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Synthesis of ribitol phosphate based wall teichoic acids

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Synthesis of Ribitol Phosphate based Wall Teichoic acids

Sara Ali

Synthesis of Ribitol Phosphate based Wall Teichoic acids

PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. dr. ir. H. Bijl,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 10 februari 2022
klokke 15:00 uur

door

Sara Ali
geboren te Al-Najaf in 1990

PROMOTIECOMMISSIE

Promotoren:	Prof. dr. G.A. van der Marel
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	Prof. dr. A. Silipo (University of Naples Federico II)

It is well to give when asked,
but it is better to give unasked,
through understanding.

- Khalil Gibran -

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LIST OF ABBREVIATIONS

4FB-OSu	4-formylbenzoate *N*-hydroxysuccinimide ester
5-BTT	5-benzylthio-1*H*-tetrazole
Ac	acetyl
ACN	acetonitrile
ADH	adipic acid dihydrazide
AgOTf	silver triflate
Ala	alanine
aq	aqueous
ASPS	automated solid phase synthesis
Bn	benzyl
BOP	benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate
BSA	bovine serum albumin
Bz	benzoyl
CAN	ceric ammonium nitrate
Cbz	carboxybenzyl
CDI	*C. difficile* infections
CNE	2-cyanoethyl
COD	1,5-cyclooctadiene
conc	concentrated
CPG	controlled pore glass
CRM_{197}	cross-reacting material 197
CSO	(1S)-(+)-(10-camphorsulfonyl)-oxaziridine
DAG	diacylglycerol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCA	dichloroacetic acid
DCC	*N,N'*-Dicyclohexylcarbodiimide
DCI	4,5-dicyanoimidazole
DCM	dichloromethane
DDQ	2,3-dichloro-4,5-dicyano-1,4-benzoquinone
DIC	*N,N'*-Diisopropylcarbodiimide
DIPEA	*N,N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMTr	dimethoxytrityl
DSG	disuccinimidyl glutarate
EDAC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
eq	molar equivalents
Et_2O	diethyl ether
EtCN	propionitrile
EtOAc	ethyl acetate
ETT	5-ethylthio-1*H*-tetrazole
F-Pse	perfluorooctylpropylsulfonyl ethyl
FA	Freund's adjuvant
Fmoc	fluorenylmethyloxycarbonyl
GalNAc	*N*-acetyl galactosamine
Glc	glucose
GlcNAc	*N*-acetyl glucosamine
GroP	glycerolphosphate
h	hour
HA	healthcare associated
HladM	detoxified α-hemolysin of *S. aureus*
hMNCs	human mononuclear cells
HPLC	High Performance Liquid Chromatography
HSA	human serum albumin
I-Pr	isopropyl
Ig	immunoglobulin
KLH	keyhole limpet hemocyanin
Lev	levulinoyl
LPS	lipopolysaccharide
LTA	lipoteichoic acid
MBL	mannose binding lectin
MeOH	methanol
MP	methoxyphenol
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	molecular sieves
NaOMe	sodium methoxide
Nap	naphtyl
NMR	nuclear magnetic resonance
NIS	*N*-Iodosuccinimide
OPIA	opsonophagocytic killing inhibition assay
p-TolSCl	*p*-toluenesulfonyl chloride
PBM	*para*-methoxybenzyl

PBS	phosphate-buffered saline
Ph	phenyl
Piv	pivaloyl
PMB	*para*-methoxybenzyl
PPTS	pyridinium *para*-toluenesulphonate
Prot	protein
PS	polysaccharides
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
pyr	pyridine
quant	quantitative
RboP	ribitol phosphate
rEPA	recombinant exotoxin A from *Psudomonas aeruginosa*
RP	reversed phase
rt	room temperature
sat	saturated
SLP	surface layer protein
sn	stereospecific numbering
TA	teichoic acid
Tar	teichoic acid ribitol
TBABr	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
tBu	*tert*-butyl
TCA	trichloroacetyl
TEA	triethylamine
TES-H	triethylsilane
Tf_2O	trifluoromethansulfonic anhydride
TFA	trifluoroacetic acid
TfOH	trifluoromethansulfonic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
Tol	*p*-toluene
Troc	2,2,2-trichloroethoxycarbonyl
Ts	*para*-toluenesulfonyl
TT	tetanus toxoid
TTBP	2,4,6-tris(tert-butyl)pyrimidine
UDP	uridine diphosphate
VRE	Vancomycin-resistant enterococcus
WTA	wall teichoic acid

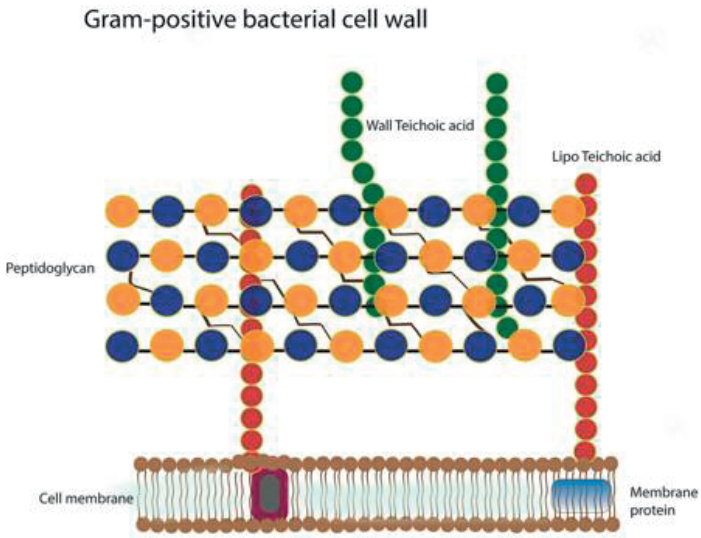
1

Synthetic teichoic acid chemistry for vaccine applications

INTRODUCTION

The cell wall of virtually all Gram-positive bacteria contains characteristic anionic carbohydrate-based polymers, called teichoic acids (from the Greek word τεῖχος, fortified wall). Teichoic acids (TAs) are alditol phosphate (predominantly glycerol or ribitol phosphate) based polymers that can be either covalently connected to the peptidoglycan or linked to the cell membrane through a glycolipid anchor. The first class of TAs is referred to as wall teichoic acids (WTAs), while the latter is called lipoteichoic acids (LTAs). Both classes can be further subdivided in different WTA- and LTA-subclasses, depending on the position of pyranosyl or furanosyl carbohydrate moieties, in or on the alditol phosphate chain, and the presence or absence of an anomeric phosphodiester linkage. Figure 1 presents a schematic drawing of the Gram-positive cell wall composition and the different subclasses of TAs.[1-5] The roles of TAs in the bacterial cell wall are equally diverse and important, as they are involved in the protection of the bacteria against the environment (for example against antimicrobial peptides), nutrient uptake, cation homeostasis and cell wall enzyme regulation as well as binding to receptors and surfaces.[6] Thus, TAs are crucial cell wall components for bacterial fitness and virulence. Protruding from the cell wall towards the environment, they represent anchor points for host cells through binding of cell surface lectins[7-8] for example, antibodies of the host immune system and they serve as recognition motifs for phage binding [9-10] and entry. TAs can be substituted with different carbohydrate and D-alanine [11] (D-Ala) appendages, generating micro-heterogeneous structures and the exact substitution patterns are important for the interactions of the TAs with the outside world. For example, phage binding has been shown to be dependent on the type of alditol phosphate polymers and glycosyl substituents [12-13], while D-alanylation is important for blocking the binding of cationic antimicrobials. [14-15] The micro-heterogeneity of TAs represents a major challenge if one aims to study the interaction of these molecules at the molecular level and therefore synthetic organic chemistry has been called upon to generate well-defined single TA molecules bearing various substitution patterns. This Chapter will describe the synthetic efforts reported to date to generate TA fragments for vaccine purposes. For a complete overview of synthetic methods to generate TAs, the reader is referred to recently published reviews on the subject. [16-18] The Chapter is divided in three subsections, each dealing with specific bacterial species, *Staphylococcus aureus*, *Enterococci faecalis* and *faecium* and *Clostridium difficile*, for which synthetic TAs have been used in the generation of conjugate vaccine modalities or diagnostic tools.

Sara Ali, Francesca Berni, Jacopo Enotarpi, Gijs A. van der Marel, Jeroen D.C. Codée, Synthetic teichoic acid chemistry for vaccine applications, *Recent Trends in Carbohydrate Chemistry*, Elsevier, Ed. Amelia Pilar Rauter, Bjorn Christensen, Laszlo Somsak, Paul Kosma, Roberto Adamo 10.1016/B978-0-12-820954-7.00006-2, (207-238), (**2020**).



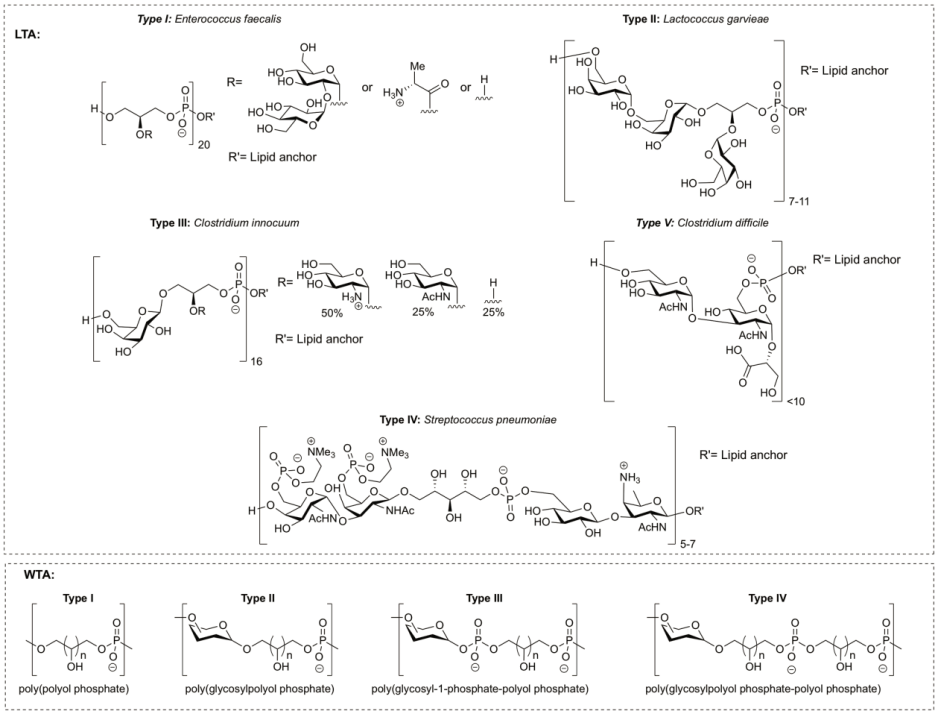


Figure 1. Schematic representation of the Gram-positive cell wall and different types of lipo- and wall teichoic acids.

SYNTHETIC TEICHOIC ACIDS

S. aureus TAs

S. aureus is an opportunistic pathogen, colonizing our skin, gastrointestinal tract, throat and anterior nares. While healthy people are commonly not at risk for *S. aureus* infections, hospitalized immunocompromised subjects are vulnerable and the bacterium can cause infections of the skin/soft tissue as well as respiratory and blood infections. Methicillin-resistant *S. aureus* (MRSA) is one of the major sources of fatal hospital acquired infections and the rise of antibiotic resistant strains represents a major challenge. Below the approaches are reviewed that have been directed at the use of synthetic LTA- and WTA-fragments of *S. aureus* in the development of vaccine modalities.

S. aureus LTA

The most common type of LTA is characterized by a glycerol phosphate (GroP) backbone randomly decorated at the C2 position of the glycerol unit with D-Ala or glycosyl moieties. Type I LTA is present in the cell wall of various Gram-positive bacteria, including *Bacillus subtilis*, *Listeria monocytogenes*, *Streptomyces hygroscopicus* and the important human pathogens *S. aureus*, *S. epidermidis*, *Enterococcus faecalis* and *E. faecium*. *S. aureus* type I LTA carries D-Ala and α-D-GlcNAc substituents, as depicted in Figure 2.

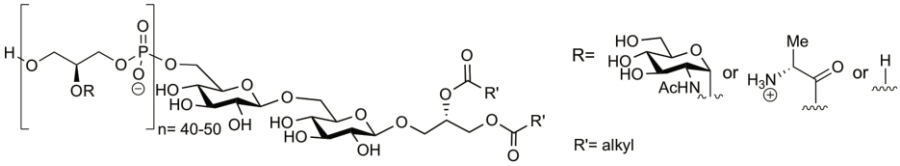
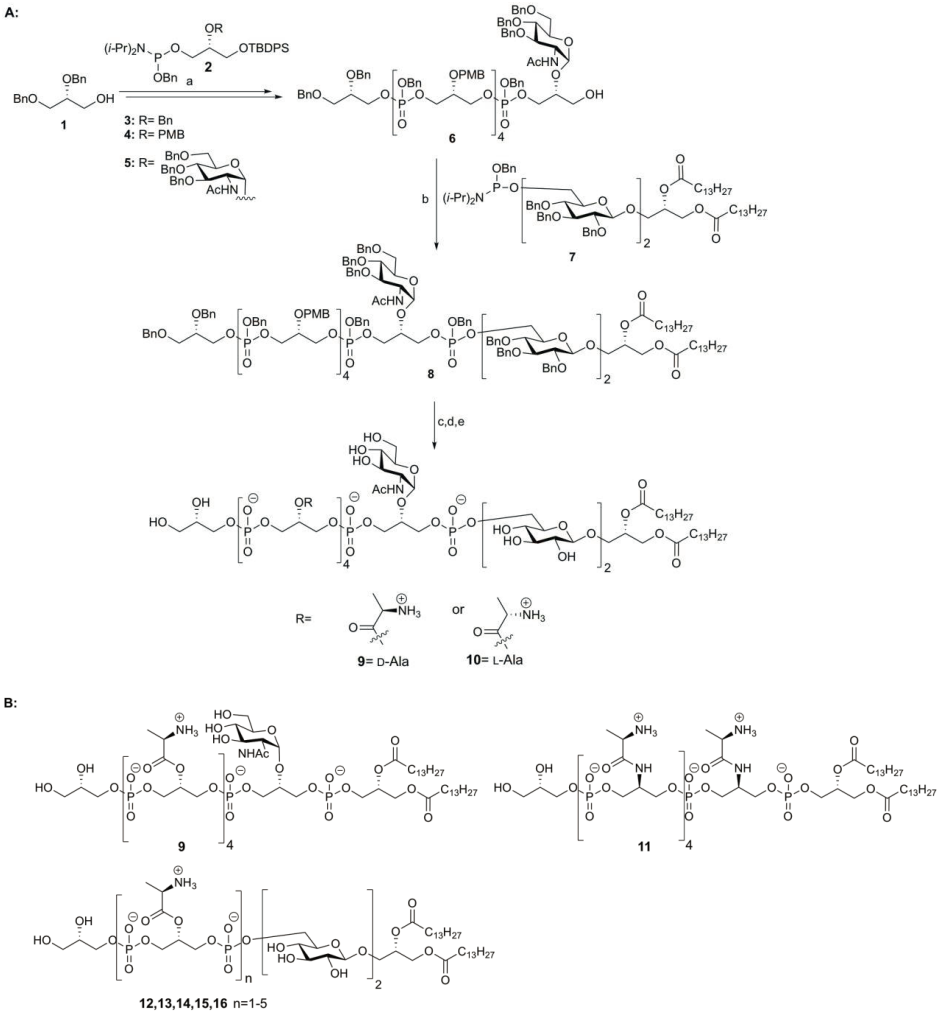


Figure 2. ***S. aureus*** LTA

A significant amount of synthetic work towards this type of LTA has been reported by Schmidt and co-workers who assembled a large set of *S. aureus* LTA oligomers to probe their innate immune-stimulating activity and establish the molecular basis of the "Gram-positive equivalent of the Gram-negative lipopolysaccharide (LPS)".[19] Notably, they reported not only on the total synthesis of LTA fragments including the glycolipid anchor, they also managed to install the labile D-Ala substituents on their synthetic fragments. As a representative example, Scheme 1A shows the synthetic strategy used to build compounds **9** and **10**, which relies on the use of benzyl phosphoramidite building blocks **4** and **5** in which the *tert*-butyldiphenylsilyl (TBDPS) group was used as a temporary protecting group for the primary alcohol. Since the required D-alanine moieties are base labile, they were introduced in a late stage of the synthesis. A *para*-methoxybenzyl (PMB) group was chosen to mask the C2-alcohols that had to be esterified, during the construction of the glycerol phosphate oligomers. The building blocks were united through coupling

cycles employing tetrazole as the activating agent for the phosphoramidites, *t*-BuOOH for the oxidation of the phosphites to the phosphotriesters and TBAF for the removal of the TBDPS groups (See Scheme 1A). After completion of the LTA chain, it was connected to the gentiobiose diacyl glycerol lipid anchor. After oxidative removal of the PMB ethers, the alanine residues were introduced before general hydrogenolysis to deliver the target LTAs **9** and **10**. Using a broad set of synthetic fragments (examples shown in Scheme 1B), the following structure-activity relationships could be established: the (glyco)lipid anchor, and the presence of positively charged alanine esters on the GroP repeating units were both required for full innate immune-stimulating activity (as assessed by cytokine production in a whole blood assay).[20] While the chirality of the alanine substituents proved to be important for activity (fragment **10**, bearing L-Ala esters proved to be 100 times less active than D-Ala LTA **9**), the labile ester linkage could be replaced by the more stable amide linkage (as in **11**). It was also shown that the diacyl glycerol anchor was indispensable for activity but that the gentiobiose core could be removed without having a large effect on the activity of the fragments. The fragments generated by Schmidt and co-workers have not been investigated for their potential effect in an adaptive immune response setting.

Snapper and co-workers have reported on the development of a synthetic *S. aureus* vaccine, employing synthetic GroP-LTA chains.[21] They used an automated solid phase synthesis strategy for the generation of the GroP oligomers as depicted in Scheme 2. Phosphoramidite building block **19** was used to build the oligomers. In line with contemporary nucleic acid chemistry, a dimethoxytrityl (DMTr) group was used for the protection of the primary alcohol to be elongated. Different from other approaches (*vide infra*), a benzoyl (Bz) group was chosen for protection of the secondary alcohol, even though this group is known to easily migrate from a secondary to a primary alcohol. Using an amino spacer functionalized glycerol controlled pore glass (CPG)-resin, a GroP-decamer was synthesized using 35-40 equivalents of the phosphoramidite per coupling cycle. Ammonia treatment released the product from the resin and cleaved all protecting groups (benzoates, cyanoethyl groups and the trifluoroacetyl (TFA) group on the amino group). Of note, the product was not purified and no spectroscopic data of the so-obtained product have been provided. The crude product was desalted before conjugation to the tetanus toxoid (TT) carrier protein using a formylbenzoate hydrazinonicotinamide conjugation couple. In order to establish whether the conjugate **24** was able to elicit a T-cell mediated immune response, mice were immunized with the TA oligomer **20** alone or conjugate **24** using a CPG-ODN (a TLR9 agonist) as adjuvant. A high IgG titer was detected when mice were immunized with the TA-conjugate and the serum raised against the conjugate was able to enhance opsonophagocytic killing of S. *aureus in vitro* and mediate protection in a bacteremia model *in vivo*.

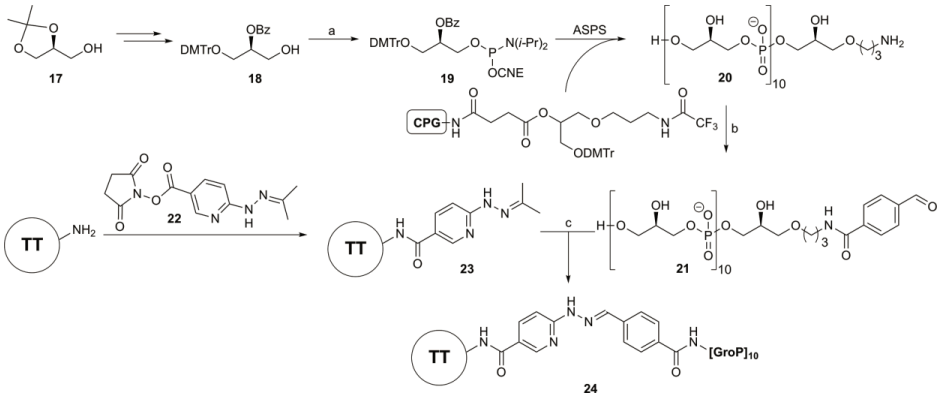


Scheme 1. A) Synthesis of *S. aureus* LTA fragments by Schmidt and co-workers;
Reagents and conditions: a) (i) **4** or **5**, tetrazole, DCM, (ii) *t*BuOOH; (iii) TBAF; b) (i) tetrazole, DCM; (ii) *t*BuOOH, 75%; c) CAN, ACN/toluene/H_2O, 67%; d) PyBOP, *N*-methylimidazole, Z-protected alanine, DCM; e) $Pd(OH)_2$-C, H_2, DCM/MeOH/H_2O, **9**: 25% over 2 steps, **10**: 33% over 2 steps. B) selection of generated LTA fragments for immunological evaluation.

S. aureus WTA

S. aureus produces a type I WTA, composed of 1→5-linked ribitol phosphate (RboP) repeats, that can be decorated with D-Ala residues at the C2 position and GlcNAc appendages at C3-β or C4-α/β, as depicted in Figure 3.

The glycosylation pattern has been shown to be critical for the fitness and virulence of the bacteria. The presence of β-GlcNAc residues, introduced on the WTA through the action of the glycosyl transferase TarS[22], has been related to β-lactam resistance[23] and



Scheme 2. Automated solid phase synthesis of a *S. aureus* LTA GroP oligomer for the development of a conjugate vaccine; *Reagents and conditions*: a) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite; b) 4FB-OSu; c) PBS, aniline.

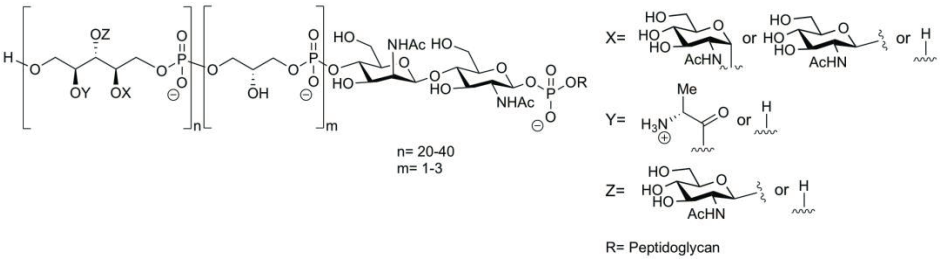


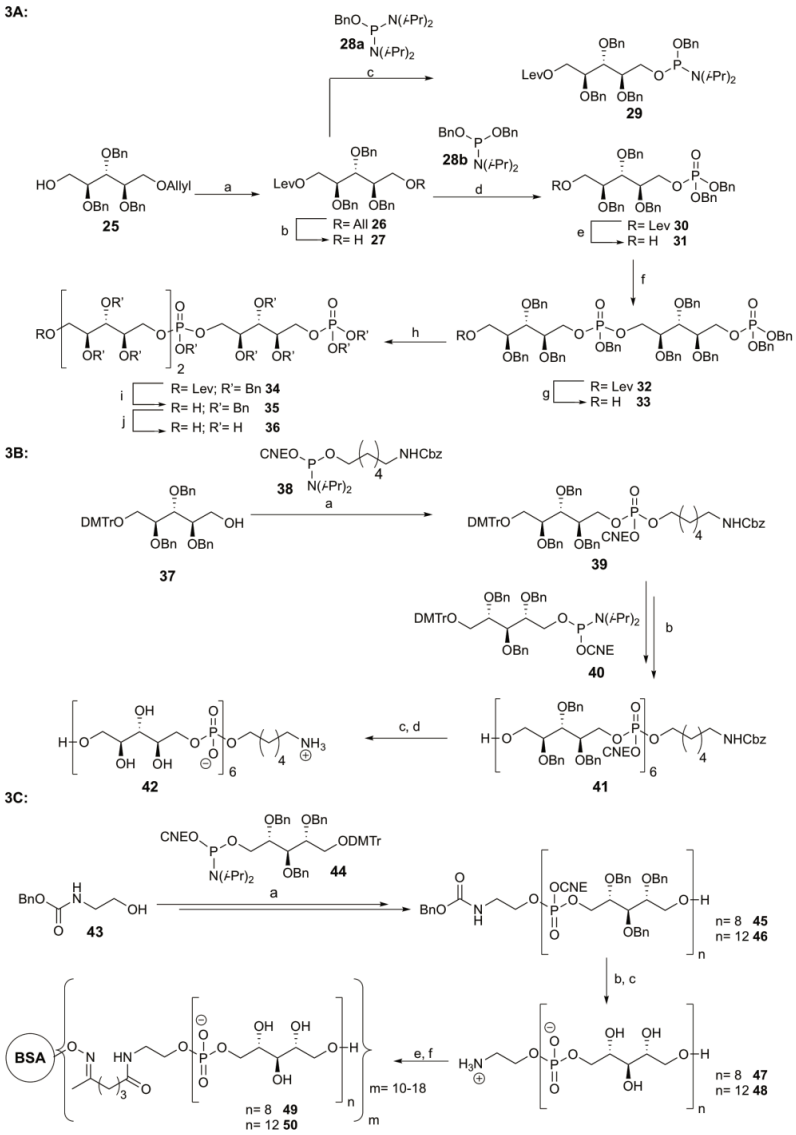
Figure 3. *S. aureus* WTA

the β-GlcNAc appendages also play a role in host colonization in the binding to human epithelial cells.[24-25] Because of the exposure to the bacterium, most humans have antibodies directed at the WTA of *S. aureus*, with C4 modified β-GlcNAc-decorated RboP WTA as the prime target. These antibodies have been shown to facilitate complement C3 deposition and subsequent opsonophagocytosis.[26] C4-β-GlcNAc-decorated RboP WTA is also recognized by human serum mannose binding lectin (MBL) to activate the lectin arm of the complement pathway.[7] Currently it is not clear why the other "carbotypes" are less virulent. Very recently, Peschel and Stehle and co-workers have shown that prominent healthcare associated (HA) MRSA-strains may escape from host immune surveillance by changing their WTA-glycosylation pattern.[27] These strains express, next to TarS, a second glycosyl transferase, TarP, that places a β-GlcNAc at the C3-OH of the RboP residues as opposed to the "normal" C4-position. Glycosylation by TarP was shown to be dominant over TarS glycosylation and this very subtle WTA modification was related to the ability of the bacterium to subvert the host immune system. Synthetic WTA fragments were used to probe the enzyme and solve the first crystal structure of a TarP glycosyl transferase in complex with a GlcNAc-UDP-pyrophosphate donor and an oligo-RboP-WTA acceptor, shedding light on the regiochemistry of the enzymatic

transformation. Two different WTA fragments were generated for this study: RboP trimer **36** (Scheme 3A) and RboP-hexamer **42** (Scheme 3B). Seeberger and co-workers generated trimer **36** using ribitol synthon **26**, featuring an allyl ether and a levulinoyl ester as a set of orthogonal protecting groups (Scheme 3A).[27] This building block was transformed into benzyl phoshoramidite **29** using reagent **28a**. Dibenzyl phosphate **31** was generated from the reaction of building block **27** and reagent **28b** after *t*BuOOH oxidation and delevulinoylation. Phosphoramidite **29** and building block **31** were coupled under the agency of tetrazole to provide, after *t*BuOOH oxidation, the levulinoyl protected RboP dimer **32**. Unmasking the primary alcohol and a subsequent coupling to building block **29** delivered the trimer **34**. Delevulinoylation and subsequent hydrogenolysis provided the desired RboP trimer **36.**

Our laboratory used an assembly strategy based on the use of cyanoethyl phosphoramidite building blocks, protected with a DMTr-group to mask the primary alcohol. This strategy, building on state-of- the-art nucleic acid chemistry and our previously described LTA-work (*vide infra*) has also been used by Pozsgay and co-workers for the assembly of longer RboP oligomers (See Scheme 3C).[28] In short, alcohol **37** was first coupled with spacer phosphoramidite **38** to provide spacer-equipped monomer **39**. Acidolysis of the DMTr group then delivered the primary alcohol for further elongation. Using phosphoramidite **40**, the hexamer **41** was obtained after 5 cycles of coupling/oxidation/deprotection. Standard deprotection conditions then provided the spacer-equipped hexamer **42** (Scheme 3B).

With the goal to investigate the role of the chain length in the immunological properties of ribitol phosphate oligomers, Pozsgay and co-workers set out to develop an automated solid phase assembly strategy using building block **44** (Scheme 3C). Initially the synthesis of a RboP-hexamer was explored. However, after six automated synthesis cycles and cleavage form the solid support, an intractable product mixture was obtained, necessitating the authors to revert to solution-phase chemistry. Starting with ethanol amine linker **43**, eight or twelve consecutive couplings led to the assembly of RboP-8-mer and RboP-12-mer **45** and **46**, which were deprotected using ammonia treatment followed by hydrogenolysis. Fragments **47** and **48** were attached to BSA through the use of a 5-ketohexanoic acid linker, the ketone functionality of which was used for conjugation to oxime groups that were installed onto the BSA carrier protein. On average 10–18 WTA oligomers were installed on the carrier protein. The immunogenicity of the conjugates has not been reported so far. Noteworthy, the aminospacer was installed on the side of the corresponding WTA fragments, to the side of the WTA that is not attached to the peptidoglycan.



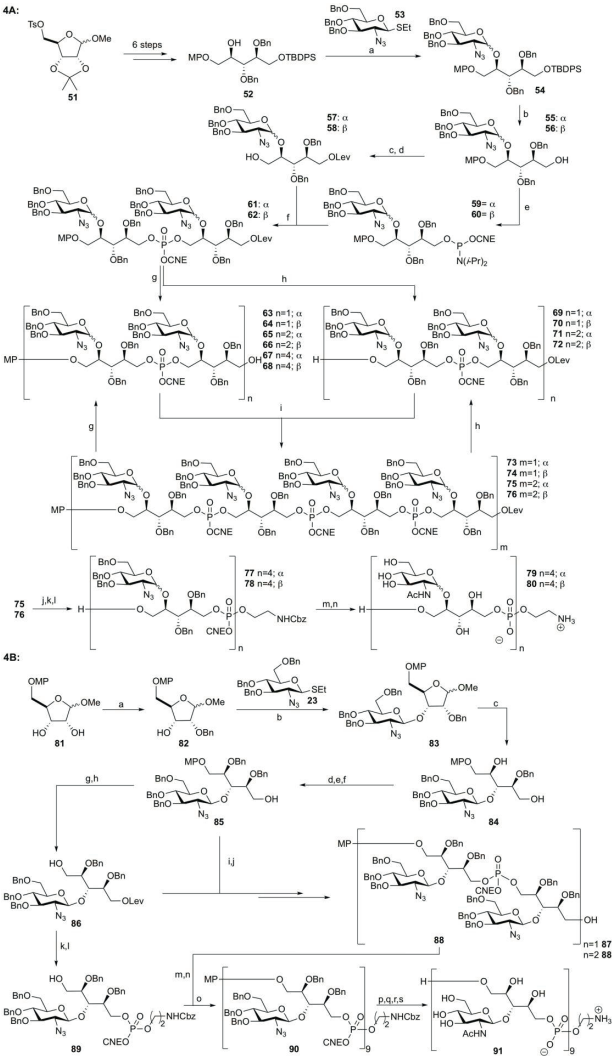
Scheme 3. A) Assembly of a *S. aureus* WTA RboP trimer; *Reagents and conditions*: a) LevOH, DMAP, DCC, DCM, 3 h, 98%; b) $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, MeOH, 40°C, 24 h, 77%; c) diisopropylammonium tetrazolide, DCM, 2 h, 98%; d) (i) 1*H*-tetrazole, ACN, 2 h (ii) *t*BuOOH, 1 h, 85%; e) hydrazine hydrate, pyridine, AcOH, DCM, 4 h, 93%; f) (i) **29**, 1*H*-tetrazole, ACN, 2 h (ii) *t*BuOOH, 1 h, 86%; g) hydrazine hydrate, pyridine, AcOH, DCM, 4 h, 94%; h) (i) **29**, 1*H*-tetrazole, ACN, 2 h (ii) *t*BuOOH, 1 h, 94%; i) hydrazine hydrate, pyridine, AcOH, DCM, 4 h, 98%; j) Pd-C, H_2, EtOAc/MeOH/H_2O, 24 h, quant.

B) Assembly of an aminospacer functionalized RboP hexamer; *Reagents and conditions*: a) (i) DCI, ACN, **38**; (ii) CSO; (iii) 3% TCA in DCM, 85%; b) (i) DCI, ACN, **40**; (ii) CSO; (iii) 3% TCA in DCM, n=2, 74%, n=3, 88%, n=4, 80%, n=5, 76%, (**41**) n=6, 91%; c) NH_3 (30-33% aqueous solution), dioxane; d) Pd black, H_2, AcOH, H_2O/dioxane, 87% over 2 steps.

C) Pozsgay's synthesis of a RboP-12-mer-BSA conjugate; *Reagents and conditions*: a) (i) 10 eq. 0.45 M tetrazole in ACN, ACN, 23°C, 1 h; (ii) 0.5 M I_2 in 2:1 THF/water; (iii) 85:10:5 AcOH/DCM,H_2O n=1, 88%; b) (i) 10 eq. 0.45 M tetrazole in ACN, ACN, 23 °C, 1 h; (ii) 0.5 M I_2 in 2:1 THF/water, n=8; 9.9%, n=12; 2.4%; c) (i) MeOH, conc. NH_4OH, 50 °C, 8 h; (ii) H_2, 10% Pd/C, 2:1 *t*BuOOH/H_2O, n=8; 66%, n=12; 80%; e) 5-ketohexanoic anhydride, Et_3N, MeOH, H_2O; f) aminooxy-BSA, PBS (pH=7.4), EDTA, glycerol.

Glycosylated RboP-oligomers have recently been assembled and evaluated as potential antigens by a team at Sanofi Pasteur.[29] A panel of RboP-protein conjugates was assembled, in which the nature of the WTA fragments varied with respect to the glycosylation pattern and the manner in which it was generated (synthetic or isolated, see Scheme 5). Two different carrier proteins were probed: the detoxified α-hemolysin of *S. aureus* (HladM) or the detoxified recombinant exotoxin A from *Pseudomonas aeruginosa* (rEPA). Three different RboP-oligomers were synthesized: two RboP-octamers featuring either α-D-GlcNAc or β-D-GlcNAc substituents at all of the C4 hydroxyls, and one RboP-nonamer bearing β-D-GlcNAc residues on each of the C3-alcohols (See Scheme 4A and 4B). The building block required for the C4-glycosylated WTAs was generated from ribose **51** in six steps. This building block **52** was coupled with thiodonor **53** under activation of NIS/TfOH to yield **54** as a 52:48 α/β mixture in 94% yield. The diastereoisomers were separated after the TBDPS deprotection in the next step delivering far advanced intermediates for both the α- and the β-substituted RboP oligomers. Installing the levulinoyl ester (Lev) and removal of the methoxyphenol (MP) group provided alcohols **57** and **58**. The phosphoramidites **59** and **60** were formed *in situ* and used directly in the condensation with **57/58**, to give, after oxidation using pyridine/I_2/H_2O, dimers **61** and **62**. To facilitate a convergent synthesis approach, the Lev-group or the MP-ether of dimers **61, 62** were removed to give two dimer building blocks that were united using the *in situ* coupling strategy delivering the tetramers **65**, **66**. Following a similar approach, the so-obtained tetramers were transformed into two different alcohols, which were combined to give the desired fully protected octamers **67, 68**. The last steps in the synthesis of the octamers **67, 68** comprised coupling with the ethanolamine spacer phosphoramidite, followed by removal of the cyanoethyl groups. Of note, the spacer in these molecules is installed on the opposite site of the WTA fragment chain, with respect to the peptidoglycan binding site (See Figure 3). Next, the azides were transformed into the required acetamides using thioacetic acid in pyridine. Birch-type reduction of all benzyl groups then delivered the fully deprotected RboP-octamers **79** and **80**. Both native and synthetic WTAs were attached to the carrier proteins using adipic acid hydrazide as a conjugation handle (Scheme 5B).

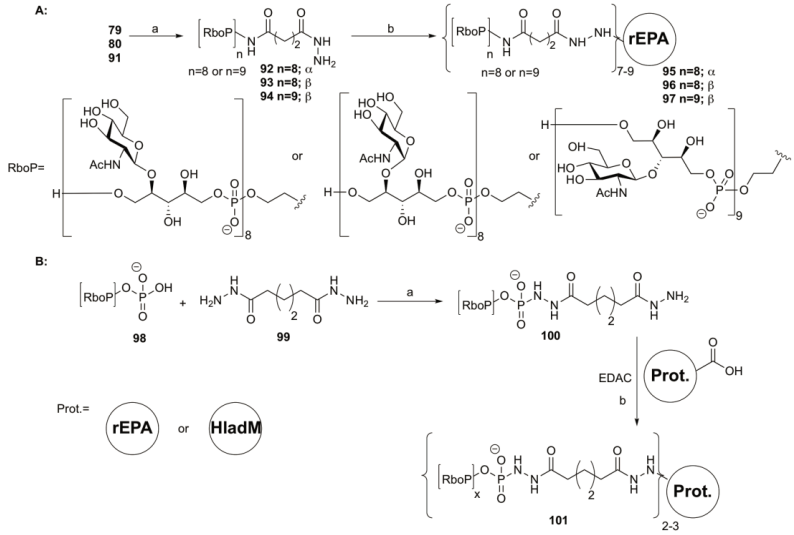
Scheme 4B depicts the synthesis of the C-3 β-GlcNAc nonamer. To regioselectively introduce the GlcNAc substituent on the ribitol chain, ribose **81** was first regioselectively benzylated at the C2-OH under phase-transfer conditions. Next the C-3 OH was glycosylated under NIS/TfOH conditions in a participating solvent mixture at low temperature (-70 °C) providing the desired β-stereoisomer **83** in excellent yield. Hydrolysis of the furanose linkage and reduction of the lactol delivered the ribitol chain. A series of protecting group manipulations then afforded the key intermediates **85** and **86**, featuring the methoxyphenol ether and levulinoyl ester protecting groups. Using the same



Scheme 4. A) Synthesis of C4-α- and β-GlcNAc RboP-octamers; *Reagents and conditions*: a) DCM/Et_2O, NIS and TfOH at -20°C for 10 min, 94%, (52:48 α/β); b) TBAF 1M/AcOH, THF, 0°C to rt 17h, 40% β, 46% α; c) Levulinic acid, DMAP, EDCI, DCM; d) CAN, ACN/H_2O, 0°C to rt 2h, 81% β; e) (i) Alcohol in ACN, cooled to 0°C, DIPEA and chloro-2-cyanoethyl-*N,N*-diisopropyl-amidite added at 0°C for 0.5 h (ii) cool to -10 °C, add alcohol then 5-ethiolthio-1*H*-tetrazole 1h at 0°C, m=1, β, 80%, m=2, β, 72%; f) step e, 69% β; g) hydrazine hydrate, pyridine/AcOH, 83% β; h) step d, 79% β; i) step e, **74** (80%), **76** (72%); j) hydrazine hydrate, pyridine/AcOH, 80% β; k) step e, benzyl *N*-(2-hydroxyethyl) carbamate, 67%; l) CAN, ACN/H_2O, 0°C to rt 2h, **78** (76%); m) thio acetic acid, pyridine, 3.5 days, β 97%; n) (i) Na, THF, NH_3, -78°C, 30 min, (ii) sat. aq. $NHCl_4$, -78°C, 1h, **80** (96%). **B) Synthesis of a C3-β-GlcNAc-RboP-nonamer;** ***Reagents and conditions*:** a) TBABr, BnBr, 10% aq. NaOH, DCM, 34%; b) ACN/propionitrile/DCM 2/1/1, NIS and TfOH, at -70°C 10 min, 84%; c) (i) 3M HCl/dioxane, reflux 7h, 73%; (ii) $NaBH_4$, MeOH, 0°C; d) TBDMSCl, DMAP, TEA, DCM; e) BnBr, DMF, NaH; f) THF, AcOH, TBAF, 0 °C to rt, 94%; g) Levulinic acid, DMAP, EDCI, DCM/dioxane 1/10 (0.08M); h) CAN, ACN/H_2O, 0°C to rt 2h, 71%; i) (i) Alcohol in ACN, cooled to 0°C, DIPEA and chloro-2-cyanoethyl-*N,N*-diisopropylamidite added at 0°C for 0.5 h (ii) cool to -10°C, add second alcohol then 5-ethiolthio-1H-tetrazole 1h at 0°C, n=1 (82%), n=2 (79%); j) hydrazine hydrate, pyridine/AcOH, **87** (86%), **88** (82%); k) step i, benzyl *N*-(2-hydroxyethyl) carbamate, 65%; l) CAN, ACN/H_2O, 0°C to rt 2h, 85%; m) step i, n=5, 77%, n) CAN, ACN/H_2O, 0°C to rt, 2h, n=5, (74%); o) step i, n=9, 27%; p) CAN, ACN/H_2O, 0°C to rt 2h, n=9, (80%); q) thio acetic acid, pyridine, 3.5 days, 90%; r) NH_4OH, MeOH, reflux 5h, 97%; s) (i) Na, THF, NH_3, -78°C, 30 min, (ii) sat. aq. $NHCl_4$, -78°C, 1h, 50%.

phosphoramidite approach used for the assembly of the C4-GlcNAc RboP-octamers, C-3 β-GlcNAc RboP tetramer **88** was generated. Building block **86** was also coupled to the ethanolamine phosphoramidite to give the spacer-functionalized monomer **89**. The union of tetramer **88** and monomer **89** then delivered the pentamer, which was coupled to a second copy of the tetramer to deliver the protected WTA nonamer **90**. Deprotection and linker installation as described above delivered the C-3 β-GlcNAc RboP-nonamer with a hydrazide linker (See Scheme 5).

The synthetic fragments as well as native TAs isolated from strains ATCC 10832 (carrying β-GlcNAc at C4), ATCC 25904 (having C4-α-GlcNAc substituents) and ATCC 55804 (with C3-β-GlcNAc monosaccharides) were conjugated to rEPA (Scheme 5A). Additionally, the ATCC 10832 TA was also conjugated to *S. aureus* alpha toxin (HladM) (Scheme 5B). To this end, the native WTAs were functionalized with a hydrazide linker using adipic acid dihydrazide (ADH) in a carbodiimide mediated condensation reaction. Using a similar coupling strategy, the hydrazide functionalized synthetic and native TA fragments were covalently linked to the carrier proteins. All conjugates, as well as the non-conjugated WTAs, were used to immunize mice, with or without adjuvant (AF04, a squalene emulsion containing the synthetic toll-like receptor 4 agonist, E6020, a hexa-acylated diphosphoryl urea), after which the IgG1 and IgG2-titers were determined after 0, 21, 35 and 42 days. It was revealed that the unconjugated WTAs were not able to induce a specific immune response against the WTA used for AF04 immunization, while immunization with the WTA-conjugates did lead to the production of IgG antibodies. The titers of the serum of the mice immunized with the adjuvant were significantly higher than those immunized without adjuvant. There was little difference in the immune response against the conjugates of the native WTAs or the synthetic fragments. The synthetic C4-β-GlcNAc WTA conjugate appeared to elicit a somewhat stronger immune response than the C4-α-GlcNAc conjugate. The cross reactivity of the sera was evaluated on 19 *S. aureus* strains and it was shown that the sera of mice immunized with the synthetic WTA-conjugates recognized homologous and heterologous strains better than sera from mice immunized with the native WTA-conjugates. The sera from mice immunized with the C4-β-GlcNAc WTA conjugate showed a higher cross reactivity than the sera raised against the C4-α-GlcNAc conjugate, while the C3-β-GlcNAc WTA conjugate serum did not appear to be cross reactive. The latter observation stands in contrast to the cross reactivity of the Nabi Pharmaceuticals PentaStaph vaccine, containing conjugates of the capsular polysaccharides 5 and 8 as well as a conjugate of antigen 336, the C3-β-GlcNAc WTA, that was shown to be cross reactive against different *S. aureus* strains as well as against *S. epidermidis*.[30] The results described by the Sanofi team, could suggest that the C4-β-GlcNAc WTA conjugate can be used as a vaccine modality rendering broad spectrum protection against a variety of *S. aureus* strains. Whether the vaccine can offer



Scheme 5. A) Conjugation chemistry and an overview of the WTA conjugates assembled by Sanofi pasteur of the synthetic WTAs; *Reagents and conditions*: a) (i) disuccinimidyl succinate, DIPEA, DMSO, 0.5 h rt (ii) hydrazine hydrate, **93**, (24%), **94**, (53%); b) rEPA, EDAC, 3h, pH= 5.7.
B) Conjugation of the native WTAs; *Reagents and conditions*: a) (i) aq. NaCl, aq. ADH, pH= 5.7, 1 M EDAC, 3h (ii) pH= 7.0 (iii) TA-AHs, rEPA or HladM, EDAC, pH= 5.7, 3h.

protection *in vivo* against different strains of *S. aureus* will have to be shown in future studies.

Enterococcal TAs

Enterococci are the third most common class of nosocomial pathogens and they can cause bacteremia, endocarditis, peritonitis, urinary tract infection and foreign-body infections.[31] Multiresistant enterococci, including Vancomycin-resistant enterococcus (VRE), represent a major health threat, especially to immunocompromised hosts. The rise of antibiotic resistance has been an important motivation for the development of alternative strategies, including active and passive immunization therapies. To this end, TAs have been investigated as potential antigens as they are prominently present in the cell wall of these bacteria.

E. faecalis and E. faecium ***LTA***

Figure 4 shows the type I LTA of *E. faecalis* and *faecium* with their characteristic glycosyl substituents. As described above poly-(1,3)-glycerol phosphate (GroP) LTA is present in the cell wall of various Gram-positive bacteria, and has therefore been probed as a universal antigen that could potentially be used in broad-spectrum vaccines targeting various Gram-positive species.[32] Initial studies conducted with isolated LTA of *E. faecalis* have shown that opsonophagocytic antibodies could be raised against type I LTA, that

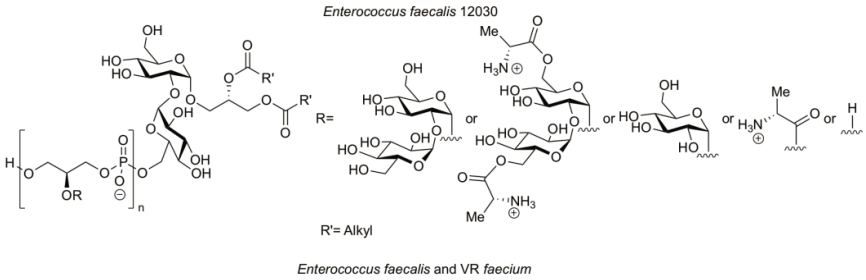


Figure 4. Structure of *E. faecalis and E. faecium* LTA.

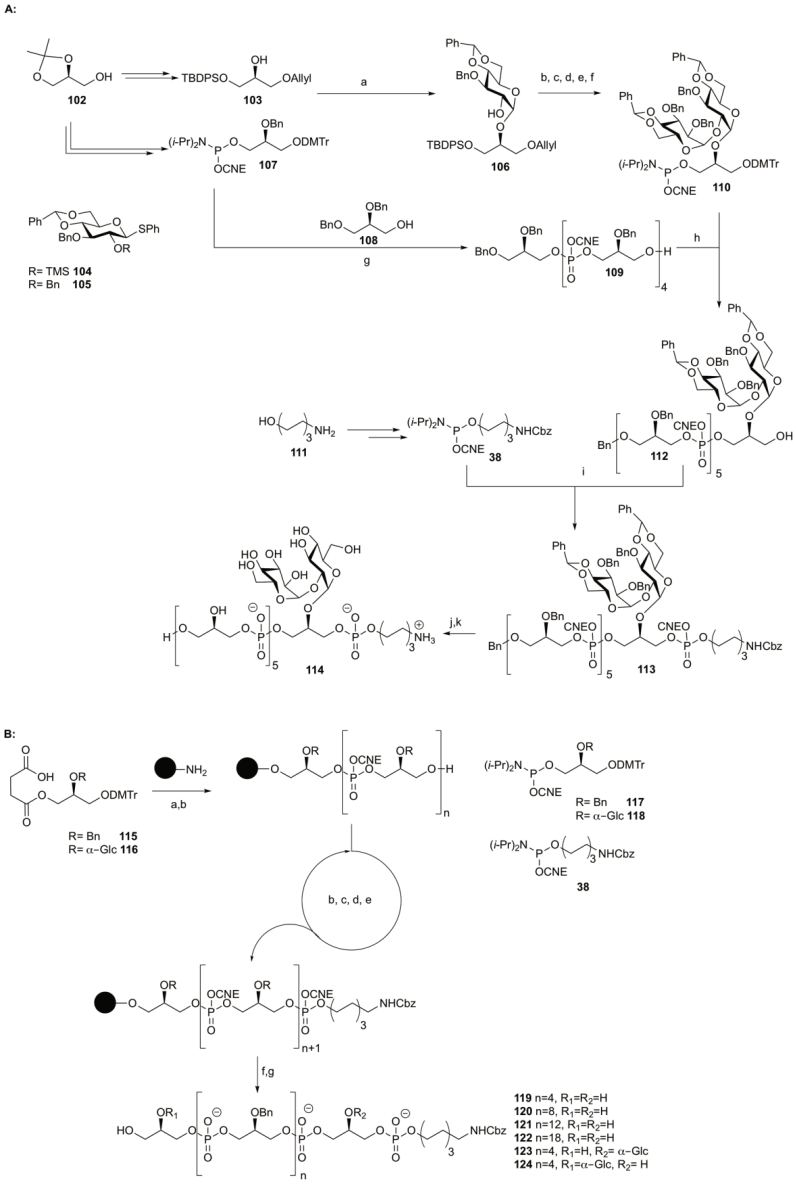
were cross reactive against different *E. faecalis* strains as well as *E. faecium* strains, including (VRE) and against *S. aureus* and *S. epidermidis*.[33]

The potential of (substituted) glycerol phosphate TA antigens in therapeutic and diagnostic applications has been an incentive to develop synthetic routes to assemble these molecules *de novo* through organic synthesis. Our group has reported several approaches to assemble *E.* faecalis LTA fragments with a special focus on the site-selective introduction of different carbohydrate moieties, the number of repeating units and the kind of functional moiety at the end of the chain, being either a hydroxyl or phosphate group. Initially, we developed 'traditional' solution-phase chemistry to assemble α-kojibiosyl GroP LTA hexamer **114** as depicted in Scheme 6A.[34] The required phosphodiesters were installed using phosphoramidite chemistry and the cyanoethyl phosphoramidite glycerol building blocks were generated starting from solketal **102**, which represents a convenient commercially available chiral starting material in which all hydroxyl groups can be addressed site specifically. Installation of the α-kojibiose (α-D-glucopyranosyl-(1→2)-α-D-glucose) moiety was achieved using alcohol **103** and a benzylidene-protected glucose donor. After condensation of donor **104** and acceptor **103** the TMS group was removed upon work-up to set the stage for the second glucosylation to complete the kojibiose moiety. A standard set of protecting group manipulations then installed the DMTr and the cyanoethyl phosphoramidite groups to give **110**. Assembly of the first target *E. faecalis* LTA fragment **114** started from dibenzyl glycerol **108**, which was elongated with four copies of building block **107** (using DCI as activating agent, I_2/H_2O/THF/pyridine for the P(III) to P(V)-oxidation, and dichloroacetic acid (DCA) and Et_3SiH for DMTr removal). Subsequently, the fully protected GroP-pentamer **109** and α-kojibiosyl-glycerol phosphoramidite **110** were coupled. DMTr cleavage form the resulting GroP-hexamer was performed using a pyridinium *para*-toluenesulphonate (PPTS)/MeOH mixture to avoid unwanted acidolysis of the benzylidene functionalities. Final coupling with spacer phosphoramidite **38** delivered the fully protected hexamer **113**. After removal of the cyanoethyl groups through ammonolysis, all the benzyl-type

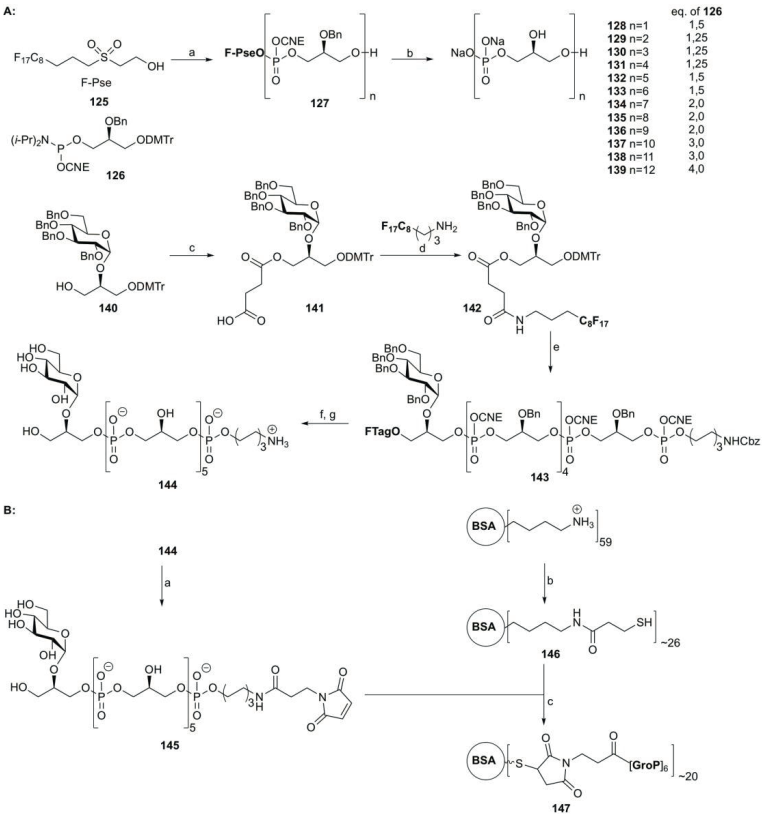
protecting groups were cleaved by hydrogenolysis, to afford the kojibiosyl TA-hexamer **114** in 76% yield.

To streamline the synthesis of substituted GroP oligomers, the established solution-phase chemistry was next translated to automated solid-phase methodology (Scheme 6B).[35] To this end, aminopropyl controlled pore glass (CPG) resin was coupled with succinyl linker building block **115** or **116**. With the first building block immobilized, the GroP chains were constructed in a fully automated manner using the commercially available ÄKTA™ oligopilot™ synthesizer. For each coupling cycle 5 equivalents of phosphoramidite **117** or **118** were used in combination with 5-benzylthiotetrazole (5-BTT) as activator. Oxidation by I_2 in pyridine/H_2O oxidized the phosphites to the phosphate triesters and was followed by a capping step of the unreacted alcohols using *N*-methylimidazole and acetic anhydride. Removal of the DMTr was performed using DCA in DCM, generally showing coupling efficiencies higher than 98% (automatic DMTr-count). Final coupling of the fragments with spacer amidite **38**, followed by oxidation, capping and detritylation afforded the complete resin-bound fragments. The target compounds were cleaved from the resin by aqueous ammonia treatment, which simultaneously removed the cyanoethyl groups. Purification of the semi-protected fragments was done by anion exchange chromatography and the final target compounds were obtained by hydrogenolysis. Using this assembly method, a small set of GroP oligomers was assembled varying in length between 6-mers and 20-mers and with different substitution pattern.

Although the latter methodology proved to be efficient in terms of synthesis time and labor, it does require the use of large amounts of phosphoramidite building blocks and can only be done on a limited scale. An alternative synthetic strategy is represented by the application of soluble supports, which combine a more rapid and effective intermediate isolation procedure (compared to solution phase synthesis) with the use of a relatively small excess of reagents (compared to solid phase chemistry) and the possibility to scale up. Based on light fluorous synthesis techniques, Hogendorf *et al.* applied two different fluorous scaffolds as soluble supports: a (perfluorooctyl)propylsulfonylethyl (F-Pse) linker for the assembly of TA fragments with a terminal phosphate monoester and a (perfluorooctyl)succinyl spacer delivering TA oligomers featuring a terminal hydroxyl group (Scheme 7A).[36] Using building blocks **126** and **127** GroP-oligomers were made up to the dodecamer level (n = 12). It was noted that a larger excess of reagents was required to push the coupling reactions to completion with growing length of the oligomers. Standard deprotection chemistry delivered the desired GroP-oligomers **128-139**. The light fluorous approach was used to scale up the synthesis of hexamer **144** using the (perfluorooctyl)succinyl linker.



Scheme 6. A) Solution phase synthesis of a kojibiosyl decorated GroP LTA fragment; *Reagents and conditions*: a) (i) **104**, Tf_2O, Ph_2SO, TTBP, DCM (α / β = 6:1) (ii) Na_2CO_3, MeOH, 46%; b) **105**, Tf_2O, Ph_2SO, TTBP, DCM, 82%; c) (i) Ir(COD)$(Ph_2MeP)_2$, THF (ii) I_2, aq $NaHCO_3$, 67%; d) DMTr-Cl, TEA, DCM, 98%; e) TBAF, THF, 91%; f) 2-cyanoethyl *N,N*-diiopropylchlorophosphoramidite, DIPEA, DCM, 72%; g) **107** (i) DCI, ACN (ii) I_2, THF, H_2O, pyridine (iii) DCA, TES-H, DCM, n=1 (80%), n=2 (82%), n=3 (81%), n=4 (62%); h) (i) **110**, DCI, ACN (ii) I_2, THF, H_2O, pyridine, 80%, (iii) pyridium-*p*-toluenesulphonate, MeOH, DCM, 70%; i) **38**, DCI, ACN (ii) I_2, THF, H_2O, pyridine, 77%; j) NH_3, H_2O, dioxane, 100%; k) Pd black, H_2, H_2O, dioxane, 76%.
B) Automated solid phase synthesis of a set of GroP oligomers; *Reagents and conditions*: a) **115** or **116**, DIC, ACN; b) 3% DCA, toluene; c) **117**, **118** or **38**, 5-BTT, ACN; d) I_2, pyridine, H_2O/ACN; e) Ac_2O, *N*-methylimidazole, 2,6-lutidine, ACN; f) conc. NH_4OH; g) Pd black, H_2, H_2O/dioxane, AcOH; **119** (65%), **120** (78%), **121** (84%), **122** (95%), **123** (68%), **124** (86%).



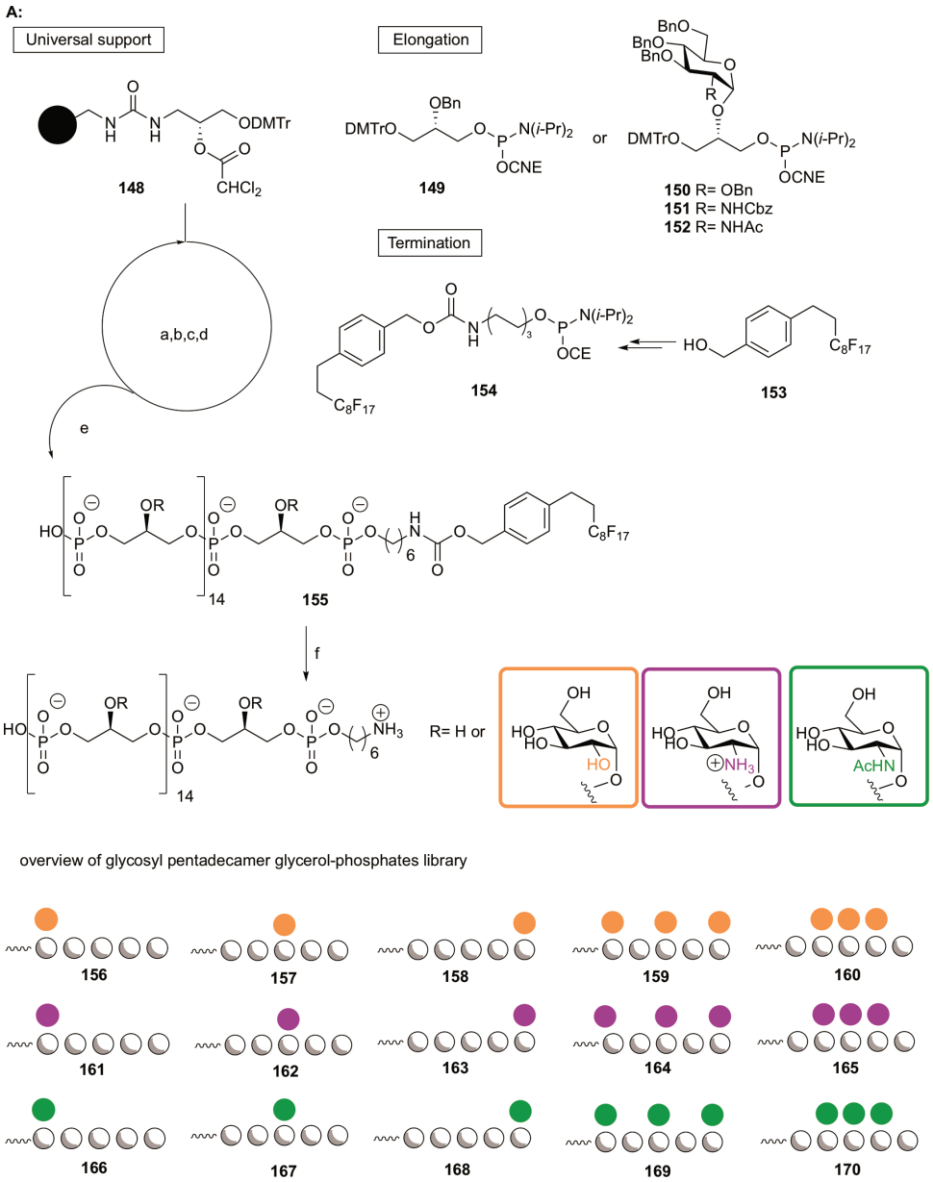
Scheme 7. A) Light fluorous assembly strategy; *Reagents and conditions*: a) (i) **126**, DCI, ACN (ii) I_2, pyridine, THF (iii) DCA, TES-H, DCM; b) (i) **126**, DCI, ACN (ii) I_2, pyridine, THF (iii) DCA, TES-H, DCM; c) succinic anhydride, TEA, DCM, 96%; d) (i) BOP, DIPEA, DMF, DCM (ii) DCA, TES-H, DCM (iii) F-SPE, 90% 3 steps; e) repetition (i) **126** or **38**, DCI, ACN (ii) I_2, pyridine, THF (iii) DCA, TES-H, DCM; f) conc. NH_4OH, 40°C; g) Pd black, H_2, H_2O, AcOH, 76-98% 2 steps. **B) Generation of a GroP$_6$-LTA BSA conjugate;** *Reagents and conditions*: a) *N*-succinimidyl-3 maleimidopropionate, aq. $NaHCO_3$, **145** (67%); b) (i) *N*-succinimidyl-3-thio-propionatehomodisulfide, PBS (pH= 8.0), DMF; (ii) dithiothreitol; c) PBS (pH= 7.2).

An opsonophagocytic killing inhibition assay (OPIA) was used to evaluate the synthetic fragments for their potency in binding to LTA-binding antibodies, present in rabbit serum raised against *E. faecalis* 12030-LTA.[35] These studies indicated a clear length dependency for the unsubstituted fragments, with better inhibition for the longer fragments. Surprisingly the fragment bearing the naturally occurring kojibiose substituent (**114**), proved to be less active than the structurally simpler mono-glucose hexamer **144**, which appeared to be the most potent synthetic antigen in the series.[37] Structures bearing the phosphate at the end of the GroP-chain all proved less active. From these studies, GroP-hexamer **144** was selected as a lead antigen to be used in the generation of a TA-protein conjugate vaccine modality for follow-up studies (Scheme 7B). Based on the approach[38] used by Verez-Bencomo and co-workers for the development of the Quimihib®-vaccine, the maleimide derivative **145** was generated and linked to thiofunctionalized BSA, to deliver the conjugate **147** with a TA-protein ratio of 20:1.[37] Rabbit serum obtained by

immunization with **147** proved to be highly opsonic not only towards *E. faecalis* strain but also *E. faecium* and S. *aureus*. While the activity against the former pathogen can be readily understood, because *E. faecium* LTA carries mono-α-D-glucose appendages, the reactivity against *S. aureus* is somewhat surprising, as the LTA of this bacterium has not been shown to carry α-D-glucose substituents. Perhaps the lead antigen **144** mimics well the naturally occurring α-D-*N*-acetyl glucosamine-GroP LTA or the α-D-*N*-acetyl galactosamine-bearing GroP-WTA of *S. aureus*. In all, these results support the idea that compound **144** is a good TA mimic and that LTAs can be used as antigen candidate for vaccine development with a broad spectrum of action.

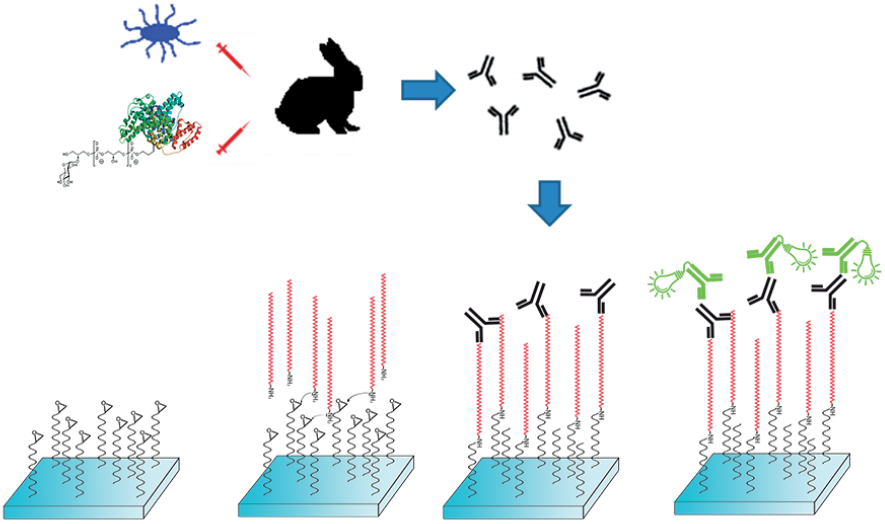
To further streamline the assembly of synthetic TAs, allowing for longer fragments, carrying more diverse substitution patterns, van der Es *et al.* developed a 'second generation' automated solid phase synthesis approach introducing a universal support, which obviates the need for linker functionalized building blocks, and the use of a fluorous tagging technique, to facilitate purification of the target structures from generated deletion sequences (See Scheme 8).[39] To this end the fluorous aminospacer phosphoramidite **154** was developed and it was shown that the fluorous tag facilitated the purification of the long fragments using a conventional reversed phase HPLC. A large GroP-15-mer library was generated bearing one or three α-D-glucose, α-D-*N*-acetyl glucosamine or α-D-glucosamine substituents, evenly distributed along the GroP chain or clustered together.

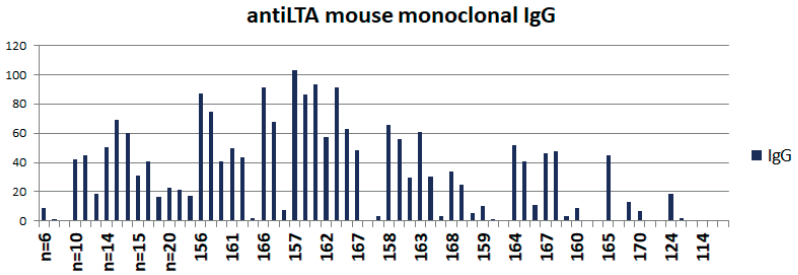
With the library of synthetic TA-fragments a TA microarray was developed to allow for the rapid screening of interactions with biomolecules using a minimal amount of analyte (Figure 5). The array was used to compare the binding specificities of antibodies and sera raised against different antigens, *i.e* purified LTA from *E. faecalis* 12030 or the synthetic BSA-mono-glucose GroP-hexamer conjugate **147**. As shown in Figure 5, the binding specificity of the sera and antibodies differed tremendously. The commercially available monoclonal antibody raised against *S. epidermidis* recognized many different GroP oligomers, with little specificity for length or substitution pattern, although the multiple substituted GroP oligomers showed least binding. This indicates that the antibody most likely binds to the GroP-backbone, providing an explanation for the cross reactivity of this antibody towards TAs derived from different bacterial species. The serum raised against the purified LTA contained both IgM and IgG-isotypes and the IgM-type antibodies showed no specificity for any substitution pattern, again indicating primarily binding to the GroP-backbone. The IgGs showed a clear preference for substituted TAs but were non-discriminative for the type of monosaccharides. Finally, the serum raised against the well-defined GroP-hexamer antigen showed highly specific binding towards mono-glucosylated structures, resembling the structure of the WTA-fragment

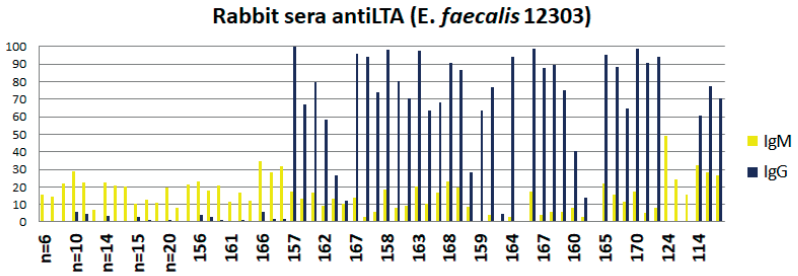


Scheme 8. 'Second-generation' automated solid phase assembly of long GroP-TA fragments; *Reagents and conditions*: a) 3% DCA, toluene; b) 5-BTT, ACN; c) I_2, pyridine, H_2O/ACN; d) Ac_2O, *N*-methylimidazole, 2,6-lutidine, ACN; e) (i) NH_3, MeOH; (ii) NH_4OH, H_2O; f) H_2, Pd^0, H_2O.

against which the serum was raised. These results have shown that the TA-microarray is a powerful analytical tool to map binding specificity for TA-interaction partners and it is expected that the technique will be very useful to study the interaction of TAs with other biomolecules, such as lectins and phage binding proteins. The results have also clearly highlighted that a very selective response against a well-defined TA antigen can







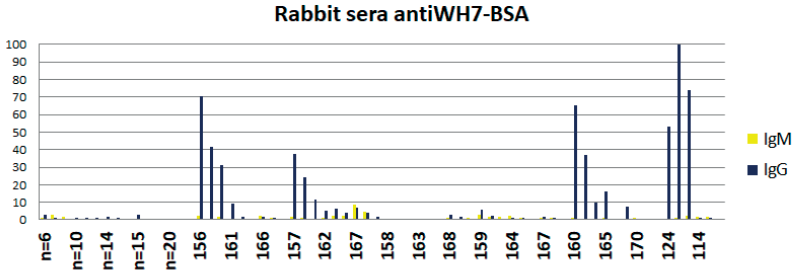


Figure 5: Evaluation of monoclonal antibodies and sera using microarray technology.

be achieved, indicating the possibility to selectively target a bacterial (sub)population if the proper antigen and vaccination method are used. Clearly such a response can not be expected from isolated LTA preparations as these are very heterogeneous highlighting a clear advantage of synthetic material over naturally sourced material.

E. faecium WTA

Besides LTA, *Enterococci* can express different types of WTA. In *E. faecalis* several type-II WTAs have been described that are involved in evasion of complement mediated phagocytosis (See Figure 6 for structures of different enterococcal WTAs).

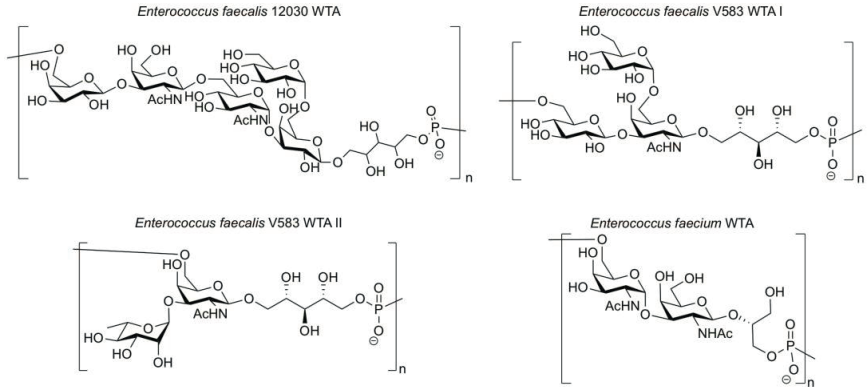


Figure 6: Structures of *E. faecalis* and *E. faecium* WTA.

E. faecium strain U0317 produces a WTA that can shield the LTA from opsonophogocytic antibodies. This WTA was shown to be built up from [→ 6-(α-D-GalNAc-(1→ 3)-β-D-GalNAc-(1→ 2)-GroP-(3→]-repeating units. As the stereochemistry of the GroP moiety was not revealed, van der Es *et al.* synthesized two sets of oligomers of the repeating units featuring either the *sn*-glycerol-1-phosphate or the *sn*-glycerol-3-phosphate constituents.[40] Scheme 9 describes the syntheses towards these oligomers. In a stereoselective glycosylation reaction, imidate donor **175** was condensed with azidogalactose **174** to give the α-linked disaccharide. Transformation of the selenoglycoside **176** into an imidate donor and subsequent coupling to either of the enantiomeric glycerol acceptors, using a solvent system of acetonitrile, propionitrile and DCM to control the stereoselectivity of the reaction, delivered the β-linked pseudo-trisaccharides **177** and **178** in high yield and as a single diastereoisomer. These were transformed into the required phosphoramidite building blocks for oligomerization **181** and **182**. First a spacer was installed for future conjugation purposes giving **183** and **184**. The employed coupling cycles used 4,5-dicyanoimidazole (DCI) as activator, (1*S*)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) as oxidant and trichloroacetic acid (TCA) to unmask the DMTr protected alcohols. The elongation proceeded uneventfully to provide the oligomers of both GroP-epimers to

eventually provide structures encompassing three repeating units. Standard deprotection provided the set of target compounds. NMR analysis of the generated compounds and comparison to the spectra of the naturally occurring WTA revealed the stereochemistry of the *E. faecium* WTA GroP to be *sn*-3-glycerol phosphate. The biosynthesis of GroP-containing WTAs[41-42] and LTAs[5, 43] generally employs different glycerolphosphate donors. While LTA is assembled using phosphatidyl glycerol, having the *sn*-1-glycerol phosphate stereochemistry, as a source of the GroP units, WTA is generated through the use of cytidine *sn*-3-glycerol phosphate. The stereochemical assignment based on the synthetic compounds described above is thus supported by biosynthesis precedent.

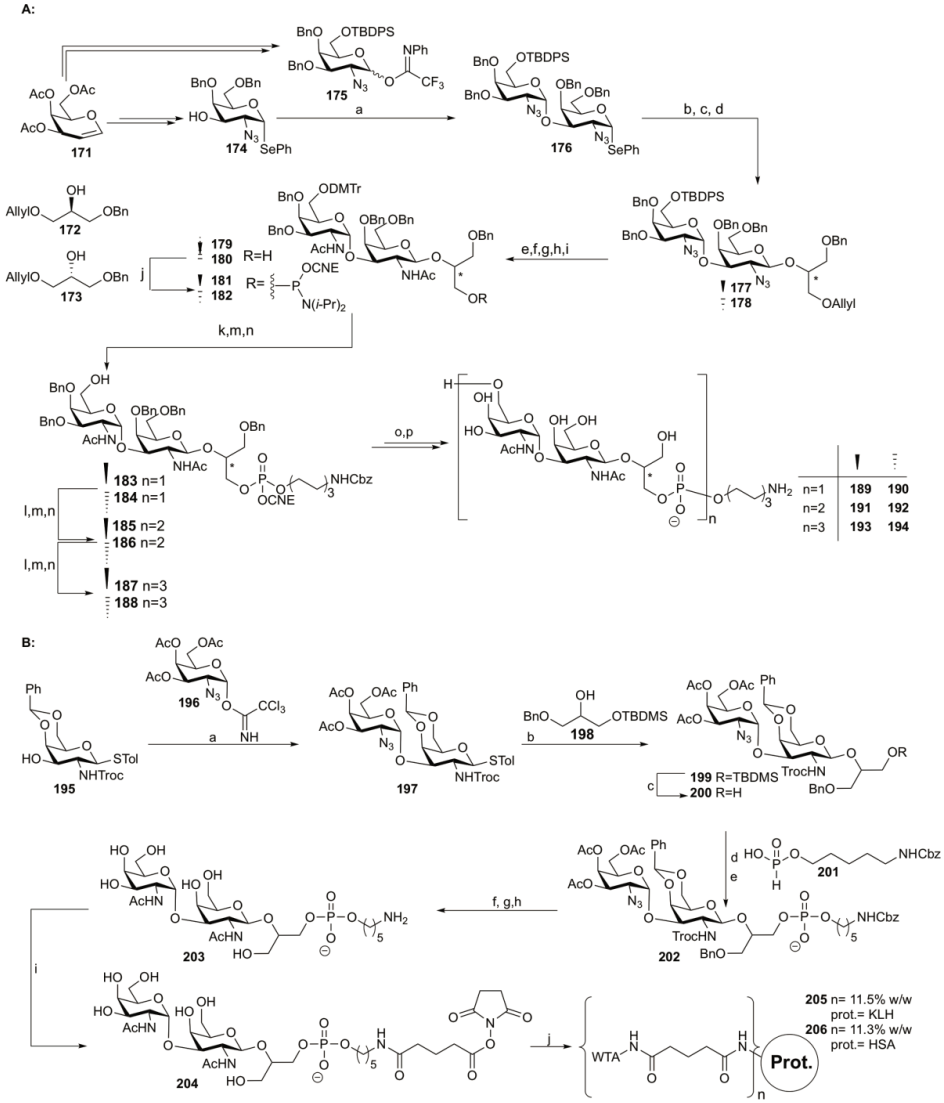
Wu and co-workers also reported on the synthesis of the *E. faecium* U0317 WTA monomer, although the stereochemistry of the glycerol moiety was not specified (See Scheme 9B).[44] The digalactosamine glycerol building block **200** was coupled to spacer **201** using H-phosphonate chemistry employing pivaloyl chloride to activate the H-phosphonate. Compound **202** was subjected to Zn/AcOH/Ac_2O to transform the azido and Troc protecting group into an acetamide, followed by deprotection of the acetyl groups using Zemplén conditions and a hydrogenolysis reaction then yielded final compound **203**. The generated monomer was conjugated to KLH and HSA as carrier proteins using a bifunctional glutaryl ester method. The KLH conjugate was used to immunize mice and the serum raised against the conjugate was evaluated by ELISA to probe recognition of the HSA conjugate and the non-conjugated monomer. High levels of IgG were observed in the sera raised against the conjugate and the antibodies were able to recognise both **205**, **206** proving that they are antigen specific. The sera will have to be further evaluated for recognition of the naturally occurring WTA and opsonic properties towards *E. faecium* bacteria.

Clostridium difficile

Clostridium difficile is a Gram-positive anaerobic bacterium, frequently found in the environment as spores that can infect humans and other animals.[45-46] The spores can survive in the stomach and intestine of the host and colonize the gastrointestinal tract. *C. difficile* infections (CDI) are responsible for nosocomial diarrhea and antibiotic-associated colitis.[47] Cell surface-associated antigen-based vaccines can protect both the colonization by *C. difficile* and the symptoms of the infection.[48] The surface-associated antigens can be divided in two main classes: surface proteins (SLP, FliD and Cwp84) and surface polysaccharide antigens (called PSI, PSII and PSIII).

Clostridium difficile LTA (PSIII)

The structure of the cell-surface glycans has been elucidated and it has been shown that PSIII is an LTA that features a triglucoside diacylglycerol (DAG) lipid anchor and a



Scheme 9. A) Synthesis of *E. faecium* WTAs; *Reagents and conditions*: a) TfOH, DCM, 0°C, 58%; b) NIS, THF/H_2O; c) CF_3(=NPh)Cl, K_2CO_3, acetone, quant; d) **172** or **173**, TfOH, ACN/EtCN/DCM, -40°C, **177** (90%), **178** (80%); e) PMe_3, dioxane/H_2O; f) Ac_2O, TEA, DCM; g) TBAF, THF; h) DMTr-Cl, TEA, DCM; i) (i). $Ir(COD)(Ph_2MeP)_2$, THF (ii). I_2, $NaHCO_3$, H_2O/THF; **179** (28% 5 steps), **180** (46% 5 steps); j) DIPEA, *N,N*-diisopropylamino-2-cyanoethyl-chlorophosphite, DCM **181** (85%), **182** (62%); k) **38**, DCI, **179**, or **180**, ACN; l) **181**, or **182**, DCI, ACN; m) CSO, ACN; n) 3% TCA, DCM, **183** (72%), **184** (85%), **185** (62%), **186** (64%), **187** (69%), **188** (67%); o) conc. NH_3, dioxane; p) Pd^0, H_2, AcOH, H_2O, **189** (73%), **190** (57%), **191** (44%), **192** (45%), **193** (49%), **194** (56%). **B) Assembly of an *E. faecium* WTA-BSA conjugate;** *Reagents and conditions:* a) DCM/diethyl ether (2:1), TMSOTf, 4Å MS, -78°C, 78% (α:β = 5:1); b) DCM, TfOH, NIS, 4Å MS, -20°C, 61%; c) TBAF:AcOH = 1:1, THF, 75%; d) pyridine, pivaloyl chloride; e) Iodine, pyridine:H_2O = 9:1, 75%; f) Zn/AcOH/Ac_2O, 67%; g) NaOMe, MeOH; h) H_2, $Pd(OH)_2$/C, 41% over 2 steps; i) DSG, DMF and PBS buffer (4:1), rt, 4h, quantitative; j) KLH or HSA, PBS buffer, rt, 3 days.

di-α-glucosamine glyceric acid repeating unit interconnected through phosphodiesters bridging the primary alcohols of the glucosamine moieties (See Figure 7).

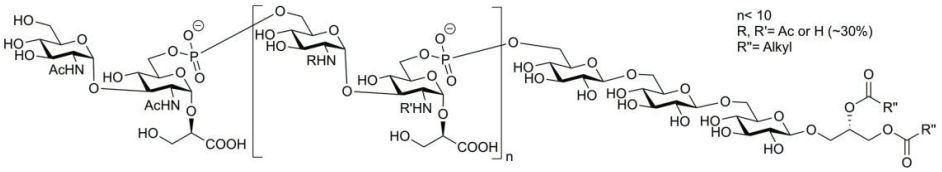
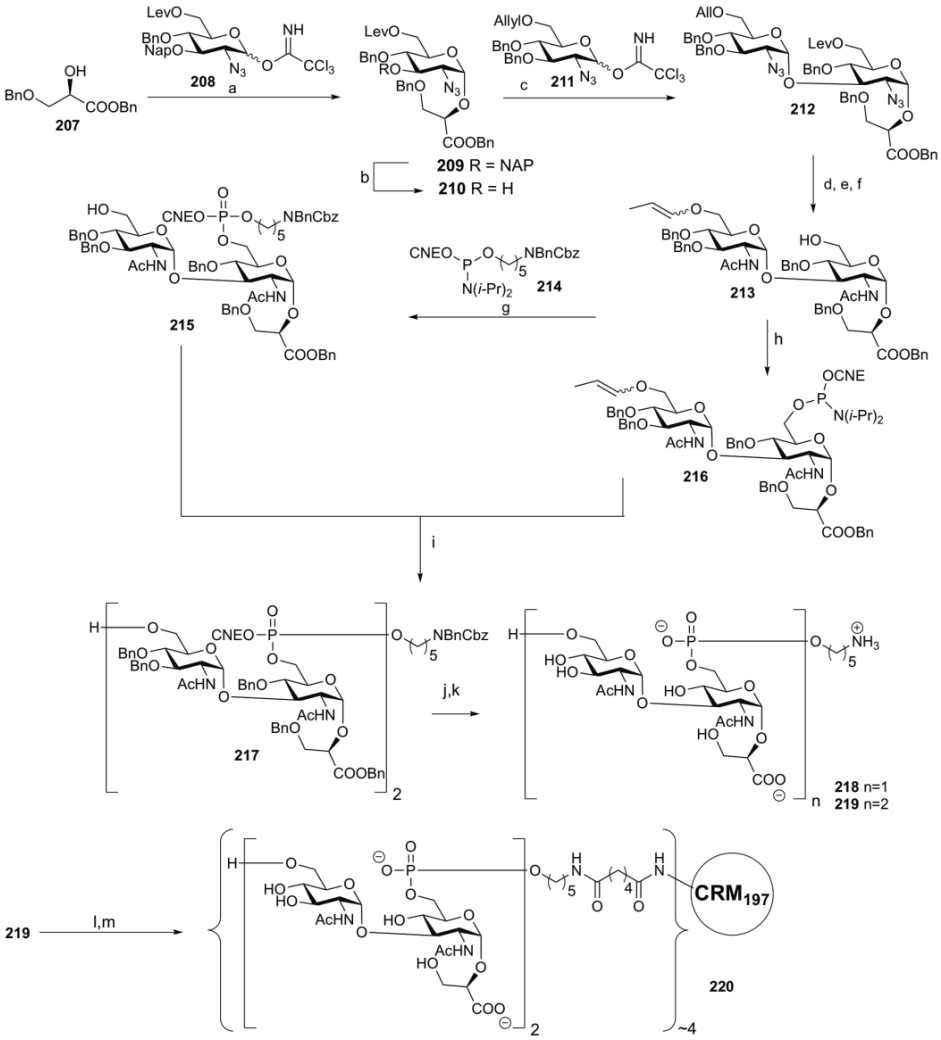


Figure 7. *C. difficile* LTA

Different synthetic approaches have been reported to generate well-defined fragments of the *C. difficile* LTA. Seeberger and co-workers successfully synthesized a monomer and a dimer of the repeating unit as depicted in scheme 10.[49] The key pseudo-trisaccharide **212** was obtained by the glycosylation of the benzyl protected (2*R*)-glyceric acid and the trichloroacetimidate **208**. Removal of the C3′-*O*-naphthyl ether and subsequent glycosylation with building block **211** then gave diglucosamine **212**. In both glycosylation reactions the desired α-selectivity was achieved through the use of low-temperature glycosylations in a dichloromethane-diethylether mixture. The azides were next transformed into the corresponding acetamides, after which the C6″-allyl ether was isomerized into the enol ether. Delevulinoylation provided the C6-OH (**213**), which was connected with the phosphoramidite aminopentanol spacer **214** using 5-ethylthio-1H-tetrazole (ETT) as the coupling agent. Notably, in the next step, iodine was not only used as oxidizing agent but also to cleave the C-6 enol ether protecting group. The monomer **215** was then elongated using phosphoramidite **216**. The monomer and dimer were then treated with triethylamine to cleave the cyanoethyl groups followed by hydrogenation to remove the remaining protecting groups to deliver the final targets **218** and **219**. The dimer repeat **219** was immobilized on a glycan microarray together with synthetic structures representing PSI and PSII and screened with sera of 12 patients with CDI. Serum IgG against PSI and PSII was detected in 10 and 11 out of 12 samples, respectively, while antibodies, recognizing LTA **219** were detected in half of the samples, indicating that *C. difficile* LTA can be a suitable antigen for a vaccine development against CDI. Encouraged by these preliminary results compound **219** was conjugated to carrier protein CRM_{197} using an adipic acid linker (Scheme 10).[50] With this glycoconjugate three different vaccination formulations were investigated. The first one using Freund's adjuvant (FA), the second one adsorbed on alum and the third without any adjuvant. Sera raised against the non-adjuvated conjugate **220** showed good opsonic killing of *C. difficile*, suggesting that the synthetic glycans provide an intrinsic adjuvant activity, while the conjugate with FA showed a weaker response. The most robust response was obtained with the alum formulation and this vaccine was used in a mouse-infection

protection model. It was shown that vaccination with the alum formulation offered protection and inhibited colonization, proving that surface antigen-based vaccines are indeed effective in reducing the colonization by *C. difficile*.

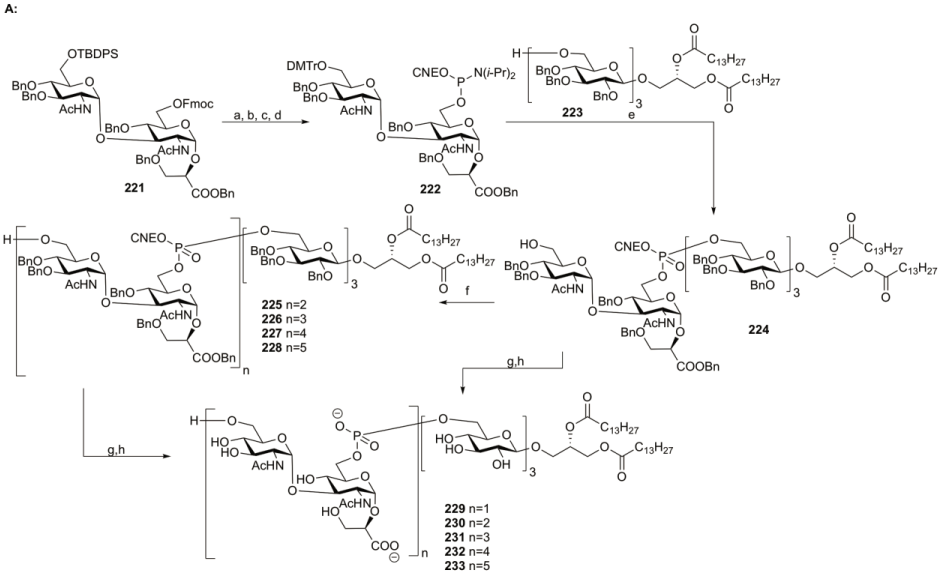


Scheme 10. Assembly of *C. difficile* LTA-conjugates;

Reagents and conditions: a) TMSOTf, DCM, Et_2O, -20°C to -10°C, 81% α/β = (9:1); b) DDQ, DCM, phosphate buffer pH= 7.2, 0°C to rt, 80%; c) TMSOTf, DCM, Et_2O, -20°C to -10°C, 69%, α/β = (8:1); d) AcSH, pyridine, 67%; e) $Ir(COD)(Ph_2MeP)_2$,THF; f) hydrazine hydrate, AcOH, pyridine, DCM, 78% over 2 steps; g) (i) 5(ethylthio)tetrazole, ACN; (ii) I_2, H_2O, THF, 98%; h) 2-cyano-ethyl bis(*N,N*-diisopropylamino)phosphoramidite, tetrazole, diisopropylamine, DCM, ACN, 82%; i) (i) 5(ethylthio)tetrazole, ACN; (ii) I_2, H_2O, THF, 78%; j) TEA; k) H_2 (4 bar), Pd/C, H_2O, AcOH; l) di-*N*-succinimidyl adipate, TEA, DMSO; m) CRM_{197}, 100 mM sodiumphosphate, pH=7.4.

Longer LTA fragments with the glycolipid anchor attached have been assembled by the group of Pedersen (see Scheme 11A).[51] Following a roughly similar approach as described

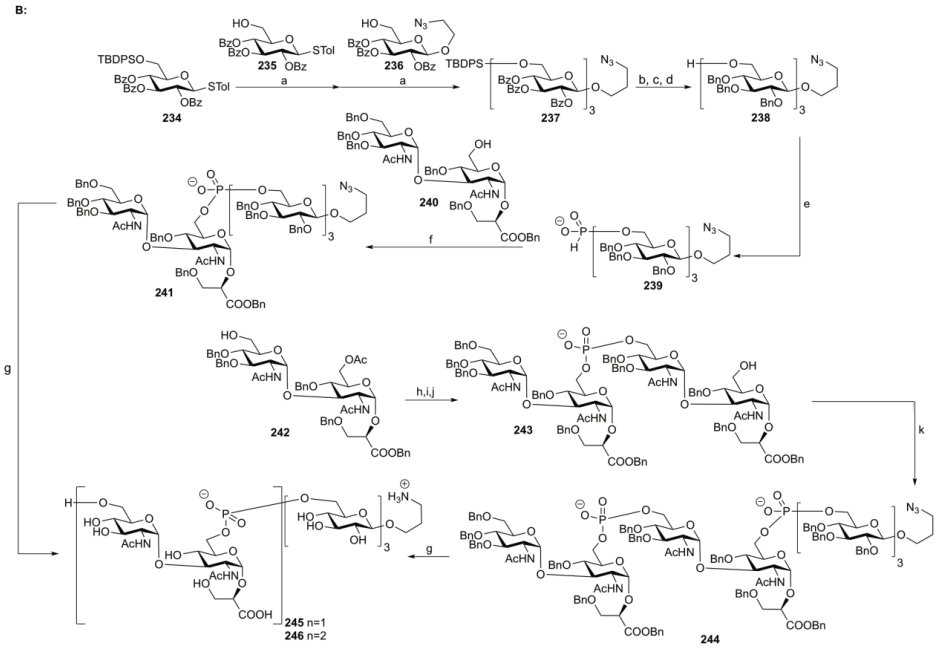
above, they assembled the di-glucosamine glyceric acid building block **221** with a C6-*O*-fluorenylmethylcarbonate and a C6'-*O*-TBDPS ether as an orthogonal set of protecting groups. This intermediate was transformed into the required LTA repeating unit synthon **222**, bearing a cyanoethyl protected phosphoramidite and a DMTr-ether as a temporary protecting group for the alcohol groups that were to be elongated. The lipid anchor **223** was first coupled to **222** using 4,5-dicyanoimidazole (DCI) as activating agent and the generated phosphite was oxidized using iodine followed by the liberation of the DMTr group using a dichloroacetic acid solution. Alcohol **224** was then further elongated with **222** to give oligomers up to 5 repeating units. It was observed that the yield of the elongation cycles dropped with growing chain length. The fragments were then treated with DBU to liberate the phosphodiesters and a final hydrogenolysis reaction cleaved all benzyl groups to deliver the target compounds **229**-**233**. The immunomodulatory properties of the LTA-fragments were investigated in human mononuclear cells (hMNCs) and in a whole blood assay but no innate immune system activation was observed.



Scheme 11. A) Total synthesis of *C. difficile* LTA; *Reagents and conditions:* a) HF, pyridine, THF, 95%; b) DMTr-Cl, pyridine, 91%; c) DBU, DCM, 94%; d) 2-cyanoethyl bis(*N,N*-diisopropylamino)phosphoramidite, tetrazole, DIPEA, DCM, 84%; e) (i) DCI, ACN; (ii) I_2, H_2O, pyridine, THF; (iii) DCA, TES-H, DCM 62%; f) (i) **222**, DCI, ACN (ii) I_2, H_2O, pyridine, THF; (iii) DCA, TES-H, DCM, n=2 (74%), n=3 (66%), n=4 (56%), n=5 (42%); g) DBU, DCM, n=1 (83%), n=2 (76%), n=3 (82%), n=4 (97%), n=5 (77%); h) Pd black, H_2, THF/H_2O, AcOH, **229** (56%), **230** (53%), **231** (mixture of LTAs, 59% product calculated, after purification by RP18 HPLC, 60%), **232** (mixture of LTAs, 26% product calculated, after purification by RP18 HPLC, 51%), **233** (mixture of LTAs, 45% product calculated).

Recently, Gu *et al.* reported an alternative strategy for the assembly of *C. difficile* LTA fragments based on H-phosphonate chemistry (see Scheme 11B).[52] They generated two target molecules, containing one or two LTA repeating unit and the lipid trisaccharide

core equipped with a conjugation handle instead of the diacyl glycerol moiety. The triglucosyl lipid anchor core was assembled in a one-pot procedure using conditions developed by Huang *et al.*[53], while the LTA repeating unit was assembled by comparable means as previously described. To conjugate the triglucoside to the repeating unit, it was transformed into H-phosphonate **239** which was then coupled to diglucosamine **240** using pivaloyl chloride (PivCl). Subsequent oxidation then generated the phosphodiester. In similar vein two repeating units were combined, to provide dimer **243**. The C6-acetate of this building block was removed to set the stage for a second coupling to H-phosphonate **239**. A single hydrogenation event transformed **241** and **244** into target compounds **245** and **246**. Conjugation to a carrier protein has been foreseen by the authors but not reported yet.



Scheme 11. B) H-phosphonate chemistry to assemble *C. difficile* LTA; *Reagents and conditions:* a) (i) *p*-TolSCl, AgOTf, TTBP, -78°C to rt (ii) **235** or **236**, 62% overall yield; b) sat NH_3, MeOH; c) BnBr, NaH, DMF; d) TBAF, THF, 98%; e) (i) 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, pyridine/dioxane (v/v 2:3); (ii) H_2O, 87%; f) (i) PivCl, pyridine; (ii) I_2, H_2O, 78%; g) H_2, 10% Pd/C, DCM/MeOH/H_2O (v/v/v 10:10:1) n=1 (75%), n=2 (69%); h) (i) 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, pyridine/dioxane (v/v 2:3); (ii) H_2O, 89%; i) **240**, (i) PivCl, pyridine (ii) I_2, H_2O, 76%; j) AcCl (cat), MeOH/DCM (1:1); k) (i) **239**, PivCl, pyridine; (ii) I_2, H_2O, 69%.

CONCLUSIONS

Teichoic acids are abundantly present in Gram-positive bacterial cell-walls and they play an all-important role in host-pathogen interactions. As such they represent attractive structures for vaccine development. Unfortunately, all attempts at using isolated TAs for vaccine purposes have failed. One of the reasons behind these failures may be the relatively ill-defined material used for generation of the vaccines. Organic synthesis can provide well-defined TA-fragments, that can be modified at will to, for example, attach conjugation handles at pre-determined sites in the molecule. It thus provides an excellent platform to tackle the problems that naturally sourced micro-heterogeneous TAs present. Over the years, several important advances have been reported regarding the synthesis of both LTA and WTA structures. To deal with the structural variety, automated synthesis techniques have been outlined that can allow for the rapid generation of libraries of TAs. Building block chemistry is now at a level that the required glycosylated building blocks can be reliably obtained through innovative stereoselective glycosylation methodology in combination with effective protecting group chemistry. Total syntheses have been reported of large and complex TAs, requiring the union of large building blocks. Labile moieties, such as the crucial D-alanine esters and functional lipid tails have been successfully incorporated. The large majority of approaches for the assembly of TAs hinges on the use of phosphoramidite building blocks to construct the phosphotriester linkages. This methodology has proven to be extremely reliable and will undoubtedly be used for the assembly of many TA targets in the future. In the future synthetic methods will further mature, to allow for the more rapid assembly of more complex and varied TAs as well as expand the library of available TAs. Interaction studies at the atomic level will unravel how TA-substitution patterns govern host-pathogen interactions and how they impact the fitness and virulence of important human pathogens. This will open up possibilities to use these molecules in vaccine formulations to neutralize the ever-growing threat of multidrug resistant super bugs.

OUTLINE OF THIS THESIS

Chapter 2 describes the synthesis of ribitol wall teichoic acid (WTA) fragments, both in solution, and on solid phase. These WTA fragments were used to screen for binding to human IgG sera and human langerin. The hexamer was used as a substrate for the enzyme TarP in crystallization studies probing the binding mode. The hexamer was enzymatically glycosylated and this product was coupled to magnetic beads and used to detect WTA-specific IgG in human serum.

Chapter 3 reports the synthesis of C-4 glycosylated WTAs using α- and β–linked C4-GlcNAc ribitol phosphoramidite building blocks. The binding affinity between human langerin and both glycosylated and non-glycosylated WTA fragments was evaluated on the micro array. This showed selective binding of C-type lectin to the WTA fragments bearing a β–GlcNAc. In addition, spacer-free trimers were synthesized for crystallization studies to probe the interaction in the active site of langerin.

Chapter 4 describes the synthesis of C-3 glycosylated WTAs. Building on the successful synthesis of unsubstituted WTAs on solid phase as described in Chapter 2, here the automated solid phase synthesis was used to assemble glycosylated WTA fragments. NMR data of the glycosylated WTA fragments were presented for structure elucidation of newly identified bacterial WTA species. The antibody binding of the C-4- and C-3 glycosylated WTAs was probed in the bead assay. This showed specific binding of the α-mAb toward α-GlcNAc WTAs, whereas β–mAb showed cross-reactive binding to both the C-3 and C-4 β–GlcNAc WTAs.

Chapter 5 describes an approach to synthesize a ribitol phosphate heptamer bearing D-alanine esters on the C-2 position. This ester modification is found in *S. aureus* WTAs, and the degree of this esterification seems to influence the susceptibility toward Vancomycin and other glycopeptide antibiotics. Isolation of these fragments is very difficult due to high lability of the esters.

Well-defined WTA fragments are required to evaluate the role of the D-alanine modification at the molecular level in structure activity studies. Due to the high hydrolytic and labile nature of the esters, benzyl protected phosphoramidites were used instead of the common cyanoethyl protected phosphoramidite which require a basic step in the deprotection stage. At the final deprotection stage it was necessary to keep the conditions acidic to protect the D-alanine esters from hydrolysis.

Chapter 6 reports a synthesis route for *E. faecalis* V583 WTA fragments composed of *N*-acetyl-β-D-galactosaminyl ribitol phosphate residues connected to an α-L-rhamnose branch at the C-3 of the galactosamine residue. The WTA repeating unit was assembled through the regioselective coupling between a rhamnose donor with a participating benzoyl group at the C-2 for α-selectivity and a C-3, C-4-diol GalNAc acceptor. Subsequent coupling with a ribitol acceptor yielded the pseudo-trissacharide.
This pseudo-trisaccharide was the key intermediate for further elongations. The intermediate was converted into an alcohol and into a phosphoramidite building block. Condensation of the alcohol with a phosphoramidite spacer yielded the monomer target

compound. This monomer was further coupled to the phosphoramidite building block to deliver the dimer target compound.

Chapter 7 provides a summary of this thesis and future prospects including crystallizations of WTA fragments with langerin and monoclonal antibodies, and the assembly of WTAs featuring both GlcNAc and D-alanine esters using a cleavable silyl linker on solid phase.

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2

Synthesis and application of *Staphylococcus aureus* ribitol phosphate fragments

INTRODUCTION

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

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

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

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

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

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

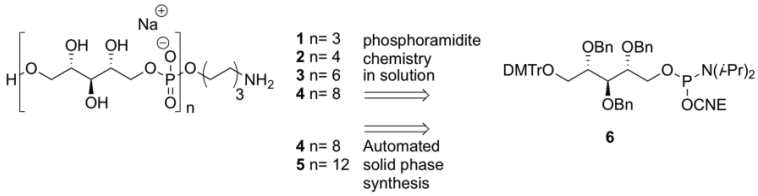
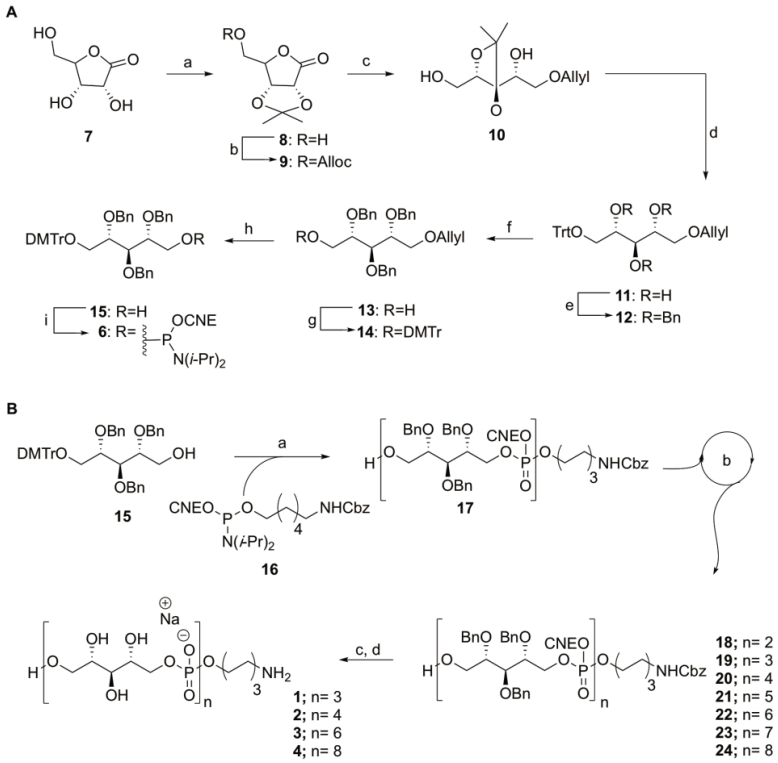


Figure 1. RboP oligomers synthesized from repeating unit **6** in both solution and solid phase.

RESULTS AND DISCUSSION

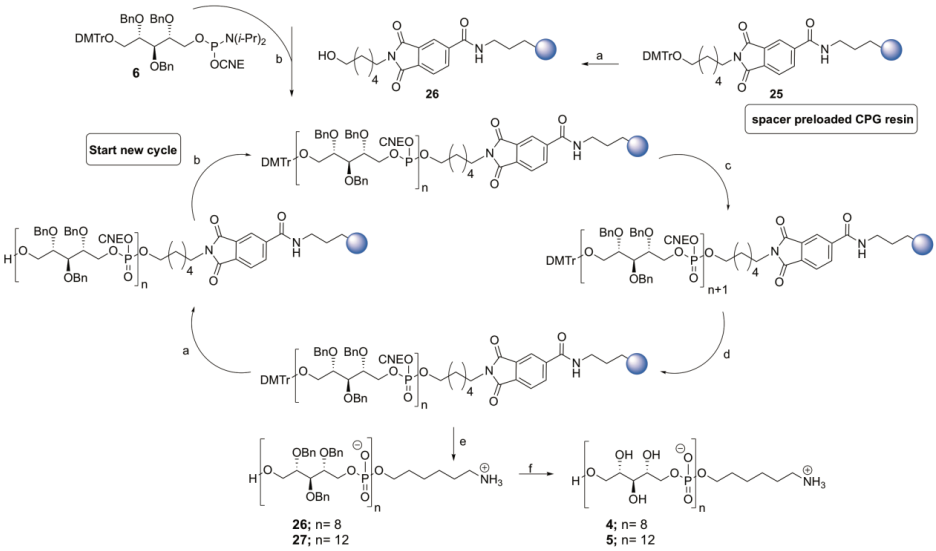
For the solution and automated solid phase assembly (ASPS) of the set of target compounds key phosphoramidite **6** was required, the synthesis of which started from lactone **7**. Following the route reported by Hermans *et al.*[22] **13** was generated as shown in Scheme 1A. Isopropylidene protection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

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



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

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



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

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

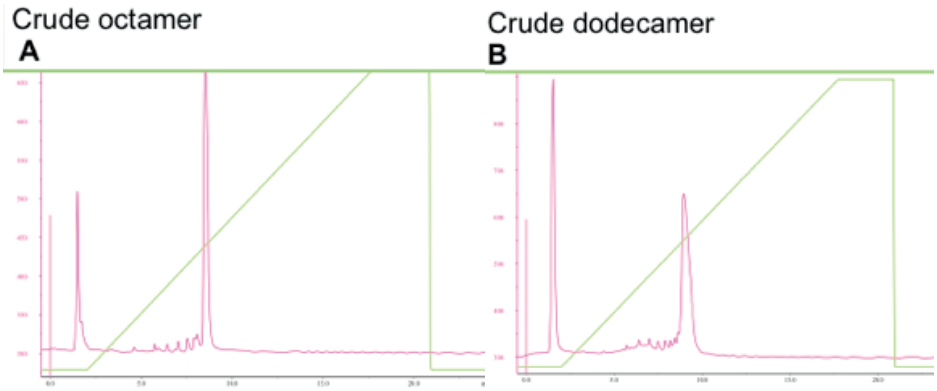


Figure 2. Anion-exchange chromatogram of the crude octamer **26** (A) and crude dodecamer **27** (B). Column type: DNA PAC PA 100, Eluent buffer A: 10 mM NaOAc + 10 mM NaCl, buffer B: 10 mM NaOAc en 1 mM NaCl, lineair gradient 1/0 to 0/1.

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

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

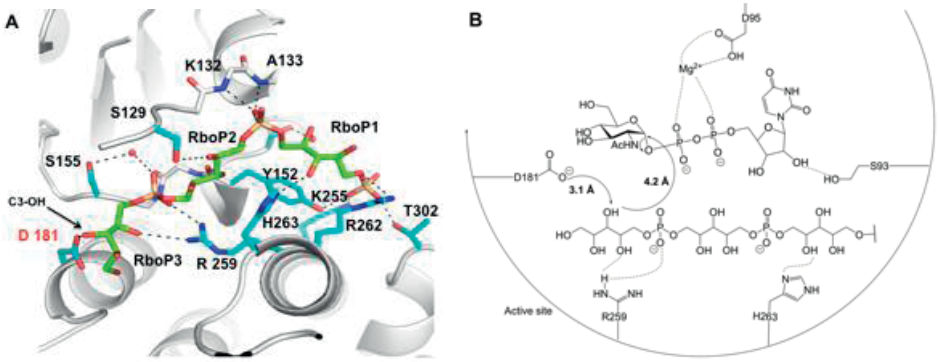


Figure 3. Crystal structure **of** the hexamer (**3**) in the active site of TarP (A), schematic representation of the hexamer in the active site. (B). Dashed bonds represent hydrogen- or ionic bonds.

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

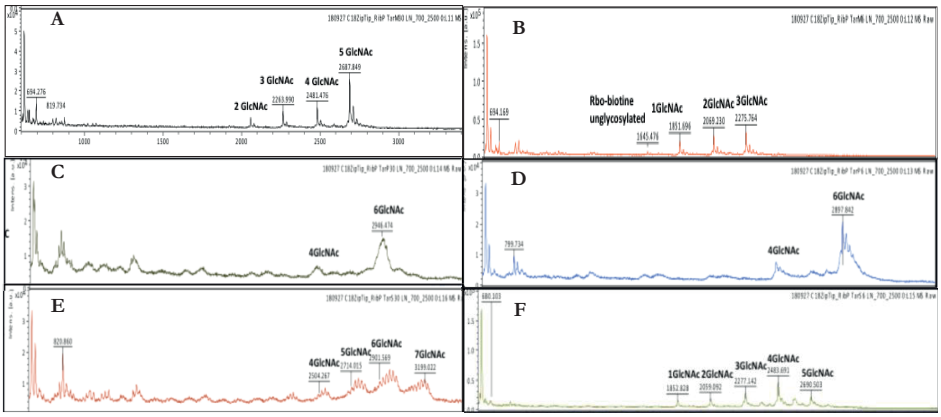


Figure 4. MALDI-MS analysis of enzymatic glycosylations performed on biotinylated hexamer **28** upon 2 different concentrations of enzyme: (A) TarM 30 μg/mL, (B) TarM 6 μg/mL, (C) TarP 30 μg/mL, (D) TarP 6 μg/mL, (E) TarS 30 μg/mL and (F) TarS 6 μg/mL.

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

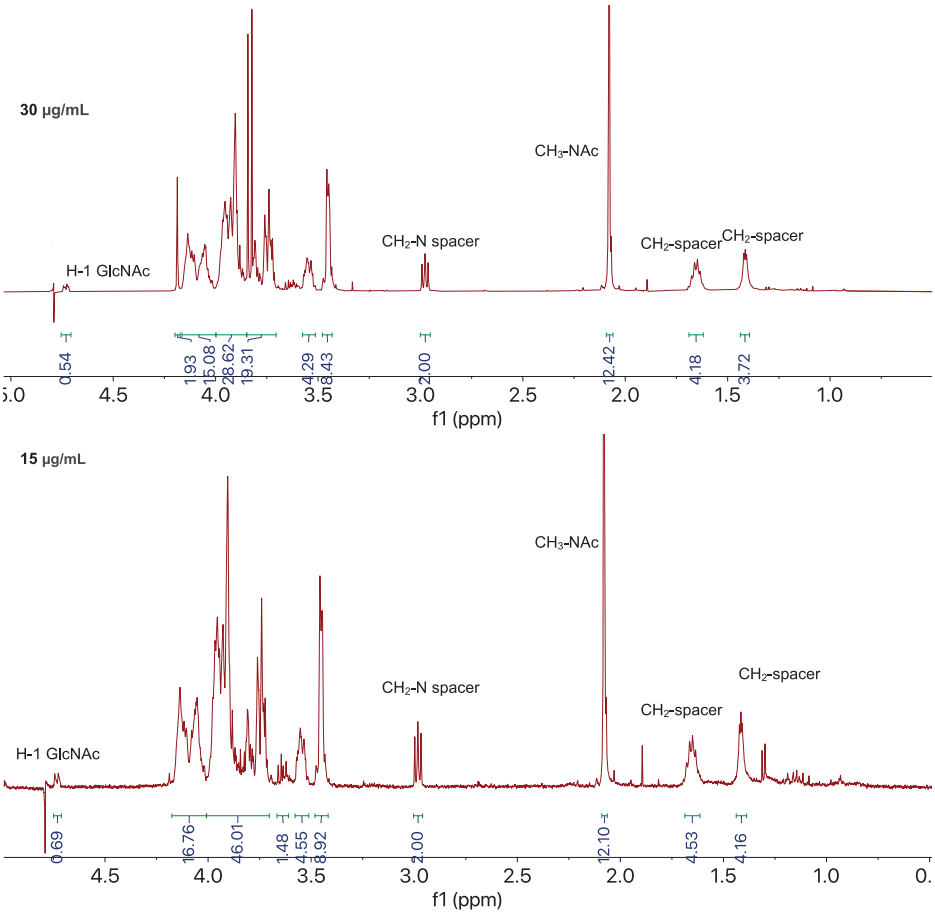


Figure 5. Partial ^{1}H NMR spectra of the 30 μg/mL- and 15 μg/mL TarS glycosylation of compound **3.** All spectra were measured in D_2O on a 500 MHz NMR at 25°C.

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

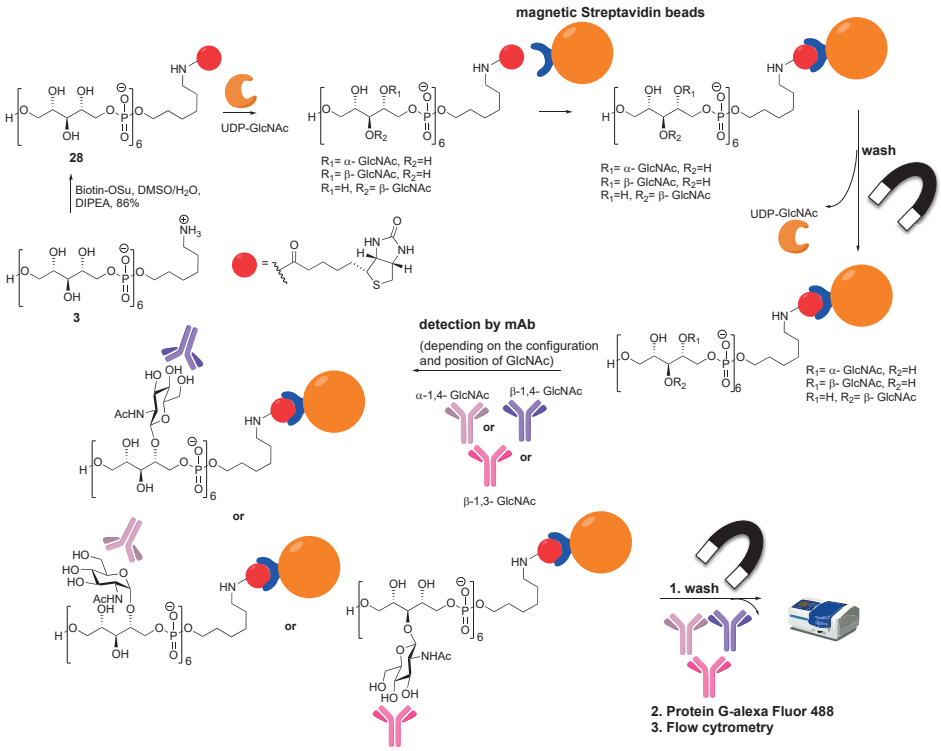


Figure 6. Schematic representation of enzymatic modification on Streptavidin beads. 1) Biotinylation of WTA fragment **3**; 2) Enzymatic glycosylation using UDP-GlcNAc and TarM/TarS or TarP; 3) Adsorption on streptavidin coated M280 Dynabeads; 4) Binding of monoclonal antibodies; 5) Alexa 488-Protein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

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

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

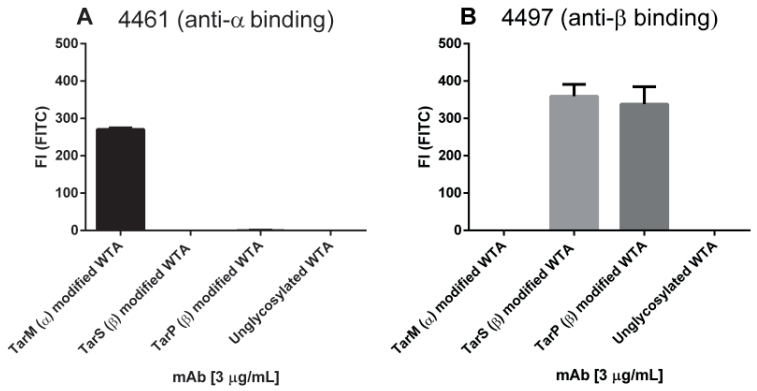


Figure 7. Monoclonal antibody detection by anti-α 1,4-GlcNAc (A) and anti-β 1,4-GlcNAc (B). Data is expressed as mean with standard error of the mean.

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

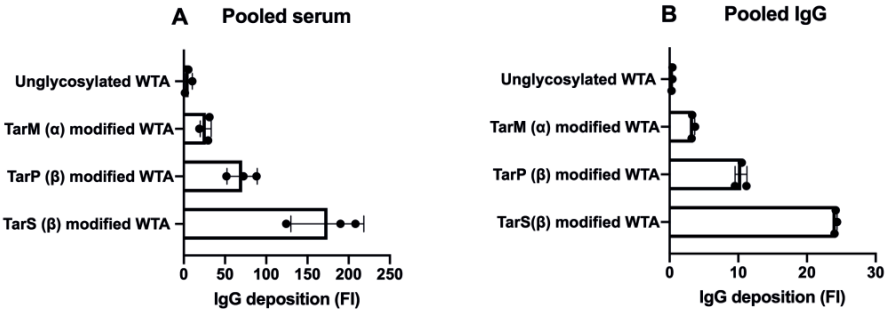


Figure 8. (A) 3% Heat Inactivated pooled human serum (B) 10 mg/mL pooled human IgG. Corrected for background binding to biotin control beads of three independent experiments are shown.

CONCLUSION AND OUTLOOK

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

EXPERIMENTAL SECTION

General information

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

(2%) and K_2CO_3 (1%) in water. Optical rotation measurements ($[\alpha]_D^{20}$) were performed on an Anton Paar Modular Circular Polarimeter MCP 100/150 with a concentration of 10 mg/mL (*c* 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300. ^{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).

Phosphoramidite coupling, oxidation, and detritylation.

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

General procedure for global deprotection

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

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

Procedure for large-scale enzymatic glycosylation

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

Procedure for enzymatic glycosylation

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

General procedure for automated solid phase synthesis

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

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

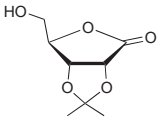
Purification method using anion-exchange chromatography

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

IgG deposition on WTA beads

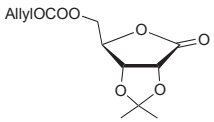
Biotinylated RboP hexamers (0.17 mM) were coated on $5x10^7$ pre-washed Dynabeads M280 Streptavidin (Thermo Fisher) in sterile PBS for 15 minutes at room temperature. The coated beads were washed three times with PBS using a plate magnet, resuspended in PBS 0.1% BSA 0.05% Tween-20 and stored at 4°C.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

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



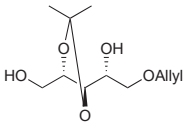
D-(+)-Ribono-1,4-lactone (50.0 g, 337.6 mmol, 1.0 eq.) was dissolved in acetone (2.0 L; 0.17 M). Concentrated HCl (20.0 mL; 1.9 eq.) was added and the reaction mixture was stirred at rt overnight. The reaction was quenched by the addition of solid $NaHCO_3$ until a neutral pH was reached. The mixture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

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



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

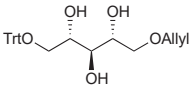
5-*O*-Allyl-2,3-*O*-isopropylidene-D-ribitol (10)



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

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

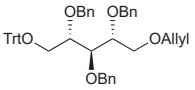
5-*O*-Allyl-1-*O*-trityl-D-ribitol (11)



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

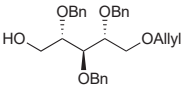
arom), 134.2 (CH_2=*C*H), 143.6 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{27}H_{30}O_5Na$ 457.19855, found 457.19833.

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



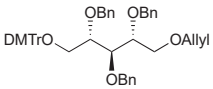
Compound **11** (22.1 g; 50.9 mmol; 1.0 eq.) was dissolved in a (v/v = 1/1) mixture of THF/DMF (150 mL, 0.34 M). The mixture was cooled to 0°C and NaH (8.1 g; 203.6 mmol; 4.0 eq., 60% in mineral oil) was added portion wise. BnBr (24.2 mL; 203.6 mmol; 4.0 eq.) was added dropwise over 30 minutes and the reaction was stirred from 0°C to rt overnight. The mixture was quenched by the addition of 10 mL MeOH at 0°C followed by the addition of 600 mL Et_2O. The organic phase was washed with 400 mL H_2O (5x) and then dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 1:0 to 89:11 pentane/EtOAc) yielded title compound **12** (24.3 g; 34.4 mmol) in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.35 – 3.69 (m, 4H, 2x CH_2-Rbo), 3.86 – 3.94 (m, 5H, H-2, H-3, H-4, C*H*$_2$-CH), 4.46 – 4.80 (m, 6H, 3x C*H*$_2$-Bn), 5.11 – 5.25 (m, 2H, C*H*$_2$=CH), 5.87 (ddt, 1H, *J*= 17.2, 10.7, 5.5 Hz, CH_2=C*H*), 7.08 – 7.46 (m, 30H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 64.1 (C-1/C-5), 70.5 (C-1/C-5), 72.3 - 73.7 (*C*H$_2$-CH, 3x *C*H$_2$-Bn), 78.8 - 79.1 (C-2, C-3, C-4), 86.8 (Cq-Trt), 116.8 (*C*H$_2$=CH), 126.1 – 129.5 (CH-arom), 135.1 (CH_2=*C*H), 138.6 - 144.3 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{48}H_{48}O_5Na$ 727.3399, found 727.3417.

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



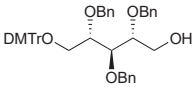
Compound **12** (24.2 g; 34.3 mmol) was dissolved in a (v/v= 9/1) mixture of AcOH/H_2O (428 mL; 0.08 M). The reaction mixture was heated to 80°C and stirred for 2 hours. After full conversion, the mixture was allowed to cool to rt. Subsequently, the mixture was concentrated under reduced pressure and diluted in Et_2O. The organic phase was washed with H_2O (1x), sat. aq. $NaHCO_3$ (2x) and brine (1x). The organic layer was dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 1:0 to 7:3 pentane/EtOAc) yielded title compound **13** (11.3 g, 24.5 mmol) in 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.40 (s, 1H, OH), 3.59 – 3.69 (m, 2H, CH_2-Rbo), 3.71 – 3.77 (m, 3H, C*H*$_2$-OH, H-2), 3.86 (td, 1H, *J*= 5.1, 3.7 Hz, H-4), 3.90 – 4.01 (m, 3H, C*H*$_2$-CH, H-3), 4.40 – 4.88 (m, 6H, 3x C*H*$_2$-Bn), 5.04 – 5.39 (m, 2H, C*H*$_2$=CH), 5.88 (ddt, 1H, *J*= 17.2, 10.7, 5.5 Hz, CH_2=C*H*), 7.06 – 7.55 (m, 15H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 61.4 (C-1), 69.7 (C-5), 71.9 (*C*H$_2$-Bn), 72.3 (*C*H$_2$-CH), 72.5 (*C*H$_2$-Bn), 74.0 (*C*H$_2$-Bn), 78.2 (C-4), 78.9 (C-2/C-3), 79.0 (C-2/C-3), 117.0 (*C*H$_2$=CH), 127.8 – 128.5 (CH-arom), 134.8 (CH_2=*C*H), 138.2 – 138.3 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{29}H_{34}O_5Na$ 485.2304, found 485.2309.

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



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

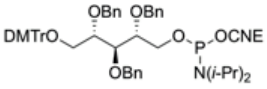
2,3,4-tri-*O*-benzyl-1-*O*-(4,4'-dimetoxytrityl)-D-ribitol (15)



Compound **14** (4.56 g; 5.96 mmol; 1.0 eq.) was dissolved in THF (30.0 mL; 0.20 M) and the solution was degassed with argon. Ir(COD)$(Ph_2MeP)_2PF_6$ (50 mg; 1 mol%) was added and the solution was degassed with argon. Then the red solution was purged with H_2 untill the color became yellow (~7 seconds) and hereafter the solution was degassed with argon to remove traces of H_2 from the solution and the reaction was stirred under argon atmosphere until the isomerization was complete according to TLC analysis. Then the solution was diluted with THF (30.0 mL) and aq. sat. $NaHCO_3$ (30.0 mL) followed by the addition of I_2 (2.27 g; 8.94 mmol; 1.5 eq.). The mixture was stirred +/- 30 minutes and was then quenched by the addition of sat. aq. NaS_2O_3. The mixture was diluted with EtOAc and washed with aq. sat. NaCl/$NaHCO_3$ (v/v= 1/1). Column chromatography using TEA neutralized silica (pentane: EtOAc 1:0 to 6:4 pentane/EtOAc) yielded title compound **15** in 79% yield (3.42 g; 4.72 mmol). $[\alpha]_D^{25}$= +16.2 (*c* 1.0, DCM); IR (neat, cm^{-1}): 3032, 2932, 2358, 1608, 1508, 1455, 1302, 1250, 1176, 1089, 1033, 829, 737, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 2.78 - 2.80 (m, 1H, O-H), 3.27 - 3.34 (m, 2H, CH_2-Rbo), 3.62 - 3.68 (m, 2H, H-3 CH-Rbo, C*H*H-OH), 3.72 - 3.79 (m, 7H, CH*H*-OH, 2 x OC*H*$_3$), 3.89 - 3.96 (m, 2H, H-2 CH-Rbo, H-4 CH-Rbo), 4.47 (d, 1H, *J*= 11.6 Hz, CH_2-Bn), 4.54 (d, 1H, *J*= 11.2 Hz, CH_2 Bn), 4.62 (d, 1H, *J*= 12.0 Hz, CH_2

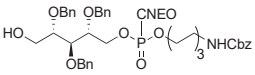
Bn), 4.67 (d, 1H, *J*= 11.6 Hz, CH_2-Bn), 4.75 (d, 1H, *J*= 11.6 Hz, CH_2 Bn), 6.77 (dd, 4H, *J*= 9.2 Hz, 2.8 Hz, H-arom), 7.15 - 7.34 (m, 24H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 55.8 (CH_3O), 61.8 (CH_2-OH), 64.7 (CH_2-Rbo), 72.6, 73.3, 74.3 (CH_2-Bn), 79.7, 79.8 (CH-Rbo), 80.8 (C-3 Rbo),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

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



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

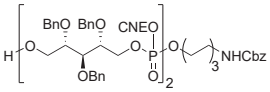
D-ribitol phosphate monomer (17)



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

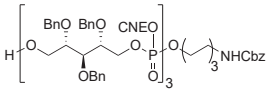
CH_2-hexylspacer), 2.67 - 2.70 (m, 2H, CH_2 cyanoethyl), 3.06 (q, 2H, *J*= 6.8 Hz, CH_2-N hexylspacer), 3.69 (dd, 1H, *J*= 10.8 Hz, *J*= 6.4 Hz, C*H*H-Rbo), 3.75 (q, 1H, *J*= 4.4 Hz, CH-Rbo), 3.79 (dd, 1H, *J*= 10.8 Hz, *J*= 3.6 Hz, CH*H*-Rbo), 3.92 (t, 1H, *J*= 4.8 Hz, CH-Rbo), 3.95 - 4.01 (m, 3H, CH-Rbo,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

D-ribitol phosphate dimer (18)



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

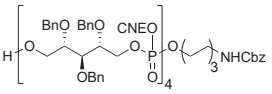
D-ribitol phosphate trimer (19)



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

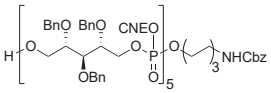
the title compound **19** in 88% yield (0.532 g; 0.285 mmol). IR (neat, cm-^{1}): 3412, 2936, 2866, 1717, 1520, 1456, 1260, 1028, 1011, 743, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.27 (m, 4H, CH_2-hexylspacer), 1.40 - 1.42 (m, 2H, CH_2-hexylspacer), 1.56 - 1.61 (m, 2H, CH_2-hexylspacer), 2.53 - 2.59 (m,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

D-ribitol phosphate tetramer (20)



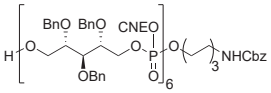
According to the general procedure described above, alcohol **19** (0.508 g; 0.273 mmol; 1.0 eq.) was coupled with phosphoramidite **6** (0.328 g; 0.355 mmol; 1.3 eq.) yielding the title compound **20** in 80% yield (0.522 g; 0.217 mmol). IR (neat, cm-^{1}): 3447, 2938, 2866, 1717, 1506, 1456, 1267,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

D-ribitol phosphate pentamer (21)



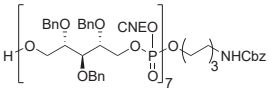
According to the general procedure described above, alcohol **20** (0.147 g; 61.0 μmol; 1.0 eq.) was coupled with phosphoramidite **6** (0.074 g; 80.0 μmol; 1.3 eq.) yielding the title compound **21** in 76% yield (0.136 g; 46.0 μmol). IR (neat, cm-^{1}): 3450, 2937, 1717, 1506, 1456, 1271, 1028, 1009,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

D-ribitol phosphate hexamer (22)



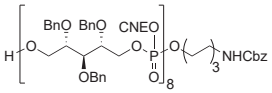
According to the general procedure described above, alcohol **21** (108 mg; 37.0 μmol; 1.0 eq.) was coupled with phosphoramidite **6** (74.0 mg; 80.0 μmol; 2.0 eq.) yielding the title compound **22** in 91% yield (0.117 g; 33.7 μmol). IR (neat, cm-^{1}): 3455, 1717, 1506, 1456, 1269, 1028, 737, 698;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

D-ribitol phosphate heptamer (23)



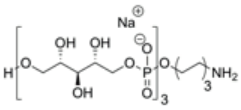
According to the general procedure described above, alcohol **22** (116 mg; 33.4 μmol; 1.0 eq.) was coupled with phosphoramidite **6** (51.0 mg; 55.1 μmol; 1.5 eq.) yielding the title compound **23** in 85% yield (115 mg; 28.7 μmol). IR (neat, cm-^{1}): 3447, 30301717, 1522, 1456, 1267, 1015, 746, 698;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

D-ribitol phosphate octamer (24)



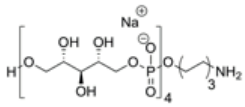
According to the general procedure described above, alcohol **23** (99 mg; 24.7 μmol; 1.0 eq.) was coupled with phosphoramidite **6** (34.2 mg; 37.0 μmol; 1.5 eq.) yielding the title compound **24** in 87% yield (98.0 mg; 21.5 μmol). IR (neat, cm-^{1}): 3447, 2934, 2872, 1717, 1522, 1456, 1271, 1028,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

Deprotected trimer (1)



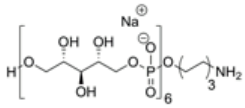
According to the general procedure described above, trimer **19** (57.0 mg; 30.6 μmol) was deprotected affording **1** in 75% yield (19.0 mg; 23.0 μmol). ^{1}H NMR (500 MHz, D_2O) δ= 1.41 - 1.46 (m, 4H, 2x CH_2 hexylspacer), 1.62 - 1.70 (m, 4H, 2x CH_2 hexylspacer), 2.99 (t, 2H, *J*= 7.5 Hz, CH_2-N hexylspacer),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

Deprotected tetramer (2)



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

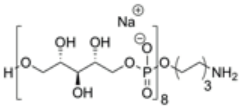
Deprotected hexamer (3)



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

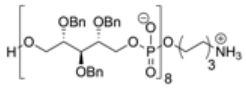
D_2O) δ= 1.8, 1.8, 1.6; MALDI-FT-ICR MS (m/z): $[M+Na]^+$ calculated for $C_{36}H_{75}NNa_7O_{43}P_6$ 1556.1417, found 1556.1335.

Deprotected octamer (4)



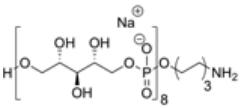
According to the general procedure described above, octamer **24** (40.0 mg; 8.79 μmol) was deprotected affording the target compound **4** in 89% yield (16.5 mg; 7.79 μmol). ^{1}H NMR (400 MHz, D_2O) δ= 1.41 (m, 4H, CH_2-hexylspacer), 1.64 (m, 4H, CH_2-hexylspacer), 2.96 (t, 2H, *J*= 7.2 Hz, CH_2-N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

Semi protected octamer (26)



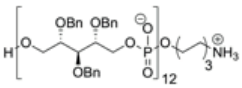
According to the general procedure described above for solid phase synthesis semi protected **26** was obtained in 15% yield (6.1 mg; 1.46 μmol). ^{1}H NMR (400 MHz, MeOD) δ= 1.16 - 1.22 (m, 4H, CH_2-hexylspacer), 1.41 (m, 4H, CH_2-hexylspacer), 2.70 (t, 2H, *J*= 7.2 Hz, CH_2-N hexylspacer), 3.64 - 4.29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

Deprotected octamer (4)



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

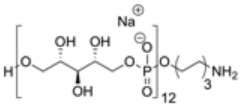
Semi protected dodecamer (27)



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

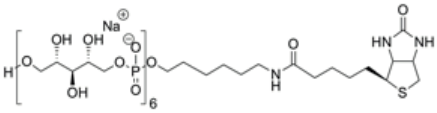
72H, CH_2-Bn), 7.10 - 7.32 (m, 180H, H-arom); ^{31}P NMR (162 MHz, MeOD) δ= 1.3, 1.2, 1.2, 1.1, 1.0.

Deprotected dodecamer (5)



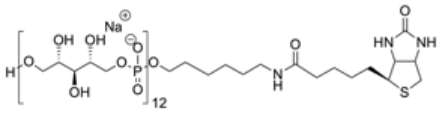
According to the general procedure described above for deprotection, compound **27** (3.36 mg; 0.54 μmol) was deprotected yielding **5** in quantitative yield (1.8 mg; 0.61 μmol). ^1H NMR (500 MHz, D_2O) δ= 1.30 - 1.35 (m, 4H, CH_2-hexylspacer), 1.63 - 1.68 (m, 4H, CH_2-hexylspacer), 2.99 (t, 2H, *J*=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

Biotin-(28)



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

Biotin-(29)



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

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

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3

Synthesis of glycosylated ribitol phosphates and their binding to human langerin

INTRODUCTION

The skin provides the first line of defense against microbes and the skin immune system relies on a rich network of professional antigen-presenting dendritic cells (DCs) on the epidermis and dermis.[1-2] Langerhans cells (LCs) are a subset of DCs, present in the epidermis and they express high levels of langerin, a CD207 C-type lectin receptor[3], which aids in the detection of invading pathogens by binding to pathogen-associated molecular patterns (PAMPs). Langerin is involved in the detection and uptake of a wide set of pathogens, including viruses like HIV[4] and measles[5], fungi[6], and (myco)bacteria.[7] Langerin is a type II C-type lectin receptor that has been shown to bind mannose, fucose, glucose, galactose-6-phosphate as well as *N*-acetyl glucosamine and sulfated heparin disaccharides, in a calcium dependent manner through its carbohydrate-recognition domain.[8]

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.

Staphylococcus aureus (*S. aureus*) is a commensal bacterium residing on our skin and LCs play a crucial role in the host defence against the bacterium. The cell wall of *S. aureus* is densely functionalized with wall teichoic acids (WTAs), ribitol phosphate (RboP) polymers decorated with *N*-acetyl glucosamine (GlcNAc) and D-alanine residues. As described in Chapter 1 and 2, WTAs are involved in host interaction, biofilm formation, cation homeostasis and autolysin activity. It has previously been shown that langerin can recognize β-GlcNAc modifications on *S. aureus* contributing to LC activation and production of Th1- and Th17-polarizing cytokines, while α-GlcNAcylation was found to impair langerin interaction, weakening the functional response of LCs.[9] This latter finding implies that *S. aureus* can modulate immune detection and subsequent inflammation in the epidermis. van Dalen *et al.*[9] reported langerin as the first human innate receptor to discriminate between the α-GlcNAc and β-GlcNAc modifications. Unraveling the interactions of *S. aureus* WTAs and langerin at the molecular level is of importance for the development of a vaccine specifically targeting skin and soft tissue infections and may also open up possibilities for the targeted delivery of vaccines.[10-11]

Since the isolation of WTA from bacterial sources results in heterogenous fragments with possible bacterial contaminations, the synthesis of well-defined fragments is of great interest. This Chapter describes the synthesis of a set of glycosylated ribitol phosphate oligomers, varying in length of the ribitol phosphate chain as well as the substitution pattern. Both C-4-α- and C-4-β-GlcNAc are incorporated (Fig 1). The GlcNAc-WTAs fragments will be equipped with an aminohexanol linker that serves as a ligation handle to attach the molecules to surfaces, biotin affinity handles or carrier proteins for example. The short trimer fragments are intended for future crystallization studies. These latter fragments lack the flexible spacer entity as its presence may hamper crystallization.

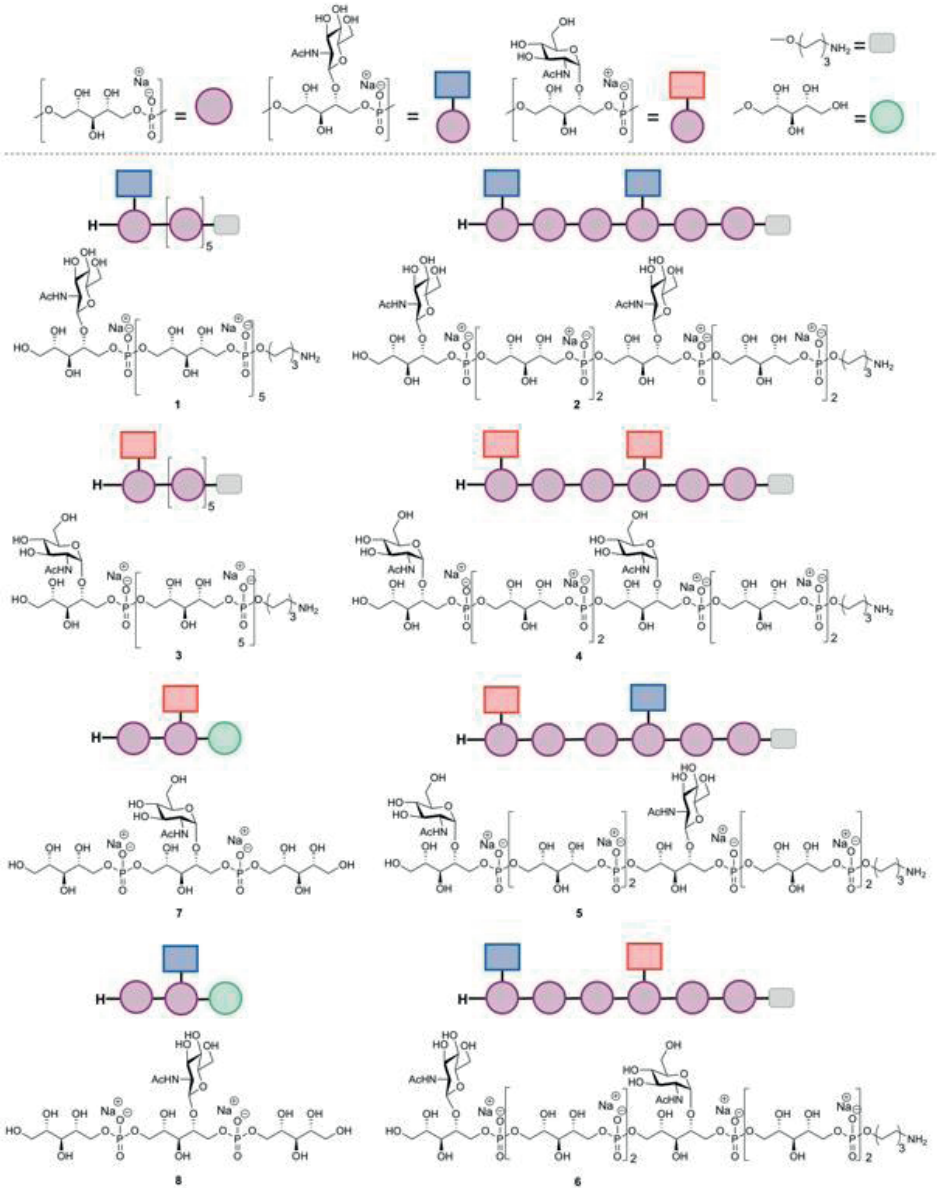


Figure 1. Library of glycosylated ribitol phosphates targeted in this Chapter.

RESULTS AND DISCUSSION

As discussed in Chapter 1, a team at Sanofi Pasteur synthesized two octamers with either an α- or a β-GlcNAc on the C-4 of each ribitol phosphate moiety and a nonamer bearing a C-3 β-GlcNAc on each ribitol phosphate repeating unit using a block coupling

approach.[12] The spacer was installed in the last coupling event to the ribitol phosphate chain, on the opposite position on the WTA chain with respect to the peptidoglycan binding site. Jung *et al.*[13] also used a block approach to generate ribitol phosphate tetramers bearing an α- or β-GlcNAc moiety at C-4 of the RboP motifs, and D-alanine amides at C-2. This Chapter outlines a strategy for the assembly of well-defined WTA fragments based on repetitive coupling cycles using monomeric RboP building blocks to allow for maximum flexibility in terms of substitution patterns that can be targeted. The spacer will be attached at the site of the WTA chain where the peptidoglycan is attached in the bacterial structures.

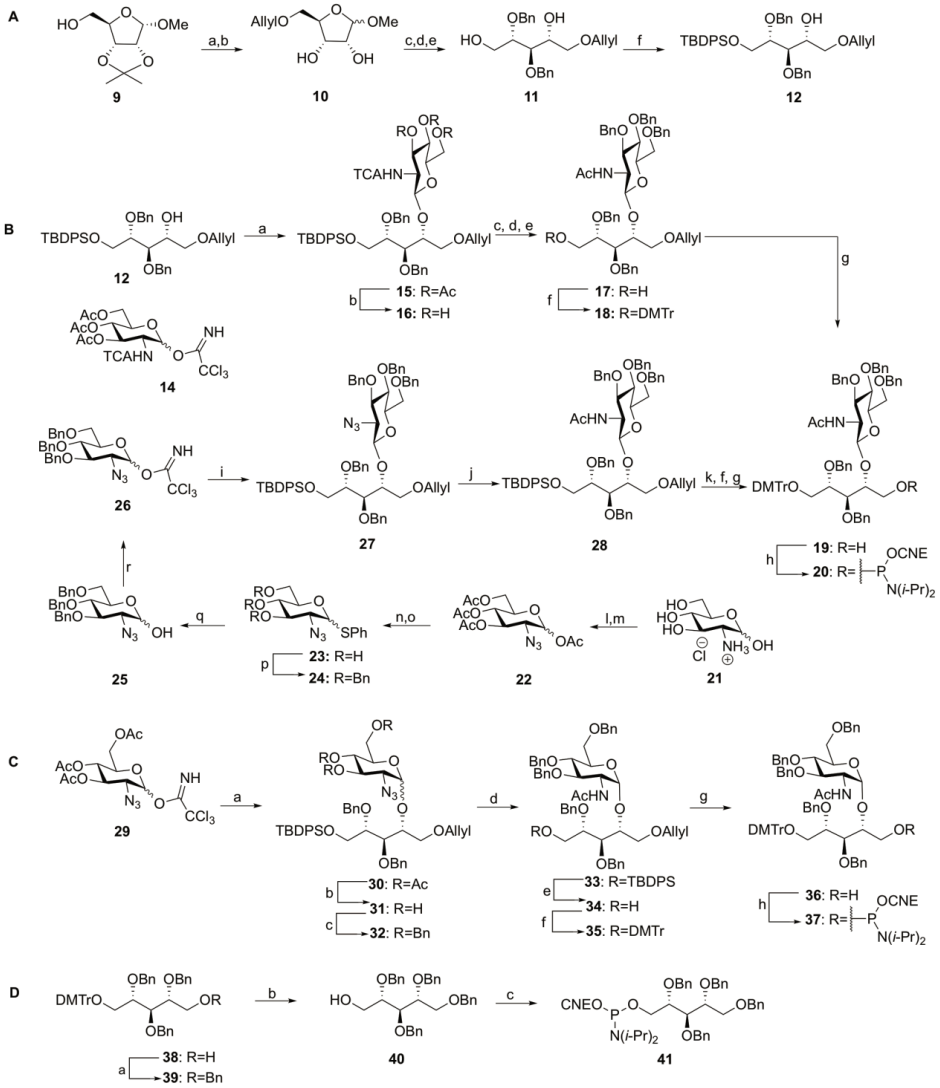
Scheme 1 depicts the synthesis of the required phosphoramidite building blocks **20**, **37**, and **41**, which will be used alongside building block **42** (Scheme 2), the synthesis and use of which has been described in Chapter 2. Scheme 1A shows the synthesis of C4-OH ribitol **12**, and starts from compound **9** by allylation of the primary alcohol, followed by isopropylidene hydrolysis to yield **10**. Benzylation and ensuing acidic hydrolysis of the methyl riboside yielded the corresponding hemi-acetal intermediate and subsequent ring opening using sodium borohydride provided primary alcohol **11**. Protection with a TBDPS group then gave acceptor **12.** Two approaches were explored to introduce the β-GlcNAc as shown in scheme 1B. In the first approach, acceptor **12** was coupled with trichloroacetimidate donor **14**. The TCA protecting group on the glucosamine donor can participate in the stabilization of the oxocarbenium ion formed upon activation of the donor, forcing the nucleophilic attack to the other side of the pyranose ring leading to the desired β-product and coupling of acceptor **12** and donor **14** afforded the desired β-product **15** in 92% yield. Subsequent deacetylation under Zemplén conditions yielded triol **16**, after which the alcohols were benzylated. To avoid benzylation of the trichloroacetamide the reaction was kept at 0°C although this also led to slower and incomplete conversion of the starting material. The TCA was removed using $CsCO_3$ in DMF at 70°C,[14] followed by acetylation of the free amine and TBAF mediated TBDPS removal to yield β-product **17** in 42% over 4 steps. The primary alcohol was protected with a DMTr group in 53% yield, after which an iridium catalyzed allyl isomerization and iodine mediated enol ether hydrolysis delivered alcohol **19** in 79%. Equipment of the alcohol with a cyanoethyl protected phosphoramidite yielded key building block **20** for the upcoming oligomerization. Although the TCA protecting group served well to provide a β-selective glycosylation reaction, its undesired reactivity in the benzylation reaction and relatively difficult removal made the assembly of **20** using donor **14** sub-optimal. To circumvent the use of a TCA group, a second route was developed in which glucose azide **26** was coupled to acceptor **12**. This donor[15] was synthesized starting from commercially available glucosamine **21**, of which the amine was masked with an azide using Stick's reagent[16] after which acetylation gave **22**. Subsequent introduction of a

thiophenol and deacetylation under Zemplén conditions led to compound **23** in 56% over 2 steps. Benzylation of the free alcohols gave **24** in 98% yield and hydrolysis of the anomeric thiophenyl gave hemiacetal **25** in 62% yield. The hemiacetal was equipped with an imidate moiety, completing the synthesis of donor **26**. The use of acetonitrile as a β-directing solvent in combination with a low reaction temperature ensured the stereoselective formation of the desired β-glucosamine linkage and **27** was obtained in 85%.[17-24] Propanedithiol mediated azide reduction, followed by acetylation delivered acetamide **28** in 59% over 2 steps and removal of the TBDPS afforded alcohol **19** in 68% yield. Overall this latter route proved significantly more efficient than the route using TCA-donor **14**.

The synthesis of amidite **37**, bearing the α-GlcNAc appendage is shown in Scheme 1C. Azide donor **29** was coupled with acceptor **12** to yield **30** as a 7:1 α/β mixture. The two anomers could be separated after Zemplén deacetylation, leading to the pure α-product **31** in 70% yield.[25] Benzylation of the liberated alcohols, followed by Staudinger reduction and subsequent acetylation of the amine yielded **33** in 89% yield over 3 steps. Removal of the TBDPS group gave **34** in 86% yield and protection of the primary alcohol with a DMTr group afforded **35**. Allyl removal as described above yielded the primary alcohol **36** which was functionalized with a cyanoethyl phosphoramidite to give the second key building block **37** in 81% yield.

Where the spacer is required for conjugation and biological purposes, it may impede crystallization studies. Therefore, a terminal building block was generated with a benzyl group at the terminating alcohol. To this end phosphoramidite **41** was assembled by benzylation of alcohol **38** which was followed by detritylation to yield intermediate **40** in 66% over 2 steps. Conversion into the amidite yielded **41**.

With all the required phosphoramidites in hand, the stage was set to assemble the target library (Fig 1). Scheme 2A schematically depicts the assembly of the fragments. For the elongation of the oligomers, the condensation procedure described in Chapter 2 was employed: in the first step the phosphoramidite is activated by DCI, after which the activated group is replaced by the incoming alcohol of the growing chain to yield the phosphite triester. Subsequent oxidation using CSO affords the phosphate triester, after which a detritylation step using 3% DCA in DCM liberates the alcohol for the next coupling event. Purification was achieved by size exclusion or silica gel column chromatography.



Scheme 1. A Building block synthesis; *Reagents and conditions:* a) AllylBr, NaH, THF/DMF (v/v= 7/1), 0°C to rt; b) AcOH/H_2O (v/v= 1/1), 50°C, 300 mbar, 62% 2 steps; c) BnBr, NaH, THF/DMF (v/v= 7/1), 0°C to rt; d) 4M HCl dioxane, 80°C; e) $NaBH_4$, MeOH, 0°C, 50% over 3 steps; f) TBDPSCl, TEA, DCM 0°C to rt, 95%.

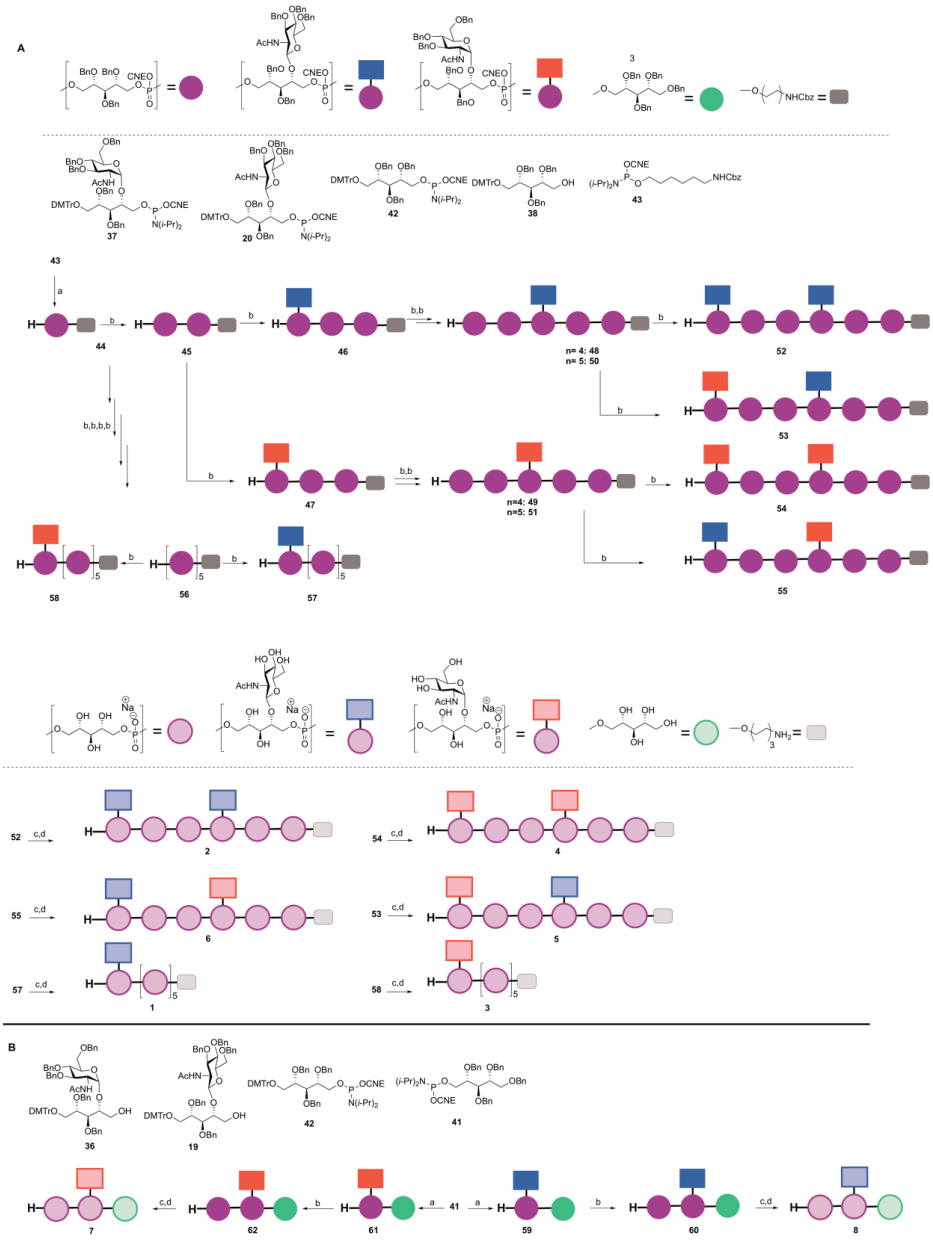
B Building block synthesis; *Reagents and conditions:* a) **14**, TMSOTf, DCM, 0°C, 92%; b) NaOMe, MeOH, 85%; c) BnBr, NaH, DMF, 0°C; d) i. $CsCO_3$, DMF, 70°C; ii. Ac_2O, pyridine; e) TBAF, THF, rt, 42% 4 steps; f) DMTrCl, TEA, DCM, 53%; g) i. Ir(COD)$(Ph_2MeP)_2PF_6$, H_2, THF, ii. I_2, sat. aq. $NaHCO_3$, THF, 79%; h) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, DIPEA, DCM, 78%; i) **12**, TMSOTf, ACN, -40°C to 0°C, 85%; j) propane dithiol, pyridine, H_2O, TEA, rt; ii. Ac_2O, pyridine, 59% 2 steps; k) TBAF, THF, rt, 68%; l) Stick reagent, K_2CO_3, $CuSO_4$·5 H_2O, MeOH; m) Ac_2O, pyridine, 99% over 2 steps; n), PhSH, BF_3·OEt_2, DCM; o) NaOMe, MeOH, 56% over 2 steps; p) BnBr, NaH, THF/DMF (v/v= 1/1), 98%; q) NBS, acetone, 62%; r) TCAN, K_2CO_3, DCM, 89%. **C Building block synthesis;** *Reagents and conditions:* a) **12**, TMSOTf, DCM, rt, 92%, α/β (7:1); b) NaOMe, MeOH, rt, 70% α-anomer; c) BnBr, NaH, THF/DMF (v/v= 7/1), 0°C to rt, 73%; d) i. PMe_3, KOH, THF; ii. Ac_2O, pyridine, 89% 2 steps; e) TBAF, THF, rt, 86%; f) DMTrCl, TEA, DCM, 78%; g) i. Ir(COD)$(Ph_2MeP)_2PF_6$, H_2, THF, ii. I_2, sat. aq. $NaHCO_3$, THF, 88%; h) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, DIPEA, DCM, 81%.

D Building block synthesis; *Reagents and conditions:* a) BnBr, NaH, THF/DMF (v/v= 7/1) 0°C to rt, 84%; b) 3% DCA in DCM, 87%; c) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, DIPEA, DCM, 82%.

As described in Chapter 2, alcohol **38** was coupled with spacer phosphoramidite **43** to give monomer **44**. Detritylation then set the stage for a second coupling cycle with amidite **42** to deliver dimer **45**, which was coupled to β-GlcNAc amidite **20** or α-GlcNAc amidite **37** to give trimers **46** and **47**. Both trimers were extended by two coupling cycles using **42** to yield pentamers **50** and **51**. Both pentamers were coupled to **20** or **37** yielding four double glycosylated hexamers **52**, **53**, **54**, **55** with a different substitution pattern. In addition, unsubstituted pentamer **56** (Chapter 2) was coupled to amidite **20** and **37** to yield hexamers **57** and **58** bearing a single terminal GlcNAc moiety. Global deprotection using aqueous ammonia and subsequent hydrogenolysis of the semi protected fragments yielded hexamers **1**, **2**, **3**, **4**, **5**, and **6**.

Scheme 2B depicts the generation of the α- and β-GlcNAc trimers, designed for crystallization studies. Phosphoramidite **41** was coupled to α- and β-GlcNAc ribitol alcohol **36** and **19** giving dimers **59** and **61,** which were coupled with amidite **42** to afford trimers **60** and **62**. Global deprotection as described above yielded trimers **7** and **8**.

To study the interactions of glycosylated and non-glycosylated WTAs with human langerin, a micro array interaction study was undertaken. As previously reported by van der Es *et al*.,[26] teichoic acid (TA) micro arrays can be employed to rapidly report on the sequence binding specificity of biomolecules interaction with TAs. Thus, amino spacer functionalized WTA fragments **1**-**5** as well as non-glycosylated trimer **63**, tetramer **64**, octamer **65**, and dodecamer **66** (Chapter 2) were coupled to epoxide functionalized micro array slides and the generated arrays were interrogated using langerin-FITC.[8, 27] As can be seen in Figure 2, the WTAs that bear a β-GlcNAc show selective binding to langerin, with fragment **2** having 2 β-GlcNAcs showing highest binding. The α-GlcNAc WTAs (**3**, **4**) did not bind to langerin, nor did the unsubstituted WTAs (**63** - **66**). WTA **5** that bears an α- and a β-GlcNAc shows binding comparable to the mono-β-GlcNAc WTA **1**. The array clearly reveals that langerin does not bind to the RboP-backbone and that a β-GlcNAc is required for binding. These results support the data of van Dalen *et al.*[9], who studied binding of langerin-FITC to a panel of *S. aureus* strains, expressing either the glycosyltransferase TarM or TarS, responsible for the introduction of WTA-α- and WTA-β-GlcNAc residues, respectively. It was shown that the knock-out of both enzymes (ΔTarS/TarM) decreased langerin binding compared to the wild-type indicating that a GlcNAc is required for binding. The ΔTarM species showed increased binding to langerin compared to the *S. aureus* wild-type, while the ΔTarS bacterium showed 7-8 fold lower binding compared to the wild type. These results showed that at the bacterial level the WTA β-GlcNAc is required for langerin binding and that the α-GlcNAc-moieties could hinder langerin binding. The micro array results confirm langerin WTA β-GlcNAc binding at the molecular level, and show that a single α-GlcNAc-residue (as in **5**) does



Scheme 2. WTA hexamer assembly **A**; *Reagents and conditions:* a) i. DCI, ACN, **38**; ii. CSO; iii. 3% DCA in DCM, **44**: 85%; b) i. DCI, ACN, phosphoramidite **20** or **37** or **42**; ii. CSO; iii. 3% DCA in DCM, **45**: 74%, **47**: 53%, **46**: 88%, **49**: 82%, **48**: 90%, **51**: 89%, **50**: 68%, **54**: 88%, **52**: 79%, **53**: 76%, **55**: 61%, **58**: 73%, **57**: 85%; c) i. NH_3 (30-33% aqueous solution), dioxane; d) Pd black, H_2, AcOH, H_2O/dioxane, **4**: 55%, **2**: 78%, **5**: 88%, **6**: 16%, **3**: 96%, **1**: 68%;
WTA trisaccharide assembly **B**: a) i. DCI, ACN, alcohol **36** or **19**, **61**: 80%, **59**: 46%; b) i. DCI, ACN, amidite **42**, **60**: 91%, **62**: 91%; c) i. NH_3 (30-33% aqueous solution), dioxane; d) Pd black, H_2, AcOH, H_2O/dioxane, **7**: 90%, **8**: 85%.

not adversely affect interaction of the C-type lectin with the WTA β-GlcNAc-moieties. The differences between the *S. aureus* study of van Dalen *et al*. and the here presented results may be explained by the different number of GlcNAc residues per RboP unit and/or the difference in density of the WTAs on the array *vs* the bacterial cell wall. In addition, D-alanine residues may also play a role in WTA-langerin interaction.

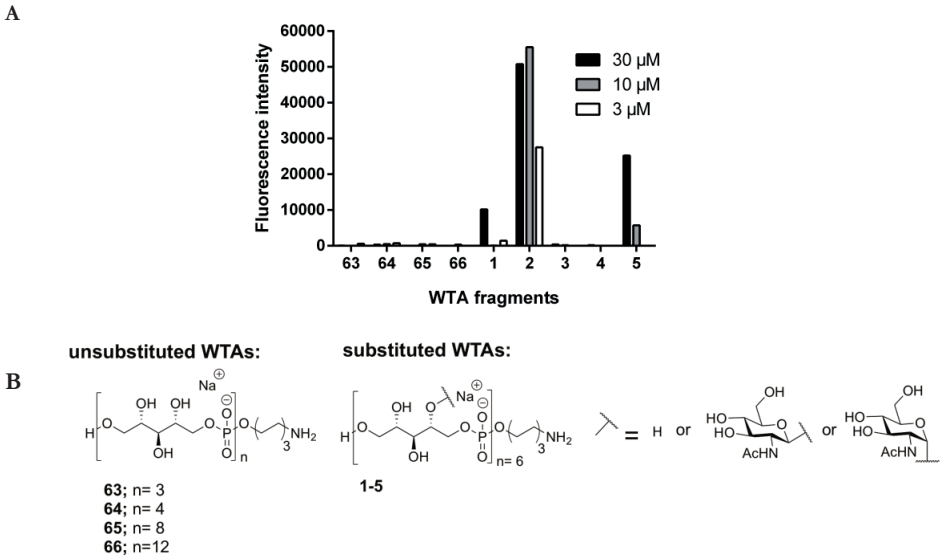


Figure 2. A) Human langerin binding on a RboP micro array. X-as represents the WTA fragments printed with 3 different concentrations on the slide; B) WTA fragments included on micro array for their langerin binding.

To further probe langerin WTA binding with fragments having a higher density of GlcNAc residues, an assay using WTA functionalized magnetic beads was employed. Thus, as described in Chapter 2, the biotinylated non-glycosylated RboP-hexamer and RboP-dodecamer were enzymatically glycosylated using the enzymes TarS, TarM and TarP generating β-(1,4)-GlcNAc-WTA, α-(1,4)-GlcNAc-WTA and β-(1,3)-GlcNAc-WTA respectively, which were then captured on streptavidin functionalized beads. Figure 3 shows langerin-FITC binding to these beads and reveals that both the β-(1,4)- and β-(1,3)-GlcNAc-WTAs are recognized by langerin, with equal binding efficiency. The dodecamer shows significantly higher binding than the hexamers. Thus, although the microarray has indicated that a single β-GlcNAc can already provide langerin binding, the presence of more copies of the sugar ligand on the RboP chains leads to stronger binding with the lectin.

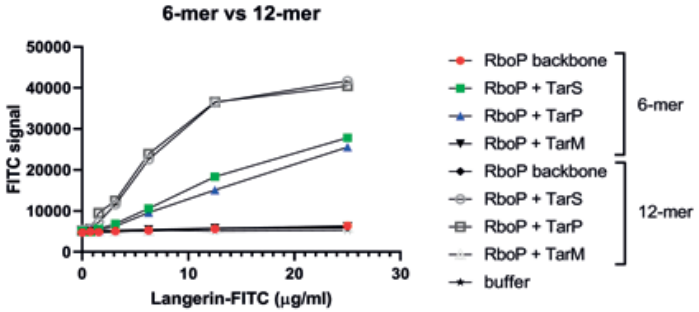


Figure 3. Magnetic beads functionalized with enzymatically glycosylated 6-mer and 12-mer can bind langerin in an anomeric configuration dependent manner.

CONCLUSION AND OUTLOOK

This Chapter described the successful synthesis of a set of C-4 glycosylated WTAs on milligram scale. Here, the phosphoramidite chemistry developed in Chapter 2 was further extended by synthesizing α- and β-linked C4-GlcNAc ribitol phosphoramidite building blocks. The synthetic route towards β-glycosylated amidites was realized following two approaches with different donors. The trichloroacetamide protecting group, chosen for the excellent beta selectivity during the glycosylation reaction, showed to be less optimal for the overall efficiency in the rest of the route. It presented an obstacle during the benzylation and its removal proved challenging. Meanwhile, the glucose azide donor, bearing a non-participating group on the C-2 gave excellent beta-selectivity in a nitrile-assisted glycosylation reaction and no further laborious steps in the synthesis route were encountered, making the approach using this latter donor the preferred one to generate the required building block on multigram scale. The activity of the synthesized WTAs towards langerin has been established on a micro array platform and it was found that langerin binds in selective manner to the β-epitope. A similar outcome was found using WTA-functionalized beads, carrying TarS, TarM or TarP modified synthetic ribitol phosphate hexa- or dodecamers. The α-GlcNAc WTA beads did not capture langerin, while the β-GlcNAc functionalized beads effectively bound the C-type lectin. The position of the GlcNAc on the ribitol phosphates seems to be of less importance for binding. These results clearly demonstrate β-GlcNAc-WTA to be an epitope for human langerin. Establishing the molecular interaction of langerin and *S. aureus* using well-defined WTA fragments is of great importance for the development of treatments against *S. aureus* soft skin and tissue infections. The activity of the glycosylated WTA-fragments against monoclonal antibodies will be presented in Chapter 4 to reveal the role of these antigens in adaptive immunity.

EXPERIMENTAL SECTION

General information

All chemicals (Acros, Fluka, Merck, Sigma-Aldrich, etc.) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040- 0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 25 g/L and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ 10 g/L, in 10% aqueous H_2SO_4 followed by charring at +/- 140°C. Some unsaturated compounds were visualized by spraying with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in water. Optical rotation measurements ($[\alpha]_D^{20}$) were performed on an Anton Paar Modular Circular Polarimeter MCP 100/150 with a concentration of 10 mg/mL (*c* 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300. ^{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).

Phosphoramidite coupling, oxidation, and detritylation

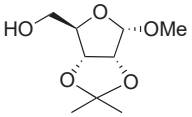
The starting alcohol was co-evaporated 2 times with toluene before being dissolved in acetonitrile (ACN, 0.15 M). 4,5-dicyanoimidazole (DCI) (1.6-2.4 eq; 0.25 M in ACN) was added and the mixture was stirred over freshly activated molecular sieves under an argon atmosphere for 20 min. Then phosphoramidite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

The organic layer was washed with saturated NaCl/$NaHCO_3$ solution (1:1), the water layer was extracted 3 times with DCM, and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by either flash chromatography (DCM/acetone) or size exclusion chromatography (sephadex LH-20, MeOH/DCM, 1/1).

General procedure for global deprotection

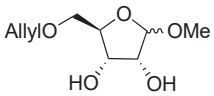
The oligomer was dissolved in a 1:1 solution of NH_3 (30-33% aqueous solution) and dioxane (1.2-2.4 mM) and stirred overnight. The mixture was concentrated *in vacuo* and loaded on a Dowex Na^+ cation-exchange resin (50WX4-200, stored on 0.5 M NaOH, flushed with H_2O and MeOH before use) column and flushed with water/dioxane (1:1). The fractions were then concentrated *in vacuo*, dissolved in water/dioxane (2 ml per 10 µmol) and 4 drops of glacial AcOH were added. After purging the mixture with argon, Pd black was added (32-59 mg), and the mixture was repurged with N_2. The mixture was stirred under hydrogen gas for 3 - 7 days, filtered over celite, and concentrated *in vacuo*. The crude product was purified by size-exclusion chromatography (Toyopearl HW-40, NH_4OAc buffer) and the fractions were concentrated. The product was co-evaporated repeatedly with MilliQ water to remove NH_4OAc/ NH_4HCO_3 traces and eluted through a Dowex Na^+ cation-exchange resin column, and lyophilized.

Methyl 2,3-*O*-isopropylidene-α-D-ribofuranoside (9)



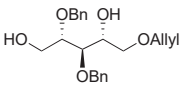
D-Ribose (40.0 g; 266 mmol; 1.0 eq.) was dissolved in MeOH (950 ml; 0.28 M) and AcCl (5.7 ml; 0.3 eq.) was added and the mixture was stirred for 2h at rt. Then the mixture was quenched with Na_2CO_3, filtrated and concentrated. The crude was dissolved in acetone (750 ml, 0.35 M), HCl (16 ml) was added and the mixture was stirred overnight at rt. The mixture was quenched with Na_2CO_3, filtrated and concentrated under reduced pressure. Column purification pentane/EtOAc 9:1 to 6:4 pentane/EtOAc afforded the title compound **9** in 72% over 2 steps (39.0 g; 190.9 mmol). IR (neat, cm-^{1}): 3431, 2988, 2940, 1456, 1373, 1089, 1040, 866; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.32 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 3.33 (dd, 1H, *J*= 10.0 Hz, 3.2 Hz, O*H*), 3.43 (d, 3H, *J*= 2.8 Hz, CH_3O), 3.59 - 3.70 (m, 2H, H-5), 4.41 (d, 1H, *J*= 2.8 Hz, H-4), 4.58 - 4.60 (m, 1H, H-3), 4.82 - 4.84 (m, 1H, H-2), 4.97 (d, 1H, *J*= 2.4 Hz, H-1); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 24.7, 26.4 (CH_3), 55.5 (CH_3O), 64.0 (C-5), 81.5 (C-2), 85.8 (C-3), 88.3 (C-4), 109.9 (C-1), 112.1 (Cq); HRMS: $[M+Na]^+$ calculated for $C_9H_{16}O_5Na$ 227.0895, found 227.0896.

Methyl 5-*O*-allyl-α/β-D-ribofuranoside (10)



Compound **9** (38.7 g; 189 mmol; 1.0 eq.) was dissolved in a mixture of THF/DMF (540 ml; 0.35 M; v/v= 7/1). The mixture was cooled to 0°C and NaH (11.3 g; 284 mmol; 1.5 eq.) was added in portions followed by dropwise addition of AllylBr (24.5 ml; 284 mmol; 1.5 eq.) and the mixture was allowed to warm up to rt and was stirred overnight. Then the mixture was quenched with MeOH at 0°C and diluted with Et_2O. The organic layer was washed 5x with H_2O, 1x with brine, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The crude was dissolved in a mixture of AcOH/H_2O (v/v= 1:1, 528 ml; 0.35M) and the mixture was stirred under a pressure of 300 mbar at 50°C. Then the mixture was concentrated under reduced pressure and the crude was purified by column chromatography 8:2 pentane/EtOAc to 2:8 pentane/EtOAc affording the title compound **10** in 62% yield over 2 steps as an α/β mixture with a ratio of 11:1 (24.1 g; 118 mmol). IR (neat, cm-^{1}): 3441, 2914, 1558, 1449, 1103, 1051, 1026, 1005, 974; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.35 (s, 3H, OCH_3 α anomer), 3.47 (s, 0.3 H, OCH_3 β anomer), 3.51 - 3.61 (m, 2.2H, H-5 α/β anomer), 3.97- 4.16 (m, 6.7H, H-2/H-3, H-4, C*H*$_2$-CH α/β anomer), 4.24 (d, 1H, *J*= 4.4 Hz, H-2/H-3), 4.83 (s, 1H, H-1 α anomer), 4.92 (d, 0.09H, *J*= 4.8 Hz, β anomer), 5.18 - 5.32 (m, 2H, CH=C*H*$_2$), 5.86 - 5.96 (m, C*H*=CH_2); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 55.1 (CH_3O α anomer), 55.5 (CH_3 β anomer), 70.0 (C-5/*C*H$_2$-CH β anomer), 70.8, 71.7 (C2/C3 β anomer), 72.0, 72.4 (C-5/*C*H$_2$-CH α anomer), 72.4, 74.7 (C-2/C-3 α anomer), 81.8 (C-4 α anomer), 83.6 (C-4 β anomer), 102.9 (C-1 β anomer), 108.2 (C-1 α anomer), 117.5 (CH=*C*H$_2$), 134.4 (*C*H=CH_2); HRMS: $[M+Na]^+$ calculated for $C_9H_{16}O_5Na$ 227.0895, found 227.0895.

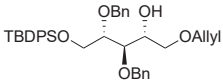
5-*O*-allyl-2,3-*O*-benzyl-D-ribitol (11)



Compound **10** (24.1 g; 118 mmol; 1.0 eq.) was co-evaporated twice with toluene before use and was dissolved in a mixture of THF/DMF (590 ml; 0.30 M; v/v= 7:1). The solution was cooled to 0°C and NaH (7.1 g; 177 mmol; 1.5 eq.) was added, followed by dropwise addition of BnBr (21.0 ml; 177 mmol; 1.5 eq.). The remaining NaH (7.1 g; 177 mmol; 1.5 eq.) was added followed by the dropwise addition of BnBr (21.0 ml; 177 mmol; 1.5 eq.) and the mixture was allowed to warm up to rt and was stirred overnight. Then the mixture was quenched with MeOH at 0°C, diluted with Et_2O and the organic layer was washed 5x with H_2O. The organic layer dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography pentane/EtOAc 9:1 to 1:1 pentane/EtOAc yielded the crude (63.2 g) with benzyl alcohol traces. The crude was dissolved in a mixture of 4M HCl (aq) /dioxane (800 ml, 0.15M v/v= 1:1) and the mixture was heated at 80°C for 2.5h and was then left stirring overnight at rt. The mixture was reheated at 80°C for 1.5 h and was then poured into 200 ml sat aq. $NaHCO_3$ after cooling down. Na_2CO_3 was added to neutralize the mixture and the mixture was diluted with EtOAc. The organic layer was washed with

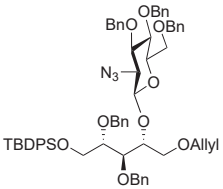
water and brine, dried over $MgSO_4$, filtrated and concentrated in vacuo. Purification by column chromatography pentane/EtOAc 8:2 to 4:6 pentane/EtOAc yielded a mixture of product and starting material. The mixture was dissolved in MeOH (375 ml; 0.20 M) and $NaBH_4$ (3.7 g; 98.0; 1.3 eq.) was added at 0°C in 2 portions and the mixture was stirred 4 days at rt. Then the reaction was quenched with EtOAc, concentrated under reduced pressure and co-evaporated with toluene. Purification by column chromatography pentane/EtOAc 1:0 to 2:8 pentane/EtOAc yielded the product **11** in 50% over 3 steps (21.7 g; 58.3 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 1): + 19.4; IR (neat, cm-^{1}): 3383, 2924, 2872, 1717, 1506, 1456, 1096, 1070, 1028, 737, 698; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.86 (bs, 1H, O*H*), 3.22 (bs, 1H, O*H*), 3.49 - 3.56 (m, 2H, *H*-C-OH, C*H*H), 3.74 - 3.87 (m, 4H, CH_2-OH, 2x C*H*-Rbo), 3.91 - 4.00 (m, 3H, C*H*H, CH-CH_2), 4.58 - 4.66 (m, 3H, CH_2-Bn), 4.73 (d, 1H, *J*= 11.2 Hz, C*H*H-Bn), 5.14 - 5.27 (m, 2H, CH=CH_2), 5.82 - 5.92 (m, 1H, C*H*=CH_2), 7.24 - 7.35 (m, 10H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 60.9 (CH_2-OH), 70.5 (CH-OH), 71.1 71.9, 72.2, 73.9 (CH_2-Rbo/CH_2-CH, 2x CH_2-Bn), 79.4 (2x CH-Rbo), 117.4 (CH=*C*H_2), 127.8, 127.9, 128.0, 128.4, 128.4 (C-arom), 134.5 (*C*H=CH_2), 138.1, 138.1 (Cq-arom); HRMS: $[M+Na]^+$ calculated for $C_{22}H_{28}O_5Na$ 395.1834, found 395.1831.

5-*O*-allyl-2,3-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (12)



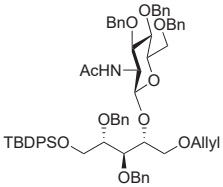
Compound **11** (17.8 g; 47.8 mmol; 1.0 eq.) was dissolved in DCM (480 ml; 0.1M) and the solution was cooled to 0°C. TEA (40 ml; 6.0 eq.) was added followed by dropwise addition of TBDPSCl (13.7 ml; 52.6 mmol: 1.1 eq.). The mixture was allowed to warm up to rt and was stirred overnight. The reaction was quenched by the addition of MeOH at 0°C and was concentrated under reduced pressure. Purification by column chromatography pentane/EtOAc 1:0 to 6:4 pentane/EtOAc yielded the product in 95% yield (27.7 g; 45.3 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 1): + 26.7; IR (neat, cm-^{1}): 3545, 2930, 2884, 1717, 1506, 1456, 1111, 1028, 824, 739, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.07 (s, 9H, *t*Bu), 2.86 (d, 1H, *J*= 4.0 Hz, O*H*), 3.52 - 3.55 (m, 1H, H-4), 3.81 - 3.82 (m, 2H, H-2, H-3), 3.89 - 4.03 (m, 6H, CH_2-CH, 2x CH_2-Rbo), 4.53 (d, 1H, *J*= 11.6 Hz, C*H*H Bn), 4.60 - 4.67 (m, 2H, CH_2-Bn), 4.70 (d, 1H, *J*= 11.6 Hz, C*H*H Bn), 5.13 - 5.26 (m, 2H, CH=CH_2), 5.84 - 5.91 (m, 1H, C*H*=CH_2), 7.19 - 7.42 (m, 15H, H-arom), 7.68 - 7.72 (m, 5H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (Cq *t*Bu), 26.7, 26.9, 27.0 (CH_3 *t*Bu), 63.3 (CH_2-Rbo), 71.2 (C4-OH), 71.3, 72.3, 72.6, 73.8 (CH_2-Bn, CH_2-Rbo, *C*H_2-CH), 78.9, 80.7 (C-2, C-3), 117.2 (CH=*C*H_2), 127.6, 127.7, 127.8, 127.8, 128.0, 128.4, 129.7, 129.8 (CH-arom), 133.3, 133.4 (Cq-arom), 134.8, 134.9, 135.6, 135.8, 135.8, 138.4 (*C*H=CH_2, C-arom), 138.5 (Cq-arom); HRMS: $[M+Na]^+$ calculated for $C_{38}H_{46}O_5SiNa$ 633.3012, found 633.3015.

O-(3,4,6-tri-*O*-benzyl-2-azido-2-deoxy-β-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (27)



Alcohol **12** (1.83 g; 3.00 mmol; 1.0 eq.) was co-evaporated with toluene under a N_2 atmosphere and dissolved in dry ACN (30.0 ml; 0.10 M). Activated molecular sieves (3Å) were added and the solution was stirred for 30 minutes under N_2 atmosphere. The mixture was cooled to -40°C and TMSTOTf (55 µl; 0.30 mmol; 0.1 eq.) was added. Imidate **26** (2.79 g; 4.5 mmol; 1.5 eq.) was co-evaporated with toluene under a N_2 atmosphere and dissolved in dry ACN (30 ml; 0.15M). The donor was added to the reaction mixture and the mixture was stirred from -40°C to 0°C in a timeframe of 3 hours. Subsequently, 3 drops TEA were added and the mixture was diluted in DCM. The organic phase was washed with sat. aq. $NaHCO_3$; NaCl (v/v= 1:1) and the water layer was extracted with DCM. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography Et_2O/pentane 2:98 to 14:86 Et_2O/pentane yielded compound **27** in 85% yield (2.71 g; 2.54 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 1): + 6.6; IR (neat, cm-^{1}): 2931, 2858, 2109, 1454, 1361, 1089, 1075, 1029, 737, 698; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.05 (s, 9H, 3x CH_3-*t*Bu), 3.34 - 4.01 (m, 14H, H-2, 2x CH_2-Rbo, C*H*$_2$-CH, 3x CH-Rbo, H-3, H-4, H-6′, H-6″), 4.38 (d, 1H, *J*= 12.1 Hz, C*H*H-Bn), 4.43 (m, 1H, H-5), 4.48 - 4.60 (m, 4H, CH_2-Bn), 4.67 (d, 1H, *J*= 8.0 Hz, H-1), 4.72 - 4.91 (m, 5H, CH_2-Bn), 5.10 - 5.28 (m, 2H, C*H*$_2$=CH), 5.90 (m, 1H, CH_2=C*H*), 7.13 - 7.42 (m, 30H, H-arom), 7.68 (m, 5H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (Cq-*t*Bu), 27.0 (CH_3-*t*Bu), 63.8 (CH_2-Rbo), 66.8 (C-2), 68.8 (C-6), 71.0 (CH_2-Rbo), 72.4, 72.7, 73.9, 74.1, 75.1 (*C*H$_2$-CH, CH_2-Bn), 75.2 (CH-Rbo, C-3, C-4), 75.5 (*C*H$_2$-CH, CH_2-Bn), 79.5 (C-5), 79.8, 83.2 (CH-Rbo, C-3, C-4), 102.5 (C-1), 116.8 (*C*H$_2$=CH), 127.5, 127.5, 127.5, 127.6, 127.6, 127.8, 127.8, 127.9, 127.9, 127.9, 128.0, 128.1, 128.1, 128.3, 128.4, 128.4, 128.5, 128.5, 128.5, 128.5, 129.7, 129.7 (CH-arom), 133.4, 133.6 (Cq-arom), 135.0 (CH_2=*C*H), 135.8, 135.9 (CH-arom), 138.2, 138.2, 138.3, 138.3, 138.7, 138.8 (Cq-arom); HRMS: $[M+Na]^+$ calculated for $C_{65}H_{73}N_3NaO_9Si$ 1090.5014, found 1090.5023.

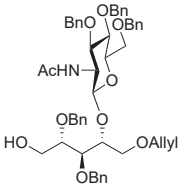
O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (28)



Compound **27** (1.65 g; 1.55 mmol; 1.0 eq.) was dissolved in pyridine/H_2O (27 ml; 0.058M; v/v= 5:1). TEA (0.1 ml) and propanedithiol (0.78 ml; 7.75 mmol; 5.0 eq.) were added and the mixture was stirred overnight at rt. Then the mixture was concentrated under reduced pressure and was 3x co-evaporated with toluene. The mixture was dissolved in pyridine/Ac_2O (27 ml; v/v= 2:1) and the mixture was stirred overnight at rt. The mixture was then quenched with MeOH, concentrated under reduced pressure and purified by column chromatography 1:0 pen-

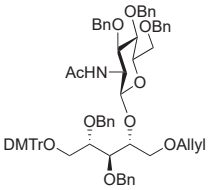
tane/EtOAc to 1:1 pentane/EtOAc affording the title compound **28** in 59% yield over 2 steps (1.00 g; 0.92 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 1): + 11.8; IR (neat, cm-^{1}): 2929, 2858, 1653, 1454, 1362, 1112, 1070, 1029, 738, 698; ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.06 (s, 9H, 3x CH_3-*t*Bu), 1.77 (s, 3H, CH_3-NAc), 3.49 (m, 1H, CH-Rbo/H-3), 3.54 - 3.64 (m, 2H, CH_2-Rbo), 3.64 - 3.81 (m, 7H, H-2, H-4, CH_2-Rbo, 2x CH-Rbo), 3.83 (m, 2H, C*H*$_2$-CH), 3.88 (dd, 1H, *J*= 11.3 Hz, 5.4 Hz, H-6′), 3.93 - 4.00 (m, 2H, H-6″, CH-Rbo/H-3), 4.30 (m, 1H, H-5), 4.42 (d, *J*= 12.1 Hz, 1H, C*H*H-Bn), 4.46 - 4.83 (m, 10H, CH_2-Bn, H-1), 5.08 - 5.22 (m, 2H, C*H*$_2$=CH), 5.59 (d, 1H, *J*= 7.0 Hz, N*H*), 5.82 (m, 1H, CH_2=C*H*), 7.08 - 7.38 (m, 30H, H-arom), 7.69 (m, 5H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (Cq-*t*Bu), 23.6 (CH_3-NAc), 27.0 (CH_3-*t*Bu), 56.4 (C-2), 63.8 (C-6), 69.1 (CH_2-Rbo), 71.3 (CH_2-Rbo), 72.1, 72.3, 73.6, 74.0, 74.5, 74.8, 75.0 (*C*H$_2$-CH, CH_2-Bn), 75.3 (CH-Rbo/C-3), 78.3, 79.5, 79.5, 82.1 (C-5, CH-Rbo/C-3, C-4, 2x CH-Rbo), 101.6 (C-1), 116.9 (CH_2=*C*H), 127.5, 127.5, 127.6, 127.7, 127.8, 127.8, 127.8, 127.9, 128.0, 128.1, 128.1, 128.3, 128.3, 128.3, 128.4, 128.5, 128.5, 129.7 (CH-arom), 133.4, 133.6 (Cq-arom), 134.7 (*C*H=CH_2), 135.8, 135.8 (CH-arom), 138.3, 138.4, 138.6, 138.7, 138.7 (Cq-arom), 170.3 (C=O); HRMS: $[M+Na]^+$ calculated for $C_{67}H_{77}NNaO_{10}Si$ 1106.5214, found 1106.5231.

O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-D-ribitol (17)



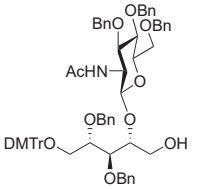
Compound **28** (0.93 g; 0.85 mmol; 1.0 eq.) was dissolved in THF (5.0 ml; 0.17M). TBAF (1M in THF: 1.7 ml; 1.70 mmol; 2.0 eq.) was added and the mixture was stirred at rt. After 1h TBAF (1M in THF: 2.6 ml; 2.60 mmol; 3.0 eq.) was added and stirring was continued until the starting material was completely converted. The mixture was concentrated under reduced pressure and purified by column chromatography pentane/EtOAc 1:0 to 4:6 pentane/EtOAc yielding the title compound **17** in 68% yield (0.49 g; 0.58 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 1): +14.4; IR (neat, cm-^{1}): 3288, 3064, 2923, 2868, 1653, 1454, 1371, 1069, 1029, 736, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.83 (s, 3H, CH_3-NAc), 2.86 (m, 1H, O*H*), 3.42 - 3.57 (m, 4H, H-3, H-4, CH_2-Rbo), 3.61 - 3.80 (m, 5H, H-2, H-6′, CH_2-Rbo, CH-Rbo), 3.81 – 3.97 (m, 5H, H-6″, C*H*$_2$-CH, 2x CH-Rbo), 4.07 (td, 1H, *J*= 7.2 Hz, 2.4 Hz, H-5), 4.43 - 4.55 (m, 3H, CH_2-Bn), 4.55 - 4.82 (m, 8H, CH_2-Bn, H-1), 5.11 - 5.25 (m, 2H, C*H*$_2$=CH), 5.75 (d, 1H, *J*= 7.9 Hz, N*H*), 5.84 (m, 1H, CH_2=C*H*), 7.16 (m, 2H, H-arom), 7.28 (m, 23H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 23.5 (CH_3-NAc), 56.3 (C-2), 61.4 (C-6), 68.9 (CH_2-Rbo), 71.4, 71.8, 72.1, 73.4, 73.9 (CH_2-Rbo, C*H*$_2$-CH, CH_2-Bn), 74.5 (C-3/C-4), 74.6, 74.7 (CH_2-Bn), 78.3 (C-3/C-4), 78.4, 78.7, 79.3, 82.0 (C-5, 3x CH-Rbo), 101.2 (C-1), 117.0 (*C*H$_2$=CH), 127.5, 127.6, 127.7, 127.8, 127.8, 128.0, 128.2, 128.3, 128.4, 128.4 (CH-arom), 134.5 (CH_2=*C*H), 137.6, 137.8, 138.3, 138.3, 138.6 (Cq-arom), 170.4 (C=O); HRMS: $[M+H]^+$ calcd for $C_{51}H_{60}NO_{10}$846.42117, found 846.42055.

O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-1-*O*-(4,4′-dimethoxytrityl)-D-ribitol (18)



Compound **17** (0.47 g; 0.55 mmol; 1.0 eq.) was dissolved in DCM (5.5 ml; 0.10M) followed by the addition of TEA (0.12 ml; 0.83 mmol; 1.5 eq.) and the mixture was cooled to 0°C. DMTrCl (0.23 g; 0.67 mmol; 1.2 eq.) was added and the mixture was allowed to warm up to rt and stirring was continued for 2 days. The reaction was then quenched with MeOH at 0°C, diluted with DCM and washed with sat. aq. $NaHCO_3$/NaCl. The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by TEA neutralized column chromatography pentane/EtOAc 1:0 to 1:1 pentane/EtOAc yielded the title **18** compound in 53% yield (0.34 g; 0.30 mmol). $[\alpha]_D^{20}$ (DCM *c* 1): + 2.7; IR (neat, cm-^{1}): 2928, 2869, 1653, 1454, 1364, 1251, 1069, 1029, 751, 737, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.85 (s, 3H, CH_3-NAc), 3.24 (d, 1H, *J*= 10.4 Hz, H-6′), 3.42 - 3.55 (m, 2H, H-6″, CH-Rbo), 3.55 - 3.75 (m, 12H, H-3, 2x CH_2-Rbo, CH-Rbo, 2x CH_3O), 3.75 - 3.91 (m, 2H, H-5, H-2), 3.91 - 4.02 (m, 3H, H-4, C*H*$_2$-CH), 4.30 - 4.33 (m, 1H, CH-Rbo), 4.41 (m, 2H, CH_2-Bn), 4.49 (d, 1H, *J*= 12.1 Hz, C*H*H-Bn), 4.60 (d, 2H, *J*= 11.2 Hz, CH_2-Bn), 4.70 - 4.86 (m, 6H, CH_2-Bn, H-1), 5.12 - 5.33 (m, 2H, C*H*$_2$=CH), 5.90 - 6.00 (m, 1H, CH_2=C*H*), 6.54 (d, 1H, *J*= 9.1 Hz, NH), 6.73 - 6.83 (m, 4H, H-arom), 7.11 (m, 2H, H-arom), 7.16 - 7.54 (m, 32H, H-arom); ^{13}C-APT NMR (126 MHz, CD_3CN) δ= 23.6 (CH_3-NAc), 55.8 (CH_3O), 56.5 (C-2), 64.2 (C-6), 70.1 (CH_2-Rbo), 71.2, 72.7, 73.2, 74.0, 74.3, 75.3, 75.5, (CH_2-Rbo, *C*H$_2$-CH, CH_2-Bn), 75.8 (CH-Rbo), 79.4, 79.5, 79.5, 80.5 (C-3, C-4, C-5, CH-Rbo), 83.6 (CH-Rbo), 86.8 (Cq-DMTr), 102.1 (C-1), 114.0 (CH-arom), 116.9 (*C*H$_2$=CH), 127.7, 128.3, 128.4, 128.4, 128.5, 128.6, 128.7, 128.7, 128.8, 128.9, 128.9, 129.0, 129.0, 129.2, 129.3, 129.3, 131.1, 131.1 (CH-arom), 136.3 (*C*H=CH$_2$), 137.0, 137.1, 139.5, 139.6, 139.8, 139.8, 139.9, 146.5, 159.5 (Cq-arom), 170.6 (C=O).

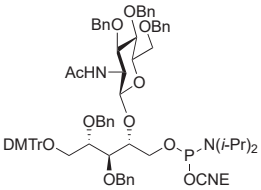
O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1-4)-2,3-di-*O*-benzyl-1-*O*-(4,4′-dimethoxytrityl)-D-ribitol (19)



A solution of compound **18** (0.27 g; 0.21 mmol) in destilled THF (2.1 ml; 0.10M) was degassed with N_2. $Ir(COD)(Ph_2MeP)_2PF_6$ (6 mg; 0.03 eq.) was added and the solution was degassed with N_2. Then the red solution was purged with H_2 until the color became yellow (~5 seconds) and hereafter the solution was degassed with argon to remove traces of H_2 from the solution and stirring was continued under N_2 atmosphere until complete conversion of the substrate occured according to TLC analysis. The mixture was diluted with THF (2.0 ml) and aq. sat. $NaHCO_3$ (2.0 ml) followed by the addition of I_2 (0.08 g; 0.31 mmol; 1.5 eq.) and stirred for +/- 30 mins. The reaction was quenched by the addition of sat. aq. Na_2SO_3, diluted with EtOAc and the organic layer was washed with sat. aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4,

filtrated and concentrated under reduced pressure. Purification by TEA neutralized column chromatography DCM/aceton 96:4 to 9:1 DCM/aceton yielded the title compound **19** in 79% yield. $[\alpha]_D^{20}$ (DCM *c* 1): + 3.6; IR (neat, cm-^{1}): 3288, 3064, 2929, 2870, 1653, 1508, 1453, 1362, 1251, 1070, 1029, 736, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.87 (s, 3H, CH_3-NAc), 3.25 (dd, 1H, *J*= 10.3 Hz, 5.3 Hz, H-6′), 3.47 - 3.50 (m, 2H, H-6″, CH-Rbo), 3.58 - 3.67 (m, 2H, C*H*H-Rbo, CH-Rbo), 3.67 - 3.77 (m, 10H, 2x CH_3O, C*H*H-Rbo, H-3, CH_2-Rbo), 3.77 - 3.90 (m, 2H, H-5, H-2), 4.03 (dd, 1H, *J*= 7.4 Hz, 3.0 Hz, H-4), 4.09 - 4.13 (m, 1H, CH-Rbo), 4.39 (dd, *J*= 11.6, 3.6 Hz, 2H, CH_2-Bn), 4.48 (d, *J*= 12.0 Hz, 1H, C*H*H-Bn), 4.59 (dd, 2H, *J*= 11.2, 5.7 Hz, CH_2-Bn), 4.70 - 4.85 (m, 6H, CH_2-Bn, H-1), 6.72 - 6.82 (m, 4H, H-arom), 6.85 (d, 1H, *J*= 9.0 Hz, N*H*), 7.09 - 7.12 (m, 2H, *J*= 6.6 Hz, 2.2 Hz, H-arom), 7.17 - 7.44 (m, 30H, H-arom), 7.47 - 7.53 (m, 2H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 23.6 (CH_3-NAc), 55.8 (CH_3O), 56.9 (C-2), 62.2 (CH_2-Rbo), 64.1 (C-6), 70.0 (CH_2-Rbo), 73.3, 74.0, 74.5, 75.3, 75.5 (CH_2-Bn), 75.7(CH-Rbo), 79.3, 79.5 (CH-Rbo, C-5), 80.4 (C-4), 82.0 (C-3), 83.4 (CH-Rbo), 86.9 (Cq-DMTr), 102.4 (C-1), 114.0, 127.7, 128.3, 128.4, 128.5, 128.5, 128.6, 128.6, 128.8, 128.9, 128.9, 128.9, 129.0, 129.1, 129.2, 129.2, 129.3, 131.1, 131.1 (CH-arom), 137.0, 137.1, 139.4, 139.5, 139.7, 139.8, 146.4, 159.5 (Cq-arom), 171.6 (C=O); HRMS: $[M+Na]^+$ calculated for $C_{69}H_{73}NNaO_{12}$ 1130.5030 found, 1130.5049.

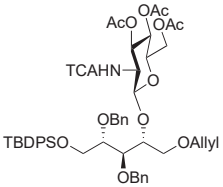
O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1-4)-2,3-di-*O*-benzyl-5-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-*O*-(4,4′-dimethoxytrityl)-D-ribitol (20)



To a solution of compound **19** (0.19 g; 0.15 mmol; 1.0 eq.) in DCM (1.5 ml; 0.10 M) was added DIPEA (43 µl; 0.25 mmol; 1.6 eq.). The mixture was stirred over activated MS 4Å for +/- 15 min. *N,N′*-di-isopropylamino-2-cyanoethyl-chlorophosphite (45 µl; 0.20 mmol; 1.3 eq.) was added and the mixture was stirred for 1h. Water was added, the mixture was diluted with DCM and the organic layer was washed with sat. aq. $NaHCO_3$:NaCl (v/v= 1:1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by TEA neutralized column chromatography pentane/EtOAc 1:0 to 6:4 pentane/EtOAc yielded phosphoramidite **20** in 61% yield (0.13 g; 0.09 mmol). ^{1}H NMR (400 MHz, CD_3CN) δ= 1.08 - 1.23 (m, 12H, 4x CH_3-isopropylamine), 1.83 (bs, 3H, CH_3-NAc), 2.54 - 2.64 (m, 2H, CH_2-cyanoethyl), 3.19 (ddd, 1H, *J*= 10.0 Hz, 8.1 Hz, 5.1 Hz, H-6′), 3.45 (m, 2H, H-6″, CH-Rbo), 3.51 - 3.75 (m, 15H, 2x CH-isopropylamine, 2x CH_2-cyanoethyl, 2x CH_3O, CH_2-Rbo, CH-Rbo, CH-Rbo/H-3/H-4), 3.75 - 4.02 (m, 5H, H-2, H-5, CH_2-Rbo, CH-Rbo/H-3/H-4), 4.23 - 4.34 (m, 1H, CH-Rbo/H-3/H-4), 4.34 - 4.81 (m, 11H, 5x CH_2-Bn, H-1), 6.40 (d, 1H, *J*= 9.2 Hz, N*H*), 6.74 - 6.78 (m, 4H, H-arom), 7.07 - 7.11 (m, 2H, H-arom), 7.12 - 7.49 (m, 32H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 21.1, 21.1 (CH_2-cyanoethyl), 23.7 (CH_3-NAc), 24.9, 25.0, 25.1, 25.2 (CH_3-isopropylamine), 43.7, 43.7, 43.8, 43.8 (CH-isopropylamine), 55.8 (CH_3O), 56.4 (C-2), 59.2,

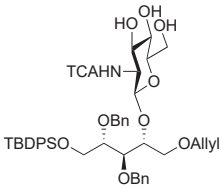
59.4, 59.6 (CH_2-cyanoethyl), 63.5, 63.6, 64.0, 64.2, 64.3, 64.4 (CH_2-Rbo, C-6), 70.0, 70.0 (CH_2-Rbo), 73.3, 73.9, 74.0, 74.2, 75.3, 75.4 (CH_2-Bn), 75.8 (CH-Rbo), 79.3, 79.3, 79.4, 79.5, 79.6, 79.7, 80.1, 80.1, 83.6, 83.6 (C-3, C-4, C-5, 2x CH-Rbo), 86.8 (Cq-DMTr), 101.8, 101.8 (C-1), 113.9 (CH-arom), 126.2, 127.6, 128.3, 128.3, 128.3, 128.5, 128.6, 128.6, 128.7, 128.7, 128.7, 128.9, 128.9, 129.0, 129.2, 129.2, 129.9, 131.1 (CH-arom), 137.1, 137.2, 139.5, 139.6, 139.7, 139.7, 139.9, 140.0, 140.0, 146.5, 159.5 (Cq-arom), 170.6 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 148.3, 147.9.

O-(3,4,6-tri-*O*-acetyl-2-trichloroacetylamino-2-deoxy-β-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (15)



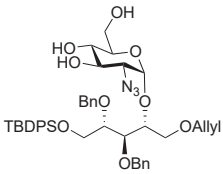
Donor **14** (3.86 g; 6.48 mmol; 1.2 eq.) and acceptor **12** (3.29 g; 5.39 mmol; 1.0 eq.) were co-evaporated with toluene twice under a N_2 atmosphere in 1 pot. The mixture was dissolved in dry DCM (65.0 ml; 0.10 M) and stirred on activated MS 4Å and cooled to 0°C. TMSOTf (125.0 μl; 0.69 mmol; 0.1 eq.) was added and the reaction was quenched with TEA after complete conversion of the acceptor according to TLC analysis. The mixture was diluted with DCM and was washed with water and brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography pentane/EtOAc 9:1 to 7:3 pentane/EtOAc. The combined eluate was concentrated *in vacuo* and purified by size exclusion chromatography affording the title compound **15** in 92% yield (5.44 g; 5.93 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 1): - 0.5; IR (neat, cm-^{1}): 2931, 2858, 1749, 1723, 1457, 1368, 1232, 1112, 1039, 701; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.06 (s, 9H, CH_3-*t*Bu), 1.97 (s, 3H, CH_3-Ac), 2.02 (d, 6H, *J*= 2.2 Hz, CH_3-Ac), 3.52 - 3.71 (m, 4H, H-5, CH-Rbo, C*H*$_2$-CH), 3.77 - 3.97 (m, 5H, 2x CH_2-Rbo, CH-Rbo), 4.04 (dd, 1H, *J*= 12.2 Hz, 2.4 Hz, H-6′), 4.14 (q, 1H, *J*= 10.4 Hz, H-2), 4.26 (dd, *J*= 12.2 Hz, 4.9 Hz, 1H, H-6″), 4.36 - 4.39 (m, 1H, H-4), 4.48 (dd, 2H, *J*= 14.3 Hz, 11.4 Hz, CH_2-Bn), 4.73 (dd, 2H, *J*= 15.2 Hz, 11.4 Hz, CH_2-Bn), 4.93 (d, 1H, *J*= 8.5 Hz, H-1), 5.10 - 5.26 (m, 4H, C*H*$_2$=CH, H-3, CH-Rbo), 5.79 - 5.89 (m, 1H, CH_2=C*H*), 7.07 (d, 1H, *J*= 8.8 Hz, N*H*), 7.16 - 7.44 (m, 16H, H-arom), 7.66 - 7.68 (m, 4H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (Cq-*t*Bu), 20.7, 20.7 (CH_3-Ac) 26.9 (CH_3-*t*Bu), 56.0 (C-2), 62.2 (C-6), 63.3 (CH_2-Rbo), 68.5 (C-3/CH-Rbo), 71.4 (CH_2-Rbo/CH_2-CH/CH_2-Bn), 72.1 (C-3/CH-Rbo), 72.2, 72.3 (CH_2-Rbo/CH_2-CH/CH_2-Bn), 72.7 (C-3/CH-Rbo), 74.2 (CH_2-Rbo/CH_2-CH/CH_2-Bn), 79.2, 79.4, 79.5 (C-4, C-5, CH-Rbo), 92.5 (CCl_3), 101.3 (C-1), 117.4 (*C*H$_2$=CH), 127.6, 127.6, 127.6, 127.7, 127.7, 127.7, 127.7, 127.8, 128.2, 128.2, 128.3, 128.3, 128.3, 129.8 (CH-arom), 133.2, 133.5 (Cq-arom), 134.5 (CH_2=*C*H), 135.7, 135.8 (CH-arom), 138.3, 138.5 (Cq-arom), 162.1, 169.4, 170.8, 171.0 (C=O); HRMS: $[M+Na]^+$ calcd for $C_{52}H_{62}Cl_3NNaO_{13}Si$ 1064.2954, found 1064.2965.

O-(2-trichloroacetylamino-2-deoxy-β-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (16)



To a solution of Compound **15** (4.23 g; 3.90 mmol; 1.0 eq.) in MeOH (39.0 ml; 0.10 M) was added NaOMe (21.0 mg; 0.39 mmol; 0.1 eq.) and the mixture was stirred overnight. A small piece of Na was added and the mixture was stirred for 3h. Then the mixture was quenched with amberlite H^+ resin, filtrated and concentrated *in vacuo*. Purification by column chromatography pentane/EtOAc 9:1 to 0:1 pentane/ EtOAc afforded fractions of the starting compound and the product. The fractions of the starting compound were combined, concentrated *in vacuo* and treated for deacetylation according to the described procedure above. The crude was purified using column chromatography pentane/EtOAc 7:3 to 3:7 pentane/ EtOAc affording the title compound **16** in a total yield of 73% (2.63 g; 2.86 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 1): + 5.0; IR (neat, cm-^{1}): 3348, 2931, 2858, 1701, 1457, 1112, 1076, 1028, 701; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.35 (ddd, 1H, *J*= 9.1 Hz, 5.2 Hz, 3.2 Hz, CH-Rbo), 3.47 (dd, 1H, *J*= 10.7, 2.5 Hz, H-6'), 3.52 - 3.97 (m, 12H, 2x CH_2-Rbo, C*H*$_2$-CH, H-6'', H-2, H-3, H-4, 2x CH-Rbo), 4.22 (dt, *J*= 9.2 Hz, 2.9 Hz, 1H, H-5), 4.48 (dd, 2H, *J*= 29.1, 11.6 Hz, CH_2-Bn), 4.65 (dd, 2H, *J*= 14.5, 11.6 Hz, CH_2-Bn), 4.78 (d, 1H, *J*= 7.7 Hz, H-1), 5.12 - 5.22 (m, 2H, C*H*$_2$=CH), 5.77 - 5.86 (m, 1H, CH_2=C*H*), 7.13 - 7.17 (m, 2H, H-arom), 7.22 - 7.35 (m, 11H, H-arom), 7.37 - 7.44 (m, 2H, H-arom), 7.49 (d, 1H, *J*= 5.1 Hz, H-arom), 7.65 - 7.48 (m, 4H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (Cq-*t*Bu), 27.0 (CH_3-*t*Bu), 59.1 (C-2), 62.4, 63.0 (CH_2-Rbo), 71.2 (C-6), 71.8 (C-3/C-4/CH-Rbo), 72.1, 72.4, 74.1 (CH_2-CH, CH_2-Bn), 75.7, 76.1 (C-3/C-4/ CH-Rbo), 78.7, 79.3, 79.5 (C-3/C-4/C-5/CH-Rbo), 101.3 (C-1), 117.6 (CH_2=CH), 127.8, 127.8, 127.8, 128.0, 128.1, 128.4, 128.5, 129.8, 129.9 (CH-arom), 133.3, 133.4 (Cq-arom), 134.4 (CH_2=C*H*), 135.8, 135.9 (CH-arom), 138.2 (Cq-arom), 164.3 (C=O); HMRS: $[M+Na]^+$ calcd for $C_{46}H_{56}Cl_3NNaO_{10}Si$ 938.2637, found 938.2653.

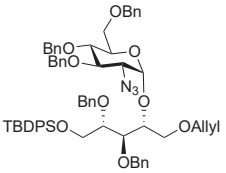
O-(2-azido-2-deoxy-α-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (31)



Donor **29** (11.5 g; 24.1 mmol; 1.0 eq.) and acceptor **12** (17.7 g; 29.0 mmol; 1.2 eq.) were co-evaporated together twice with toluene under an N_2 atmosphere. The mixture was dissolved in DCM (240 ml; 0.10 M) and stirred on activated MS 4Å for +/- 30 min. TMSOTf (0.44 ml; 2.41 mmol; 0.1 eq.) was added at rt and the reaction was stirred until full conversion of the donor was achieved according to TLC analysis. The reaction was quenched with TEA, concentrated under reduced pressure and purified by column chromatography pentane/EtOAc 1:0 to 8:2 pentane/EtOAc yielding the product as an α/β mixture (7:1). The mixture was dissolved in MeOH (165 ml; 0.13 M) followed by addition of 4.3M NaOMe (0.82 ml; 0.15

eq.) and the mixture was stirred for 2h. The reaction was neutralized with amberlite H^+, filtrated and concentrated under reduced pressure. Purification by column chromatography pentane/EtOAc 85:15 to 30:70 pentane/EtOAc yielded the product in 64% over 2 steps as the α anomer (12.3 g; 15.4 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 4.2): + 57.1; IR (neat, cm-^{1}): 3352, 2930, 2857, 2108, 1454, 1362, 1103, 1024, 741, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.05 (s, 9H, CH_3-*t*Bu), 2.87 (bs, 1H, O*H*), 3.32 (dd, 1H, *J*= 10.4 Hz, 3.6 Hz, H-2), 3.52 (dd, 1H, *J*= 10.8 Hz, 2.4 Hz, H-6′), 3.60 - 4.00 (m, 12H, H-3, H-4, H-6″, 3x CH-Rbo, 2x CH_2-Rbo, CH_2-CH), 4.17 (bs, 1H, O*H*), 4.23 - 4.25 (m, 1H, H-5), 4.31 (bs, 1H, O*H*), 4.47 (d, 1H, *J*= 11.6 Hz, C*H*H-Bn), 4.58 (d, 1H, *J*= 11.2 Hz, C*H*H-Bn), 4.67 (d, 1H, *J*= 11.6 Hz, C*H*H-Bn), 4.82 (d, 1H, *J*= 11.2 Hz, C*H*H-Bn), 5.09 - 5.19 (m, 3H, H-1, CH=CH_2), 5.77 - 5.84 (m, 1H, C*H*=CH_2), 7.19 - 7.40 (m, 15H, H-arom), 7.65 - 7.69 (m, 5H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (Cq-*t*Bu), 27.0 (CH_3-*t*Bu), 61.8, 62.9 (CH_2-Rbo, *C*H_2-CH), 63.6 (C-2), 70.4 (C-6), 70.7 (C-4), 71.2 (C-3/CH-Rbo), 72.1 (CH_2 Bn/CH_2-Rbo), 72.3, (C-3/CH-Rbo), 72.3 (CH_2 Bn/CH_2-Rbo), 74.0 (CH_2 Bn/CH_2-Rbo), 77.6 (C-5), 78.8, 78.9 (CH-Rbo), 96.8 (C-1), 117.3 (CH=*C*H_2), 127.6, 127.8, 127.8, 127.8, 127.9, 128.1, 128.4, 129.8 (CH-arom), 133.3, 133.6 (Cq-arom), 134.7 (*C*H=CH_2), 135.7, 135.9 (CH-arom), 138.4, 138.5 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{44}H_{55}N_3NaO_9Si$ 820.3605, found 820.3616.

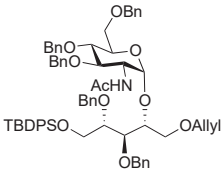
O-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (32)



To a solution of compound **31** (12.7 g; 15.9 mmol; 1.0 eq.) in THF/DMF (160 ml; 0.1M; v/v= 7:1) at 0°C was added NaH (2.50 g; 63.6 mmol; 4.0 eq.) in portions followed by BnBr (9.5 ml; 79.5 mmol; 5.0 eq.) and the mixture was allowed to warm up to rt and stirred for 2 days. The mixture was then quenched with MeOH at 0°C, diluted with EtOAc (260 ml), washed with water (5x 150 ml) and brine. Column chromatography afforded the product and partly benzylated intermediate (3.00 mmol) that was recovered and dissolved in THF/DMF (30.0 ml; 0/1 M; v/v= 7:1) followed by addition of NaH (0.16 g; 4.1 mmol; 1.4 eq.) and BnBr (0.46 ml; 3.8 mmol; 1.3 eq.) at 0°C and the reaction was allowed to warm up to rt and was stirred overnight. The mixture was quenched with MeOH at 0°C and worked up as described above leading to a total yield of the title compound in 73% (12.36 g; 11.57 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 4.2): + 48.3; IR (neat, cm-^{1}): 2928, 2857, 2106, 1454, 1362, 1105, 1074, 1028, 737, 698; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.06 (s, 9H, CH_3-*t*Bu), 3.55 (dd, 1H, *J*= 9.2 Hz, 3.6 Hz, H-2), 3.59 - 3.65 (m, 3H, H-6′, CH_2-Rbo), 3.77 - 3.84 (m, CH_2-CH, H-6″, 2x CH-Rbo), 3.91 (dd, 1H, *J*= 11.2 Hz, 4.4 Hz, C*H*H-Rbo), 3.98 - 4.03 (m, 3H, C*H*H-Rbo, H-3, H-4), 4.17 - 4.20 (m, 1H, CH-Rbo), 4.25 - 4.27 (m, 1H, H-5), 4.44 - 4.88 (m, 10H, CH_2-Bn), 7.18 - 7.39 (m, 30H, H-arom), 7.67 - 7.71 (m, 5H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.3 (Cq-*t*Bu), 26.9 (CH_3-*t*Bu), 62.9 (CH_2-Rbo), 64.3 (C-2), 68.4 (C-6), 70.3 (CH_2-Rbo), 70.7 (CH-Rbo), 72.0, 72.2 (CH_2-CH, CH_2-Bn), 73.6,

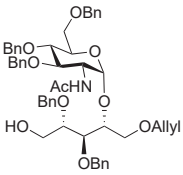
73.9, 74.9, 75.4 (CH_2-Bn), 77.8, 78.5, 78.8, 79.1, 80.6 (C-5, C-3, C-4, 2x CH-Rbo), 97.2 (C-1), 116.8 (*C*H_2=CH), 127.4, 127.5, 127.7, 127.8, 127.9, 127.9, 128.0, 128.2, 128.3, 128.4, 128.4, 128.5, 128.8, 129.1, 129.7 (CH-arom), 133.3, 133.6 (Cq-arom), 134.9 (*C*H=CH_2), 135.7, 135.7, 135.9 (Cq-arom), 138.1, 138.3, 138.6, 138.6 (Cq-arom); HRMS: $[M+Na]^+$ calculated for $C_{65}H_{73}N_3NaO_9Si$ 1090.5014, found 1090.5040.

O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (33)



Compound **32** (12.4 g; 11.6 mmol; 1.0 eq.) was dissolved in pyridine/H_2O (200 ml; 0.058M; v/v= 5:1) followed by the addition of TEA (0.93 ml) and 1,3-propaandithiol (5.80 ml; 57.8 mmol; 5.0 eq.) and the mixture was stirred overnight at rt. Then the mixture was concentrated under reduced pressure, co-evaporated with toluene (3x), dissolved in pyr/Ac_2O (200 ml; v/v= 2:1) and stirred overnight. The mixture was then quenched with MeOH, concentrated under reduced pressure and purified by column chromatography pentane/EtOAc 1:0 to 1:1 pentane/EtOAc affording the title compound **27** in 92% yield (11.66 g; 10.8 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 2.6): + 53.3; IR (neat, cm^{-1}): 2930, 2857, 1684, 1454, 1271, 1111, 1070, 1028, 741, 700; ^1H NMR (400 MHz, $CDCl_3$) δ= 1.05 (s, 9H, CH_3-*t*Bu), 1.43 (s, 3H, CH_3-NAc), 3.54 - 5.06 (m, 14H, 3x CH-Rbo, 2x CH_2-Rbo, H-3, H-4, H-5, H-6′, H-6″, C*H*$_2$-CH), 4.19 - 4.25 (m, 1H, H-2), 4.41 (d, 1H, *J*= 11.6 Hz, C*H*H-Bn), 4.64 - 4.67 (m, 8H, CH_2-Bn), 4.81 (d, 1H, *J*= 10.8 Hz, C*H*H-Bn), 4.92 (d, 1H, *J*= 3.2 Hz, H-1), 5.08 - 5.20 (m, 2H, CH=C*H*$_2$), 5.59 (d, 1H, *J*= 9.2 Hz, N*H*), 5.77 - 5.84 (m, 1H, C*H*=CH_2), 7.17 - 7.38 (m, 30H, H-arom), 7.62 - 7.68 (m, 5H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 19.2 (Cq-*t*Bu), 22.9 (*C*H_3-NAc), 26.8 (CH_3-*t*Bu), 52.8 (C-2), 63.2 (*C*H_2-CH), 68.6, 69.5 (CH_2-Rbo, C-6), 71.4 (CH-Rbo), 71.9, 72.4, 73.4, 74.7, 74.9 (CH_2-Rbo, 5x CH_2-Bn), 78.1, 78.2, 79.3, 79.4, 80.6 (2x CH-Rbo, C-3, C-4, C-5), 99.3 (C-1), 116.7 (*C*H_2=CH), 127.6, 127.7, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 128.9 129.8, 129.8 (C-arom), 133.0, 133.2 (Cq-arom), 134.7 (*C*H=CH_2), 135.5, 135.6 (C-arom), 138.0, 138.0, 138.1, 138.4 (Cq-arom), 169.7 (C=O); HRMS: $[M+H]^+$ calculated for $C_{67}H_{78}NO_{10}Si$ 1084.5395, found 1084.5394.

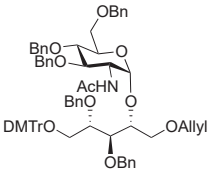
O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-D-ribitol (34)



Compound **33** (11.2 g; 10.4 mmol; 1.0 eq.) was dissolved in THF (61 ml; 0.17 M) and to this solution was added TBAF (15.5 ml; 15.5 mmol; 1.5 eq.) and the mixture was stirred at rt. When TLC analysis showed a small amount of starting material, TBAF (5.2 ml; 5.2 mmol; 0.5 eq.) was added and the reaction was stirred for 40 min, after which the mixture was concentrated under reduced pressure. Purification

by column chromatography pentane/EtOAc 1:0 to 3:7 pentane/EtOAc yielded the title compound **34** in 86% yield (7.93 g; 8.9 mmol). $[\alpha]_D^{20}$ ($CHCl_3$ *c* 1.6): + 42.4; IR (neat, cm-^{1}): 3447, 3420, 2920, 2862, 1684, 1558, 1456, 1097, 1070, 1047, 1028, 737, 698; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.51 (s, 3H, CH_3-NAc), 2.59 (bs, 1H, O*H*), 3.48 (dd, 1H, *J*= 10.4 Hz, 5.6 Hz, H-6′), 3.57 (dd, 1H, *J*= 10.4 Hz, 5.6 Hz, H-6″), 3.62 - 3.83 (m, 9H, 2x CH_2-Rbo, CH_2-CH, H-3, 2x CH-Rbo), 3.92 - 3.95 (m, 2H, H-4, CH-Rbo), 4.01 (q, 1H, *J*= 3.6 Hz, H-5), 4.22 (ddd, *J* = 10.5 Hz, 9.0 Hz, 3.6 Hz, H-2), 4.48 - 4.68 (m, 8H, CH_2-Bn), 4.82 (dd, *J*= 11.2 Hz, 3.3 Hz, 2H, CH_2-Bn), 4.94 (d, *J*= 3.6 Hz, 1H, H-1), 5.09 - 5.20 (m, 2H, CH=CH_2), 5.77 (m, 1H, C*H*=CH_2), 5.86 (d, *J*= 9.1 Hz, 1H, N*H*), 7.17 - 7.38 (m, 25H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 22.9 (CH_3-NAc), 52.9 (C-2), 61.9 (CH_2-CH), 68.7 (CH_2-Rbo), 69.3 (C-6), 71.6 (CH-Rbo), 71.7, 72.2, 73.6, 74.2, 74.8, 75.2 (CH_2-Rbo, 5x CH_2-Bn), 78.0, 78.4, 78.9, 79.2, 80.1, (2x CH-Rbo, C-3, C-4, C-5), 100.1 (C-1), 117.3 (CH=CH_2), 127.8, 127.8, 127.9, 127.9, 128.0, 128.0, 128.0, 128.0, 128.1, 128.1, 128.2, 128.2, 128.5, 128.5, 128.5, 128.6, 128.6, 128.7, 128.7 (C-arom), 134.5 (*C*H=CH_2), 137.7, 137.9, 138.1, 138.2, 138.4 (Cq-arom), 170.3 (C=O); HRMS: $[M+H]^+$ calcd for $C_{51}H_{60}NO_{10}$846.4217, found 846.4230.

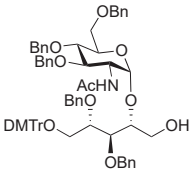
O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1-4)-5-*O*-allyl-2,3-di-*O*-benzyl-1-*O*-(4,4′-dimethoxytrityl)-D-ribitol (35)



To a solution of compound **34** (7.61 g; 9.0 mmol; 1.0 eq.) in DCM (60.0 ml; 0.15 M) was added DMTrCl (3.66 g; 10.8 mmol; 1.2 eq.) and TEA (2.0 ml; 13.5 mmol; 1.5 eq.) and the reaction was stirred for 2h at rt. The reaction was then quenched with MeOH at 0°C, diluted with DCM and washed with sat. aq. $NaHCO_3$/NaCl. The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by TEA neutralized column chromatography pentane/EtOAc 1:0 to 1:1 pentane/EtOAc yielded the title compound **35** in 78% yield (8.05 g; 7.00 mmol). $[\alpha]_D^{20}$ (DCM *c* 1): + 44.1; IR (neat, cm-^{1}): 2909, 2868, 1684, 1508, 1454, 1250, 1088, 1072, 1029, 829, 737, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.69 (s, 3H, CH_3-NAc), 3.21 (dd, 1H, *J*= 10.2 Hz, 4.9 Hz, C*H*H-Rbo), 3.47 (dd, 1H, *J*= 10.2 Hz, 2.9 Hz, CH*H*-Rbo), 3.59 - 3.77 (m, 13H, H-6′, H-6″, H-3, 2x CH_3O, CH_2-Rbo, 2x CH-Rbo), 3.87 (ddt, 2H, *J*= 8.1 Hz, 5.3 Hz, 1.6 Hz, CH_2-CH), 3.95 (dd, 1H, *J*= 7.2, 2.6 Hz, H-4), 4.01 - 4.10 (m, 2H, H-2, CH-Rbo), 4.14 - 4.17 (m, 1H, H-5), 4.42 (d, 1H, *J*= 11.1 Hz, C*H*H-Bn), 4.49 (d, 1H, *J*= 12.0 Hz, C*H*H-Bn), 4.53 - 4.85 (m, 8H, CH_2-Bn), 4.93 (d, 1H, *J*= 3.6 Hz, H-1), 5.05 - 5.26 (m, 2H, CH_2=CH), 5.85 (m, 1H, CH_2=C*H*), 6.30 (d, 1H, *J*= 9.0 Hz, N*H*), 6.74 - 6.83 (m, 4H, H-arom), 7.08 - 7.50 (m, 34H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 23.3 (CH_3-NAc), 54.1 (C-2), 55.8 (CH_3O), 63.6 (CH_2-Rbo), 70.0, 70.7 (C-6, CH_2-Rbo), 71.8 (CH-Rbo), 72.5, 73.3, 73.9, 74.4, 75.4, 75.5 (CH_2-CH, 5x CH_2-Bn), 77.8 (C-5), 79.2, 79.2, 79.6, 81.2 (2x CH-Rbo, C-3, C-4), 86.8 (Cq-DMTr), 97.7 (C-1), 114.0 (CH-arom), 116.8 (CH_2=CH), 127.7, 128.5, 128.5, 128.5, 128.6, 128.8, 128.8, 128.8, 128.9, 129.2, 129.3, 129.3, 131.0, 131.0 (CH-arom), 136.2 (*C*H=CH_2), 137.0, 137.1 (Cq-arom), 139.5, 139.5,

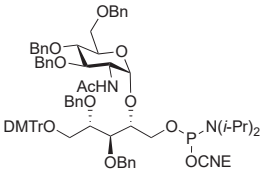
139.6, 139.7, 139.9, 146.4, 159.5 (Cq-arom), 170.3 (C=O); HRMS: $[M+Na]^+$ calculated for $C_{72}H_{77}NNaO_{12}$ 1170.5343, found 1170.5337.

O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1-4)-2,3-di-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol (36)



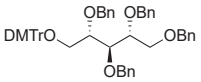
A solution of compound **35** (0.85 g; 0.74 mmol; 1.0 eq.) in destilled THF (7.4 ml; 0.10 M) was degassed with N_2. $Ir(COD)(Ph_2MeP)_2PF_6$ (14 mg; 0.02 eq.) was added and the solution was degassed with N_2. Then the red solution was purged with H_2 until the color became yellow (~25 seconds) and hereafter the solution was degassed with argon to remove traces of H_2 from the solution and the reaction was warmed up to 30°C for 5 mins under argon atmosphere. The mixture was diluted with THF (7.4 ml) and aq. sat. $NaHCO_3$ (7.4 ml) followed by the addition of I_2 (0.28 g; 1.12 mmol; 1.5 eq.) and stirred for +/- 30 min. The reaction was quenched by the addition of sat. aq. Na_2SO_3, diluted with EtOAc and the organic layer was washed with sat. aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by TEA neutralized column chromatography pentane/EtOAc 1:0 to 1:1 pentane/EtOAc yielded the title compound **36** in 77% yield (0.70 g; 0.63 mmol). $[\alpha]_D^{20}$ (DCM *c* 1): + 31.7; IR (neat, cm^{-1}): 3567, 3064, 3031, 2931, 1668, 1508, 1454, 1368, 1251, 1069, 1029, 737, 698; ^1H NMR (400 MHz, CD_3CN) δ= 1.66 (s, 3H, CH_3-NAc), 3.25 (t, 1H, *J*= 6.3 Hz, O*H*), 3.30 (dd, 1H, *J*= 10.2 Hz, 5.0 Hz, H-6'), 3.50 (dd, 1H, *J*= 10.1 Hz, 2.8 Hz, H-6''), 3.58 (dd, 1H, *J*= 10.1, 8.9 Hz, CH-Rbo), 3.64 - 3.81 (m, 11H, 2x CH_2-Rbo, 2x CH_3O, H-3), 3.87 (m, 1H, H-5), 3.93 - 4.02 (m, 2H, H-4, CH-Rbo), 4.09 (ddd, 1H, *J*= 10.2 Hz, 5.5 Hz, 2.0 Hz, CH-Rbo), 4.16 (ddd, 1H, *J*= 10.7 Hz, 9.1 Hz, 3.7 Hz, H-2), 4.45 - 4.86 (m, 10H, CH_2-Bn), 4.94 (d, 1H, *J*= 3.7 Hz, H-1), 6.33 (d, 1H, *J*= 9.1 Hz, N*H*), 6.77 - 6.84 (m, 4H, H-arom), 7.14 - 7.23 (m, 3H, H-arom), 7.24 (s, 27H, H-arom), 7.46 (dt, 2H, *J*= 6.5 Hz, 1.5 Hz, H-arom), 7.49 - 7.54 (m, 2H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 23.3 (CH_3-NAc), 54.0 (C-2), 55.8, 55.8 (CH_3O), 62.7 (CH_2-Rbo), 64.2 (C-6), 70.0 (CH_2-Rbo), 72.1 (CH-Rbo), 73.4, 73.9, 74.2, 75.5, 75.6 (CH_2-Bn), 79.3, 79.5, 79.7 (C-4, C-5, CH-Rbo), 81.4 (C-3), 82.3 (C-4, C-5, CH-Rbo), 86.9 (Cq-DMTr), 98.8 (C-1), 114.0 (CH-arom), 127.7, 128.5, 128.5, 128.5, 128.6, 128.6, 128.7, 128.8, 128.8, 128.9, 129.0, 129.2, 129.2, 129.3, 129.4, 131.0, 131.1 (CH-arom), 137.0, 137.1, 139.2, 139.4, 139.4, 139.5, 139.8, 146.4, 159.5 (Cq-arom), 170.4 (C=O); HRMS: $[M+Na]^+$ calculated for $C_{69}H_{73}NNaO_{12}$ 1130.50250, found 1130.50183.

O-(2-acetylamino-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1-4)-2,3-di-*O*-benzyl-5-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-*O*-(4,4′-dimethoxytrityl)-D-ribitol (37)



To a solution of alcohol **36** (0.70 g; 0.63 mmol; 1.0 eq.) in DCM (6.3 ml; 0.10 M) was added DIPEA (0.16 ml; 0.94 mmol; 1.5 eq.). The mixture was stirred over activated MS 4Å for +/- 30 min. *N,N′*-di-isopropylamino-2-cyanoethyl-chlorophosphite (0.17 ml; 0.75 mmol; 1.2 eq.) was added and the mixture was stirred for 1h. Water was added, the mixture was diluted with DCM and the organic layer was washed with sat. aq. $NaHCO_3$:NaCl (v/v= 1:1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by TEA neutralized column chromatography pentane/EtOAc 1:0 to 1:1 pentane/EtOAc yielded phosphoramidite **37** in 81% yield (0.67 g; 0.51 mmol). ^{1}H NMR (400 MHz, CD_3CN) δ= 1.07 - 1.18 (m, 12H, 4x CH_3-isopropylamine), 1.72 (d, 3H, *J*= 14.7 Hz, CH_3-NAc), 2.37 - 2.51 (m, 2H, CH_2-cyanoethyl), 3.22 (dt, 1H, *J*= 9.8 Hz, 4.7 Hz, H-6′), 3.45 (dd, 1H, *J*= 10.3 Hz, 2.9 Hz, H-6″), 3.52 - 3.96 (m, 15H, 2x CH_3O, 2x CH_2-Rbo, 2x CH-isopropylamine, H-5, H-3, CH-Rbo), 3.98 - 4.22 (m, 4H, H-2, H-4, 2x CH-Rbo), 4.45 (dd, 1H, *J*= 11.0 Hz, 4.3 Hz, C*H*H-Bn), 4.52 (dd, 1H, *J*= 12.0 Hz, 5.3 Hz, C*H*H-Bn), 4.56 - 4.67 (m, 4H, CH_2-Bn), 4.70 - 4.84 (m, 4H, CH_2-Bn), 4.98 (dd, 1H, *J*= 9.8 Hz, 3.6 Hz, H-1), 6.36 (dd, 1H, *J*= 16.4 Hz, 9.0 Hz, N*H*), 6.74 - 6.83 (m, 4H, H-arom), 7.11 - 7.22 (m, 3H, H-arom), 7.22 - 7.51 (m, 31H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.8, 20.9, 21.0, 21.0 (CH_2-cyanoethyl), 23.3, 23.3 (CH_3-NAc), 24.9, 25.0, 25.1, 25.2, 25.2, 25.3 (CH_3-isopropylamine), 43.7, 43.7, 43.8, 43.8, 43.9 (CH-isopropylamine), 54.1 (C-2), 55.8 (CH_3O), 59.3, 59.4, 59.5, 59.6 (CH_2-cyanoethyl), 63.6, 63.7, 63.7, 63.8, 63.9, 64.0 (CH_2-Rbo, C-6), 69.8, 69.9 (CH_2-Rbo), 71.8 (CH-Rbo), 73.3, 73.3, 73.9, 74.0, 74.3, 74.5, 75.4, 75.5, 75.5, 75.6 (CH_2-Bn), 78.1, 78.1, 78.4, 78.5, 78.6, 79.0, 79.3, 79.4, 79.6, 79.7, 81.0 (2x CH-Rbo, C-3, C-4, C-5), 86.9 (Cq-DMTr), 97.6, 97.7 (C-1), 114.0 (CH-arom), 127.7, 128.5, 128.5, 128.6, 128.6, 128.6, 128.7, 128.7, 128.7, 128.8, 128.9, 128.9, 129.0, 129.0, 129.2, 129.2, 129.3, 129.3, 131.0, 131.0, 131.0 (CH-arom), 137.0, 137.1, 137.1, 139.5, 139.5, 139.6, 139.6, 139.8, 139.9, 140.0, 146.4, 159.5, 159.5 (Cq-arom), 170.3, 170.4 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 148.9.

2,3,4,5-tetra-*O*-benzyl-1-*O*-(4,4′-dimethoxytrityl)-D-ribitol (39)

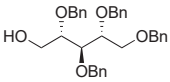


To a solution of compound **38** (887 mg; 1.16 mmol; 1.0 eq.) in a mixture of THF/DMF (10.0 ml; 0.12 M: v/v= 7:1) at 0°C was added NaH (100 mg; 2.32 mmol, 2.0 eq.) followed by the addition of BnBr (0.20 ml; 1.74 mmol; 1.5 eq.) and the mixture was allowed to warm up to rt and was stirred overnight. The mixture was quenched with MeOH at 0°C, was diluted with Et_2O and the organic layer was washed with 4x water and brine. The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by column chromatography

pentane/EtOAc 1:0 to 8:2 pentane/EtOAc yielded compound **39** in 84% yield (836 mg; 0.98 mmol). $[\alpha]_D^{25}$ (DCM *c* 1): +11.9; IR (neat, cm-^{1}): 3567, 2931, 2355, 1608, 1508, 1454, 1251, 1176, 1093, 829, 736, 697; ^{1}H NMR (400 MHz, CD_3CN) δ= 3.25 - 3.38 (m, 2H, CH_2-Rbo), 3.66 (dd, 1H, *J*= 10.5 Hz, 5.6 Hz, C*H*H-Rbo), 3.71 (d, 6H, *J*= 1.4 Hz, CH_3O), 3.76 (dd, 1H, *J*= 10.6 Hz, 3.1 Hz, C*H*H-Rbo), 3.84 - 3.95 (m, 3H, CH-Rbo), 4.46 (s, 2H, CH_2-Bn), 4.48 - 4.61 (m, 3H, CH_2-Bn), 4.61 - 4.67 (m, 2H, CH_2-Bn), 4.72 (d, 1H, *J*= 11.6 Hz, C*H*H-Bn), 6.77 (dd, 4H, *J*= 9.0 Hz, 2.7 Hz, H-arom), 7.09 - 7.48 (m, 29H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 55.8 (CH_3O), 64.7 (CH_2-Rbo), 71.0 (CH_2-Rbo), 72.9, 73.3, 73.7, 74.2 (CH_2-Bn), 79.6, 79.8 (CH-Rbo), 86.8 (Cq-DMTr), 113.9 (CH-arom), 127.6, 128.3, 128.4, 128.6, 128.6, 128.7, 128.8, 129.0, 129.1, 129.2, 129.3, 129.3, 131.0, 131.0 (CH-arom), 137.1, 137.2, 139.6, 139.7, 139.9, 146.4, 159.5 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{54}H_{54}O_7Na$ 837.3767, found 837.3784.

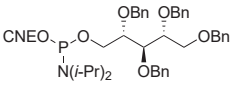
3

2,3,4,5-tetra-*O*-benzyl-D-ribitol (40)



Compound **39** (1.04 g, 1.27 mmol; 1.0 eq.) was dissolved in a solution of 3% DCA in DCM (23 ml, 0.18 M, 3.3 eq.) and the reaction mixture was stirred for 1h at rt. A mixture of MeOH/H_2O (23 ml; v/v= 1:1) was added, and the reaction mixture was stirred for 45 minutes. The mixture was diluted in DCM, and 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 $MgSO_4$, filtrated and concentrated *in vacuo*. Column chromatography pentane/EtOAc 1:0 to 8:2 pentane/EtOAc yielded title compound **40** (0.56 g, 1.08 mmol) in 87% yield. $[\alpha]_D^{25}$ ($CHCl_3$ *c* 1.0): -9.8; IR (neat, cm-^{1}): 3031, 2866, 2354, 1507, 1454, 1098, 1029, 736, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.32 (s, 1H, O*H*), 3.61 - 3.79 (m, 5H, H-1, H-3, H-5), 3.88 (td, 1H, *J*= 5.1, 3.7 Hz, H-4), 3.94 (t, 1H, *J*= 4.7 Hz, H-2), 4.37 - 4.85 (m, 8H, 4x CH_2-Bn), 7.23 - 7.47 (m, 20H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 61.5 (C-1), 69.8 (C-5), 72.0 - 74.1 (4x CH_2-Bn), 78.3 (C-4), 78.9 (C-3), 79.2 (C-2), 127.8 - 128.5 (C-arom), 138.2 - 138.4 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{33}H_{36}O_5Na$ 535.2460, found 535.2435.

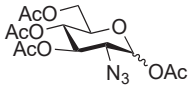
2,3,4,5-tetra-*O*-benzyl-1-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-D-ribitol (41)



To a solution of alcohol **40** (388 mg; 0.76 mmol; 1.0 eq.) in DCM (7.6 ml; 0.10 M) was added DIPEA (0.20 ml; 1.14 mmol; 1.5 eq.). The mixture was stirred over activated MS 4Å for +/- 30 min. *N,N'*-di-isopropylamino-2-cyanoethyl-chlorophosphite (0.20 ml; 0.91 mmol; 1.2 eq.) was added and the mixture was stirred for 1h. Water was added, the mixture was diluted with DCM and the organic layer was washed with sat. aq. $NaHCO_3$:NaCl (v/v= 1:1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by TEA neutralized column chromatography pentane/EtOAc 1:0 to 8:2 pentane/EtOAc yielded phosphoramidite **41** in 82% yield (442 mg; 0.62 mmol). ^{1}H NMR (400 MHz, CD_3CN) δ= 1.15 - 1.19 (m, 12H, 4x

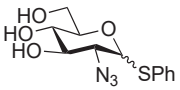
CH_3 isopropylamino), 2.53 - 2.60 (m, 2H, CH_2-cyanoethyl), 3.59 - 4.04 (m, 11H, 2x CH-isopropylamino, 2x CH_2-Rbo, 3x CH-Rbo, CH_2-cyanoethyl), 4.49 - 4.74 (m, 8H, 4x CH_2 -Bn), 7.28 - 7.38 (m, 20H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 21.0, 21.0 (CH_2-cyanoethyl), 24.9, 25.0, 25.0, 25.1 (CH_3-isopropylamino), 59.2, 59.3, 59.4, 59.5 (CH_2-cyanoethyl), 63.7, 63.8 (CH_2-Rbo), 71.0 (CH_2-Rbo), 72.8, 72.8, 72.9, 73.7, 74.5 (CH_2-Bn), 79.4, 79.6, 80.0, 80.1 (CH-Rbo), 119.5 (Cq-cyanoethyl), 128.3 - 129.2 (CH-arom), 139.7-139.9 (Cq-arom 4x); ^{31}P NMR (162 MHz, CD_3CN) δ= 149.1, 149.0.

1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranose (22)



Glucosamine · HCl (17.8 g, 82.8 mmol; 1.0 eq.) was dissolved in MeOH (410 mL, 0.2 M). K_2CO_3 (30.8 g, 223 mmol, 2.7 eq.), $CuSO_4 \cdot 5 H_2O$ (0.21 g, 1.32 mmol, 0.02 eq.), and the stick reagent (31.3 g, 99.3 mmol, 1.2 eq.) were added at rt. The reaction mixture was stirred for 3 hours, and the mixture was filtrated over celite. The mixture was concentrated *in vacuo*, co-evaporated with toluene (2x), and continued without purification to give the crude glucoseazide. The crude compound (17.0 g) was dissolved in pyridine (410 mL, 0.2 M). Ac_2O (62.6 mL, 662 mmol, 8.0 eq.) was added at 0°C and the reaction mixture was stirred from 0°C to rt overnight, followed by the addition of MeOH at 0°C. The mixture was diluted in EtOAc, and washed with 3M HCl (3x), sat. aq. $NaHCO_3$ (2x), and brine (1x). The organic layer was dried over $MgSO_4$, filtrated, and concentrated *in vacuo* to give title compound **22** (30.6 g, 81.9 mmol) as an α:β mixture with a ratio of 1 : 2.3 in 99% yield over 2 steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.97 – 2.25 (m, 12H, 4x C*H*$_3$-Acetyl), 3.63 – 3.76 (m, 1H, H-2), 3.88 (ddd, *J*= 9.8, 4.4, 2.1 Hz, 1H, H-5β), 4.01 – 4.35 (m, 2H, H-6), 4.97 – 5.20 (m, 2H, H-3β, H-4), 5.45 (dd, *J*= 10.6, 9.4 Hz, 0.44H, H-3 α), 5.62 (d, *J*= 8.6 Hz, 1H, H-1β), 6.31 (d, *J*= 3.6 Hz, 0.44H, H-1 α); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 20.2 – 20.6 (4x *C*H$_3$- Acetyl α, 4x *C*H$_3$-Acetyl β), 59.9 (C- 2α), 61.2 (C-6), 62.3 (C-2β), 67.6 (C-4β, C-4 α), 69.5 (C-5 α), 70.5 (C-3 α), 72.3 (C-3β, C-5β), 89.7 (C-1α), 92.2 (C-1β), 168.3 – 170.2 (4x C=O- α, 4x C=O- β); HRMS: $[M+Na]^+$ calcd for $C_{14}H_{19}N_3O_9Na$ 396.1019, found 396.1021.

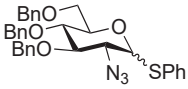
Phenyl 2-azido-2-deoxy-thio-D-glucopyranose (23)



Compound **22** (30.6 g, 81.9 mmol; 1.0 eq.) was dissolved in dry DCM (275 mL, 0.3 M). Thiophenol (8.35 mL, 81.9 mmol, 1.0 eq.) and $BF_3 \cdot OEt_2$ (31.1 mL, 246 mmol, 3.0 eq.) were added and the reaction mixture was refluxed for 7 days. TEA was added, and the organic layer was washed with sat. aq. $NaHCO_3$ (3x), 1M NaOH (3x), and brine (1x). The organic layer was dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. Column chromatography (100% toluene to 32% Et_2O in toluene) yielded the crude product. The crude compound (25.0 g) was dissolved in MeOH (300 mL, 0.2 M), followed by the dropwise addition of NaOMe (5.4 M) in MeOH (4.4 mL, 23.6 mmol, 0.4 eq.). The reaction mixture was stirred at rt overnight, followed

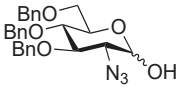
by the addition of H^+ amberlite. The H^+ amberlite was filtered off, and the mixture was concentrated *in vacuo*. Column chromatography (100% DCM to 9% MeOH in DCM) yielded triol **23** (13.7 g, 46.0 mmol) as an α:β mixture with a ratio of 2.9 : 1 in 56% yield over 2 steps. ^{1}H NMR (400 MHz, MeOD) δ= 3.30 – 3.40 (m, 1H, H-4α), 3.53 – 3.60 (m, 1H, H-5α), 3.62 – 3.70 (m, 3H, H-2α, H-6), 3.99 (dt, *J* = 10.0, 3.6 Hz, 1H, H-3α), 4.43 (d, *J* = 10.1 Hz, 0.34H, H-1β), 5.46 (d, *J* = 5.2 Hz, 1H, H-1α), 6.95 – 7.90 (m, 5H, H-arom); ^{13}C-APT NMR (101 MHz, MeOD) δ= 62.2 (C-6α), 65.5 (C-2α), 71.9 (C-4α), 74.8 – 75.0 (C-3α, C-5α), 87.3 (C-1β), 89.2 (C-1α), 128.8 – 133.9 (C-arom), 135.5 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{12}H_{15}N_3O_4SNa$ 320.0681, found 320.0685.

Phenyl 3,4,6-tri-*O*-benzyl-2-azido-2-deoxy-thio-D-glucopyranose (24)



Triol **23** (13.7 g, 46.0 mmol; 1.0 eq.) was co-evaporated with toluene, and dissolved in a (v/v= 1:1) mixture of DMF/THF (130 mL, 0.35 M). The mixture was cooled to 0°C and NaH (8.3 g, 207 mmol, 4.5 eq., 60% in mineral oil) was added portion wise. BnBr (21.3 mL, 180 mmol, 3.9 eq.) was added dropwise, and the reaction was stirred from 0°C to rt overnight, followed by the slow addition of a small amount of MeOH at 0°C. The mixture was diluted in Et_2O, and the organic phase was washed with H_2O (4x), and brine (1x). The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Column chromatography (100% pentane to 12% EtOAc in pentane) yielded title compound **24** (25.7 g, 45.2 mmol) as an α/β mixture with a ratio of 2:3 in 98% yield. IR (neat, cm^{-1}): 3595, 3064, 2550, 2108, 1457, 1054, 1027, 738, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.29 – 3.39 (m, 1H, H- 2β), 3.41 – 3.55 (m, 2H, H-3β, H-5β), 3.55 – 3.67 (m, 2.3H, H-4β, H-6α), 3.68 – 3.87 (m, 4H, H-6β, H-3α, H-4α, H-5α), 3.94 (dd, *J*= 10.1, 5.3 Hz, 1H, H-2α), 4.40 (d, *J*= 10.2 Hz, 1H, H-1β), 4.33 – 4.95 (m, 10H, 3x C*H*$_2$-Cq α, 3x C*H*$_2$-Cq β), 5.60 (d, *J*= 5.3 Hz, 1H, H-1α), 6.81 – 7.88 (m, 33H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 64.1 (C-2α), 65.1 (C-2β), 68.4 (C-6α), 68.8 (C-6β), 71.9 (C-3α/C-3β/C-5α/C-5β), 77.6 (C-4β), 78.3 (C-4α), 79.4 (C-3α/C-3β/C-5α/C-5β), 81.9 (C-3α/C-3β/C-5α/C-5β), 85.1 (C-3α/C-3β/C-5α/C-5β), 86.0 (C-1β), 87.3 (C-1α), 127.6 – 133.7 (C-arom), 131.2 – 138.3 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{33}H_{33}N_3O_4SNa$ 590.2089, found 590.2094.

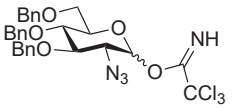
3,4,6-tri-*O*-benzyl-2-azido-2-deoxy-D-glucopyranose (25)



Compound **24** (17.9 g, 31.5 mmol; 1.0 eq.) was dissolved in acetone (650 mL, 0.05 M), followed by the addition of NBS (22.5 g, 126 mmol, 4.0 eq.). The reaction mixture was stirred for 3 hours, and after full conversion a small amount of sat. aq. $Na_2S_2O_3$ was added. The mixture was concentrated under reduced pressure and diluted in EtOAc. The organic phase was washed with sat. aq. $Na_2S_2O_3$ (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

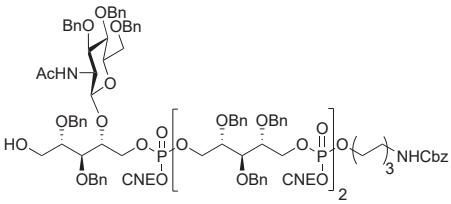
to 30% EtOAc in pentane) yielded hemiacetal **25** (9.31 g, 19.6 mmol) as an α/β mixture with a ratio of 1.3:1 in 62% yield. IR (neat, cm^{-1}): 3410, 2345, 2106, 1457, 1120, 1052, 1027, 736, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.96 (dd, *J*= 3.4, 1.3 Hz, 1H, OH), 3.33 – 3.53 (m, 4H, H-2α, H-2β, H-3β, H-5β), 3.54 – 3.74 (m, 6H, H-4α, H- 4β, H-6α, H-6β), 4.02 (dd, *J*= 10.2, 8.9 Hz, 1H, H-3α), 4.08 (ddd, *J*= 10.0, 4.4, 2.2 Hz, 1H, H-5α), 4.41 – 4.95 (m, 12, H-1β, 3x C*H*$_2$-Bn α, 3x C*H*$_2$-Bn β), 5.33 (t, *J*= 3.4 Hz, 1H, H-1α), 6.94 – 8.16 (m, 25H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 64.1 (C-2α), 68.6 (C-6α), 70.8 (C-5α), 73.6 – 75.7 (3x *C*H$_2$-Bn), 78.6 (C-4α), 80.2 (C-3α), 92.2 (C-1α), 96.3 (C-1β), 127.9 – 128.6 (C-arom), 137.8 – 137.9 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{27}H_{29}N_3O_5Na$ 498.2005, found 498.1999.

O-(3,4,6-tri-*O*-benzyl-2-azido-2-deoxy-α/β-D-glucopyranosyl) trichloroacetimidate (26)



Hemiacetal **25** (4.3 g, 9.0 mmol; 1.0 eq.) was co-evaporated with toluene (2x) under a N_2 atmosphere and dissolved in dry DCM (45 mL, 0.2 M). The mixture was cooled to 0°C and K_2CO_3 (3.7 g, 27 mmol, 3.0 eq.) and TCAN (5.4 mL, 54 mmol, 6.0 eq.) were added. The reaction mixture was stirred from 0°C to rt overnight. K_2CO_3 was filtered off after full conversion and the mixture was concentrated *in vacuo* at 30°C. Column chromatography with neutralized silica (100% pentane to 15% EtOAc in pentane) yielded title compound **26** (4.9 g, 8.0 mmol) as an α/β mixture with a ratio of 1:8.3 in 89% yield. IR (neat, cm^{-1}): 3336, 2866, 2360, 2110, 1457, 1057, 1029, 737, 697; ^{1}H NMR (400 MHz, CD_3CN) δ= 3.51 – 3.80 (m, 7H, H-2, H-3, H-4, H-5β, H-6), 3.88 – 4.00 (0.13 H, H-5α), 4.35 – 5.17 (m, 7H, 3x C*H*$_2$-Bn), 5.69 (d, *J*= 7.6 Hz, 1H, H-1β), 6.37 (d, *J*= 3.4 Hz, 0.13H, H-1α), 7.06 – 7.57 (m, 19H, H-arom), 9.07 (s, 0.13H, NH-α), 9.17 (s, 1H, NH-β); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 66.6 (C-2β), 69.2 (C-6β), 73.8 – 76.1 (3x *C*H$_2$-Bn), 76.6 (C-5β), 78.4 (C-4β), 83.6 (C-3β), 95.6 (C-1α), 97.3 (C-1β), 128.7 – 129.4 (C-arom), 139.2 (Cq-arom), 161.1 (NH=*Cq*); HRMS: $[M+Na]^+$ calcd for $C_{27}H_{29}N_3O_5Na$ (hydrolysed form of compound **26**) 498.2005, found 498.2000.

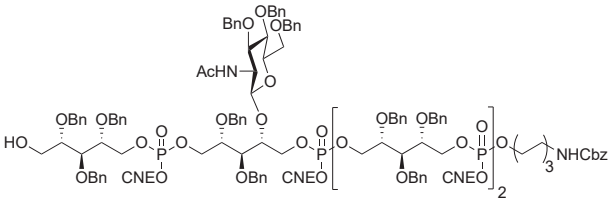
Trimer (46)



According to the general procedure above, alcohol **45** (276 mg; 0.208 mmol; 1.0 eq.) was coupled with phosphoramidite **20** (431 mg; 0.305 mmol; 1.5 eq.) and the title compound was synthesized in 88% yield (432 mg; 0.184 mmol). IR (neat, cm^{-1}): 3546, 2931, 2868, 1717, 1560, 1453, 1262, 1025, 1005, 736, 697; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.22 - 1.29 (m, 4H, CH_2-hexylspacer), 1.37 - 1.44 (m, 2H, CH_2-hexylspacer), 1.53 - 1.59 (m, 2H, CH_2-hexylspacer), 1.86 (d, 3H, *J*= 4.6 Hz, CH_3-NAc), 2.50 – 2.70 (m, 6H, CH_2-

cyanoethyl), 2.89 - 2.93 (m, 1H, OH), 3.04 (q, 2H, *J*= 4.5 Hz, CH_2-N hexylspacer), 3.40 - 4.12 (m, 24H, CH_2-Rbo, CH_2-O, 3x CH_2 cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, CH-Rbo), 4.12 - 4.32 (m, 11H, 5x CH_2-Rbo, CH-Rbo/H-3/H-4/H-5), 4.40 - 4.79 (m, 23H, CH_2-Bn, H-1), 5.03 (s, 2H, CH_2-Cbz), 5.67 (bs, 1H, N*H*), 6.72 (dd, 1H, *J*= 9.1 Hz, 2.3 Hz, N*H*Ac), 7.21 - 7.36 (m, 60H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1, 20.2, 20.2, 20.2, (CH_2 cyanoethyl), 23.7 (CH_3-NAc), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 56.4 (C-2), 61.5 (CH_2-Rbo), 63.1, 63.1, 63.3, 63.4 (CH_2 cyanoethyl), 66.6 (CH_2-Cbz), 67.5, 67.8, 68.6, 68.9, 69.0, 70.0 (CH_2-Rbo, C-6), 72.6, 73.1, 74.0, 74.5, 75.3, 75.5, (CH_2-Bn), 75.6, 78.3, 78.6, 79.3, 79.4, 79.7, 80.3, 83.6, 83.8 (CH-Rbo, C-3, C-4, C-5), 101.7, 101.8 (C-1), 118.5, 118.5, 118.6, 118.7 (Cq-cyanoethyl), 128.4, 128.5, 128.5, 128.6, 128.7, 128.7, 128.8, 128.9, 128.9, 129.2, 129.2, 129.2, 129.3, 129.3, 129.3, 129.4 (CH-arom), 139.1, 139.3, 139.4, 139.6, 139.8 (Cq-arom), 171.0 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.2, 0.2, -0.1, -0.1, -0.2, -0.2; HRMS: $[M+2H]^{2+}$ calculated for $C_{123}H_{144}N_5O_{29}P_3$ 1124.4591, found 1124.4622.

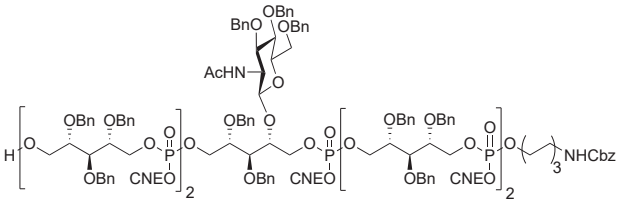
Tetramer (48)



According to the general procedure above, alcohol **46** (225 mg; 0.100 mmol; 1.0 eq.) was coupled with phosphoramidite **42** (109 mg g; 0.15 mmol; 1.5 eq.) and the title compound was synthesized in 90% yield (250 mg; 89.7 μmol). IR (neat, cm-^{1}): 3567, 2935, 2868, 1717, 1560, 1454, 1265, 1092, 1025, 1003, 734, 697; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.22 - 1.31 (m, 4H, CH_2-hexylspacer), 1.37 - 1.42 (m, 2H, CH_2-hexylspacer), 1.55 - 1.60 (m, 2H, CH_2-hexylspacer), 2.47 - 2.71 (m, 8H, 4x CH_2-cyanoethyl), 3.05 (q, 2H, *J*= 6.7 Hz, CH_2-N hexylspacer), 3.43 - 4.13 (m, 29H, CH_2-Rbo, CH_2-O, 4x CH_2-cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, 11x CH-Rbo), 4.13 - 4.45 (m, 15H, 7x CH_2-Rbo, H-1), 4.45 - 4.78 (m, 29H, 14x CH_2-Bn, H-1), 5.03 (s, 2H, CH_2-Cbz), 5.72 (t, 1H, *J*= 6.2 Hz, N*H*), 6.80 - 6.86 (m, 1H, N*H*Ac), 7.18 – 7.42 (m, 75H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.0, 20.1, 20.1, 20.1, 20.2, 20.2, 20.2 (CH_2-cyanoethyl), 23.7 (CH_3-NAc), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 56.3, 56.5 (C-2), 61.5 (CH_2-Rbo), 63.1, 63.1, 63.2, 63.2, 63.3, 63.4, 63.5, 63.5 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.5, 67.5, 67.8, 67.9, 68.2, 68.2, 68.9, 68.9, 69.0, 69.0, 70.0 (CH_2-Rbo, C-6), 72.7, 72.9, 73.0, 73.0, 73.1, 73.1, 73.2, 73.9, 74.4, 74.5, 74.5, 74.6, 75.3, 75.5, 75.5 (CH_2-Bn), 75.7, 75.7, 78.3, 78.3, 78.3, 78.4, 78.6, 78.7, 78.8, 78.9, 78.9, 79.0, 79.1, 79.2 (CH-Rbo, C-3, C-4, C-5), 80.6 (CH-Rbo), 83.5, 83.7 (CH-Rbo, C-3, C-4, C-5), 101.3, 101.4 (C-1), 118.5, 118.5, 118.6, 118.7, 118.7 (Cq-cyanoethyl), 128.4, 128.4, 128.4, 128.5, 128.5, 128.6, 128.7, 128.7, 128.7, 128.8, 128.8, 128.8, 128.9, 129.0, 129.1, 129.2, 129.2, 129.2, 129.3, 129.3, 129.4, 129.4 (CH-arom), 138.5, 139.1, 139.2, 139.3, 139.3, 139.5, 139.7, 139.8, 139.8 (Cq-arom), 157.3, 171.1 (C=O); ^{31}P NMR (162 MHz, CD_3CN)

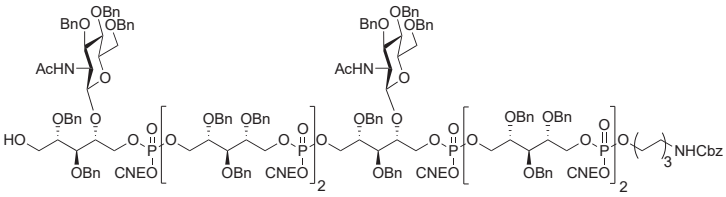
δ= 0.5, 0.3, 0.3, 0.0, 0.0, 0.0; HRMS: $[M+2H]^{2+}$ calculated for $C_{152}H_{176}N_6O_{36}P_4$ 1393.0549, found 1393.0590.

Pentamer (50)



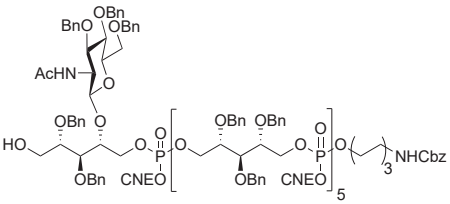
According to the general procedure above, alcohol **48** (210 mg; 75.4 μmol; 1.0 eq.) was coupled with phosphoramidite **42** (71.0 mg; 98.0 μmol; 1.3 eq.) and the title compound was synthesized in 68% yield (171 mg; 51.5 μmol). IR (neat, cm^{-1}): 3567, 2935, 2868, 1717, 1560, 1457, 1262, 1093, 1027, 1009, 747, 698; ^{1}H NMR (500 MHz, CD_3CN) δ= 1.20 - 1.31 (m, 4H, CH_2-hexylspacer), 1.38 - 1.42 (m, 2H, CH_2-hexylspacer), 1.55 - 1.60 (m, 2H, CH_2-hexylspacer), 1.84 - 1.85 (m, 3H, CH_3-NAc), 2.46 - 2.70 (m, 10H, 5x CH_2-cyanoethyl), 3.05 (q, 2H, *J*= 6.9 Hz, CH_2-N hexylspacer), 3.42 (td, 1H, *J*= 13.9 Hz, 13.3 Hz, 6.7 Hz, CH-Rbo/H-3/H-4/H-5), 3.55 (t, 1H, *J*= 9.2 Hz, CH-Rbo/H-3/H-4/H-5), 3.60 - 4.12 (m, 35H, 1x CH_2-Rbo, CH_2-O, 5x CH_2-cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, CH-Rbo), 4.17 - 4.45 (m, 19H, 9x CH_2-Rbo, C*H*H-Bn, CH-Rbo/H-3/H-4/H-5), 4.45 - 4.77 (m, 34H, 16.5x CH_2-Bn, H-1), 5.03 (s, 2H, CH_2-Cbz), 5.66 (s, 1H, N*H*), 6.74 (d, 1H, *J*= 9.3 Hz, N*H*Ac), 7.19 - 7.35 (m, 90H, H-arom); ^{13}C-APT NMR (126 MHz, CD_3CN) δ= 20.1, 20.1, 20.2, 20.2, 20.2, 20.2, 20.3 (CH_2-cyanoethyl), 23.7 (CH_3-NAc), 25.7, 26.8, 30.4, 30.8, 30.8 (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 56.4, 56.4 (C-2), 61.6 (CH_2-Rbo), 63.1, 63.1, 63.2, 63.2, 63.2, 63.3, 63.4, 63.5 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.5, 67.9, 68.2, 68.3, 68.9, 69.0, 70.1 (CH_2-Rbo, C-6), 72.8, 73.0, 73.0, 73.1, 73.1, 73.2, 73.2, 74.0, 74.5, 74.5, 74.6, 74.6, 75.3, 75.5, 75.5 (CH_2-Bn), 75.8, 75.8, 78.3, 78.3, 78.4, 78.4, 78.5, 78.6, 78.7, 78.8, 78.9, 78.9, 79.0, 79.1, 79.1, 79.1, 79.2, 79.2, 79.3, 79.3, 79.3 (CH-Rbo, C-3, C-4, C-5), 80.6, 80.6 (CH-Rbo), 83.6, 83.7 (CH-Rbo, C-3, C-4, C-5), 101.3, 101.5 (C-1), 118.4, 118.5, 118.5, 118.5, 118.6, 118.6, 118.7, 118.7 (Cq-cyanoethyl), 128.4, 128.4, 128.5, 128.5, 128.5, 128.6, 128.6, 128.7, 128.7, 128.7, 128.7, 128.8, 128.8, 128.8, 128.9, 128.9, 129.0, 129.0, 129.1, 129.2, 129.2, 129.3, 129.3, 129.4 (CH-arom), 139.1, 139.2, 139.2, 139.2, 139.3, 139.4, 139.6, 139.7, 139.8, 139.8, 139.8 (Cq-arom), 157.3, 171.0 (C=O); ^{31}P NMR (202 MHz, CD_3CN) δ= 0.5, 0.4, 0.3, 0.2, 0.1, 0.1, 0.0, 0.0; HRMS: $[M+2H]^{2+}$ calculated for $C_{181}H_{208}N_7O_{43}P_5$ 1661.6508, found 1661.6587.

Hexamer (52)



According to the general procedure above, alcohol **50** (150 mg; 45.0 μmol; 1.0 eq.) was coupled with phosphoramidite **20** (75.9. mg; 58.0 μmol; 1.3 eq.) and the title compound was synthesized in 79% yield (151 mg; 35.6 μmol). IR (neat, cm-^{1}): 3567, 2933, 2866, 1717, 1558, 1454, 1262, 1070, 1025, 1004, 737, 697; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.23 - 1.31 (m, 4H, CH_2-hexylspacer), 1.36 - 1.45 (m, 2H, CH_2-hexylspacer), 1.55 - 1.61 (m, 2H, CH_2-hexylspacer), 1.86 - 1.89 (m, 6H, CH_3-NAc), 2.47 - 2.70 (m, 12H, CH_2-cyanoethyl), 3.06 (q, 2H, *J*= 6.8 Hz, CH_2-N hexylspacer), 3.38 – 4.14 (m, 41H, 1x CH_2-Rbo, CH_2-O, 5x CH_2 cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, 17x CH-Rbo), 4.14 – 4.41 (m, 23H, 11x CH_2-Rbo, CH-Rbo/H-3/H-4/H-5), 4.43 - 4.78 (m, 46H, 22x CH_2-Bn, 2x H-1), 5.04 (s, 2H, CH_2-Cbz), 5.72 (s, 1H, NH), 6.75 - 6.79 (m, 2H, N*H*Ac), 7.21 - 7.35 (m, 115H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.0, 20.1, 20.1, 20.1, 20.2, 20.2, 20.2 (CH_2-cyanoethyl), 23.7 (CH_3-NAc), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2 hexylspacer), 41.4 (CH_2-N hexylspacer), 55.8, 56.3, 56.4 (C-2), 61.5 (CH_2-Rbo), 63.0, 63.1, 63.2, 63.4, 63.4 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.4, 67.7, 67.8, 68.5, 68.9, 69.0, 70.0 (CH_2-Rbo, C-6), 72.6, 73.0, 73.1, 73.0, 74.5, 74.5, 75.3, 75.5 (CH_2-Bn), 75.7, 77.6, 78.2, 78.3, 78.6, 79.2, 79.4, 79.7, (CH-Rbo, C-3, C-4, C-5), 80.3 (CH-Rbo), 83.5, 83.6, 83.7 (CH-Rbo, C-3, C-4, C-5), 101.3, 101.6, 101.8 (C-1), 118.4, 118.5, 118.5, 118.5, 118.6, 118.6 (Cq-cyanoethyl), 127.6, 128.4, 128.5, 128.5, 128.6, 128.7, 128.7, 128.8, 128.9, 129.2, 129.3, 129.4 (CH-arom), 138.5, 139.1, 139.1, 139.2, 139.4, 139.5, 139.6, 139.7, 139.8 (Cq-arom), 157.3, 159.4, 170.9, 171.0 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.4, 0.2, 0.2, 0.1, -0.1, -0.1, -0.2, -0.2; HRMS: $[M+2H]^{2+}$ calculated for $C_{232}H_{265}N_9O_{55}P_6$ 2122.3349, found 2122.3276.

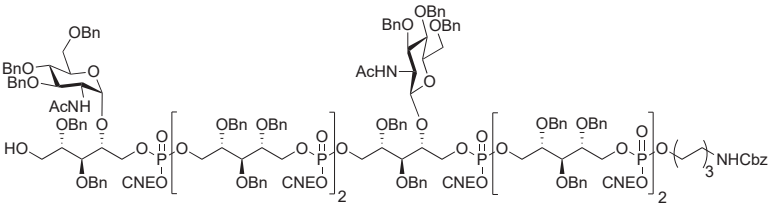
Hexamer (57)



According to the general procedure above, alcohol **56** (29.0 mg; 9.89 μmol; 1.0 eq.) was coupled with phosphoramidite **20** (16.7 mg; 12.8 μmol; 1.3 eq.) and the title compound was synthesized in 85% yield (32.3 mg; 8.36 μmol). IR (neat, cm-^{1}): 3567, 2935, 2865, 1717, 1560, 1457, 1275, 1262, 1095, 1027, 750, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.23 - 1.30 (m, 4H, CH_2-hexylspacer), 1.36 - 1.41 (m, 2H, CH_2-hexylspacer), 1.53 - 1.59 (m, 2H, CH_2-hexylspacer), 1.83 - 1.85 (m, 3H, CH_3-NAc), 2.49 - 2.69 (m, 12H, CH_2-cyanoethyl), 3.03 (m, 2H, *J*= 6.5 Hz, CH_2-N hexylspacer), 3.39 - 4.10 (CH_2-Rbo, CH_2-O, 6x

CH_2 cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, 18x CH-Rbo), 4.12 - 4.34 (m, 22H, 11x CH_2-Rbo), 4.40 - 4.77 (m, 42H, CH_2-Bn, H-1, CH-Rbo/H-3/H-4/H-5), 5.02 (s, 2H, CH_2-Cbz), 5.65 (bs, 1H, N*H*), 6.73 (d, *J*= 9.1 Hz, 1H, N*H*Ac), 7.18 – 7.35 (m, 105H); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1, 20.1, 20.2, 20.2, 20.2, 20.3 (CH_2-cyanoethyl), 23.7 (CH_3-NAc), 25.7, 26.8, 30.4, 30.8, 30.8 (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 56.3 (C-2), 61.5 (CH_2-Rbo), 63.1, 63.1, 63.3, 63.4 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.7, 67.7, 67.7, 67.7, 67.8, 67.8, 67.8, 67.8, 67.9, 68.9, 69.0, 70.0 (CH_2-Rbo, C-6), 72.6, 73.0, 73.1, 74.0, 74.5, 74.6, 75.3 (CH_2-Bn), 75.6, 78.3, 78.6, 79.3, 79.4 (CH-Rbo, C-3, C-4, C-5), 80.3 (CH-Rbo), 83.6, 83.8 (CH-Rbo, C-3, C-4, C-5), 101.7 (C-1), 118.5, 118.5 (Cq-cyanoethyl), 128.4, 128.5, 128.6, 128.7, 128.7, 128.8, 128.8, 128.9, 129.0, 129.3, 129.3, 129.3, 129.3, 129.4 (CH-arom), 139.2, 139.2, 139.5, 139.7 (Cq-arom), 171.1 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.2, 0.2, 0.2, 0.1, -0.1, -0.2, -0.2, -0.2, -0.2, -0.2; HRMS: $[M+2H]^{2+}$ calculated for $C_{210}H_{240}N_8O_{50}P_6$ 1930.7483, found 1930.7478.

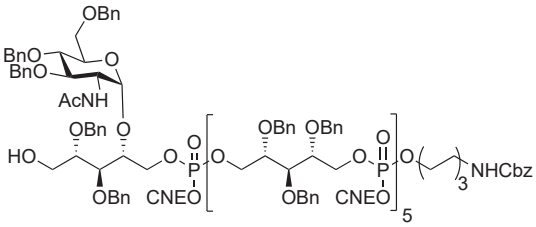
Hexamer (53)



According to the general procedure above, alcohol **50** (129 g; 39.0 μmol; 1.0 eq.) was coupled with phosphoramidite **37** (76.6 mg; 58.5 μmol; 1.5 eq.) and the title compound was synthesized in 76% yield (126 mg; 29.5 μmol). IR (neat, cm-^{1}): 2932, 2869, 1717, 1560, 1455, 1274, 1262, 1093, 1027, 1007, 748, 698; ^{1}H NMR (500 MHz, CD_3CN) δ= 1.22 - 1.29 (m, 4H, CH_2-hexylspacer), 1.36 - 1.41 (m, 2H, CH_2-hexylspacer), 1.54 - 1.59 (m, 2H, CH_2-hexylspacer), 1.72 (s, 3H, CH_3-NAc), 1.85 (m, 3H, CH_3-NAc), 2.45 - 2.58 (m, 12H, CH_2-cyanoethyl), 3.03 - 3.06 (m, 2H, CH_2-N hexylspacer), 3.41 - 4.41 (m, 68H, 12x CH_2-Rbo, CH_2-O, 6x CH_2-cyanoethyl, 2x H-2, 2x H-3, 2x H-4, 2x H-5, 4x H-6, 18x CH-Rbo), 4.42 - 4.79 (m, 45H, 22x CH_2-Bn, H-1 β), 4.99 - 5.03 (m, 3H, CH_2-Cbz, H-1 α), 5.68 (bs, 1H, N*H*), 6.40 (d, 0.6H, *J*= 9.0 Hz, N*H*Ac), 6.47 (d, 0.3 H, *J*= 9.0 Hz, N*H*Ac), 6.76 (bs, 1H, N*H*Ac), 7.21 - 7.37 (m, 115H, H-arom); ^{13}C-APT NMR (126 MHz, CD_3CN) δ= 20.1, 20.2, 20.2, 20.2, 20.3 (CH_2-cyanoethyl), 23.2, 23.7 (CH_3-NAc), 25.7, 26.8, 30.4, 30.8, 30.8 (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 54.0, 56.4 (C-2), 60.9 (CH_2-Rbo), 63.1, 63.1, 63.2, 63.2, 63.4, 63.5 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.5, 67.7, 67.8, 68.2, 68.6, 69.0, 69.0, 69.7, 70.0 (CH_2-Rbo, C-6), 72.0, 72.1 (CH-Rbo/C-3/C-4/C-5), 72.7, 72.7, 73.0, 73.1,73.1, 73.9, 74.0, 74.4, 74.5, 74.6, 74.6, 75.3, 75.5, 75.6, 75.6 (CH_2-Bn), 75.8, 76.9, 77.6, 77.6, 78.3, 78.4, 78.6, 79.2, 79.4, 79.9, 80.0, (CH-Rbo, C-3, C-4, C-5), 80.6, 81.0, 81.0 (CH-Rbo), 83.6, 83.7, (CH-Rbo, C-3, C-4, C-5), 97.3, 97.9 (C-1 α), 101.3, 101.4 (C-1 β), 118.5, 118.5, 118.6, 118.6, 118.7 (Cq-cyanoethyl), 128.4, 128.5, 128.5, 128.6, 128.6, 128.7, 128.7, 128.8, 128.8, 128.9, 128.9,

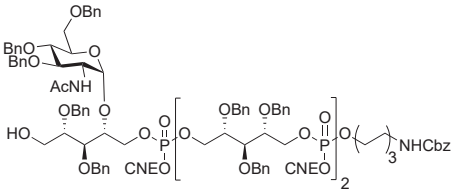
129.0, 129.0, 129.2, 129.2, 129.3, 129.3, 129.4 (CH-arom), 139.1, 139.2, 139.2, 139.3, 139.5, 139.5, 139.8, 139.9 (Cq-arom), 157.3, 170.6, 171.0 (C=O); ^{31}P NMR (202 MHz, CD_3CN) δ= 0.5, 0.5, 0.4, 0.3, 0.3, 0.2, 0.1, 0.0, 0.0, 0.0; HRMS: $[M+2H]^{2+}$ calculated for $C_{232}H_{265}N_9O_{55}P_6$ 2122.3349, found 2122.3345.

Hexamer (58)



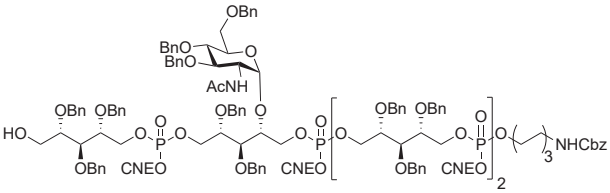
According to the general procedure above, alcohol **56** (361 mg; 0.272 mmol; 1.0 eq.) was coupled with phosphoramidite **37** (569 mg; 0.435 mmol; 1.6 eq.) and the title compound was synthesized in 53% yield (325 mg; 0.144 mmol). IR (neat, cm-^{1}): 3580, 2933, 2865, 1717, 1560, 1454, 1262, 1093, 1025, 1002, 736, 697; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.20 - 1.32 (m, 4H, CH_2-hexylspacer), 1.36 - 1.41 (m, 2H, CH_2-hexylspacer), 1.52 - 1.59 (m, 2H, CH_2-hexylspacer), 1.72 (s, 3H, CH_3-NAc), 2.38 (td, 1H, *J*= 6.0 Hz, 2.6 Hz, CH_2-cyanoethyl), 2.45 (t, 1H, *J*= 5.9 Hz, CH_2-cyanoethyl), 2.51 - 2.59 (m, 2H, CH_2-cyanoethyl), 2.60 - 2.70 (m, 2H, CH_2-cyanoethyl), 3.03 (q, 1H, *J*= 8.3 Hz, CH_2-N hexylspacer), 3.54 - 4.39 (m, 35H, 6x CH_2-Rbo, CH_2-O, 3x CH_2-cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, 9x CH-Rbo), 4.39 - 4.82 (m, 22H, CH_2-Bn), 4.99 (d, 1H, *J*= 3.6 Hz, H-1), 5.02 (s, 2H, CH_2-Cbz), 5.66 (s, 1H, N*H*), 6.43 (d, 0.5H, *J*= 8.9 Hz, N*H*Ac), 6.52 (d, 0.3H, *J*= 9.5 Hz, N*H*Ac), 7.20 - 7.37 (m, 60H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 19.9, 20.0, 20.0, 20.1, 20.1, 20.2, 20.2, 20.2 (CH_2-cyanoethyl), 23.1 (CH_3-NAc), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 54.0 (C-2), 60.8, 60.9 (CH_2-Rbo), 63.1, 63.1, 63.2, 63.2, 63.2, 63.3 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.5, 67.8, 68.6, 68.9, 69.0, 69.6, 69.7 (CH_2-Rbo, C-6), 72.0, 72.0 (CH-Rbo/C-3/C-4/C-5), 72.6, 72.7, 73.0, 73.0, 73.1, 73.1, 73.8, 74.5, 74.6, 74.6, 75.5, 75.6, 75.6 (CH_2-Bn), 76.7, 76.7, 77.4, 77.5, 78.2, 78.2, 78.3, 78.5, 78.6, 78.6, 79.2, 79.3, 79.8, 79.9, 80.9, 81.0 (CH-Rbo, C-3, C-4, C-5), 97.1, 97.8 (C-1), 118.5, 118.5, 118.5, 118.6 (Cq-cyanoethyl), 128.4, 128.5, 128.5, 128.6, 128.7, 128.7, 128.7, 128.8, 128.9, 128.9, 128.9, 129.0, 129.0, 129.1, 129.3, 129.4 (CH-arom), 139.0, 139.0, 139.0, 139.1, 139.1, 139.2, 139.4, 139.4, 139.5, 139.9 (Cq-arom), 157.3, 170.6, 170.6 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.5, 0.3, 0.0; HRMS: $[M+2H]^{2+}$ calculated for $C_{210}H_{240}N_8O_{50}P_6$ 1930.7483, found 1930.7495.

Trimer (47)



According to the general procedure above, alcohol **45** (24.0 mg; 8.17 μmol; 1 eq.) was coupled with phosphoramidite **37** (21.4 mg; 16.3 μmol; 2.0 eq.) and the title compound was synthesized in 73% yield (23.0 mg; 5.96 μmol). IR (neat, cm-^{1}): 3567, 2928, 2865, 1717, 1560, 1457, 1262, 1093, 1026, 1007, 738, 698; ^{1}H NMR (500 MHz, CD_3CN) δ= 1.25 - 1.27 (m, 4H, CH_2-hexylspacer), 1.39 (m, 2H, CH_2-hexylspacer), 1.52 - 1.58 (m, 2H, CH_2-hexylspacer), 1.71 (s, 3H, CH_3-NAc), 2.45 - 2.64 (m, 12H, CH_2-cyanoethyl), 3.02 - 3.04 (m, 2H, CH_2-N hexylspacer), 3.55 - 4.35 (m, 62H, 12x CH_2-Rbo, CH_2-O, 6x CH_2-cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, 18x CH-Rbo), 4.44 - 4.80 (m, 40H, CH_2-Bn), 4.96 - 5.02 (m, 3H, CH_2-Cbz, H-1), 5.63 (bs, 1H, N*H*), 6.35 (d, 0.4H, *J*= 8.5 Hz, N*H*Ac), 6.42 (d, 0.3H, *J*= 9.0 Hz, N*H*Ac), 7.19 - 7.36 (m, 105H, H-arom); ^{13}C-APT NMR (126 MHz, CD_3CN) δ= 20.0, 20.1, 20.2, 20.3, 20.3, (CH_2-cyanoethyl), 23.2 (CH_3-NAc), 25.8, 26.9, 30.5, 30.8, 30.8 (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 54.0 (C-2), 60.9, (CH_2-Rbo), 63.1, 63.2, 63.2, 63.3, 63.3 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.5, 67.7, 67.8, 68.6, 69.0, 69.0, 69.7 (CH_2-Rbo, C-6), 72.1 (CH-Rbo/C-3/C-4/C-5), 72.7, 73.1, 73.1, 73.2, 73.9, 74.6, 74.6, 75.5, 75.6, 75.7 (CH_2-Bn), 77.6, 77.7, 78.3, 78.4, 78.7, 78.7, 78.8, 79.4, 79.9, 80.0, 81.0, 81.0 (CH-Rbo, C-3, C-4, C-5), 97.4, 98.0 (C-1), 118.5, 118.6 (Cq-cyanoethyl), 128.5, 128.5, 128.6, 128.7, 128.8, 128.8, 128.8, 128.9, 129.0, 129.1, 129.2, 129.4, 129.4 (CH-arom), 139.2, 139.2, 139.5, 139.6, 139.9 (Cq-arom), 170.5 (C=O); ^{31}P NMR (202 MHz, CD_3CN) δ= 0.5, 0.3, 0.3, 0.0, 0.0, -0.1; HRMS: $[M+2H]^{2+}$ calculated for $C_{123}H_{144}N_5O_{29}P_3$ 1124.4591 found 1124.4624.

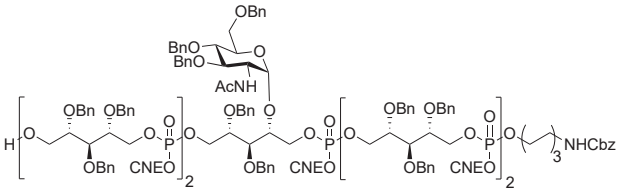
Tetramer (49)



According to the general procedure above, alcohol **47** (298 mg; 0.133 mmol; 1.0 eq.) was coupled with phosphoramidite **42** (215 mg; 0.231 mmol; 1.7 eq.) and the title compound was synthesized in 82% yield (302 mg; 0.108 mmol). IR (neat, cm-^{1}): 3587, 2935, 2866, 1717, 1560, 1457, 1262, 1095, 1026, 1009, 748, 698; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.21 - 1.30 (m, 4H, CH_2-hexylspacer), 1.38 - 1.43 (m, 2H, CH_2-hexylspacer), 1.54 - 1.61 (m, 2H, CH_2-hexylspacer), 1.74 - 1.75 (m, 3H, CH_3-NAc), 2.36 - 2.71 (m, 8H, CH_2-cyanoethyl), 3.06 (q, 2H, *J*= 6.7 Hz, CH_2-N hexylspacer), 3.64 - 4.40 (m, 44H, 8x CH_2-Rbo, CH_2-O, 4x CH_2-cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, 12x CH-Rbo), 4.40 - 4.86 (m, 28H, CH_2-Bn), 5.04 - 5.09 (m, 3H, CH_2-Cbz, H-1), 5.74 (t, 1H, *J*= 6.1 Hz, NH), 6.64 (d, 0.4H, *J*= 8.8 Hz, N*H*Ac), 6.72 (d, 0.3H, *J*= 8.8 Hz, N*H*Ac), 7.22 - 7.38 (m, 75H, H-arom); ^{13}C-APT NMR (101

MHz, CD_3CN) δ= 20.0, 20.0, 20.1, 20.1, 20.1, 20.2, 20.2, 20.2 (CH_2-cyanoethyl), 23.2 (CH_3-NAc), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 54.1 (C-2), 61.5, 61.5 (CH_2-Rbo), 63.1, 63.1, 63.2, 63.2, 63.3, 63.3 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.0, 67.1, 67.1, 67.5, 67.5, 67.7, 67.7, 67.8, 67.9, 67.9, 68.2, 68.3, 68.3, 68.4, 68.4, 68.5, 68.9, 69.0, 69.6, 69.7 (CH_2-Rbo, C-6), 72.1, 72.1 (CH-Rbo/C-3/C-4/C-5), 72.7, 72.9, 73.0, 73.1, 73.1, 73.8, 74.4, 74.5, 74.5, 74.6, 75.5, 75.6 (CH_2-Bn), 77.3, 77.9, 77.9, 78.0, 78.1, 78.1, 78.3, 78.6, 78.6, 78.8, 78.9, 79.0, 79.1, 79.4, 80.5, 80.8, 80.8 (CH-Rbo, C-3, C-4, C-5), 97.5, 97.6, 98.2, 98.2 (C-1), 118.5, 118.5, 118.6 (Cq-cyanoethyl), 128.4, 128.5, 128.5, 128.6, 128.7, 128.7, 128.8, 128.9, 129.0, 129.1, 129.3, 129.3, 129.4, 129.4 (CH-arom), 138.5, 138.8, 138.9, 139.0, 139.0, 139.1, 139.1, 139.2, 139.3, 139.4, 139.5, 139.5, 139.5, 139.7, 139.9, 139.9 (Cq-arom), 157.3, 170.7, 170.7, 170.7 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.5, 0.3, 0.3, 0.2, 0.0, 0.0, 0.0; HRMS: $[M+2H]^{2+}$ calculated for $C_{152}H_{176}N_6O_{36}P_4$ 1393.0549, found 1393.0587.

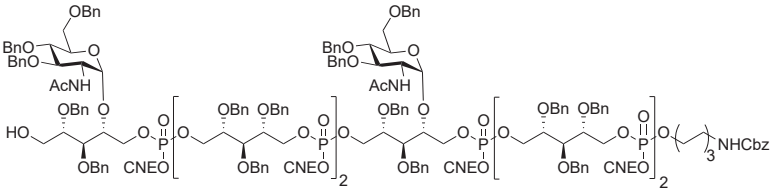
Pentamer (51)



According to the general procedure above, alcohol **49** (270 mg; 97.0 μmol; 1.0 eq.) was coupled with phosphoramidite **42** (153 mg; 0.165 mmol; 1.7 eq.) and the title compound was synthesized in 89% yield (287 mg; 86.4 μmol). IR (neat, cm^{-1}): 3562, 2931, 2865, 1717, 1560, 1457, 1274, 1262, 1096, 1027, 748, 698; ^1H NMR (400 MHz, CD_3CN) δ= 1.20 - 1.33 (m, 4H, CH_2-hexylspacer), 1.38 - 1.44 (m, 2H, CH_2-hexylspacer), 1.54 - 1.61 (m, 2H, CH_2-hexylspacer), 1.74 - 1.75 (m, 3H, CH_3-NAc), 2.33 - 2.71 (m, 10H, CH_2-cyanoethyl), 3.06 (q, 2H, *J*= 6.7 Hz, CH_2-N hexylspacer), 3.62 - 4.40 (m, 53H, 10x CH_2-Rbo, CH_2-O, 5x CH_2-cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, 15x CH-Rbo), 4.40 - 4.86 (m, 34H, CH_2-Bn), 5.03 - 5.11 (m, 3H, CH_2-Cbz, H-1), 5.74 (t, 1H, *J*= 6.0 Hz, N*H*), 6.58 - 6.67 (m, 1H, N*H*Ac), 7.21 - 7.37 (m, 90H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.0, 20.0, 20.1, 20.1, 20.2, 20.2, 20.3 (CH_2-cyanoethyl), 23.2 (CH_3-NAc), 25.7, 26.8, 30.4, 30.7, 30.8 (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 54.1 (C-2), 61.5 (CH_2-Rbo), 63.1, 63.1, 63.1, 63.2, 63.2, 63.2, 63.3, 63.3, 63.4 (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.2, 67.5, 67.7, 67.7, 67.8, 68.2, 68.2, 68.9, 69.0, 69.6 (CH_2-Rbo, C-6), 72.1, 72.1 (CH-Rbo/C-3/C-4/C-5), 72.7, 72.9, 73.0, 73.0, 73.1, 73.1, 73.9, 74.5, 74.5, 74.5, 74.6, 75.5, 75.5, 75.5, 75.6 (CH_2-Bn), 78.1, 78.2, 78.3, 78.6, 78.6, 78.6, 78.8, 78.9, 79.0, 79.1, 79.1, 79.1, 79.4, 80.5, 80.6, 80.8, 80.8 (CH-Rbo, C-3, C-4, C-5), 97.6, 97.7, 98.2, 98.3 (C-1), 118.5, 118.5, 118.6 (Cq-cyanoethyl), 128.4, 128.5, 128.5, 128.6, 128.6, 128.6, 128.7, 128.7, 128.7, 128.8, 128.9, 128.9, 129.0, 129.0, 129.2, 129.2, 129.3, 129.3, 129.3, 129.3, 129.4, 129.4, 129.4 (CH-arom), 138.5, 138.8, 138.9, 139.0, 139.1, 139.1, 139.1, 139.2, 139.2, 139.3, 139.4, 139.5, 139.5, 139.5, 139.7, 139.7, 139.9, 139.9 (Cq-arom), 157.3, 170.6, 170.6, 170.7 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.7,

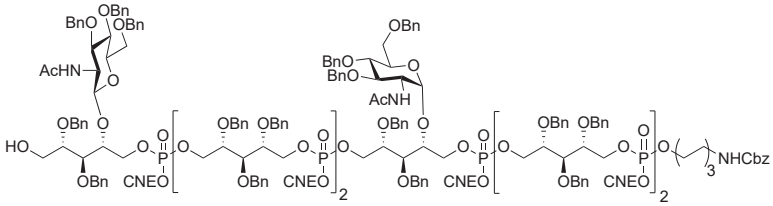
0.6, 0.6, 0.4, 0.3, 0.3, 0.3, 0.1, 0.1, 0.0, 0.0; HRMS: $[M+2H]^{2+}$ calculated for $C_{181}H_{208}N_7O_{43}P_5$ 1661.6508, found 1661.6509.

Hexamer (54)



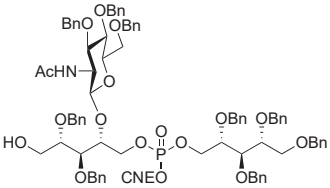
According to the general procedure above, alcohol **51** (257 mg; 77.0 μmol; 1.0 eq.) was coupled with phosphoramidite **37** (182 mg; 0.139 mmol; 1.8 eq.) and the title compound was synthesized in 88% yield (290 mg; 68.0 μmol). IR (neat, cm^{-1}): 3553, 2931, 2866, 1717, 1560, 1454, 1264, 1093, 1070, 1024, 1005, 737, 697; ^{1}H NMR (500 MHz, CD_3CN) δ= 1.27 (m, 4H, CH_2-hexylspacer), 1.41 - 1.42 (m, 2H, CH_2-hexylspacer), 1.58 - 1.59 (m, 2H, CH_2-hexyl-spacer), 1.74 (s, 3H, CH_3-NAc), 1.75 (3H, CH_3-NAc), 2.41 - 2.68 (m, 12H, CH_2-cyanoethyl), 3.06 - 3.07 (m, 2H, CH_2-N hexylspacer), 3.60 - 4.37 (m, 68H, 12x CH_2-Rbo, CH_2-O, 6x CH_2-cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, 18x CH-Rbo), 4.42 - 4.84 (m, 44H, CH_2-Bn), 5.05 - 5.09 (m, 4H, CH_2-Cbz, 2x H-1), 5.75 (bs, 1H, N*H*), 6.47 (d, 0.6H, *J*= 9.0 Hz, N*H*Ac), 6.55 - 6.57 (m, 0.4H, N*H*Ac), 6.63 - 6.70 (m, 1H, N*H*Ac), 7.25 - 7.40 (m, 115H, H-arom); ^{13}C-APT NMR (126 MHz, CD_3CN) δ= 20.0, 20.1, 20.1, 20.1, 20.2, 20.2, 20.3 (CH_2-cyanoethyl), 23.2, 23.2 (CH_3-NAc), 25.7, 26.8, 30.4, 30.8, 30.8, (CH_2-hexylspacer), 41.4 (CH_2-N hexylspacer), 54.0, 54.1 (C-2), 60.9, 60.9 (CH_2-Rbo), 63.1, 63.1, 63.2, 63.3, 63.3, 63.4, (CH_2-cyanoethyl), 66.6 (CH_2-Cbz), 67.2, 67.5, 67.8, 68.2, 68.6, 69.0, 69.0, 69.7 (CH_2-Rbo, C-6), 72.0, 72.1, 72.1, 72.1 (CH-Rbo/C-3/C-4/C-5), 72.7, 72.7, 73.0,73.1, 73.1, 73.1, 73.9, 73.9, 75.5, 75.6, 75.6, 75.5, 75.6, 75.6 (CH_2-Bn), 76.8, 76.8, 77.3, 77.5, 77.6, 78.0, 78.2, 78.2, 78.3, 78.4, 78.6, 78.6, 78.8, 79.4, 79.8, 80.0, 80.1, 80.9, 81.0, 81.0 (CH-Rbo, C-3, C-4, C-5), 97.2, 97.7, 97.9, 98.3 (C-1), 118.5, 118.5, 118.6 (Cq-cyanoethyl), 128.5, 128.5, 128.5, 128.6, 128.7, 128.8, 128.8, 128.8, 128.9, 128.9, 128.9, 129.0, 129.0, 129.1, 129.3, 129.4, 129.4, 138.5, 138.8, 138.9, 139.0, 139.1, 139.2, 139.2, 139.4, 139.4, 139.5, 139.5, 139.9 (Cq-arom), 157.3, 170.6, 170.6, 170.6, 170.7 (C=O); ^{31}P NMR (202 MHz, CD_3CN) δ= 0.7, 0.6, 0.6, 0.6, 0.4, 0.4, 0.3, 0.3, 0.1, 0.0, 0.0; HRMS: $[M+3H]^{3+}$ calculated for $C_{232}H_{266}N_9O_{55}P_6$ 1415.22569, found 1415.22566.

Hexamer (55)



According to the general procedure above, alcohol **51** (25.0 mg; 7.5 μmol; 1.0 eq.) was coupled with phosphoramidite **20** (21.2 mg; 15.0 μmol; 2.0 eq.) and the title compound was synthesized in 61% yield (19.6 mg; 4.6 μmol). IR (neat, cm-^{1}): 3567, 2926, 2858, 1717, 1560, 1457, 1266, 1095, 1027, 1009, 747, 698; ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.26 - 1.28 (m, 4H, CH_2-hexylspacer), 1.44 (m, 2H, CH_2-hexylspacer), 1.57 - 1.60 (m, 8H, CH_2-hexylspacer, CH_3-NAc), 2.26 - 2.57 (m, 12H, CH_2-cyanoethyl), 3.13 - 3.14 (m, 2H, CH_2-N hexylspacer), 3.43 – 4.35 (m, 68H, 12x CH_2-Rbo, CH_2-O, 6x CH_2-cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6, 18x CH-Rbo), 4.35 – 4.85 (m, 45H, CH_2-Bn, H-1), 5.05 – 5.15 (m, 3H, CH_2-Cbz, H-1), 5.69 (m, 1H, N*H*), 5.82 - 5.83 (m, 0.3H, N*H*Ac), 5.95 - 6.00 (m, 0.1H, N*H*Ac), 6.06 - 6.09 (m, 0.2H, N*H*Ac), 6.50 - 6.53 (m, 0.2H, N*H*Ac), 7.12 - 7.35 (m, 115H, H-arom); ^{13}C-APT NMR (126 MHz, $CDCl_3$) δ= 19.2, 19.3, 19.3, 19.3, 19.5, 19.5, 19.6, 19.6 (CH_2-cyanoethyl), 23.0, 23.0 (CH_3-NAc), 25.1, 26.2, 29.8, 29.9, 30.1, 30.2 (CH_2-hexylspacer), 41.0 (CH_2-N hexylspacer), 53.1, 53.1, 53.2, 53.6 (C-2), 61.2, 61.2 (CH_2-Rbo), 61.8, 61.8, 61.8, 61.9, 62.0, 62.0, 62.0, 62.1 (CH_2-cyanoethyl), 66.7 (CH_2-Cbz), 66.8, 66.9, 67.0, 67.1, 67.1, 67.2, 67.3, 67.4, 67.4, 67.5, 67.6, 68.3, 68.4, 68.4 (CH_2-Rbo, C-6), 71.5, 71.6 (CH-Rbo/C-3/C-4/C-5), 72.2, 72.4, 72.5, 72.5, 72.6, 72.6, 72.7, 73.5, 73.6, 73.9, 73.9, 73.9, 74.0, 74.1, 74.1, 74.6, 74.7, 74.9, 75.1, 75.3 (CH_2-Bn), 77.3, 77.5, 77.6, 77.7, 77.7, 77.8, 77.9, 78.0, 78.1, 78.2, 78.2, 78.9, 78.9, 79.6, 79.7 (CH-Rbo, C-3, C-4, C-5), 98.2, 98.2 (C-1 α), 100.8, 101.1 (C-1 β), 116.5, 116.6, 116.7, 116.7, 116.9, 116.9, 116.9 (Cq-cyanoethyl), 127.8, 127.9, 128.0, 128.0, 128.0, 128.1, 128.2, 128.2, 128.3, 128.6, 128.6, 128.6, 128.7 (CH-arom), 136.8, 137.4, 137.5, 137.6, 137.7, 137.8, 137.9, 137.9, 137.9, 138.0, 138.1, 138.2, 138.3, 138.6 (Cq-arom), 156.5, 170.2, 170.2, 170.3 (C=O); ^{31}P NMR (202 MHz, $CDCl_3$) δ= 0.1, -0.1, -0.3, -0.3, -0.3, -0.4, -0.7, -0.7, -0.8, -0.8, -0.9, -0.9, -1.0, -1.4.

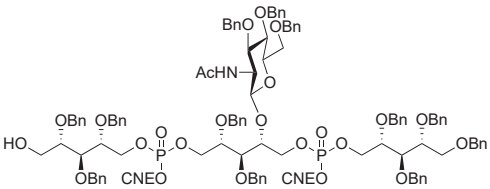
Dimer (59)



According to the general procedure above, alcohol **19** (179 mg; 0.162 mmol; 1.0 eq.) was coupled with phosphoramidite **41** (162 mg; 0.227 mmol; 1.4 eq.) and the title compound was synthesized in 46% yield (107 mg; 74.6 μmol). IR (neat, cm-^{1}): 3567, 2919, 2866, 1717, 1560, 1454, 1266, 1069, 1027, 737, 697; ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.90 (d, 3H, *J*= 2.5 Hz, CH_3-NAc), 2.26 - 2.30 (m, 2H, CH_2-cyanoethyl),

3.40 – 3.53 (m, 3H, C*H*H-Rbo, H-3/H-4/H-5/CH-Rbo), 3.56 - 3.76 (m, 6H, C*H*H-Rbo, CH_2-Rbo, 2x H-6, H-3/H-4/H-5/CH-Rbo), 3.76 - 3.97 (m, 10H, CH_2-cyanoethyl, H-2, CH-Rbo, H-3/H-4/H-5), 4.04 - 4.38 (m, 6H, CH_2-Rbo, H-3/H-4/H-5/CH-Rbo), 4.41 - 4.78 (m, 19H, CH_2-Bn, H-1), 6.61 (d, 0.7H, *J*= 8.6 Hz, N*H*Ac), 6.65 6.61 (d, 0.5H, *J*= 8.6 Hz, N*H*Ac), 7.10 - 7.35 (m, 45H, H-arom); ^{13}C-APT NMR (126 MHz, $CDCl_3$) δ= 19.2, 19.3, 19.3 (CH_2-cyanoethyl), 23.5, 23.5 (CH_3-NAc), 55.9, 56.1 (C-2), 61.3, 61.9, 61.9, 61.9, 62.0 (C-6, CH_2-cyanoethyl), 67.8, 67.8, 68.2, 68.2, 68.4, 68.4, 69.1, 69.1, 69.4, 69.5 (CH_2-Rbo), 72.1, 72.4, 72.5, 72.5, 72.6, 73.4, 73.5, 73.5, 73.8, 73.8, 73.9 (CH_2-Bn), 74.5, 74.6 (CH-Rbo/C-3/C-4/C-5), 74.8, 74.8, 75.0 (CH_2-Bn), 77.0, 77.0, 77.2 (CH-Rbo/C-3/C-4/C-5), 77.9, 78.0, 78.0, 78.1, 78.1, 78.1, 78.2, 78.2, 78.3, 78.9, 79.1, 79.2, 82.8, 82.9 (CH-Rbo/C-3/C-4/C-5), 100.8, 101.0 (C-1), 116.6, 116.7 (Cq-cyanoethyl), 127.6, 127.6, 127.7, 127.7, 127.7, 127.8, 127.8, 127.8, 127.8, 127.9, 127.9, 127.9, 128.0, 128.0, 128.0, 128.1, 128.1, 128.2, 128.4, 128.4, 128.4, 128.5, 128.5, 128.5 (CH-arom), 137.6, 137.6, 137.9, 137.9, 138.0, 138.0, 138.1, 138.1, 138.2, 138.2, 138.3, 138.3, 138.4, 138.4, 138.5, 138.6 (Cq-arom), 171.0, 171.0 (C=O); ^{31}P NMR (202 MHz, $CDCl_3$) δ= -0.8, -1.4; HRMS: $[M+H]^+$ calculated for $C_{84}H_{94}N_2O_{17}P$ 1433.62846, found 1433.62819.

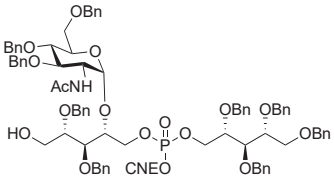
Trimer (60)



According to the general procedure above, alcohol **59** (88.4 mg; 61.7 μmol; 1.0 eq.) was coupled with phosphoramidite **42** (85.7 mg; 92.6 μmol; 1.5 eq.) and the title compound was synthesized in 91% yield (111 mg; 56.3 μmol). IR (neat, cm-^{1}): 3567, 2919, 2865, 1684, 1560, 1507, 1457, 1261, 1093, 1029, 750, 698; ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.87 (d, 3H, *J*= 2.2 Hz, CH_3-NAc), 2.18 - 2.31 (m, 4H, CH_2-cyanoethyl), 3.47 - 4.37 (m, 32H, 6x CH_2-Rbo, 9x CH-Rbo, 2x CH_2-cyanoethyl, H-2, H-3, H-4, H-5, H-6′, H6″), 4.39 - 4.77 (m, 25H, CH_2-Bn, H-2), 6.67 (dd, 1H, *J*= 17.3, 8.8 Hz, N*H*Ac), 7.12 – 7.34 (m, 60H, H-arom); ^{13}C-APT NMR (126 MHz, $CDCl_3$) δ= 19.1, 19.1, 19.1, 19.2, 19.2, 19.3, 19.3 (CH_2-cyanoethyl), 23.4 (CH_3-NAc), 55.8, 55.9, 56.0 (C-2), 61.1, 61.2, 61.7, 61.8, 61.8, 61.9, 61.9, 62.0 (C-6, CH_2-cyanoethyl), 66.8, 67.3, 67.3, 67.5, 67.5, 67.8, 67.8, 68.2, 69.2, 69.2, 69.3, 69.5 (CH_2-Rbo), 72.1, 72.4, 72.4, 72.4, 72.5, 72.5, 72.5, 72.5, 73.3, 73.4, 73.4, 73.5, 73.7, 73.8, 73.8, 73.9, 74.0, 74.8, 74.8, 74.9, 74.9, 74.9 (CH_2-Bn), 75.2, 75.2, 76.3, 76.3, 77.8, 77.9, 78.0, 78.0, 78.0, 78.0, 78.1, 78.1, 78.2, 78.2, 78.3, 78.8, 78.9, 82.7, 82.7, 83.0, (CH-Rbo/C-3/C-4/C-5), 100.0, 100.1, 100.1 (C-1), 116.6, 116.6, 116.7, 116.7, 116.8, 116.8 (Cq-cyanoethyl), 127.6, 127.6, 127.6, 127.6, 127.7, 127.7, 127.7, 127.8, 127.8, 127.8, 127.9, 127.9, 128.0, 128.0, 128.0, 128.0, 128.1, 128.1, 128.4, 128.5, 128.5, 128.7 (CH-arom), 137.8, 137.8, 137.8, 137.9, 137.9, 138.0, 138.1, 138.1, 138.1, 138.2, 138.2, 138.2, 138.3, 138.3, 138.3, 138.3, 138.3, 138.4, 138.5, 138.5 (Cq-arom),170.7, 170.8 (C=O); ^{31}P NMR (202 MHz,

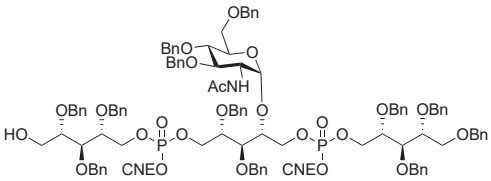
$CDCl_3$) δ= -0.2, -0.3, -0.6, -0.6, -0.7, -0.7, -1.1; HRMS: $[M+H]^+$ calculated for $C_{113}H_{126}N_3O_{24}P_2$ 1971.82346, found 1971.82551.

Dimer (61)



According to the general procedure above, alcohol **36** (528 mg; 0.476 mmol; 1.0 eq.) was coupled with phosphoramidite **41** (435 mg; 0.611 mmol; 1.3 eq.) and the title compound was synthesized in 80% yield (538 mg; 0.375 mmol). IR (neat, cm-^{1}): 3567, 2915, 2868, 1684, 1560, 1457, 1275, 1261, 1096, 1043, 1027, 747, 697; ^{1}H NMR (500 MHz, CD_3CN) δ= 1.73 (s, 3H, CH_3-NAc), 2.40 (t, 1H, *J*= 5.1 Hz, CH_2-cyanoethyl), 2.46 (t, 1H, *J*= 5.6 Hz, CH_2-cyanoethyl), 2.92 - 2.99 (m, 1H, OH), 3.56 – 3.91 (m, 12H, H-6, 2x CH_2-Rbo, H-3/H-4/H-5, CH-Rbo), 4.03 (m, 4H, CH_2-cyanoethyl, H-2, H-3/H-4/H-5/CH-Rbo), 4.18 – 4.39 (m, 5H, 2x CH_2-Rbo, H-3/H-4/H-5/CH-Rbo), 4.39 – 4.83 (m, 18H, CH_2-Bn), 5.03 (dd, *J* = 11.6, 3.6 Hz, 1H, H-1), 6.45 (dd, *J*= 33.2, 9.0 Hz, 1H, N*H*Ac), 7.21 – 7.40 (m, 45H, H-arom); ^{13}C-APT NMR (126 MHz, CD_3CN) δ= 20.0, 20.1, 20.1, 20.1 (CH_2-cyanoethyl), 23.2, 23.2 (CH_3-NAc), 54.0 (C-2), 60.9, 60.9, 63.2, 63.2, 63.2, 63.3 (C-6, CH_2-cyanoethyl), 68.0, 68.0, 68.2, 68.2, 68.5, 68.5, 68.6, 69.7, 69.7 (CH_2-Rbo), 70.6, 70.7 (CH_2-Bn), 72.0, 72.1 (C-3/C-4/C-5/CH-Rbo), 72.7, 72.7, 72.9, 72.9, 73.0, 73.8, 73.9, 74.5, 74.6, 74.6, 75.5, 75.5, 75.6, 75.6 (CH_2-Bn), 76.9, 76.9, 77.5, 77.6, 78.6, 78.7, 78.8, 78.9, 78.9, 78.9, 79.1, 79.2, 79.2, 79.4, 79.4, 79.9, 80.0, 81.0, 81.0 (CH-Rbo/C-3/C-4/C-5), 97.3, 97.9 (C-1), 118.5, 118.5 (Cq-cyanoethyl), 128.4, 128.5, 128.5, 128.5, 128.6, 128.6, 128.6, 128.6, 128.7, 128.7, 128.8, 128.8, 128.8, 128.9, 128.9, 129.0, 129.0, 129.1, 129.2, 129.3, 129.3, 129.3 (CH-arom), 139.2, 139.3, 139.5, 139.5, 139.5, 139.5, 139.5, 139.5, 139.6, 139.6, 139.6, 139.7, 139.8, 139.9 (Cq-arom), 170.6 (C=O); ^{31}P NMR (202 MHz, CD_3CN) δ= 0.5, 0.3; HRMS: $[M+H]^+$ calculated for $C_{84}H_{94}N_2O_{17}P$ 1433.62846, found 1433.62744.

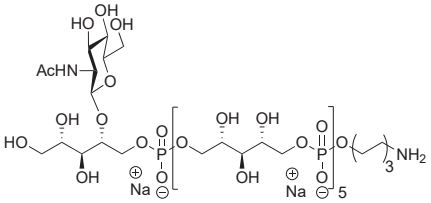
Trimer (62)



According to the general procedure above, alcohol **61** (508 mg; 0.354 mmol; 1.0 eq.) was coupled with phosphoramidite **42** (426 mg; 0.461 mmol; 1.3 eq.) and the title compound was synthesized in 91% yield (636 mg; 0.323 mmol). IR (neat, cm-^{1}): 3567, 2928, 2866, 1684, 1560, 1457, 1265, 1093, 1070, 1027, 731, 697; ^{1}H NMR (500 MHz, CD_3CN) δ= 1.71 - 1.73 (m, 3H, CH_3-NAc), 2.40 (q, 1H, *J*= 6.0 Hz, CH_2-cyanoethyl), 2.45 (q, 1H, *J*= 5.6 Hz, CH_2-cyanoethyl), 2.54 (td, 1H, *J*= 6.0 Hz, 2.4 Hz, CH_2-cyanoethyl), 2.59 (td, 1H, *J*= 6.0 Hz, 2.5 Hz, CH_2-cyanoethyl), 2.89 (bs, 1H, OH), 3.60 - 4.35 (m, 33H, 6x CH_2-Rbo, 9x CH-Rbo, 2x CH_2-cyanoethyl, H-2, H-3, H-4, H-5, 2x H-6),

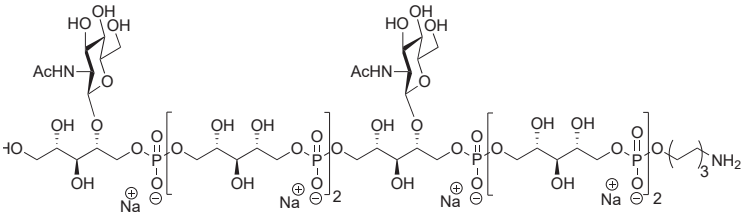
4.40 - 4.83 (m, 24H, CH_2-Bn), 5.01 – 5.07 (m, 1H, H-1), 6.56 (d, 0.5H, *J*= 8.9 Hz, N*H*Ac), 6.61 (dd, 0.5H, *J*= 8.9 Hz, 2.6 Hz, N*H*Ac), 7.19 - 7.36 (m, 60H, H-arom); ^{13}C-APT NMR (126 MHz, CD_3CN) δ= 20.0, 20.1, 20.1, 20.2 (CH_2-cyanoethyl), 23.2 (CH_3-NAc), 54.1, 54.1 (C-2), 61.6, 61.6, 63.2, 63.3, 63.3, 63.3, 63.4 (C-6, CH_2-cyanoethyl), 67.1, 67.1, 67.1, 67.1, 67.2, 67.2, 67.2, 68.1, 68.2, 68.2, 68.2, 68.4, 68.4, 68.5, 68.5, 69.7, 69.7, 70.7, 70.8, (CH_2-Rbo), 72.1, 72.2 (CH-Rbo/C-3/C-4/C-5), 72.8, 72.9, 73.0, 73.0, 73.0, 73.1, 73.1, 73.8, 73.9, 74.5, 74.5, 74.5, 74.6, 74.6, 74.6, 75.5, 75.5, 75.6, 75.6, 75.6 (CH_2-Bn), 76.7, 76.7, 76.8, 76.8, 77.4, 77.4, 77.4, 77.5, 77.9, 77.9, 78.0, 78.0, 78.1, 78.1, 78.2, 78.9, 78.9, 78.9, 79.0, 79.0, 79.1, 79.1, 79.1, 79.2, 79.2, 79.2, 79.4, 80.6, 80.8, 80.8, 80.9, 80.9 (CH-Rbo, C-3, C-4, C-5), 97.7, 97.8, 98.3, 98.3 (C-1), 118.5, 118.5 (Cq-cyanoethyl), 128.5, 128.5, 128.5, 128.6, 128.6, 128.7, 128.7, 128.8, 128.8, 128.9, 128.9, 128.9, 129.0, 129.0, 129.1, 129.3, 129.3, 129.3, 129.4, 129.4, 129.4 (CH-arom), 138.9, 138.9, 139.1, 139.1, 139.2, 139.3, 139.4, 139.4, 139.5, 139.5, 139.5, 139.5, 139.6, 139.6, 139.6, 139.7, 139.8, 139.8, 139.9, 140.0 (Cq-arom), 170.6, 170.7, 170.7 (C=O); ^{31}P NMR (202 MHz, CD_3CN) δ= 0.6, 0.5, 0.3, 0.3; HRMS: $[M+2H]^{2+}$ calculated for $C_{113}H_{127}N_3O_{24}P_2$ 986.41537, found 986.41536.

Hexamer (1)



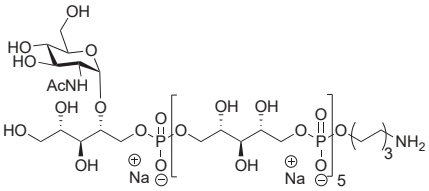
According to the general procedure described above, compound **57** (26.0 mg; 6.74 µmol) was deprotected affording the target compound in 67% yield (8.0 mg; 4.5 µmol). ^{1}H NMR (500 MHz, D_2O) δ= 1.41 - 1.42 (m, 4H, 2x CH_2-hexylspacer), 1.64 - 1.67 (m, 4H, 2x CH_2-hexylspacer), 2.08 (s, 3H, CH_3-NAc), 2.99 (t, 3H, *J*= 7.5 Hz, CH_2-N hexylspacer), 3.43 - 3.45 (m, 2H, CH-Rbo/CH-GlcNAc), 3.54 - 3.57 (m, 1H, CH-Rbo/CH-GlcNAc), 3.59-3.63 (m, 1H, C*H*H), 3.71- 3.76 (m, 2H, H-2, C*H*H), 3.78-4.14 (m, 47H, 2x C*H*H, 12x CH_2, 2x H-6, 17x CH-Rbo/CH-GlcNAc, CH_2O), 4.72 (d, 1H, *J*= 8.5 Hz, H-1); ^{13}C-APT NMR (126 MHz, D_2O) δ= 22.4 (CH_3-NAc), 24.5, 25.2, 26.7, 29.4, 29.5 (CH_2-hexylspacer), 39.5 (CH_2-N hexylspacer), 55.7 (C-2), 60.7, 62.7, 65.0, 66.2, 66.2, 66.4, 66.5, 66.5 (CH_2-Rbo, C-6, CH_2O), 70.0, 70.9, 70.9, 71.2, 71.3, 71.6, 71.7, 73.8, 75.8 (CH-Rbo/C-GlcNAc), 79.4, 79.5 (*C*-O-C-1), 101.4 (C-1), 175.1 (C=O); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 2.0, 1.8, 1.7; HRMS: $[M+H]^+$ calculated for $C_{44}H_{95}N_2O_{48}P_6$ 1605.3475, found 1605.3480.

Hexamer (2)



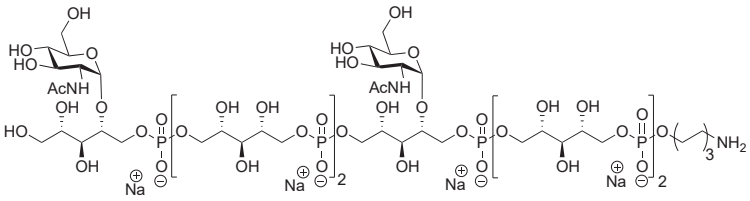
According to the general procedure described above, compound **52** (120 mg; 28.2 µmol) was deprotected affording the target compound in 78% yield (43.0 mg; 22.3 µmol).
^{1}H NMR (500 MHz, D_2O) δ= 1.39 - 1.40 (m, 4H, 2x CH_2-hexylspacer), 1.61 - 1.65 (m, 4H, 2x CH_2-hexylspacer), 2.06 (s, 6H, 2x CH_3-NAc), 2.97 (t, 2H, *J*= 7.5 Hz, CH_2-N hexylspacer), 3.41 - 3.44 (m, 4H, CH-Rbo/CH-GlcNAc), 3.51 - 3.53 (m, 2H, CH-Rbo/CH-GlcNAc), 3.56 - 3.61 (m, 1H, C*H*H), 3.69 - 4.12 (m, 53H, 12x CH-Rbo, 12x CH_2, 10x CH-GlcNAc, 4x H-6, 1x CH*H,* CH_2O), 4.70 (dd, 2H, *J*= 8.0 Hz, *J*= 4.0 Hz, H-1); ^{13}C-APT NMR (126 MHz, D_2O) δ= 22.4 (CH_3-NAc), 24.5, 25.2, 26.6, 29.4, 29.5 (CH_2-hexylspacer), 39.5 (CH_2-N hexylspacer), 55.7, 55.7 (C-2), 60.6, 60.7, 62.6, 65.0, 66.2, 66.2, 66.4, 66.4, 66.5, 66.7 (CH_2-Rbo, CH_2-O, C-6), 69.9, 70.2, 70.2, 70.9, 70.9, 71.0, 71.2, 71.6, 71.7, 73.8, 73.9, 75.8, 75.8 (CH-Rbo, C-3, C-4, C-5), 79.4, 79.5, 79.7, 79.7 (*C*-O-C-1), 101.4, 101.6 (C-1), 175.1, 175.1 (C=O); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 2.0, 1.8, 1.7; HRMS: $[M+H]^+$ calculated for $C_{52}H_{108}N_3O_{53}P_6$ 1808.42682, found 1808.42555.

Hexamer (3)



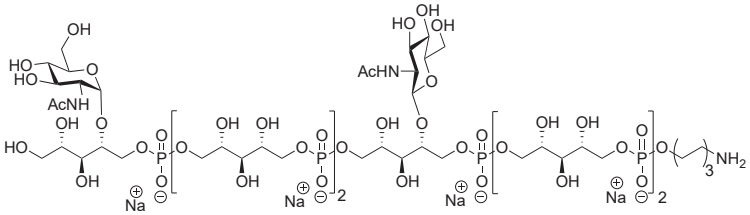
According to the general procedure described above, compound **58** (18.0 mg; 4.66 µmol) was deprotected affording the target compound in 96% yield (7.74 mg; 4.45 µmol).
^{1}H NMR (500 MHz, D_2O) δ= 1.41 - 1.43 (m, 4H, 2x CH_2-hexylspacer), 1.64 - 1.69 (m 4H, 2x CH_2-hexylspacer), 2.05 (s, 3H, CH_3-NAc), 2.99 (t, 2H, *J*= 7.5 Hz, CH_2-N hexylspacer), 3.48 (t, 1H, *J*= 9.5 Hz, CH-Rbo/CH-GlcNAc), 3.61 - 3.63 (m, 1H, C*H*H), 3.77 - 4.12 (m, 48H, 11x CH_2-Rbo, 1x CH*H*, 21x CH-Rbo/CH-GlcNAc, 2x H-6, CH_2O), 5.03 (d, 1H, *J*= 3.5 Hz, H-1); ^{13}C-APT NMR (126 MHz, D_2O) δ= 22.0 (CH_3-NAc), 24.5, 25.2, 26.7, 29.4, 29.5 (CH_2-hexylspacer), 39.5 (CH_2-N hexylspacer), 53.9 (C-2), 60.6, 62.7, 64.4, 66.2, 66.2, 66.4, 66.4, 66.5, 66.5, 66.6 (CH_2-Rbo, C-6, CH_2-O), 70.1, 70.9, 70.9, 71.1, 71.2, 71.3, 72.0 (CH-Rbo/CH-GlcNAc), 77.6, 77.6 (*C*-O-C-1), 96.4 (C-1), 174.5 (C=O); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 2.0, 1.8, 1.6; HRMS: $[M+H]^+$ calculated for $C_{44}H_{95}N_2O_{48}P_6$ 1605.34745, found 1605.34696.

Hexamer (4)



According to the general procedure described above, compound **54** (145 mg; 34.0 µmol was deprotected affording the target compound in 49% yield (34.4 mg; 16.7 µmol). ^{1}H NMR (500 MHz, D_2O) δ= 1.41 - 1.43 (m, 4H, 2x CH_2-hexylspacer), 1.64 - 1.67 (m, 4H, 2x CH_2-hexylspacer), 2.05 (s, 3H, CH_3-NAc), 2.07 (s, 3H, CH_3-NAc), 2.99 (t, 2H, *J*= 7.5 Hz, CH_2-N hexylspacer), 3.49 (t, 2H, *J*= 9.0 Hz, CH-Rbo/CH), 3.63 (q, 1H, *J*= 5.5 Hz, C*H*H-Rbo), 3.77 - 4.12 (m, 53H, CH-GlcNAc, CH-Rbo, CH_2-Rbo, CH_2O), 5.03 (d, 1H, *J*= 3.5 Hz, H-1), 5.06 (d, 1H, *J*= 3.5 Hz, H-1); ^{13}C-APT NMR (126 MHz, D_2O) δ= 22.0, 22.1 (CH_3-NAc), 24.5, 25.2, 26.6, 29.4, 29.5 (CH_2-hexylspacer), 39.5 (CH_2-N hexylspacer), 53.9 (C-2), 60.6, 62.7, 64.4, 64.5, 66.2, 66.2, 66.4, 66.4, 66.5, 66.5, 66.6 (CH_2-Rbo, CH_2-O, C-6), 69.9, 69.9, 70.1, 70.1, 70.9, 70.9, 71.1, 71.2, 71.2, 71.3, 72.0 (CH-Rbo, C-3/C-4/C-5), 77.6, 77.7, 77.8 (*C*-O-C-1 GlcNAc), 96.4, 96.5 (C-1), 174.5 (C=O); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 2.0, 1.9, 1.8, 1.6, 1.6; HRMS: $[M+2Na]^{2+}$ calculated for $C_{52}H_{107}N_3O_{53}P_6$ Na_2 926.6990, found 926.7034.

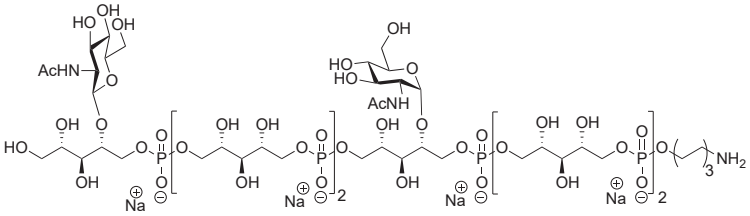
Hexamer (5)



According to the general procedure described above, compound **53** (106 mg; 25.0 µmol) was deprotected affording the target compound in 88% yield (42.5 mg; 21.9 µmol). ^{1}H NMR (500 MHz, D_2O) δ= 1.41 - 1.42 (m, 4H, 2x CH_2-hexylspacer), 1.63 - 1.68 (m, 4H, 2x CH_2-hexylspacer), 2.04 (s, 3H, CH_3-NAc), 2.08 (s, 3H, CH_3-NAc), 2.99 (t, 3H, *J*= 7.5 Hz, CH_2-N hexylspacer), 3.45 - 3.50 (m, 3H, CH-Rbo/CH-GlcNAc), 3.53 - 3.57 (m, 1H, CH-Rbo/CH-GlcNAc), 3.59 - 3.66 (m, 2H, CH_2), 3.72 - 4.15 (m, 52H, 14x CH_2, 22x CH-Rbo/CH-GlcNAc, CH_2-O), 4.73 (d, 1H, *J*= 8.0 Hz, H-1 β), 5.03 (d, 1H, *J*= 3.5 Hz, H-1 α); ^{13}C-APT NMR (126 MHz, D_2O) δ= 22.0, 22.4 (CH_3-NAc), 24.5, 25.2, 26.6, 29.4, 29.5 (CH_2-hexylspacer), 39.5 (CH_2-N hexylspacer), 53.9, 55.7 (C-2), 60.6, 60.6, 62.4, 62.7, 64.4, 64.5, 64.9, 66.2, 66.2, 66.4, 66.4, 66.5, 66.6 (CH_2-Rbo, C-6, CH_2-O), 69.9, 70.1, 70.3, 70.9, 70.9, 71.0, 71.1, 71.2, 71.3, 71.7, 72.0, 72.1, 73.9, 75.8 (CH-Rbo/CH-GlcNAc), 77.6, 77.7 (*C*-O-C-1 α), 79.7, 79.7 (*C*-O-C-1 β),

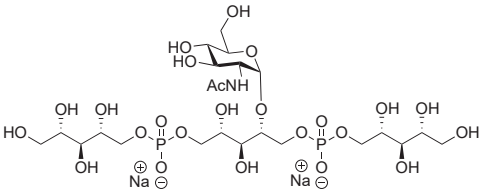
96.4 (C-1 α), 101.6 (C-1 β), 174.5, 175.1 (C=O); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 2.0, 1.8, 1.7, 1.6; HRMS: $[M+2H]^{2+}$ calculated for $C_{52}H_{109}N_3O_{53}P_6$ 904.7172, found 904.7208.

Hexamer (6)



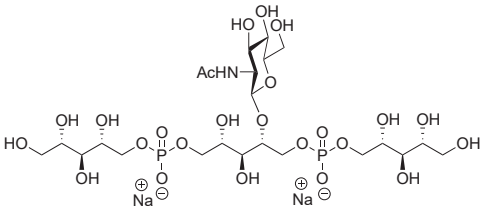
According to the general procedure described above, compound **55** (19.6 mg; 4.6 µmol) was deprotected affording the target compound in 16% yield (1.4 mg; 0.73 µmol). ^{1}H NMR Presat D_2O (500 MHz, D_2O) δ= 1.39 - 1.42 (m, 4H, CH_2-hexylspacer), 1.61 - 1.69 (m, 4H, CH_2-hexylspacer), 2.06 (d, 6H, *J*= 7.3 Hz, CH_3-NHAc), 2.96 - 3.01 (m, 2H, CH_2-N hexylspacer), 3.40 - 4.15 (m, 56H, CH_2-Rbo, CH-Rbo, 2x H-2, 2x H-3, 2x H-4, 2x H-5, 2x H-6′, 2x H-6″, CH_2-O), 4.72 (d, 0.25H, *J*= 8.6 Hz, H-1 β), 5.05 (d, 1H, *J*= 3.7 Hz, H-1 α); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 2.0, 1.9, 1.8, 1.7, 1.6; HRMS: $[M+2H]^{2+}$ calculated for $C_{52}H_{109}N_3O_{53}P_6$ 904.7171, found 904.7202.

Trimer (7)



According to the general procedure described above, compound **62** (318 mg; 0.161 mmol) was deprotected affording the target compound in 90% yield (120.3 mg; 0.145 mmol). ^{1}H NMR (500 MHz, D_2O) δ= 2.05 (s, 3H, CH_3-NAc), 3.48 (dd, 1H, *J*= 10.1 Hz, 9.0 Hz, CH-Rbo), 3.63 (dd, 2H, *J*= 11.9 Hz, 7.2 Hz, CH_2-Rbo), 3.73 (t, *J*= 6.2 Hz, 2H, CH-Rbo), 3.75 – 4.12 (m, 22H, 5x CH_2-Rbo, 6x CH-Rbo, H-2, H-3, H-4, H-5, 2x H-6), 5.04 (d, 1H, *J*= 3.7 Hz, H-1); ^{13}C-APT NMR (126 MHz, D_2O) δ= 22.0 (CH_3-NAc), 53.8 (C-2), 60.5, 62.3, 64.4, 64.4, 66.5, 66.6, 66.6 (CH_2-Rbo, C-6), 69.8, 69.9, 70.1, 70.9, 70.9, 71.0, 71.0, 71.7, 71.7, 72.0, 72.1, 72.1 (CH-Rbo, C-3, C-4, C-5), 77.7, 77.7 (*C*O-C1), 96.5 (C-1), 174.5 (C=O); ^{31}P NMR (202 MHz, D_2O) δ= 1.9, 1.6; HRMS: $[M+H]^+$ calculated for $C_{23}H_{48}NO_{24}P_2$ 784.20360, found 784.20354.

Trimer (8)



According to the general procedure described above, compound **60** (105 mg; 53.2 μmol) was deprotected affording the target compound in 85% yield (37.5 mg; 45.3 μmol). ^{1}H NMR presat D_2O (400 MHz, D_2O) δ= 2.06 (s, 3H, CH_3-NAc), 3.43 - 3.47 (m, 2H, CH-ribitol/CH-GlcNAc), 3.49 - 3.56 (m, 1H, CH-Rbo/CH-GlcNAc), 3.62 (dd, 2H, *J*= 11.6 Hz, 7.2 Hz, CH_2-Rbo), 3.70 - 4.14 (m, 24H, 5x CH_2-Rbo, 11x CH-Rbo/CH-GlcNAc), 4.71 (d, 1H, *J*= 8.4 Hz, H-1); ^{13}C-APT NMR (101 MHz, D_2O) δ= 22.4 (CH_3-NAc), 55.7 (C-2), 60.6, 62.3, 64.9, 64.9, 66.5, 66.5, 66.6, 66.7 (CH_2-ribitol/C-6), 69.8, 70.1, 70.2, 70.9, 70.9, 71.0, 71.0, 71.0, 71.7, 71.7, 72.0, 72.1, 73.9, 75.7 (CH-Rbo, C-3, C-4, C-5), 79.7, 79.7 (*C*-O-C-1), 101.6 (C-1), 175.0 (C=O); ^{31}P NMR (162 MHz, D_2O) δ= 2.0, 1.7; HRMS: $[M+H]^+$ calculated for $C_{23}H_{48}NO_{24}P_2$ 784.2036, found 784.2062.

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4

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).

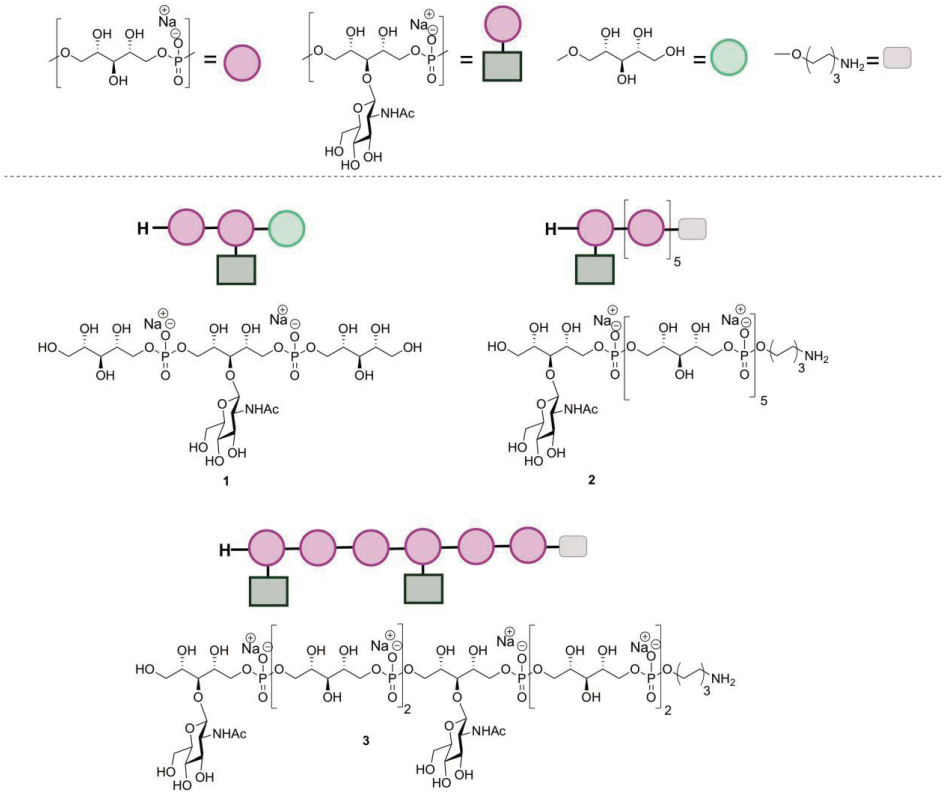


Figure 1. C-3 glycosylated compounds **1**, **2** and **3**, described in this Chapter.

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.

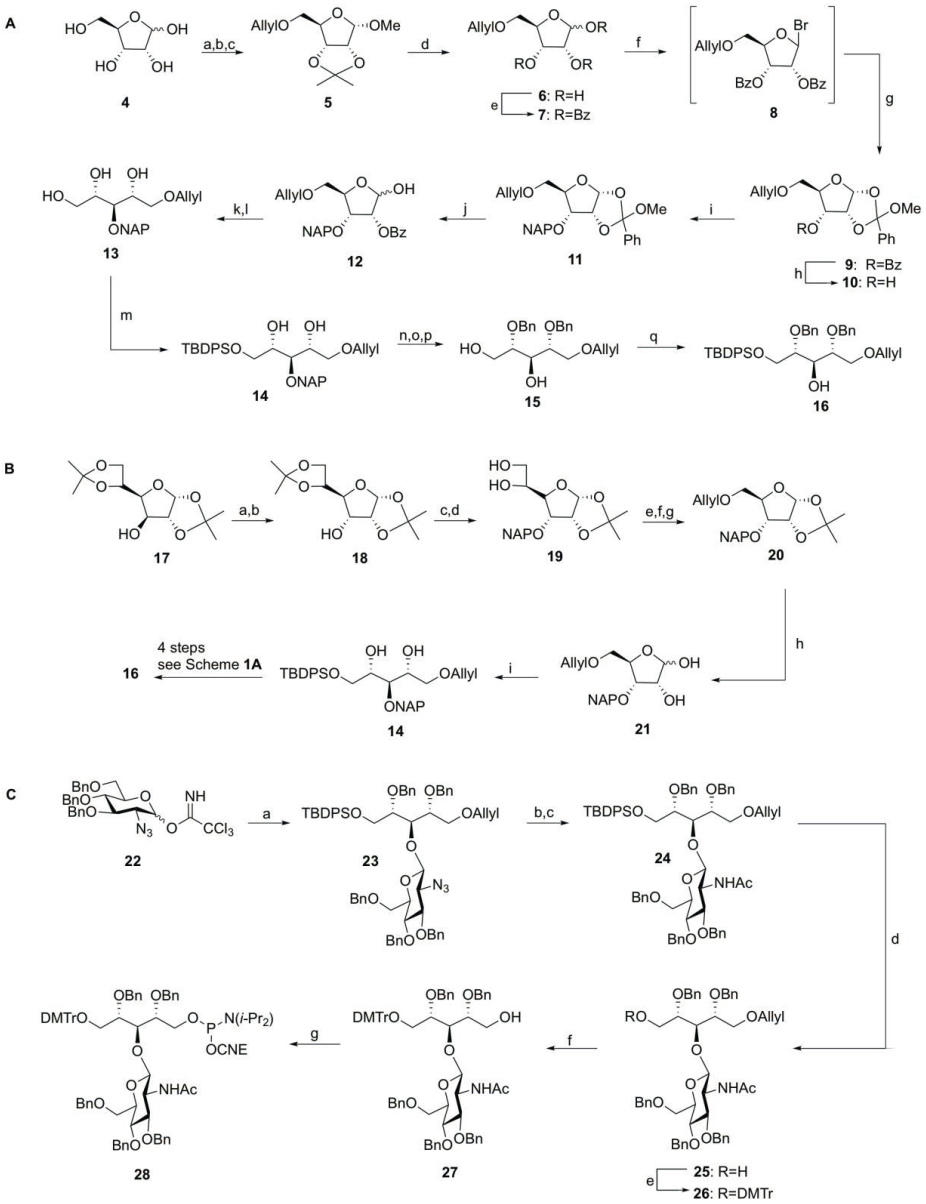
The synthesis of key phosphoramidite **28** is presented in Scheme 1C. First, ribitol alcohol **16** was glycosylated with glucosazide donor **22**, described in Chapter 3. The desired β-glycosidic bond was introduced by the use of ACN as solvent under activation of

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**.

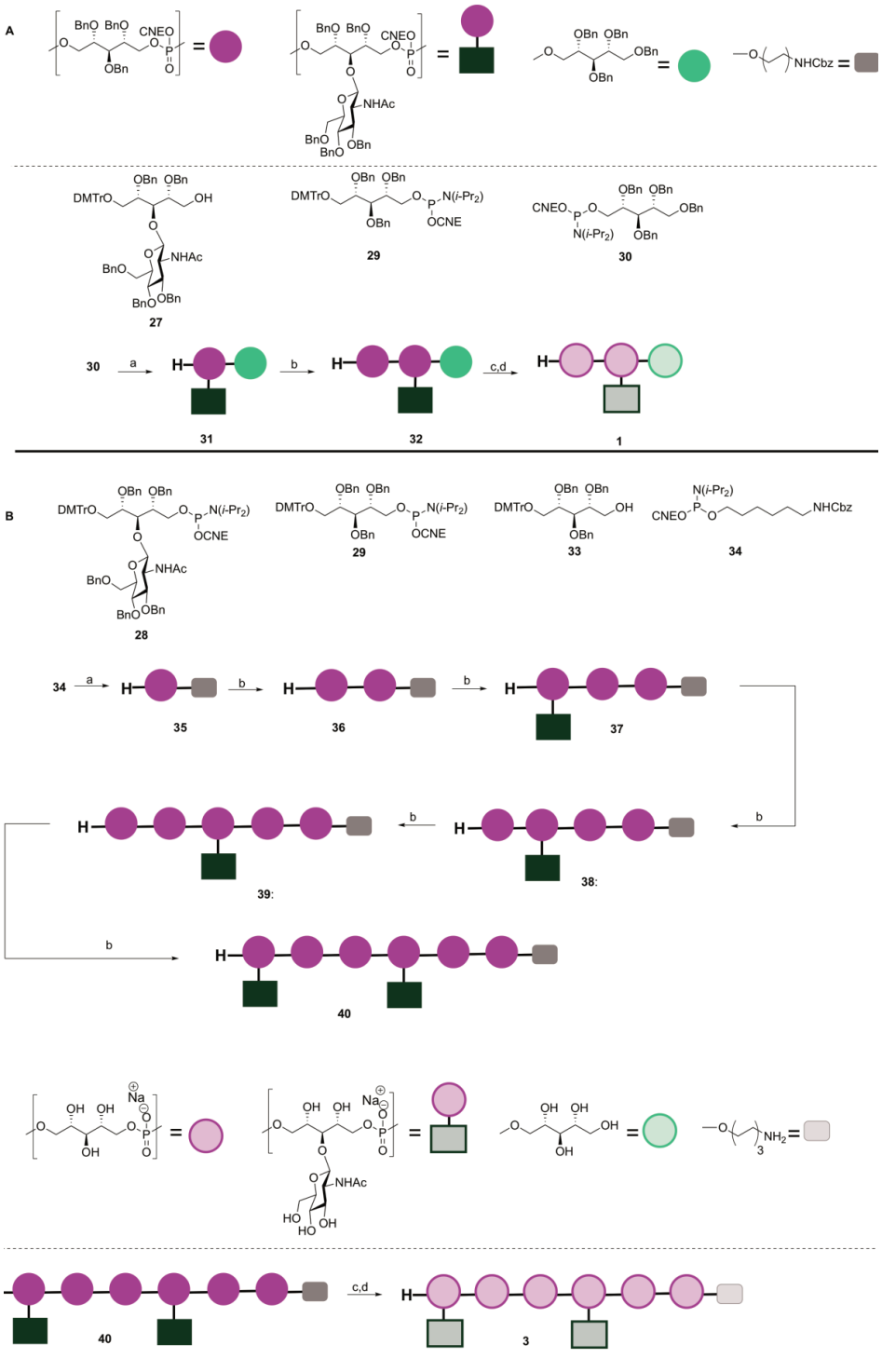
Next, the C-3-β-GlcNAc WTA fragments **1**-**3** were assembled with key amidites **28** and **29** (See Chapter 2 and 3) as schematically depicted in Scheme 2 and 3. Hexamer **2** and **3** are equipped with a chemoselective handle for conjugation purposes while trimer **1** was designed for crystallization 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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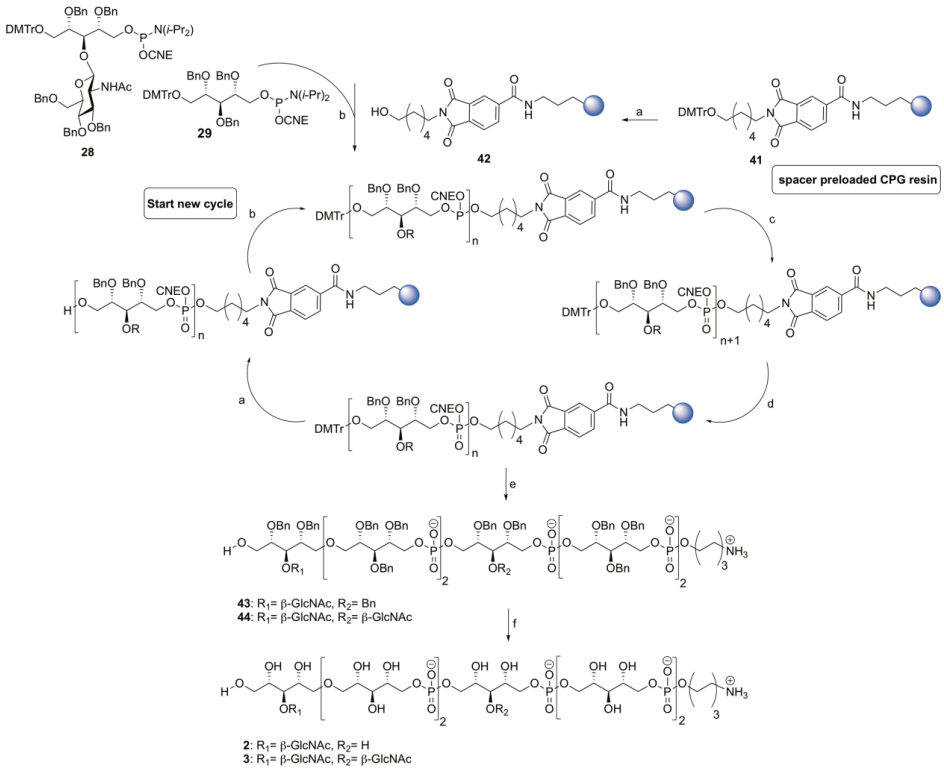
← **Scheme 2.** A. WTA assembly of glycosylated trimer **1** and glycosylated hexamers **3**; *Reagents and conditions:* a) i. DCI, ACN, **33**; ii. CSO; iii. 3% DCA in DCM, **31:** 68%; b) i. DCI, ACN, phosphoramidite **29**; ii. CSO; iii. 3% DCA in DCM, **32:** 78%; c) i. NH_3 (30-33% aqueous solution), 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]

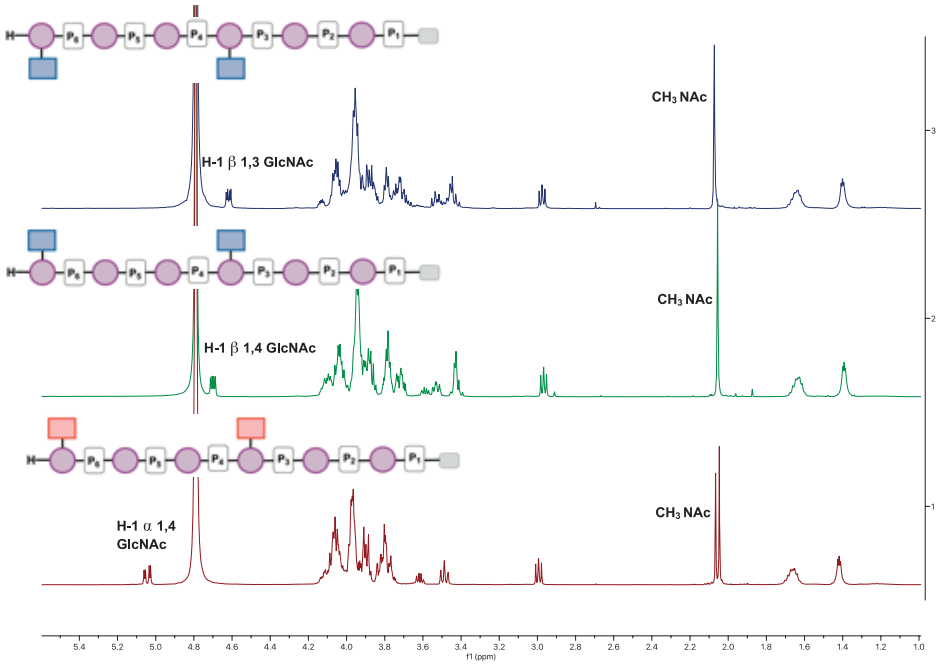


Figure 2. ^{1}H NMR spectra of the synthetic α-1,4-, β-1,4- and β-1,3 glycosylated WTAs.

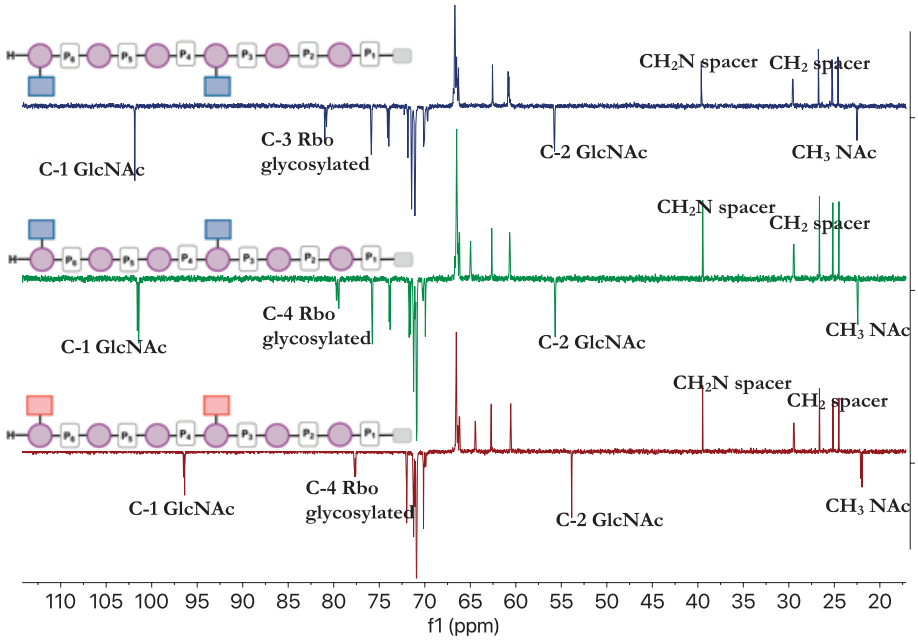


Figure 3. ^{13}C NMR spectra of the synthetic α-1,4-, β-1,4- and β-1,3 glycosylated WTAs.

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.

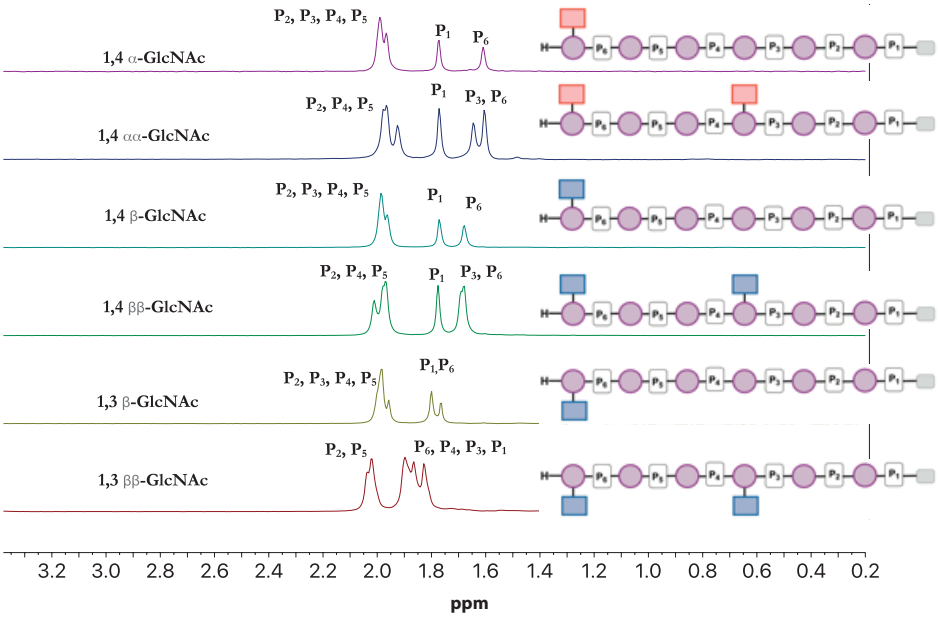
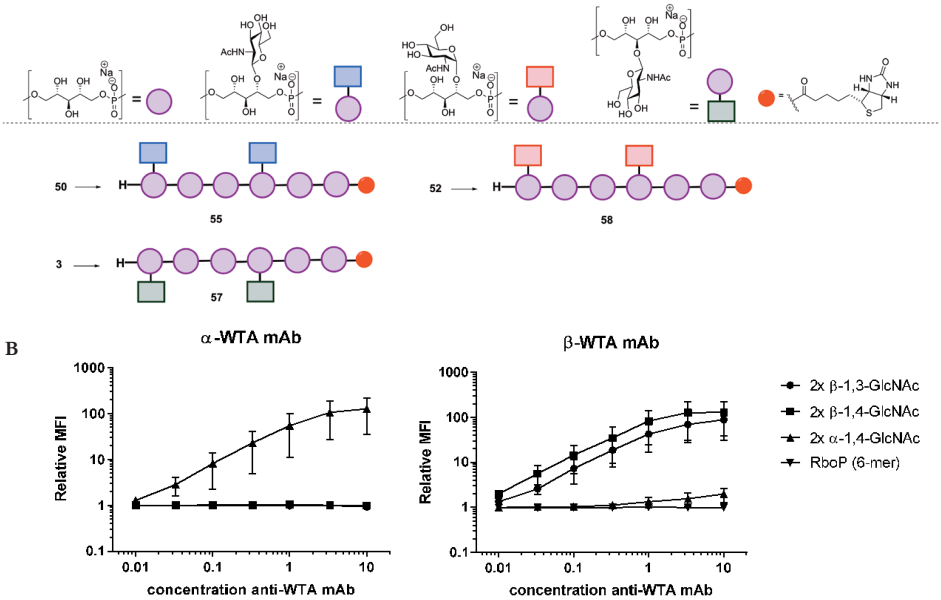


Figure 4. ^{31}P NMR spectra of the synthetic mono- and di-α-1,4-, -β-1,4- and -β-1,3 glycosylated WTAs.

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

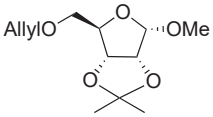
Chapter 2 described the enzymatic glycosylation using the glycosyltransferases TarS and TarM in the presence of UDP-GlcNAc and unglycosylated 6-mer and 12-mer as substrates. In order to explore if these enzymes are also able to glycosylate synthetic glycosylated WTAs, whether in α- or β conformation,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

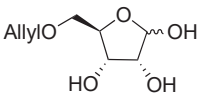
column, dimensions: 16/60 mm, eluent 0.15M NH_4OAc). After repeated co-evaporation (7-10 x) with miliQ water to remove NH_4OAc, the product was eluted through a small column containing Dowex Na^+ cation-exchange resin (type 50WX8-50-100, stored on 0.5M NaOH in H_2O, flushed with H_2O and MeOH before 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; ^{1}H 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 (O*C*H$_3$), 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.

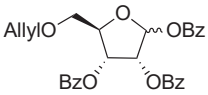
5-*O*-allyl-D-ribofuranoside (6)



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)

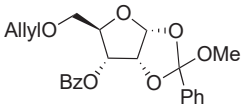
as an α:β mixture with a ratio of ±2:1 in 76% yield. IR (neat, cm^{-1}): 2943, 2360, 1654, 1507, 1049; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.49 – 3.72 (m, 2.3 H, H-5 α,β), 3.94 - 4.23 (m, 5.6H, H-2 α, H-3 α, H-4 α, H-4 β, H-2 β, H-3 β, C*H*$_2$-CH α,β), 5.11 – 5.39 (m, 3.5H, H-1 α, H-1 β, C*H*$_2$=CH), 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, C*H*$_2$-CH α), 4.06 – 4.17 (m, 2H C*H*$_2$-CH β), 4.61 – 4.80 (m, 2H, H-3α, H-3β), 5.04 – 5.45 (m, 4H, C*H*$_2$=CH α, C*H*$_2$=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.

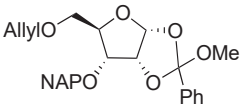
5-*O*-allyl-3-*O*-benzoyl-(1,2-*O*-methylorthobenzoyl)-α-D-ribofuranoside (9)



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

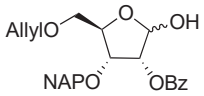
dissolved in dry DCM (12.1 mL, 0.4 M), and the mixture was cooled to 0°C. *N,N*-dimethylformamide dimethyl acetal (0.97 mL, 7.28 mmol, 1.5 eq.) was added dropwise and the reaction was stirred from 0°C to rt for 3 days. The reaction mixture was concentrated *in vacuo* at 30°C and column chromatography (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)



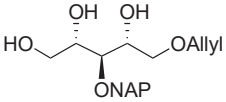
Compound **9** (13.3 g, 32.3 mmol, 1.0 eq.) was dissolved in MeOH (160 mL, 0.2 M). NaOMe (5.4 M) in MeOH (0.6 mL, 3.23 mmol, 0.1 eq.) was added dropwise, and the reaction was stirred at rt for 2 hours. The mixture was concentrated *in vacuo* and continued without purification to give the crude alcohol. 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, C*H*$_2$-CHβ, C*H*$_2$-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, C*H*$_2$=CHβ), 5.19 – 5.27 (m, 2H, C*H*$_2$=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.

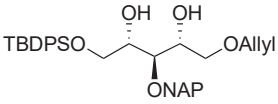
5-*O*-allyl-3-*O*-(2-naphtylmethyl)-D-ribitol (13)



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, C*H*$_2$-CH, OH), 4.77 (q, 2H, *J*= 20.0, 11.5 Hz, C*H*$_2$-Cq), 4.94 – 5.34 (m, 2H, C*H*$_2$=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),

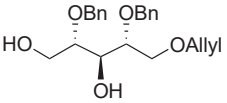
134.3 (CH_2=*C*H), 135.4 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{19}H_{24}O_5Na$ 355.1516, found 355.1526.

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 CH_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, CH_2-CH), 4.07 (p, 1H, *J*= 2.8 Hz, H-4), 4.75 (q, 2H, *J*= 32.0, 11.5 Hz, CH_2-NAP), 5.07 – 5.35 (m, 2H, CH_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-*Cq*), 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.

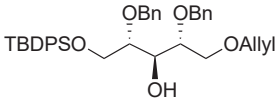
5-*O*-allyl-2,4-di-*O*-benzyl-D-ribitol (15)



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

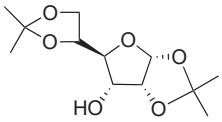
chromatography (100% pentane to 20% EtOAc in pentane) yielded the product (3.61 g) still with small traces of the byproduct. The crude product was dissolved in dry THF (35 mL, 0.17 M), followed by the addition of TBAF (1.0 M) in THF (8.84 mL, 8.84 mmol, 1.5 eq.). The reaction mixture was stirred at rt 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; ^{1}H 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 (CH_2-Bn), 71.9 (C-3) 72.1 (CH_2-Bn), 72.3 (CH_2-CH), 77.9 (C-4), 78.4 (C-2), 117.2 (CH_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; ^{1}H 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.

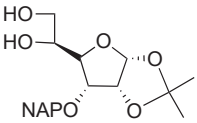
1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose (18)



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

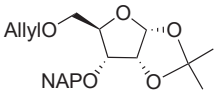
cooled to 0°C and $NaBH_4$ (26.0 g, 687 mmol, 3.4 eq.) was added portion wise. The reaction mixture was stirred from 0°C to rt overnight. The mixture was diluted with EtOAc, and washed with H_2O and brine. The water layer was extracted with EtOAc (5x), and the combined organic layers were dried over $MgSO_4$, 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; ^{1}H 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)



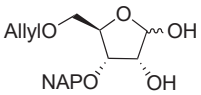
Compound **18** (36.5 g, 140 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved in a (v/v= 7:1) mixture of THF/DMF (470 mL, 0.3 M). The mixture was cooled to 0°C, and NaH (11.2 g, 260 mmol, 2.0 eq., 60% in mineral oil) and TBAI (5.2 g, 20 mmol, 0.1 eq.) were added portion wise. NAPBr (40.4 g, 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; ^{1}H 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 (CH$_2$-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, CH_3-Cq), 1.60 (s, 3H, CH_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, CH_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, CH_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 CH_3-Cq), 67.9 (C-5), 72.3 (CH_2-NAP, CH_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 (CH_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.

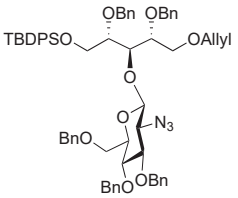
5-*O*-allyl-3-*O*-(2-naphtylmethyl)-D-ribofuranoside (21)



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 (CH_2=CH α), 117.9 (CH_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.

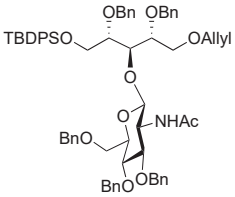
O-(3,4,6-tri-*O*-benzyl-2-azido-2-deoxy-β-D-glucopyranosyl)-(1-3)-5-*O*-allyl-2,4-di-*O*-benzyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (23)



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-*Cq*), 27.0 (3x *C*H_3-Cq), 65.8 (C-1 Rbo), 67.2 (C-2

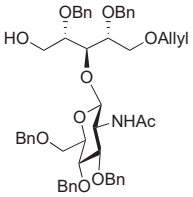
GlcNAc), 68.7 (C-6 GlcNAc), 69.8 (C-5 Rbo), 72.3 (CH_2-CH), 72.3 – 75.6 (5x CH_2-Bn), 75.2 (C-5 GlcNAc), 77.1 (C-3 Rbo), 77.9 (C-4 GlcNAc), 78.6 (C-4 Rbo), 81.4 (C-2 Rbo), 83.3 (C-3 GlcNAc), 101.1 (C-1 GlcNAc), 116.9 (CH_2=CH), 127.4 – 129.7 (C-arom), 133.6 – 133.8 (Cq-arom), 135.0 (CH_2=CH), 135.7 – 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)



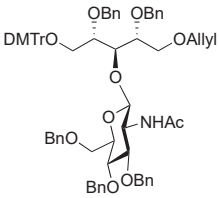
Compound **23** (3.42 g, 3.20 mmol, 1.0 eq.) was dissolved in a (v/v= 5:1) mixture of pyridine/H_2O (55 mL, 0.058 M), followed by the addition of TEA (0.2 mL). Propane dithiol (1.60 mL, 16.0 mmol, 5.0 eq.) was added, and the reaction mixture was stirred at rt overnight. The mixture was concentrated under 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)

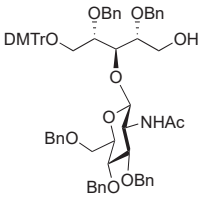


Compound **25** (2.23 g, 2.64 mmol, 1.0 eq.) was co-evaporated with toluene under a N_2 atmosphere, and dissolved in dry DCM (26.4 mL, 0.1 M). The mixture was cooled to 0°C. TEA (0.55 mL, 3.96 mmol, 1.5 eq.) and DMTrCl (1.08 g, 3.17 mmol, 1.2 eq.) were added, and the reaction mixture was stirred from 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.

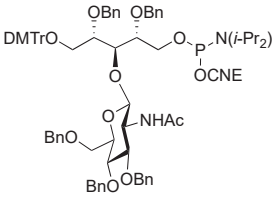
4

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)



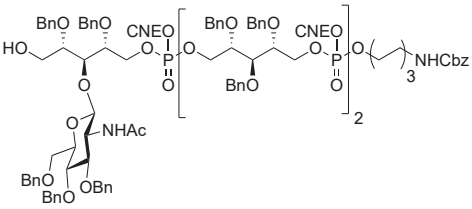
Compound **26** (1.83 g, 1.60 mmol, 1.0 eq.) was co-evaporated with distilled toluene (2x) under a N_2 atmosphere, and dissolved in freshly distilled dry THF (16 mL, 0.1 M). The mixture was degassed with N_2. Next,(1,5-Cyclooctadiene)bis(methyldiphenylphosphine) iridium(I) hexafluorophosphate (0.015 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 C*H*$_3$-CH), 1.85 (d, 3H, *J*= 9.3 Hz, C*H*$_3$-Acetyl), 2.55 (dt, 2H, *J*= 35.9, 6.0 Hz, P-O-C*H*$_2$), 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 C*H*$_3$O), 3.66 – 3.90 (m, 5H, NC-C*H*$_2$, 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 C*H*$_2$-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$_3$O), 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 (N*Cq*-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.

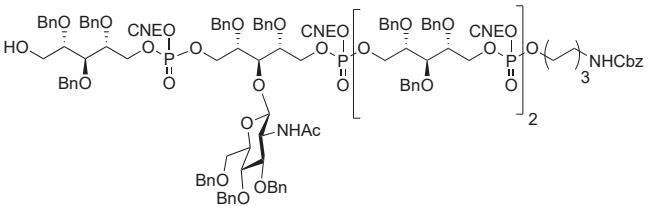
Trimer (37)



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, C*H*$_2$-3 linker and C*H*$_2$-4 linker), 1.44 (qd, 2H, *J*= 9.5, 8.8, 4.6 Hz, C*H*$_2$-2 linker), 1.60 (p, 2H, *J*= 6.7 Hz, C*H*$_2$-5 linker), 1.89 (d, 3H, *J*= 3.1 Hz, C*H*$_3$-NHAc), 2.55 – 2.78 (m, 6H, 3x NC-C*H*$_2$), 3.09 (q, 2H, *J*= 6.7 Hz, NH-C*H*$_2$-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, C*H*$_2$-6 linker, H-2 GlcNAc, 3x P-O-C*H*$_2$), 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 C*H*$_2$-Bn),

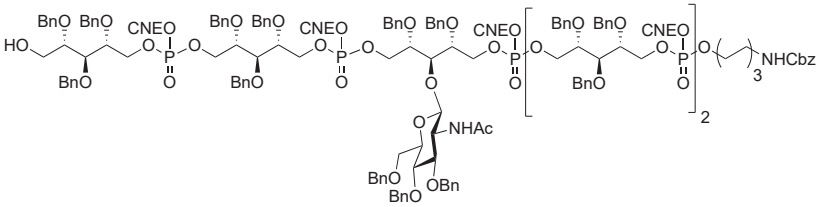
5.08 (s, 2H, CH_2-Cbz), 5.83 (t, 1H, *J*= 6.1 Hz, N*H*), 6.78 (t, 1H, *J*= 7.0 Hz, N*H*Ac), 7.03 – 7.59 (m, 60H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 20.1 – 20.3 (3x NC-*C*H_2), 23.8 (*C*H_3-NHAc), 25.8 – 26.9 (C-3 linker, C-4 linker), 30.5 (C-2 linker), 30.9 (C-5 linker), 41.5 (NH-*C*H_2 (C-1 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)



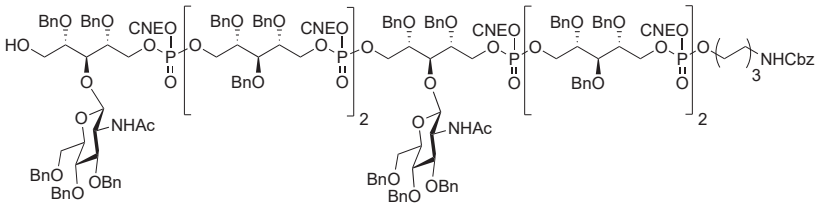
Tetramer **38** was synthesized based on the general procedure for phosphoramidite coupling (starting with 0.28 mmol (1.0 eq.) of trimer **37** and 0.42 mmol (1.5 eq.) of phosphoramidite **29.** Size exclusion column chromatography yielded title compound (0.72 g, 0.26 mmol) in 92% yield. IR (neat, cm^{-1}): 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-*C*H_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.

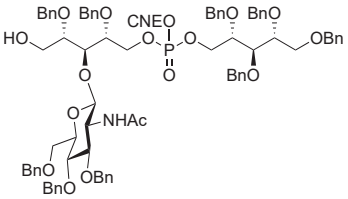
Hexamer 40



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

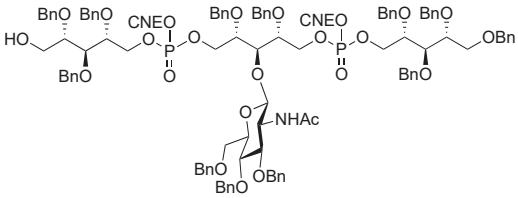
40 (0.339 g, 0.080 mmol) in a quantitative yield. IR (neat, cm^{-1}): 3546, 2909, 2314, 1717, 1550, 1506, 1457, 1266, 1027, 740, 697; ^{1}H NMR (400 MHz, CD3CN) δ= 1.20 – 1.34 (m, 4H, CH_2-3 linker, CH_2-4 linker), 1.37 – 1.47 (m, 2H, CH_2-2 linker), 1.59 (h, 2H, *J*= 6.6 Hz, CH_2-5 linker), 1.86 (d, 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-CH_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-CH_2 (C-1 linker)), 56.1 – 56.2 (2x C-2 GlcNAc), 60.6 (C-1 Rbo), 63.1 – 63.6 (6x P-O-CH_2), 66.7 (CH_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 CH_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)



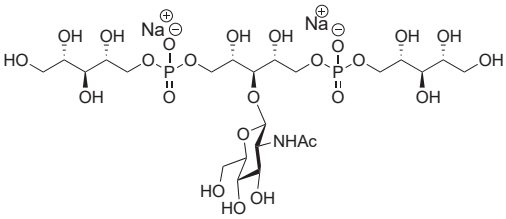
According to the general procedure for phosphoramidite coupling, alcohol **27** (602 mg; 0.54 mmol; 1.0 eq.) was coupled with phosphoramidite **30** (543 mg; 0.76 mmol; 1.4 eq.) and the title compound was synthesized in 68% yield (530 mg; 0.37 mmol). IR (neat, cm^{-1}): 3736, 2872, 2360, 1717, 1654, 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)



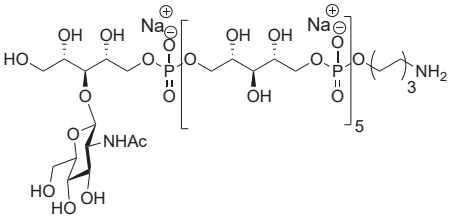
According to the general procedure for phosphoramidite coupling, alcohol **31** (530 mg; 0.37 mmol; 1.0 eq.) was coupled with phosphoramidite **29** (481 mg; 0.52 mmol; 1.4 eq.) and the title compound was synthesized in 78% yield (567 mg; 0.288 mmol). IR (neat, cm^{-1}): 3736, 2883, 2355, 1717, 1560, 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)



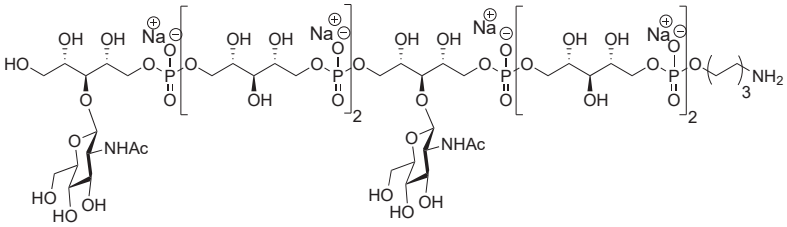
Compound **32** (0.200 g; 0.101 mmol) was deprotected according to the general procedure for global deprotection affording the target compound in 77% yield (63.8 mg; 77.1 µmol). ^{1}H NMR (400 MHz, D_2O) δ= 1.96 (s, 3H, CH_3-NAc), 3.25 - 4.08 (m, 27H, 6x CH_2-Rbo, 9x CH-Rbo, H-2, H-3, H-4, H-5, 2x H-6), 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)



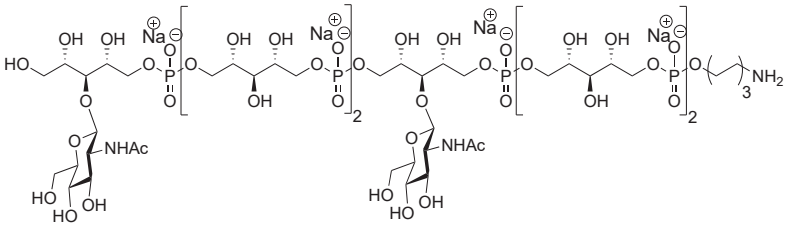
Hexamer **43** (6.9 mg, 1.94 μmol) was deprotected according to the general procedure for global deprotection yielding compound **2** (3.0 mg, 1.73 μmol) in 88% yield. ^{1}H NMR (500 MHz, D_2O) δ= 1.38 – 1.44 (m, 4H, 2x CH_2-hexylspacer), 1.66 (h, 4H, *J*= 7.4 Hz, 2x CH_2-hexylspacer), 2.08 (s, 3H, C*H*$_3$-NAc), 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.

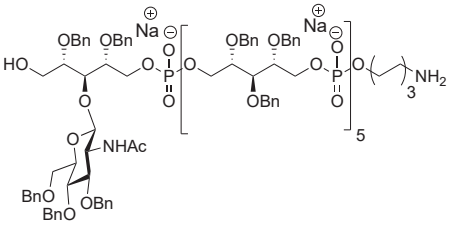
Hexamer (3)



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

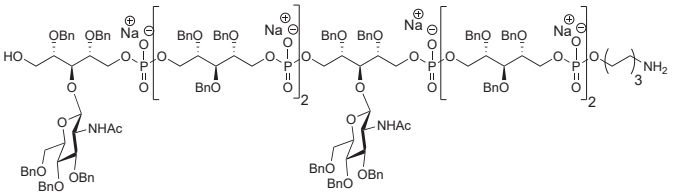
was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with MiliQ water and MeOH before use), yielding title compound **3** (0.0545 g, 0.0281 mmol) in 70% yield over 2 steps. ^{1}H NMR (500 MHz, D_2O) δ= 1.33 – 1.44 (m, 4H, 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.

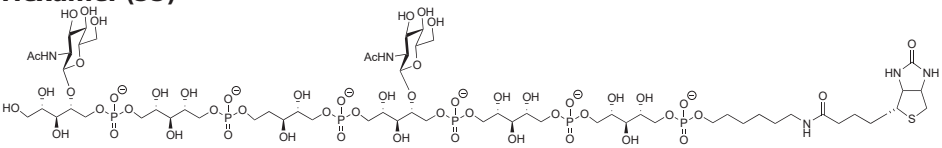
Hexamer (44)



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,

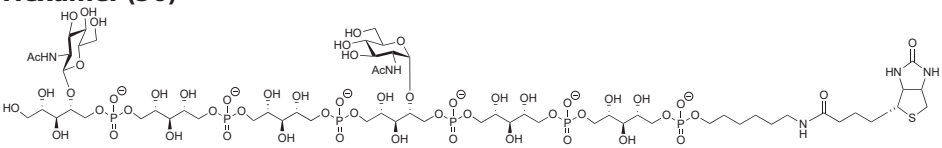
12x CH_2-Rbo, 6x H-2 Rbo, 6x H-3 Rbo, 6x H-4 Rbo, 6x H-6 Rbo, 20x C*H*$_2$-Bn), 7.10 – 7.31 (m, 101H, N*H*Ac, H-arom); ^{31}P NMR (162 MHz, CD_3CN) δ= 1.0, 1.2, 1.5, 1.6; HRMS: $[M+2H]^{2+}$ calculated for $C_{206}H_{241}N_3O_{53}P_6$ 1896.23686, found 1896.23710.

Hexamer (55)



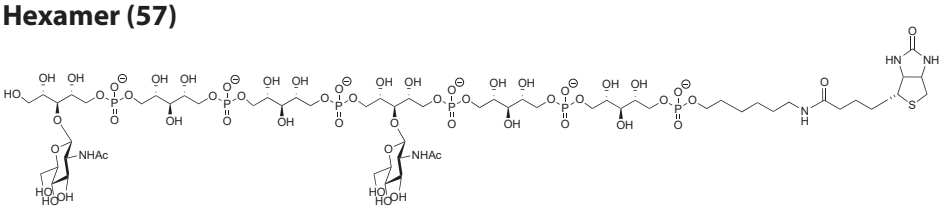
The title compound was synthesized according to the general procedure for biotinylation yielding (0.55 mg; 0.25 µmol) the product in 51% yield. ^{1}H NMR (500 MHz, D_2O) δ=1.36 – 1.42 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.50 – 1.53 (m, 2H, CH_2-hexylspacer/CH_2-biotin), 1.61 – 1.69 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 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.

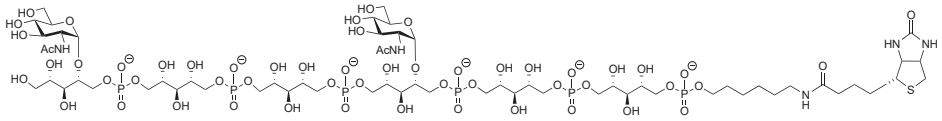
Hexamer (57)



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.

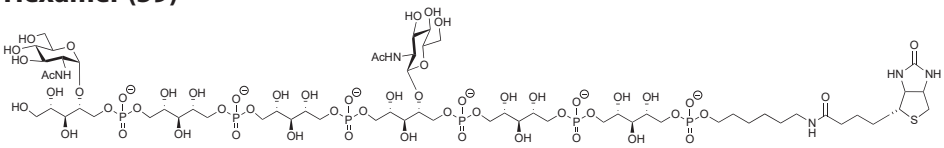
^{1}H NMR (500 MHz, D_2O) δ= 1.27 – 1.43 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.46 – 1.54 (m, 2H, 2H, CH_2-hexylspacer/CH_2-biotin), 1.53 – 1.75 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 2.07 (d, 6H, *J*= 1.0 Hz, CH_3 NAc), 2.22 (t, 2H, *J*= 7.2 Hz, CH_2-C=O), 2.74 – 2.77 (m, 1H, S-C*H*H), 2.97 (ddd, 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)



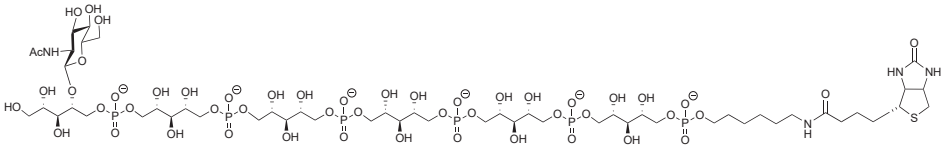
The title compound was synthesized according to the general procedure for biotinylation yielding (0.74 mg; 0.34 μmol) the product in 68% yield. ^{1}H NMR (500 MHz, D_2O) δ= 1.29 – 1.46 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.46 – 1.55 (m, 2H, CH_2-hexylspacer/CH_2-biotin), 1.55 – 1.77 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 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)



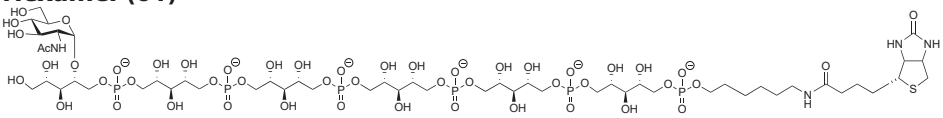
The title compound was synthesized according to the general procedure for biotinylation yielding (0.70 mg; 0.32 µmol) the product in 65% yield. ^{1}H NMR (500 MHz, D_2O) δ=1.31 – 1.44 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 1.51 (m, 2H, CH_2-hexylspacer/CH_2-biotin), 1.55 – 1.76 (m, 6H, CH_2-hexylspacer/CH_2-biotin), 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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5

A synthetic approach towards an alanylated ribitol phosphate

INTRODUCTION

Antibiotic resistance, caused by widespread use of antibiotics, leads to bacterial infections that are difficult, if not impossible, to treat and is a major worldwide health concern. Various mechanisms can be used by bacteria to counteract antibiotic action, including blockage of antibiotic entry, increasing efflux of the drugs, changing the structure of the antibiotic target, or development of antibiotic annihilating activity.[1-2] Methicillin-resistant *Staphylococcus aureus* (*MRSA)* is a multi-drug resistant pathogen, and the causative agent of a large and growing amount of hospital acquired infections. The cell wall decoration of this bacterium plays an important role in escaping our immune system and blocking antibiotic action. Wall teichoic acids (WTAs) that are covalently attached to the peptidoglycan layer represent an important component of the cell wall of Gram-positive bacteria, including *S. aureus*. WTAs are built up from repeating ribitol phosphate (RboP) units and are highly negatively charged. They are essential for viability and are involved in the control of cell shape, autolytic enzymes, and regulating the cation concentrations within the cell envelope.[3-4] The *S. aureus* ribitol phosphate WTA-backbone can carry various modifications as mentioned in previous Chapters, including *N*-acetylglucosamine (GlcNAc) moieties on the RboP C-4 in α- or β conformation or a β-GlcNAc on the C-3 position. Another important modification is the placement of D-alanine esters on the C-2 position. This modification introduces positively charged amino groups in the WTA chains, thereby altering the properties of these biopolymers.

The role of D-alanine esters has been explored by knocking out the dlt operons (DltA, DltB, DltC and DltD) involved in the introduction of the D-alanine moieties into the staphylococcal cell envelope and it was found that these mutants were more sensitive to antimicrobial peptides such as defensins and other host defense peptides.[5] It was further revealed that human α-definsin HNP1-3, which belong to the alpha defensin family of antimicrobial peptides, were able to inhibit the growth of an *S. aureus* Dlt-mutant but this effect was not found on wild type bacteria.[5] This can be explained by the fact that the D-alanine modification into the cell envelope causes a decrease of the net negative charge of the bacteria leading to the repulsion of positively charged antimicrobial peptides. It was further found that the absence of D-alanine esters in a Dlt-mutant also led to an increased susceptibility to Vancomycin and other glycopeptide antibiotics.[6] Vancomycin and teicoplanin glycopeptides are often being used as last option in the treatment of bacterial infections,[7] but clinical *S. aureus* isolates have developed reduced susceptibility towards these antibiotics.[8-9] Vancomycin-resistant *Enterococcus faecium* strains were found to bear twice the amount of D-alanine on their lipoteichoic acids as compared to non-resistant strains.[10] Overall, it is clear that D-alanine plays a role in protecting the bacteria against these antimicrobial peptides.

In order to better understand the role of the D-alanine modification at the molecular level, synthetic fragments will enable for structure-activity studies. The microheterogeneity of teichoic acids hampers the isolation of well-defined specimens and the high hydrolytic lability of the D-alanine ester can easily lead to loss of these residues during isolation from bacterial sources.[11]

Previous chapters have described the site- and stereoselective introduction of GlcNAc residues in WTA chains. This chapter focuses on the development of a synthesis route towards D-alanine-containing RboP-oligomers. To do so, heptamer **1** (Fig 1) bearing a D-alanine substituent at the third and sixth repeat was selected as a target compound. In previous chapters, GlcNAc-residues were introduced at the third and sixth residue and the generated sequences proved to be efficient tools to probe binding partners, including monoclonal antibodies and C-type lectin receptors. Target heptamer **1** contains a terminal seventh repeat to prevent the labile D-alanine ester to migrate to the primary alcohol at the terminus of the chain, after complete assembly.

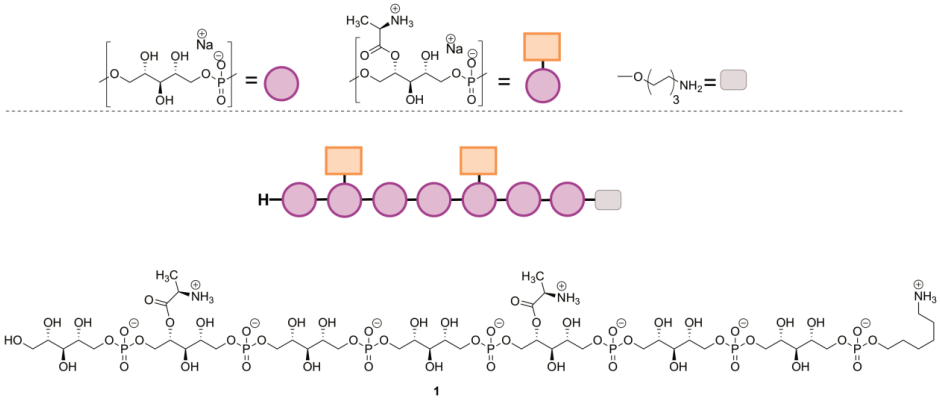


Figure 1. Target compound **1** of this chapter.

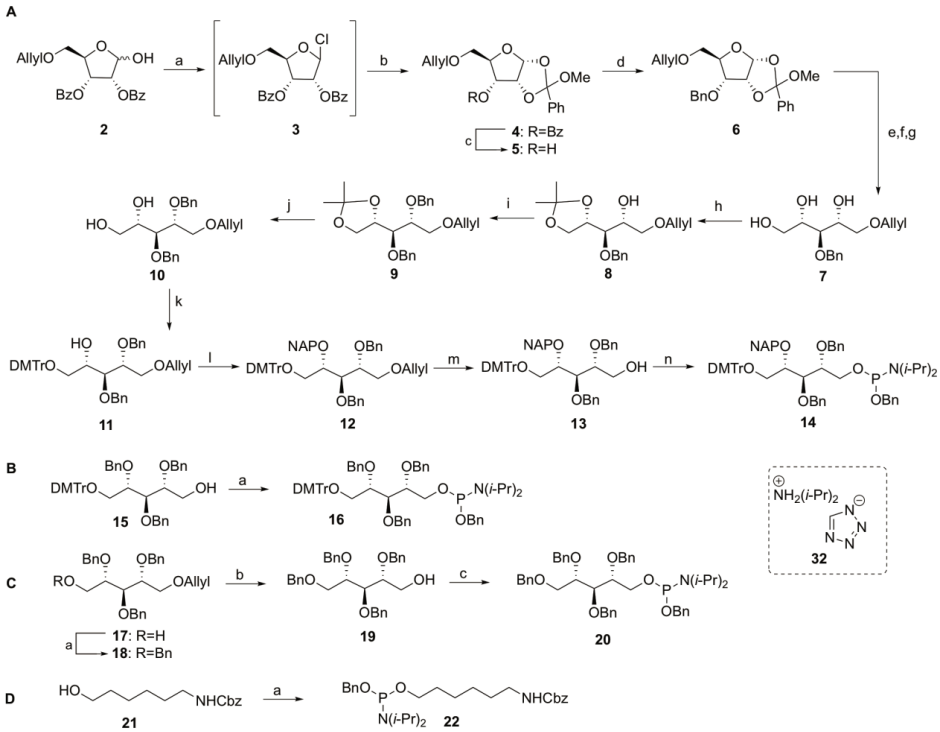
RESULTS AND DISCUSSION

Considering the base labile nature of the alanines, benzyl protected phosphoramidite building blocks were utilized instead of the commonly used cyanoethyl building blocks that require a basic deprotection step after assembly of the oligomers. The group of Schmidt has reported on the synthesis of an *S. aureus* LTA[12] fragment, composed of a glycerol phosphate hexamer, bearing four D-alanines and a GlcNAc-residue, using benzyl protected phosphoramidites. Later, a LTA fragment of *Streptococcus* species DSM 8747 was synthesized and Schmidt and co-workers also aimed to introduce four D-alanine esters in this glycerol phosphate based LTA. After global deprotection by hydrogenolysis and purification, the LTA fragment was obtained with an average of only two D-alanine esters, illustrating the challenge posed to the synthesis of these fragments by the lability of D-alanine esters.[13]

The synthesis of a *S. aureus* WTA ribitol phosphate substituted with a D-alanine ester at the C-2 of the third and sixth RboP-repeat is shown in Scheme 1. The heptamer was assembled bearing temporary naphthylmethyl (NAP) ethers on the C-2 positions, which can be selectively removed prior to the introduction of the benzylcarbamate (Cbz)-protected D-alanine moieties.

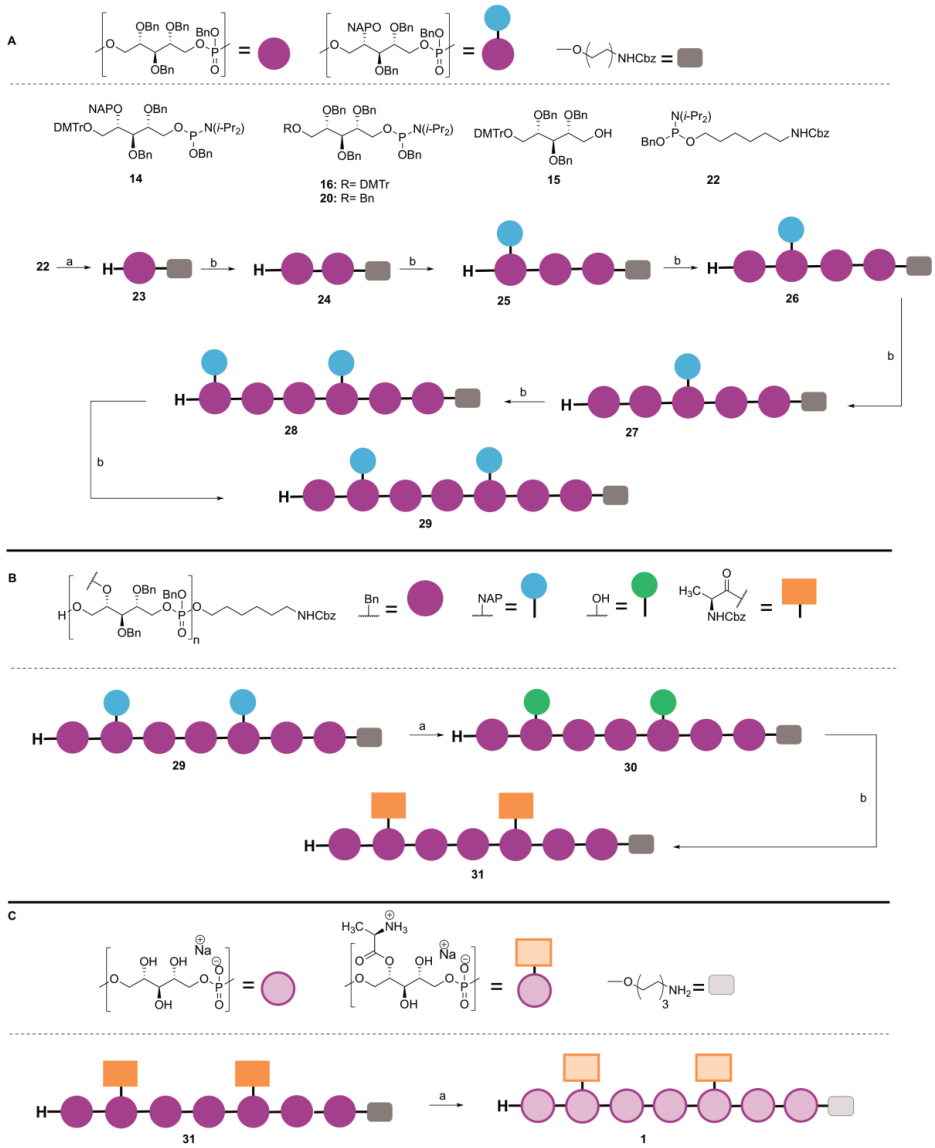
The target heptamer was assembled from the key amidite building blocks **14**, **16**, **20** and **22**, the synthesis of which is depicted in Scheme 1A-D. Starting from intermediate **2** (obtained as described in Chapter 3), the anomeric chloride **3** was obtained by treatment with dry HCl in dioxane. The chloride serves as an intermediate towards the required orthoester **4**, that will allow the regioselective modification of the C-3 OH. Therefore, chloride **3** was reacted with *N,N*-dimethylformamide dimethyl acetal, as a

source of methanol that can attack the dioxolenium ion, formed upon expulsion of the anomeric chloride by the C-2 benzoate. As mentioned in Chapter 3, the formation of the orthoester was troublesome, due to the lability of the chloride and the product, which had to be handled with care to prevent decomposition. The formed orthoester **4** was subjected to Zemplén deacetylation giving alcohol **5** in 30% yield over 3 steps. Even though the overall yield for these three steps was low, a sufficient amount of intermediate **5** was obtained to reach the final building block **14**. The free alcohol in **5** was benzylated giving compound **6** in 96% yield. Next, the orthoester was hydrolyzed using acidic conditions, ensuing removal of the resulting benzoyl group using NaOMe in MeOH and sodium borohydride mediated reduction of the lactol delivered ribitol **7** in 72% over 3 steps. Isopropylidene protection of the primary and secondary alcohols gave a mixture of products, out of which the desired product **8** could be isolated in 41% yield. Benzylation of the remaining alcohol gave **9** in quantitative yield and isopropylidene cleavage using formic acid in a mixture of THF and water then provided diol **10** in 61% yield. Installation of a DMTr group on the primary alcohol gave **11** in quantitative yield and the secondary alcohol group was protected with a temporary NAP-ether giving **12** in 85% yield. Allyl isomerization using an iridium catalyst was followed by I_2 mediated hydrolysis to give alcohol **13** in 85% yield. In the next step the benzyl phosphoramidite function was installed using BnO-P-(N-(*i*-Pr_2))$_2$ (synthesized according to the literature procedure[14]) under activation of tetrazole salt **32**[15] giving the first key amidite **14** in 71% yield. Amidite **16** was synthesized in 83% yield from alcohol **15** (Chapter 2) as shown in scheme 1B. To provide phosphoramidite **20** for the chain terminus, ribitol **17** (Chapter 2) was benzylated to give intermediate **18** in 92% yield (Scheme 1C). Allyl removal was again effected using an iridium catalyzed isomerization and iodine mediated hydrolysis to give alcohol **19**, which was converted into amidite **20** in 61% yield. Spacer **22** was finally synthesized in 71% yield from **21** as a potential handle for conjugation application (Scheme 1D).



Scheme 1. A Building block synthesis; *Reagents and conditions:* a) HCl in dioxane; b) *N,N*-dimethylformamide dimethyl acetal, DCM; c) K_2CO_3, MeOH, 30% over 3 steps; d) BnBr, NaH, THF/DMF, 0°C to rt, 96%; e) THF/H_2O/Formic acid (0.10M; v/v/v= 6/3/1), 70°C; f) NaOMe, MeOH; g) $NaBH_4$, MeOH 0°C to rt, 72% over 3 steps; h) DMP, cat. *p*TsOH, DCM, 0°C, 41% yield; i) BnBr, NaH, THF/DMF, 0°C to rt, quantitative; j) formic acid/ H_2O/THF (0.10M; v/v/v= 6/2/2) rt to 55°C, 61%; k) DMTrCl, TEA, DCM, quantitative; l) NAPBr, NaH, THF/DMF, 0°C to rt, 85%; m) i. $Ir(COD)(Ph_2MeP)_2PF_6$, H_2, THF, ii. I_2, sat. aq. $NaHCO_3$, THF, 85%; n) BnO-P-(N-(*i*-Pr_2))$_2$, tetrazole salt **32**, DCM, 71%; **B Building block synthesis; *Reagents and conditions:*** a) BnO-P-(N-(*i*-Pr_2))$_2$, tetrazole salt **32**, ACN, 83%. **C Building block synthesis; *Reagents and conditions:*** a) BnBr, NaH, THF/DMF 0°C to rt, 92%; b) i. $Ir(COD)(Ph_2MeP)_2PF_6$, H_2, THF, ii. I_2, sat. aq. $NaHCO_3$, THF, 77%; c) BnO-P-(N-(*i*-Pr_2))$_2$, tetrazole salt **32**, DCM, 61%. **D Building block synthesis; *Reagents and conditions:*** a) BnO-P-(N-(*i*-Pr_2))$_2$, tetrazole salt **32**, DCM, 71%.

Scheme 2 shows the assembly of the target heptamer using the generated building blocks. The coupling of the phosphoramidites proceeded by activation using dicyanoimidazole (DCI) by protonation of the di-*iso*-propylamine moiety leading to displacement by the incoming alcohol affording the phosphite intermediate or substitution of the protonated di-*iso*-propylamine moiety by DCI leading to a new activated reagent.[15] Nucleophilic displacement of the DCI moiety by the incoming alcohol forms the intermediate phosphite, which was immediately oxidized by the use of CSO. After a detritylation step the (n+1) oligomers were purified by size exclusion or silica column chromatography to set the stage for the next coupling cycle. In the first coupling on way to the heptamer, spacer amidite **22** was coupled to ribitol alcohol **15** to give spacer equipped monomer **23** in 43% yield. Fragment **23** was further elongated with amidite **16** giving dimer **24** in 73% yield. Next amidite **14** was used to provide trimer **25**, bearing an orthogonal NAP-group on the C-2 of the terminal repeat. Two couplings with



Scheme 2. A Heptamer assembly; *Reagents and conditions:* a) i. DCI, ACN, **15**; ii. CSO; iii. 3% TCA in DCM, **23: 43%;** b) i. DCI, ACN, phosphoramidite **14** or **16** or **20**; ii. CSO; iii. 3% TCA in DCM, **24: 73%, 25: 96%, 26: 88%, 27: 61%, 28: 91%, 29: 65%; B DDQ mediated napthyl removal and Z-D-alanine coupling;** *Reagents and conditions:* a) DDQ, β-pinene, DCM/H_2O, *t*-BuOH (v/v/v/= 2/2/1, 0.05M), **30**: 52%; b) Z-D-alanine, PyBOP, NMI, DCM, **31**: 48%; **C Heptamer deprotection:** *Reagents and conditions:* a) H_2, Pd black dioxane/H_2O, AcOH, **1:** quantitative.

amidite **16** were performed to give tetramer **26** and pentamer **27** in 88% and 61% yield respectively. Pentamer **27** was elongated using amidite **14** yielding hexamer **28** in 91%. A final coupling with terminal amidite **20** gave heptamer **29** in 65% yield. Throughout the assembly of the target heptamer, a gradually increasing amount of phosphoramidite building block was used with the growing of the chain to ensure high yielding coupling steps. To introduce the D-alanine esters, the naphthyls were removed using DDQ and β-pinene[16] as proton scavenger in DCM/H_2O/*t*-BuOH to ensure solubility of DDQ and the substrate.[17] It proved difficult to follow the progress of the reaction because several striping spots were observed by TLC analysis. After a difficult separation of the desired product by silica gel column chromatography the desired diol **30** was isolated in 52% yield. Now, heptamer **30** could be coupled with protected D-alanine using PyBOP as coupling agent in the presence of NMI giving **31** in 48% yield. The final hydrogenation required several days to afford the target heptamer, which was directly analyzed by NMR after filtration and concentration, to ascertain the presence of the alanine esters. The heptamer was then lyophilized to obtain the target heptamer in quantitative yield. Part of the material was transferred into its Na^+ salt by the use of a dialysis membrane, stirring on NaCl for several days followed by stirring on miliQ water for several days to desalt. Lyophilization afforded the compound in quantitative yield.
Figure 2 shows the ^{1}H NMR spectrum of the product obtained after dialysis.

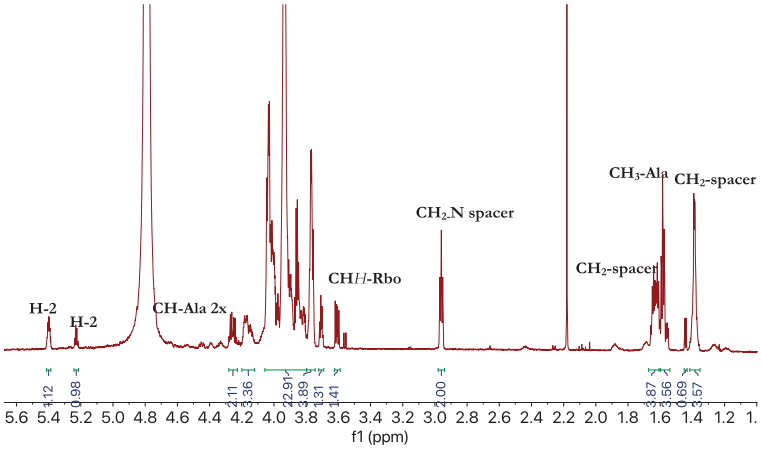


Figure 2. ^{1}H NMR of target compound **1**. (measured at a 500 MHz, at 25°C)

The protons at the alanylated positions show shifts at 5.40 and 5.23 ppm, which is close to the shift reported by Gerlach *et al.* (5.44 ppm).[18] Other characteristic resonances can be seen for the Hα of the D-alanines at 4.23 - 4.28 ppm and the D-alanine methyl groups in the region 1.55 - 1.60 pm, where they overlap with the spacer CH_2-signals. The spacer CH_2-N protons can be found as a triplet at 2.96 ppm. Calibration of this latter signal to

account for two protons, leads to integrals of the signals at 5.30 and 5.23 ppm of 1.12 and 0.98 respectively, accounting for the presence of two alanyl esters on the heptamer.

CONCLUSION

To conclude, this chapter presents a synthetic route towards an alanylated ribitol phosphate heptamer. The synthesis approach was based on the use of benzyl protected phosphoramidites because of the base lability of the alanyl esters. In the generation of the orthogonally protected C2-NAP RboP building block the synthesis of the ribose orthoester intermediate posed an obstacle, but despite the low yield in the formation of this species, enough material was produced to complete the synthesis route. At the end of the synthesis the removal of the naphthyl groups proved troublesome. Changing the NAP-ethers for more labile *para*-methoxy benzyl (PMB) ethers may allow for more efficient unmasking of the Rbo C2-hydroxyls. Further improvements in the synthesis of D-alanine-containing WTA fragments can be made to the final purification steps. An alternative size exclusion-based purification method using a neutral aqueous eluent containing NaCl, followed by a rapid desalination could prove effective. Heptamer **1** features an aminohexanol spacer. No attempts have been made to derivatize the primary amine of the spacer in the presence of the two D-alanine amino functionalities, but it may be challenging to address the spacer-amine regioselectively. Therefore, novel spacers have to be considered in the future. With chemistry in place to assembly RboP WTA fragments with D-alanine and GlcNAc substituents at predetermined sites, the effect of these substituents on the binding with various interaction partners, such as antibodies and lectins, can be probed.

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. High resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

Phosphoramidite coupling, oxidation, and detritylation

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

ganic layer was washed with saturated NaCl/$NaHCO_3$ solution (1/1), the water layer was extracted 3 times with DCM, and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was further purified by either flash chromatography (DCM/acetone) or size exclusion chromatography (sephadex LH-20, MeOH/DCM, 1/1).

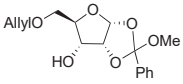
General procedure for global deprotection

The oligomer was dissolved in a 1:1 solution of NH_3 (30-33% aqueous solution) and dioxane (1.2-2.4 mM) and stirred overnight. The mixture was concentrated *in vacuo* and loaded on a Dowex Na^+ cation-exchange resin (50WX4-200, stored on 0.5 M NaOH, flushed with H_2O and MeOH before use) column and flushed with water/dioxane (1:1). The fractions were then concentrated *in vacuo*, dissolved in water/dioxane (2 ml per 10 µmol) and 4 drops of glacial AcOH were added. After purging the mixture with argon, Pd black was added (32-59 mg), and the mixture was repurged with N_2. The mixture was stirred under hydrogen gas for 3 - 7 days, filtered over celite, and concentrated *in vacuo*. The crude product was purified by size-exclusion chromatography (Toyopearl HW-40, NH_4OAc buffer) and the fractions were concentrated. The product was co-evaporated repeatedly with MilliQ water to remove NH_4OAc/ NH_4HCO_3 traces and eluted through a Dowex Na^+ cation-exchange resin column, and lyophilized.

Procedure dialysis

After global deprotection, the title compound was dissolved in 2.0 ml miliQ water and transferred to a dialysis tubing bag with dimensions (100-500D, 31MM, 1M). The dialysis tubing bag was then placed in a beaker containing 500 ml miliQ water and 5.5 g NaCl. After slowly stirring the solution for 5 days, the sample was desalted by placing the dialysis tubing bag in a beaker containing 500 ml miliQ water and stirred overnight. This desalting process was repeated 2 times. Finally, the compound was removed from the dialysis tubing, concentrated under reduced pressure, analysed by NMR and lyophilized.

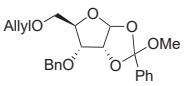
5-*O*-allyl-(1,2-*O*-methylorthobenzoyl)-α-D-ribofuranoside (5)



Compound **2** (45.6 g; 90.8 mmol; 1.0 eq.) was dissolved in dry DCM (45.0 m: 2.0M) and cooled to 0°C. A 2M solution of HCl in dioxane (45.0 ml; 2.0 eq.) was added slowly and the mixture was stirred at 7°C overnight. After two subsequent additions of 2M solution of HCl in dioxane (45.0 ml; 2.0 eq. and 23.0 ml, 1 eq. respectively) over the course of 3 hours, the reaction mixture was stirred at r.t. for one hour. The mixture was then diluted with DCM and washed 2x with sat. aq. $NaHCO_3$ and 1x with brine.

The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The intermediate was then dissolved in DCM (230 ml; 0.40M) and *N,N*-dimethylformamidedimethyl acetal (18.0 ml; 136 mmol; 1.5 eq.) was added dropwise at rt and the mixture was stirred overnight. *N,N*-dimethylformamidedimethyl acetal (12.0 ml; 90.7 mmol; 1.0 eq.) was added and the mixture was stirred for 3h. The mixture was concentrated under reduced pressure and purified by column chromatography (1:0 pentane/EtOAc to 6:4 pentane/EtOAc) yielding impure fractions. The fractions were collected and used in the next step without further purification. To a solution of the crude (19.0 g; 46.0 mmol; 1.0 eq.) in MeOH (230 ml; 0.20 M) was added K_2CO_3 (0.64 g; 4.60 mmol; 0.1 eq.) and the mixture was stirred for 1 hour at rt. Then K_2CO_3 (0.64 g; 4.60 mmol; 0.1 eq.) was added and the mixture was stirred until complete conversion was achieved according to TLC analysis. The mixture was then concentrated under reduced pressure and co evaporated with toluene. Purification by column chromatography (1:0 pentane/EtOAc to 1:1 pentane/EtOAc) yielded the product in 30% yield over 3 steps (4.30 g; 13.9 mmol). IR (neat, cm^{-1}):
3466, 3068, 2945, 2912, 1451, 1291, 1130, 1075, 1039, 967, 767; $[\alpha]_D^{20}$ = +32.4 ° (*c* 1.0, DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.24 (s, 3H, CH_3O), 3.49 (dd, 1H, *J*= 10.9 Hz, 4.7 Hz H-5), 3.56 (ddd, 1H, *J*= 8.8 Hz, 4.7 Hz, 2.3 Hz, H-4), 3.66 (dd, 1H, *J*= 10.9 Hz, 2.3 Hz, H-5), 3.94 – 4.01 (m, 3H, C*H*$_2$-CH, H-3), 4.80 (dd, 1H, *J*= 5.3 Hz, 4.1 Hz, H-2), 5.14 – 5.27 (m, 2H, C*H*$_2$=CH), 5.82 - 5.91 (m, 1H, CH_2=C*H*), 6.08 (d, 1H, *J*= 4.0 Hz, H-1), 7.37 - 7.42 (m, 3H, H-arom), 7.63 – 7.69 (m, 2H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 50.5 (CH_3O), 68.1 (C-5), 71.6 (C-3), 72.4 (*C*H$_2$-CH), 79.4, 80.0 (C-2, C-4), 104.2 (C-1), 117.4 (*C*H$_2$=CH), 123.7 (Cq), 126.0, 128.3, 129.4 (C-arom), 134.3 (CH_2=*C*H), 136.5 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{16}H_{20}O_6$ Na 331.11521, found 331.11490.

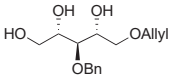
5-*O*-allyl-3-*O*-benzyl-(1,2-*O*-methylorthobenzoyl)-α-D-ribofuranoside (6)



To a solution of compound **5** (4.25 g; 13.8 mmol; 1.0 eq.) in a mixture of THF/DMF (40.0 ml; 0.35M; v/v= 7:1) at 0°C was added NaH (1.10 g; 27.6 mmol; 2.0 eq) followed by BnBr (2.20 ml; 20.7 mmol; 1.5 eq.). The mixture was allowed to warm up to rt and was stirred overnight. The mixture was quenched by addition of MeOH at 0°C, diluted with Et_2O and washed with H_2O 3x and brine. The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by TEA neutralized column chromatography (1:0 pentane/EtOAc to 6:4 pentane/EtOAc) yielded the product in 96% yield (5.30 g; 13.3 mmol). IR (neat, cm^{-1}): 3065, 2942, 1452, 1289, 1135, 1086, 1046, 1027, 970, 766, 700; $[\alpha]_D^{20}$ = +128.3 ° (*c* 1.0, DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.21 (s, 3H, CH_3O), 3.41 (dd, 1H, *J*= 11.4, 4.1 Hz, H-5), 3.58 (dd, 1H, *J*= 11.3, 2.0 Hz, H-5), 3.76 (ddd, 1H, *J* = 9.1, 4.1, 1.9 Hz, H-4), 3.80 – 3.85

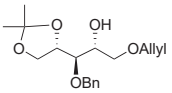
(m, 1H, H-3), 3.85 – 3.95 (m, 2H, CH_2-CH), 4.55 (d, 1H, *J*= 11.7 Hz, C*H*H-Bn), 4.72 – 4.81 (m, 2H, C*H*H-Bn, H-2), 5.08 – 5.23 (m, 2H, CH_2=CH), 5.74 – 5.84 (m, 1H, CH_2=C*H*), 6.01 (d, 1H, *J*= 4.2 Hz, H-1), 7.23 – 7.38 (m, 8H, H-arom), 7.68 – 7.73 (m, 2H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 49.8 (CH_3O), 67.5 (C-5), 71.7, 71.9 (CH_2-Bn, CH_2-CH), 77.1, 77.6, 78.0 (C-2, C-3, C-4), 104.2 (C-1), 116.7 (CH_2=CH), 123.7 (Cq), 125.9, 127.6, 127.8, 128.1, 128.8 (CH-arom), 134.2 (CH_2=CH), 136.9, 137.3 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{23}H_{26}O_6Na$ 421.16216, found 421.16163.

5-*O*-allyl-3-*O*-benzyl-D-ribitol (7)



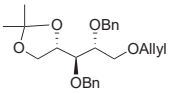
Compound **6** (5.25 g; 13.2 mmol; 1.0 eq.) was dissolved in a mixture of THF/H_2O/Formid acid (130 ml; 0.10M; v/v/v= 6:3:1) and the mixture was heated to 70°C until complete conversion of the starting material was achieved according to TLC analysis. The mixture was then diluted with EtOAc, washed with water and 3x with sat. aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was dissolved in MeOH (70.0 ml; 0.19M), 4.8M NaOMe was added (0.3ml; 0.1 eq) and the mixture was stirred overnight at rt. Amberlite H^+ was added to quench the reaction and the mixture was filtrated and concentrated under reduced pressure. The crude was co-evaporated with toluene and dissolved in MeOH (66 ml; 0.20M). $NaBH_4$ (600 mg; 15.8 mmol; 1.2 eq) was added at 0°C. To speed up the conversion additional $NaBH_4$ (600 mg; 15.8 mmol; 1.2 eq) was added. After 2h, still starting material was present according to TLC analysis, and $NaBH_4$ (600 mg; 15.8 mmol; 1.2 eq) was added to complete the reaction. The reaction was quenched with acetone, concentrated *in vacuo* and co-evaporated with MeOH. The crude was dissolved in MeOH (66.0 ml; 0.20M) and $NaBH_4$ (1.75 g; 46.2 mmol; 3.5 eq) and the mixture was stirred for 2h. The reaction was quenched with acetone, concentrated under reduced pressure and co-evaporated with MeOH. Purification by column chromatography (1:0 DCM/MeOH to 9:1 DCM/MeOH) yielded the product and starting material fractions. The starting material fractions were collected, concentrated and were subjected to the reduction conditions described above. After complete conversion, the reaction was quenched and worked up as described above and the crude was purified using column chromatography 1:0 DCM/MeOH to 9:1 DCM/MeOH yielding the title compound in a total yield of 72% over 3 steps (2.68 g; 9.49 mmol). IR (neat, cm^{-1}): 3647, 3567, 2357, 1560, 1506, 1456, 771, 668; $[\alpha]_D^{20}$ = -0.3 ° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.53 – 3.82 (m, 6H, H-3, H-2/H-4, 2x CH_2-Rbo), 3.89 – 4.04 (m, 3H, H-2/H-4, CH_2-CH), 4.58 – 4.69 (m, 2H, CH_2-Bn), 5.14 – 5.29 (m, 2H, CH_2=CH), 5.83 - 5.93 (m, 1H, CH_2=C*H*), 7.24 – 7.35 (m, 5H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 63.3, 71.1 (CH_2-Rbo), 71.4 (C-2/C-4), 72.3 (CH_2-CH), 72.8 (C-2/C-4), 73.8 (CH_2-Bn), 79.4 (C-3), 117.6 (CH_2=CH), 127.8, 128.0, 128.4 (CH-arom), 134.4 (CH_2=C*H*), 138.0 (Cq-arom); HRMS: $[M+H]^+$ calcd for $C_{15}H_{23}O_5$ 283.15400, found 283.15392.

5-*O*-allyl-3-*O*-benzyl-1,2-*O*-isopropylidene-D-ribitol (8)



Compound **7** (2.63 g; 9.30 mmol; 1.0 eq.) was dissolved in DCM (38.0 ml; 0.24M) and at 0°C DMP (9.4 ml; 1.0M) and *p*-TsOH (0.24 g; 1.40 mmol; 0.15 eq.) were added. After complete conversion (+/- 20 min.) the reaction was quenched with TEA and concentrated under reduced pressure. Column chromatography (pentane/EtOAc 1:0 to 7:3 pentane/EtOAc) afforded the title compound and mixed fractions, yielding the side product due to isopropylidene installment on the C-2 hydroxyl. The side product was treated with 1M HCl solution in EtOAc (v/v= 1/10, 0.20M) and the mixture was stirred until the sideproduct was completely converted into the product. The mixture was then further diluted with EtOAc, washed with sat. aq. $NaHCO_3$ and brine. The organic layer was filtrated over Na_2SO_4, concentrated under reduced pressure, yielding the title compound in a total yield of 41% (1.22 g; 3.78 mmol). IR (neat, cm^{-1}): 3735, 3567, 2988, 2908, 2355, 1457, 1215, 1070, 1029, 930, 853, 747, 700; $[\alpha]_D^{20}$ = -4.8 ° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.34 (s, 3H, CH_3-Cq), 1.41 (s, 3H, CH_3-Cq), 3.12 (bs, 1H, OH), 3.48 – 3.58 (m, 2H, C*H*$_2$-OAllyl), 3.70 (t, 1H, *J*= 5.3 Hz, H-3), 3.85 – 3.88 (m, 1H, *J*= 6.1 Hz, 3.5 Hz, H-2), 3.91 (dd, 1H, *J*= 8.2 Hz, 6.9 Hz, H-5), 3.94 – 3.98 (m, 2H, C*H*$_2$-CH), 4.03 (dd, 1H, *J*= 8.2 Hz, 6.5 Hz, H-5), 4.31 (td, 1H, *J*= 6.7 Hz, 5.1 Hz, H-4), 4.66 – 4.77 (m, 2H, CH_2-Bn), 5.13 – 5.28 (m, 2H, C*H*$_2$=CH), 5.83 – 5.92 (m, 1H, CH_2=C*H*), 7.22 – 7.34 (m, 5H, H-arom); ^{13}C-APT NMR (126 MHz, $CDCl_3$) δ= 25.1, 26.3 (*C*H$_3$-Cq), 65.7 (C-5), 70.8 (*C*H$_2$-OAllyl), 71.1 (C-2), 72.1 (*C*H$_2$-CH), 74.0 (CH_2-Bn), 75.9 (C-4), 78.9 (C-3), 108.7 (Cq), 117.0 (*C*H$_2$=CH), 127.6, 127.8, 128.2 (CH-arom), 134.4 (CH_2=*C*H), 138.2 (Cq-arom); HRMS: $[M+NH_4]^+$ calcd for $C_{18}H_{30}O_5N$ 340.21185, found 340.21185.

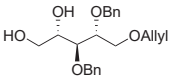
5-*O*-allyl-3,4-di-*O*-benzyl-1,2-*O*-isopropylidene-D-ribitol (9)



To a solution of compound **8** (1.18 g; 3.60 mmol; 1.0 eq.) in THF/DMF (18.0 ml; 0.20M; v/v= 7/1) at 0°C NaH (0.22 g; 5.50 mmol; 1.5 eq.) was added, followed by BnBr (0.60 ml; 4.70 mmol; 1.3 eq.) and the mixture was allowed to warm up to rt and stirred 2.5h. The reaction was quenched with MeOH, diluted with Et_2O, washed with H_2O 2x and brine. The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by column chromatography pentane/EtOAc 1:0 to pentane/EtOAc 9:1 yielded the title compound in quantitative yield (1.48 g; 3.59 mmol). IR (neat, cm^{-1}): 2986, 2873, 2322, 1457, 1209, 1072, 1027, 923, 853, 736, 697; $[\alpha]_D^{20}$ = -27.8 ° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.33 (s, 3H, CH_3-Cq), 1.38 (s, 3H, CH_3-Cq), 3.59 – 3.71 (m, 2H, C*H*$_2$-OAllyl), 3.75 – 3.84 (m, 2H, H-2, H-3), 3.88 – 3.94 (m, 2H, H-5), 3.97 (dt, 2H, *J*= 5.5 Hz, 1.5 Hz, C*H*$_2$-CH), 4.27 (td, 1H, *J*= 6.4 Hz, 5.0 Hz, H-4), 4.58 – 4.76 (m, 4H, CH_2-Bn), 5.12 – 5.31 (m, 2H, C*H*$_2$=CH), 5.84 – 5.94 (m, 1H, CH_2=C*H*), 7.21 – 7.38 (m, 10H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 25.3, 26.6 (*C*H$_3$-Cq), 66.2 (C-5), 69.9 (*C*H$_2$-OAllyl), 72.2, 72.7, 73.9 (CH_2-Bn, *C*H$_2$-CH), 75.6 (C-4), 78.4, 79.4 (C-2, C-3), 108.8 (Cq),

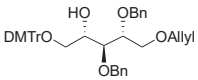
116.8 (CH_2=CH), 127.6, 127.7, 127.9, 128.4 (CH-arom), 134.8 (CH_2=*C*H), 138.5 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{25}H_{32}O_5Na$ 435.21420, found 435.21420.

5-*O*-allyl-3,4-di-*O*-benzyl-D-ribitol (10)



Compound **9** (1.58 g; 3.82 mmol) was dissolved in a mixture of formic acid/ H_2O/THF (38.2 ml; 0.10M; v/v/v= 6/2/2) and was stirred for 30 min at rt. Then the mixture was heated to 50°C, when no more conversion of the starting material took place according to TLC analysis, the mixture was diluted in EtOAc, washed with water and sat. aq. $NaHCO_3$, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The crude was dissolved in formic acid/H_2O/THF (38.2 ml; 0.10M; v/v/v= 6/2/2) and the mixture was heated to 55°C until complete conversion into the product was achieved according to TLC analysis. Purification by column chromatography (pentane/EtOAc 1:0 to pentane/EtOAc 6:4) yielded the title compound along with the half deprotected isopropyl-intermediate. This intermediate was collected, concentrated and re-dissolved in formic acid/H_2O (20.0 ml; v/v= 1/1) and the mixture was heated to 55°C. The reaction was diluted in EtOAc, washed with water and sat. aq. $NaHCO_3$, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by column chromatography (pentane/EtOAc 1:0 to pentane/EtOAc 1:1) yielded the title compound in a total yield of 61% (872 mg; 2.34 mmol). IR (neat, cm^{-1}): 3397, 2916, 2872, 2354, 2322, 1456, 1209, 1089, 1073, 1027, 927, 737, 698; $[\alpha]_D^{20}$ = -30.0 ° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.99 (bs, 1H, OH), 3.60 – 3.77 (m, 5H, 2x CH_2-Rbo, H2/H3), 3.80 - 3.84 (m, 1H, H-4), 3.88 (q, 1H, *J*= 4.5 Hz, H2/H3), 3.97 (dt, 2H, *J*= 5.7 Hz, 1.5 Hz, C*H*$_2$-CH), 4.57 – 4.75 (m, 4H, CH_2-Bn), 5.13 – 5.31 (m, 2H, C*H*$_2$=CH), 5.83 – 5.93 (m, 1H, CH_2=C*H*), 7.21 – 7.37 (m, 10H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 63.6, 69.2 (CH_2-Rbo), 71.9 (C-4), 72.2, 72.4, 73.8 (CH_2-Bn, *C*H$_2$-CH), 79.0, 79.2 (C-2, C-3), 117.2 (*C*H$_2$=CH), 127.7, 127.7, 127.8, 128.0, 128.4 (CH-arom), 134.4 (CH_2=*C*H), 138.0, 138.1 (Cq-arom); HRMS: $[M+H]^+$ calcd for $C_{22}H_{29}O_5$ 373.20095, found 373.20079.

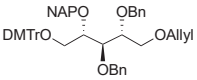
5-*O*-allyl-3,4-di-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol (11)



To a solution of compound **10** (848 mg; 2.28 mmol; 1.0 eq.) in DCM (23.0 ml; 0.10M), at 0°C TEA (0.50 ml; 3.42 mmol; 1.5 eq.) and DMTCl (850 mg; 2.50 mmol; 1.1 eq.) were added and the mixture was allowed to warm up to rt. The reaction was quenched with MeOH at 0°C and concentrated under reduced pressure. Purification by TEA neutralized column chromatography (pentane/EtOAc 1:0 to pentane/EtOAc 6:4) yielded the title compound in quantitative yield (1.61 g; 2.38 mmol). IR (neat, cm^{-1}): 2931, 2836, 2354, 1608, 1521, 1508, 1457, 1249, 1176, 1073, 1033, 829, 698; $[\alpha]_D^{20}$ = -5.4 (*c* 1.0, DCM); ^{1}H NMR (400 MHz, CD_3CN) δ= 3.16 – 3.31 (m, 2H, DMTO-C*H*$_2$), 3.60 – 3.80 (m, 9H, CH_2-Rbo, 2x CH_3O, H2/H3), 3.88 – 3.93 (m, 1H, H-2/H-3), 3.98 (dt, 2H, *J*= 5.4 Hz, 1.7 Hz, C*H*$_2$-CH, H-4), 4.46 (d, 1H, *J*= 11.2 Hz, C*H*H-Bn),

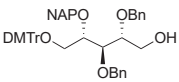
4.55 (d, 1H, *J*= 11.8 Hz, C*H*H-Bn), 4.65 (dd, 2H, *J*= 16.4, 11.5 Hz, CH_2-Bn), 5.12 – 5.35 (m, 2H, C*H*$_2$=CH), 5.88 – 5.98 (m, 1H, CH_2=C*H*), 6.79 – 6.87 (m, 4H, H-arom), 7.10 – 7.39 (m, 17H, H-arom), 7.48 – 7.54 (m, 2H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 55.8 (CH_3O), 66.2 (DMTO-C*H*$_2$), 70.8 (CH_2-Rbo), 71.7 (C-4), 72.6, 72.9, 74.2 (CH_2-Bn, *C*H$_2$-CH), 79.9, 80.6 (C-2, C-3), 86.7 (Cq-DMT), 113.9 (CH-arom), 116.8 (*C*H$_2$=CH), 127.6, 128.3, 128.4, 128.6, 128.7, 128.8, 129.1, 129.1, 129.2, 130.0, 131.1 (CH-arom), 136.2 (CH_2=*C*H), 137.1, 137.1, 139.6, 139.9, 146.4, 159.5 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{43}H_{46}O_7$ Na 697.31357, found 697.31343.

5-*O*-allyl-3,4-di-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-2-*O*-(2-naphtylmethyl)-D-ribitol (12)



To a solution of compound **11** (1.58 g; 2.34 mmol; 1.0 eq.) in THF/DMF (23.0 ml; 0.10M; v/v= 7:1) at 0°C, NaH (140 mg; 3.51 mmol; 1.5 eq.) and NAPBr (674 mg; 3.05 mmol; 1.3 eq.) were added. The mixture was allowed to warm up to rt and was stirred overnight. The reaction was quenched with MeOH at 0°C, diluted with Et_2O, washed with H_2O 2x, and brine. The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by TEA neutralized column chromatography (pentane/EtOAc 1:0 to pentane/EtOAc 8:2) yielded the title compound in 85% yield (1.63 g; 1.99 mmol). IR (neat, cm^{-1}): 2931, 2870, 2355, 2320, 1608, 1521, 1508, 1457, 1249, 1175, 1090, 1035, 827, 698; $[\alpha]_D^{20}$ = +16.7 ° (*c* 1.0, DCM); ^{1}H NMR (400 MHz, CD_3CN) δ= 3.29 – 3.37 (m, 2H, DMTO-C*H*$_2$), 3.60 (dd, 1H, *J*= 10.6 Hz, 5.8 Hz, C*H*H), 3.68 (d, 7H, *J*= 1.6 Hz, C*H*H, 2x CH_3O), 3.80 – 3.85 (m, 1H, H-2), 3.87 – 3.93 (m, 3H, H-3, C*H*$_2$-CH), 3.96 – 4.00 (m, 1H, H-4), 4.46 – 4.91 (m, 6H, CH_2-Bn), 5.07 – 5.26 (m, 2H, C*H*$_2$=CH), 5.82 – 5.92 (m, 1H, CH_2=C*H*), 6.69 – 6.76 (m, 4H, H-arom), 7.13 – 7.31 (m, 18H, H-arom), 7.40 – 7.55 (m, 5H, H-arom), 7.78 – 7.90 (m, 4H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 55.8 (CH_3O), 64.8 (DMTO-CH_2), 70.8 (CH_2-Rbo), 72.6, 72.9, 73.3, 74.3 (CH_2-Bn), 79.5, 79.8, 79.9 (CH-Rbo), 86.8 (Cq-DMT), 113.9 (CH-arom), 116.7 (*C*H$_2$=CH), 126.9, 127.1, 127.2, 127.3, 127.6, 128.3, 128.4, 128.6, 128.6, 128.7, 128.8, 128.9, 129.0, 129.1, 129.2, 131.0, 131.0 (CH-arom), 133.9, 134.2 (Cq-arom), 136.3 (CH_2=CH), 137.1, 137.2, 137.5, 139.7, 139.9, 146.4, 159.5 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{54}H_{54}O_7$ Na 837.37618, found 837.37620.

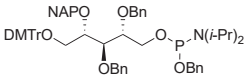
3,4-di-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-2-*O*-(2-naphtylmethyl)-D-ribitol (13)



A solution of compound **12** (613 mg; 0.75 mmol; 1.0 eq.) in distilled THF (7.5 ml; 0.10M) was degassed with N_2. $Ir(COD)(Ph_2MeP)_2PF_6$ (13 mg; 0.02 eq.) was added and the solution was degassed with N_2. Then the red solution was purged with H_2 until the color became yellow (~6 seconds) and hereafter the solution was degassed with N_2 to remove traces of H_2 from the solu-

tion and the mixture was stirred under N_2 atmosphere until complete conversion was achieved according to TLC analysis. The mixture was diluted with THF (7.5 ml) and aq. sat. $NaHCO_3$ (7.5 ml) followed by the addition of I_2 (0.29 g; 1.13 mmol; 1.5 eq.) and stirred for +/- 30 min. The reaction was quenched by the addition of sat. aq. Na_2SO_3, diluted with EtOAc and the organic layer was washed with sat. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by TEA neutralized column chromatography (pentane/EtOAc 1:0 to pentane/EtOAc 6:4) yielded the title compound in (492 mg; 0.63 mmol.) 85% yield. IR (neat, cm^{-1}): 2932, 2875, 2360, 2312, 1607, 1521, 1521, 1508, 1457, 1249, 1175, 1073, 1032, 827, 698; $[\alpha]_D^{20}$ = +1.6 ° (*c* 1.0, DCM); ^{1}H NMR (400 MHz, CD_3CN) δ= 2.79 (s, 1H, OH), 3.28 – 3.35 (m, 2H, DMTO-C*H*$_2$), 3.62 – 3.72 (m, 8H, 2x CH_3O, C*H*H-OH, H-2), 3.76 – 3.81 (m, 1H, C*H*H-OH), 3.92 (t, 1H, *J*= 4.9 Hz, H-3), 3.99 – 4.03 (m, 1H, H-4), 4.45 (d, 1H, *J*= 11.6 Hz, C*H*H-Bn), 4.54 – 4.64 (m, 3H, CH_2-Bn), 4.86 (q, 2H, *J*= 10.0 Hz CH_2-Bn), 6.70 – 6.75 (m, 4H, H-arom), 7.15 – 7.30 (m, 17H, H-arom), 7.40 – 7.45 (m, 2H, H-arom), 7.46 – 7.56 (m, 3H, H-arom), 7.80 – 7.91 (m, 4H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 55.8 (CH_3O), 61.8 (CH_2-OH), 64.9 (DMTO-CH_2), 72.7, 73.3, 74.3 (CH_2-Bn), 79.8, 79.9 (C-3, C-4), 80.8 (C-2), 86.9 (Cq-DMT), 113.9, 126.9, 127.1, 127.2, 127.3, 127.6, 128.3, 128.4, 128.6, 128.7, 128.8, 128.9, 129.0, 129.2, 129.2, 131.0 (CH-arom), 133.9, 134.2, 137.1, 137.2, 137.6, 139.7, 139.8, 146.4, 159.5, 159.5 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{51}H_{50}O_7$ Na 797.34488, found 797.34491.

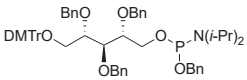
2-Benzyl [3,4-di-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-2-*O*-(2-naphtylmethyl)-1-D-ribityl] *N,N*-diisopropylphosphoramidite (14)



To a stirred solution of compound **13** (402 mg; 0.52 mmol; 1.0 eq.) in DCM (5.2 ml; 0.10M) was added (2.1 ml; 0.52 mmol; 1.0 eq., (0.25 M in dry DCM)) BnO-P-(N-(*i*-Pr_2))$_2$ stock solution followed by tetrazole salt **32** (44 mg; 0.26 mmol; 0.5 eq.). After 1.5h (1.7 ml; 0.42 mmol; 0.8 eq.) BnO-P-(N-(*i*-Pr_2))$_2$ stock solution and tetrazole salt **32** (50 mg; 0.29 mmol; 0.6 eq.) were added to speed up the conversion. 3 ml DCM were added to improve the solubility and the reaction was stirred in a waterbath at 40°C. (0.6 ml; 0.16 mmol; 0.3 eq.) BnO-P-(N-(*i*-Pr_2))$_2$ stock solution was further added to convert the minor amount of starting material, afterwhich the reaction was quenched by the addition of water. The mixture was diluted with DCM, washed with sat. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by TEA neutralized column chromatography pentane/EtOAc 1:0 to pentane/EtOAc 8:2 yielded the title compound in 71% yield. ^{1}H NMR (400 MHz, CD_3CN) δ= 1.05 – 1.32 (m, 12H, 4x CH_3-isopropylamine), 3.28 – 3.73 (m, 10H, CH_2-Rbo, CH-isopropylamine, 2x CH_3O), 3.73 – 4.09 (m, 5H, 3x CH-Rbo, CH_2-Rbo), 4.44 – 5.04 (m, 8H, 3x CH_2-Bn, CH_2-NAP), 6.71 (ddd, 4H, *J*= 8.8, 4.2, 1.6 Hz, H-arom), 7.11 – 7.54 (m, 27H, H-arom), 7.73 – 7.89 (m, 4H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 25.0, 25.0, 25.1, 25.2 (CH_3-isopropylamine), 43.6, 43.7,

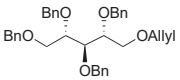
43.7, 43.8, 43.9, 43.9 (CH-isopropylamine), 55.8 (CH_3O), 63.6, 63.7, 63.8, 63.9 (CH_2-Rbo), 64.9, 65.0 (CH_2-Rbo), 65.8, 65.9, 66.0, 66.0, 66.1, 73.0, 73.3, 74.2, 74.2 (CH_2-Bn, CH_2-NAP), 79.7, 79.9, 80.1, 80.2 (CH-Rbo), 86.8 (Cq-DMT), 113.9, 126.3, 127.1, 127.1, 127.2, 127.2, 127.6, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6, 128.7, 128.7, 128.8, 128.9, 129.0, 129.1, 129.2, 129.2, 129.2, 131.0, 131.0 (CH-arom), 133.8, 134.2, 137.1, 137.2, 137.6, 139.6, 139.8, 139.8, 146.4, 159.4 (Cq-arom); ^{31}P NMR (162 MHz, CD_3CN) δ= 148.7, 148.7.

2-Benzyl [2,3,4-tri-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-1-D-ribityl] *N,N*-diisopropylphosphoramidite (16)



To a stirred solution of compound **15** (1.17 g; 1.61 mmol; 1.0 eq.) in ACN (11.6 ml; 0.1M), BnO-P-(N-(*i*-Pr_2))$_2$ (6.4 mL; 1.61 mmol; 1.0 eq., (0.25 M in dry DCM)) was added, followed by tetrazole salt **32** (138 mg; 0.81 mmol; 0.5 eq.). After 3h, (2.0 ml; 0.50 mmol; 0.3 eq.) BnO-P-(N-(*i*-Pr_2))$_2$ stock solution was added to speed up the conversion. Then the mixture was diluted with DCM, washed with a solution of sat. aq. $NaHCO_3$: NaCl (v/v= 1:1). The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo* at 30°C. Purification by TEA neutralized column chromatography (pentane/EtOAc 1:0 to pentane/EtOAc 8:2) yielded the title compound in 70% (1.14 g; 1.12 mmol) yield. ^{1}H NMR (400 MHz, CD_3CN) δ= 1.08 – 1.31 (m, 12H, 4x CH_3-isopropylamine), 3.39 – 3.46 (m, 2H, CH_2-Rbo), 3.66 – 3.72 (m, 8H, 2x CH_3O, CH_2-Rbo), 3.82 – 4.14 (m, 5H, 3x CH-Rbo, CH_2-Rbo), 4.50 – 4.84 (m, 8H, 4x CH_2-Bn), 6.80 (ddd, 4H, *J* = 8.9, 3.3, 1.9 Hz, H-arom), 7.13 – 7.58 (m, 33H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 25.0, 25.1, 25.2, 25.2 (CH_3-isopropylamine), 43.7, 43.8, 43.8, 43.9 (CH-isopropylamine), 55.8 (CH_3O), 63.7, 63.8, 63.9, 63.9 (CH_2-Rbo), 64.7, 64.8 (CH_2-Rbo), 65.8, 65.9, 66.0, 66.1 (CH_2-Bn), 73.0, 73.3, 74.2, 74.2 (CH_2-Bn), 79.7, 79.8, 79.9, 80.1, 80.2, 80.3 (CH-Rbo), 86.9 (Cq-DMT), 113.9, 127.6, 127.9, 128.1, 128.2, 128.3, 128.4, 128.5, 128.5, 128.7, 128.7, 128.7, 129.0, 129.1, 129.1, 129.2, 129.2, 129.2, 131.0, 131.0 (CH-arom), 137.1, 137.1, 139.6, 139.6, 139.8, 139.8, 139.9, 146.4, 159.5 (Cq-arom); ^{31}P NMR (162 MHz, CD_3CN) δ= 148.8, 148.7.

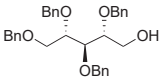
5-*O*-allyl-1,2,3,4-tetra-*O*-benzyl-D-ribitol (18)



To a solution of compound **17** (412 mg; 0.89 mmol; 1.0 eq.) in THF/DMF (4.5 ml; 0.20M; v/v= 7:1) at 0°C, NaH (55 mg; 1.34 mmol; 1.5 eq.) and BnBr (0.13 mL; 1.16 mmol; 1.3 eq.) were added. The mixture was allowed to warm up to rt and was stirred overnight. Then NaH was added (53 mg; 1.34 mmol; 1.5 eq.) at 0°C and the mixture was allowed to warm up to rt and the mixture was stirred for 2 days. The reaction was quenched with MeOH at 0°C, diluted with Et_2O, washed with H_2O 4x, and brine. The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by TEA neutralized column chromatography (pentane/EtOAc 1:0 to pentane/EtOAc 7:3) yielded the title compound (453 mg; 0.82 mmol)

in 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 3.60 – 3.76 (m, 4H, 2x CH_2-Rbo), 3.87 – 3.95 (m, 5H, 3x CH-Rbo, C*H*$_2$-CH), 4.44 – 4.75 (m, 8H, 4x CH_2-Bn), 5.10 – 5.28 (m, 2H, C*H*$_2$=CH), 5.88 (ddt, 1H, *J* = 17.3, 10.7, 5.5 Hz, CH_2=C*H*), 7.19 – 7.38 (m, 20H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 70.2, 70.2 (CH_2-Rbo), 72.2, 72.3, 72.4, 72.5, 73.3, 73.9 (CH_2-Bn), 78.5, 78.6, 78.8 (CH-Rbo), 116.8 (*C*H$_2$=CH), 127.5, 127.6, 127.6, 127.7, 127.7, 127.8, 127.9, 127.9, 128.0, 128.3, 128.4, 128.5 (CH-arom), 135.0 (CH_2=*C*H), 138.5, 138.6, 138.7 (Cq-arom).

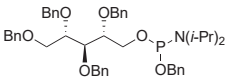
1,2,3,4-tetra-*O*-benzyl-D-ribitol (19)



A solution of compound **18** (453 mg; 0.82 mmol; 1.0 eq.) in distilled THF (8.0 ml; 0.10M) was degassed with N_2.

$Ir(COD)(Ph_2MeP)_2PF_6$ (7 mg; 0.01 eq.) was added and the solution was degassed with N_2. Then the red solution was purged with H_2 until the color became yellow (~8 seconds) and hereafter the solution was degassed with N_2 to remove traces of H_2 from the solution and the mixture was stirred under N_2 atmosphere until complete conversion was achieved according to TLC analysis. The mixture was diluted with THF (8.0 ml) and aq. sat. $NaHCO_3$ (8.0 ml) followed by the addition of I_2 (0.31 g; 1.22 mmol; 1.5 eq.) and stirred for +/- 30 min. The reaction was quenched by the addition of sat. aq. Na_2SO_3, diluted with EtOAc and the organic layer was washed with sat. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification by TEA neutralized column chromatography pentane/EtOAc 1:0 to pentane/EtOAc 6:4 yielded the title compound (324 mg; 0.63 mmol) in 77% yield. IR (neat, cm^{-1}): 2928, 2872, 2377, 2312, 1560, 1507, 1457, 1272, 1096, 1070, 1027, 738, 697; $[\alpha]_D^{20}$ = +3.1 ° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ= 2.36 (m, 1H, O*H*), 3.66 - 3.75 (m, 5H, 2x CH_2-Rbo, CH-Rbo), 3.88 (td, 1H, *J*= 5.1, 3.8 Hz, CH-Rbo), 3.94 (t, 1H, *J*= 4.8 Hz, CH-Rbo), 4.44 – 4.74 (m, 8H, 4x CH_2-Bn), 7.22 – 7.34 (m, 20H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 61.4 (CH_2-Rbo), 69.8 (CH_2-Rbo), 72.0, 72.5, 73.4, 74.0 (CH_2-Bn), 78.3, 78.9, 79.1 (CH-Rbo), 127.7, 127.7, 127.8, 127.8, 127.8, 127.9, 127.9, 128.1, 128.4, 128.4, 128.4, 128.5 (CH-arom), 138.2, 138.2, 138.3, 138.4 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{33}H_{36}O_5$ Na 535.24550, found 535.24496.

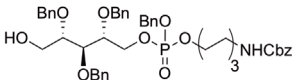
2-Benzyl [1,2,3,4-tetra-*O*-benzyl-1-D-ribityl] *N,N*-diisopropylphosphoramidite (20)



To a stirred solution of compound **19** (303 mg; 0.59 mmol; 1.0 eq.) in DCM (5.9 ml; 0.10M) was added (2.8 ml; 0.71 mmol; 1.2 eq. (0.25 M in dry DCM)) BnO-P-(N-(*i*-Pr$_2$))$_2$ stock solution followed by tetrazole salt **32** (51 mg; 0.30 mmol; 0.5 eq.). After 2h the mixture was warmed up in a water bath at 40°C for 10 min. Then 60 mg (0.35 mmol; 0.6 eq.) tetrazole salt **32** was added followed by 2.1 mL (0.53 mmol; 0.75 eq.) BnO-P-(N-(*i*-Pr$_2$))$_2$ stock solution to

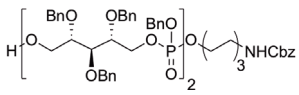
speed up the conversion. After complete conversion of the reaction according to TLC analysis, the reaction was filtrated and concentrated *in vacuo*. Purification by TEA neutralized column chromatography pentane/EtOAc 1:0 to pentane/EtOAc 9:1 yielded the title compound in 61% (270 mg; 0.36 mmol). ^{1}H NMR (400 MHz, CD_3CN) δ= 1.13 – 1.21 (m, 12H, 4x CH_3-isopropylamine), 3.61 -3.71 (m, 3H, 2x CH-isopropylamine, C*H*H-Rbo), 3.73 – 4.07 (m, 6H, 3x CH-Rbo, CH_2-Rbo, CH*H*-Rbo), 4.47 (d, 2H, *J*= 3.3 Hz, CH_2-Bn), 4.49 – 4.77 (m, 8H, 4x CH_2-Bn), 7.22 – 7.36 (m, 25H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 25.0, 25.0, 25.1, 25.2 (CH_3-isopropylamine), 43.7, 43.8, 43.8, 43.9 (CH-isopropylamine), 63.6, 63.7, 63.8, 63.9 (CH_2-Rbo), 65.8, 65.8, 66.0, 66.0 (CH_2-Bn), 71.1 (CH2-Rbo), 72.8, 72.9, 72.9, 73.7, 74.5 (CH_2-Bn), 79.5, 79.6, 79.7, 80.0, 80.1, 80.2 (CH-Rbo), 127.9, 128.2, 128.2, 128.3, 128.4, 128.4, 128.6, 128.6, 128.8, 128.8, 129.2, 129.2 (CH-arom), 139.7, 139.8, 139.9, 139.9, 140.7, 140.8 (Cq-arom); ^{31}P NMR (162 MHz, CD_3CN) δ= 148.8, 148.7.

D-ribitol phosphate monomer (23)



According to the general procedure above, alcohol **15** (0.05M in ACN; 40.0 mL; 1.55 g; 2.14 mmol; 1.0 eq.) was coupled with phosphoramidite **22** (1.31 g; 2.68 mmol; 1.3 eq.). Column chromatography yielded the title compound in 43% yield (0.76 g; 0.92 mmol); IR (neat, cm^{-1}): 3347, 3032, 2935, 2863, 2377, 2312, 1717, 1700, 1558, 1539, 1457, 1252, 1098, 999, 734, 695; ^{1}H NMR (400 MHz, CD_3CN) δ= 1.18 – 1.29 (m, 4H, CH_2-hexylspacer), 1.36 –1.42 (m, 2H, CH_2-hexylspacer), 1.50 – 1.57 (m, 2H, CH_2-hexylspacer), 3.05 (q, 2H, *J*= 6.6 Hz, CH_2N hexylspacer), 3.69 – 3.84 (m, 3H, CH_2-Rbo, CH-Rbo), 3.90 – 3.99 (m, 4H, 2x CH-Rbo, CH_2O), 4.18 – 4.25 (m, 1H, C*H*H-Rbo), 4.34 - 4.39 (m, 1H, C*H*H-Rbo), 4.56 – 4.74 (m, 6H, 3x CH_2-Bn), 4.98 – 5.08 (m, 4H, CH_2-Bn, CH_2-Cbz), 5.78 (t, 1H, *J*= 5.9 Hz, NH), 7.26 – 7.40 (m, 25H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 25.8, 26.8, 30.4, 30.8, 30.8 (CH_2-hexylspacer), 41.4 (CH_2N hexylspacer), 61.6 (CH_2-Rbo), 66.6 (CH_2-Cbz), 67.8, 67.8 (CH_2-Rbo), 68.6, 68.7 (CH_2O), 69.7, 69.8, 69.8, 69.8, 72.7, 72.9, 74.4, 74.4 (CH_2-Bn), 78.9, 79.1, 79.2, 80.6 (CH-Rbo), 128.4, 128.5, 128.5, 128.6, 128.7, 128.8, 128.8, 128.8, 129.2, 129.3, 129.3, 129.4, 129.4, 129.5, 129.5 (CH-arom), 137.3, 137.4, 138.5, 139.4, 139.6, 139.7 (Cq-arom), 157.3 (C=O); ^{31}P NMR (162 MHz, CD_3CN) δ= 0.6; HRMS: $[M+H]^+$ calcd for $C_{47}H_{57}NO_{10}P$ 826.37146, found 826.37138.

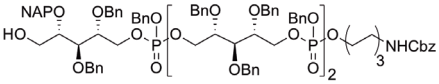
D-ribitol phosphate dimer (24)



According to the general procedure above, alcohol **23** (0.05M in ACN; 17.6 mL; 727 mg; 0.88 mmol; 1.0 eq.) was coupled with phosphoramidite **16** (1.10 g; 1.14 mmol; 1.3 eq.). Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1, v/v) yielded the title compound in 73% yield (898 mg; 0.64 mmol); IR (neat, cm^{-1}): 3032, 2938, 2865, 2377, 2312, 1717, 1700, 1560, 1540, 1457, 1264, 1096, 999, 733, 695; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.12 –

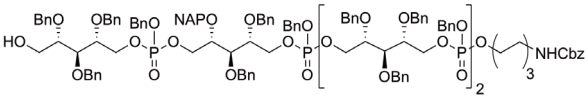
1.28 (m, 4H, CH_2-hexylspacer), 1.32 – 1.44 (m, 2H, CH_2-hexylspacer), 1.47 – 1.54 (m, 2H, CH_2-hexylspacer), 3.09 (q, 2H, *J*= 7.6 Hz, CH_2N hexylspacer), 3.62 – 3.73 (m, 3H, CH_2-Rbo, CH-Rbo), 3.75 – 3.95 (m, 7H, 5x CH-Rbo, CH_2O), 4.11 – 4.41 (m, 6H, 3x CH_2-Rbo), 4.45 – 4.67 (m, 12H, 6x CH_2-Bn), 4.88 – 5.04 (m, 4H, 2x CH_2-Bn), 5.06 (s, 2H, CH_2-Cbz), 7.20 – 7.33 (m, 45H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 24.9, 26.1, 29.7, 29.9, 30.0, 30.0, 30.0 (CH_2-hexylspacer), 40.8 (CH_2N hexylspacer), 61.0 (CH_2-Rbo), 66.4 (CH_2-Cbz), 66.5, 66.6, 66.9, 67.0, 67.0, 67.6, 67.7, 67.7, 67.7 (CH_2-Rbo), 69.0, 69.0, 69.1, 69.1, 69.1, 69.2 (CH_2O), 72.0, 72.3, 72.4, 72.5, 73.7, 73.8, 73.9, 73.9 (CH_2-Bn), 77.4, 77.5, 77.6, 77.6, 77.7, 77.8, 77.9, 77.9, 78.1, 78.2, 78.8, 78.8 (CH-Rbo), 127.6, 127.6, 127.7, 127.8, 127.8, 127.9, 127.9, 128.0, 128.0, 128.3, 128.3, 128.4, 128.4, 128.4, 128.5, 128.5, 128.5 (CH-arom), 135.7, 135.8, 135.8, 135.9, 135.9, 135.9, 135.9, 136.7, 137.7, 137.8, 137.8, 138.0, 138.0 (Cq-arom), 156.4 (C=O); ^{31}P NMR (162 MHz, $CDCl_3$) δ= 0.3, 0.1, 0.1; HRMS: $[M+H]^+$ calcd for $C_{80}H_{92}NO_{17}P_2$ 1400.58350, found 1400.58296.

D-ribitol phosphate trimer (25)



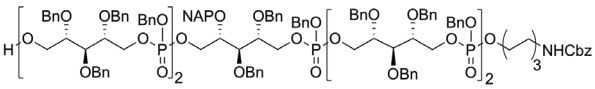
According to the general procedure above, alcohol **24** (0.05M in ACN; 7.4 mL; 522 mg; 0.37 mmol; 1.0 eq.) was coupled with phosphoramidite **14** (0.47 g; 0.44 mmol; 1.2 eq.). Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1, v/v) yielded the title compound in 96% yield (720 mg; 0.36 mmol). IR (neat, cm^{-1}): 3032, 2933, 2865, 2377, 2320, 1717, 1700, 1560, 1540, 1457, 1261, 1096, 999, 734, 695; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.13 – 1.30 (m, 4H, CH_2-hexylspacer), 1.30 – 1.42 (m, 2H, CH_2-hexylspacer), 1.47 - 1.54 (m, 2H, CH_2-hexylspacer), 2.33 (bs, 1H, O*H*), 3.09 (q, 2H, *J*= 6.9 Hz, CH_2N hexylspacer), 3.65 – 3.94 (m, 13H, 9x CH-Rbo, CH_2-Rbo, CH_2O), 4.10 – 5.03 (m, 36H, CH_2-Rbo, 11x CH_2-Bn, CH_2-NAP, CH_2-Cbz), 7.12 – 7.44 (m, 64H, CH-arom), 7.67 – 7.79 (m, 3H, CH-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 25.0, 26.1, 29.7, 29.7, 30.0, 30.0, 30.1 (CH_2-hexylspacer), 40.8 (CH_2N hexylspacer), 61.2 (CH_2-Rbo), 66.5 (CH_2-Cbz), 66.6, 66.7, 66.8, 66.9, 67.0, 67.1 (CH_2-Rbo), 67.7, 67.7 (CH_2-O), 69.0, 69.1, 69.1, 69.1, 69.2, 69.2, 72.0, 72.1, 72.4, 72.4, 72.5, 72.5, 73.7, 73.8, 73.8, 73.9, 73.9 (CH_2-Bn, CH_2-NAP), 76.8, 77.2, 77.4, 77.4, 77.5, 77.5, 77.7, 77.7, 77.8, 77.9, 77.9, 77.9, 78.0, 78.2, 78.2, 78.8, 78.9 (CH-Rbo), 125.8, 125.9, 126.1, 126.6, 127.6, 127.7, 127.7, 127.7, 127.7, 127.8, 127.8, 127.8, 127.9, 127.9, 128.0, 128.0, 128.0, 128.1, 128.1, 128.2, 128.3, 128.4, 128.4, 128.4, 128.5 (CH-arom), 132.9, 133.2, 135.5, 135.5, 135.7, 135.8, 135.9, 135.9, 136.0, 136.7, 137.7, 137.8, 137.8 (Cq-arom), 156.4 (C=O); ^{31}P NMR (162 MHz, $CDCl_3$) δ= 0.4, 0.3, 0.2, 0.1, 0.1, 0.0; HRMS: $[M+2H]^{2+}$ calcd for $C_{117}H_{130}NO_{24}P_3$ 1012.90924, found 1012.90956.

D-ribitol phosphate tetramer (26)



According to the general procedure above, alcohol **25** (0.05M in ACN; 6.9 mL; 700 mg; 0.35 mmol; 1.0 eq.) was coupled with phosphoramidite **16** (0.50 g; 0.52 mmol; 1.5 eq.). Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1, v/v) yielded the title compound in 88% yield (794 mg; 0.31 mmol). IR (neat, cm^{-1}): 3032, 2938, 2870, 2377, 2312, 1717, 1700, 1560, 1540, 1457, 1261, 1096, 1009, 736, 697; ^{1}H NMR (400 MHz, Acetone-d_6) δ= 1.21 – 1.31 (m, 4H, CH_2-hexylspacer), 1.40 – 1.50 (m, 2H, CH_2-hexylspacer), 1.53 – 1.58 (m, 2H, CH_2-hexylspacer), 3.11 (q, 2H, *J*= 6.7 Hz, CH_2N hexylspacer), 3.74 – 3.80 (m, 2H, C*H*H-Rbo, CH-Rbo), 3.85 – 4.08 (m, 14H, 11x CH-Rbo, CH_2O), 4.18 – 5.09 (m, 48H, 6.5x CH_2-Rbo, 15x CH_2-Bn, CH_2-NAP, CH_2-Cbz), 6.40 (t, 1H, *J*= 5.9 Hz, N*H*), 7.15 – 7.51 (m, 84H, CH-arom), 7.73 – 7.84 (m, 3H, CH-arom); ^{13}C-APT NMR (101 MHz, Acetone) δ= 25.7, 26.8, 29.3, 29.5, 29.6, 29.8, 30.0, 30.2, 30.4, 30.5, 30.8, 30.8 (CH_2-hexylspacer), 41.4 (CH_2N hexylspacer), 61.6 (CH_2-Rbo), 66.3 (CH_2-Cbz), 67.1, 67.3, 67.4, 67.5, 67.5, 68.0, 68.0, 68.1, 68.2, 68.2, 68.3, 68.3 (CH_2-Rbo, CH_2O), 69.4, 69.5, 69.6, 69.6, 69.7, 72.6, 72.8, 72.9, 72.9, 73.0, 73.0, 74.3, 74.3, 74.4, 74.4 (CH_2-Bn, CH_2-NAP), 78.6, 78.7, 78.8, 78.9, 78.9, 80.7, 80.8 (CH-Rbo), 126.6, 126.8, 126.9, 126.9, 127.2, 127.3, 128.1, 128.1, 128.2, 128.2, 128.2, 128.3, 128.3, 128.3, 128.4, 128.4, 128.4, 128.5, 128.5, 128.5, 128.5, 128.5, 128.6, 128.6, 128.6, 128.7, 128.7, 128.9, 129.0, 129.0, 129.0, 129.1, 129.1, 129.1, 129.2, 129.2, 129.2, 129.3 (CH-arom), 133.8, 134.1, 136.7, 137.2, 137.2, 137.3, 137.3, 137.3, 137.4, 138.5, 139.1, 139.1, 139.1, 139.2, 139.4, 139.4, 139.5, 139.7 (Cq-arom), 157.1 (C=O); ^{31}P NMR (162 MHz, Acetone) δ= 1.4, 1.4, 1.4, 1.2, 1.2, 1.2, 1.1, 1.1; HRMS: $[M+2H]^{2+}$ calcd for $C_{150}H_{165}NO_{31}P_4$ 1300.01526, found 1300.01560.

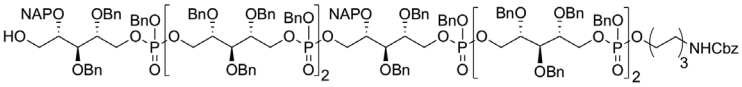
D-ribitol phosphate pentamer (27)



According to the general procedure above, alcohol **26** (0.05 M in DCM; 6.0 mL; 754 mg; 0.29 mmol; 1.0 eq.) was coupled with phosphoramidite **16** (0.48 g; 0.50 mmol; 1.7 eq.). Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1, v/v) yielded the title compound in 61% yield (564 mg; 0.18 mmol). IR (neat, cm^{-1}): 3032, 2941, 2869, 2377, 2312, 1717, 1560, 1540, 1457, 1261, 1096, 1009, 736, 695; ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.17 – 1.27 (m, 4H, CH_2-hexylspacer), 1.32 – 1.42 (m, 2H, CH_2-hexylspacer), 1.48 – 1.54 (m, 2H, CH_2-hexylspacer), 2.35 – 2.39 (bs, 1H, O*H*), 3.09 (q, 2H, *J*= 6.9 Hz, CH_2N hexylspacer), 3.65 – 3.70 (m, 3H, CH_2-Rbo, CH-Rbo), 3.71 – 3.94 (m, 16H, 14x CH-Rbo, CH_2O), 4.09 – 4.38 (m, 18H, 9x CH_2-Rbo), 4.38 – 5.13 (m, 42H, CH_2-Cbz, 19x CH_2-Bn, CH_2-NAP), 7.03 – 7.40 (m, 104H, H-arom), 7.61 – 7.72 (m, 3H, H-arom); ^{13}C-APT NMR (126 MHz, $CDCl_3$) δ= 24.9, 26.0, 29.7, 29.9, 29.9, 30.0, 30.0 (CH_2-hexylspacer), 40.8 (CH_2N hexylspacer), 61.0 (CH_2-Rbo),

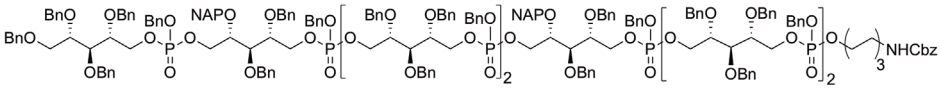
66.4 (CH_2-Cbz), 66.5, 66.5, 66.6, 66.7, 66.7, 66.8, 66.8, 66.9, 66.9, 67.0 (CH_2-Rbo), 67.6, 67.6, 67.6, 67.7 (CH_2O), 68.9, 69.0, 69.0, 69.0, 69.1, 69.1, 71.9, 72.1, 72.3, 72.3, 72.3, 72.4, 72.4, 72.5, 73.6, 73.7, 73.7, 73.7, 73.8, 73.8, 73.9 (CH_2-Bn, CH_2-NAP), 76.9, 77.2, 77.2, 77.4, 77.4, 77.5, 77.5, 77.7, 77.8, 77.8, 78.0, 78.1, 78.1, 78.7, 78.8 (CH-Rbo), 125.7, 125.8, 125.8, 126.0, 126.4, 126.5, 127.5, 127.5, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 127.9, 127.9, 127.9, 128.0, 128.0, 128.1, 128.2, 128.3, 128.3, 128.3, 128.4, 128.4, 128.4, 128.4, 128.5 (CH-arom), 132.8, 133.1, 135.3, 135.7, 135.7, 135.8, 135.8, 135.8, 135.9, 135.9, 136.6, 137.7, 137.8, 137.8, 137.9, 137.9 (Cq-arom), 156.3 (C=O); ^{31}P NMR (202 MHz, $CDCl_3$) δ= 0.4, 0.4, 0.3, 0.2, 0.1; HRMS: $[M+2H]^{2+}$ calcd for $C_{183}H_{200}NO_{38}P_5$ 1587.12128, found 1587.12108.

D-ribitol phosphate hexamer (28)



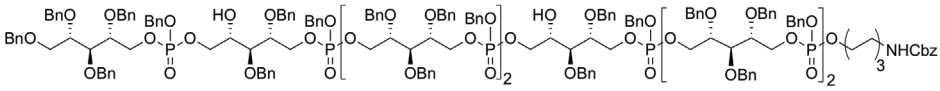
According to the general procedure above, alcohol **27** (0.15 M in ACN; 1.1 mL; 532 mg; 0.17 mmol; 1.0 eq.) was coupled with phosphoramidite **14** (0.27 g; 0.25 mmol; 1.5 eq.). Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1, v/v) yielded the title compound in 91% yield (580 mg; 0.15 mmol). IR (neat, cm^{-1}): 3032, 2945, 2870, 2377, 2312, 1717, 1560, 1540, 1457, 1266, 1096, 1012, 738, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.09 – 1.15 (m, 4H, CH_2-hexylspacer), 1.25 – 1.31 (m, 2H, CH_2-hexylspacer), 1.40 – 1.45 (m, 2H, CH_2-hexylspacer), 3.00 (q, 2H, *J*= 7.0 Hz, CH_2N hexylspacer), 3.57 – 3.86 (m, 22H, 18x CH-Rbo, CH_2-Rbo, CH_2O), 3.97 – 4.27 (m, 22H, 11x CH_2-Rbo), 4.28 – 4.92 (m, 48H, 22x CH_2-Bn, 2x CH_2-NAP), 4.97 (s, 2H, CH_2-Cbz), 6.95 – 7.35 (m, 123H, H-arom), 7.49 – 7.69 (m, 6H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 25.0, 26.1, 29.8, 30.0, 30.1 (CH_2-hexylspacer), 40.9 (CH_2N hexylspacer), 61.2 (CH_2-Rbo), 66.5 (CH_2-Cbz), 66.6, 66.8, 66.8 (CH_2-Rbo), 67.7, 67.7 (CH_2O), 69.0, 69.1, 69.1, 69.1, 69.1, 69.2, 69.2, 72.1, 72.4, 72.4, 72.4, 72.5, 73.8, 73.8, 73.9, 73.9, 74.0 (CH_2-Bn, CH_2-NAP), 77.6, 77.6, 77.6, 77.7, 77.7, 77.8, 77.9, 78.0, 78.2, 78.3, 78.8, 78.9 (CH-Rbo), 126.0, 126.0, 126.0, 126.1, 126.2, 126.5, 126.6, 126.6, 127.6, 127.6, 127.7, 127.7, 127.7, 127.7, 127.8, 127.8, 127.8, 127.9, 127.9, 128.0, 128.0, 128.0, 128.0, 128.1, 128.1, 128.3, 128.3, 128.3, 128.4, 128.4, 128.5, 128.5 (CH-arom), 132.9, 133.0, 133.2, 133.2, 135.3, 135.5, 135.8, 135.8, 135.8, 135.9, 135.9, 136.7, 137.8, 137.9 (Cq-arom), 156.4 (C=O); ^{31}P NMR (202 MHz, $CDCl_3$) δ= 0.3, 0.3, 0.2, 0.1, 0.0, 0.0, -0.1; HRMS: $[M+2H]^{2+}$ calcd for $C_{220}H_{237}NO_{45}P_6$ 1899.2351, found 1899.2278.

D-ribitol phosphate heptamer (29)



According to the general procedure above, alcohol **28** (0.10 M in ACN, 0.8 mL; 295 mg; 78.0 μmol; 1.0 eq.) was coupled with phosphoramidite **20** (87.7 mg; 0.12 mmol; 1.5 eq.). Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1, v/v) yielded the title compound in 65% yield (223 mg; 51.0 μmol). IR (neat, cm^{-1}): 3032, 2928, 2865, 2377, 2312, 1717, 1560, 1457, 1261, 1093, 1008, 737, 697; ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.15 – 1.28 (m, 4H, CH_2-hexylspacer), 1.36 – 1.41 (m, 2H, CH_2-hexylspacer), 1.50 – 1.55 (m, 2H, CH_2-hexylspacer), 3.08 – 3.11 (m, 2H, CH_2N hexylspacer), 3.58 – 3.60 (m, 2H, CH_2-Rbo), 3.67 – 3.94 (m, 23H, 21x CH-Rbo, CH_2O), 4.07 – 4.35 (m, 26H, 13x CH_2-Rbo), 4.35 – 5.12 (m, 58H, 27x CH_2-Bn, 2x CH_2-NAP), 5.07 (s, 2H, CH_2-Cbz), 7.07 – 7.41 (m, 148H, H-arom), 7.60 – 7.71 (m, 6H, H-arom); ^{13}C-APT NMR (126 MHz, $CDCl_3$) δ= 25.1, 26.2, 29.8, 29.9, 30.1, 30.1, 30.2 (CH_2-hexylspacer), 41.0 (CH_2N hexylspacer), 66.6, 66.7, 66.8, 66.9, 67.0, 67.4, 67.5 (CH_2-Cbz, CH_2-Rbo), 67.8, 67.8 (CH_2O), 69.1, 69.1, 69.1, 69.2, 69.2, 69.2, 69.3 (CH_2-Bn, CH_2-NAP), 69.9 (CH_2-Rbo), 72.5, 72.5, 72.5, 72.6, 72.7, 73.3, 73.8, 73.9, 73.9, 73.9, 73.9 (CH_2-Bn, CH_2-NAP), 77.6, 77.7, 77.7, 77.8, 77.8, 77.9, 78.0, 78.0, 78.1, 78.1, 78.2, 78.3, 78.3 (CH-Rbo), 125.9, 126.0, 126.0, 126.0, 126.0, 127.5, 127.5, 127.6, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 127.8, 127.8, 127.9, 127.9, 128.0, 128.1, 128.1, 128.4, 128.4, 128.5, 128.5, 128.6, 128.6, 128.6 (CH-arom), 133.0, 133.3, 135.5, 135.5, 135.9, 135.9, 136.0, 136.0, 136.8, 137.9, 138.0, 138.2, 138.3, 138.4, 138.6 (Cq-arom), 156.5 (C=O); ^{31}P NMR (202 MHz, $CDCl_3$) δ= 0.4, 0.3, 0.3, 0.3, 0.0, 0.0, -0.1.

D-ribitol phosphate heptamer (30)

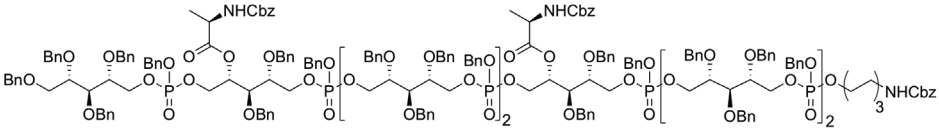


To a solution of compound **29** (60.0 mg; 13.7 μmol; 1.0 eq.) in a mixture of DCM/H_2O, *t*-BuOH (0.04 M; 0.38 mL; v/v/v/= 4/2/1) was added β-pineen (7.5 mg; 55.0 μmol; 4.0 eq.) and then DDQ (12.4 mg; 55.0 μmol; 4.0 eq.) at rt. The mixture was then warmed up in a waterbath at 40°C for 1.5 h. Then the mixture was quenched by the addition of sat. aq. $Na_2S_2O_3$, diluted in DCM and washed with a solution of sat. aq. $NaHCO_3$; NaCl (v/v= 1:1). The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by column chromatography DCM/aceton 1:0 to DCM/aceton 6:4 yielded the title compound in 52% yield (29.9 mg; 7.15 μmol). IR (neat, cm^{-1}): 3567, 2923, 2378, 2321, 1717, 1560, 1540, 1457, 1261, 1105, 1026, 741, 697; ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.16 – 1.25 (m, 4H, CH_2-hexylspacer), 1.34 – 1.44 (m, 2H, CH_2-hexylspacer), 1.54 (s, 2H, CH_2-hexylspacer), 3.07 – 3.13 (m, 2H, CH_2N hexylspacer), 3.47 – 3.59 (m, 4H, CH-Rbo),

3.59 – 3.66 (m, 2H, CH_2-Rbo), 3.69 – 3.93 (m, 19H, 17x CH-Rbo, CH_2O), 4.00 – 4.37 (m, 26H, 13x CH_2-Rbo), 4.37 – 4.68 (m, 38H, 19x CH_2-Bn), 4.83 – 5.04 (m, 16H, 8x CH_2-Bn), 5.08 (s, 2H, CH_2-Cbz), 7.09 – 7.35 (m, 140H, H-arom); ^{13}C-APT NMR (126 MHz, $CDCl_3$) δ= 25.1, 26.3, 29.8, 29.9, 30.1, 30.2, (CH_2-hexylspacer), 41.0 (CH_2N hexylspacer), 66.7, 66.9, 66.9, 66.9, 67.0, 67.1, 67.2, 67.5, 67.6, 67.6, 67.6, 67.8, 67.8, 67.8, 67.8 (CH_2-Cbz, CH_2-Rbo, CH_2O), 69.2, 69.2, 69.2, 69.2, 69.3, 69.3, 69.4, 69.5, 69.5, 69.8, 69.8, 69.8 (CH_2-Rbo, CH_2-Bn), 70.4, 70.4, 70.4 (CH-Rbo), 72.5, 72.5, 72.6, 72.6, 72.7, 73.4, 73.9, 73.9, 74.0, 74.0 (CH_2-Bn), 77.5, 77.6, 77.7, 77.7, 77.8, 77.8, 78.0, 78.1, 78.4, 78.5, 78.5 (CH-Rbo), 127.6, 127.7, 127.7, 127.7, 127.8, 127.8, 127.8, 127.8, 127.9, 127.9, 127.9, 127.9, 127.9, 127.9, 127.9, 128.0, 128.0, 128.0, 128.1, 128.1, 128.1, 128.2, 128.2, 128.4, 128.5, 128.5, 128.6, 128.6, 128.6, 128.6, 128.7 (CH-arom), 135.8, 135.8, 135.9, 135.9, 135.9, 135.9, 136.0, 136.0, 136.8, 137.8, 137.9, 137.9, 138.0, 138.0, 138.0, 138.2, 138.2, 138.3, 138.4, 138.4, 138.5, 138.5, 138.5 (CH-arom), 156.5 (C=O); ^{31}P NMR (202 MHz, $CDCl_3$) δ= 1.5, 1.5, 1.4, 1.4, 1.0, 1.0, 1.0, 0.3, 0.3, 0.1, 0.0, 0.0, -0.1, -0.1, -0.3, -0.3.

5

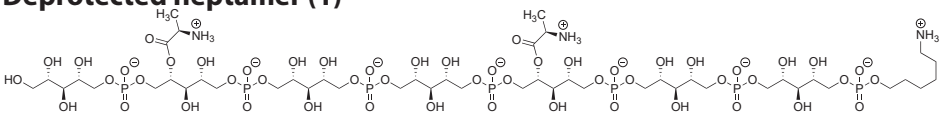
D-ribitol phosphate heptamer (31)



To a solution of diol **30** (28.0 mg; 6.7 μmol; 1.0 eq.) in DCM (0.75 mL; 8.9 mM) followed by the addition of Z-D-Ala (15 mg; 66.9 μmol; 10.0 eq.) and PyBOP (35 mg; 66.9 μmol; 10.0 eq.). Then NMI was added (5 μL; 66.9 μmol; 10.0 eq.) and the mixture was stirred for 7 days at rt under N_2 atmosphere. The mixture was then diluted with DCM, washed with sat. aq. NH_4Cl, filtrated and concentrated *in vacuo*. Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1, v/v) yielded the title compound in 48% yield (14.6 mg; 3.2 μmol). IR (neat, cm^{-1}): 3649, 3032, 2923, 2853, 2378, 2312, 1717, 1560, 1540, 1457, 1261, 1096, 1016, 738, 697; ^{1}H NMR (500 MHz, CD_3CN) δ= 1.14 – 1.40 (m, 12H, CH_2-hexylspacer, CH_3-D-Ala), 1.48 – 1.52 (s, 2H, CH_2-hexylspacer), 2.99 – 3.02 (m, 2H, CH_2N hexylspacer), 3.54 – 3.93 (m, 25H, 21x CH-Rbo, CH_2O, CH_2Rbo), 4.00 – 4.31(m, 28H, 13x CH_2-Rbo, 2x CH-D-Ala), 4.35 – 5.02 (m, 60H, 27x CH_2-Bn, 3x CH_2-Cbz), 5.41 (s, 1H, N*H*), 5.62 (s, 1H, N*H*), 6.15 (s, 1H, N*H*), 7.07 – 7.36 (m, 150H, H-arom); ^{13}C-APT NMR (126 MHz, CD_3CN) δ= 18.0 (CH_3-D-Ala), 25.8, 26.8, 30.3, 30.5, 30.8, 30.9 (CH_2-hexylspacer), 41.4 (CH_2N hexylspacer), 50.9 (CH-D-Ala), 66.6, 67.1, 67.3, 67.6, 68.1, 68.6, 68.7 (CH_2-Cbz, CH_2-Rbo, CH_2O), 70.0, 70.7, 72.9, 73.0, 73.1, 73.1, 73.1, 73.1, 73.7, 73.8, 73.8, 74.4, 74.5, 74.5, 74.5, 74.5, 74.5, 74.6 (CH_2-Bn, CH_2-Rbo), 77.9, 77.9, 78.0, 78.0, 78.2, 78.4, 78.5, 78.5, 78.6, 78.7, 78.8, 78.9, 78.9, 79.0, 79.1, 79.2, 79.2 (CH-Rbo), 128.4, 128.4, 128.5, 128.5, 128.6, 128.6, 128.6, 128.6, 128.7, 128.7, 128.7, 128.7, 128.8, 128.8, 128.8, 128.8, 128.9, 128.9, 128.9, 128.9, 129.0, 129.0, 129.0, 129.1, 129.3, 129.4, 129.4, 129.5, 129.5, 129.5, 129.6 (CH-arom), 137.1, 137.2, 137.2, 137.2, 138.0, 138.0,

138.8, 138.8, 138.9, 138.9, 139.2, 139.2, 139.2, 139.3, 139.5, 139.5, 139.7, 139.8 (Cq-arom), 156.9, 173.2 (C=O); ^{31}P NMR (202 MHz, CD_3CN) δ= 0.9, 0.8, 0.7, 0.6, 0.6, 0.3, 0.2.

Deprotected heptamer (1)



Compound **31** (12.0 mg; 2.6 μmol; 1.0 eq.) was dissolved in a mixture of dioxane/H_2O (0.9 mM; 2.9 mL; v/v/= 1:1) and 3 drops of AcOH were added. The mixture was degassed with N_2 followed by the addition of a scoop Pd black and the mixture was degassed with N_2 for the second time. Then H_2 was purged through the mixture and the mixture was left for stirring under a H_2 atmosphere for 3 days. Then mixture was purged with N_2, filtrated over a Whatman filter and concentrated *in vacuo*. The compound was lyophilized and purified using dialysis as mentioned in the general procedure yielding the product **1** in 50% yield (2.3 mg; 1.3 μmol). ^{1}H NMR (850 MHz, D_2O) δ= 1.41 – 1.46 (m, 4H, CH_2-hexylspacer), 1.59 – 1.73 (m, 10H, CH_2-hexylspacer, CH_3-D-Ala), 3.01 (t, 2H, *J*= 7.6 Hz, CH_2N hexylspacer), 3.64 – 4.12 (m, 43H, 17x CH-Rbo, 13x CH_2-Rbo), 4.17 – 4.25 (m, 4H, 2x CH-Rbo, CH_2-Rbo), 4.27 – 4.34 (m, 2H, CH-D-Ala), 5.27 – 5.29 (m, 1H, CH-Rbo), 5.45 (ddt, 1H, *J*= 7.4 Hz, 4.8 Hz, 2.8 Hz, CH-Rbo); ^{13}C-APT NMR (214 MHz, D_2O) δ= 16.0, 16.0, 16.2 (CH_3-D-Ala), 25.3, 26.0, 27.5, 30.3, 30.3 (CH_2-hexylspacer), 40.3 (CH_2N hexylspacer), 49.8, 49.8, 49.8, 49.8 (CH-D-Ala), 60.9, 61.2, 61.3, 63.2, 63.4, 64.4, 64.4, 64.4, 66.5, 66.5, 66.5, 66.8, 66.9, 66.9, 67.1, 67.1, 67.3, 67.3, 67.3, 67.3, 67.4, 67.5, 68.5 (CH_2-Rbo), 70.0, 70.1, 70.1, 71.7, 71.7, 71.7, 71.7, 71.7, 71.8, 71.8, 71.8, 71.8, 72.0, 72.0, 72.1, 72.1, 72.1, 72.1 (CH-Rbo), 72.5 (CH_2-Rbo), 72.6, 72.6, 73.0, 73.0 (CH-Rbo), 73.2 (CH_2-Rbo), 76.0, 76.7, 76.7, 76.7, 76.8, 76.8 (CH-Rbo), 170.5, 170.8, 170.9 (C=O); ^{31}P NMR (202 MHz, D_2O) δ= 2.0, 1.9, 1.8, 1.6, 1.5, 1.5, 1.4; HRMS: $[M+2H]^{2+}$ calcd for $C_{47}H_{104}N_3O_{52}P_7$ 879.68691, found 879.68626.

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6

Synthesis of *E. faecalis* wall teichoic acid fragments

INTRODUCTION

Enterococci are gram-positive bacteria that form part of the intestinal flora of both humans and animals. For a long time they have been considered as commensal and harmless but they are a source of nosocomial infections and a frequent cause of infection in critically ill patients.[1] They can cause invasive infections endocarditis, blood- and urinary tract infections in immunocompromised patients, suffering from malignancy, neutropenia, or are receiving antineoplastic chemotherapy, and immunosuppressive medication.[2-4]

Enterococcus species are the second most common pathogen causing hospital acquired infections (HAI) and these species are associated with almost 30% of transplant surgical site infections (SSIs), of which *E. faecalis* accounted for 6.4% and *E. faecium* for 14.5%. The extensive use of antibiotics led to multi-resistant strains that are difficult to treat with commonly used antibiotics causing a major health threat for hospitals and society. It has been reported that 14% of all HAIs that occurred in acute care hospitals in the US in 2014 were caused by multi-resistant bacteria. As an example, 29.5% of enterococcal infections were resistant to vancomycin (VRE).[5] The growing concern for rising antibiotic resistance urges new treatment options and vaccination with bacterial polysaccharides may be a promising way to combat these pathogens.[6-7]

The cell wall of a gram-positive bacterium is built up from a thick peptidoglycan layer which is decorated with anionic carbohydrate-based polymers called teichoic acids (TAs). These teichoic acids are built up from repeating glycerol- and ribitol phosphate units, which in turn are substituted with carbohydrates or D-alanyl ester residues along the chain and this substitution pattern seems to occur randomly. TAs occur in two types: wall teichoic acids (WTAs) that are covalently attached to the peptidoglycan, and lipoteichoic acids (LTAs), anchored in the lipid bilayer and these teichoic acids have a variety of functions within the cell envelope,[8] such as autolysin activity, cell division, scaffolding of surface proteins, cation homeostasis and attachment to host cell and abiotic surfaces.[9-10] The cell wall polymers of *E. faecalis* were found to be critical for resistance to complement activation via mannose-binding lectin.[11]

WTA isolation from bacterial sources delivers heterogenous mixtures potentially contaminated with bacterial impurities. Organic synthesis, on the other hand is a powerful tool to generate WTA fragments with a defined length and substitution pattern of choice, allowing the detailed study of their immunological properties by probing interactions with biomolecules for their possible incorporation as antigens for future vaccine applications. The group of Theilacker elucidated the structures of teichoic acids of *E. faecalis* V583 wild type strains, the first vancomycin-resistant isolate from a human bloodstream infection, and one of the structures is presented in Fig 1A.[11-12] It is a structurally more complex WTA than the commonly encountered polyribitol phosphates and is composed of repeating units built up from a *N*-acetyl-β-D-galactosaminyl ribitol phosphate residues having an α-L-rhamnose branch at the C3 of the galactosamine residue. This Chapter reports on the assembly of *E. faecalis* WTA structures including a mono- and dimer repeat bearing an aminohexanol spacer as ligation handle for future conjugation purposes.

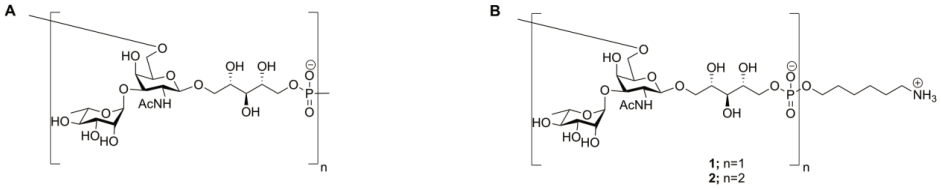


Figure 1. Structure of *E. faecalis* WTA (A) and the target structures of this Chapter (B).

RESULTS & DISCUSSION

The previous Chapters described WTA syntheses based on well-established DNA chemistry, utilizing phosphoramidite chemistry for the installation of phosphate moieties. This Chapter will adopt this chemistry as well and Figure 2 shows the retrosynthetic analysis for the assembly of WTA fragments **1** and **2**. The dimer **2** will be generated using pseudo-trisaccharide **3**, which can be assembled from monomeric building blocks **5**, **6** and **7**. Donor **6** bears a benzoyl at the C-2 position that assist in the stereoselective formation of the desired α-glycosidic linkage to the galactosamine. To minimize protecting group manipulations, diol **5** was chosen to use, as the difference in reactivity between the equatorial and the axial alcohol can be exploited in a regioselective glycosylation reaction, preferentially occurring at the desired equatorial site. The β-galactosamine linkage was introduced using a trichloroacetyl (TCA) protecting group on the amine, as the use of an acetamide could lead to oxazoline formation during the glycosylation. While in the previous Chapters a DMTr ether was used as protecting group for the alcohol to be elongated, it was here chosen to use the more stable TBDPS protecting group, as it can resist the required (Lewis) acidic glycosylation reaction conditions.

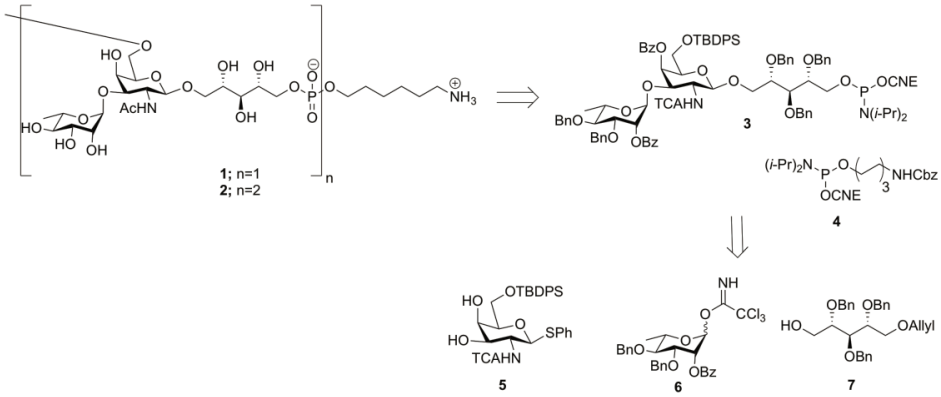
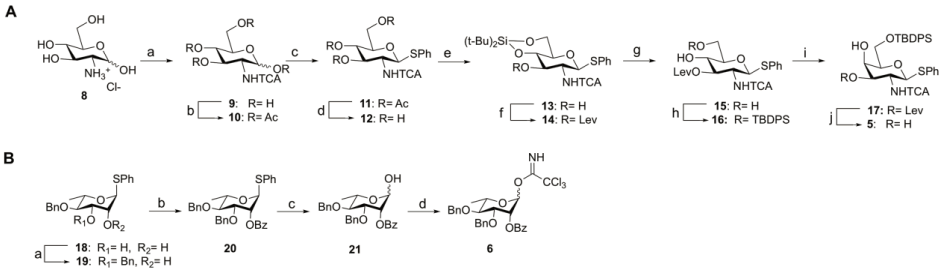


Figure 2. Retrosynthetic analysis for the assembly of WTA fragments.

The synthesis of the building blocks **5** and **6**, required for the assembly of intermediate **3**, is shown in Scheme 1. Ribitol **7** was obtained as described in previous Chapters. The synthesis of building block **5** started from D-glucosamine by trichloroacetylation of the amine to give compound **9** (Scheme 1A). The route was continued with acetylation to give **10**. Thiophenylation followed by deacetylation then gave triol **12**. The C-4 and C-6-OH were protected with a silylidene group followed by a placement of a levulinoyl ester on the C-3, after which the silylidene was cleaved off using HF in pyridine. The primary alcohol was protected with a TBDPS and at this stage the stereochemistry of the C-4 position was inverted[13] by triflation of the hydroxyl, and subsequent treatment with $NaNO_2$ giving **17** in 68% yield. Removal of the levulinoyl liberated the C-3-hydroxyl for glycosylation with rhamnosyl donor **6**. The rhamnose building block was synthesized from known diol **18**[14-15] (Scheme 1B). Regioselective benzylation of **18** using cyclic tin ketal chemistry[16] gave **19** and subsequent benzoylation on the C-2-OH then provided compound **20**. Hemiacetal **21** was formed in 80% by NBS driven hydrolysis and subsequent treatment with trichloroacetonitrile (TCAN) and K_2CO_3 gave imidate **6** in 83% yield.

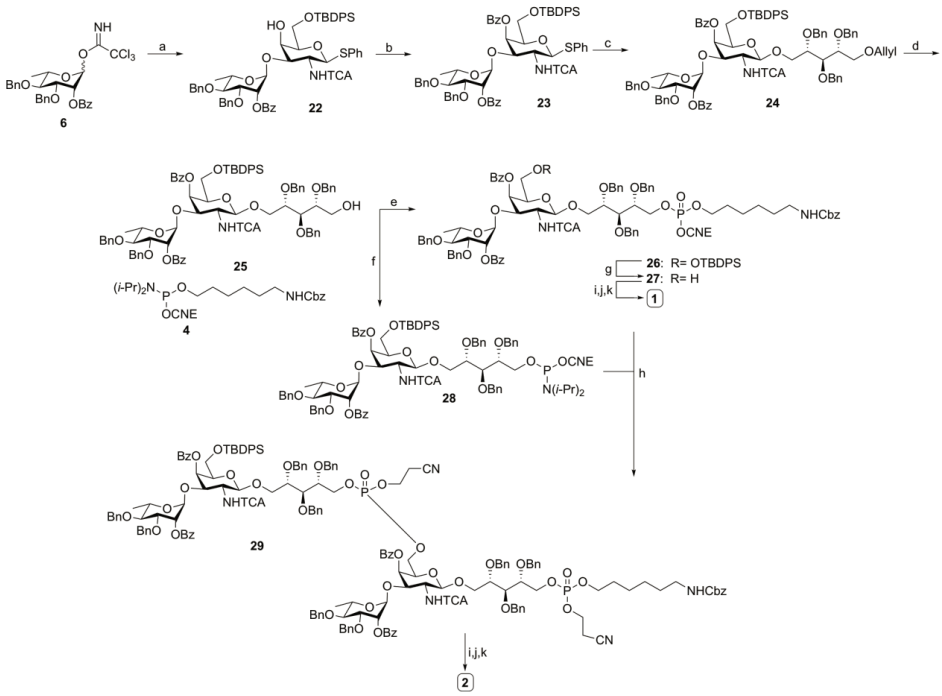


Scheme 1. A Building block synthesis; *Reagents and conditions:* a) TCACl, TEA, MeOH 0ºC to rt, 86%; b) Ac_2O, pyr 0ºC to rt, 57%; c) C_6H_5SH, $BF_3.Et_2O$, DCM, -40ºC to rt, 57%; d) NaOMe, MeOH, rt, quant.; e) di-*tert*-butylsilanediyl bistriflate, DMF, -40ºC, 40%; f) LevOH, EDC, DMAP, DCM, quant.; g) HF/pyridine, THF, 81%; h) TBDPSCl, TEA, DCM, 57%; i) i. Tf_2O, pyr, DCM, ii. 10 eq $NaNO_2$, DMF, rt, 2 hours, 68%; j) Hydrazine, DCM, 84%.
B Building block synthesis; *Reagents and conditions:* a) i. Bu_2SnO, toluene, reflux; ii CsF, BnBr, DMF, 86%; b) BzCl, pyr, quant; c) NBS, THF/water, 80%; d) TCAN, K_2CO_3, DCM, 0ºC to rt, 83%.

With all building blocks in hand, the WTA repeat unit was assembled. Coupling of donor **6** at 0ºC with diol acceptor **5** gave disaccharide **22** in 36% (Scheme 2). After the work up, some acceptor was recovered but no C-4-glycosylated product was observed. The disaccharide was benzoylated in the next step to mask the axial alcohol position. Coupling of disaccharide **23** with ribitol **7** then gave pseudo-trisaccharide **24** in 45% yield. Iridium catalyzed isomerization of the allyl ether followed by iodine mediated enol ether hydrolysis provided alcohol **25**, which was equipped with a phosphoramidite moiety giving building block **28.** Beside, coupling of alcohol **25** and spacer phosphoramidite **4** under activation of 4,5-dicyanoimidazole (DCI) followed by oxidation of the phosphite intermediate using (10-camphorsulfonyl)oxaziridine (CSO) gave **26** in 68% yield**.** Cleavage

of the TBDPS group using HF in pyridine gave building block **27** containing an alcohol function for the assembly of the WTA-dimer. The two trisaccharide building blocks **27** and **28** were condensed using DCI as activation agent forming the phosphite intermediate, which in turn was oxidized *in situ* using CSO to give the fully protected dimer **29**.

Dimer **29** was subjected to a deprotection sequence consisting of TBDPS removal, followed by elimination of the cyanoethyl groups, benzoyl hydrolysis and finally hydrogenation of the benzyl group and concomitant transformation of the TCA groups into the acetamides. After this deprotection sequence, the crude compound **2** was purified using HW-40 Sephadex size exclusion chromatography, providing two compounds having a different retention time. The first compound corresponds to be target dimer **2** (2.9 mg) while the longer retention time of the second compound (6.5 mg) suggested that it could be a smaller product. During the reduction of the TCA groups, HCl was formed which might have caused hydrolysis of the glycosidic linkages. NMR analysis of the first compound showed the presence of minor aromatic residues and therefore this product was subjected to a second hydrogenation reaction using 4 eq. of $NaHCO_3$



Scheme 2. WTA assembly; *Reagents and conditions:* a) **5**, TMSOTf, DCM, 0ºC, 36%; b) BzCl 20 eq, DMAP, pyr, 65 ºC, quant; c) **7**, NIS, TMSOTf, DCM, 0ºC, 45%; d) i. $Ir(COD)(Ph_2MeP)_2PF_6$, H_2, THF; ii. I_2, sat. aq. $NaHCO_3$, THF, 54%; e) i. **4**, DCI, ACN; ii. CSO, 68%; f) CNEO-P-(N-(*i*-Pr_2))$_2$, tetrazole salt, 70%; g) HF, pyr, DCM, 81%; h) i. DCI, ACN; ii. CSO, 45%; i) HF/pyr, DCM; j) NH_4OH/dioxane; k) Pd black/ H_2, dioxane/H_2O **1**: 32%, **2**: 17%. CNE: cyanoethyl.

delivering 1,5 mg of pure target dimer (1.5 mg; 17%) after a size exclusion purification step. The protecting groups in monomer **27** were globally removed using the following sequence of reactions: first, NH_4OH in dioxane removed the cyanoethyl group, and next the benzoyls were cleaved using NaOMe in methanol. Removal of the benzyl ethers and reduction of the TCA group was accomplished by hydrogenation in the presence of 2 eq. $NaHCO_3$ to prevent cleavage of the glycosidic linkages. The target monomer **1** was obtained in 32% yield.

CONCLUSION

This Chapter reports on the exploration of a route of synthesis towards two *E. faecalis* WTA-fragments. Both a monomeric repeating unit **1** and a dimer of two repeating units **2** were assembled. The dimer was obtained using phosphoramidite chemistry to couple two trisaccharide repeating units. The individual building blocks to generate the protected trisaccharide repeating unit were synthesized in good yields, but the reactions to assemble this trimer proceeded in moderate yield and require further optimization. The global deprotection also proved sub-optimal, as cleavage of glycosidic linkages was observed, likely as the result of HCl that was released upon reduction of the TCA-group. The addition of an appropriate base to neutralize the generated acid can prevent this undesirable side-reaction. At present, low amounts of the WTA fragments, (1.1 mg and 1.5 mg of the mono- and dimer repeats) were obtained, which can be sufficient for initial biological evaluation. In principle the strategy developed here can be used to generate longer fragments, which can then be evaluated as synthetic antigens for vaccine development.

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 aluminum 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 $(NH4)_4Ce(SO_4)_4{\cdot}2H_2O$ 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).

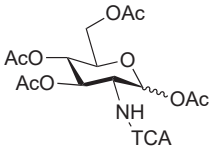
Phosphoramidite coupling and oxidation

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

General procedure for global deprotection

The oligomer was dissolved in a 1:1 solution of NH_3 (30-33% aqueous solution) and dioxane (1.2-2.4 mM) and stirred overnight. The mixture was concentrated *in vacuo* and loaded on a Dowex Na^+ cation-exchange resin (50WX4-200, stored on 0.5 M NaOH, flushed with H_2O and MeOH before use) column and flushed with water/dioxane (1:1). The fractions were then concentrated *in vacuo*, dissolved in water/dioxane (2 ml per 10 µmol) and 4 drops of glacial AcOH were added. After purging the mixture with argon, Pd black was added (32-59 mg), and the mixture was repurged with N_2. The mixture was stirred under hydrogen gas for 3 - 7 days, filtered over celite, and concentrated *in vacuo*. The crude product was purified by size-exclusion chromatography (Toyopearl HW-40, NH_4OAc buffer) and the fractions were concentrated. The product was co-evaporated repeatedly with MilliQ water to remove NH_4OAc/ NH_4HCO_3 traces and eluted through a Dowex Na^+ cation-exchange resin column, and lyophilized.

1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloroacetamide-α/β-D-glucopyranoside (10)

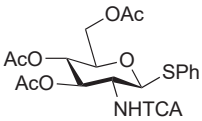


D-glucosamine hydrochloride **8** (42.9 g, 200 mmol, 1.0 eq.) was suspended in MeOH (343 mL, 0.9M) and TEA (83.2 mL, 1 mol, 5.0 eq.) was added. The suspension was cooled to 0°C and trichloroacetylchloride (24.7 mL, 220 mmol, 1.1 eq.) was added dropwise to the solution. It was allowed to warm up to rt and vigorously stirring was continued for 3 days. The reaction mixture was then filtered over silica and concentrated. The intermediate was obtained as yellow solid. The intermediate (65.5 g, 200 mmol, 1.0 eq.) was dissolved in pyridine (454 mL, 0.44M) and cooled to 0°C. Acetic anhydride (113.4 mL, 1.2 mol, 6.0 eq.) was slowly added and the solution was left stirring at rt overnight. The reaction mixture was quenched with MeOH (80 mL) at 0°C and dissolved in EtOAc. The solution was washed with HCl (3M, until pH was acidic), sat. aq. $NaHCO_3$ (2x 200 mL) and sat. aq. NaCl (200 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated. The crude was dissolved in EtOH and heated up in order to crystallize. Compound **10** was obtained as white crystals (50.12 g, 101.6 mmol, 51%, α-product). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 2.07 (s, 6H, 2x C*H*$_3$-Acetyl), 2.11 (s, 3H, C*H*$_3$-Acetyl), 2.20 (s, 3H, C*H*$_3$-Acetyl), 4.02 - 4.11 (m, 2H, H-5, H-6), 4.29 - 4.38 (m, 2H, H-2, H-6), 5.26 (t, 1H, *J* = 9.7 Hz, H-4), 5.36 (t, 1H, *J* = 10.2 Hz, H-3), 6.32 (d, 1H, *J* = 3.7 Hz, H-1), 6.81 (d, 1H, *J* = 8.4 Hz, NH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ = 53.5 (C-2), 61.5 (C-6), 67.1 (C-4), 70.0 (C-5), 70.2 (C-3), 89.7 (C-1).

The motherlayer was concentrated under reduced pressure and dissolved in EtOAc. Celite was added to the solution and the solvent was removed *in vacuo*. The pulver was purified over silica column (10% to 60% EtOAc in PE). Compound **10** was obtained as

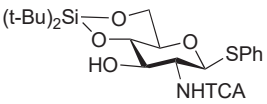
white solid (34.8 g, 70.6 mmol, 35%) as an α/β mixture with a ratio of 54/46. ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 2.06 (m, 14H, C*H*$_3$-Acetyl), 2.09 - 2.15 (m, 11H, C*H*$_3$-Acetyl), 2.20 (s, 3H, C*H*$_3$-Acetyl), 3.84 - 4.19 (m, 4H, 2x H-5, 2x H-6), 4.22 - 4.40 (m, 4H, 2x H-2, 2x H-6), 5.15 - 5.41 (m, 4H, 2x H-3, 2x H-4), 5.80 (d, 1H, *J*= 8.7 Hz, H-1 β), 6.31 (d, *J*= 3.7 Hz, H-1α), 6.82 (d, 1H, *J*= 8.5 Hz, -N*H* β), 6.91 (d, 1H, *J*= 9.4 Hz, -NH α); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 53.5, 54.9 (2x C-2), 61.5, 61.7 (2x C-6), 67.1, 67.6, 70.0, 70.2, 71.8, 73.3 (2x C-3, 2x C-5, 2x C-4), 89.7 (C-2 β), 92.2 (C-1α). The overall yield is 69.8 g (172.2 mmol, 86%) and the anomeric ratio is α/β is 81:19.

3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-2-(2,2,2-trichloroacetamide)-1-β-D-phenyl-glucopyranoside (11)



Compound **10** (84.93 g, 172.17 mmol, 1.0 eq.) was dissolved in dry DCM (603 mL, 0.3M). Thiophenol (17.6 mL, 172.2 mmol, 1.0 eq.) and $BF_3{\cdot}OEt_2$ (65.5 mL, 516.5 mmol, 3.0 eq.) were added to the solution and stirred over night at rt. The reaction mixture was quenched with Et_3N and washed with sodium bicarbonate (3x), NaOH (1M, 3x) and brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated. The residue was purified over a silica column (pentane/EtOAc, 9/1 to 6/4). Compound **11** was obtained as a yellow solid (53.2 g, 98.0 mmol, 57%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 2.00 (s, 3H, CH_3-Acetyl), 2.01 (s, 3H, CH_3-Acetyl), 2.10 (s, 3H, CH_3-Acetyl), 3.76 (m, 1H, H-5), 4.05 - 3.95 (m, 1H, H-2), 4.22 (m, 2H, H-6), 4.86 (d, 1H, *J*= 10.4 Hz, H-1), 5.09 (t, 1H, *J*= 9.7 Hz, H-4), 5.31 (dd, 1H, *J*= 10.5 Hz, *J*= 9.5 Hz, H-3), 6.82 (d, 1H, *J*= 9.1 Hz, N*H*), 7.37 - 7.29 (m, 3H, H-arom), 7.52 (dd, 2H, *J*= 7.5 Hz, *J*= 2.0 Hz, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 20.7 (CH_3-Acetyl), 20.9 (CH_3-Acetyl), 54.8 (C-2), 62.3 (C-6), 68.2 (C-4), 73.1 (C-3), 76.2 (C-5), 86.6 (C-1), 128.8, 129.2, 135.5, (C-arom), 171.0 (C=O, Acetyl); HRMS: $[M+Na]^+$ calcd for $C_{20}H_{22}Cl_3NO_8SNa$ 564.00239, found 564.00260.

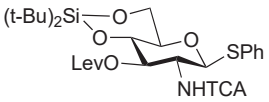
4,6-*O*-*tert*-butylsilylanediyl-2-deoxy-1-thio-2-(2,2,2-trichloroacetamide)-β-D-phenyl-glucopyranoside (13)



Compound **12** (50.6 g, 93.2 mmol, 1.0 eq.) was suspended in dry MeOH and NaOMe (5.5 g, 102.5 mmol, 1.1 eq.) and stirred for 2 days. The reaction mixture was quenched with Amberlite H^+ resin IR-120 and filtered off. The solvent was removed *in vacuo*. The crude (18.2 g, mmol, 43.6 mmol, 1.0 eq.) was dissolved in DMF (450 mL, 0.1M) and cooled to -40°C. Di-*tert*-butylsilanediyl bistriflate (13.5 mL, 41.4 mmol, 0.95 eq.) was added dropwise and stirring was continued for 1h and then the reaction mixture was quenched with pyridine (10.5 mL, 130.8 mmol, 3.0 eq.). Thereafter, the mixture was diluted in Et_2O (500 mL) and washed with water (5x 200 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated. The residue was purified over

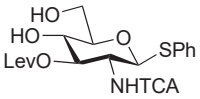
silica column (PE/EtOAc 1/0 to 7/3) yielding compound **13** as a white solid (9.8 g, 17.6 mmol, 40%). IR (neat): 2936, 2859, 1767, 1684, 1528, 1474, 1242, 1069, 823, 748, 656; $[\alpha]_D^{20}$ -14.4° (*c* 0.63, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 0.98 (s, 9H, *t*-Bu), 1.08 - 1.02 (m, 16H, *t*-Bu), 3.57 - 3.47 (m, 2H, H-2, H-5), 3.71 - 3.65 (m, 1H, H-3), 3.93 (t, 1H, *J*= 10.2 Hz, H-6), 4.04 (t, 1H, *J*= 9.6 Hz, H-3), 4.23 (dd, 1H, *J*= 10.2 Hz, *J*= 5.1 Hz, H-6), 5.17 (d, 1H, *J*= 10.2 Hz, H-1), 6.86 (d, 1H, *J*= 7.8 Hz, -N*H*), 7.35 - 7.30 (m, 3H, H-arom), 7.49 (dd, 2H, *J*= 6.6 Hz, *J*= 3.1 Hz, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 27.1 (*t*-Bu), 27.6 (*t*-Bu), 56.9 (C-2), 66.1 (C-6), 74.2 (C-3), 74.6 (C-5), 77.7 (C-4), 85.6 (C-1), 110.1 (CCl_3), 128.7 - 133.4 (C-arom); HRMS: $[M+H]^+$ calcd for $C_{22}H_{33}Cl_3NO_5SSi$ 556.09088, found 556.09088.

4,6-*O*-*tert*-butylsilylanediyl-2-deoxy-4-*O*-levulinoyl-1-thio-2-(2,2,2-trichloroacetamide)-β-D-phenyl-glucopyranoside (14)



Compound **13** (9.83 g, 17.6 mmol, 1.0 eq.) was dissolved in DCM (110 mL, 0.16M) and was cooled to 0°C. Levulinic acid (5.0 mL, 49.3 mmol, 2.8 eq.) was added, followed by DIC (3.8 mL, 24.6 mmol, 1.4 eq.) and catalytic amount of DMAP (0.1 g, 0.9 mmol, 0.05 eq.). Stirring at 0°C was continued for 4h and at rt overnight. The reaction mixture was then filtered over celite and concentrated and the residue was purified over silica column (PE/EtOAc 1/0 to 6/4). Compound **14** was obtained as a colourless solid (11.9 g, 18.1 mmol, quant.). IR (neat): 3327, 2934, 1721, 1526, 1474, 1169, 1072, 764, 654; $[\alpha]_D^{20}$ -26.1° (c 0.88, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 0.95 (s, 9H, *t*-Bu), 1.05 (d, 13H, *J*= 7.6 Hz, *t*-Bu), 2.15 (s, 3H, CH_3-Lev), 2.59 (t, 2H, *J*= 7.1 Hz, CH_2-Lev), 2.72 (t, 2H, *J*= 7.0 Hz, CH_2-Lev), 3.53 (m, 2H, H-5), 3.99 - 3.87 (m, 3H, H-6, H-4, H-2), 4.24 (dd, 1H, *J*= 10.3 Hz, *J*= 5.1 Hz, H-6), 4.91 (d, 1H, *J*= 10.4 Hz, H-1), 5.18 (dd, 1H, *J*= 10.2 Hz, 9.2 Hz, H-3), 6.88 (d, 1H, *J*= 9.1 Hz, -N*H*), 7.35 - 7.30 (m, 3H, H-arom), 7.47 (dd, 2H, *J*= 6.5 Hz, *J*= 3.1 Hz, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 27.0, 27.5 (*t*-Bu), 28.1 (CH_2 Lev), 29.9 (CH_3 Lev), 38.2 (CH_2 Lev), 54.8 (C-2), 66.2 (C-6), 74.7 (C-4), 75.1 (C-3), 75.2 (C-5), 87.5 (C-1), 128.6, 129.3, 132.1, 133.2 (C-arom), 172.8 (C=O Lev), 206.0 (C=O Lev).

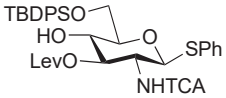
2-deoxy-4-*O*-levulinoyl-1-thio-2-(2,2,2-trichloroacetamide)-β-D-phenyl-glucopyranoside (15)



Compound **14** (1.01 g, 1.54 mmol, 1.0 eq.) was dissolved in THF (15.5 mL, 0.1M) and HF.pyridine (0.12 mL, 4.62 mmol, 3.0 eq.) was added. The solution was stirred for 1.5h at rt. Thereafter, the reaction mixture was diluted in EtOAc (15 mL), washed with water (15 mL) and brine (15 mL). The organic layer was then dried over $MgSO_4$, filtrated and concentrated. The residue was purified over silica column (PE/EtOAC, 4/6 to 1/9) yielding compound **15** as a white solid (0.64 g, 1.24 mmol, 81%). IR (neat) 3335, 2924, 1705, 1526, 1159, 1047, 822, 748; $[\alpha]_D^{20}$ -32.2° (*c* 1.22, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 2.15 (s, 3H,

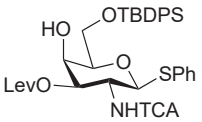
CH_3 Lev), 2.39 - 2.61 (m, 2H, CH_2 Lev), 2.70 - 2.88 (m, 2H, CH_2 Lev), 3.55 - 3.60 (m, 1H, H-5), 3.63 (s, 1H, OH), 3.75 - 3.88 (m, 2H, H-6, H-4), 3.94 - 4.04 (m, 2H, H-2, H-6), 4.89 (d, 1H, *J*= 10.4 Hz, H-1), 5.23 (dd, 1H, *J*= 10.3 Hz, *J*= 9.1 Hz, H-3), 7.04 (d, 1H, *J*= 9.3 Hz, N*H*), 7.32 (dd, 3H, *J*= 4.9 Hz, *J*= 1.8 Hz, H-arom), 7.47 (dd, 2H, *J*= 6.6 Hz, *J*= 3.0 Hz, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 28.4, 38.6 (CH_2 Lev), 54.4 (C-2), 62.6 (C-6), 69.5 (C-4), 76.7 (C-3), 79.6 (C-5), 86.5 (C-1), 128.4, 129.3, 132.7 (C-arom).

2-deoxy-4-*O*-levulinoyl-6-*O*-*tert*-butyl-diphenylsilyl-1-thio-2-(2,2,2-trichloroacetamide)-β-D-phenyl-glucopyranoside (16)



A solution of compound **15** (6.5 g, 12.7 mmol, 1.0 eq.) in DCM (127 mL, 0.1M) was cooled to 0°C. Triethylamine (10.6 mL, 76.2 mmol, 6.0 eq.) was added followed by dropwise addition of *tert*-butyl(chloro)diphenylsilane (4.3 mL, 16.5 mmol, 1.3 eq.) and the reaction was stirred for 3 days at rt. The reaction mixture was then quenched with MeOH, diluted in DCM and washed with sat. aq. $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtrated and concentrated. The residue was purified over silica column (PE/EtOAc, 9/1 to 7/3) affording compound **16** as a solid (5.5 g, 7.2 mmol, 57%). IR (neat): 3345, 2930, 1717, 1522, 1157, 1113, 1069, 822, 743, 702; $[\alpha]_D^{20}$ -18.3° (*c* 0.73, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 1.07 (s, 9H, *t*-Bu), 2.10 (s, 3H, CH_3 Lev), 2.41 - 2.59 (m, 2H, CH_2 Lev), 2.74 (t, 2H, *J*= 6.6 Hz, CH_2 Lev), 3.57 (m, 1H, H-5), 3.83 (t, 1H, *J*= 9.3 Hz, H-4), 3.90 - 4.02 (m, 3H, H-2, H-6), 4.83 (d, 1H, *J*= 10.3 Hz, H-1), 5.25 (dd, 1H, *J*= 10.2 Hz, *J*= 9.2 Hz, H-3), 7.02 (d, 1H, *J*= 9.3 Hz, -N*H*), 7.13 - 7.28 (m, 11H, H-arom), 7.33 - 7.44 (m, 6H, H-arom), 7.51 (dd, 2H, *J*= 7.7 Hz, *J*= 1.7 Hz, H-arom), 7.72 (m, 4H, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 26.9 (*t*-Bu), 28.4 (CH_2 Lev), 29.8 (CH_3 Lev), 38.5 (CH_2 Lev), 54.2 (C-2), 63.6 (C-6), 69.4 (C-4), 76.9 (C-3), 79.8 (C-5), 86.3 (C-1), 125.4 - 135.8 (C-arom), 161.8 (C=O), 173.5, 207.9 (2x C=O Lev); HRMS: $[M+Na]^+$ calcd for $C_{35}H_{40}Cl_3NO_7SSiNa$ 774.12525, found 774.12547.

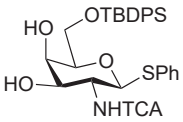
2-deoxy-4-*O*-levulinoyl-6-*O*-*tert*-butyl-diphenylsilyl-1-thio-2-(2,2,2-trichloroacetamide)-β-D-phenyl-glalactopyranoside (17)



A stirred solution of trifluoroacetic anhydride (TFAA) (2.4 g, 14.5 mmol, 2.0 eq.) in dry DCM (23.4 mL, 0.62M) was cooled to -18°C. A solution of pyridine (2.3 mL, 29.0 mmol, 4.0 eq.) in dry DCM (0.8 mL, 36.3M) was added dropwise followed by a solution of compound **16** (5.5 g, 7.2 mmol, 1.0 eq.) in dry DCM (35 mL). After 30 min stirring the reaction mixture was diluted in DCM (280 mL), washed with 2M HCl (200 mL), saturated aqueous $NaHCO_3$ (200 mL) and water (200 mL), dried over $MgSO_4$, filtrated and concentrated. $NaNO_2$ (5.4 g, 78.9 mmol, 10.9 eq.) in DMF (9.3 mL) was added at rt to the residue containing the triflate-intermediate and stirred overnight. Insoluble material was filtered off and washed with DCM. The filtrate and washings were combined and washed with

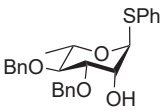
water, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was purified over silica column (PE/EtOAc, 4/1 to 1/1). Compound **17** was obtained as a white solid (3.7 g, 4.9 mmol, 68%). IR (neat): 3322, 2930, 2489, 1717, 1526, 1152, 1113, 822, 743, 702; $[\alpha]_D^{20}$ +12.0° (*c* 1.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 1.06 (s, 9H, *t*-Bu), 2.15 (s, 3H, CH_3 Lev), 2.49 - 2.67 (m, 2H, CH_2 Lev), 2.74 (t, 2H, *J*= 6.3 Hz, CH_2 Lev), 3.25 (d, 1H, *J*= 3.5 Hz, 4-OH), 3.66 (t, 1H, *J*= 4.8 Hz, H-5), 3.96 (m, 2H, H-6), 4.34 - 4.22 (m, 2H, H-2, H-4), 4.97 (d, 1H, *J*= 10.4 Hz, H-1), 5.19 (dd, 1H, *J*= 10.6 Hz, *J*= 2.9 Hz, H-3), 6.78 (d, 1H, *J*= 8.9 Hz, N*H*), 7.35 - 7.47 (m, 7H, H-arom), 7.25 (s, 4H, arom.), 7.52 (dd, 2H, *J*= 7.5 Hz, *J*= 1.9 Hz, H-arom), 7.70 (m, 4H, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 26.9 (*t*Bu), 28.2 (CH_2 Lev), 29.9 (CH_3 Lev), 38.1 (CH_2 Lev), 51.3 (C-2), 64.2 (C-6), 67.8 (C-4), 73.7 (C-3), 78.1 (C-5), 86.7 (C-1), 128.0, 128.2, 129.2, 130.1, 132.6, 132.8, 135.7, 135.8 (C-arom.), 161.8 (C=O), 172.5 (C=O Lev), 207.2 (C=O Lev); HRMS: $[M+Na]^+$ calcd for $C_{35}H_{40}Cl_3NO_7SSiNa$ 774.12525, found 774.12547.

2-deoxy-6-*O*-*tert*-butyl-diphenylsilyl-1-thio-2-(2,2,2-trichloroacetamide)-β-D-phenyl-galactopyranoside (5)



Compound **17** (3.7 g, 4.9 mmol, 1.0 eq.) was dissolved in AcOH/pyridine (49 mL, 1/4, 0.1M) and a hydrazine solution (0.26 mL, 5.4 mmol, 1.1 eq.) was added. After 30 min the reaction mixture was quenched with acetone, diluted in EtOAc, washed with 1M HCl, sat. aq. sodium bicarbonate, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was purified over silica column (PE/EtOAc, 9/1 to 1/1) affording compound **5** as a white foam (2.7 g, 4.1 mmol, 84%). IR (neat): 3327, 2930, 1694, 1526, 1427, 822, 741, 702; $[\alpha]_D^{20}$ +6.9° (*c* 1.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 1.06 (s, 9H, *t*-Bu), 3.32 (d, 1H, *J*= 6.4 Hz, 3-OH), 3.37 - 3.45 (m, 1H, 4-OH), 3.58 (t, 1H, *J*= 4.8 Hz, H-5), 3.80 - 3.98 (m, 4H, H-6, H-3, H-2), 4.12 (s, 1H, H-4), 4.93 (d, 1H, *J*= 10.1 Hz, H-1), 7.00 (d, 1H, *J*= 7.5 Hz, N*H*), 7.13 - 7.28 (m, 5H, H-arom), 7.35 - 7.46 (m, 6H, H-arom), 7.52 (dd, 2H, *J*= 7.5 Hz, *J*= 1.9 Hz, H-arom), 7.71 (m, 4H, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 26.5 (*t*-Bu), 54.5 (C-2), 64.4 (C-6), 69.6 (C-4), 72.3 (C-3), 78.1 (C-5), 85.8 (C-1), 128.0, 128.2, 129.2, 130.1, 132.4, 132.6, 132.7, 132.8, 135.7,135.8 (C-arom), 162.6 (C=O).

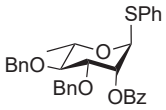
Phenyl 3,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (19)



Diol **18** (3.7 g, 10.8 mmol, 1.0 eq.) was dissolved in toluene (108 mL, 0.1M). Dibutyl tin oxide (3.2 g, 13.0 mmol, 1.2 eq.) was added and the reaction mixture was refluxed at 111°C. The yellowish clear solution obtained after 3 hours was concentrated and redissolved in dry DMF (108 mL, 0.1M). Cesium fluoride (3.3 g, 21.6 mmol, 2.0 eq.) and benzyl bromide (1.7 mL, 14.0 mmol, 1.3 eq.) were added and the mixture was stirred overnight. The reaction mixture was diluted in Et_2O, washed with water and brine and dried over $MgSO_4$, filtered

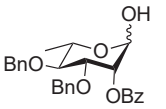
and concentrated. The residue was purified over a silica column (PE/EtOAc, 1/0 to 13/7). Compound **18** was obtained as colourless oil (3.9 g, 9.0 mmol, 83%). Analytical data are identical to literature precendence.[16] HRMS: $[M+Na]^+$ calcd for $C_{26}H_{28}O_4SNa$ 459.16005, found 459.15995.

Phenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (20)



Compound **19** (3.9 g, 9.0 mmol, 1.0 eq.) was dissolved in DCM/pyridine (36 mL, 4:1, 0.2M) and benzoyl chloride (3.1 mL, 27.0 mmol, 3.0 eq.) was slowly added followed by DMAP (0.22 g, 1.8 mmol, 0.2 eq.). After 3 hours the reaction mixture was quenched with MeOH, diluted in DCM and washed with 1M HCl (2x) sat. aq. $NaHCO_3$ and sat. aq. NaCl. The solution was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified over silica column (PE/EtOAc, 1/0 to 17/3) yielding the title compound as colourless oil (6.6 g, quant.). ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 1.39 (d, 3H, *J*= 6.2 Hz, H-6), 3.64 (t, 1H, *J*= 9.4 Hz, H-4), 4.04 (dd, 1H, *J*= 9.3 Hz, *J*= 3.1 Hz, H-3), 4.35 - 4.26 (m, 1H, H-5), 4.59 (d, 1H, *J*= 11.3 Hz, CH_2 Bn), 4.66 (d, 1H, *J*= 10.9 Hz, CH_2 Bn), 4.80 (d, 1H, *J*= 11.3 Hz, CH_2 Bn), 4.94 (d, 1H, *J*= 10.9 Hz, CH_2 Bn), 5.57 (d, 1H, *J*= 1.5 Hz, H-1), 5.87 (dd, 1H, *J*= 3.1 Hz, *J*= 1.7 Hz, H-2), 7.57 - 7.20 (m, 25H, H-arom), 8.15 - 8.00 (m, 6H, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 18.2 (C-6), 69.2 (C-5), 71.2 (C-2), 71.8 (CH_2 Bn), 75.6 (CH_2 Bn), 78.6 (C-3), 80.2 (C-4), 86.3 (C-1), 127. 7 - 138. 4 (C-arom); $[\alpha]_D^{20}$ -59.4 (c 1.71, $CHCl_3$); IR (neat, cm^{-1}): 710, 743, 1096, 1267, 1452, 1720, 2903; HRMS: $[M+Na]^+$ calcd for $C_{33}H_{32}O_5SNa$ 563.18627, found 563.18633.

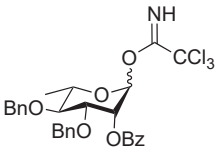
2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-α/β-L-rhamnopyranoside (21)



Compound **20** (4.9 g, 9.0 mmol, 1.0 eq.) was dissolved in DCM (90 mL, 0.1M) and NIS (2.2 g, 9.9 mmol, 1.1 eq.) was added followed by cooling to 0 °C. TFA (0.8 mL, 9.9 mmol, 1.1 eq.) was dropped to the solution and the mixture was allowed to warm up to rt. After 3 hours major conversion was visible on TLC and piperidine (2.7 mL, 27.0 mmol, 3.0 eq.) was added at 0°C and the mixture was warmed up to rt. After 1 hour $Na_2S_2O_3$ (s) was added. The solution was washed with sat. aq. $Na_2S_2O_3$, 1M HCl, water and sat. aq. NaCl, dried over $MgSO_4$, filtered and concentrated. The residue was purified over silica column (PE/EtOAc, 9/1 to 1/1) yielding compound **21** as white solid (3.2 g, 7.2 mmol, 80%) (α/β = 2:9) β-anomer; ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 1.34 (d, 3H, *J*= 6.2 Hz, H-6), 3.58 - 3.46 (m, 1H, H-4), 4.13 - 4.00 (m, 2H, H-5, H-3), 4.52 - 4.94 (m, 6H, CH_2-Bn), 5.25 (d, 1H, *J*= 1.6 Hz, H-1), 5.61 (dd, 1H, *J*= 3.2 Hz, 1.9 Hz, H-2), 7.19 - 7.62 (m, 18H, H-arom), 8.05 - 8.14 (m, 3H, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 18.3 (C-6), 68.0 (C-5), 69.9 (C-2), 71.7 (CH_2 Bn), 75.5 (CH_2 Bn), 77.7 (C-3), 80.3 (C-4), 92.6 (C-1), 127.7 - 138.5 (C-arom), 166.0 (C=O); IR (neat, cm^{-1}) 712,

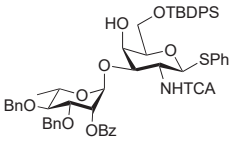
1098, 1271, 1452, 1719, 2932, 3422; HRMS: $[M+Na]^+$ calcd for $C_{27}H_{28}O_6$ Na 471.17781, found 471.17770.

2-*O*-benzoyl-3,4-di-*O*-benzyl-1-*O*-trichloroacetimidoyl-α/β-L-rhamnopyranoside (6)



To a solution of compound **21** (1.47 g; 3.28 mmol; 1.0 eq.) in DCM (33 mL; 0.1M) was added K_2CO_3 (1.81 g; 13.1 mmol; 4.0 eq.) followed by the addition of TCAN (2.0 mL; 19.7 mmol; 6.0 eq.) at 0°C and the mixture was then allowed to warm up to rt overnight. Then K_2CO_3 (0.90 g; 6.6 mmol; 2.0 eq.) and TCAN (1.0 mL; 9.9 mmol; 3.0 eq.) were added to complete the conversion. After complete conversion according to TLC analysis, the mixture was filtrated and concentrated *in vacuo*. TEA neutralized silica column purification (PE/EtOAc, 1/0 to 7/3) afforded compound **6** in 83% yield (1.62 g; 2.72 mmol) (α/β ratio= 1: 0.08). NMR assignment for the α-product. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ= 1.38 (d, 3H, *J*= 6.2 Hz, H-6), 3.71 (t, 1H, *J*= 9.6 Hz, H-4), 3.91 – 4.01 (m, 1H, H-5), 4.06 (dd, 1H, *J*= 9.4, 3.2 Hz, H-3), 4.62 (d, 1H, *J*= 11.4 Hz, C*H*H-Bn), 4.69 (d, 1H, *J*= 10.9 Hz, C*H*H-Bn), 4.77 (d, 1H, *J*= 11.4 Hz, C*H*H-Bn), 4.90 (d, 1H, *J*= 10.9 Hz, C*H*H-Bn), 5.77 (t, 1H, *J*= 2.5 Hz, H-2), 6.34 (s, 1H, H-1), 7.21 – 7.41 (m, 10H, H-arom), 7.55 (dd, 2H, *J*= 8.4, 7.1 Hz, H-arom), 7.63 – 7.71 (m, 1H, H-arom), 8.12 (dd, 2H, *J*= 7.6, 1.4 Hz, H-arom), 9.07 (s, 1H, N*H*); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 18.5 (C-6), 68.7 (C-2), 71.6 (C-5), 72.3, 75.9 (CH_2-Bn), 78.0 (C-3), 80.1 (C-4), 91.5 (CCl_3), 95.9 (C-1), 128.6, 128.7, 129.1, 129.2, 129.2, 129.3, 129.7 (C-arom), 130.6 (Cq-arom), 134.5 (C-arom), 138.7, 139.5 (Cq-arom), 160.2, 166.1 (C=O).

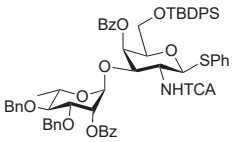
Phenyl 3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-2-deoxy-6-*O*-*tert*-butyl-diphenylsilyl-1-thio-2-(2,2,2-trichloroacetamide)-β-L-galactopyranoside (22)



To a stirred mixture of donor **6** (1.00 g; 1.70 mmol; 1.0 eq.) and acceptor **5** (1.34 g; 2.05 mmol; 1.2 eq.) in DCM (31 mL; 0.05M) on MS 3Å at 0°C was activated by the addition of TMSOTf (62 μL; 71.2 μg; 0.2 eq.). After 1.5h complete conversion of the donor was achieved and the reaction was quenched with 6 drops TEA, DCM was added and the organic layer was washed with aq. sat. NaCl/$NaHCO_3$ (v/v= 1/1), dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Size exclusion chromatography yielded disaccharide **22** in 44% yield (0.82 g; 0.76 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.07 (s, 9H, CH_3-*t*Bu), 1.34 (d, 3H, *J*= 6.3 Hz, H-6), 2.71 (d, 1H, *J*= 2.5 Hz, O*H*), 3.52 (t, 1H, *J*= 9.4 Hz, H-4 ram), 3.63 (t, 1H, *J*= 5.3 Hz, H-5 gal), 3.79 – 3.87 (m, 1H, H-2 gal), 3.87 – 3.93 (m, 1H, H-6 gal), 3.97 - 4.04 (m, 3H, H-6 gal, H-3 ram, H-5 ram), 4.26 (t, 1H, *J*= 2.6 Hz, H-4 gal), 4.32 (dd, 1H, *J*= 10.3, 2.9 Hz, H-3 gal), 4.52 (d, 1H, *J*= 11.1 Hz, C*H*H-Bn), 4.60 (d, 1H,

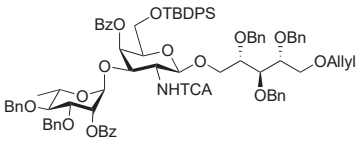
J= 10.9 Hz, C*H*H-Bn), 4.75 (d, 1H, *J*= 11.1 Hz, C*H*H-Bn), 4.88 (d, 1H, *J*= 10.8 Hz, C*H*H-Bn), 4.99 (d, 1H, *J*= 1.8 Hz, H-1 ram), 5.21 (d, 1H, *J*= 10.3 Hz, H-1 gal), 5.64 (dd, 1H, *J*= 3.3, 1.8 Hz, H-2 ram), 6.82 (d, 1H, *J*= 7.6 Hz, N*H*), 7.18 – 7.48 (m, 21H, H-arom), 7.51 – 7.60 (m, 3H, H-arom), 7.71 (ddt, 4H, *J*= 14.3, 6.5, 1.7 Hz, H-arom), 8.00 – 8.05 (m, 2H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 17.8 (C-6), 19.3 (Cq-*t*Bu), 26.9 (CH_3-*t*Bu), 53.0 (C-2 gal), 63.7 (C-6 gal), 68.9, 69.1, 69.2 (C-2 ram, C-4 gal, C-5 ram/C-3 ram), 71.8, 75.5 (CH_2-Bn), 78.0, 78.3, 78.6 (C-3 gal, C-5 gal, C-5 ram/C-3 ram), 79.7 (C-4 ram), 84.7 (C-1 gal), 92.4 (CCl_3), 100.1 (C-1 ram), 127.7, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.5, 129.2 (C-arom), 129.9 (Cq-arom), 130.0, 130.1 (CH-arom), 132.0, 132.7, 132.9 (Cq-arom), 133.1, 133.4, 135.7, 135.8 (C-arom), 138.1, 138.4 (Cq-arom), 161.9, 165.5 (C=O); HRMS: $[M+ NH_4]^+$ calcd for $C_{57}H_{64}Cl_3N_2O_{10}SSi$ 1101.31110, found 1101.31117.

Phenyl 3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-4-*O*-benzoyl-2-deoxy-6-*O*-*tert*-butyl-diphenylsilyl-1-thio-2-(2,2,2-trichloroacetamide)-β-D-galactopyranoside (23)



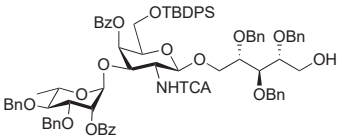
To a solution of compound **22** (0.80 g; 0.74 mmol; 1.0 eq.) in pyridine (10.0 mL; 0.10M) was added BzCl (1.7 mL; 14.8 mmol; 20.0 eq.) and DMAP (110 mg; 0.9 mmol; 1.2 eq.) and the mixture was heated overnight at 65°C. Then the mixture was cooled to rt and quenched with MeOH at 0°C. The organic layer was diluted with DCM and washed with sat. aq. $NaCl/NaHCO_3$ (v/v= 1/1). The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by size exclusion chromatography yielded compound **23** (0.90 g; 0.76 mmol) in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.04 (s, 9H, CH_3-*t*Bu), 1.31 (d, 3H, *J*= 6.2 Hz, H-6), 3.48 (t, 1H, *J*= 9.4 Hz, H-4 ram), 3.72 – 3.84 (m, 4H, H-3 ram, H-2 gal, 2x H-6 gal), 3.94 (t, 1H, *J*= 6.5 Hz, H-5 gal), 4.16 (dq, 1H, *J*= 9.5, 6.2 Hz, H-5 ram), 4.25 (d, 1H, *J*= 11.2 Hz, C*H*H-Bn), 4.50 (d, 1H, *J*= 11.2 Hz, C*H*H-Bn), 4.58 (d, 1H, *J*= 11.6 Hz, C*H*H-Bn), 4.63 (dd, 1H, *J*= 10.4, 3.1 Hz, H-3 gal), 4.81 (d, 1H, *J*= 11.6 Hz, C*H*H-Bn), 4.93 (d, 1H, *J*= 1.7 Hz, H-1 ram), 5.31 (d, 1H, *J*= 10.2 Hz, H-1 gal), 5.45 (dd, 1H, *J*= 3.3, 1.7 Hz, H-2 ram), 5.83 (d, 1H, *J*= 3.0 Hz, H-4 gal), 6.87 (d, 1H, *J*= 7.6 Hz, N*H*), 7.08 – 7.45 (m, 22H, H-arom), 7.52 – 7.70 (m, 9H, H-arom), 7.85 – 7.90 (m, 2H, H-arom), 7.95 – 7.99 (m, 2H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 18.1 (C-6 ram), 19.2 (Cq-*t*Bu), 26.9 (CH_3-*t*Bu), 54.2 (C-2 gal), 61.9 (C-6 gal), 68.3, 69.4, 69.7 (C-5 ram, C-2 ram, C-4 gal), 71.8, 73.9 (CH_2-Bn), 74.7 (C-3 gal), 77.7 (C-3 ram), 78.6, 79.0 (C-5 gal, C-4 ram), 83.6 (C-1 gal), 92.3 (CCl_3), 99.7 (C-1 ram), 127.4, 127.5, 127.8, 127.9, 127.9, 127.9, 128.2, 128.2, 128.5, 128.5, 128.7, 129.3 (C-arom), 129.7, (Cq-arom), 129.8 (C-arom), 129.9 (Cq-arom), 129.9, 130.0 (CH-arom), 130.9 (Cq-arom), 133.0, 133.1 (Cq-arom), 133.3, 133.3, 134.2, 135.6, 135.7 (C-arom), 138.1, 138.9 (Cq-arom), 161.9, 165.2, 165.5 (C=O); HRMS: $[M+ NH_4]^+$ calcd for $C_{64}H_{68}Cl_3N_2O_{11}SSi$ 1205.33732, found 1205.33743.

1-*O*-(3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-4-*O*-benzoyl-2-deoxy-6-*O*-*tert*-butyl-diphenylsilyl-2-(2,2,2-trichloroacetamide)-β-D-galactopyranosyl)-5-*O*-allyl-2,3,4-tri-*O*-benzyl-D-ribitol (24)



To a stirring mixture of acceptor **7** (60 mg; 0.13 mmol; 1.3 eq.) and donor **23** (119 mg; 0.10 mmol; 1.0 eq.) in DCM (0.1M; 1.0 mL) on MS 3Å was added NIS (27 mg; 0.12 mmol; 1.2 eq.) at 0°C. Then the mixture was cooled to -42°C and the reaction was activated by the addition of TMSOTf (5 μL; 0.02 mmol; 0.2 eq.). The mixture was gradually warmed up to 0°C and quenched with TEA. The mixture was then diluted with DCM, washed with sat. aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$/NaCl (v/v= 1/1). The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by silica chromatography (DCM/acetone 100/0 to 95/5 DCM acetone) yielded target compound **24** (70 mg; 45 μmol) in 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.05 (s, 9H, CH_3-*t*Bu), 1.33 (d, 3H, *J*= 6.2 Hz, H-6), 3.50 (t, 1H, *J*= 9.5 Hz, H-4 ram), 3.58 – 4.14 (m, 13H, 2x CH_2-Rbo, H-3 ram, CH_2-allyl, 3x CH-Rbo, H-5 gal, 2x H-6 gal), 4.18 (dq, 1H, *J*= 9.6, 6.1 Hz, H-5 ram), 4.24 – 4.33 (m, 2H, H-3 gal, C*H*H-Bn), 4.52 – 4.70 (m, 8H, C*H*H-Bn), 4.75 (dd, 1H, *J*= 10.0, 1.7 Hz, H-1 gal), 4.81 (d, 1H, *J*= 11.6 Hz, C*H*H-Bn), 4.94 (m, 1H, *J*= 1.7 Hz H-1 ram), 5.12 – 5.27 (m, 2H, CH_2=CH), 5.51 (dd, 1H, *J*= 3.2, 1.8 Hz, H-2 ram), 5.78 (d, 1H, *J*= 3.2 Hz, H-4 gal), 5.87 (ddt, *J* = 17.3, 10.7, 5.5 Hz, CH_2=C*H*), 6.75 (d, 1H, *J*= 7.8 Hz, N*H*), 7.09 – 7.48 (m, 35H, H-arom), 7.50 – 7.60 (m, 4H, H-arom), 7.60 – 7.69 (m, 2H, H-arom), 8.02 (ddt, 4H, *J*= 11.7, 7.1, 1.4 Hz, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 18.1 (C-6 ram), 19.2 (Cq-), 26.8 (CH_3-*t*Bu), 56.2 (C-2 gal), 61.7 (C-6 gal), 68.8, 69.0, 69.4, 69.5 (2x CH_2-Rbo, H-4 gal, H-2 ram, H-5 ram, CH*H*-Rbo), 70.2, 71.8, 72.3, 72.4, 72.5, 73.3, 74.0, 74.3, 74.8, 77.7, 78.0, 78.6, 78.9, 79.1 (5x CH_2-Bn, CH_2-allyl, H-3 gal, H-3 ram, 3x CH-Rbo, CH_2-Rbo, CH*H*-Rbo, H-5 gal, 2x H-6 gal, H-2 gal, H-4 ram, 92.4 (CCl_3), 99.8, 99.9 (C-1 ram, C-1 gal), 117.0 (CH_2=CH), 127.4, 127.5, 127.7, 127.8, 127.9, 127.9, 128.0, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 129.8, 129.9, 129.9, 130.0 (C-arom), 132.9, 133.2 (Cq-arom), 133.2 C-arom), 134.9, 135.6, 135.6 (C-arom), 138.1, 138.5, 138.6, 138.9, 138.9 (Cq-arom), 162.2, 165.4, 165.4 (C=O); HRMS: $[M+ NH_4]^+$ calcd for $C_{87}H_{96}Cl_3N_2O_{16}Si$ 1557.55892, found 1557.55907.

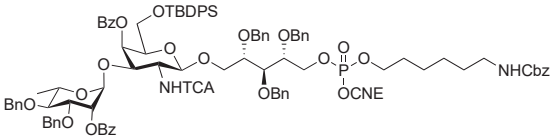
1-*O*-(3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-4-*O*-benzoyl-2-deoxy-6-*O*-*tert*-butyl-diphenylsilyl-2-(2,2,2-trichloroacetamide)-β-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-D-ribitol (25)



A solution of compound **24** (244 mg; 0.16 mmol; 1.0 eq.) in distilled THF (3.2 ml; 0.05M) was degassed with N_2. $Ir(COD)(Ph_2MeP)_2PF_6$ (5 mg; 0.04 eq.) was added and the solution was degassed with N_2. Then the red solution was purged with H_2 until the color became yellow (~5 seconds) and hereafter the solution was degassed with argon to remove traces of H_2 from the solution and stirring was continued under N_2 atmosphere until complete conversion of the substrate occured according to TLC analysis. The mixture was diluted with THF (3.2 ml) and aq. sat. $NaHCO_3$ (3.2 ml) followed by the addition of I_2 (0.06 g; 0.24 mmol; 1.5 eq.) and stirred for +/- 30 mins. The reaction was quenched by the addition of sat. aq. Na_2SO_3, diluted with EtOAc and the organic layer was washed with sat. aq. $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtrated and concentrated under reduced pressure. Purification by TEA neutralized column chromatography (pentane/EtOAc, 1/0 to 4/6) yielded **25** (128 mg; 85.4 μmol) in 54% yield.

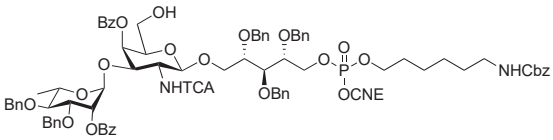
^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.04 (s, 9H, CH_3-*t*Bu), 1.33 (d, 3H, *J*= 6.2 Hz, H-6 ram), 2.31 (s, 1H, O*H*), 3.50 (t, 1H, *J*= 9.5 Hz, H-4 ram), 3.67 – 3.84 (m, 9H, C*H*H-Rbo, CH_2-Rbo, 3x CH-Rbo, 2x H-6, H-3 ram), 3.92 (t, 1H, *J*= 5.0 Hz, H-5 gal), 3.94 – 3.99 (m, 1H, H-2 gal), 4.12 (dd, 1H, *J*= 10.6, 4.3 Hz, C*H*H-Rbo), 4.17 (dq, 1H, *J*= 9.5, 6.2 Hz, H-5 ram), 4.30 (d, 1H, *J*= 11.2 Hz, C*H*H-Bn), 4.33 (dd, 1H, *J*= 10.9, 3.2 Hz, H-3 gal), 4.49 – 4.70 (m, 8H, CH_2-Bn), 4.81 (t, 2H, *J*= 10.1 Hz, C*H*H-Bn, H-1 gal), 4.95 (d, 1H, *J*= 1.8 Hz, H-1 ram), 5.50 (dd, 1H, *J*= 3.2, 1.8 Hz, H-2 ram), 5.79 (d, 1H, *J*= 3.2 Hz, H-4 gal), 6.82 (d, 1H, *J*= 7.8 Hz, N*H*), 7.06 – 7.46 (m, 35H, H-arom), 7.54 (ddt, 4H, *J*= 14.2, 7.6, 1.4 Hz, H-arom), 7.59 – 7.68 (m, 2H, H-arom), 8.01 (ddt, 4H, *J*= 11.7, 6.9, 1.4 Hz, H-arom); ^{13}C-APT NMR (126 MHz, $CDCl_3$) δ= 18.1 (C-6 ram), 19.2 (Cq-*t*Bu), 26.9 (CH_3-*t*Bu), 56.3 (C-2 gal), 61.5, 61.7 (CH_2-Rbo, C-6 gal), 68.3 (CH_2-Rbo), 69.1, 69.3, 69.5 (C-4 gal, C-2 ram, C-5 ram), 71.8, 72.0, 72.4, 73.9, 74.2 (CH_2-Bn), 74.3, 74.6 (C-3 gal, C-5 gal/CH-Rbo), 77.6, 77.7, 79.0, 79.1, 79.2 (2x CH-Rbo, C-3 ram, C-4 ram, CH-Rbo/C-5 gal), 92.4 (CCl_3), 99.8 C-1 ram, C-1 gal), 127.4, 127.5, 127.8, 127.9, 127.9, 127.9, 127.9, 128.0, 128.0, 128.0, 128.2, 128.2, 128.3, 128.3, 128.4, 128.4, 128.5, 128.5, 128.6, 128.6, 129.8, 129.9, 129.9, 130.0 (C-arom), 132.9, 133.2 (Cq-arom), 133.3, 135.6, 135.6 (C-arom), 138.1, 138.1, 138.3, 138.9 (Cq-arom), 162.3, 165.4, 165.5 (C=O). HRMS: $[M+H]^+$ calcd for $C_{84}H_{89}Cl_3NO_{16}Si$ 1500.50107, found 1500.50197.

1-*O*-(3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-4-*O*-benzoyl-2-deoxy-6-*O*-*tert*-butyl-diphenylsilyl-2-(2,2,2-trichloroacetamide)-β-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-D-ribitol-1-[2-cyanoethylphosphate])-*N*-benzyloxycarbonyl-6-aminohexanol (26)



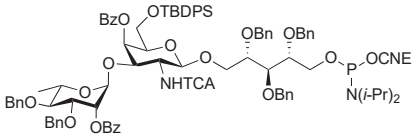
According to the general procedure described above, alcohol **25** (62 mg; 41.0 μmol; 1.0 eq.) was coupled with phosphoramidite **4** (28.0 mg; 62.0 μmol; 1.5 eq.) yielding the title compound **26** in 68% yield (52.2 mg; 27.9 μmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.03 (d, 9H, *J*= 1.5 Hz, CH_3-*t*Bu), 1.18 – 1.35 (m, 7H, H-6 ram, 2x CH_2-hexylspacer), 1.42 (dt, 2H, *J*= 13.9, 7.3 Hz, CH_2-hexylspacer), 1.58 (dp, 2H, *J*= 21.2, 6.8 Hz, CH_2-hexylspacer), 2.35 – 2.55 (m, 2H, CH_2-cyanoethyl), 3.07 – 3.16 (m, 2H, CH_2-N hexylspacer), 3.49 (td, 1H, *J*= 9.4, 3.3 Hz, H-5 ram), 3.68 – 4.44 (m, 19H, CH_2O-hexylspacer, 3x CH-Rbo, 2x CH_2-Rbo, H-3 ram, H-4 ram, H-2 gal, H-3 gal, H-5 gal, 2x H-6 gal, CH_2-cyanoethyl, C*H*H-Bn), 4.44 – 4.70 (m, 8H, 4x CH_2-Bn), 4.79 (dd, 1H, *J*= 11.6, 5.0 Hz, C*H*H-Bn), 4.86 (dd, 1H, *J*= 19.4, 8.4 Hz, H-1 gal), 4.99 (dd, 1H, *J*= 5.6, 1.8 Hz, H-1 ram), 5.08 (s, 2H, CH_2-Cbz), 5.52 (dd, 1H, *J*= 3.2, 1.8 Hz, H-2 ram), 5.80 (dd, 1H, *J*= 7.6, 3.2 Hz, H-4 gal), 7.10 – 7.45 (m, 40H, H-arom), 7.50 – 7.58 (m, 4H, H-arom), 7.64 (ddt, 2H, *J*= 8.5, 4.4, 2.2 Hz, H-arom), 7.95 – 8.05 (m, 4H, H-arom); ^{13}C-APT NMR (126 MHz, $CDCl_3$) δ= 18.1 (C-6 ram), 19.2, 19.4, 19.5, 19.5, 19.6 (Cq-*t*Bu), 25.0, 25.1, 26.2 (CH_2-hexylspacer), 26.8 (CH_3-*t*Bu), 29.8, 29.8, 30.0, 30.1, 30.1 (CH_2-hexylspacer), 40.9 (CH_2-N hexylspacer), 55.8, 56.0 (C-2 gal), 61.7, 61.7, 61.7, 61.8 (CH_2-Rbo), 66.7, 66.7, 67.5, 67.9, 68.2, 68.3, 68.3, 68.3 (CH_2-Rbo, C-6 gal, CH_2O-hexylspacer, CH_2-Cbz), 69.0, 69.3, 69.4, 69.5, 69.5 (C-4 gal, C-2 ram), 71.7, 71.7, 72.4, 72.5, 72.6, 72.7, 73.6, 73.8 (CH_2-Bn), 74.3, 74.4, 74.7, 75.1, 77.7, 77.7, 77.8, 78.1, 78.1, 78.2, 78.2, 78.3 (CH-Rbo, C-3 gal, C-5 gal, C-3 ram, C-4 ram), 79.0 (C-5 ram), 92.5, 92.6 (CCl_3), 99.9, 99.9, 100.0, 100.2 (C-1 ram, C-1 gal), 116.7, 117.1 (Cq-cyanoethyl), 127.4, 127.5, 127.7, 127.8, 127.8, 127.9, 127.9, 127.9, 127.9, 128.0, 128.0, 128.2, 128.4, 128.4, 128.5, 128.5, 128.6, 128.6, 129.8, 129.9, 129.9, 130.0, 130.0, (C-arom), 132.9, 132.9, 133.2 (Cq-arom), 133.2, 135.6, 135.6 (C-arom), 136.7, 138.1, 138.1, 138.2, 138.2, 138.3, 138.9, 138.9 (Cq-arom), 156.5, 162.4, 162.4, 165.3, 165.4, 165.4, 165.4 (C=O); ^{31}P NMR (202 MHz, $CDCl_3$) δ= -0.4, -1.0.

1-*O*-(3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-4-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroacetamide)-β-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-D-ribitol-1-[2-cyanoethylphosphate])-*N*-benzyloxycarbonyl-6-aminohexanol (27)



To a solution of compound **26** (52.2 mg; 27.9 μmol; 1.0 eq.) in a mixture of THF/pyr. (2.0 mL; v/v= 1/1; 0.01M) was added 50 μL HF/pyridine. After 2h additional HF/pyridine (50 μL) was added at 0°C to speed up the conversion. After complete conversion, the mixture was quenched with slow addition of sat. aq. $NaHCO_3$ at 0°C. Then the mixture was diluted with DCM and the water layer was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by size exclusion chromatography yielded compound **27** in 81% yield (37 mg; 22.7 μmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.14 (d, 3H, *J*= 6.1 Hz, H-6), 1.18 – 1.35 (m, 4H, CH_2-hexylspacer), 1.42 (ddt, 2H, *J*= 17.7, 11.5, 6.0 Hz, CH_2-hexylspacer), 1.60 (q, 2H, *J*= 6.9 Hz, CH_2-hexylspacer), 2.40 – 2.56 (m, 2H, CH_2-cyanoethyl), 3.07 – 4.48 (m, 21H, 2x CH_2-Rbo, 3x CH-Rbo, CH_2-N hexylspacer, CH_2O-hexylspacer, H-3 ram, H-4 ram, H-2 gal, H-3 gal, H-5 gal, 2x H-6 gal, CH_2-cyanoethyl, C*H*H-Bn), 4.57 – 4.71 (m, 8H, 4x CH_2-Bn), 4.78 – 4.84 (m, 2H, C*H*H-Bn, H-1 gal), 5.01 (dd, 1H, *J*= 5.4, 1.9 Hz, H-1 ram), 5.08 (s, 2H, CH_2-Cbz), 5.56 (d, 2H, *J*= 3.2 Hz, H-2 ram, H-4 gal), 6.97 – 7.03 (m, 2H, H-arom), 7.15 (q, 5H, *J*= 5.1 Hz, H-arom), 7.18 – 7.63 (m, 29H, H-arom), 7.94 – 8.03 (m, 2H, H-arom), 8.07 – 8.15 (m, 2H, H-arom); ^{31}P NMR (202 MHz, $CDCl_3$) δ= -0.6, -1.2; HRMS: $[M+H]^+$ calcd for $C_{85}H_{94}Cl_3N_3O_{21}P$ 1628.51775, found 1628.51880.

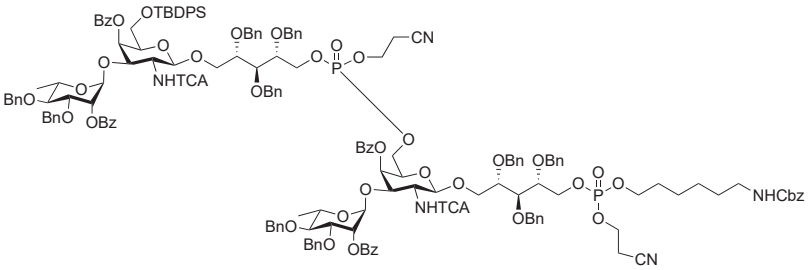
1-*O*-(3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-4-*O*-benzoyl-2-deoxy-6-*O*-*tert*-butyl-diphenylsilyl-2-(2,2,2-trichloroacetamide)-β-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-5-*O*-([*N,N'*-di-isopropylamino]-2-cyanoethyl-phosphite)-D-ribitol (28)



Compound **25** (6.2 mg; 42.6 mmol; 1.0 eq.) was co-evaporated with toluene twice and was then dissolved in DCM (1.0 mL; 0.05 M), DIPEA was added (16 μL; 1.5 eq.) and the mixture was stirred over activated molecular sieves for +/- 20 minuts. 2-Cyanoethyl *N,N*-(diisopropyl)$_2$phosphoramidite (0.18 mL; 1.3 eq.) was added followed by tetrazole salt (18 mg; 0.11 mmol; 2.5 eq.) and the mixture was stirred until TLC showed complete conversion of the starting material. The reaction was then quenched with a few drops of water and diluted with DCM. The organic layer was washed with sat. aq. $NaHCO_3$/NaCl (v/v= 1/1). The organic layer was dried over Na_2SO_4, filtrated and concentrated *in*

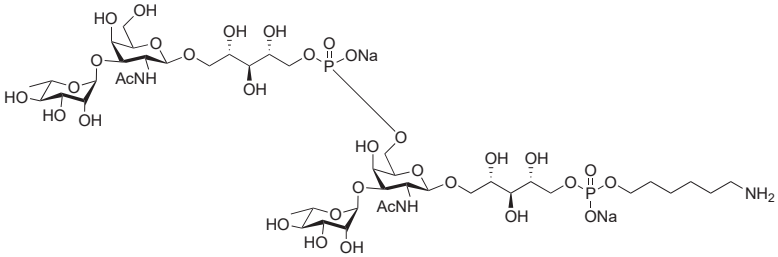
vacuo. Column chromatography using TEA neutralized silica (pentane/EtOAc 1/0 to 7/3 pentane/EtOAc) afforded phosphoramidite **28** in 70% yield (51 mg; 29.2 μmol).

Dimer (29)



According to the general procedure described above, alcohol **27** (27 mg; 16.6 μmol; 1.0 eq.) was coupled with phosphoramidite **28** (48.0 mg; 27.5 μmol; 1.7 eq.) yielding the title compound **29** in 45% yield (24.3 mg; 7.5 μmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.14 – 1.65 (m, 23H, H-6 ram, 4x CH_2-hexylspacer, CH_3-*t*Bu), 2.19 – 2.59 (m, 4H, 2x CH_2-cyanoethyl), 3.10 – 3.13 (m, 2H, CH_2-N hexylspacer), 3.40 (ddd, 2H, *J*= 10.8, 8.3, 2.3 Hz, 2x H-5 ram), 3.50 – 4.99 (m, 58H, 6x CH-Rbo, 4x CH_2-Rbo, 2x H-1 gal, 2x H-2 gal, 2x H-3 gal, 2x H-5 gal, 4x H-6 gal, 2x H-1 ram, 2x H-3 ram, 2x H-4 ram, 2x CH_2-cyanoethyl, CH_2O-hexylspacer, 10x CH_2-Bn), 5.07 (s, 2H, CH_2-Cbz), 5.33 – 5.35 (m, 1H, H-2 ram), 5.50 (dt, 1H, *J*= 4.3, 2.5 Hz, H-2 ram), 5.57 (dt, 1H, *J*= 10.0, 2.9 Hz, H-4 gal), 5.75 – 5.81 (m, 1H, H-4 gal), 7.06 – 8.05 (m, 75H, H-arom); ^{13}C-APT NMR (126 MHz, $CDCl_3$) δ= 18.1, 18.1 (C-6 ram), 19.2, 19.3, 19.5, 19.6 (Cq-*t*Bu), 23.1, 24.6, 25.1, 26.2 (CH_2-hexylspacer), 26.9 (CH_3-*t*Bu), 29.1, 29.8, 30.1 (CH_2-hexylspacer), 41.0 (CH_2-N hexylspacer), 61.7, 62.1 (CH_2-Rbo), 66.7, 67.6, 68.3, 68.3 (CH_2-Rbo, C-6 gal, CH_2O-hexylspacer, CH_2-Cbz), 68.8, 69.0, 69.3, 70.0 (C-4 gal, C-2 ram), 71.7, 72.4, 72.5, 72.6, 72.7, 73.7, 73.8, 73.9, 74.2 (CH_2-Bn), 77.8, 78.1, 79.1, 79.2 (CH-Rbo, C-3 gal, C-5 gal, C-3 ram, C-4 ram, C-5 ram), 92.3 (CCl_3), 98.6, 99.9, 99.9, 100.0, 100.4 (C-1 gal, C-1 ram), 116.8, 117.2 (Cq-cyanoethyl), 127.5, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 127.9, 127.9, 127.9, 128.0, 128.0, 128.1, 128.1, 128.2, 128.2, 128.3, 128.4, 128.4, 128.5, 128.5, 128.5, 128.5, 128.6, 128.6, 128.6, 128.7, 129.2, 129.9, 129.9, 129.9, 130.1 (C-arom), 132.9, 133.2, 133.2 (Cq-arom), 133.3, 133.3, 133.3, 135.6, 135.6 (C-arom), 136.8, 136.9, 138.0, 138.1, 138.2, 138.2, 138.3, 138.8, 138.9 (Cq-arom), 162.4, 162.5, 164.7, 165.4, 165.8, 165.9 (C=O); ^{31}P NMR (202 MHz, $CDCl_3$) δ= -0.5, -0.6, -0.8, -0.9, -1.0, -1.2, -1.5, -1.6.

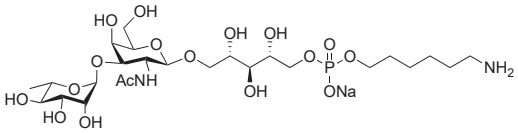
Deprotected dimer (2)



To a solution of compound **29** (22.0 mg; 6.8 μmol; 1.0 eq.) in a mixture of THF/pyr. (2.0 mL; v/v= 1/1; 3.4 mM) was added HF/pyridine (0.1 mL) at 0°C for 1h. Then the mixture was left stirring at rt overnight. The mixture was quenched by the addition of sat. aq. $NaHCO_3$ at 0°C. The mixture was diluted with EtOAc, washed with sat. aq. $NaHCO_3$, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was dissolved in a mixture of NH_4OH/dioxane (3.2 mL; v/v= 1/1; 2.1 mM) and the mixture was stirred overnight at rt. Then the mixture was concentrated *in vacuo*, and the crude was redissolved in a mixture of dioxane/MeOH (2 mL; v/v= 1/1; 3.4 mM) and 7 drops of a 5.8 M NaOMe in MeOH was added and the mixture was stirred overnight at rt. The mixture was quenched with AcOH, then with NH_4OH, afterwhich the mixture was concentrated *in vacuo*. The crude was purified using size exclusion chromatography. The product was obtained along with fractions corresponding to uncomplete benzoyl removal. These latter fractions were re-subjected for benzoyl removal by dissolving in dioxane/miliQ (6.0 mL; v/v= 1/1) and 8 drops of a 5.8 M NaOMe in MeOH was added and the mixture was stirred for 2.5 days at rt. The mixture was quenched and worked up as described above. The collected pure fractions were dissolved in a mixture of dioxane/miliQ (4.5 mL; v/v= 5/4), 2 drops of AcOH were added and the solution was degassed with N_2. Two scoops of Pd black were added and the mixture was repurged with N_2. The mixture was then purged with H_2 and then left stirring under H_2 atmosphere for 4 days. The mixture was purged with N_2 to remove excess of H_2, filtrated over celite and concentrated *in vacuo*. ^{1}H NMR analysis showed presence of benzoyls and therefore the compound was dissolved in MeOH and 5 drops of a 5.8 M NaOMe in MeOH were added and the mixture was stirred overnight. The mixture was quenched with AcOH, and then with NH_4OH and then concentrated *in vacuo*. Purification by HW-40 size exclusion chromatography followed by concentration under reduced pressure yielded the product. The product was dissolved in water and eluted through a Dowex Na^+ cation-exchange resin column, and concentrated under reduced pressure. Lyophilization yielded the product in 17% yield (1.5 mg; 1.16 μmol). ^{1}H NMR (500 MHz, D_2O) δ= 1.25 (dd, 6H, *J*= 6.3, 4.2 Hz, H-6), 1.35 – 1.45 (m, 4H, 2x CH_2-hexylspacer), 1.59 – 1.71 (m, 4H, 2x CH_2-hexylspacer), 2.04 (d, *J* = 2.3 Hz, 6H, CH_3-NHAc), 2.98 (t, 2H, *J*= 7.5 Hz, CH_2-N hexylspacer), 3.32 – 4.13 (m, 36H, 6x CH-Rbo, 4x CH_2-Rbo, CH_2O-hexylspacer, 2x H-2 gal, 2x H-3 gal, 2x H-4 gal, 2x H-5 gal, 4x H-6 gal, 2x H-2 ram, 2x

H-3 ram, 2x H-4 ram, 2x H-5 ram), 4.57 (dd, 2H, *J*= 12.5, 8.6 Hz, 2x H-1 gal), 4.84 – 4.88 (m, 2H, 2x H-1 ram): ^{13}C-APT NMR (214 MHz, D_2O) δ= 17.4, 17.5 (C-6 ram), 23.0 (CH_3-NHAc), 25.0, 25.2, 25.9, 27.4, 29.8, 30.2, 40.2 (CH_2-hexylspacer), 52.3 (C-2 gal), 61.3, 61.8, 64.8, 66.9, 67.3, 67.5, 67.9, 68.0, 68.5, 68.6 (4x CH_2-Rbo, 2x C-6 gal, CH_2O-hexylspacer), 69.0, 70.1, 70.8, 71.5, 72.3, 72.8, 73.4, 74.3, 75.9, 79.6, 79.9 (CH-Rbo, C-3 gal, C-4 gal, C-5 gal, C-2 ram, C-3 ram, C-4 ram, C-5 ram), 102.0 (C-1 gal), 103.1 (C-1 ram), 175.6 (C=O); ^{31}P NMR (202 MHz, D_2O) δ= 1.8, 1.5; HRMS: $[M+H]^+$ calcd for $C_{44}H_{84}N_3O_{33}P_2$ 1244.44568, found 1244.44655.

1-*O*-(1-*O*-[3-*O*-{α-L-rhamnopyranosyl}-2-*N*-acetamido-β-D-galactopyranosyl]-D-ribitol-3-phosphate)-6-aminohexanol (1)



A solution of compound **24** (8.0 mg; 4.9 μmol; 1.0 eq.) in a mixture of NH_4OH/dioxane (2.3 mL; v/v= 1/1; 2.1 mM) was stirred overnight at rt. The mixture was then concentrated *in vacuo* and redissolved in MeOH (2.0 mL; 2.5 μmol) and 12 drops of a 5.8M NaOMe in MeOH were added and the mixture was left to stir for 3 days at rt. The mixture was quenched with AcOH followed by NH_4OH and the mixture was concentrated *in vacuo*. The crude was purified using size exclusion chromatography and the collected fractions were concentrated under reduced pressure. The compound was eluted through a Dowex Na^+ cation-exchange resin column, and concentrated under reduced pressure. The compound was dissolved in a mixture of dioxane/water (2.3 mL; v/v= 1/1; 2.1 mM) and $NaHCO_3$ (1 mg; 9.8 μmol; 2.0 eq.) was added. The solution was purged with N_2, and a scoop of Pd black was added. The solution was then re-purged with N_2 and was then purged with H_2 and was left stirring under H_2 atmosphere for 9 days. The mixture was purged with N_2, filtrated over celite and concentrated *in vacuo*. ^{1}H NMR analysis showed presence of benzoyl intermediate and therefore the compound was dissolved in a mixture of water/MeOH (1.5 mL; v/v/= 2/1; 3.3 mM) and 8 drops of a 5.8M NaOMe in MeOH were added and the mixture was stirred overnight at rt. The mixture was quenched a solution of AcOH/water (v/v= 1/10) to pH= 6. The mixture was then quenched with NH_4OH and concentrated *in vacuo*. The compound was purified using HW-40 size exclusion chromatography, concentrated under reduced pressure, dissolved in water and eluted through a Dowex Na^+ cation-exchange resin column, and concentrated under reduced pressure. Lyophilization yielded the product in 32% yield (1.1 mg; 1.57 μmol). ^{1}H NMR (850 MHz, D_2O) δ= 1.29 (d, 3H, *J*= 6.3 Hz, H-6), 1.43 – 1.44 (m, 4H, 2x CH_2-hexylspacer), 1.66 – 1.71 (m, 4H, 2x CH_2-hexylspacer), 2.05 – 2.09 (m, 3H, CH_3-NHAc), 3.00 – 3.03 (t, 2H, *J*= 7.7 Hz, CH_2-N hexylspacer), 3.44 – 4.14 (m, 19H, 3x CH-Rbo, 2x CH_2-Rbo, CH_2O-hexylspacer, H-2 gal, H-3 gal, H-4 gal, H-5 gal, 2x H-6 gal, H-2 ram, H-3 ram, H-4 ram, H-5 ram), 4.59 (d, 1H, *J*= 8.5 Hz, H-1 gal), 4.89 (d, 1H, *J*= 1.7 Hz, H-1 ram);

^{13}C-APT NMR (214 MHz, D_2O) δ= 17.5 (C-6 ram), 23.1 (CH_3-NHAc), 25.3, 25.9, 26.0, 27.5, 30.3, 30.3, 40.3, (CH_2-hexylspacer), 52.5 (C-2 gal), 61.4, 61.8, 63.2, 67.0, 67.0, 67.3, 67.3, 67.4, 67.4, 68.5 (4x CH_2-Rbo, 2x C-6 gal, CH_2O-hexylspacer), 70.2, 70.9, 70.9, 71.0, 71.3, 71.3, 71.4, 71.6, 71.7, 71.8, 71.8, 72.3, 72.6, 72.6, 72.8, 73.0, 73.0, 76.0, 76.1, 79.9 (CH-Rbo, C-3 gal, C-4 gal, C-5 gal, C-2 ram, C-3 ram, C-4 ram, C-5 ram),102.1 (C-1 gal),103.2 (C-1 ram),175.6 (C=O); ^{31}P NMR (202 MHz, D_2O) δ= 1.9, 1.8, 1.8, 1.6; HRMS: $[M+H]^+$ calcd for $C_{25}H_{50}N_2O_{17}P$ 681.28416, found 681.28425.

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7

Summary and future prospects

The Gram-positive bacterial peptidoglycan is densely functionalized with carbohydrate-based anionic cell wall polymers, known as wall teichoic acids (WTAs). These WTAs are composed of repeating ribitol phosphates (RboP), that can be randomly modified with carbohydrate appendages and/or D-alanine (D-ala) substituents. WTAs fulfil important roles in cell shape, cell division, biofilm formation, phage infectivity, and pathogenesis as well as resistance to cationic antimicrobial peptides.

This Thesis describes the synthesis of various WTA fragments of *Staphylococcus aureus* and *Enterococcus faecalis*. Multi-drug-resistant strains of both bacteria have developed, including the 'hospital bugs' Methicillin-resistant *S. aureus* (MRSA) and Vancomycin-resistant enterococci (VRE). Novel strategies to combat these bacteria are urgently required and passive or active vaccines may contribute to protection against these opportunistic pathogens. This Thesis describes the development of synthetic methodologies to generate both non-substituted and substituted RboP oligomers. The pursued substitution patterns include α- and β-*N*-acetyl glucosamine (GlcNAc) residues on the RboP C-4, β-*N*-acetyl glucosamine on the RboP C-3 and D-alanine (D-ala) at the RboP C-2, as found in the WTA of *S. aureus*. The synthetic strategy followed relies on the use of phosphoramidite chemistry both in solution and on an automated solid phase synthesizer. The WTA of *E. faecalis* consists of *N*-acetyl-β-D-galactosaminyl ribitol phosphate residues having a α-L-rhamnose branch at the C3 of the galactosamine residue and a ribitol phosphate linkage to the C-1 of the galactosamine. The isolation of these WTA structures from bacterial sources presents problems with contaminations and obtaining mixtures of structures with different lengths and substitution patterns. Organic synthesis on the other hand can deliver the target compounds with the length and substitution pattern of choice and both in higher purity and in larger amounts, allowing detailed immunological studies that can aid in future vaccine development.

Chapter 1 reviews the synthetic efforts in the field of teichoic acid (TA) chemistry to generate well-defined TAs for vaccine development. This Chapter describes the synthesis of WTA fragments of *S. aureus*, *E. faecalis* and *faecium,* and *Clostidrium difficile*. Besides these syntheses, this Chapter also introduces TA micro-arrays as a diagnostic tool to study the interaction between TAs and relevant molecules, like human sera or phage proteins. Synthetic TAs can also serve as substrates for bacterial biosynthesis enzymes.[1] The manipulation of these processes will enable studies unravelling the function of WTAs at the cellular level.

Chapter 2 describes the synthesis of non-substituted ribitol phosphates in solution with a length ranging from a trimer to an octamer from readily available building blocks. The same building blocks were applied for the assembly of an octa- and dodecamer

using automated solid phase synthesis. The synthesized WTA hexamer has been used as a substrate to probe the enzyme glycosyltransferase TarP, which was recently discovered to be responsible for the β-GlcNAcylation on the C-3 position of the RboP chain. In another application, the WTA hexamer was coupled to magnetic beads to generate WTA-functionalized beads. The WTA-oligomers were equipped with a biotin affinity handle and subsequently enzymatically glycosylated using the glycosyltransferases TarM, which introduces α-GlcNAc residues, TarS to attach β-GlcNAc residues at the RboP C-4, and TarP. The glycosylated-WTAs could next be bound to streptavidin-coated magnetic beads and used as diagnostic tools to detect WTA-specific IgG antibodies in human serum. The beads represent rapid and clean diagnostic tools to measure antibody levels and provide the opportunity to study the interaction of WTAs with other relevant biological samples. The enzymatic glycosylation using TarS was performed on a larger scale to provide 0.5 mg enzymatically glycosylated WTA. Future research will be directed to further scaling up this process for all three glycosyl transferases for the rapid assembly of larger amounts glycosylated WTAs.

Chapter 3 describes the synthesis of a set of WTA hexamers, glycosylated at the C-4 of the RboP repeating units, with one or two α- or β-GlcNAc moieties on the RboP chain. A micro-array was used to investigate the binding of the β-GlcNAc WTAs with human langerin. In addition, a RboP hexamer- and dodecamer (Chapter 2) were enzymatically modified, bound to magnetic beads and interrogated for binding with human langerin. Langerin binding was observed for the β-GlcNAc functionalized WTA fragments using the micro array, this powerful tool can also be used to investigate WTA binding of (monoclonal) antibodies (mAbs) and human sera to discover immunogenic promising antigen candidates.
To enable crystallization studies with biological binding partners, such as antibodies and lectins two WTA trimers were synthesized bearing a single α-GlcNAc or β-GlcNAc at the second RboP repeat. Future studies will be directed to the crystallization of these fragments with langerin as well as monoclonal antibodies.

Chapter 4 describes the synthesis of C-3 β-GlcNAc-RboP hexamers. The introduction of C-3 β-GlcNAc residues, was recently discovered to be mediated by the glycosyltransferase TarP, and TarP modified WTA was found on clinically relevant *S. aureus* strains.[2-4] The fragments were synthesized both in solution and on solid phase. The multi-milligram quantities of material that were obtained made it possible to fully characterize the fragments using NMR spectroscopy, corroborating the structure determination of WTA-species isolated from bacterial strains. The WTAs fragments bearing two α-GlcNAc or β-GlcNAc residues were coupled to magnetic beads as previously described (Chapter 2). The beads were screened against monoclonal anti-α-1,4-GlcNAc WTA antibodies

(anti-α) and anti-1,4-β-GlcNAc-WTA antibodies (anti-β). The anti-α-GlcNAc WTA mAb selectively bound the α-1,4-GlcNAc WTA in a concentration-dependent manner, and the anti-β-GlcNAc WTA mAb bound both the 1,4-β-GlcNAc-WTA and 1,3-β-GlcNAc-WTA functionalized beads, with slightly better binding of the 1,4-β-GlcNAc-WTA. The mAbs were also screened against the unsubstituted WTAs (Chapter 2), and the substituted C-4 α- and β-glycosylated WTAs using the WTA micro-array. Figure 1 shows that the mAbs bind only to the WTA fragments bearing a GlcNAc appendage, showing that these mAbs do not bind to the RboP-backbone. The graph also shows that mAbs bind very specifically to the configurational epitope to which they are raised and that only a single GlcNAc moiety is required for effective binding. As expected, the WTA fragment that bears both an α- and β-GlcNAc binds to both mAbs.

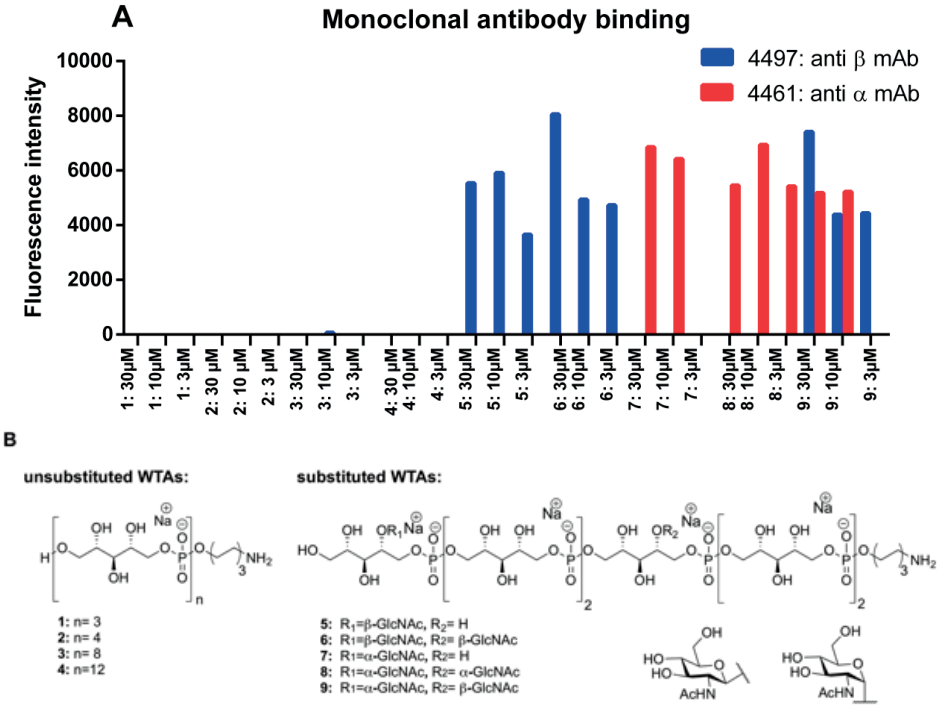


Figure 1. Binding specificity mAbs 4461 and 4497 (A) to synthetic WTAs on micro array (B).

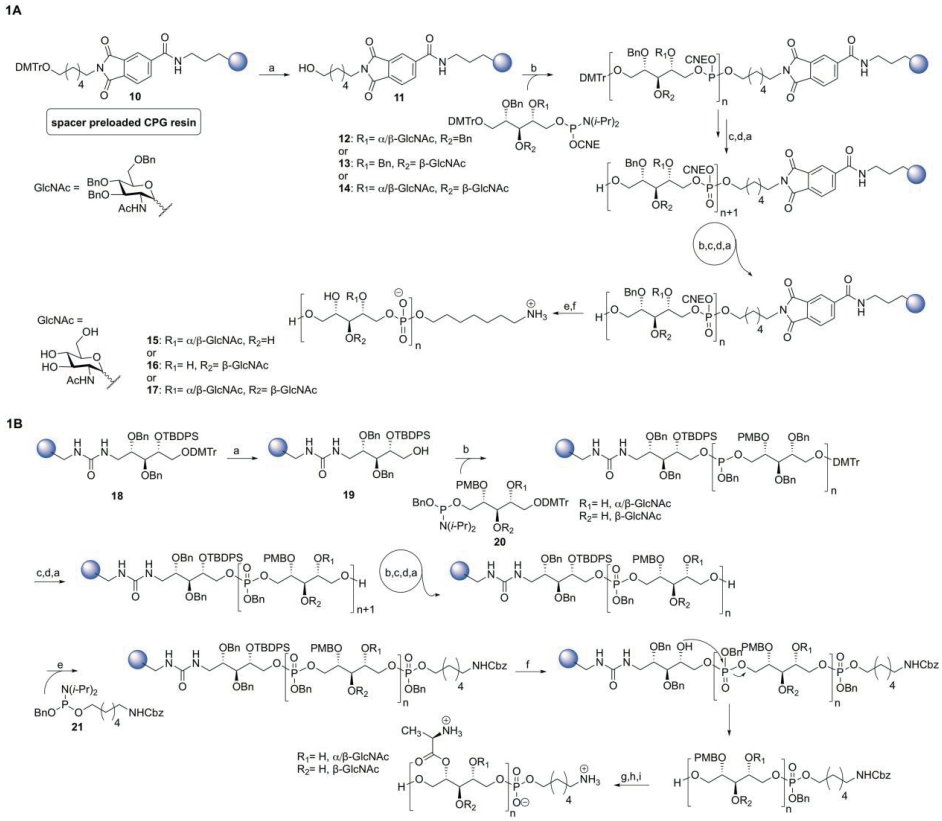
In an effort to further streamline the synthesis of GlcNAc WTAs, solid phase synthesis can be used to assemble these fragments of various length and featuring different substitution patterns, rapidly delivering a broad library of WTA fragments. An approach is depicted in Scheme 1A. Starting from commercially available spacer preloaded CPG resin **10**, an acid mediated step using DCA in toluene liberates the alcohol functionality. Condensation of alcohol **11** with either building blocks **12**, **13** or **14** under activation

of 5-(Benzylthio)-1*H*-tetrazole (BHT) will yield the phosphite intermediate, wich upon oxidation will be delivering the phosphate fragment. A capping step using Ac_2O, *N*-methylimidazole and 2,6-lutidine prevents any unreacted alcohol functionalities to react further in the upcoming cycles. Acidic detritylation then allows the next coupling cycle to start using one of the available phosphoramidite building blocks to assemble the library. Final deprotection steps include removal of the cyanoethyl groups by β-elimination using aqueous ammonia and the release from the CPG resin followed by palladium-catalyzed hydrogenolysis to afford the target fragments.

Chapter 5 has described an approach to synthesize an alanylated WTA oligomer. The presence of D-alanine esters in WTA fragments plays an important role in the biological activity of WTAs and it has been found that mutants lacking D-alanine esters are not only more sensitive to antimicrobial peptides like defensins but also show an increased susceptibility to Vancomycin and other glycopeptide antibiotics.[5-6] To explore the role of the D-alanine ester modification further, well-defined synthetic fragments are indispensable for biological activity studies. However, their synthesis poses a great challenge. Chapter 5 has described the synthesis of a heptamer carrying two D-alanine esters. It proved to be important to keep the final deprotection conditions slightly acidic because of the high lability of the D-alanine esters under basic conditions. A specific purification protocol was needed not to jeopardize the labile esters.

Scheme 1B presents a possible automated solid phase assembly procedure. Since most linkers require a basic step for cleavage, the novel silyl linker **18** is proposed, which requires mild fluoride-mediated conditions for cleavage. The phosphotriester group of the building blocks are protected with a benzyl group that is removable by hydrogenolysis, instead of the commonly used cyanoethyl group, which requires a basic step for removal. Starting from linker **18**, an acidic step removes the trityl group and the first coupling can take place. The first coupling cycle can start with building block **20**, which bears a PMB group that masks hydroxyl group that is to be functionalized with a D-alanine ester in the penultimate stage of the synthesis. Cleavage of the PBM ethers requires less harsh conditions than the removal of the NAP, described in Chapter 5. To allow for more variation in substitution patterns, the C-4 and C-3 positions of **20** can be modified with an α- or β-GlcNAc at the C-4 or β-GlcNAc on the C-3 yielding both an D-alanine and a glycoside substituent within the same RboP repeating unit. Oxidation, capping and DMTr cleavage complete the coupling cycle. Repetition of these steps will deliver the protected resin-bound fragments, which can be functionalized with a spacer using phosphoramidite **21**. In the next step the TBDPS group is cleaved off using TBAF leading to cleavage of the target fragment form the resin. Next the PMB groups can

be removed using DDQ, after which the coupling with D-alanine can take place. A final hydrogenation step can then deliver the target compounds.



Scheme 1A. Assembly of glycosylated WTAs using ASPS approach; *Reagents and conditions:* a) 3% DCA, toluene; b) phosphoramidite **12, 13,** or **14**, 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); f) Pd black, H_2, dioxane H_2O, AcOH. **Scheme 1B.** Assembly of alanylated WTAs using cleavable linker **18** based on ASPS approach; *Reagents and conditions:* a) 3% DCA, toluene; b) phosphoramidite **20**, 5-(Benzylthio)-1*H*-tetrazole, ACN; c) I_2, pyridine, H_2O, ACN; d) Ac_2O, *N*-methylimidazole, 2,6-lutidine, ACN; e) phosphoramidite **21**, 5-(Benzylthio)-1*H*-tetrazole, ACN; f) TBAF/THF; g) DDQ, β-pinene, DCM/H_2O/*t*-BuOH; h) Z-D-alanine, PyBOP, NMI, DCM; i) H_2, Pd black dioxane/H_2O, AcOH.

Chapter 6 describes the synthesis of Enterococcus faecalis (*E. faecalis)* WTA fragments. *E. faecalis,* a Gram-positive bacterium commonly found in human and animal intestines, is responsible for a wide range of infections like endocarditis, wound- and urinary tract infections. These infections are treated with antibiotics, however the widespread use of antibiotics has led to the emergence of resistant strains such as Vancoymicin Resistant Enterocuccus (VRE), which poses a great danger for our healthcare system. Chapter 6 is focused on the synthesis of two well-defined WTA fragments belonging to *E. faecalis* which can serve for the development of a possible conjugate vaccine. The WTA is built

up from a -6-[((GalNAc)-α(3-1)-L-Rha)-β(1-1)-RboP]- repeating unit (Fig 2). The trisaccharide repeating unit was assembled using a regioselective glycosylation of a rhamnose donor, carrying a participating benzoyl group at the C-2 to ensure the stereoselective formation of the α-glycosidic linkage, and a C3, C4-diol GalNAc acceptor. The C-4 OH was masked with a benzoyl group at the dimer stage and the resulting building block was used in the next step in a glycosylation with the ribitol acceptor.

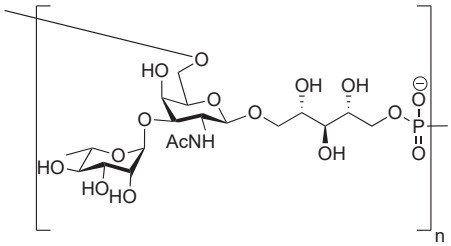


Figure 2. Structure of *E. faecalis* WTA.

Unfortunately, this coupling resulted only in 45% yield. Optimization was hampered by the low quantities of building blocks available. Converting the thiodonor into an imidate donor may increase the reactivity leading to a higher yield. Two repeating units were united through a phosphoramidite condensation to deliver the target hexasaccharide in 45% yield. This yield can possibly be increased by using more equivalents of the phosphoramidite. It was found that in the final hydrogenation step, the reduction of the trichloroacetyl groups led to the formation of HCl, which was responsible for partial cleavage of the rhamnosyl bonds. The hydrogenation steps therefore have to be optimized using non-acidic conditions. Currently, both fragments are available in sufficient quantities for use in biological studies. Whether they are suitable as components for a vaccine has yet to be determined using for example micro-array, competitive ELISA, and opsonophagocytic inhibition assays.

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Nederlandse samenvatting

Antibioticaresistentie is wereldwijd een groeiend medisch probleem. Door veelvuldig gebruik van antibiotica zijn multiresistente stammen ontstaan van *Staphylococcus aureus* en *Enterococcus faecalis* zoals, Methicillin-resistant *S. aureus* (MRSA) en Vancomycin-resistant enterococci (VRE). Deze multiresistente stammen zijn moeilijk te bestrijden met de huidige antibiotica. Daarnaast wordt het lastiger en risicovoller om medische interventies die afhankelijk zijn van antibiotica uit te voeren, zoals operaties en transplantaties. Daarom bestaat er een grote behoefte om nieuwe behandelingen te ontwikkelen. Een veelbelovende aanpak behelst het gebruik van passieve of actieve vaccinatie. Voor het opwekken van een immuunrespons tegen bacteriën zijn bacterie-specifieke structuren nodig. Uit eerdere studies is gebleken dat teichoïnezuren (teichoic acids, TAs) als antigen kunnen fungeren voor een vaccin. Celwand teichoïnezuren (wall teichoic acids, WTAs) zijn negatief geladen polymeren die op de gehele bacteriële celwand aanwezig zijn en die covalent gebonden zijn aan de peptidoglycaanlaag. Ze spelen een fundamentele rol in de fysiologie van Gram-positieve bacteriën zoals celvorming en celdeling. Ze moduleren de gevoeligheid voor antibiotica en zijn bijvoorbeeld vereist voor β-lactam resistentie. WTAs zijn opgebouwd uit repeterende ribitolfosfaat (RboP) monomeren, die willekeurig gemodificeerd zijn met *N*-acetyl glucosamine (GlcNAc) residuen en/of D-alanine esters. Voor *S. aureus* zijn drie glycosyltransferases bekend namelijk, TarP, TarM en TarS die verantwoordelijk zijn voor de GlcNAc modificatie op de RboP keten. TarP introduceert een β-GlcNAc op de RboP C-3 positie van de repeterende eenheid. TarM en TarS zijn verantwoordelijk voor de introductie van respectievelijk een α-GlcNAc en β-GlcNAc op de RboP C-4. D-alanine esters spelen een belangrijke rol in de biologische activiteit van WTAs. Uit voorgaande studies is gebleken dat bacteriële stammen met teichoïnezuren zonder D-alanine esters niet alleen gevoeliger zijn voor antimicrobiële peptiden, maar ook gevoeliger zijn voor Vancomycin en andere glycopeptide antibiotica.

Dit proefschrift beschrijft de synthese van verschillende teichoïnezuur fragmenten van *Staphylococcus aureus* en *Enterococcus faecalis*. De isolatie van deze teichoïnezuren uit de bacteriën is lastig, omdat dit resulteert in polyribitolketens van verschillende lengte met verschillende substitutiepatronen terwijl bovendien nog biologische verontreinigingen aanwezig kunnen zijn. Het is dus technisch zeer moeilijk om specifieke polyribitol fragmenten met een gedefinieerde structuur te isoleren. Dit proefschrift beschrijft methoden voor de organische synthese van ribitolfosfaat fragmenten met een bekende moleculaire structuur. Het is mogelijk gebleken om deze ribitolfosfaat fragmenten in voldoende hoeveelheden te verkrijgen voor immunologische studies. Deze verbindingen werden gesynthetiseerd met en zonder *N*-acetyl glucosamine (GlcNAc) residuen en/of D-alanine esters (D-ala). Het substitutiepatroon van deze fragmenten bestond uit α- en β-GlcNAc residuen op de RboP C-4, β-GlcNAc op de RboP C-3 en D-ala esters op de

RboP C-2, naar analogie van het substitutiepatroon van WTAs zoals deze voorkomen in *S. aureus*. De uitgevoerde synthese methode maakte gebruik van fosforamidiet chemie, die eertijds ontwikkeld is voor de synthese van DNA-fragmenten. De syntheses zijn niet alleen uitgevoerd in oplossing maar er zijn ook automatische vaste-drager syntheses ontwikkeld. Verder zijn er ook twee complexe teichoïnezuur fragmenten die voorkomen in *E. faecalis* gesynthetiseerd. Deze fragmenten hebben als repeterende eenheid een *N*-acetyl-β-D-galactosaminyl ribitol fosfaat residu met een α-L-rhamnose vertakking op de C-3 van het galactosamine residu.

Hoofdstuk 1 geeft een overzicht van de ontwikkelingen in de synthese van goed-gedefinieerde teichoïnezuurfragmenten voor vaccin toepassingen. Dit hoofdstuk behandelt de synthese van teichoïnezuren die voorkomen in de celwand van *S. aureus*, *E. Faecalis*, *E. faecium,* en *Clostidrium difficile*. Voor het bestuderen van de biologische activiteit van deze teichoïnezuren is onder andere gebruik gemaakt van micro-arrays, waarmee de affiniteit onderzocht is van de verschillende teichoïnezuurfragmenten met antilichamen in serum.

Hoofdstuk 2 beschrijft de synthese van niet-gesubstitueerde ribitolfosfaat oligomeren met een lengte van drie tot acht repeterende eenheden en voorzien van een 6-amino-hexanol-spacer om verdere biologische studies mogelijk te maken. De bouwstenen, die ontwikkeld werden voor de synthese in oplossing, konden ook worden gebruikt voor de synthese van een octa- en dodecameer met behulp van een automatische vaste-drager synthesizer. Het gesynthetiseerde hexameer is gebruikt om de werking van het recent ontdekte *S. aureus* glycosyltransferase TarP te onderzoeken en heeft het mogelijk gemaakt een kristalstructuur te verkrijgen van het enzym met het substraat om zo de regiochemie van de glycosylering te begrijpen. In een andere toepassingen werden de fragmenten gekoppeld aan magnetische beads, waarvoor de fragmenten eerst werden voorzien van een biotine label. Vervolgens werden de gebiotinyleerde fragmenten enzymatisch geglycosyleerd door gebruik te maken van TarM, TarS en TarP. De geglycosyleerde fragmenten werden vervolgens gekoppeld aan streptavidine-gecoate magnetische beads en daarna toegepast om teichoïnezuur-specifieke IgG antilichamen te detecteren in humaan serum. De methode is een snelle en schone methode om antilichaam levels te meten en biedt de mogelijkheid om de interactie te bestuderen tussen teichoïnezuren en andere relevante biologische samples. De enzymatische glycosylering met behulp van TarS is ook uitgevoerd op grotere schaal waarbij er 0.5 mg geglycosyleerd teichoïnezuur fragment werd verkregen.

Hoofdstuk 3 beschrijft de synthese van een set ribitol fosfaat fragmenten die geglycosyleerd zijn op de C-4 van de RboP repeterende eenheid, met een enkele of dubbele

α- of β-GlcNAc substituent aan de RboP-keten. Met behulp van een micro-array werd de binding onderzocht tussen de geglycosyleerde fragmenten met humaan langerin, een belangrijke receptor die zich bevindt op Langerhans cellen. Daarnaast werden het hexameer en dodecameer (Hoofdstuk 2) enzymatisch geglycosyleerd, gebonden aan magnetische beads, waarmee ook de binding met humaan langerin werd onderzocht. Uit beide technieken is gebleken dat langerin specifiek bindt aan teichoïnezuurfragmenten met de β-GlcNAc configuratie. Tot slot werden er twee trimeren gesynthetiseerd met een enkele α-GlcNAc of β-GlcNAc op de tweede RboP eenheid zonder een spacer. Deze trimeren kunnen worden toegepast voor kristallisatiestudies met langerin en monoklonale antilichamen.

Hoofdstuk 4 beschrijft de synthese van ribitolfosfaat hexameren gemodificeerd met zowel een enkele- als een dubbele β-GlcNAc op de C-3 positie van de repeterende eenheid. Voor de synthese van deze fragmenten werd gebruik gemaakt van zowel oplossings- als vaste-drager synthese. De fragmenten werden verkregen op multi-miligram schaal. Deze fragmenten en de fragmenten uit hoofdstuk 3 zijn volledig gekarakteriseerd met behulp van NMR zodat, ze als referentie kunnen dienen voor structuurbepaling van teichoïnezuren afkomstig van bacteriële stammen. De gesynthetiseerde teichoïnezuur fragmenten met twee α-GlcNAc of β-GlcNAc groepen werden gekoppeld aan magnetische beads zoals beschreven in hoofdstuk 2. Vervolgens werden de beads gebruikt om binding met anti-α-1,4-GlcNAc monoklonale teichoïnezuur antilichamen (anti-α) en anti-1,4-β-GlcNAc teichoïnezuur antilichamen (anti-β) te onderzoeken. Hieruit is gebleken dat het anti-α-GlcNAc mAb concentratie-afhankelijk en selectief het α-1,4-GlcNAc teichoïnezuurfragment bond. Verder bond het anti-β-GlcNAc mAb concentratie-afhankelijk en selectief zowel het 1,4-β-GlcNAc- als het 1,3-β-GlcNAc fragment. De binding was sterker met het 1,4-β-GlcNAc fragment. De binding met monoklonale antilichamen werd ook onderzocht met de ongesubstitueerde fragmenten en de C-4 α- en β-geglycosyleerde fragmenten met behulp van de micro-array. Uit de resultaten van de micro-array bleek dat dat de monoklonale antilichamen alleen de teichoïnezuurfragmenten herkennen die een GlcNAc groep dragen. Verder werd bevestigd dat de monoklonale antilichamen heel specifiek aan de GlcNAc configuratie (α of β) binden waartegen ze zijn opgewekt. Een enkele GlcNAc groep was voldoende voor effectieve binding. Zoals verwacht bond het fragment, dat zowel een α- als een β-GlcNAc substituent bevat, aan beide antilichamen.

Hoofdstuk 5 behandelt een syntheseroute naar een teichoïnezuur ribitolfosfaat heptameer met een D-alanine esters op de C-2 positie van twee repeterende eenheden. Om het effect van D-alanine ester modificatie op biologische activiteit te onderzoeken zijn goed gedefinieerde synthetische fragmenten nodig. Uit de synthese is gebleken dat het

erg belangrijk is om de laatste ontschermingscondities licht zuur te houden vanwege de hoge labiliteit van de D-alanine esters. Een specifiek zuiveringsprotocol was nodig om het risico op hydrolyse van de labiele D-alanine esters te voorkomen.

Hoofdstuk 6 behandelt de synthese van twee goed gedefinieerde fragmenten van een specifiek teichoïnezuur dat voorkomt in de celwand van *Enterococcus faecalis*, een Gram-positieve bacterie die onderdeel is van de darmflora van mensen en dieren. *E. faecalis* is onschadelijk voor gezonde mensen, maar bij patiënten met een verminderde afweer kan het ernstige infecties veroorzaken zoals endocarditis, alsook wond- en urineweginfecties. Deze infecties worden behandeld met antibiotica, maar door het grote gebruik heeft dit geleid tot resistente stammen zoals Vancoymicin Resistente Enterococci (VRE). De gesynthetiseerde teichoïnezuur fragmenten zijn opgebouwd uit een -6-[((GalNAc)-α(3-1)-L-Rha)-β(1-1)-RboP]- repeterende eenheid en voorzien van een 6-aminohexanol-spacer voor toekomstige immunologische studies. De trimeer repeterende eenheid werd opgebouwd doormiddel van een regioselectieve glycosylering tussen een rhamnose donor met een participerende benzoyl groep op de C-2 voor de stereoselective vorming van de α-glycosidische band en een C-3, C-4-diol GalNAc acceptor. De C-4 hydroxyl groep werd vervolgens gemaskeerd met een benzoyl groep, waarna er koppeling plaatsvond met een ribitol acceptor. Het verkregen trimeer werd na de verwijdering van een allyl groep omgezet in een fosforamidiet bouwsteen. Condensatie van het fosforamidiet trimeer met een trimeer alcohol vormde het hexameer fragment. De hydrogenering van het beschermde tri- en hexameer resulteerde in de isolatie van beide doelfragmenten in een relatief lage opbrengst. Dit kan verklaard worden door de gedeeltelijke afbraak van de zuur labiele rhamnose banden door HCl dat gevormd werd bij de reductie van de trichlooracetyl groepen. Desalniettemin zijn de fragmenten in voldoende hoeveelheden verkregen voor toekomstige immunologische studies.

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Curriculum Vitae

Sara Ali werd op 2 mei 1990 geboren te Al-Najaf, Irak. Na het behalen van het HAVO diploma (profiel Natuur en Techniek & Natuur en Gezondheid) aan het Krimpenerwaard college in 2007, werd begonnen aan de bacheloropleiding Chemie aan de hogeschool Rotterdam. Als onderdeel van het programma in het derde jaar, werd een stage uitgevoerd bij TNO Defensie & Veiligheid en onderzoek verricht naar de "reactivering van verouderd acetylcholine-esterase onder leiding van dr. M.C. de Koning. In het vierde jaar werd de afstudeerstage uitgevoerd bij de vakgroep Bio-organische Synthese groep van de Leidse universiteit. In dit project werd onderzoek verricht naar de profilering van de ziekte van Gaucher onder begeleiding van dr. C.S Wong, prof. dr. G.A. van der Marel en prof. dr. J.D.C. Codée. In 2011 werd begonnen met de masteropleiding Chemistry (Ontwerp & Synthese) aan de Universiteit Leiden. Tijdens de research stage dat werd voortgezet bij de vakgroep Bio-organische Synthese groep werd gewerkt aan de synthese van de repeterende eenheid van *S. aureus* type 5 onder begeleiding van dr. B. Hagen.

In december 2013 werd begonnen met het in dit proefschrift beschreven onderzoek, dat is verricht in de vakgroep Bio-organische Synthese groep onder supervisie van prof. dr. G.A. van der Marel en prof. dr. J.D.C. Codée. Delen van dit onderzoek werden gepresenteerd middels een posterpresentatie op de NWO-CHAINS conferentie 2015 te Veldhoven, op het 19e European Carbohydrate Symposium 2017 te Barcelona en op het 29e internationale Carbohydrate Symposium 2018 te Lissabon. Mondelinge presentaties werden gegeven op het 19^{e} European Carbohydrate Symposium 2017 te Barcelona en op de NWO-CHAINS conferentie 2018. In de periode 2015-2017 werd deelgenomen aan de educatieve master op grond waarvan de eerste graad onderwijsbevoegdheid in de scheikunde werd behaald. Deze master werd parttime gevolgd als onderdeel van het traject "Doctors voor de klas". Hiervoor werd in 2015-2016 stage gelopen op Wolfert Lyceum onder begeleiding van N. Folmer en dhr. G. Nieborg en onder supervisie van Drs. J.M. Espinola y Vázquez. In 2016-2017 werd een overstap gemaakt naar het Johan de Witt Scholengroep onder begeleiding van dhr. H. El Mimouni.