Synthesis of *Neisseria meningitidis* X capsular polysaccharide fragments

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Dedicated to Professor Richard R. Schmidt on the occasion of his 78th anniversary

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Abstract

Serotype X of *Neisseria meningitidis* bacterium (Men X) recently emerged as a substantial threat to public health. Since anti-meningococcal vaccines currently available or under investigation do not contain antigenic components of Men X capsular polysaccharide, there is the need to develop more comprehensive conjugate vaccines capable to offer higher protection. As a preliminary step towards this goal, the synthesis of three conjugatable Men X capsular polysaccharide fragments is described. The installation of the crucial α-glycosyl phosphodiester linkages is based on the hydrogenphosphonate methodology using pure α-glycosyl hydrogenphosphonates **10** and **12** obtained from hemiacetals **9** and **11**, respectively.

Keywords: Carbohydrates, glycosyl phosphates, hydrogenphosphonates, *Neisseria meningitidis*

Introduction

Bacterial meningitis causes 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 a 20% in the developing world.[1] *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib) and *Neisseria meningitidis* are responsible for most of the cases of bacterial meningitis worldwide.[2] In particular, thirteen different serogroups of *N. meningitidis* have so far been defined, but about 90% of the infections are due to serogroups A, B, C, Y and W135.[3] However, in the past 20 years sporadic cases or clusters of meningitis due to other *N. meningitidis* serogroups have emerged. *N. meningitidis* type X (Men X), first described in the 1960s,[4] has been found to cause a few cases of invasive disease across North America, Europe, Australia and Africa.[5] In 2006, owing to an unprecedented incidence of meningitis caused by Men X in Niger,[6] the World Health Organization (WHO) recognized Men X as a substantial threat and epidemic potential.

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Vaccination is considered by the WHO to be the most cost-effective strategy for controlling infectious disease, since it should confer long-term protective immunity in the population. In particular, carbohydrate-based vaccines have recently emerged as a powerful tool with enormous potential benefits for human health.[7] They are designed to target pathogen-specific cell surface saccharide structures, such as the carbohydrate capsule (capsular polysaccharide, CPS), which represent a major virulence factor of encapsulated bacteria, including *N. meningitidis*. Saccharide vaccines currently present on the market, or under development, are based on carbohydrate-protein conjugates,[8] and they comprise also different formulations against *H. influenzae* type b, *S. pneumoniae* and several meningococcal serogroups. However, none of them include antigenic components of Men X, and therefore they do not offer protection against infections caused by this emerging serogroup. Although Men X currently causes only a small proportion of meningococcal disease,[5,6] it is possible that repeated vaccination against some serogroups (especially A and C) has the potential to select meningococci of other serogroups (for example, Men X) and might result in a changed profile of meningococcal disease. This possibility should be considered when conjugate vaccines carrying limited ranges of serogroups are introduced.[5b] The development of more comprehensive conjugate vaccines including Men X CPS fragments could therefore become an urgent issue in the near future.

Carbohydrate-based antigens needed for inclusion in a vaccine, however, are not readily available from natural sources. The isolation and purification of the polysaccharide from the bacterial source is indeed a challenging task, leading to the presence of biological contaminants and impurities, thus raising severe issues of quality assurance. On the other hand, synthetic carbohydrate-based vaccines have important advantages, including their well-defined chemical structures and a better safety profile. Consequently, a variety of pathogen-associated saccharide antigens have been synthesized in the last decade, coupled to carrier proteins and proved to elicit protective antibodies in animal models.[9] In addition, synthetic oligosaccharides can help elucidate the structural moieties of the native bacterial polysaccharide that are essential to induce antibodies.[10] This step is crucial for the design of a new generation of improved and safer vaccines obtained either from chemical synthesis or bacterial source.

On the basis of these considerations, we report herein the synthesis of the monomer, dimer and trimer of Men X CPS repeating unit (compounds **1-3**, Figure 1). The synthetic fragments are provided with a phosphodiester-linked aminopropyl spacer at their reducing end, suitable for their eventual conjugation to a carrier protein.

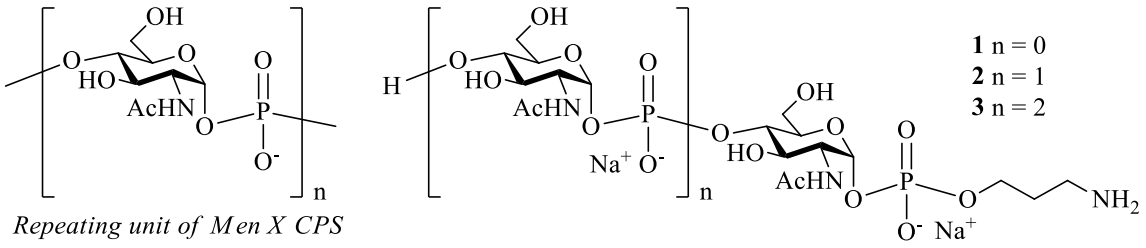

Figure 1. Structures of repeating unit of Men X CPS and target oligomers **1-3**.

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Results and Discussion

The CPS of *N. meningitides* X is a homopolymer of (1→4)-linked 2-acetamido-2-deoxy- α-D-glucopyranosyl phosphate residues (Figure 1).[11] There is no doubt that the major challenge in the preparation of our target oligomers is the stereochemical control in the synthesis of the α-phosphodiester linkages. Among other methodologies available in the literature for the formation of phosphodiester linkages, we relied on the well-established hydrogenphosphonate (H-phosphonate) protocol.[12] The same strategy was employed by Shibaev and co-workers who, to the best of our knowledge, reported the first synthesis of Men X dimer.[13] The Russian group synthesized an anomerically pure α-H-phosphonate (glycosyl phosphite) of a *N*-acetylglucosamine (GlcNAc) derivative which was then coupled with the 4-OH of the *O*-*p*-nitrophenylglycoside of a second GlcNAc residue. On the other hand, our fragments are intended for the preparation of the corresponding protein conjugates, and each compound contains a phosphodiester-linked spacer available for this purpose. An additional advantage of our approach is the higher flexibility, since the protecting groups pattern of the synthetic precursors was designed to allow further elongation and synthesis of oligomers with variable length, as demonstrated by the preparation of trimer **3**. Accordingly, suitably protected azide-containing monosaccharides **4** and **5** (Scheme 1) were employed as key building-blocks. In particular, compound **5** was selected as elongation block, since the 4-OH is easily available by Zemplén transesterification, whereas we envisaged intermediate **4** as a capping block to be introduced at the non-reducing terminus of each oligomer. Compound **4** -precursor of **5**- was initially synthesized in four linear steps from D-glucosamine hydrochloride as described by Martin-Lomas *et al*.[14] This procedure resulted however unsuitable for large laboratory scale (over 20 g of starting material), and we applied the longer but more reliable reactions sequence outlined in Scheme 1, which provided intermediates **4** and **5** in good overall yield and much higher purity. Briefly, D-glucosamine was first converted into the 2-azidoglucose derivative **7** by diazotransfer reaction followed by standard *O*-acetylation (Ac_2O, pyridine, *N*,*N*-dimethylaminopyridine, DMAP) in 77% yield. The crucial diazotransfer reaction was carried out using the recently described[15] imidazole-1-sulfonyl azide diazo donor as a cheap and safe alternative to the more popular trifluoromethanesulfonyl azide (TfN_3).[16] Besides the high cost of trifluoromethanesulfonic anhydride, used in its preparation, neat TfN_3 has indeed explosive nature and its poor shelf life requires its preparation in solution just prior to use.[17] On the contrary, the imidazole-1-sulfonyl azide can be easily prepared from relatively inexpensive starting materials and its hydrochloride salt (compound **6** in Scheme 1) is a crystalline solid that can be stored at 4°C for weeks without loss of efficiency.

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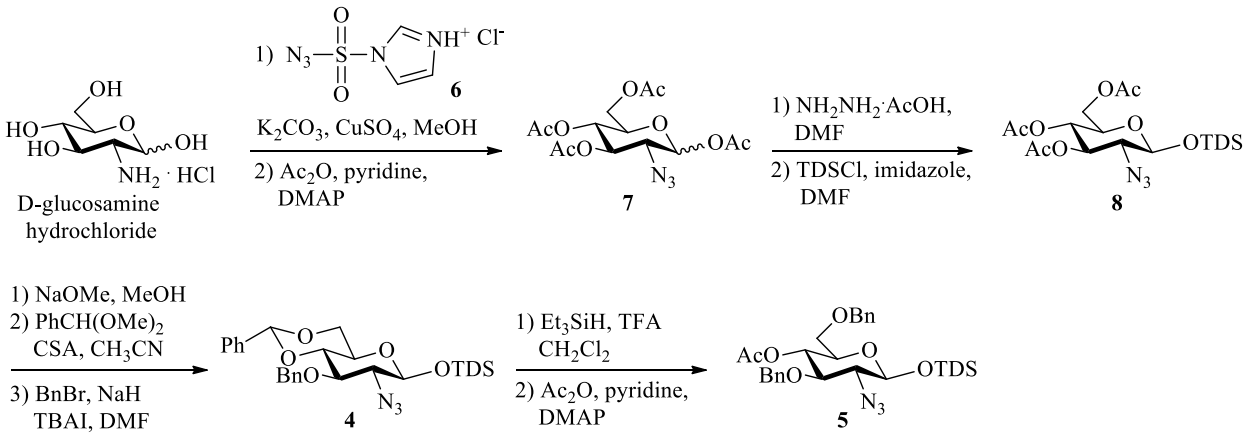

Scheme 1. Synthesis of key building-blocks **4** and **5**.

Regioselective 1-*O*-deacetylation followed by 1-*O*-silylation with thexyldimethylsilyl chloride (TDSCl) and imidazole in DMF afforded intermediate **8** in good yield (66% yield over two steps). This compound was converted into building-block **4** by Zemplén *O*-deacetylation, 4,6-*O*-benzylidenation and final 3-*O*-benzylation (45% yield over three steps). Eventually, regioselective reductive opening of the benzylidene acetal in **4** (Et_3SiH and trifluoroacetic acid), followed by standard 4-*O*-acetylation provided monosaccharide **5** in high yield (78% yield over two steps).

Next stage of the synthesis of the target oligomers was the crucial installation of the anomeric phosphodiester linkages using the H-phosphonate protocol. We reasoned that the most reliable way to obtain the stereochemical control in the synthesis of anomerically pure α-glycosyl phosphodiesters is the condensation of a glycosyl α-H-phosphonate with the free hydroxyl of a sugar acceptor, followed by oxidation of the H-phosphonate diester intermediate. The preparation of anomerically pure glycosyl α-H-phosphonate is however a challenging task. Accordingly, no examples have been found in the literature describing the synthesis of exclusively α-anomeric H-phosphonate from 2-azido-2-deoxy-glucopyranosyl derivatives.

Literature data suggest however that the treatment of a sugar hemiacetal with a proper phosphitylating agent in the presence of phosphorous acid could lead to equilibration of the initially formed α,β mixture of the anomeric H-phosphonates into the pure, more thermodynamically stable α anomer.[18] Compound **4** was therefore 1-*O*-desilylated using tetrabutylammonium fluoride and acetic acid in THF at -40°C to afford hemiacetal **9** in 80% yield (Scheme 2). The synthesis of the α-H-phosphonate from **9** was next thoroughly investigated testing different reaction conditions, varying the phosphitylating agent, the reaction solvent and temperature, and the stoichiometry of the reagents. Eventually, we found that the treatment of **9** with 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one (commonly named salicylchlorophosphite) and H_3PO_3 in 1:1.5:3 molar ratio in pyridine from room temperature to 40°C (see the Experimental Section) provided exclusively α-H-phosphonate **10**, as evinced by ^{1}H- and ^{31}P-NMR spectra. The formation of the H-phosphonate was confirmed by the diagnostic

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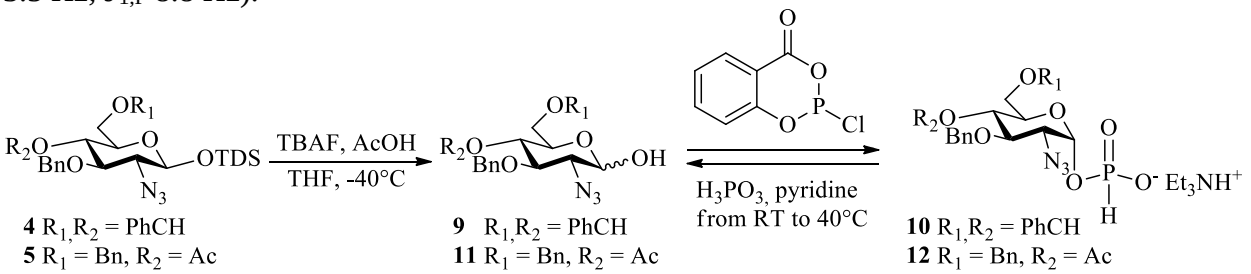
doublet at 7.01 ppm in the ^{1}H-NMR spectrum with the characteristic value of $^1J_{H,P}$ 640.4 Hz (δ_P 1.84), while the α-configuration was ascertained by the signal corresponding to H-1 (δ_H 5.70, $J_{1,2}$ 3.5 Hz, $J_{1,P}$ 8.8 Hz).



Scheme 2. Synthesis of α-H-phosphonates **10** and **12**.

The long reaction time (8-9 days are needed) represents a significant drawback of this procedure. The reaction progress could be however easily monitored by ^{1}H- and ^{31}P-NMR analysis. As shown in Figure 2 (part a), as the reaction proceeds one can observe the progressive disappearance of the signals corresponding to the β-glycosyl H-phosphonate with the concomitant formation of the hemiacetal **9** (see the doublet at δ 5.32). Figure 2b shows the same trend in ^{31}P-NMR spectra. According to the literature,[18] a reasonable explanation of the observed behaviour is that the more reactive β-glycosyl H-phosphonate is converted to either the α-anomer **10** (due to a S_N2 displacement by H_3PO_3) or the hemiacetal **9** as a result of acid-catalyzed cleavage of the H-phosphonate group. In accordance with this mechanism, the α-H-phosphonate **10** was obtained in 41% yield along with 45% of the hemiacetal **9**, which could be easily separated by chromatography and recycled.

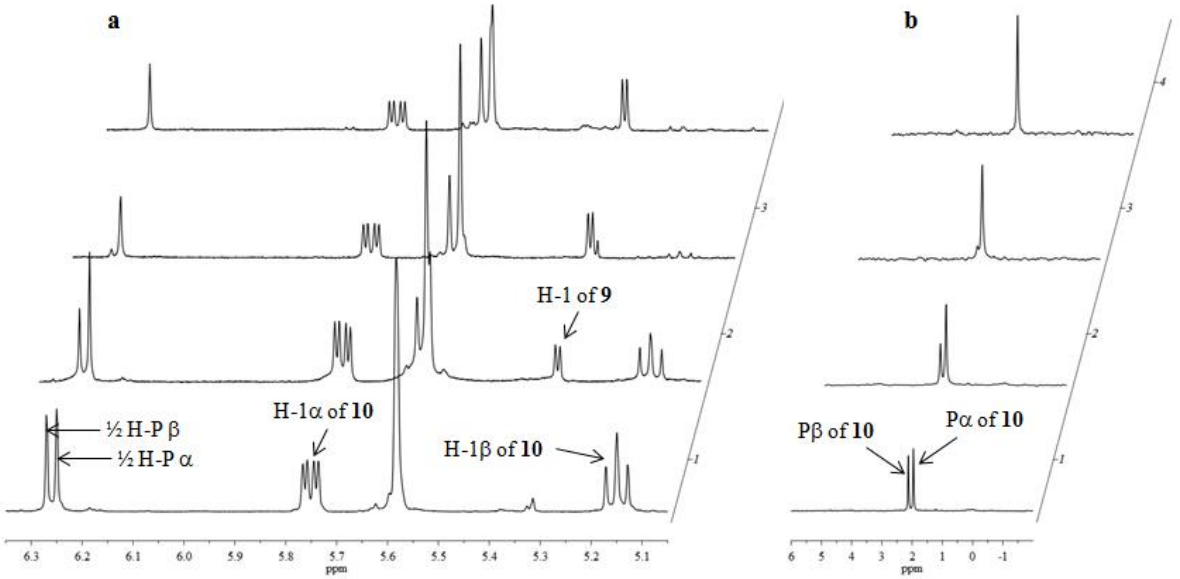


Figure 2. ^{1}H-NMR (part a) and ^{31}P-NMR spectra (part b) showing the reaction progress from **9** to **10**.

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The same protocol was successfully applied to hemiacetal **11**, obtained by 1-*O*-desilylation of compound **5**, affording the α-glycosyl H-phosphonate **12** in 52% yield (along with 38% of recovered **11**, Scheme 2). The formation of α-H-phosphonate **12** from **11** was slightly faster (6-7 days), and its structure was confirmed by ^{31}P- and ^{1}H-NMR data (δ_P 2.24, δ_H 7.03 for H-P with $^{1}J_{H,P}$ 642.0 Hz, δ_H 5.73 for H-1, with $J_{1,2}$ 3.0 Hz and $J_{1,P}$ 8.7 Hz). NMR spectra of the reaction mixture during the conversion of **11** into **12** are illustrated in Figure 3.

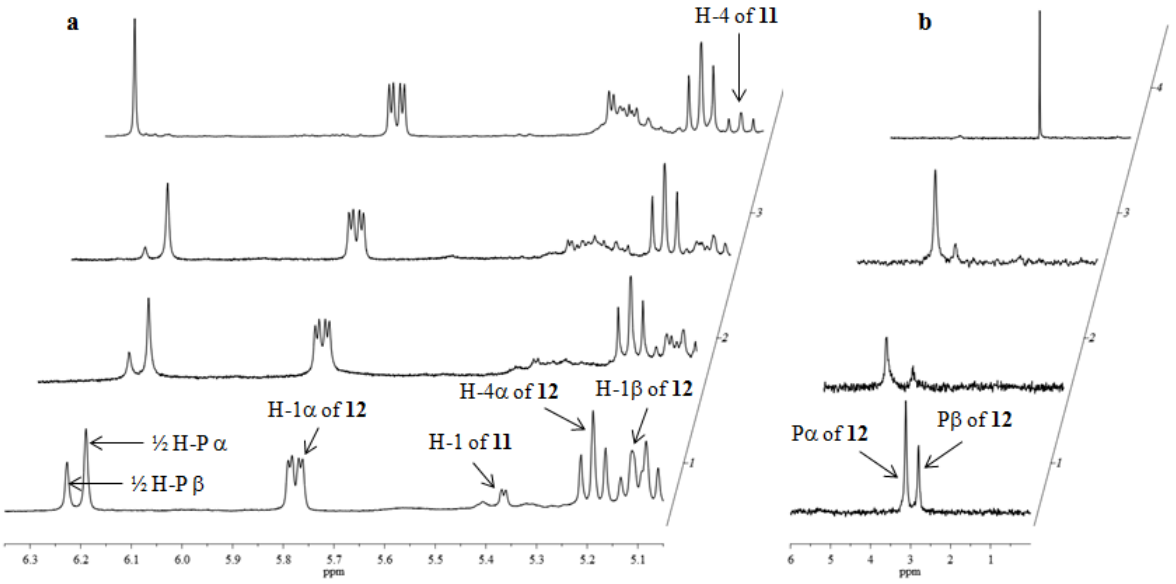

Figure 3. ^{1}H-NMR (part a) and ^{31}P-NMR spectra (part b) showing the reaction progress from **11** to **12**.

Now the stage was set for the assembly of the target oligomers. First, glycosyl H-phosphonate **10** was coupled with commercially available benzyl *N*-(3-hydroxypropyl)carbamate using pivaloyl chloride as condensing agent (Scheme 3).[12,19] In situ oxidation of the H-phosphonate diester intermediate was carried out with iodine in aqueous pyridine, providing phosphodiester **13** in 62% yield as triethylammonium salt after quenching the reaction mixture with 1 M aq. triethylammonium hydrogen carbonate (TEAB) buffer solution (pH 7). The structure of compound **13** was confirmed by NMR and mass spectrometry. The ^{31}P-NMR spectrum exhibited a single signal with δ_P -0.84 characteristic for glycoside-linked phosphodiesters.

The subsequent azide reduction turned out much more difficult than expected. While the classical Staudinger reaction[20] failed, all other attempts (1,3-propanethiol, PMe_3, Zn-Cu couple in THF-Ac_2O-AcOH) gave disappointing results, with only traces of the desired product. Finally, the azide was converted into the acetamide using a combination of $NiCl_2$ and $NaBH_4$ followed by treatment with Ac_2O,[21] affording compound **14** in 29% yield. In spite of this unsatisfactory result, we deemed this yield acceptable at this stage of the research, and the obtained acetamido

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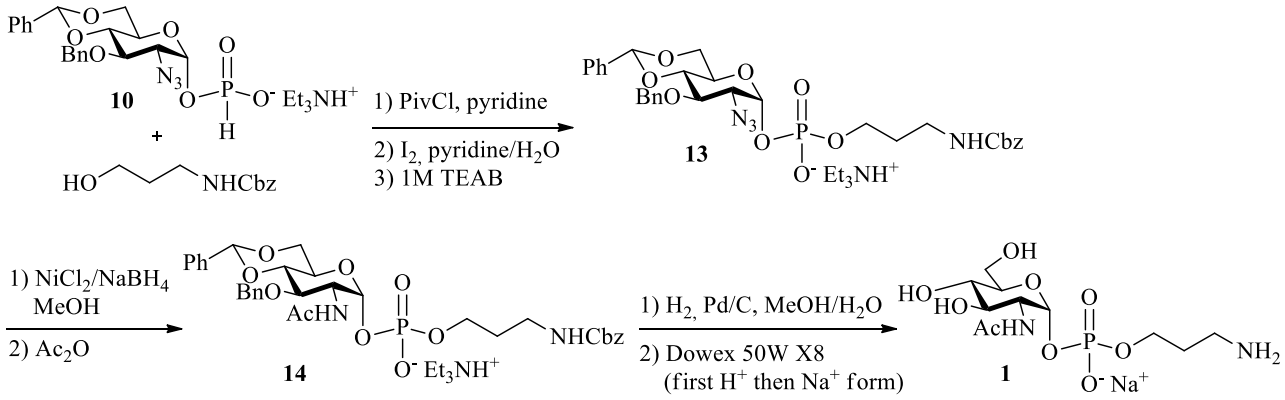
intermediate **14** was submitted to final deprotection. The remaining protecting groups were removed via hydrogenolysis over Pd on carbon. Final purification was accomplished by eluting a water solution of deprotected compound over a column filled with Dowex 50W X8 resin (H^+ form), followed by a second ion exchange on the same resin in Na^+ form. Lyophilization of the eluates provided the spacer-linked monomer **1** as sodium salt in 93% yield (Scheme 3).



Scheme 3. Synthesis of the spacer-linked monomer **1**.

The syntheses of dimer and trimer required the condensation of the glycosyl H-phosphonate **12** with benzyl *N*-(3-hydroxypropyl)carbamate in the presence of pivaloyl chloride, followed by in situ oxidation to phosphodiester **15** (64%) and careful Zemplén 4-*O*-deacetylation (96% yield, Scheme 4). The resulting alcohol **16** was coupled with the capping residue **10** to afford, after oxidation, phosphodisaccharide **17** in 45% yield. The azide reduction was accomplished using the $NiCl_2/NaBH_4$ protocol, followed by *N*-acetylation and hydrogenolytical removal of the protecting groups, furnishing after ion exchange fully deprotected dimer **2** as bis-sodium salt in 36% overall yield (from **17**, Scheme 4).

On the other hand, the synthesis of trimer **3** was achieved by condensation of alcohol **16** with the elongation block **12** followed by oxidation, which gave phosphodisaccharide **18** in 40% yield (Scheme 5).

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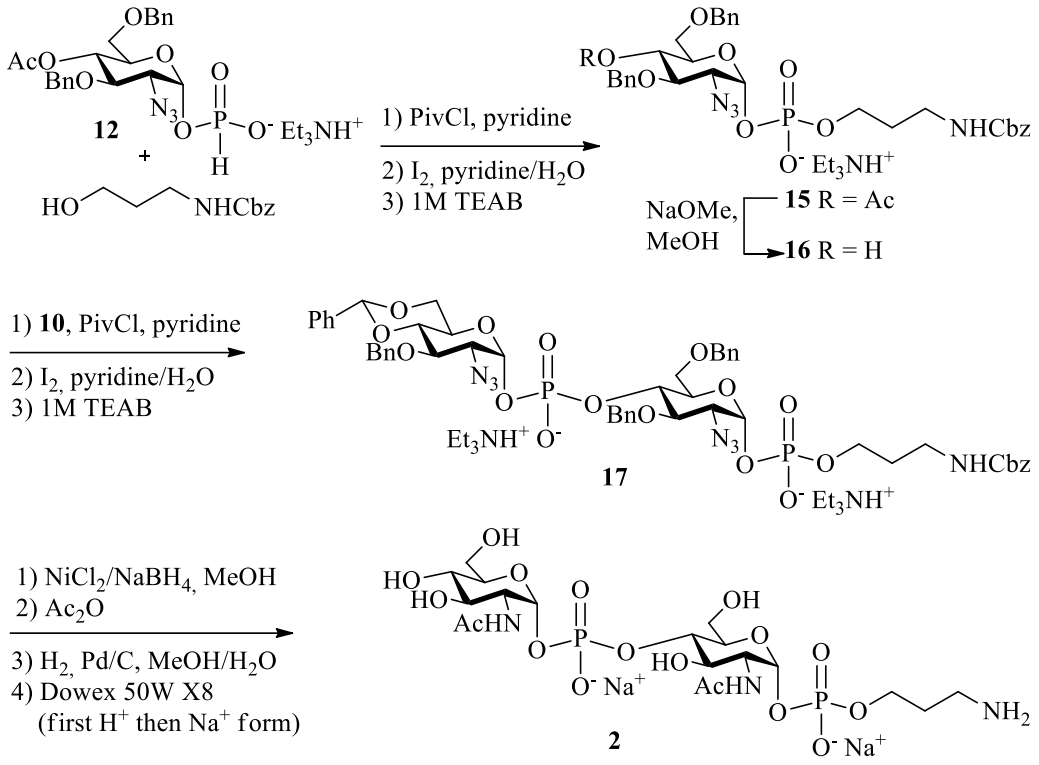
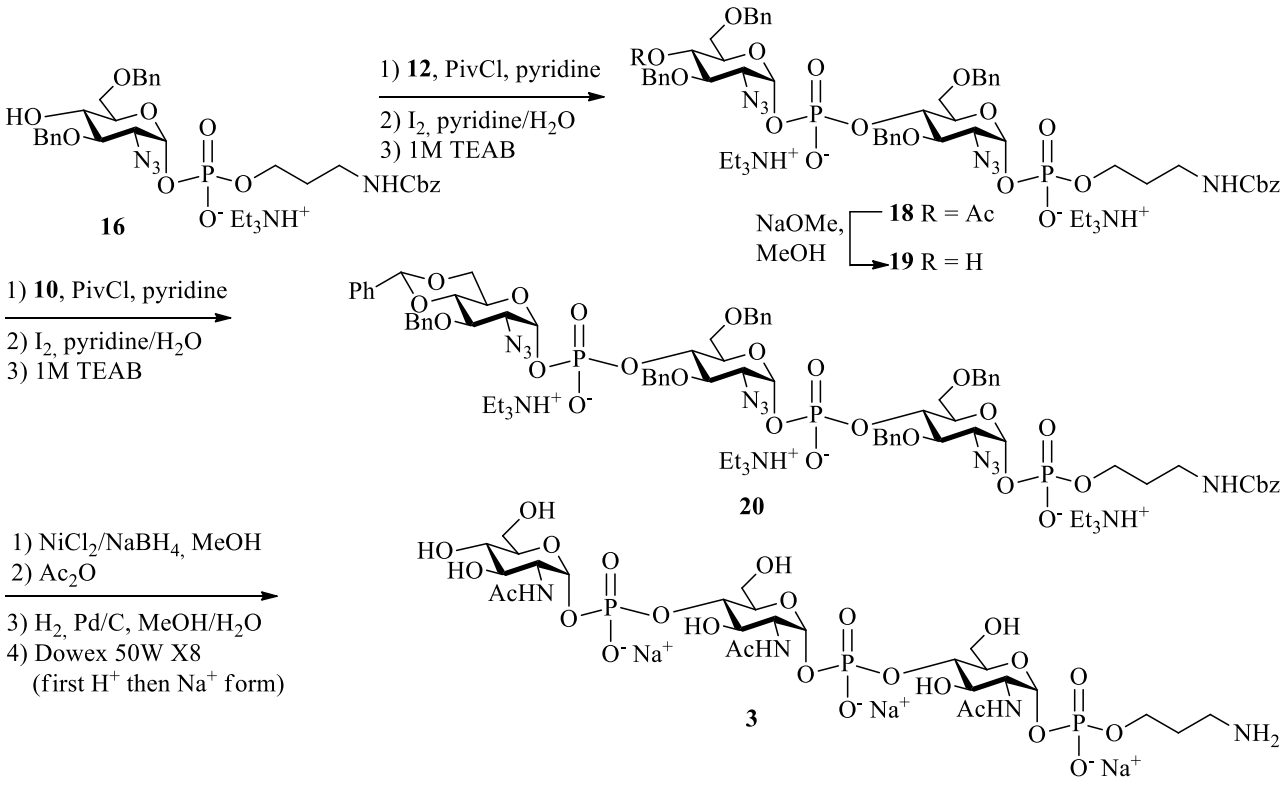


Scheme 4. Synthesis of the spacer-linked dimer **2**.



Scheme 5. Synthesis of the spacer-linked trimer **3**.

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Zemplén deacetylation of **18** and subsequent coupling with **10** provided phosphotrisaccharide **20** in 43% overall yield after oxidation. Reduction of the three azides and *N*-acetylation was carried out with $NiCl_2/NaBH_4$ followed by Ac_2O addition. Eventually, hydrogenolysis of the remaining protecting groups and ion exchange performed as described above afforded trimer **3** as tris-sodium salt in 33% overall yield from **20** (Scheme 5).

Oligomers **2** and **3** were fully characterized by NMR spectroscopy and mass spectrometry. ^{1}H- and ^{13}C-NMR spectra, using both mono- and bidimensional experiments, were in excellent agreement with the proposed structures (see the Experimental Section). In addition, ^{31}P-NMR spectra showed two peaks at δ 0.90 and 0.60 for dimer **2**, and three peaks (δ -0.31, -0.65 and -0.71) for trimer **3**.

Conclusions

In conclusion, as a preliminary step towards the development of more comprehensive anti-meningococcal conjugate vaccines including new emerging serotypes, we described the synthesis of three conjugatable fragments (monomer **1**, dimer **2** and trimer **3**) with structures corresponding to the natural CPS of *N. meningitidis* type X. The challenging synthesis of anomerically pure α-hydrogenphosphonate, a crucial aspect for our strategy, has been accomplished for the first time on 2-azido-2-deoxy derivatives using a combination of H_3PO_3 and salicylchlorophosphite. Our approach is featured by high flexibility and, in principle, it allows the synthesis of even longer oligomers by iteration of the 4-*O*-deacetylation-hydrogenphosphonate condensation sequence either using the capping residue **10** or the elongation block **12**. On the other hand, a major drawback of our approach are the disappointing results obtained in the reduction of the azido functions, which caused a significant drop of the chemical yields. The use of the azido group was due to its non-participating and electron-withdrawing properties, that were expected to enhance the stability of the anomeric phosphodiester linkages. The difficulties encountered in the final deprotection of the oligomers however induced us to take into account a different approach, based on GlcNAc instead of azido glucose building blocks, which is currently under investigation.

The chemical conjugation of oligomers **1**-**3** to immunogenic carrier proteins, and the biological evaluation of the resulting *neo*-glycoconjugates will be reported in due course.

Experimental Section

General. All commercially available reagents including dry solvents were used as received. The only exception is H_3PO_3, which was coevaporated three times with dry toluene, and dried by high vacuum pump prior to use. 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

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sulfate (1 g) and ammonium heptamolybdate tetrahydrate (27 g) in water (469 mL) and concentrated sulfuric acid (31 mL). Chromatographic purifications have been performed either using the flash purification apparatus Biotage® SP1™ by gradient elution, or by standard column chromatography on Fluka silica gel 60. NMR spectra were recorded at 300K 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). In ^{13}C-NMR spectra, signals corresponding to aromatic carbons are omitted and, apart quaternary carbons, signals attribution was derived by HSQC experiment. Signals attribution in NMR spectra are designated using indexes a, b and c, and referred to ring a (reducing end, directly linked to the spacer), ring b (either the non-reducing end or the inner ring), and ring c (the non-reducing terminus). 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). ESI-MS spectra were recorded on a JEOL AX-505 spectrometer.

1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranose (7). Imidazole-1-sulfonyl azide hydrochloride **6**[15] (11.75 g, 56.04 mmol) was added at 0 °C to a solution of D-glucosamine hydrochloride (10.07 g, 46.7 mmol), K_2CO_3 (17.43 g, 126.1 mmol) and $CuSO_4$ pentahydrate (140 mg, 0.467 mmol) in MeOH (234 ml) and the mixture was stirred at room temperature for 3 h. The solution was concentrated and co-evaporated with toluene (2x100 ml). Acetic anhydride (35.6 ml, 373.6 mmol) and a catalytic amount of DMAP were added to the residue dissolved in pyridine (120 ml) and the mixture was stirred for 3 h, then concentrated, diluted with water (500 ml) and extracted three times with CH_2Cl_2 (3x500 ml). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. Column flash chromatography [hexane (H)/ethyl acetate (EA) 6/4] gave the azide **7** (13.41 g, 77%). The spectroscopic characterization data of compound **7** were in agreement with those previously reported.[22]

Thexyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranoside (8). A freshly prepared 2 M solution of hydrazine acetate [$NH_2NH_2 \cdot H_2O$ (1.5 ml, 29.20 mmmol), AcOH (1.4 ml, 22.46 mmol) and dry MeOH (15.6 ml)] was added dropwise to a solution of **7** (8.38 g, 22.46 mmol) in dry DMF (100 ml) at 0°C. The mixture was stirred at rt for 2 hours. The mixture was concentrated, diluted with EA (300 ml) and extracted with water (3 × 300 ml). The organic layer was dried (Na_2SO_4), filtered and concentrated to dryness. The obtained residue was dissolved in dry DMF (100 ml) and cooled to 0°C, then imidazole (4.19 g, 60.6 mmol) and TDSCl (5.94 ml, 30.31 mmol) were added. The mixture was stirred for 24 hours at 0°C. The reaction was concentrated to reduce the DMF amount, then diluted with EA (150 ml) and washed with water (2 × 200 mL) and brine (200 mL). The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. Flash chromatography (H/EA 7/3) gave **8** as a yellow solid

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(7.02 g, 66% from **7**). The spectroscopic characterization data of compound **8** were in agreement with those previously reported.[23]

Thexyldimethylsilyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (4). Compound **8** (6.11 g, 12.9 mmol) was treated with a 0.1 M solution of NaOMe in dry MeOH and stirred at rt under nitrogen atmosphere. Deacetylation was confirmed by TLC (H/EA 7/3), then the reaction mixture was neutralized by ion-exchange resin (Amberlite IR120, H^+ form), filtered and concentrated. Complete *O*-deacetylation was further confirmed by ^{1}H-NMR spectrum of the crude residue. This compound (4.48 g, 12.9 mmol) was suspended in dry CH_3CN (130 ml) and benzaldehyde dimethylacetal (5.8 ml, 38.7 mmol) was added, followed by addition of a catalytic amount of camphorsulfonic acid. The reaction mixture was vigorously stirred at rt for 72 h, then it was quenched by addition of Et_3N until neutral pH and solvents were removed under vacuum. The crude was purified by flash chromatography using the Biotage system (H/EA gradient) to yield 3.56 g of the 4,6-*O*-benzylidene intermediate. This compound (3.56 g, 8.17 mmol) was dissolved in dry DMF (70 ml), then BnBr (1.9 ml, 16.2 mmol) and a catalytic amount of TBAI (0.75 g) were added. 95% NaH (293 mg, 12.2 mmol) was slowly added and the reaction mixture was stirred at rt for 1 h. The reaction was quenched by MeOH addition, then the solvents were removed under vacuum. The residue was dissolved in EA (60 ml) and washed with 5% aq. solution of HCl (2 × 200 ml) and brine (1 × 200 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography using the Biotage system (H/EA gradient) affording **4** (3.2 g, 45% yield over three steps) as a white solid. The spectroscopic characterization data of compound **4** were in agreement with those previously reported.[24]

Thexyldimethylsilyl 4-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (5). Compound **4** (4.07 g, 7.74 mmol) was dissolved in dry CH_2Cl_2 (35 ml) and Et_3SiH (6.15 ml, 38.7 mmol) was added dropwise. After 30 min, the mixture is cooled to 0 °C, and trifluoroacetic acid (2.87 ml, 38.7 mmol) was slowly added. The reaction mixture was stirred at rt and, after completion (TLC H/EA 95/5), diluted with CH_2Cl_2, quenched by addition of a satd aq. solution of $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2, the combined organic phases were dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude product was purified by column flash chromatography (H/EA 9/1) to give the alcohol intermediate as a white solid (3.49 g, 6.61 mmol, 85% yield), whose NMR data were in agreement with those previously reported.[25]

The alcohol (2.73 g, 5.18 mmol) was dissolved in dry pyridine (50 ml) and the solution was cooled to 0°C. Acetic anhydride (987 µl, 10.36 mmol) was slowly added, followed by a catalytic amount of DMAP. The mixture was stirred at rt until completion (TLC H/EA 85/15). Then, the reaction mixture was concentrated under reduced pressure and the obtained residue was diluted with EA (100 ml), washed with aq. 5% solution of HCl (1 × 100 ml), aq. satd $NaHCO_3$ (1 × 100 ml) and brine (1 × 100 ml). The first aqueous phase was extracted with CH_2Cl_2 (1 × 100 ml) and the combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography using the Biotage system

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(H/EA gradient) affording **5** as a white amorphous solid (2.70 g, 92% yield). $[\alpha]_D$ -29.0 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.38–7.24 (m, 10H, Ar), 5.03–4.91 (m, 1H, H-4), 4.81 (d, *J* 11.5 Hz, 1H, ½ C*H*$_2$Ph), 4.62 (d, *J* 11.5 Hz, 1H, ½ C*H*$_2$Ph), 4.53 (d, $J_{1,2}$ 8.4 Hz, 1H, H-1), 4.51 (s, 2H, C*H*$_2$Ph), 3.57–3.47 (m, 3H, H-6, H-6', H-5), 3.41–3.34 (m, 2H, H-3, H-2), 1.85 (s, 3H, CH_3CO), 1.68 (hept, *J* 7.0 Hz, 1H, CH TDS), 0.92 (s, 3H, CH_3 TDS), 0.90 (s, 9H, CH_3 TDS), 0.22 (s, 3H, CH_3Si TDS), 0.21 (s, 3H, CH_3Si TDS). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 169.8 (CO), 97.1 (C-1), 80.5 (C-3), 74.9 (CH_2Ph), 73.8 (CH_2Ph), 73.6 (C-5), 71.2 (C-4), 70.1 (C-6), 68.7 (C-2), 34.1 (CH TDS), 25.0 (C_q TDS), 20.9 (*C*H_3CO), 20.1, 20.0, 18.7, 18.6 (4 CH_3 TDS), -1.89, -3.09 (2 CH_3Si TDS). HRMS (ES) *m/z* calcd for $C_{30}H_{43}N_3O_6SiNa$ 592.2819; Found 592.28077 $[M+Na]^+$.

General procedure A. Desilylation. An equimolar amount of glacial acetic acid and a 1 M solution of tetrabutylammonium fluoride in THF (1.5 mmol of TBAF per silylglycoside mmol) were sequentially added to a solution of the silylglycoside in anhydrous THF at -40°C. After completion of the reaction (TLC), the mixture was warmed up to r.t., poured in CH_2Cl_2, and washed three times with brine. The organic layer was dried (Na_2SO_4), filtered and concentrated. The residue was purified by column chromatography.

2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α/β-D-glucopyranose (9). Compound **4** (5.47 g, 9.97 mmol) was treated as described in the General Procedure A. Flash chromatography (H/EA 7/3) gave hemiacetal **9** as an oil (3.06 g, 80% yield). The spectroscopic characterization data of compound **9** were in agreement with those previously reported.[14,26]

4-*O*-Acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranose (11). Compound **5** (2.70 g, 4.74 mmol) was treated as described in the General Procedure A. Flash chromatography (H/EA 7/3) gave hemiacetal **11** as a colourless oil (1.90 g, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$, selected NMR signals of the anomeric mixture): δ_H 7.38–7.24 (m, 10H, Ar), 5.31 (br t, $J_{1,2}$ $J_{1,OH}$ 2.9 Hz, 1H, H-1 α), 5.01 (dd, $J_{4,3}$ 10.1, $J_{4,5}$ 9.3 Hz, 1H, H-4 α), 4.95 (m, 1H, H-4 β), 4.83 (d, *J* 11.1 Hz, 1H, ½ C*H*$_2$Ph α), 4.81 (d, *J* 11.4 Hz, 1H, ½ C*H*$_2$Ph β), 4.64 (d, *J* 11.1 Hz, 1H, ½ C*H*$_2$Ph α), 4.61 (d, *J* 11.4 Hz, 1H, ½ C*H*$_2$Ph β), 4.60–4.55 (br d, 1H, H-1 β), 4.51 (s, 4H, C*H*$_2$Ph β, C*H*$_2$Ph α), 4.19–4.10 (m, 1H, H-5 α), 4.04–3.92 (m, 1H, H-3 α), 3.60–3.37 (m, 8H, H-5 β, 2 H-6 β, 2 H-6 α, H-2 β, H-2 α, H-3 β), 1.87 (s, 3H, CH_3CO α), 1.84 (s, 3H, CH_3CO β). ^{13}C NMR (100.6 MHz, $CDCl_3$, selected NMR signals of the anomeric mixture): δ_C 169.8 (2 CO α + β), 96.3 (C-1 β), 92.1 (C-1 α), 80.5 (C-3 β), 77.8 (C-3 α), 75.0, 73.7 (CH_2Ph β, CH_2Ph α), 73.7 (C-5 β), 71.3 (C-4 α), 70.8 (C-4 β), 69.3 (C-5 α), 69.3 (C-6 β, C-6 α), 67.2 (C-2 β), 63.7 (C-2 α), 20.9 (CH_3CO β, CH_3CO α). MS (ES) *m/z* calcd for $C_{22}H_{25}N_3O_6Na$ 450.16; Found 450.5 $[M+Na]^+$.

General procedure B. Synthesis of α-hydrogenphosphonate. A 2 M solution of H_3PO_3 in pyridine (3:1 molar ratio as to the hemiacetal) was added dropwise to a solution of the hemiacetal in dry pyridine (4 ml/mmol), and thereafter the solution was cooled to 0°C and salicylchlorophosphite (1.5:1 molar ratio as to the hemiacetal) was slowly added. The reaction was stirred at rt and, after disappearance of the starting material (TLC), the reaction temperature was raised up to +40°C. The reaction mixture was stirred under argon until complete

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disappearance of the β anomer (evidenced by ^{1}H- and ^{31}P-NMR spectra). A 1 M solution of TEAB (4 ml/mmol) was added to the reaction mixture at rt, then it was diluted with CH_2Cl_2, washed three times with cold TEAB solution (0.5 M), dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 8/2 + 1% Et_3N). The obtained compound was further washed with cold TEAB solution (0.25M) to afford the α-H-phosphonate as a triethylammonium salt.

2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl hydrogenphosphonate triethylammonium salt (10). Compound **9** (168 mg, 0.44 mmol) was treated as described in the General Procedure B, providing α-H-phosphonate **10** (98 mg, 41% yield). $[\alpha]_D$ +10.4 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.55-7.22 (m, 10H, Ar), 7.01 (d, $J_{H,P}$ 640.4 Hz, 1H, H-P), 5.70 (dd, $J_{1,P}$ 8.8, $J_{1,2}$ 3.5 Hz, 1H, H-1), 5.55 (s, 1H, C*H*Ph), 4.92 (d, *J* 11.1 Hz, 1H, ½ C*H*$_2$Ph), 4.76 (d, *J* 11.1 Hz, 1H, ½ C*H*$_2$Ph), 4.25 (dd, $J_{6,6'}$ 9.8, $J_{6,5}$ 4.9 Hz, 1H, H-6), 4.21–4.14 (m, 1H, H-5), 4.11 (t, $J_{3,2}J_{3,4}$ 9.8 Hz, 1H, H-3), 3.74-3.68 (m, 2H, H-4, H-6'), 3.47 (ddd, $^{3}J_{2,P}$ 1.3 Hz, 1H, H-2). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 101.6 (*C*HPh), 93.8 (C-1), 82.9 (C-4), 76.3 (C-3), 74.9 (*C*H_2Ph), 69.0 (C-6), 63.6 (C-2), 63.5 (C-5). ^{31}P NMR (162 MHz, $CDCl_3$): δ_P 1.84. MS (ES) *m/z* calcd for $C_{20}H_{21}N_3O_7P$ 446.11; Found 446.3 $[M]^-$.

4-*O*-Acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl hydrogenphosphonate triethylammonium salt (12). Compound **11** (368 mg, 0.86 mmol) was treated as described in the General Procedure B, providing α-H-phosphonate **12** (265 mg, 52% yield). $[\alpha]_D$ +9.7 (c 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.38–7.19 (m, 10H, Ar), 7.03 (d, $J_{H,P}$ 642.0 Hz, 1H, H-P), 5.73 (dd, $J_{1,P}$ 8.7, $J_{1,2}$ 3.0 Hz, 1H, H-1), 5.14 (t, $J_{4,3}$ $J_{4,5}$ 9.7 Hz, 1H, H-4), 4.79 (d, *J* 11.1 Hz, 1H, ½ C*H*$_2$Ph), 4.60 (d, *J* 11.1 Hz, 1H, ½ C*H*$_2$Ph), 4.49 (d, *J* 11.8 Hz, 1H, ½ C*H*$_2$Ph), 4.44 (d, *J* 11.8 Hz, 1H, ½ C*H*$_2$Ph), 4.19 (m, 1H, H-5), 4.05 (t, $J_{3,2}$ 9.7 Hz, 1H, H-3), 3.57–3.38 (m, 3H, H-6, H-6', H-2), 1.83 (s, 3H, C*H*$_3$CO). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 169.7 (CH_3*C*O), 93.1 (C-1), 78.0 (C-3), 74.8 (*C*H_2Ph), 73.6 (*C*H_2Ph), 71.0 (C-4), 70.2 (C-5), 69.0 (C-6), 63.6 (C-2), 20.8 (*C*H_3CO). ^{31}P NMR (162 MHz, $CDCl_3$): δ_P 2.24. MS (ES) *m/z* calcd for $C_{22}H_{25}N_3O_8P$ 490.14; Found 490.3 $[M]^-$.

General procedure C. Hydrogenphosphonate coupling and oxidation. The alcohol and the H-phosphonate (**10** or **12**) were co-evaporated with dry pyridine for three times under high vacuum. The residue was then dissolved in dry pyridine(10 ml/mmol), and PivCl (2.5:1 molar ratio as to the H-phosphonate) was added. The mixture was stirred under nitrogen atmosphere for 45 min-1 h (TLC in CH_2Cl_2/MeOH mixtures). After cooling to -40°C, a freshly prepared 0.5 M solution of I_2 (2.5:1 molar ratio as to the H-phosphonate) in a 19:1 mixture of pyridine-H_2O was added. The oxidation was completed at 0°C and quenched by dropwise addition of a 0.5 M solution of $Na_2S_2O_3 \cdot 5H_2O$ (10% w/v). The reaction mixture was diluted with $CHCl_3$, and the organic layer was washed two times with a 0.5 M solution of $Na_2S_2O_3 \cdot 5H_2O$ (10% w/v), then 0.5 M TEAB, dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by flash chromatography (CH_2Cl_2/MeOH + 1% of Et_3N), providing the phosphodiester derivative. The obtained compound was further washed with cold TEAB solution (0.25 M) to afford the corresponding triethylammonium salt.

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General procedure D. Azide reduction and N-acetylation. To a solution of the azide-containing compound in MeOH (10 ml/mmol), first $NiCl_2 \cdot 6H_2O$ (3:1 molar ratio as to the number of azido groups) was added under stirring and N_2 atmosphere, then $NaBH_4$ (8:1 molar ratio as to the number of azido groups) portionwise (1 hour) at 0 °C. A black precipitate indicated the formation of a Ni-B species. The mixture was stirred at 0 °C and, after consumption of the starting material (amine formation is monitored by ninhydrin-detection), Ac_2O (20:1 molar ratio as to the azide-containing compound) was added. The mixture was concentrated under reduced pressure, diluted with CH_2Cl_2 and washed three times with water. The organic phase was dried (Na_2SO_4), filtered and concentrated, and purified by flash chromatography to give the acetamide. The obtained compound was further washed with cold TEAB solution (0.25 M) to afford the corresponding triethylammonium salt.

General procedure E: Hydrogenolysis and ion-exchange. The protected acetamide was hydrogenolysed over Pd/C (10%) in a 5:1 mixture of MeOH and H_2O at room temperature for 24-48 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-(*N*-Carbobenzyloxy)aminopropyl 1-*O*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl phosphate) triethylammonium salt (13). Benzyl *N*-(3-hydroxypropyl)carbamate (136 mg, 0.65 mmol) was condensed with H-phosphonate **10** (145 mg, 0.26 mmol) in pyridine (1.3 ml) in the presence of PivCl (0.04 ml, 0.32 mmol), and *in situ* oxidized with I_2 in pyridine-H_2O (2 ml) according to General Procedure C. Flash chromatography (CH_2Cl_2/MeOH 8/2 + 1% of Et_3N) and subsequent TEAB washing afforded glycosyl phosphodiester **13** as a colourless oil (122 mg, 62% yield). $[\alpha]_D$ +11.1 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.50–7.43 (m, 2H, Ar), 7.42–7.23 (m, 13H, Ar), 6.18 (br t, *J* 5.3 Hz, 1H, N*H*Cbz), 5.65 (dd, $J_{H,P}$ 7.5, $J_{1,2}$ 3.5 Hz, 1H, H-1), 5.55 (s, 1H, C*H*Ph), 5.06 (s, 2H, C*H*$_2$Ph), 4.93 (d, *J* 11.1 Hz, 1H, ½ C*H*$_2$Ph), 4.76 (d, *J* 11.1 Hz, 1H, ½ C*H*$_2$Ph), 4.24 (dd, $J_{6,6'}$ 10.1, $J_{6,5}$ 4.9 Hz, 1H, H-6), 4.20–3.94 (m, 4H, H-5, H-3, OC*H*$_2$CH$_2$CH$_2$N), 3.743.66 (m, 2H, H-4, H-6'), 3.47 (ddd, $J_{2,3}$ 9.8, $J_{2,P}$ 2.3 Hz, 1H, H-2), 3.35 (dd, *J* 11.7, 5.9 Hz, 2H, OCH$_2$CH$_2$C*H*$_2$N), 1.83–1.72 (m, 2H, OCH$_2$C*H*$_2$CH$_2$N). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 156.7 (CO), 101.6 (*C*HPh), 94.6 (C-1), 82.8 (C-4), 76.3 (C-3), 74.9 (C-6), 69.1 (*C*H$_2$Ph), 66.5 (*C*H$_2$Ph), 63.7 (C-2), 63.6 (C-5), 63.04 (O*C*H$_2$CH$_2$CH$_2$N), 37.46 (OCH$_2$CH$_2$*C*H$_2$N), 30.45 (OCH$_2$*C*H$_2$CH$_2$N). ^{31}P NMR (162 MHz, $CDCl_3$): δ_P -0.84. HRMS (ES) *m/z* calcd for $C_{31}H_{34}N_4O_{10}P$ 653.2018; Found 653.20169 $[M]^-$.

3-(*N*-Carbobenzyloxy)aminopropyl 1-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl phosphate), triethylammonium salt (14). Compound **13** (97 mg, 0.13 mmol) was converted into acetamide **14** as described in the General Procedure D. Flash chromatography (EA/MeOH from 9/1 to 8/2 + 1% of Et_3N) and subsequent TEAB washing afforded glycosyl phosphodiester **14** (29 mg, 29% yield). $[\alpha]_D$ +31.2 (c 0.4, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.53–7.44 (m, 2H, Ar), 7.44–7.20 (m, 13H, Ar), 6.76 (d, *J* 9.9 Hz, 1H,

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N*H*Ac), 5.99 (t, *J* 5.5 Hz, 1H, N*H*Cbz), 5.56 (s, 1H, C*H*Ph), 5.47 (dd, $J_{1,P}$ 7.2, $J_{1,2}$ 3.5 Hz, 1H, H-1), 5.05 (s, 2H, CH_2Ph), 4.88 (d, *J* 12.1 Hz, 1H, ½ CH_2Ph), 4.66 (d, *J* 12.1 Hz, 1H, ½ CH_2Ph), 4.36–4.27 (m, 1H, H-2), 4.21 (dd, $J_{6,6'}$ 10.2, $J_{6,5}$ 4.9 Hz, 1H, H-6), 4.10–4.02 (m, 1H, H-4), 4.02–3.87 (m, 2H, OCH_2CH_2CH_2N), 3.86–3.68 (m, 3H, H-3, H-5, H-6'), 3.32 (dd, *J* 12.3, 6.2 Hz, 2H, OCH_2CH_2CH_2N), 1.91 (s, 3H, NHCOCH_3), 1.81–1.71 (m, 2H, OCH_2CH_2CH_2N). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 156.7 (CO), 101.6 (*C*HPh), 94.6 (C-1), 82.8 (C-4), 76.3 (C-3), 74.9 (C-6), 69.1 (*C*H_2Ph), 66.5 (*C*H_2Ph), 63.7 (C-2), 63.6 (C-5), 63.0 (O*C*H_2CH_2CH_2N), 37.46 (OCH_2CH_2*C*H_2N), 30.45 (OCH_2*C*H_2CH_2N). ^{31}P NMR (162 MHz, $CDCl_3$): δ_P -0.49. MS (ES) *m/z* calcd for $C_{33}H_{38}N_2O_{11}P$ 669.22; Found 669.4 $[M]^-$.

3-Aminopropyl 1-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate), sodium salt (1). The acetamide **14** (26 mg, 0.034 mmol) was hydrogenolysed over Pd/C in MeOH:H_2O (4 mL) and subjected to ion exchange according to General Procedure E, affording monomer **1** as a foam (12 mg, 93% yield). $[\alpha]_D$ +66.0 (*c* 0.1, H_2O). ^{1}H NMR (400 MHz, D_2O): δ_H 5.43 (dd, $J_{1,P}$ 7.4, $J_{1,2}$ 3.3 Hz, 1H, H-1), 4.03–3.73 (m, 7H, OCH_2CH_2CH_2N, H-2, H-6, H-6', H-3, H-5), 3.51 (t, $J_{4,3}$ $J_{4,5}$ 9.3 Hz, 1H, H-4), 3.20–3.04 (m, 2H, OCH_2CH_2CH_2N), 2.08–2.01 (m, 3H, NHCOCH_3), 2.01–1.87 (m, 2H, OCH_2CH_2CH_2N). ^{13}C NMR (100.6 MHz, D_2O): δ_C 94.2 (C-1), 72.9 (C-3), 70.1 (C-5), 69.4 (C-4), 63.3 (O*C*H_2CH_2CH_2N), 60.2 (C-6), 53.7 (C-2), 36.9 (OCH_2CH_2*C*H_2N), 27.6 (OCH_2*C*H_2CH_2N), 21.7 (NHCO*C*H_3). ^{31}P NMR (162 MHz, D_2O): δ_P -0.94. HRMS (ES) *m/z* calcd for $C_{11}H_{22}N_2O_9P$ 357.1068; Found 357.10632 $[M]^-$; calcd for $C_{11}H_{23}N_2O_9PNa$ 381.1044; Found 381.10368 $[M+H+Na]^+$; calcd for $C_{11}H_{23}N_2O_9PNa_2$ 404.0942; Found 404.08561 $[M+H+2Na]^{2+}$.

3-(*N*-Carbobenzyloxy)aminopropyl 1-*O*-(4-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl phosphate), triethylammonium salt (15). Benzyl *N*-(3-hydroxypropyl)carbamate (136 mg, 0.65 mmol) was condensed with H-phosphonate **12** (76 mg, 0.13 mmol) in pyridine (1.3 ml) in the presence of PivCl (0.04 ml, 0.32 mmol), and *in situ* oxidized with I_2 in pyridine-H_2O (2 ml) according to General Procedure C. Flash chromatography (CH_2Cl_2/MeOH from 9/1 to 8/2 + 1% of Et_3N) and subsequent TEAB washing afforded glycosyl phosphodiester **15** as a colourless oil (66 mg, 64% yield). $[\alpha]_D$ +35.8 (c 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.33–7.17 (m, 15H, Ar), 6.20 (t, *J* 5.5 Hz, 1H, N*H*), 5.62 (dd, $J_{1,P}$ =7.8, $J_{1,2}$ 3.1 Hz, 1H, H-1), 5.06 (t, $J_{4,3}$ $J_{4,5}$ 9.7 Hz, 1H, H-4), 5.02 (s, 2H, CH_2Ph), 4.74 (d, *J* 11.1 Hz, 1H, ½ CH_2Ph), 4.56 (d, *J* 11.1 Hz, 1H, ½ CH_2Ph), 4.51 (d, *J* 11.8 Hz, 1H, ½ CH_2Ph), 4.48 (d, *J* 11.8 Hz, 1H, ½ CH_2Ph) 4.20–4.09 (m, 1H, H-5), 4.05–3.90 (m, 3H, H-3, OCH_2CH_2CH_2N), 3.56–3.46 (m, 3H, H-2, H-6, H-6'), 3.27 (dd, *J* 11.5, 5.7 Hz, 2H, OCH_2CH_2CH_2N), 1.81 (s, 3H, CH_3CO), 1.73–1.55 (m, 2H, OCH_2CH_2CH_2N). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 169.7, 156.6 (CO), 93.7 (C-1), 77.7 (C-3), 74.7 (*C*H_2Ph), 73.5 (*C*H_2Ph), 70.9 (C-4), 69.9 (C-5), 69.0 (C-6), 66.3 (*C*H_2Ph), 63.7 (C-2), 62.8 (O*C*H_2CH_2CH_2N), 37.28 (OCH_2CH_2*C*H_2N), 30.2 (OCH_2*C*H_2CH_2N), 20.79 (*C*H_3CO). ^{31}P NMR (162 MHz, $CDCl_3$): □δ_P -0.52. HRMS (ES) *m/z* calcd for $C_{33}H_{38}N_4O_{11}P$ 697.2280; Found 697.22780 $[M]^-$.

3-(*N*-Carbobenzyloxy)aminopropyl 1-*O*-[2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl phosphate 4-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl phos-

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phate)], triethylammonium salt (17). A solution of **15** (120 mg, 0.15 mmol) in dry MeOH was treated with 0.05 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 (CH_2Cl_2/MeOH 8/2 + 1% of Et_3N), providing the corresponding deacetylated product **16** (110 mg), which was condensed with H-phosphonate **10** (66 mg, 0.12 mmol) in pyridine (1.5 ml) in the presence of PivCl (0.045 ml, 0.36 mmol), and *in situ* oxidized with I_2 in pyridine-H_2O (2 ml) according to General Procedure C. Flash chromatography (CH_2Cl_2/MeOH 8/2 + 1% of Et_3N) and subsequent TEAB washing afforded glycosyl phosphodiester **17** (71 mg, 45% yield). $[\alpha]_D$ +12.7 (c 1.15, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.58–7.41 (m, 5H, Ar), 7.41–7.13 (m, 20H, Ar), 6.34 (t, *J* 5.9 Hz, 1H, N*H*Cbz), 5.78–5.67 (m, 2H, H-1a, H-1b), 5.56 (s, 1H, C*H*Ph), 5.34 (d, *J* 11.0 Hz, 1H, ½ C*H*$_2$Ph), 5.06 (s, 2H, C*H*$_2$Ph), 4.82 (d, 1H, ½ C*H*$_2$Ph), 4.79 (d, 1H, ½ C*H*$_2$Ph), 4.67□4.55 (m, 3H, ½ C*H*$_2$Ph, C*H*$_2$Ph), 4.42 (t, $J_{4a,3a}$ = $J_{4a,5a}$ 10.0 Hz, 1H, H-4a), 4.34–4.16 (m, 3H, H-6b, H-5a, H-5b), 4.13–3.93 (m, 5H, H-6a, H-3b, H-3a, OC*H*$_2$CH$_2$CH$_2$N), 3.87 (dd, $J_{6'a,6a}$ 10.9, $J_{6'a,5a}$ 6.2 Hz, 1H, H-6'a), 3.77–3.65 (m, 2H, H-6'b, H-4b), 3.52–3.35 (m, 2H, H-2a, H-2b), 3.30 (dd, *J* 10.9, 5.2 Hz, 2H, OCH$_2$CH$_2$C*H*$_2$N), 1.69–1.52 (m, 2H, OCH$_2$C*H*$_2$CH$_2$N). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 101.5 (*C*HPh), 94.5 (C-1b), 93.8 (C-1a), 82.8 (C-4b), 79.0 (C-3b), 76.5 (C-3a), 74.8 (*C*H$_2$Ph), 74.7 (C-4a), 74.4 (*C*H$_2$Ph), 73.4 (*C*H$_2$Ph), 72.2 (C-5a), 69.9 (C-6a), 69.0 (C-6b), 66.2 (*C*H$_2$ Cbz), 64.0 (C-2b), 63.7 (C-2a), 63.3 (C-5b), 62.7 (O*C*H$_2$CH$_2$CH$_2$N), 37.25 (OCH$_2$CH$_2$*C*H$_2$N), 30.17 (OCH$_2$*C*H$_2$CH$_2$N). ^{31}P NMR (162 MHz, $CDCl_3$): δ_P -0.23, -1.99. HRMS (ES) *m/z* calcd for $C_{51}H_{55}N_7O_{17}P_2Na$ 1122.3033; Found 1122.30177 $[M+Na]^-$; calcd for $C_{51}H_{55}N_7O_{17}P_2$ 1099.3135; Found 549.65634 $[M]^{2-}$.

3-Aminopropyl 1-*O*-[2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate 4-(2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate)], bis-sodium salt (2). Compound **17** (95 mg, 0.073 mmol) was converted into acetamide as described in the General Procedure D. Flash chromatography (CH_2Cl_2/MeOH from 8/2 to 7/3 + 1% of Et_3N) and subsequent TEAB washing afforded the bis-acetamido glycosyl phosphodisaccharide, which was hydrogenolysed over Pd/C in MeOH:H_2O (4 ml) and subjected to ion exchange according to General Procedure E, affording dimer **2** as a foam (18 mg, 36% yield over two steps). $[\alpha]_D$ +19.0 (c 0.07, H_2O). ^{1}H NMR (400 MHz, D_2O): δ_H 5.17 (dd, $J_{1a,P}$ 7.5 Hz, $J_{1a,2a}$ 3.3 Hz, 1H, H-1a), 4.70 (dd, $J_{1b,P}$ 8.3 Hz, $J_{1b,2b}$ 3.8 Hz, 1H, H-1b), 4.12–3.60 (m, 10H, H-2a or H-2b, H-3a, H-3b, H-4a, H-5a, H-5b, H-6a, H-6'a, OC*H*$_2$CH$_2$CH$_2$N), 3.54–3.34 (m, 4H, H-2b or H-2a, H-4b, H-6b, H-6'b), 3.16□3.10 (m, 2H, OCH$_2$CH$_2$C*H*$_2$N), 2.01 (br s, 6H, 2 × NHCOC*H*$_3$), 1.97□1.91 (m, 2H, OCH$_2$C*H*$_2$CH$_2$N). ^{13}C NMR (100.6 MHz, D_2O): δ_C 94.8, 90.7 (C-1a, C-1b), 75.8, 73.7 (C-3a, C-3b), 71.4, 70.5 (C-5a, C-5b), 70.4, 70.0 (C-4a, C-4b), 68.3 (O*C*H$_2$CH$_2$CH$_2$N), 60.6, 60.4 (C-6a, C-6b), 56.5, 53.9 (C-2a, C-2b), 22.0 (NHCO*C*H$_3$), 21.7 (OCH$_2$CH$_2$*C*H$_2$N), 19.9 (OCH$_2$*C*H$_2$CH$_2$N). ^{31}P NMR (162 MHz, D_2O): δ_P 0.90, 0.60. HRMS (ES) *m/z* calcd for $C_{19}H_{34}N_3O_{17}P_2Na$ 661.1267; Found 661.77595 $[M+Na]^-$.

3-(*N*-Carbobenzyloxy)aminopropyl 1-*O*-[2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl phosphate 4-(4-*O*-acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl phos-

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phate)], bis-triethylammonium salt (18). Alcohol **16** (425 mg, 0.56 mmol) was condensed with H-phosphonate **12** (366 mg, 0.62 mmol) in pyridine (2.5 ml) in the presence of PivCl (0.14 ml, 1.11 mmol), and *in situ* oxidized with I_2 in pyridine-H_2O (2 ml) according to General Procedure C. Flash chromatography (CH_2Cl_2/MeOH 85/15 + 1% of Et_3N) and subsequent TEAB washing afforded glycosyl phosphodiester **18** (290 mg, 40% yield). The identity of compound **18** was confirmed by ^{1}H- and ^{31}P-NMR, and it was used in the following steps without further characterization. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.53–7.15 (m, 25H, Ar), 6.29 (br t, *J* 5.8 Hz, 1H, NHCbz), 5.72 (dd, $J_{1,P}$ 7.5 Hz, $J_{1,2}$ 3.3 Hz, 1H, H-1a or H-1b), 5.67 (dd, $J_{1,P}$ 8.2 Hz, $J_{1,2}$ 3.4 Hz, 1H, H-1b or H-1a), 5.28 (d, *J* 11.0 Hz, 1H, ½ C*H*$_2$Ph), 1H), 5.14 (t, $J_{4b,3b} = J_{4b,5b}$ 9.7 Hz, 1H, H-4b), 5.02 (br s, 2H, C*H*$_2$Ph), 4.75 (d, *J* 11.0 Hz, 1H, ½ C*H*$_2$Ph), 4.63 (d, *J* 11.5 Hz, 1H, ½ C*H*$_2$Ph), 4.58 (br s, 2H, C*H*$_2$Ph), 4.47–4.36 (m, 4H, H-4a, ½ C*H*$_2$Ph, C*H*$_2$Ph), 4.24 (br t, 1H, H-5a or H-5b), 4.17 (dt, $J_{5,4}$ 10.2 Hz, $J_{5,6}$ 3.5 Hz, 1H, H-5b or H-5a), 4.11–3.89 (m, 5H, H-3a, H-3b, H-6a, OC*H*$_2$CH$_2$CH$_2$N), 3.85 (dd, $J_{6'a,6a}$ 11.1 Hz, $J_{6'a,5a}$ 6.5 Hz, 1H, H-6'a), 3.49–3.37 (m, 4H, H-2a, H-2b, H-6b, H-6'b), 3.25 (dd, *J* 11.8, 6.1 Hz, 2H, OCH$_2$CH$_2$C*H*$_2$N), 1.75 (s, 3H, C*H*$_3$CO), 1.61–1.52 (m, 2H, OCH$_2$C*H*$_2$CH$_2$N).). ^{31}P NMR (162 MHz, $CDCl_3$): δ_P -0.90, -2.77.

3-(*N*-Carbobenzyloxy)aminopropyl 1-*O*-{2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl phosphate 4-[2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl phosphate 4-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl phosphate)]}, tris-triethylammonium salt (20). A solution of **18** (290 mg, 0.22 mmol) in dry MeOH was treated with 0.05 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 (CH_2Cl_2/MeOH 85/15 + 1% of Et_3N), providing the corresponding deacetylated product **19** (228 mg, 0.16 mmol), which was condensed with H-phosphonate **10** (142 mg, 0.24 mmol) in pyridine (2.5 ml) in the presence of PivCl (0.074 ml, 0.6 mmol), and *in situ* oxidized with I_2 in pyridine-H_2O (2 ml) according to General Procedure C. Flash chromatography (CH_2Cl_2/MeOH 8/2 + 1% of Et_3N) and subsequent TEAB washing afforded glycosyl phosphodiester **20** as an amorphous solid (190 mg, 43% yield from **18**). $[\alpha]_D$ +12.8 (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.59–7.04 (m, 35H, Ar), 6.32 (s, 1H, N*H*Cbz), 5.78 (dd, $J_{1a,P}$ 7.0, $J_{1a,2a}$ =2.4 Hz, 1H, H-1a), 5.74–5.58 (m, 2H, H-1b, H-1c), 5.52 (s, 1H, C*H*Ph), 5.41–5.16 (m, 2H, 2 × ½ C*H*$_2$Ph), 5.03 (s, 2H, C*H*$_2$Ph), 4.83–4.70 (m, 2H, 2 × ½ C*H*$_2$Ph), 4.68–4.52 (m, 5H, 3 × ½ C*H*$_2$Ph, C*H*$_2$Ph), 4.52–4.32 (m, 2H, H-4b, H-4a), 4.32–3.76 (m, 12H, H-6c, H-5a, H-5b, H-6a, H-3b, H-3a, H-6b, H-3c, OC*H*$_2$CH$_2$CH$_2$N, H-6'a, H-6'b), 3.76–3.53 (m, 2H, H-6'c, H-4c), 3.47□3.28 (m, 3H, H-2a, H-2b, H-2c), 3.25 (d, *J* 5.2 Hz, 2H, OCH$_2$CH$_2$C*H*$_2$N), 1.62–1.45 (m, 2H, OCH$_2$C*H*$_2$CH$_2$N). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C 101.56 (*C*HPh), 94.6 (C-1c), 94.1, 93.9 (C-1a, C-1b), 82.9 (C-4c), 79.5, 79.0 (C-3a, C-3b), 76.7 (C-3c), 75.1 (C-4a), 74.9 (*C*H$_2$Ph), 74.7 (C-4b), 74.5 (*C*H$_2$Ph), 73.5, 73.4 (*C*H$_2$Ph), 72.3 (C-5a), 72.3 (C-5b), 70.0 (C-6b), 69.7 (C-6a), 69.0 (C-6c), 66.3 (*C*H$_2$Ph), 64.1, 64.0 (C-2a, C-2b), 63.8 (C-2c), 63.3 (C-5c), 62.8 (O*C*H$_2$CH$_2$CH$_2$N), 37.3 (OCH$_2$CH$_2$*C*H$_2$N), 29.8 (OCH$_2$*C*H$_2$CH$_2$N). ^{31}P NMR (162 MHz, $CDCl_3$): δ_P -0.30, -2.26 (2 P). HRMS (ES) *m/z* calcd for $C_{71}H_{77}N_{10}O_{24}P_3$ 1546.4336; Found 773.21635 $[M+H]^{2-}$; calcd for $C_{71}H_{76}N_{10}O_{24}P_3Na$ 1568.4150;

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Found 784.20616 $[M+Na]^{2-}$; calcd for $C_{71}H_{76}N_{10}O_{24}P_3Na_2$ 1591.4048; Found 1591.39910 $[M+2Na]^-$.

3-Aminopropyl 1-*O*-[2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate 4-[2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate 4-(2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate)]], tris-sodium salt (3). Compound **20** (80 mg, 0.043 mmol) was converted into acetamide as described in the General Procedure D. Flash chromatography (CH_2Cl_2/MeOH 7/3 + 1% of Et_3N) and subsequent TEAB washing afforded the tris-acetamido glycosyl phosphotrisaccharide, which was hydrogenolysed over Pd/C in MeOH:H_2O (4 ml) and subjected to ion exchange according to General Procedure E, affording trimer **3** as a foam (14 mg, 33% yield over two steps). $[\alpha]_D$ +86.6 (c 0.4, H_2O). ^{1}H NMR (400 MHz, D_2O): δ_H 5.55–5.45 (m, 2H, H-1a, H-1b), 5.45–5.35 (m, 1H, H-1c), 4.16–3.61 (m, 19H, O*CH*$_2$$CH_2CH_2N$, H-2a, H-2b, H-2c, H-6a, H-6b, H-6c, H-6'a, H-6'b, H-6'c, H-3a, H-3b, H-3c, H-5a, H-5b, H-5c, H-4a, H-4b), 3.51 (t, $J_{4c,3c} = J_{4c,5c}$ 9.5 Hz, 1H, H-4c), 3.10 (t, *J* 7.0 Hz, 1H, $OCH_2CH_2CH_2N$), 2.07–1.88 (m, 11H, 3 × NHCO*CH*$_3$, $OCH_2CH_2CH_2N$). ^{13}C NMR (100.6 MHz, D_2O): δ_C 174.6 (2 CO),175.8 (CO), 94.5 (C-1b), 94.2 (C-1c), 93.6 (C-1a), 74.0 (C-3a, C-3b), 73.1 (C-3c), 72.2 (C-5a, C-5b), 70.9 (C-5c), 70.1, 69.6 (C-4a, C-4b, C-4c), 63.4 ($OCH_2CH_2CH_2N$), 60.3 (C-6a, C-6b, C-6c), 53.7 (C-2a, C-2b, C-2c), 37.2 ($OCH_2CH_2CH_2N$), 27.7 ($OCH_2CH_2CH_2N$), 22.2 (3 × NHCO*CH*$_3$). ^{31}P NMR (162 MHz, D_2O): δ_P -0.31, -0.65, -0.71. HRMS (ES) *m/z* calcd for $C_{27}H_{48}N_4O_{25}P_3Na_2$ 967.1632; Found 967.16133 $[M+2Na]^-$; calcd for $C_{27}H_{49}N_4O_{25}P_3Na$ 945.1707; Found 945.17800 $[M+H+Na]^-$; calcd for $C_{27}H_{48}N_4O_{25}P_3Na$ 944.1729; Found 472.08681 $[M+Na]^{2-}$; calcd for $C_{27}H_{49}N_4O_{25}P_3$ 922.1904; Found 461.09538 $[M+H]^{2-}$.

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