Hz, 1H; CH*H*Ph), 4.74 (d, $J=10.9$ Hz, 1H; CH*H*Ph), 4.57 (d, $J=11.8$ Hz, 1H; CH*H*Ph), 4.47 (d, 1H; CH*H*Ph), 4.45 (d, 1H; CH*H*Ph), 4.43 (d, 1H; CH*H*Ph), 4.28 (m, 1H; H-1), 4.03 (dd, $J_{3,4}=$ 7.9 Hz, 1H; H-3), 3.80–3.77 (m, 2H; H-5, H-6a), 3.70–3.66 (m, 2H; H-4, H-6b), 3.60 (d, $J_{Me,P}=10.3$, 3H; OMe), 3.04 (q, $J=7.3$, 6H; 3×CH_2CH_3), 2.10 (ddd, $J_{7a,1}=9.7$, $J_{7a,7b}=14.7$, $J_{7a,P}=17.6$ Hz, 1H; H-7a), 1.96 (s, 3H; $COCH_3$), 1.95 (dt, $J_{7b,1}=4.9$, $J_{7b,P}=14.7$ Hz, 1H; H-7b), 1.30 ppm (t, $J=$ 7.3 Hz, 9H; CH_2CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta=170.01$ (CO), 77.31 (C-3), 74.21, 73.36, 71.55 (3×CH_2Ph), 73.85 (C-4), 72.75 (C-5, C-1), 68.90 (C-6), 51.60 (d, $J_{Me,P}\approx5$ Hz, OMe), 49.37 (C-2), 45.28 (3×CH_2CH_3), 28.00 (d, $J_{7,P}\approx100$ Hz, C-7), 23.54 (CH_3CO), 8.49 ppm (3×CH_2CH_3); ^{31}P NMR (162 MHz, $CDCl_3$): $\delta=20.61$ ppm; HRMS (MALDI): *m/z* (%):

622.3303 (100) $[M-Et_3N+K]^+$, 606.3513 (40) $[M-Et_3N+Na]^+$; elemental analysis calcd (%) for $C_{37}H_{53}N_2O_8P$ (684.8): C 64.89, H 7.80, N 4.09; found: C 65.02, H 7.78, N 4.08.

Methyl *C*-(2-acetamido-6-O-acetyl-3,4-di-O-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate triethylammonium salt (4): TEA (6.75 mL, 48.72 mmol) and thiophenol (3.34 mL, 32.48 mmol) were added under nitrogen to a solution of compound **15** (2.23 g, 4.06 mmol) in dry THF (22.0 mL). The reaction mixture was stirred at room temperature for 24 h with monitoring of the course of the reaction by TLC (MeOH/$CHCl_3$ 2:8). The reaction mixture was diluted with TEA and concentrated to give a crude residue, which was purified by silica gel chromatography ($CHCl_3$/MeOH 8:2→6:4→0:10 with 1% TEA) to provide compound **4** (2.30 g, 89%) as an oil. R_f=0.43 (MeOH/$CHCl_3$ 2:8); $[\alpha]_D^{25}$=+14.7 (c=1.1 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.35–7.26 (m, 10H; arom.), 6.71 (brs, 1H; NHAc), 4.78 (d, J=11.4 Hz, 1H; CH*H*Ph), 4.73–4.68 (m, 1H; H-2), 4.70 (d, J=11.0 Hz, 1H; CH*H*Ph), 4.51 (d, 1H; CH*H*Ph), 4.46 (d, 1H; CH*H*Ph), 4.38 (dd, $J_{6a,6b}$=11.8, $J_{6a,5}$=6.0 Hz, 1H; H-6a), 4.29–4.22 (m, 2H; H-1, H-6b), 4.09 (dd, $J_{3,2}$=3.5 Hz, 1H; H-3), 3.92–3.87 (m, 1H; H-5), 3.60 (d, $J_{Me,P}$=10.5 Hz, 3H; OMe), 3.47 (t, $J_{4,3}$=$J_{4,5}$=6.8 Hz, 1H; H-4), 3.06 (q, J=7.2 Hz, 6H; 3×CH_2CH_3), 2.09–1.92 (m, 2H; H-7a, H-7b), 2.03 (s, 3H; CH_3CO), 2.00 (s, 3H; CH_3CO), 1.33 ppm (t, 9H; 3×CH_2CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=170.73, 169.99 (2×CO), 76.43 (C-3), 73.56 (CH_2Ph, C-4), 72.15 (CH_2Ph), 72.09 (C-5), 71.02 (C-1), 63.18 (C-6), 51.67 (d, $J_{Me,P}\approx$5 Hz, OMe), 50.10 (C-2), 45.44 (3×CH_2CH_3), 29.68 (d, $J_{7,P}\approx$139 Hz, C-7), 22.53 (CH_3CO), 20.86 (CH_3CO), 8.52 ppm (3×CH_2CH_3); ^{31}P NMR (162 MHz, $CDCl_3$): δ=21.57 ppm; HRMS (ESI): m/z (%): 534.1890 (100) $[M]^-$; elemental analysis calcd (%) for $C_{32}H_{49}N_2O_9P$: (636.7): C 60.36, H 7.76, N 4.40; found: C 60.23, H 7.74, N 4.41.

***N*-(Benzyloxycarbonyl)aminopropyl 3,4,6-tri-O-benzyl-β-D-glucopyranoside (17)**: Trimethylsilyl triflate (0.11 mL, 0.59 mmol) was added to a solution of **7** (1.0 g, 1.98 mmol) and benzyl *N*-(3-hydroxypropyl)carbamate (3.31 g, 15.8 mmol) in CH_2Cl_2 (8 mL), cooled at 0°C. The reaction was monitored by TLC (hexane/AcOEt 7:3) and after 2.5 h the mixture was neutralised with TEA and diluted with CH_2Cl_2, and the organic layer was washed with water and dried over Na_2SO_4. Removal of the solvent afforded a mixture of 2-hydroxy and 2-*O*-acetylated derivatives (1.35 g, 1.98 mmol); this was dissolved in methanol (5 mL) and a solution of MeONa in dry MeOH (0.9 M, 0.5 mL, 0.43 mmol) was added. Monitoring of the reaction by TLC (hexane/AcOEt 6:4) indicated complete deacetylation in 4 h. After neutralisation with Amberlite IR-120 resin (H^+ form) and filtration, the crude residue was purified by silica gel chromatography (hexane/AcOEt 7:3) to give alcohol **17** as a yellow oil (1.12 g, 85%). R_f=0.38 (hexane/AcOEt 6:4); $[\alpha]_D^{25}$=+18.3 (c=1.0 in methanol); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.34–7.15 (m, 20H; arom.), 5.38 (brs, 1H; N*H*Z), 5.12 (s, 2H; C*H*$_2$ Z), 4.93 (d, J=11.3 Hz, 1H; CH*H*Ph), 4.85 (d, J=11.8 Hz, 1H; CH*H*Ph), 4.82 (d, 1H; CH*H*Ph), 4.54 (d, 1H; J=12.2 Hz, CH*H*Ph), 4.51 (d, 1H; CH*H*Ph), 4.49 (d, 1H; CH*H*Ph), 4.24 (d, $J_{1,2}$=7.4 Hz, 1H; H-1), 3.95 (ddd, $J_{7a,8a}$=4.3, $J_{7a,8b}$=7.3, $J_{7a,7b}$=10.1 Hz, 1H; H-7a), 3.70 (dd, $J_{6a,5}$=2.1, $J_{6a,6b}$=11.1 Hz, 1H; H-6a), 3.67 (m, 1H; H-7b), 3.62 (dd, $J_{6b,5}$=5.4 Hz, 1H; H-6b), 3.58–3.47 (m, 5H; H-2, H-3, H-4, H-5, H-9a), 3.25 (m,1H; H-9b), 2.78 (s, 1H; OH), 1.87–1.71 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=156.72 (CO Z), 102.77 (C-1), 84.60 (C-3), 77.59 (C-2), 75.10, 74.97, 73.35 (3×CH_2Ph), 74.89 (C-4), 74.57 (C-5), 68.79 (C-7), 66.63 (CH_2Ph Z), 37.78 (C-9), 29.57 ppm (C-8); HRMS (MALDI): m/z (%): 664.2883 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{38}H_{43}NO_8$ (641.7): C 71.12, H 6.75, N 2.18; found: C 71.01, H 6.76, N 2.18.

***N*-(Benzyloxycarbonyl)aminopropyl 2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-mannopyranoside (18)**: Pyridine (1.22 mL, 15.16 mmol) was added under argon to a solution of **17** (2.43 g, 3.79 mmol) in dry CH_2Cl_2 (20 mL). The mixture was cooled to 0°C and after 15 min triflic anhydride (1.87 mL, 11.37 mmol) was added dropwise. The reaction was monitored by TLC (hexane/AcOEt 6:4). After 30 min, the reaction mixture was diluted with CH_2Cl_2 and the organic layer was washed with aq. HCl solution (5%) and then with satd. aq. $NaHCO_3$ solution until neutralisation, dried over Na_2SO_4 and concentrated under reduced pressure. The 2-*O*-triflate intermediate was dissolved under nitrogen in dry toluene (60 mL), after which $Bu_4N^+N_3^-$ (2.69 g, 9.47 mmol) was quickly added and the solution was stirred at 55°C with monitoring of the course of the reaction by TLC (hexane/AcOEt 7:3). After 24 h the reaction mixture was concentrated and the crude residue was purified by silica gel chromatography (hexane/AcOEt 7:3) to afford compound **18** as a yellow oil (1.62 g, 64%). R_f=0.33 (hexane/AcOEt 6:4); $[\alpha]_D^{25}$=−49.9 (c=1.0 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.37–7.15 (m, 20H; arom.), 5.49 (brs, 1H; N*H*Z), 5.09 (d, J=12.2 Hz, 1H; CH*H*Ph-Z), 5.06 (d, 1H; CH*H*Ph-Z), 4.83 (d, J=10.8 Hz, 1H; CH*H*Ph), 4.74 (d, J=11.8 Hz, 1H; CH*H*Ph), 4.68 (d, 1H; CH*H*Ph), 4.54 (d, J=12.3 Hz, 1H; CH*H*Ph), 4.50 (d, 1H; CH*H*Ph), 4.49 (d, 1H; CH*H*Ph), 4.45 (brs, 1H; H-1), 3.95 (d, $J_{2,3}$=3.7 Hz, 1H; H-2), 3.91 (ddd, $J_{7a,8a}$=4.4, $J_{7a,8b}$=8.3, $J_{7a,7b}$=9.8 Hz, 1H; H-7a), 3.71–3.59 (m, 5H; H-3, H-4, H-6a, H-6b, H-7b), 3.41–3.37 (m, 2H; H-5, H-9a), 3.35–3.25 (m, 1H; H-9b), 1.83–1.73 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=156.58 (CO Z), 99.54 (C-1), 80.91 (C-3), 75.37 (C-5), 75.26, 73.32, 72.12 (3×CH_2Ph), 74.43 (C-4), 68.87 (*C*-6), 67.19 (C-7), 66.48 (CH_2Ph Z), 61.70 (C-2), 37.89 (C-9), 29.59 ppm (C-8); HRMS (MALDI): m/z (%): 689.3080 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{38}H_{42}N_4O_7$ (666.8): C 68.45, H 6.35, N 8.40; found: C 68.38, H 6.34, N 8.39.

***N*-(Benzyloxycarbonyl)aminopropyl 2-acetamido-3,4,6-tri-O-benzyl-2-deoxy-β-D-mannopyranoside (19)**: A solution of compound **18** (0.2 g, 0.3 mmol) and PPh_3 (0.17 g, 0.66 mmol) in dry THF (1.5 mL) was stirred at room temperature for 12 h, after which H_2O (21 μL, 1.2 mmol) was added and the solution was stirred at room temperature for an additional 20 h and finally concentrated. The crude amine was dissolved in MeOH (1.5 mL), acetic anhydride (0.48 mL, 5.1 mmol) was added, and the solution was stirred at room temperature for 1 h and then concentrated to dryness. The residue was purified by silica gel chromatography (toluene/acetone 3:1) to afford **19** as an oil (0.19 g, 93%). R_f=0.48 (hexane/AcOEt 6:4); $[\alpha]_D^{25}$=−7.7 (c=1.0 in chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.41–7.16 (m, 20H; arom.), 5.82 (d, 1H; N*H*Ac), 5.48–5.37 (m, 1H; N*H* Z), 5.10 (s, 2H; C*H*$_2$Ph Z), 4.91 (s, 1H; CH*H*Ph), 4.71 (s, 1H; C*H*HPh), 4.86–4.77 (m, 1H; H-2), 4.56–4.41 (m, 5H; 2×C*H*$_2$Ph, H-1), 3.92–3.80 (m, 1H; H-7a), 3.62–3.55 (m, 5H; H-3, H-4, H-5, H-6a, H-6b), 3.48–3.39 (m, 1H; H-7b), 3.37–3.21 (m, 2H; H-9a, H-9b), 2.01 (s, 3H; C*H*$_3$CO), 1.84–1.73 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.02 (CO), 156.52 (CO Z), 99.62 (C-1), 80.13 (C-3), 74.96 (C-5), 74.96, 73.42, 71.17 (3×CH_2Ph), 74.03 (C-4), 68.68 (C-6), 67.52 (C-7), 66.83 (CH_2Ph Z), 49.16 (C-2), 38.50 (C-9), 29.69 (C-8), 23.44 ppm (CH_3CO); HRMS (MALDI): m/z (%): 705.3084 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{40}H_{46}N_2O_8$ (682.8): C 70.36, H 6.79, N 4.10; found: C 70.47, H 6.78, N 4.11.

***N*-(Benzyloxycarbonyl)aminopropyl 2-acetamido-3,4-di-O-benzyl-2-deoxy-β-D-mannopyranoside (5) and its α anomer (6)**

***Alcohol* 5**: A solution of freshly fused zinc chloride (0.55 g, 4.08 mmol) in Ac_2O/AcOH 2:1 (3 mL) was added to a solution of **19** (0.35 g, 0.51 mmol) in Ac_2O/AcOH 2:1 (3 mL). The mixture was stirred at room temperature with monitoring of the course of the reaction by TLC (hexane/AcOEt 1:9). After disappearance of the starting material (2–4 h), the reaction mixture was quenched with water, diluted with AcOEt, washed with satd. aq. $NaHCO_3$ until neutralisation and then with water, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was dissolved in dry MeOH (5 mL), after which a solution of MeONa in dry MeOH (1 M, 3 mL) was added dropwise. Monitoring of the reaction (TLC, hexane/AcOEt 1:9) indicated complete deacetylation after 1 h. The reaction mixture was neutralised with Amberlite IR-120 resin (H^+ form), filtered and concentrated to dryness. The residue was purified by silica gel chromatography (AcOEt) to give the β alcohol **5** as a white solid (0.23 g, 74%).

***Alcohol* 6**: Compound **19** (0.40 g, 0.59 mmol) was dissolved in Ac_2O/AcOH (2:1, 3 mL) and treated with a solution of freshly fused $ZnCl_2$ (0.64 g, 4.69 mmol) in Ac_2O/AcOH (2:1, 4 mL) as described above. After 24 h the reaction mixture was quenched (see above) and the crude residue was dissolved in dry MeOH (4 mL), after which a solution of MeONa in dry MeOH (1 M, 0.59 mL) was added dropwise. After 1 h, the reaction mixture was neutralised with Amberlite IR-120 resin (H^+ form), filtered and concentrated to dryness. The residue was purified by silica

gel chromatography (AcOEt) to give the β-alcohol **5** (0.07 g, 20%). Further elution provided α-alcohol **6** (0.17 g, 50%) as a white solid.

Alcohol 5: R_f=0.43 (hexane/AcOEt 1:9); $[\alpha]_D^{25}$=+34.6 (c=1.0 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.35–7.25 (m, 15H; arom.), 6.09 (d, J=9.5 Hz, 1H; NHAc), 5.31 (brs, 1H; NHZ), 5.11 (s, 2H; CH_2Ph-Z), 4.93 (d, J=10.9 Hz, 1H; CHPh), 4.92–4.85 (m, 1H; H-2), 4.83 (d, J=11.3 Hz, 1H; CHPh), 4.61 (d, J=10.9 Hz, 1H; CHPh), 4.53 (s, 1H; H-1), 4.49 (d, J=11.3 Hz, 1H; CHPh), 3.84–3.77 (m, 3H; H-6a, H-6b, H-7a), 3.68 (dd, $J_{3,2}$=4.2, $J_{3,4}$=9.2 Hz, 1H; H-3), 3.65–3.60 (m, 2H; H-4, H-7b), 3.38–3.23 (m, 3H; H-5, H-9a, H-9b), 2.71 (brs, 1H; OH), 1.98 (s, 3H; CH_3CO), 1.76–1.74 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=171.13 (CO), 156.58 (CO-Z), 99.64 (C-1), 80.06 (C-3), 75.72 (C-5), 75.16 (CH_2Ph), 71.11 (CH_2Ph), 73.75 (C-4), 67.15 (C-7), 66.71 (CH_2Ph-Z), 61.58 (C-6), 49.28 (C-2), 38.33 (C-9), 29.55 (C-8), 23.44 ppm (CH_3CO); HRMS (MALDI): m/z (%): 615.2679 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{33}H_{40}N_2O_8$ (592.7): C 66.87, H 6.80, N 4.73; found: C 66.80, H 6.81, N 4.74.

Alcohol 6: R_f=0.25 (hexane/AcOEt 1:9); $[\alpha]_D^{25}$=+15.65 (c=1.5 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.40–7.28 (m, 15H; arom.), 6.07 (d, J=8.1 Hz, 1H; NHAc), 5.13 (brt, 1H; NHZ), 5.09 (s, 2H; CH_2Ph-Z), 4.93 (d, J=10.9 Hz, 1H; CHPh), 4.85 (brs, 1H; H-1), 4.70 (d, J=10.9 Hz, 1H; CHPh), 4.65–4.62 (m, 1H; H-2), 4.62 (d, 1H; CHPh), 4.47 (d, 1H; CHPh), 4.07 (dd, $J_{3,2}$=4.8, $J_{3,4}$=8.7 Hz, 1H; H-3), 3.87–3.76 (m, 2H; H-7a, H-7b), 3.77–3.67 (m, 1H; H-6), 3.75–3.59 (m, 3H; H-4, H-5, H-6a), 3.50–3.42 (m, 1H; H-6b), 3.32–3.27 (m, 2H; H-9a, H-9b), 2.55 (brs, 1H; OH), 2.05 (s, 3H; CH_3CO), 1.85–1.70 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=170.72 (CO), 156.50 (CO-Z), 99.17 (C-1), 77.61 (C-3), 75.16 (CH_2Ph), 73.78, 71.25 (C-4, C-5), 71.30 (CH_2Ph), 66.66 (CH_2Ph-Z), 65.81 (C-6), 61.78 (C-7), 49.59 (C-2), 38.56 (C-9), 29.55 (C-8), 23.38 ppm (CH_3CO); HRMS (ESI): m/z (%): 615.2663 (100) $[M+Na]^+$, 1207.5446 (20) $[2\times M+Na]^+$; elemental analysis calcd (%) for $C_{33}H_{40}N_2O_8$ (592.7): C 66.87, H 6.80, N 4.73; found: C 66.98, H 6.83, N 4.71.

Aminopropyl 2-acetamido-2-deoxy-β-D-mannopyranoside (20): Mannopyranoside **5** (0.15 g, 0.25 mmol) was dissolved in a MeOH/H_2O mixture (1:1, 6 mL), Pd/C catalyst (10%, 0.11 g) was added, and the reaction mixture was vigorously stirred under hydrogen at room temperature. After 24 h, a second portion of the catalyst (10%) was added and the mixture was stirred under hydrogen for an additional 12 h. The reaction mixture was diluted with MeOH/H_2O and filtered over a Celite pad, concentrated under reduced pressure to remove MeOH and finally lyophilised to give **20** (0.066 g, 95%) as a white foam. $[\alpha]_D^{25}$=−37.1 (c=1.0 in water); ^{1}H NMR (400 MHz, D_2O, 35°C): δ=4.82 (d, $J_{1,2}$=1.5 Hz, 1H; H-1), 4.55 (brdd, 1H; H-2), 4.06–3.99 (m, 1H; H-7a), 3.96 (dd, $J_{6a,6b}$=12.2, $J_{6a,5}$=2.3 Hz, 1H; H-6a), 3.87 (dd, $J_{3,2}$=4.3 Hz, 1H; H-3), 3.85–3.78 (m, 2H; H-6b, H-7b), 3.58 (t, $J_{4,3}$=$J_{4,5}$=9.8 Hz, 1H; H-4), 3.45 (ddd, 1H; H-5), 3.19–3.11 (m, 2H; H-9a, H-9b), 2.10 (s, 3H; CH_3CO), 2.01–1.97 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, D_2O, 35°C): δ=175.26 (CO), 99.11 (C-1), 76.32 (C-5), 71.63 (C-3), 67.25 (C-7), 66.71 (C-4), 60.37 (C-6), 52.95 (C-2), 37.55 (C-9), 26.45 (C-8), 21.92 ppm (CH_3CO); HRMS (ESI): m/z (%): 279.1548 (100) $[M+H]^+$, 301.1368 (20) $[M+Na]^+$; elemental analysis calcd (%) for $C_{11}H_{22}N_2O_6$ (278.3): C 47.47, H 7.97, N 10.07; found: C 47.61, H 7.96, N 10.11.

Aminopropyl 2-acetamido-2-deoxy-α-D-mannopyranoside (21): Mannopyranoside **6** (0.08 g, 0.13 mmol) was dissolved in a MeOH/H_2O mixture (1:1, 4 mL), Pd/C catalyst (10%, 0.06 g) was added, and the reaction mixture was vigorously stirred under hydrogen at room temperature. After 24 h, a second portion of the catalyst (10%) was added and the mixture was stirred under hydrogen for an additional 12 h. The reaction mixture was diluted with MeOH/H_2O and filtered over a Celite pad, concentrated under reduced pressure to remove MeOH and finally lyophilised to give **21** (0.035 g, 96%) as a white foam. $[\alpha]_D^{25}$=+1.35 (c=0.5 in water); ^{1}H NMR (400 MHz, D_2O, 35°C): δ=4.85 (brs, 1H; H-1), 4.39 (dd, $J_{2,1}$=1.1, $J_{2,3}$=4.5 Hz, 1H; H-2), 4.05 (dd, $J_{3,4}$=9.6 Hz, 1H; H-3), 3.94–3.82 (m, 3H; H-6a, H-6b, H-7a), 3.72–3.61 (m, 3H; H-4, H-5, H-7b), 3.22–3.17 (m, 2H; H-9a, H-9b), 2.09 (s, 3H; CH_3CO), 2.06–2.00 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, D_2O, 35°C): δ=174.96 (CO), 98.86 (C-1), 65.12 (C-7), 60.59 (C-6), 72.17, 66.84 (C-4, C-5), 69.15 (C-3), 52.66 (C-2), 37.69 (C-9), 26.62 (C-8), 22.03 ppm (CH_3CO); HRMS (ESI): m/z (%): 301.1370 (100) $[M+Na]^+$, 279.1550 (80) $[M+H]^+$; elemental analysis calcd (%) for $C_{11}H_{22}N_2O_6$ (278.15): C 47.47, H 7.97, N 10.07; found: C 47.58, H 7.94, N 10.09.

[*N*-(Benzyloxycarbonyl)aminopropyl (2-acetamido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosidyl)]methyl *C*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (22): Phosphonate **16** (0.13 g, 0.19 mmol), alcohol **5** (0.12 g, 0.20 mmol) and freshly recrystallised triphenylphosphine (0.08 g, 0.41 mmol) were dissolved in dry THF (5.5 mL) under nitrogen. The solution was cooled at 0°C and DIAD (62 μL, 0.41 mmol) was added. The mixture was stirred at room temperature for 24 h with monitoring of the course of the reaction by TLC (hexane/AcOEt 1:9 and *i*PrOH/$CHCl_3$ 1:9). The solution was concentrated under reduced pressure to give a crude residue, which was purified by silica gel chromatography (hexane/AcOEt 1:9→CH_3OH/AcOEt 1:9) to give **22** (0.21 g, 97%) as a mixture of phosphorus diastereoisomers (**22a** and **22b**). An analytical sample of each diastereoisomer was characterised by mass spectrometry: HRMS (MALDI): m/z (%): **22a**: 1180.4910 (100) $[M+Na]^+$, 1196.4611 (10) $[M+K]^+$; **22b**: 1180.4979 (100) $[M+Na]^+$, 1196.4673 (20) $[M+K]^+$.

No further characterisation was performed on compounds **22**, and the diastereoisomeric mixture was employed in the following steps.

[*N*-(Benzyloxycarbonyl)aminopropyl (2-acetamido-3,4-di-O-benzyl-2-deoxy-β-D-mannopyranosidyl)]methyl *C*-(2-acetamido-3,4-di-O-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (23)

From compound 22: A solution of freshly fused zinc chloride (0.04 g, 0.29 mmol) in Ac_2O/AcOH 2:1 (1 mL) was added to a solution of **22** (mixture of phosphorus diastereoisomers, 0.034 g, 0.03 mmol) in Ac_2O/AcOH 2:1 (1 mL). The mixture was stirred at room temperature with monitoring of the course of the reaction by TLC (AcOEt/*i*PrOH 20:1). After 36 h the reaction mixture was quenched with water, diluted with $CHCl_3$, washed with satd. aq. $NaHCO_3$ until neutralisation and then with water, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was dissolved in dry MeOH (1 mL), after which a solution of MeONa in dry MeOH (0.1 M, 20 μL) was added. Monitoring of the reaction (TLC, AcOEt/*i*PrOH 15:1) indicated complete deacetylation after 18 h. The reaction mixture was neutralised with Amberlite IR-120 resin (H^+ form), filtered and concentrated to dryness. The residue was purified by silica gel chromatography (AcOEt/*i*PrOH 40:1→30:1) to give alcohol **23** as a glassy solid (mixture of phosphorus diastereoisomers, 0.022 g, 70%).

From compound 24: A solution of MeONa in dry MeOH (0.1 M, 4.7 mL) was added under nitrogen to a solution of compound **24** (mixture of phosphorus diastereoisomers, 1.60 g, 1.44 mmol) in dry MeOH (6.0 mL). The mixture was stirred at room temperature for 14 h with monitoring of the course of the reaction by TLC (AcOEt/*i*PrOH 15:1). The solution was neutralised with Amberlite IR-120 resin (H^+ form), filtered and concentrated to dryness. The residue was purified by silica gel chromatography (AcOEt/*i*PrOH 20:1) to give alcohol **23** (mixture of phosphorus diastereoisomers, 1.17 g, 76%) as a glassy solid; R_f (HPTLC)=0.36 and 0.18 (AcOEt/*i*PrOH 15:1). A pure analytical sample containing a single diastereoisomer of **23** was isolated during column chromatography and fully characterised.[31] $[\alpha]_D^{25}$=+11.2 (c=2.0 in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.52 (d, J=9.2 Hz, 1H; NHAc), 7.41–7.24 (m, 25H; arom.), 5.66 (d, J=9.2 Hz, 1H; NHAc), 5.33 (brt, 1H; NHZ), 5.12 (s, 2H; CH_2Ph-Z), 4.95 (d, J=10.9 Hz, 1H; CHPh), 4.92 (d, J=11.3 Hz, 1H; CHPh), 4.90–4.84 (m, 1H; H-2), 4.64 (d, J=12.4 Hz, 1H; CHPh), 4.58 (d, J=11.1 Hz, 1H; CHPh), 4.57 (d, 1H; CHPh), 4.54 (d, 1H; CHPh), 4.49 (d, 1H; CHPh), 4.45 (brs, 1H; H-1), 4.40–4.12 (m, 6H; H-6a, H-6b, H-7a, H-2′, H-3′, CHPh), 4.09–3.97 (m, 1H; H-1′), 3.86–3.80 (m, 2H; H-4, H-6′a), 3.78 (d, $J_{Me,P}$=11.2 Hz, 3H; OMe), 3.67 (dd, $J_{3,4}$=9.3, $J_{3,2}$=3.7 Hz, 1H; H-3), 3.64 (brt, 1H; H-4′), 3.59–3.53 (m, 1H; H-6′b), 3.42 (brd, $J_{5',6'}$=2.7 Hz, 1H; H-5′), 3.37 (d, $J_{5,4}$=9.3 Hz, 1H; H-5), 3.29–3.22 (m, 3H; H-7b, H-9a, H-9b), 2.26–2.02 (m, 2H; H-7′a, H-7′b), 2.11 (s, 3H; CH_3CO), 1.81 (s, 3H; CH_3CO), 1.79–1.74 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=172.49 (CO), 170.40 (CO), 157.25 (CO-Z), 100.43 (C-1), 80.93 (C-3), 77.74 (C-3′), 76.50 (C-4′), 75.87 (CH_2Ph), 74.96 (C-5), 73.79 (C-4), 73.40 (CH_2Ph), 72.60 (CH_2Ph),

72.16 (C-5′), 71.51 (CH_2Ph), 67.87 (C-6′), 67.13 (CH_2Ph-Z), 66.91 (C-6), 65.29 (C-1′), 58.91 (C-7), 54.67 (OMe), 49.48 (C-2), 49.23 (brd, C-2′), 39.06 (C-9), 30.97 (d, $J_{7',P}\approx156$ Hz, C-7′), 30.19 (C-8), 23.96 (CH_3CO), 23.78 ppm (CH_3CO); ^{31}P NMR (162 MHz, $CDCl_3$): $\delta=33.48$ ppm; HRMS (ESI): m/z (%): 1090.4430 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{57}H_{70}N_3O_{15}P$ (1068.1): C 64.09, H 6.61, N 3.93; found: C 64.17, H 6.60, N 3.94.

[*N*-(Benzyloxycarbonyl)aminopropyl (2-acetamido-3,4-di-O-benzyl-2-deoxy-β-D-mannopyranosidyl)]methyl *C*-(2-acetamido-6-O-acetyl-3,4-di-O-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (24): Phosphonate **4** (1.02 g, 1.60 mmol), alcohol **5** (0.95 g, 1.60 mmol) and freshly recrystallised Ph_3P (0.92 g, 3.52 mmol) were dissolved under nitrogen in dry THF (53 mL). The solution was cooled at 0 °C and DIAD (0.68 mL, 3.52 mmol) was added. The mixture was stirred at room temperature for 24 h with monitoring of the course of the reaction by TLC (MeOH/CH_2Cl_2 1:9 and AcOEt/*i*PrOH 20:1). The solution was concentrated under reduced pressure to give a crude residue, which was purified by silica gel chromatography (AcOEt/*i*PrOH 20:1) to give **24** (1.60 g, 90 %) as a mixture of phosphorus diastereoisomers; R_f (HPTLC) = 0.42 and 0.24 (AcOEt/*i*PrOH 20:1). A pure analytical sample containing a single diastereoisomer of **24** was isolated during column chromatography and fully characterised. $[\alpha]_D^{25}=+14.1$ ($c=0.4$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.96$ (d, $J=10.0$ Hz, 1H; N*H*Ac), 7.42–7.24 (m, 25H; arom.), 5.69 (brd, $J=9.1$ Hz, 1H; N*H*Ac), 5.38 (brt, 1H; N*H*Z), 5.11 (brs, 2H; CH_2Ph-Z), 4.96 (d, $J=10.8$ Hz, 1H; CH*H*Ph), 4.90 (m, 1H; H-2), 4.89 (d, $J=11.2$ Hz, 1H; CH*H*Ph), 4.62–4.58 (m, 3H; 3× CH*H*Ph), 4.50 (d, $J=12.0$ Hz, 1H; CH*H*Ph), 4.49–4.47 (m, 2H; H-1, H-7a), 4.38–4.18 (m, 6H; H-6a, H-6b, H-7b, H-2′, 2×CH*H*Ph), 4.16–4.07 (m, 2H; H-1′, H-3′), 3.90–3.82 (m, 2H; H-4, H-6a), 3.79 (d, $J_{Me,P}=11.0$ Hz, 3H; OMe), 3.70–3.67 (m, 2H; H-3, H-5′), 3.59–3.57 (m, 2H; H-6b, H-4′), 3.38 (brd, 1H; H-5), 3.31–3.19 (m, 2H; H-9a, H-9b), 2.15–2.05 (m, 2H; H-7′a, H-7′b), 2.10 (s, 3H; CH_3CO), 2.00 (s, 3H; CH_3CO), 1.81 (s, 3H; CH_3CO), 1.80–1.75 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta=171.84$, 170.56, 169.87 (3×CO), 156.55 (CO-Z), 99.92 (C-1), 80.10 (C-3), 75.32 (C-5′), 75.19 (CH_2Ph), 74.47 (C-5), 73.28 (C-3′), 73.15 (C-4), 72.53 (CH_2Ph), 72.41 (CH_2Ph), 71.86 (C-4′), 70.67 (CH_2Ph), 67.43 (C-6′), 67.09 (C-1′), 66.51 (CH_2Ph-Z), 66.35 (C-6), 61.94 (C-7), 53.76 (OMe), 48.62 (C-2), 48.41 (C-2′), 38.53 (C-9), 29.74 (d, $J_{7',P}\approx149$ Hz, C-7′), 29.55 (C-8), 23.32 (CH_3CO), 22.92 (CH_3CO), 20.77 ppm (CH_3CO); ^{31}P NMR (162 MHz, $CDCl_3$): $\delta=30.87$ ppm; HRMS (ESI): m/z (%): 1132.4515 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{59}H_{72}N_3O_{16}P$ (1110.2): C 63.83, H 6.54, N 3.78; found: C 63.61, H 6.56, N 3.77.

[*N*-(Benzyloxycarbonyl)aminopropyl (2-acetamido-3,4-di-O-benzyl-2-deoxy-α-D-mannopyranosidyl)]methyl *C*-(2-acetamido-6-O-acetyl-3,4-di-O-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (25): Compound **4** (0.15 g, 0.24 mmol), alcohol **6** (0.12 g, 0.20 mmol) and freshly recrystallised triphenylphosphine (0.12 g, 0.45 mmol) were dissolved under nitrogen in dry THF (6 mL). The solution was cooled at 0 °C and DIAD (87 μL, 0.45 mmol) was added. The mixture was stirred at room temperature for 24 h with monitoring of the course of the reaction by TLC (AcOEt/*i*PrOH 15:1). The solution was concentrated under reduced pressure to give a crude residue, which was purified by silica gel chromatography (AcOEt/*i*PrOH 15:1→15:2) to give **25** (0.19 g, 84 %) as a mixture of phosphorus diastereoisomers (**25a** and **25b**). An analytical sample of each diastereoisomer was characterised by mass spectrometry: HRMS (ESI): m/z (%): **25a**: 1132.4557 (100) $[M+Na]^+$; **25b**: 1132.4581 (100) $[M+Na]^+$.

No further characterisation was performed on compounds **25**, and the diastereoisomeric mixture was employed in the following steps.

[*N*-(Benzyloxycarbonyl)aminopropyl (2-acetamido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosidyl)] *C*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate sodium salt (26): TEA (39 μL, 0.282 mmol) and thiophenol (19 μL, 0.19 mmol) were added under nitrogen to a solution of compound **22** (mixture of phosphorus diastereoisomers, 0.050 g, 0.04 mmol) in dry toluene (1.0 mL). The reaction mixture was stirred at 110 °C for 36 h with monitoring of the course of the reaction by TLC ($CH_3OH/CHCl_3$ 1:9). The reaction mixture was diluted with TEA and the solvent was evaporated to give a crude residue that was purified by silica gel chromatography ($CH_3OH/CHCl_3$ 0:100→50:50 with 1 % TEA). The obtained residue was dissolved in MeOH and eluted through a column filled with Amberlite IR-120 resin (Na^+ form). The eluate was concentrated to dryness to yield **26** (sodium salt) as a white foam (0.048 g, 95 %). $R_f=0.15$ ($CH_3OH/CHCl_3$ 1:9); $[\alpha]_D^{25}=-40.5$ ($c=1.0$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.78$ (brs, 1H; NH), 7.34–7.20 (m, 30H; arom.), 5.92 (d, $J=8.9$ Hz, 1H; NH), 5.14 (s, 2H; CH_2Ph-Z), 5.00–4.95 (m, 2H; 2×CH*H*Ph), 4.80 (brdd, 1H; H-2), 4.74 (d, $J=11.3$ Hz, 1H; CH*H*Ph), 4.62 (d, $J=10.8$ Hz, 1H; CH*H*Ph), 4.58–4.45 (m, 7H; H-1, H-2′, H-6a, CH_2Ph, 2×CH*H*Ph), 4.42 (d, $J=11.8$ Hz, 1H; CH*H*Ph), 4.32 (d, 1H; CH*H*Ph), 4.31–4.24 (m, 1H; H-1′), 4.18 (brdd, $J_{6a,6b}=11.4$ Hz, 1H; H-6b), 3.98–3.87 (m, 3H; H-4, H-7a, H-5′), 3.83–3.79 (m, 3H; H-3′, H-4′, H-6′a), 3.73 (dd, $J_{6'b,6'a}=9.7$, $J_{6'b,5}=2.9$ Hz, 1H; H-6′b), 3.68 (dd, $J_{3,2}=3.5$, $J_{3,4}=9.4$ Hz, 1H; H-3), 3.55–3.48 (m, 1H; H-7b), 3.39 (brd, $J_{5,4}=9.5$ Hz, 1H; H-5), 3.37–3.31 (m, 1H; H-9a), 3.17–3.07 (m, 1H; H-9b), 2.32–2.20 (m, 1H; H-7′a), 2.12–2.02 (m, 4H; H-7′b, CH_3CO), 1.90 (s, 3H; CH_3CO), 1.76–1.68 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta=171.79$ (CO), 169.88 (CO), 159.46 (CO-Z), 97.27 (C-1), 80.12 (C-3), 76.82 (C-3′), 75.38 (CH_2Ph), 73.86 (CH_2Ph), 73.30 (CH_2Ph), 71.37 (CH_2Ph), 70.74 (CH_2Ph), 73.86 (C-5), 73.10 (C-4′), 72.74 (C-4, C-5′), 71.05 (C-1′), 68.40 (C-6′), 67.62 (CH_2Ph-Z), 65.13 (C-6), 62.09 (C-7), 49.47 (C-2), 48.90 (d, $J_{2',P}\approx15$ Hz, C-2′), 37.40 (C-9), 28.76 (d, $J_{7',P}\approx147$ Hz, C-7′), 27.21 (C-8), 23.42 (CH_3CO), 22.94 ppm (CH_3CO); ^{31}P NMR (162 MHz, $CDCl_3$): $\delta=24.83$ ppm; HRMS (MALDI): m/z (%): 1166.4669 (100) $[M+H]^+$, 1188.4552 (40) $[M+Na]^+$; elemental analysis calcd (%) for $C_{63}H_{73}N_3NaO_{15}P$ (1166.2): C 64.88, H 6.31, N 3.60; found: C 65.05, H 6.30, N 3.58.

[*N*-(Benzyloxycarbonyl)aminopropyl (2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosidyl)] *C*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate sodium salt (27): Dimer **25** (mixture of phosphorus diastereoisomers, 0.12 g, 0.11 mmol) was dissolved in dry MeOH (5 mL), and a solution of MeONa in dry MeOH (0.1 M, 0.11 mL) was added under nitrogen. The mixture was stirred at room temperature for 6 h, with monitoring of the course of the reaction by TLC (AcOH/*i*PrOH 15:2). The solution was neutralised with Amberlite IR-120 resin (H^+ form), filtered and concentrated to dryness. ^{1}H NMR of the crude residue confirmed the 6′-*O*-deacetylation, and the following step was performed without further purification. The crude residue was dissolved under nitrogen in dry THF (5.0 mL), after which DBU (65 μL, 0.43 mmol) and thiophenol (33 μL, 0.32 mmol) were added. The reaction mixture was stirred at room temperature for 7 h with monitoring of the course of the reaction by TLC (MeOH/$CHCl_3$ 2:8). The solvent was evaporated to give a crude residue that was purified by silica gel chromatography (MeOH/$CHCl_3$ 0.5:9.5→1:9). The obtained foam was dissolved in MeOH and eluted through a column filled with Amberlite IR-120 resin (Na^+ form). The eluate was concentrated to dryness to yield **27** (sodium salt) as a white foam (0.09 g, 75 %). $R_f=0.32$ (MeOH/$CHCl_3$ 2:8); $[\alpha]_D^{25}=+6.2$ ($c=1.0$ in methanol); ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.45$–7.20 (m, 25H; arom), 5.07 (brs, 2H; CH_2Ph-Z), 4.85 (d, $J=10.9$ Hz, 1H; CH*H*Ph), 4.74 (d, 1H; CH*H*Ph), 4.71 (d, $J=11.2$ Hz, 1H; CH*H*Ph), 4.66 (d, 1H, CH*H*Ph), 4.61 (d, $J=11.5$ Hz, 1H; CH*H*Ph), 4.59 (brdd, 1H; H-2), 4.57 (d, 1H; CH*H*Ph), 4.51 (d, $J=11.6$ Hz, 1H; CH*H*Ph), 4.49 (brt, 1H; H-2′), 4.44 (d, 1H; CH*H*Ph), 4.34–4.26 (m,1H; H-1′), 4.18–4.14 (m, 2H; H-6a, H-6b), 4.03–3.96 (m, 2H; H-3, H-6′a), 3.89 (dd, $J_{3',2'}=4.1$ Hz, 1H; H-3′), 3.85–3.79 (m, 2H; H-4, H-5′), 3.75–3.69 (m, 2H; H-5, H-7a), 3.63–3.59 (m, 1H; H-6′b), 3.60 (t, $J_{4',5'}=J_{4',3'}=6.2$ Hz, 1H; H-4′), 3.45–3.37 (m, 1H; H-7b), 3.25–3.19 (m, 2H; H-9a, H-9b), 2.05 (s, 3H; CH_3CO), 2.01–1.90 (m, 2H; H-7′a, H-7′b), 1.95 (s, 3H; CH_3CO), 1.80–1.72 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, CD_3OD): $\delta=172.99$ (CO), 172.17 (CO), 158.61 (CO-Z), 99.90 (C-1), 78.31 (C-3), 77.10 (C-3′), 75.48, 74.72 (C-4, C-5′), 75.32 (CH_2Ph), 74.27 (C-4′), 73.74 (CH_2Ph), 72.24 (CH_2Ph), 71.71 (C-5), 71.48 (CH_2Ph), 70.53 (C-1′), 66.66 (CH_2Ph-Z), 65.88 (C-7), 63.97 (C-6), 60.77 (C-6′), 50.77 (d, $J_{2',P}\approx11$ Hz, C-2′), 50.19 (C-2), 38.52 (C-9), 29.33 (C-8), 29.30 (d, $J_{7',P}\approx134$ Hz, C-7′), 22.06, 21.91 ppm (2×CH_3CO); ^{31}P NMR (162 MHz, CD_3OD, 35 °C): $\delta=22.46$ ppm; HRMS (ESI): m/z (%): 1052.4268 (100) $[M\text{-}Na^+]$; elemental

analysis calcd (%) for $C_{56}H_{67}N_3O_{15}NaP$ (1076.1): C 62.50, H 6.28, N 3.90; found: C 62.47, H 6.29, N 3.91.

[Aminopropyl (2-acetamido-2-deoxy-β-D-mannopyranosidyl)] *C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate sodium salt (2): Dimer **26** (0.05 g, 0.04 mmol) was dissolved in a MeOH/H_2O mixture (3:2, 5 mL), Pd/C catalyst (10%, 0.03 g) was added, and the reaction mixture was vigorously stirred under hydrogen at room temperature. After 48 h, a second portion of the catalyst (0.03 g) was added and the mixture was stirred under hydrogen for an additional 12 h. The reaction mixture was diluted with MeOH/H_2O and filtered over a Celite pad, concentrated under reduced pressure to remove MeOH and finally lyophilised to give an amorphous solid. This was dissolved in H_2O and first eluted through a column filled with Dowex 50W X8 resin (H^+ form), and then through a column filled with the same resin in Na^+ form. The eluate was lyophilised to afford **2** as a foam (0.024 g, 96%). $[\alpha]_D^{25} = -17.8$ ($c = 1.0$ in water); ^{1}H NMR (400 MHz, D_2O): $\delta = 4.94$ (d, $J_{1,2} = 1.7$ Hz, 1H; H-1), 4.69 (m, 1H; H-2), 4.51 (br dd, 1H; H-2′), 4.38–4.23 (m, 3H; H-1′, H-6a, H-6b), 4.17–4.08 (m, 2H; H-3′, H-7a), 4.02–3.93 (m, 4H; H-3, H-7b, H-6′a, H-6′b), 3.81–3.73 (m, 3H; H-4, H-4′, H-5′), 3.66 (m, 1H; H-5), 3.26 (br t, J = 7.5 Hz, 2H; H-9a, H-9b), 2.40–2.27 (m, 2H; H-7′a, H-7′b), 2.22 (s, 3H; CH_3CO), 2.20 (s, 3H; CH_3CO), 2.11 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, D_2O): $\delta = 175.73$ (CO), 174.60 (CO), 99.73 (C-1), 75.63 (C-5), 74.44, 67.70, 66.90 (C-4, C-4′, C-5′), 72.84 (C-1′), 71.86 (C-3), 69.57 (C-3′), 67.97 (C-7), 63.38 (C-6), 60.77 (C-6′), 53.27 (C-2), 53.05 (d, $J_{2',P} \approx 10$ Hz, C-2′), 38.02 (C-9), 27.80 (d, $J_{7',P} \approx 135$ Hz, C-7′), 26.85 (C-8), 22.31 ppm (2×*C*H_3CO); ^{31}P NMR (162 MHz, D_2O): $\delta = 22.95$ ppm; HRMS (ESI): *m/z* (%): 582.2036 (100) $[M+H]^+$, 604.1857 (70) $[M+Na]^+$; elemental analysis calcd (%) for $C_{20}H_{37}N_3NaO_{15}P$ (581.5): C 41.31, H 6.41, N 7.23; found: C 41.25, H 6.42, N 7.22.

[Aminopropyl (2-acetamido-2-deoxy-α-D-mannopyranosidyl)] *C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate sodium salt (3): Dimer **27** (0.08 g, 0.07 mmol) was dissolved in a MeOH/H_2O mixture (1:1, 2 mL), Pd/C catalyst (10%, 0.05 g) was added, and the reaction mixture was vigorously stirred under hydrogen at room temperature. After 24 h, a second portion of the catalyst (10%) was added and the mixture was stirred under hydrogen for an additional 12 h. The reaction mixture was diluted with MeOH/H_2O and filtered over a Celite pad, concentrated under reduced pressure to remove MeOH and finally lyophilised to give an amorphous solid. This was dissolved in H_2O and first eluted through a column filled with Dowex 50W X8 resin (H^+ form), and then through a column filled with the same resin in Na^+ form. The eluate was lyophilised to afford dimer **3** (0.04 g, 92%, sodium salt) as a white foam. $[\alpha]_D^{25} = +8.6$ ($c = 0.5$ in water); ^{1}H NMR (400 MHz, D_2O): $\delta = 4.74$ (br s, 1H; H-1), 4.33–4.28 (m, 2H; H-2, H-2′), 4.20–4.13 (m, 1H; H-1′), 4.11–4.05 (m, 2H; H-6a, H-6b), 3.98–3.92 (m, 2H; H-3, H-3′), 3.84–3.72 (m, 3H; H-7a, H-6′a, H-6′b), 3.70–3.66 (m, 1H; H-5), 3.63 (t, J = 9.5 Hz, 1H; H-4 or H-4′), 3.59–3.49 (m, 3H; H-7b, H-4′ or H-4, H-5′), 3.12–3.02 (m, 2H; H-9a, H-9b), 2.14–2.09 (m, 2H; H-7′a, H-7′b), 1.99 (br s, 6H; 2×CH_3CO), 1.97–1.90 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, D_2O): $\delta = 174.97$ (CO), 174.39 (CO), 98.94 (C-1), 74.19, 67.39, 66.59 (C-4, C-4′, C-5′), 72.66 (C-1′), 71.62 (d, $J_{5,P} \approx 5$ Hz, C-5), 69.35, 68.94 (C-3, C-3′), 65.28 (C-7), 63.34 (C-6), 60.50 (C-6′), 52.89 (d, $J_{2',P} \approx 10$ Hz, C-2′), 52.64 (C-2), 37.62 (C-9), 27.34 (d, $J_{7',P} \approx 141$ Hz, C-7′), 26.62 (C-8), 22.09, 21.99 ppm (2×CH_3CO); ^{31}P NMR (162 MHz, D_2O, 35 °C): $\delta = 24.40$ ppm; HRMS (ESI): *m/z* (%): 558.2042 (100) $[M-Na]^+$; elemental analysis calcd (%) for $C_{20}H_{37}N_3O_{13}NaP$ (581.5): C 41.31, H 6.41, N 7.23; found: C 41.37, H 6.40, N 7.21.

[*N*-(Benzyloxycarbonyl)aminopropyl (2-acetamido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosidyl) methyl *C*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonyl]methyl *C*-(2-acetamido-6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate (28): Compound **4** (0.056 g, 0.09 mmol), alcohol **23** (0.076 g, 0.07 mmol, mixture of phosphorus diastereoisomers) and freshly recrystallised Ph_3P (0.041 g, 0.16 mmol) were dissolved under nitrogen in dry THF (2.4 mL). The solution was cooled at 0 °C and DIAD (0.030 mL, 0.16 mmol) was added. The mixture was stirred at room temperature for 24 h with monitoring of the course of the reaction by TLC (AcOEt/*i*PrOH 15:1 and AcOEt/*i*PrOH 19:4) and the solution was concentrated under reduced pressure to give a crude residue, which was purified by silica gel chromatography (AcOEt/*i*PrOH 20:3) to give trimer **28** (0.096 g, 83%) as a mixture of phosphorus diastereoisomers. A fraction containing a single diastereoisomer of **28** was isolated during column chromatography and fully characterised. $[\alpha]_D^{25} = +14.8$ ($c = 1.6$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.91$ (br d, 1H; N*H*Ac), 7.87 (br d, 1H; N*H*Ac), 7.39–7.23 (m, 35H; arom.), 5.74 (d, J = 9.2 Hz, 1H; N*H*Ac), 5.44 (br t, 1H; N*H*Z), 5.11 (s, 2H; CH_2Ph-Z), 4.95 (d, J = 11.0 Hz, 1H; CH*H*Ph), 4.90–4.85 (m, 3H; H-2, 2×CH*H*Ph), 4.71 (d, J = 11.3 Hz, 1H; CH*H*Ph), 4.64–4.58 (m, 6H; H-2′, CH_2Ph, 3×CH*H*Ph), 4.53–4.45 (m, 4H; H-1, H-7a, 2×CH*H*Ph), 4.34–4.19 (m, 8H; H-6a, H-6b, H-7b, H-1′, H-2″, H-6″a, H-6″b, CH*H*Ph), 4.15–4.03 (m, 2H; H-1″, H-3″), 3.88–3.72 (m, 4H; H-4, H-3′, H-5′, H-6′a), 3.76 (d, $J_{Me,P}$ = 11.2 Hz, 6H; 2×OMe), 3.71–3.67 (m, 3H; H-3, H-4′, H-5″), 3.60–3.54 (m, 2H; H-6′b, H-4″), 3.39 (br d, 1H; H-5), 3.33–3.20 (m, 2H; H-9a, H-9b), 2.10–1.99 (m, 4H; H-7′a, H-7′b, H-7″a, H-7″b), 2.07 (s, 3H; CH_3CO), 2.02 (s, 3H; CH_3CO), 2.01 (s, 3H; CH_3CO), 1.82 (s, 3H; CH_3CO), 1.81–1.75 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 171.68$, 170.55, 169.84 (4×CO), 156.57 (CO-Z), 99.99 (C-1), 80.06 (C-3), 76.74, 72.94, 72.87 (C-4, C-3′, C-5′), 75.40, 72.56 (C-4′, C-5″), 75.05 (*C*H_2Ph), 74.70 (*C*H_2Ph), 74.40 (C-5), 73.21 (C-3″), 72.56 (*C*H_2Ph), 72.38 (*C*H_2Ph), 71.82 (C-1′, C-4″), 71.19 (*C*H_2Ph), 70.68 (*C*H_2Ph), 67.53 (C-6′), 67.06 (C-1″), 66.63, 65.75 (C-6, C-6″), 66.48 (*C*H_2Ph-Z), 61.96 (C-7), 54.00 (d, $J_{Me,P} \approx 6$ Hz; OMe), 53.70 (d, $J_{Me,P} \approx 6$ Hz; OMe), 48.61 (C-2), 48.44, 48.25 (C-2′, C-2″), 38.51 (C-9), 30.06 (d, $J \approx 149$ Hz; C-7′ or C-7″), 29.54 (C-8), 28.67 (d, $J \approx 149$ Hz; C-7″ or C-7′), 23.32 (*C*H_3CO), 22.99 (*C*H_3CO), 22.89 (*C*H_3CO), 20.80 ppm (*C*H_3CO); ^{31}P NMR (162 MHz, $CDCl_3$): $\delta = 31.16$, 28.78 ppm; HRMS (ESI): *m/z* (%): 1607.6332 (100) $[M+Na]^+$, 815.3120 (20) $[M+2Na]^{2+}$; elemental analysis calcd (%) for $C_{83}H_{102}N_4O_{23}P_2$ (1585.7): C 62.87, H 6.48, N 3.53; found: C 62.63, H 6.47, N 3.53.

[*N*-(Benzyloxycarbonyl)aminopropyl (2-acetamido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosidyl) *C*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonyl] *C*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-mannopyranosyl)methanephosphonate disodium salt (30): A solution of MeONa in dry MeOH (0.07 M, 0.26 mL) was added under nitrogen to a solution of compound **28** (mixture of phosphorus diastereoisomers, 0.28 g, 0.18 mmol) in dry MeOH (3 mL). The mixture was stirred at room temperature for 6 h, with monitoring of the course of the reaction by TLC (AcOEt/*i*PrOH 10:2). The solution was neutralised with Amberlite IR-120 resin (H^+ form), filtered and concentrated to dryness. ^{1}H NMR and HRMS (ESI) of the crude residue confirmed 6″-O-deacetylation: *m/z* (%): 1565.6237 (100) $[M+Na]^+$, 794.3066 (30) $[M+2Na]^{2+}$.

Crude **29** (mixture of phosphorus diastereoisomers, 0.22 g, 0.14 mmol) was dissolved under nitrogen in dry toluene (6.00 mL) and DBU (0.11 mL, 0.70 mmol) and thiophenol (0.06 mL, 0.56 mmol) were added. The reaction mixture was stirred at room temperature for 8 h with monitoring of the course of the reaction by TLC (MeOH/$CHCl_3$ 3:8, AcOEt/*i*PrOH 10:2). The solvent was evaporated and the residue was purified by silica gel chromatography (MeOH/$CHCl_3$ 1:19→1:9); The obtained compound was dissolved in MeOH and eluted through a column filled with Amberlite IR-120 resin (Na^+ form). The eluate was concentrated to dryness to yield **30** (disodium salt) as a white foam (0.21 g, 76% over two steps). $R_f = 0.26$ (MeOH/$CHCl_3$ 3:8); $[\alpha]_D^{25} = -16.3$ ($c = 1.0$ in chloroform); ^{1}H NMR (400 MHz, CD_3OD, 30 °C): $\delta = 7.34$–7.20 (m, 35H; arom.), 5.07 (s, 2H; CH_2Ph-Z), 4.81–4.76 (m, 3H; H-2, 2×CH*H*Ph), 4.71–4.60 (m, 6H; CH_2Ph, 4×CH*H*Ph), 4.58–4.48 (m, 6H; H-1, H-2′, H-2″, 3×CH*H*Ph), 4.45 (d, J = 11.1 Hz, 1H; CH*H*Ph), 4.36–4.27 (m, 2H; H-1′, H-1″), 4.24–4.11 (m, 2H; H-6a, H-6b), 4.00 (br dd, $J_{7a,7b}$ = 11.9, $J_{7a,8}$ = 7.8 Hz, 1H; H-7a), 3.92–3.76 (m, 6H; H-3′, H-4′, H-6′a, H-3″, H-4″, H-6″a), 3.73 (dd, $J_{3,2}$ = 3.9 Hz, 1H; H-3), 3.67 (t, $J_{4,3} = J_{4,5}$ = 9.4 Hz, 1H; H-4), 3.63–3.50 (m, 5H; H-7b, H-5′, H-6′b, H-5″, H-6″b), 3.49–3.42 (m, 1H; H-5), 3.21–3.16 (m, 2H; H-9a, H-9b), 2.06–1.90 (m, 4H; H-7′a, H-7′b, H-7″a, H-7″b), 2.04 (s, 3H; CH_3CO), 1.98 (s, 6H; 2×CH_3CO), 1.76–1.70 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, CD_3OD, 30 °C): $\delta = 172.79$, 171.53 (3×CO), 157.60 (CO-Z), 99.44 (C-1), 80.43 (C-3), 76.37, 75.11, 74.90 (C-5, C-4′, C-4″), 74.46 (*C*H_2Ph), 74.13, 73.55 (C-4, C-3′, C-5′, C-3″, C-5″), 73.01, 71.60, 71.23, 70.73 (5×*C*H_2Ph), 69.60 (C-1′, C-1″), 66.81 (C-6′, C-6″), 65.93 (*C*H_2Ph-Z), 63.46 (C-6), 60.01 (C-7), 49.99, 49.51 (C-2, C-

2′, C-2″), 37.72 (C-9), 29.37 (C-8), 29.27, 28.00 (C-7′, C-7″), 21.41 ppm ($3\times CH_3CO$); ^{31}P NMR (162 MHz, CD_3OD, 30 °C): δ = 22.58, 21.80 ppm; HRMS (ESI): *m/z* (%): 1535.5751 (100) $[M^{2-}+Na^+]^-$, 756.2922 (40) $[M]^{2-}$; elemental analysis calcd (%) for $C_{79}H_{94}N_4O_{22}Na_2P_2$ (1559.5): C 60.84, H 6.08, N 3.59; found: C 61.02, H 6.07, N 3.59.

[Aminopropyl (2-acetamido-2-deoxy-β-D-mannopyranosidyl) *C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonyl] *C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate disodium salt (1): Trimer **30** (0.090 g, 0.06 mmol) was dissolved in a MeOH/H_2O mixture (1:1, 4 mL), Pd/C catalyst (10%, 0.10 g) was added, and the reaction mixture was vigorously stirred under hydrogen at room temperature. After 27 h a second portion of the catalyst (10%) was added and the mixture was stirred under hydrogen for an additional 12 h. The reaction was diluted with H_2O, filtered over a Celite pad and concentrated under reduced pressure and finally lyophilised to give an amorphous solid. This was dissolved in H_2O and first eluted through a column filled with Dowex 50W X8 resin (H^+ form) and then through a column filled with the same resin in Na^+ form. The eluate was lyophilised to afford **1** (0.043 g, 84%, disodium salt) as a white foam. $[\alpha]_D^{25}=-6.1$ (c = 1.1 in chloroform); ^{1}H NMR (400 MHz, D_2O, 45 °C): δ = 4.96 (d, $J_{1,2}$ = 1.2 Hz, 1H; H-1), 4.68 (br dd, 1H; H-2), 4.60 (br dd, 1H; H-2′ or H-2″), 4.53 (br dd, 1H; H-2″ or H-2′), 4.42–4.15 (m, 8H; H-6a, H-6b, H-1′, H-3′, H-6′a, H-6′b, H-1″, H-3″), 4.13 (br dd, $J_{7a,7b}$ = 11.2, $J_{7a,8}$ = 5.2 Hz, 1H; H-7a), 4.03 (dd, $J_{6''a,6''b}$ = 12.5, $J_{6''a,5''}$ = 4.7 Hz, 1H; H-6″a), 3.99–3.93 (m, 3H; H-3, H-7b, H-6″b), 3.91–3.74 (m, 5H; H-4, H-4′, H-5′, H-4″, H-5″), 3.68 (br ddd, $J_{5,4}$ = 9.5 Hz, 1H; H-5), 3.34–3.24 (m, 2H; H-9a, H-9b), 2.36–2.26 (m, 4H; H-7′a, H-7′b, H-7″a, H-7″b), 2.24 (s, 3H; CH_3CO), 2.22 (s, 6H; $2\times CH_3CO$), 2.16–2.10 ppm (m, 2H; H-8a, H-8b); ^{13}C NMR (100.6 MHz, D_2O, 45 °C): δ = 175.75, 174.65, 174.59 ($3\times$CO), 99.74 (C-1), 75.67 (d, $J_{5,P}\approx$6 Hz; C-5), 74.42 (C-5″), 74.01, 72.90 (C-1′, C-1″), 73.14 (d, $J\approx$6 Hz, C-5′), 71.93 (C-3), 69.62, 69.40 (C-3′, C-3″), 67.98 (C-7), 67.77, 67.20, 66.98 (C-4, C-4′, C-4″), 63.45 (C-6, C-6′), 60.84 (C-6″), 53.30, 53.19, 53.00 (C-2, C-2′, C-2″), 38.00 (C-9), 27.86, 27.77 ($2\times$d, $J\approx$133 Hz, C-7′, C-7″), 26.94 (C-8), 22.39 ppm ($3\times CH_3CO$); ^{31}P NMR (162 MHz, D_2O, 45 °C): δ = 23.79, 23.24 ppm; HRMS (ESI): *m/z* (%): 839.2717 (100) $[M]^-$, 861.2536 (20) $[M^{2-}+Na^+]^-$; elemental analysis calcd (%) for $C_{29}H_{52}N_4O_{20}Na_2P_2$ (884.7): C 39.37, H 5.92, N 6.33; found: C 39.44, H 5.91, N 6.35.

Competitive ELISA assay: Ninety-six-well flat-bottomed plates were incubated overnight at 4 °C with a mixture of MenA CPS (1 mg mL^{-1}) and methylated human serum albumin (1 mg mL^{-1}). A solution of foetal calf serum (5%) in phosphate buffered saline supplemented with Brij-35 (0.1%) and sodium azide (0.05%) was applied to the plates for blocking of nonspecific binding sites. The plates were incubated overnight at 4 °C with a solution (1:1000) of human anti-MenA, used as reference serum. This serum was obtained from human volunteers vaccinated with an anti-meningococcal A+C+W135+Y polysaccharide vaccine. When saccharide competitors were tested, they were added to each well just before the addition of the reference serum. The plates were then incubated with alkaline phosphatide-conjugated antibody to human IgG, stained with *p*-nitrophenylphosphate, and the absorbance was measured at 405 nm with an Ultramark microplate reader (Bio-Rad Laboratories S.r.l., Milan, Italy).

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[31] The characterisation of the other phosphorus diastereoisomer of **23** is reported in ref. [16].
[32] **Note added in proof**: A similar synthesis of all α-linked phosphonoester-bridged oligomers of *N*-acetyl-D-mannosamine has recently been reported by S. Oscarson et al.: P. Teodorović, R. Slättegård, S. Oscarson, *Org. Biomol. Chem.* **2006**, *4*, 4485–4490.

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