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Synthesis of *E. faecium* wall teichoic acid fragments

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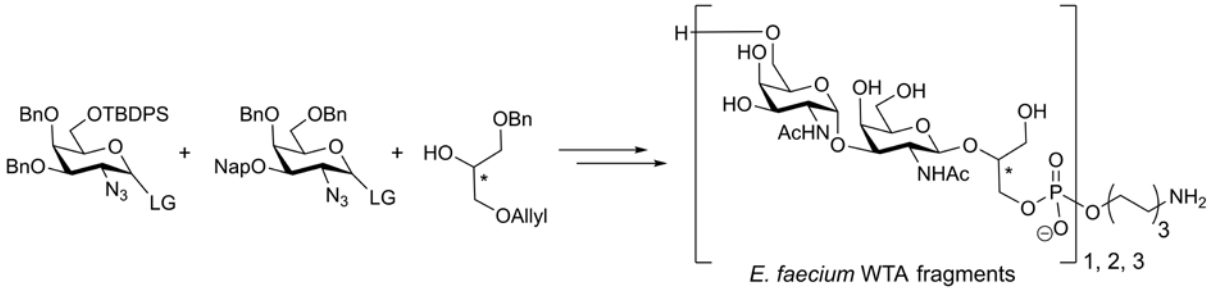
Keywords

E. faecium; wall teichoic acid; carbohydrate; glycosylation; phosphoramidite.

Abstract

The first synthesis of different E. faecium wall teichoic acids (WTA) fragments is presented. The structure of these major cell wall components was elucidated recently and it was shown that these glycerolphosphate (GroP) based polymers are built up from -6-(GalNAc-α(1-3)-GalNAc-β(1-2)-GroP)- repeating units. We assembled WTA fragments up to three repeating units in length, in two series that differ in the stereochemistry of the glycerolphosphate moiety. The key GalNAc-GalNAc-GroP synthons, required for the synthesis, were generated from galactosazide building blocks that were employed in highly stereoselective glycosylation reactions to furnish both the α- and β-configured linkages. By comparing the NMR spectra of the synthesized fragments with the isolated material it appears that the hereto undefined stereochemistry of the glycerol phosphate moiety is sn-glycerol-3-phosphate. The generated fragments will be valuable tools to study their immunological activity at the molecular level.

Graphical Abstract



Introduction

The rise of multi-drug resistant bacteria is a great threat to public health and the development of novel therapeutic or prophylactic approaches represents a grand challenge to the medical and scientific community. Amongst the most resistant bacterial species are enterococci, of which the vancomycin resistant enterococci (VRE) are especially difficult to treat with currently available antibiotics. *Enterococcus faecium* and *Enterococcus faecalis* are the most prevalent enterococci cultured from humans, accounting for 90% of all clinical isolates and they are responsible for a large part of nosocomial infections (14% in the USA in 2009-2010[1]) *E. faecium*, a commensal bacterium inhabiting the gastrointestinal tract,[2] is responsible for most VRE infections. Novel ways to combat these bacteria are urgently needed and therefore different vaccination strategies are currently being developed.[3,4] Teichoic acids (TAs), poly-alditol phosphate based glycopolymers, are major components of the cell wall of Gram-positive bacteria, accounting for up to 70% of the dry weight of the bacterial cell wall. Two types of TAs are commonly present in the Gram-positive cell wall. Lipoteichoic acids (LTAs) are generally built up from a poly-glycerol phosphate backbone with D-alanyl and carbohydrate residues randomly appended to the glycerol's C-2-OH. They are functionalized with a glycolipid anchor, which inserts into the phospholipid cell membrane underneath the peptidoglycan (PG) layer. Wall teichoic acids (WTAs) are covalently attached to the PG layerand the structure of WTAs varies considerably between species and even different strains from the same bacterium.[5] LTA is a target for opsonic (*i.e.* protective) antibodies[6] and we have recently shown that synthetic fragments of *E. faecalis* LTA can be used as antigens in combination with a BSA-carrier protein to provide a semi-synthetic vaccine modality.[7] Serum raised against this conjugate showed effective cross reactivity towards different *E. faecalis* and *E. faecium* strains indicating than an LTA based vaccine could provide broad protection against different enterococci and also several other gram-positive pathogens (such as group-B streptococci, *S. aureus* and *S. epidermidis*).[6] Some strains of *E. faecium* however are not

opsonized by anti-LTA antibodies. One possible explanation for this lack of killing is that the *E. faecium* LTA is not or less available for antibodies, probably due to capsular polysaccharides that cover LTA epitopes and prevent binding of antibodies and activation of complement. WTAs are generally longer than LTAs and may also obscure the targets for the opsonic antibodies.[8] Very recently, the first structure of an enterococcal WTA was elucidated. This structure was isolated from an *E. faecium* strain, resistant to anti-LTA opsonic antibodies[9] and is shown in Figure 1. It is built up from pseudotrisaccharide repeats, composed of an α-*N*-acetyl galactosamine that is linked to a β-*N*-acetyl galactosamine which in turn is attached to a glycerol phosphate residue. The stereochemistry of the glycerol moiety has not been established.

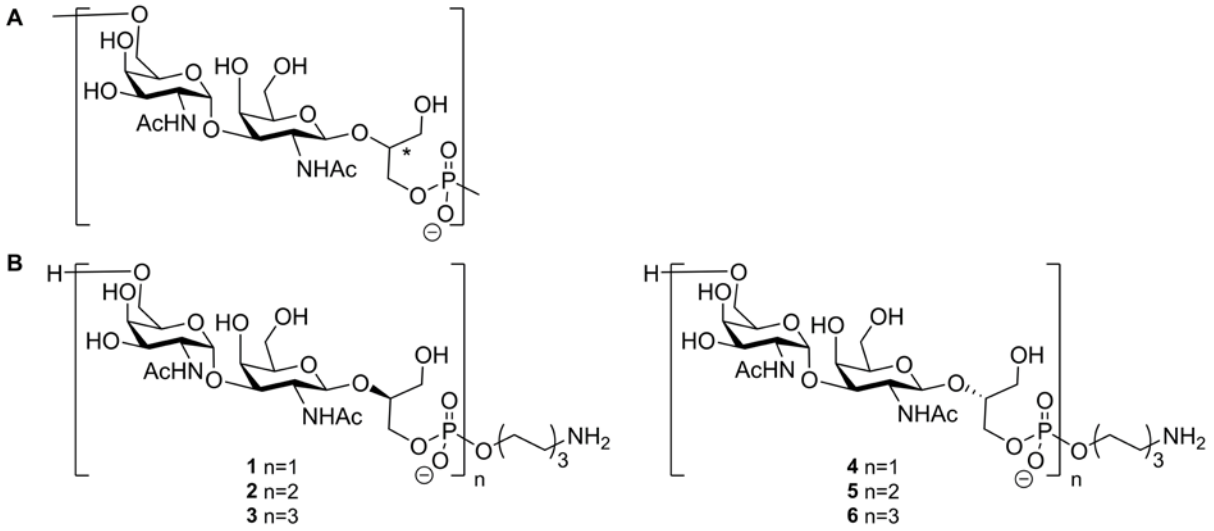


Figure 1. Structure of *E. faecium* WTA (A) and the target structures of this study (B).

Synthetic teichoic acid fragments can be powerful tools to unravel the immunological mode of action of these molecules. Not only can they be used as components in (semi)-synthetic vaccines and employed for diagnostic purposes, they can also be used to probe the interaction with other players of the immune system, such as C-type lectin receptors (CLRs), carbohydrate receptors playing an important role in shaping the immune system, and Toll-like receptors (TLRs), receptors responsible for detecting pathogen associated molecular patterns (PAMPs). Therefore the structure depicted in Figure 1 is an attractive synthetic target. In addition synthetic fragments may be used to assign the stereochemistry of the as-yet unknown glycerol C-2. Here we report the assembly of a small library of *E. faecium* WTA structures, encompassing mono-, di- and trimeric repeats of both glycerol stereoisomers. Compounds **1**-

3 represent members of the *sn*-glycerol-1-phosphate family, where compound **4**-**6** are *sn*-glycerol-3-phosphate based targets).

Results and discussion

We, and others, have described a number of approaches to generate synthetic TA fragments[10], including traditional solution phase studies[11], light fluorous approaches[12] and fully automated solid phase[13] assemblies. All of our syntheses hinged on the use of well-established synthetic "DNA-chemistry", employing phosphoramidite building blocks bearing a dimethoxytrityl ether as a temporary protecting group for the nascent oligomer chain. To assemble the set of target compounds in Figure 1, we again resorted to this strategy here. All target structures were equipped with an aminohexanol spacer for conjugation purposes in the future. Thus, for the assembly of WTAs **1**-**6**, spacer phosphoramidite **9** and diastereomeric building blocks **7** and **8** were required. To minimize protecting group manipulations on far-advanced building blocks, we reasoned that the use of galactosazide building blocks as precursors for the α- and β-GalNAc-moieties would be beneficial. We decided to explore two routes towards key pseudo trisaccharides **10** and **11**. The first route builds up the target structure from the glycerol end, first introducing the β-galactosamine linkage and subsequently installing the α-$GalN_3$-$GalN_3$ linkage. The alternative route proceeds in opposite direction and it installs the Gal-α-(1-3)-Gal linkage first, after which the glycerol moieties are attached.

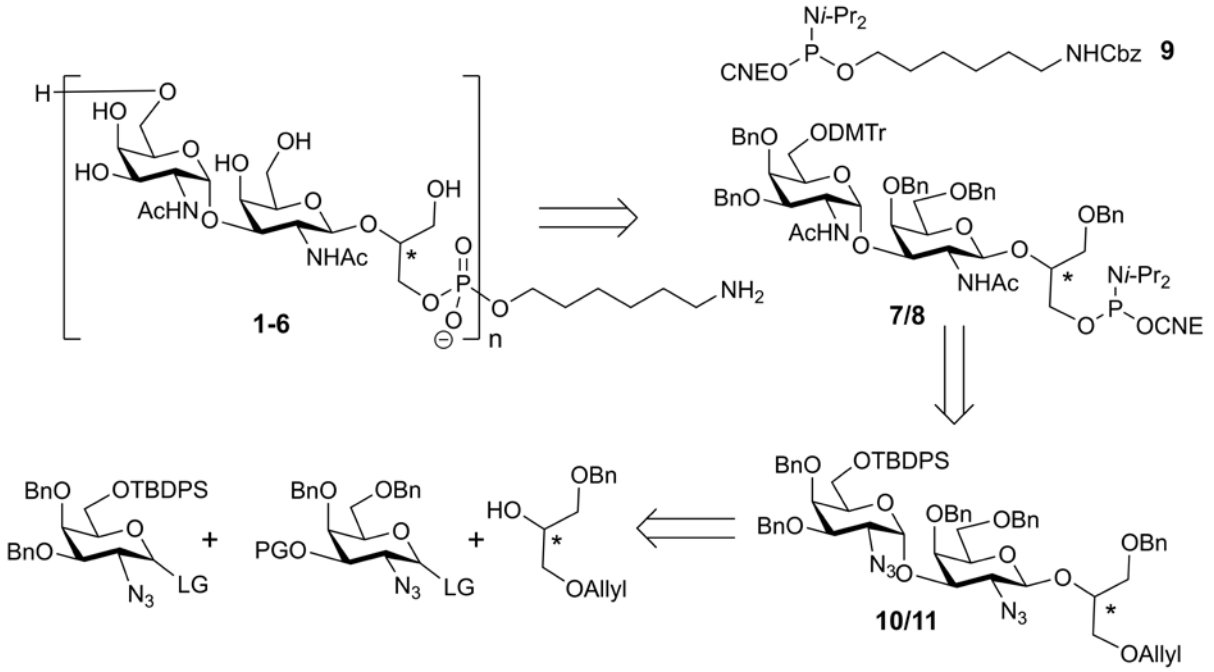
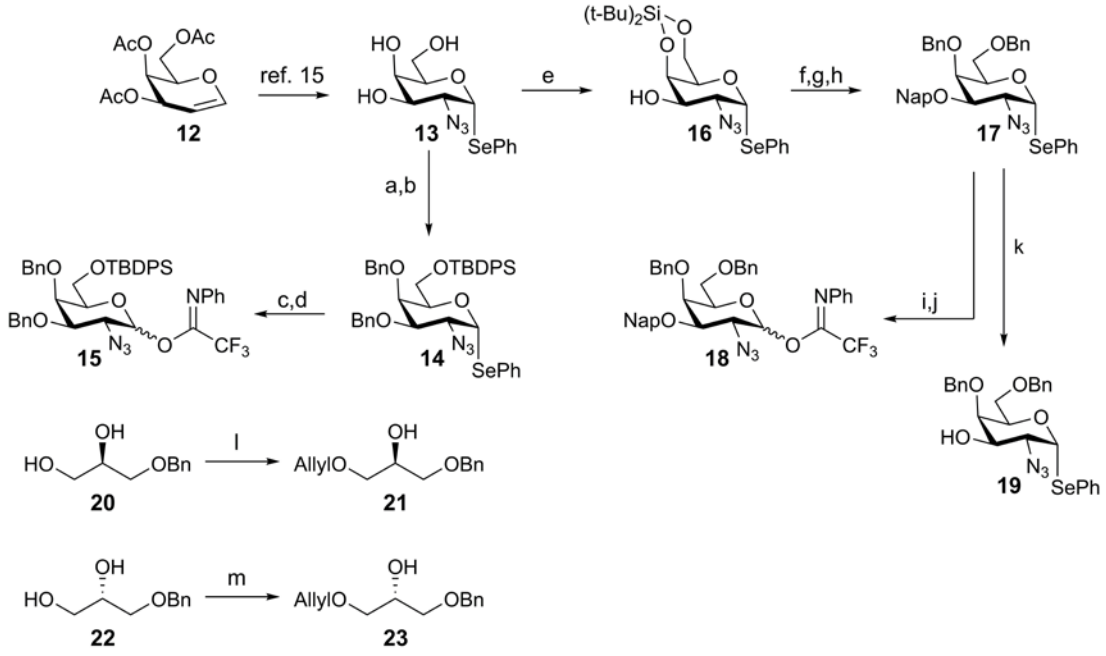


Figure 2. Retrosynthetic analysis

The synthesis of the building blocks required for the assembly of the central glycerol-disaccharide intermediate is depicted in Scheme 1. Both galactosazide synthons were accessed from azidoselenogalactoside **13**, which was readily obtained through an azidoselenylation[14] of commercially available 3,4,6-triacetylgalactal **12**. To facilitate the installation of the α-galactosamine bond, we investigated donors **14** and **15**, featuring a bulky TBDPS-ether at C-6.[15–18] To synthesize these galactosides, triol **13** was selectively protected on C-6 with a TBDPS group and the remaining alcohols were benzylated yielding selenoglycoside **14**. Hydrolysis of the anomeric selenoacetal and subsequent introduction of the trifluoroimidate[19] furnished donor **15** in 87% yield over 2 steps.

To install the β-galactosamine bond we aimed to exploit the nitrile assisted glycosylation system.[20] We thus equipped building block **18** with a "permanent" benzyl ether at C-4 and C-6 and a 2-naphthylmethyl (Nap) ether, which can be chemoselectively removed, at C-3. To introduce the Nap at C-3, the C-4 and C-6 alcohols in **13** were first masked with a silylidene-ketal yielding compound **16** in good yield. Subsequent naphthylation, desilylation and double benzylation yielded **17** in 54% yield from **16**. Donor **18** was obtained from **17** by executing the above described hydrolysis-imidate introduction sequence in 80% yield. Acceptor **19** was synthesized by selectively removing the naphthylmethyl group from **17** in 78% yield using DDQ in a biphasic solvent system.



Scheme 1. Building block synthesis.
Reagents and conditions: a) TBDPSCl, imidazole, DMF, 90%; b) BnBr, NaH, DMF, quant; c) NIS, THF/H_2O; d) $CF_3C(=NPh)Cl$, K_2CO_3, acetone, 87% over 2 steps; e) $(t\text{-}Bu)_2Si(OTf)_2$, DMF, 97%; f) NapBr, NaH, DMF, 71%; g) NEt_3•3HF, THF, 94%; h) BnBr, NaH, DMF, 83%; i) NIS, THF/H_2O; j) $CF_3C(=NPh)Cl$, K_2CO_3, acetone, 80% over 2 steps; k) DDQ, DCM:H_2O, 78%; l) 2-aminoethyl diphenylborinate, KI, K_2CO_3, allylbromide, MeCN, 66%; m) 2-aminoethyl diphenylborinate, KI, K_2CO_3, allylbromide, MeCN, 62%.

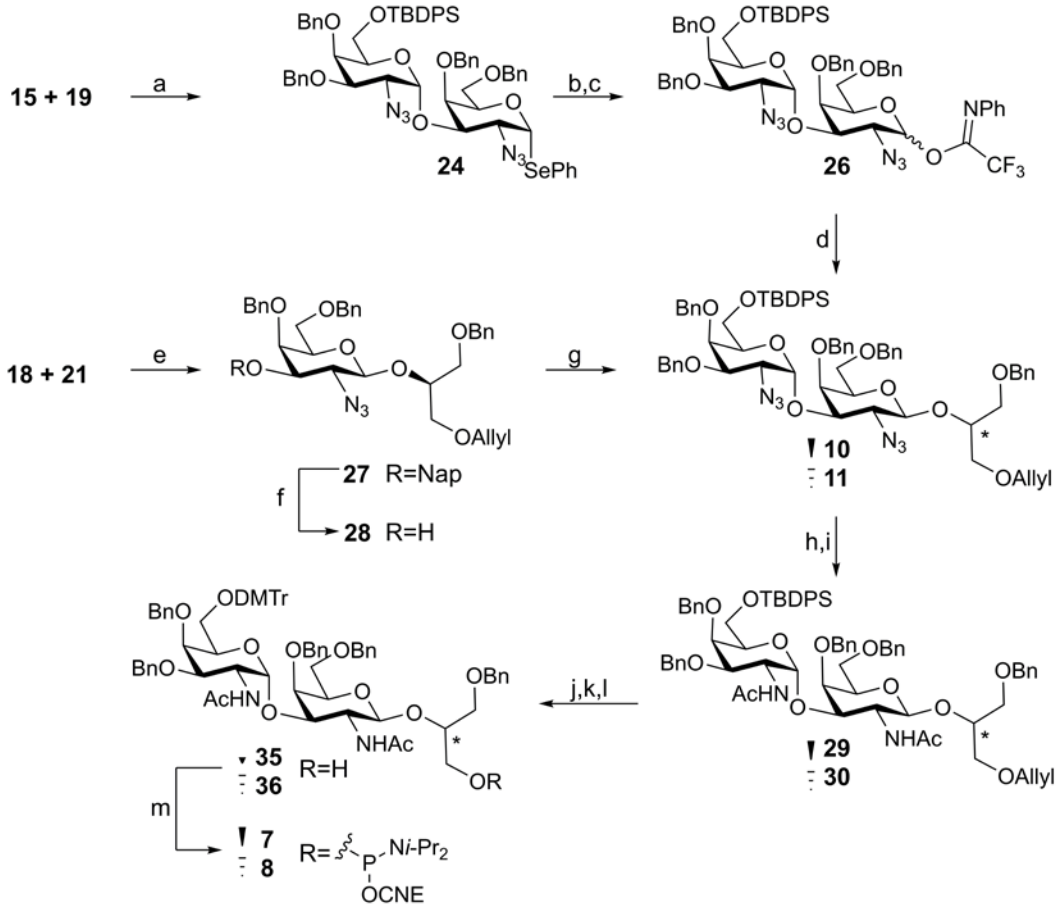
Glycerol acceptors **21** and **23** were synthesized by the regioselective introduction of an allyl-ether on the primary alcohol of commercially available diols **20** and **22**. Using a palladium-tin bimetallic catalysis system as reported by Onomura[21] we obtained **21** from **20** with excellent regioselectivity in 84% yield. Scaling up this reaction proved troublesome and therefore we investigated alternative procedures. Using a tin-ketal mediated allylation, **21** was obtained in 66% as a single regioisomer. A similar yield was obtained by employing a 2-aminoethyl diphenylborinate-catalyzed allylation as described by Taylor and co-workers.[22–24] Although this procedure also gave 20% of the undesired C-2-allyl ether, the regioisomers were readily separated and the reaction could be easily scaled up (62% yield on 6 mmol scale).

With all building blocks in hand we attended to the assembly of the glycerol-disaccharide building blocks **35** and **36** (Scheme 2). We first explored the combination of selenophenyl galactosides **14** and **16** in a pre-activation based glycosylation reaction. However, we found that activation of seleno donor **14** using the diphenyl sulfoxide (Ph_2SO)-triflic anhydride (Tf_2O) couple and the subsequent addition of selenogalactoside **16** led to a complex reaction mixture. We therefore switched to a chemoselective approach in which imidate donor **15** was paired with **16**. This led to the desired disaccharide in good yield but with poor stereoselectivity. Even though we employed diethyl ether, a commonly employed solvent to promote the formation of *cis*-glycosidc linkages, the β-linked disaccharide was predominantly formed (α/β = 1 : 3). Using dibenzyl acceptor **19** instead of **16**, and changing the solvent to dichloromethane significantly improved the stereoselectivity of the reaction and disaccharide **24** was obtained as a single diastereomer in 58% yield. For the crucial condensation of the disaccharide donor and the glycerol acceptor we changed the anomeric seleno group into an imidate donor (**24** to **26**) to stay close to the condensation condition devised by Mong and co-workers. Gratifyingly, we found that condensation of the disaccharide imidate donor **26** with glycerol acceptor **21** under the agency of a catalytic amount of triflic acid (TfOH) in the presence of acetonitrile and propionitrile provided the target glycerol-disaccharide **10** as a single diastereoisomer in 90% yield.

In the alternative route towards the glycerol-disaccharide building block donor **18** was coupled to glycerol acceptor **21** using the aforementioned conditions to provide **27** with good stereoselectivity (α/β = 1 : 19) and in good yield (Scheme 2). To unmask the C-3-napthyl ether we subjected **27** to a catalytic amount of HCl in hexafluoro-*iso*-propanol (HFIP),[25] which produced galactosazide **28** in 63%. When DDQ was used in a biphasic DCM/H_2O

solvent system, the primary benzyl ether on the glycerol moiety was also partly cleaved. This side reaction could be circumvented by executing the oxidative cleavage in a phosphate buffer[26] at pH=7.4 to yield glycerol-galactoside acceptor **28** in 75% yield. To complete the assembly of the glycerol-disaccharide intermediate, donor **15** was coupled to alcohol **28** to provide the key building block **10** as a single anomer in 88%.

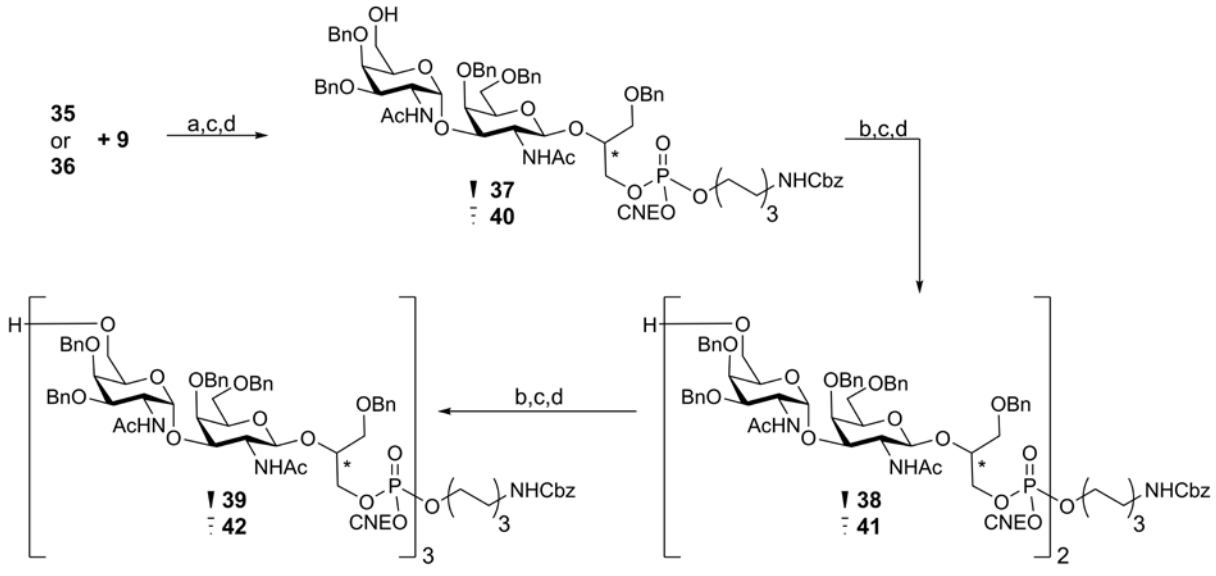
Overall, both assembly routes feature highly stereoselective glycosylation reactions and both deliver the target glycerol-disaccharide in similar yields (Scheme 2). Because the first route allows the introduction of both glycerol epimers from a single far-advanced synthon we proceeded with this route to provide the diastereomeric glycerol-disaccharide **11**. To this end donor **26** and glycerol **23** were united using the acetonitrile/propionitrile glycosylation conditions to provide glycerol-disaccharide **11** in good yield and stereoselectivity.



Scheme 2. Glycerol-disaccharide building block synthesis.
Reagents and conditions: a) TfOH, DCM, 0°C, 58%; b) NIS, THF/H_2O; c) CF_3(=NPh)Cl, K_2CO_3, acetone, quant over 2 steps; d) **21** or **23,** TfOH, MeCN/EtCN/DCM, -40°C, **10**:90% **11**:80%; e) TfOH, MeCN/EtCN/DCM, -40°C, 90%; f) DDQ, phosphate buffer, DCM, 75%; g) **15**, TfOH, DCM, 0°C, 88%; h) PMe_3, dioxane/H_2O; i) Ac_2O, NEt_3 , DCM, **29**:59% over 2 steps, **30**:76% over 2 steps; j) TBAF, THF, **31:**77%, **32**:89%; k) DMTrCl, NEt_3, DCM, **33**:89%, **34**:86%; l) 1.Ir(COD)($Ph_2MeP)_2$, THF; 2.$NaHCO_3$, I_2, H_2O/THF, **35**:70%, **36**:80%; m) di-isopropylethylamine, *N*,*N*-di-isopropylamino-2-cyanoethyl-chlorophosphite, DCM, **7**: 85% **8**:62%.

To convert **10** and **11** into the necessary phosphoramidite building blocks **7** and **8** we transformed the azide functionalities using a Staudinger reduction and subsequent *N*-acetylation provided **29** and **30**. Removal of the silyl ether in **29** and **30** was followed by installation of the dimethoxytrityl group to set the stage for the final de-allylation (Scheme 2). To this end we employed $Ir(COD)(Ph_2MeP)_2$, briefly activated with hydrogen gas, to isomerize the allyl ether into the enol ether and I_2 in combination with aqueous $NaHCO_3$ to unmask the alcohol. These mild conditions do not affect the acid labile dimethoxytrityl ether. Following this sequence of reactions glycerol-disaccharide **35** and epimeric glycerol-disaccharide **36** were obtained in 50% and 60% yield over 3 steps from **29** and **30**, respectively. Finally, the alcohols were transformed into phosphoramidites **7** and **8**.

With the building blocks in hand, we turned to the assembly of the oligomers. To this end we first coupled the phosphoramidite functionalized spacer **9**[11] with alcohols **35** and **36** (Scheme 3) using dicyanoimidazole as an activator. Subsequently we oxidized the intermediate phosphite to the phosphotriester and unmasked the labile dimethoxytrityl ether with trichloroacetic acid (TCA). Because we found that the use of pyridine/H_2O in combination with I_2 for the oxidation of the phosphite intermediate led to partial removal of the cyanoethyl group, we employed the (10-camphorsulfonyl)oxaziridine (CSO) oxidation method[27,28] to yield spacer-coupled products **37** and **40** in 72% and 80%yield.

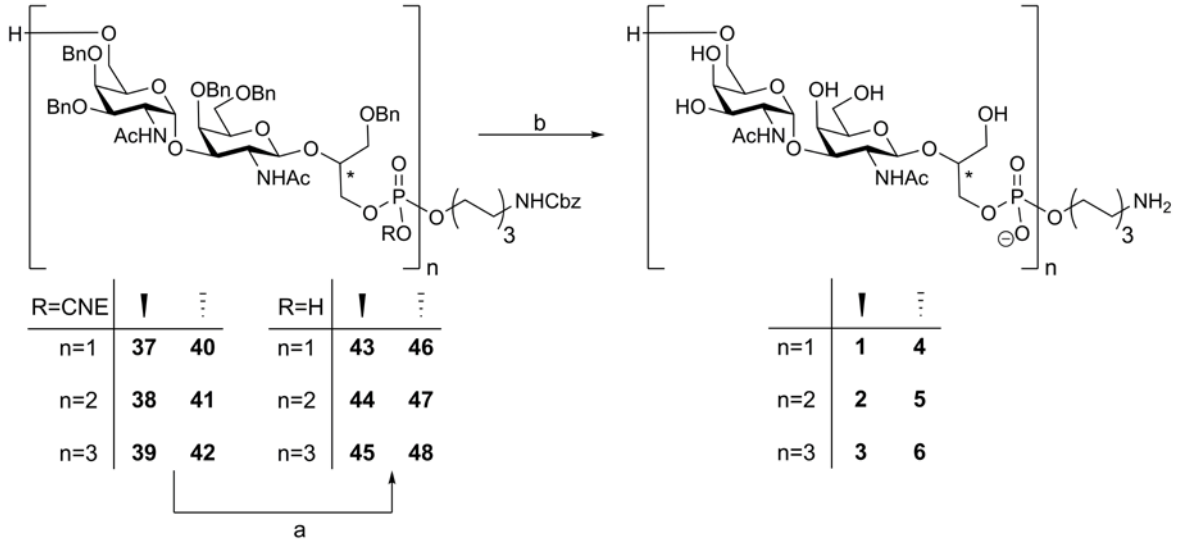


Scheme 3. Assembly of spacer equipped oligomers.
Reagents and conditions: a) DCI, **9**, MeCN; b) DCI, **7** or **8**, MeCN; c) CSO, MeCN; d) 3% TCA, DCM, **37**:72%, **40**:80%, **38**:62%, **41**:64%, **39**:69%, **42**:67%.

Elongation of these molecules with phosphoramidites **7** and **8**, gave the protected spacer functionalized dimers **38** and **41**. Because we could not separate the target products from the

used excess of phosphoramidite by size exclusion chromatography or conventional flash column chromatography we used reversed phase automated flash column chromatography (20% – 100% MeOH in H_2O) to purify dimers **38** and **41**. These were obtained in 62% and 64%, respectively. The trimers **39** and **42** were obtained in a similar fashion and isolated in 69% and 67% yield.

The fully protected compounds were deprotected by a treatment with concentrated ammonia and dioxane to remove the cyanoethyl protecting groups from the phosphotriesters followed by a hydrogenation of the so-formed phosphodiesters (Scheme 4). To this end the oligomers were treated with H_2 using palladium black as the catalyst in a mixture of water/dioxane under slightly acidic conditions. Progress of the reactions was monitored by NMR spectroscopy. If the crude reaction mixtures still contained aromatic functionalities the products were resubjected to the deprotection conditions. Final purification of the fully deprotected oligomers was affected by HW-40 gel filtration, after which the compounds were transformed into the sodium salts.



Scheme 4. Deprotection of the oligomers
Reagents and conditions: a) NH_3(conc), dioxane; b) Pd(0), H_2, AcOH, H_2O, **1**:73%, **2**:57%, **3**:44%, **4**:45%, **5**:49%, **6**:56%.

The spectra of the diastereomeric mono- di- and trimer repeats are shown in Figure 3, alongside the spectrum for the *E. faecium* WTA obtained from natural sources as reported by Bychowska *et al.*[9] When the spectra are compared it becomes clear that the spectrum of the *sn*-3-phosphate trimer **6** resembles the spectrum of the natural compound better than the spectrum of the corresponding *sn*-1-phosphate **3**. Especially the set of signal belonging to the β-anomeric galactosamine residues (δ = 4.65 ppm) is indicative. Also the overall shape of the multiplet between 3.6-4.1 ppm is matched better to the spectrum of the natural WTA in the

spectrum of **6** than of its diastereomer **3**. Based on the similarities between the spectrum of **6** and the spectrum of the isolated WTA we tentatively assign the stereocenter in *E. faecium* WTA as *R* and the stereochemistry of the glycerol as *sn*-glycerol-3-phosphate. Of note, the biosynthesis of WTA[29–31] and LTA[32] use different glycerolphosphate donors. Where LTA is assembled using phosphatidyl glycerol as building block, WTA is synthesized from cytidine diphosphate glycerol. In the latter process, the glycerol (or PG) alcohol of the nascent WTA chain attacks the pyrophosphate moiety and expels cytidine monophosphate as the leaving group.[33] The stereochemistry is thus defined by the CDP-glycerol building block that is used. The natural configuration of this biological synthon is *sn*-glycerol-3-phosphate, which corroborates the assignment of the stereocenter using the NMR data presented above.

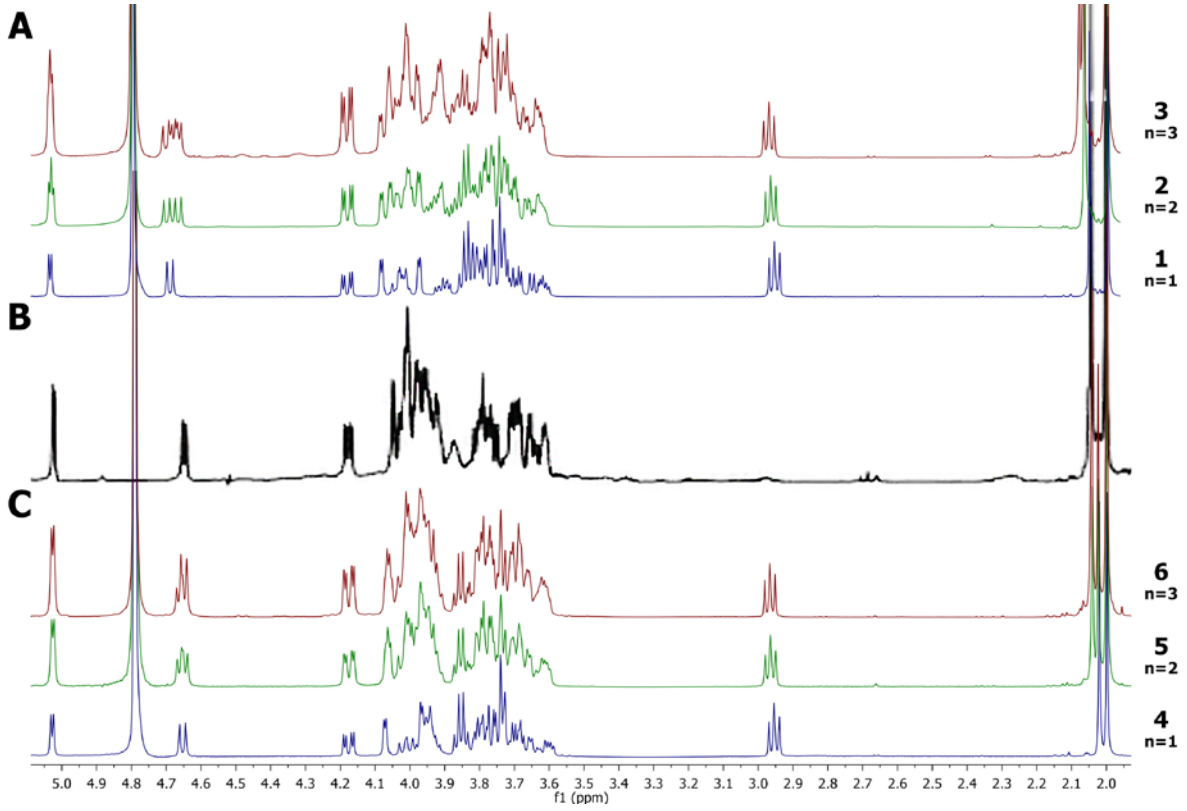


Figure 3. Part of the NMR spectra (solvent D_2O) of WTA fragments 1-6 and WTA isolated from *E. faecalis*. (A) *sn*-glycerol-1-phosphate tri-, di- and monomer, (B) natural WTA, (C) *sn*-glycerol-3-phosphate tri-, di- and monomer. The triplet signal at δ = 2.95 ppm is the resonance of the spacer's CH_2-NH_2 moiety.

Conclusion

In conclusion, we have assembled three *E. faecalis* WTA fragments alongside their three glycerol epimers. The fragments were built using a synthetic strategy that relied on the use of DMT-phosphoramidite based building blocks. The repeating unit building block, that featured both an α- and a β-linked GalNAc moiety was assembled using galactosazide donors with excellent stereoselectivity. Based on the NMR spectra of the assembled structures and

biosynthesis arguments we assign the stereochemistry of the glycerol moiety in *E. faecium* WTA as *sn*-glycerol-3-phosphate. The synthesized structures will be evaluated in immunological assays in which they will be probed as synthetic antigens. Discovery of a potent antigen will pave the way to use the structures in (semi)-synthetic vaccine modalities.

Experimental

General

All chemicals (Acros, Fluka, Merck, Sigma-Aldrich, etc.) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 25 g/l and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ 10 g/l, in 10% aqueous H_2SO_4 followed by charring at +/- 140 ^oC. Some unsaturated compounds were visualized by spraying with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in water. Optical rotation measurements ($[\alpha]_D^{20}$) were performed on a Propol automated polarimeter (Sodium D-line, λ = 589 nm) with a concentration of 10 mg/ml (c = 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300. ^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 ^oC) 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).

Experimental procedures

General procedure for phosphoramidite coupling

The alcohol was coevaporated with acetonitrile four times, DCI (0.25M in MeCN) (1.5-3 eq), acetonitrile (0.06 M) and 3Å molsieves were added and the mixture was stirred for 15 minutes under an argon atmosphere. The phosphoramidite (1.5-3 eq) was added and the reaction was stirred for 1-4 h at RT. CSO (0.5M in MeCN) (1.5-3 eq) was added and the reaction was

stirred for 5 minutes. The mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was taken up in DCM (0.03 M). TCA (3% in DCM) (5 eq) was added and the mixture was stirred for 20 min. H_2O was added and the mixture was stirred for 15 minutes. The reaction mixture was diluted with DCM and was washed with a mixture of sat. aq. $NaHCO_3$ and brine (1/1 v/v). The aqueous layer was extracted with DCM and the combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*.

General procedure for global deprotection

The oligomer was dissolved in a mixture of ammonia (conc.) and dioxane (2 mM, 1/1 v/v). The mixture was stirred for 1h and was concentrated *in vacuo* yielding the phosphodiester intermediate. The residue was flushed over a Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with H_2O and MeOH before use) column to end up in a mixture of water and dioxane (2 mM, 2/1 v/v). ~0.1ml AcOH was added and the mixture was purged of oxygen. Pd-black (~20 mg) was added and subsequently, the reaction mixture was treated with Hydrogen gas for 3 days. Celite was added to the mixture and after short sonication (20-30 sec) the mixture was filtered over celite and Chelex 100 resin and concentrated *in vacuo* The residue was purified by size-exclusion chromatography (HW40, dimensions: 16/60 mm, eluent: 0.15 M NH_4OAc). After repeated lyophilization, the product was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with H_2O and MeOH before use).

Phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-seleno-6-*O*-(*tert*-butyl-di-phenylsilyl)- α-D-galactopyranoside (14)

To a cooled (0°C) solution of **13** (4.7 g, 13.5 mmol) in DMF (50 ml) imidazole (1.4 g, 20.3 mmol) and TBDPSCl (3.7 ml, 14 mmol) were added. After stirring for 10 minutes, TLC analysis showed complete conversion of the starting material. H_2O was added and the mixture was diluted with Et_2O. The organic layer was washed with H_2O (5x), brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Column chromatography yielded intermediate 2-azido-2-deoxy-1-seleno-6-O-(tert-butyl-di-phenylsilyl)-α-D-galacto-pyranoside (7.03 g, 12.1 mmol) in 90% yield. TLC: R_f 0.8 (30% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 1): +172; IR (neat, cm^{-1}): 3432, 2929, 2889, 2110, 1113, 1055, 738, 702; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.42 – 7.15 (m, 15H), 5.96 (d, *J* = 5.2 Hz, 1H), 4.26 – 4.23 (m, 2H), 4.16 – 4.13 (m, 1H), 3.89 – 3.87 (m, 2H), 3.78 – 3.74 (m, 1H), 1.05 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.7,

135.6, 134.4, 132.7, 132.2, 130.2, 130.1, 129.2, 128.6, 128.0, 127.9, 127.8, 85.5,71.8, 71.6, 69.9, 64.4, 62.1, 26.9; HRMS: [$C_{28}H_{33}N_3O_4SeSi$ + Na] requires 606.12997, found 606.12976. To a cooled (0°C) solution of the intermediate (6.1 g, 10.5 mmol) and BnBr (3.1 ml, 26.3 mmol) in DMF (70 ml) NaH (60% disp.) (1.1 g, 26.3 mmol) was added in portions over 20 minutes. The mixture was allowed to warm up to RT while stirring overnight. The mixture was cooled to 0°C and H_2O was added. The mixture was diluted with Et_2O, the organic layer was washed with H_2O (5x) and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded compound **14** (8.0 g, 10.5 mmol) in >98% yield. TLC: R_f 0.9 (20% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 1): +131.6; IR (neat, cm^{-1}): 3066, 2929, 2856, 2110, 1103, 1062, 738, 700; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41 – 7.05 (m, 25H), 5.82 (d, *J* = 5.2 Hz, 1H), 4.92 (d, *J* = 11.2 Hz, 1H), 4.78 (q, *J* = 11.6 Hz, 2H), 4.59 (d, *J* = 11.2 Hz, 1H), 4.34 (dd, *J* = 5.2 Hz, 10.2 Hz, 1H), 4.23 (t, *J* = 7.6 Hz, 1H), 4.10 (s, 1H), 3.85 – 3.80 (m, 1H), 3.72 (dd, *J* = 2.4 Hz, 10.2 Hz, 1H), 3.58 – 3.54 (m, 1H), 1.05 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.4, 137.6, 135.6, 135.1, 133.2, 133.1, 130.0, 129.9, 129.1, 128.7, 128.4, 128.2, 128.1, 127.9, 127.8, 85.7, 80.4, 75.2, 73.3, 73.2, 72.7, 61.7, 61.2, 27.0 ; HRMS: [$C_{42}H_{45}N_3O_4SeSi$ + Na] requires 786.22411, found 786.22388.

2-azido-3,4-di-*O*-benzyl-2-deoxy-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-6-*O*-(*tert*-butyl-di-phenylsilyl)-α/β-D-galactopyranoside (15)

To a cooled (0°C) solution of **14** (7.3 g, 9.5 mmol) in a mixture of H_2O/THF (1:1 v/v, 85 ml) NIS (2.4 g, 10.5 mmol) was added. The reaction was allowed to warm up towards RT and was stirred for 1h. The reaction mixture was diluted with EtOAc, the organic layer was washed with H_2O, brine, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded intermediate 2-azido-3,4-di-*O*-benzyl-2-deoxy -6-*O*-(*tert*-butyl-di-phenylsilyl)-α/β- D-galactopyranose (5.1 g, 8.3 mmol) in 87% yield. TLC: R_f 0.3 (10% EtOAc/pentane); IR (neat, cm^{-1}): 3389, 2956, 2929, 2856, 2110, 1112, 1060, 738, 700; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.62 – 7.14 (m, 45H), 5.18 (d, *J* = 3.2 Hz, 1H), 4.93 – 4.90 (m, 2H), 4.74 – 4.54 (m, 6H), 4.34 (d, *J* = 8 Hz, 1H), 4.11 – 4.01 (m, 2H), 3.99 – 3.81 (m, 4H), 3.75 – 3.65 (m, 4H), 3.60 – 3.29 (m, 2H), 1.05 (s, 16H); ^{13}C NMR (101 MHz, $CDCl_3$): δ =138.5, 138.4, 137.7, 127.6, 135.6, 135.5, 133.2, 133.0, 129.9, 129.8, 129.1, 128.6, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 96.5, 92.4, 80.7, 77.2, 74.9, 74.8, 73.6, 72.6, 72.4, 70.8, 64.9, 61.1, 61.8, 60.4, 26.9; HRMS: [$C_{36}H_{41}N_3O_5Si$ + Na] requires 646.27077, found 646.27081. To a stirred solution of the intermediate (0.31 g, 0.5 mmol) in acetone (5 ml) K_2CO_3 (82 mg, 0.6 mmol) and *N*-phenyl trifluoroacetimidoyl chloride (0.12 ml, 0.75 mmol) were added. After stirring for 2 days at RT

the reaction mixture was diluted with EtOAc and H_2O. The organic layer was washed with brine (2x), dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded compound **15** (0.4 g, 0.5 mmol) in >98% yield. TLC: R_f 0.8 (10% EtOAc/pentane); IR (neat, cm^{-1}): 2953, 2858, 2113, 1716, 1209, 1163, 1112, 738, 696; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.63 – 6.74 (m, 43H), 6.25 (s, 1H), 5.49 (s, 0.7H), 4.92 – 4.87 (m, 2H), 4.80 – 4.69 (m, 4H), 4.59 – 4.55 (m, 2H), 4.07 – 4.04 (m, 1H), 4.01 – 3.99 (m, 1H), 3.96 – 3.93 (m, 1H), 3.89 – 3.87 (m, 3H), 3.82 – 3.72 (m, 4H), 3.38 – 3.35 (m, 2H),1.04 (s, 15H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 143.6, 143.5, 138.5, 137.6, 135.7, 133.5, 133.4, 130.0, 129.9, 129.4, 128.8, 128.7, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 124.5, 120.8, 119.7, 119.6, 96.4, 95.1, 81.1, 77.8, 76.4, 75.1, 75.0, 74.1, 73.5, 73.1, 72.9, 72.7, 62.7, 62.5, 62.4, 59.6, 27.1; HRMS: [$C_{44}H_{45}F_3N_4O_5Si$ + Na+] requires 817.30035, found 817.30042.

Phenyl 2-azido-4,6-*O*-silylidene-2-deoxy-1-seleno-α-D-galactopyranoside (16)

To a cooled (-40°C) solution of **13** (0.17 g, 0.5 mmol) in dry DMF (2 ml) di-tert-butylsilyl bis(trifluoromethanesulphonate) (0.17 ml, 0.5 mmol) was added. The mixture was stirred for 10 minutes, pyridine (0.12 ml, 1.5 mmol) was added and the mixture was stirred for 20 additional minutes at -40°C. Et_2O and H_2O were added and the organic layer was washed with H_2O (5x) and brine (2x). The aqueous layers were extracted with Et_2O, and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded compound **16** (0.24 g, 0.49 mmol) in 97% yield. TLC: R_f 0.7 (10% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$): +196; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.56 – 7.53 (m, 2H), 7.29 – 7.25 (m, 3H), 5.93 (m, *J* = 5.2 Hz, 1H), 4.48 – 4.47 (m, 1H), 4.30 – 4.26 (m, 1H), 4.18 (s, 1H), 4.04 – 4.00 (m, 2H), 3.81 – 3.77 (m, 1H), 2.81 (d, *J* = 10.4 Hz, 1H), 1.06 (s, 9H), 1.03 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 134.4, 134.0, 129.2, 128.4, 127.9, 85.4, 72.3, 71.8, 69.8, 66.7, 62.1, 27.6, 27.3; HRMS: [$C_{20}H_{31}N_3O_4SeSi$ + NH_4] requires 501.15983, found 501.15244.

Phenyl 2-azido-4,6-di-*O*-benzyl-2-deoxy-3-*O*-naphthylmethyl-1-seleno-α-D-galactopyranoside (17)

To a cooled (0°C) solution of **16** (0.24 g, 0.49 mmol) in DMF (2 ml) napthyl bromide (0.12 g, 0.54 mmol) and NaH (60% disp.) (0.03 g, 0.68 mmol) were added. After stirring for 2h, H_2O and Et_2O were added, the organic layer was washed with H_2O (5x) and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded naphthylated intermediate 2-azido-4,6-*O*-silylidene-2-deoxy-3-*O*-napthyl-1-seleno-α-D-galacto-pyranoside (0.35 g, 0.22 mmol) in 71% yield. TLC: R_f 0.82 (10% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$): +154; IR (neat, cm^{-1}): 3055, 2931, 2856, 2112, 1473, 1080, 823, 731, 690; ^1H NMR (400 MHz,

$CDCl_3$): δ = 7.89 – 7.78 (m, 4H), 7.56 – 7.32 (m, 3H), 7.48 – 7.46 (m, 2H), 7.27 – 7.22 (m, 4H), 5.95 (d, *J* =5.2 Hz, 1H), 4.78 (q, *J* = 11.6 Hz, 2H), 4.59 – 4.57 (m, 1H), 4.35 (dd, *J* = 5.2 Hz, 10 Hz, 1H), 4.21 – 4.18 (m, 1H), 4.01, 3.96 (m, 2H), 3.68 (dd, *J* = 2.8 Hz, 10.2 Hz, 1H), 1.06 (s, 9H), 1.04 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.2, 134.5, 133.4, 133.2, 129.4, 129.2, 128.6, 128.5, 128.3, 128.0, 127.9, 127.8, 126.7, 126.4, 126.3, 126.1, 125.9, 125.6, 85.9, 78.8, 71.0, 70.5, 70.3, 67.0, 59.8, 28.0, 27.4 ; HRMS: [$C_{31}H_{39}N_3O_4SeSi$ + Na] requires 648.17697, found 648.17651. To a stirred solution of the intermediate (0.22 g, 0.35 mmol) in THF (7 ml) was added Et_3N●3HF (0.17 ml, 1.05 mmol) . The mixture was stirred for 2h at RT, after which the reaction was diluted with EtOAc and washed with H_2O (1x). The aqueous layer was extracted with EtOAc (2x), and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded intermediate diol 2-azido-2-deoxy-3-*O*-napthyl-1-seleno-α-D-galacto-pyranoside (0.16 g, 0.33 mmol) in 94% yield. TLC: R_f 0.4 (40% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 1): +228; IR (neat, cm^{-1}): 3419, 2885, 2108, 1080, 1066, 817, 740, 690; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.78 – 6.99 (m, 13H), 5.90 (d, *J* = 5.2 Hz, 1H), 4.86 – 4.78 (m, 2H), 4.21 (dd, *J* = 5.2 Hz, 10 Hz, 1H), 4.16 – 4.13 (m, 1H), 4.12 – 4.02 (m, 1H), 3.82 – 3.76 (m, 1H), 3.71 – 3.65 (m, 1H), 2.98 (s, 1H), 2.18 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 134.4, 133.3, 133.2, 133.1, 129.3, 128.7, 128.2, 128.1, 127.9, 127.1, 126.5, 126.5, 125.7, 84.7, 78.8, 72.3, 72.2, 67.2, 62.8, 60.3; HRMS: [$C_{23}H_{23}N_3O_4Se$ + Na] requires 508.07474, found 508.07443. To a cooled (0°C) solution of the intermediate (1.4 g, 3.4 mmol) and BnBr (1.0 ml, 8.5 mmol) in DMF (23 ml) NaH (60% disp.) (0.34 g, 8.5 mmol) was added. After stirring overnight, H_2O was added at 0°C and the mixture was diluted with Et_2O. The organic layer was washed with H_2O (2x) and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded compound **17** (1.67 g, 2.8 mmol) in 83% yield. TLC: R_f 0.8 (10% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 1): +172; IR (neat, cm^{-1}): 3057, 3030, 2908, 2866, 2108, 1099, 1066, 817, 738, 696; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.86 – 7.15 (m, 23H), 5.94 (d, *J* = 5.2 Hz, 1H), 4.97 – 4.55 (m, 4H), 4.43 – 4.36 (m, 4H), 4.08 – 4.07 (m, 1H), 4.77 (dd, *J* = 2.4 Hz, 10.6 Hz, 1H), 3.63 – 3.58 (m, 1H), 3.47 – 3.41 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): 138.3, 137.9, 135.0, 134.8, 133.4, 133.2, 129.1, 128.5, 128.4, 128.1, 127.9, 127.8, 127.7, 126.6, 126.3, 126.2, 125.7, 85.5, 80.3, 75.0, 73.5, 73.2, 72.5, 72.0, 68.4, 61.2; HRMS: [$C_{37}H_{35}N_3O_4Se$ + Na] requires 688.16883, found 688.16858.

2-azido-4,6-di-*O*-benzyl-2-deoxy-3-*O*-naphthylmethyl-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-galactopyranoside (18)

To a cooled (0°C) solution of **17** (0.84 g, 1.4 mmol) in a mixture of THF/H_2O (14 ml, 1/1, v/v) NIS (0.35 g, 1.54 mmol) was added. The reaction was allowed to warm up to RT and stirred overnight. The mixture was diluted with EtOAc, washed with $Na_2S_2O_3$ (10% solution in H_2O), H_2O, brine, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded intermediate 2-azido-4,6-di-*O*-benzyl-2-deoxy-3-*O*-napthyl-α/β-D-galactopyranose (0.64 g, 1.4 mmol) in >98% yield. TLC: R_f 0.4 (30% EtOAc/pentane); IR (neat, cm^{-1}): 3389, 3059, 2916, 2868, 2108, 1096, 1059, 818, 746, 696; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.80 – 7.01 (m, 29H), 5.28 (d, *J* = 2.8 Hz, 1H), 4.87 – 4.30 (m, 10H), 4.15 – 4.12 (m, 1H), 3.99 – 3.82 (m, 4H), 3.77 – 3.76 (m, 1H), 3.56 – 3.52 (m, 2H), 3.48 – 3.43 (m, 1H), 3.37 – 3.34 (m, 1H), 3.28 – 3.25 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.0, 137.9, 137.4, 137.1, 135.0, 134.9, 133.2, 133.1, 132.9, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127., 127.3, 126.8, 126.4, 126.3, 126.1, 126.0, 125.9, 125.6, 96.2, 92.1, 80.6, 77.2, 74.5, 73.5, 73.4, 73.3, 73.2, 72.3, 72.2, 72.1, 69.3, 69.2, 68.5, 64.3, 60.2; HRMS: [$C_{31}H_{31}N_3O_5$ + Na] requires 548.21559, found 548.21535. To a stirred solution of the intermediate (0.31 g, 0.5 mmol) in acetone (5 ml) K_2CO_3 (0.082 g, 0.6 mmol) and *N*-phenyl trifluoroacetimidoyl chloride (0.12 ml, 0.75 mmol) were added. After stirring for 4 days at RT, $MgSO_4$ was added, the mixture was filtered and concentrated *in vacuo*. Column chromatography yielded imidate **18** (0.32 g, 0.4 mmol) in 80% yield. TLC: R_f 0.75 (10% EtOAc/pentane); IR (neat, cm^{-1}): 3061, 3030, 2918, 2886, 2113, 1716, 1209, 1161, 1112, 736, 696; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.84 – 7.90 (m, 10H), 7.51 – 7.46 (m, 8H), 7.31 -7.21 (m, 28H), 7.08 – 7.03 (m, 3H), 6.81 – 6.79 (m, 4H), 6.32 (s, 1H), 5.42 (d, *J* = 7.2 Hz, 1H), 4.93 – 4.84 (m, 6H), 4.62 – 4.40 (m, 6H), 4.17 – 4.00 (m, 5H), 3.94 – 3.93 (m, 1H), 3.68 – 3.36 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 135.4, 133.6, 129.4, 129.1, 128.8, 128.4, 128.0, 127.8, 127.0, 126.5, 126.1, 124.7, 124.4 119.8, 119.5, 96.4, 96.1, 81.1, 77.9, 75.2, 75.1, 75.0, 73.9, 73.8, 73.6, 73.1, 72.9, 72.6, 62.7, 59.8 ; HRMS: [$C_{39}H_{35}F_3N_4O_5$ + Na] requires 719.24518, found 719.24518.

Phenyl 2-azido-4,6-di-*O*-benzyl-2-deoxy-1-seleno-α-D-galactopyranoside (19)

To a stirred solution of **17** (0.71 g, 1.2 mmol) in a mixture of DCM/H_2O (14 ml, 4/1, v/v), DDQ (0.63 g, 2.8 mmol) was added. The mixture was stirred for 2h, after which TLC analysis showed conversion of the starting material and formation of a lower running byproduct. $Na_2S_2O_3$ (10% solution in H_2O) was added, the mixture was diluted with DCM and the layers were separated. The organic layer was washed with sat. aq. $NaHCO_3$ (2x), dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded compound **19** (0.49 g, 0.94 mmol) in 78% yield. TLC: R_f 0.61 (20% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 1): +174; IR (neat,

cm- 1): 3442, 3062, 2922, 2106, 1089, 1055, 731, 692; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30 – 6.79 (m, 15H), 5.90 (d, *J* = 5.2 Hz, 1H), 4.71 – 4.59 (m, 2H), 4.52 – 4.40 (m, 3H), 4.04 (dd, *J* = 5.2 Hz, 10.4 Hz, 1H), 3.89 – 3.88 (m, 1H), 3.76 – 3.70 (m, 1H), 3.67 – 3.62 (m, 1H), 3.51 – 3.45 (m, 1H), 2.52 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 137.9, 137.7, 134.7, 134.3, 129.3, 129.1, 128.6, 128.5, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8, 125.3, 85.2, 76.1, 75.4, 73.4, 68.3, 68.1, 62.6 ; HRMS: [$C_{26}H_{27}N_3O_4Se$ + Na] requires 548.10607, found 548.10583.

1-*O*-allyl-3-*O*-benzyl-*sn*-glycerol (21)

Bimetalic catalysis: To a stirred solution of **20** (0.09 g, 0.5 mmol), DPPB (0.05 g, 0.13 mmol), $Pd(OAc)_2$ (0.011 g, 0.05 mmol), Me_2SnCl_2 (0.011 g, 0.05 mmol), and Cs_2CO_3 (0.24 g, 0.75 mmol) in DCM (3 ml), allylacetate (0.16 ml, 1.5 mmol) was added. After stirring overnight at RT, H_2O was added and the mixture was extracted with EtOAc. The organic layer was washed with H_2O (2x), brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography, yielding compound **21** (0.09 g, 0.42 mmol) in 84% yield.

Tin ketal: A mixture of **20** (0.09 g, 0.5 mmol) and dibutyltinoxide (0.12 g, 0.5 mmol) was refluxed in toluene (10 ml) for 3h. The mixture was concentrated *in vacuo*, taken up in DMF (3 ml) and CsF (0.11 g, 0.725 mmol) and allylbromide (0.056 ml, 0.65 mmol) were added. The reaction was stirred for 1h, and refluxed for 1h, after which TLC analysis indicated complete conversion of the starting material. The mixture was concentrated *in vacuo*, taken up in EtOAc, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography, yielding compound **21** (0.073 g, 0.33 mmol) in 66% yield.

Borinate catalysis: To a stirred solution of **20** (0.09 g, 0.5 mmol), 2-aminoethyl diphenylborinate (0.011 g, 0.05 mmol), KI (0.08 g, 0.5 mmol), K_2CO_3 (0.08 g, 0.55 mmol) in CH_3CN (2.5 ml) under an argon atmosphere, allyl bromide (0.065 ml, 0.75 mmol) was added, and the mixture was heated to 60°C. After stirring overnight, TLC analysis indicated complete conversion of the starting material. The mixture was diluted with EtOAc, washed with H_2O (2x), brine, dried over $MgSO_4$ and concentrated *in vacuo.* The residue was purified by column chromatography, yielding compound **21** (0.073 g, 0.33 mmol) in 66% yield. TLC: R_f 0.6 (50% EtOAc/pentane); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35 – 7.24 (m, 5H), 5.89 (ddd, *J* = 22.5 Hz, 10.8 Hz, 5.6 Hz, 1H), 5.27 – 5.15 (m, 2H), 4.53 (s, 2H), 3.94 (d, *J* = 5.6 Hz, 3H), 3.55 – 3.43 (m, 4H), 2.89 (s, 1H, OH); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.0, 134.5, 128.4, 127.7, 117.2, 73.4, 72.3, 71.4, 71.3, 69.5.

3-*O*-allyl-1-*O*-benzyl-*sn*-glycerol (23)

To a stirred solution of **22** (1.8 g, 9.9 mmol), 2-aminoethyl diphenylborinate (0.22 g, 0.99 mmol), KI (1.64 g, 9.9 mmol), K_2CO_3 (1.5 g, 10.9 mmol) in CH_3CN (50 ml) under an argon atmosphere, allyl bromide (1.3 ml, 14.8 mmol) was added, and the mixture was heated to 60°C. After stirring overnight, TLC analysis indicated complete conversion of the starting material. The mixture was diluted with EtOAc, washed with H_2O (2x), brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography, yielding compound **23** (1.4 g, 6.1 mmol) in 62% yield. TLC: R_f 0.6 (50% EtOAc/pentane); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35 – 7.24 (m, 5H), 5.89 (ddd, *J* = 22.5 Hz, 10.8 Hz, 5.6 Hz, 1H), 5.27 – 5.15 (m, 2H), 4.53 (s, 2H), 3.94 (d, *J* = 5.6 Hz, 3H), 3.55 – 3.43 (m, 4H), 2.89 (s, 1H, OH); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.0, 134.5, 128.4, 127.7, 117.2, 73.4, 72.3, 71.4, 71.3, 69.5.

Phenyl 3-*O*-(2-azido-3,4-*O*-di-benzyl-2-deoxy-6-*O*-(*tert*-butyl-di-phenylsilyl)-α-D-galactopyranosyl)-2-azido-4,6-*O*-di-benzyl-2-deoxy-1-seleno-α-D-galactopyranoside (24)

Donor **15** (6.86 g, 8.6 mmol) and acceptor **19** (5.43 g, 10.4 mmol) were coevaporated with toluene (2x) and dissolved in DCM (85 ml). Molecular sieves (3Å) were added and the mixture was stirred at 0°C for 10 minutes. TfOH (0.15 ml, 1.7 mmol) was added and the mixture was stirred for 30 minutes. After addition of NEt_3, DCM was added and the organic layer was washed with H_2O, brine, was dried over $MgSO_4$ and concentrated in vacuo. Column chromatography yielded compound **24** (5.7 g, 5.0 mmol) in 58% yield as a single anomer. TLC: R_f 0.4 (10% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 1): +102; IR (neat, cm^{-1}): 2927, 2856, 2110, 1726, 1112, 1051, 738, 700; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68 – 7.15 (m, 35H), 5.98 (d, *J* = 5.2 Hz, 1H), 5.20 (d, *J* = 3.6 Hz, 1H), 5.05 – 4.73 (m, 4H), 4.58 – 4.52 (m, 2H), 4.44 – 4.41 (m, 1H), 4.37 (s, 2H), 4.31 – 4.72 (m, 1H), 4.22 – 4.15 (m, 3H), 4.08 – 4.00 (m, 2H), 3.95 – 3.90 (m, 1H), 3.87 – 3.81 (m, 2H), 3.60 – 3.56 (m, 1H), 3.38 – 3.32 (m, 1H), 1.10 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.5, 138.3, 137.8, 137.6, 135.6, 135.0, 134.9, 133.4, 133.0, 131.0, 129.9, 129.1, 128.9, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 127.5, 94.4 (C-1'), 84.5 (C- 1), 77.5, 75.2, 75.0 (C-3'), 74.9, 73.7, 73.4, 72.7, 71.7, 71.6, 71.5, 68.0, 61.9, 61.0, 59.6, 27.2; HRMS: $[C_{62}H_{66}N_6O_8SeSi + 2H]^{2+}$ requires 566.20158, found 566.19305.

3-*O*-(2-azido-3,4-*O*-di-benzyl-2-deoxy-6-*O*-(*tert*-butyl-di-phenylsilyl)-α-D-galactopyranosyl)-2-azido-4,6-*O*-di-benzyl-2-deoxy-α/β-D-galactopyranose (25)

To a cooled (0°C) solution of **24** (0.06 g, 0.05 mmol) in THF/H_2O (0.5 ml, 1/1, v/v,) was added NIS (0.012 g, 0.05 mmol) . The mixture was allowed to warm up to RT and stirred 2h, after which $Na_2S_2O_3$ (10% solution in H_2O) was added to the reaction and it was diluted with EtOAc. The organic layer was washed with H_2O (2x), brine, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded compound **25** (0.05 g, 0.05 mmol) in >98% yield. TLC: R_f 0.2 (20% EtOAc/pentane); IR (neat, cm^{-1}): 3390, 3030, 2927, 2856, 2108, 1496, 1103, 1047, 1026, 734, 696; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 – 7.15 (m, 80H), 5.34 (d, *J* = 3.2 Hz, 1H), 5.13 (d, *J* = 3.2 Hz, 2H), 5.03 – 5.01 (m, 2H), 4.91 – 4.87 (m, 2H), 4.76 – 4.73 (m, 4H), 4.56 – 4.52 (m, 5H), 4.47 – 4.33 (m, 3H), 4.18 – 4.02 (m, 8H), 3.91 – 3.72 (m, 6H), 3.59 – 3.40 (m, 4H) 1.05 (s, 20H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.5, 138.4, 138.3, 138.1, 137.7, 137.5, 135.6, 133.4, 133.3, 133.1, 129.9, 129.8, 128.9, 128.7, 129.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 96.6, 95.7, 94.3, 92.3, 77.7, 77.4, 75.5, 75.1 – 74.6, 74.0, 73.7, 73.6, 73.5, 73.4, 72.7, 72.6, 71.9, 70.5, 69.6, 69.0, 62.7, 62.0, 60.3, 60.0, 59.7, 27.0; HRMS: [$C_{56}H_2N_6O_9Si$ + Na] requires 952.95447, found 952.42582.

3-*O*-(2-azido-3,4-*O*-di-benzyl-2-deoxy-6-*O*-(*tert*-butyl-di-phenylsilyl)-α-D-galactopyranosyl)-2-azido-4,6-*O*-di-benzyl-2-deoxy-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-galactopyranoside (26)

To a stirred solution of **25** (0.05 g, 0.05 mmol) in acetone (0.5 ml) was added K_2CO_3 (8 mg, 0.06 mmol) and *N*-phenyl trifluoroacetimidoyl chloride (0.01 ml, 0.07 mmol) were added. The reaction mixture was stirred for 3 days under a dry atmosphere at RT. The reaction mixture was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded compound **26** (0.056 g, 0.05 mmol) in >98% yield. TLC: R_f 0.76 (20% EtOAc/pentane); IR (neat, cm^{-1}): 3311, 2954, 2924, 2854, 2112, 1714, 1207, 1151, 1112, 731, 696; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 – 6.78 (m, 35H), 5.17 – 5.16 (m, 1H), 5.06 – 5.03 (m, 1H), 4.94 – 4.89 (m, 1H), 4.80 – 4.75 (m, 3H), 4.59 – 4.55 (m, 3H), 4.46 – 4.37 (m, 1H), 4.15 – 3.91 (m, 6H), 3.78 – 3.58 (m, 5H), 1.07 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.3, 138.2, 137.6, 137.5, 135.6, 133.3, 133.1, 129.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 124.5, 119.4, 94.5, 77.53, 75.6, 75.1, 74.9 74.5, 73.6, 73.6, 72.7, 72.0, 70.4, 67.8, 62.7, 61.7, 59.7, 27.0; HRMS: [$C_{64}H_{66}F_3N_7O_9Si$ + Na] requires 1184.45356, found 1184.45410.

2-*O*-(2-azido-4,6-di-*O*-benzyl-2-deoxy-3-*O*-naphthylmethyl-β-D-galactopyranosyl)-1-*O*-allyl-3-*O*-benzyl-*sn*-glycerol (27)

Donor **18** (1.67 g, 2.4 mmol) and acceptor **21** (0.80 g, 3.6 mmol) were coevaporated with toluene (2x) and dissolved in a mixture of acetonitrile, propionitrile and DCM (24 ml, 2:1:1 v/v/v). Molecular sieves (3Å) were added and the mixture was stirred at -40°C for 10 minutes. TfOH (45 µl, 0.5 mmol) was added and the mixture was stirred for 20 minutes. After addition of NEt_3, DCM was added and the organic layer was washed with H_2O, brine, was dried over $MgSO_4$ and concentrated in vacuo. Size exclusion chromatography yielded compound **27** (1.6 g, 2.2 mmol) in 90% yield (α/β = 1 : 19). TLC: R_f 0.85 (20% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 1): -8; IR (neat, cm^{-1}): 3061, 2922, 2860, 2110, 1454, 1064, 817, 732, 696; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 – 7.80 (m, 4H), 7.51 – 7.46 (m, 3H), 7.32 – 7.22 (m, 16H), 5.90 (ddd, *J* = 22.7 Hz, 10.4 Hz, 5.6 Hz, 1H), 5.29 – 5.24 (m, 1H), 5.16 – 5.13 (m, 1H), 4.93 – 4.90 (m, 1H), 4.82 (s, 2H), 4.67 – 4.37 (m, 6H),4.06 – 4.00 (m, 3H), 3.89 – 3.84 (m, 2H), 3.71 – 3.45 (m, 7H), 3.32 (dd, *J* = 2.8 Hz, 10.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 139.1, 138.9, 138.1, 127.8, 133.3, 131.9, 131.7, 128.6, 128.4, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.6, 126.6, 126.3, 126.1, 125.8, 116.9, 102.7, 80.6, 78.1, 74.4, 73.6, 73.5, 73.4, 72.7, 72.5, 72.4, 71.0, 70.3, 68.6, 63.5; HRMS: [$C_{44}H_{47}N_3O_7$ + Na] requires 752.33062, found 752.33026.

2-*O*-(2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-1-*O*-allyl-3-*O*-benzyl-*sn*-glycerol (28)

HCl/HFIP method: To a stirred solution of **27** (0.46 g, 0.6 mmol) in a mixture of HFIP/DCM (7 ml, 1/1 v/v) was added a solution of HCl (3.5 ml, 0.2M, 0.7 mmol) in HFIP. The mixture was stirred for 30 min, after which TLC analysis indicated complete conversion of the starting material. The mixture was poured into sat. aq. $NaHCO_3$, DCM was added and the layers were separated. The organic layer was washed with sat. aq. $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded compound **28** (0.21 g, 0.35 mmol) in 63% yield.

Buffered DDQ method: To a cooled (0°C) solution of **27** (0.42 g, 0.57 mmol) in DCM (20 ml) a phosphate buffer (2 ml, pH=7.5, 10 mM) was added. DDQ (0.41 g, 1.8 mmol) was added over 1h in small portions, after which the mixture was allowed to warm up to RT and was stirred for 30 min. The mixture was diluted with $NaHCO_3$ and the aqueous layer was extracted with DCM (2x). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded compound **28** (0.25 g, 0.42 mmol) in 75% yield. TLC: R_f 0.8 (20% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 1): +42; IR (neat, cm^{-1}): 3447, 2916, 2863, 2110, 1454, 1064, 910, 732, 696; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34 –

7.21 (m, 15H), 5.90 (ddd, *J* =22.6 Hz, 10.6 Hz, 5.6 Hz, 1H), 4.71 – 4.65 (m, 2H), 4.55 – 4.41 (m, 5H), 4.13 – 3.96 (m, 3H), 3.78 (d, *J* = 3.2 Hz, 1H), 3.72 – 3.35 (m, 7H), 3.43 -3.35 (m, 1H), 2.41 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.3, 138.2, 137.7, 134.4, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 116.9, 102.6, 78.0, 75.2, 75.2, 73.5, 73.4, 73.3, 72.6, 71.0, 70.2, 68.3, 64.8; HRMS: [$C_{33}H_{39}N_3O_7$ + Na] requires 612.28602, found 612.28746.

2-*O*-(3-*O*-(2-azido-3,4-*O*-di-benzyl-2-deoxy-6-*O*-(*tert*-butyl-di-phenylsilyl)-α-D-galactopyranosyl)-2-azido-4,6-*O*-di-benzyl-2-deoxy-β-D-galactopyranosyl)-1-*O*-allyl-3-*O*-benzyl-*sn*-glycerol (10)

From 28: Donor **15** (0.75 g, 1.3 mmol) and acceptor **28** (1.35 g, 1.7 mmol) were coevaporated with toluene (2x) and dissolved in DCM (15 ml). Molecular sieves (3Å) were added and the mixture was stirred at 0°C for 10 minutes. TfOH (23 µl, 0.26 mmol) was added and the mixture was stirred for 30 minutes. After addition of NEt_3, DCM was added and the organic layer was washed with H_2O, brine, was dried over $MgSO_4$ and concentrated in vacuo. Column chromatography yielded compound **10** (1.36 g, 1.14 mmol) in 88% yield as α-anomer only.

From 26: Donor **26** (0.06 g, 0.05 mmol) and acceptor **21** (0.02 g, 0.1 mmol) were coevaporated with toluene (2x) and dissolved in a mixture of acetonitrile, propionitrile and DCM (0.5 ml, 2:1:1 v/v/v). Molecular sieves (3Å) were added and the mixture was stirred at -40°C for 10 minutes. TfOH (1 µl, 10 µmol) was added and the mixture was stirred for 20 minutes. After addition of NEt_3, DCM was added and the organic layer was washed with H_2O, brine, was dried over $MgSO_4$ and concentrated in vacuo. Size exclusion chromatography yielded compound **10** (0.37 g, 0.03 mmol) in 70% yield (α/β = 1 : 19). TLC: R_f 0.6 (10% EtOAc/toluene); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 1): +54; IR (neat, cm^{-1}): 2929, 2910, 2856, 2110, 1454, 1103, 1078, 1028, 736, 700; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 – 7.62 (m, 4H), 7.43 – 7.20 (m, 31H), 5.87 (ddd, *J* = 22.4 Hz, 10.4 Hz, 5.6 Hz, 1H), 5.25 – 5.21 (m, 1H), 5.14 -5.01 (m, 2H), 5.02 (d, *J* = 12 Hz, 1H), 4.88 (d, *J* = 11.2 Hz, 1H), 4.76 (s, 2H), 4.56 – 4.51 (m, 4H), 4.40 – 4.38 (m, 3H), 4.22 – 4.20 (m, 1H), 4.13 – 4.10 (m, 1H), 4.04 – 3.98 (m, 5H), 3.90 – 3.82 (m,2H), 3.76 – 3.47 (m, 9H), 3.44 – 3.41 (m, 1H), 1.06 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 138.5, 138.4, 137.9, 137.7, 135.7, 135.6, 134.9, 133.5, 133.2, 129.9, 129.8, 128.8, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.4, 116.9, 102.6, 94.5, 78.4, 77.7, 75.6, 75.0, 74.7, 73.7, 73.6, 73.4, 73.3, 72.6, 72.5, 71.8, 70.8, 70.4,

70.3, 68.4, 62.8, 62.7, 59.9, 27.0; HRMS: [$C_{69}H_{78}N_6O_{11}Si$ + Na] requires 1217.53900, found 1217.53846.

2-*O*-(3-*O*-[2-*N*-acetamido-3,4-di-*O*-benzyl-6-*O*-*tert*-butyl-di-phenylsilyl-α-D-galactopyranosyl]-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-1-*O*-allyl-3-*O*-benzyl-*sn*-glycerol (29)

Di-azido-pseudotrisaccharide **10** (0.93 g, 0.78 mmol) was dissolved in a mixture of dioxane (4.5 ml) and water (0.9 ml). PMe_3 (1M in THF, 3 ml, 3 mmol) was added to the mixture and it was stirred for 0.5h. The mixture was concentrated, coevaporated with toluene and dissolved in DCM (10 ml). Triethylamine (0.33 ml, 2.3 mmol) and Ac_2O (0.14 ml, 1.5 mmol) were added and the mixture was stirred for 1.25h. The organic layer was washed with sat.aq. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded di-acetylated pseudotrisaccharide **29** (0.56 g, 0.46 mmol) in 59% yield. TLC: R_f=0.3 (50% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 0.2): +113; IR (neat, cm^{-1}): 2859, 1661, 1454, 1112, 1056, 1027, 696, 667; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 – 7.54 (m, 6H), 7.48 – 7.37 (m, 8H), 7.37 – 7.21 (m, 44H), 7.18 (s, 5H), 5.82 (ddd, *J* = 22.7, 10.0, 4.7 Hz, 3H), 5.55 (d, *J* = 8.1 Hz, 1H), 5.26 – 5.17 (m, 2H), 5.15 – 5.09 (m, 3H), 4.94 (d, *J* = 11.7 Hz, 1H), 4.86 – 4.68 (m, 4H), 4.64 (d, *J* = 12.1 Hz, 1H), 4.55 (d, *J* = 12.2 Hz, 1H), 4.52 – 4.44 (m, 9H), 4.44 – 4.33 (m, 3H), 4.31 – 4.19 (m, 1H), 3.97 – 3.86 (m, 8H), 3.81 (s, 4H), 3.74 – 3.57 (m, 9H), 3.57 – 3.41 (m, 10H), 1.86 (s, 3H), 1.51 (s, 3H), 1.08 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.1, 170.0, 138.3, 138.0, 137.5, 135.6, 135.4, 135.3, 134.3, 132.9, 132.9, 129.8, 129.8, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 127.2, 126.7, 126.6, 117.1, 102.1, 93.9, 78.5, 77.9, 77.3, 77.0, 76.7, 75.7, 74.1, 73.9, 73.2, 73.1, 73.1, 72.9, 72.3, 72.1, 71.8, 71.1, 71.1, 69.9, 67.9, 63.5, 51.9, 48.7, 26.9, 23.5, 22.7, 19.1; HRMS: [$C_{73}H_{86}N_2O_{13}Si$ +H] requires 1227.59719, found 1227.59871.

2-*O*-(3-*O*-[2-*N*-acetamido-3,4-di-*O*-benzyl-α-D-galactopyranosyl]-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-1-*O*-allyl-3-*O*-benzyl-*sn*-glycerol (31)

Silyl-protected pseudotrisaccharide **29** (0.6 g, 0.5 mmol) was coevaporated with toluene and was dissolved in THF (10 ml). TBAF (1M in THF, 0.54 ml, 0.54 mmol) was added and the reaction was stirred for 30min. The mixture was concentrated and subsequent column chromatography yielded alcohol **31** (0.37 g, 0.37 mmol) in 77% yield. TLC: R_f=0.1 (50% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 0.2): +61; IR (neat, cm^{-1}): 3331, 3063, 2912, 2870, 1699, 1645, 1538, 1118, 1014, 734, 695; ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.47 – 7.21 (m, 32H), 7.16 (dd, *J* = 21.5, 9.0 Hz, 2H), 5.96 – 5.84 (m, 1H), 5.26 (dd, *J* = 17.8, 2.4 Hz, 2H), 5.12 (d,

J = 10.5 Hz, 1H), 4.95 (dd, *J* = 21.3, 11.5 Hz, 2H), 4.79 (d, *J* = 11.5 Hz, 1H), 4.74 – 4.66 (m, 2H), 4.66 – 4.58 (m, 2H), 4.56 – 4.42 (m, 4H), 4.39 – 4.28 (m, 1H), 4.15 (s, 1H), 4.10 (t, *J* = 6.3 Hz, 1H), 4.03 – 3.92 (m, 6H), 3.87 (dd, *J* = 11.2, 2.2 Hz, 1H), 3.81 (dd, *J* = 10.6, 7.0 Hz, 1H), 3.75 – 3.57 (m, 7H), 3.57 – 3.49 (m, 4H), 1.97 (s, 3H), 1.70 (s, 3H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 170.7, 170.3, 140.1, 140.1, 139.7, 139.7, 139.4, 136.2, 129.0, 129.0, 128.9, 128.9, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 116.4, 103.0, 95.7, 78.6, 78.3, 77.0, 75.1, 75.0, 74.6, 74.0, 73.6, 73.5, 73.4, 72.6, 72.6, 72.5, 71.1, 70.8, 69.7, 62.4, 52.2, 50.3, 30.4, 30.2, 30.0, 29.8, 29.6, 29.5, 23.7, 23.0; HRMS: [$C_{57}H_{68}N_2O_{13}$ +H] requires 989.47942, found 989.48077.

2-*O*-(3-*O*-[2-*N*-acetamido-3,4-di-*O*-benzyl-6-*O*-{4,4'-di-methoxy-tri-phenylmethyl}-α-D-galactopyranosyl]-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-1-*O*-allyl-3-*O*-benzyl-*sn*-glycerol (33)

Pseudotrisaccharide **31** (0.26 g, 0.26 mmol) was coevaporated with toluene three times and was dissolved in DCM (2.6 ml). Triethylamine (0.06 ml, 0.4 mmol) and 4,4'-dimethoxytrityl-chloride (0.098 g, 0.3 mmol) were added and the mixture was stirred for 23h. Water was added and the organic phase was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography with neutralized silica yielded protected pseudotrisaccharide **33** (0.30 g, 0.24 mmol) in 89% yield. TLC: R_f=0.2 (50% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 0.2): +43; IR (neat, cm^{-1}): 3032,2861, 1669, 1508, 1247, 1061, 1027, 736, 696; ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.53 (d, *J* = 7.5 Hz, 2H), 7.46 (d, *J* = 7.3 Hz, 5H), 7.42 – 7.18 (m, 39H), 7.12 (dd, *J* = 6.5, 2.9 Hz, 3H), 6.91 (dd, *J* = 8.9, 5.4 Hz, 4H), 6.59 (d, *J* = 8.8 Hz, 1H), 5.92 – 5.79 (m, 1H), 5.35 (d, *J* = 3.5 Hz, 1H), 5.24 (dd, *J* = 17.3, 1.8 Hz, 1H), 5.09 (dd, *J* = 10.5, 1.7 Hz, 1H), 4.99 (d, *J* = 11.5 Hz, 1H), 4.93 – 4.86 (m, 1H), 4.83 (d, *J* = 10.3 Hz, 1H), 4.79 (s, 1H), 4.74 (ddd, *J* = 12.1, 9.0, 3.5 Hz, 2H), 4.68 (d, *J* = 11.7 Hz, 1H), 4.61 (d, *J* = 11.5 Hz, 1H), 4.53 (s, 2H), 4.52 – 4.41 (m, 3H), 4.36 (q, *J* = 8.7 Hz, 1H), 4.31 – 4.24 (m, 1H), 4.19 (s, 1H), 4.15 (dd, *J* = 10.9, 2.3 Hz, 1H), 4.10 – 4.03 (m, 3H), 3.98 – 3.90 (m, 3H), 3.77 (s, 9H), 3.73 – 3.65 (m, 4H), 3.65 – 3.53 (m, 7H), 3.26 (s, 2H), 3.14 (dd, *J* = 9.1, 4.7 Hz, 1H), 1.92 (s, 3H), 1.72 (s, 3H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 170.4, 170.1, 159.4, 146.0, 139.9, 139.8, 139.6, 139.6, 139.2, 136.8, 136.4, 136.0, 131.0, 130.9, 129.0, 129.0, 128.9, 128.9, 128.7, 128.6, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.5, 116.7, 113.9, 113.8, 102.9, 94.8, 86.9, 79.0, 78.4, 76.2, 75.1, 74.8, 74.7, 74.3, 73.6, 73.5, 72.8, 72.6, 72.6, 71.4, 71.1, 70.8, 69.5, 64.4, 55.5, 52.6, 50.0, 30.2, 30.0, 29.8, 29.6, 29.5, 23.8, 23.0; HRMS: [$C_{78}H_{86}N_2O_{15}$ +H] requires 1291.61010, found 1291.61161.

2-*O*-(3-*O*-[2-*N*-acetamido-3,4-di-*O*-benzyl-6-*O*-{4,4'-di-methoxy-tri-phenylmethyl}-α-D-galactopyranosyl]-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-3-*O*-benzyl-*sn*-glycerol (35)

Allyl-protected pseudotrisaccharide **33** (0.44, 0.34 mmol) was coevaporated with toluene three times and was dissolved in distilled THF (3.5 ml). The mixture was purged of oxygen by bubbling through argon for 10 minutes. (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (0.035 g, 0.04 mmol) was added and H_2 gas was bubbled through for 5 seconds. Argon was applied and the reaction mixture was stirred for 2.5h, yielding the isomerised intermediate. The reaction mixture of was diluted with THF (5.5 ml) and sat. aq. $NaHCO_3$ (6 ml) was added. Iodine (0.13 g, 0.5 mmol) was added and the reaction was stirred for 1h. The mixture was diluted with EtOAc, washed with sat. aq. $Na_2S_2O_3$ and brine, dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography yielded deprotected pseudotrisaccharide **35** (0.30 g, 0.24 mmol) in 70 % yield. TLC: R_f=0.1 (75% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 0.2): +25; IR (neat, cm^{-1}): 3100, 2929, 2910, 1699, 1509, 1247, 829, 732, 697; ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.52 (d, *J* = 7.4 Hz, 2H), 7.49 – 7.42 (m, 6H), 7.42 – 7.19 (m, 42H), 7.11 (dd, *J* = 6.6, 2.9 Hz, 3H), 6.99 (d, *J* = 8.3 Hz, 1H), 6.91 (dd, *J* = 8.9, 6.8 Hz, 4H), 5.36 (d, *J* = 3.6 Hz, 1H), 5.00 (d, *J* = 3.4 Hz, 1H), 4.98 (s, 1H), 4.83 (dd, *J* = 17.9, 11.6 Hz, 2H), 4.78 – 4.69 (m, 2H), 4.67 (s, 0H), 4.60 (d, *J* = 11.5 Hz, 1H), 4.51 (s, 2H), 4.46 (d, *J* = 6.7 Hz, 2H), 4.44 – 4.40 (m, 1H), 4.39 – 4.30 (m, 1H), 4.27 (t, *J* = 6.0 Hz, 1H), 4.22 – 4.17 (m, 1H), 4.16 – 4.09 (m, 1H), 4.09 – 4.04 (m, 2H), 4.02 – 3.94 (m, 5H), 3.76 (s, 10H), 3.74 – 3.65 (m, 4H), 3.65 – 3.52 (m, 5H), 3.34 (s, 2H), 3.15 (dd, *J* = 9.0, 5.0 Hz, 1H), 1.96 (s, 3H), 1.71 (s, 3H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 171.2, 170.2, 159.4, 159.4, 145.9, 139.9, 139.7, 139.6, 139.5, 139.2, 136.8, 136.4, 132.6, 132.5, 130.9, 130.8, 129.4, 129.3, 129.0, 129.0, 128.9, 128.9, 128.7, 128.6, 128.4, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 127.9, 127.5, 113.9, 113.8, 103.0, 94.7, 86.9, 80.8, 78.3, 76.2, 75.0, 74.8, 74.7, 74.3, 73.6, 73.5, 72.8, 72.5, 71.4, 70.8, 69.5, 64.3, 63.5, 55.4, 52.9, 49.9, 30.2, 30.0, 29.8, 29.6, 29.5, 23.7, 23.0; HRMS: [$C_{75}H_{82}N_2O_{15}$ +H] requires 1251.57880, found 1251.57979.

2-*O*-(3-*O*-[2-*N*-acetamido-3,4-di-*O*-benzyl-6-*O*-{4,4'-di-methoxy-tri-phenylmethyl}-α-D-galactopyranosyl]-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl)- 3-*O*-benzyl -1-*O*-([*N, N'*-di-isopropylamino]-2-cyanoethyl-phosphite)-*sn*-glycerol (7)

Pseudotrisaccharide **35** (0.37 g, 0,29 mmol) was dissolved in DCM (3 ml). Diisopropylethylamine (0.077 ml, 0.44 mmol) and 3Å molsieves were added and the resulting

solution was stirred for 45 minutes. *N*, *N*'-di-isopropylamino-2-cyanoethyl-chlorophosphite (0.078 ml, 0.35 mmol) was added and the reaction mixture was stirred for 2h. Water was added, the organic phase was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography with neutralized silica yielded phosphoramidite **7** (0.36 g, 0.25 mmol) in 85% yield. TLC: R_f=0.8 (80% EtOAc/pentane); ^{1}H NMR (400 MHz, CD_3CN) δ 7.50 (d, *J* = 7.7 Hz, 2H), 7.45 – 7.22 (m, 42H), 7.13 – 7.03 (m, 3H), 6.93 – 6.76 (m, 4H), 6.39 – 6.29 (m, 1H), 5.44 (d, *J* = 2.4 Hz, 1H), 5.21 (s, 1H), 4.85 (dd, *J* = 11.1, 2.1 Hz, 1H), 4.81 – 4.71 (m, 3H), 4.59 (d, *J* = 11.6 Hz, 1H), 4.55 – 4.40 (m, 7H), 4.35 (d, *J* = 11.3 Hz, 1H), 4.19 – 4.14 (m, 1H), 4.14 – 4.03 (m, 3H), 4.03 – 3.92 (m, 3H), 3.83 – 3.53 (m, 17H), 3.47 (q, *J* = 8.0, 7.2 Hz, 1H), 3.04 (td, *J* = 8.9, 4.4 Hz, 1H), 2.64 – 2.47 (m, 5H), 1.93 (d, *J* = 5.7 Hz, 3H), 1.70 (s, 3H), 1.26 – 1.09 (m, 12H); ^{13}C NMR (101 MHz, CD_3CN) δ 171.2, 170.8, 159.6, 146.1, 139.7, 139.6, 139.3, 136.9, 136.6, 131.2, 131.1, 129.4, 129.3, 129.3, 129.2, 129.1, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.4, 127.8, 119.6, 118.3, 114.1, 114.0, 102.4, 94.8, 87.1, 79.1, 78.3, 76.1, 76.0, 75.1, 75.0, 74.9, 74.3, 73.8, 73.7, 73.0, 72.7, 71.5, 70.6, 70.4, 69.6, 64.6, 61.0, 59.6, 59.4, 59.3, 59.1, 55.9, 52.4, 49.8, 43.9, 43.7, 25.1, 25.0, 23.9, 23.2, 21.0, 14.6; ^{31}P NMR (162 MHz, CD_3CN) δ 147.8, 147.7.

2-*O*-(3-*O*-[2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*tert*-butyl-di-phenylsilyl-α-D-galactopyranosyl]-2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-3-*O*-allyl-1-*O*-benzyl-*sn*-glycerol (11)

Disaccharide donor **26** (2.33 g, 2.0 mmol) and glycerol acceptor **23** (0.67 g, 3.0 mmol) were coevaporated with toluene (2x) and dissolved in a mixture of acetonitrile, propionitrile and DCM (10 ml, 2:1:1 v/v/v). Molecular sieves (3Å) were added and the mixture was stirred at -40°C for 10 minutes. TfOH (35 µl, 0.4 mmol) was added and the mixture was stirred for 30 minutes. After addition of NEt_3, DCM was added and the organic layer was washed with H_2O, brine, was dried over $MgSO_4$ and concentrated in vacuo. Size exclusion chromatography yielded compound pseudotrisaccharide **11** (1.92 g, 1.61 mmol) in 80% yield as a single anomer. TLC: R_f 0.6 (15% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, c 0.2): +48; IR (neat, cm^{-1}): 2915, 2856, 2109, 1454, 1207, 1097, 1028, 736; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 – 7.56 (m, 4H), 7.47 – 7.13 (m, 28H), 5.21 (dd, J = 17.2, 1.6 Hz, 1H), 5.17 – 5.07 (m, 2H), 5.03 (d, J = 11.2 Hz, 1H), 4.89 (d, J = 11.2 Hz, 1H), 4.76 (s, 1H), 4.61 – 4.49 (m, 4H), 4.40 (s, 2H), 4.24 (t, J = 6.6 Hz, 1H), 4.16 (dd, J = 10.6, 2.4 Hz, 1H), 4.09 – 3.99 (m, 3H), 3.96 (d, J = 5.3 Hz, 3H), 3.93 – 3.84 (m, 2H), 3.84 – 3.76 (m, 1H), 3.76 – 3.67 (m, 2H), 3.67 – 3.62 (m, 2H), 3.62 – 3.49 (m, 3H), 3.46 – 3.41 (m, 1H), 1.06 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.2, 138.1,

137.5, 137.3, 135.4, 135.3, 134.6, 133.1, 132.8, 129.7, 129.6, 128.6, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.3, 116.6, 102.2, 94.2, 78.0, 77.4, 77.3, 77.0, 76.7, 75.4, 74.7, 74.4, 73.4, 73.3, 73.2, 73.0, 72.3, 72.1, 71.5, 70.5, 70.2, 69.9, 68.0, 62.5, 59.6, 26.7, 26.7, 19.1; HRMS: [$C_{69}H_{78}N_6O_{11}Si$ + NH4] requires 1213.58658, found 1213.58828.

2-*O*-(3-*O*-[2-*N*-acetamido-3,4-di-*O*-benzyl-6-*O*-*tert*-butyl-di-phenylsilyl-α-D-galactopyranosyl]-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-3-*O*-allyl-1-*O*-benzyl-*sn*-glycerol (30)

Di-azido-pseudotrisaccharide **11** (1.92 g, 1.6 mmol) was dissolved in a mixture of dioxane (9 ml) and water (1.8 ml). PMe_3 (1M in THF, 6.4 ml, 6.4 mmol) was added to the mixture and it was stirred for 1.7h. The mixture was concentrated, coevaporated with toluene and dissolved in DCM (20 ml). Triethylamine (0.7 ml, 4.8 mmol) and Ac_2O (0.45 ml, 4.8 mmol) were added and the mixture was stirred for 47h. The organic layer was washed with sat.aq. $NaHCO_3$, water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography yielded di-acetylated pseudotrisaccharide **30** (1.49 g, 1.2 mmol) in 76% yield. TLC: R_f=0.3 (50% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, c 0.2): +44; IR (neat, cm^{-1}): 3067, 2930, 2857, 1663, 1305, 1112, 1027, 732, 696; ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.69 – 7.52 (m, 7H), 7.50 – 6.94 (m, 67H), 5.97 – 5.65 (m, 3H), 5.44 (d, *J* = 8.3 Hz, 1H), 5.29 – 5.04 (m, 5H), 4.93 (d, *J* = 11.7 Hz, 1H), 4.77 (td, *J* = 10.6, 3.4 Hz, 3H), 4.70 (d, *J* = 11.7 Hz, 1H), 4.63 (d, *J* = 12.1 Hz, 1H), 4.56 – 4.44 (m, 11H), 4.41 (d, *J* = 6.7 Hz, 3H), 4.30 – 4.19 (m, 2H), 4.08 (d, *J* = 7.1 Hz, 0H), 3.98 – 3.86 (m, 8H), 3.86 – 3.78 (m, 4H), 3.71 (td, *J* = 9.9, 8.8, 3.6 Hz, 5H), 3.68 – 3.39 (m, 19H), 1.74 (s, 3H), 1.51 (s, 3H), 1.07 (s, 9H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 169.9, 138.2, 138.2, 137.9, 137.8, 137.5, 135.4, 135.2, 134.4, 132.8, 129.8, 129.7, 128.2, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 127.3, 127.2, 126.6, 126.5, 116.6, 101.7, 94.0, 78.2, 77.7, 77.3, 77.0, 76.7, 75.6, 74.0, 73.9, 73.1, 73.1, 73.0, 72.8, 72.2, 72.0, 71.7, 71.3, 70.7, 69.8, 67.9, 63.4, 51.8, 48.7, 26.8, 23.3, 22.6, 19.0; HRMS: [$C_{73}H_{87}N_2O_{13}Si$ + H] requires 1227.59719, found 1227.59868.

2-*O*-(3-*O*-[2-*N*-acetamido-3,4-di-*O*-benzyl-α-D-galactopyranosyl]-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-3-*O*-allyl-1-*O*-benzyl-*sn*-glycerol (32)

Silyl-protected pseudotrisaccharide **30** (1.53 g, 1.25 mmol) was coevaporated with toluene and was dissolved in THF (15 ml). TBAF (1M in THF, 1.4 ml, 1.4 mmol) was added and the reaction was stirred for 45 min. The mixture was concentrated and subsequent column

chromatography yielded alcohol **32** (1.1 g, 1.1 mmol) in 89% yield. TLC: R_f=0.1 (50% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 0.2): +39; IR (neat, cm^{-1}): 3282, 3029, 2869, 2865, 1652, 1539, 1553, 1370, 1061, 735, 696; ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.41 (d, *J* = 7.2 Hz, 6H), 7.38 – 7.21 (m, 43H), 7.14 (dd, *J* = 17.1, 9.0 Hz, 2H), 5.84 (ddt, *J* = 17.2, 10.6, 5.3 Hz, 1H), 5.22 (d, *J* = 3.1 Hz, 2H), 5.19 (q, *J* = 1.7 Hz, 1H), 5.06 (dq, *J* = 10.5, 1.4 Hz, 2H), 4.95 (dd, *J* = 19.9, 11.5 Hz, 3H), 4.83 – 4.71 (m, 2H), 4.67 (ddd, *J* = 11.9, 8.6, 3.7 Hz, 3H), 4.61 (dd, *J* = 11.4, 7.8 Hz, 3H), 4.54 (s, 3H), 4.51 (d, *J* = 3.3 Hz, 2H), 4.48 (d, *J* = 7.3 Hz, 2H), 4.20 – 4.12 (m, 3H), 4.07 (t, *J* = 6.2 Hz, 2H), 4.02 (d, *J* = 2.3 Hz, 2H), 3.99 – 3.91 (m, 7H), 3.86 (dd, *J* = 11.2, 2.6 Hz, 2H), 3.78 (dd, J = 10.6, 7.0 Hz, 2H), 3.72 – 3.65 (m, 4H), 3.65 – 3.61 (m, 3H), 3.58 (m, 7H), 3.49 (dd, J = 10.0, 6.5 Hz, 1H), 3.32 (s, 2H), 3.07 (s, 3H), 1.90 (s, 3H), 1.69 (s, 3H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 206.2, 170.7, 170.2, 140.2, 140.2, 139.8, 139.7, 139.4, 136.2, 129.1, 129.0, 128.9, 128.7, 128.6, 128.5, 128.5, 128.3, 128.3, 128.2, 128.1, 116.3, 103.0, 95.8, 78.6, 78.3, 77.1, 75.1, 75.1, 74.7, 74.1, 73.7, 73.7, 73.5, 72.7, 72.5, 72.5, 71.2, 70.7, 69.7, 62.5, 52.3, 50.3, 30.4, 30.2, 30.0, 29.8, 29.6, 29.5, 29.3, 23.7, 23.0; HRMS: [$C_{57}H_{68}N_2O_{13}$ +H] requires 989.47942, found 989.48061.

2-*O*-(3-*O*-[2-*N*-acetamido-3,4-di-*O*-benzyl-6-*O*-{4,4'-di-methoxy-tri-phenylmethyl}-α-D-galactopyranosyl]-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-3-*O*-allyl-1-*O*-benzyl-*sn*-glycerol (34)

Pseudotrisaccharide **32** (0.9 g, 0.9 mmol) was coevaporated with toluene three times and was dissolved in DCM (9 ml). Triethylamine (0.2 ml, 1.4 mmol) and 4,4'-dimethoxytrityl-chloride (0.34 g, 1.0 mmol) were added and the mixture was stirred for 2h. Water was added and the organic phase was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography with neutralized silica yielded protected pseudotrisaccharide **34** (1.01 g, 0.78 mmol) in 86% yield. TLC: R_f=0.2 (50% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 0.2): +40; IR (neat, cm^{-1}): 2930, 1699, 1508, 1247, 1060, 1027, 828, 736, 696; ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.56 (d, *J* = 7.7 Hz, 2H), 7.50 – 7.20 (m, 40H), 7.17 – 7.10 (m, 3H), 7.05 (d, *J* = 8.6 Hz, 1H), 6.92 (dd, *J* = 8.6, 5.9 Hz, 4H), 6.54 (d, *J* = 8.7 Hz, 1H), 5.95 – 5.82 (m, 1H), 5.38 (d, *J* = 3.4 Hz, 1H), 5.33 – 5.21 (m, 1H), 5.11 (d, *J* = 10.5 Hz, 1H), 4.98 (t, *J* = 9.5 Hz, 2H), 4.91 – 4.82 (m, 1H), 4.81 – 4.72 (m, 2H), 4.66 (dd, *J* = 16.4, 11.7 Hz, 2H), 4.55 – 4.44 (m, 6H), 4.43 – 4.27 (m, 2H), 4.20 (d, *J* = 8.3 Hz, 2H), 4.07 (d, *J* = 7.3 Hz, 2H), 4.01 – 3.91 (m, 4H), 3.81 (t, *J* = 6.3 Hz, 2H), 3.76 (s, 6H), 3.73 – 3.50 (m, 10H), 3.16 (dd, *J* = 9.0, 4.7 Hz, 1H), 1.85 (s, 3H), 1.75 (s, 3H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 170.1, 170.0, 159.3, 145.9, 139.8, 139.7, 139.5, 139.4, 139.2, 136.8, 136.3, 136.0, 130.9, 130.8, 129.0, 128.9,

128.9, 128.7, 128.5, 128.5, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.4, 116.4, 113.8, 113.8, 102.7, 95.0, 86.9, 78.8, 78.3, 76.3, 74.8, 74.7, 74.6, 74.2, 73.5, 72.8, 72.6, 72.4, 71.3, 70.9, 70.6, 69.5, 64.3, 55.4, 52.6, 50.0, 30.2, 30.0, 29.8, 29.6, 29.5, 23.7, 23.0; HRMS: [$C_{78}H_{86}N_2O_{15}$ +H] requires 1291.61010, found 1291.61152.

2-*O*-(3-*O*-[2-*N*-acetamido-3,4-di-*O*-benzyl-6-*O*-{4,4'-di-methoxy-tri-phenylmethyl}-α-D-galactopyranosyl]-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-1-*O*-benzyl-*sn*-glycerol (36)

Allyl-protected pseudotrisaccharide **34** (1.01 g, 0.78 mmol) was coevaporated with toluene three times and was dissolved in distilled THF (5 ml). The mixture was purged of oxygen by bubbling through argon for 10 minutes. (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (0.02 g, 0.02 mmol) was added and H_2 gas was bubbled through for 5 seconds. Argon was applied and the reaction mixture was stirred for 2h, yielding the isomerised intermediate. The reaction mixture of was diluted with THF (12.5 ml) and sat. aq. $NaHCO_3$ (13.5 ml) was added. Iodine (0.30 g, 1.2 mmol) was added and the reaction was stirred for 1.5h. The mixture was diluted with EtOAc, washed with sat. aq. $Na_2S_2O_3$ and brine, dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography yielded deprotected pseudotrisaccharide **36** (0.78 g, 0.63 mmol) in 80 % yield. TLC: R_f=0.1 (75% EtOAc/pentane); $[\alpha]_D^{20}$ ($CHCl_3$, *c* 0.2): +28; IR (neat, cm^{-1}): 3061, 2862, 1674, 1508, 1248, 1053, 1026, 826, 735, 697; ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.51 (d, *J* = 7.6 Hz, 2H), 7.44 (d, *J* = 7.4 Hz, 4H), 7.40 – 7.19 (m, 33H), 7.14 – 7.09 (m, 2H), 6.93 – 6.87 (m, 4H), 6.74 – 6.68 (m, 1H), 5.34 (d, *J* = 3.4 Hz, 1H), 4.97 (d, *J* = 11.4 Hz, 1H), 4.90 – 4.76 (m, 3H), 4.77 – 4.69 (m, 1H), 4.67 (d, *J* = 11.7 Hz, 1H), 4.58 (d, *J* = 11.4 Hz, 1H), 4.53 – 4.42 (m, 5H), 4.42 – 4.33 (m, 2H), 4.26 – 4.20 (m, 1H), 4.20 – 4.10 (m, 2H), 4.06 (s, 1H), 3.97 – 3.87 (m, 3H), 3.87 – 3.78 (m, 3H), 3.76 (s, 5H), 3.69 – 3.59 (m, 6H), 3.57 (d, *J* = 5.5 Hz, 2H), 3.53 – 3.42 (m, 2H), 3.35 (d, *J* = 4.9 Hz, 4H), 3.25 – 3.16 (m, 1H), 3.12 (dd, *J* = 9.1, 4.6 Hz, 1H), 1.85 (s, 3H), 1.72 (s, 3H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 189.9, 189.4, 178.6, 165.1, 158.9, 158.8, 158.6, 158.3, 156.0, 155.6, 150.1, 150.0, 148.2, 148.2, 148.1, 147.9, 147.7, 147.7, 147.6, 147.6, 147.4, 147.4, 147.3, 147.3, 147.1, 146.7, 133.0, 133.0, 122.3, 114.0, 106.1, 101.8, 97.6, 95.4, 94.2, 94.0, 93.9, 93.6, 92.8, 92.7, 92.0, 91.7, 90.6, 90.1, 88.8, 83.7, 82.7, 74.6, 71.7, 69.1, 68.9, 49.5, 49.4, 49.2, 49.0, 48.8, 48.6, 48.4, 42.8, 42.2; HRMS: [$C_{75}H_{82}N_2O_{15}$ +H] requires 1251.57880, found 1251.58015.

2-*O*-(3-*O*-[2-*N*-acetamido-3,4-di-*O*-benzyl-6-*O*-{4,4'-di-methoxy-tri-phenylmethyl}-α-D-galactopyranosyl]-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl)- 1-*O*-benzyl -3-*O*-([*N, N'*-di-isopropylamino]-2-cyanoethyl-phosphite)-*sn*-glycerol (8)

Pseudotrisaccharide **36** (0.19 g, 0,15 mmol) was dissolved in DCM (1.5 ml). Diisopropylethylamine (0.045 ml, 0.26 mmol) and 3Å molsieves were added and the resulting solution was stirred for 45 minutes. *N,N*-di-isopropylamino-2-cyanoethyl-chlorophosphite (0.043 ml, 0.20 mmol) was added and the reaction mixture was stirred for 1.6h. Water was added, the organic phase was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography with neutralized silica yielded phosphoramidite **8** (0.13 g, 0.09 mmol) in 62% yield. TLC: R_f=0.8 (80% EtOAc/pentane); ^{1}H NMR (400 MHz, CD_3CN) δ 7.48 (d, *J* = 7.5 Hz, 2H), 7.44 – 7.20 (m, 45H), 7.07 (dd, *J* = 6.3, 2.7 Hz, 3H), 6.93 – 6.82 (m, 4H), 6.64 (d, *J* = 9.1 Hz, 1H), 6.17 (dd, *J* = 9.1, 2.4 Hz, 1H), 5.19 (d, *J* = 3.6 Hz, 1H), 4.84 (d, *J* = 11.1 Hz, 1H), 4.78 – 4.68 (m, 3H), 4.62 – 4.41 (m, 9H), 4.34 (d, *J* = 11.3 Hz, 1H), 4.13 – 4.02 (m, 2H), 4.02 – 3.90 (m, 5H), 3.79 – 3.66 (m, 16H), 3.65 – 3.42 (m, 10H), 2.99 (dd, *J* = 9.2, 4.2 Hz, 1H), 2.63 – 2.38 (m, 5H), 1.78 (s, 3H), 1.66 (d, *J* = 1.6 Hz, 3H), 1.26 – 1.08 (m, 12H); ^{13}C NMR (101 MHz, CD_3CN) δ 171.0, 170.7, 159.6, 146.0, 139.8, 139.6, 137.0, 136.6, 131.2, 131.0, 129.4, 129.3, 129.1, 128.9, 128.8, 128.6, 128.5, 128.5, 128.4, 127.8, 118.3, 114.1, 102.7, 95.0, 87.1, 78.3, 76.3, 75.1, 75.0, 74.9, 74.3, 73.9, 73.8, 72.9, 71.4, 70.6, 69.5, 64.6, 59.3, 55.9, 52.4, 49.8, 43.9, 43.8, 24.9, 23.8, 23.2, 21.0, 14.5, 1.9, 1.7, 1.5, 1.3, 1.1, 0.9; ^{31}P NMR (162 MHz, CD_3CN) δ 148.0, 147.9.

1-*O*-(2-*O*-[3-*O*-{2-*N*-acetamido-3,4-di-*O*-benzyl-α-D-galactopyranosyl}-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl]-3-*O*-benzyl-*sn*-glycerol-1-[2-cyanoethylphosphate])-*N*-benzyloxycarbonyl-6-aminohexanol (37)

Phosphoramidite **9** (0.2M in MeCN, 1.1 ml, 227µmol) and alcohol **35** (189mg,151µmol) were coupled using the general procedure for phosphoramidite coupling. Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1 v/v) column chromatography yielded spacer equipped monomer **37** (120mg, 91µmol) in 61% yield. IR (neat, cm^{-1}): 3420, 3275, 2914, 1699, 1653, 1539, 1454, 1248, 1026, 736, 696; ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.56 – 7.19 (m, 30H), 6.38 (d, J = 6.3 Hz, 1H), 5.25 (d, J = 3.6 Hz, 1H), 5.06 (s, 2H), 4.96 (dd, J = 22.6, 11.4 Hz, 2H), 4.78 (t, J = 10.5 Hz, 2H), 4.74 – 4.60 (m, 3H), 4.54 – 4.49 (m, 3H), 4.49 – 4.43 (m, 2H), 4.25 (dt, J = 11.1, 5.4 Hz, 2H), 4.18 – 4.13 (m, 1H), 4.13 – 4.02 (m, 4H), 3.90 (ddd, J = 28.1, 11.0, 2.5 Hz, 2H), 3.80 (t, J = 9.1 Hz, 1H), 3.75 – 3.65 (m, 3H), 3.65 – 3.52 (m, 3H), 3.33 (s, 1H), 3.21 (s, 5H), 3.18 – 3.10 (m, 2H), 2.89 (t, J = 5.8 Hz, 2H), 2.02 (s, 3H), 1.72 – 1.62 (m, 5H), 1.56 –

1.46 (m, 2H), 1.45 – 1.29 (m, 5H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 171.3, 170.4, 159.5, 140.1, 140.0, 139.7, 139.4, 129.1, 129.0, 129.0, 128.9, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.1, 118.3, 102.5, 102.2, 95.1, 78.3, 76.5, 75.1, 74.8, 74.3, 73.7, 73.6, 72.9, 69.6, 68.9, 66.3, 63.3, 62.4, 51.9, 50.1, 41.4, 30.5, 30.2, 30.0, 29.8, 29.6, 29.5, 29.3, 26.8, 25.7, 23.7, 23.0, 19.9; ^{31}P NMR (162 MHz, Acetone-d_6) δ -1.5; HRMS: [$C_{71}H_{87}N_4O_{18}P$ +H] requires 1315.58257, found 1315.58397.

1-*O*-di(2-*O*-[3-*O*-{2-*N*-acetamido-3,4-di-*O*-benzyl-α-D-galactopyranosyl}-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl]-3-*O*-benzyl-*sn*-glycerol-1-[2-cyanoethylphosphate])-*N*-benzyloxycarbonyl-6-aminohexanol (38)

Phosphoramidite **7** (0.05M in MeCN, 2.3 ml, 114μmol) and alcohol **37** (115mg, 87μmol) were coupled using the general procedure for phosphoramidite coupling. Automated reversed phase (C18, 10% → 100% MeOH/H_2O) column chromatography yielded spacer equipped dimer **38** (128mg, 54μmol) in 62% yield. IR (neat, cm^{-1}): 3520, 3275, 2915, 2870, 1641, 1549, 1454, 1250, 1026, 736, 697; ^{1}H NMR (400 MHz, MeOD) δ 7.41 – 7.19 (m, 55H), 5.29 – 5.22 (m, 2H), 5.05 (s, 2H), 4.80 – 4.68 (m, 6H), 4.68 – 4.52 (m, 11H), 4.52 – 4.46 (m, 4H), 4.46 – 4.33 (m, 13H), 4.33 – 4.12 (m, 10H), 4.12 – 3.95 (m, 12H), 3.86 – 3.46 (m, 24H), 3.14 – 3.05 (m, 2H), 2.85 – 2.73 (m, 5H), 2.11 – 1.99 (m, 7H), 1.68 (d, J = 3.0 Hz, 8H), 1.53 – 1.42 (m, 2H), 1.42 – 1.23 (m, 4H); ^{31}P NMR (162 MHz, MeOD) δ -0.7, -0.8, -0.8, -0.8, -1.2, -1.3; HRMS: [$C_{128}H_{153}N_7O_{33}P_2$ +2H]$^{2+}$ requires 1190.50815, found 1190.50915

1-*O*-tri(2-*O*-[3-*O*-{2-*N*-acetamido-3,4-di-*O*-benzyl-α-D-galactopyranosyl}-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl]-3-*O*-benzyl-*sn*-glycerol-1-[2-cyanoethylphosphate])-*N*-benzyloxycarbonyl-6-aminohexanol (39)

Phosphoramidite **7** (0.05M in MeCN, 2.1 ml, 107μmol) and alcohol **38** (85mg, 36μmol) were coupled using the general procedure for phosphoramidite coupling. Automated reversed phase (C18, 10% → 100% MeOH/H_2O) column chromatography yielded spacer equipped trimer **39** (83mg, 24μmol) in 67% yield. IR (neat, cm^{-1}): 3522, 3285, 2920, 2868, 1653, 1557, 1454, 1254, 1024, 737, 696; ^{1}H NMR (500 MHz, CD_3CN) δ 7.39 – 7.19 (m, 80H), 5.26 – 5.07 (m, 2H), 5.02 (s, 1H), 4.88 – 4.69 (m, 5H), 4.69 – 4.33 (m, 28H), 4.33 – 4.04 (m, 20H), 4.04 – 3.86 (m, 12H), 3.84 – 3.70 (m, 6H), 3.70 – 3.41 (m, 17H), 3.10 – 3.00 (m, 2H), 2.85 – 2.66 (m, 6H), 1.96 (s, 9H), 1.66 – 1.48 (m, 12H), 1.46 – 1.35 (m, 2H), 1.35 – 1.18 (m, 6H); ^{31}P NMR (202 MHz, CD_3CN) δ -1.8, -1.9, -2.2, -2.3, -2.3, -2.4, -2.5; HRMS: [$C_{185}H_{219}N_{10}O_{48}P_3$ +2H]$^{2+}$ requires 1722.72132, found 1722.72021.

1-*O*-(2-*O*-[3-*O*-{2-*N*-acetamido-3,4-di-*O*-benzyl-α-D-galactopyranosyl}-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl]-1-*O*-benzyl-*sn*-glycerol-3-[2-cyanoethylphosphate])-*N*-benzyloxycarbonyl-6-aminohexanol (40)

Phosphoramidite **9** (0.2M in MeCN, 0.721 ml, 146µmol) and alcohol **36** (122mg, 97µmol) were coupled using the general procedure for phosphoramidite coupling. Size exclusion (Sephadex LH-20, DCM/MeOH, 1/1 v/v) column chromatography yielded spacer equipped monomer **40** (80mg, 61µmol) in 61% yield. IR (neat, cm^{-1}): 3499, 3283, 2934, 2866, 1659, 1549, 1454, 1254, 1026, 737, 698; ^{1}H NMR (400 MHz, CD_3CN) δ 7.41 – 7.24 (m, 30H), 6.58 (dd, J = 52.6, 9.1 Hz, 2H), 5.70 (s, 1H), 5.11 (d, J = 3.7 Hz, 1H), 5.03 (s, 2H), 4.82 (dd, J = 14.9, 11.1 Hz, 2H), 4.71 (d, J = 11.5 Hz, 1H), 4.61 – 4.37 (m, 11H), 4.18 – 4.01 (m, 10H), 4.01 – 3.87 (m, 9H), 3.79 (d, J = 11.0 Hz, 1H), 3.76 – 3.47 (m, 12H), 3.06 (q, J = 6.7 Hz, 2H), 2.63 – 2.54 (m, 2H), 1.86 (s, 3H), 1.62 (s, 3H), 1.57 (q, J = 7.0, 6.1 Hz, 2H), 1.42 (p, J = 6.9 Hz, 2H), 1.27 (s, 4H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 171.1, 171.0, 170.4, 157.2, 140.1, 140.0, 139.7, 139.4, 138.5, 129.1, 129.1, 128.9, 128.6, 128.6, 128.5, 128.5, 128.3, 128.3, 128.2, 128.1, 118.4, 118.3, 102.7, 102.3, 95.7, 78.2, 77.6, 77.5, 77.3, 76.9, 75.3, 75.3, 75.1, 74.7, 74.1, 73.9, 73.8, 73.8, 73.3, 72.8, 72.5, 69.6, 68.6, 68.5, 68.1, 68.0, 66.3, 63.0, 62.5, 55.5, 52.1, 50.3, 41.4, 30.8, 30.7, 30.5, 30.4, 30.2, 30.0, 29.8, 29.6, 29.5, 29.3, 26.8, 25.7, 23.6, 23.0, 19.9, 19.8; ^{31}P NMR (162 MHz, CD_3CN) δ -1.8, -1.9; HRMS: $[C_{71}H_{87}N_4O_{18}P$ +H] requires 1315.58257, found 1315.58421.

1-*O*-di(2-*O*-[3-*O*-{2-*N*-acetamido-3,4-di-*O*-benzyl-α-D-galactopyranosyl}-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl]-1-*O*-benzyl-*sn*-glycerol-3-[2-cyanoethylphosphate])-*N*-benzyloxycarbonyl-6-aminohexanol (41)

Phosphoramidite **8** (73mg, 29µmol) and alcohol **40** (33mg, 25µmol) were coupled using the general procedure for phosphoramidite coupling. Automated reversed phase (C18, 10% → 100% MeOH/H_2O) column chromatography yielded spacer equipped dimer **41** (38mg, 16µmol) in 64% yield. IR (neat, cm^{-1}): 3275, 2926, 2866, 1637, 1558, 1456, 1252, 1026, 737, 698; ^{1}H NMR (500 MHz, CD_3CN) δ 7.40 – 7.20 (m, 55H), 6.66 (t, J = 9.3 Hz, 1H), 6.41 (t, J = 9.5 Hz, 1H), 6.31 (dd, J = 21.0, 8.7 Hz, 1H), 5.68 (s, 1H), 5.16 (dd, J = 5.8, 3.5 Hz, 1H), 5.10 (t, J = 3.3 Hz, 1H), 5.02 (s, 2H), 4.85 – 4.74 (m, 4H), 4.74 – 4.68 (m, 2H), 4.61 – 4.32 (m, 24H), 4.30 – 3.87 (m, 27H), 3.80 (d, J = 11.0 Hz, 1H), 3.76 – 3.40 (m, 21H), 3.05 (q, J = 6.7 Hz, 2H), 2.64 – 2.48 (m, 4H), 1.90 – 1.81 (m, 6H), 1.64 – 1.47 (m, 8H), 1.45 – 1.36 (m, 2H), 1.26 (d, J = 15.4 Hz, 4H); ^{31}P NMR (202 MHz, CD_3CN) δ -0.4, -1.1, -1.3; HRMS: $[C_{128}H_{153}N_7O_{33}P_2$ +2H$]^{2+}$ requires 1190.50815, found 1190.50890.

1-*O*-tri(2-*O*-[3-*O*-{2-*N*-acetamido-3,4-di-*O*-benzyl-α-D-galactopyranosyl}-2-*N*-acetamido-4,6-di-*O*-benzyl-β-D-galactopyranosyl]-1-*O*-benzyl-*sn*-glycerol-3-[2-cyanoethylphosphate])-*N*-benzyloxycarbonyl-6-aminohexanol (42)

Phosphoramidite **8** (42mg, 29µmol) and alcohol **41** (31mg, 13µmol) were coupled using the general procedure for phosphoramidite coupling. Automated reversed phase (C18, 10% → 100% MeOH/H_2O) column chromatography yielded spacer equipped trimer **42** (30mg, 8.7µmol) in 67% yield. IR (neat, cm^{-1}): 3287, 3063, 2924, 2860, 1663, 1557, 1454, 1254, 1028, 739, 698; ^{1}H NMR (400 MHz, CD_3CN) δ 7.41 – 7.14 (m, 80H), 6.74 (s, 3H), 6.46 – 6.26 (m, 3H), 5.68 (s, 1H), 5.20 – 5.06 (m, 3H), 5.02 (s, 2H), 4.87 – 4.65 (m, 12H), 4.62 – 4.29 (m, 40H), 4.29 – 3.85 (m, 40H), 3.85 – 3.42 (m, 35H), 3.05 (q, J = 6.4 Hz, 2H), 2.63 – 2.43 (m, 6H), 1.84 (dd, J = 8.3, 6.1 Hz, 9H), 1.63 – 1.44 (m, 11H), 1.40 (q, J = 9.1, 8.1 Hz, 2H), 1.25 (d, J = 18.9 Hz, 4H); ^{31}P NMR (162 MHz, CD_3CN) δ -0.6, -0.6, -1.3, -1.3, -1.4, -1.5, -1.5; HRMS: $[C_{185}H_{219}N_{10}O_{48}P_3 +2H]^{2+}$ requires 1722.72132, found 1722.72097.

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

Spacer equipped pseudotrisaccharide monomer **37** was deprotected using the general procedure for global deprotection. Purification yielded deprotected **1** in 73% yield. Intermediate **43**: ^{31}P NMR (202 MHz, MeOD) δ 0.65; HRMS: $[C_{68}H_{84}N_3O_{18}P +H]$ requires 1262.55603, found 1262.55694. Product **1:** ^{1}H NMR (500 MHz, D_2O) δ 5.03 (d, J = 3.8 Hz, 1H), 4.68 (d, J = 8.5 Hz, 1H), 4.18 (dd, J = 11.0, 3.7 Hz, 1H), 4.08 (d, J = 3.1 Hz, 1H), 4.06 – 3.99 (m, 2H), 3.97 (d, J = 2.9 Hz, 1H), 3.94 – 3.87 (m, 1H), 3.86 – 3.66 (m, 11H), 3.66 – 3.59 (m, 2H), 2.95 (t, J = 7.1 Hz, 2H), 2.05 (s, 3H), 2.00 (s, 3H), 1.68 – 1.57 (m, 4H), 1.42 – 1.33 (m, 4H); ^{31}P NMR (202 MHz, D_2O) δ 0.7; HRMS: $[C_{25}H_{48}N_3O_{16}P +H]$ requires 678.28450, found 678.28411.

1-*O*-di(2-*O*-[3-*O*-{2-*N*-acetamido-α-D-galactopyranosyl}-2-*N*-acetamido-β-D-galactopyranosyl]-*sn*-glycerol-1-phosphate)-6-aminohexanol (2)

Spacer equipped pseudotrisaccharide dimer **38** was deprotected using the general procedure for global deprotection. Purification yielded deprotected **2** in 57% yield. Intermediate **44**: ^{31}P NMR (162 MHz, MeOD) δ 0.50, 0.33; HRMS: $[C_{122}H_{147}N_5O_{33}P_2 +2H]^{2+}$ requires 1137.48161, found 1137.48890. Product **2**: ^{1}H NMR (500 MHz, D_2O) δ 5.02 (t, J = 3.4 Hz, 2H), 4.68 (dd, J = 16.5, 8.5 Hz, 2H), 4.18 (dd, J = 11.0, 3.7 Hz, 2H), 4.11 – 3.58 (m, 37H), 2.96 (t, J = 7.4 Hz, 2H), 2.06 (s, 6H), 2.00 (s, 6H), 1.69 – 1.58 (m, 4H), 1.44 – 1.34 (m, 4H);

^{31}P NMR (202 MHz, D_2O) δ 0.7, 0.2; HRMS: [$C_{44}H_{81}N_5O_{31}P_2$ +H] requires 1238.44635, found 1238.44631.

1-*O*-tri(2-*O*-[3-*O*-{2-*N*-acetamido-α-D-galactopyranosyl}-2-*N*-acetamido-β-D-galactopyranosyl]-*sn*-glycerol-1-phosphate)-6-aminohexanol (3)

Spacer equipped pseudotrisaccharide trimer **39** was deprotected using the general procedure for global deprotection. Purification yielded deprotected **3** in 44% yield. Intermediate **45**: ^{31}P NMR (162 MHz, MeOD) δ 0.34, 0.25, 0.15; HRMS: [$C_{176}H_{210}N_7O_{48}P_3$ +2H]$^{2+}$ requires 1643.18151, found 1643.18103. Product **3**: ^{1}H NMR (500 MHz, D_2O) δ 5.08 – 4.97 (m, 3H), 4.73 – 4.63 (m, 3H), 4.18 (dd, *J* = 11.0, 3.7 Hz, 3H), 4.12 – 3.56 (m, 55H), 2.97 (t, *J* = 7.6 Hz, 2H), 2.07 (d, *J* = 6.1 Hz, 9H), 2.00 (s, 9H), 1.69 – 1.57 (m, 4H), 1.46 – 1.33 (m, 4H); ^{13}C NMR (151 MHz, D_2O) δ 174.8, 174.7, 174.7, 174.5, 174.5, 174.5, 100.8, 93.6, 93.5, 93.2, 79.1, 79.1, 79.0, 78.9, 78.9, 75.1, 74.9, 74.8, 74.5, 71.2, 70.0, 70.0, 68.2, 67.8, 67.8, 67.6, 67.5, 67.4, 66.0, 65.9, 64.7, 64.4, 64.3, 64.0, 63.7, 63.6, 63.4, 61.2, 61.0, 60.7, 50.8, 50.7, 49.2, 39.3, 29.4, 29.3, 26.5, 25.0, 24.3, 22.3, 22.3, 21.9, 21.9; ^{31}P NMR (202 MHz, D_2O) δ 0.7, 0.2, 0.2; HRMS: [$C_{63}H_{114}N_7O_{46}P_3$ +2H]$^{2+}$ requires 899.80774, found 899.80953.

1-*O*-(2-*O*-[3-*O*-{2-*N*-acetamido-α-D-galactopyranosyl}-2-*N*-acetamido-β-D-galactopyranosyl]-*sn*-glycerol-3-phosphate)-6-aminohexanol (4)

Spacer equipped pseudotrisaccharide monomer **40** was deprotected using the general procedure for global deprotection. Purification yielded deprotected **4** in 45% yield. Intermediate **46**: ^{31}P NMR (202 MHz, MeOD) δ 0.46; HRMS: [$C_{68}H_{84}N_3O_{18}P$ +H] requires 1262.55603, found 1262.55687. Product **4**: ^{1}H NMR (500 MHz, D_2O) δ 5.03 (d, J = 3.8 Hz, 1H), 4.65 (d, J = 8.5 Hz, 1H), 4.18 (dd, J = 11.0, 3.7 Hz, 1H), 4.07 (d, J = 3.0 Hz, 1H), 4.05 – 3.90 (m, 5H), 3.85 (q, J = 6.4 Hz, 2H), 3.83 – 3.57 (m, 11H), 2.95 (t, J = 7.6 Hz, 2H), 2.02 (s, 3H), 2.00 (s, 3H), 1.69 – 1.57 (m, 4H), 1.44 – 1.34 (m, 4H); ^{31}P NMR (202 MHz, D_2O) δ 0.7; HRMS: [$C_{25}H_{48}N_3O_{16}P$ +H] requires 678.28450, found 678.28431.

1-*O*-di(2-*O*-[3-*O*-{2-*N*-acetamido-α-D-galactopyranosyl}-2-*N*-acetamido-β-D-galactopyranosyl]-*sn*-glycerol-3-phosphate)-6-aminohexanol (5)

Spacer equipped pseudotrisaccharide dimer **41** was deprotected using the general procedure for global deprotection. Purification yielded deprotected **5** in 49% yield. Intermediate **47**: ^{31}P NMR (202 MHz, MeOD) δ 0.43, 0.21; HRMS: [$C_{122}H_{147}N_5O_{33}P_2$ +2H]$^{2+}$ requires 1137.48161, found 1137.48287. Product **5**: ^{1}H NMR (500 MHz, D_2O) δ 5.03 (d, *J* = 3.6 Hz, 2H), 4.70 – 4.62 (m, 2H), 4.17 (dd, *J* = 11.0, 3.6 Hz, 2H), 4.09 – 3.89 (m, 15H), 3.89 – 3.58

(m, 20H), 2.96 (t, *J* = 7.5 Hz, 2H), 2.04 (s, 3H), 2.02 (s, 3H), 2.00 (s, 6H), 1.70 – 1.58 (m, 4H), 1.45 – 1.35 (m, 4H); ^{31}P NMR (202 MHz, D_2O) δ 0.7, 0.3; HRMS: $[C_{44}H_{81}N_5O_{31}P_2 + H]$ requires 1238.44635, found 1238.44620.

1-*O*-tri(2-*O*-[3-*O*-{2-*N*-acetamido-α-D-galactopyranosyl}-2-*N*-acetamido-β-D-galactopyranosyl]-*sn*-glycerol-3-phosphate)-6-aminohexanol (6)

Spacer equipped pseudotrisaccharide trimer **42** was deprotected using the general procedure for global deprotection. Purification yielded deprotected **6** in 56% yield. Intermediate **48**: ^{31}P NMR (162 MHz, MeOD) δ 0.41; HRMS: $[C_{176}H_{210}N_7O_{48}P_3 + 2H]^{2+}$ requires 1643.18151, found 1643.18546. Product **6**: ^{1}H NMR (500 MHz, D_2O) δ 5.03 (d, *J* = 3.7 Hz, 3H), 4.69 – 4.63 (m, 3H), 4.17 (dd, *J* = 11.0, 3.6 Hz, 3H), 4.09 – 3.89 (m, 27H), 3.89 – 3.57 (m, 30H), 2.97 (t, *J* = 7.5 Hz, 2H), 2.04 (s, 6H), 2.02 (s, 3H), 2.00 (s, 9H), 1.69 – 1.57 (m, 4H), 1.43 – 1.37 (m, 4H); ^{13}C NMR (151 MHz, D_2O) δ 174.7, 174.5, 174.5, 174.5, 101.3, 101.2, 101.2, 93.4, 93.3, 79.6, 79.6, 79.4, 79.4, 75.1, 75.0, 74.9, 74.8, 74.7, 71.2, 70.1, 68.2, 67.9, 67.6, 67.5, 67.5, 66.1, 66.0, 64.5, 64.3, 63.6, 63.5, 61.0, 60.8, 60.5, 60.4, 50.9, 50.8, 49.3, 49.2, 39.3, 29.3, 29.3, 26.5, 25.0, 24.3, 22.3, 22.1, 21.9; ^{31}P NMR (202 MHz, D_2O) δ 0.7, 0.3; HRMS: $[C_{63}H_{114}N_7O_{46}P_3 + 2H]^{2+}$ requires 899.80774, found 899.80953.

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Supplementary data

Supplementary data (^{1}H, ^{13}C, ^{31}P NMR spectra) associated with this article can be found, in the online version, at ...

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