Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 6673

www.rsc.org/obc

PAPER

Synthesis and preliminary biological evaluation of *carba* analogues from *Neisseria meningitidis* A capsular polysaccharide†

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Received 30th January 2012, Accepted 11th June 2012
DOI: 10.1039/c2ob25222h

The Gram-negative encapsulated bacterium *Neisseria meningitidis* type A (MenA) is a major cause of meningitis in developing countries, especially in the sub-Saharan region of Africa. The development and manufacture of an efficient glycoconjugate vaccine against MenA is greatly hampered by the poor hydrolytic stability of its capsular polysaccharide, consisting of (1→6)-linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate repeating units. The replacement of the ring oxygen with a methylene group to get a carbocyclic analogue leads to the loss of the acetalic character of the phosphodiester and consequently to the enhancement of its chemical stability. Here we report the synthesis of oligomers (mono-, di- and trisaccharide) of *carba*-*N*-acetylmannosamine-1-*O*-phosphate as candidates for stabilized analogues of the corresponding fragments of MenA capsular polysaccharide. Each of the synthesized compounds contains a phosphodiester-linked aminopropyl spacer at its reducing end to allow for protein conjugation. The inhibition abilities of the synthetic molecules were investigated by a competitive ELISA assay, showing that only the *carba*-disaccharide is recognized by a polyclonal anti-MenA serum with an affinity similar to a native MenA oligosaccharide with average polymerization degree of 3.

Introduction

Bacterial meningitis is a severe inflammation of the membranes that surround the brain and the spinal cord, causing approximately 170 000 annual deaths upon more than 1 200 000 cases, with at least a 5–10% of case fatality in industrialized countries and 20% in the developing world.[1] *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib) and *Neisseria meningitidis*[2] are responsible for most of the cases of bacterial meningitis worldwide although, with the advent of conjugate vaccines for Hib and for the pneumococcus, the meningococcus is the remaining major bacterial pathogen causing meningitis in children and adults. Among thirteen clinically significant capsular serogroups of the Gram-negative bacterium *N. meningitidis*, serotype A (MenA) is the main cause of meningitis epidemics and outbreaks in developing countries, predominantly throughout what is known as the African meningitis belt.[3,4] The carbohydrate capsule (capsular polysaccharide, CPS) represents a major virulence factor of MenA and consists of (1→6)-linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate repeating units (Fig. 1).[5] The first effective anti-MenA vaccine was based on purified natural CPS and was licensed in the seventies in combination with other meningococcal CPSs.[6] Although polysaccharide-based vaccines were demonstrated to be highly effective in preventing disease in adults and older children, it is well-established that polysaccharide immunogenicity is strongly enhanced by chemical conjugation to carrier proteins (typically, CRM197, tetanus or diphtheria toxoid, Protein D).[2a,7] In this way, immunological memory is established, raising a strong, durable and protective immune response from early childhood.[8] In 2005, a tetravalent meningococcal vaccine containing *N. meningitidis* serogroup A, C, Y and W-135 CPS conjugated individually to diphtheria toxoid was approved in the United States.[9] Recently, a second anti-meningococcal glycoconjugate vaccine, where meningococcal CPS oligosaccharides are covalently linked to CRM197, has been licensed.[10]

An additional feature of MenA CPS is its chemical lability in water, mainly due to the inherent instability of the anomeric glycosyl phosphodiesters.[7h,11] This could hamper the development of glycoconjugate vaccines against MenA, that are mainly intended for use in the "meningitis belt" countries. Thus, we became interested in the design and synthesis of novel and hydrolytically stable analogues of the MenA CPS repeating unit, such as 1-*C*-phosphono analogues.[12] These could be incorporated into a saccharide chain in order to obtain oligomers endowed with enhanced shelf-life. On the other hand, their conjugation to a proper immunogenic protein carrier should elicit

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†Electronic supplementary information (ESI) available: Additional experimental data and copies of ^{1}H-NMR spectra of known compounds **6–11**. Copies of ^{1}H, ^{13}C and ^{31}P NMR spectra for all new compounds. See DOI: 10.1039/c2ob25222h

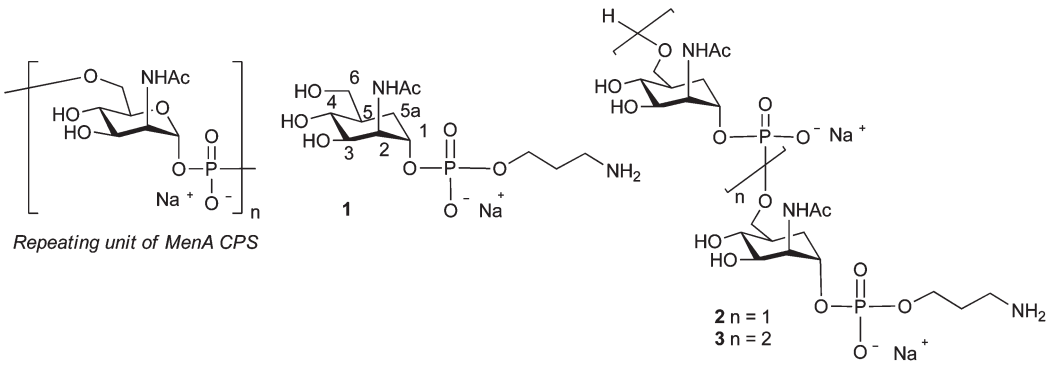
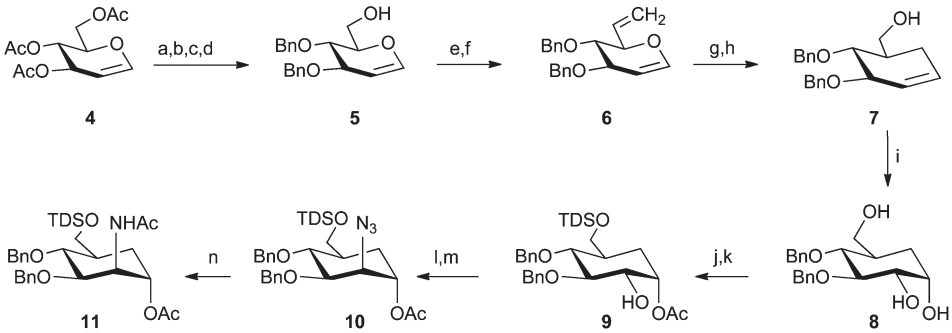


Fig. 1 Structures of repeating unit of MenA CPS and target *carba*-oligomers **1–3**.



Scheme 1 *Reagents and reaction conditions*: (a) NaOMe, MeOH, rt, 3 h. (b) TDSCl, imidazole, THF, 20 °C → rt, 24 h. (c) BnBr, NaH, TBAI, THF, rt, 2 h. (d) TBAF, THF, 0 °C → rt, 2 h, 67% over 4 steps. (e) IBX, EtOAc, 75 °C, 4 h. (f) PPh_3CH_3I, KHMDS, THF, −78 °C → rt, 3 h, 77%, over 2 steps. (g) 1,6-dichlorobenzene, 240 °C, 2 h. (h) $NaBH_4$, THF–EtOH 4 : 1, rt, 15 min, 86% over 2 steps. (i) OsO_4, Me_3NO, acetone–H_2O 4 : 1, rt, 48 h, 86%. (j) TDSCl, imidazole, THF, 15 °C → rt, 24 h, 93%. (k) $(MeO)_3CMe$, CH_3CN, PTSA, rt, 15 min, then 80% HOAc, rt, 15 min, 91%. (l) Tf_2O, CH_2Cl_2–pyridine 5 : 1, −10 °C → 0 °C, 1 h. (m) NaN_3, DMF–H_2O 19 : 1, 40 °C, 12 h, 79% over 2 steps. (n) PPh_3, THF, 60 °C, 12 h, then H_2O, 60 °C, 24 h, then MeOH, Ac_2O, rt, 24 h, 95%.

protective antibodies that will cross-react with the bacterial capsule.[7,8,13]

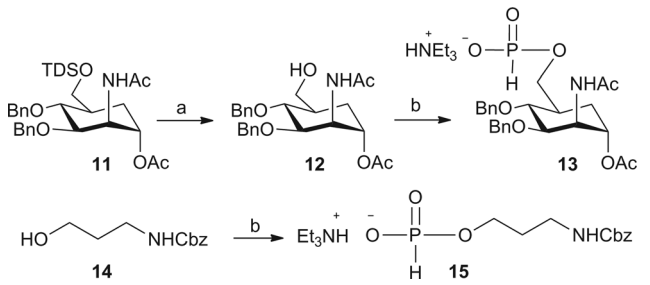
Stabilization of glycosyl 1-*O*-phosphates can also be achieved using carbasugar analogues, where a methylene group replaces the pyranose oxygen atom.[14] This structural modification leads to the loss of the acetalic character of the phosphodiester, and is expected to gain improved stability towards hydrolysis.[15] We recently showed that a *carba*-rhamnose is able to preserve the conformation and the biological activity – in terms of antibody affinity – of the natural rhamnopyranose when inserted into the *Streptococcus pneumoniae* type 19F CPS trisaccharide repeating unit.[16] Prompted by this encouraging result, we synthesized the *carba*-*N*-acetylmannosamine-1-*O*-phosphate and compared its geometrical and conformational properties with the naturally occurring *N*-acetylmannosamine-1-*O*-phosphate, the MenA CPS repeating unit.[17] The results showed they have similar conformational behavior, so that *carba*-*N*-acetylmannosamine-1-*O*-phosphate can be regarded as a potential mimic of the MenA CPS repeating unit and can be used in the construction of *carba* oligomers endowed with enhanced stability in comparison with the native polymer.

In the present work, we report on the synthesis of mono-, di- and trisaccharide *carba* analogues of MenA CPS (compounds **1–3**, Fig. 1). The synthetic fragments are provided with a phosphodiester-linked aminopropyl spacer at their reducing end, for their eventual conjugation to a carrier protein.

Moreover, the relative affinities of synthetic compounds **1–3** were investigated by a competitive ELISA assay using a polyclonal anti-MenA serum derived from immunization of mice with a native MenA oligosaccharide–protein conjugate.

Results and discussion

Our synthetic route towards compounds **1–3** is based on the *H*-phosphonate methodology[18] for the formation of the phosphodiester bridges. Moreover, we envisaged carbasugar **11** (Scheme 1) as the key precursor of our strategy. The first synthesis of carbasugar **11** has been recently reported by our group.[17] In that work, commercial glucal **4** was first converted into triol **8** as described in the literature,[19] and finally transformed into intermediate **11** in a five further steps. Herein, we briefly describe an improved preparation of **11** which, although it is based on our same route, allowed this key intermediate to be produced in gram scale for the synthesis of *carba* oligomers (Scheme 1, see ESI† for the experimental procedures). Tri-*O*-acetyl glucal **4** was converted into alcohol **5**[20] in four linear steps – Zemplén deacetylation, regioselective 6-*O*-silylation, benzylation of the C-3 and C-4 hydroxyls, removal of the silyl ether – in 67% overall yield. The primary hydroxy group of **5** was oxidized by refluxing in EtOAc in the presence of 2-iodoxybenzoic acid (IBX), and the resulting aldehyde was submitted to



Scheme 2 *Reagents and reaction conditions*: (a) TBAF, THF, rt, 3 h, 91%. (b) 2-Chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one, CH_3CN–pyridine 3 : 1, rt, 45 min (**13**: 82%; **15**: 98%).

Wittig reaction (methyltriphenylphosphonium iodide and potassium hexamethyldisilylamide), furnishing **6** in 77% yield over 2 steps.

The formation of the carbocycle was achieved by Claisen rearrangement. Glucal **6** was heated in a sealed tube in *p*-dichlorobenzene at 240 °C to yield the unstable 5-formyl cyclohexene intermediate, which was immediately reduced with $NaBH_4$, giving **7** in 86% yield. Stereoselective OsO_4-mediated *syn*-dihydroxylation of the double bond in **7** produced **8** in 86% yield. Triol **8** was regioselectively 6-*O*-silylated (93% yield) and 1-*O*-acetylated (91% yield), providing alcohol **9**. The 2-azido intermediate **10** was obtained in 79% yield on a 10 g scale by preliminary conversion of **9** into the 2-*O*-trifluoromethanesulfonate ester, followed by nucleophilic displacement with sodium azide in a 19 : 1 DMF–H_2O mixture at 40 °C.

Eventually, reduction of the azide under Staudinger's conditions with triphenylphosphine and water, followed by *N*-acetylation with acetic anhydride, gave **11** in 95% yield. Altogether, the key precursor **11** was obtained in 14 steps on a multigram scale in 17% overall yield, to be compared with 8.6% reported in our previous synthesis.[17]

The removal of orthogonal protecting groups from building block **11** provided access to the phosphodiester-linked oligomers. Compound **11** was desilylated with tetrabutylammonium fluoride (TBAF) in THF (91%), and the resulting alcohol **12** was treated with 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one (commonly named salicylchlorophosphite) in a mixture of dry acetonitrile and pyridine at room temperature to give *H*-phosphonate **13** in 82% yield as a triethylammonium salt (Scheme 2).

Following a similar procedure, commercial benzyl *N*-(3-hydroxypropyl)carbamate **14** was activated as the *H*-phosphonate **15**[21] (98% yield) in order to be inserted at the reducing end of the oligomers.

Having building blocks **11**, **13** and **15** in our hands, the stage was set for the synthesis of oligomers **1–3** (Scheme 3). First, deacetylation of **11** under Zemplén conditions afforded **16** in 84% yield. Then, according to a standard *H*-phosphonate protocol, alcohol **16** and compound **15** were condensed in the presence of trimethylacetyl (pivaloyl) chloride in pyridine. The resulting *H*-phosphonate diester intermediate was oxidized *in situ* by iodine in a 19 : 1 mixture of pyridine and water to afford the glycosyl phosphodiester **17** in 81% yield. Protected dimer **18** was achieved under the same conditions by condensation of **16** with *H*-phosphonate **13**, followed by oxidation (82% yield). Next, dimer **18** was deacetylated under Zemplén conditions and condensed with *H*-phosphonate **15** affording the spacer-bearing dimer **19** in 45% yield after oxidation. This modest yield was however significantly improved up to 85% by increasing the amount (5 equivalents) of the *H*-phosphonate **15**.

On the other hand, deacetylation of **18** followed by condensation with *H*-phosphonate **13** and subsequent oxidation, led to trimer **20** in 81% yield. Compound **20** was next deacetylated, coupled with **15** and oxidized, furnishing fully protected trimer **21** in moderate yield (57%), which could not be increased even when using a large excess of **15**.

Global removal of the protecting group on oligomers **17**, **19**, and **21** was accomplished in two steps. First, the TDS ethers were removed in the presence of TBAF in THF. Thereafter, the remaining protecting groups (benzyls and benzyloxycarbonyl) were cleaved by hydrogenolysis over 10% Pd on carbon in a methanol–water mixture. Final purification was completed 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 eluted compounds provided oligomers **1** (95% yield), **2** (77% yield), and **3** (78% yield) as their sodium salts. The identity and purity of the target compounds was ascertained by ^1H, ^{13}C, and ^{31}P NMR spectroscopic analyses (see ESI†), including two-dimensional techniques.

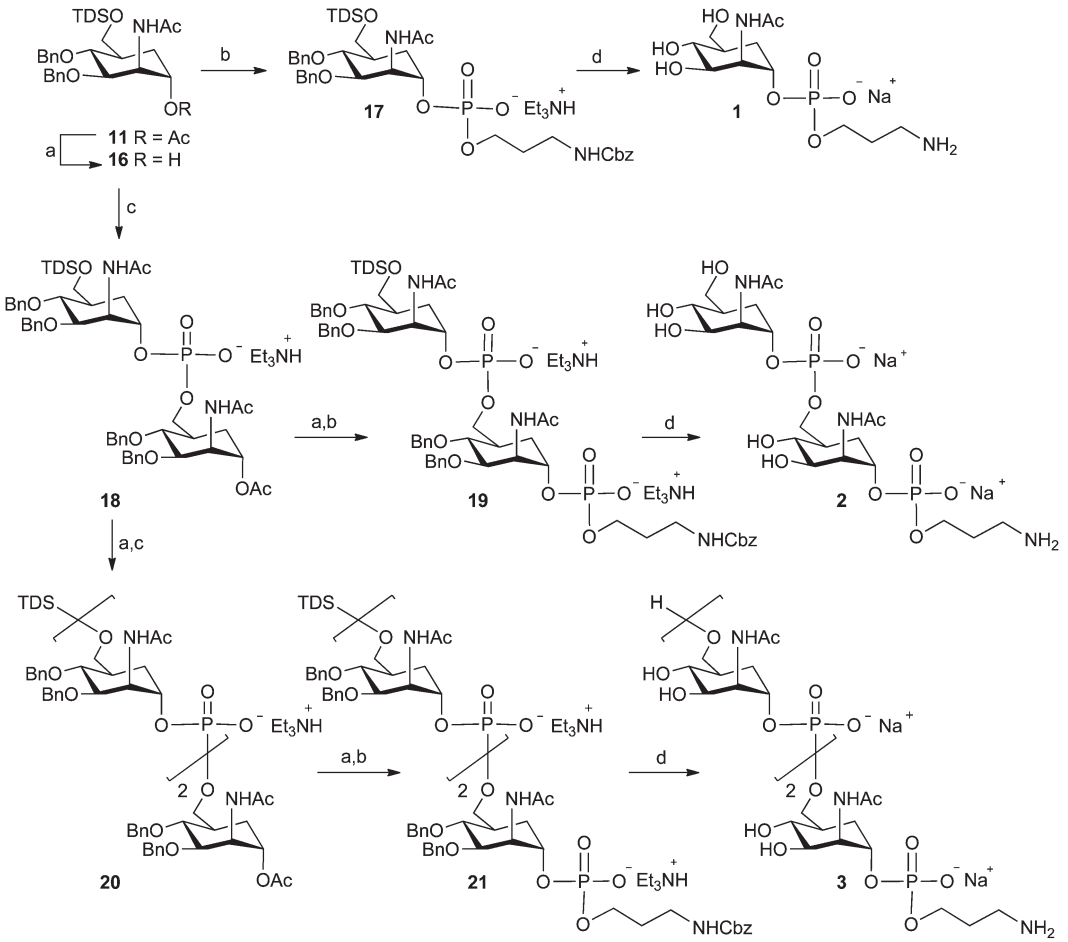
Competitive ELISA assay of synthetic *carba* oligomers

The ability of increasing concentrations (from 0.5×10^{-7} mM to 0.5 mM) of the *carba* oligomers **1–3** to inhibit the binding between the MenA CPS, coated onto plates, and a polyclonal anti-MenA serum was evaluated by competitive ELISA assay using native MenA CPS and native MenA *av*DP15 (MenA oligosaccharide with average polymerization degree of 15) as positive controls, and Laminarin as negative control. The inhibition capacity of the synthetic compounds was determined in comparison with a native MenA oligosaccharide with average polymerization degree of 3 (native MenA *av*DP3). The results showed that the best inhibition was given by native MenA CPS and by native MenA *av*DP15 (IC_{50} of 5.15×10^{-6} and 4.3×10^{-3} mM, respectively, and a 98% of inhibition of serum, Table 1 and Fig. 2). Only dimer **2** was able to induce a 90% of serum inhibition with an IC_{50} of 0.16–0.091 mM (Table 1). In contrast, compounds **1** and **3** were only poor competitors, with an inhibition around 30% of the antibody binding. *Carba* disaccharide **2** showed a similar trend to native MenA *av*DP3 oligosaccharide, which reached 93% of serum inhibition with an IC_{50} of 4.3×10^{-2} mM.

These data showed that the synthetic molecule **2**, containing the unnatural *carba*-*N*-acetylmannosamine units, is still recognized by the specific anti-Men A serum. Interestingly, the inhibition ability of compound **2** seems to be similar to the native MenA *av*DP3 oligosaccharide.

Conclusions

Taking advantage of our synthesis of the *carba*-*N*-acetylmannosamine building block,[17] we developed a straightforward strategy for the preparation of phosphodiester-linked oligomers of *carba*



Scheme 3 *Reagents and reaction conditions*: (a) NaOMe, MeOH, rt, 4 h: 84% from **11**, 87% from **18**, 70% from **20**. (b) **15**, pyridine, PivCl, rt, 45 min, then I_2, pyridine–H_2O 19 : 1, rt, 15 min: **17** (81%), **19** (85%), **21** (57%). (c) **13**, pyridine, PivCl, rt, 45 min, then I_2, pyridine–H_2O 19 : 1, rt, 15 min: **18** (82%), **20** (81%). (d) TBAF, THF, RT, 8–12 h, then MeOH–H_2O 1 : 1, 10% Pd/C, H_2, rt, 48 h, then H_2O, Dowex 50W X8 resin (H^+ form), then Dowex 50W X8 resin (Na^+ form): **1** (95%), **2** (77%), **3** (78%).

Table 1 Competitive ELISA IC_{50} values (mM)

Meningococcal A antigens	IC_{50} (mM)
1 = monosaccharide	—
2 = disaccharide	0.16–0.091
3 = trisaccharide	—
Native MenA CPS	5.15×10^{-6}
Native *av*DP15	4.3×10^{-3}
Native *av*DP3	4.3×10^{-2}

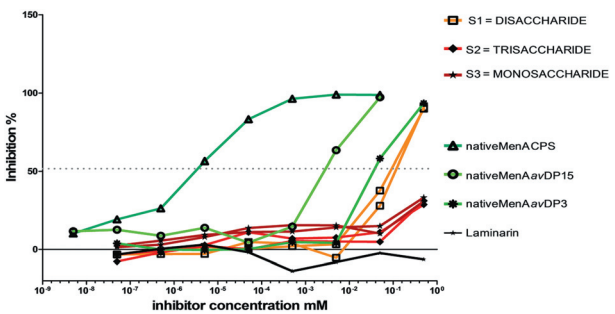


Fig. 2 Competitive ELISA between the native MenA CPS and oligosaccharide inhibitors for anti-MenA CPS polyclonal serum. S1, S2 and S3 are the synthetic inhibitors compared with the native molecules MenA CPS, *av*DP15 and *av*DP3 oligosaccharides. The non correlated polysaccharide Laminarin was used as negative control.

analogues of *Neisseria meningitidis* A capsular polysaccharide fragments. Owing to the chemical and enzymatic lability of the anomeric phosphodiester linkages occurring in the native polysaccharide, these compounds are proposed as stabilised analogues of the corresponding phosphate-bridged oligomers. The synthetic oligomers have 1-*O*-phosphodiester-linked aminopropyl spacer arms to allow conjugation with a carrier protein. The installation of each phosphodiester linkage was carried out by using the classical *H*-phosphonate methodology,[18] using pivaloyl chloride as a condensing agent and I_2 in a pyridine–water mixture for the oxidation step. It is noteworthy that our strategy is suitable for further elongation and synthesis of longer oligomers by iteration of the deacetylation step followed by coupling with the key *H*-phosphonate building block **13**.

A competitive ELISA assay was employed to assess the abilities of the synthetic molecules to bind the specific anti-MenA serum, showing that only dimer **2** is able to significantly

inhibit the antibody binding. Further investigations are needed to provide a rational interpretation of the differences in the inhibition potency between the three synthetic molecules **1**, **2** and **3**. In particular, it would be intriguing to understand why the dimer **2**, and not the trimer **3**, seems to possess the structural features to mimic a native meningococcal A CPS epitope. Moreover, further insight can be gained by chemical conjugation of the synthetic molecules with a carrier protein to test the resulting glycoconjugates for the stimulation of appropriate immune responses. These studies are currently in progress and will be reported elsewhere.

Experimental

Chemical synthesis

General procedures. All commercially available reagents including dry solvents were used as received. Nonvolatile materials were dried under high vacuum. Reactions were monitored by thin-layer chromatography on pre-coated Merck silica gel 60 F254 plates and visualized by staining with a solution of cerium sulfate (1 g) and ammonium heptamolybdate tetrahydrate (27 g) in water (469 mL) and concentrated sulfuric acid (31 mL). Flash chromatography was performed on Fluka silica gel 60. NMR spectra were recorded at 300 K (unless otherwise stated) on spectrometer operating at 400 MHz. Proton chemical shifts are reported in ppm (δ) with the solvent reference relative to tetramethylsilane (TMS) employed as the internal standard ($CDCl_3$ δ = 7.26 ppm). *J* values are given in Hz. Carbon chemical shifts are reported in ppm (δ) relative to TMS with the respective solvent resonance as the internal standard ($CDCl_3$, δ = 77.0 ppm). Apart from quaternary carbons, signal attribution in ^{13}C-NMR spectra was derived by HSQC experiment. Optical rotations values are given in 10^{-1} deg cm^2 g^{-1} and were measured at 25 °C on a polarimeter at 589 nm using a 5 mL cell with a length of 1 dm. High resolution mass spectra (HRMS) were performed at CIGA (Centro Interdipartimentale Grandi Apparecchiature), with Mass Spectrometer *APEX II* & *Xmass* software (Bruker Daltonics).

2-Acetamido-1-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranose (12). A stirred solution of **11** (1.05 g, 1.8 mmol) in dry THF (30 mL) was treated with TBAF (1 M solution in THF, 2.2 mL, 2.2 mmol), and the mixture was stirred at room temperature under nitrogen atmosphere for 2 h. The mixture was then diluted with CH_2Cl_2 (50 mL), the organic layer was washed with a saturated aqueous solution of NH_4Cl (50 mL), dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by flash chromatography (MeOH–CH_2Cl_2 5 : 95), affording **12** (722 mg, 91%). $[\alpha]_D$ +32.3 (*c* 1.0 in $CHCl_3$); Found: C, 68.15; H, 7.1; N, 3.21. Calc for $C_{25}H_{31}NO_6$: C, 68.01; H, 7.08; N, 3.17%; δ_H (400 MHz; $CDCl_3$) 7.51–7.20 (10H, m, H_{Ar}), 5.80 (1H, d, *J* 8.5, NH), 5.20–5.15 (1H, m, 1-H), 4.74 (1H, d, *J* 11.4, C*H*HPh), 4.62–4.58 (2H, m, CH_2Ph), 4.48–4.41 (2H, m, CH*H*Ph, 2-H), 3.94–3.90 (1H, m, 3-H), 3.82–3.78 (1H, m, 6-H), 3.72–3.64 (2H, m, 4-H, 6′-H), 2.20–2.12 (1H, m, 5-H), 2.03 (3H, s, OAc), 2.00–1.96 (1H, m, 5a-H), 1.95 (3H, s, *Ac*NH), 1.85–1.77 (1H, m, 5a′-H); δ_C (100.6 MHz; $CDCl_3$) 170.9 (C═O), 170.1 (C═O), 138.1–127.9 (CH_{Ar}), 78.9 (3-C), 76.7 (4-C), 72.9 (*C*H_2Ph), 72.8 (*C*H_2Ph), 68.3 (1-C), 63.6 (6-C), 50.7 (2-C), 39.8 (5-C), 27.2 (5a-C), 23.3 (NHAc), 21.1 (OAc); ESI-HRMS ([M + Na]$^+$) *m*/*z* calc 464.20436 for $C_{25}H_{31}NO_6Na$, found 464.20417.

6-*O*-(2-Acetamido-1-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl) hydrogenphosphonate, triethylammonium salt (13). To a solution of **12** (70 mg, 0.16 mmol) in dry CH_3CN (2 mL) and pyridine (0.7 mL), a 0.4 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one in dry CH_3CN (0.5 mL, 0.2 mmol) was added at room temperature. The mixture was stirred at room temperature under nitrogen atmosphere for 45 min, then a 1 : 1 mixture of pyridine–H_2O (1 mL) was added and the mixture was diluted with $CHCl_3$ (20 mL). The organic layer was washed with H_2O (10 mL) and 1 M TEAB (10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (MeOH–CH_2Cl_2 5 : 95, 1% TEA), providing a clear oil, that was diluted with $CHCl_3$ (20 mL), then washed again with 0.5 M TEAB (10 mL), dried (Na_2SO_4), filtered, and concentrated to get product **13** (80 mg, 82%) as a syrup. The formation of the *H*-phosphonate intermediate was ascertained by ^{1}H NMR analysis, which showed the diagnostic doublet at δ 6.03 ($J_{H,P}$ = 635 Hz). Compound **13** was used directly in the following steps without further characterization.

3-(Benzyloxycarbonyl)aminopropyl hydrogenphosphonate, triethylammonium salt (15). To a stirred solution of benzyl 3-hydroxypropylcarbamate **14** (13 mg, 0.06 mmol) in dry CH_3CN (0.3 mL) and pyridine (0.1 mL), a 0.4 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one in dry CH_3CN was added (0.2 mL, 0.08 mmol) at room temperature. The mixture was stirred at room temperature under nitrogen atmosphere for 45 min. Then a 1 : 1 mixture of pyridine–H_2O (1 mL) was added. TEAB (triethylammonium bicarbonate buffer, 1 M solution in H_2O, 0.5 mL) was then added to the mixture, and the mixture was diluted with $CHCl_3$ and concentrated without phase separation. The residue was purified by flash chromatography (MeOH–CH_2Cl_2 5 : 95 → 10 : 90, 1% TEA), providing **15** (22 mg, 98%). The spectroscopic characterization data of hydrogenphosphonate **15** were in agreement with those previously reported.[20]

General procedure A: deacetylation, *H*-phosphonate coupling and oxidation

A solution of precursor (**11**, **18** or **20**) in dry MeOH was treated with 0.08 M NaOMe in MeOH and stirred under nitrogen atmosphere. After reaction completion, the mixture was diluted with MeOH, neutralized with Amberlite IR 120 (H^+) resin, filtered, and concentrated. The crude was purified by flash chromatography, providing the corresponding deacetylated product.

The deacetylated acceptor and the *H*-phosphonate (**13** or **15**) were co-evaporated with pyridine three times under high vacuum. The residue was then dissolved in dry pyridine, and PivCl was added. The mixture was stirred under nitrogen atmosphere for 40 min, then a freshly prepared solution of I_2 in a 19 : 1 mixture of pyridine–H_2O was added and the mixture was stirred for another 15 min. The reaction mixture was diluted with $CHCl_3$, and the organic layer was washed with a 1 M aqueous

solution of $Na_2S_2O_3$ and 0.5 M TEAB, dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by flash chromatography, providing the phosphodiester derivative.

Synthesis of phosphodiesters 17 and 18 from 11

Compound **11** (600 mg, 1.02 mmol) was dissolved in MeOH (20 mL) and deacetylated with NaOMe in MeOH (5 mL) according to general procedure A. The crude was purified by flash chromatography (MeOH–CH_2Cl_2 5 : 95), providing alcohol **16** (470 mg, 84%).

3-(Benzyloxycarbonyl)aminopropyl 1-*O*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-manno-pyranosyl phosphate), triethylammonium salt (17). Alcohol **16** (204 mg, 0.38 mmol) was condensed with *H*-phosphonate **15** (282 mg, 0.75 mmol) in pyridine (5 mL) in the presence of PivCl (0.1 mL, 0.79 mmol), and *in situ* oxidized with a solution of I_2 (155 mg, 0.61 mmol) in pyridine–H_2O (4 mL) according to general procedure A. Purification by flash chromatography (MeOH–CH_2Cl_2 5 : 95, 1% TEA) yielded compound **17** (281 mg, 81%) as a colourless oil. $[\alpha]_D$ +14.7 (*c* 1.0 in $CHCl_3$); Found: C, 62.91; H, 8.34; N, 4.54. Calc for $C_{48}H_{76}N_3O_{10}PSi$: C, 63.06; H, 8.38; N, 4.60%; δ_H (400 MHz; $CDCl_3$) 7.40–7.23 (15H, m, H_{Ar}), 5.15–5.05 (2H, m, CH_2Ph), 4.78 (1H, d, *J* 11.4, C*H*HPh), 4.63–4.38 (5H, m, 1-H, 2-H, CH_2Ph, C*H*HPh), 4.17 (1H, dd, $J_{3,2}$ 4.4, $J_{3,4}$ 6.4, 3-H), 4.07–3.91 (2H, m, OCH_2CH$_2$CH$_2$N), 3.83–3.80 (1H, m, 6-H), 3.63 (1H, br t, 4-H), 3.59–3.56 (1H, m, 6′-H), 3.45–3.27 (2H, m, OCH$_2$CH$_2$CH_2N), 3.03 (6H, q, 3 CH_2 Et), 2.15–2.11 (1H, m, 5-H), 1.95 (3H, s, *Ac*NH), 1.90–1.87 (2H, m, 5a-H, 5a′-H), 1.79 (2H, br t, OCH$_2$CH_2CH$_2$N), 1.62 (1H, h, CH thexyl), 1.29 (9H, t, *J* 7.3, 3 CH_3 Et), 0.90 (6H, d, *J* 6.8, 2 CH_3CH thexyl), 0.84 and 0.83 (6H, 2s, 2 CH_3 thexyl), 0.07 (3H, s, CH_3Si), 0.05 (3H, s, CH_3Si); δ_C (100.6 MHz; $CDCl_3$) 181.8 (C═O), 167.4 (C═O), 156.7 (C_{Ar}), 138.9 (C_{Ar}), 138.2 (C_{Ar}), 133.6–116.0 (CH_{Ar}), 78.2 (3-C), 76.5 (4-C), 72.4 (CH_2Ph), 72.5 (CH_2Ph), 70.4 (1-C), 66.3 (CH_2Ph), 62.7 (6-C, OCH_2CH$_2$CH$_2$N), 52.8 (2-C), 45.6 (3 CH_2 Et), 40.0 (5-C), 37.2 (OCH$_2$CH$_2$CH_2N), 34.3 (CH thexyl), 30.4 (OCH$_2$CH_2CH$_2$N), 29.7 (5a-C), 25.1 (C thexyl), 23.3 (NHAc), 20.4 (2 CH_3 thexyl), 18.6 (2 CH_3CH thexyl), 8.5 (3 CH_3 Et), −3.5 (CH_3Si thexyl), −3.7 (CH_3Si thexyl); δ_P (162 MHz; $CDCl_3$) 1.28; ESI-HRMS $[M]^-$ *m*/*z* calc 811.37603 for $C_{42}H_{60}N_2O_{10}PSi$, found 811.37373.

1-*O*-Acetyl-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl 6-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranosyl phosphate), triethylammonium salt (18). Alcohol **16** (40 mg, 0.073 mmol) was condensed with *H*-phosphonate **13** (48 mg, 0.079 mmol) in pyridine (2 mL) in the presence of PivCl (23 μL, 0.18 mmol), and *in situ* oxidized with a solution of I_2 (60 mg, 0.24 mmol) in pyridine–H_2O (1.2 mL) according to general procedure A. Purification by flash chromatography (MeOH–CH_2Cl_2 10 : 90, 1% TEA) yielded compound **18** (68 mg, 82%) as a colourless oil. $[\alpha]_D$ +17.3 (*c* 1.0 in $CHCl_3$); Found: C, 64.71; H, 8.04; N, 3.61. Calc for $C_{62}H_{92}N_3O_{13}PSi$: C, 64.95; H, 8.09; N, 3.67%; δ_H (400 MHz; $CDCl_3$) 7.42–7.20 (20H, m, H_{Ar}), 5.15 (1H, br t, 1-H), 4.83–4.76 (2H, m, 2 C*H*HPh), 4.73–4.49 (8H, m, 2-H, 2′-H, 3 CH_2Ph), 4.38–4.30 (1H, m, 6′-Ha), 4.22–4.17 (1H, m, 3′-H), 3.95–3.80 (5H, m, 3-H, 1′-H, 6′-Hb, 6-Ha, 4-H), 3.73–3.65 (1H, m, 4′-H), 3.61–3.57 (m, 1H, 6-Hb), 3.03 (6H, q, 3 CH_2 Et), 2.18–2.11 (2H, m, 5-H, 5′-H), 2.00 (3H, s, OAc), 1.95 (6H, br s, 2 *Ac*NH), 1.92–1.83 (2H, m, 5a′-Ha, 5a′-Hb), 1.62 (1H, h, CH thexyl), 1.28–1.22 (11H, m, 5a-Ha, 5a-Hb, 3 CH_3 Et), 0.89 (6H, d, *J* 6.8, 2 CH_3CH thexyl), 0.82 (6H, 2s, 2 CH_3 thexyl), 0.07 (3H, s, CH_3Si), 0.06 (3H, s, CH_3Si); δ_C (100.6 MHz; $CDCl_3$) 170.5 (C═O), 170.3 (C═O), 170.0 (C═O), 138.9 (C_{Ar}), 138.3 (C_{Ar}), 128.7–127.4 (CH_{Ar}), 79.1, 78.4 (3-C, 3′-C), 75.8, 74.1 (4-C, 4′-C), 74.2 (CH_2Ph), 72.3 (CH_2Ph), 72.0 (CH_2Ph), 71.8 (CH_2Ph), 70.3, 67.5 (1-C, 1′-C), 65.2, 62.7 (6-C, 6′-C), 52.0, 49.6 (2-C, 2′-C), 45.3 (3 CH_2 Et), 39.7, 38.3 (5-C, 5′-C), 34.3 (CH thexyl), 29.7, 28.5 (5a-C, 5a′-C), 25.1 (C thexyl), 23.5, 23.2 (2 NHAc), 21.2 (OAc), 20.4 (2 CH_3 thexyl), 18.7 (2 CH_3CH thexyl), 8.5 (3 CH_3 Et), −3.5 (CH_3Si thexyl), −3.7 (CH_3Si thexyl); δ_P (162 MHz; $CDCl_3$) 1.46; ESI-HRMS $[M]^-$ *m*/*z* calc 1043.48598 for $C_{56}H_{76}N_2O_{13}PSi$, found 1043.48326.

Synthesis of oligomers 19 and 20 from 18, and 21 from 20

Compound **18** (200 mg, 0.17 mmol) was dissolved in MeOH (5 mL) and deacetylated with NaOMe in MeOH (3.5 mL) according to general procedure A. The crude residue was purified by flash chromatography (MeOH–CH_2Cl_2 20 : 80, 1% TEA), providing the corresponding deacetylated intermediate (166 mg, 87%).

3-(Benzyloxycarbonyl)aminopropyl 1-*O*-[2-acetamido-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranosyl phosphate)], bis-sodium salt (19). The deacetylated derivative of **18** (48 mg, 0.043 mmol) was condensed with *H*-phosphonate **15** (80 mg, 0.215 mmol) in pyridine (2 mL) in the presence of PivCl (13 μL, 0.1 mmol), and *in situ* oxidized with a solution of I_2 (29 mg, 0.12 mmol) in pyridine–H_2O (2 mL) according to general procedure A. Purification by flash chromatography (MeOH–CH_2Cl_2 20 : 80, 1% TEA) yielded compound **19** as a colourless foam. This 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 **19** as a bis-sodium salt (54 mg, 85%). $[\alpha]_D$ +7.9 (*c* 1.0 in $CHCl_3$); Found: C, 59.35; H, 6.71; N, 3.56. Calc for $C_{65}H_{87}N_3Na_2O_{17}P_2Si$: C, 59.21; H, 6.65; N, 3.49%; δ_H (400 MHz; CD_3OD, T = 313 K) 7.40–7.15 (25H, m, H_{Ar}), 5.05 (2H, br s, CH_2Ph), 4.78–4.65 (8H, m, 4 CH_2Ph), 4.59–4.42 (4H, m, 1-H, 1′-H, 2-H, 2′-H), 4.28–4.23 (1H, m, 6′-Ha), 4.17–3.90 (6H, m, 3-H, 3′-H, 6-Ha, 6′-Hb, OCH_2CH$_2$CH$_2$N), 3.81–3.65 (3H, m, 4-H, 4′-H, 6-Hb), 3.25 (2H, br t, OCH$_2$CH$_2$CH_2N), 2.30–2.20 (2H, m, 5-H, 5′-H), 2.15–1.95 (4H, m, 5a-Ha, 5a-Hb, 5a′-Ha, 5a′-Hb), 2.02, 1.98 (6H, 2 s, 2 Ac), 1.88–1.82 (2H, m, OCH$_2$CH_2CH$_2$N), 1.62 (1H, h, CH thexyl), 0.90 (6H, d, *J* 6.8, 2 CH_3CH thexyl), 0.85 (6H, 2s, 2 CH_3 thexyl), 0.08 (3H, s, CH_3Si), 0.06 (3H, s, CH_3Si); δ_C (100.6 MHz; CD_3OD, T = 313 K) 171.9 (C═O), 172.0 (C═O), 138.9 (C_{Ar}), 138.4 (C_{Ar}), 128.0–127.0 (CH_{Ar}), 78.6, 78.5 (3-C, 3′-C), 75.5 (4-C, 4′-C), 71.8 (4 CH_2Ph), 70.5 (1-C, 1′-C), 66.0 (CH_2Ph), 65.5, 63.3 (6-C, 6′-C), 62.8 (OCH_2CH$_2$CH$_2$N), 52.1, 51.7 (2-C, 2′-C),

39.8, 38.6 (5-C, 5′-C), 37.3 ($OCH_2CH_2CH_2N$), 34.2 (CH thexyl), 30.8 ($OCH_2CH_2CH_2N$), 29.3, 28.6 (5a-C, 5a′-C), 25.0 (C thexyl), 21.5, 21.4 (2 NHAc), 19.7 (2 CH_3 thexyl), 17.8 (2 CH_3CH thexyl), −4.5 (2 CH_3Si thexyl); δ_P (162 MHz; CD_3OD) 0.85, 0.33; ESI-HRMS $[M]^{2-}$ *m/z* calc 635.76455 for $C_{65}H_{87}N_3O_{17}P_2Si$, found 635.76322; $[M + Na]^-$ *m/z* calc 1294.51832 for $C_{65}H_{87}N_3O_{17}P_2SiNa$, found 1294.51018.

1-*O*-Acetyl-2-acetamido-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl 6-[2-acetamido-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranosyl phosphate)], bis-triethylammonium salt (20). The deacetylated derivative of **18** (80 mg, 0.072 mmol) was condensed with *H*-phosphonate **13** (48 mg, 0.079 mmol) in pyridine (2 mL) in the presence of PivCl (23 μL, 0.18 mmol), and *in situ* oxidized with a solution of I_2 (60 mg, 0.24 mmol) in pyridine–H_2O (1.2 mL) according to general procedure A. Purification by flash chromatography (MeOH–CH_2Cl_2 15 : 85, 1% TEA) yielded compound **20** (100 mg, 81%) as a colourless oil. $[\alpha]_D$ +12.4 (*c* 1.0 in $CHCl_3$); Found: C, 63.55; H, 7.91; N, 4.02. Calc for $C_{91}H_{135}N_5O_{20}P_2Si$: C, 63.95; H, 7.96; N, 4.10%; δ_H (400 MHz; CD_3OD, *T* = 313 K) 7.37–7.19 (30H, m, CH_{Ar}), 5.03 (1H, m, 1-H), 4.84–4.67 (7H, m, 2″-H, 6 C*H*HPh), 4.64–4.45 (10H, m, 1′-H, 1″-H, 2-H, 2′-H, 6 C*H*HPh), 4.32–4.28 (1H, m, 6″-Ha), 4.22–4.18 (1H, m, 6′-Ha), 4.10–4.02 (2H, m, 3″-H, 6′-Hb), 3.99–3.92 (1H, m, 6″-Hb), 3.89–3.78 (6H, m, 4-H, 4′-H, 4″-H, 3-H, 3′-H, 6-Ha), 3.70 (1H, dd, $J_{6,5}$ 5.5, $J_{6a,6b}$ 9.4, 6-Ha), 3.13 (12H, q, 6 CH_2 Et), 2.28–2.12 (3H, m, 5-H, 5′-H, 5″-H), 2.01–1.92 (18H, m, 5a-Ha, 5a-Hb, 5a′-Ha, 5a′-Hb, 5a″-Ha, 5a″-Hb, 4 Ac), 1.62 (1H, h, CH thexyl), 1.28 (18H, t, *J* 7.6, 6 CH_3 Et), 0.90 (6H, d, *J* 6.8, 2 CH_3CH thexyl), 0.85 (6H, 2 s, 2 CH_3 thexyl), 0.06 (3H, s, CH_3Si), 0.03 (3H, s, CH_3Si); δ_C (100.6 MHz; CD_3OD, *T* = 313 K) 172.0, 171.8, 171.6, 170.3 (4 C═O), 139.3–138.2 (C_{Ar}), 128.0–126.8 (CH_{Ar}), 79.1, 78.6, 78.5 (3-C, 3′-C, 3″-C), 75.8 (4-C, 4′-C, 4″-C), 73.8, 71.9, 71.5, 71.4 (6 CH_2Ph), 70.0 (1-C, 1′-C, 1″-C), 65.4, 65.3, 63.4 (6-C, 6′-C, 6″-C), 52.6, 50.8, 49.7 (2-C, 2′-C, 2″-C), 46.3 (6 CH_2 Et), 40.2, 38.6, 38.2 (5-C, 5′-C, 5″-C), 34.2 (CH thexyl), 29.6, 28.9, 27.0 (5a-C, 5a′-C, 5a″-C), 24.5 (C thexyl), 21.5, 21.4, 21.3 (4 Ac), 19.6 (2 CH_3 thexyl), 17.8 (2 CH_3CH thexyl), 7.9 (6 CH_3 Et), −4.0 (2 CH_3Si); δ_P (162 MHz; CD_3OD, *T* = 313 K) 0.77, 0.18; ESI-HRMS $[M]^{2-}$ *m/z* calc 751.81952 for $C_{79}H_{103}N_3O_{20}P_2Si$, found 751.82014; $[M + Na]^-$ *m/z* calc 1526.62826 for $C_{79}H_{103}N_3O_{20}P_2SiNa$, found 1526.62836; $[M + H]^-$ *m/z* calc 1504.64632 for $C_{79}H_{104}N_3O_{20}P_2Si$, found 1504.64788.

3-(Benzyloxycarbonyl)aminopropyl 1-*O*-{2-acetamido-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-[2-acetamido-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranosyl phosphate)]}, tris-triethylammonium salt (21). Compound **20** (100 mg, 0.059 mmol) was dissolved in MeOH (5 mL) and deacetylated with NaOMe in MeOH (3 mL) according to general procedure A. The crude residue was purified by flash chromatography (MeOH–CH_2Cl_2 25 : 75, 1% TEA), providing the corresponding deacetylated intermediate (68 mg, 70%). This compound (68 mg, 0.041 mmol) was condensed with *H*-phosphonate **15** (90 mg, 0.24 mmol) in pyridine (2 mL) in the presence of PivCl (17 μL, 0.13 mmol), and *in situ* oxidized with a solution of I_2 (27 mg, 0.11 mmol) in pyridine–H_2O (2 mL) according to general procedure A. Purification by flash chromatography (MeOH–CH_2Cl_2 35 : 65, 1% TEA) yielded compound **21** (47 mg, 57%) as a colourless oil. $[\alpha]_D$ +13 (*c* 1.0 in $CHCl_3$); Found: C, 62.67; H, 8.10; N, 4.87. Calc for $C_{106}H_{162}N_7O_{24}P_3Si$: C, 62.43; H, 8.01; N, 4.81%; δ_H (400 MHz; CD_3OD) 7.40–7.15 (m, 35H, H_{Ar}), 5.05 (2H, br s, CH_2Ph), 4.83–4.44 (18H, m, 1-H, 1′-H, 1″-H, 2-H, 2′-H, 2″-H, 6 CH_2Ph), 4.31–4.22 (2H, m, $OCH_2CH_2CH_2N$), 4.10–3.91 (7H, m, 3-H, 3′-H, 3″-H, 6-Ha, 6-Hb, 6″-Ha, 6″-Hb), 3.88–3.79 (4H, m, 4-H, 4′-H, 4″-H, 6′-Ha), 3.69 (1H, dd, $J_{6',5'}$ 5.7, $J_{6'a,6'b}$ 9.8, 6′-Hb), 3.28 (2H, t, *J* 6.7, $OCH_2CH_2CH_2N$), 3.05 (18H, q, *J* 7.3, 9 CH_2 Et), 2.28–1.95 (16H, m, 5-H, 5′-H, 5″-H, 3 OAc, 5a-Ha, 5a-Hb, 5a′-Ha, 5a′-Hb), 1.87–1.82 (2H, m, $OCH_2CH_2CH_2N$), 1.63 (1H, h, CH thexyl), 1.37–1.25 (29H, m, 5a″-Ha, 5a″-Hb, 9 CH_3 Et), 0.90 (6H, d, *J* 6.6, 2 CH_3CH thexyl), 0.85 (6H, 2 s, 2 CH_3 thexyl), 0.08, 0.03 (6H, 2 s, 2 CH_3Si); δ_C (100.6 MHz; CD_3OD) 172.0, 171.7, 171.6 (3 C═O), 139.2–138.3 (C_{Ar}), 128.0–127.0 (CH_{Ar}), 79.2, 79.0, 78.5 (3-C, 3′-C, 3″-C), 76.5 (4-C, 4′-C, 4″-C), 74.0 (CH_2Ph), 71.9, 71.4 (5 CH_2Ph), 70.5 (1-C, 1′-C, 1″-C), 65.8 (CH_2Ph), 65.5 ($OCH_2CH_2CH_2N$, 6-C or 6″-C), 63.4 (6′-C), 62.7 (6-C or 6″-C), 52.2, 52.1, 50.5 (2-C, 2′-C, 2″-C), 46.0 (9 CH_2 Et), 39.9, 39.7, 38.2 (5-C, 5′-C, 5″-C), 37.2 ($OCH_2CH_2CH_2N$), 34.1 (CH thexyl), 30.5 ($OCH_2CH_2CH_2N$), 29.6, 29.3, 29.0 (5a-C, 5a′-C, 5a″-C), 25.1 (C thexyl), 21.6, 21.4 (3 Ac), 19.6 (2 CH_3 thexyl), 17.8 (2 CH_3CH thexyl), 8.5 (9 CH_3 Et), −4.5 (2 CH_3Si); δ_P (162 MHz; CD_3OD) 1.11, 0.68, 0.50; ESI-HRMS $[M]^{3-}$ *m/z* calc 577.22739 for $C_{88}H_{114}N_4O_{24}P_3Si$, found 577.22718; $[M + Na]^{2-}$ *m/z* calc 877.33569 for $C_{88}H_{114}N_4O_{24}P_3SiNa$, found 877.33524; $[M + 2Na]^-$ *m/z* calc 1777.66060 for $C_{88}H_{114}N_4O_{24}P_3SiNa_2$, found 1777.66537.

General procedure B: global removal of protecting groups

The protected oligomer (**17**, **19** or **21**) was dissolved in dry THF and treated with a 1 M solution of TBAF in THF under nitrogen atmosphere for 4 h. The mixture was then diluted with $CHCl_3$, and the organic layer was washed with a saturated aqueous solution of NH_4Cl and 0.5 M TEAB, dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by flash chromatography, providing the corresponding desilylated derivative. This intermediate was hydrogenolysed over Pd–C in a 1 : 1 mixture of MeOH and H_2O at room temperature for 48 h. The mixture was filtered over a Celite pad and the filtrate was concentrated. Then the residue 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 concentrated and lyophilized to afford the target compound as sodium salt.

3-Aminopropyl 1-*O*-(2-acetamido-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate), sodium salt (1). Compound **17** (36 mg, 0.039 mmol) was dissolved in THF (2 mL) and treated with TBAF in THF (0.4 mL, 0.4 mmol) according to general procedure B. The crude residue was purified by flash chromatography (MeOH–CH_2Cl_2 25 : 75, 1% TEA), providing the

desilylated compound. This intermediate (30 mg) was hydrogenolysed over Pd/C (60 mg) in MeOH : H_2O (4 mL) and subjected to ion exchange according to general procedure B, affording monomer **1** as a white solid (14 mg, 95% over two steps). $[\alpha]_D$ +7.6 (*c* 1.0 in H_2O); δ_H (400 MHz; D_2O, *T* = 313 K) 4.52 (1H, br t, 2-H), 4.45 (1H, br dd, 1-H), 4.13 (2H, q, *J* 6.1, $OCH_2CH_2CH_2N$), 4.06 (1H, dd, $J_{3,2}$ 4.8, $J_{3,4}$ 9.7, 3-H), 3.90–3.64 (3H, m, 4-H, 6-Ha, 6-Hb), 3.28–3.20 (2H, m, $OCH_2CH_2CH_2N$), 2.16 (3H, s, *Ac*NH), 2.15–2.02 (4H, m, 5-H, 5a-Ha, $OCH_2CH_2CH_2N$), 1.77 (1H, br t, 5a-Hb); δ_C (100.6 MHz; D_2O, *T* = 313 K) 176.0 (C═O), 72.8 (1-C), 71.0 (3-C), 70.9 (4-C), 64.0 ($OCH_2CH_2CH_2N$), 62.8 (6-C), 54.1 (2-C), 39.1 (5-C), 37.7 ($OCH_2CH_2CH_2N$), 28.6 (5a-C), 28.4 ($OCH_2CH_2CH_2N$), 22.4 (Ac); δ_P (162 MHz; D_2O, *T* = 313 K) 1.10; ESI-HRMS $[M]^-$ *m/z* calc 355.12758 for $C_{12}H_{24}N_2O_8P$, found 355.12727; $[M + H + Na]^+$ *m/z* calc 379.12407 for $C_{12}H_{25}N_2O_8PNa$, found 379.12374; $[M + 2Na]^+$ *m/z* calc 401.10602 for $C_{12}H_{24}N_2O_8PNa_2$, found 401.10579.

3-Aminopropyl 1-*O*-[2-acetamido-2-deoxy-5a-carba-α-D-manno-pyranosyl phosphate 6-(2-acetamido-2-deoxy-5a-carba-α-D-manno-pyranosyl phosphate)], disodium salt (2). Compound **19** (53 mg, 0.040 mmol) was dissolved in THF (4 mL) and treated with TBAF in THF (0.60 mL, 0.60 mmol) according to general procedure B. The crude residue was purified by flash chromatography (MeOH–CH_2Cl_2 30 : 70, 1% TEA), providing the desilylated compound. This intermediate (38 mg) was hydrogenolysed over Pd/C (80 mg) in MeOH : H_2O (4 mL) and subjected to ion exchange according to general procedure B, affording dimer **2** as a white solid (21 mg, 77% over two steps). $[\alpha]_D$ +10.8 (*c*1.0 in H_2O); δ_H (400 MHz; D_2O, *T* = 313 K) 4.58–4.52 (2H, m, 2-H, 2′-H), 4.48–4.42 (2H, m, 1-H, 1′-H), 4.23–4.18 (1H, m, 6′-Ha), 4.14–4.03 (5H, m, 3-H, 3′-H, 6-Ha, $OCH_2CH_2CH_2N$), 3.89–3.81 (2H, m, 6-Hb, 6′-Hb), 3.75 (1H, t, *J* 10.0, 4-H), 3.68 (1H, t, *J* 9.8, 4′-H), 3.26 (2H, t, *J* 7.2, $OCH_2CH_2CH_2N$), 2.15 (6H, s, 2 *Ac*NH), 2.17–2.05 (6H, m, 5-H, 5′-H, 5a-Ha, 5a′-Ha,) $OCH_2CH_2CH_2N$), 1.89 (1H, br t, *J* 13.4, 5a-Hb), 1.77 (1H, br t, *J* 13.4, 5a′-Hb); δ_C (100.6 MHz; D_2O, *T* = 313 K) 175.0, 174.9 (2 C═O), 72.4, 72.3 (1-C, 1′-C), 70.9 (4′-C), 70.6, 70.5 (3-C, 3′-C), 69.5 (4-C), 66.0 (6-C), 63.6 ($OCH_2CH_2CH_2N$), 62.4 (6′-C), 53.8, 53.7 (2-C, 2′-C) 38.9, 37.9 (5-C, 5′-C), 37.5 ($OCH_2CH_2CH_2N$), 28.3, 28.1 (5a-C, 5a′-C), 22.3 (2 Ac); δ_P (162 MHz; D_2O, *T* = 313 K) 0.93, 0.61; ESI-HRMS $[M + H]^-$ *m/z* calc 636.19401 for $C_{21}H_{40}N_3O_{15}P_2$, found 636.19368; $[M + Na]^-$ *m/z* calc 658.17596 for $C_{21}H_{39}N_3O_{15}P_2Na$, found 658.17583.

3-Aminopropyl 1-*O*-{2-acetamido-2-deoxy-5a-carba-α-D-manno-pyranosyl phosphate 6-[2-acetamido-2-deoxy-5a-carba-α-D-manno-pyranosyl phosphate 6-(2-acetamido-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate)]}, trisodium salt (3). Compound **21** (45 mg, 0.022 mmol) was dissolved in THF (5 mL) and treated with TBAF in THF (0.45 mL, 0.45 mmol) according to general procedure B. The crude was purified by flash chromatography (MeOH–CH_2Cl_2 50 : 50, 1% TEA), providing the desilylated compound. This intermediate (32 mg) was hydrogenolysed over Pd/C (80 mg) in MeOH : H_2O (4 mL) and subjected to ion exchange according to general procedure B, affording trimer **3** as a white solid (17 mg, 78% over two steps). $[\alpha]_D$ +8.2 (*c* 1.0 in H_2O); δ_H (400 MHz; D_2O, *T* = 313 K) 4.60–4.58 (1H, m, 2-H), 4.57–4.52 (2H, m, 2′-H, 2″-H), 4.48–4.42 (3H, m, 1-H, 1′-H, 1″-H), 4.29–4.01 (8H, m, 4-H, 4′-H, 6-Ha, 6-Hb, 6′-Ha, 6′-Hb, $OCH_2CH_2CH_2N$), 3.87–3.84 (2H, m, 6″-Ha, 6″-Hb), 3.69–3.72 (3H, m, 3-H, 3′-H, 3″-H), 3.68 (1H, t, *J* 10, 4″-H) 3.27 (2H, t, *J* 7.2, $OCH_2CH_2CH_2N$), 2.15 (9H, s, 3Ac), 2.14–2.04 (5H, m, 5-H, 5′-H, 5″-H, $OCH_2CH_2CH_2N$), 1.97–1.74 (6H, m, 5a-Ha, 5a-Hb, 5a′-Ha, 5a′-Hb, 5a″-Ha, 5a″-Hb); δ_C (100.6 MHz; D_2O, *T* = 308 K) 174.9 (3 C═O), 72.3 (1-C, 1′-C, 1″-C), 70.5, 70.4 (4-C, 4′-C, 4″-C), 69.5, 69.3 (3-C, 3′-C, 3″-C), 66.0, 65.7 (6-C, 6′-C), 63.5 ($OCH_2CH_2CH_2N$), 62.3 (6″-C), 53.7 (2-C, 2′-C, 2″-C), 38.9, 37.8 (5-C, 5′-C, 5″-C), 37.4 ($OCH_2CH_2CH_2N$), 28.2 ($OCH_2CH_2CH_2N$), 27.8, 27.5, 26.8 (5a-C, 5a′-C, 5a″-C), 22.2 (3 Ac); δ_P (162 MHz; D_2O, *T* = 308 K) 0.95, 0.86, 0.60; ESI-HRMS $[M + 2H]^-$ *m/z* calc 917.26045 for $C_{30}H_{56}N_4O_{22}P_3$, found 917.26045; $[M + H + Na]^-$ *m/z* calc 939.24240 for $C_{30}H_{55}N_4O_{22}P_3Na$, found 939.24192; $[M + 2Na]^-$ *m/z* calc 961.22434 for $C_{30}H_{54}N_4O_{22}P_3Na_2$, found 961.22486; $[M + H]^{2-}$ *m/z* calc 458.12659 for $C_{30}H_{55}N_4O_{22}P_3$, found 458.12739.

Competitive ELISA assay

Native MenA CPS and oligosaccharides were provided by Novartis Vaccines and Diagnostics, Siena, Italy. Polyclonal serum derived from immunization of mice with a native meningococcal A oligosaccharide–protein conjugate was provided by Preclinical Serology Laboratory in Novartis Vaccines and Diagnostics, Siena, Italy.

96-Well Maxisorp plates (Nunc, Thermo Fisher Scientific) were coated overnight at +4 °C with meningococcal A native capsular polysaccharide (MenA CPS), 5 μg per well in Phosphate Saline Buffer (PBS) pH 8.2. After coating, the plates were washed three times with 300 μl per well of PBS with 0.05% Tween 20 (TPBS) at pH 7.4. Plates were blocked with 3% bovine serum albumin (Fraction V, Sigma-Aldrich) in TPBS for 1 h at 37 °C and then washed again. 50 μl of polyclonal immune mouse sera pre-diluted in TPBS were put on the plate and mixed with 50 μl of inhibitor previously diluted with ten fold serial dilution on another plate. On the column without inhibitor 50 μl of immune mouse sera pre-diluted in TPBS were mixed with 50 μl of TPBS. Polyclonal serum obtained from native meningococcal A immunization was used at the final dilution of 1 : 1600. After 2 h of incubation at 37 °C and washing with TPBS, 100 μl per well of 1 : 10 000 of anti-mouse IgG alkaline phosphatase conjugated (Sigma-Aldrich) were added and plates were incubated for 1 h at 37 °C. After, plates were developed for 30 min at room temperature with 100 μl per well of 1 mg ml^{-1} *p*-nitrophenyl phosphate disodium (Sigma-Aldrich) in 1 M diethanolamine (pH 9.8) and read at 405 nm with a microplate spectrophotometer (Biorad).

The plate was designed to contain: (a) blank column with TPBS alone, without serum and inhibitors, (b) column with serum alone, without inhibitors (b0); the other columns contained both, the serum and the inhibitors which included also MenA CPS, native MenA oligosaccharides and the not correlated Laminarin polysaccharide as positive and negative controls respectively.

The different competitors (compounds **1** = monosaccharide, **2** = disaccharide, **3** = trisaccharide and native *av*DP3 = native MenA oligosaccharide average polymerization degree 3) were pre-diluted to obtain the starting concentration of 0.5 mM and ten fold dilutions were performed eight times on the plate. Native MenA CPS and *av*DP15 oligosaccharide used as positive control were pre-diluted to obtain a starting concentration of 0.05 mM, instead Laminarin of 1 mM and was used as negative control.

All OD values were subtracted from the mean value of the blank column (b). The inhibition percentage was expressed as follows: % inhibition = [(B0 − ODx)/B0] × 100, where B0 is the mean values of the b0 column (serum without inhibitor) and ODx is the optical density corresponding to each inhibitor concentration.

IC_{50} was defined as the inhibitor concentration resulting in 50% inhibition of the main reaction. Fitting of inhibition curves and calculation of IC_{50} values was performed on the GraphPad Prism software using variable slope model (GraphPad Prism Inc.).

Acknowledgements

We gratefully acknowledge MIUR-Italy (PRIN 2008, contract 2008CZ3NP3) for financial support.

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