Synthesis of D-alanylated wall teichoic acids from staphylococcus aureus

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Chapter

5

Chemical synthesis of the wall teichoic acid linkage unit of *Staphylococcus aureus* and *Bacillus subtilis*

Introduction

Wall teichoic acids (WTAs) are a crucial cell wall component in Gram-positive bacteria. Initially, they were thought to have ancillary roles in bacterial physiology, but it is now widely recognised that WTAs play pivotal roles with diverse functions. For instance, in the Gram-positive bacterium *Bacillus subtilis*, they determine cell shape[1]. For *Staphylococcus aureus*, WTAs are integral to host colonisation, coordination of peptidoglycan synthesis, and confer resistance to β-lactam antibiotics.

The structure of WTAs is composed of two primary constituents: a disaccharide linkage unit and a main chain polymer consisting of ribitol phosphate repeating units connected through phosphodiester linkages. The disaccharide linkage unit, which is broadly conserved across bacterial species, is constructed from an *N*-acetylmannosamine (ManNAc), (β1→4)-linked to an *N*-acetylglucosamine-1-phosphate (GlcNAc-1P)[1][2][3]. One or two glycerol-3-phosphate (GroP) units are attached to the C-4 hydroxyl of the ManNAc, as shown in Figure 1[3]. The anomeric phosphate of the linkage unit forms a phosphodiester bond with the C-6 hydroxyl of *N*-acetylmuramic acid, thereby linking it to the peptidoglycan. Furthermore, the phosphodiester-linked polyol repeats extend from the GroP-end of the linkage unit.

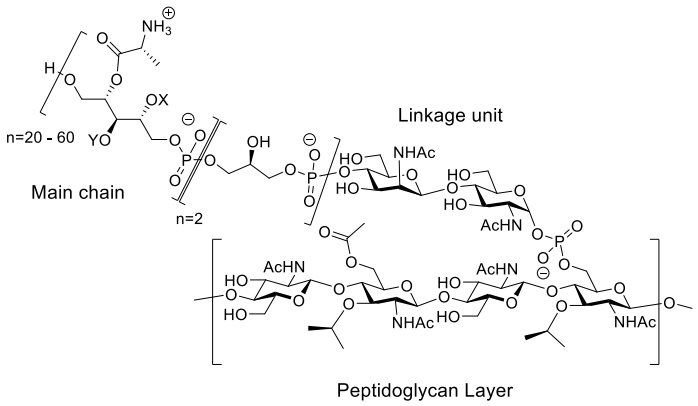


Figure 1: Overview of wall teichoic acid from S. aureus and B. subtilis W23 consisting of the peptidoglycan, linkage unit and Poly ribitol phosphate main chain. X = H, α-GlcNAc or β-GlcNAc; Y = H or or β-GlcNAc

Regardless of the main chain polymer, the biosynthesis of wall teichoic acids is a complex and intricate process that begins intracellularly on an undecaprenyl-phosphate lipid carrier anchored to the cytoplasmic face of the cellular membrane[4][5]. TarO initiates the process by transferring an *N*-acetylglucosamine-1-phosphate moiety from UDP-*N*-acetylglucosamine to the lipid carrier. This is followed by TarA, which connects an *N*-acetylmannosamine residue to the C-4 hydroxyl of GlcNAc. Finally, TarB completes the linkage unit by attaching an *sn*-glycerol-3-phosphate unit to the C-4 hydroxyl of ManNAc. In *B. subtilis*, the glycerol residues serve as a substrate for TarK, which attaches a ribitol-5-phosphate unit, followed by elongation by TarL to form a poly(ribitol-phosphate) polymer. *S. aureus*, on the other hand, first attaches an additional sn-glycerol-3-phosphate unit, catalyzed by TarF, followed by elongation with ribitol-5-phosphate units by TarL to form a poly(ribitol-phosphate) polymer.

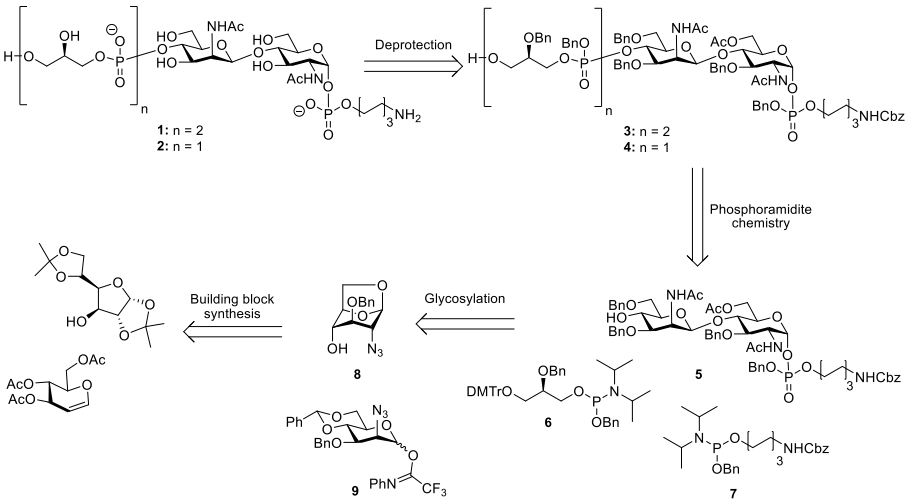
Isolating this linkage unit from bacterial sources is laborious, often resulting in a heterogeneous mixture potentially contaminated with bacterial impurities. While the biosynthesis of the linkage unit has been reconstructed *in vitro* using recombinantly expressed biosynthesis enzymes, this has not provided a scalable synthesis. Organic synthesis offers an alternative approach to producing a linkage unit. It can deliver the linkage unit in larger quantities and equip the molecule with reporter groups, such as a fluorophore for visualisation. As such, synthetic liker units may be used as

substrates to probe the interaction with biosynthesis enzymes and as substrates in developing inhibition assays. This chapter presents the first chemical synthesis of the WTA-linkage unit of *B. subtilis* and *S. aureus*.

Results and discussion

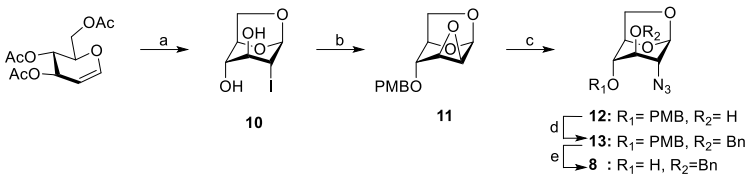
The linkage unit of interest comprises *N*-acetylmannosamine (ManNAc)-(β1→4)-*N*-acetylglucosamine-1-phosphate (GlcNAc-1P), supplemented by one to two glycerol-3-phosphate (GroP) units appended to the C4 hydroxyl position, and equipped with a ligation handle at the anomeric phosphate. The synthetic plan to generate these target compounds is shown in Scheme 1. To install the required phosphotriester bonds, phosphoramidite chemistry will be employed. Literature precedent has shown that the condensation of a GlcNAc lactol and a phosphoramidite can stereoselectively provide the desired α-linked phosphates after oxidation of the intermediate phosphite triester[4][5]. Using phosphoramidite reagent **7,** the spacer will be introduced featuring an amine for the projected conjugation purposes. Functionalisation of the ManNAc-C4 hydroxyl will be achieved using *sn*-glycerol-3 phosphoramidite **6**.

The synthesis of the β-mannosamine-linkage presents a notable challenge. Still, previous studies have shown that 4,6-*O*-benzylidene-protected mannosamine donors can be used for the stereoselective construction of the desired β-linkage [6][7]. This donor can be obtained in six steps from diacetone glucose, a precursor chosen over D-mannosamine·HCl as a starting compound because of cost and time considerations. The C-4 hydroxyl of *N*-acetylglucosamine acceptors is notoriously unreactive in glycosylation reactions, and to overcome this issue, it is proposed to use an 1,6-anhydro-2-azido-D-glucopyranose acceptor, which features a more reactive C-4 hydroxyl group due to steric effects[8][9]. This acceptor can be obtained from D-glucal in four steps[10].



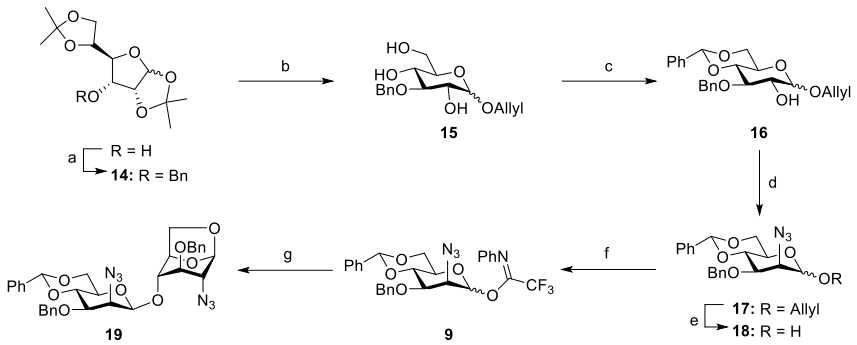
*Scheme 1: Retrosynthetic analysis of target compounds **1** and **2**.*

The synthesis of the acceptor **8** is depicted in Scheme 2. First, the acetyl groups were cleaved using a mixture of methanol, triethylamine, and water, with subsequent removal of the solvents. The resulting glucal was then reacted with bis(tributyltin) oxide under reflux conditions in acetonitrile[10]. Then, iodine was introduced at room temperature to transform the O-stannylated glucal into 1,6-anhydro-2-deoxy-2-iodo-glucopyranose **10**. Initial efforts to substitute the iodine at the C-2 position with an azide utilising sodium azide at elevated temperatures yielded unsatisfactory results. This reaction led to retention of stereochemistry at C2, through the formation of an epoxide, that is subsequently opened by the azide nucleophile. To increase the yield of the epoxide formation and subsequent nucleophilic opening, anhydroglucopyranose **10** was treated with sodium hydride (NaH) in the presence of 4-methoxybenzyl (PMB) chloride. These conditions facilitated the epoxide formation between C-2 and C-3, thereby enabling regioselective alkylation of the C-4 hydroxyl. The epoxide was then regioselective opened at the C-2 using sodium azide at elevated temperature to obtain compound **12.** The liberated hydroxyl group was alkylated using benzyl bromide and NaH. Finally, the PMB ether was removed in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to obtain acceptor **8**.



*Scheme 5: The synthesis of acceptor **8:** a) i. MeOH:H_2O:TEA (8/1/1), ii. bis(tributyltin)oxide, ACN, reflux, iii. Iodine, 97% over 3 steps; b) PMB-Cl, NaH, DMF; c) NaN_3, DMF/H_2O (9/1), 72% over 2 steps; d) BnBr, NaH, DMF, 86%; e) DDQ, DCM/H_2O (9/1), 89%.*

The synthesis of donor **9** started with the alkylation of the glucose C3 hydroxyl using BnBr and NaH (Scheme 3). The crude product underwent Fischer glycosylation, employing allyl alcohol as the solvent in combination with hydrochloric acid. This procedure resulted in the removal of the isopropylidene groups and facilitated the glycosylation reaction. Next, the benzylidene was installed using benzaldehyde dimethyl acetal and camphor sulfonic acid (CSA) under reduced pressure and elevated temperatures. The C-2 hydroxyl was converted into a triflate using trifluoromethanesulfonic anhydride, followed by inversion with sodium azide to obtain compound **17** having the desired mannose configuration[11]. Subsequently, the allyl group was removed by isomerisation employing an iridium catalyst followed by iodine-mediated enol ether hydrolysis to obtain compound **18**. Lastly, the hydroxyl was converted into the corresponding *N*-phenyl trifluoroacetimidate using 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride in the presence of potassium carbonate (K_2CO_3), yielding donor **9**. The glycosylation performed with acceptor **8** and donor **9**, catalysed by trimethylsilyl trifluoromethanesulfonate, led to the formation of the β-mannose linkage in a yield of 60%. The α-product could be readily separated via column chromatography.

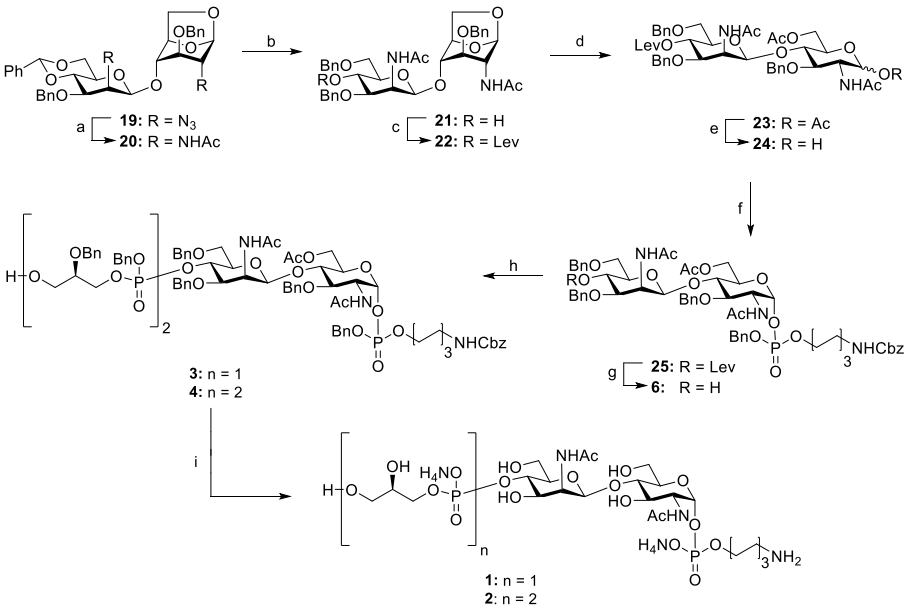


*Scheme 3: The synthesis of disaccharide **19:** a) BnBr, NaH, DMF; b) AllylOH, HCl (37%), reflux; c) benzaldehyde dimethyl acetal, CSA, 56% over 3 steps; d) i. Tf_2O, DCM/Pyridine (4/1) ii. NaN_3, DMF/H_2O (9/1), 69% over 2 steps; e) i. $Ir(COD)(Ph_2MeP)_2PF_6$, THF, ii. I_2, $NaHCO_3$ (aq) 79% over 2 steps; f) N-phenyl trifuoroacetimidoyl chloride, K_2CO_3, DCM, 74%; f) **8,** TMSOTf, DCM, -78°C, 60%.*

Initially, the azides in compound **19** underwent reduction to the corresponding amines, utilising Lindlar's catalyst. Following filtration and solvent removal, the resulting product was dissolved in a mixture of acetic anhydride and trifluoroacetic acid to effectuate acetylation of the amine and ringopening of the 1,6-anhydro-2-azido-D-glucopyranose, all in a one-pot fashion[12]. Unfortunately, this protocol led to the unintentional elimination of the benzylidene moiety. Therefore, an alternative reaction pathway was selected, as depicted in Scheme 4. First, both azides were reduced and acetylated, utilising activated zinc in a mixture of tetrahydrofuran, acetic acid, and acetic anhydride to obtain compound **20**. This was followed by the regioselective opening of the benzylidene acetal employing sodium cyanoborohydride in combination with hydrogen chloride in diethyl ether. A levulinic ester was installed at the liberated C4 hydroxyl using levulinic anhydride, generated *in situ* with levulinic acid and *N,N'*-diisopropylcarbodiimide. Subsequently, acidolysis of the 1,6-anhydro-bridge in a mixture of trifluoroacetic acid and acetic anhydride yielded compound **23**. The anomeric acetyl group was selectively unmasked in the presence of piperidine. Next, the anomeric hydroxyl group was treated with phosphoramidite **7** and 4,5-dicyanoimidazole (DCI). This was succeeded by oxidation of the phosphite intermediate utilising (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO), resulting in the stereoselective formation of the α-configured phosphotriester **25**. The levulinic ester was removed using hydrazine monohydrate in a blend of pyridine and acetic acid.

However, this procedure led to unidentified by-products, resulting in a low overall yield. By modifying the reaction conditions employing hydrazine acetate in a solvent mixture of ethanol and toluene, the yield of **6** was increased to 53%, an improvement deemed satisfactory for further progression. Subsequently, the liberated hydroxyl group underwent additional modification with one or two glycerophosphate (GroP) moieties. This was achieved by utilising phosphoramidite building block **6** in a coupling cycle employing DCI as an activator, CSO for oxidation, and trichloroacetic acid (TCA) to cleave the 4,4-dimethoxytrityl ether, releasing the hydroxyl group for the subsequent coupling cycle.

The deprotection process started with removing the acetyl group employing sodium methoxide in methanol. After the deacetylation, the benzyl ethers were removed using palladium-catalysed hydrogenolysis conditions. Unfortunately, this resulted in the unintended cleavage of the anomeric phosphate. Because of the susceptibility of the anomeric phosphate to the slightly acidic reaction conditions, the use of a phosphate buffer was next explored. Unfortunately, this modification resulted in unpractically long reaction times. Consequently, an alternative deprotection strategy was pursued, employing sodium in liquid ammonia with *tert*-butanol as a proton source. This led to the smooth removal of the benzyl ethers, and the desired products were purified with gel filtration using an NH_4OAc buffer (0.15M) to obtain compound **1** in 34% and compound **2** in 33% yield.



*Scheme 6: a) Zn, AcOH/Ac_2O/THF (1/2/3, v/v/v), 72%; b) NaCNBH_3, HCl (2M in Et_2O), 95%; c) LevOH, DCC, DMAP, 95%; d) Ac_2O/AcOH/TFA (12/70/18, v/v/v) 76%; e) 6% piperidine in THF, 74%; f) i. DCI, ACN, **7**; ii. CSO; iii. 3% TCA in DCM, 64%; g) hydrazine acetate, toluene/EtOH (1/2), 53%; h) i. DCI, ACN, **6**; ii. CSO; iii. 3% TCA in DCM, **3:** 61%, **4**: 92% i) i. NaOMe, MeOH; ii. Na, NH_3, tert-butanol, **1**: 33%, **2**: 34%.*

Conclusion

In conclusion, this Chapter has outlined a synthesis to form the WTA-linkage unit of *B. subtilis* and *S. aureus*. Hereto, a synthetic strategy has been employed, whereby donor **9** is synthesised through an eight-step process, necessitating merely four purifications and achieving an overall yield of 23%. Disaccharide **19** was obtained in gram-scale utilising the 4,6-*O*-benzylidene-protected mannosamine donor **9** and acceptor **8** in combination with TMSOTf. Benzyl-protected phosphoramidites **6** and **7** facilitated the introduction of the phosphotriester groups. Notably, the incorporation of the phosphotriester at the anomeric position was achieved in a stereoselective manner. Global deprotection was accomplished using liquid ammonia and sodium, yielding compounds **1** and **2** in milligram quantities without affecting the acid-labile anomeric phosphate.

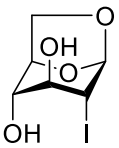
The synthesised linkage unit enables the exploration of binding interactions with antibodies, immune cell receptors, and biosynthesis enzymes. The linkage units can be employed as a substrate to enhance understanding of the activities of TarL and TarK transferases. Structural and biochemical analyses facilitated by this approach can further elucidate the catalytic mechanisms of these transferases, potentially supporting the design and development of novel antimicrobial agents.

Experimental

General information

All chemicals (Acros, Fluka, Merck, Sigma-Aldrich, etc.) were used as received, and reactions were carried out dry, under a nitrogen atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualised by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 25 g/l and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ 10 g/l, in 10% aqueous H_2SO_4 or with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in water followed by charring at +/- 140 °C. ^1H, ^{13}C and ^{31}P NMR spectra were recorded with a Bruker WB 400 (400, 101 and 162 MHz, respectively), a Bruker AV 500 (500, 125 and 202 MHz, respectively) or a Bruker DMX 850 (850, 214 and 344 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane for ^1H and ^{13}C. When D_2O or CD_3CN were used, ^1H-NMR was recorded with chemical shift (δ) relative to the proton of residual solvent (4.75 ppm and 1.94 ppm, respectively). ^{13}C-NMR spectra were recorded with chemical shift (δ) relative to TMS (external standard) in the case of D_2O and 1.32 ppm as a residual solvent in CD_3CN. The ^{31}P- NMR spectra were recorded with chemical shift (δ) relative to H3PO4. (external standard). High-resolution mass spectra were recorded by direct injection (2 µl of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1 % formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a lock mass. High resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

1,6-Anhydro-2-deoxy-2-iodo-β-D-glucopyranose (**10**)

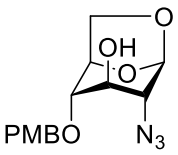


Tri-acetyl-D-glucal (13.6 g, 50 mmol) was dissolved in a mixture of methanol:H_2O:TEA (8/2/2, 250 ml, 0.2M). The mixture was stirred at room temperature ON, and the volatiles were removed *in vacuo.* The crude product was co-evaporated thrice with ACN and dissolved in ACN (250 ml, 0.2M). Subsequently,

molecular sieves were added. Bis(tributyltin)oxide (25 ml, 50 mmol, 1 eq) was added, and the reaction was refluxed for 3 hours. The reaction was cooled to 0°C, followed by adding iodine (19.0 g, 75 mmol, 1.5 eq) and stirring ON at RT. The reaction was quenched with $Na_2S_2O_3$ (aq) and filtered over Celite®. The mixture was washed twice with pentane, followed by a five-time extraction of the product with ethyl acetate. The product was purified by column chromatography (100/0 to 7/3 DCM/acetone) to obtain an oil (13.2 g, 48.6 mmol, 97%).

The spectroscopic data were in agreement with the reported data[13].

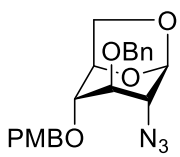
1,6-Anhydro-2-azido-2-deoxy-4-O-p-methoxybenzy-β-D-glucopyranose (**12**)



Compound **10** (13.2 g, 48.8 mmol) was co-evaporated twice with ACN and dissolved in DMF (250 ml, 0.2M). The mixture was cooled to 0°C, and PMBCl (16.6 ml, 122 mmol, 2.5 eq) was added, followed by the portions-wise addition of NaH (60% dispersion in mineral oil, 4.96 g, 124 mmol, 2.5 eq). The reaction was stirred ON at RT and quenched with H_2O. The product was extracted eight times with Et_2O, and the combined organic layers were dried with $MgSO_4$. The product was concentrated *in vacuo* and re-dissolved in DMF/H_2O (9:1, 250 ml, 0.2M). NaN_3 (16.4 g, 252 mmol, 5.2 eq) was added to the mixture, and the reaction was stirred ON at 120°C. $NaHCO_3$ (aq) and EtOAc were added to the mixture, and the product was extracted eight times with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The product was purified by column chromatography (9/1 to 6/4 pentane/EtOAc) to obtain a solid (10.8g, 35 mmol, 72%).

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

1,6-Anhydro-2-azido-3-O-benzyl-2-deoxy-4-O-p-methoxybenzy-β-D-glucopyranose (**13**)

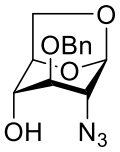


Compound **12** (10.8 g, 35 mmol) was co-evaporated twice with toluene and dissolved in DMF (200 ml, 0.2M). Benzyl bromide (5.4 ml, 46 mmol, 1.3 eq) was added to the mixture and was cooled to 0°C, followed by the portion-wise addition of NaH (60% dispersion in mineral oil, 2.1 g,

53 mmol, 1.5 eq). The reaction was stirred overnight at RT and quenched with H_2O. The product was extracted thrice with Et_2O, and the combined organic layers were dried $MgSO_4$. The product was concentrated *in vacuo* and purified by column chromatography (9/1 to 7/3 pentane/ EtOAc) to obtain a solid (12 g, 30.2 mmol, 86%).

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

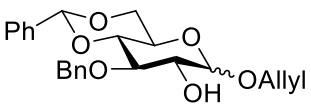
1,6-Anhydro-2-azido-3-O-benzyl-2-deoxy-β-D-glucopyranose (**8**)



Compound (12g, 30.2 mmol) **13** was dissolved in DCM/H_2O (9/1, 300 ml, 0.1M). The mixture was cooled to 0°C, and DDQ (14.1 g, 62 mmol, 2 eq) was added. The reaction was stirred for 3 hours at RT and quenched with $NaHCO_3$ (aq). The product was extracted with EtOAc, and the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The product was purified by column chromatography (8/2 to 7/3 pentane/ EtOAc) to obtain a solid (7.49 g, 27.0 mmol, 89%).

The spectroscopic data were in agreement with the reported data[15].

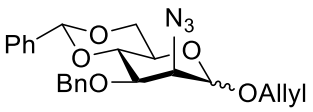
Allyl 3-O-benzyl-4,6-O-benzylidene-D-glucopyranoside (**16**)



Diacetone glucose (13 g, 50.8 mmol) was co-evaporated thrice with toluene and dissolved in DMF (250 ml, 0.2M). Benzyl bromide (8 ml, 67 mmol, 1.3 eq) was added, and the mixture was cooled to 0°C, followed by the portion-wise addition of NaH (60% dispersion in mineral oil, 3.0 g, 75 mmol, 1.5 eq). The reaction was stirred ON at RT and quenched with H_2O. The product was extracted thrice with Et_2O, and the combined organic layers were dried $MgSO_4$. The product was concentrated *in vacuo* and re-dissolved in allyl alcohol (250 ml, 0.2M). Concentrated HCl (5.5 ml) was added, and the reaction was heated to 90 °C for 30 minutes. The reaction was cooled to RT and concentrated *in vacuo*. The crude product was co-evaporated thrice with ACN and dissolved in ACN (170 ml, 0.3M). Benzaldehyde dimethyl acetal (10 ml, 66 mmol, 1.3 eq) and camphorsulfonic acid (1.24 g, 5.3 mmol, 0.1 eq) were added, and the reaction was stirred at 50 °C at a pressure of 250 mbar for 5 hours. The reaction was quenched with TEA and concentrated *in vacuo*. The product was purified by column

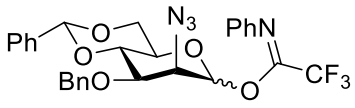
chromatography (9/1 to 6/4 pentane/EtOAc) to obtain the product as a mixture of anomers (10.4 g, 28.6, 56%, 9/1 α/β). α anomer: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.70 – 7.24 (m, 10H, CH_{arom0}), 6.04 – 5.85 (m, 1H, CH_{allyl}), 5.61 (s, 1H, $CH_{benzylidene}$), 5.48 – 5.21 (m, 2H, $CH_{2\ allyk}$), 5.00 – 4.98 (m, 2H, H-1', $CH_{2\ benzyl}$), 4.84 (d, *J* = 11.5 Hz, 1H, $CH_{2\ benzyl}$), 4.45 – 4.18 (m, 2H, $CH_{2\ allyl}$, H-6), 4.15 – 4.06 (m, 1H, $CH_{2\ allyl}$), 3.98 – 3.50 (m, 4H, H-4, H-5, H-2, H-6, H-3), 2.36 (d, *J* = 8.0 Hz, 1H, OH). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.6, 137.4(C_{arom}), 133.4(CH_{allyl}), 129.0, 128.5, 128.5, 128.3, 128.1, 127.8, 126.1(CH_{arom}), 118.4($CH_{2\ allyl}$), 101.3($C_{benzylidene}$), 98.0(C-1), 82.0(C-3), 79.1(C-4), 74.9($CH_{2\ benzyl}$), 72.5(C-2), 69.0(C-6), 68.8($CH_{2\ allyl}$), 62.9 (C-5).

Allyl 2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-D-mannopyranoside (**17**)



Compound **16** (5.2 g, 13 mmol) was co-evaporated twice with toluene and dissolved in DCM/Pyridine (4/1, 90 ml, 0.2M). The mixture was cooled to -40°C, and Tf_2O (2.5 ml, 28 ml, 2.2 eq) was added. The reaction was allowed to warm up to -10°C before quenching it with $NaHCO_3$ (aq). The product was extracted thrice with DCM, and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was dissolved in DMF:H_2O (9/1, 100, 0.1M), and NaN_3 (4.3 g, 66 mmol, 5.1 eq) was added. The reaction was stirred overnight at 80°C and quenched with $NaHCO_3$ (aq). The product was extracted thrice with EtOAc, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The product was purified by column chromatography (9/1 to 7/3) to obtain a white solid (3.83, 9 mmol, 69%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.69 – 7.24 (m, 10H, CH_{arom}), 5.92 (dddd, *J* = 16.9, 10.3, 6.3, 5.2 Hz, 1H, CH_{allyl}), 5.68 (s, 1H, $CH_{benzylidene}$), 5.41 – 5.24 (m, 2H, $CH_{2\ allyl}$), 4.95 (d, *J* = 12.1 Hz, 1H, $CH_{2\ benzyl}$), 4.86 (d, *J* = 1.5 Hz, 1H, H-1), 4.79 (d, *J* = 12.1 Hz, 1H, $CH_{2\ benzyl}$), 4.30 (d, *J* = 5.3 Hz, 1H, H-6), 4.25 – 4.17 (m, 3H, $CH_{2\ allyl}$, H-3, H-4), 4.06 (dd, *J* = 3.3, 1.6 Hz, 1H, H2), 4.01 (ddt, *J* = 12.8, 6.3, 1.4 Hz, 1H, $CH_{2\ allyl}$), 3.94 – 3.82 (m, 2H, H-6, H-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.2, 137.5 (C_{arom}), 133.2(CH_{allyl}), 129.1, 128.5, 128.4, 127.9, 127.6, 126.2(CH_{arom}), 118.3($CH_{2\ allyl}$), 101.7 ($C_{benzylidene}$), 98.4(C-1), 79.2(C-3), 75.8(C-4), 73.4($CH_{2\ benzyl}$), 68.8(C-6), 68.4($CH_{2\ allyl}$), 64.0(C-5), 62.9(C-2).2-Azido-3-O-benzyl-4,6-

O-benzylidene-2-deoxy-D-mannopyranosyl N-phenyltrifluoroacetimidate (**9**)



Compound **17** (5.29, 12.5 mmol) was co-evaporated once with toluene and dissolved in THF (100 ml 0.1M). The solution was purged with N_2 for 30 minutes, followed by the addition of $Ir(COD)(Ph_2MeP)_2PF_6$ (186 mg, 0.22 mmol, 0.02 eq). The mixture was purged with N_2 for 30 minutes and then 5 seconds with H_2. The mixture was purged with N_2 to remove the excess of H_2. The reaction was stirred for 90 minutes, followed by adding $NaHCO_3$ (aq) and I_2 (4.83g, 19 mmol, 1.5eq). The reaction was stirred overnight and quenched with $Na_2S_2O_4$ (aq). The product was extracted thrice with EtOAc and washed once with $NaHCO_3$ (aq) and brine. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The product was purified by column chromatography (9/1 > 6/4 pentane/EtOAc) to obtain a solid (3.81g, 9.93 mmol, 79%). The compound (1.62 g, 4.22 mmol) was co-evaporated thrice with toluene and dissolved in acetone (50 ml, 0.1M). K_2CO_3 (1.23 g, 8.9 mmol, 2.1 eq) and *N*-phenyl trifluoroacetimidoyl chloride (1.1 ml, 6.8 mmol, 1.5 eq) were added and the reaction was stirred overnight. The reaction was filtered over Celite® and concentrated *in vacuo*. The product was purified by column chromatography (9/1 to 7/3 pentane/ EtOAc) to obtain a solid (1.73 g, 3.12 mmol, 74%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.66 – 7.39 (m, 12H, CH_{arom}), 7.35 – 7.20 (m, 1H, CH_{arom}), 7.06 – 6.90 (m, 2H, CH_{arom}), 6.07 – 5.85 (m, 1H, H-1), 5.68 (s, 1H, $CH_{benzylidene}$), 5.00 (d, *J* = 12.2 Hz, 1H, $CH_{2\ benzyl}$), 4.85 (d, *J* = 12.2 Hz, 0H, $CH_{2\ benzyl}$), 4.42 (m, 1H, H-6), 4.29 – 4.16 (m, 2H, H-2, H-3), 4.03 – 3.85 (m, 2H, H4, H-6), 3.57 – 3.39 (m, 1H, H-5). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 143.0, 137.7, 137.2 (C_{arom}), 129.4, 129.3, 129.3, 129.2, 129.0, 128.9, 128.9, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 126.4, 126.2, 124.9, 120.8, 120.7, 119.4(CH_{arom}), 101.8($C_{benzylidene}$), 94.5(C-1), 78.1(C-3), 76.5(C-4), 73.3($CH_{2\ benzyl}$), 68.2(C-6), 68.1(C-5), 62.3(C-2).

Disaccharide (**19**)

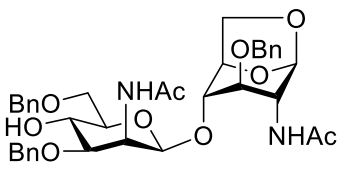
Acceptor **8** (893 mg, 3.22 mmol) was co-evaporated thrice with toluene and dissolved in DCM (10 ml, 0.3M). Activated molecular sieves were added, and the mixture was stirred for 30 min. The mixture was cooled to -78°C, followed by the addition of TMSOTf (0.1 ml, 0.48 mmol, 0.15 eq). Donor **9** (2.54 g, 6.7 mmol, 2 eq) was co-evaporated thrice with toluene and dissolved in DCM (20 ml, 0.13M). The mixture was added dropwise to the mixture of the acceptor with a cannula over 15 minutes. The reaction was allowed to warm up and stirred for 2 hours. The reaction was quenched with TEA, followed by the addition of DCM and $NaHCO_3$ (aq). The product was extracted thrice with DCM and washed with brine. The combined organic layers were dried with $MgSO_4$, and the product was concentrated *in vacuo.* The product was purified by column chromatography (100/0 to 90 toluene/ EtOAc) to obtain an oil (1.25 g, 1.94 mmol, 60%). The alpha product was not collected. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.49 (m, 2H, CH_{arom}), 7.45 – 7.30 (m, 13H, CH_{arom}), 5.60 (s, 1H, $CH_{aromaro}$), 5.54 (s, 1H, H-1), 4.91 – 4.80 (m, 2H, H-1', $CH_{2\ benzyl}$), 4.76 (m, 1H, $CH_{2\ benzyl}$), 4.71 – 4.64 (m, 1H, H-5), 4.62 (s, 2H, $CH_{2\ benzyl}$), 4.26 (dd, *J* = 10.5, 4.8 Hz, 1H, H-6'), 4.18 – 4.11 (m, 2H, H-6, H-2'), 4.06 (t, *J* = 9.5 Hz, 1H, H-4'), 3.94 (s, 1H, H-4), 3.87 (t, *J* = 10.3 Hz, 1H, H-6'), 3.85 – 3.77 (m, 2H, H-6, H-3), 3.74 (dd, *J* = 9.6, 3.7 Hz, 1H, H-3'), 3.36 – 3.26 (m, 1H, H-5'), 3.20 (s, 1H, H-2). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.7, 137.4, 137.2(C_{arom}), 129.2, 128.8, 128.7, 128.4, 128.1, 128.1, 127.8, 127.7, 126.1(CH_{arom}), 101.8($C_{benzylidene}$), 101.1 (C-1), 97.0(C-1'), 78.5(C-4'), 77.9(C-3), 76.1(C-3'), 73.1(C-4), 72. 9($CH_{2\ benzyl}$), 72.7($CH_{2\ benzyl}$), 71.9(C-5), 68.4(C-6'), 67.8(C-5'), 64.9(C-6), 63.0(C-2'), 58.9(C-2).**HRMS:** $[C_{33}H_{34}N_6O_8 + Na]^+$ calculated 665.23303, found 665.23383.

Disaccharide (**20**)

Zinc was activated with HCl (3M), rinsed with H_2O twice, dioxane and Et_2O. The activated was dried *in vacuo* at 40°C and placed under a nitrogen atmosphere. Compound **19** (642 mg, 1.23 mmol) was co-evaporated twice with toluene and dissolved in $AcOH/Ac_2O$/THF (20 ml

1/2/3 v/v/v, 0.1M). Activated zinc (3.1 g, 40 mmol, 33 eq) was added and the mixture was stirred for 4 hours. The reaction mixture was filtrated, followed by the addition of EtOAc and $NaHCO_3$ (aq). The product was extracted thrice with EtOAc and washed with brine. The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The product was purified by column chromatography (100/0 to 95/5 DCM/MeOH) to obtain a foam (936 mg, 1.39 mmol, 72%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 – 7.25 (m, 15H, CH_{arom}), 6.43 (d, *J* = 9.6 Hz, 1H, NH), 5.93 (s, 1H, NH), 5.56 (s, 1H, $CH_{benzylidene}$), 5.43 (s, 1H, H-1), 4.93 – 4.84 (m, 1H, H-2'), 4.84 – 4.74 (m, 3H, H-1', $CH_{2\ benzyl}$), 4.66 (d, *J* = 12.0 Hz, 1H, $CH_{2\ benzyl}$), 4.63 – 4.57 (m, 1H, H-5), 4.52 (d, *J* = 11.8 Hz, 1H $CH_{2\ benzyl}$), 4.37 – 4.24 (m, 3H, H-2, H-6', H-6), 3.89 – 3.77 (m, 4H, H-6, H-4', H-3', H-4), 3.70 (t, *J* = 10.4 Hz, 1H, H-6'), 3.47 – 3.35 (m, 2H, H-3, H-5'), 2.09 (s, 3H, $CH_{3\ acetyl}$), 2.05 (s, 3H, $CH_{3\ acetyl}$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.1, 170.2($C_{carbonyl}$), 137.8, 137.3, 137.0(C_{arom}), 129.3, 128.6, 128.5, 128.4, 128.1, 127.9, 127.9, 127.8, 127.7, 126.1(CH_{arom}), 101.8($C_{benzylidene}$), 101.2(C-1), 96.7(C-1'), 78.9(C-4'), 77.6(C-3), 74.8(C-3'), 73.3(C-4), 72.1($CH_{2\ benzyl}$), 71.8($CH_{2\ benzyl}$), 71.7(C-5), 68.7(C-6'), 66.9(C-5'), 64.6(C-6), 49.9(C-2'), 47.3(C-2), 23.5($CH_{3\ acetyl}$), 23.0($CH_{3\ acetyl}$). **HRMS:** $[C_{37}H_{42}N_2O_{10} + H]^+$ calculated 675.29122, found 675.29214.

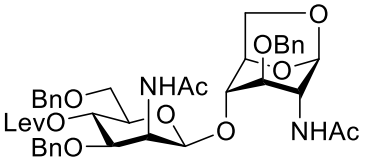
Disaccharide (**21**)



Compound **20** (1.50 g, 2,22 mmol) was co-evaporated twice with toluene and dissolved in THF (45 ml, 0.05M). $NaCNBH_3$ (12.8 g, 44 mmol, 20 eq) and molecular sieves were added to the mixture and stirred for 30 minutes. HCl (2M in Et_2O, 22.2 ml, 44 mmol, 20 eq) was added dropwise, and the reaction was stirred for 1 hour. The reaction was quenched with $NaHCO_3$ (aq), and the product was extracted thrice using EtOAc. The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The product was purified by column chromatography (7/3 to 5/5 DCM/acetone) to obtain a foam (1.43 g, 2.11 mmol, 95%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.43 – 7.21 (m, 15H, CH_{arom}), 6.39 (d, *J* = 9.4 Hz, 1H, NH), 5.91 (d, *J* = 9.6 Hz, 1H, NH), 5.41 (s, 1H, H-1), 4.88 (ddd, *J* = 9.6, 4.5, 1.6 Hz, 1H, H-2'), 4.77 (dd, *J* = 11.4, 6.3 Hz, 2H, $CH_{2\ benzyl}$), 4.66 (d, *J* = 1.6 Hz, 1H, H-1'), 4.65 – 4.58 (m, 1H, H-5), 4.60 – 4.41 (m, 4H, 2xCH_{2benyl}), 4.31 – 4.24 (m, 2H, H-2, H-6a), 3.88 (d, *J* = 1.8 Hz, 1H, H-4), 3.82 – 3.65 (m, 4H, H-6b, H-6', H-3), 3.54 (dd, *J* = 9.4, 4.6

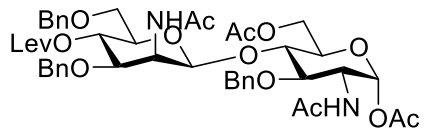
Hz, 1H, H-3'), 3.47 (t, *J* = 1.5 Hz, 1H, H-4'), 3.35 (dt, *J* = 9.6, 3.8 Hz, 1H, H-5'), 2.07 (s, 3H, $CH_{3\ acetyl}$), 2.03 (s, 3H, $CH_{3\ acetyl}$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.5, 170.4($C_{\ carbonyl}$), 137.9, 137.7, 137.2($C_{\ arom}$), 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7($CH_{\ arom}$), 101.0(C-1), 96.4(C-1'), 79.0(C-3'), 77.5(C-4'), 75.3(C-5'), 73.8($CH_{2\ benzyl}$), 72.9(C-4), 71.9($CH_{2\ benzyl}$), 71.8(C-5), 71.0($CH_{2\ benzyl}$), 69.3(C-6'), 67.2(C-3), 64.7(C-6), 49.0(C-2'), 47.7(C-2), 23.6($CH_{3\ acetyl}$), 23.1($CH_{3\ acetyl}$). **HRMS:** $[C_{37}H_{44}N_2O_{10}+ H]+$ calculated 677.30687, found 677.30752.

Disaccharide (**22**)



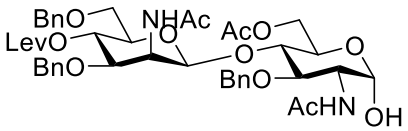
Compound **21** (1. 42 g, 2.10 mmol) was co-evaporated twice with toluene and dissolved in DCM (20 ml, 0.21 M). LevOH (0.45 ml, 4.41 mmol, 2.1 eq), DCC (867 mg, 4.2 mmol, 2 eq) and DMAP (523 mg, 4.28 mmol, 2 eq) were added and the reaction was stirred for 1 hour. The reaction was filtrated over Celite®, and $NaHCO_3$ (aq) was added. The product was extracted thrice with DCM, and the combined organic layers were dried with MgSO4 and concentrated *in vacuo*. The product was purified by column chromatography (100/0 to 93/7 DCM/ MeOH) to obtain a foam (1.54 g, 1.99 mmol, 95%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.48 – 7.16 (m, 15H, $CH_{\ arom}$), 6.36 (d, *J* = 9.6, 2.5 Hz, 1H, NH), 6.08 – 5.97 (m, 1H, NH), 5.40 (s, 1H, H-1), 5.14 (t, *J* = 9.2 Hz, 1H, H-4'), 4.86 (ddd, *J* = 9.6, 4.8, 1.7 Hz, 1H, H-2'), 4.78 – 4.64 (m, 3H, $CH_{2\ benzyl}$, H-1), 4.62 – 4.57 (m, 1H, H-5), 4.57 – 4.39 (m, 4H, 2x$CH_{2\ benzyl}$), 4.31 – 4.21 (m, 2H, H-2, H-6a), 3.86 (d, *J* = 1.9 Hz, 1H, H-4), 3.76 (t, *J* = 7.3, 5.9 Hz, 1H, H-6b), 3.69 – 3.59 (m, 2H, H-3', H-6a'), 3.56 – 3.42 (m, 3H, H-6b', H-5', H-3), 2.79 – 2.61 (m, 2H, $CH_{2\ lev}$), 2.54 – 2.39 (m, 2H, $CH_{2\ lev}$), 2.17 (s, 3H CH_{3ev}), 2.03 (s, 3H $CH_{3\ acetyl}$), 2.00 (s, 3H, $CH_{3\ acetyl}$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 206.5, 171.9, 171.4, 170.2($C_{\ carbonyl}$), 137.8, 137.8, 137.3($C_{\ arom}$), 128.6, 128.5, 128.5, 128.1, 128.0, 127.9, 127.8, 127.7($CH_{\ arom}$), 101.1(C-1), 96.1(C-1'), 77.5(C-3), 75.8(C-3'), 73.9(C-5'), 73.7($CH_{2\ benzyl}$), 73.0(C-4), 71.8($CH_{2\ benzyl}$), 71.8(C-5), 70.7($CH_{2\ benzyl}$), 68.9(C-6'), 67.7(C-4'), 64.6(C-6), 49.0(C-2'), 47.6 (C-2), 37.9($CH_{2\ lev}$), 29.9($CH_{3\ lev}$), 28.0($CH_{2\ lev}$), 23.5($CH_{3\ acetyl}$), 23.1($CH_{3\ acetyl}$). **HRMS:** $[C_{42}H_{50}N_2O_{12}+ H]^+$ calculated 755.34365, found 755.34399.

Disaccharide (**23**)



Compound **22** (976 mg, 1.26 mmol) was co-evaporated thrice with toluene and dissolved in AcOH/Ac_2O (15/85, 11 ml, 0.11M). The mixture was cooled to 0°C, and TFA (2.5 ml, 34 mmol, 27 eq) were added. The reaction was stirred overnight at RT and quenched with $NaHCO_3$ (aq). Brine was added to the mixture, and the product was extracted thrice using DCM. The combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo*. The product was purified by column chromatography (100/0 to 95/5 DCM/MeOH) to obtain a foam (812mg, 0.93 mmol, 76%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.44 – 7.11 (m, 15H, CH_{arom}), 6.09 (d, *J* = 3.4 Hz, 1H, H-1), 5.16 – 5.04 (m, 1H, H-4'), 5.02 – 4.81 (m, 2H, H-2, $CH_{2\ benzyl}$), 4.79 – 4.67 (m, 1H, $CH_{2\ benzyl}$), 4.69 – 4.56 (m, 2H, H-1', $CH_{2\ benzyl}$), 4.50 – 4.39 (m, 3H, $CH_{2\ benzyl}$), 4.37 – 4.19 (m, 3H, H-2', H-6), 4.01 – 3.84 (m, 2H, H-4, H-5), 3.74 – 3.37 (m, 5H, H-3, H-3', H-5', H-6'), 2.79 – 2.56 (m, 2H, $CH_{2\ lev}$), 2.56 – 2.38 (m, 3H, $CH_{2\ lev}$), 2.15 (s, 3H, $CH_{3\ lev}$), 2.10 (s, 3H, $CH_{3\ acetyl}$), 2.09 (s, 3H, $CH_{3\ acetyl}$), 1.94 (s, 3H, $CH_{3\ acetyl}$), 1.79 (s, 3H, $CH_{3\ acetyl}$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 203.9, 171.8, 171.0, 170.8, 170.1, 169.0($C_{carbonyl}$), 138.5, 137.8(C_{arom}), 128.7, 128.5, 128.5, 128.5, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8(CH_{arom}), 99.7(C-1'), 90.9(C-1), 77.1, 76.7, 76.4, 74.3, 74.0, 73.7, 71.1(C-6), 70.8, 67.7(C-4'), 62.2(C-6'), 50.9(C-2'), 49.2(C-2), 36.8($CH_{2\ lev}$), 29.9($CH_{3\ lev}$), 28.0($CH_{2\ lev}$), 23.5($CH_{3\ acetyl}$), 23.2($CH_{3\ acetyl}$), 21.0($CH_{3\ acetyl}$), 21.0($CH_{3\ acetyl}$). **HRMS:** $[C_{46}H_{56}N_2O_{15}+ H]^+$ calculated 877.37535, found 877.37614.

Disaccharide (**24**)



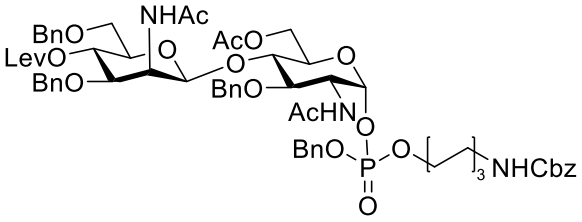
Compound **23** (723 mg, 0.83 mmol) was co-evaporated twice with ACN and dissolved in THF with 6% piperidine (0.6 ml piperidine in 10 ml THF). The reaction was stirred overnight, and EtOAc was added and washed once with HCl (1M), $NaHCO_3$ (aq) and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The product was purified by column chromatography (100/0 to 95/5 DCM/MeOH) to obtain a foam (512 mg, 0.61 mmol, 74%). **^{1}H NMR** (400 MHz, CD_3CN) δ 7.42 – 7.22 (m, 10H, CH_{arom}), 6.61 – 6.52 (m, 2H, NH), 6.39 (d, *J* = 9.4 Hz, 1H, NH), 5.06 – 4.92 (m, 3H, H-1, H-4',

$CH_{2\ benzyl}$), 4.90 – 4.84 (m, 1H, H-2'), 4.67 – 4.39 (m, 4H, $CH_{2\ benzyl}$), 4.38 – 4.30 (m, 2H, H-6, $CH_{2\ benzyl}$), 4.24 – 4.10 (m, 1H, H-6), 4.04 – 3.92 (m, 2H, H-2, H-5), 3.91 – 3.82 (m, 1H, H-3), 3.73 – 3.64 (m, 1H, H-4), 3.65 – 3.58 (m, 1H, H-3'), 3.53 – 3.50 (m, 3H, H-5', H-6'), 2.70 – 2.61 (m, 2H, $CH_{2\ lev}$), 2.49 – 2.37 (m, 2H, $CH_{2\ lev}$), 2.03 (s, 3H, $CH_{3\ lev}$), 1.97 (s, 3H, $CH_{3\ acetyl}$), 1.91 (s, 3H, $CH_{3\ acetyl}$), 1.81 (2, 3H, $CH_{3\ acetyl}$). **^{13}C NMR** (101 MHz, CD_3CN) δ 206.9($C_{\ carbonyl}$), 172.1, 172.0, 171.3, 170.7, 170.6, 170.0, 139.7, 138.7, 138.4($C_{\ arom}$), 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4($CH_{\ arom}$), 99.2(C-1'), 92.1(C-1), 78.5(C-4), 77.4, 77.3(C-3/C-3'), 74.5($CH_{2\ benzyl}$), 74.4(C-5'), 73.1($CH_{2\ benzyl}$), 70.5($CH_{2\ benzyl}$), 69.6(C-6'), 68.6(C-5), 68.3(C-4'), 62.9(C-6), 53.1(C-2'), 49.1(H-2), 49.1($CH_{2\ lev}$), 37.5($CH_{3\ lev}$), 29.1($CH_{2\ lev}$), 28.0($CH_{3\ acetyl}$), 27.9($CH_{3\ acetyl}$), 22.3($CH_{3\ acetyl}$), 20.3($CH_{3\ acetyl}$). **HRMS**: $[C_{44}H_{54}N_2O_{14}+H]^+$ calculated 835.36478, found 835.36602.

General procedure for phosphoramidite coupling, oxidation and detritylation

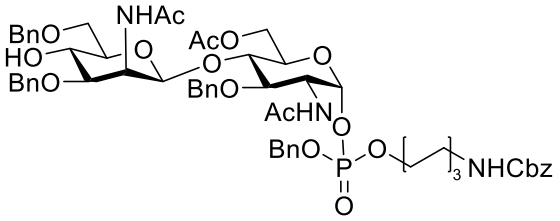
The starting alcohol was co-evaporated thrice with ACN, followed by the addition of DCI (0.25M in ACN, 1.5 eq). The mixture was added freshly activated molecular sieves (MA3Å) and stirred for 20 minutes. Phosphoramidite (+/- 0.18M in ACN, 1.3-2.1 eq) was added to the mixture and stirred until TLC showed complete conversion of the starting alcohol. CSO (0.5M in ACN, 3 eq) was added to the reaction and stirred for 15 minutes. The reaction was quenched with $NaHCO_3$ (aq), and the product was extracted thrice with DCM. The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The crude was treated with TCA (0.18M in DCM, 5 eq) and stirred overnight. The reaction was quenched with $NaHCO_3$ (aq), and the product was extracted thrice with DCM. The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified with flash chromatography (DCM/Aceton) and, if needed, with size exclusion chromatography (Sephadex LH-20, MeOH/DCM, 1:1)

(6-(Benzyloxycarbonylamino)hexyl)-6-O-acetyl-2-N-acetylamino-3-O-benzyl-4-O-(2-N-acetylamino-3,6-Di-O-benzyl-4-O-levulinoyl-2-deoxy-β-D-mannopyrosyl)-(1->4)-2-deoxy-α-D-glucopyranose (**25**)



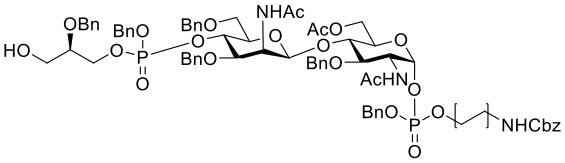
Compound **24** (300 mg, 0.36 mmol) was coupled to phosphoramidite **7** (2.6 ml, 0.18M in ACN, 0.23 mmol, 1.3 eq), oxidised and detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/acetone) obtaining the product as an oil (290 mg, 0.23 mmol, 64%). **^{1}H NMR** (400 MHz, CD_3CN) δ 7.53 – 7.19 (m, 30H, CH_{arom}), 6.73 – 6.39 (m, 2H, 2xNH), 6.05 – 5.84 (m, 1H, NH), 5.62 – 5.52 (m, 1H, H-1), 5.21 – 4.91 (m, 7H, 2,5x$CH_{2\ benzyl}$, H-4', H-2'), 4.83 – 4.74 (m, 1H, H-14.72 – 4.33 (m, 6H, 2.5 $CH_{2\ benzyl}$, H-6a), 4.31 – 3.84 (m, 8H, H-6b, $CH_{2linker}$, H-2, H-3 H-5, H-6'), 3.73 – 3.51 (m, 4H, H-3', H-5', H-3), 3.12 (q, *J* = 6.7 Hz, 2H, $CH_{2linker}$), 2.78 – 2.62 (m, 2H, $CH_{2\ lev}$), 2.54 – 2.32 (m, 2H, $CH_{2\ lev}$), 2.13 – 1.90 (m, 12H, $CH_{3\ lev,}$ 3x $CH_{3\ acetyl}$), 1.83 – 1.76 (m, 3H, $CH_{3\ acetyl}$), 1.70 – 1.60 (m, 2H, $CH_{2linker}$), 1.52 – 1.43 (m, 2H, $CH_{2linker}$), 1.39 – 1.29 (m, 4H, 2x$CH_{2linker}$). **^{13}C NMR** (101 MHz, CD_3CN) δ 206.9($C_{carbonyl}$), 172.1, 172.0, 170.5, 170.5, 170.1, 156.5, 139.5, 139.4, 138.7, 138.4, 138.4, 137.7, 136.3(C_{arom}), 128.8, 128.8, 128.7, 128.6, 128.4, 128.4, 128.4, 128.3, 128.2, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6(CH_{arom}), 99.4(C-1'), 96.1(C-1), 77.7, 77.6, 77.5, 77.4, 77.3, 77.1, 76.7, 76.7, 76.6, 76.5(CH), 74.7($CH_{2\ benzyl}$), 74.6(CH), 73.2($CH_{2\ benzyl}$), 72.7, 70.9(CH), 70.6($CH_{2\ benzyl}$), 69. 3(C-6'), 68.2($CH_{2\ benzyl}$), 65.8($CH_{2\ benzyl}$), 62.2 (C-6), 52.6(C-2'), 49.3(C-2'), 40.6($CH_{2linker}$), 37.5($CH_{2\ lev}$), 30.0($CH_{2linker}$), 29.6($CH_{2linker}$), 29.1($CH_{3\ lev}$), 28.0($CH_{2\ lev}$), 26.0($CH_{2linker}$), 25.0($CH_{2linker}$), 22.4($CH_{3\ acetyl}$), 22.2($CH_{3\ acetyl}$), 20.3($CH_{3\ acetyl}$). **^{31}P NMR** (162 MHz, CD_3CN) δ -2.4, -2.4.

(6-(benzyloxycarbonylamino)hexyl)-6-O-acetyl-2-N-acetylamino-3-O-benzyl-4-O-(2-N-acetylamino-3,6-Di-O-benzyl-2-deoxy-β-D-mannopyrosyl)-(1->4)-2-deoxy-α-D-glucopyranose (**26**)



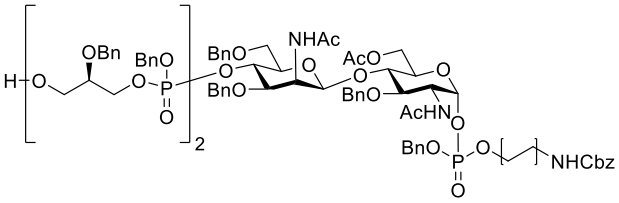
Compound **25** (430 mg, 0.35 mmol) was dissolved in toluene/ethanol 1:2 (7.5 ml, 0.05M), and hydrazine acetate (323 mg, 3.5 mmol, 10 eq) was added. The reaction was stirred for 10 minutes, followed by adding water, and the product was extracted thrice with EtOAc. The combined organic layers were washed with HCl (1M), $NaHCO_3$ (aq) and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Th product was purified by flash chromatography (100/0 to 95/5 DCM/MeOH) to obtain an oil (210 mg, 0.18 mmol, 53%). **^{1}H NMR** (400 MHz, CD_3CN) δ 7.46 – 7.07 (m, 25H, CH_{arom}), 6.47 – 6.21 (m, 2H, 2xNH), 5.79 (d, *J* = 30.8 Hz, 1H, NH), 5.51 (m, 1H, H-1), 5.20 – 4.94 (m, 5H, 2.5c $CH_{2\ benzyl}$), 4.90 – 4.77 (m, 1H, H-2'), 4.74 – 4.25 (m, 7H, 2.5x $CH_{2\ benzyl}$, H-1', H-6a), 4.25 – 4.09 (m, 1H, H-6b), 4.08 – 3.82 (m, 5H, $CH_{2linker}$, H-2, H-4, H-5), 3.77 (m, 1H, H-6a'), 3.67 – 3.57 (m, 2H, H-6b', H-3), 3.47 – 3.33 (m, 3H, H-4', H-3', H-5'), 3.06 (q, *J* = 6.6 Hz, 2H, $CH_{2linker}$), 2.02 – 1.84 (m, 6H, 2x$CH_{3acetul}$), 1.78 – 1.71 (m, 3H, $CH_{3\ acetyl}$), 1.66 – 1.56 (m, 2H, $CH_{2linker}$), 1.43 (t, *J* = 7.0 Hz, 2H, $CH_{2linker}$), 1.32 – 1.24 (m, 4H, 2x $CH_{2linker}$). **^{13}C NMR** (101 MHz, CD_3CN) δ 170.5, 170.3, 170.1($C_{carbonyl}$), 139.5, 139.0, 138.7(C_{arom}), 130.0, 129.1, 129.0, 128.8, 128.7, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 126.3, 126.1(CH_{arom}), 99.3(C-1'), 96.1(C-1), 80.1, 79.9, 77.7, 77.6, 76.8, 76.1, 76.1, 74.9(CH), 74.6, 74.5, 73.0($CH_{2\ benzyl}$), 71.0(CH), 70.9, 70.5(CH_2), 70.0(C-6'), 69.3, 69.2, 68.2, 68.1, 68.1(CH_2), 67.0, 66.7(CH), 65.7(CH_2), 62.1(C-6'), 52.6(C-2), 48.7(C-2'), 40.5($CH_{2linker}$), 30.0, 29.9, 29.6, 26.0, 24.9, 24.9($CH_{2linker}$), 22.4, 22.1, 20.2($CH_{3\ acetyl}$). **^{31}P NMR** (162 MHz, CD_3CN) δ -2.4, -2.4.

Compound (**3**)



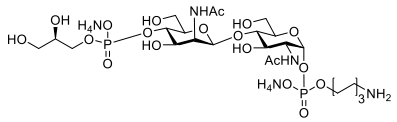
Compound **26** (349 mg, 0.31 mmol) was coupled to phosphoramidite **6** (2.0 ml, 0.23M in ACN, 0.46 mmol, 1.4 eq), oxidised and detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/acetone) obtaining the product as an oil (269 mg, 0.18 mmol, 61%). **^{1}H NMR** (400 MHz, CD_3CN) δ 7.52 – 7.14 (m, 35H, CH_{arom}), 6.62 – 6.51 (m, 1H, NH), 6.42 (dd, *J* = 17.0, 8.7 Hz, 1H, NH), 5.81 (d, *J* = 29.8 Hz, 1H, NH), 5.57 – 5.52 (m, 1H, H-1'), 5.18 – 4.71 (m, 16H, H-1', H-2',4x $CH_{2\ benzyl}$), 4.65 – 4.27 (m, 9H, 3x$CH_{2\ benzyl}$, H-6a), 4.24 – 3.32 (m, 16H, H-6b, $CH_{2linker,}$ 2xCH_{2gly}, 1x CH_{gly}, H-3', H-4', H-5', H-6', H-2, H-3, H-4, H-5), 3.07 (q, *J* = 6.7 Hz, 3H, $CH_{2linker}$), 2.78 (s, 1H, OH), 2.06 – 1.80 (m, 6H, 2x$CH_{3\ acetyl}$), 1.81 – 1.70 (m, 3H, $CH_{3\ acetyl}$), 1.66 – 1.55 (m, 2H, $CH_{2linker}$), 1.43 (t, *J* = 7.0 Hz, 2H, $CH_{2linker}$), 1.38 – 1.20 (m, 4H, 2x$CH_{2linker}$). **^{13}C NMR** (101 MHz, CD_3CN) δ 171.4, 171.3, 170.9, 157.3, 140.3, 139.6, 138.9, 138.9, 137.1, 129.9, 129.6, 129.5, 129.5, 129.4, 129.4, 129.3, 129.2, 129.1, 129.1, 129.0, 129.0, 128.9, 128.8, 128.8, 128.8, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 127.1, 127.0, 100.0, 96.9, 79.0, 78.9, 78.4, 77.2, 75.4, 73.9, 73.8, 72.3, 72.2, 71.7, 71.6, 71.2, 70.1, 70.0, 69.9, 69.8, 69.0, 69.0, 68.9, 67.3, 67.2, 66.5, 63.0, 61.1, 61.0, 53.4, 49.6, 41.3, 30.8, 30.7, 30.4, 26.8, 25.7, 25.7, 23.2, 22.9, 21.1. **^{31}P NMR** (162 MHz, CD_3CN) δ -1.6, -1.6, -2.4, -2.4.

Compound (**4**)



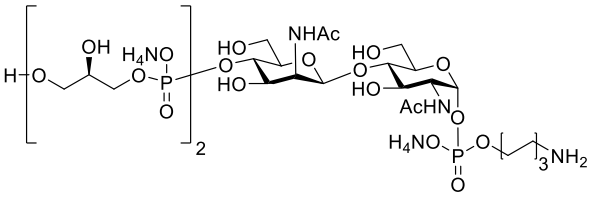
Compound **3** (234 mg, 0.16 mmol) was coupled to phosphoramidite **6** (1.5 ml, 0.23M in ACN, 0.35 mmol, 2.1 eq), oxidised and detritylated using the general procedure as described above. The crude was purified by flash chromatography (DCM/acetone) obtaining the product as an oil (262 mg, 0.15 mmol, 92%). **^{1}H NMR** (400 MHz, CD_3CN) δ 7.77 – 7.08 (m, 45H, CH_{arom}), 7.07 – 6.64 (m, 1H, NH), 6.53 – 6.29 (m, 1H, NH), 5.97 – 5.80 (m, 1H, NH), 5.64 – 5.46 (m, 1H, H-1'), 5.23 – 4.69 (m, 12H, $xCH_{2benyzl}$, H-1', H-2'), 4.67 – 4.32 (m, 11H, $4xCH_{2\ benzyl}$, H-6a), 4.22 – 3.44 (m, 21H, H-6b, $CH_{2linker,}$ $4xCH_{2gly}$, 2x CH_{gly}, H-3', H-4', H-5', H-6', H-2, H-3, H-4, H-5), 3.10 (q, *J* = 7.0 Hz, 2H, $CH_{2linker}$), 2.08 – 1.88 (m, 6H, $2xCH_{3\ acetyl}$), 1.84 – 1.77 (m, 3H, $CH_{3\ acetyl}$), 1.69 – 1.60 (m, 2H, $CH_{2linker}$), 1.53 – 1.42 (m, 2H, $CH_{2linker}$), 1.38 – 1.28 (m, 4H, $2xCH_{2linker}$). **^{13}C NMR** (101 MHz, CD_3CN) δ 170.5, 170.0, 139.5, 138.8, 138.2, 136.3, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.6, 127.4, 127.4, 99.2, 96.1, 78.3, 78.2, 77.7, 76.4, 75.8, 74.6, 73.7, 73.1, 71.7, 71.6, 70.9, 70.8, 70.5, 69.5, 69.3, 69.2, 68.2, 68.1, 68.1, 66.8, 66.7, 65.7, 65.5, 65.0, 62.1, 60.3, 60.3, 60.2, 52.6, 48.8, 40.5, 29.9, 29.6, 26.0, 24.9, 24.9, 22.4, 22.1, 20.2. **^{31}P NMR** (162 MHz, CD_3CN) δ -0.9, -1.0, -1.1, -2.1, -2.1, -2.2, -2.4, -2.4, -2.4.

Compound (**1**)



Compound **3** (10 mg, 6.9 µmol) was dissolved in MeOH (1 ml, 6.9 mM) and NaOMe in MeOH (10 µl, 4.8M, 48 µmol, 7 eq) was added. The reaction was stirred for 30 minutes and quenched with NH_4Cl (aq). The product was extracted thrice with DCM, and the combined organic layers were concentrated *in vacuo.* In an empty flask, liquid ammonia was condensed at a temperature ranging from -85°C to -65°C while maintaining a gentle flow of ammonia. Upon reaching approximately 2 ml of liquid ammonia, the flask was sealed off and placed under a nitrogen atmosphere. Solid sodium (25 mg, 1.1 mmol, 192 eq) was added to the liquid ammonia at -80°C. The de-acetylated compound was co-evaporated twice with toluene and dissolved in THF (1mL, 5.5 mM), followed by the addition of *tert*-butanol (0.1 ml, 1.1 mmol, 192 eq). The mixture was added to the solution of liquid ammonia and sodium and left to stir for 1 hour. The reaction was quenched with NH_4Cl (aq) and warmed to RT. The product was concentrated *in vacuo* and purified by HW40 gel filtration (NH_4OAc) to obtain a white solid (1.8 mg, 2.3 µmol, 34%). **^{1}H NMR** (500 MHz, D_2O) δ 5.42 (d, *J* = 7.3 Hz, 1H, H-1), 4.94 (s, 1H, H-1'), 4.61 – 4.56 (m, 1H, H-2'), 4.13 – 3.77 (m, 14H), 3.73 – 3.55 (m, 4H), 3.04 – 2.97 (m, 2H, $CH_{2\ linker}$), 2.08 (s, 3H, $CH_{3\ acetyl}$), 2.06 (s, 3H, $CH_{3\ acetyl}$),1.70 – 1.62 (m, 4H, 2x$CH_{2\ linker}$), 1.41 (d, *J* = 5.0 Hz, 4H, 2x$CH_{2\ linker}$). **^{13}C NMR** (214 MHz, D2O) δ 175.4, 174.5, 99.4, 93.4, 93.3, 78.7, 76.5, 75.5, 75.4, 72.0, 71.3, 71.1, 71.1, 69.0, 66.6, 66.2, 66.2, 65.2, 65.2, 65.0, 65.0, 60.3, 59.7, 54.1, 53.3, 39.3, 29.5, 26.5, 25.0, 24.3, 22.0, 21.9. **^{31}P NMR** (202 MHz, D2O) δ 0.4, -1.3.

Compound (**2**)



Compound **4** (10 mg, 5.5 µmol) was dissolved in MeOH (1 ml, 5.5 mM) and NaOMe in MeOH (10 µl, 4.8M, 48 µmol, 9 eq) was added. The reaction was stirred for 30 minutes and quenched with NH_4Cl (aq). The product was extracted thrice with DCM, and the combined organic layers were concentrated *in vacuo.* In an empty flask, liquid ammonia was condensed at a temperature ranging from -85°C to -65°C while maintaining a gentle flow of ammonia. Upon reaching approximately 2 ml of liquid ammonia, the flask was sealed off and placed under a nitrogen atmosphere. Solid sodium (25 mg, 1.1 mmol, 192 eq) was added to the liquid ammonia at -80°C. The de-acetylated compound was co-evaporated twice with toluene and dissolved in THF (1m, 5.5 mM), followed by the addition of *tert*-butanol (0.1 ml, 1.1 mmol, 192 eq). The mixture was added to the solution of liquid ammonia and sodium and left to stir for 1 hour. The reaction was quenched with NH_4Cl (aq) and warmed to RT. The product was concentrated *in vacuo* and purified by HW40 gel filtration (NH_4OAc) to obtain the product as a white solid with a 75% purity (1.9 mg, 1.8 µmol, 35%). **^{1}H NMR** (850 MHz, D_2O) δ 5.29 – 5.27 (m, 1H, H-1), 4.81 (d, *J* = 1.7 Hz, 1H, H-1'), 4.46 – 4.45 (m, 1H, H-2), 3.99 – 3.71 (m, 17H), 3.69 – 3.62 (m, 3H), 3.55 (dd, *J* = 11.8, 4.3 Hz, 1H), 3.47 (dd, *J* = 11.8, 6.2 Hz, 1H), 3.44 – 3.42 (m, 1H), 2.87 (t, 2H, $CH_{2\ linker}$), 1.95 (s, 3H, $CH_{3\ acetyl}$), 1.92 (s, 3H, $CH_{3\ acetyl}$), 1.57 – 1.51 (m, 2H, $CH_{2\ linker}$), 1.51 – 1.47 (m, 2H, $CH_{2\ linker}$), 1.27 (dd, J = 7.1, 3.5 Hz, 4H, 2x $CH_{2\ linker}$). **^{13}C NMR** (214 MHz, D_2O) δ 175.3, 174.4, 99.4, 93.3, 78.7, 75.6, 71.7, 71.3, 71.1, 70.7, 70.6, 69.4, 68.8, 66.2, 66.1, 66.0, 62.0, 60.2, 59.7, 53.6, 53.1, 39.3, 29.4, 26.5, 25.1, 24.3, 21.9.

Literature

[1] Y. Araki and E. Ito, “Linkage Units in Cell Walls of Gram-Positive Bacteria,” *Crit. Rev. Microbiol.*, vol. 17, no. 2, pp. 121–135, Jan. 1989, doi: 10.3109/10408418909105745.

[2] N. KOJIMA, Y. ARAKI, and E. ITO, “Structural studies on the linkage unit of ribitol teichoic acid of Lactobacillus plantarum,” *Eur. J. Biochem.*, vol. 148, no. 1, pp. 29–34, Apr. 1985, doi: https://doi.org/10.1111/j.1432-1033.1985.tb08802.x.

[3] N. Kojima, Y. Araki, and E. Ito, “Structure of the linkage units between ribitol teichoic acids and peptidoglycan,” *J. Bacteriol.*, vol. 161, no. 1, pp. 299–306, Jan. 1985, doi: 10.1128/jb.161.1.299-306.1985.

[4] V. Torgov, L. Danilov, N. Utkina, V. Veselovsky, and I. Brockhausen, “Synthesis of P1-(11-phenoxyundecyl)-P2-(2-acetamido-2-deoxy-3-O-α-D-rhamnopyranosyl-α-D-glucopyranosyl) diphosphate and P1-(11-phenoxyundecyl)-P2-(2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-α-D-galactopyranosyl) diphosphate for the investigation of bios,” *Carbohydr. Res.*, vol. 453–454, pp. 19–25, 2017, doi: https://doi.org/10.1016/j.carres.2017.10.016.

[5] M. Bosco *et al.*, “Synthesis and biological evaluation of chemical tools for the study of Dolichol Linked Oligosaccharide Diphosphatase (DLODP),” *Eur. J. Med. Chem.*, vol. 125, pp. 952–964, 2017, doi: https://doi.org/10.1016/j.ejmech.2016.10.013.

[6] M. Heuckendorff, P. S. Bols, C. B. Barry, T. G. Frihed, C. M. Pedersen, and M. Bols, “β-Mannosylation with 4,6-benzylidene protected mannosyl donors without preactivation,” *Chem. Commun.*, vol. 51, no. 68, pp. 13283–13285, 2015, doi: 10.1039/C5CC04716A.

[7] R. E. J. N. Litjens, L. J. van den Bos, J. D. C. Codée, R. J. B. H. N. van den Berg, H. S. Overkleeft, and G. A. van der Marel, “Sulfonium Triflate Mediated Glycosidations of Aryl 2-Azido-2-deoxy-1-thio-D-mannosides,” *European J. Org. Chem.*, vol. 2005, no. 5, pp. 918–924, Mar. 2005, doi: https://doi.org/10.1002/ejoc.200400784.

[8] D. Shapiro, A. J. Acher, and A. Diver-Haber, “Ganglioside series. IV. Preparation of 2,3-di-O-acetyl-1, 6-anhydro-.beta.-D-glucopyranose and its

utilization in the synthesis of oligosaccharides," *J. Org. Chem.*, vol. 35, no. 5, pp. 1464–1467, May 1970, doi: 10.1021/jo00830a044.

[9] D. Crich and V. Dudkin, "Why Are the Hydroxy Groups of Partially Protected N-Acetylglucosamine Derivatives Such Poor Glycosyl Acceptors, and What Can Be Done about It? A Comparative Study of the Reactivity of N-Acetyl-, N-Phthalimido-, and 2-Azido-2-deoxy-glucosamine Derivatives ," *J. Am. Chem. Soc.*, vol. 123, no. 28, pp. 6819–6825, Jul. 2001, doi: 10.1021/ja010086b.

[10] D. Tailler, J.-C. Jacquinet, A.-M. Noirot, and J.-M. Beau, "An expeditious and stereocontrolled preparation of 2-azido-2-deoxy-β-D-glucopyranose derivatives from D-glucal," *J. Chem. Soc. Perkin Trans. 1*, no. 23, pp. 3163–3164, 1992, doi: 10.1039/P19920003163.

[11] C. Alex, S. Visansirikul, Y. Zhang, J. P. Yasomanee, J. Codee, and A. V Demchenko, "Synthesis of 2-azido-2-deoxy- and 2-acetamido-2-deoxy-D-manno derivatives as versatile building blocks," *Carbohydr. Res.*, vol. 488, p. 107900, 2020, doi: https://doi.org/10.1016/j.carres.2019.107900.

[12] D. Crich and S. Sun, "Direct chemical synthesis of β-mannopyranosides and other glycosides via glycosyl triflates," *Tetrahedron*, vol. 54, no. 29, pp. 8321–8348, 1998, doi: https://doi.org/10.1016/S0040-4020(98)00426-8.

[13] G. Malik, A. Ferry, X. Guinchard, T. Cresteil, and D. Crich, "N□O Bond as a Glycosidic-Bond Surrogate: Synthetic Studies Toward Polyhydroxylated N-Alkoxypiperidines," *Chem. – A Eur. J.*, vol. 19, no. 6, pp. 2168–2179, Feb. 2013, doi: https://doi.org/10.1002/chem.201202374.

[14] S. Arndt and L. C. Hsieh-Wilson, "Use of Cerny Epoxides for the Accelerated Synthesis of Glycosaminoglycans," *Org. Lett.*, vol. 5, no. 22, pp. 4179–4182, Oct. 2003, doi: 10.1021/ol035606h.

[15] J. K. Fairweather, T. Karoli, L. Liu, I. Bytheway, and V. Ferro, "Synthesis of a heparan sulfate mimetic disaccharide with a conformationally locked residue from a common intermediate," *Carbohydr. Res.*, vol. 344, no. 17, pp. 2394–2398, 2009, doi: https://doi.org/10.1016/j.carres.2009.09.012.