Synthesis of D-alanylated wall teichoic acids from staphylococcus aureus

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Chapter

2

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Introduction

Most soft tissue infections in humans are caused by *Staphylococcus aureus*[1]. However, the severity of these infections has escalated, with *S. aureus* now being the source of invasive and life-threatening conditions, such as pneumonia, endocarditis, or sepsis[2]. Treating these infections has become increasingly more challenging in recent years, primarily due to the emergence of methicillin-resistant strains. These strains exhibit resistance against multiple antibiotics and are responsible for ±20.000 deaths annually [3][4]. This alarming trend necessitates a deeper understanding of antibiotic resistance mechanisms in *S. aureus*.

The bacterial cell wall decoration plays a crucial role in evading the host immune system and inhibiting the action of antibiotics[2][3]. A prominent component of the cell wall of *S. aureus* are the wall teichoic acids (WTAs), which are covalently attached to the peptidoglycan and consist of repeating ribitol phosphate units. The poly ribitol phosphate chain can be decorated with α- or β-*N*-acetylglucosamine moieties on the C-4 hydroxyl or a β-*N*-acetylglucosamine on the C-3 hydroxyl of the ribitol in a (seemingly) random manner. Moreover, the C-2 hydroxyl of the ribitol moieties can be decorated with D-alanine esters. The interplay between the negatively charged phosphodiesters and the positively charged D-alanine esters provides a partial zwitterionic character. It influences the hydrophilicity of the cell wall and the binding of a plethora of extracellular molecules[4]. As such, it plays a vital role in protecting the bacteria against diverse threats and unfavourable conditions. The contribution of the D-alanine substituents to these processes has been explored at the bacterial level through knockout of

the *dlt* operon, which encodes for the enzymes responsible for integrating D-alanine esters into TAs[5]. These mutants showed an increased susceptibility to antimicrobial peptides, providing evidence for the role of the D-alanine substituents in protecting bacteria against these antibiotics. It has been hypothesised that the enhanced susceptibility resulted from the modification in the electrostatic interaction between the peptides and the mutant cells, as decreased susceptibility was solely observed for cationic peptides. Moreover, the absence of D-alanine substituents in TAs resulted in a three-fold increase in sensitivity towards glycopeptide antibiotics, such as vancomycin and teicoplanin[6]. Overall, these data clearly show the importance of the D-alanine substituents in protection against antimicrobial peptides.

The use of well-defined WTAs is imperative to understand better the role of the D-alanine residues at the molecular level. However, the microheterogeneity of WTAs poses a substantial obstacle in isolating well-defined structures. Furthermore, the high hydrolytic lability of the D-alanine substituents can easily result in their loss during isolation from bacterial sources. Chemical synthesis therefore emerges as a viable solution to these challenges, as it enables the production of WTAs with a well-defined length and pre-defined substitution patterns[7].

Synthetic WTA molecules have recently been used to elucidate the interactions with WTA-targeting antibodies. It was observed that the GlcNAc substituent serves as the prime recognition element, which is inserted into an antibody binding pocket formed by the heavy and light chains[8]. Additionally, synthetic WTA molecules have contributed to a deeper understanding of the binding requirements between different WTA glycoforms and langerin, a C-type lectin present in Langerhans cells in the epidermis, where they play a crucial role in immune surveillance [9]. While these studies have highlighted the value of synthetic WTA molecules, it is worth noting that these structures lack the common D-alanine substituents. Therefore, the effect of the D-alanine substituents on antibody-mediated epitope recognition and receptor binding remains unclear[10]. This chapter focuses on developing a synthetic route towards ribitol phosphate (RboP) oligomers with a predetermined D-alanylation pattern to elucidate their role in antibody-mediated epitope recognition. An effective synthesis route towards the required ribitol building block is presented, an essential

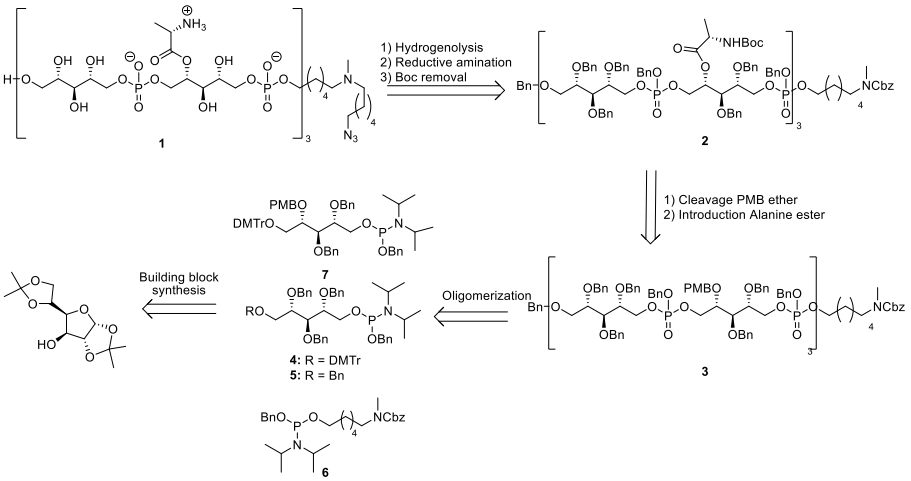
component to achieve the well-defined D-alanylation pattern. It has been used to generate an RboP-hexamer carrying three alanine esters and an azido spacer for future conjugation purposes, as illustrated in Scheme 1.

Results and discussion

Previous studies have demonstrated that glycosylated ribitol phosphate hexamers can be adequate binding partners for antibodies elicited against *S. aureus*[11]. Consequently, the decision was made here to generate ribitol phosphate hexamers featuring a precisely defined alanylation arrangement. Additionally, the ratio of alanine substituents to phosphorus has been established to be one to two [5], and therefore, three alanine substituents will be incorporated in the hexamer. The retrosynthesis of target hexamer **1**, carrying a chemoselective azide ligation handle, is depicted in Scheme 1. The previously generated synthetic TA fragments were all furnished with a linker with an orthogonal amino group. The presence of the D-alanine residues on the target compound obviously precludes using primary amines as a linkage moiety. Therefore, an azide was projected to be introduced as a conjugation handle in the D-alanylated target compounds. As seen in the retrosynthetic analysis, this ligation handle will be introduced via a reductive amination after removing all benzyl-type protecting groups. During the reductive amination reaction, the D-alanine substituents remain protected with a *tert*-butyloxycarbonyl (Boc) group, which can be removed after the installation of the linker.

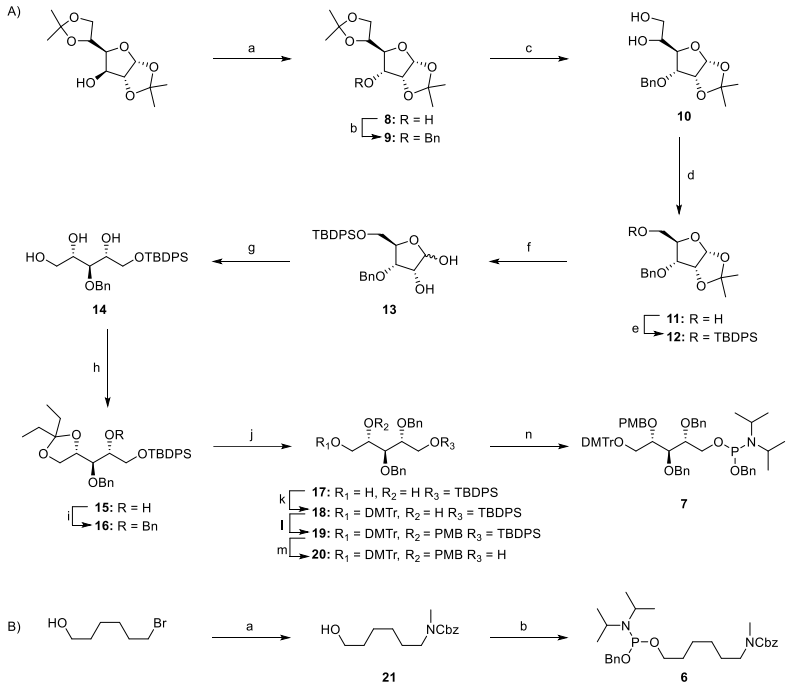
Considering the labile nature of the alanines, benzyl-protected phosphoramidite building blocks are utilised instead of the commonly used cyanoethyl-protected phosphoramidites, which necessitate basic deprotection conditions. The benzyl-protected phosphotriesters can be deprotected under mild conditions [12]. The alanine esters will be introduced on the fully assembled ribitol phosphate backbone. During the assembly of the hexamer, a *para*-methoxylbenzyl (PMB)-ether was incorporated as a temporary protecting group that can be selectively removed using oxidative conditions to set the stage for introducing the alanine moieties. The fully protected backbone can be deconstructed into essential building blocks **4, 5, 6,** and **7** for use in the phosphoramidite coupling cycles. Herein, the dimethoxytrityl group is a temporary protection group that can be selectively removed to liberate the primary hydroxyl group for chain elongation in the

subsequent coupling cycle[13]. The phosphoramidite building blocks **4, 5,** and **6** can be obtained from the commercially available 5-*O*-allyl-2,3,4-tri-*O*-benzyl-D-ribitol and 6-bromo hexanol. Furthermore, phosphoramidite ribitol building block **7**, outfitted with a C-2-PMB ether, can be obtained in 15 synthetic steps from di-acetone-D-glucose.



*Scheme 1: Retrosynthetic analysis of target compound **1.***

Following the synthetic approach described by Ali *et al*., the synthesis of building block **7** started from commercially available di-acetone-D-glucose, which underwent Albright-Goldman oxidation to provide the C-3 ketone, which was subsequently reduced using sodium borohydride ($NaBH_4$) in a stereoselective manner to yield allose **8** (Scheme 2) [14]. The hydroxyl group in **8** was protected through benzylation using benzyl bromide (BnBr), sodium hydride (NaH), tetra n-butylammonium iodide (TBAI). Subsequently, the 5,6-isopropylidene moiety was regioselectively cleaved using a catalytic amount of *para*-toluenesulfonic acid (PTSA) in methanol (MeOH). The obtained diol **10** was cleaved using sodium periodate ($NaIO_4$), after which the resulting aldehyde was reduced by sodium borohydride ($NaBH_4$) to obtain ribose **11**. The primary hydroxyl in **11** was silylated with tert-butyl(chloro)diphenylsilane (TBDPSCl) in the presence of imidazole in dimethylformamide (DMF) to obtain ribose **12**. Initial efforts to remove the isopropylidene with PTSA in MeOH led to the loss of the silyl ether. However, using aqueous trifluoracetic acid (TFA) in dichloromethane (DCM) effectively resulted in its selective removal to obtain hemiacetal **13**.



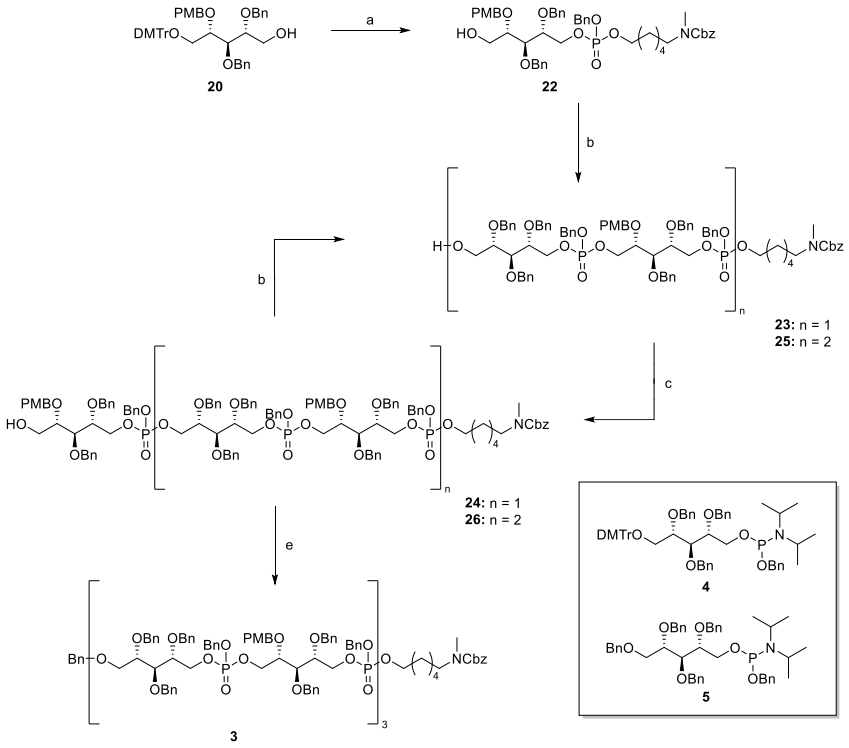
Scheme 2: ***A Building block synthesis.*** *Reagents and conditions: a) i. DMSO, Ac_2O; ii. $NaBH_4$, $EtOH/H_2O$, 0°C to RT (v/v= 7/3), 73% over 2 steps; b) BnBr, NaH, TBAI, DMF, 0°C to RT 86%; c) PTSA, MeOH, 70%; d) i. $NaIO_4$, MeOH, 0°C to RT; ii) $NaBH_4$, MeOH, 0°C to RT, 97% over 2 steps; e) TBDPSCl, imidazole, DMF; f) TFA(aq), DCM, 0°C, 74% over 2 steps; g) $NaBH_4$, MeOH, 0°C to RT; h) 3-pentanone, PTSA, 71% over 2 steps; i) BnBr, TBAI, NaH, -40°C, 78%; j) TFA(aq), DCM, 0°C, Q?; k) DMTr, TEA, DCM, 0°C to RT 91%; l) PMBBr, THF:DMF (7:1), 0°C to RT, 81%; m) TBAF, THF, 0°C to RT, 87%, n); 1-(Benzyloxy)-N,N,N',N'-tetraisopropylphosphinediamine, diisopropylammonium tetrazolide, DCM, 91%* ***B Linker Synthesis.*** *Reagents and conditions: a) i. methylamine, THF; ii. benzyl chloroformate, $NaHCO_3$, Et_2O/H_2O, 71% over 2 steps; b) 1-(Benzyloxy)-N,N,N',N'-tetraisopropylphosphinediamine, diisopropylammonium tetrazolide, DCM.*

The hemiacetal was reduced by utilising $NaBH_4$ to obtain triol **14**. It was initially probed to protect the terminal diol with an isopropylidene ketal regioselectively. However, these attempts did not provide sufficient regioselectivity. Hence, 3-pentanon was utilised to protect the terminal diol with an isopentyl ketal to obtain compound **15**, preventing the formation of the undesired six-membered ring ketal[15]. Next, the hydroxyl group was transformed in benzyl ether using BnBr and NaH. When this reaction was executed, starting at 0 °C and warming to room temperature, a complex mixture was produced, likely due to migration and cleavage of the silyl ether.

Reducing the reaction temperature to -25 °C prevented the formation of the side products and yielded a single product **16**. Subsequently, the pentonide was removed using aqueous TFA in DCM, and the liberated primary hydroxyl was regioselectively protected with a dimethoxytrityl (DMTr) group to obtain compound **18**. An attempt to introduce a PMB-ether at the C-2 position led to the formation of a complex mixture when employing 4-methoxybenzyl chloride (PMB-Cl) using NaH as a base. Interestingly, this was not observed in a trial experiment in which 2-methylnapthyl bromide (Nap-Br) was used as an alkylating agent. Therefore, the corresponding 4-methoxybenzyl bromide (PMB-Br) was prepared from p-anisyl alcohol and phosphorus tribromide. This freshly prepared reagent produced a clean reaction, delivering **19** in excellent yield. In the final steps, the silyl ether was selectively removed using tetra-n-butylammonium fluoride (TBAF) to yield building block **20**, which was readily converted into amidite building block **7** by treatment with benzyl phosphordiamidite using di-isopropylammonium tetrazole-2-ide. It is important to note that using the latter reagent prevents activation of the amidite product **7**[16]. The linker amidite building block was synthesised from the commercially available 6-bromohexanol, as depicted in Scheme 2. First, the bromide was substituted with methylamine in tetrahydrofuran, followed by the introduction of the benzyl carbamate. The methyl group on the amine will prevent over-alkylation in the reductive amination step used to introduce the linker in the fragment. Lastly, the hydroxyl was transformed into the amidite building block **6** using benzyl phosphordiamidite in combination with di-isopropylammonium tetrazole-2-ide.

The assembly of hexamer **1** commenced with introducing the linker, which was coupled to PMB-carrying ribitol **20** using 4,5-dicyanoimidazole (DCI) as the activating agent (Scheme 3). Subsequently, the intermediate phosphite was oxidised to a phosphate triester using (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO). Following oxidation, the DMTr-group was removed under mild acidic conditions employing trichloroacetic acid (TCA) to obtain compound **22**, thereby liberating the hydroxyl for the subsequent coupling cycle. Importantly, the PMB ether remained untouched under these conditions. A subsequent coupling cycle with phosphoramidite **4**, synthesized using the same strategy as for **6** and **7** with 2,3,4-*O*-di-benzyl-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol as the starting material, produced ribitol phosphate dimer **23.** Repetition of these two coupling cycles led to the longer

oligomers and eventually pentamer **26**. The final coupling step involved the utilisation of phosphoramidite **5**, which is capped with a benzyl ether instead of a DMTr group to prevent alanylation at this position, yielding the target hexamer **3**. Hexamer **3** bears three PMB-protected hydroxyl moieties, facilitating the subsequent introduction of D-alanine esters.



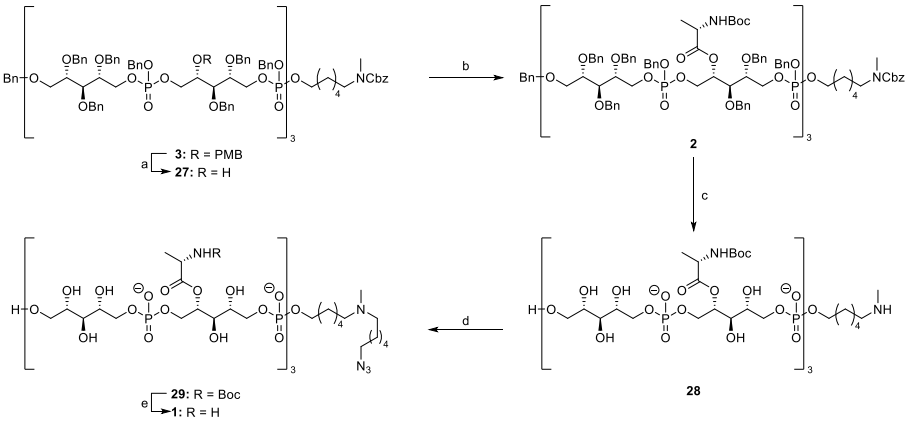
Scheme 3: ***Hexamer assembly.*** *Reagents and conditions a) i. DCI, ACN,* ***6****; ii. CSO; iii. 3% TCA in DCM, 70%; b) i. DCI, ACN,* ***4****; ii. CSO; iii. 3% TCA in DCM,* ***23****: 91%,* ***25:*** *94%; c) i. DCI, ACN,* ***7****; ii. CSO; iii. 3% TCA in DCM,* ***24:*** *71%,* ***26:*** *90%; e) i. DCI, ACN,* ***5****; ii. CSO; iii. 3% TCA in DCM, 90%*

To introduce the D-alanine substituents, the PMB ethers were removed using cerium ammonium nitrate (CAN) in a mixture of acetonitrile and water to obtain triol **27** in 66% yield (Scheme 4). 2,3-Dichloro-5,6-dicyanoquinone (DDQ) was also explored, but this did not provide consistent results. Next, the incorporation of D-alanine substituents was achieved by coupling Boc-protected D-alanine using benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBop) and N-methylimidazole (NMI), to deliver the

fully protected alanylated hexamer **2**[12]. After introducing the D-alanine substituents, the benzyl ethers were removed by hydrogenolysis. Initial attempts to reduce the ethers using palladium in a mixture of dioxane and miliQ, acidified with three drops of acetic acid, resulted in partial loss of the Boc groups. Therefore, sodium acetate was added to the reduction medium to circumvent the acidic pH arising from the phosphate diesters. Unfortunately, adding sodium acetate resulted in an exceedingly long reaction time. Moreover, adding acetic acid combined with sodium acetate decreased the reaction time. However, this reaction mixture facilitated the migration of the D-alanine substituent from the C-2 position to the C-3 position of the ribitol. Fewer equivalents of sodium acetate were used to prevent the migration, but this, unfortunately, was insufficient to prevent the migration completely. In pursuit of enhanced pH control, sodium acetate was replaced by a monopotassium phosphate buffer for the hydrogenation. This resulted in the effective removal of the benzyl ethers, thereby obtaining hexamer **28** without jeopardising the Boc groups or the alanine substituents.

After successfully removing the benzyl ethers, the azide linker was introduced. Initially, an attempt was made using a dimeric test substrate to transform the amine of the linker into the corresponding azide utilising fluorosulfuryl azide. [17][18]. Regrettably, migration of the D-alanine substituents was observed even under the relatively mild basic conditions and the short reaction time. Also using the less basic sodium acetate instead of sodium bicarbonate proved ineffective in preventing the migration. Considering that the basic reaction conditions facilitate the migration, it was anticipated that the D-alanine substituents would remain stable and not undergo migration during a reductive amination, which can be achieved at neutral or slightly acidic pH[19]. Therefore, a reductive amination was performed with hexamer **28** and 6-azidohexanal in a mixture of dimethyl sulfoxide (DMSO) and acetic acid, using sodium triacetoxy borohydride as a reducing agent. The presence of the methyl group at the amine of the linker prevents double alkylation of the spacer. Purification of the crude hexamer was attempted using gel filtration using 100 mM NaCl as a buffer. This, however, necessitated an additional purification step to remove the NaCl, which again led to a partial migration of substituents. To avoid the additional purification step, the eluent system was switched to 1% acetic acid in a mixture of MiliQ and ACN (9:1). However, this also led to partial migration of

the D-alanine substituents. Eventually, switching from HW40 gel filtration to C18-chromatography, using acetonitrile and MiliQ as mobile phase, resulted in a consistent purification protocol and gave the target hexamer a 33% yield. Finally, the Boc-groups were removed using a combination of HCl and hexafluoroisopropanol (HFIP), followed by the removal of all volatiles by lyophilisation, resulting in the target hexamer in 95% yield.



Scheme 4: ***Hexamer modification****, Reagents and conditions a) CAN, ACN/H_2O, 66%; b) Boc-Ala-OH, Pybop, NMI, DCE, 96%; c) H_2, Pb_{black}, KH_2PO_4 buffer, AcOH, Dioxane/H_2O; d) 6-azidohexanal, Na[$(CH_3COO)_3BH$], DMSO/AcOH, 33% over 2 steps; e) HCl in HFIP, 96%.*

Conclusion

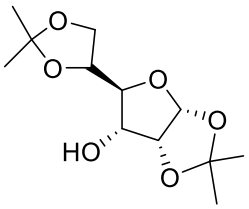
In conclusion, this study has outlined a synthetic pathway for producing an alanylated ribitol phosphate hexamer. A phosphoramidite ribitol building block with a PMB ether at the 2-position hydroxyl group was synthesised and used for hexamer assembly. The selective removal of the PMB ether was followed by introducing Boc-protected alanine residues to introduce the alanine esters. The hydrogenolysis required for removing the benzyl ethers required using a phosphate buffer to ensure the integrity of the labile alanine substituents and the unintended cleavage of Boc protections. A reductive amination strategy incorporated a linker bearing an azide functionality. Despite purification challenges, notably the migration of substituents during elution, the adoption of C18-chromatography consistently yields the desired hexamer in satisfactory quantity. The synthesis was then completed by removing the Boc groups using HCl in HFIP. Overall, the first synthesis of an alanylated ribitol phosphate WTA fragment has been achieved, and the chemistry developed can now be used to generate libraries of WTA fragments with different alanylation and glycosylation patterns. These can be used for further investigation of their biological functions.

Experimental

General information

All chemicals (Acros, Fluka, Merck, Sigma-Aldrich, etc.) were used as received, and reactions were carried out dry, under a nitrogen 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 visualised 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 or with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in water followed by charring at +/- 140 °C. ^1H, ^{13}C and ^{31}P NMR spectra were recorded with a Bruker WB 400 (400, 101 and 162 MHz, respectively), a Bruker AV 500 (500, 125 and 202 MHz, respectively) or a Bruker DMX 850 (850, 214 and 344 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane for ^1H and ^{13}C. When D_2O or CD_3CN were used, ^1H-NMR was recorded with chemical shift (δ) relative to the proton of residual solvent (4.75 ppm and 1.94 ppm, respectively). ^{13}C-NMR spectra were recorded with chemical shift (δ) relative to TMS (external standard) in the case of D_2O and 1.32 ppm as a residual solvent in CD_3CN. The ^{31}P- NMR spectra were recorded with chemical shift (δ) relative to H3PO4. (external standard). 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).

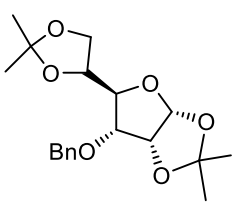
1,2-5,6-Di-*O*-isopropylidene-α-D-allofuranose (**8**)



Diacetone-D-glucose (78.1 g, 300 mmol) was dissolved in a mixture of DMSO:Ac_2O 3:2 (1.5 L, 0,2 M) and stirred for 72 hours. The reaction mixture was concentrated *in vacuo* and dissolved in EtOH:H_2O 7:3 (1.5 L, 0.2M). The mixture was cooled to 0°C, followed by the portion-wise addition of $NaBH_4$ (23.7 g, 627 mmol, 2.1 eq) and stirred for 75 minutes. The product was extracted thrice with EtOAc and concentrated *in vacuo.* The crude product was dissolved in MTBE and extracted thrice with H_2O. The combined water layers were extracted thrice with DCM. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to obtain the product as an oil (56.7g, 218 mmol, 73%).

The spectroscopic data were in agreement with the reported data[14].

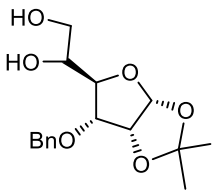
3-*O*-Benzyl-1,2-5,6-Di-*O*-isopropylidene-α-D-allofuranose (**9**)



Compound **8** (52.8 g, 203 mmol) was dissolved in DMF (800 ml, 0.25M) and cooled to 0°C. Benzyl bromide (30 ml, 244 mmol, 1.2 eq) and TBAI (6.55 g, 20.3 mmol, 0.1 eq) were added, followed by a portion-wise addition of NaH (60% dispersion in mineral oil, 12.2 g, 305 mmol, 1.5 eq). The reaction was stirred ON and quenched at 0°C with MeOH. H_2O was added, and the product was extracted thrice with Et_2O. The combined organic layers were washed with NaHCO3 (aq.) and brine, then dried over $MgSO_4$ and concentration *in vacuo*. The product was purified by flash chromatography (pentane: Et_2O 10:0 to 7:3) to obtain the product as an oil (61.2 g, 175 mmol, 86%).

The spectroscopic data were in agreement with the reported data[20].

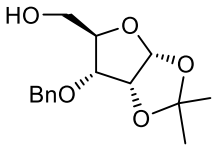
3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-allofuranose (**10**)



Compound **9** (36.6g, 105 mmol) was dissolved in MeOH (2.1 L, 0.05M), and PTSA monohydrate (2.0 g, 10.5 mmol, 0.1 eq) was added. The reaction was stirred for four hours, followed by adding TEA and concentrated *in vacuo*. The product was purified by flash chromatography (10/0 to 7/3 DCM/ acetone) to obtain the product as an oil (22.7 g, 73 mmol, 70%).

The spectroscopic data were in agreement with the reported data[21].

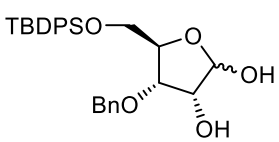
3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-ribofuranoside (**11**)



Compound **10** (22.7 g, 73 mmol) was dissolved in MeOH (365 ml, 0,2M) and cooled to 0°C. $NaIO_4$ (0.22 M in water, 400 ml, 88 mmol, 1.2 eq) was added and stirred for 3 hours, followed by the addition of ethylene glycol (8.3 ml, 148 mmol, 2 eq). The mixture was filtrated and diluted with DCM. The organic layer was washed once with H_2O and brine. The crude product was dissolved in MeOH (730 ml, 0,1M) and cooled to 0°C. $NaBH_4$ (3.3 g, 88 mmol, 1.2 eq) was added portion-wise, and the reaction was stirred ON. The reaction was quenched with acetone and concentrated *in vacuo.* The crude product was dissolved in DCM and washed once with NH_4Cl(aq) and brine, dried over $MgSO_4$ and concentrated *in vacuo* to obtain the product as an oil (19.5 g, 71 mmol, 97%).

The spectroscopic data were in agreement with the reported data[22].

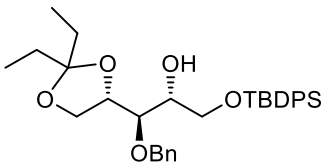
3-*O*-Benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-D-ribofuranoside (**12**)



Compound **11** (20.5 g, 73.3 mmol) was co-evaporated thrice with toluene and dissolved in dry DMF (400 ml, 0.2 M). Imidazole (15.1g, 222 mmol, 3 eq) and TBDPSCl (24.6 g, 90 mmol, 1.2 eq) were added, and the mixture was stirred for 72 hours. The reaction was quenched with H_2O, and the product was extracted thrice using Et_2O. The combined organic layers were washed with twice $NaHCO_3$(aq) and brine,

dried over $MgSO_4$ and concentrated *in vacuo.* The crude product was dissolved in DCM (740 ml, 0.1M) and cooled to 0°C. TFA (84% in water, 71.5 ml, 779 mmol, 11 eq) was added and stirred for four hours. The reaction was quenched with $NaHCO_3$(aq) and the product was extracted thrice with DCM. The combined organic layers were washed thrice with $NaHCO_3$(aq) and once with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The product was purified by flash chromatography (10/0 to 5/5 pentane/EtOAc) to obtain an oil (26.1g , 54.5 mmol, 74%). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.75 – 7.54 (m, 4H, CH_{arom}), 7.51 – 7.25 (m, 11H, CH_{arom}), 5.30 (s, 2H, H-1), 4.66 (d, *J* = 2.9 Hz, 2H, $CH_{2\ benzyl}$), 4.63 – 4.48 (m, 2H, $CH_{2\ benzyl}$), 4.38 (dd, *J* = 6.1, 4.7 Hz, 1H, H-3α), 4.31 – 4.23 (m, 1H, H-5α), 4.21 (d, *J* = 4.8 Hz, 1H, H-2α), 4.18 – 4.15 (m, 1H, H-5β), 4.14 – 4.09 (m, 2H, H-2β, H-5β), 3.90 – 3.85 (m, 1H, H-6β), 3.74 – 3.61 (m, 3H, H-6α/β), 3.24 (s, 1H, OH), 2.97 (s, 1H, OH), 2.74 (s, 1H, OH), 1.07 (s, 9H, $CH_{3\ tert\text{-butyl}}$). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 137.0(CH_{arom}), 135.8, 135.7, 135.7, 135.6(CH_{arom}), 133.1, 132.9, 132.5(C_{arom}), 130.2, 130.1, 130.0, 130.0, 128.8, 128.5, 128.4, 128.1, 128.1, 128.0, 127.9, 127.9(CH_{arom}), 102.3(C-1β), 97.3(C-1α), 82.4, 82.0(C-5), 78.1, 77.9(C-3), 74.4(C-2β), 73.1, 73.0($CH_{2\ benzyl}$), 71.2(C-2α), 63.9, 63.8(C-6), 27.0, 26.9($CH_{3\ tert\text{-butyl}}$), 19.3($C_{tert\text{-butyl}}$).

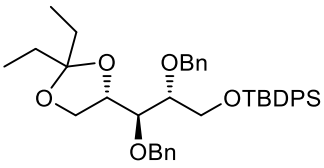
3-*O*-Benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-1,2-*O*-pentonide-D-ribitol (**15**)



Compound **12** (30.3 g, 63.3 mmol) was dissolved in MeOH (500 ml, 0.13M) and cooled to 0°C. $NaBH_4$ (4.79 g, 127 mmol, 2 eq) was added portion-wise, and the reaction was stirred ON. The reaction was diluted with EtOAc and $NaHCO_3$(aq), and the product was extracted twice with EtOAc. The combined organic layers were washed with once $NaHCO_3$(aq) and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was dissolved in 3-pentanone (350 ml, 0.18M), and PTSA monohydrate (1.19, 6.3 mmol, 0.1 eq) was added. The reaction was stirred for 6 hours and quenched with TEA, followed by the addition of H_2O. The product was extracted thrice with EtOAc and washed once with $NaHCO_3$ (aq) and brine, dried over $MgSO_4$ and concentrated *in vacuo* to obtain an oil (24.7 g, 45.0 mmol, 71%). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.71 – 7.64 (m, 4H, CH_{arom}), 7.46 – 7.35 (m, 5H, CH_{arom}), 7.33 – 7.26 (m, 4H, CH_{arom}), 7.26 – 7.19 (m, 2H, CH_{arom}), 4.76 (d, *J* = 11.2 Hz,

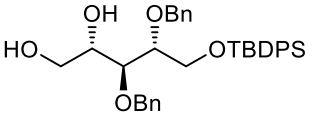
1H, $CH_{2\ benzyl}$), 4.65 (d, *J* = 11.2 Hz, 1H, $CH_{2\ benzyl}$), 4.30 (ddd, *J* = 7.8, 6.3, 4.9 Hz, 1H, H-2), 4.07 (dd, *J* = 7.9, 6.3 Hz, 1H, H-1), 3.94 – 3.67 (m, 5H, H-1, H-5,H-3,H-4), 2.69 (dd, *J* = 2.6, 1.1 Hz, 1H. OH), 1.71 – 1.61 (m, 4H, 2xCH_2), 1.11 (s, 9H, $CH_{3\ tert\text{-butyl}}$), 0.91 (q, *J* = 7.5 Hz, 6H, 2xCH_3). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.3(C_{arom}), 135.8(CH_{arom}), 135.7(C_{arom}), 133.2, 133.1, 130.0, 130.0, 128.5, 128.0, 128.0(, 127.9, 127.8(CH_{arom}), 113.0($C_{pentonide}$), 78.8(C-3), 76.4(C-2), 74.5($CH_{2\ benzyl}$), 72.9(C-4), 66.6(C-1), 64.6(C-5), 29.8($CH_{2\ pentonide}$), 29.0($CH_{2\ pentonide}$), 27.0($CH_{3\ tert\text{-butyl}}$), 19.4($CH_{pentonide}$), 8.4($CH_{3\ pentonide}$), 8.3($CH_{3\ pentonide}$).

3,4-*O*-Di-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-1,2-*O*-pentonide-D-ribitol (**16**)



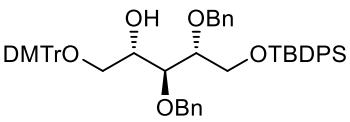
Compound **15** (4.3 g, 7.7 mmol) was co-evaporated thrice with toluene and dissolved in DMF (80ml, 0.1M). TBAI (0.29 g, 0.79 mmol, 0.1 eq) and benzyl bromide (1.4 ml, 11.8 mmol, 1.5 eq) were added to the mixture. The mixture was cooled to -45˚C, and NaH (60% dispersion in mineral oil, 0,48 g, 11.9 mmol, 1.5 eq) was added to the mixture in two portions in 30 min. The reaction was stirred ON at -40 ˚C and quenched with H_2O, and then the product was extracted thrice with ether. The combined organic layers were washed once with $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The product was purified by flash chromatography (97/3 to 90/10 pentane/Et_2O) to obtain an oil (3.9 g, 6.1 mmol, 78%). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.74 – 7.67 (m, 4H, CH_{arom}), 7.45 – 7.20 (m, 16H, CH_{arom}), 4.69 (d, *J* = 12.1 Hz, 2H, $CH_{2\ benyl}$), 4.55 (d, *J* = 11.9 Hz, 1H, $CH_{2\ benyl}$), 4.29 (ddd, *J* = 7.4, 6.5, 5.1 Hz, 1H, H-2), 3.97 – 3.84 (m, 5H, H-1, H-3, H-5), 3.72 (td, *J* = 5.1, 3.7 Hz, 1H, H-4), 1.86 – 1.54 (m, 4H, 2x$CH_{2\ pentonide}$), 1.09 (s, 9H, $CH_{3\ tert\text{-butyl}}$), 0.90 (td, *J* = 7.5, 1.8 Hz, 6H, 2x$CH_{3\ pentonide}$).**^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.6,(C_{arom}) 135.8, 135.8, 129.8, 128.4, 128.4, 127.9, 128.0, 128.0, 127.8, 127.7(CH_{arom}), 112.7($C_{pentonide}$), 80.3(C-4), 79.3(C-3), 76.0(C-2), 74.0($CH_{2\ benzyl}$), 72.8($CH_{2\ benzyl}$), 66.7(C-1), 63.3(C-5), 29.8($CH_{2\ pentonide}$), 29.0($CH_{2\ pentonide}$), 27.0($CH_{3\ tert\text{-butyl}}$), 19.3($C_{\ tert\text{-butyl}}$), 8.4($CH_{3\ pentonide}$), 8.3($CH_{3\ pentonide}$). **HRMS:** [$C_{40}H_{50}O_5Si$ + $Na]^+$ calculated 661.33197, found 661.33195.

3,4-*O*-Di-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-D-ribitol (**17**)



Compound **16** (2.31 g, 3.6 mmol) was dissolved in DCM (130 ml, 0.028M) and cooled to 0˚C. To the mixture was added TFA (50% in water, 5.2 ml, 35.1 mmol, 10 eq) and stirred at 0˚C for 1 hour. The reaction was quenched with $NaHCO_3$(aq), and the product was extracted thrice using EtOAc. The combined organic layers were washed twice with $NaHCO_3$(aq) and once with brine. Dried over $MgSO_4$ and concentrated *in vacuo*. The product was obtained without any further purification as an oil (2.07g, 3.6 mmol, Quantitative). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.73 (ddt, *J* = 7.9, 6.6, 1.4 Hz, 4H, CH_{arom}), 7.53 – 7.21 (m, 16H, CH_{arom}), 4.76 – 4.61 (m, 3H, $CH_{2\ benzyl}$), 4.50 (d, *J* = 11.6 Hz, 1H, $CH_{2\ benzyl}$), 4.02 (dd, *J* = 11.3, 3.9 Hz, 1H, H-5), 3.91 (m, *J* = 10.6, 8.7, 4.3 Hz, 2H, H-5, H-2), 3.84 (dd, *J* = 6.6, 5.6 Hz, 1H, H-3), 3.81 – 3.73 (m, 3H, H-4, H-1), 3.29 (d, *J* = 4.2 Hz, 1H, OH), 2.31 (t, *J* = 6.2 Hz, 1H, OH), 1.13 (s, 9H, $CH_{3\ tert\text{-butyl}}$). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.0, 137.8(C_{arom}), 135.9, 135.8(CH_{arom}), 133.2, 133.0(C_{arom}), 130.0, 130.0, 128.6 , 128.0, 128.0 , 128.0 , 127.9, 127.9(CH_{arom}), 81.0(C-4), 78.9(C-3), 74.0($CH_{2\ benzyl}$), 72.6($CH_{2\ benzyl}$), 72.4(C-2), 63.6(C-1), 62.8(C-5), 27.0($CH_{3\ tert\text{-butyl}}$), 19.3($C_{tert\text{-butyl}}$). **HRMS:** $[C_{35}H_{42}O_5Si + Na]^+$ calculated 593.26937, found 593.26885.

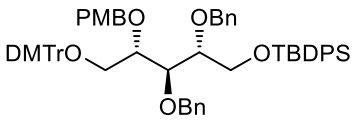
3,4-*O*-Di-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol (**18**)



Compound **17** (3.1 g, 5.5 mmol) was co-evaporated twice with toluene and dissolved in DCM (12 ml, 0.46M). The mixture was cooled to 0˚C and added TEA (1.9 ml, 13.7 mmol, 2.5 eq) and DMTrCl (2.23 g, 6.6 mmol, 1.2 eq). The reaction was stirred ON at RT and quenched with MeOH. Brine and $NaHCO_3$(aq) were added to the solution, and the product was extracted thrice using DCM. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The product was purified by flash chromatography (10/0 to 6/4 pentane/ ether) to obtain an oil (4.4 g, 5.0 mmol, 91%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.73 – 7.64 (m, 4H, CH_{arom}), 7.51 – 7.15 (m, 25H, CH_{arom}), 7.04 – 6.95 (m, 2H, CH_{arom}), 6.83 – 6.72 (m, 4H, CH_{arom}), 4.67 – 4.52 (m, 2H, $CH_{2\ benzyl}$), 4.50 – 4.35 (m, 2H, $CH_{2\ benzyl}$), 4.05 – 3.94 (m, 2H, H-2, H-5), 3.92 – 3.83 (m, 2H, H-5, H-3), 3.74 (s, 7H, 2x OCH_3, H-4), 3.38 (dd, *J* = 9.6, 3.2 Hz, 1H, H-1), 3.28 (dd, *J* = 9.6,

5.9 Hz, 1H, H-1), 2.98 (d, *J* = 4.2 Hz, 1H, OH), 1.07 (s, 9H, $CH_{3\ tert\text{-butyl}}$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 158.4, 145.0, 138.4, 138.4, 136.2, 136.1(C_{arom}), 135.8, 135.8(CH_{arom}), 133.4, 133.2(C_{arom}), 130.2, 130.20, 130.1, 129.8, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0 , 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 126.8, 113.1, 113.1(CH_{arom}), 86.0(C_{dmt}), 80.9(C-4), 78.7(C-3), 73.6($CH_{2\ benzyl}$), 72.4($CH_{2\ benzyl}$), 71.7(C-2), 64.6(C-1), 63.1(C-5), 55.2(OCH_3), 27.0 ($CH_{3\ tert\text{-butyl}}$), 19.3($C_{tert\text{-butyl}}$). **HRMS:** $[C_{56}H_{60}O_7Si + Na]^+$ calculated 895.40005, found 895.39986.

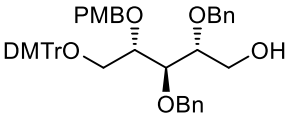
3,4-*O*-Di-benzyl-5-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-(4-metoxybenzyl)-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol (**19**)



4-methoxybenzyl bromide was freshly prepared by dissolving 4-Methoxybenzyl alcohol (1.9 ml, 15 mmol) in Et_2O (15 ml, 1M). The mixture was cooled to 0°C and PBr_3 (0.5 ml, 5 mmol, 0.3 eq) was added. The reaction as stirred for 2 hours at 0°C followed by the addition of $NaHCO_3$ (aq). The product was extracted twice with Et_2O and the combined organic layers were washed with $NaHCO_3$ (aq), dried over $MgSO_4$ and dried *in vacuo.* 4-methoxybenzyl bromide was used without any further purification. Compound **18** (4.58 g, 5.25 mmol) was co-evaporated twice with toluene and dissolved in THF:DMF 7:1 (14.8 ml, 0.35M). The solution was cooled to 0˚C, followed by the addition of 4-methoxybenzyl bromide (1.2 ml, 8.33 mmol, 1.6 eq) and NaH (60% dispersion in mineral oil, 0.42 g, 10.5 mmol, 2 eq) and stirred ON at RT. The reaction was quenched with H_2O, and the product was extracted thrice with ether. The combined organic layers were washed once with $NaHCO_3$(aq) and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The product was purified by flash chromatography (95/5 to 70/30 pentane/ether) to obtain an oil (4.2 g, 4.23 mmol, 81%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.71 – 7.60 (m, 5H, CH_{arom}), 7.49 – 7.42 (m, 2H, CH_{arom}), 7.38 – 7.16 (m, 23H, CH_{arom}), 7.06 – 6.97 (m, 2H, CH_{arom}), 6.87 – 6.76 (m, 3H, CH_{arom}), 6.76 – 6.69 (m, 4H, CH_{arom}), 4.71 – 4.61 (m, 2H, $CH_{2\ benzyl}$), 4.54 – 4.47 (m, 4H, $CH_{2\ benzyl}$), 3.98 – 3.88 (m, 4H, H-2, H-3, H-5), 3.83 – 3.80 (m, 1H, H-4), 3.78 (s, 3H, $OCH_{3\ pmb}$), 3.75 – 3.71 (m, 6H, $OCH_{3\ dmt}$), 3.41 (dd, *J* = 10.2, 2.7 Hz, 1H, H-1), 3.32 (dd, *J* = 10.1, 5.7 Hz, 1H, H-1), 1.05 (s, 9H, $CH_{3\ tert\text{-butyl}}$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 159.0, 158.4, 145.4, 139.0, 138.7, 136.5(C_{arom}), 135.8, 135.80(CH_{arom}), 133.7, 133.6, 131.1(C_{arom}),

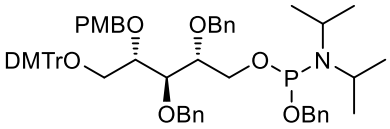
130.3, 130.2, 129.7, 129.6, 129.44, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.4, 127.3, 126.7, 113.7, 113.2, 113.1(CH_{arom}), 86.1(C_{dmt}), 80.6(C-4), 78.9(C-2), 78.7(C-3), 73.6($CH_{2\ benzyl}$), 72.7($CH_{2\ pmb}$), 72.3($CH_{2\ benzyl}$), 64.1(C-1), 63.9(C-5), 55.3($CH_{3\ OMe}$), 27.0(CH_3 *tert*-butyl), 19.3(C *tert*-butyl). **HRMS:** $[C_{64}H_{68}O_8Si + Na]^+$ calculated 1015.45757, found 1015.45679.

3,4-*O*-Di-benzyl-2-*O*-(4-metoxybenzyl)-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol (**20**)



Compound **19** (4.49 g, 4.52 mmol) was dissolved in THF (45 ml, 0.1M) and cooled to 0°C. TBAF (1M in THF, 6 ml, 6 mmol, 1.3eq) was added, and the reaction was stirred ON at RT. The reaction was quenched with $NaHCO_3$(aq), and the product was extracted thrice with EtOAc. The combined organic layers were washed once with brine and concentrated *in vacuo*. The product was purified by flash chromatography (8/2 to 6/4 pentane/EtOAc) to obtain a colourless oil (2.96 g, 3.92 mmol, 87%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 – 7.41 (m, 2H, CH_{arom}), 7.41 – 7.12 (m, 19H, CH_{arom}), 7.01 – 6.69 (m, 6H, CH_{arom}), 4.88 – 4.44 (m, 6H, $CH_{2\ benzyl}$), 4.08 – 3.97 (m, 1H, H-4), 3.96 – 3.71 (m, 13H, H-2, H-3, H-3, 3x$CH_{3\ OMe}$), 3.47 – 3.34 (m, 2H, H-1), 2.47 (t, *J* = 5.8 Hz, 1H, OH). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 159.2, 158.4, 145.1, 138.3, 138.1, 136.2, 136.2, 130.4(C_{arom}), 130.2, 129.6, 129.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 126.7, 113.8, 113.8, 113.6, 113.1, 113.0(CH_{arom}), 86.2(C_{dmt}), 79.4(C-4), 79.0(C-3), 78.2(C-2), 73.8($CH_{2\ benzyl}$), 72.4($CH_{2\ benzyl}$), 71.9($CH_{2\ benzyl}$), 63.4(C-1), 61.5(C-5), 55.3($CH_{3\ OMe}$), 55.2($CH_{3\ OMe}$). **HRMS:** $[C_{48}H_{50}O_8 + Na]+$ calculated 777.33979, found 777.33974.

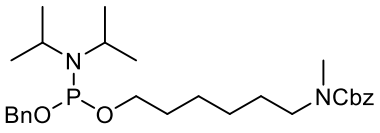
3,4-*O*-Di-benzyl-2-*O*-(4-metoxybenzyl)-1-*O*-(4,4'-dimethoxytrityl)-5-*O*-((*N,N*-diisopropylamino)-*O*-2-benzyl-phosphoramidite))-D-ribitol (**7**)



Compound **20** (1.1 g, 1.4 mmol) was co-evaporated thrice with toluene and dissolved in DCM (2 ml, 0.7M). Activated molecular sieves (MA3Å) and diisopropylammonium tetrazolide (263 mg, 1.54 mmol, 1.1 eq) were added, and the mixture was stirred for 30 minutes. 1-(benzyloxy)-*N,N,N',N'*-

tetraisopropylphosphanediamine (0.18 M, 9.5 ml, 1.2 eq) was added, and the reaction was stirred for 3 hours, followed by the addition of $NaHCO_3$(aq). brine was added, and the product was extracted thrice using DCM. The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The product was purified by flash chromatography (neutralised silica, 98/2 to 8/2 pentane/ Et_2O) to obtain an oil (1.3g, 1.3 mmol, 91%). **^{1}H NMR** (400 MHz, CD_3CN) δ 7.50 – 7.43 (m, 2H, CH_{arom}), 7.37 – 7.13 (m, 26H, CH_{arom}), 6.90 (dd, *J* = 8.5, 5.6 Hz, 2H, CH_{arom}), 6.79 (dt, *J* = 8.9, 1.8 Hz, 4H, CH_{arom}), 4.79 – 4.43 (m, 8h, $CH_{2\ benzyl}$), 4.07 – 3.63 (m, 15H, H-2, H-3, H-4, H-5, 3x$CH_{3\ OMe}$), 3.37 – 3.24 (m, 1H, H-1), 1.30 – 1.14 (m, 12H, 4xCH_3).**^{13}C NMR** (101 MHz, CD_3CN) δ 159.3, 158.6, 145.6, 139.0, 139.0, 138.8, 138.8, 136.4, 136.3, 136.3, 131.0, 130.2, 130.2, 129.6, 129.5, 128.4, 128.4, 128.3, 128.3, 128.2, 127.9, 127.9, 127.7, 127.7, 127.5, 127.4, 127.4, 127.3, 127.1, 127.1, 126.8, 117.4, 86.0, 79.4, 79.4, 79.3, 78.9, 78.8, 78.6, 78.6, 73.3, 73.3, 72.2, 72.1, 72.1, 72.1, 65.2, 65.1, 65.0, 65.0, 64.0, 63.9, 63.1, 62.9, 55.0, 55.0, 43.0, 43.0, 42.9, 42.9, 24.3, 24.2, 24.2, 24.1.**^{31}P NMR** (162 MHz, CD_3CN) δ 147.2, 147.1.

Benzyl(6(((benzyloxy)(diisopropylamino)phosphanyl)oxy)hexyl)(methyl) carbamate (**6**)



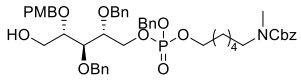
6-Bromo hexanol (0.5 g, 1.9 mmol) was co-evaporated thrice with toluene and dissolved in methylamine (5 ml, 2.0M in THF, 10 mmol, 5 eq). The reaction was stirred ON and diluted with Et_2O, filtrated and concentrated. The crude product was dissolved in Et_2O:$NaHCO_3$(aq) 3:5 (8 ml, 0.2 M) and Carbobenzyloxychloride (0.8 ml, 5.6 mmol, 3 eq) was added. The reaction was stirred vigorously for 2 hours and diluted with Et_2O. The organic layer was washed once with Brine and concentrated *in vacuo*. The crude product was dissolved in DCM (3 ml, 0.6M), followed by the addition of activated molecular sieves (MA3Å) and diisopropylammonium tetrazolide (355 mg, 2.0 mmol, 1.1 eq). The mixture was stirred for 30 minutes and 1-(benzyloxy)-*N,N,N',N'*-tetraisopropylphosphanediamine (0.18 M, 9.5 ml, 1.2 eq) was added. The reaction was stirred for 3 hours, followed by the addition of $NaHCO_3$(aq). Brine was added, and the product was extracted thrice using DCM. The combined organic layers were dried with MgSO4 and concentrated in vacuo. The product was purified by flash chromatography (neutralised silica, 98/2 to 8/2 pentane/ Et2O) to obtain an oil (0.7 g, 1.4 mmol, 75%). **^{1}H**

NMR (400 MHz, CD_3CN) δ 7.43 – 7.25 (m, 10H, CH_{arom}), 5.11 (s, 2H, $CH_{2\ Cbz}$), 4.79 – 4.63 (m, 2H, $CH_{2\ benzyl}$), 3.74 – 3.59 (m, 4H, 2x $CH_{iso\text{-}prop}$, $CH_{2\ linker}$), 3.31 – 3.22 (m, 2H, $CH_{2\ linker}$), 2.88 (s, 3H, NMe), 1.65 – 1.47 (m, 4H, 2x $CH_{2\ linker}$), 1.43 – 1.07 (m, 16H, 2x$CH_{2\ linker}$, 4x $CH_{3\ iso\text{-}prop}$). **^{13}C NMR** (101 MHz, CD_3CN) δ 156.0, 140.0, 140.0, 137.7 (C_{arom}), 128.5, 128.4, 127.9, 127.8, 127.7, 127.3, 127.1(CH_{arom}), 66.4($CH_{2\ Cbz}$), 65.1, 64.9($CH_{2\ Bn}$), 63.4, 63.2($CH_{2\ linker}$), 48.7, 48.3($CH_{2\ linker}$), 42.9, 42.8($CH_{iso\text{-}prop}$), 34.0, 33.4 (NMe),31.2, 31.1, 27.8, 26.2, 25.6($CH_{2\ linker}$), 24.2, 24.1($CH_{3\ iso\text{-}prop}$). **^{31}P NMR** (162 MHz, CD_3CN) δ 146.5.

General procedure for phosphoramidite coupling, oxidation and detritylaton

The starting alcohol was co-evaporated thrice with ACN, followed by the addition of DCI (0.25M in ACN, 1.5 eq). To the mixture were added freshly activated molecular sieves (MA3Å) and stirred for 20 minutes. Phosphoramidite (+/- 0.18M in ACN, 1.3-1.5 eq) was added to the mixture and stirred until TLC showed complete conversion of the starting alcohol. CSO (0.5M in ACN, 3 eq) was added to the reaction and stirred for 15 minutes. The reaction was quenched with $NaHCO_3$(aq), and the product was extracted thrice with DCM. The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The crude was treated with TCA (0.18M in DCM, 5 eq) and stirred ON, followed by quenching with $NaHCO_3$(aq). The product was extracted thrice with DCM. The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified with flash chromatography (DCM/acetone) and, if needed, size exclusion chromatography (Sephadex LH-20, MeOH/DCM 1:1)

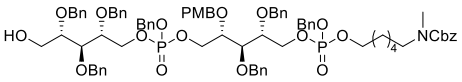
Monomer (**22**)



Compound **20** (0.41g, 0.54 mmol) was coupled to phosphoramidite **6** (4 ml, 0.18M in ACN, 0.72 mmol, 1.3 eq), oxidised and detritylated using the general procedure as described above. The crude was purified by fash chromatography (DCM/aceton) obtaining the product as an oil (0.33 g, 0.38 mol, 70%). **^{1}H NMR** (500 MHz, CD_3CN) δ 7.50 – 7.18 (m, 22H, CH_{arom}), 6.91 – 6.79 (m, 2H, CH_{pmb}), 5.15 – 4.90 (m, 4H, $CH_{2\ cbz}$, $CH_{2\ Benzyl}$), 4.74 – 4.43 (m, 6H, 3x$CH_{2\ benzyl}$), 4.34 – 4.25 (m, 1H, H-5a), 4.20 – 4.07 (m, 1H, H-5b), 3.96 – 3.82 (m, 4H, $CH_{2\ linker}$, H-4, H-3), 3.78 – 3.56 (m, 6H, $CH_{3\ OMe}$, H-2, H-1), 3.25 – 3.14 (m, 2H, $CH_{2\ linker}$), 2.86 – 2.78 (m, 3H, NMe), 1.52 (s, 4H, 2x$CH_{2\ linker}$), 1.32 – 1.14 (m, 4H, 2x$CH_{2\ linker}$). **^{13}C NMR** (126 MHz, CD_3CN) δ 160.2, 156.9($C_{carbonyl}$),

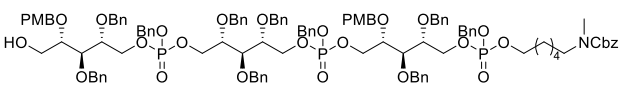
139.8, 139.6, 139.5, 131.7, 131.4, 130.6(C_{arom}), 130.5, 129.5, 129.5, 129.4, 129.4, 129.3, 129.3, 129.3, 128.9, 128.9, 128.8, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 114.6(CH_{arom}), 80.7, 80.2, 79.3, 79.2, 79.0, 78.8, 78.7(CH), 74.4, 72.9, 72.8, 72.5, 72.4, 69.8, 69.8, 69.7, 69.7($CH_{2benzyl}$), 68.6, 68.6($CH_{2\ linker}$), 67.8, 67.8, 67.7(C-5), 67.3($CH_{2\ benzyl}$), 61.6(C-1), 61.6(C-1), 55.8(C_{OMe}), 30.9, 30.8, 26.8, 25.9($CH_{2\ linker}$). **^{31}P NMR** (202 MHz, CD_3CN) δ -0.9, -0.9.**HRMS:** $[C_{49}H_{60}NO_{11}P + Na]^+$ calculated 892.37962, found 892.38054.

Dimer (**23**)



Compound **22** (0.38 g, 0.44 mmol) was coupled to phosphoramidite **4** (3.2 ml, 0.18M in ACN, 0.56 mmol, 1.3 eq), oxidised and detritylated using the general procedure as described above. The crude was purified by fash chromatography (DCM/acetone) obtaining the product as an oil (0.57 g, 0.40 mol, 91%). **^{1}H NMR** (500 MHz, CD_3CN) δ 7.44 – 7.04 (m, 60H, CH_{arom}), 6.86 – 6.73 (m, 4H, CH_{pmb}), 5.09 – 4.89 (m, 8H, $CH_{2\ Cbz}$, $CH_{2\ benzyl}$), 4.69 – 4.39 (m, 18H, 9x$CH_{2\ benzyl}$), 4.39 – 4.07 (m, 10H, 2xH-1, 3xH-5), 3.95 – 3.82 (m, 8H, $CH_{2\ linker,}$ 3xH3, 3xH-4), 3.81 – 3.76 (m, 2H, 2xH-2), 3.75 – 3.59 (m, 9H, H-2, H-1, 2x$CH_{3\ OMe}$), 3.23 – 3.16 (m, 2H, $CH_{2\ linker}$), 2.83 (s, 3H, NMe), 2.77 – 2.67 (m, 1H, OH), 1.52 (m, 2H, $CH_{2\ linker}$), 1.42 (m, 2H, $CH_{2\ linker}$), 1.29 – 1.11 (m, 4H, 2x$CH_{2\ linker}$).**^{13}C NMR** (126 MHz, CD_3CN) δ 139.3(C_{arom}), 130.6, 130.4, 129.5, 129.4, 129.2, 128.8, 128.8, 128.6, 128.5, 128.5, 114.5(CH_{arom}), 80.1, 79.2, 78.9, 78.8, 78.5(CH), 74.4, 73.0, 72.7, 72.3, 69.8($CH_{2\ benzyl}$), 68.6($CH_{2\ linker}$), 68.0($CH_{2\ rib}$), 67.5($CH_{2\ rib}$), 67.2($CH_{2\ benzyl}$), 61.5($CH_{2\ rib}$), 55.8($CH_{3\ OMe}$), 49.6, 30.4, 26.8, 25.8($CH_{2\ linker}$). **^{31}P NMR** (202 MHz, CD_3CN) δ -0.6, -0.6, -0.6, -0.7, -0.8, -0.9, -0.9. **HRMS:** $[C_{82}H_{95}NO_{18}P_2 + Na]^+$ calculated 1466.59166, found 1466.59068.

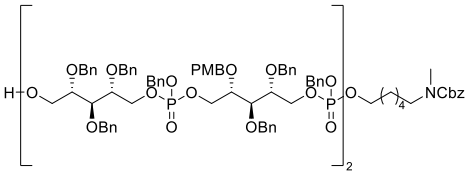
Trimer (**24**)



Compound **23** (0.55 g, 0.37 mmol) was coupled to phosphoramidite **7** (3.0 ml, 0.17M in ACN, 0.51 mmol, 1.3 eq), oxidised and detritylated using the general procedure as described above. The crude was purified by fash chromatography (DCM/acetone) obtaining the product as an oil (0.55 g, 0.27 mol, 71%). **^{1}H NMR** (500 MHz, CD_3CN) δ 7.44 – 7.04 (m, 60H, CH_{arom}), 6.86 – 6.73 (m, 4H, CH_{pmb}), 5.09 – 4.89 (m, 8H, $CH_{2\ Cbz}$, $CH_{2\ benzyl}$), 4.69

– 4.39 (m, 18H, 9x$CH_{2\ benzyl}$), 4.39 – 4.07 (m, 10H, 2xH-1, 3xH-5), 3.95 – 3.82 (m, 8H, $CH_{2\ linker,}$ 3xH3, 3xH-4), 3.81 – 3.76 (m, 2H, 2xH-2), 3.75 – 3.59 (m, 9H, H-2, H-1, 2x$CH_{3\ OMe}$), 3.23 – 3.16 (m, 2H, $CH_{2\ linker}$), 2.83 (s, 3H, NMe), 2.77 – 2.67 (m, 1H, OH), 1.52 (m, 2H, $CH_{2\ linker}$), 1.42 (m, 2H, $CH_{2\ linker}$), 1.29 – 1.11 (m, 4H, 2x$CH_{2\ linker}$). **^{13}C NMR** (126 MHz, CD_3CN) δ 139.3($C_{\ arom}$), 130.6, 130.4, 129.5, 129.4, 129.2, 128.8, 128.8, 128.6, 128.5, 128.5, 114.5($CH_{\ arom}$), 80.1, 79.2, 78.9, 78.8, 78.5(CH), 74.4, 73.0, 72.7, 72.3, 69.8($CH_{2\ benzyl}$), 68.6($CH_{2\ linker}$), 68.0($CH_{2\ rib}$), 67.5($CH_{2\ rib}$), 67.2($CH_{2\ benzyl}$), 61.5($CH_{2\ rib}$), 55.8($CH_{3\ OMe}$), 49.6, 30.4, 26.8, 25.8($CH_{2\ linker}$). **^{31}P NMR** (202 MHz, CD_3CN) δ -0.6, -0.6, -0.6, -0.7, -0.8, -0.9, -0.9. **HRMS:** $[C_{116}H_{132}NO_{26}P_3 + H]^+$ calculated 2048.83232, found 2048.83196.

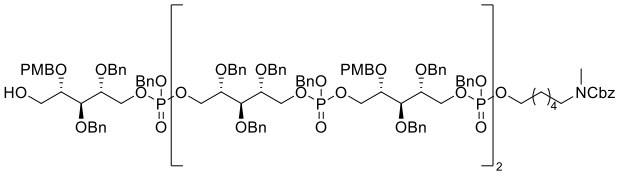
Tetramer (**25**)



Compound **24** (0.53 g, 0.26 mmol) was coupled to phosphoramidite **4** (2.0 ml, 0.18M in ACN, 0.36 mmol, 1.4 eq), oxidised and detritylated using the general procedure as described above. The crude was purified by fash chromatography (DCM/acetone) and size exclusion chromatography (Sephadex LH-20, MeOH/DCM 1:1) obtaining the product as an oil (0.63 g, 0.24 mol, 94%). **^{1}H NMR** (500 MHz, CD_3CN) δ 7.46 – 7.07 (m, 79H, $CH_{\ arom}$), 6.84 – 6.70 (m, 4H, $CH_{\ pmb}$), 5.06 (s, 2H, $CH_{2\ Cbz}$), 4.99 – 4.89 (m, 6H, $CH_{2\ benzyl}$), 4.68 – 4.32 (m, 24H, $CH_{2\ benzyl}$), 4.32 – 4.06 (m, 14H, 4xH-5, 3xH-1) 4.00 – 3.74 (m, 13H, $CH_{2\ linker,}$ 4xH4, 4xH3, 3xH-2), 3.74 – 3.58 (m, 5H, 2x$CH_{3\ OMe,}$ H-2, H-1), 3.18 (t, *J* = 7.4 Hz, 2H, $CH_{2\ linker}$), 2.86 – 2.75 (m, 4H, NMe, OH), 1.47 (m, 4H, 2x$CH_{2\ linker}$), 1.30 – 1.13 (m, 4H, 2x$CH_{2\ linker}$). **^{13}C NMR** (126 MHz, CD_3CN) δ 160.2, 160.1, 156.8($C_{\ carbonyl}$), 139.7, 139.7, 139.5, 139.5, 139.3, 139.3, 139.3, 139.2, 139.2, 139.1, 139.1, 137.2, 137.2, 131.0($C_{\ arom}$), 130.6, 130.6, 130.5, 129.5, 129.5, 129.5, 129.4, 129.4, 129.3, 129.3, 129.3, 129.2, 129.2, 129.2, 129.2, 129.0, 128.9, 128.9, 128.9, 128.8, 128.8, 128.8, 128.8, 128.7, 128.7, 128.7, 128.7, 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 114.5($CH_{\ arom}$), 80.8, 80.7, 80.6, 80.6, 80.5, 79.3, 79.2, 79.2, 79.1, 79.1, 79.0, 79.0, 78.9, 78.9, 78.8, 78.7, 78.7, 78.7, 78.5, 78.4, 78.4, 78.4, 78.3, 78.3, 78.2(CH), 74.5, 74.5, 74.4, 74.4, 73.1, 73.1, 73.0, 73.0, 72.9, 72.8, 72.8, 72.7, 72.7, 72.6, 69.9, 69.9, 69.9, 69.8, 69.8, 69.7($CH_{2\ benzyl}$), 68.6($CH_{2\ linker}$), 68.6($CH_{2\ linker}$), 68.0, 68.0, 67.6, 67.6, 67.5, 67.5, 67.5, 67.5, 67.4($CH_{2\ rib}$), 67.3($CH_{2\ linker}$), 65.9, 65.9, 65.8, 65.8($CH_{2\ rib}$),

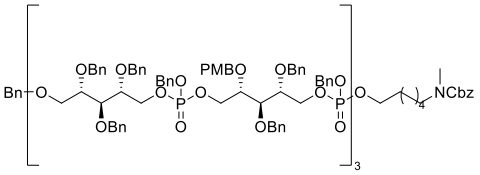
61.5(C-1), 55.8($CH_{3\ OMe}$), 49.4, 49.0, 30.8, 30.8, 26.8, 25.8($CH_{2\ linker}$).**^{31}P NMR** (202 MHz, CD_3CN) δ -0.6, -0.6, -0.7, -0.8, -0.9, -0.9. **HRMS:** $[C_{149}H_{167}NO_{33}P_4 + H]^+$ calculated 2623.04436, found 2623.05107.

Pentamer (**26**)



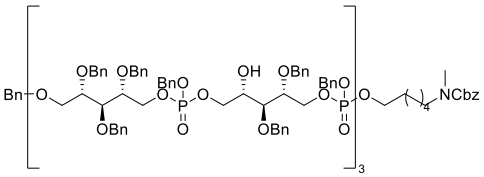
Compound **25** (0.63 g, 0.24 mmol) was coupled to phosphoramidite **7** (1.9 ml, 0.17M in ACN, 0.31 mmol, 1.3 eq), oxidised and detritylated using the general procedure as described above. The crude was purified by fash chromatography (DCM/acetone) and size exclusion chromatography (Sephadex LH-20, MeOH/DCM 1:1) obtaining the product as an oil (0.70 g, 0.22 mol, 90%). **^{1}H NMR** (500 MHz, CD_3CN) δ 7.50 – 7.01 (m, 96H, CH_{arom}), 6.85 – 6.69 (m, 6H, CH_{pmb}), 5.06 (s, 2H, $CH_{2\ Cbz}$), 5.03 – 4.90 (m, 10H, 5x$CH_{2\ benzyl}$), 4.69 – 4.35 (m, 30H, 15x $CH_{2\ benzyl}$), 4.35 – 4.08 (m, 18H, 5xH-5, 4xH-1), 3.98 – 3.75 (m, 16H, $CH_{2\ linker}$, 5xH-4,5xH-3,4xH-2), 3.75 – 3.58 (m, 12H, 3x$CH_{3\ OMe}$, H-2, H-1), 3.19 (t, *J* = 7.3 Hz, 2H, $CH_{2\ linker}$), 2.82 (s, 3H, NMe), 1.59 – 1.38 (m, 4H, 2x$CH_{2\ linker}$), 1.36 – 1.07 (m, 4H, 2x$CH_{2\ linker}$).**^{13}C NMR** (126 MHz, CD_3CN) δ 159.3, 159.3, 159.3, 156.0($C_{carbonyl}$), 138.7, 138.6, 138.4, 138.3, 138.3, 136.4, 136.3, 130.8, 130.2, 130.2(C_{arom}), 129.8, 129.7, 129.7, 129.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 113.7, 113.7(CH_{arom}), 79.3, 79.3, 78.2, 78.1, 77.9, 77.9, 77.6, 77.6, 77.5, 77.4(CH), 73.7, 73.6, 73.6, 73.6, 72.2, 72.2, 72.2, 72.1, 71.9, 71.9, 71.8, 71.8, 71.5, 69.1, 69.1, 69.0, 69.0, 68.9, 68.9, 68.9, 67.8($CH_{2\ benzyk}$), 67.8($CH_{2\ linker}$), 67.3, 67.2, 66.7($CH_{2\ rib}$), 66.4($CH_{2\ linker}$), 60.7(C-1), 54.9($CH_{3\ OMe}$), 54.9($CH_{3\ OMe}$), 48.8, 47.8, 30.0, 25.9, 25.0($CH_{2\ linker}$).**^{31}P NMR** (202 MHz, CD_3CN) δ -0.6, -0.6, -0.7, -0.7, -0.9, -0.9. **HRMS:** $[C_{183}H_{204}NO_{41}P_5 + NH_4]^+$ calculated 3244.29351, found 3244.30918.

Hexamer (**3**)



Compound **26** (0.69 g, 0.22 mmol) was coupled to phosphoramidite **5** (1.9 ml, 0.17M in ACN, 0.31 mmol, 1.5 eq), oxidised and detritylated using the general procedure as described above. The crude was purified by fash chromatography (DCM/acetone) and size exclusion chromatography (Sephadex LH-20, MeOH/DCM 1:1) obtaining the product as an oil (0.75 g, 0.19 mol, 90%). **^{1}H NMR** (500 MHz, CD_3CN) δ 7.47 – 6.98 (m, 111H, $CH_{2\ benzyl}$), 6.82 – 6.68 (m, 6H, $CH_{2\ pmb}$), 5.06 (s, 2H, $CH_{2\ Cbz}$), 5.02 – 4.88 (m, 12H, $CH_{2\ benzyl}$), 4.65 – 4.35 (m, 38H, 19x$CH_{2\ benzyl}$), 4.31 – 4.07 (m, 22H, 6xH-5, 4xH-1), 3.94 – 3.75 (m, 17H, 6xH-4, 6xH3,5xH2), 3.74 – 3.60 (m, 12H, 3x$CH_{3\ OMe}$, H-2, H-1), 3.18 (t, *J* = 7.4 Hz, 2H, $CH_{2\ linker}$), 2.82 (s, 3H, NMe), 1.47 (m, 4H, 2x$CH_{2\ linker}$), 1.31 – 1.10 (m, 4H, 2x$CH_{2\ linker}$).**^{13}C NMR** (126 MHz, CD_3CN) δ 159.3($C_{carbonyl}$), 138.9, 138.8, 138.6, 138.4, 138.3, 138.3, 138.3, 136.4, 136.4, 130.2, 130.2(C_{arom}), 129.7, 129.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 113.7(CH_{arom}), 78.4, 78.3, 78.3, 78.2, 78.2, 78.0, 77.9, 77.9, 77.9, 77.7, 77.6, 77.5, 77.4(CH), 73.7, 73.6, 73.6, 72.9, 72.2, 72.2, 72.1, 72.1, 72.1, 71.9, 71.8($CH_{2\ benzyl}$), 69.8(C-1), 69.1, 69.0, 69.0, 69.0, 68.9, 68.9, 67.8($CH_{2\ linker}$), 67.7($CH_{2\ linker}$), 67.0, 67.0, 66.7, 66.7, 66.6($CH_{2\ rib}$), 66.4($CH_{2\ benzyl}$), 54.9($CH_{3\ OMe}$), 29.9, 25.9, 25.0($CH_{2\ linker}$).**^{31}P NMR** (202 MHz, CD_3CN) δ -0.6, -0.6, -0.7, -0.7, -0.9, -0.9. **HRMS:** $[C_{223}H_{245}NO_{48}P_6 + H]^{2+}$ calculated 1946.26661, found 1946.29653.

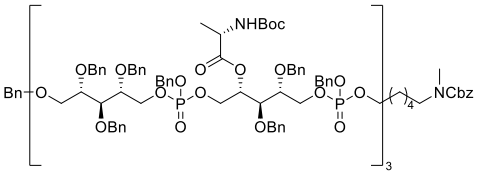
Hexamer (**27**)



Compound **3** (0.46 g, 0.12 mmol) was dissolved in ACN:H_2O 19:1 (2 ml, 0.055 M) and cooled to 0°C. CAN (0.96 g, 1.75 mmol, 15 eq) was added, and the reaction was warmed to RT. After 1 hour, the reaction was quenched with $NaHCO_3$(aq), and the product was extracted thrice with DCM. The combined organic layers were washed with brine and concentrated *in vacuo*. The product was purified by flash chromatography (DCM/aceton) and size exclusion chromatography (Sephadex LH-20, MeOH/DCM 1:1) to obtain an oil (0.27 g, 77 µmol, 66%). **^{1}H**

NMR (500 MHz, CD_3CN) δ 7.54 – 7.12 (m, 115H, CH_{arom}), 5.12 – 4.89 (m, 14H, $CH_{2\ benzyl}$), 4.67 – 3.55 (m, 76H, 16x$CH_{2\ benzyl}$, 6xH-5, 6xH-4, 6xH-3, 6xH-2, 6xH-1, $CH_{2\ linker}$), 3.19 (t, *J* = 7.4 Hz, 2H, $CH_{2\ linker}$), 2.82 (s, 3H, NMe), 1.53 (s, 2H, $CH_{2\ linker}$), 1.42 (m, 2H, $CH_{2\ linker}$), 1.31 – 1.08 (m, 4H, 2x $CH_{2\ linker}$).**^{13}C NMR** (126 MHz, CD_3CN) δ 138.8, 138.7, 138.6, 138.4, 138.3, 138.3, 138.2, 136.3, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 78.6, 78.2, 78.1, 78.1, 78.0, 77.8, 77.5, 73.6, 73.5, 73.4, 72.8, 72.2, 72.2, 72.2, 72.1, 72.1, 72.0, 72.0, 69.8, 69.7, 69.7, 69.4, 69.1, 69.0, 69.0, 68.9, 68.8, 67.8, 67.7, 67.0, 66.6, 66.4, 48.1, 29.9, 29.8, 25.8, 24.9 **31P NMR** (202 MHz, CD3CN) δ -0.1, -0.1, -0.1, -0.1, -0.2, -0.2, -0.2, -0.2, -0.3, -0.3, -0.3, -0.3, -0.4, -0.6, -0.6, -0.7, -0.7, -0.8, -0.8. **HRMS:** $[C_{199}H_{221}NO_{45}P_6+ 2H]^+$ calculated 1767.18368, found 1767.18389.

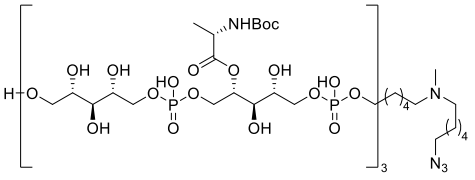
Hexamer(**2**)



Compound **27** (0.14 g, 39 µmol) was co-evaporated thrice with toluene and dissolved in DCE (1 ml, 0.04M). Pybop (0.32, 0.62 mmol, 16 eq), (*tert*-butoxycarbonyl)alanine (0.10 g, 0.54 mmol, 15 eq) and NMI (0.1 ml, 1.25 mmol, 32 eq) were added and the reaction was stirred 3 hours. The reaction was quenched with $NH_4Cl(aq)$, and the product was extracted thrice with DCM. The combined organic layers were concentrated *in vacuo*. The product was purified by size exclusion chromatography (Sephadex LH-20, MeOH/DCM 1:1) to obtain an oil (0.15 g, 36 µmol, 93%). **^{1}H NMR** (500 MHz, CD_3CN) δ 7.53 – 7.11 (m, 115H, CH_{arom}), 5.73 (s, 3H, 3xNH), 5.46 (m, 3H, 3xH-2), 5.13 – 4.87 (m, 14H, $CH_{2\ benzyl}$), 4.70 – 3.50 (m, 76H, 16x$CH_{2\ benzyl}$, 6xH-5, 6xH-4, 6xH-3, 3xH-2, 6xH-1, $CH_{2\ linker}$, 3xCH_{ala}), 3.19 (t, *J* = 7.1 Hz, 2H, $CH_{2\ linker}$), 2.84 (s, 3H, NMe), 1.62 – 1.11 (m, 44H, 3x $CH_{2\ linker}$, 3xCH_3 *tert*-butyl, 3x$CH_{3\ ala}$). **^{13}C NMR** (126 MHz, CD_3CN) δ 172.6, 138.8, 138.5, 138.4, 138.2, 137.9, 136.2, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 78.9, 78.2, 78.1, 77.7, 77.5, 77.0, 73.6, 73.5, 72.8, 72.6, 72.1, 72.1, 72.0, 71.9, 69.7, 69.0, 67.8, 67.1, 66.7, 66.3, 65.8, 65.6, 49.5, 29.8, 27.6, 25.8, 24.9, 17.1. **31P NMR** (202 MHz, CD3CN) δ -0.6, -0.6, -0.7, -0.8, -0.8, -0.8, -0.9, -0.9, -0.9, -1.0,

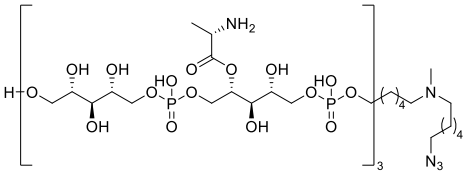
-1.1, -1.1. **HRMS:** $[C_{183}H_{204}NO_{41}P_5 + 2H]^{2+}$ calculated 2022.81466, found 2022.82118.

Hexamer (**29**)



Compound **2** (10 mg, 2.5 µmol) was dissolved in dioxane:MiliQ 3:1 (4 ml, 0.6 mM), AcOH (0.1 ml) and KH_2PO_4 buffer (0.1 M, 0.1 ml) were added. The mixture was purged with N_2, and a catalytic spoon of Pd Black was added. The mixture was purged with N_2 and subsequently with H_2 and left stirring in an H_2 atmosphere for three days. The reaction was filtrated over a Whatman® filter and contracted *in vacuo* at RT. The crude product was dissolved in DMSO:AcOH 8:2 (0.1 ml, 25 mM) and 6-azidohexanal (5.3 µL, 38 µmol, 15 eq) was added. The reaction was shaken for 1 hour before adding sodium triacetoxy borohydride (8 mg, 38 µmol, 15eq). The reaction was shaken for 10 minutes and quenched with MiliQ. The MiliQ was washed twice with EtOAc and concentrated *in vacuo* at RT. The product was purified by C-18 column (BAKERBOND) (1% to 10% ACN in miliQ) to obtain a white solid (1.7 mg, 0.83 µmol, 33%). **^{1}H NMR** (850 MHz, D_2O) δ 5.40 – 5.18 (m, 3H, 3xH2), 4.22 – 3.70 (m, 42H), 3.67 (t, *J* = 6.3 Hz, 1H), 3.58 (dd, *J* = 11.9, 7.0 Hz, 1H), 3.26 (t, *J* = 6.7 Hz, 2H, $CH_{2\ linker}$), 3.14 – 3.08 (m, 2H, $CH_{2\ linker}$), 3.03 – 2.96 (m, 2H, $CH_{2\ linker}$), 2.76 (s, 3H, NMe), 1.68 – 1.49 (m, 10H, $5xCH_{2\ linker}$), 1.41 – 1.21 (m, 45H, $3xCH_{3\ ala}$, $3xCH_{3\ tert\text{-}butyl}$, $3xCH_{2\ linker}$). **^{13}C NMR** (214 MHz, D_2O) δ 72.0, 71.7, 71.1, 70.8, 70.2, 66.5, 66.1, 63.4, 62.3, 55.8, 55.8, 51.0, 49.6, 48.9, 39.6, 29.6, 29.4, 27.7, 27.6, 25.4, 25.2, 24.5, 23.4, 23.3, 16.6. **HRMS:** $[C_{67}H_{133}N_7O_{52}P_6 + 2H]^+$ calculated 1027.82746, found 1027.82896.

Hexamer (**1**)



Compound **29** (1.7 mg, 0.83 µmol) was dissolved in HFIP (0.1 ml, 8.3 mM), and HCl (1M in HFIP, 42 µL, 50 eq) was added. The reaction was shaken for 20 minutes and concentrated *in vacuo* at RT to obtain a white solid (1.4 mg, 0.80 µmol, 96%). **^{1}H NMR** (850 MHz, D_2O) δ 5.36 (s, 3H, 3xH-2), 4.25 – 3.66 (m, 43H), 3.58 (dd, *J* = 12.0, 7.0 Hz, 1H), 3.26 (t, *J* = 6.8 Hz, 2H, $CH_{2\ linker}$), 3.16 – 3.08 (m, 1H, $CH_{2\ linker}$), 3.05 – 2.97 (m, 1H, $CH_{2\ linker}$), 2.76 (s, 2H, NMe), 1.70 – 1.46 (m, 20H, 4x $CH_{2\ linker}$, 3xCH_{3ala}), 1.38 – 1.32 (m, 8H, 4x$CH_{2\ linker}$). **^{13}C NMR** (214 MHz, D_2O) δ 75.8, 72.1, 72.0, 71.7, 71.1, 70.8, 70.2, 69.2, 69.1, 68.2, 68.0, 67.9, 66.5, 66.4, 66.2, 65.9, 63.5, 62.3, 55.9, 55.8, 51.0, 48.9, 48.9, 48.8, 39.6, 29.4, 27.7, 25.4, 25.2, 24.5, 23.4, 23.3, 15.3, 15.1 **^{31}P NMR** (122 MHz, D2O) δ 1.9, 1.8, 1.5. **HRMS:** $[C_{67}H_{133}N_7O_{58}P_6 + 2H]^+$ calculated 877.74882, found 877.74839.

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