Synthesis of ribitol phosphate based wall teichoic acids

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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.

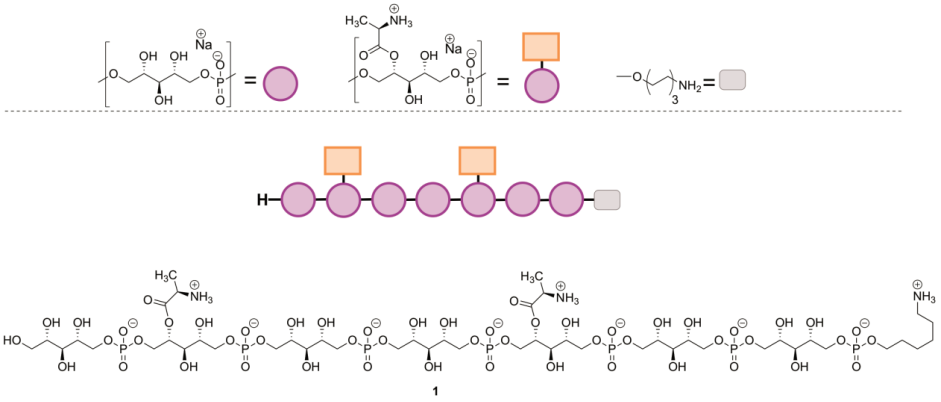


Figure 1. Target compound **1** of this chapter.

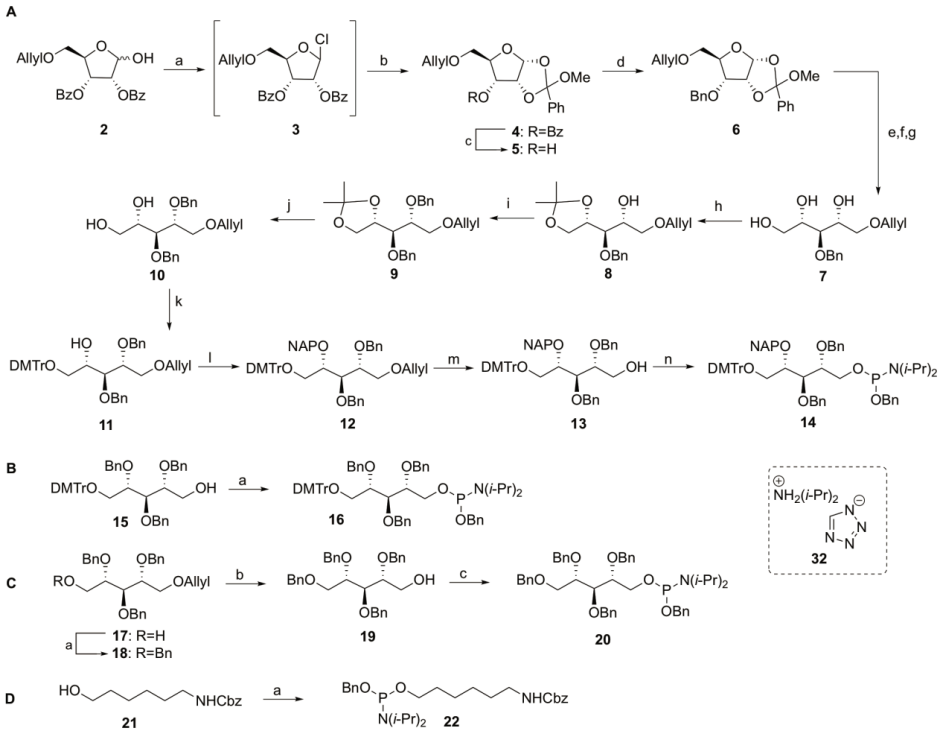
RESULTS AND DISCUSSION

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.

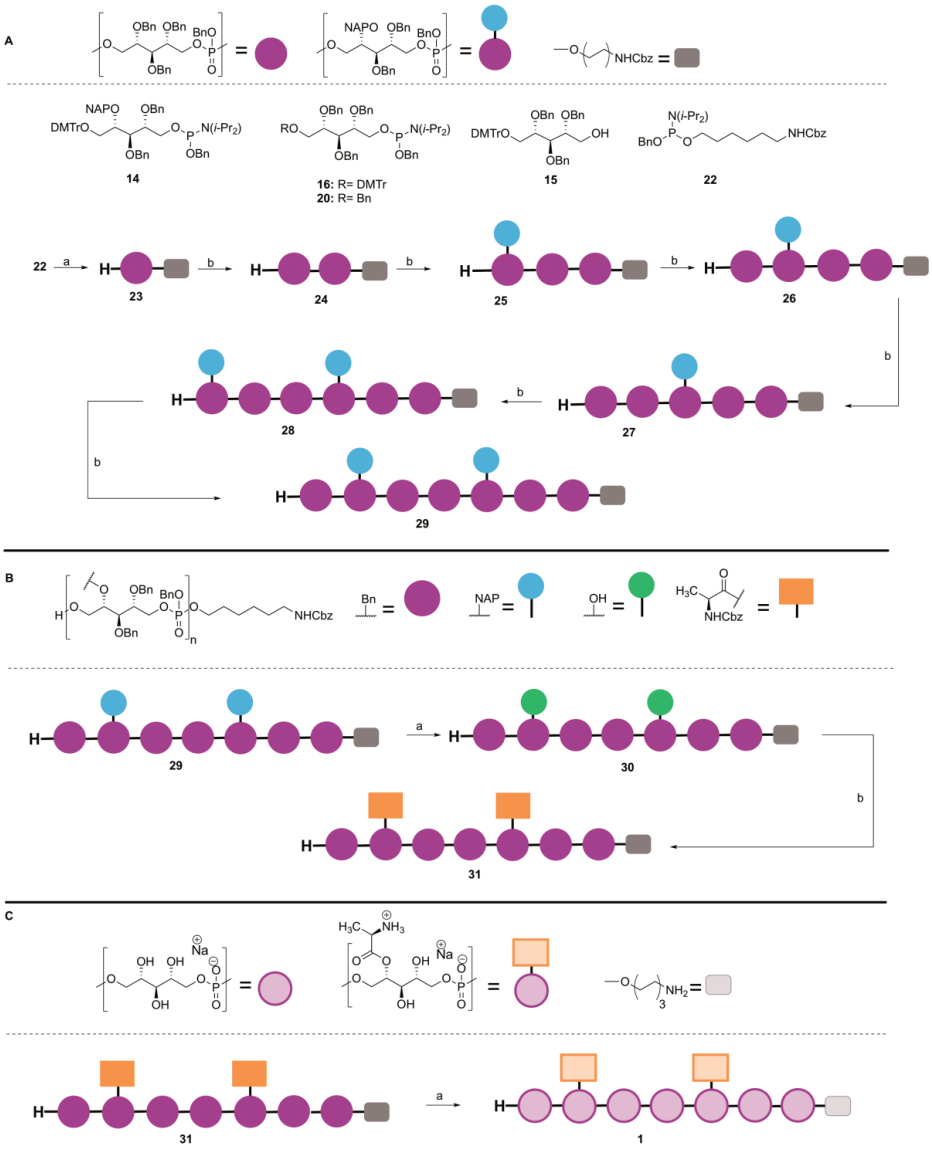
The target heptamer was assembled from the key amidite building blocks **14**, **16**, **20** and **22**, the synthesis of which is depicted in Scheme 1A-D. Starting from intermediate **2** (obtained as described in Chapter 3), the anomeric chloride **3** was obtained by treatment with dry HCl in dioxane. 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.

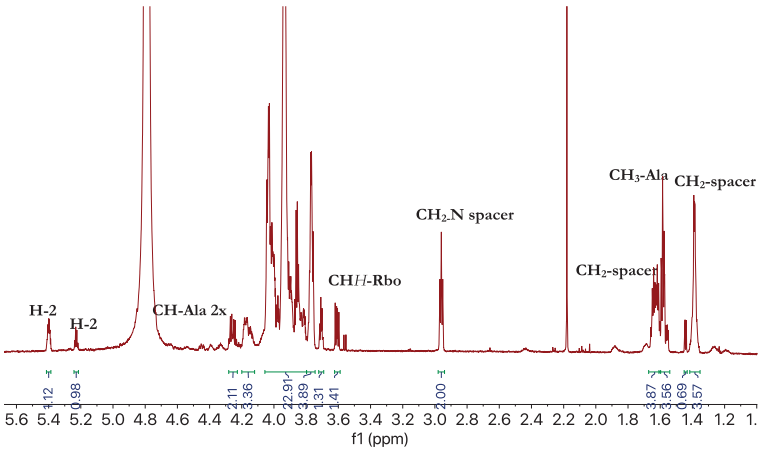


Figure 2. ^{1}H NMR of target compound **1**. (measured at a 500 MHz, at 25°C)

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, $\lambda = 589$ nm) with a concentration of 10 mg/mL (c= 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300. ^1H, ^{13}C and ^{31}P NMR spectra were recorded with a Bruker AV 400 (400, 101 and 162 MHz respectively), a Bruker AV 500 (500 and 202 MHz respectively) or a Bruker DMX 600 (600 and 151 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane, unless stated otherwise. High resolution mass spectra were recorded by direct injection (2 µl of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1 % formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250°C) with resolution R = 60000 at *m/z* 400 (mass range *m/z* = 150-2000) and dioctylphthalate (*m/z* = 391.28428) as a lock mass. 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).

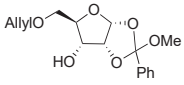
General procedure for global deprotection

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.

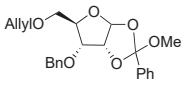
5-*O*-allyl-(1,2-*O*-methylorthobenzoyl)-α-D-ribofuranoside (5)



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.

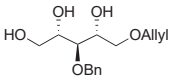
5-*O*-allyl-3-*O*-benzyl-(1,2-*O*-methylorthobenzoyl)-α-D-ribofuranoside (6)



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

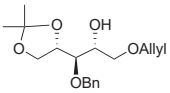
(m, 1H, H-3), 3.85 – 3.95 (m, 2H, C*H*$_2$-CH), 4.55 (d, 1H, *J*= 11.7 Hz, C*H*H-Bn), 4.72 – 4.81 (m, 2H, C*H*H-Bn, H-2), 5.08 – 5.23 (m, 2H, C*H*$_2$=CH), 5.74 – 5.84 (m, 1H, CH$_2$=C*H*), 6.01 (d, 1H, *J*= 4.2 Hz, H-1), 7.23 – 7.38 (m, 8H, H-arom), 7.68 – 7.73 (m, 2H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 49.8 (CH_3O), 67.5 (C-5), 71.7, 71.9 (CH_2-Bn, *C*H_2-CH), 77.1, 77.6, 78.0 (C-2, C-3, C-4), 104.2 (C-1), 116.7 (*C*H_2=CH), 123.7 (Cq), 125.9, 127.6, 127.8, 128.1, 128.8 (CH-arom), 134.2 (CH$_2$=*C*H), 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)



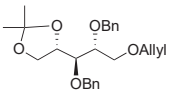
Compound **6** (5.25 g; 13.2 mmol; 1.0 eq.) was dissolved in a mixture of THF/H_2O/Formid acid (130 ml; 0.10M; v/v/v= 6:3:1) and the mixture was heated to 70°C until complete conversion of the starting material was achieved according to TLC analysis. The mixture was then diluted with EtOAc, washed with 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, C*H*$_2$-CH), 4.58 – 4.69 (m, 2H, CH_2-Bn), 5.14 – 5.29 (m, 2H, C*H*$_2$=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 (C*H*$_2$-CH), 72.8 (C-2/C-4), 73.8 (CH_2-Bn), 79.4 (C-3), 117.6 (*C*H_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, $CHCl_3$); ^1H 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, CH_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, CH_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, CH_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.

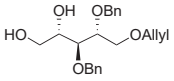
5-*O*-allyl-3,4-di-*O*-benzyl-1,2-*O*-isopropylidene-D-ribitol (9)



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, $CHCl_3$); ^1H 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, CH_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, CH_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, CH_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),

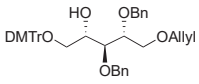
116.8 (CH_2=CH), 127.6, 127.7, 127.9, 128.4 (CH-arom), 134.8 (CH_2=CH), 138.5 (Cq-arom); HRMS: $[M+Na]^+$ calcd for $C_{25}H_{32}O_5Na$ 435.21420, found 435.21420.

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, CH_2-CH), 4.57 – 4.75 (m, 4H, CH_2-Bn), 5.13 – 5.31 (m, 2H, CH_2=CH), 5.83 – 5.93 (m, 1H, CH_2=CH), 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, CH_2-CH), 79.0, 79.2 (C-2, C-3), 117.2 (CH_2=CH), 127.7, 127.7, 127.8, 128.0, 128.4 (CH-arom), 134.4 (CH_2=CH), 138.0, 138.1 (Cq-arom); HRMS: $[M+H]^+$ calcd for $C_{22}H_{29}O_5$ 373.20095, found 373.20079.

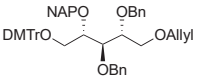
5-*O*-allyl-3,4-di-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-D-ribitol (11)



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-CH_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, CH_2-CH, H-4), 4.46 (d, 1H, *J*= 11.2 Hz, CHH-Bn),

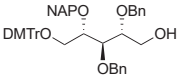
4.55 (d, 1H, *J*= 11.8 Hz, C*H*H-Bn), 4.65 (dd, 2H, *J*= 16.4, 11.5 Hz, CH_2-Bn), 5.12 – 5.35 (m, 2H, CH_2=CH), 5.88 – 5.98 (m, 1H, CH_2=C*H*), 6.79 – 6.87 (m, 4H, H-arom), 7.10 – 7.39 (m, 17H, H-arom), 7.48 – 7.54 (m, 2H, H-arom); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 55.8 (CH_3O), 66.2 (DMTO-CH_2), 70.8 (CH_2-Rbo), 71.7 (C-4), 72.6, 72.9, 74.2 (CH_2-Bn, CH_2-CH), 79.9, 80.6 (C-2, C-3), 86.7 (Cq-DMT), 113.9 (CH-arom), 116.8 (CH_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=CH), 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-CH_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, CH_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, CH_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 (CH_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.

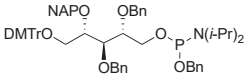
3,4-di-*O*-benzyl-1-*O*-(4,4'-dimethoxytrityl)-2-*O*-(2-naphtylmethyl)-D-ribitol (13)



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.

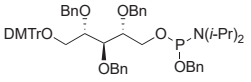
2-Benzyl [3,4-di-*O*-benzyl-1-*O*-(4,4′-dimethoxytrityl)-2-*O*-(2-naphtylmethyl)-1-D-ribityl] *N,N*-diisopropylphosphoramidite (14)



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,

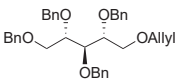
43.7, 43.8, 43.9, 43.9 (CH-isopropylamine), 55.8 (CH_3O), 63.6, 63.7, 63.8, 63.9 (CH_2-Rbo), 64.9, 65.0 (CH_2-Rbo), 65.8, 65.9, 66.0, 66.0, 66.1, 73.0, 73.3, 74.2, 74.2 (CH_2-Bn, CH_2-NAP), 79.7, 79.9, 80.1, 80.2 (CH-Rbo), 86.8 (Cq-DMT), 113.9, 126.3, 127.1, 127.1, 127.2, 127.2, 127.6, 127.9, 128.0, 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.

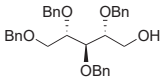
5-*O*-allyl-1,2,3,4-tetra-*O*-benzyl-D-ribitol (18)



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, CH_2-CH), 4.44 – 4.75 (m, 8H, 4x CH_2-Bn), 5.10 – 5.28 (m, 2H, CH_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).

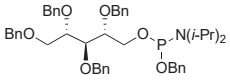
1,2,3,4-tetra-*O*-benzyl-D-ribitol (19)



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.

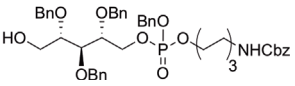
2-Benzyl [1,2,3,4-tetra-*O*-benzyl-1-D-ribityl] *N,N*-diisopropylphosphoramidite (20)



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

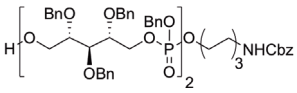
speed up the conversion. After complete conversion of the reaction according to TLC analysis, the reaction was filtrated and concentrated *in vacuo*. Purification by TEA neutralized column chromatography pentane/EtOAc 1:0 to pentane/EtOAc 9:1 yielded the title compound in 61% (270 mg; 0.36 mmol). ^{1}H 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.

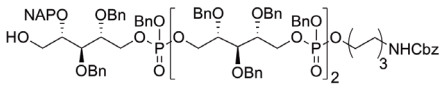
D-ribitol phosphate dimer (24)



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 –

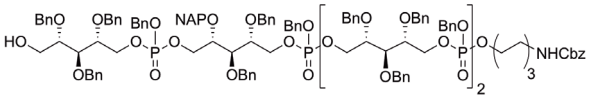
1.28 (m, 4H, CH_2-hexylspacer), 1.32 – 1.44 (m, 2H, CH_2-hexylspacer), 1.47 – 1.54 (m, 2H, CH_2-hexylspacer), 3.09 (q, 2H, *J*= 7.6 Hz, CH_2N hexylspacer), 3.62 – 3.73 (m, 3H, CH_2-Rbo, CH-Rbo), 3.75 – 3.95 (m, 7H, 5x CH-Rbo, CH_2O), 4.11 – 4.41 (m, 6H, 3x CH_2-Rbo), 4.45 – 4.67 (m, 12H, 6x CH_2-Bn), 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)



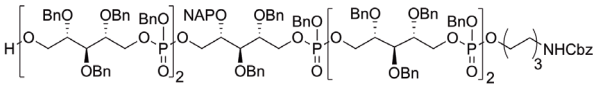
According to the general procedure above, alcohol **24** (0.05M in ACN; 7.4 mL; 522 mg; 0.37 mmol; 1.0 eq.) was coupled with phosphoramidite **14** (0.47 g; 0.44 mmol; 1.2 eq.). Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1, v/v) yielded the title compound in 96% yield (720 mg; 0.36 mmol). IR (neat, 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.

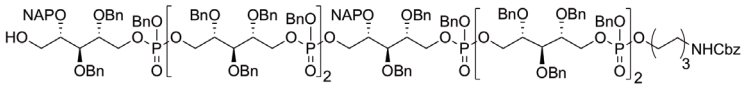
D-ribitol phosphate pentamer (27)



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),

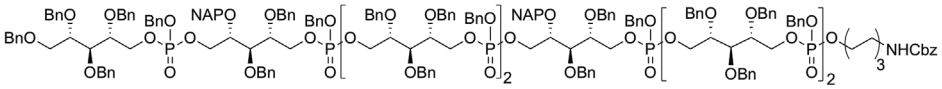
66.4 (CH_2-Cbz), 66.5, 66.5, 66.6, 66.7, 66.7, 66.8, 66.8, 66.9, 66.9, 67.0 (CH_2-Rbo), 67.6, 67.6, 67.6, 67.7 (CH_2O), 68.9, 69.0, 69.0, 69.0, 69.1, 69.1, 71.9, 72.1, 72.3, 72.3, 72.3, 72.4, 72.4, 72.5, 73.6, 73.7, 73.7, 73.7, 73.8, 73.8, 73.9 (CH_2-Bn, CH_2-NAP), 76.9, 77.2, 77.2, 77.4, 77.4, 77.5, 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)



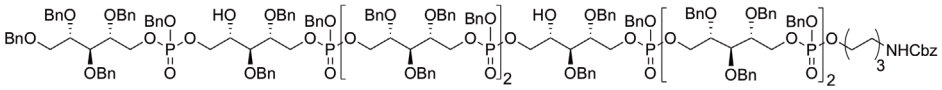
According to the general procedure above, alcohol **27** (0.15 M in ACN; 1.1 mL; 532 mg; 0.17 mmol; 1.0 eq.) was coupled with phosphoramidite **14** (0.27 g; 0.25 mmol; 1.5 eq.). Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1, v/v) yielded the title compound in 91% yield (580 mg; 0.15 mmol). IR (neat, 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.

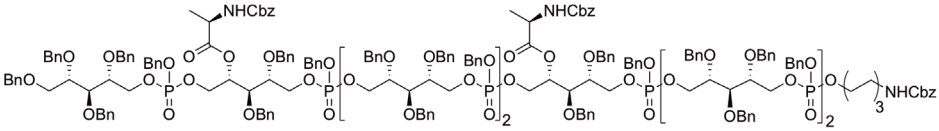
D-ribitol phosphate heptamer (30)



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.

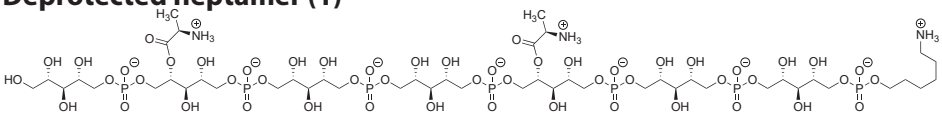
D-ribitol phosphate heptamer (31)



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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