Synthesis of ribitol phosphate based wall teichoic acids

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Synthesis and application of *Staphylococcus aureus* ribitol phosphate fragments

INTRODUCTION

The Gram-positive bacterium *Staphylococcus aureus (S. aureus)* is a commensal pathogen that is part of the human microbiome and is commonly found on the skin and in the nasal nares. *S. aureus* usually does not cause infections, however, when entering the blood stream or internal tissues, the bacteria can cause serious infections, for which immunocompromized patients especially are at risk.[1] Extensive use of antibiotics has led to increasing resistance among *S. aureus* strains against commonly used antibiotics leading to infections that are difficult to treat. Currently Methicillin-resistant Staphylococcus aureus (MRSA) is the most commonly identified antibiotic-resistant pathogen in clinical medicine worldwide.[2] The spread of MRSA highlights the urgent need for alternative therapies, such as vaccination.[3]

Ali, S., Hendriks, A., van Dalen, R., Bruyning, T., Meeuwenoord, N., Overkleeft, H., Filippov, D., van der Marel, G., van Sorge, N., Codée, J.D.C., (Automated) Synthesis of Well-defined Staphylococcus Aureus Wall Teichoic Acid Fragments. *Chem. Eur. J.* **2021**, 27 (40): 10461-10469.

Wall teichoic acids (WTAs), prime constituents of the Gram-positive cell wall, can function as effective antigenic epitopes and are therefore promising candidates for the development of a conjugate vaccine against *S. aureus* infections.[4-6] As described in Chapter 1, WTAs are anionic poly-ribitol phosphate (RboP) chains attached to the peptidoglycan of the bacterial cell wall. WTAs are involved in host interaction, biofilm formation, autolysin activity and their overexpression can increase bacterial virulence.[7] The RboP residues can be substituted in a seemingly random manner with either D-alanine (D-Ala) on the C-2 position, α- or β-N-acetylglucosamine (GlcNAc) on the C-4 position or a β-GlcNAc on C-3 position. The GlcNAc residues are introduced by three different glycosyltransferases, TarS[8] (1,4-β-GlcNAc), TarM[9] (1,4-α-GlcNAc), and the recently discovered TarP[10] (1,3-β-GlcNAc), respectively. The substitution pattern of WTAs is varied and is highly influenced by environmental conditions. A study on a panel of 24 invasive infection causing *S. aureus* strains, revealed that most strains express TarS and produced the C-4 β-GlcNAc WTA[11]. When both TarS and TarM were present and the bacteria were grown under stress-inducing conditions, glycosylation with β-GlcNAc was predominant. Strains that produce exclusively 1,3-β-GlcNAc modified RboPs under non-stressed conditions, switched to β-GlcNAcylation at both C-3 and C-4 under high NaCl concentration growth medium.

In a study, in which sera of human adults were screened for the presence of anti α- or β-GlcNAc WTA antibodies, it was found that predominantly anti β-GlcNAc WTA antibodies were present, with an average of 76% of the total anti-WTA IgG while 4% of the IgGs was specific to α-GlcNAc WTA.[12] In the same study, it was shown that 70% of IgG in infant sera was directed against β-GlcNAc-WTA. A plausible explanation for the high level of anti β-GlcNAc WTA is that these antibodies might be transferred maternally, or that these infants produce mainly anti β-GlcNAc WTA antibodies when their adaptive immune system starts to develop. Recently, TarP has been detected in healthcare- (HA) and livestock-associated (LA) MRSA clones CC5[13-14] and CC398[15] as a prominent glycosyltransferase.[10] It has been suggested that the subtle switch in WTA-glycosylation patterns from 1,4-β-GlcNAc to 1,3-β-GlcNAc may be a strategy of the bacteria to escape from host immune responses.

To unravel the roles of WTAs in biology at the molecular level well-defined fragments are indispensable tools. Since isolation from the bacteria leads to heterogenous mixtures of fragments and bacterial contaminations, organic synthesis is the method of choice to generate WTA-fragments with pre-defined substitution patterns. As the WTA fragments are built up from repeating units interconnected through phosphodiester linkages, the use of a solid phase DNA synthesizer would be particularly suitable. This Chapter reports on the development of chemistry that allows for the generation of well-defined

unsubstituted RboP oligomers, using both solution and automated solid phase synthesis (ASPS) techniques. All fragments are equipped with a 6-aminohexanol spacer for conjugation purposes. Taking into account that the bacterial WTA is covalently attached to the peptidoglycan at the RboP C1-position, this should also be the attachment site for the synthetic fragments. This Chapter describes the synthesis of WTA fragments **1**-**4** in solution up to the octamer level and applies ASPS for the WTA assembly of octa- and dodecamer **4** and **5** (Fig. 1). The assembly of the fragments builds on contemporary DNA/RNA synthesis, which has previously been used to generate various lipoteichoic acid fragments.[16-20] The generated RboP oligomers have been used for the structural and functional analysis of TarP, and as substrates for glycosylation reactions employing TarS/TarM/TarP. The binding of the enzymatically glycosylated WTA fragments to antibodies is also described.[21]

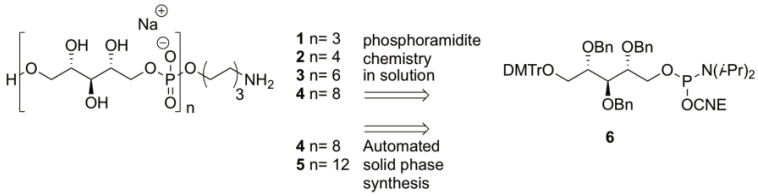
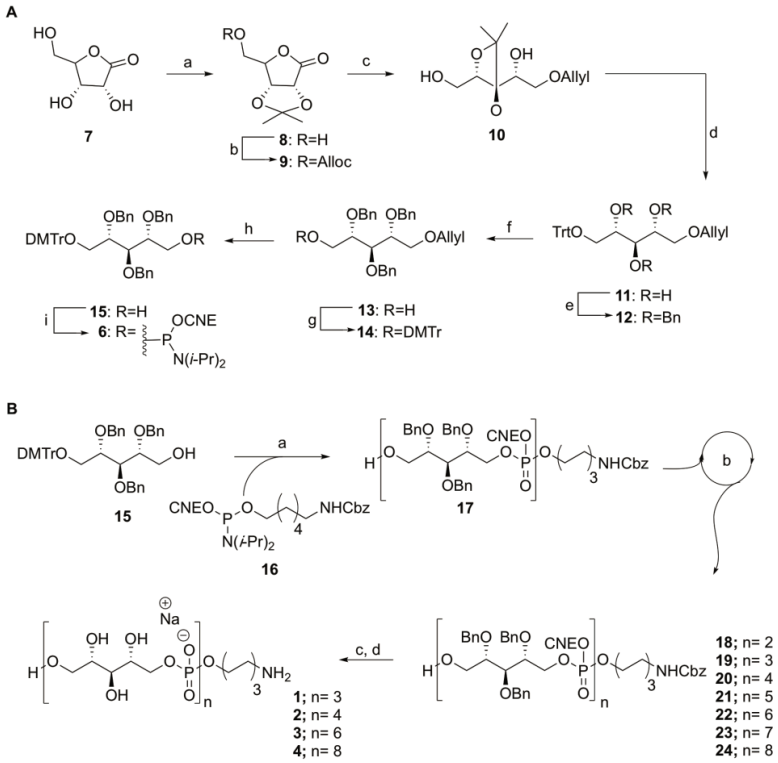


Figure 1. RboP oligomers synthesized from repeating unit **6** in both solution and solid phase.

RESULTS AND DISCUSSION

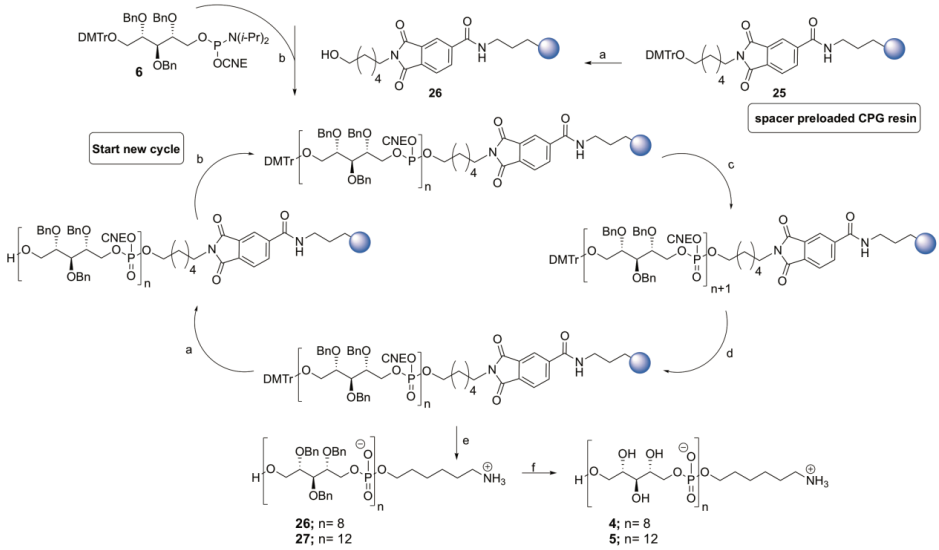
For the solution and automated solid phase assembly (ASPS) of the set of target compounds key phosphoramidite **6** was required, the synthesis of which started from lactone **7**. Following the route reported by Hermans *et al.*[22] **13** was generated as shown in Scheme 1A. Isopropylidene protection of the secondary alcohols in **7** proceeded with a yield of 77% (on 300 mmol scale) and was followed by Alloc protection of the primary alcohol to afford **9** in 80% yield. Decarboxylation using $Pd(PPh_3)_4$ and an ensuing ring opening by carbonyl reduction using sodium borohydride delivered primary alcohol **10** in 85% over 2 steps. $AcOH/H_2O$ mediated hydrolysis cleaved the isopropylidene group and subsequent tritylation of the primary alcohol yielded **11** in quantitative yield. Benzylation of the remaining alcohol and subsequent detritylation provided **13**. The primary alcohol was protected with a 4,4'-Dimethoxytrityl (DMTr) giving **14**, which was then subjected to iridium catalyzed allyl isomerization and a subsequent iodine mediated enol ether hydrolysis to yield **15** in 79%. Introduction of the phosphoramidite afforded the required key building block **6** for oligomerization in 79%.

The assembly of the oligomers using the solution phase approach is shown in Scheme 1B. First, alcohol **15** was coupled with phosphoramidite spacer **16**, obtained according to the procedure described by Hogendorf *et al*. [16] The RboP-chain elongation steps using the phosphoramidite couplings consisted of 3 steps. In the first step the amidite group was activated by 4,5-dicyanoimidazole (DCI) to enable attack by the primary ribitol alcohol to form the phosphite intermediate, which was oxidized in the next step using (10-camphorsulfonyl)oxaziridine (CSO). Detritylation using 3% Dichloroacetic acid (DCA) in DCM liberated the primary alcohol and silica gel column chromatography yielded the pure ribitol phosphate fragment, ready for the next elongation step. This way, monomer **17** was obtained in 85% yield. From alcohol **17**, the coupling cycles were repeated seven times to yield **18**-**24**, all in good yield. The cyanoethyl group was removed under aqueous ammonia conditions and subsequent hydrogenation of the benzyl groups yielded **1**, **2**, **3**, and **4** in 87%, 75%, 87% and 89% yield respectively.



Scheme 1A. Ribitol building block synthesis; *Reagents and conditions*: a) HCl, acetone, 77%; b) AllocCl, pyridine/ACN, 80%; c) i. $Pd(PPh_3)_4$, dioxane, reflux, ii. $NaBH_4$,THF, 55°C, MeOH, 85%; d) i. $AcOH/H_2O$, 50°C, ii. TrtCl, pyridine, 99%; e) BnBr, NaH, THF/DMF 68%; f) $AcOH/H_2O$, 80°C, 70%; g) DMTrCl, TEA, DCM, quantitative; h) i. $Ir(COD)(Ph_2MeP)_2PF_6$, H_2, THF, ii. I_2, sat. aq. $NaHCO_3$, THF, 79%; i) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, DIPEA, DCM, 79%; **Scheme 1B.** Assembly of aminospacer functionalized RboP WTAs; *Reagents and conditions*: a) i. DCI, ACN, 16; ii. CSO; iii. 3% DCA in DCM, 85%; b) i. DCI, ACN, **6**, ii. CSO, iii. 3% DCA in DCM, **18**; 74%, **19**; 88%, **20**: 80%, **21**; 76%, **22**: 91%, **23**: 85%, **24**: 86%; c) NH_3 (30-33% aqueous solution), dioxane; d) Pd black, H_2, AcOH, H_2O/dioxane, **1**: 87%, **2**: 75%, **3**: 87%, **4**: 89%.

Next, the assembly of longer fragments was investigated using ASPS. Hoogerhout *et al.* previously described an attempt to synthesize an RboP-octa- and dodecamer using a solid phase synthesis approach,[23] but they reported that an intractable mixture was obtained after cleavage of the product from the resin. As suggested by the authors, this could have been caused by the high concentration of TCA used to remove the DMTr. In the solution phase assembly of **1**-**4**, a milder acid, DCA, was used for the removal of the DMTr group and these conditions were applied to the solid phase synthesis. The syntheses were performed on an Äkta oligopilot plus™ synthesizer and started on 10 μmol scale using a commercially available spacer-preloaded resin **25** (Scheme 2). The DMTr group was cleaved from resin **25** using 3% DCA in toluene and the coupling with cyanoethyl (CNE) amidite **6** under 5-(benzylthio)-1*H*-tetrazole activation then provided the resin bound phosphite. Oxidation using I_2 in pyridine/H_2O yielded the phosphate triester after which a capping step took place to prevent any unreacted alcohol functionalities to react in the next step, which could lead to byproducts that may be difficult to separate. Removal of the DMTr group allowed a new cycle to start and the coupling cycles were repeated 7 to 11 times to reach the target octa- and dodecamer. Treatment of the resin with 3% DCA unmasked the primary alcohol and subsequent treatment with aqueous 25% NH_3 cleaved the cyanoethyl groups and released the oligomer from the resin. Figure 2 depicts the LCMS chromatograms of the crude products **26** and **27**, indicating highly efficient syntheses of these oligomers. Purification of the crude oligomers



Scheme 2. Assembly of RboP WTAs using ASPS approach; *Reagents and conditions:* a) 3% DCA, toluene; b) phosphoramidite **6**, 5-(Benzylthio)-1*H*-tetrazole, ACN; c) I_2, pyridine, H_2O, ACN; d) Ac_2O, *N*-methylimidazole, 2,6-lutidine, ACN; e) i. 3% DCA, toluene; ii. 25% NH_3 (aq) n=8; **26**: 6.1 mg; 15%, n=12; **27**: 3.4 mg; 11%; f) Pd black, H_2, dioxane H_2O, AcOH, n=8; **4**: 3.5 mg; quant, n=12; **5**: 1.8 mg; quant.

by reversed phase HPLC and desalination afforded **26** and **27** in 15% and 11% yield respectively. Hydrogenation of the semi-protected octa- and dodecamer yielded the targets **4** and **5** both in quantitative yields.

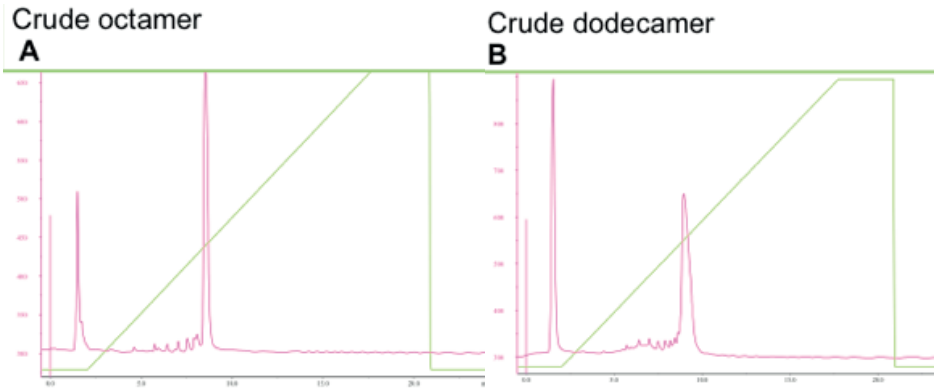


Figure 2. Anion-exchange chromatogram of the crude octamer **26** (A) and crude dodecamer **27** (B). Column type: DNA PAC PA 100, Eluent buffer A: 10 mM NaOAc + 10 mM NaCl, buffer B: 10 mM NaOAc en 1 mM NaCl, lineair gradient 1/0 to 0/1.

As described before, the enzymes TarM and TarS perform both glycosylation on the C-4 position, but their products differ in anomeric configuration. The crystal structure of TarS has been elucidated explaining the mode of action. TarP however, glycosylates in β-manner but on the C-3 position instead. To evaluate how the orientation of the WTA substrate in the active site influences the outcome of the glycosylation at the C-3 position, Gerlach *et al*. used hexamer **3** as a model WTA substrate to soak TarP crystals. Figure 3A shows the crystal structure of compound **3** in the active site of TarP showing 3 RboP repeating units. The dashed lines represent the hydrogen bonds between the RboP units and the key amino acids. Figure 3B sketches the interaction of the key amino acids, RboP and UDP-GlcNAc. The enzyme is proposed to glycosylate the RboP alcohol using an S_N2-type displacement of the anomeric pyrophosphate, and it uses asparagine 181, found at a distance of 3.1 Å to the C-3 hydroxyl, as the catalytic base. In a ternary complex, in which also the UDP-GlcNAc was bound, the distance between C-1 of UDP-GlcNAc and the RboP C-3 OH is 4.2 Å and the C-3 OH is well oriented for attack on the GlcNAc C-1 on the β-side to yield the β-product.

Next, the synthetic structures were evaluated as substrates for glycosylations using the three different WTA GlcNAc transferases. Glycosylation of the substrates was evaluated using MALDI-MS and the products of the reactions were used to probe for antibody binding. To this end hexamer **3** was equipped with a biotin handle to capture the glycosylated oligomers by streptavidin coated magnetic beads (See Figure 6). Two different enzyme concentrations were used for each modification: 30 μg/mL and 6 μg/mL, and

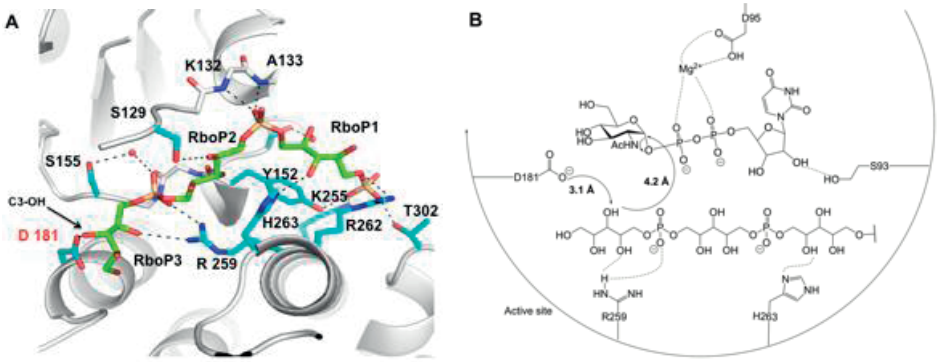


Figure 3. Crystal structure **of** the hexamer (**3**) in the active site of TarP (A), schematic representation of the hexamer in the active site. (B). Dashed bonds represent hydrogen- or ionic bonds.

the MALDI analyses, shown in Fig. 4 indicate different outcomes of the glycosylations using the different enzymes. TarM glycosylation using the high enzyme concentration leads to the formation of products carrying up to 5 GlcNAc-residues (Fig. 4A), while the lower concentration maximally introduces 3 GlcNAc's (Fig. 4B). The use of TarP shows a similar outcome for both concentrations, reaching the maximum of 6-GlcNAc-transfers (Fig 4C, D). At low enzyme concentration, TarS introduces one to five GlcNAc's to the RboP hexamer (Fig 4F), while at higher concentrations more GlcNAc transfer takes place and it appears that a RboP structure is formed that contains 7 GlcNAcs (Fig 4E), indicating that higher concentrations of enzyme may lead to overglycosylation.

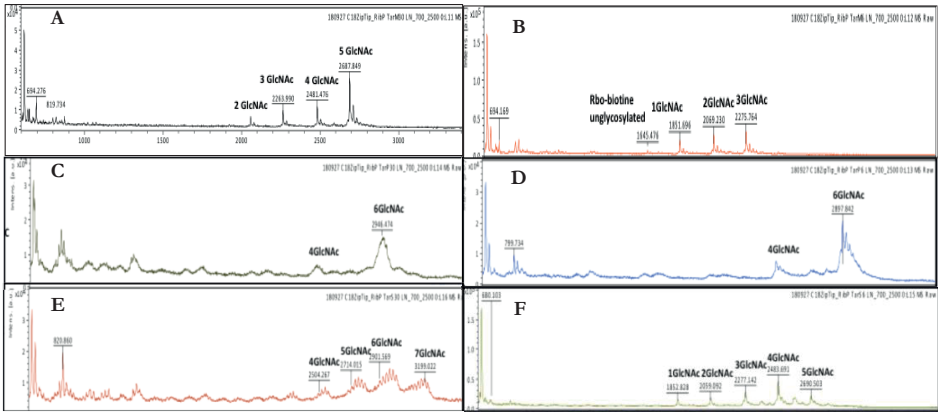


Figure 4. MALDI-MS analysis of enzymatic glycosylations performed on biotinylated hexamer **28** upon 2 different concentrations of enzyme: (A) TarM 30 μg/mL, (B) TarM 6 μg/mL, (C) TarP 30 μg/mL, (D) TarP 6 μg/mL, (E) TarS 30 μg/mL and (F) TarS 6 μg/mL.

Having established that the three transferases are capable of glycosylating the biotin-RboP-hexamers, a reaction on 0.5 mg scale using the TarS enzyme was performed using RboP hexamer **3** as a substrate. This chemoenzymatic glycosylation strategy can open a door towards the efficient assembly of fully glycosylated RboP fragments, without the need for glycosylated RboP phosphoramidite building blocks, which are more difficult to synthesize (as discussed in Chapter 3). As the use of 6 μg/mL of TarS gave incomplete GlcNAc transfer, TarS was used at a concentration of 15 μg/mL and 30 μg/mL to glycosylate **3** on 0.5 mg scale. Glycosylating **3** with 10 mM UDP-GlcNAc for 6 hours, gave after purification by HW-40 size exclusion chromatography 0.65 mg (82%) product for the reaction run with 15 μg/ml and 0.75 mg (93%) product for the reaction using of 30 μg/mL TarS. Figure 5 shows the NMR spectra of the generated glycosylated hexamers indicating the presence of 4 GlcNAc residues per RboP-hexamer chain.

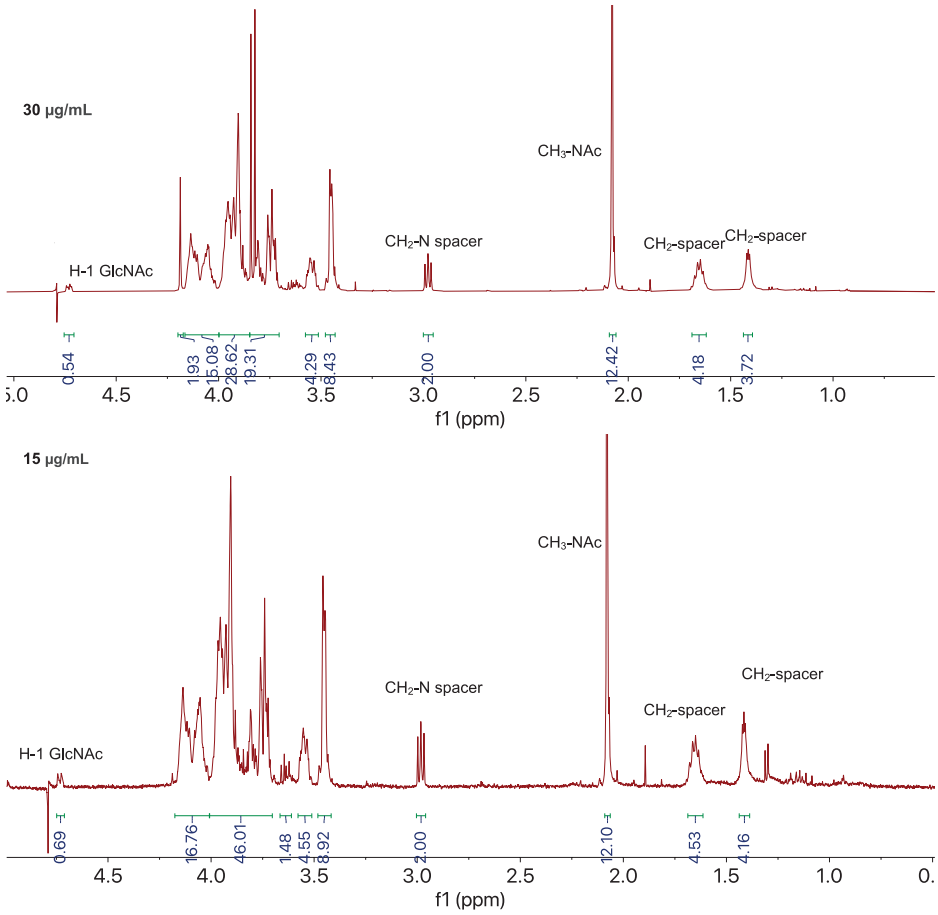


Figure 5. Partial ^{1}H NMR spectra of the 30 μg/mL- and 15 μg/mL TarS glycosylation of compound **3.** All spectra were measured in D_2O on a 500 MHz NMR at 25°C.

Next, the enzymatic glycosylation was applied to obtain hexamers that could be coupled to Streptavidin magnetic beads to probe antibody binding as depicted in Fig 6. First the biotinylated substrate **28** was glycosylated using UDP-GlcNAc and TarS/TarM or TarP for 2h. Streptavidin coated dynabeads M280 were then added to capture the biotin-substrates. The WTA-coated beads were washed with PBS and then used to detect IgG in human serum.

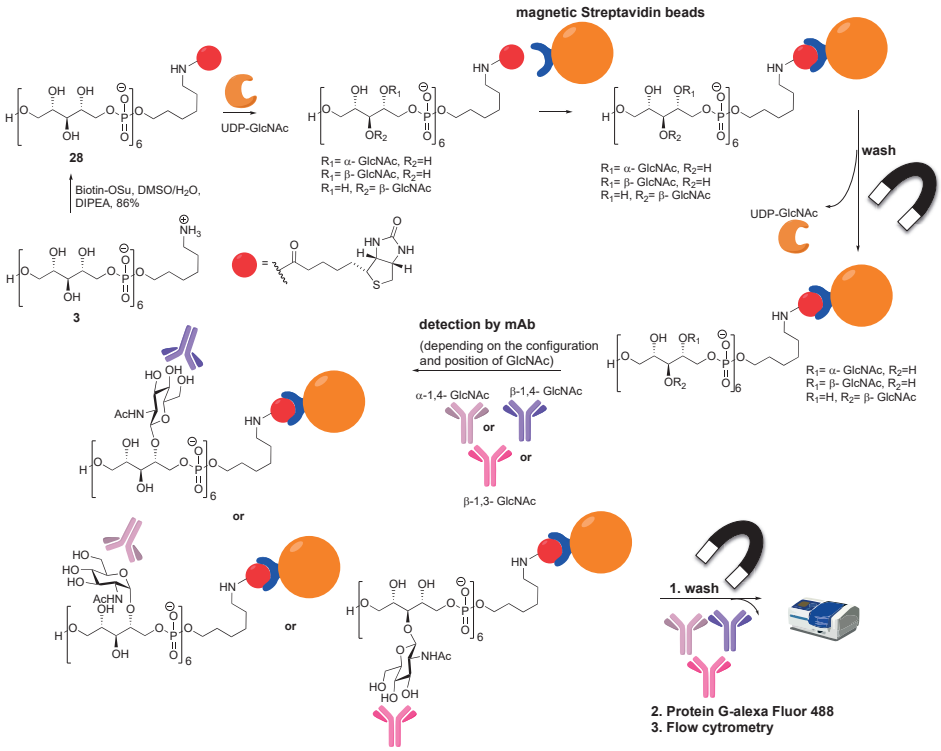


Figure 6. Schematic representation of enzymatic modification on Streptavidin beads. 1) Biotinylation of WTA fragment **3**; 2) Enzymatic glycosylation using UDP-GlcNAc and TarM/TarS or TarP; 3) Adsorption on streptavidin coated M280 Dynabeads; 4) Binding of monoclonal antibodies; 5) Alexa 488-Protein G conjugation; 6) Readout of fluorescent beads.

To validate the WTA-bead model, binding of recombinantly expressed monoclonal antibodies 4497 (an anti β-GlcNAc-RboP Ab) and 4461 (an anti α-GlcNAc-RboP Ab) were screened using the enzymatic modified WTA-beads. These mAbs have previously been shown to bind to GlcNAc-ylated WTA and activate complement leading to efficient uptake[24] of *S. aureus* by phagocytosis. Figure 7A shows specific binding of the anti α-GlcNAc mAbs to the WTA glycosylated by TarM. The WTA fragments glycosylated by the other transferases were not recognized, nor was the "naked" WTA RboP backbone. On the other hand, the anti β-GlcNAc mAb bound both to TarS-WTA and TarP-WTA, indicat-

ing that this antibody is cross-reactive for both β-GlcNAc-WTAs. This antibody did not bind to the backbone or the epimeric GlcNAc-WTA.

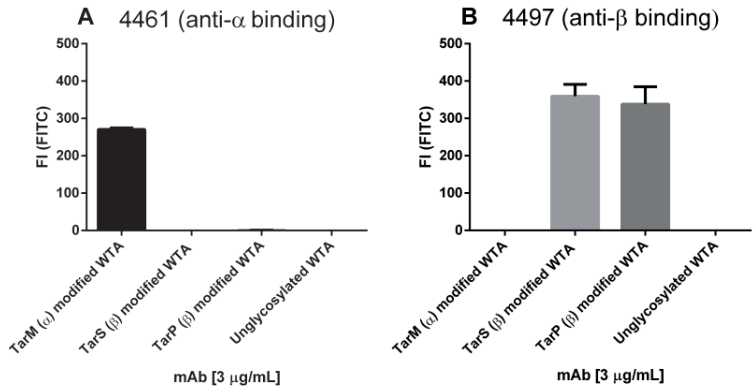


Figure 7. Monoclonal antibody detection by anti-α 1,4-GlcNAc (A) and anti-β 1,4-GlcNAc (B). Data is expressed as mean with standard error of the mean.

Next, the WTA beads were used to detect WTA-specific IgG antibodies in human serum to elucidate which antigens can be detected by antibodies in human serum. Figure 8 shows that TarS-WTA is best recognized by IgG in human serum, while RboP-specific IgG seems not to be present. The levels of IgG reactive towards TarP-WTA were higher than the anti-TarM-WTA IgG levels, but approximately two-fold lower than anti TarS-WTA IgG levels (Fig 7A, B). Whether recognition of the TarP-WTA is due to cross-reactivity of 1,4-β-GlcNAc-WTA antibodies or results from specific 1,3-β-GlcNAc-WTA antibodies remains to be established. This experiment demonstrates that 1,4 β-GlcNAc WTA is the most dominant WTA-antigen of *S. aureus* followed by the regio-isomeric 1,3 β-GlcNAc WTA.

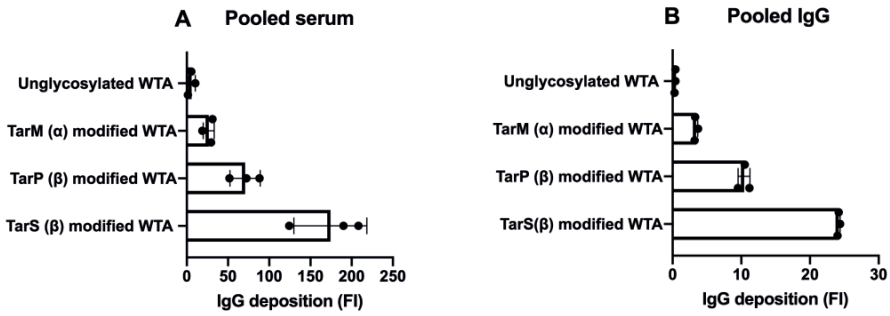


Figure 8. (A) 3% Heat Inactivated pooled human serum (B) 10 mg/mL pooled human IgG. Corrected for background binding to biotin control beads of three independent experiments are shown.

CONCLUSION AND OUTLOOK

This chapter has described the successful synthesis of a set of well-defined WTA ribitol phosphates. Employing two approaches, WTA fragments up to an octamer were synthesized in solution and an octa- and dodecamer-RboP WTA were assembled using an automated solid phase synthesis for the first time. The ASPS approach allows the rapid assembly of WTA fragments and is also suitable for the application of N-acetylglucosamine substituted ribitol phosphoramidites for the generation of a WTA library with a variation of substitution patterns. On the other hand, the solution phase synthesis afforded WTA fragments on multi-milligram scale for biological activity studies. The RboP-hexamer served as substrate for the recently discovered TarP enzyme, aiding in the elucidation of the interaction of the substrate with the enzyme and clarify the function of the probed TarP glycosyltransferase. The hexamer was used as a substrate for enzymatic modifications and the formed glycosylated hexamers was attached to beads and used to detect reactive IgG in human serum. It was found that the β-1,4-GlcNAc epitope on WTA represents the most reactive antigen toward human sera, but also the β-1,3-GlcNAc WTA was found to bind to antibodies. These findings present β-GlcNAc WTAs as promising candidates for vaccine development and proves the relevance of synthetic well-defined α/β-GlcNAc WTAs for immunological evaluation. The WTA bead assay proved to be a valuable tool to probe IgG for binding and with the generation of more TAs, which will be further described in chapter 3 and 4, this model can be included to study the interaction of synthetic WTA with sera or lectins. Finally, the enzymatic glycosylation of the synthetic ribitol phosphate hexamer was established to generate glycosylated WTA-hexamers. Considering the relative ease of this enzymatic glycosylation and the use of readily available building blocks, this method opens the door for a rapid production of glyco-WTAs.

EXPERIMENTAL SECTION

General information

All chemicals (Acros, Fluka, Merck, Sigma-Aldrich, etc.) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040- 0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 25 g/L and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ 10 g/L, in 10% aqueous H_2SO_4 followed by charring at +/- 140°C. Some unsaturated compounds were visualized by spraying with a solution of $KMnO_4$

(2%) and K_2CO_3 (1%) in water. Optical rotation measurements ($[\alpha]_D^{20}$) were performed on an Anton Paar Modular Circular Polarimeter MCP 100/150 with a concentration of 10 mg/mL (*c* 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300. ^1H, ^{13}C and ^{31}P NMR spectra were recorded with a Bruker AV 400 (400, 101 and 162 MHz respectively), a Bruker AV 500 (500 and 202 MHz respectively) or a Bruker DMX 600 (600 and 151 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane, unless stated otherwise. High resolution mass spectra were recorded by direct injection (2 µl of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1 % formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250°C) with resolution R = 60000 at *m/z* 400 (mass range *m/z* = 150-2000) and dioctylphthalate (*m/z* = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

Phosphoramidite coupling, oxidation, and detritylation.

The starting alcohol was co-evaporated 2 times with dry toluene before being dissolved in dry acetonitrile (ACN, 0.15 M). 4,5-dicyanoimidazole (DCI) (1.6-2.4 eq; 0.25 M in ACN) was added and the mixture was stirred over freshly activated molecular sieves under an argon atmosphere for 20 min. Then phosphoramidite (1.3-2.0 eq; 0.20 M) was added and the mixture was stirred at rt until total conversion of the starting material (15 - 45 min). Subsequently, (10-camphorsulfonyl)oxaziridine (CSO) (2.0 eq; 0.5 M in ACN) was added and the stirring was continued for 15 min. The mixture was diluted with DCM and washed with a 1:1 solution of saturated NaCl/$NaHCO_3$. The water layer was extracted 3 times with DCM and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was dissolved in DCM, DCA was added (5 eq; 0.18 M in DCM), and the mixture was stirred at rt. After 40 – 60 min an aqueous solution of methanol (1:1) was added, stirred for an additional 30-40 min and diluted with DCM. The organic layer was washed with saturated NaCl/$NaHCO_3$ solution (1:1), the water layer was extracted 3 times with DCM, and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was further purified by either flash chromatography (DCM/acetone) or size exclusion chromatography (sephadex LH- 20, MeOH/DCM, 1:1).

General procedure for global deprotection

The oligomer was dissolved in a 1:1 solution of NH_3 (30-33% aqueous solution) and dioxane (1.2-2.4 mM) and stirred overnight. The mixture was concentrated *in vacuo* and loaded on a Dowex Na^+ cation-exchange resin (50WX4-200, stored on 0.5 M NaOH, flushed with H_2O and MeOH before use) column and flushed with water/dioxane (1:1).

The fractions were then concentrated *in vacuo*, dissolved in water/dioxane (2 mL per 10 μmol) and 4 drops of glacial AcOH were added. After purging the mixture with argon, Pd black was added (32-59 mg), and the mixture was repurged with N_2. The mixture was stirred under hydrogen atmosphere for 3 - 7 days, filtered over celite, and concentrated *in vacuo*. The crude product was purified by size-exclusion chromatography (Toyopearl HW-40, NH_4OAc buffer)) and the fractions were concentrated. The product was co-evaporated repeatedly with MiliQ water to remove NH_4OAc/ NH_4HCO_3 traces and eluted through a Dowex Na^+ cation-exchange resin column, and lyophilized.

Procedure for large-scale enzymatic glycosylation

Compound **3** was glycosylated with two different concentrations of TarS enzyme (30 μg/mL or 15 μg/mL). Both were incubated for 6h with 10 mM UDP-GlcNAc and 0.5 mg of compound **3** in a total volume of 500 μl. Afterwards, the enzymes were heat killed and the residue was purified by size-exclusion chromatography (HW40, dimensions: 16/60 mm, eluent: 0.15 M NH_4OAc or NH_4HCO_3). After repeated lyophilization, the product was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with MiliQ water and MeOH before use) and lyophilized affording 0.65 mg (82%) of the glycosylated product for the concentration of 15 μg/mL TarS and 0.75 mg (93%) of the glycosylated product for the concentration of 30 μg/mL TarS. Yield is determined based on a MW 2448.12 average of 3.5 GlcNAc.

Procedure for enzymatic glycosylation

Biotinylated RboP hexamer (6RboP-$(CH_2)_6$NH-biotin; 0.17 nM) was enzymatically glycosylated by recombinant TarM, TarS or TarP (6.3 μg/mL) in glycosylation buffer (15 mM HEPES, 20 mM NaCl, 1 mM EGTA, 0.02% Tween 20, 10 mM $MgCl_2$, 0.1% BSA, pH 7.4) with 2 mM UDP-GlcNAc (Merck) as the substrate. After 2 hours incubation at rt, $5x10^7$ pre-washed Dynabeads M280 Streptavidin (Thermo Fisher) or screen MAG beads (Chemicell) were added and incubated for 15 minutes at rt. Control beads were produced by incubation of Dynabeads M280 Streptavidin with 10nM biotin-LPETG. The coated beads were washed three times in PBS using a plate magnet, resuspended in PBS 0.1% BSA and stored at 4°C.

General procedure for automated solid phase synthesis

A small column containing highly cross-linked polystyrene based universal support resin (USP III PS, Glen research) was loaded in an automated synthesizer (Äkta oligopilot plus, GE healthcare). The resin was flushed with a solution of 3% DCA in toluene (15 ml, 3 min) followed by ACN (5 ml, 1 min). A solution of phosphoramidite (0.1M in ACN, 0.5 ml, 2x 30 μmol) and a solution of 5-(Benzylthio)-1*H*-tetrazole (0.3M in ACN, 0.75 ml,

0.2 mmol) were added to the column and the mixture was recycled over the resin for 5 minutes. The resin was flushed with ACN (1 ml, 5x) and a solution of I_2 (0.05M in a mixture of pyridine and H_2O (v/v = 7:1), 2 ml, 1 min) subsequently. The resin was flushed with ACN (1 ml, 5x) and a capping mixture (1/1 mixture of cap A (0.5M Ac_2O in ACN) and cap B (*N*-methylimidazole, 2,6-lutidine, ACN, v/v/v= 1:1:9, 1 ml, 0.2 min) subsequently. The system was flushed with ACN (1 ml, 5x), and a detritylation step was performed using the reaction conditions mentioned before. The molecule was further elongated following the same set of reactions (coupling, oxidation, capping, detritylation). When the desired length was obtained, the column was removed from the system and NH_3 (25% in H_2O, 10 ml) was added and the mixture was rested for 1 hour. The mixture was passed over a filter and the resin was flushed with ACN, H_2O, a mixture of (*t*-BuOH, ACN and H_2O, v/v/v= 1:1:1, 10 ml), ACN and DMF. The combined eluate was concentrated *in vacuo* and the residue was purified using reversed phase HPLC (C4, NH_4OAc). After repeated lyophilization, the product was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with MiliQ water and MeOH before use).

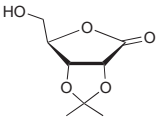
Purification method using anion-exchange chromatography

The semi-protected oligomer was purified using a column. Eluent buffer A: 10 mM NaOAc + 10 mM NaCl, buffer B: 10 mM NaOAc en 1 mM NaCl, lineair gradient 1/0 to 0/1 followed by desalination using size-exclusion chromatography (Sephadex G10/G25), GE healthcare, dimensions: 26/60 mm, eluent: 0.15M NH_4HCO_3. The purified oligomer was lyophilized several times before it was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with MiliQ water and MeOH before use) yielding the semi-protected oligomer.

IgG deposition on WTA beads

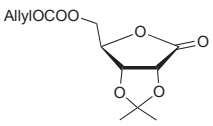
Biotinylated RboP hexamers (0.17 mM) were coated on $5x10^7$ pre-washed Dynabeads M280 Streptavidin (Thermo Fisher) in sterile PBS for 15 minutes at room temperature. The coated beads were washed three times with PBS using a plate magnet, resuspended in PBS 0.1% BSA 0.05% Tween-20 and stored at 4°C. $5x10^5$ beads were incubated with monoclonal antibodies 4461, 4624, 4497 and 6292-Vk3 (0.03-30 µg/ml) for 20 minutes at 4°C in PBS 0.1% BSA 0.05% Tween-20, washed and stained with Protein G-Alexa Fluor 488 (1 µg/ml, Thermo Fisher) for 20 minutes at 4°C. After a final washing cycle, beads were analyzed by flow cytometry on a FACSverse (BD Biosciences). Per sample, 10,000 gated events were collected and data was analyzed using FlowJo 10 (FlowJo, LLC).

2,3-*O*-isopropylidene-D-ribonolactone (8)



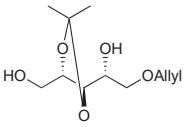
D-(+)-Ribono-1,4-lactone (50.0 g, 337.6 mmol, 1.0 eq.) was dissolved in acetone (2.0 L; 0.17 M). Concentrated HCl (20.0 mL; 1.9 eq.) was added and the reaction mixture was stirred at rt overnight. The reaction was quenched by the addition of solid $NaHCO_3$ until a neutral pH was reached. The mixture was filtered and concentrated under reduced pressure. Then the mixture was diluted in EtOAc and the organic layer was washed with sat. aq. $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Crystallization from acetone/pentane at -30°C afforded title compound **8** (49.0 g, 260 mmol) as white crystals in 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.39 (s, 3H, C*H*$_3$-C$_q$), 1.48 (s, 3H, C*H*$_3$-Cq), 2.77 (t, *J* = 5.6 Hz, 1H, OH), 3.80 (ddd, *J*= 7.2 Hz, 5.6, 1.6, 1H, H-5), 3.99 (ddd, *J* = 7.6, 5.2, 2.0 Hz, 1H, H-5), 4.64 (t, *J* = 2.0 Hz, 1H, H-4), 4.79 (d, *J*= 5.6 Hz, 1H, H-2/H-3), 4.85 (d, *J*=5.2 Hz, 1H, H-2/H-3); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ=25.6 (*C*H$_3$-C$_q$), 26.8 (*C*H$_3$-C$_q$), 62.0 (C-5), 75.8 (C-2/C-3), 78.4 (C-2/C-3), 83.0 (C-4), 113.3 (CH$_3$-*C$_q$*), 175.3 (C=O); HRMS: [M+Na]$^+$ calcd for $C_8H_{12}O_5Na$ 211.0582, found 211.0582.

5-*O*-(Allyloxycarbonyl)-2,3-*O*-isopropylidene-D-ribonolactone (9)



Compound **8** (14.4 g, 76.7 mmol; 1.0 eq.) was dissolved in dry ACN (36.5 mL; 2.1 M) and dry pyridine (12.4 mL; 153 mmol; 2.0 eq.), and the mixture was cooled to 0°C. Allyl chloroformate (16.3 mL; 153 mmol; 2.0 eq.) was dissolved in dry ACN (36.5 mL; 4.2 M) and added dropwise in ±30 minutes. The reaction mixture was stirred for 2 hours and ice was added after full conversion. The mixture was diluted in Et_2O and the organic phase was washed with H_2O (2x) and brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 1:0 to 6:4 pentane/EtOAc) yielded title compound **9** (16.7 g, 61.4 mmol) in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ=1.39 (s, 3H, C*H*$_3$-C$_q$), 1.49 (s, 3H, C*H*$_3$-C$_q$), 4.32 (dd, *J*= 12.0 Hz, 2.0 Hz, 1H, H-5), 4.48 (dd, 1H, *J*= 12.0 Hz, *J*= 2.8 Hz, 1H, H-5), 4.63 (dt, *J* = 6.0, 1.3 Hz, 2H, C*H*$_2$-CH), 4.71 – 4.80 (m, 2H, H-2, H-3), 4.85 (d, *J*= 5.6 Hz, 1H, H-4), 5.18 – 5.47 (m, 2H, C*H2*=CH), 5.92 (ddt, *J*= 17.3, 10.4, 5.9 Hz, 1H, CH2=C*H*); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ=25.6 (*C*H$_3$-C$_q$), 26.8 (*C*H$_3$-C$_q$), 66.7 (C-5), 69.3 (*C*H2-CH), 75.2 (C-4), 77.7 (C-2/C-3), 79.4 (C-2/C-3), 113.8 (CH$_3$-*C$_q$*), 119.8 (*C*H$_2$=CH), 131.0 (CH$_2$=*C*H), 154.1 (C=O), 173.6 (C=O–Alloc); HRMS: [M+Na]$^+$ calcd for $C_{12}H_{16}O_7Na$ 295.0794, found 295.0793.

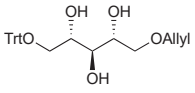
5-*O*-Allyl-2,3-*O*-isopropylidene-D-ribitol (10)



Compound **9** (16.7 g; 61.4 mmol; 1.0 eq.) was co-evaporated with toluene under a N_2 atmosphere and dissolved in freshly distilled dioxane (66.7 mL, 0.92 M). The mixture was degassed with N_2, followed by the addition of $Pd(PPh)_4$ (0.050 g; 0.04 mmol; 0.0007 eq.).

The mixture was degassed with N_2 and the reaction mixture was refluxed for 35 minutes at 110°C. After full conversion the mixture was allowed to cool to rt and concentrated under reduced pressure. The crude compound (13.6 g) was co-evaporated with distilled toluene under N_2 atmosphere and dissolved in dry THF (240 mL; 0.25 M). $NaBH_4$ (5.42 g; 143 mmol; 2.4 eq.) was added and the reaction mixture was heated to 55°C under a continuous N_2 flow. Dry MeOH was added dropwise over ±40 minutes and the reaction mixture was stirred for 1 hour. The mixture was concentrated under reduced pressure and co-evaporated with MeOH (3x). Subsequently, the product was diluted in DCM and the organic phase was washed with 90% sat. aq. NH_4Cl. The water layer was extracted with DCM (2x) and the combined organic layers were dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (100% DCM/MeOH 1:) to DCM/MeOH 94:6) yielded title compound **10** (11.75 g, 50.6 mmol) in 85% yield over 2 steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.34 (s, 3H, CH_3-C_q), 1.40 (s, 3H, CH_3-C_q), 3.51 – 3.55 (m, 1H, H-5), 3.70 – 3.77 (m, 2H, H-1, H-5), 3.84 – 3.89 (m, 1H, H-1), 3.91 – 3.97 (m, 1H, H-4), 4.02 – 4.16 (m, 3H, H-3, CH_2-CH), 4.33 (ddd, *J*= 7.6, 5.0, 3.3 Hz, 1H, H-2), 5.12 – 5.39 (m, 2H, CH_2=CH), 5.92 (ddt, *J*= 17.2, 10.4, 5.7 Hz, 1H, CH_2=C*H*); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 25.3 (*C*H_3-C_q), 27.9 (*C*H_3-C_q), 60.7 (C-1), 68.6 (C-4), 71.7 (C-5), 72.4 (*C*H_2-CH), 76.7 (C-3), 77.4 (C-2), 108.5 (CH_3-*C*$_q$), 117.5 (*C*H_2=CH), 134.3 (CH_2=*C*H); HRMS: $[M+Na]^+$ calcd for $C_{11}H_{20}O_5Na$ 255.1208, found 255.1208.

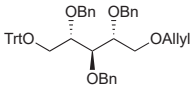
5-*O*-Allyl-1-*O*-trityl-D-ribitol (11)



Compound **10** (11.9 g; 51.3 mmol; 1.0 eq.) was dissolved in a (v/v = 5/2) mixture of AcOH/H_2O (266 mL; 0.19 M) and the reaction mixture was stirred at 50°C for 2 hours. The mixture was concentrated under reduced pressure, co-evaporated with 50 mL toluene (3x) and used without further purification. The crude ribitol was dissolved in pyridine (75 mL; 0.7 M). TrtCl (14.3 g; 51.3 mmol; 1.0 eq.) was added and the reaction was stirred at rt overnight. Then 10 mL MeOH was added and the mixture was concentrated under reduced pressure and co-evaporated with 50 mL toluene (4x). The product was diluted in DCM and the organic phase was washed with sat. aq. $NaHCO_3$ and H_2O. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Column chromatography using TEA neutralized silica (DCM/MeOH 1:0 to 95:5 DCM/MeOH) afforded title compound **11** (22.1 g; 50.9 mmol) in 99% yield over 2 steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.18 - 3.24 (m, 3H, OH), 3.36 (dd, 1H, *J*= 9.6, 5.2 Hz, H-1), 3.47 (dd, 1H, *J*= 9.6 Hz, 4.4 Hz, H-1), 3.54 – 3.66 (m, 2H, H-5), 3.71 (m, 1H, H-3), 3.75 – 3.88 (m, 2H, H-2, H-4), 3.99 (dd, *J*= 5.6, 1.2 Hz, 2H, CH_2-CH), 5.05 – 5.30 (m, 2H, CH_2=CH), 5.84 (ddt, *J*= 17.3, 10.4, 5.7 Hz, 1H, CH_2=C*H*), 7.09 – 7.52 (m, 15H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 65.3 (C-1), 71.3 (C-2/C-4), 71.5 (C-5), 71.8 (C-2/C-4), 72.4 (*C*H_2-CH), 73.4 (C-3), 87.2 (C_q-Trt), 117.7 (*C*H_2=CH), 127.2 – 128.6 (CH-

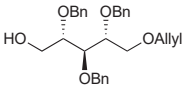
arom), 134.2 (CH_2=*C*H), 143.6 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{27}H_{30}O_5Na$ 457.19855, found 457.19833.

5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-trityl-D-ribitol (12)



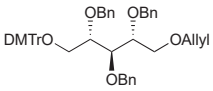
Compound **11** (22.1 g; 50.9 mmol; 1.0 eq.) was dissolved in a (v/v = 1/1) mixture of THF/DMF (150 mL, 0.34 M). The mixture was cooled to 0°C and NaH (8.1 g; 203.6 mmol; 4.0 eq., 60% in mineral oil) was added portion wise. BnBr (24.2 mL; 203.6 mmol; 4.0 eq.) was added dropwise over 30 minutes and the reaction was stirred from 0°C to rt overnight. The mixture was quenched by the addition of 10 mL MeOH at 0°C followed by the addition of 600 mL Et_2O. The organic phase was washed with 400 mL H_2O (5x) and then dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 1:0 to 89:11 pentane/EtOAc) yielded title compound **12** (24.3 g; 34.4 mmol) in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.35 – 3.69 (m, 4H, 2x CH_2-Rbo), 3.86 – 3.94 (m, 5H, H-2, H-3, H-4, CH_2-CH), 4.46 – 4.80 (m, 6H, 3x CH_2-Bn), 5.11 – 5.25 (m, 2H, CH_2=CH), 5.87 (ddt, 1H, *J*= 17.2, 10.7, 5.5 Hz, CH_2=C*H*), 7.08 – 7.46 (m, 30H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 64.1 (C-1/C-5), 70.5 (C-1/C-5), 72.3 - 73.7 (CH_2-CH, 3x CH_2-Bn), 78.8 - 79.1 (C-2, C-3, C-4), 86.8 (Cq-Trt), 116.8 (CH_2=CH), 126.1 – 129.5 (CH-arom), 135.1 (CH_2=*C*H), 138.6 - 144.3 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{48}H_{48}O_5Na$ 727.3399, found 727.3417.

5-*O*-Allyl-2,3,4-tri-*O*-benzyl-D-ribitol (13)



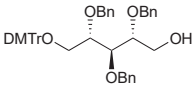
Compound **12** (24.2 g; 34.3 mmol) was dissolved in a (v/v= 9/1) mixture of AcOH/H_2O (428 mL; 0.08 M). The reaction mixture was heated to 80°C and stirred for 2 hours. After full conversion, the mixture was allowed to cool to rt. Subsequently, the mixture was concentrated under reduced pressure and diluted in Et_2O. The organic phase was washed with H_2O (1x), sat. aq. $NaHCO_3$ (2x) and brine (1x). The organic layer was dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 1:0 to 7:3 pentane/EtOAc) yielded title compound **13** (11.3 g, 24.5 mmol) in 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.40 (s, 1H, OH), 3.59 – 3.69 (m, 2H, CH_2-Rbo), 3.71 – 3.77 (m, 3H, CH_2-OH, H-2), 3.86 (td, 1H, *J*= 5.1, 3.7 Hz, H-4), 3.90 – 4.01 (m, 3H, CH_2-CH, H-3), 4.40 – 4.88 (m, 6H, 3x CH_2-Bn), 5.04 – 5.39 (m, 2H, CH_2=CH), 5.88 (ddt, 1H, *J*= 17.2, 10.7, 5.5 Hz, CH_2=C*H*), 7.06 – 7.55 (m, 15H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 61.4 (C-1), 69.7 (C-5), 71.9 (CH_2-Bn), 72.3 (CH_2-CH), 72.5 (CH_2-Bn), 74.0 (CH_2-Bn), 78.2 (C-4), 78.9 (C-2/C-3), 79.0 (C-2/C-3), 117.0 (CH_2=CH), 127.8 – 128.5 (CH-arom), 134.8 (CH_2=*C*H), 138.2 – 138.3 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{29}H_{34}O_5Na$ 485.2304, found 485.2309.

5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol (14)



Compound **13** (4.6 g, 10.0 mmol; 1.0 eq.) was co-evaporated with toluene under a N_2 atmosphere and dissolved in dry DCM (100 mL; 0.1 M). The mixture was cooled to 0°C. TEA (2.1 mL; 15 mmol; 1.5 eq.) and DMTrCl (4.1 g; 12 mmol; 1.2 eq.) were added and the reaction mixture was stirred from 0°C to rt overnight. MeOH was added and the mixture was diluted in DCM. The organic phase was washed with sat. aq. $NaHCO_3$:brine v/v= 1:1). The water layer was extracted with DCM (3x), and the combined organic layers were dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. Column chromatography using TEA neutralized silica (pentane/EtOAc to 1:0 to 89:11 pentane/EtOAc) yielded title compound **14** (7.65 g; 10.0 mmol) in quantitative yield. $[\alpha]_D^{25}$ = +11.2 (*c* 1.0, DCM); IR (neat, cm-^{1}): 3032, 2932, 1608, 1508, 1455, 1302, 1250, 1176, 1093, 1034, 830, 737, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 3.29 – 3.45 (m, 2H, C*H*$_2$-OAllyl), 3.58 – 3.76 (m, 8H, DMTrO-C*H*$_2$, 2x C*H*$_3$-O), 3.82 – 3.91 (m, 1H, H-2), 3.92 – 3.98 (m, 4H, H-3, H-4, C*H*$_2$-CH), 4.41 – 4.86 (m, 6H, 3x C*H*$_2$-Bn), 5.08 – 5.37 (m, 2H, C*H*$_2$=CH), 5.86 – 5.99 (m, 1H, CH_2=C*H*), 6.60 – 7.67 (m, 28H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 55.9 (2x *C*H$_3$O), 64.8 (C-5), 71.0 (C-1), 72.7 (*C*H$_2$-CH), 73.0 – 74.3 (3x *C*H$_2$-Bn), 79.6 – 79.9 (C-2, C-3, C-4), 86.9 (Cq-DMTr), 114.0 (CH-arom), 116.8 (*C*H$_2$=CH), 127.7 – 131.1 (CH-arom), 136.4 (CH_2=*C*H), 137.2 (Cq-arom), 137.2 (Cq-arom), 139.7 – 140.0 (Cq, arom), 146.5, 159.6 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{50}H_{52}O_7Na$ 787.3611, found 787.3634.

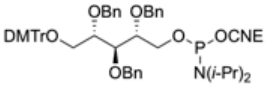
2,3,4-tri-*O*-benzyl-1-*O*-(4,4'-dimetoxytrityl)-D-ribitol (15)



Compound **14** (4.56 g; 5.96 mmol; 1.0 eq.) was dissolved in THF (30.0 mL; 0.20 M) and the solution was degassed with argon. Ir(COD)$(Ph_2MeP)_2PF_6$ (50 mg; 1 mol%) was added and the solution was degassed with argon. Then the red solution was purged with H_2 untill the color became yellow (~7 seconds) and hereafter the solution was degassed with argon to remove traces of H_2 from the solution and the reaction was stirred under argon atmosphere until the isomerization was complete according to TLC analysis. Then the solution was diluted with THF (30.0 mL) and aq. sat. $NaHCO_3$ (30.0 mL) followed by the addition of I_2 (2.27 g; 8.94 mmol; 1.5 eq.). The mixture was stirred +/- 30 minutes and was then quenched by the addition of sat. aq. $Na_2S_2O_3$. The mixture was diluted with EtOAc and washed with aq. sat. NaCl/$NaHCO_3$ (v/v= 1/1). Column chromatography using TEA neutralized silica (pentane: EtOAc 1:0 to 6:4 pentane/EtOAc) yielded title compound **15** in 79% yield (3.42 g; 4.72 mmol). $[\alpha]_D^{25}$= +16.2 (*c* 1.0, DCM); IR (neat, cm^{-1}): 3032, 2932, 2358, 1608, 1508, 1455, 1302, 1250, 1176, 1089, 1033, 829, 737, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 2.78 - 2.80 (m, 1H, O-H), 3.27 - 3.34 (m, 2H, CH_2-Rbo), 3.62 - 3.68 (m, 2H, H-3 CH-Rbo, C*H*H-OH), 3.72 - 3.79 (m, 7H, CH*H*-OH, 2 x OC*H*$_3$), 3.89 - 3.96 (m, 2H, H-2 CH-Rbo, H-4 CH-Rbo), 4.47 (d, 1H, *J*= 11.6 Hz, CH_2-Bn), 4.54 (d, 1H, *J*= 11.2 Hz, CH_2 Bn), 4.62 (d, 1H, *J*= 12.0 Hz, CH_2

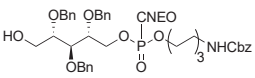
Bn), 4.67 (d, 1H, *J*= 11.6 Hz, CH_2-Bn), 4.75 (d, 1H, *J*= 11.6 Hz, CH_2 Bn), 6.77 (dd, 4H, *J*= 9.2 Hz, 2.8 Hz, H-arom), 7.15 - 7.34 (m, 24H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 55.8 (CH_3O), 61.8 (CH_2-OH), 64.7 (CH_2-Rbo), 72.6, 73.3, 74.3 (CH_2-Bn), 79.7, 79.8 (CH-Rbo), 80.8 (C-3 Rbo), 86.8 (Cq-DMTr), 113.9 (CH-arom), 127.6, 128.3, 128.4, 128.7, 128.8, 129.0, 129.2, 129.2, 129.3, 131.0, 131.0 (CH-arom), 137.1, 137.1, 139.6, 139.8, 139.9, 146.4, 159.5 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{47}H_{48}O_7Na$ 747.3298, found 747.3308.

2-Cyanoethyl [2,3,4-tri-*O*-benzyl-5-*O*-(4,4'-dimethoxytrityl)-1-D-ribityl] *N,N*-diisopropylphosphoramidite (6)



Compound **15** (1.77 g; 2.44 mmol; 1.0 eq.) was co evaporated with toluene twice under a N_2 atmosphere and was then dissolved in DCM (24 mL; 0.1 M), DIPEA was added (0.64 mL; 1.5 eq.) and the mixture was stirred over activated molecular sieves for +/- 20 minuts. 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.65 mL; 1.2 eq) was added and the mixture was stirred until TLC showed complete conversion of the starting material. The reaction was then quenched with a few drops of water and diluted with DCM. The organic layer was washed with sat. aq. $NaHCO_3$/NaCl (v/v= 1:1). The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Column chromatography using TEA neutralized silica (pentane/EtOAc 1:0 to 8:2 pentane/EtOAc) afforded phosphoramidite **6** in 79% yield (1.79 g; 1.94 mmol). ^{1}H NMR (400 MHz, CD_3CN) δ= 1.12 - 1.22 (m, 12H, 4x CH_3-isopropylamine), 2.50 - 2.59 (m, 2H, CH_2-cyanoethyl), 3.28 - 3.35 (m, 2H, CH_2-Rbo), 3.58 - 3.69 (m, 2H, CH-isopropylamine), 3.72 - 4.16 (13H, 3x CH-Rbo, CH_2-Rbo, 2x CH_3O, CH_2 cyanoethyl), 4.49 (d, 1H, *J*= 11.6 Hz, CH_2-Bn), 4.56 (dd, 1H, *J*= 10.8 Hz, *J*= 4.0 Hz, CH_2-Bn), 4.58 - 4.75 (m, 4H, CH_2-Bn), 6.77 - 6.79 (m, 4H, H-arom), 7.16 - 7.46 (m, 24H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 21.0, 21.0 (CH_2 cyanoethyl), 24.9, 25.0, 25.0, 25.1 (CH_3 isopropylamine), 43.7, 43.8, 43.9, 43.9 (CH isopropylamine), 55.8 (CH_3O), 59.2, 59.3, 59.4, 59.5 (CH_2-cyanoethyl), 63.7, 63.9, 64.8, 64.8 (CH_2-Rbo), 73.0, 73.3, 74.2, 74.2 (CH_2-Bn), 79.6, 79.7, 79.8, 80.0, 80.1, 80.1 (CH-Rbo), 86.8 (Cq-DMTr), 113.8 (CH-arom), 127.6, 127.7, 128.3, 128.4, 128.6, 128.6, 128.7, 128.8, 128.8, 129.0, 129.1, 129.2, 129.3, 130.0, 131.0, 131.0 (CH-arom), 137.1, 137.1, 139.6, 139.7, 139.8, 139.9, 146.4, 159.5 (Cq-arom); ^{31}P NMR (162 MHz, CD_3CN) δ= 148.9, 149.0.

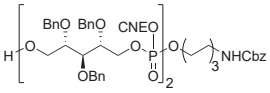
D-ribitol phosphate monomer (17)



According to the general procedure described above, alcohol **15** (0.523 g; 0.721 mmol; 1.0 eq.) was coupled with phosphoramidite **16** (0.423 g; 0.937 mmol; 1.3 eq.) yielding the title compound **17** in 85% yield (0.486 g; 0.616 mmol). IR (neat, cm-^{1}): 3426, 2936, 2866, 1709, 1528, 1454, 1256, 1009, 1028, 739, 698. ^{1}H NMR (400 MHz, CD_3CN) δ= 1.21 -1.32 (m, 4H, CH_2-hexylspacer), 1.35 - 1.45 (m, 2H, CH_2-hexylspacer), 1.56 - 1.61 (m, 2H,

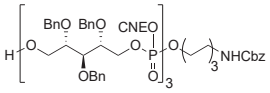
CH_2-hexylspacer), 2.67 - 2.70 (m, 2H, CH_2 cyanoethyl), 3.06 (q, 2H, *J*= 6.8 Hz, CH_2-N hexylspacer), 3.69 (dd, 1H, *J*= 10.8 Hz, *J*= 6.4 Hz, C*H*H-Rbo), 3.75 (q, 1H, *J*= 4.4 Hz, CH-Rbo), 3.79 (dd, 1H, *J*= 10.8 Hz, *J*= 3.6 Hz, CH*H*-Rbo), 3.92 (t, 1H, *J*= 4.8 Hz, CH-Rbo), 3.95 - 4.01 (m, 3H, CH-Rbo, CH_2-O hexylspacer), 4.04 - 4.12 (m, 2H, CH_2 cyanoethyl), 4.19 - 4.25 (m, 1H, C*H*H-Rbo), 4.35 - 4.41 (m, 1H, CH*H*-Rbo), 4.60 - 4.73 (m, 6H, CH_2-Bn), 5.04 (s, 2H, CH_2-Cbz), 5.71 (bs, 1H, N-H), 7.27 - 7.39 (m, 20H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.2, 20.2, 20.2, 20.3 (CH_2 cyanoethyl), 25.7, 26.8, 30.4, 30.8, 30.8 (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 61.6 (CH_2-Rbo), 63.1, 63.1 (CH_2 cyanoethyl), 66.6 (CH_2 Cbz), 68.1, 68.1 (CH_2 Rbo), 68.9, 69.0 (CH_2-O hexylspacer), 72.8, 72.9, 74.5 (CH_2 Bn), 78.9, 79.0, 79.1, 79.1, 79.2, 80.6 (CH-Rbo), 118.3 (Cq-cyanoethyl), 128.5, 128.6, 128.6, 128.6, 128.6, 128.8, 128.8, 128.9, 128.9, 129.3, 129.4 (CH-arom), 139.4, 139.6, 139.8 (Cq-arom), 157.4 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= -0.2, -0.2; HRMS: $[M+H]^+$ calculated for $C_{43}H_{54}N_2O_{10}P$ 789.3516, found 789.3527.

D-ribitol phosphate dimer (18)



According to the general procedure described above, alcohol **17** (0.397 g; 0.503 mmol; 1.0 eq.) was coupled with phosphoramidite **6** (0.605 g; 0.654 mmol; 1.3 eq) yielding the title compound **18** in 74% yield (0.494 g; 0.374 mmol). IR (neat, cm^{-1}): 3422, 2941, 1717, 1701, 1522, 1456, 1258, 1028, 1007, 739, 698. ^{1}H NMR (400 MHz, CD_3CN) δ= 1.21 - 1.27 (m, 4H, CH_2-hexylspacer), 1.40 - 1.43 (m, 2H, CH_2-hexylspacer), 1.56 - 1.61 (m, 2H, CH_2-hexylspacer), 2.55 - 2.61 (m, 2H, CH_2 cyanoethyl), 2.63 - 2.70 (m, 2H, CH_2 cyanoethyl), 3.06 (q, 2H, *J*= 6.4 Hz, CH_2-N hexylspacer), 3.65 - 3.80 (m, 3H, CH-Rbo, CH_2-Rbo), 3.87 - 4.13 (m, 12H, 6 x CH-Rbo, 2x CH_2 cyanoethyl, CH_2-O hexylspacer), 4.17 - 4.43 (m, 6H, 3x CH_2-Rbo), 4.55 - 4.70 (m, 12H, 6x CH_2-Bn), 5.05 (s, 2H, CH_2-Cbz), 5.73 (bs, 1H, N-H), 7.26 - 7.36 (m, 35H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.0, 20.1, 20.1, 20.2, 20.2, 20.2 (CH_2 cyanoethyl), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2 hexylspacer), 41.4 (CH_2-N hexylspacer), 61.5 (CH_2-Rbo), 63.1, 63.1, 63.1, 63.2 (CH_2 cyanoethyl), 66.6 (CH_2-Cbz), 67.5, 67.7, 68.2, 68.3, 68.3 (CH_2-Rbo), 68.9, 69.0 (CH_2-O hexylspacer), 72.7, 72.9, 73.0, 73.0, 73.1, 73.1, 74.5 (CH_2-Bn), 78.3, 78.6, 78.8, 78.9, 79.0, 79.0, 79.1, 79.1, 80.5, 80.6 (CH-Rbo), 118.3, 118.5 (Cq-cyanoethyl), 128.4, 128.5, 128.6, 128.6, 128.7, 128.8, 128.9, 128.9, 129.3, 129.3, 129.4 (CH-arom), 138.5, 139.1, 139.2, 139.3, 139.5, 139.7 (Cq-arom), 157.3 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.2, -0.0, -0.2, -0.2; HRMS: $[M+H]^+$ calculated for $C_{72}H_{86}N_3O_{17}P_2$ 1326.5432, found 1326.5441.

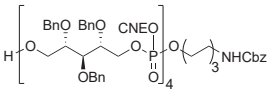
D-ribitol phosphate trimer (19)



According to the general procedure described above, alcohol **18** (0.432 g; 0.326 mmol; 1.0 eq.) was coupled with phosphoramidite **6** (0.392 g; 0.424 mmol; 1.3 eq.) yielding

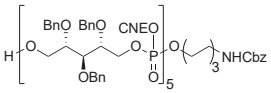
the title compound **19** in 88% yield (0.532 g; 0.285 mmol). IR (neat, cm-[1]): 3412, 2936, 2866, 1717, 1520, 1456, 1260, 1028, 1011, 743, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.27 (m, 4H, CH_2-hexylspacer), 1.40 - 1.42 (m, 2H, CH_2-hexylspacer), 1.56 - 1.61 (m, 2H, CH_2-hexylspacer), 2.53 - 2.59 (m, 4H, CH_2 cyanoethyl), 2.63 - 2.68 (m, 2H, CH_2 cyanoethyl), 3.06 (q, 1H, *J*= 6.4 Hz, CH_2-N hexylspacer), 3.67 - 3.78 (m, 3H, CH-Rbo, CH_2-Rbo), 3.84 - 4.10 (m, 17H, 9x CH-Rbo, 3x CH_2 cyanoethyl, CH_2-O hexylspacer), 4.20 - 4.39 (m, 10H, 5x CH_2-Rbo), 4.53 - 4.59 (m, 18H, 9x CH_2-Bn), 5.04 (s, 2H, CH_2-Cbz), 5.72 (bs, 1H, N-H), 7.25 - 7.35 (m, 50H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1, 20.1, 20.2, 20.2 (CH_2 cyanoethyl), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2 hexylspacer), 41.4 (CH_2-N hexylspacer), 61.5 (CH_2-Rbo), 63.1, 63.1, 63.2, 63.2 (CH_2 cyanoethyl), 66.6 (CH_2 Cbz), 67.5, 67.7, 67.7, 67.8, 68.2 (CH_2-Rbo), 68.9, 69.0 (CH_2-O hexylspacer), 72.7, 72.9, 73.0, 73.0, 73.1, 74.5, 74.5, 74.6 (CH_2 Bn), 78.3, 78.6, 78.8, 78.9, 79.0, 79.1, 80.5 (CH-Rbo), 118.3 -118.5 (Cq-cyanoethyl), 128.4, 128.5, 128.6, 128.7, 128.7, 128.8, 128.9, 128.9, 129.3, 129.3, 129.4 (CH-arom), 139.1, 139.3, 139.5, 139.7 (Cq-arom), 157.1; ^{31}P NMR (162 MHz, CD_3CN) δ= 0.2, 0.2, -0.0, -0.1, -0.1, -0.2, -0.2; HRMS: $[M+Na]^+$ calculated for $C_{101}H_{117}N_4O_{24}NaP_3$ 1885,7168, found 1885.7172.

D-ribitol phosphate tetramer (20)



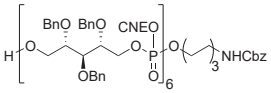
According to the general procedure described above, alcohol **19** (0.508 g; 0.273 mmol; 1.0 eq.) was coupled with phosphoramidite **6** (0.328 g; 0.355 mmol; 1.3 eq.) yielding the title compound **20** in 80% yield (0.522 g; 0.217 mmol). IR (neat, cm-[1]): 3447, 2938, 2866, 1717, 1506, 1456, 1267, 1028, 1009, 746, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.27 (m, 4H, CH_2-hexylspacer), 1.40 - 1.41 (m, 2H, CH_2-hexylspacer), 1.58 - 1.59 (m, 2H, CH_2-hexylspacer), 2.52 - 2.59 (m, 8H, 4x CH_2-cyanoethyl), 3.06 (q, 2H, *J*= 6.4 Hz, CH_2-N hexylspacer), 3.67 - 3.79 (m, 3H, CH-Rbo, CH_2-Rbo), 3.84 - 4.13 (m, 22H, 12x CH-Rbo, CH_2-O hexylspacer, 4x CH_2 cyanoethyl), 4.17 - 4.40 (m, 14H, 7x CH_2-Rbo), 4.50 - 4.69 (m, 24H, 12x CH_2-Bn), 5.05 (s, 2H, CH_2-Cbz), 5.72 (bs, 1H, N-H), 7.25 - 7.35 (m, 65H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1, 20.2, 20.2 (CH_2 cyanoethyl), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2 hexylspacer), 41.4 (CH_2-N hexylspacer), 61.5 (CH_2-Rbo), 63.1, 63.1, 63.2 (CH_2 cyanoethyl), 66.6 (CH_2-Cbz), 67.5, 67.7, 67.8, 68.3 (CH_2-Rbo), 68.9, 69.0 (CH_2-O hexylspacer), 72.7, 73.0, 73.1, 73.1, 74.5, 74.5, 74.6 (CH_2-Bn), 78.3, 78.6, 78.9, 78.9, 79.0, 79.1, 80.6 (CH-Rbo), 118.3 - 118.6 (Cq-cyanoethyl), 128.4, 128.6, 128.6, 128.7, 128.8, 128.9, 128.9, 129.3, 129.3, 129.4 (CH-arom), 139.1, 139.2, 139.3, 139.5, 139.7 (Cq-arom), 158.0 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.2, 0.2, 0.2, -0.0, -0.1, -0.1, -0.2, -0.2; HRMS: $[M+H]^+$ calculated for $C_{101}H_{119}N_4O_{24}P_3$ 2401.9343, found 2401.9241.

D-ribitol phosphate pentamer (21)



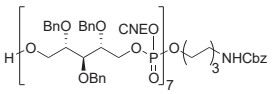
According to the general procedure described above, alcohol **20** (0.147 g; 61.0 μmol; 1.0 eq.) was coupled with phosphoramidite **6** (0.074 g; 80.0 μmol; 1.3 eq.) yielding the title compound **21** in 76% yield (0.136 g; 46.0 μmol). IR (neat, cm-[1]): 3450, 2937, 1717, 1506, 1456, 1271, 1028, 1009, 745, 698. ^{1}H NMR (400 MHz, CD_3CN) δ= 1.27 (m, 4H, CH_2-hexylspacer), 1.40 - 1.41 (m, 2H, CH_2-hexylspacer), 1.56 - 1.59 (m, 2H, CH_2-hexylspacer), 2.54 - 2.59 (m, 8H, 4x CH_2-cyanoethyl), 2.64 - 2.70 (m, 2H, CH_2-cyanoethyl), 3.06 (q, 2H, *J*= 6.4 Hz, CH_2-N hexylspacer), 3.66 - 3.78 (m, 3H, CH-Rbo, CH_2-Rbo), 3.84 - 4.13 (m, 27H, 15x CH-Rbo, CH_2-O hexylspacer, 5x CH_2 cyanoethyl), 4.16 - 4.39 (m, 30H, 15x CH_2-Bn), 5.04 (s, 2H, CH_2-Cbz), 5.7 (bs, 1H, N-H), 7.26 - 7.34 (m, 80H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1, 20.1 (CH_2 cyanoethyl), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2 hexylspacer), 41.4 (CH_2-N hexylspacer), 61.5 (CH_2-Rbo), 63.1, 63.1, 63.2 (CH_2 cyanoethyl), 66.6 (CH_2 Cbz), 67.5, 67.7, 68.3 (CH_2-Rbo), 68.9, 69.0 (CH_2-O hexylspacer), 72.7, 72.9, 73.0, 73.1 74.5, 74.5, 74.6 (CH_2-Bn), 78.3, 78.6, 78.8, 79.1, 80.6 (CH-Rbo), 118.3 - 118.6 (Cq-cyanoethyl), 128.4, 128.6, 128.6, 128.7, 128.8, 128.9, 128.9, 129.3, 129.3, 129.4 (CH-arom), 139.1, 139.2, 139.3, 139.5 (Cq-arom), 157.5 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.2, 0.2, 0.2, -0.1, -0.1, -0.2, -0.2, -0.2; HRMS: $[M+H]^+$ calculated for $C_{159}H_{183}N_6O_{38}P_5$ 2939.1260, found 2939.1348.

D-ribitol phosphate hexamer (22)



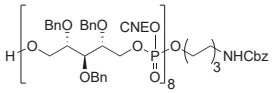
According to the general procedure described above, alcohol **21** (108 mg; 37.0 μmol; 1.0 eq.) was coupled with phosphoramidite **6** (74.0 mg; 80.0 μmol; 2.0 eq.) yielding the title compound **22** in 91% yield (0.117 g; 33.7 μmol). IR (neat, cm-[1]): 3455, 1717, 1506, 1456, 1269, 1028, 737, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.26 - 1.28 (m, 4H, CH_2-hexylspacer), 1.41 (m, 2H, CH_2-hexylspacer), 1.55 - 1.59 (m, 2H, CH_2-hexylspacer), 2.53 - 2.58 (m, 10H, 5x CH_2-cyanoethyl), 2.63 - 2.69 (m, 2H, CH_2-cyanoethyl), 3.05 (q, 2H, *J*= 6.4 Hz, CH_2-N hexylspacer), 3.69 - 3.77 (m, 3H, CH-Rbo, CH_2-Rbo), 3.83 - 4.09 (m, 32H, 18x CH-Rbo, CH_2-O hexylspacer, 6x CH_2 cyanoethyl), 4.16 - 4.32 (m, 22H, 11x CH_2-Rbo), 4.48 - 4.68 (m, 36H, 18x CH_2-Bn), 5.04 (s, 2H, CH_2-Cbz), 5.70 (bs, 1H, N-H), 7.28 - 7.34 (m, 95H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1, 20.1, 20.1, 20.2, 20.2, 20.3 (CH_2 cyanoethyl), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2 hexylspacer), 41.4 (CH_2-N hexylspacer), 61.5 (CH_2-Rbo), 63.1, 63.1, 63.2, 63.2, 63.3 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.7 - 67.9 (CH_2-Rbo), 68.9, 69.0 (CH_2-O hexylspacer), 72.7, 72.9, 73.0, 73.1, 74.5, 74.5, 74.6 (CH_2-Bn), 78.3, 78.6, 78.6, 78.9, 78.9, 80.6 (CH-Rbo), 117.4 - 117.7 (Cq-cyanoethyl), 128.4, 128.4, 128.6, 128.6, 128.7, 128.8, 128.9, 128.9, 129.2, 129.3, 129.3, 129.4 (CH-arom), 139.1, 139.2, 139.2, 139.3 (Cq-arom), 156.0 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.2, 0.2, 0.2, -0.1, -0.1, -0.2, -0.2; HRMS: $[M+2H]^{2+}$ calculated for $C_{188}H_{215}N_7O_{45}P_6$ 1739.1616, found 1739.1575.

D-ribitol phosphate heptamer (23)



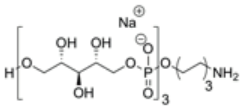
According to the general procedure described above, alcohol **22** (116 mg; 33.4 μmol; 1.0 eq.) was coupled with phosphoramidite **6** (51.0 mg; 55.1 μmol; 1.5 eq.) yielding the title compound **23** in 85% yield (115 mg; 28.7 μmol). IR (neat, cm-^{1}): 3447, 30301717, 1522, 1456, 1267, 1015, 746, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.26 (m, 4H, CH_2-hexylspacer), 1.40 (m, 2H, CH_2-hexylspacer), 1.54 - 1.58 (m, 2H, CH_2-hexylspacer), 2.51 - 2.67 (m, 14H, 7x CH_2-cyanoethyl), 3.04 (q, 2H, *J*= 6.4 Hz, CH_2-N hexylspacer), 3.64 - 3.75 (m, 3H, CH-Rbo, CH_2-Rbo), 3.81 - 4.08 (m, 37H, 21x CH-Rbo, CH_2-O hexylspacer, 7x CH_2 cyanoethyl), 4.13 - 4.36 (m, 26H, 13x CH_2-Rbo), 4.47 - 4.67 (m, 42H, 21x CH_2-Bn), 5.02 (s, 2H, CH_2-Cbz), 5.65 (bs, 1H, N-H), 7.24 - 7.37 (m, 110H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1, 20.2 (CH_2 cyanoethyl), 25.7, 26.8, 30.4, 30.8, (CH_2 hexylspacer), 41.4 (CH_2-N hexylspacer), 61.5 (CH_2-Rbo), 63.2 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.7, 68.3, 68.9, 69.0 (CH_2-Rbo), 72.7, 72.7, 73.1, 73.1, 74.5, 74.6 (CH_2-Bn), 78.3, 78.7, 78.9, 80.6 (CH-Rbo), 118.3 (cq-cyanoethyl), 128.7, 128.8, 128.9, 129.3 (C-arom), 139.1, 139.2 (Cq-arom); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.2, 0.2, 0.1, -0.1, -0.1, -0.2, -0.2, -0.2; HRMS: $[M+2H]^{2+}$ calculated for $C_{217}H_{247}N_8O_{52}P_7$ 2007.7574, found 2007.7588

D-ribitol phosphate octamer (24)



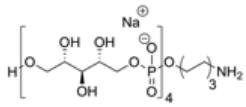
According to the general procedure described above, alcohol **23** (99 mg; 24.7 μmol; 1.0 eq.) was coupled with phosphoramidite **6** (34.2 mg; 37.0 μmol; 1.5 eq.) yielding the title compound **24** in 87% yield (98.0 mg; 21.5 μmol). IR (neat, cm-^{1}): 3447, 2934, 2872, 1717, 1522, 1456, 1271, 1028, 1009, 746, 698. ^{1}H NMR (400 MHz, CD_3CN) δ= 1.25 (m, 4H, CH_2-hexylspacer), 1.39 - 1.40 (m, 2H, CH_2-hexylspacer), 1.54 - 1.57 (m, 2H, CH_2-hexylspacer), 2.50 - 2.68 (m, 16H, 7x CH_2-cyanoethyl), 2.83 (m, 1H, OH), 3.04 (q, 2H, *J*= 6.4 Hz, CH_2-N hexylspacer), 3.67 - 3.85 (m, 3H, 3H, CH-Rbo, CH_2-Rbo), 3.81 - 4.09 (m, 42H, 24x CH-Rbo, CH_2-O hexylspacer, 8x CH_2 cyanoethyl), 4.11 - 4.36 (m, 30H, 15x CH_2-Rbo), 4.46 - 4.67 (m, 48H, 24x CH_2-Bn), 5.02 (s, 2H, CH_2-Cbz), 5.65 (bs, 1H, N-H), 7.19 - 7.33 (m, 125H, H-arom); ^{13}C-APT NMR (126 MHz, CD_3CN) δ= 20.1, 20.2, 20.2 (CH_2 cyanoethyl), 25.7, 26.8, 30.4, 30.8, (CH_2 hexylspacer), 41.4 (CH_2-N hexylspacer), 61.5 (CH_2-Rbo), 63.1, 63.1, 63.2, 63.2, 63.3 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.7, 67.7, 68.9, 69.0 (CH_2-Rbo), 72.8, 73.0, 73.0, 73.1, 73.1, 74.5, 74.5, 74.6 (CH_2-Bn), 78.3, 78.3, 78.6, 78.7, 78.7, 78.9, 78.9, 80.6, 80.6 (CH-Rbo), 118.3, 118.5, 118.5 (Cq-cyanoethyl), 128.5, 128.6, 128.6, 128.8, 128.8, 128.9, 129.0, 129.3, 129.4 (C-arom), 139.1, 139.2 (Cq-arom); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.2, 0.2, 0.1, -0.1, -0.1, -0.1, -0.2, -0.2, -0.2; HRMS: $[M+2H]^{2+}$ calculated for $C_{246}H_{279}N_9O_{59}P_8$ 2276.3533, found 2276.3547.

Deprotected trimer (1)



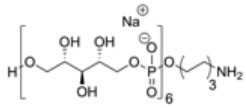
According to the general procedure described above, trimer **19** (57.0 mg; 30.6 μmol) was deprotected affording **1** in 75% yield (19.0 mg; 23.0 μmol). ^{1}H NMR (500 MHz, D_2O) δ= 1.41 - 1.46 (m, 4H, 2x CH_2 hexylspacer), 1.62 - 1.70 (m, 4H, 2x CH_2 hexylspacer), 2.99 (t, 2H, *J*= 7.5 Hz, CH_2-N hexylspacer), 3.63 (dd, 1H, *J*= 11.5 Hz, *J*= 7.0 Hz, CH_2-ribitol), 3.74 (t, 1H, *J*= 6.0 Hz, CH-ribitol), 3.78 - 3.94 (m, 17H, CH/CH_2-ribitol, CH_2-O hexylspacer), 4.02 - 4.09 (m, 5H, CH_2-ribitol); ^{13}C-APT NMR (126 MHz, D_2O) δ= 24.5, 25.1, 26.6, (3x CH_2-hexylspacer), 29.4 (d, *J*= 7.6 Hz, CH_2-hexylspacer), 39.5 (CH_2-N hexylspacer), 62.3 (CH_2-ribitol), 66.1 - 66.5 (5x CH_2-ribitol/CH_2-O hexylspacer), 70.8 - 72.1 (8x CH-ribitol); 31P NMR (202 MHz, D_2O) δ= 2.0, 1.8; HRMS: $[M+H]^+$ calculated for $C_{21}H_{49}NO_{22}P_3$ 760.1959, found 760.1958.

Deprotected tetramer (2)



According to the general procedure described above, tetramer **20** (69.0 mg; 28.8 μmol) was deprotected affording **2** in 84% yield (28.6 mg; 23.9 μmol). ^{1}H NMR (600 MHz, D_2O) δ= 1.39 - 1.40 (m, 4H, 2x CH_2 hexylspacer), 1.55 - 1.68 (m, 4H, 2x CH_2-hexylspacer), 2.97 (t, 2H, *J*= 7.2 Hz, CH_2-N hexylspacer), 3.62 (dd, 1H, *J*= 12.0 Hz, *J*= 7.2 Hz, CH_2-ribitol), 3.72 (t, 1H, *J*= 6.6 Hz, CH-ribitol), 3.76 - 3.82 (m, 4H, CH/CH_2-ribitol), 3.82 - 3.84 (m, 1H, CH/CH_2-ribitol), 3.85 - 3.96 (m, 16H, 14 CH/CH_2-ribitol, CH_2-O hexylspacer), 3.97 - 4.06 (m, 7H, CH/CH_2-ribitol); ^{13}C-APT NMR (151 MHz, D_2O) δ= 25.4, 26.0, 27.5 (3x CH_2-hexylspacer), 30.3 (d, *J*= 7.6 Hz, CH_2-hexylspacer), 40.3 (CH_2-N hexylspacer), 63.2 (CH_2-ribitol), 67.0 - 67.4 (7x CH_2-ribitol/CH_2-O hexylspacer), 71.7 - 73.0 (10x CH-ribitol); ^{31}P NMR (162 MHz, D_2O) δ= 1.8, 1.6; HRMS: $[M+H]^+$ calculated for $C_{26}H_{60}NO_{29}P_4$ 974.2201, found 974.2202.

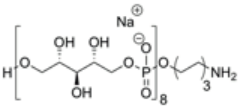
Deprotected hexamer (3)



According to the general procedure described above, hexamer **22** (53.0 mg; 16.8 μmol) was deprotected affording the target compound **3** in 87% yield (22.5 mg; 14.7 μmol). ^{1}H NMR (600 MHz, D_2O) δ= 1.40 - 1.41 (m, 4H, CH_2-hexylspacer), 1.62 - 1.67 (m, 4H, CH_2-hexylspacer), 2.98 (t, 2H, *J*= 7.2 Hz, CH_2-N hexylspacer), 3.62 (dd, 1H, *J*= 12.0 Hz, *J*= 7.2 Hz, CH_2-ribitol), 3.73 (t, 1H, *J*= 6.0 Hz, CH-ribitol), 3.77 - 3.90 (m, 7H, CH/CH_2-ribitol, CH_2-O hexylspacer), 3.90 - 4.01 (m, 22H, CH/CH_2-ribitol), 4.02 - 4.07 (m, 11H, CH/CH_2-ribitol); ^{13}C-APT NMR (151 MHz, D_2O) δ= 25.4, 26.0, 27.5 (3x CH_2-hexylspacer), 30.3 (d, *J*= 7.6 Hz, CH_2- hexylspacer), 40.3 (CH_2-N hexylspacer), 63.2 (CH_2-ribitol), 67.0 - 67.4 (5x CH_2 ribitol/CH_2-O hexylspacer), 71.7 - 73.0 (8x CH-ribitol); ^{31}P NMR (162 MHz,

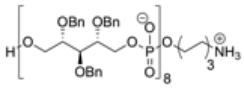
D_2O) δ= 1.8, 1.8, 1.6; MALDI-FT-ICR MS (m/z): $[M+Na]^+$ calculated for $C_{36}H_{75}NNa_7O_{43}P_6$ 1556.1417, found 1556.1335.

Deprotected octamer (4)



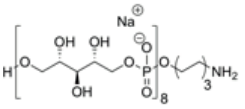
According to the general procedure described above, octamer **24** (40.0 mg; 8.79 μmol) was deprotected affording the target compound **4** in 89% yield (16.5 mg; 7.79 μmol). ^{1}H NMR (400 MHz, D_2O) δ= 1.41 (m, 4H, CH_2-hexylspacer), 1.64 (m, 4H, CH_2-hexylspacer), 2.96 (t, 2H, *J*= 7.2 Hz, CH_2-N hexylspacer), 3.63 (dd, 1H, *J*= 12.0 Hz, 7.2 Hz, C*H*H), 3.75 (t, 1H, *J*= 6.0 Hz, CH-ribitol), 3.78 - 4.03 (m, 56H, CH*H,* 23x CH-ribitol, 15x CH_2-Rbo, CH_2-O hexylspacer); ^{31}P NMR (162 MHz, D_2O) δ= 1.8, 1.8, 1.8, 1.6; HRMS: $[M+2H]^{2+}$ calculated for $C_{46}H_{105}NO_{57}P_8$ 915.66192, found 915.66135.

Semi protected octamer (26)



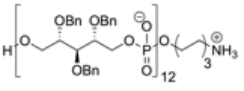
According to the general procedure described above for solid phase synthesis semi protected **26** was obtained in 15% yield (6.1 mg; 1.46 μmol). ^{1}H NMR (400 MHz, MeOD) δ= 1.16 - 1.22 (m, 4H, CH_2-hexylspacer), 1.41 (m, 4H, CH_2-hexylspacer), 2.70 (t, 2H, *J*= 7.2 Hz, CH_2-N hexylspacer), 3.64 - 4.29 (m, 58H, CH-Rbo, CH_2-Rbo, CH_2-O hexylspacer), 4.41 (m, 48H, CH_2-Bn), 7.10 - 7.32 (m, 120H, H-arom); ^{31}P NMR (162 MHz, MeOD) δ= 1.5, 1.3, 1.2, 1.0; HRMS: $[M+2H]^{2+}$ calculated for $C_{214}H_{249}NO_{57}P_8$ 1997.22865, found 1997.23325.

Deprotected octamer (4)



According to the general procedure described above for deprotection, compound **26** (6.1 mg; 1.46 μmol) was deprotected yielding octamer **4** in quantitative yield (3.5 mg; 1.74 μmol). ^{1}H NMR (500 MHz, D_2O) δ= 1.42 (m, 4H, CH_2-hexylspacer), 1.60 - 1.65 (m, 4H, CH_2-hexylspacer), 2.99 (t, 2H, *J*= 7.0 Hz, CH_2-N hexylspacer), 3.64 (dd, 1H, *J*= 12.0 Hz, 7.0 Hz, C*H*H), 3.73 - 3.79 (m, 1H, CH-ribitol), 3.80 - 3.99 (m, 56H, CH*H,* CH-Rbo, CH_2-Rbo, CH_2-O hexylspacer); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 1.8; HRMS: $[M+2H]^{2+}$ calculated for $C_{46}H_{105}NO_{57}P_8$ 915.66192, found 915.66135.

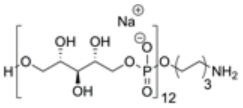
Semi protected dodecamer (27)



According to the general procedure described above for solid phase synthesis, semi protected **27** was obtained in 11% yield (3.4 mg; 1.14 μmol). ^{1}H NMR (400 MHz, MeOD) δ= 1.10 - 1.19 (m, 4H, CH_2-hexylspacer), 1.40 - 1.45 (m, 4H, CH_2-hexylspacer), 2.67 - 2.68 (m, 2H, CH_2-N hexylspacer), 3.64 - 4.25 (m, 86H, CH-Rbo, CH_2-Rbo, CH_2-O hexylspacer), 4.41 - 4.63 (m,

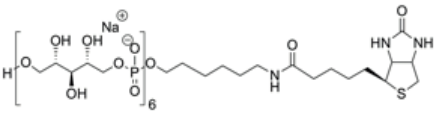
72H, CH_2-Bn), 7.10 - 7.32 (m, 180H, H-arom); ^{31}P NMR (162 MHz, MeOD) δ= 1.3, 1.2, 1.2, 1.1, 1.0.

Deprotected dodecamer (5)



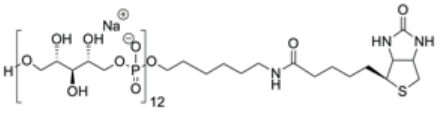
According to the general procedure described above for deprotection, compound **27** (3.36 mg; 0.54 μmol) was deprotected yielding **5** in quantitative yield (1.8 mg; 0.61 μmol). ^{1}H NMR (500 MHz, D_2O) δ= 1.30 - 1.35 (m, 4H, CH_2-hexylspacer), 1.63 - 1.68 (m, 4H, CH_2-hexylspacer), 2.99 (t, 2H, *J*= 7.5 Hz, CH_2-N hexylspacer), 3.63 (dd, 1H, *J*= 12.0 Hz, 7.0 Hz, C*H*H), 3.74 (t, 1H, *J*= 6.0 Hz, CH-ribitol), 3.78 - 3.96 (m, 84H, CH*H,* CH-ribitol, CH_2-Rbo, CH_2-O hexylspacer); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 2.0, 2.0, 1.8, 1.8, 1.6, 1.5; HRMS: $[M+2H]^{2+}$ calculated for $C_{66}H_{149}NO_{85}P_{12}$ 1343.71039, found 1343.71394.

Biotin-(28)



Compound **3** (1.5 mg; 0.98 μmol; 1.0 eq.) was dissolved in DMSO (2.0 mM; 0.50 mL) and water (3.3 mM; 0.30 mL). DIPEA (6 μL) and Biotin-OSu (0.70 mg; 2.1 μmol; 2.1 eq) dissolved in 40 μL DMSO were added and the mixture was shaken overnight at rt. Then 3 drops water were added and the mixture was centrifuged and purified by size exclusion chromatography (HW-40 column, dimensions: 16/60 mm, eluent 0.15M NH_4OAc). After repeated co-evaporation (7-10 x) with miliQ water to remove NH_4OAc, the product was eluted through a small column containing Dowex Na^+ cation-exchange resin (type 50WX8-50-100, stored on 0.5M NaOH in H_2O, flushed with H_2O and MeOH before use). Lyophilization yielded the product (1.5 mg; 0.85 μmol) in 86% yield. ^{1}H NMR (500 MHz, D_2O) δ= 1.36 – 1.41 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.50 – 1.56 (m, 2H, CH_2-hexylspacer/CH_2-biotin), 1.61 – 1.72 (m, 6H, CH_2, CH_2-hexylspacer/CH_2-biotin), 2.24 (t, 2H, *J*= 7.1 Hz, CH_2-C=O), 2.77 (d, 1H, *J*= 13.0 Hz, S-C*H*H), 2.99 (dd, 1H, *J*= 13.1 Hz, *J*= 5.0 Hz, S-CH*H),* 3.17 (hept, 2H, *J*= 6.7 Hz, CH_2-N), 3.33 (dt, 1H, *J*= 9.8 Hz, *J*= 5.1 Hz, S-CH), 3.64 (dd, 1H, *J*= 11.9 Hz, *J*= 7.2 Hz, C*H*H-Rbo), 3.74 (t, 1H, *J*= 6.1 Hz, CH-Rbo), 3.76 – 4.12 (m, 42H, CH-Rbo/CH_2-Rbo/CH_2-O- hexylspacer), 4.42 (dd, 1H, *J*= 7.9 Hz, *J*= 4.5 Hz, S-CH-C*H*), 4.60 (dd, 1H, *J*= 8.2 Hz, *J*= 4.9 Hz, S-CH_2-C*H*); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 1.9, 1.8; HRMS: $[M+2H]^{2+}$ calculated for $C_{46}H_{97}N_3O_{45}P_6S$ 814.67648, found 814.67728.

Biotin-(29)



Compound **5** (4.1 mg; 1.39 μmol) was dissolved in 73 μL H_2O to which was added 36 μL (5.4 μmol) Biotin-Osu (0.15M) and the mixture was shaken overnight at rt. 0.5 mL

was added to the mixture, centrifuged and purified by size exclusion chromatography (HW-40 column, dimensions: 16/60 mm, eluent 0.15M NH_4OAc). After repeated co-evaporation (7-10 x) with miliQ water to remove NH_4OAc, the product was eluted through a small column containing Dowex Na^+ cation-exchange resin (type 50WX8-50-100, stored on 0.5M NaOH in H_2O, flushed with H_2O and MeOH before use). Lyophilization yielded the product in 63% yield (2.79 mg; 0.88 μmol). ^{1}H NMR (400 MHz, D_2O) δ= 1.29 - 1.43 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.45 - 1.75 (m, 8H, CH_2-hexylspacer/CH_2-biotin), 2.23 (t, 2H, *J*= 7.1 Hz, CH_2-C=O), 2.76 (d, *J*= 13.0 Hz, 1H, S-C*H*H), 2.98 (dd, 1H, *J*= 13.1 Hz, 5.0 Hz, S-CH*H*), 3.14 - 3.18 (m, 2H, CH_2-N), 3.31 (dt, 1H, *J*= 9.6, 5.2 Hz, S-CH), 3.59 - 4.11 (m, 86H, CH-Rbo, CH_2-Rbo, CH_2-O- hexylspacer), 4.40 (dd, *J*= 8.0, 4.5 Hz, 1H, S-CH-C*H*), 4.56 - 4.63 (m, 1H, S-CH_2-C*H*); ^{31}P NMR (202 MHz, D_2O) δ= 1.8, 1.8, 1.6.

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