Synthesis of ribitol phosphate based wall teichoic acids

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Synthesis of *Staphylococcus aureus* C-3 glycosylated ribitol phosphates

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a commensal bacterium that colonizes approximately 30% of the human population.[1] It is also a major human pathogen that can cause a wide variety of infections to the skin and respiratory system, as well as endocarditis and post-operative infections.[2] Especially the antibiotic resistant strains, commonly designated MRSA (methicillin-resistant *S. aureus*), are a growing health threat causing 20-25% of all hospital acquired bacterial infections.[3] MRSA was first reported in 1960,[4] however recently[4] it became clear that the first MRSA strains emerged already in the mid-1940s long before the introduction of methicillin in 1959. Resistance against vancomycin, the antibiotic of last resort against multi-drug resistant *S. aureus*, has also emerged in so called VRSA (vancomycin resistant *S. aureus*) strains that have acquired the *vanA* operon from vancomycin resistant enterococci (VRE).[5-6] The continuous development of resistance against antibiotics urges the development of alternative ways to treat infections, for example through passive or active immunization.

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.

The bacterial cell wall of *S. aureus* carries wall teichoic acids (WTAs) that are covalently attached to the peptidoglycan. WTAs are built up from repeating ribitol phosphate (RboP) units that can be decorated with *N*-acetylglucosamine (GlcNAc) through the action of TarS and TarM at the C-4 position in either an α- or β-configuration respectively. In addition, the C-2 can be modified with a D-alanine ester and this latter modification is involved in bacterial resistance to cationic antimicrobial peptides (CAMPs).[7,8,9] These WTA modifications play a crucial role in cell division, phage infectivity and pathogenicity of *S. aureus*.[10,11] Recently, the healthcare-associated MRSA (HA-MRSA) strain, CC5[12] and livestock associated MRSA (LS-MRSA) strains CC398[13] and CC5[14] strains were found to carry an unique C-3 β-GlcNAc modification. These strains were found to express an additional glycosyltransferase TarP, which was shown to be responsible for this C-3 modification.[15]

Because of exposure to bacteria, humans carry protective antibodies against *S. aureus*. Previous studies have shown high levels of antibodies directed to the (1,4)-β-GlcNAc, while the amount of antibodies directed against α-GlcNAc modified WTA was significantly lower.[16] To unravel antibody specificity at the molecular level and provide well-defined material for conjugate vaccine generation, synthetic WTA fragments are invaluable tools. Chapter 2 of this Thesis reported the assembly of synthetic RboP oligomers up to the dodecamer level and showed the successful application of automated solid phase synthesis (ASPS) for unsubstituted WTAs. Chapter 3 presented methods for the generation of C4-modified WTAs carrying α- or β-GlcNAc residues. This chapter describes the synthesis of C-3 β-glycosylated WTAs for antibody binding studies. In line with the set of WTA-fragments generated in the previous Chapter, the set of targeted C-3 β-GlcNAc WTAs comprises a symmetric trimer with a single C-3 β-GlcNAc in the middle RboP residue (**1**), intended for crystallization studies and two hexamers carrying either one or two β-GlcNAc-residues and a hexylamine spacer for conjugation purposes (**2** and **3**, see Figure 1).

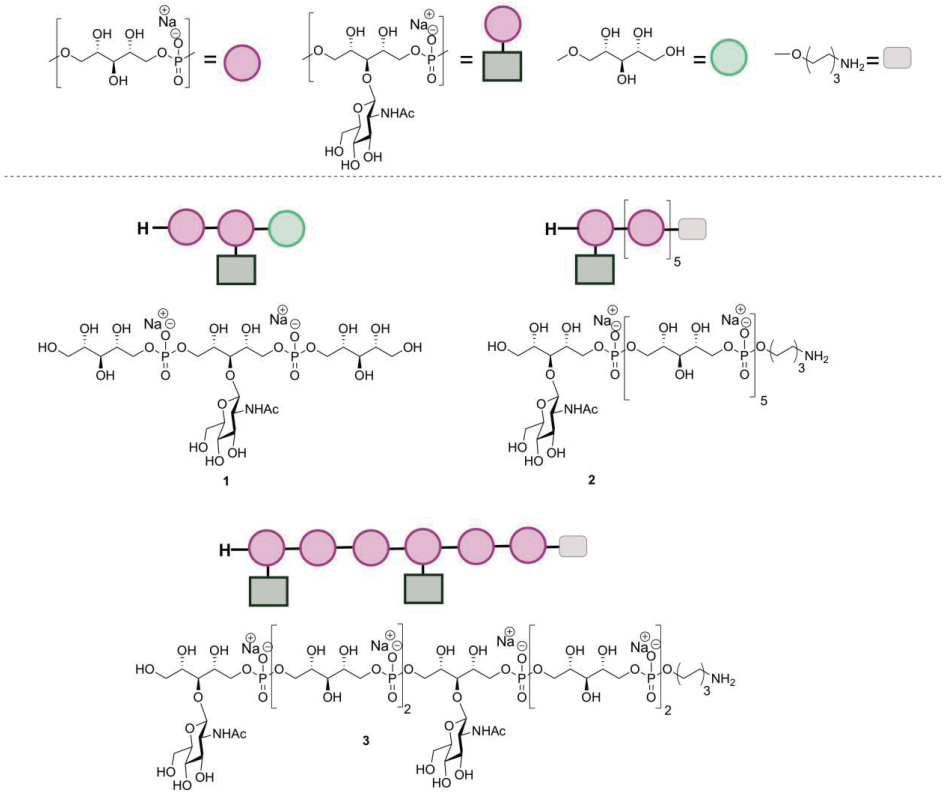


Figure 1. C-3 glycosylated compounds **1**, **2** and **3**, described in this Chapter.

RESULTS AND DISCUSSION

In line with the synthetic approach taken in Chapter 2 and 3, the synthesis of the C-3 β-glycosylated WTAs employs a monomeric assembly strategy, and the synthesis of the required building blocks is depicted in Scheme 1. The synthesis of the C3-OH ribitol acceptor **14** is more challenging compared to the C4-OH ribitol acceptor discussed in Chapter 3 and two synthetic pathways were explored to generate this building block. Scheme 1A depicts the first synthesis route with the formation of the orthoester **10**[17] as a key intermediate giving access to the orthogonal protection of the C-3-OH. Starting from commercially available D-ribose **4**, a Fisher glycosylation followed by isopropylidene protection of the secondary alcohols and subsequent allylation of the primary alcohol, delivered compound **5** in 84% over 3 steps. Acidic hydrolysis of the methyl acetal and isopropylidene ketal then yielded the corresponding triol **6**. Benzoylation of the free alcohols and subsequent HBr/AcOH treatment formed the bromide **8**,[17] which was subjected to a reaction with *N,N*-dimethylformamide dimethyl acetal. Initially, the bromide with acetyl groups on the 2- and 3-position was used to synthesize the 1,2-or-

thoester instead of the benzoylated compound **7**. However, lower yields were obtained with the acetylated derivative compared to the benzoylated compound. On 4.85 mmol scale, the desired the 1,2-orthoester **9** was formed in 80%, but scale up of the reaction to 85.7 mmol resulted in a much lower yield (33%). Direct S_N2 type displacement of the bromide to provide the methyl riboside and ribose hemiacetal formation occurred as major competing side reactions. In the next step the benzoyl at the C-3 position was removed under Zemplén conditions followed by naphthylation of the resulting alcohol, yielding compound **11** in 72% over 2 steps. Hydrolysis of the orthoester gave lactol **12**, which was reduced using $NaBH_4$ followed by the removal of the benzoyl ester to yield ribitol triol **13** in 60% over 2 steps. Protection of the primary alcohol with a TBDPS group gave **14**, of which the remaining alcohols were benzylated using BnBr and NaH. During this alkylation step a byproduct formed, due to TBDPS migration and this product could not be separated from the desired product at this stage. Therefore, the naphthyl ether and TBDPS ethers were removed to provide **15**, which could be purified from the formed byproduct at this stage yielding product **15** in 62% yield over 3 steps. Reinstallation of the TBDPS group on the primary alcohol furnished the C3-OH ribitol building block **16**.

To circumvent the laborious orthoester formation step, a second synthesis route was established as depicted in Scheme 1B. This route started from diacetone-D-glucose[18] which can be transformed into the corresponding allose **18**, having the required ribose stereochemistry, through a well-established[18] oxidation-reduction sequence in 69% yield. Naphthylation of the C-3 hydroxyl gave the fully protected allofuranose. The selective removal of the 5,6-isopropylidene was first tried using the conditions reported by Kiss *et al*[19], using 0.05M H_2SO_4 in H_2O/THF (v/v= 5:1), however these conditions led to solubility issues. Adding more THF to increase the solubility of the starting material unfortunately led to an increase of reaction time and the removal of both isopropylidene groups, which in turn resulted in a poor 15% yield of compound **19**. Switching to the use of *p*-TsOH in MeOH did cleave the 5,6-isopropylidene selectively to form diol **19** with a yield of 75% over 2 steps. Next, oxidative cleavage of the 5,6-diol with $NaIO_4$ gave the aldehyde which was reduced to form the primary alcohol. Allylation of this alcohol, afforded ribose **20** with a yield of 88% over 3 steps. Subsequently, the 1,2-isopropylidene was cleaved under acidic conditions to give **21**. Reductive opening of hemiacetal **21**, and TBDPS protection of the primary alcohol then provided **14**. The first synthetic pathway (Scheme 1A) to synthon **14** proceeded with an overall yield of 7%, while the second synthesis route (Scheme 1B) delivered **14** in an overall yield of 22%, making the second synthesis route clearly favorable over the first one.

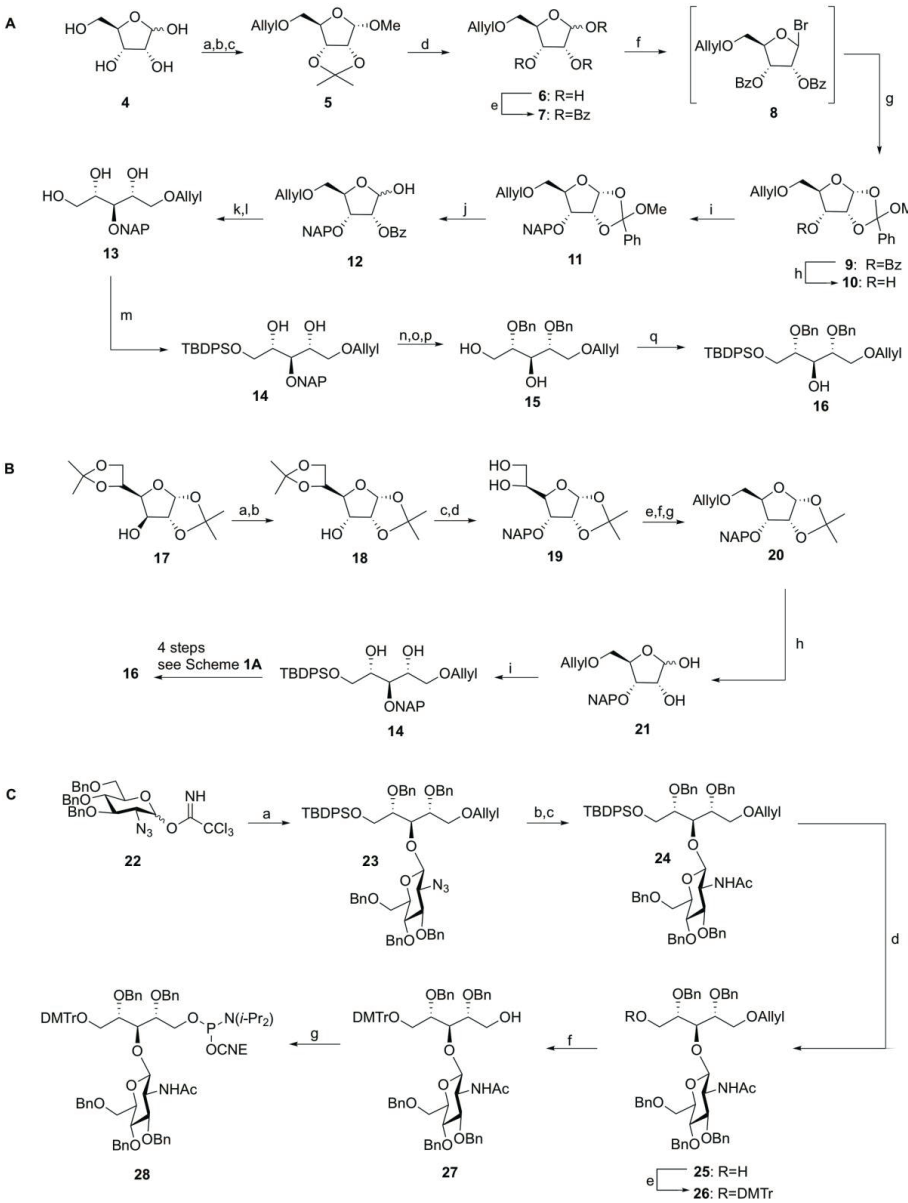
The synthesis of key phosphoramidite **28** is presented in Scheme 1C. First, ribitol alcohol **16** was glycosylated with glucosazide donor **22**, described in Chapter 3. The desired β-glycosidic bond was introduced by the use of ACN as solvent under activation of

TMSOTf.[20] ACN can coordinate to the intermediately formed oxocarbenium ion in axial manner, driving the acceptor to react on the β-face of the glucosazide. The desired β-$GlcN_3$ ribitol **23** was obtained as the sole anomer in 80% yield. The following protecting groups manipulations were required to arrive at amidite **28**: reduction of the azide group using propanedithiol and subsequent acetylation gave acetamide **24** in 86% yield over 2 steps. Removal of the TBDPS group using TBAF gave alcohol **25**, which was protected with a dimethoxytrityl (DMTr) group. Isomerization of the allyl ether by an iridium catalyst and subsequent iodine mediated enol ether hydrolysis provided alcohol **27**. Coupling of the alcohol to the 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite resulted in amidite **28**.

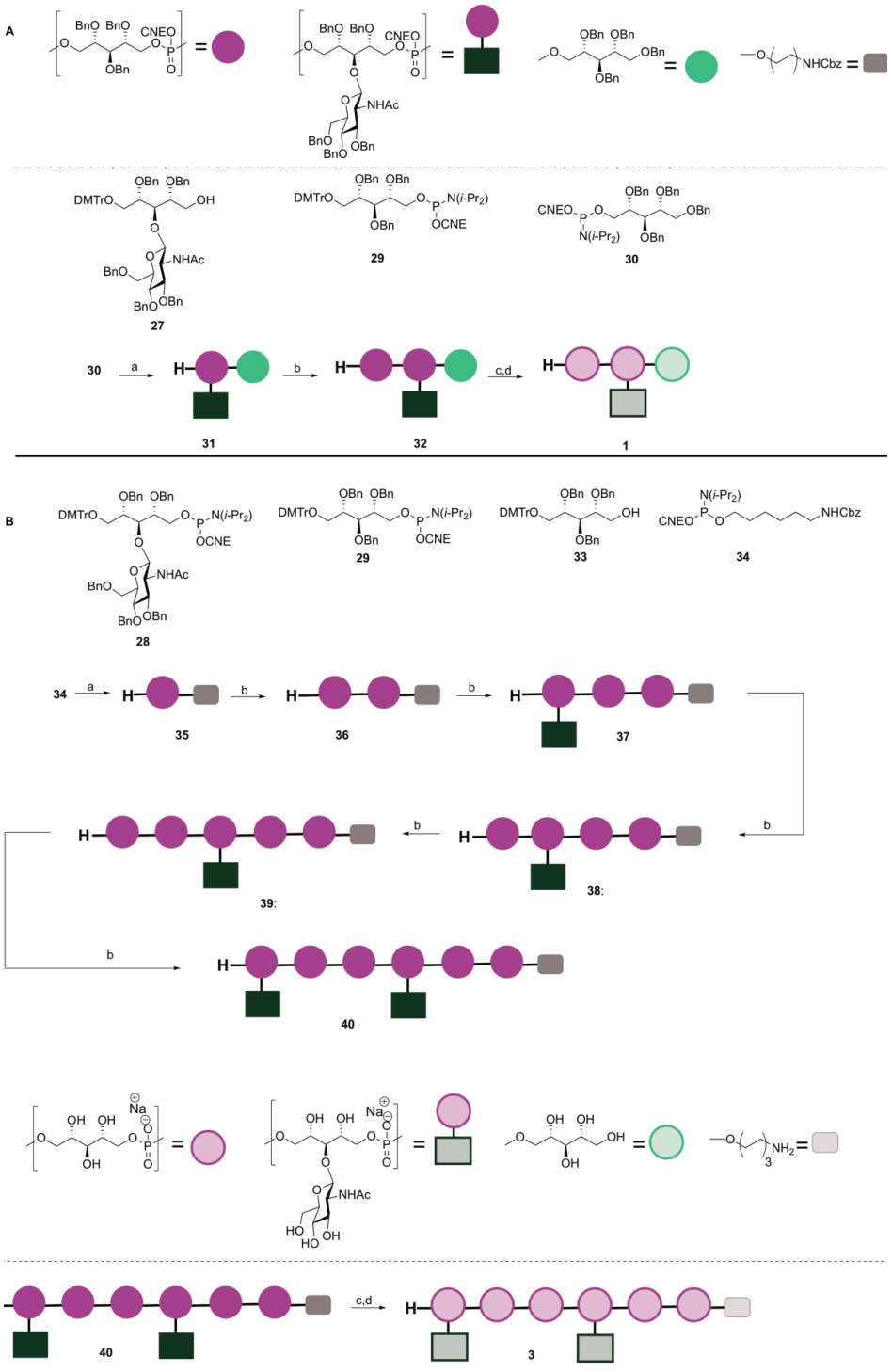
Next, the C-3-β-GlcNAc WTA fragments **1**-**3** were assembled with key amidites **28** and **29** (See Chapter 2 and 3) as schematically depicted in Scheme 2 and 3. Hexamer **2** and **3** are equipped with a chemoselective handle for conjugation purposes while trimer **1** was designed for crystallization studies and therefore lacks this handle. The syntheses were first explored using solution phase chemistry (for trimer **1** and hexamer **3**) and next translated to an automated solid phase synthesis approach (for hexamers **2** and **3**).

The assembly of trimer **1** started with the union of ribitol phosphoramidite **30** (See Chapter 3) and C-3-β-GlcNAc ribitol **27** (Scheme 2A). Condensation of these two building blocks occurred under the agency of dicyanoimidazole to provide the intermediate phosphite, which was oxidized using (10-camphorsulfonyl)oxaziridine (CSO) to deliver the phosphotriester. Unmasking the primary alcohol by removal of the DMTr-group using dichloroacetic acid gave dimer **31** in 68% yield. In the next coupling-oxidation-deprotection cycle, comprising the same three steps, trimer **32** was formed in 78% yield. Deprotection of the trimer was accomplished by removal of the cyanoethyl esters under aqueous ammonia conditions and subsequent hydrogenation of the semi protected trimer to yield **1** in 77% yield over 2 steps.

Next the assembly of the longer hexamer **3** was undertaken, which started with the coupling of the spacer amidite **34** and ribitol alcohol **33** using the above described coupling-oxidation-deprotection cycle (Scheme 2B). The resulting spacer functionalized monomer **35**, obtained in 72% yield, was then elongated using phosphoramidite **29** to provide dimer **36** (88%). Ensuing coupling with C-3-β-GlcNAc phosphoramidite **28** then gave, after oxidation and DMTr removal, trimer **37** in similar yield. Trimer **37** was next elongated towards hexamer **40** by three coupling-oxidation-deprotection cycles involving amidite **29** (2x) and C-3-β-GlcNAc amidite **28**, which all proceeded in good yield. Global deprotection of the fully protected hexamer was accomplished using the same conditions as described for the trimer to deliver C-3-β-GlcNAc WTA fragment **3** in 77% yield over 2 steps.



Scheme 1. A Building block synthesis; *Reagents and conditions:* a) AcCl, MeOH; b) acetone, HCl; c) AllylBr, NaH, THF/DMF, 84% over 3 steps, d) formic acid/H_2O/THF (v/v/v= 6/2/2), 50°C, 76%; e) BzCl, pyridine, quant.; f) HBr, AcOH; g) *N,N* dimethylformamide dimethyl acetal, DCM, 80%; h) NaOMe, MeOH; i) NAPBr, NaH, TBAI, THF, 72% over 2 steps; j) formic acid/H_2O/THF (v/v/v= 2/2/6), 76%; k) $NaBH_4$, MeOH; l) NaOMe, MeOH, 60% over 2 steps; m) TBDPSCl, TEA, DCM, 81%; n) BnBr, NaH, THF/DMF (v/v= 7/1); o) DDQ, DCM/H_2O (v/v= 4/1) p) TBAF, THF, 62% over 2 steps; q) TBDPSCl, TEA, DCM, 98%; **B Building block synthesis**; *Reagents and conditions:* a) DMSO, Ac_2O; b) $NaBH_4$, EtOH/H_2O (v/v= 7/3), 69% over 2 steps; c) NAPBr, NaH, TBAI, THF; d) *p*-TsOH·H_2O, MeOH, 75% over 2 steps; e) 0.2 M $NaIO_4$ in H_2O, MeOH; f) $NaBH_4$, MeOH; g) AllylBr, NaH, THF/DMF (v/v= 7/1), 88%; h) THF/H_2O/formic acid (v/v/v= 2/2/6) 85%; i) i. $NaBH_4$, MeOH; ii. TBDPSCl, TEA, DCM, 57% over 2 steps; **C Building block synthesis**; *Reagents and conditions:* a) **16**, TMSOTf, ACN, -40°C to 0°C, 80%; b) propane dithiol, TEA, pyridine/H_2O; c) Ac_2O, pyridine, 86% over 2 steps; d) TBAF, THF, 96%; e) DMTrCl, TEA, DCM, 61%; f) i. $Ir(COD)(Ph_2MeP)_2PF_6$, H_2, THF, ii. I_2, sat. aq. $NaHCO_3$, THF, 94%; g) 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite, DIPEA, DCM, 85%.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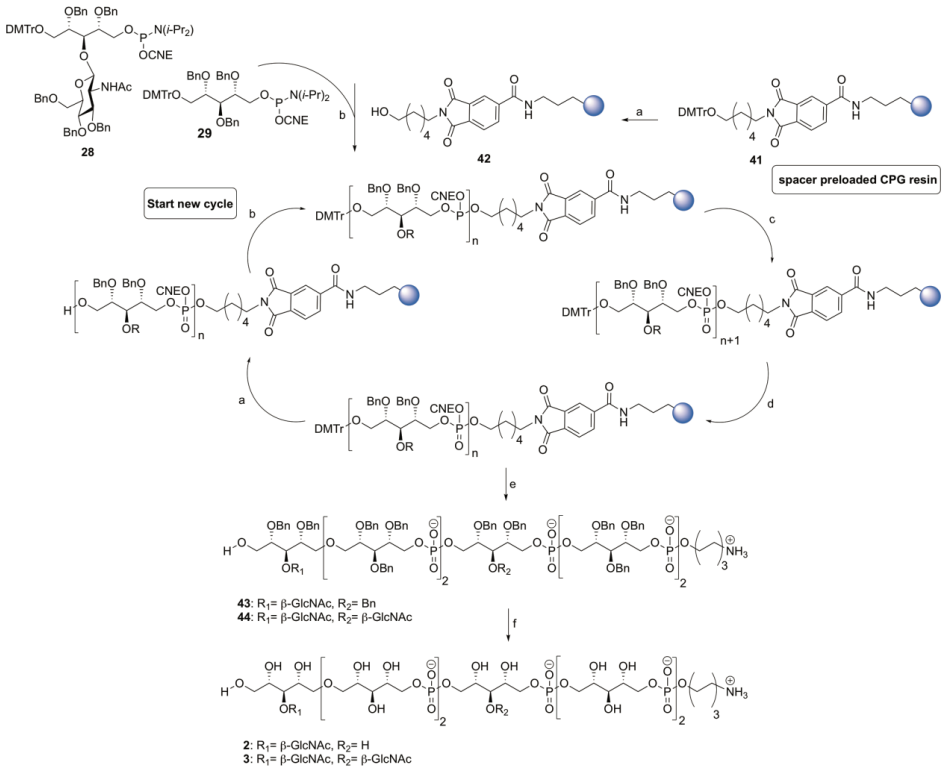
← **Scheme 2.** A. WTA assembly of glycosylated trimer **1** and glycosylated hexamers **3**; *Reagents and conditions:* a) i. DCI, ACN, **33**; ii. CSO; iii. 3% DCA in DCM, **31:** 68%; b) i. DCI, ACN, phosphoramidite **29**; ii. CSO; iii. 3% DCA in DCM, **32:** 78%; c) i. NH_3 (30-33% aqueous solution), dioxane; d) Pd black, H_2, AcOH, H_2O/dioxane, **1:** 77%; B. *Reagents and conditions:* a) i. DCI, ACN, **33**; ii. CSO; iii. 3% DCA in DCM, **35:** 72%; b) i. DCI, ACN, phosphoramidite **28** or **29**; ii. CSO; iii. 3% DCA in DCM, **36:** 88%, **37:** 88%; **38:** 92%; **39:** 87%; **40:** quant.; c) i. NH_3 (30-33% aqueous solution), dioxane; d) Pd black, H_2, AcOH, H_2O/dioxane, **3:** 70%.

As discussed in Chapter 2, automated solid phase synthesis (ASPS) was applied for the synthesis of unsubstituted WTAs and being encouraged by the synthesis of glycosylated TAs as reported by Hogendorf *et al.*[21-23] and van der Es *et al.*[24] the synthesis of glycosylated WTAs was attempted (Scheme 3). To ensure spacer installation at the "peptidoglycan attachment site", commercial CPG resin **41** was used, featuring a phthalimide protected aminohexanol spacer moiety. ASPS was performed on 10 μmol scale resin and a DMTr cleavage using 3% DCA in toluene liberated the primary alcohol **42** on which the first coupling could take place. To this end the resin was reacted with amidite **29** under the agency of 5-(Benzylthio)-1*H*-tetrazole to give the phosphite intermediate, which was oxidized to the corresponding phosphate using I_2 and pyridine. Afterwards a capping step took place to prevent alcohol functionalities to react in the next step, which could lead to difficult to separate byproducts. Liberation of the primary alcohol then allowed for a new coupling cycle with an amidite of choice. *En route* to target hexamer **2**, 4 additional couplings cycle with amidite **29** were performed and for the last coupling β-glycosylated amidite **28** was used. For target hexamer **3**, featuring two C-3-β-GlcNAc RboP residues, the second cycle used amidite **29** and the third cycle β-glycosylated amidite **28**. Two ensuing coupling cycles with amidite **29** and a last coupling cycle with β-glycosylated amidite **28** were performed to arrive at the hexamer stage. The primary alcohol was unmasked using 3% DCA followed by treatment with aqueous 25% NH_3 that removed the cyanoethyls and released the oligomers from the resin. The crude hexamers were purified using reversed HPLC and a desalting step afforded **43** and **44** in 20% and 11% yield respectively. Final hydrogenations of the semi-protected hexamers gave the targets **2** and **3** in 87% and quantitative yield.



Scheme 3. Assembly of glycosylated WTAs **2** and **3** using ASPS approach; *Reagents and conditions:* a) 3% DCA, toluene; b) phosphoramidite **28** or **29**, 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) **43:** 6.9 mg; 20%; **44:** 4.2 mg; 11%; f) Pd black, H_2, dioxane H_2O, AcOH, **2:** 3.0 mg; 87%; **3:** 2.5 mg; 1.30 μmol; quant.

Figure 2 depicts the ^{1}H NMR spectra of the α-1,4-, β-1,4- and the β-1,3-GlcNAc WTAs. The NMR spectra of these well-defined WTAs can be very useful for the structure determination of new WTA-species isolated from bacterial strains, and in particular the position and configuration of the modifications along the chain. As shown in Fig 2, the anomeric protons of the β-linked GlcNAc are present at a different chemical shift value than the anomeric protons of the α-GlcNAc. The β-1,3-GlcNAc anomeric protons appear at 4.62 ppm, slightly lower than the β-1,4-GlcNAc anomeric protons with resonances at 4.70 ppm. These values are in accordance with those reported by Sanofi Pasteur[25] for β-1,3-GlcNAc modified WTA, isolated from strain ATC 55804 with a anomeric value of 4.65 and with β-1,4-GlcNAc WTA isolated from strain wood 46 showing anomeric signals at 4.75 ppm for. The reported anomeric signals for α-1,4-GlcNAc WTA from Newman D2C (at 5.07 ppm) are also well in agreement with the values for the α-1,4-GlcNAc WTA (5.03 ppm and 5.06 ppm). They are also in line with the TarP and TarM modified WTAs described by Gerlach *et al.*[15]

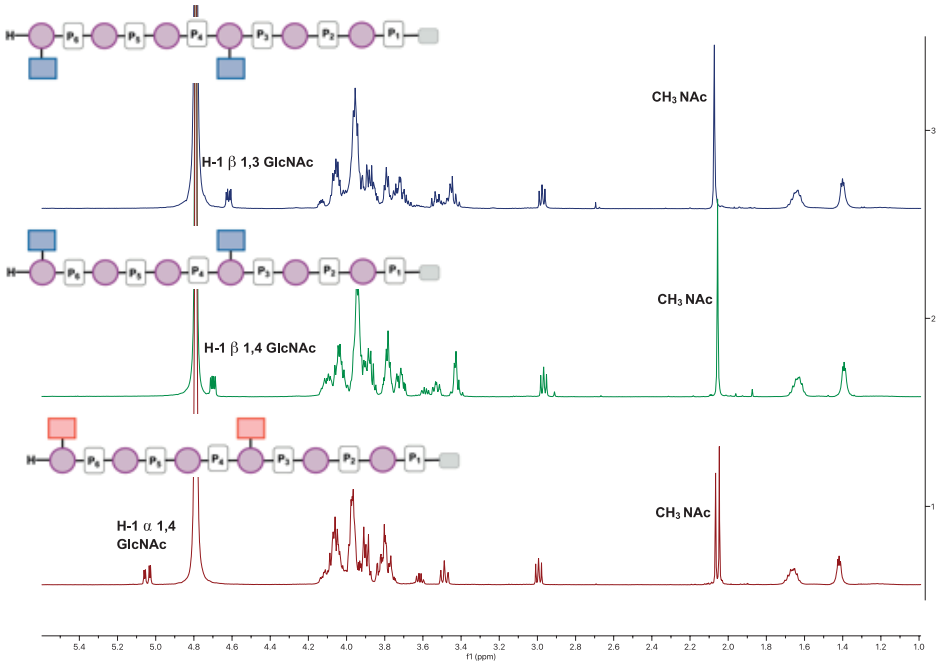


Figure 2. ^{1}H NMR spectra of the synthetic α-1,4-, β-1,4- and β-1,3 glycosylated WTAs.

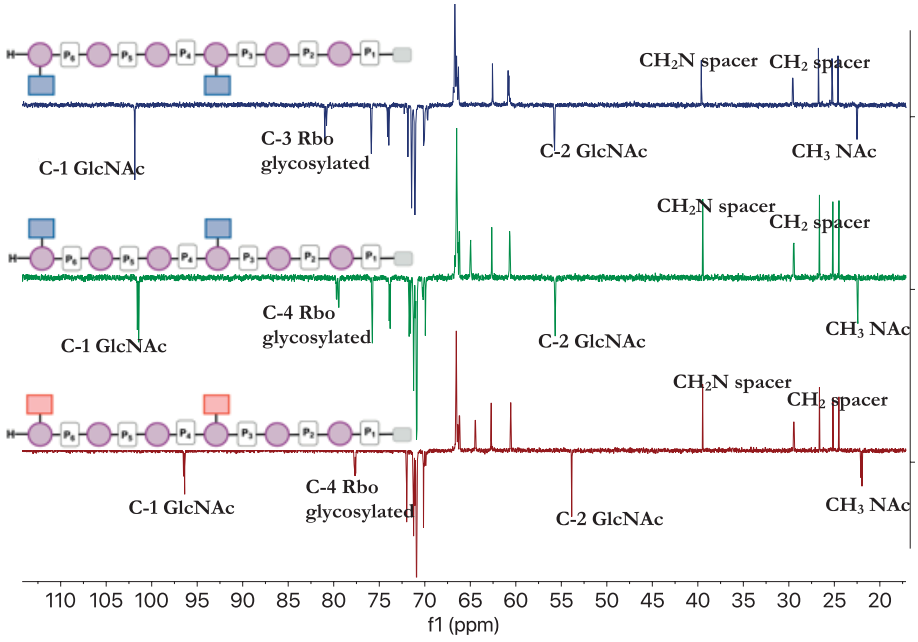


Figure 3. ^{13}C NMR spectra of the synthetic α-1,4-, β-1,4- and β-1,3 glycosylated WTAs.

Figure 3 shows the ^{13}C NMR of the β-1,3-, β-1,4- and α-1-4- GlcNAc WTAs. The anomeric signals of the β-1,3-WTA appear at 101.6 and the C-3 glycosylated Rbo shows a shift at 80.8 and 81.0 which is in agreement with previously reported data at 81.8 for C-3 glycosylated Rbo position, appearing at higher ppm values than the non-glycosylated ribitol positions[15]. The β-1,4-WTA anomeric shifts are at 101.4 and 101.6 comparable to the β-1,3-GlcNAc anomeric signals. The C-4 Rbo glycosylated appears around 79.4 - 79.9 and is closely in accordance with 80.8 ppm for C-4 glycosylated Rbo position[15]. The anomeric signals corresponding to α-1-4- GlcNAc WTAs appear at 96.4 and 96.5, lower in ppm shift as expected for α-glycosidic linkages and the glycosylated C-4 position ppm values are at 77.6 - 77.8.

Next, the ^{31}P NMR spectra of the α-1,4-GlcNAc WTA, β-1,4-GlcNAc WTA and β-1,3-GlcNAc WTA hexamers were compared, and it appears that the ^{31}P-chemical shift is diagnostic for the type of GlcNAc appendage (Figure 4). The ^{31}P signals are assigned $\mathbf{P_1}$ to $\mathbf{P_6}$, as shown in the schematic structure diagrams next to the spectra. The spectrum of the αα-1,4-GlcNAc hexamer shows three types of signals: three around 2 ppm, a single peak at 1.8 ppm and two peaks around 1.7 ppm. Considering that the phosphate next to the spacer will be different from the other phosphate diesters that are all flanked by two ribitol residues the single peak likely corresponds to the phosphate diester attached to the spacer. When the spectra of the α-1,4-GlcNAc and αα-1,4-GlcNAc hexamers are compared it becomes clear that one peak has shifted to a lower ppm value. This phosphorous resonance thus likely corresponds to $\mathbf{P_3}$. This analysis also holds for the β-1,4-GlcNAc and ββ-1,4-GlcNAc hexamer which shows a similar chemical shift pattern. The ^{31}P-spectrum of the β-1,3 glycosylated WTA shows similarity to the β-1,4- and α-1,4-GlcNAc WTAs. The introduction of the second GlcNAc substituent at the third ribitol residue causes the resonance of the phosphate diesters $\mathbf{P_3}$ and $\mathbf{P_4}$ (which are equally close to the GlcNAc residue in the middle of the RboP moiety) to shift to a lower ppm value: the peaks around 1.8-1.9, corresponding to four P signals, belong to $\mathbf{P_1}$, $\mathbf{P_3}$, $\mathbf{P_4}$ and $\mathbf{P_6}$. The phosphodiesters, flanked by two non-substituted ribitols are found around 2 ppm. In all, this analysis shows that ^{31}P-NMR chemical shifts can be diagnostic for the substitution pattern along the RboP chain, and the relative intensity of the signals indicative for the degree of glycosylation.

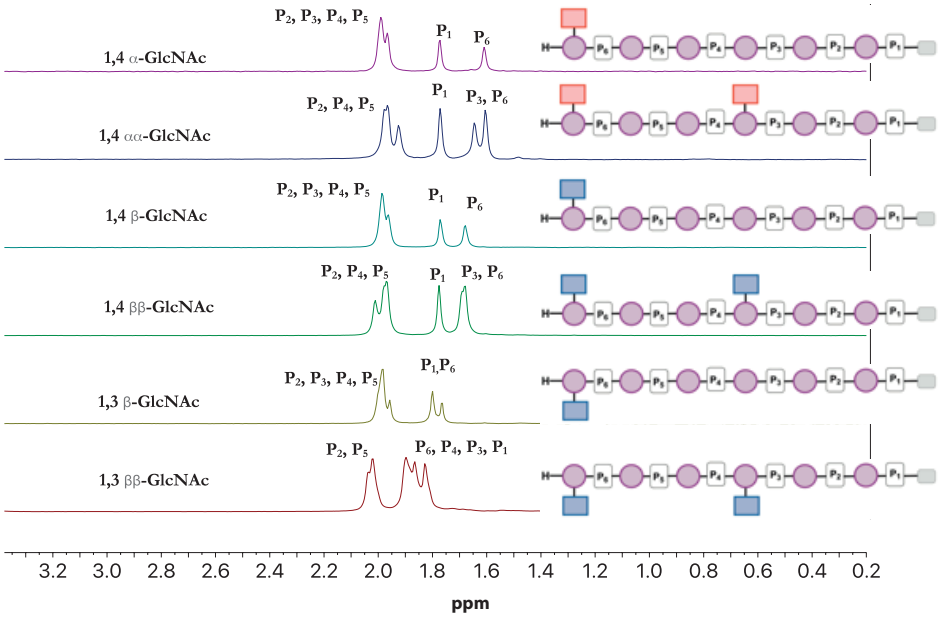
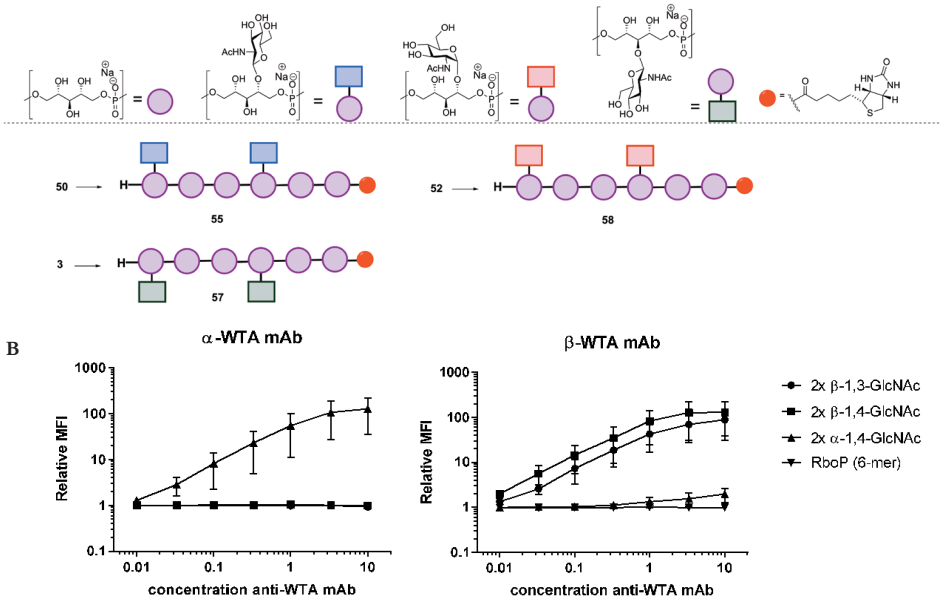


Figure 4. ^{31}P NMR spectra of the synthetic mono- and di-α-1,4-, -β-1,4- and -β-1,3 glycosylated WTAs.

To probe Ab binding to the generated WTA-hexamers, the fragments were evaluated in the previously described magnetic bead assay (See Chapter 2) using two monoclonal antibodies (mAbs): 4461, a recombinantly expressed anti α-1,4-GlcNAc-WTA antibody, and 4497, which recognizes 1,4-β-GlcNAc-WTA. To this end the synthesized glycosylated WTA hexamers (ββ-1,3-GlcNAc WTA **3**, ββ-1,4-GlcNAc WTA **50**, and αα-1,4-GlcNAc WTA **52**) were equipped with a biotin handle to couple them to Streptavidin-coated magnetic beads (Scheme 4A). Figure 4B depicts the binding of the bead-bound hexamers with the monoclonal antibodies used in increasing concentration. As can be seen from the left graph the anti α-mAb 4461 selectively binds to the αα-1,4-GlcNAc WTA in a concentration dependent manner. The anti β-mAb 4497 on the other hand (See right panel in Figure 4B), shows binding to both the ββ-1,4- and the ββ-1,3-GlcNAc WTAs, with the former being recognized slightly better than the latter. This shows that this mAb, raised against β-1,4-GlcNAc WTA, can cross react with β-1,3-GlcNAc WTA. It is thus not unlikely that IgG in human serum is also capable of interacting with both TarS-WTA and TarP-WTA, as described by Van Dalen *et al.*[26] More detailed binding studies are required to pinpoint the differences in binding between the two different epitopes and the (recombinantly expressed) monoclonal antibodies and human sera.



Scheme 4. A. Biotinylation of hexamer **50**, **52** and **3**; *Reagents and conditions*: Biotin-OSu, DIPEA, DMSO, **55:** 51%, **58:** 68%, **57:** 93%; **B.** Concentration dependent assay of mAbs 4461 and 4497 against WTA hexamers, **55**, **57** and **58.**

CONCLUSION

This Chapter described the successful synthesis of C-3 glycosylated ribitol phosphate WTA fragments. A solution phase synthesis approach has enabled the synthesis of well-defined β-1,3-GlcNAc WTA fragments on large scale yielding sufficient amounts for various activity and binding studies. The automated solid phase assembly afforded lower amounts but it does allow the rapid assembly of WTA fragments with a diverse substitution pattern, without the need for purification steps after each coupling cycle. NMR analysis of the full set of WTA fragments, generated in this and the previous chapter, showed characteristic chemical shifts for the different GlcNAc epimers and regioisomers in both ^{1}H, ^{13}C and ^{31}P spectra, indicating and corroborating how these NMR techniques can be used in structural elucidation studies performed on ribitol phosphate WTA. Binding of the synthesized fragments with mAbs raised against either α- or β-GlcNAc WTAs, was evaluated using the magnetic bead model and it was shown only binding to the WTA-type against which the mAbs were raised could be detected. Noteworthy, the binding of the mAbs directed to the β-GlcNAc which showed binding to both the C-4 and the C-3 glycosylated WTA. The magnetic bead assay allows the sensitive and specific detection of antibodies using well-defined synthetic WTA fragments and presents a reliable way to detect WTA specific antibodies in serum. In the future it can be used to screen larger cohorts to show how adaptive immunity develops or fails to develop upon

exposure to different *S. aureus* infections. Similarly, the assembled library of WTAs can be used to generate a TA-microarray platform to screen serum and used to identify infections by different strains of *S. aureus*. This will require a lower amount of the fragments and would not require the attachment of a biotin affinity handle as used in the magnetic bead assay. Both platforms would be expertly suited to also interrogate other relevant biomolecules, such as C-type lectin receptors or phage proteins. Finally, the synthetic structures reported here may be explored as antigens to generate synthetic vaccines or antibodies against *S. aureus*.

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 a Propol automated polarimeter (Sodium D- line, λ = 589 nm) with a concentration of 10 mg/mL (c= 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300. ^{1}H, ^{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).

General procedure for phosphoramidite synthesis

The alcohol was co-evaporated with distilled toluene two times under a N_2 atmosphere, and dissolved in dry DCM (0.1 M). DIPEA (1.5 eq.) and activated molecular sieves (3Å)

were added and the solution was stirred for 30 minutes. 2-Cyanoethyl-*N,N*-diisopropyl-chlorophosphoramidite (1.2 eq.) was added and the reaction mixture was stirred for 2.5 hours. Next, a few drops of H_2O were added and the mixture was diluted in DCM. The organic phase was washed with sat. aq. $NaHCO_3$/brine (1:1)(v/v). The water layer was extracted with DCM (3x), and the combined organic layers were dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. Purification was performed by neutralized column chromatography to give the corresponding phosphoramidite.

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 minutes. 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 minutes). Subsequently, (10-camphorsulfonyl)oxaziridine (CSO) (2.0 eq; 0.5 M in ACN) was added and the stirring was continued for 15 minutes. 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 minutes an aqueous solution of methanol (1:1) was added, stirred for an additional 30-40 minutes 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 fully protected oligomer was dissolved in a (v/v= 1:1) mixture of NH_4OH/dioxane (3.33 mM) and the reaction mixture was stirred at rt overnight. The mixture was concentrated under reduced pressure, and the residue was flushed over a Dowex Na^+ cation-exchange resin (type: 50WX4-50-100, stored on 0.5 M NaOH in H_2O, flushed with MiliQ water and MeOH before use). The crude product was dissolved in a (v/v= 1:1) mixture of dioxane/H_2O (0.013 M), and 3 drops of AcOH were added. The mixture was purged with N_2, Pd black (±50 mg) was added and the mixture was repurged with N_2. Then the mixture was purged with H_2 and was stirred under a H_2 atmosphere multiple days. The mixture was filtered over celite and concentrated *in vacuo*. The residue was purified by size-exclusion chromatography (HW40, dimensions: 16/60 mm, eluent: 0.15 M NH_4OAc or NH_4HCO_3). The product was co-evaporated repeatedly with MiliQ water to remove

NH_4OAc/NH_4HCO_3 traces, and 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), to give the deprotected ribitol phosphate oligomer.

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).

Biotinylation

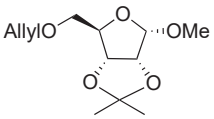
Chapter 2 described the enzymatic glycosylation using the glycosyltransferases TarS and TarM in the presence of UDP-GlcNAc and unglycosylated 6-mer and 12-mer as substrates. In order to explore if these enzymes are also able to glycosylate synthetic glycosylated WTAs, whether in α- or β conformation, the set of glycosylated WTAs was subjected to the biotinylation conditions as described below.

General procedure biotinylation

0.5 µmol of the GlcNAc-RboP-hexamer was dissolved in DMSO (250 µL; 2.0 mM). 105 µL of 0.075 M Biotin-OSu in DMSO was added (0.85 µmol; 1.7 eq) followed by DIPEA (104.5 µL) and the mixture was shaken overnight at rt. 250 µL of magic and 250 µL were added and the mixture was centrifuged and purified by size exclusion chromatography (HW-40

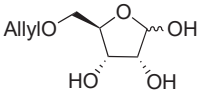
column, dimensions: 16/60 mm, eluent 0.15M NH_4OAc). After repeated co-evaporation (7-10 x) with miliQ water to remove NH_4OAc, the product was eluted through a small column containing Dowex Na^+ cation-exchange resin (type 50WX8-50-100, stored on 0.5M NaOH in H_2O, flushed with H_2O and MeOH before use). Lyophilization yielded the product.

Methyl 5-*O*-allyl-2,3-*O*-isopropylidene-α-D-ribofuranoside (5)



D-ribose (37.5 g, 250 mmol, 1.0 eq.) was dissolved in MeOH (930 mL, 0.27 M). AcCl (5.45 mL, 76.4 mmol, 0.31 eq.) was added dropwise and the reaction mixture was stirred for 2 hours at rt. After full conversion solid $NaHCO_3$ was added until the mixture reached a neutral pH. The $NaHCO_3$ was filtered, and the solution was concentrated under reduced pressure. The crude product was dissolved in acetone (1240 mL, 0.20 M). Concentrated HCl (37%) (14.9 mL, 2.0 eq.) was added and the reaction mixture was stirred at rt overnight. Solid $NaHCO_3$ was added until the mixture was pH neutral. The $NaHCO_3$ was filtered, and the solution was concentrated under reduced pressure. Subsequently, the crude product was dissolved in a (v/v= 7:1) mixture of THF/DMF (715 mL, 0.35 M) and the mixture was cooled to 0°C. NaH (15.0 g, 375 mmol, 1.5 eq., 60% in mineral oil) was added in portions. Allyl bromide (25.9 mL, 300 mmol, 1.2 eq.) was added dropwise and the reaction mixture was stirred from 0°C to rt overnight, followed by the slow addition of MeOH at 0°C. The mixture was diluted in Et_2O and the organic phase was washed with H_2O (5x). The organic layer was dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. Column chromatography (100% pentane to 20 % EtOAc in pentane) yielded the pure α title compound **5** (51.3 g, 210 mmol) in 84% over 3 steps. IR (neat, cm^{-1}): 2939, 2362, 1373, 1211, 1090, 1049, 962, 870; ^1H NMR (400 MHz, $CDCl_3$) δ=1.32 (s, 3H, C*H*$_3$-Acetyl), 1.48 (s, 3H, C*H*$_3$-Acetyl), 3.31 (s, 3H, OC*H*$_3$), 3.37 – 3.56 (m, 2H, H-5), 4.01 (dd, 2H, *J*= 5.6, 1.4 Hz, C*H*$_2$-CH), 4.33 (t, 1H, *J*= 6.8 Hz, H-4), 4.57 (d, 1H, *J*= 6.0 Hz, H-2), 4.68 (d, 1H, *J*= 6.1 Hz, H-3), 4.96 (s, 1H, H-1), 5.14 – 5.41 (m, 2H, C*H*$_2$=CH), 5.90 (ddt, 1H, *J*= 17.3, 10.3, 5.6 Hz, CH_2=C*H*); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 25.0 (*C*H$_3$-Acetyl), 26.5 (*C*H$_3$-Acetyl), 54.8 (OCH$_3$), 71.0 (C-5), 72.2 (*C*H$_2$-CH), 81.2 (C-3), 85.2 (C-2, C-4), 109.3 (C-1), 112.3 (CH$_3$-*Cq*), 117.2 (*C*H$_2$=CH), 134.6 (CH_2=*C*H); HRMS: $[M+Na]^+$ calcd for $C_{12}H_{20}O_5Na$ 267.1203, found 267.1213.

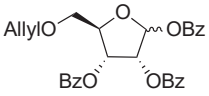
5-*O*-allyl-D-ribofuranoside (6)



Compound **5** (50.0 g, 205 mmol) was dissolved in a (v/v/v= 6:2:2) mixture of formic acid/H_2O/THF (1.4 L, 0.15 M), and the reaction mixture was stirred at 50°C overnight. The mixture was concentrated *in vacuo* and the product was co-evaporated with toluene two times. Column chromatography (100% DCM to 15% MeOH in DCM) yielded triol **6** (29.7 g, 156 mmol)

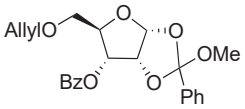
as an α:β mixture with a ratio of ±2:1 in 76% yield. IR (neat, cm^{-1}): 2943, 2360, 1654, 1507, 1049; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.49 – 3.72 (m, 2.3 H, H-5 α,β), 3.94 - 4.23 (m, 5.6H, H-2 α, H-3 α, H-4 α, H-4 β, H-2 β, H-3 β, CH_2-CH α,β), 5.11 – 5.39 (m, 3.5H, H-1 α, H-1 β, CH_2=CH), 5.81 – 5.97 (ddt, 1H, *J*= 17.2, 10.4, 5.7 Hz, CH_2=C*H*); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 70.3 (C-5), 71.4 (C-2, C-3), 71.2 (C-5), 71.7 (C-2/C-3), 72.5, 72.5 (*C*H_2-CH), 75.7(C-2/C-3), 81.9, 82.4 (C-4), 96.6 (C-1 α), 101.9 (C-1 β), 117.6, 118.0 (*C*H_2=CH), 134.1, 134.3 (CH_2=*C*H); HRMS: $[M+Na]^+$ calcd for $C_8H_{14}O_5Na$ 213.0733, found 213.0741.

5-*O*-allyl-1,2,3-tri-*O*-benzoyl-D-ribofuranoside (7)



Triol **6** (16.3 g, 85.7 mmol, 1.0 eq.) was dissolved in pyridine (430 mL, 0.20 M) and the mixture was cooled to 0°C. BzCl (44.8 mL, 386 mmol, 4.5 eq.) was added and the reaction was stirred for 1.5 hours from 0°C to rt. 250 mL H_2O was added at 0°C and the mixture was stirred for 15 minutes at 0°C. The mixture was diluted in EtOAc, and the organic phase was washed with H_2O (1x), 3M HCl (2x), sat. aq. $NaHCO_3$ (1x), and brine (1x). The organic layer was dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. Column chromatography (100% pentane to 35% EtOAc in pentane) yielded title compound **7** (43.1 g, 85.7 mmol) as an α:β mixture with a ratio of ±3:2 in quantitative yield. IR (neat, cm^{-1}): 2860, 1724, 1261, 1108, 1066, 1025, 707; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.63 – 3.93 (m, 5H, H-4α, H-4β, H-5α, H-5β), 4.02 (dt, 2H, *J*= 5.6, 1.6 Hz, CH_2-CH α), 4.06 – 4.17 (m, 2H CH_2-CH β), 4.61 – 4.80 (m, 2H, H-3α, H-3β), 5.04 – 5.45 (m, 4H, CH_2=CH α, CH_2=CH β), 5.61 – 6.11 (m, 6H, H-2α, H-2β, CH_2=C*H* α, CH_2=C*H* β), 6.68 (d, *J*= 1.2 Hz, 1H, H-1α), 6.93 (d, 1H, *J*= 4.5 Hz, H-1β), 7.14 – 8.29 (m, 30H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 69.4 – 69.8 (C-5 α, β), 71.5 – 71.8 (C-4 α, β), 72.6 – 72.7 (*C*H_2-CH α, β), 75.3 (C-2 α, β), 82.1 (C-3 α), 84.5 (C-3 β), 95.2 (C-1 β), 99.2 (C-1 α), 117.2 – 117.6 (*C*H_2=CH α, β), 128.4 – 130.1 (C-arom), 133.4 – 133.5 (CH_2=*C*H α, β), 165.0 – 165.9 (6x C=O); HRMS: $[M+Na]^+$ calcd for $C_{29}H_{26}O_8Na$ 525.1520, found 525.1529.

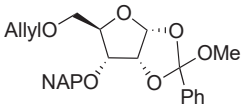
5-*O*-allyl-3-*O*-benzoyl-(1,2-*O*-methylorthobenzoyl)-α-D-ribofuranoside (9)



Compound **7** (4.9 g, 9.7 mmol, 1.0 eq.) was dissolved in dry DCE (32 mL, 0.3 M) and the mixture was cooled to 0°C. 33% HBr in AcOH (2.4 mL, 14.6 mmol, 1.5 eq.) was added dropwise, and the mixture was stirred for 10 minutes at 0°C, and 1 hour at rt. The reaction mixture was diluted in DCM and the organic phase was washed with ice cold sat. aq. $NaHCO_3$. The water layer was extracted with DCM, and the combined organic layers were dried over Na_2SO_4, filtrated, and concentrated under reduced pressure at 30°C to give the crude anomeric bromide intermediate *in situ*, which was used in the next step without further purification. The crude (2.24 g, 4.85 mmol, 1.0 eq.) was

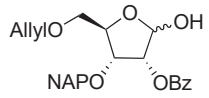
dissolved in dry DCM (12.1 mL, 0.4 M), and the mixture was cooled to 0°C. *N,N*-dimethylformamide dimethyl acetal (0.97 mL, 7.28 mmol, 1.5 eq.) was added dropwise and the reaction was stirred from 0°C to rt for 3 days. The reaction mixture was concentrated *in vacuo* at 30°C and column chromatography (100% pentane to 35% EtOAc in pentane) yielded title compound **9** (1.61 g, 3.89 mmol) in 80% yield. $[\alpha]_D^{25}$= +115.5° (*c* 1.0, DCM); IR (neat, cm^{-1}): 2943, 2360, 1724, 1457, 1272, 1095, 1055, 766, 712, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.24 (s, 3H, OCH_3), 3.48 – 3.73 (m, 2H, H-5), 3.92 – 4.03 (m, 3H, H-4, C*H*$_2$-CH), 5.02 (dd, 1H, *J*= 9.1, 5.4 Hz, H-3), 5.08 – 5.29 (m, 3H, H-2, C*H*$_2$=CH), 5.83 (ddt, 1H, *J*= 17.3, 10.4, 5.7 Hz, CH_2=C*H*), 6.21 (d, 1H, *J*= 4.2 Hz, H-1), 7.30 – 8.15 (m, 10H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 50.1 (Cq-O-*C*H3), 67.8 (C-5), 72.5 (C-3), 72.6 (*C*H$_2$-CH), 77.8 – 78.1 (C-2, C-4), 104.7 (C-1), 117.5 (*C*H$_2$=CH), 126.1 (*Cq*-O-CH_3), 128.1 – 129.2 (C-arom), 129.9 (CH_2=*C*H), 137.4 (Cq-arom), 165.7 (C=O); HRMS: $[M+Na]^+$ calcd for $C_{23}H_{24}O_7Na$ 435.1414, found 435.1419.

5-*O*-allyl-(1,2-*O*-methylorthobenzoyl)-3-*O*-(2-naphtylmethyl)-α-D-ribofuranoside (11)



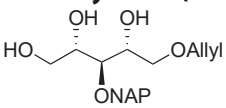
Compound **9** (13.3 g, 32.3 mmol, 1.0 eq.) was dissolved in MeOH (160 mL, 0.2 M). NaOMe (5.4 M) in MeOH (0.6 mL, 3.23 mmol, 0.1 eq.) was added dropwise, and the reaction was stirred at rt for 2 hours. The mixture was concentrated *in vacuo* and continued without purification to give the crude alcohol. The crude compound (9.96 g) was co-evaporated with toluene and dissolved in THF (110 mL, 0.3 M). The mixture was cooled to 0°C, followed by the portion wise addition of NaH (2.60 g, 64.6 mmol, 2.0 eq., 60% in mineral oil) and TBAI (1.19 g, 3.23 mmol, 0.1 eq.). NAPBr (9.30 g, 42.0 mmol, 1.3 eq.) was added and the reaction mixture was stirred from 0°C to rt overnight. A small amount of MeOH was added to the reaction mixture at 0°C. The mixture was diluted in EtOAc and the organic phase was washed with H_2O (3x), sat. aq. $NaHCO_3$ (1x), and brine (1x). The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Column chromatography (100% pentane to 45% EtOAc in pentane) yielded title compound **11** (10.4 g, 23.1 mmol) in 72% yield over 2 steps. $[\alpha]_D^{25}$= +77.8° (*c* 1.0, DCM); IR (neat, cm^{-1}): 2911, 2360, 1734, 1288, 1089, 1047, 973, 766; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.22 (s, 3H, Cq-O-C*H*$_3$), 3.42 – 3.71 (m, 2H, H-5), 3.78 – 3.99 (m, 4H, H-2, H-4, C*H*$_2$-CH), 4.84 (t, 1H, *J*= 4.4 Hz, H-3), 4.69 – 5.05 (m, 2H, C*H*$_2$-NAP), 5.07 – 5.23 (m, 2H, C*H*$_2$=CH), 5.78 (ddt, 1H, *J*= 17.2, 10.3, 5.7 Hz, CH_2=C*H*), 6.05 (d, 1H, *J*= 4.1 Hz, H-1), 7.30 – 7.98 (m, 12H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 50.6 (Cq-O-*C*H$_3$), 67.6 (C-5), 72.4 (*C*H$_2$-CH), 72.5 (*C*H$_2$-NAP), 77.3 (C-2/C-4), 78.0 (C-3), 78.4 (C-2/C-4), 104.6 (C-1), 117.4 (*C*H$_2$=CH), 124.1 (*Cq*-O-CH_3), 126.0 – 129.3 (C-arom), 133.3 (Cq-arom), 134.5 (CH_2=*C*H), 135.2 (Cq-arom), 136.9 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{27}H_{28}O_6Na$ 471.1778, found 471.1782.

5-*O*-allyl-2-*O*-benzoyl-3-*O*-(2-naphtylmethyl)-D-ribofuranoside (12)



Orthoester **11** (10.4 g, 23.1 mmol) was dissolved in a (v/v/v= 2:2:6) mixture of formic acid/H_2O/THF (230 mL, 0.1 M), and the reaction mixture was stirred at rt for 1.5 hours. DCM was added and 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 (100% DCM to 3% acetone in DCM) yielded title compound **12** (5.77 g, 17.5 mmol) as an β:α mixture with a ratio of ±3:1 in in 76% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.29 – 3.69 (m, 2H, H-5), 3.90 – 4.02 (m, 3H, CH_2-CHβ, CH_2-CH α, H-2 α), 4.25 – 4.37 (m, 1H, H-4β), 4.42 – 4.52 (m, 1H, H-3β, H-4α), 4.60 (d, 1H, *J*= 11.8 Hz, C*H*H-NAP), 4.80 (d, 1H, *J*= 10.8 Hz, CH*H*-NAP), 5.00 – 5.18 (m, 2H, CH_2=CHβ), 5.19 – 5.27 (m, 2H, CH_2=CHα), 5.44 (d, 1H, *J*= 6.7 Hz, H-1β), 5.51 (d, 1H, *J*= 4.5 Hz, H-2β), 5.52 – 5.56 (m, 1H, H-1α), 5.68 (ddt, 1H, *J*= 16.7, 10.0, 5.7 Hz, CH_2=C*H*β), 5.77 – 5.86 (m, 1H, CH_2=C*H*α), 7.28 – 8.47 (m, 14H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 69.2 (C-5β), 69.9 (C-5α), 72.4 (*C*H_2-CHβ), 72.5 (*C*H_2-CHα), 72.9 (C-2α), 73.2 (*C*H_2-NAP), 75.8 (C-2β), 76.8 (C-3β), 77.6 (C-3α), 81.0 (C-4β), 81.5 (C-4α), 96.3 (C-1α), 100.6 (C-1β), 117.4 (*C*H_2=CHα), 118.0 (*C*H_2=CHβ), 126.0 – 129.0 (C-arom), 129.8 (Cq-arom), 133.1 (Cq-arom), 133.2 (Cq-arom), 133.4 (CH_2=*C*H), 133.6, 133.7 (C-arom), 134.3, 134.6, 135.0 (Cq-arom), 165.8 (C=O); HRMS: $[M+Na]^+$ calcd for $C_{26}H_{26}O_6Na$ 457.1622, found 457.1627.

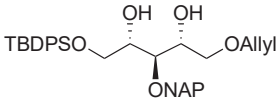
5-*O*-allyl-3-*O*-(2-naphtylmethyl)-D-ribitol (13)



Compound **12** (5.77 g, 17.5 mmol, 1.0 eq.) was dissolved in MeOH (88 mL, 0.2 M). The mixture was cooled to 0°C, followed by the portion wise addition of $NaBH_4$ (0.79 g, 21.0 mmol, 1.2 eq.). The reaction mixture was stirred for 30 minutes, followed by the addition of a small amount of EtOAc at 0°C. Subsequently, the mixture was concentrated under reduced pressure and co-evaporated with toluene. Column chromatography (100% pentane to 70% EtOAc in pentane) yielded the benzoylated product as crude (4.77 g). The crude compound was dissolved in MeOH (55 mL, 0.32 M), and NaOMe (5.4 M) in MeOH (0.35 mL, 1.75 mmol, 0.1 eq.) was added dropwise. The reaction was stirred at rt overnight, followed by the addition of H^+ amberlite. The H^+ amberlite was filtered off, and the mixture was concentrated *in vacuo*. Column chromatography (100% DCM to 10% MeOH in DCM) yielded triol **13** (3.51 g, 10.6 mmol) in 60% yield over 2 steps. $[\alpha]_D^{25}$ = +7.5° (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 3400, 2928, 2356, 1457, 1078; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.52 – 3.65 (m, 3H, H-3, H-5), 3.71 – 3.87 (m, 4H, H-1, 2x OH), 3.91 – 4.06 (m, 5H, H-2, H-4, CH_2-CH, OH), 4.77 (q, 2H, *J*= 20.0, 11.5 Hz, CH_2-Cq), 4.94 – 5.34 (m, 2H, CH_2=CH), 5.84 (ddt, 1H, *J*= 17.1, 10.2, 5.8 Hz, CH_2=C*H*), 7.30 – 7.96 (m, 7H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 63.4 (C-1), 71.1 (C-5), 71.4 (C-2/C-4), 72.4 (*C*H2-CH), 72.8 (C-2/C-4), 73.9 (*C*H_2-Cq), 79.5 (C-3), 117.7 (*C*H_2=CH), 126.0 – 128.3 (C-arom), 133.0 (Cq-arom), 133.3 (Cq-arom),

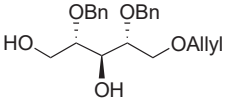
134.3 (CH_2=*C*H), 135.4 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{19}H_{24}O_5Na$ 355.1516, found 355.1526.

5-*O*-allyl-3-*O*-(2-naphtylmethyl)-1-*O*-(tert-butyldiphenylsilyl)-D-ribitol (14)



Compound **13** (3.51 g, 10.6 mmol, 1.0 eq.) was dissolved in dry DCM (106 mL, 0.1 M) and cooled to 0°C. TEA (13.4 mL, 95.4 mmol, 9.0 eq.) was added, followed by the addition of TBDPSCl (3.6 mL, 13.8 mmol, 1.3 eq.). The reaction mixture was stirred from 0°C to rt for 2 days. Next, MeOH was added at 0°C and the mixture was concentrated *in vacuo*. Column chromatography with neutralized silica (100% DCM to 3% MeOH in DCM) yielded title compound **14** (4.87 g, 8.53 mmol) in 81% yield. $[\alpha]_D^{25}$ = 6.2° (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 2931, 2364, 1684, 1507, 1457, 1112, 703; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.08 (s, 9H, 3x C*H*$_3$-Cq), 2.95 (d, 1H, *J*= 4.7 Hz, OH-2), 3.03 (d, 1H, *J*= 3.9 Hz, OH-4), 3.57 – 3.68 (m, 2H, H-5), 3.73 (t, 1H, *J*= 6.2 Hz, H-3), 3.79 – 3.92 (m, 2H, H-1), 3.93 – 3.97 (m, 1H, H-2), 4.00 (ddt, 2H, *J*= 5.5, 3.9, 1.4 Hz, C*H*$_2$-CH), 4.07 (p, 1H, *J*= 2.8 Hz, H-4), 4.75 (q, 2H, *J*= 32.0, 11.5 Hz, C*H*$_2$-NAP), 5.07 – 5.35 (m, 2H, C*H*$_2$=CH), 5.89 (ddt, 1H, *J*= 17.3, 10.4, 5.7 Hz, CH_2=C*H*), 7.24 – 7.89 (m, 17H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (CH_3-*C*q), 27.0 (3x *C*H$_3$-Cq), 64.9 (C-1), 71.0 (C-5), 71.8 (C-4), 72.4 (*C*H$_2$-CH), 72.8 (C-2), 74.0 (*C*H$_2$-Cq), 78.9 (C-3), 117.4 (*C*H$_2$=CH), 126.0 – 129.9 (Carom), 133.0 (Cq-arom), 133.1 (Cq-arom), 133.3 (Cq-arom), 135.6 (Cq-arom), 135.7 (CH_2=*C*H); HRMS: $[M+Na]^+$ calcd for $C_{35}H_{42}O_5SiNa$ 593.2694, found 593.2705.

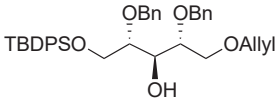
5-*O*-allyl-2,4-di-*O*-benzyl-D-ribitol (15)



Compound **14** (4.54 g, 7.95 mmol, 1.0 eq.) was dissolved in a (v/v= 7:1) mixture of dry THF/dry DMF (22.7 mL, 0.35 M). The mixture was cooled to 0°C, followed by the addition of BnBr (2.83 mL, 23.9 mmol, 3.0 eq.). NaH (0.95 g, 23.9 mmol, 3.0 eq., 60% in mineral oil) was added portion wise and the reaction mixture was stirred at 0°C for 3 hours. The mixture was diluted in Et_2O, and washed carefully with sat. aq. NH_4Cl (1x), H_2O (3x), and brine (1x). The organic phase was dried over $MgSO_4$, filtrated, concentrated under reduced pressure, and column chromatography (100% pentane to 8% EtOAc in pentane) yielded the crude benzylated compound (5.37 g) with small traces byproduct of the migrated TBDPS group to the second position. The crude product (4.98 g) was dissolved in a (v/v= 4:1) mixture of DCM/H_2O (66.3 mL, 0.1 M), followed by the addition of DDQ (2.26 g, 9.94 mmol, 1.5 eq.). The reaction mixture was stirred for 30 minutes at rt, followed by the addition of a small amount of sat. aq. $Na_2S_2O_3$. The mixture was diluted in DCM, and the organic phase was washed with sat. aq. $Na_2S_2O_3$ (2x), sat. aq. $NaHCO_3$ (2x), and brine (1x). The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Column

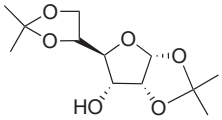
chromatography (100% pentane to 20% EtOAc in pentane) yielded the product (3.61 g) still with small traces of the byproduct. The crude product was dissolved in dry THF (35 mL, 0.17 M), followed by the addition of TBAF (1.0 M) in THF (8.84 mL, 8.84 mmol, 1.5 eq.). The reaction mixture was stirred at rt for 3 hours, and concentrated *in vacuo*. Column chromatography (100% DCM to 10% acetone in DCM) yielded title compound **15** (1.68 g, 4.51 mmol) with a yield of 62% over 3 steps. $[\alpha]_D^{25}$ = +0.3° (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 3430, 2872, 2364, 1684, 1560, 1507, 1457, 1070, 1027, 697; ^1H NMR (400 MHz, $CDCl_3$) δ= 2.90 (t, 1H, *J*= 6.1 Hz, OH-1), 3.42 (d, 1H, *J*= 4.9 Hz, OH-3), 3.55 – 3.70 (m, 3H, H-2, H-5), 3.72 – 3.78 (m, 3H, H-1, H-4), 3.94 (dd, 2H, *J*= 5.6, 1.4 Hz, CH_2-CH), 4.05 (dt, 1H, *J*= 6.0, 5.1 Hz, H-3), 4.45 – 4.75 (m, 4H, 2x CH_2-Bn), 5.05 – 5.32 (m, 2H, CH_2=CH), 5.86 (ddt, 1H, *J*= 17.3, 10.4, 5.6 Hz, CH_2=C*H*), 7.24 – 7.45 (m, 10H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 61.2 (C-1), 69.8 (C-5), 71.5 (*C*H_2-Bn), 71.9 (C-3) 72.1 (*C*H_2-Bn), 72.3 (*C*H_2-CH), 77.9 (C-4), 78.4 (C-2), 117.2 (*C*H_2=CH), 127.8 – 128.4 (C-arom), 134.5 (CH_2=*C*H), 138.1 (Cq-arom); HRMS: $[M+Na]^+$ calculated for $C_{22}H_{28}O_5Na$ 395.1834, found 395.1835.

5-*O*-allyl-2,4-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (16)



Compound **15** (1.68 g, 4.51 mmol, 1.0 eq.) was dissolved in dry DCM (45 mL, 0.1 M) and cooled to 0°C. TEA (3.77 mL, 27.1 mmol, 6.0 eq.) and TBDPSCl (1.28 mL, 4.91 mmol, 1.1 eq.) were added. The reaction mixture was stirred from 0°C to rt overnight, followed by the addition of MeOH at 0°C. The mixture was concentrated *in vacuo*, and column chromatography (100% pentane to 20% EtOAc in pentane) yielded title compound **16** (2.69 g, 4.40 mmol) in 98% yield. $[\alpha]_D^{25}$ = +4.7° (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 2858, 2360, 1654,1507, 1457, 1112, 740, 700; ^1H NMR (400 MHz, $CDCl_3$) δ= 1.07 (s, 9H, 3x CH_3-Cq), 3.12 (d, 1H, *J*= 5.3 Hz, O*H*), 3.60 – 3.81 (m, 4H, H-2, H-4, 2x H-5), 3.86 – 3.99 (m, 4H, 2x H-1, CH_2-CH), 4.05 – 4.11 (m, 1H, H-3), 4.46 – 4.75 (m, 4H, 2x CH_2-Bn), 5.11 – 5.33 (m, 2H, CH_2=CH), 5.87 (ddt, 1H, *J*= 17.3, 10.8, 5.6 Hz, CH_2=C*H*), 7.20 – 7.80 (m, 20H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (CH_3-*Cq*), 26.9 (3x *C*H_3-Cq), 64.3 (C-1), 70.4 (C-5), 71.9 (C-3), 72.1 – 72.2 (2x *C*H_2-Bn), 72.4 (*C*H_2-CH), 78.3 (C-4), 79.4 (C-2), 117.1 (*C*H_2=CH), 127.8 – 129.8 (C-arom), 133.1 (Cq-arom), 134.7 (CH_2=*C*H), 135.8 (C-arom), 138.6 (Cq-arom); HRMS: $[M+Na]^+$ calculated for $C_{38}H_{46}O_5SiNa$ 633.3012, found 633.3019.

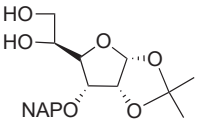
1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose (18)



1,2;5,6-di-O-isopropylidene-α-D-glucofuranose (**17**) (52.1 g, 200 mmol) was dissolved in a (v/v= 3:2) mixture of DMSO/Ac_2O (1.0 L, 0.2 M), and the reaction mixture was stirred at rt overnight. The mixture was concentrated *in vacuo* to give the crude ketone (51.7 g), which was used in the next step without further purification. The crude compound was dissolved in a (v/v= 7:3) mixture of EtOH/H_2O (1.0 L, 0.2 M). The mixture was

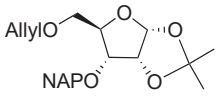
cooled to 0°C and $NaBH_4$ (26.0 g, 687 mmol, 3.4 eq.) was added portion wise. The reaction mixture was stirred from 0°C to rt overnight. The mixture was diluted with EtOAc, and washed with H_2O and brine. The water layer was extracted with EtOAc (5x), and the combined organic layers were dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. Column chromatography (100% DCM to 11% acetone in DCM) yielded title compound **18** (35.7 g, 137 mmol) in 69% yield over 2 steps. $[\alpha]_D^{25}$ = +31.7° (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 2986, 2360, 1684, 1507, 1457, 1215, 1059, 1017, 856; ^1H NMR (400 MHz, $CDCl_3$) δ= 1.38 (s, 3H, C*H*$_3$-Cq), 1.39 (s, 3H, C*H*$_3$-Cq), 1.47 (s, 3H, C*H*$_3$-Cq), 1.58 (s, 3H, C*H*$_3$-Cq), 2.65 (d, 1H, *J*= 8.4 Hz, O*H*), 3.83 (dd, 1H, *J*= 8.5, 4.6 Hz, H-4), 3.98 – 4.13 (m, 3H, H-3, 2x H-6), 4.32 (td, 1H, *J*= 6.6, 4.6 Hz, H-5), 4.62 (dd, 1H, *J*= 5.2, 3.8 Hz, H-2), 5.82 (d, 1H, *J*= 3.8 Hz, H-1); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 25.3 – 26.6 (4x *C*H_3-Cq), 65.9 (C-6), 72.5 (C-3), 75.6 (C-5), 79.1 (C-4), 79.7 (C-2), 104.0 (C-1), 109.9 (CH_3-*Cq*), 112.9 (CH_3-*Cq*); HRMS: $[M+Na]^+$ calcd for $C_{12}H_{20}O_6Na$ 283.1152, found 283.1163.

1,2-*O*-isopropylidene-3-*O*-(2-naphtylmethyl)-α-D-allofuranose (19)



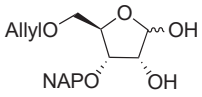
Compound **18** (36.5 g, 140 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved in a (v/v= 7:1) mixture of THF/DMF (470 mL, 0.3 M). The mixture was cooled to 0°C, and NaH (11.2 g, 260 mmol, 2.0 eq., 60% in mineral oil) and TBAI (5.2 g, 20 mmol, 0.1 eq.) were added portion wise. NAPBr (40.4 g, 182 mmol, 1.3 eq.) was added, and the reaction mixture was stirred from 0 °C to rt overnight. Subsequently, MeOH was added slowly at 0°C, and the mixture was concentrated under reduced pressure. The mixture was diluted in EtOAc, and the organic phase was washed with H_2O (5x), sat. aq. $NaHCO_3$ (1x), and brine (1x). The organic phase was dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The resulting crude was continued without purification. The crude compound (51.3 g) was dissolved in MeOH (2.56 L, 0.05 M), followed by the addition of *p*-TsOH·H_2O (2.43 g, 12.8 mmol, 0.1 eq.). The reaction mixture was stirred at rt for 3 hours, after which the reaction was quenched by the addition of TEA. The mixture was concentrated *in vacuo*, and column chromatography (100% DCM to 30% acetone in DCM) yielded title compound **19** (34.4 g, 95.4 mmol) in 75% yield over 2 steps. $[\alpha]_D^{25}$ = +69.4° (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 3439, 2935, 2360, 1653, 1560, 1507, 1457, 1020; ^1H NMR (400 MHz, $CDCl_3$) δ= 1.31 (s, 3H, C*H*$_3$-Cq), 1.57 (s, 3H, C*H*$_3$-Cq), 3.27 (s, 1H, OH-6), 3.51 (d, 1H, *J*= 3.9 Hz, OH-5), 3.66 (t, 2H, *J*= 4.5 Hz, H-6), 3.92 (dd, 1H, *J*= 8.9, 4.3 Hz, H-3), 3.99 (tt, 1H, *J*= 6.2, 3.3 Hz, H-5), 4.10 (dd, 1H, *J*= 8.8, 3.2 Hz, H-4), 4.46 (t, 1H, *J*= 4.1 Hz, H-2), 4.71 (d, 1H, *J*= 11.5 Hz, C*H*H-NAP), 4.91 (d, 1H, *J*= 11.5 Hz, CH*H*-NAP, 5.67 (d, 1H, *J*= 3.7 Hz, H-1), 7.35 – 7.91 (m, 7H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 26.5 – 26.8 (2x *C*H_3-Cq), 63.1 (C-6), 71.1 (C-5), 72.2 (*C*H_2-Cq), 77.0 (C-3), 77.4 (C-2), 78.9 (C-4), 104.1 (C-1), 113.0 (CH_3-*Cq*), 125.9 – 128.3 (C-arom), 133.10 (Cq-arom), 134.51 (Cq-arom); HRMS: $[M+Na]^+$ calculated for $C_{20}H_{24}O_6Na$ 383.1471, found 383.1468.

5-*O*-allyl-1,2-*O*-isopropylidene-3-*O*-(2-naphtylmethyl)-α-D-ribofuranoside (20)



Compound **19** (34.4 g, 95.4 mmol, 1.0 eq.) was dissolved in MeOH (950 mL, 0.1 M), and cooled to 0°C. A 0.2 M aqueous solution of $NaIO_4$ (600 mL, 0.16 M) was added and the reaction was stirred at 0°C for 1 hour. Subsequently, 200 mL ethylene glycol was added, and the solid side product was filtered off. The filtrate was diluted in DCM, and the organic phase was washed with H_2O, dried over $MgSO_4$, filtrated, concentrated *in vacuo* and continued without purification to give the crude aldehyde intermediate *in situ*. The crude compound was dissolved in MeOH (950 mL, 0.1 M), and cooled to 0°C. $NaBH_4$ (4.70 g, 124 mmol, 1.3 eq.) was added and the reaction mixture was stirred from 0°C to rt overnight. Subsequently, a small amount of acetone was added, and the mixture was concentrated under reduced pressure. The product was diluted in DCM, and washed with sat. aq. NH_4Cl. The water layer was extracted with DCM, and the combined organic layers were dried over $MgSO_4$, filtrated, concentrated *in vacuo*, and continued without purification to give the crude alcohol. The crude compound (36.9 g) was dissolved in a (v/v= 7:1) mixture of THF/DMF (320 mL, 0.35 M), and cooled to 0°C. NaH (6.70 g, 168 mmol, 1.5 eq., 60% in mineral oil) was added in portions and allyl bromide (11.6 mL, 134 mmol, 1.2 eq.) was added dropwise. The reaction was stirred from 0°C to rt for 4 hours, followed by the slow addition of a small amount of MeOH at 0°C. The reaction mixture was diluted in 200 mL Et_2O, and the organic phase was washed with 300 mL H_2O (5x). The organic layer was dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. Column chromatography (5% EtOAc in pentane to 20% EtOAc in pentane) yielded title compound **20** (36.3 g, 98.0 mmol) in 88% yield over 3 steps. $[\alpha]_D^{25}$ = +60.6° (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 2931, 2360, 1653, 1560, 1507, 1132, 1098, 873; ^1H NMR (400 MHz, $CDCl_3$) δ= 1.34 (s, 3H, C*H*$_3$-Cq), 1.60 (s, 3H, C*H*$_3$-Cq), 3.44 – 3.74 (m, 2H, H-5), 3.84 (ddd, 1H, *J*= 9.2, 4.5, 1.0 Hz, H-3), 3.87 – 4.00 (m, 2H, C*H*$_2$-CH), 4.19 (ddd, 1H, *J*= 9.1, 4.0, 2.1 Hz, H-4), 4.52 (t, 1H, *J*= 4.1 Hz, H-2), 4.70 (d, 1H, *J*= 12.2 Hz, C*H*H-NAP) 4.88 (d, 1H, *J*= 12.2 Hz, CH*H*-NAP), 5.04 – 5.23 (m, 2H, C*H*$_2$=CH), 5.71 (d, 1H, *J*= 3.7 Hz, H-1), 5.78 (ddt, 1H, *J*= 17.3, 10.4, 5.6 Hz, CH$_2$=C*H*), 7.36 – 8.34 (m, 7H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 26.4 – 26.7 (2x *C*H$_3$-Cq), 67.9 (C-5), 72.3 (*C*H$_2$-NAP, *C*H$_2$-CH), 76.9 (C-3), 77.2 (C-2), 77.8 (C-4), 104.0 (C-1), 112.7 (CH$_3$-*Cq*), 117.0 (*C*H$_2$=CH), 125.8 – 128.2 (C-arom), 133.0 – 133.1 (Cq-arom), 134.4 (CH$_2$=*C*H), 135.0 (Cq-arom); HRMS: $[M+Na]^+$ calculated for $C_{22}H_{26}O_5Na$ 393.1678, found 393.1670.

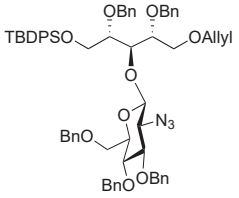
5-*O*-allyl-3-*O*-(2-naphtylmethyl)-D-ribofuranoside (21)



Compound **20** (32.3 g, 87.2 mmol) was dissolved in a (v/v/v= 2:2:6) mixture of THF/H_2O/formic acid (1.0 L, 0.087 M). The reaction mixture was stirred at rt for 4 hours. Subsequently, the mixture was diluted in DCM, and the organic layer was washed with H_2O (1x), sat. aq. $NaHCO_3$ (3x),

and brine (1x). The first H_2O layer was extracted with DCM. The organic phase was dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. Column chromatography (100% DCM to 16% acetone in DCM) yielded diol **21** (24.5 g, 74.1 mmol) as an α:β mixture with a ratio of ±1:1 in 85% yield. IR (neat, cm^{-1}): 2968, 2345, 1684, 1560, 1507, 1053; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.96 (d, 1H, *J*= 3.5 Hz, O*H*-2 α/β), 3.22 (d, 1H, *J*= 8.2 Hz, O*H*-2 α/β), 3.28 – 3.61 (m, 4H, H-5 α, β), 3.81 – 3.91 (m, 4H, CH_2-CH α, β), 3.92 – 4.01 (m, 1H, H-2 α/β), 4.08 (td, 1H, *J*= 4.0, 3.1 Hz, H-2 α/β), 4.10 – 4.15 (m, 1H, H-4 α/β), 4.20 (dt, 1H, *J*= 6.3, 3.5 Hz, H-4 α/β), 4.26 (td, 2H, *J*= 4.8, 1.4 Hz, H-3 α, β), 4.58 – 4.87 (m, 4H, CH_2-NAP α, β), 5.01 – 5.22 (m, 4H, CH_2=CH α, β), 5.21 – 5.33 (m, 2H, H-1 α, β), 5.74 (dddt, 2H, *J*= 18.6, 17.3, 10.4, 5.7 Hz, CH_2=C*H* α, β), 7.34 – 8.47 (m, 14H, H-arom α, β); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 69.7 – 70.0 (C-5 α, β), 70.8 (C-4 α), 72.3 – 73.1 (*C*H2-NAP α, β), 74.4 (C-2 β), 77.6 (C-3 β), 78.2 (C-2 α), 80.4 (C-3 α), 80.8 (C-4 β), 96.9 (C-1 α), 102.5 (C-1 β), 117.3 (*C*H_2=CH α), 117.9 (*C*H_2=CH β), 125.8 – 128.6 (C-arom), 133.2 (Cq-arom), 133.8 (CH_2=*C*H β), 134.3 (CH_2=*C*H α), 134.5 – 134.6 (Cq-arom); HRMS: $[M+Na]^+$ calculated for $C_{19}H_{22}O_5Na$ 353.1365, found 353.1367.

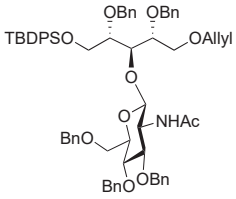
O-(3,4,6-tri-*O*-benzyl-2-azido-2-deoxy-β-D-glucopyranosyl)-(1-3)-5-*O*-allyl-2,4-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (23)



Alcohol **16** (2.35 g, 3.85 mmol, 1.0 eq.) was co-evaporated with toluene under a N_2 atmosphere, and dissolved in dry ACN (38 mL, 0.1 M). Activated molecular sieves (3Å) were added and the solution was stirred for 30 minutes. The mixture was cooled to -40°C and TMSOTf (70 μL, 0.39 mmol, 0.1 eq.) was added. Imidate **22** (3.58 g, 5.78 mmol, 1.5 eq.) was co-evaporated with toluene under a N_2 atmosphere and dissolved in dry ACN (2 mL, 0.15 M). The imidate stock solution was added to the reaction mixture and the mixture was stirred from -40°C to 0°C in a timeframe of 3 hours. Subsequently, a few drops of TEA were added and the mixture was diluted in DCM. The organic phase was washed with sat. aq. $NaHCO_3$:brine (1:1)(v/v), and the water layer was extracted with DCM. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Column chromatography (100% pentane to 14% Et_2O in pentane) yielded title compound **23** (3.29 g, 3.08 mmol) in 80% yield. $[\alpha]_D^{25}$ = -9.5° (*c* 1.0, $CDCl_3$); IR (neat, cm^{-1}): 2858, 2361, 2109, 1560, 1507, 1457, 1112, 1029, 737, 698; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.05 (s, 9H, 3x CH_3-Cq), 3.17 – 3.31 (m, 2H, H-2 GlcNAc, H-5 GlcNAc), 3.37 (t, 1H, *J*= 9.4 Hz, H-3 GlcNAc), 3.49 – 3.62 (m, 3H, H-4 GlcNAc, 2x H-6 GlcNAc), 3.77 (qd, 2H, *J*= 10.7, 4.3 Hz, 2x H-5 Rbo), 3.87 (dd, 1H, *J*= 10.2, 6.5 Hz, H-1 Rbo), 3.93 – 4.01 (m, 4H, H-1 Rbo, H-2 Rbo, CH_2-CH), 4.00 – 4.08 (m, 1H, H-4 Rbo), 4.20 (t, 1H, *J*= 4.6 Hz, H-3 Rbo), 4.26 – 4.94 (m, 11H, H-1 GlcNAc, 5x CH_2-Bn), 5.07 – 5.34 (m, 2H, CH_2=CH), 5.89 (ddt, 1H, *J*= 17.2, 10.4, 5.7 Hz, CH_2=C*H*), 7.15 – 7.75 (m, 35H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (CH_3-*C*q), 27.0 (3x *C*H_3-Cq), 65.8 (C-1 Rbo), 67.2 (C-2

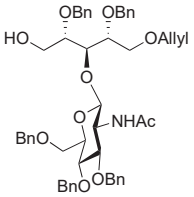
GlcNAc), 68.7 (C-6 GlcNAc), 69.8 (C-5 Rbo), 72.3 (*C*H_2-CH), 72.3 – 75.6 (5x *C*H_2-Bn), 75.2 (C-5 GlcNAc), 77.1 (C-3 Rbo), 77.9 (C-4 GlcNAc), 78.6 (C-4 Rbo), 81.4 (C-2 Rbo), 83.3 (C-3 GlcNAc), 101.1 (C-1 GlcNAc), 116.9 (*C*H_2=CH), 127.4 – 129.7 (C-arom), 133.6 – 133.8 (Cq-arom), 135.0 (CH_2=*C*H), 135.7 – 135.8 (C-arom), 138.0 – 139.0 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{65}H_{73}N_3O_9SiNa$ 1091.5014, found 1091.5054.

O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1-3)-5-*O*-allyl-2,4-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (24)



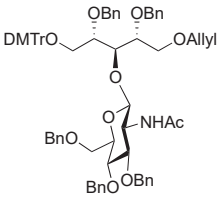
Compound **23** (3.42 g, 3.20 mmol, 1.0 eq.) was dissolved in a (v/v= 5:1) mixture of pyridine/H_2O (55 mL, 0.058 M), followed by the addition of TEA (0.2 mL). Propane dithiol (1.60 mL, 16.0 mmol, 5.0 eq.) was added, and the reaction mixture was stirred at rt overnight. The mixture was concentrated under reduced pressure, and co-evaporated with toluene (3x). The crude compound was dissolved in a (v/v= 2:1) mixture of pyridine/Ac_2O (55 mL, 0.058 M), and the reaction mixture was stirred at rt overnight. A small amount of MeOH was added at 0°C and the mixture was diluted in EtOAc. The organic phase was washed with aq. $CuSO_4$ (1x), sat. aq. $NaHCO_3$ (2x), and brine (1x). The organic layer was dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. Column chromatography (100% pentane to 50% EtOAc in pentane) yielded title compound **24** (2.99 g, 2.76 mmol) in 86% yield over 2 steps. $[\alpha]_D^{25}$ = +5.5° (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 2858, 1560, 1457, 1112, 1070, 1029, 738, 698; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.05 (s, 9H, 3x C*H*$_3$-Cq TBDPS), 1.74 (s, 3H, C*H*$_3$-Acetyl), 3.72 – 3.81 (m, 1H, H-2 GlcNAc), 3.86 – 3.94 (m, 2H, C*H*$_2$-CH), 3.16 – 4.29 (m, 12H, H-3 GlcNAc, H-4 GlcNAc, H-5 GlcNAc, 2x H-6 GlcNAc, 2x H-1 Rbo, H-2 Rbo, H-3 Rbo, H-4 Rbo, 2x H-5 Rbo), 4.40 – 4.94 (m, 11H, H-1 GlcNAc, 5x C*H*$_2$-Bn), 5.07 – 5.33 (m, 2H, C*H*$_2$=CH), 5.66 (d, 1H, *J*= 8.4 Hz, N*H*), 5.86 (ddt, 1H, *J*= 17.3, 10.6, 5.5 Hz, CH_2=C*H*), 6.96 – 7.74 (m, 35H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (CH_3-*Cq* TBDPS), 23.5 (*C*H_3-Acetyl), 27.0 (3x *C*H_3-Cq), 55.6 (C-2 GlcNAc), 65.6 – 70.0 (C-6 GlcNAc, C-1 Rbo, C-5 Rbo), 72.3 (*C*H_2-CH), 73.1 – 74.9 (5x *C*H_2-Bn), 75.5 – 83.4 (C-3 GlcNAc, C-4 GlcNAc, C-5 GlcNAc, C-2 Rbo, C-3 Rbo, C-4 Rbo), 102.8 (C-1 GlcNAc), 116.8 (*C*H_2=CH), 127.5 – 135.8 (C-arom), 134.9 (CH_2=*C*H), 133.5 – 138.8 (Cq-arom), 170.2 (C=O); HRMS: $[M+Na]^+$ calculated for $C_{67}H_{77}NO_{10}SiNa$ 1106.5214, found 1106.5228.

O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1-3)-5-*O*-allyl-2,4-di-*O*-benzyl-D-ribitol (25)



Compound **24** (2.99 g, 2.76 mmol, 1.0 eq.) was dissolved in dry THF (17 mL, 0.17 M). TBAF (1M in THF) (8.4 mL, 8.28 mmol, 3.0 eq.) was added dropwise, and the reaction mixture was stirred at rt overnight. The mixture was concentrated *in vacuo*, and column chromatography (10% EtOAc pentane to 80% EtOAc in pentane) yielded title compound **25** (2.23 g, 2.64 mmol) in 96% yield. $[\alpha]_D^{25}$ = +12.4° (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}): 2866, 2360, 1550, 1507, 1457, 1072, 737, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.69 (s, 3H, C*H*$_3$-Acetyl), 3.21 (dd, 1H, *J*= 10.1, 8.0 Hz, H-4 GlcNAc), 3.32 – 3.50 (m, 6H, H-3 Rbo, H-3 GlcNAc, H-5 GlcNAc, H-6 GlcNAc, O*H*), 3.54 – 3.63 (m, 2H, 2x H-1 Rbo), 3.78 – 3.97 (m, 5H, 2x H-5 Rbo, H-2 GlcNAc, C*H*$_2$-CH), 4.07 (ddt, 1H, *J*= 6.8, 4.2, 2.5 Hz, H-4 Rbo), 4.22 (dd, 1H, *J*= 9.9, 2.2 Hz, H-2 Rbo), 4.36 – 4.78 (m, 11H, H-1 GlcNAc, 5x C*H*$_2$-Bn), 5.10 – 5.30 (m, 2H, C*H*$_2$=CH), 5.50 (d, 1H, *J*= 8.6 Hz, N*H*), 5.86 (ddt, 1H, *J*= 17.3, 10.6, 5.4 Hz, CH_2=C*H*), 7.12 – 7.81 (m, 25H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 23.3 (*C*H$_3$-Acetyl), 54.9 (C-2 GlcNAc), 57.9 (C-5 Rbo), 68.7 (C-1 Rbo), 69.5 (C-6 GlcNAc), 70.7 (*C*H$_2$-Bn), 72.1 (*C*H$_2$-CH), 73.1 – 75.0 (4x *C*H$_2$-Bn), 74.7 (C-3 Rbo), 77.3 – 78.2 (C-3 GlcNAc, C-5 GlcNAc), 78.9 (C-2 Rbo), 79.1 (C-4 Rbo), 83.7 (C-4 GlcNAc), 103.8 (C-1 GlcNAc), 116.9 (*C*H$_2$=CH), 127.7 – 128.8 (C-arom), 134.7 (CH_2=*C*H), 137.6 – 138.4 (5x Cq-arom), 170.2 (C=O); HRMS: $[M+Na]^+$ calcd for $C_{51}H_{59}NO_{10}Na$ 868.4037, found 868.4061.

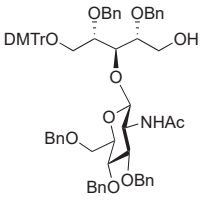
O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1-3)-5-*O*-allyl-2,4-di-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol (26)



Compound **25** (2.23 g, 2.64 mmol, 1.0 eq.) was co-evaporated with toluene under a N_2 atmosphere, and dissolved in dry DCM (26.4 mL, 0.1 M). The mixture was cooled to 0°C. TEA (0.55 mL, 3.96 mmol, 1.5 eq.) and DMTrCl (1.08 g, 3.17 mmol, 1.2 eq.) were added, and the reaction mixture was stirred from 0°C to rt overnight, after which a small amount of MeOH was added at 0°C. The mixture was diluted in DCM, and the organic phase was washed with sat. aq. $NaHCO_3$:brine (1:1). The water layer was extracted with DCM, and the combined organic layers were dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. Column chromatography with neutralized silica (100% pentane to 50% EtOAc in pentane) yielded title compound **26** (1.83 g, 1.60 mmol) in 61% yield. $[\alpha]_D^{25}$ = +0.9° (*c* 1.0, DCM); IR (neat, cm^{-1}): 2866, 2368, 1560, 1508, 1457, 1067, 736, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.87 (s, 3H, C*H*$_3$-Acetyl), 3.25 – 3.45 (m, 3H, 2x H-1 Rbo, H-3 Rbo), 3.52 (t, 1H, *J*= 9.1 Hz, H-4 GlcNAc), 3.57 – 3.64 (m, 3H, H-5 GlcNAc, 2x H-6 GlcNAc), 3.64 – 3.75 (m, 2H, 2x H-5 Rbo), 3.72 (d, 6H, *J*= 2.0 Hz, 2x C*H*$_3$O), 3.74 – 3.89 (m, 1H, H-2 GlcNAc), 3.90 (dt, 1H, *J*= 6.5, 3.2 Hz, H-3 GlcNAc), 3.96 (dd, 2H, *J*= 5.3, 1.6 Hz, C*H*$_2$-CH), 4.00 – 4.07 (m, 2H, H-2 Rbo,

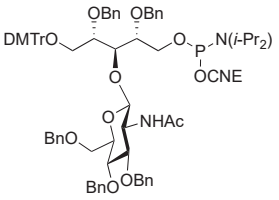
H-4 Rbo), 4.28 – 4.93 (m, 11H, H-1 GlcNAc, 5x CH_2-Bn), 5.10 – 5.39 (m, 2H, CH_2=CH), 5.93 (ddt, 1H, *J*= 17.3, 10.5, 5.3 Hz, CH_2=C*H*), 6.58 (d, 1H, *J*= 9.3 Hz, N*H*), 6.73 – 7.59 (m, 38H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 23.7 (*C*H_3-Acetyl), 55.9 (2x *C*H_3O), 56.6 (C-2 GlcNAc), 66.4 (C-1 ribitol), 70.3 (C-6 GlcNAc), 71.2 (C-5 Rbo), 72.7 (*C*H_2-CH), 73.1 – 75.6 (5x *C*H_2-Bn), 75.8 (C-3 Rbo), 78.9 (C-2 Rbo/C-4 Rbo), 79.5 – 79.6 (C-3 GlcNAc, C-4 GlcNAc), 80.8 (C-2 Rbo/C-4 Rbo), 83.7 (C-5 GlcNAc), 87.0 (Cq-DMTr), 102.2 (C-1 GlcNAc), 114.0 (C-arom), 116.7 (*C*H_2=CH), 128.4 – 129.3 (C-arom), 131.19 (C-arom), 136.4 (CH_2=*C*H), 137.1 (Cq-arom), 137.3 (Cq-arom), 139.5 – 140.1 (Cq- arom), 146.6 (Cq-arom), 159.5 (Cq-arom), 170.6 (C=O); HRMS: $[M+Na]^+$ calcd for $C_{72}H_{77}NO_{12}Na$ 1170.5343, found 1170.5374.

O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1-3)-2,4-di-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol (27)



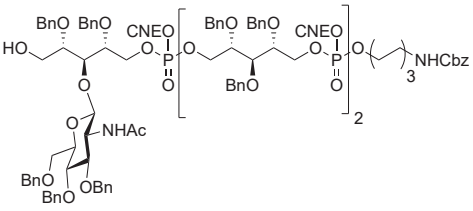
Compound **26** (1.83 g, 1.60 mmol, 1.0 eq.) was co-evaporated with distilled toluene (2x) under a N_2 atmosphere, and dissolved in freshly distilled dry THF (16 mL, 0.1 M). The mixture was degassed with N_2. Next,(1,5-Cyclooctadiene)bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate (0.015 g, 0.01 eq.) was added, and the mixture was degassed with N_2. The reaction mixture was then purged with H_2 gas for ±7 seconds. Then the mixture was degassed with N_2 to remove the excess of H_2 gas. The mixture was stirred at rt under a N_2 atmosphere for 75 minutes. THF (8.0 mL), sat. aq. $NaHCO_3$ (8.0 mL), and iodine (0.61 g, 2.40 mmol, 1.5 eq.) were added and the reaction was stirred for 15 minutes. Next, sat. aq. $Na_2S_2O_3$ was added to the reaction mixture until the dark colour disappeared. The mixture was diluted in EtOAc, and the organic phase was washed with sat. aq. $NaHCO_3$:brine (1:1). Column chromatography with neutralized silica (100% pentane to 95% EtOAc in pentane) yielded title compound **27** (1.67 g, 1.51 mmol) in 94% yield. $[\alpha]_D^{25}$ = +1.7° (*c* 1.0, DCM); IR (neat, cm^{-1}): 3278, 2931, 2355, 1560, 1507, 1457, 1066, 736, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.88 (s, 3H, C*H*$_3$-Acetyl), 3.03 (s, O*H*), 3.17 – 3.45 (m, 3H, 2x H-1 Rbo, H-3 Rbo), 3.54 (t, 1H, *J*= 9.2 Hz, H-4 GlcNAc), 3.60 - 3.84 (m, 12H, H-5 GlcNAc, 2x H-6 GlcNAc, 2x H-5 Rbo, H-2 GlcNAc, 2x C*H*$_3$-O), 3.99 (t, 1H, *J*= 4.6 Hz, H-4 Rbo), 4.18 (dt, 1H, *J*= 7.4, 3.5 Hz, H-2 Rbo), 4.32 – 4.94 (m, 11H, H-1 GlcNAc, 5x C*H*$_2$-Bn), 6.73 (d, 1H, *J*= 9.3 Hz, N*H*), 6.70 – 7.77 (m, 38H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 23.7 (*C*H_3-Acetyl), 55.9 (2x *C*H_3O), 56.6 (C-2 GlcNAc), 61.4 (C-5 Rbo), 66.4 (C-1 Rbo), 70.2 (C-6 GlcNAc), 72.7 – 75.6 (5x *C*H_2-Bn), 75.8 (C-3 Rbo), 79.4 (C-4 GlcNAc), 79.8 (C-4 Rbo), 80.5 (C-3 GlcNAc), 81.4 (C-2 Rbo), 83.8 (C-5 GlcNAc), 86.9 (Cq-DMTr), 102.8 (C-1 GlcNAc), 114.0 (C-arom), 127.7 – 131.2 (C-arom), 137.1 (Cq-arom), 139.6 – 140.2 (Cq-arom), 146.6 (Cq-arom), 159.5 (Cq-arom), 171.0 (C=O); HRMS: $[M+Na]^+$ calculated for $C_{69}H_{73}NO_{12}Na$ 1130.5030, found 1130.5054.

O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1-3)-2,4-di-*O*-benzyl-5-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol (28)



Phosphoramidite **28** was prepared based on the general procedure for phosphoramidite synthesis (starting with 0.90 mmol, 1.2 eq. of alcohol **27** and 1.08 mmol, 1.2 eq. 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite). Column chromatography with neutralized silica (100% pentane to 40% EtOAc in pentane) yielded title compound **28** (1.00 g, 0.76 mmol) in 85% yield. $[\alpha]_D^{25}$ = - 0.8° (*c* 1.0, DCM); IR (neat, cm^{-1}): 2931, 2314, 1684, 1560, 1507, 1457, 1066, 737, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 0.91 – 1.33 (m, 12H, 4x CH_3-CH), 1.85 (d, 3H, *J*= 9.3 Hz, CH_3-Acetyl), 2.55 (dt, 2H, *J*= 35.9, 6.0 Hz, P-O-CH_2), 3.14 – 3.34 (m, 2H, 2x H-1 Rbo), 3.34 – 3.43 (m, 1H, H-3 Rbo), 3.45 – 3.54 (m, 1H, H-4 GlcNAc), 3.54 – 3.65 (m, 5H, 2x CH_3-C*H*, H-5 GlcNAc, 2x H-6 GlcNAc), 3.71 (d, 6H, *J*= 1.5 Hz, 2x CH_3O), 3.66 – 3.90 (m, 5H, NC-CH_2, H-2 GlcNAc, 2x H-5 Rbo), 3.90 – 4.15 (m, 3H, H-3 GlcNAc, H-2 Rbo, H-4 Rbo), 4.24 – 4.92 (m, 11H, H-1 GlcNAc, 5x CH_2-Bn), 6.43 (t, 1H, *J*= 10.0 Hz, N*H*), 6.64 – 7.58 (m, 38H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 21.1 (NC-*C*H_2), 24.9 – 25.3 (4x *C*H_3-CH), 43.8 – 43.9 (2x CH_3-C*H*), 55.9 (2x *C*H_3O), 56.5 (C-2 GlcNAc), 59.1 – 59.4 (C-5 Rbo), 66.5 (C-1 Rbo), 70.28 (C-6 GlcNAc), 73.1 – 75.6 (5x *C*H_2-Bn), 75.9 (C-3 Rbo), 79.0 (C-3 GlcNAc), 79.5 (C-4 GlcNAc), 80.9 – 81.2 (C-2 Rbo, C-4 Rbo), 83.74 (C-5 GlcNAc), 86.93 (Cq-DMTr), 102.2 – 102.5 (C-1 GlcNAc), 114.0 (C-arom), 119.8 (NC*q*-CH_2), 127.6 – 131.2 (C-arom), 137.1 – 137.3 (Cq-arom), 139.7 (Cq-arom), 140.2 (Cq-arom), 146.6 (Cq-arom), 159.5 (Cq-arom), 170.5 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 149.2, 149.8.

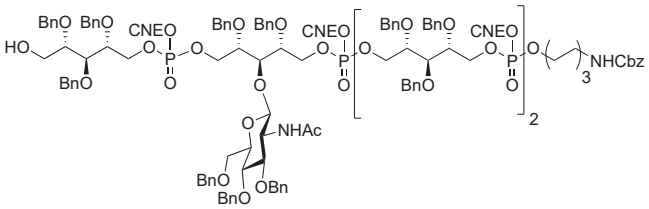
Trimer (37)



Trimer **37** was synthesized based on the general procedure for phosphoramidite coupling (starting with 0.40 mmol (1.0 eq.) of dimer **36** and 0.56 mmol (1.4 eq.) of phosphoramidite **28**). Size exclusion column chromatography yielded title compound **37** (0.75 g, 0.33 mmol) in 88% yield. IR (neat, cm^{-1}): 3567, 2915, 2360, 1717, 1684, 1570, 1456, 1266, 1027, 740, 697; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.38 – 1.26 (m, 4H, CH_2-3 linker and CH_2-4 linker), 1.44 (qd, 2H, *J*= 9.5, 8.8, 4.6 Hz, CH_2-2 linker), 1.60 (p, 2H, *J*= 6.7 Hz, CH_2-5 linker), 1.89 (d, 3H, *J*= 3.1 Hz, CH_3-NHAc), 2.55 – 2.78 (m, 6H, 3x NC-CH_2), 3.09 (q, 2H, *J*= 6.7 Hz, NH-CH_2-1 linker), 3.33 (td, 1H, *J*= 6.8, 2.8 Hz, OH Rbo), 3.57 – 3.81 (m, 2H, 2x H-6 GlcNAc), 3.86 – 4.19 (m, 9H, CH_2-6 linker, H-2 GlcNAc, 3x P-O-CH_2), 3.48 – 4.19 (m, 12H, H-3 GlcNAc, H-4 GlcNAc, H-5 GlcNAc, 3x H-2 Rbo, 3x H-3 Rbo, 3x H-4 Rbo), 4.21 – 4.46 (m, 10H, 4x H-1 Rbo, 6x H-5 Rbo), 4.49 – 4.90 (m, 23H, H-1 GlcNAc, 11x CH_2-Bn),

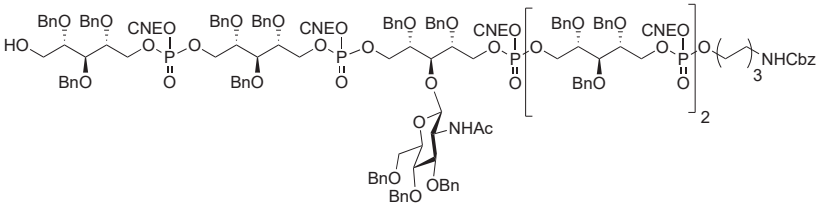
5.08 (s, 2H, CH_2-Cbz), 5.83 (t, 1H, *J*= 6.1 Hz, N*H*), 6.78 (t, 1H, *J*= 7.0 Hz, N*H*Ac), 7.03 – 7.59 (m, 60H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1 – 20.3 (3x NC-*C*H_2), 23.8 (*C*H_3-NHAc), 25.8 – 26.9 (C-3 linker, C-4 linker), 30.5 (C-2 linker), 30.9 (C-5 linker), 41.5 (NH-*C*H_2 (C-1 linker)), 56.1 (C-2 GlcNAc), 60.54 (C-1 Rbo), 63.1 – 63.3 (3x P-O-*C*H_2), 66.6 (*C*H_2-Cbz), 67.5 – 68.4 (2x C-1 Rbo, 3x C-5 Rbo), 69.0 (C-6 linker), 70.1 (C-6 GlcNAc), 72.4 – 75.7 (11x *C*H_2-Bn), 75.3 – 80.2 (C-3 GlcNAc, C-4 GlcNAc, C-5 GlcNAc, 3x C-2 Rbo, 3x C-3 Rbo, 3x C-4 Rbo), 102.9 (C-1 GlcNAc), 118.7 (N*Cq*-CH_2), 128.5 – 129.4 (C-arom), 139.2 – 139.7 (Cq-arom), 157.4 (C=O Cbz), 170.9 (C=O Ac); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.0, 0.0, 0.1, 0.2, 0.4; HRMS: $[M+2H]^{2+}$ calculated for $C_{123}H_{144}N_5O_{29}P_3$ 1124.4591, found 1124.4628.

Tetramer (38)



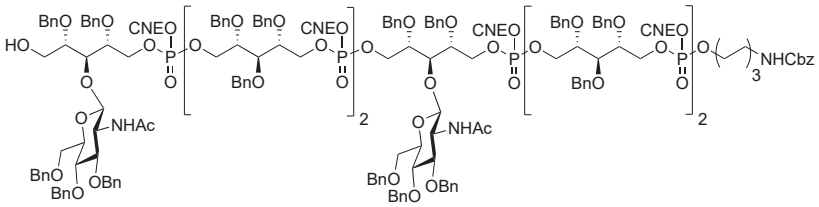
Tetramer **38** was synthesized based on the general procedure for phosphoramidite coupling (starting with 0.28 mmol (1.0 eq.) of trimer **37** and 0.42 mmol (1.5 eq.) of phosphoramidite **29.** Size exclusion column chromatography yielded title compound (0.72 g, 0.26 mmol) in 92% yield. IR (neat, cm^{-1}): 3567, 2931, 2360, 1717, 1684, 1540, 1507, 1457, 1026, 740, 697; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.19 – 1.36 (m, 4H, CH_2-3 linker, CH_2-4 linker), 1.39 – 1.51 (m, 2H, CH_2-2 linker), 1.61 (q, 2H, *J*= 6.9 Hz, CH_2-5 linker), 1.93 (s, 3H, CH_3-NHAc), 2.44 – 2.74 (m, 8H, 4x NC-CH_2), 3.09 (q, 3H, *J*= 6.8 Hz, NH-*CH*$_2$ (CH_2-1 linker), OH Rbo), 3.68 – 3.86 (m, 4H, 2x H-6 GlcNAc, 2x H-1 Rbo), 3.86 – 4.20 (m, 11H, H-2 GlcNAc, CH_2-6 linker, 4x P-O-CH_2), 3.39 – 4.17 (m, 15H, H-3 GlcNAc, H-4 GlcNAc, H-5 GlcNAc, 4x H-2 Rbo, 4x H-3 Rbo, 4x H-4 Rbo), 4.18 – 4.49 (m, 14H, 6x H-1 Rbo, 8x H-5 Rbo), 4.50 – 4.87 (m, 29H, H-1 GlcNAc, 14x CH_2-Bn), 5.08 (s, 2H, CH_2-Cbz), 5.83 (t, 1H, *J*= 6.0 Hz, N*H*-Cbz), 6.91 – 7.09 (m, 1H, N*H*Ac), 7.16 – 7.53 (m, 75H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1 – 20.3 (4x NC-*C*H_2), 23.7 (*C*H_3-NHAc), 25.8 – 26.9 (C-3 linker, C-4 linker), 30.5 (C-2 linker), 30.9 (C-5 linker), 41.5 (NH-*C*H_2 (C-1 linker)), 56.2 (C-2 GlcNAc), 61.6 (C-1 Rbo), 63.1 – 63.6 (4x P-O-*C*H_2), 66.7 (*C*H_2-Cbz), 67.5 – 68.3 (3x C-1 Rbo, 4x C-5 Rbo), 69.1 (C-6 linker), 70.0 (C-6 GlcNAc), 72.8 – 75.6 (14x *C*H_2-Bn), 75.9 – 83.9 (C-3 GlcNAc, C-4 GlcNAc, C-5 GlcNAc, 4x C-2 Rbo, 4x C-3 Rbo, 4x C-4 Rbo), 103.1 (C-1 GlcNAc), 118.5 – 118.6 (4x N*Cq*-CH_2), 128.5 – 129.4 (C-arom), 138.5 – 139.8 (Cq-arom), 157.4 (C=O Cbz), 170.9 (C=O Ac); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.0, 0.1, 0.1, 0.1, 0.4, 0.4, 0.4, 0.8; HRMS: $[M+2H]^{2+}$ calculated for $C_{152}H_{176}N_6O_{36}P_4$ 1393.0549, found 1393.0594.

Pentamer (39)



Pentamer **39** was synthesized based on the general procedure for phosphoramidite coupling (starting with 0.26 mmol (1.0 eq.) of tetramer **38** and 0.39 mmol (1.5 eq.) of phosphoramidite **29**). Size exclusion column chromatography yielded title compound **39** (0.75 g, 0.23 mmol) in 87% yield. IR (neat, cm^{-1}): 3567, 2921, 2355, 1717, 1550, 1507, 1457, 1266, 1027, 740, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.22 – 1.37 (m, 4H, CH_2-3 linker and CH_2-4 linker), 1.40 – 1.51 (m, 2H, CH_2-2 linker), 1.62 (h, 2H, *J*= 6.7 Hz, CH_2-5 linker), 1.95 (d, 3H, *J*= 1.6 Hz, CH_3-NHAc), 2.47 – 2.76 (m, 10H, 5x NC-CH_2), 3.11 (q, 2H, *J*= 6.7 Hz, NH-CH_2 (CH_2-1 linker)), 3.19 (s, 1H, OH Rbo), 3.72 – 3.87 (m, 4H, 2x H-6 GlcNAc, 2x H-1 Rbo), 3.87 – 4.21 (m, 13H, H-2 GlcNAc, CH_2-6 linker, 5x P-O-CH_2), 3.42 – 4.21 (m, 18H, H-3 GlcNAc, H-4 GlcNAc, H-5 GlcNAc, 5x H-2 Rbo, 5x H-3 Rbo, 5x H-4 Rbo), 4.20 – 4.50 (m, 18H, 8x H-1 Rbo, 10x H-5 Rbo), 4.50 – 4.89 (m, 35H, H-1 GlcNAc, 17x CH_2-Cq), 5.09 (s, 2H, CH_2- Cbz), 5.86 (t, 1H, *J*= 6.0 Hz, N*H* linker), 7.04 (dt, 1H, *J*= 26.4, 7.1 Hz, N*H*Ac), 7.16 – 7.47 (m, 90H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1 – 20.3 (5x NC-CH_2), 23.7 (CH_3-NHAc), 25.8 – 26.9 (C-3 linker, C-4 linker), 30.5 (C-2 linker), 30.9 (C-5 linker), 41.5 (NH-CH_2 (C-1 linker)), 56.2 (C-2 GlcNAc), 61.6 (C-1 Rbo), 63.1 – 63.6 (5x P-O-CH_2), 66.6 (CH_2-Cbz), 67.5 – 68.4 (4x C-1 Rbo, 5x C-5 Rbo), 69.0 (C-6 linker), 70.0 (C-6 GlcNAc), 72.8 – 75.6 (17x CH_2-Bn), 75.9 – 83.9 (C-3 GlcNAc, C-4 GlcNAc, C-5 GlcNAc, 5x C-2 Rbo, 5x C-3 Rbo, 5x C-4 Rbo), 103.1 (C-1 GlcNAc), 118.6 (N*Cq*-CH_2), 128.5 – 129.4 (C-arom), 138.5 – 139.8 (Cq-arom), 157.4 (C=O Cbz), 170.8 (C=O Ac); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.0, 0.1, 0.1, 0.1, 0.2, 0.2, 0.4, 0.5, 0.5, 0.5, 0.8, 0.8; HRMS: $[M+2H]^{2+}$ calculated for $C_{181}H_{208}N_7O_{43}P_5$ 1661.6508, found 1661.6534.

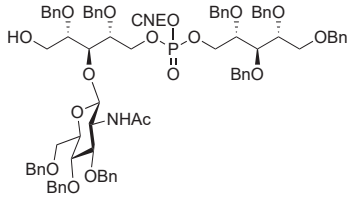
Hexamer 40



Hexamer **40** was synthesized based on the general procedure for phosphoramidite coupling (starting with 0.080 mmol (1.0 eq.) of pentamer **39** and 0.110 mmol (1.5 eq.) of phosphoramidite **28**). Size exclusion column chromatography yielded title compound

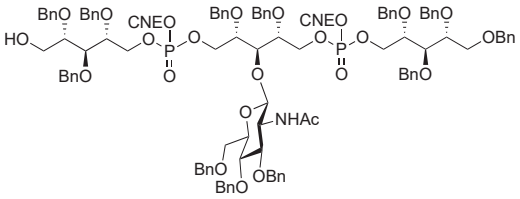
40 (0.339 g, 0.080 mmol) in a quantitative yield. IR (neat, cm^{-1}): 3546, 2909, 2314, 1717, 1550, 1506, 1457, 1266, 1027, 740, 697; ^{1}H NMR (400 MHz, CD3CN) δ= 1.20 – 1.34 (m, 4H, CH_2-3 linker, CH_2-4 linker), 1.37 – 1.47 (m, 2H, CH_2-2 linker), 1.59 (h, 2H, *J*= 6.6 Hz, CH_2-5 linker), 1.86 (d, 3H, *J*= 3.0 Hz, CH_3-NHAc), 1.90 (s, 3H, CH_3-NHAc), 2.47 – 2.74 (m, 12H, 6x NC-CH_2), 3.07 (q, 2H, *J*= 6.7 Hz, CH_2-1 linker), 3.28 (t, 1H, *J*= 5.0 Hz, OH Rbo), 3.54 – 3.80 (m, 4H, 4x H-6 GlcNAc), 3.68 – 3.75 (m, 2H, 2x H-1 Rbo), 3.81 – 4.16 (m, 16H, H_2-6 linker, 2x H-2 GlcNAc, 6x P-O-CH_2), 3.39 – 4.16 (m, 24H, 2x H-3 GlcNAc, 2x H-4 GlcNAc, 2x H-5 GlcNAc, 6x H-2 Rbo, 6x H-3 Rbo, 6x H-4 Rbo), 4.16 – 4.44 (m, 22H, 10x H-1 Rbo, 12x H-5 Rbo), 4.43 – 4.83 (m, 46H, 2x H-1 GlcNAc, 22x CH_2-Bn), 5.06 (s, 2H, CH_2-Cbz), 5.77 (t, 1H, *J*= 6.1 Hz, N*H* linker), 6.72 (t, 1H, *J*= 8.0 Hz, NHAc), 6.89 – 7.04 (m, 1H, NHAc), 7.11 – 7.54 (m, 115H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1 – 20.3 (6x NC-*C*H_2), 23.7 (2x NHAc), 25.8 – 26.9 (C-3 linker, C-4 linker), 30.5 (C-2 linker), 30.9 (C-5 linker), 41.5 (NH-*C*H_2 (C-1 linker)), 56.1 – 56.2 (2x C-2 GlcNAc), 60.6 (C-1 Rbo), 63.1 – 63.6 (6x P-O-*C*H_2), 66.7 (*C*H_2-Cbz), 67.5 – 68.4 (5x C-1 Rbo, 6x C-5 Rbo), 69.01 (C-6 linker), 70.1 (2x C-6 GlcNAc), 72.4 – 75.7 (22x *C*H_2-Bn), 75.9 – 83.9 (2x C-3 GlcNAc, 2x C-4 GlcNAc, 2x C-5 GlcNAc, 6x C-2 Rbo, 6x C-3 Rbo, 6x C-4 Rbo), 103.0 – 103.2 (2x C-1 GlcNAc), 118.5 – 118.7 (6x N*Cq*-CH_2), 128.5 – 129.5 (C-arom), 138.6 – 139.8 (Cq-arom), 157.36 (C=O Cbz), 170.8 – 170.9 (2x C=O Ac); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.0, 0.0, 0.0, 0.1, 0.1, 0.2, 0.2, 0.4, 0.4, 0.5, 0.8, 0.8; HRMS: $[M+3H]^{3+}$ calculated for $C_{232}H_{266}N_9O_{55}P_6$ 1415.2257, found 1415.2283.

Dimer (31)



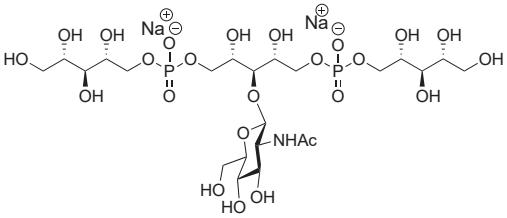
According to the general procedure for phosphoramidite coupling, alcohol **27** (602 mg; 0.54 mmol; 1.0 eq.) was coupled with phosphoramidite **30** (543 mg; 0.76 mmol; 1.4 eq.) and the title compound was synthesized in 68% yield (530 mg; 0.37 mmol). IR (neat, cm^{-1}): 3736, 2872, 2360, 1717, 1654, 1560, 1521, 1457, 1042, 740, 697; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.84 (d, 3H, *J*= 2.9 Hz, CH_3-NAc), 2.58 (t, 2H, *J*= 6.0 Hz, CH_2-cyanoethyl), 3.19 (t, 1H, *J*= 5.6 Hz, O*H*), 3.53 – 3.62 (m, 1H, H-3 GlcNAc), 3.67 – 3.76 (m, 2H, CH_2-Rbo), 3.82 - 3.87 (m, 1H, H-2 GlcNAc), 3.98 – 4.08 (m, 2H, CH_2-cyanoethyl), 3.43 – 4.34 (m, 16H, H-4 GlcNAc, H-5 GlcNAc, 2x H-6 GlcNAc, 3x CH_2-Rbo, 2x H-2 Rbo, 2x H-3 Rbo, 2x H-4 Rbo), 4.42 – 4.81 (m, 19H, H-1 GlcNAc, 9x CH_2-Bn), 6.63 (d, 1H, *J*= 9.4 Hz, N*H*), 7.16 – 7.52 (m, 45H, H-arom); ^{13} C NMR (101 MHz, CD_3CN) δ= 20.2 (CH_2-cyanoethyl), 23.7 (CH_3-NAc), 56.1 (C-2 GlcNAc), 63.3 (CH_2-cyanoethyl), 60.6 – 70.6 (C-6 GlcNAc, 2x C-1 Rbo, 2x C-5 Rbo), 72.4 – 75.7 (9x CH_2-Bn), 75.4 -83.8 (C-3 GlcNAc, C-4 GlcNAc, C-5 GlcNAc, 2x C-2 Rbo, 2x C-3 Rbo, 2x C-4 Rbo), 103.0 (C-1 GlcNAc), 118.7 (Cq-cyanoethyl), 128.5 – 129.4 (C-arom), 139.3 – 139.7 (Cq-arom), 170.9 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.4, 0.2; HRMS: $[M+H]^+$ calcd for $C_{84}H_{94}N_2O_{17}P$ 1433.6285, found 1433.6323.

Trimer (32)



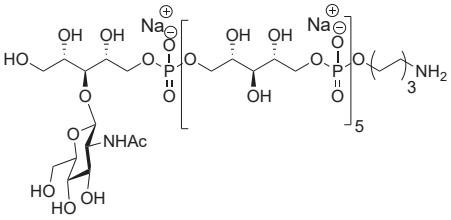
According to the general procedure for phosphoramidite coupling, alcohol **31** (530 mg; 0.37 mmol; 1.0 eq.) was coupled with phosphoramidite **29** (481 mg; 0.52 mmol; 1.4 eq.) and the title compound was synthesized in 78% yield (567 mg; 0.288 mmol). IR (neat, cm^{-1}): 3736, 2883, 2355, 1717, 1560, 1457, 1027, 740, 697; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.90 (d, *J*= 2.4 Hz, 3H, CH_3-NAc), 2.48 - 2.62 (m, 4H, 2x CH_2-cyanoethyl), 3.01 (s, 1H, OH), 3.45 (ddt, 1H, *J*= 9.5 Hz, 6.5 Hz, 3.1 Hz, H-4 GlcNAc), 3.53 – 3.62 (m, 1H, H-5 GlcNAc), 3.63 – 3.81 (m, 5H, H-3 GlcNAc, 2x H-6 GlcNAc, CH_2-Rbo), 3.85 – 4.15 (m, 14H, H-2 GlcNAc, 2x CH_2-cyanoethyl, 3x H-2 Rbo, 3x H-3 Rbo, 3x H-4 Rbo), 3.96 – 4.39 (m, 10H, 5x CH_2-Rbo), 4.38 – 4.99 (m, 25H, H-1 GlcNAc, 12x CH_2-Bn), 6.84 – 7.03 (m, 1H, N*H*Ac), 7.19 – 7.45 (m, 60H, H-arom); ^{13}C NMR (101 MHz, CD_3CN) δ= 20.1 – 20.3 (2x CH_2-cyanoethyl), 23.7 (CH_3-NAc), 56.2 (C-2 GlcNAc), 70.0 (C-6 GlcNAc), 61.6 – 70.6 (2x CH_2-cyanoethyl, 6x CH_2-Rbo), 72.8 – 75.7 (12x CH_2-Bn), 75.9 (C-4 GlcNAc), 78.4 – 80.2 (3x C-2 Rbo, 3x C-3 Rbo, 3x C-4 Rbo), 80.7 (C-3 GlcNAc), 84.0 (C-5 GlcNAc), 103.2 (C-1 GlcNAc), 118.6 (2x Cq-cyanoethyl), 128.5 – 129.4 (C-arom), 139.1 – 139.8 (Cq-arom), 170.8 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.0, 0.3, 0.4, 0.4, 0.4, 0.8, 0.8; HRMS: $[M+H]^+$ calcd for $C_{113}H_{126}N_3O_{24}P_2$ 1971.8235, found 1971.8245.

Trimer (1)



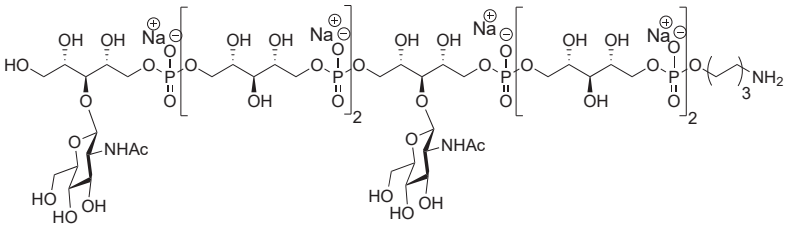
Compound **32** (0.200 g; 0.101 mmol) was deprotected according to the general procedure for global deprotection affording the target compound in 77% yield (63.8 mg; 77.1 µmol). ^{1}H NMR (400 MHz, D_2O) δ= 1.96 (s, 3H, CH_3-NAc), 3.25 - 4.08 (m, 27H, 6x CH_2-Rbo, 9x CH-Rbo, H-2, H-3, H-4, H-5, 2x H-6), 4.51 (d, 1H, *J*= 8.4 Hz, H-1); ^{13}C NMR (101 MHz, D_2O) δ= 22.28 (CH_3-NAc), 55.55 (C-2), 60.46 - 66.64 (C-6, 6x CH_2-Rbo), 69.35 - 80.50 (C-3, C-4, C-5, CH-Rbo), 101.68 (C-1), 174.69 (C=O); ^{31}P NMR (162 MHz, D_2O) δ= 1.9, 1.8; HRMS: $[M+H]^+$ calcd for $C_{23}H_{48}NO_{24}P_2$ 784.2036, found 784.2042.

Hexamer (2)



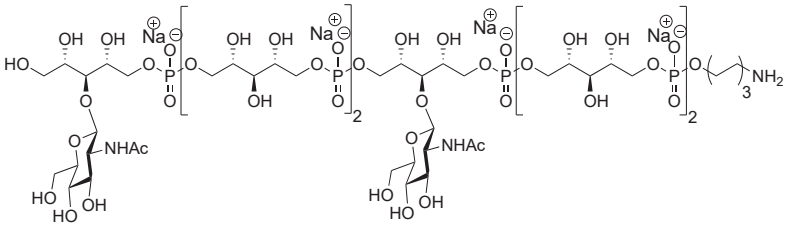
Hexamer **43** (6.9 mg, 1.94 μmol) was deprotected according to the general procedure for global deprotection yielding compound **2** (3.0 mg, 1.73 μmol) in 88% yield. ^{1}H NMR (500 MHz, D_2O) δ= 1.38 – 1.44 (m, 4H, 2x CH_2-hexylspacer), 1.66 (h, 4H, *J*= 7.4 Hz, 2x CH_2-hexylspacer), 2.08 (s, 3H, CH_3-NAc), 2.98 (t, 2H, *J*= 7.6 Hz, CH_2-N hexylspacer), 3.41 – 4.11 (m, 50H, 12x CH_2-Rbo, 18x CH-Rbo, CH_2-O, H-2, H-3, H-4, H-5, 2x H-6), 4.63 (d, 1H, *J*= 8.4 Hz, H-1); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 2.0, 1.8, 1.8; HRMS: $[M+2H]^{2+}$ calculated for $C_{44}H_{96}N_2O_{48}P_6$ 803.17736, found 803.17766.

Hexamer (3)



Hexamer **44** (4.2 mg, 1.07 μmol) was deprotected according to the general procedure for global deprotection yielding compound **3** (2.5 mg, 1.30 mol) in quantitative yield. NMR data is in agreement with the reported data for hexamer **3**. HRMS: $[M+2NH_4]^{2+}$ calculated for $C_{52}H_{114}N_5NaO_{53}P_6$ 932.73457, found 932.17575.

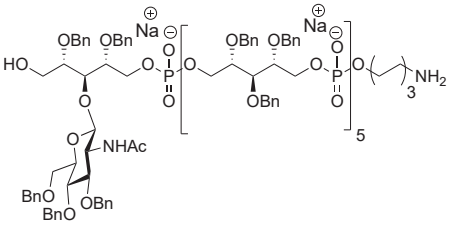
Hexamer (3)



Hexamer **40** (0.040 mmol) was deprotected according to general procedure for global deprotection. All aromatic groups were removed after the reaction mixture was stirred 3x for a full week. The first two times, after work-up, NMR still showed aromatic signals. After all aromatic groups were removed, the product was purified by size-exclusion chromatography (HW40, dimensions: 16/60 mm, eluent: 0.15 M NH_4HCO_3), and the product was co-evaporated 3 times with MiliQ water to remove NH_4HCO_3 traces. The product

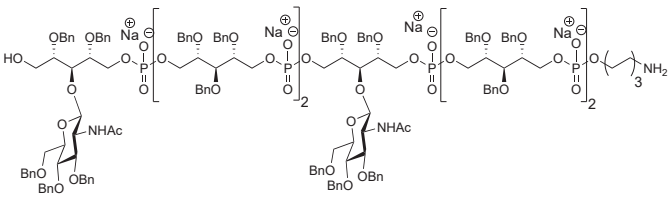
was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with MiliQ water and MeOH before use), yielding title compound **3** (0.0545 g, 0.0281 mmol) in 70% yield over 2 steps. ^{1}H NMR (500 MHz, D_2O) δ= 1.33 – 1.44 (m, 4H, CH_2-3 linker, CH_2-4 linker), 1.57 – 1.71 (m, 4H, CH_2-2 linker, CH_2-5 linker), 2.07 (s, 6H, 2x CH_3-NAc), 2.98 (t, 2H, *J*= 7.5 Hz, NH-CH_2 (CH_2-1 linker)), 3.66 – 3.76 (m, 2H, 2x H-2 GlcNAc), 3.84 – 3.90 (m, 2H, CH_2-6 linker), 3.36 – 4.16 (m, 54H, 2x H-3 GlcNAc, 2x H-4 GlcNAc, 2x H-5 GlcNAc, 4x H-6 GlcNAc, CH_2-Cbz, 12x CH_2-Rbo, 6x H-2 Rbo, 6x H-3 Rbo, 6x H-4 Rbo), 4.61 (d, 1H, *J*= 3.7 Hz, H-1 GlcNAc), 4.63 (d, 1H, *J*= 3.7 Hz, H-1 GlcNAc); ^{13}C-APT NMR (126 MHz, D_2O) δ= 22.5 (2x CH_3-NAc), 24.6 – 25.3 (C-3 linker, C-4 linker), 26.8 – 29.6 (C-2 linker, C-5 linker), 39.6 (NH-CH_2 (C-1 linker)), 55.7 – 55.8 (2x C-2 GlcNAc), 60.7 – 66.7 (CH_2-Cbz, 2x C-6 GlcNAc, 12x CH_2-Rbo), 69.7 – 81.0 (2x C-3 GlcNAc, 2x C-4 GlcNAc, 2x C-5 GlcNAc, 6x C-2 Rbo, 6x C-3 Rbo, 6x C-4 Rbo), 101.9 (2x C-1 GlcNAc), 175.0 (2x C=O); ^{31}P NMR (202 MHz, D_2O) δ= 1.8, 1.9, 1.9, 2.0; HRMS: $[M+2H]^{2+}$ calculated for $C_{52}H_{109}N_3O_{53}P_6$ 904.7170, found 904.7176.

Hexamer (43)



Hexamer **43** was synthesized based on the general procedure for solid phase synthesis (starting with 10.0 μmol of the universal linker **41**). Title compound **43** (6.9 mg, 1.95 μmol) was successfully synthesized with a total yield of 20%. ^{1}H NMR (400 MHz, MeOD) δ= 1.24 - 1.49 (m, 8H, 4x CH_2-hexylspacer), 1.95 (s, 3H, CH_3-NHAc), 2.73 (t, 2H, *J*= 7.6 Hz, CH_2-N hexylspacer), 3.47 – 4.79 (m, 91H, 12x CH_2-Rbo, 18x CH-Rbo, CH_2-O, H-1, H-2, H-3, H-4, H-5, 2x H-6, 20x CH_2-Bn), 7.10 – 7.35 (m, 99H, H-arom); ^{31}P NMR (162 MHz, MeOD) δ= 0.3, 0.1, -0.2, -0.4; HRMS: $[M+2H]^{2+}$ calculated for $C_{184}H_{216}N_2O_{48}P_6$ 1704.65018, found 1704.64992.

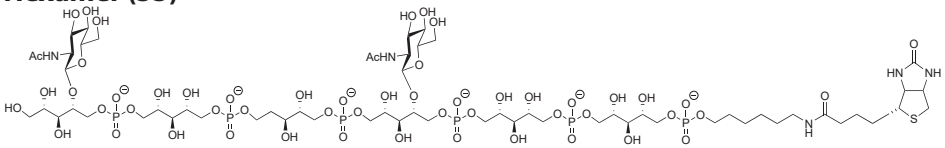
Hexamer (44)



Hexamer **44** was synthesized based on the general procedure for solid phase synthesis (starting with 10.0 μmol of the universal linker **41**). Title compound **44** (4.2 mg, 1.07 μmol) was successfully synthesized with a total yield of 11%. ^{1}H NMR (400 MHz, CD_3CN) δ= 1.22 – 1.48 (m, 8H, CH_2-2 linker, CH_2-3 linker, CH_2-4 linker, CH_2-5 linker), 1.93 (s, 3H, CH_3-NAc), 2.66 – 4.75 (m, 84H, NH-CH_2 (CH_2-1 linker), H-1 GlcNAc, H-2 GlcNAc, H-3 GlcNAc, H-4 GlcNAc, H-5 GlcNAc, H-6 GlcNAc,

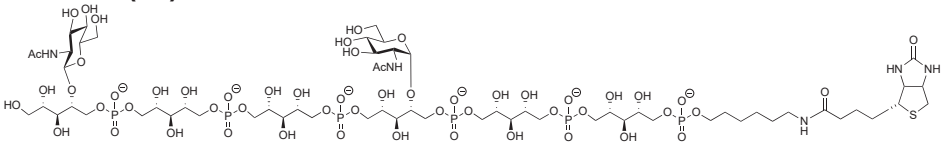
12x CH_2-Rbo, 6x H-2 Rbo, 6x H-3 Rbo, 6x H-4 Rbo, 6x H-6 Rbo, 20x C*H*$_2$-Bn), 7.10 – 7.31 (m, 101H, N*H*Ac, H-arom); ^{31}P NMR (162 MHz, CD_3CN) δ= 1.0, 1.2, 1.5, 1.6; HRMS: $[M+2H]^{2+}$ calculated for $C_{206}H_{241}N_3O_{53}P_6$ 1896.23686, found 1896.23710.

Hexamer (55)



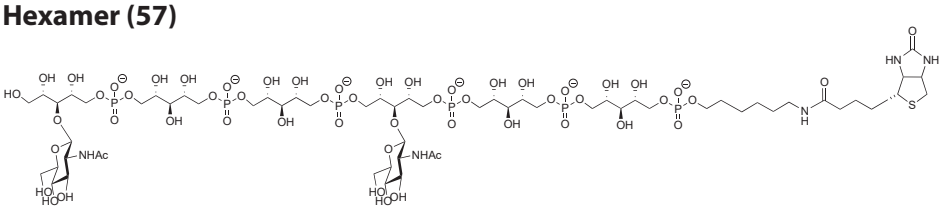
The title compound was synthesized according to the general procedure for biotinylation yielding (0.55 mg; 0.25 µmol) the product in 51% yield. ^{1}H NMR (500 MHz, D_2O) δ=1.36 – 1.42 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.50 – 1.53 (m, 2H, CH_2-hexylspacer/CH_2-biotin), 1.61 – 1.69 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 2.08 (s, 6H, CH_3 NAc), 2.24 (t, 2H, *J*= 7.0 Hz, CH_2-C=O), 2.77 (d, 1H, *J*= 13.0 Hz, S-C*H*H), 2.98 - 3.01 (m, 2H, S-C*HH),* 3.17 (hept, 2H, *J*= 6.7 Hz, CH_2-N), 3.33 (dt, 1H, *J*= 9.7 Hz, *J*= 5.2 Hz, S-CH), 3.40 - 3.49 (m, 4H, CH-Rbo, CH-GlcNAc), 3.51 - 3.64 (m, 3H, CH-Rbo, CH-GlcNAc, C*H*H-Rbo), 3.70 - 4.17 (m, 51H, CH-Rbo, CH_2-Rbo CH-GlcNAc, H-6, 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*= 8.01 Hz, *J*= 4.9 Hz, S-CH_2-C*H),* 4.73 (dd, 2H, *J*= 10.0 Hz, *J*= 5.0 Hz, H-1 βGlcNAc); ^{31}P NMR (202 MHz, D_2O) δ= 1.7, 1.8, 2.0.

Hexamer (56)



The title compound was synthesized according to the general procedure for biotinylation yielding (0.70 mg; 0.32 µmol) the product in 44% yield. ^{1}H NMR (500 MHz, D_2O) δ=1.28 – 1.42 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.42 – 1.69 (m, 10H, CH_2-hexylspacer/CH_2-biotin), 2.04 (s, 3H, CH_3 NAc) - 2.06 (s, 3H, CH_3 NAc), 2.21 - 2.26 (m, 2H, CH_2-C=O), 2.71 – 2.78 (d, 1H, J= 13.2 Hz, S-C*H*H), 2.97 (dd, 1H, *J*= 13.1 Hz, 5.0 Hz, S-C*HH*), 3.15 (hept, 2H, *J*= 6.7 Hz, CH_2-N), 3.29 - 3.33 (m, 1H, S-C*HH),* 3.37 - 4.15 (56H, CH-Rbo, CH_2-Rbo CH-GlcNAc, H-6, CH_2-O-hexylspacer), 4.40 (dd, 1H, *J*= 7.9, 4.5 Hz, S-CH-C*H*), 4.58 (dd, 1H, *J*= 7.9, 4.8 Hz, S-CH_2-C*H*), 4.70 (d, 1H, *J*= 8.6 Hz, H-1 βGlcNAc), 5.03 (d, *J*= 3.6 Hz, 1H, H-1 αGlcNAc); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 1.9, 1.8, 1.6.

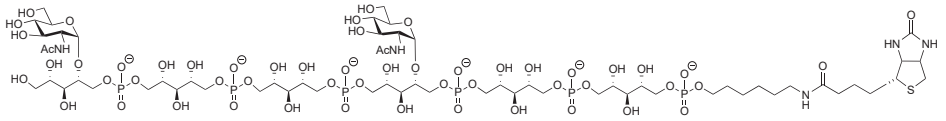
Hexamer (57)



The title compound was synthesized according to the general procedure for biotinylation starting with 4.92 mg; 2.54 μmol of the hexamer yielding (5.2 mg; 2.40 μmol) the product in 93% yield.

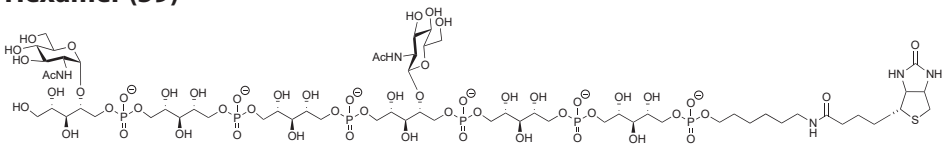
^{1}H NMR (500 MHz, D_2O) δ= 1.27 – 1.43 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.46 – 1.54 (m, 2H, 2H, CH_2-hexylspacer/CH_2-biotin), 1.53 – 1.75 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 2.07 (d, 6H, *J*= 1.0 Hz, CH_3 NAc), 2.22 (t, 2H, *J*= 7.2 Hz, CH_2-C=O), 2.74 – 2.77 (m, 1H, S-C*H*H), 2.97 (ddd, 2H, *J*= 13.1, 5.0, 2.0 Hz, S-C*HH*), 3.11 – 3.20 (m, 2H, CH_2-N), 3.31 (ddd, 1H, *J*= 13.5, 6.7, 4.1 Hz, S-CH), 3.38 - 3.55 (m, 2H, CH-Rbo/CH-GlcNAc/C*H*H-Rbo), 3.64 - 4.13 (m, 56H, CH-Rbo, CH_2-Rbo, CH-GlcNAc, H-6, CH_2-O-hexylspacer), 4.41 (td, *J* = 7.4, 4.4 Hz, 1H, S-CH-C*H*), 4.55 – 4.65 (m, 3H, S-CH_2-C*H,* 2x H-1); ^{31}P NMR (202 MHz, D_2O) δ= 1.3, 1.3, 1.2, 1.1, 1.1, 1.0, 0.9, 0.8.

Hexamer (58)



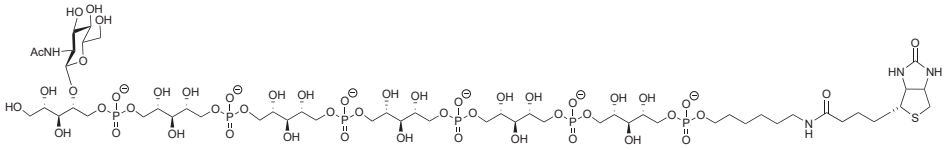
The title compound was synthesized according to the general procedure for biotinylation yielding (0.74 mg; 0.34 μmol) the product in 68% yield. ^{1}H NMR (500 MHz, D_2O) δ= 1.29 – 1.46 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.46 – 1.55 (m, 2H, CH_2-hexylspacer/CH_2-biotin), 1.55 – 1.77 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 2.05 (s, 3H, CH_3 NAc), 2.06 (s, 3H, CH_3 NAc), 2.24 (t, 2H, *J*= 7.1 Hz, CH_2-C=O), 2.78 (d, 1H, *J*= 13.1 Hz, S-C*H*H), 2.96 – 3.02 (m, 2H, S-C*HH*), 3.18 (h, 2H, *J*= 6.8 Hz, CH_2-N), 3.33 (dt, 1H, *J*= 10.0, *J*= 5.3 Hz, S-CH), 3.45 – 3.52 (m, 4H, CH-Rbo, CH-GlcNAc), 3.58 – 3.65 (m, 2H, C*H*H-Rbo/CH-Rbo/CH-GlcNAc), 3.71 – 4.17 (m, 52H, CH-Rbo, CH_2-Rbo, CH-GlcNAc, H-6, CH_2-O-hexylspacer), 4.42 (dd, 1H, *J*= 8.0, *J*= 4.5 Hz, S-CH-C*H*), 4.60 (dd, 1H, *J*= 7.9 Hz, *J*= 4.9 Hz, S-CH_2-C*H*), 5.03 (d, 1H, *J*= 3.6 Hz, H-1 αGlcNAc), 5.06 (d, 1H, *J*= 3.7 Hz, H-1 αGlcNAc); ^{31}P NMR (202 MHz, D_2O) δ= 1.6, 1.6, 1.8, 2.0.

Hexamer (59)



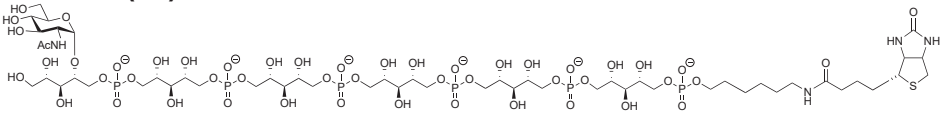
The title compound was synthesized according to the general procedure for biotinylation yielding (0.70 mg; 0.32 µmol) the product in 65% yield. ^{1}H NMR (500 MHz, D_2O) δ=1.31 – 1.44 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.51 (m, 2H, CH_2-hexylspacer/CH_2-biotin), 1.55 – 1.76 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 2.05 (s, 3H, CH_3 NAc), 2.08 (s, 3H, CH_3 NAc), 2.24 (t, 2H, *J* = 7.1 Hz, CH_2-C=O), 2.78 (d, 1H, *J*= 13.0 Hz, S-C*H*H), 2.96 – 3.02 (m, 2H, S-C*HH*), 3.18 (h, 2H, *J* = 6.7 Hz, CH_2-N), 3.33 (dt, 1H *J*= 10.0, *J*= 5.3 Hz, S-CH), 3.42 – 3.51 (m, 4H, CH-Rbo, CH-GlcNAc), 3.52 – 3.66 (m, 3H, CH-Rbo, CH-GlcNAc, C*H*H-Rbo), 3.72 – 4.17 (m, 51H, CH-Rbo, CH_2-Rbo CH-GlcNAc, H-6, CH_2-O-hexylspacer), 4.42 (dd, 1H, *J*= 8.0, *J*= 4.5 Hz, S-CH-C*H*), 4.60 (dd, 1H, *J*= 8.0, *J*= 4.9 Hz, S-CH_2-C*H*), 4.74 (d, 1H, *J*= 5.0 Hz, H-1 βGlcNAc) 5.03 (d, 1H, *J*= 3.7 Hz, H-1 αGlcNAc); ^{31}P NMR (202 MHz, D_2O) δ= 1.6, 1.7, 1.8, 2.0.

Hexamer (60)



The title compound was synthesized according to the general procedure for biotinylation yielding (0.87 mg; 0.44 µmol) the product in 89% yield. ^{1}H NMR (500 MHz, D_2O) δ=1.31 – 1.44 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.49 – 1.54 (m, 2H, CH_2-hexylspacer/CH_2-biotin), 1.59 – 1.67 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 2.08 (s, 3H, CH_3 NAc), 2.24 (t, 2H, *J*= 7.2 Hz, CH_2-C=O), 2.78 (d, 1H, *J*= 13.1 Hz, S-C*H*H), 2.97 – 3.02 (m, 2H, S-C*HH*), 3.14 – 3.20 (m, 2H, CH_2-N), 3.33 (dd, 1H, *J*= 9.4 Hz, *J*= 4.9 Hz, S-CH), 3.41 – 3.48 (m, 3H, CH-Rbo, CH-GlcNAc), 3.56 – 3.63 (m, 3H, CH-Rbo, CH-GlcNAc, C*H*H-Rbo), 3.70 – 4.16 (m, 52H, CH-Rbo, CH_2-Rbo CH-GlcNAc, H-6, 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*= 8.0, *J*= 5.0 Hz, S-CH_2-C*H*), 4.73 (d, 1H, *J*= 5.0 Hz, H-1 βGlcNAc); ^{31}P NMR (202 MHz, D_2O) δ= 1.7, 1.8, 2.0.

Hexamer (61)



The title compound was synthesized according to the general procedure for biotinylation yielding (0.75 mg; 0.38 µmol) the product in 76% yield. ^{1}H NMR (500 MHz, D_2O) δ= 1.29 – 1.45 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.49 – 1.54 (m, 2H, CH_2-hexylspacer/CH_2-biotin), 1.55 – 1.76 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 2.05 (s, 3H, CH_3 NAc), 2.24 (t, 2H, *J*= 7.1 Hz, CH_2-C=O), 2.78 (d, 1H, *J*= 13.1 Hz, S-C*H*H), 2.96 – 3.03 (m, 2H, S-C*HH*), 3.17 (hept, 2H, *J*= 6.7 Hz, CH_2-N), 3.33 (dt, 1H, *J*= 9.8 Hz, *J*= 5.2 Hz, S-CH), 3.48 (t, 1H, *J*= 9.6 Hz, CH-Rbo/CH-GlcNAc/C*H*H-Rbo), 3.60 – 3.65 (m, 1H, CH-Rbo/CH-GlcNAc/C*H*H-Rbo), 3.72 – 4.16 (m, 56H, CH-Rbo, CH_2-Rbo, CH-GlcNAc, H-6, CH_2-O-hexylspacer), 4.42 (dd, 1H, *J*= 8.0 Hz, *J*= 4.5 Hz, S-CH-C*H*), 4.61 (dd, 1H, *J*= 7.9 Hz, *J*= 4.9 Hz, S-CH_2-C*H*), 5.03 (d, 1H, *J*= 3.6 Hz, H-1 αGlcNAc); ^{31}P NMR (202 MHz, D_2O) δ= 1.6, 1.8, 2.0.

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