Synthesis of ribitol phosphate based wall teichoic acids

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Synthesis of *E. faecalis* wall teichoic acid fragments

INTRODUCTION

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

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

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

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

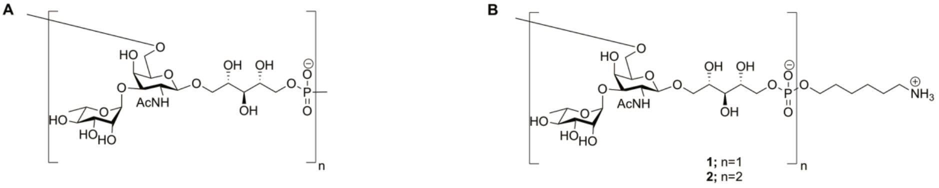


Figure 1. Structure of *E. faecalis* WTA (A) and the target structures of this Chapter (B).

RESULTS & DISCUSSION

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

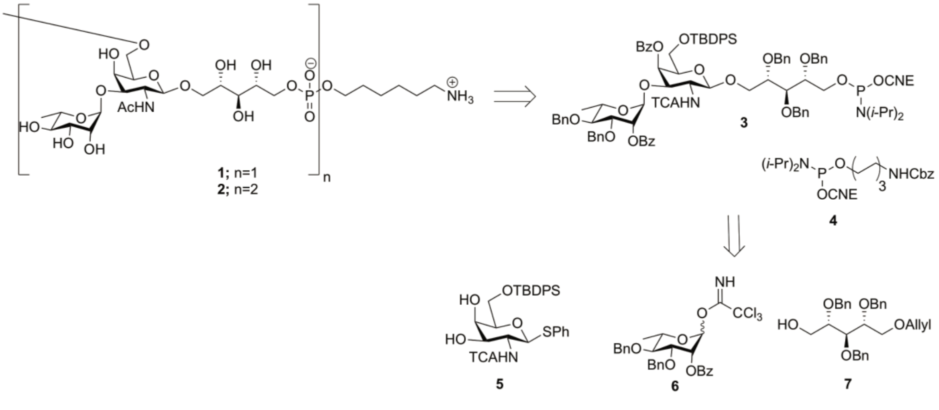
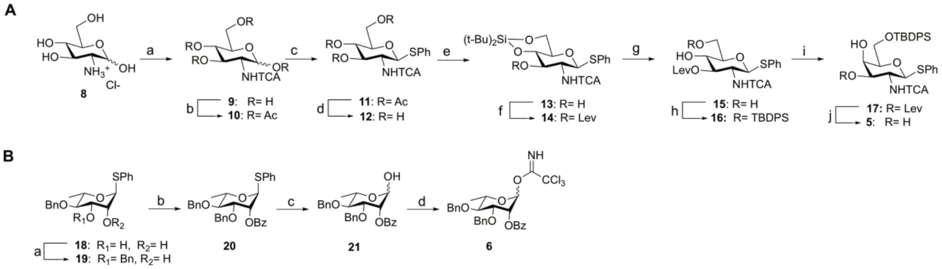


Figure 2. Retrosynthetic analysis for the assembly of WTA fragments.

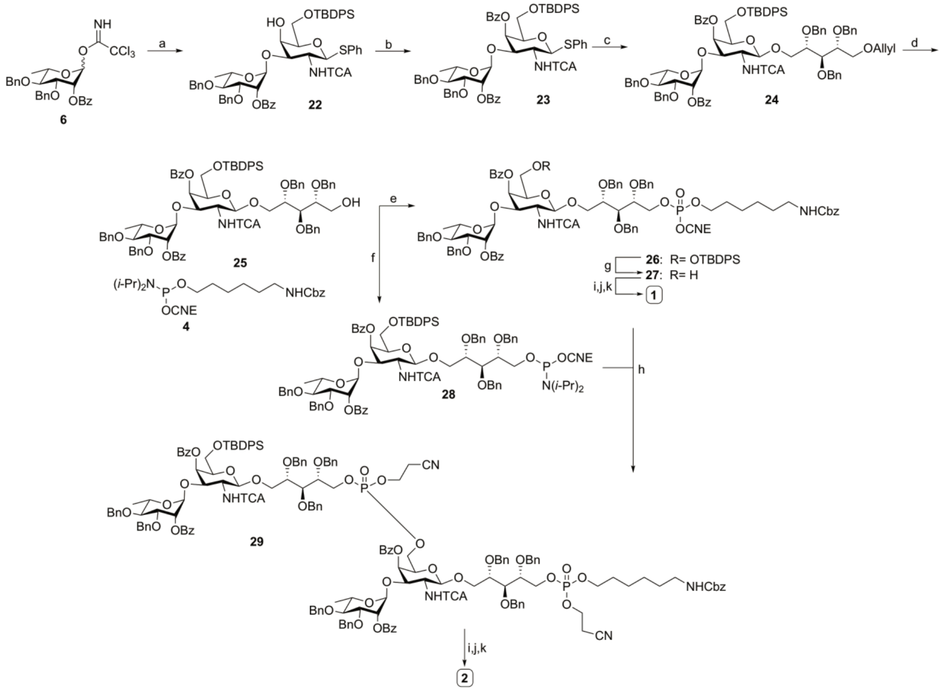
The synthesis of the building blocks **5** and **6**, required for the assembly of intermediate **3**, is shown in Scheme 1. Ribitol **7** was obtained as described in previous Chapters. The synthesis of building block **5** started from D-glucosamine by trichloroacetylation of the amine to give compound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



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

With all building blocks in hand, the WTA repeat unit was assembled. Coupling of donor **6** at 0ºC with diol acceptor **5** gave disaccharide **22** in 36% (Scheme 2). After the work up, some acceptor was recovered but no C-4-glycosylated product was observed. The disaccharide was benzoylated in the next step to mask the axial alcohol position. Coupling of disaccharide **23** with ribitol **7** then gave pseudo-trisaccharide **24** in 45% yield. Iridium catalyzed isomerization of the allyl ether followed by iodine mediated enol ether hydrolysis provided alcohol **25**, which was equipped with a phosphoramidite moiety giving building block **28.** Beside, coupling of alcohol **25** and spacer phosphoramidite **4** under activation of 4,5-dicyanoimidazole (DCI) followed by oxidation of the phosphite intermediate using (10-camphorsulfonyl)oxaziridine (CSO) gave **26** in 68% yield**.** Cleavage of the TBDPS group using HF in pyridine gave building block **27** containing an alcohol function for the assembly of the WTA-dimer. The two trisaccharide building blocks **27** and **28** were condensed using DCI as activation agent forming the phosphite intermediate, which in turn was oxidized *in situ* using CSO to give the fully protected dimer **29**.

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



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

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

CONCLUSION

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

EXPERIMENTAL SECTION

General information

All chemicals (Acros, Fluka, Merck, Sigma-Aldrich, etc.) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040- 0.063 mm). TLC analysis was conducted on HPTLC aluminum sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}\cdot4H_2O$ 25 g/L and $(NH4)_4Ce(SO_4)_4\cdot2H_2O$ 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. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

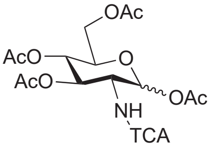
Phosphoramidite coupling and oxidation

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

General procedure for global deprotection

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

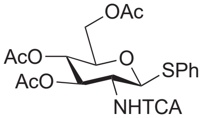
1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloroacetamide-α/β-D-glucopyranoside (10)



D-glucosamine hydrochloride **8** (42.9 g, 200 mmol, 1.0 eq.) was suspended in MeOH (343 mL, 0.9M) and TEA (83.2 mL, 1 mol, 5.0 eq.) was added. The suspension was cooled to 0°C and trichloroacetylchloride (24.7 mL, 220 mmol, 1.1 eq.) was added dropwise to the solution. It was allowed to warm up to rt and vigorously stirring was continued for 3 days. The reaction mixture was then filtered over silica and concentrated. The intermediate was obtained as yellow solid. The intermediate (65.5 g, 200 mmol, 1.0 eq.) was dissolved in pyridine (454 mL, 0.44M) and cooled to 0°C. Acetic anhydride (113.4 mL, 1.2 mol, 6.0 eq.) was slowly added and the solution was left stirring at rt overnight. The reaction mixture was quenched with MeOH (80 mL) at 0°C and dissolved in EtOAc. The solution was washed with HCl (3M, until pH was acidic), sat. aq. $NaHCO_3$ (2x 200 mL) and sat. aq. NaCl (200 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated. The crude was dissolved in EtOH and heated up in order to crystallize. Compound **10** was obtained as white crystals (50.12 g, 101.6 mmol, 51%, α-product). ^1H NMR ($CDCl_3$, 400 MHz) $\delta = 2.07$ (s, 6H, 2x CH_3-Acetyl), 2.11 (s, 3H, CH_3-Acetyl), 2.20 (s, 3H, CH_3-Acetyl), 4.02 - 4.11 (m, 2H, H-5, H-6), 4.29 - 4.38 (m, 2H, H-2, H-6), 5.26 (t, 1H, $J = 9.7$ Hz, H-4), 5.36 (t, 1H, $J = 10.2$ Hz, H-3), 6.32 (d, 1H, $J = 3.7$ Hz, H-1), 6.81 (d, 1H, $J = 8.4$ Hz, NH); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) $\delta = 53.5$ (C-2), 61.5 (C-6), 67.1 (C-4), 70.0 (C-5), 70.2 (C-3), 89.7 (C-1).

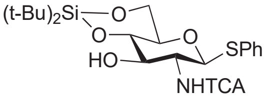
The motherlayer was concentrated under reduced pressure and dissolved in EtOAc. Celite was added to the solution and the solvent was removed *in vacuo*. The pulver was purified over silica column (10% to 60% EtOAc in PE). Compound **10** was obtained as white solid (34.8 g, 70.6 mmol, 35%) as an α/β mixture with a ratio of 54/46. ^{1}H NMR ($CDCl_3$, 400 MHz) δ= 2.06 (m, 14H, C*H*$_3$-Acetyl), 2.09 - 2.15 (m, 11H, C*H*$_3$-Acetyl), 2.20 (s, 3H, C*H*$_3$-Acetyl), 3.84 - 4.19 (m, 4H, 2x H-5, 2x H-6), 4.22 - 4.40 (m, 4H, 2x H-2, 2x H-6), 5.15 - 5.41 (m, 4H, 2x H-3, 2x H-4), 5.80 (d, 1H, *J*= 8.7 Hz, H-1 β), 6.31 (d, *J*= 3.7 Hz, H-1α), 6.82 (d, 1H, *J*= 8.5 Hz, -N*H* β), 6.91 (d, 1H, *J*= 9.4 Hz, -NH α); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 53.5, 54.9 (2x C-2), 61.5, 61.7 (2x C-6), 67.1, 67.6, 70.0, 70.2, 71.8, 73.3 (2x C-3, 2x C-5, 2x C-4), 89.7 (C-2 β), 92.2 (C-1α). The overall yield is 69.8 g (172.2 mmol, 86%) and the anomeric ratio is α/β is 81:19.

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



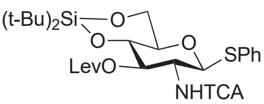
Compound **10** (84.93 g, 172.17 mmol, 1.0 eq.) was dissolved in dry DCM (603 mL, 0.3M). Thiophenol (17.6 mL, 172.2 mmol, 1.0 eq.) and $BF_3{\cdot}OEt_2$ (65.5 mL, 516.5 mmol, 3.0 eq.) were added to the solution and stirred over night at rt. The reaction mixture was quenched with Et_3N and washed with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

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



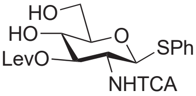
Compound **12** (50.6 g, 93.2 mmol, 1.0 eq.) was suspended in dry MeOH and NaOMe (5.5 g, 102.5 mmol, 1.1 eq.) and stirred for 2 days. The reaction mixture was quenched with Amberlite H^+ resin IR-120 and filtered off. The solvent was removed *in vacuo*. The crude (18.2 g, mmol, 43.6 mmol, 1.0 eq.) was dissolved in DMF (450 mL, 0.1M) and cooled to -40°C. Di-*tert*-butylsilanediyl bistriflate (13.5 mL, 41.4 mmol, 0.95 eq.) was added dropwise and stirring was continued for 1h and then the reaction mixture was quenched with pyridine (10.5 mL, 130.8 mmol, 3.0 eq.). Thereafter, the mixture was diluted in Et_2O (500 mL) and washed with water (5x 200 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated. The residue was purified over silica column (PE/EtOAc 1/0 to 7/3) yielding compound **13** as a white solid (9.8 g, 17.6 mmol, 40%). IR (neat): 2936, 2859, 1767, 1684, 1528, 1474, 1242, 1069, 823, 748, 656; $[\alpha]_D^{20}$ -14.4° (*c* 0.63, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ= 0.98 (s, 9H, *t*-Bu), 1.08 - 1.02 (m, 16H, *t*-Bu), 3.57 - 3.47 (m, 2H, H-2, H-5), 3.71 - 3.65 (m, 1H, H-3), 3.93 (t, 1H, *J*= 10.2 Hz, H-6), 4.04 (t, 1H, *J*= 9.6 Hz, H-3), 4.23 (dd, 1H, *J*= 10.2 Hz, *J*= 5.1 Hz, H-6), 5.17 (d, 1H, *J*= 10.2 Hz, H-1), 6.86 (d, 1H, *J*= 7.8 Hz, -N*H*), 7.35 - 7.30 (m, 3H, H-arom), 7.49 (dd, 2H, *J*= 6.6 Hz, *J*= 3.1 Hz, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 27.1 (*t*-Bu), 27.6 (*t*-Bu), 56.9 (C-2), 66.1 (C-6), 74.2 (C-3), 74.6 (C-5), 77.7 (C-4), 85.6 (C-1), 110.1 (CCl_3), 128.7 - 133.4 (C-arom); HRMS: $[M+H]^+$ calcd for $C_{22}H_{33}Cl_3NO_5SSi$ 556.09088, found 556.09088.

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



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

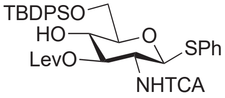
2-deoxy-4-*O*-levulinoyl-1-thio-2-(2,2,2-trichloroacetamide)-β-D-phenyl-glucopyranoside (15)



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

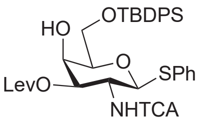
CH_3 Lev), 2.39 - 2.61 (m, 2H, CH_2 Lev), 2.70 - 2.88 (m, 2H, CH_2 Lev), 3.55 - 3.60 (m, 1H, H-5), 3.63 (s, 1H, OH), 3.75 - 3.88 (m, 2H, H-6, H-4), 3.94 - 4.04 (m, 2H, H-2, H-6), 4.89 (d, 1H, *J*= 10.4 Hz, H-1), 5.23 (dd, 1H, *J*= 10.3 Hz, *J*= 9.1 Hz, H-3), 7.04 (d, 1H, *J*= 9.3 Hz, N*H*), 7.32 (dd,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

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



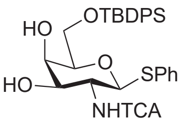
A solution of compound **15** (6.5 g, 12.7 mmol, 1.0 eq.) in DCM (127 mL, 0.1M) was cooled to 0°C. Triethylamine (10.6 mL, 76.2 mmol, 6.0 eq.) was added followed by dropwise addition of *tert*-butyl(chloro)diphenylsilane (4.3 mL, 16.5 mmol, 1.3 eq.) and the reaction was stirred for 3 days at rt. The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

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



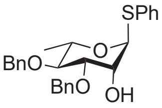
A stirred solution of trifluoroacetic anhydride (TFAA) (2.4 g, 14.5 mmol, 2.0 eq.) in dry DCM (23.4 mL, 0.62M) was cooled to -18°C. A solution of pyridine (2.3 mL, 29.0 mmol, 4.0 eq.) in dry DCM (0.8 mL, 36.3M) was added dropwise followed by a solution of compound **16** (5.5 g, 7.2 mmol, 1.0 eq.) in dry DCM (35 mL). After 30 min stirring the reaction mixture was diluted in DCM (280 mL), washed with 2M HCl (200 mL), saturated aqueous $NaHCO_3$ (200 mL) and water (200 mL), dried over $MgSO_4$, filtrated and concentrated. $NaNO_2$ (5.4 g, 78.9 mmol, 10.9 eq.) in DMF (9.3 mL) was added at rt to the residue containing the triflate-intermediate and stirred overnight. Insoluble material was filtered off and washed with DCM. The filtrate and washings were combined and washed with water, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was purified over silica column (PE/EtOAc, 4/1 to 1/1). Compound **17** was obtained as a white solid (3.7 g, 4.9 mmol, 68%). IR (neat): 3322, 2930, 2489, 1717, 1526, 1152, 1113, 822, 743, 702; $[\alpha]_D^{20}$ +12.0° (*c* 1.08, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ= 1.06 (s, 9H, *t*-Bu), 2.15 (s, 3H, CH_3 Lev), 2.49 - 2.67 (m, 2H, CH_2 Lev), 2.74 (t, 2H, *J*= 6.3 Hz, CH_2 Lev), 3.25 (d, 1H, *J*= 3.5 Hz, 4-OH), 3.66 (t, 1H, *J*= 4.8 Hz, H-5), 3.96 (m, 2H, H-6), 4.34 - 4.22 (m, 2H, H-2, H-4), 4.97 (d, 1H, *J*= 10.4 Hz, H-1), 5.19 (dd, 1H, *J*= 10.6 Hz, *J*= 2.9 Hz, H-3), 6.78 (d, 1H, *J*= 8.9 Hz, N*H*), 7.35 - 7.47 (m, 7H, H-arom), 7.25 (s, 4H, arom.), 7.52 (dd, 2H, *J*= 7.5 Hz, *J*= 1.9 Hz, H-arom), 7.70 (m, 4H, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 26.9 (*t*Bu), 28.2 (CH_2 Lev), 29.9 (CH_3 Lev), 38.1 (CH_2 Lev), 51.3 (C-2), 64.2 (C-6), 67.8 (C-4), 73.7 (C-3), 78.1 (C-5), 86.7 (C-1), 128.0, 128.2, 129.2, 130.1, 132.6, 132.8, 135.7, 135.8 (C-arom.), 161.8 (C=O), 172.5 (C=O Lev), 207.2 (C=O Lev); HRMS: $[M+Na]^+$ calcd for $C_{35}H_{40}Cl_3NO_7SSiNa$ 774.12525, found 774.12547.

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



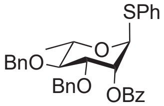
Compound **17** (3.7 g, 4.9 mmol, 1.0 eq.) was dissolved in AcOH/pyridine (49 mL, 1/4, 0.1M) and a hydrazine solution (0.26 mL, 5.4 mmol, 1.1 eq.) was added. After 30 min the reaction mixture was quenched with acetone, diluted in EtOAc, washed with 1M HCl, sat. aq. sodium bicarbonate, dried over $MgSO_4$,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

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



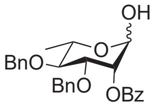
Diol **18** (3.7 g, 10.8 mmol, 1.0 eq.) was dissolved in toluene (108 mL, 0.1M). Dibutyl tin oxide (3.2 g, 13.0 mmol, 1.2 eq.) was added and the reaction mixture was refluxed at 111°C. The yellowish clear solution obtained after 3 hours was concentrated and redissolved in dry DMF (108 mL, 0.1M). Cesium fluoride (3.3 g, 21.6 mmol, 2.0 eq.) and benzyl bromide (1.7 mL, 14.0 mmol, 1.3 eq.) were added and the mixture was stirred overnight. The reaction mixture was diluted in Et_2O, washed with water and brine and dried over $MgSO_4$, filtered and concentrated. The residue was purified over a silica column (PE/EtOAc, 1/0 to 13/7). Compound **18** was obtained as colourless oil (3.9 g, 9.0 mmol, 83%). Analytical data are identical to literature precendence.[16] HRMS: $[M+Na]^+$ calcd for $C_{26}H_{28}O_4SNa$ 459.16005, found 459.15995.

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



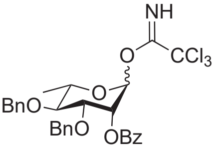
Compound **19** (3.9 g, 9.0 mmol, 1.0 eq.) was dissolved in DCM/pyridine (36 mL, 4:1, 0.2M) and benzoyl chloride (3.1 mL, 27.0 mmol, 3.0 eq.) was slowly added followed by DMAP (0.22 g, 1.8 mmol, 0.2 eq.). After 3 hours the reaction mixture was quenched with MeOH, diluted in DCM and washed with 1M HCl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

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



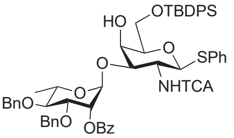
Compound **20** (4.9 g, 9.0 mmol, 1.0 eq.) was dissolved in DCM (90 mL, 0.1M) and NIS (2.2 g, 9.9 mmol, 1.1 eq.) was added followed by cooling to 0 °C. TFA (0.8 mL, 9.9 mmol, 1.1 eq.) was dropped to the solution and the mixture was allowed to warm up to rt. After 3 hours major conversion was visible on TLC and piperidine (2.7 mL, 27.0 mmol, 3.0 eq.) was added at 0°C and the mixture was warmed up to rt. After 1 hour $Na_2S_2O_3$ (s) was added. The solution was washed with sat. aq. $Na_2S_2O_3$, 1M HCl, water and sat. aq. NaCl, dried over $MgSO_4$, filtered and concentrated. The residue was purified over silica column (PE/EtOAc, 9/1 to 1/1) yielding compound **21** as white solid (3.2 g, 7.2 mmol, 80%) (α/β = 2:9) β-anomer; ^1H NMR ($CDCl_3$, 400 MHz) δ= 1.34 (d, 3H, *J*= 6.2 Hz, H-6), 3.58 - 3.46 (m, 1H, H-4), 4.13 - 4.00 (m, 2H, H-5, H-3), 4.52 - 4.94 (m, 6H, CH_2-Bn), 5.25 (d, 1H, *J*= 1.6 Hz, H-1), 5.61 (dd, 1H, *J*= 3.2 Hz, 1.9 Hz, H-2), 7.19 - 7.62 (m, 18H, H-arom), 8.05 - 8.14 (m, 3H, H-arom); ^{13}C-APT NMR ($CDCl_3$, 100 MHz) δ= 18.3 (C-6), 68.0 (C-5), 69.9 (C-2), 71.7 (CH_2 Bn), 75.5 (CH_2 Bn), 77.7 (C-3), 80.3 (C-4), 92.6 (C-1), 127.7 - 138.5 (C-arom), 166.0 (C=O); IR (neat, cm^{-1}) 712, 1098, 1271, 1452, 1719, 2932, 3422; HRMS: $[M+Na]^+$ calcd for $C_{27}H_{28}O_6$ Na 471.17781, found 471.17770.

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



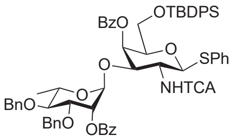
To a solution of compound **21** (1.47 g; 3.28 mmol; 1.0 eq.) in DCM (33 mL; 0.1M) was added K_2CO_3 (1.81 g; 13.1 mmol; 4.0 eq.) followed by the addition of TCAN (2.0 mL; 19.7 mmol; 6.0 eq.) at 0°C and the mixture was then allowed to warm up to rt overnight. Then K_2CO_3 (0.90 g; 6.6 mmol; 2.0 eq.) and TCAN (1.0 mL; 9.9 mmol; 3.0 eq.) were added to complete the conversion. After complete conversion according to TLC analysis, the mixture was filtrated and concentrated *in vacuo*. TEA neutralized silica column purification (PE/EtOAc, 1/0 to 7/3) afforded compound **6** in 83% yield (1.62 g; 2.72 mmol) (α/β ratio= 1: 0.08). NMR assignment for the α-product. ^{1}H NMR (400 MHz, Acetonitrile-*d*$_3$) δ= 1.38 (d, 3H, *J*= 6.2 Hz, H-6), 3.71 (t, 1H, *J*= 9.6 Hz, H-4), 3.91 – 4.01 (m, 1H, H-5), 4.06 (dd, 1H, *J*= 9.4, 3.2 Hz, H-3), 4.62 (d, 1H, *J*= 11.4 Hz, C*H*H-Bn), 4.69 (d, 1H, *J*= 10.9 Hz, C*H*H-Bn), 4.77 (d, 1H, *J*= 11.4 Hz, C*H*H-Bn), 4.90 (d, 1H, *J*= 10.9 Hz, C*H*H-Bn), 5.77 (t, 1H, *J*= 2.5 Hz, H-2), 6.34 (s, 1H, H-1), 7.21 – 7.41 (m, 10H, H-arom), 7.55 (dd, 2H, *J*= 8.4, 7.1 Hz, H-arom), 7.63 – 7.71 (m, 1H, H-arom), 8.12 (dd, 2H, *J*= 7.6, 1.4 Hz, H-arom), 9.07 (s, 1H, N*H*); ^{13}C-APT NMR (101 MHz, CD_3CN) δ= 18.5 (C-6), 68.7 (C-2), 71.6 (C-5), 72.3, 75.9 (CH_2-Bn), 78.0 (C-3), 80.1 (C-4), 91.5 (CCl_3), 95.9 (C-1), 128.6, 128.7, 129.1, 129.2, 129.2, 129.3, 129.7 (C-arom), 130.6 (Cq-arom), 134.5 (C-arom), 138.7, 139.5 (Cq-arom), 160.2, 166.1 (C=O).

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



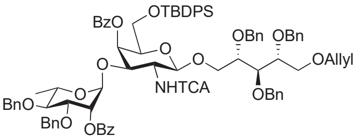
To a stirred mixture of donor **6** (1.00 g; 1.70 mmol; 1.0 eq.) and acceptor **5** (1.34 g; 2.05 mmol; 1.2 eq.) in DCM (31 mL; 0.05M) on MS 3Å at 0°C was activated by the addition of TMSOTf (62 μL; 71.2 μg; 0.2 eq.). After 1.5h complete conversion of the donor was achieved and the reaction was quenched with 6 drops TEA, DCM was added and the organic layer was washed with aq. sat. NaCl/$NaHCO_3$ (v/v= 1/1), dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Size exclusion chromatography yielded disaccharide **22** in 44% yield (0.82 g; 0.76 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.07 (s, 9H, CH_3-*t*Bu), 1.34 (d, 3H, *J*= 6.3 Hz, H-6), 2.71 (d, 1H, *J*= 2.5 Hz, O*H*), 3.52 (t, 1H, *J*= 9.4 Hz, H-4 ram), 3.63 (t, 1H, *J*= 5.3 Hz, H-5 gal), 3.79 – 3.87 (m, 1H, H-2 gal), 3.87 – 3.93 (m, 1H, H-6 gal), 3.97 - 4.04 (m, 3H, H-6 gal, H-3 ram, H-5 ram), 4.26 (t, 1H, *J*= 2.6 Hz, H-4 gal), 4.32 (dd, 1H, *J*= 10.3, 2.9 Hz, H-3 gal), 4.52 (d, 1H, *J*= 11.1 Hz, C*H*H-Bn), 4.60 (d, 1H, *J*= 10.9 Hz, C*H*H-Bn), 4.75 (d, 1H, *J*= 11.1 Hz, C*H*H-Bn), 4.88 (d, 1H, *J*= 10.8 Hz, C*H*H-Bn), 4.99 (d, 1H, *J*= 1.8 Hz, H-1 ram), 5.21 (d, 1H, *J*= 10.3 Hz, H-1 gal), 5.64 (dd, 1H, *J*= 3.3, 1.8 Hz, H-2 ram), 6.82 (d, 1H, *J*= 7.6 Hz, N*H*), 7.18 – 7.48 (m, 21H, H-arom), 7.51 – 7.60 (m, 3H, H-arom), 7.71 (ddt, 4H, *J*= 14.3, 6.5, 1.7 Hz, H-arom), 8.00 – 8.05 (m, 2H, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 17.8 (C-6), 19.3 (Cq-*t*Bu), 26.9 (CH_3-*t*Bu), 53.0 (C-2 gal), 63.7 (C-6 gal), 68.9, 69.1, 69.2 (C-2 ram, C-4 gal, C-5 ram/C-3 ram), 71.8, 75.5 (CH_2-Bn), 78.0, 78.3, 78.6 (C-3 gal, C-5 gal, C-5 ram/C-3 ram), 79.7 (C-4 ram), 84.7 (C-1 gal), 92.4 (CCl_3), 100.1 (C-1 ram), 127.7, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.5, 129.2 (C-arom), 129.9 (Cq-arom), 130.0, 130.1 (CH-arom), 132.0, 132.7, 132.9 (Cq-arom), 133.1, 133.4, 135.7, 135.8 (C-arom), 138.1, 138.4 (Cq-arom), 161.9, 165.5 (C=O); HRMS: $[M+ NH_4]^+$ calcd for $C_{57}H_{64}Cl_3N_2O_{10}SSi$ 1101.31110, found 1101.31117.

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



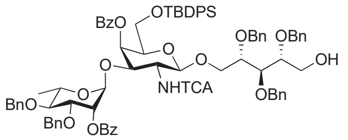
To a solution of compound **22** (0.80 g; 0.74 mmol; 1.0 eq.) in pyridine (10.0 mL; 0.10M) was added BzCl (1.7 mL; 14.8 mmol; 20.0 eq.) and DMAP (110 mg; 0.9 mmol; 1.2 eq.) and the mixture was heated overnight at 65°C. Then the mixture was cooled to rt and quenched with MeOH at 0°C. The organic layer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

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



To a stirring mixture of acceptor **7** (60 mg; 0.13 mmol; 1.3 eq.) and donor **23** (119 mg; 0.10 mmol; 1.0 eq.) in DCM (0.1M; 1.0 mL) on MS 3Å was added NIS (27 mg; 0.12 mmol; 1.2 eq.) at 0°C. Then the mixture was cooled to -42°C and the reaction was activated by the addition of TMSOTf (5 μL; 0.02 mmol; 0.2 eq.). The mixture was gradually warmed up to 0°C and quenched with TEA. The mixture was then diluted with DCM, washed with sat. aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$/NaCl (v/v= 1/1). The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Purification by silica chromatography (DCM/acetone 100/0 to 95/5 DCM acetone) yielded target compound **24** (70 mg; 45 μmol) in 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.05 (s, 9H, CH_3-*t*Bu), 1.33 (d, 3H, *J*= 6.2 Hz, H-6), 3.50 (t, 1H, *J*= 9.5 Hz, H-4 ram), 3.58 – 4.14 (m, 13H, 2x CH_2-Rbo, H-3 ram, CH_2-allyl, 3x CH-Rbo, H-5 gal, 2x H-6 gal), 4.18 (dq, 1H, *J*= 9.6, 6.1 Hz, H-5 ram), 4.24 – 4.33 (m, 2H, H-3 gal, C*H*H-Bn), 4.52 – 4.70 (m, 8H, C*H*H-Bn), 4.75 (dd, 1H, *J*= 10.0, 1.7 Hz, H-1 gal), 4.81 (d, 1H, *J*= 11.6 Hz, C*H*H-Bn), 4.94 (m, 1H, *J*= 1.7 Hz H-1 ram), 5.12 – 5.27 (m, 2H, C*H*$_2$=CH), 5.51 (dd, 1H, *J*= 3.2, 1.8 Hz, H-2 ram), 5.78 (d, 1H, *J*= 3.2 Hz, H-4 gal), 5.87 (ddt, *J* = 17.3, 10.7, 5.5 Hz, CH_2=C*H*), 6.75 (d, 1H, *J*= 7.8 Hz, N*H*), 7.09 – 7.48 (m, 35H, H-arom), 7.50 – 7.60 (m, 4H, H-arom), 7.60 – 7.69 (m, 2H, H-arom), 8.02 (ddt, 4H, *J*= 11.7, 7.1, 1.4 Hz, H-arom); ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ= 18.1 (C-6 ram), 19.2 (Cq-), 26.8 (CH_3-*t*Bu), 56.2 (C-2 gal), 61.7 (C-6 gal), 68.8, 69.0, 69.4, 69.5 (2x CH_2-Rbo, H-4 gal, H-2 ram, H-5 ram, CH*H*-Rbo), 70.2, 71.8, 72.3, 72.4, 72.5, 73.3, 74.0, 74.3, 74.8, 77.7, 78.0, 78.6, 78.9, 79.1 (5x CH_2-Bn, CH_2-allyl, H-3 gal, H-3 ram, 3x CH-Rbo, CH_2-Rbo, CH*H*-Rbo, H-5 gal, 2x H-6 gal, H-2 gal, H-4 ram, 92.4 (CCl_3), 99.8, 99.9 (C-1 ram, C-1 gal), 117.0 (C*H*$_2$=CH), 127.4, 127.5, 127.7, 127.8, 127.9, 127.9, 128.0, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 129.8, 129.9, 129.9, 130.0 (C-arom), 132.9, 133.2 (Cq-arom), 133.2 C-arom), 134.9, 135.6, 135.6 (C-arom), 138.1, 138.5, 138.6, 138.9, 138.9 (Cq-arom), 162.2, 165.4, 165.4 (C=O); HRMS: $[M+ NH_4]^+$ calcd for $C_{87}H_{96}Cl_3N_2O_{16}Si$ 1557.55892, found 1557.55907.

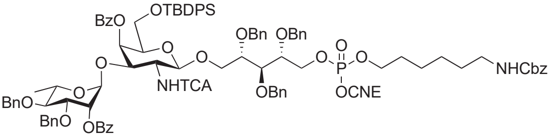
1-*O*-(3-*O*-(2-*O*-benzoyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-4-*O*-benzoyl-2-deoxy-6-*O*-*tert*-butyl-diphenylsilyl-2-(2,2,2-trichloroacetamide)-β-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-D-ribitol (25)



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

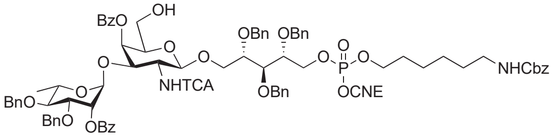
^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.04 (s, 9H, CH_3-*t*Bu), 1.33 (d, 3H, *J*= 6.2 Hz, H-6 ram), 2.31 (s, 1H, O*H*), 3.50 (t, 1H, *J*= 9.5 Hz, H-4 ram), 3.67 – 3.84 (m, 9H, C*H*H-Rbo, CH_2-Rbo, 3x CH-Rbo, 2x H-6, H-3 ram), 3.92 (t, 1H, *J*= 5.0 Hz, H-5 gal), 3.94 – 3.99 (m, 1H, H-2 gal), 4.12 (dd, 1H,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

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



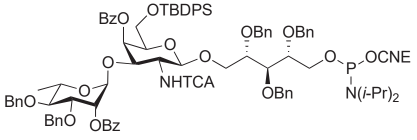
According to the general procedure described above, alcohol **25** (62 mg; 41.0 μmol; 1.0 eq.) was coupled with phosphoramidite **4** (28.0 mg; 62.0 μmol; 1.5 eq.) yielding the title compound **26** in 68% yield (52.2 mg; 27.9 μmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ= 1.03 (d, 9H, *J*= 1.5 Hz, CH_3-*t*Bu),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

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



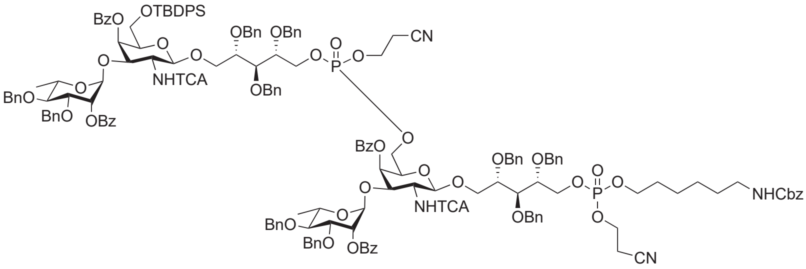
To a solution of compound **26** (52.2 mg; 27.9 μmol; 1.0 eq.) in a mixture of THF/pyr. (2.0 mL; v/v= 1/1; 0.01M) was added 50 μL HF/pyridine. After 2h additional HF/pyridine (50 μL) was added at 0°C to speed up the conversion. After complete conversion, the mixture was quenched with slow addition of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

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



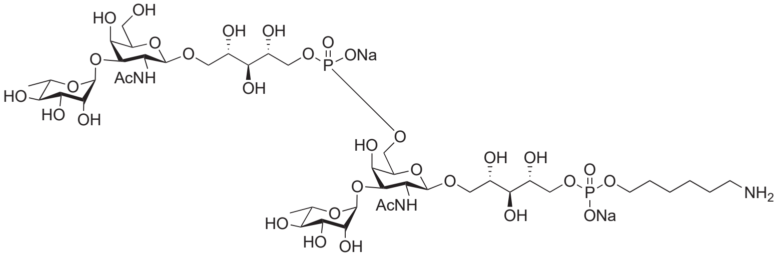
Compound **25** (6.2 mg; 42.6 mmol; 1.0 eq.) was co-evaporated with toluene twice and was then dissolved in DCM (1.0 mL; 0.05 M), DIPEA was added (16 μL; 1.5 eq.) and the mixture was stirred over activated molecular sieves for +/- 20 minuts. 2-Cyanoethyl *N,N*-(diisopropyl)$_2$phosphoramidite (0.18 mL; 1.3 eq.) was added followed by tetrazole salt (18 mg; 0.11 mmol; 2.5 eq.) and the mixture was stirred until TLC showed complete conversion of the starting material. The reaction was then quenched with a few drops of water and diluted with DCM. The organic layer was washed with sat. aq. $NaHCO_3$/NaCl (v/v= 1/1). The organic layer was dried over Na_2SO_4, filtrated and concentrated *in* *vacuo*. Column chromatography using TEA neutralized silica (pentane/EtOAc 1/0 to 7/3 pentane/EtOAc) afforded phosphoramidite **28** in 70% yield (51 mg; 29.2 μmol).

Dimer (29)



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

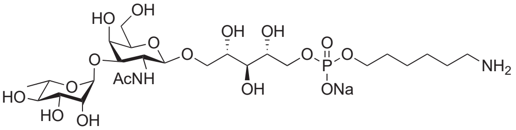
Deprotected dimer (2)



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

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

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



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

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

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