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Synthesis, structure and epitope mapping of well-defined Staphylococcus aureus capsular polysaccharides

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Chapter 5

Synthesis of a set of *Staphylococcus aureus* capsular polysaccharide type 1 oligosaccharides carrying taurine esters

Introduction

The cell wall of *Staphylococcus aureus* (*S. aureus*) comprises different cell-wall components including different types of capsular polysaccharides (CPs), which have already been thoroughly elaborated on in Chapters 2-4. Besides the previously described CP type 5 and 8, also CP1 has been identified and characterized. From a synthetic perspective, CP1 is one of the most studied *S. aureus* CPs, together with CP5 and CP8.[1] It differs from other types of CPs from *S. aureus* due to the presence of 2-acetamido-2-deoxy-α-D-galactopyranosyl uronic acid (α-D-GalNAcA) residues. The first isolation of CP1 was reported by Smith in 1962 and the structure was later characterized by Scott in 1969 and designated as *S. aureus* Strain M.[2] In 1974, Liau *et al.* further investigated the chemical components of strain M and concluded that the surface antigen consists of three components, taurine, α-D-GalNAcA and D-fucosamine (D-FucNAc).[3] However the ratio of the components remained unclear until 1977, when Liau *et al.* reported the CP1 structure to contain D-GalNAcA, D-FucNAc and taurine in a 4:2:1 ratio.[4] The complete structure of strain M was determined by Murthy *et al.* in 1983 to comprise the repeating unit →4)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl uronic acid)-(1→4)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl uronic acid)-(1→3)-*O*-2-acetamido-2-deoxy-α-D-fucopyranosyl-(1→, where taurine is linked to every fourth D-GalNAcA unit via an amide bond as shown in Figure 1A.[5] A second strain of CP1, called strain D, was established in 1982 and found to consist of the same repeating unit without the taurine substituents.[6,7]

The polysaccharide capsule of strain M has been associated with increased virulence and in mouse models found to increase resistance to phagocytosis.[8] To understand how the polysaccharide interacts with receptors of the immune system or to explore the biosynthesis pathways, synthetic fragments of bacterial polysaccharide can be excellent tools. Also, for generation of well-defined synthetic vaccines these can be valuable molecules,[9] as described in Chapters 2-4, which have shown the application of synthetic *S. aureus* CP fragments in the establishment of 3D structures, for epitope mapping and the construction of synthetic vaccine modalities. It follows that similar tools for CP1 would be very valuable.

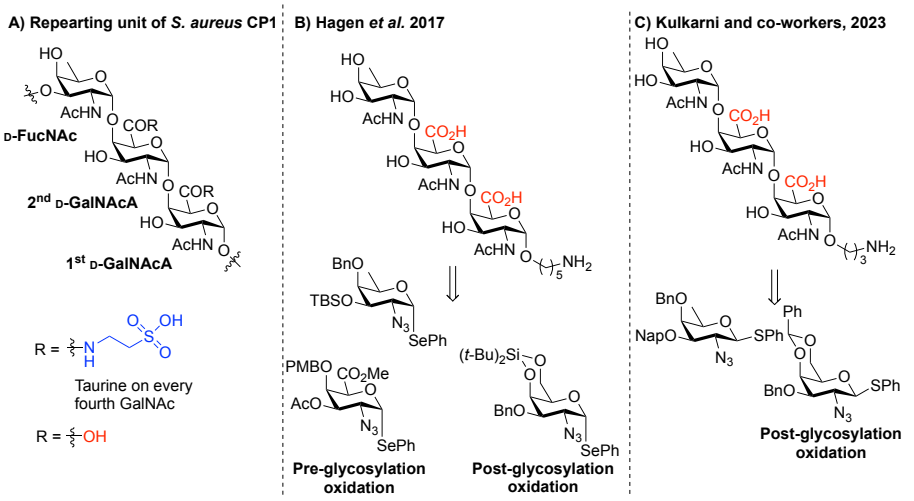


Figure 1: A) A schematic representation of the repeating unit of CP1 and the possible taurine pattern. B) Previous synthetic work of the CP1-trisaccharide by Hagen *et al.* C) Previous synthetic work of the CP1-trisaccharide by Kulkarni and co-workers.

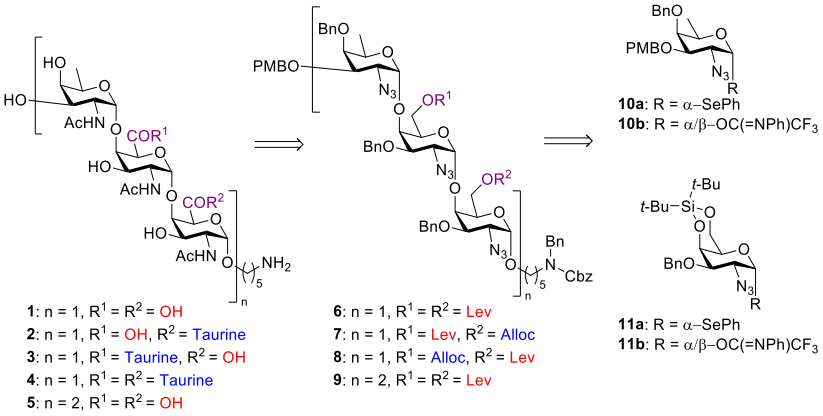
In 2017, the first reported synthesis of CP1 strain M was reported by Hagen *et al.* (Figure 1B).[10] The synthesis of a trisaccharide repeating unit (RU) relied on a post glycosylation oxidation strategy to ensure the 1,2-*cis* glycosylic linkages, through the use of silylene-protected galactosazide ($GalN_3$) synthons. A pre-glycosylation oxidation was also investigated, however using $GalN_3A$ building blocks the α-selectivity was difficult to control. The trisaccharide was built from the reducing end by using a post-glycosylation oxidation method and oxidation was executed at both a monosaccharide and at a disaccharide level. Difficulties with oxidation of the disaccharide were encountered, and these were overcome by implementing a two-step, one-pot 2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl (TEMPO)/ (diacetoxyiodo)benzene (BAIB)-Pinnick oxidation protocol. In 2023, Kulkarni and co-workers reported a synthesis of the trisaccharide repeating unit[11] relying on a 4,6-benzylidene protected D-galactosazide thioglycoside donor (Figure 1C). To ensure 1,2-*cis* glycosylation with the linker, a dimethylformamide (DMF) modulated pre-activation method[12] was implemented, while for the disaccharide glycosylation, solvent participation using diethyl ether (Et_2O) was used to ensure 1,2-*cis* linkage. Instead of a step-wise oxidation, a double oxidation on the disaccharide using TEMPO/BAIB/$NaHCO_3$ was implemented,[13] followed by alkylation to provide the benzyl esters. The protected trisaccharide was deprotected using a 2-step deprotection strategy. Both synthesized trisaccharides were equipped with a linker which allowed for conjugation, however no immunological evaluation of the synthetic material has been published to date.

Neither of the so fare published trisaccharides are equipped with the characteristic taurine. This Chapter describes the synthesis of the trisaccharides with all the possible taurine substitution patterns (*i.e.,* none, one or two taurines per repeating unit) obtaining four different trisaccharides as well as a non-taurinated hexasaccharide. The trisaccharides are constructed with the possibility for both elongation and taurine substitution of either of the two D-GalNAcA motifs. By using an orthogonal protecting group strategy on the C-6-OH of the $GalN_3A$ residues several CP1 fragments can be provided. Opposite to the strategy in Chapters 2-4, the oxidations are now performed at a late stage on more complicated molecules to allow different taurine substitution patterns to be incorporated. The saccharides will be equipped with linker functionalities for future conjugation purposes.

Results and discussion

The retrosynthetic analysis is depicted in Scheme 1. For the synthesis of the CP1 trisaccharides, the implemented strategy relied on building of the saccharides from the reducing end and installing the linker on the monosaccharide level. For the stereoselective introduction of the 1,2-*cis* $GalN_3$ linkages Kiso's di-*tert*-butylsilylene (DTBS) protecting group strategy was used.[14,15] This system can even overwrite neighboring group participation from a C-2 acyl group. Hagen *et al.* implemented this strategy with an azide moiety on the C-2 and found excellent α-selectivity.[10] In addition, a direct glycosylation using a galacturonic acid donor was found difficult by Hagen *et al.*[10] and to open up for taurine substitution, a post-glycosylation oxidation of the C-6-OH of the GalN residues on the trisaccharide level was implemented. Therefore, two different temporary C-6-OH protecting groups were used – a levulinoyl (Lev) group as precursor for the carboxylic acids and an allyloxycarbonyl (Alloc) group as precursor for the taurine esters. Extensive work by Zhang *et al.* has shown that regioselective *O*-acylation of the primary alcohol after glycosylation and desilylation is effective.[16] For the D-FucNAc residue, the C-3-OH was equipped with a *p*-methoxybenzyl (PMB) ether allowing for elongation. In all the building blocks, non-participating azides were used as precursors for the product acetamides, ensuring the formation of the 1,2-*cis* linkages, while benzyl-like protecting groups were used for permanent protection of all other groups, allowing for a single global deprotection step. For the hexasaccharide only a non-taurinated hexasaccharide was targeted, implementing a [3+3] glycosylation approach. The acceptor trisaccharide was built using the same protocol as for the non-taurinated trisaccharide, while the donor

trisaccharide was constructed with a temporary phenylselenyl protecting group on the anomeric center, again building on the protecting group strategies described above.

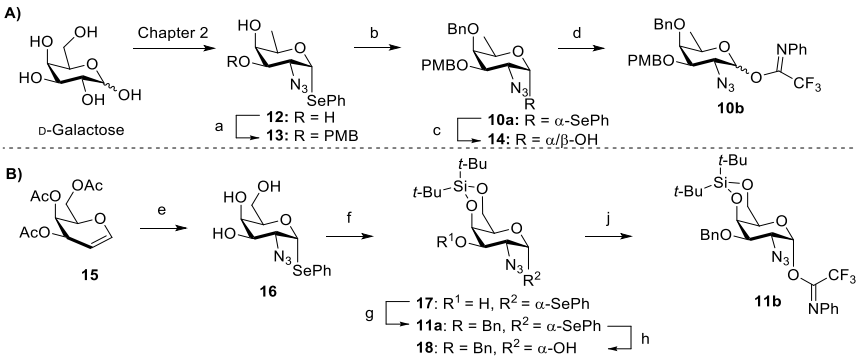


Scheme 1: Retrosynthetic analysis of the four different trisaccharides and one hexasaccharide.

For the D-FucN_3 residue a route starting from intermedia **12** (see Chapter 2 for its synthesis) was developed as shown in Scheme 2A. First, the C-3-OH was protected with a PMB ether via a tin-acetal intermediate,[17] followed by benzylation of the free C-4-OH giving fully protected **10a**.[iv] Next, the selenophenyl group was hydrolyzed with *N*-iodosuccinimide (NIS) giving hemiacetal **14** in 93%, followed by installation of a *N*-phenyl trifluoroacetimidate to provide donor **10b**.[18] The D-GalN_3 building block was synthesized following a route published by Hagen *et al.*[10] giving phenylselenyl donor **11a** in 49% yield over 4 steps and imidate donor **11b** in 44% over 6 steps as shown Scheme 2B.

The assembly of the three target trisaccharides started with a glycosylation between donor **11a** and acceptor **19** in the presence of NIS and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), which proceeded in 80% yield and delivered only the α-anomer (Scheme 3). The yield was improved to 98% yield by switching to the imidate donor **11b** without affecting the α-selectivity. The newly formed α-linkage was confirmed by ^{1}H-NMR and ^{13}C-NMR, with the anomeric proton and carbon having a CH-coupling constant of $J_{C1,H1}$ = 171.5 Hz.

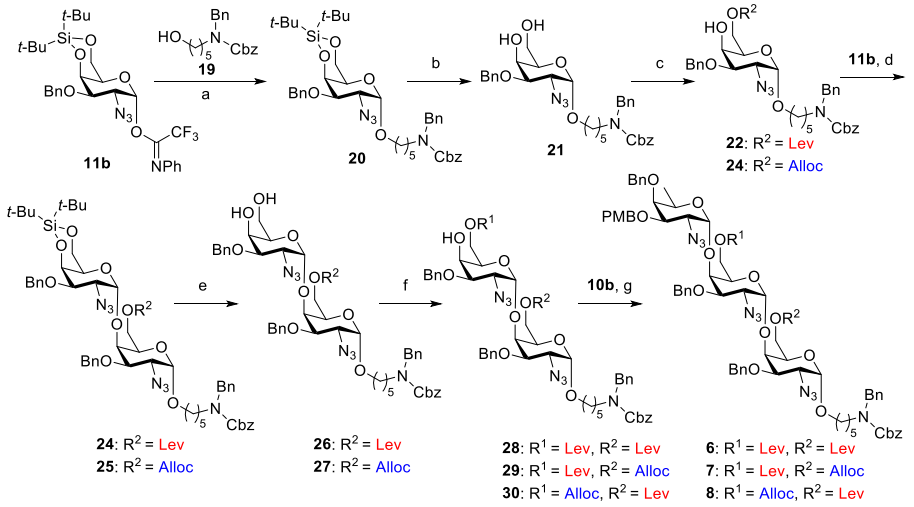
[iv] It was found that shorter reaction times (from overnight to 4 h) improved the yield from 56% to 78% of the benzylation due to hydrolysis of the anomeric seleno acetal.



Scheme 2: Synthesis of the building blocks **10** (A) and **11** (B). *Reaction conditions*: A) a) Bu_2SnO, PMBCl, CsF, Bu_4NBr, toluene, 87%, b) BnBr, NaH, DMF, 78%, c) NIS, acetone/H_2O, 93%, d) ClC(=NPh)CF_3, K_2CO_3, acetone, 91%. B) e) i) $(SePh)_2$, $TMSN_3$, BAIB, DCM, -30 to -20 ºC, 56% ii) NaOMe, MeOH, quant. f) (*t*-Bu)$_2$Si(OTf)$_2$, pyridine, DMF, 96%, g) BnBr, NaH, DMF, 92%, h) NIS, acetone/H_2O, 90% yield, j) ClC(=NPh)CF_3, K_2CO_3, acetone, 99%.

The DTBS group was removed with tetra-butylammonium fluoride (TBAF) followed by regioselective *O*-acylation on the newly liberated C-6-OH with either a Lev or an Alloc group. The Lev protection using levulinic acid (LevOH), *N*,*N*′-diisopropylcarbodiimide (DIC) and 4-dimethylaminopyridine (DMAP) afforded a mixture of C-4 and C-6 protected product, but switching to a procedure using the intermediate tin-acetal, formed using dibutyltin oxide (Bu_2SnO) in toluene, followed by addition of levulinic anhydride (Lev_2O) gave **22** in excellent 93% yield. For the Alloc protection, allyl chloroformate and pyridine were used to give **25** in 84% yield. Next, the Lev-**24** and Alloc-**25** disaccharides were generated using the phenylselenyl-donor **11a** in moderate yields (42% for **24** and 46% for **25**). These yields improved drastically by changing to imidate-donor **11b** (82% for **24** and 86% for **25**), in line with the findings of Zhang *et al.*[16] Desilylation with TBAF buffered by acetic acid (AcOH) and regioselective *O*-acylation of the C-6-OH with either a Lev or a Alloc group gave the three disaccharide acceptors **28**, **29** and **30**. Glycosylation with imidate-donor **10b** and TBSOTf gave the trisaccharides **6**, **7** and **8** in excellent 97%, 92% and 90% yield respectively.[v] All newly formed α-linkages were confirmed by ^{1}H-NMR, ^{13}C-NMR and CH-coupling constants.

[v]Also here, the imidate donor performed significantly better than the corresponding phenylselenyl donor **10a** (**6** was formed in 47% when using **10a**).



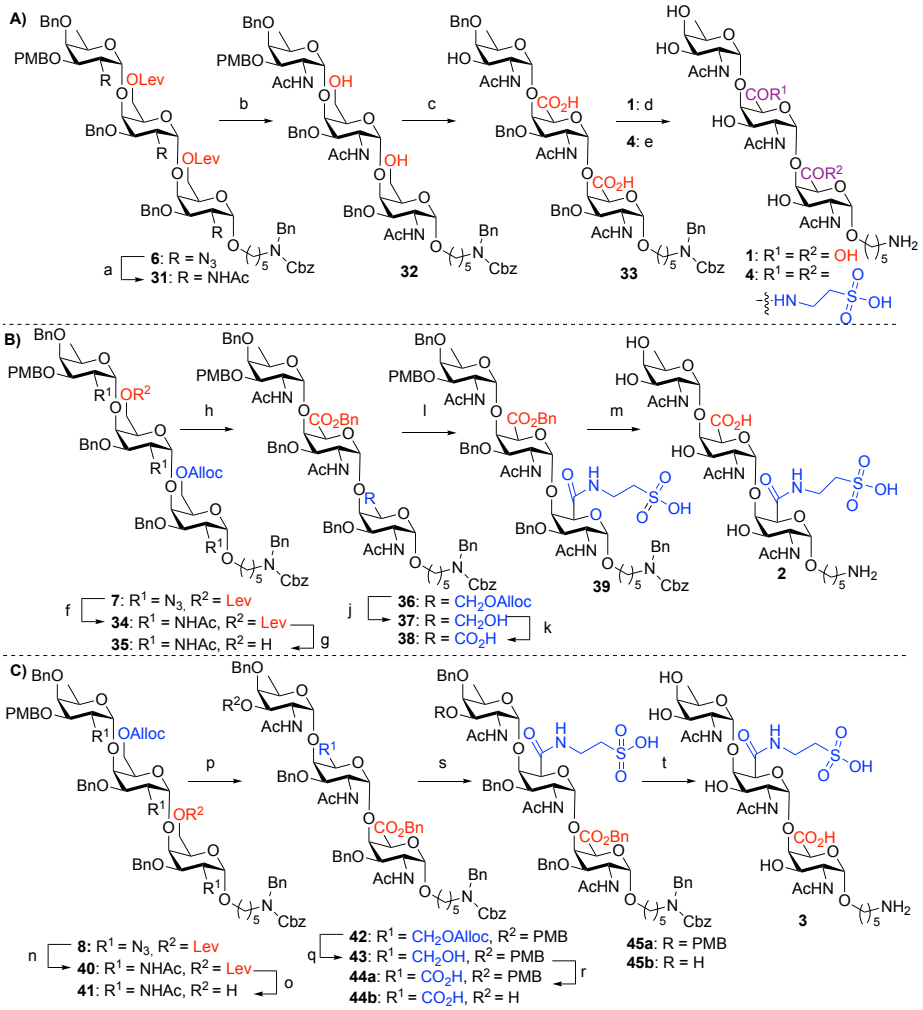
Scheme 3: Synthesis of the three different trisaccharide intermediates **6**, **7** and **8**. *Reaction conditions*: a) TBSOTf, DCM, 0 ºC, 98%, b) TBAF, THF, 91%, c) **22**: Bu_2SnO, toluene, Lev_2O, DCM, 93%; **23**: Alloc-Cl, pyridine, DCM, 84%. d) TBSOTf, DCM, 0 ºC, **24**: 82%, **25**: 86%, e) TBAF, AcOH, THF, **26**: 91%, **27**: 92%, f) **28**: Bu_2SnO, toluene, Lev_2O, DCM, 92%; **29**: Alloc-Cl, pyridine, DCM, 74%; **40**: Bu_2SnO, toluene, Lev_2O, DCM, 71%, g) TBSOTf, DCM, 0 ºC, **6**: 97%; **7**: 92%; **8**: 90%.

The oxidation and deprotection strategy used to furnish the trisaccharides depended on the taurination pattern of the products, as seen in Scheme 4. First, in all three trisaccharides (**6**, **7** and **8**), the azides where reduced and the resulting amines acetylated in a one-pot fashion using the same method used in Chapters 2-4. For the non-taurinated trisaccharide (Scheme 4A), the Lev groups in **31** were removed with hydrazine-acetate followed by a double oxidation. First, a TEMPO/BAIB oxidation was investigated,[19,20] but these conditions led to cleavage of the glycosidic linkages, as also reported by Hagen *et al.*[10] In the literature, it has more often been reported that multiple oxidations on larger saccharides can be difficult. Zhang *et al.* found that multiple oxidations could best be achieved using TEMPO and BAIB together with $NaHCO_3$ in a mixture of EtOAc/*t*-BuOH/H_2O at 4 ºC to afford the desired oxidized products in good yields,[13] as the basic conditions accelerate the formation of the hydrate from the newly formed aldehyde. These conditions were implemented, forming **33** in good yield (54%). It was observed however that a long reaction time was required (12 days), which led to partial or complete cleavage of the PMB ether. The oxidation was monitored by LC-MS to reveal a fast oxidation to the aldehyde and a slow subsequent oxidation to the carboxylic acid. Gao and co-workers have developed an oxidation strategy for complex long oligosaccharides using a minimal amount of water,[21]

by first oxidizing the alcohol to the aldehyde using TEMPO and BAIB in dry dichloromethane (DCM) followed by oxidation to the carboxylic acid by adding wet DCM[vi] and extra BAIB. This protocol was implemented on **32** and the double oxidated product **33** was achieved in significantly shorter reaction time (1 day), however degradation of the starting material was observed and the yield not improved. Finally, hydrogenation afforded the first target trisaccharide **1** in 44% yield.

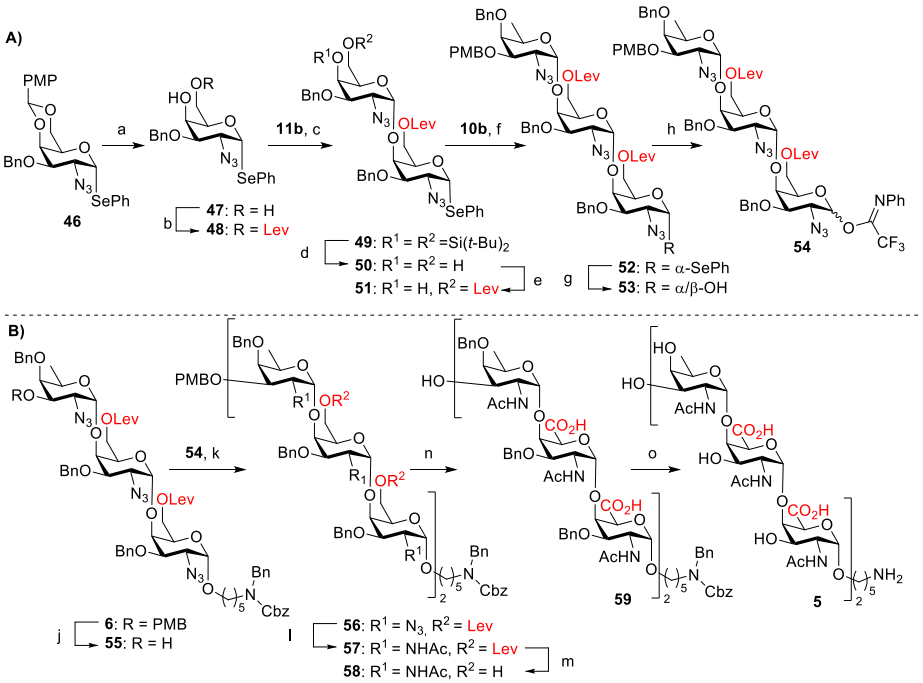
The second trisaccharide with taurines on both GalNAcA residues was obtained from intermediate **33** by installation of the taurine amide on both carboxylic acids using taurine in the presence of hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) and *N*,*N*-diisopropylethylamine (DIPEA). Hydrogenation and size exclusion gel-filtration chromatography gave target **4** in 17% yield over two steps. For the trisaccharide with the taurine on the first GalNAc residue, after the reduction of the azides, the Lev group was removed, followed by oxidation using the above described, basic TEMPO/BAIB conditions (Scheme 4B). The carboxylic acid intermediate was obtained after 4 days stirring at 4 ºC, with the PMB ether intact and alkylation then gave **36**. The Alloc-group was removed using palladium catalysis and the basic oxidation conditions provided the oxidized product **38** after 12 days of stirring at 4 ºC. The long reaction time again led to partial PMB cleavage, resulting in an inseparable mixture, which was nonetheless used for the following transformations. For installation of the taurine amide, a similar coupling was performed as described for the double taurinated trisaccharide. After aqueous work-up the crude product was immediately hydrogenated and after size exclusion gel-filtration chromatography **2** was obtained in 21% yield. The position of the taurine was confirmed with ^{1}H-NMR and ^{13}C-NMR. The same steps were implemented on **8** to obtain the trisaccharide with taurine on the 2nd GalNAcA residue, as can be seen in Scheme 4C. During the second oxidation the PMB was again partially cleaved, giving 51% of **44a** with the PMB and 27% of **44b** without PMB. The taurine was installed on the mixture of **44a/b** and after hydrogenation and gel-filtration **3** was obtained in 35% yield over two steps and hydrolysis of the taurine amide was not observed.

[vi] Wet DCM was obtained by shaking DCM with water and then adding only the DCM layer.



Scheme 4: Scheme 3: A) Deprotection towards **1** and **4**. B) Deprotection towards **2**. C) Deprotection towards **3**. *Reaction conditions*: A) a) zinc, AcOH, Ac_2O, THF, 50 °C, 92%; b) hydrazine acetate, toluene/EtOH, 96%; c) TEMPO, BAIB, $NaHCO_3$, EtOAc/*t*-BuOH/H_2O, 4 °C, 54%; d) $Pd(OH)_2$, *t*-BuOH, H_2O, AcOH, H_2, 44%; e) i) Taurine, HATU, DIPEA, DMF, ii) $Pd(OH)_2$, *t*-BuOH, H_2O, AcOH, H_2, 17% over two steps. B) f) zinc, AcOH, Ac_2O, THF, 50 °C, 80%; g) hydrazine acetate, toluene/EtOH, 89%; h) i) TEMPO, BAIB, $NaHCO_3$, EtOAc/*t*-BuOH/H_2O, 4 °C, ii) BnBr, $CsCO_3$, DMF, 92% over two steps; j) $Pd(PPh_3)_4$, Bu_3SnH, DCM, 0 °C, 76%; k) TEMPO, BAIB, $NaHCO_3$, EtOAc/*t*-BuOH/H_2O, 4 °C, 65%; l) taurine, HATU, DIPEA, DMF, m) $Pd(OH)_2$, *t*-BuOH, H_2O, AcOH, H_2, 21% over two steps; C) n) zinc, AcOH, Ac_2O, THF, 50 °C, 94%; o) hydrazine acetate, toluene/EtOH, 86%; p) TEMPO, BAIB, $NaHCO_3$, EtOAc/*t*-BuOH/H_2O, 4 °C, ii) BnBr, $CsCO_3$, DMF, 71% over two steps, q) $Pd(PPh_3)_4$, Bu_3SnH, DCM, 0 °C, 86%; r) TEMPO, BAIB, $NaHCO_3$, EtOAc/*t*-BuOH/H_2O, 4 °C, 51% with PMB and 27% without PMB; s) Taurine, HATU, DIPEA, DMF; t) $Pd(OH)_2$, *t*-BuOH, H_2O, AcOH, H_2, 35% over two steps.

Next, the construction of the hexasaccharide without taurines was undertaken, to investigate whether multiple oxidations could be executed on the hexasaccharide precursor. A [3+3] glycosylation was implemented, necessitating two different trisaccharides – one acceptor and one donor. To this end a trisaccharide donor was synthesized having a phenylselenyl on the anomeric position to minimize modification steps. Donor formation started with formation of diol **47** from monosaccharide **46** by removal of the *p*-methoxy-benzylidene, which was selectively levulinoylated with LevOH, DIC and DMAP giving acceptor **48** (Scheme 5). Glycosylation with donor **11b** afforded disaccharide **49** in 65% yield as the sole α-anomer. The lower yields found for the product with the anomeric phenylselenyl group can be explained by partial hydrolysis of the acceptor. Removal of the DTBS group followed by regioselective levulinoylation of the diol gave acceptor **51**. Here, also 8% double levulinoylated product was found. Glycosylation between donor **10b** and acceptor **51** gave trisaccharide **52** in 56% yield. Trisaccharide **52** was then converted into imidate donor **54** by hydrolysis of the phenylselenyl acetal, followed by *N*-phenyl trifluoroacetimidate installation. Hexasaccharide **56** was obtained by the glycosylation of acceptor **55** (which was synthesized by oxidative cleavage of the PMB ether in **6**) and donor **54** in 58% yield and excellent α-selectivity. The newly formed α-linkage was confirmed by ^{1}H-NMR and ^{13}C-NMR, with the $J_{C1\text{-}H1}$ coupling constants all around 170 Hz, indicating the presence of only α-linkages. The same deprotection protocol as for the non-taurinated trisaccharide was followed and first the azides were reduced and the concomitant one-pot acetylation, was followed by deprotection of the Lev-esters. The four newly liberated primary alcohols were oxidized using the basic oxidation protocol by stirring the reaction at 4 ºC for 12 days, to give the desired tetra-carboxylic acid **59** in 65% with cleavage of the PMB ether. The oxidation was also carried out under the same conditions at room temperature, which gave **59** after 6 days in 75%. Hydrogenation then gave hexasaccharide **5** in 26% yield.



Scheme 5: A) Synthesis of trisaccharide donor **54**, B) Synthesis of the non-taurinated hexasaccharide **4**. *Reaction conditions*: A) a) CSA, MeOH, 82%, b) LevOH, DIC, DMAP, DCM, 0 ºC, 92%, c) TBSOTf, DCM, 0 ºC, 65%, d) TBAF, AcOH, THF, 86%, e) LevOH, DIC, DMAP, DCM, 0 ºC, 90%, f) TBSOTf, DCM, 0 ºC, 56%, g) NIS, THF/H_2O, 99%, h) ClC(=NPh)CF_3, K_2CO_3, acetone, 80%, B) j) DDQ, DCM/H_2O 20:1, 86%, k) TBSOTf, DCM, 0 ºC, 58% l) zinc, AcOH, Ac_2O, THF, 50 °C, 100%, m) hydrazine acetate, toluene/EtOH, 96%, n) TEMPO, BAIB, $NaHCO_3$, EtOAc/*t*-BuOH/H_2O, 4 °C, 65%, o) $Pd(OH)_2$, *t*-BuOH, H_2O, AcOH, H_2, 26%. `

Conclusion

This Chapter described the construction of several CP1 strain M and D oligosaccharides with varying taurine substitution patterns and length. To be able to vary the taurine substitution pattern, different protecting groups were installed on the C-6-OH of the $GalN_3$ residues. The saccharides were constructed from the reducing end, relying on a DBST group on the $GalN_3$ donors for the 1,2-*cis* linkages. For the CP1 fragments, late-stage modification of the larger saccharides was required to install the wanted taurine substitution patterns, necessitating the use for multiple oxidations on larger saccharides. Basic TEMPO/BAIB oxidation conditions at low temperature, in combination with long reaction times were found to provide the carboxylic acids in good yields. The taurine amides could easily be introduced via a peptide coupling followed by hydrogenation to give the wanted

taurine saccharides. A non-taurinated hexasaccharide was also synthetized, corresponding to a capsular oligosaccharide fragment of strain D. By implementing a [3+3] strategy, the hexasaccharide was obtained in good yield and excellent α-selectivity. Notably the four-fold oxidation to introduce four carboxylates in a single transformation using the basic oxidation conditions proceeded well to effectively deliver the target hexasaccharide. The generated fragments can now be used for antigen mapping studies for the construction of synthetic vaccine modalities. The chemistry described can be applied to generate larger structures and generate different taurine substitution patterns as well as in the synthesis of other structurally related bacterial oligosaccharides.

Experimental

General experimental procedures

All reagents were of commercial grade and used as received unless otherwise noted. All moisture sensitive reactions were performed under an argon or nitrogen (N_2) atmosphere. Dried solvents (DCM, DMF, THF, toluene, Et_2O) were stored over flame-dried 3 or 4Å molecular sieves. Reactions were monitored by thin layer chromatography (TLC) analysis conducted with Merck aluminum sheets with 0.20 mm of silica gel 60. The plates were detected by UV (254 nm) and were applicable by spraying with 20% sulfuric acid in EtOH 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% sulfuric acid (aq.) followed by charring at ~150 °C. Flash column chromatography was performed with silica gel (40-63µm). Size-exclusion chromatography was carried out using SephadexTM (LH-20, GE Healthcare Life Sciences) by isocratic elution with DCM/MeOH (1:1, v:v). High-resolution mass spectra were recorded on a Thermo Finigan LTQ Orbitrap mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275 ºC) with resolution R=60.000 at m/z=400 (mass range 150-4000). ^1H and ^{13}C spectra were recorded on a Bruker AV-400 (400 and 101 MHz respectively), Bruker AV-500 (500 and 126 MHz respectively), Bruker AV-600 (600 and 151 MHz respectively), Bruker AV-850 (800 and 214 MHz respectively) or a Bruker AV-1200 (1200 and 302 MHz respectively). Chemical shifts (δ) are given in ppm relative to the residual signal of the deuterated solvent (^1H-NMR: 7.26 ppm for $CDCl_3$, 3.31 ppm for MeOD, 1.94 for $CNCD_3$ or 4.79 for D_2O. ^{13}C-NMR: 77.16 ppm for $CDCl_3$, 49.00 ppm for MeOD, 1.32 for $CNCD_3$). Coupling constants (*J*) are given in Hz. All ^{13}C spectra are proton decoupled. NMR peak assignments were made using COSY and HSQC experiments, where applicable, HMBC and GATED experiments were used to further elucidate the structure. The anomeric product ratios were analyzed through integration of proton NMR signals.

General glycosylation procedure A with imidate donor

Acceptor (1 equiv.) and donor (1.5 equiv.) were co-evaporated with toluene (3x), dissolved in dry DCM (0.1 M), added 3Å molecular sieves and stirred for 30 min under argon. The solution was cooled to 0 ºC, added TBSOTf (0.2 equiv.) and stirred until TLC showed full conversion at 0 ºC. The reaction was quenched with Et_3N, dissolved in EtOAc, washed with $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography gave the want product.

General azide reduction procedure B

The starting material was dissolved in THF (3 mL) and added zinc powder (300 equiv.), AcOH (1 mL) and Ac_2O (0.5 mL). The reaction was stirred overnight at 50 ºC until TLC (DCM/MeOH 95:5) showed full conversion of the starting material. The reaction was cooled to rt, filtered through Celite, evaporated *in vacuo* and co-evaporated with toluene x3. Column chromatography gave the wanted product.

General oxidation procedure C with TEMPO, BAIB and $NaHCO_3$

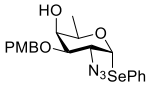
The starting material was dissolved in EtOAc/*t*-BuOH/H_2O (1:1:1), cooled to 0 ºC, added TEMPO (0.8 equiv. pr. hydroxy group) and $NaHCO_3$ (5 equiv. pr. hydroxy group) and stirred at 0 ºC for 10 min before adding BAIB (4 equiv. pr. hydroxy group). The reaction was stirred at 4 ºC until LC-MS showed full conversion from the hydroxy(s) over the aldehyde(s) to the acid group(s). The solution was quenched with $Na_2S_2O_3$ (aq., sat.) and diluted in EtOAc. NaH_2PO_4 (0.5 mL, aq., sat.) and brine (1 mL) was added and the aqueous phase was extracted with EtOAc (x3) and the combined organic layers was dried with Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (DCM/MeOH + 1% AcOH) gave the want product.

General hydrogenation procedure D

The starting material was dissolved in *t*-BuOH (1.5 mL) and added AcOH (1 mL, 0.1 mL in 100 mL MilliQ). Another 1 mL *t*-BuOH was added to dissolve the compound. The solution was birched with argon for 20 min and added $Pd(OH)_2/C$ (catalytic amount). The reaction was again birched with argon for 5 minutes before the atmosphere was changed for H_2. The mixture was stirred under H_2 atmosphere for three days or until completion by NMR was observed. The mixture was filtered over a Whatman filter and lyophilized. Purification by a HW40 column with NH_4OAc followed by lyophilization gave the wanted product.

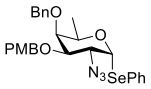
Synthesis of the building blocks

Phenyl 2-azido-2-deoxy-3-*O*-(*p*-methoxybenzyl)-1-seleno-α-D-fucopyranoside (13)



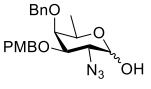
Phenyl 2-azido-2-deoxy-1-seleno-α-D-fucopyranoside **12** (3.61 g, 10.96 mmol) was co-evaporated with toluene (x3) and dissolved in dry toluene (50 ml, 0.2 M). Bu_2SnO (2.784 g, 11.18 mmol, 1.02 equiv.) was added. The flask was equipped with a Dean-Stark and the reaction was heated to 140 ºC for 3 h. The now clear solution was cooled to 60 ºC before adding Bu_4NBr (3.711 g, 11.51 mmol, 1.05 equiv.), CsF (1.699 g, 11.18 mmol, 1.02 equiv.) and PMBCl (1.6 mL, 11.51 mmol, 1.05 equiv.). The reaction was heated to 120 ºC for 1 h until TLC (pentane/EtOAc 3:2) showed full conversion. The reaction was allowed to cool to rt before a 10% KF solution was added and the reaction was stirred for 30 min. The aqueous phase was extracted with EtOAc (x3) and the combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated. Column chromatography (pentane/EtOAc 9:1 → 7:3) gave **13** in 87% yield (4.278 g, 9.525 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.60 – 7.54 (m, 2H, Ar-*H*), 7.36 – 7.26 (m, 5H, Ar-*H*), 6.95 – 6.88 (m, 2H, Ar-*H*), 5.88 (d, *J* = 5.3 Hz, 1H, H-1), 4.73 – 4.59 (m, 2H, Ar-CH_2), 4.29 (qd, *J* = 6.5, 1.4 Hz, 1H, H-5), 4.15 (dd, *J* = 10.2, 5.3 Hz, 1H, H-2), 3.86 (dd, *J* = 3.1, 1.3 Hz, 1H, H-4), 3.82 (s, 3H, PMB-OCH_3), 3.69 (dd, *J* = 10.2, 3.1 Hz, 1H, H-3), 2.37 (s, 1H, OH), 1.26 (d, *J* = 6.6 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 159.83 (Ar-C_q), 134.52 (Ar-*C*), 129.93 (Ar-*C*), 129.24 (Ar-*C*), 128.79 (Ar-C_q), 128.68 (Ar-C_q), 127.91 (Ar-*C*), 114.24 (Ar-*C*), 85.38 (C-1), 78.99 (C-3), 71.95 (Ar-*C*H_2), 68.70 (C-4/ C-5), 68.61 (C-4/ C-5), 60.23 (C-2), 55.44 (PMB-OCH_3), 16.20 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{20}H_{23}N_3O_4SeNa$: 472.07515; found 472.07463

Phenyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-1-seleno-α-D-fucopyranoside (10a)



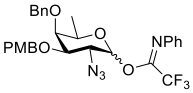
13 (4.278 g, 9.525 mmol) was dissolved in DMF (95 mL, 0.1 M) and cooled to 0 ºC. NaH (60% suspension in mineral oil, 495 mg, 12.38 mmol, 1.3 equiv.) and BnBr (1.5 mL, 12.38 mmol, 1.3 equiv.) were added and the solution was warmed to rt and stirred under N_2 for 4 h until TLC (pentane/EtOAc 9:1) showed full conversion. The reaction was quenched with H_2O and extracted with Et_2O (x3). The combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 85:15) gave **10a** in 78% yield (3.983 g, 7.388 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.58 – 7.54 (m, 2H, Ar-*H*), 7.40 – 7.26 (m, 9H, Ar-*H*), 6.96 – 6.89 (m, 2H, Ar-*H*), 5.92 (d, *J* = 5.3 Hz, 1H, H-1), 4.95 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.70 (d, *J* = 1.6 Hz, 2H, Ar-CH_2), 4.60 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.33 (dd, *J* = 10.2, 5.3 Hz, 1H, H-2), 4.21 (dd, *J* = 7.0, 5.9 Hz, 1H, H-5), 3.83 (s, 3H, PMB-OCH_3), 3.76 – 3.66 (m, 2H, H-3, H-4), 1.12 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 160.04 (Ar-C_q), 138.08 (Ar-C_q), 135.15 (Ar-*C*), 134.47 (Ar-*C*), 129.73 (Ar-*C*), 129.16 (Ar-*C*), 128.45 (Ar-*C*), 128.34 (Ar-*C*), 127.91 (Ar-*C*), 127.78 (Ar-*C*), 114.12 (Ar-*C*), 85.76 (C-1), 80.45 (C-3), 75.93 (C-4), 75.11 (Ar-*C*H$_2$), 72.37 (Ar-*C*H$_2$), 69.53 (C-5), 60.97 (C-2), 55.44 (PMB-O*C*H$_3$), 16.68 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{27}H_{29}N_3O_4SeNa$: 562.12210; found 562.12173

2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α/β-D-fucopyranose (14)



10a (1.592 g, 2.953 mmol) was dissolved in acetone/H_2O (10:1, 60 mL, 0.05 M) and cooled to 0 ºC. NIS (1.329 g, 5.907 mmol, 2 equiv.) was added and the solution stirred for 20 min until TLC (pentane/EtOAc 8:2) showed full conversion. The reaction was quenched with $Na_2S_2O_3$ (sat. aq.) and the acetone was evaporated. The residue was dissolved in EtOAc and washed with $Na_2S_2O_3$ (x1, sat. aq.), $NaHCO_3$ (x1, sat. aq.) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 8:2 → 6:4) gave **14** in 93% yield (1.0938 g, 2.738 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.38 – 7.27 (m, 12H), 6.94 – 6.89 (m, 3H), 5.30 (t, *J* = 2.8 Hz, 1H), 4.94 (d, *J* = 11.5 Hz, 2H), 4.70 – 4.56 (m, 5H), 4.45 (td, *J* = 7.6, 1.6 Hz, 1H), 4.09 (dd, *J* = 7.2, 5.9 Hz, 1H), 3.99 – 3.89 (m, 2H), 3.82 (d, *J* = 0.9 Hz, 5H), 3.77 – 3.70 (m, 1H), 3.70 – 3.67 (m, 1H), 3.56 – 3.50 (m, 1H), 3.48 (q, *J* = 6.1 Hz, 1H), 3.35 (ddd, *J* = 10.3, 2.8, 1.0 Hz, 1H), 2.83 (ddd, *J* = 35.8, 18.3, 8.7 Hz, 1H), 1.19 (d, *J* = 6.5 Hz, 2H), 1.15 (d, *J* = 6.5 Hz, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 160.15, 140.15, 139.95, 129.74, 129.68, 128.59, 128.52, 128.44, 127.96, 127.93, 114.09, 114.07, 96.53, 92.55, 80.88, 77.55, 76.12, 74.96, 74.93, 74.87, 72.45, 72.16, 71.17, 66.94, 64.81, 60.26, 55.43, 17.05, 16.97. **HRMS**: $[M+Na]^+$ calculated for $C_{21}H_{25}N_3O_5Na$: 422.16191; found 422.22625

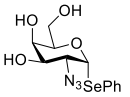
2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-fucopyranose (10b)



14 (1.10 g, 2.754 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (14 mL, 0.2 M). K_2CO_3 (571 mg, 4.132 mmol, 1.5 equiv.) and ClC(=NPh)CF_3 (0.7 mL, 4.132 mmol, 1.5 equiv.) and was added and the reaction was stirred at rt under N_2 overnight until TLC (pentane/EtOAc,) showed full conversion. The reaction was filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 85:15) **10b** in 91% yield (1.436 g, 2.517 mmol). **^{1}H NMR (400 MHz,**

CD_3CN) δ 7.42 – 7.26 (m, 8H), 7.19 – 7.09 (m, 1H), 6.97 – 6.89 (m, 2H), 6.93 – 6.83 (m, 2H), 6.26 (s, 0H), 4.88 (dd, *J* = 11.1, 3.2 Hz, 1H), 4.74 (dd, *J* = 24.8, 11.0 Hz, 1H), 4.67 – 4.54 (m, 2H), 4.07 – 3.93 (m, 2H), 3.82 (d, *J* = 8.4 Hz, 1H), 3.78 (d, *J* = 1.4 Hz, 3H), 1.25 – 1.16 (m, 3H). **^{13}C NMR (101 MHz, CD_3CN)** δ 138.90, 129.97, 128.99, 128.97, 128.38, 128.22, 128.19, 127.80, 127.76, 124.57, 119.22, 113.85, 113.83, 80.27, 76.96, 75.77, 75.07, 71.73, 71.62, 71.20, 69.67, 61.90, 58.78, 55.00, 16.06, 15.99, 0.49, 0.29.

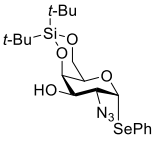
Phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-seleno-α-D-galactopyranoside (16)



3,4,6-Tri-*O*-acetyl-D-galactal **15** (28.35 g, 104.1 mmol) and $(PhSe)_2$ (33.4 g, 104.1 mmol, 1 equiv.) was dissolved in DCM (350 mL, 0.3 M) and degassed under argon at rt for 30 min. The reaction was cooled to -30 ºC, and added BAIB (33.5 g, 104.1 mmol, 1 equiv.) and $TMSN_3$ (28 mL, 208.3 mmol, 2 equiv.) and stirred at -20 ºC overnight until TLC (toluene/EtOAc 9:1) showed full conversion. Cyclohexene (50 mL) was added and the reaction was stirred at rt for 30 min before concentration. The lipophilic by products were removed by Column chromatography (pentane/EtOAc 98:2 → 0:100) were all the carbohydrate positive fraction was collected. The crude residue was recrystallized in hot EtOAc/*i*-PrOH 1:4 to give the acetylated-**16** in 56% yield (27.65 g, 58.70 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.65 – 7.54 (m, 2H, Ar-*H*), 7.35 – 7.26 (m, 3H, Ar-*H*), 6.00 (d, *J* = 5.4 Hz, 1H, H-1), 5.47 (dd, *J* = 3.3, 1.3 Hz, 1H, H-4), 5.11 (dd, *J* = 10.9, 3.2 Hz, 1H, H-3), 4.67 (ddd, *J* = 7.3, 5.7, 1.3 Hz, 1H, H-5), 4.26 (dd, *J* = 10.9, 5.4 Hz, 1H, H-2), 4.10 – 3.99 (m, 2H, H-6), 2.15 (s, 3H, COC*H*$_3$), 2.06 (s, 3H, COC*H*$_3$), 1.97 (s, 3H, COC*H*$_3$). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.48 (C=O), 170.08 (C=O), 169.76 (C=O), 134.90 (Ar-*C*), 129.34 (Ar-*C*), 128.34 (Ar-*C*), 127.63 (Ar-*C*$_q$), 85.07 (C-1), 77.48 (C-3), 77.16 (C-5), 76.84 (C-4), 71.28 (C-6), 69.06 (C-2), 66.18 (CO*C*H$_3$), 61.64 (CO*C*H$_3$), 58.82 (CO*C*H$_3$), 20.76 (CO*C*H$_3$). **HRMS**: $[M+Na]^+$ calculated for $C_{18}H_{21}N_3O_7SeNa$: 494.04424; found 494.04380

The acetylated-**16** (25.55 g, 54.33 mmol) was dissolved in MeOH (180 mL, 0.3 M) and added NaOMe (2.5 mL, 10.87 mmol, 0.2 equiv.). The reaction was stirred at rt for 1 h until TLC (pentane/EtOAc 1:1) showed full conversion and thus neutralized with Amberlite IR-120 H^+ resins, filtered and concentrated *in vacuo*. The crude product **16** (18.711 g, 54.33 mmol) was used without further purification. **^{1}H NMR (400 MHz, MeOD)** δ 7.67 – 7.61 (m, 2H, Ar-*H*), 7.32 – 7.25 (m, 3H, Ar-*H*), 5.94 (d, *J* = 5.2 Hz, 1H, H-1), 4.23 (td, *J* = 6.1, 1.3 Hz, 1H, H-5), 4.07 (dd, *J* = 10.4, 5.3 Hz, 1H, H-2), 3.96 (dd, *J* = 3.2, 1.3 Hz, 1H, H-4), 3.73 – 3.67 (m, 2H, H-3, H-6), 3.60 (dd, *J* = 11.3, 6.4 Hz, 1H, H-6). **^{13}C NMR (101 MHz, MeOD)** δ 136.02 (Ar-*C*), 130.04 (Ar-*C*), 128.81 (Ar-*C*), 87.35 (C-1), 74.94 (C-5), 72.48 (C-3), 70.19 (C-4), 63.07 (C-2), 62.01 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{12}H_{15}N_3O_4SeNa$: 368.01225; found 368.01205

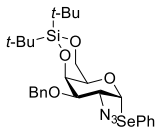
Phenyl 2-azido-2-deoxy-4,6-O-(di-*tert*-butylsilyene)-1-seleno-D-α-galactopyranoside (17)



16 (6.377 g, 18.53 mmol) was co-evaporated with toluene (x3) and dissolved in dry DMF (75 mL, 0.25 M) and cooled to -40 ºC. (*t*-Bu)$_2$Si(OTf)$_2$ (7.2 mL, 22.23 mmol, 1.2 equiv.) and pyridine (3.7 mL, 46.32 mmol, 2.5 eq) was added and the reaction was stirred at rt under argon for 2 h until TLC (pentane/EtOAc 8:2) showed full conversion. The reaction was quenched with H_2O and extracted with Et_2O (x3). The combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography

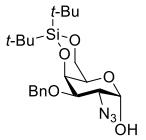
(pentane/EtOAc 100:0 → 85:15) gave **17** in 96% yield (8.665 g, 17.85 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.58 – 7.52 (m, 2H, Ar-*H*), 7.32 – 7.27 (m, 3H, Ar-*H*), 5.93 (d, *J* = 5.2 Hz, 1H, H-1), 4.49 (dd, *J* = 3.4, 1.2 Hz, 1H, H-4), 4.30 (dd, *J* = 12.7, 2.3 Hz, 1H, H-6), 4.20 (q, *J* = 1.8 Hz, 1H, H-5), 4.05 – 3.98 (m, 2H, H-2, H-6), 3.79 (ddd, *J* = 11.0, 10.3, 3.4 Hz, 1H, H-3), 2.76 (d, *J* = 10.8 Hz, 1H, OH), 1.06 (s, 9H, (CH_3)$_3$CSi), 1.02 (s, 9H, (CH_3)$_3$CSi). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 134.52 (Ar-*C*), 129.33 (Ar-*C*), 128.50 (Ar-C_q), 128.05 (Ar-*C*), 85.50 (C-1), 72.38 (C-4), 71.92 (C-3), 69.90 (C-5), 66.81 (C-6), 62.26 (C-2), 27.67 ((*C*H$_3$)$_3$CSi), 27.36 ((*C*H$_3$)$_3$CSi), 23.47 ((CH$_3$)$_3$*C*Si), 20.88 ((CH$_3$)$_3$*C*Si). **HRMS**: [M+Na]$^+$ calculated for $C_{20}H_{31}N_3O_4SeSiNa$: 508.11467; found 508.11423

Phenyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(di-*tert*-butylsilyene)-1-seleno-D-α-galactopyranoside (11a)



17 (8.665 g, 17.85 mmol) was co-evaporated with toluene (x3) and dissolved in dry DMF (109 mL, 0.1 M) and cooled to 0 ºC. BnBr (4.5 mL, 38.05 mmol, 2 equiv.) and NaH (913 mg, 22.83 mmol, 1.2 eq) were added and the reaction was stirred at rt under N_2 for 2 h until TLC (pentane/EtOAc 95:5) showed full conversion. The reaction was quenched with H_2O and extracted with Et_2O (x3). The combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 100:0 → 90:10) gave **11a** in 92% yield (9.3752 g, 16.31 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.57 – 7.53 (m, 2H, Ar-*H*), 7.46 – 7.26 (m, 9H, Ar-*H*), 5.94 (d, *J* = 5.3 Hz, 1H, H-1), 4.77 (d, *J* = 11.5 Hz, 1H, Ar-CH_2), 4.69 (d, *J* = 11.6 Hz, 1H, Ar-CH_2), 4.61 – 4.56 (m, 1H, H-4), 4.32 (ddd, *J* = 10.2, 5.3, 1.2 Hz, 1H, H-2), 4.24 (dd, *J* = 12.5, 2.2 Hz, 1H, H-6), 4.07 – 3.97 (m, 2H, H-5, H-6), 3.64 (ddd, *J* = 10.3, 3.0, 1.2 Hz, 1H, H-3), 1.06 (s, 9H, (CH_3)$_3$CSi), 1.03 (s, 9H, (CH_3)$_3$CSi). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 137.71 (Ar-C_q), 134.61 (Ar-*C*), 129.28 (Ar-*C*), 128.70 (Ar-*C*), 128.10 (Ar-*C*), 128.03 (Ar-*C*), 127.98 (Ar-*C*), 86.08 (C-1), 78.89 (C-3), 70.75 (Ari*C*H$_2$), 70.13 (C-5), 69.36 (C-4), 67.16 (C-6), 59.73 (C-2), 27.75 ((*C*H$_3$)$_3$CSi), 27.44 ((*C*H$_3$)$_3$CSi), 23.55 ((CH$_3$)$_3$*C*Si), 20.80 ((CH$_3$)$_3$*C*Si). **HRMS**: [M+Na]$^+$ calculated for $C_{27}H_{37}N_3O_4SeSiNa$: 598.16162; found 598.20610

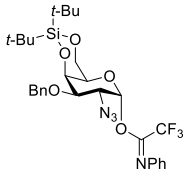
2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(di-*tert*-butylsilyene)-D-α-galactopyranoside (18)



11b (5.028 g, 8.749 mmol) was dissolved in acetone/H_2O (10:1, 175 mL, 0.05 M) and cooled to 0 ºC. NIS (3.937 g, 17.497 mmol, 2 equiv.) were added and the reaction was stirred for 15 min until TLC (pentane/EtOAc, 4:1) showed full conversion. The reaction mixture was quenched with $Na_2S_2O_3$ (aq., sat.) and acetone was evaporated. The residue was diluted in EtOAc and was washed with $Na_2S_2O_3$ (x1, aq., sat.), $NaHCO_3$ (xa, aq., sat.) and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 7:3 → 5:5) yielded **18** in 90% yield (3.432 g, 7.861 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.46 – 7.41 (m, 2H, Ar-*H*), 7.39 – 7.27 (m, 3H, Ar-*H*), 5.34 (t, *J* = 3.2 Hz, 1H, H-1), 4.77 (d, *J* = 11.7 Hz, 1H, Ar-CH_2), 4.68 (d, *J* = 11.7 Hz, 1H, Ar-CH_2), 4.56 (dd, *J* = 2.8, 1.1 Hz, 1H, H-4), 4.26 (dd, *J* = 12.6, 2.2 Hz, 1H, H-6), 4.18 – 4.11 (m, 1H, H-6), 3.95 – 3.82 (m, 3H, H-2, H-3, H-5), 2.78 – 2.69 (m, 1H, OH), 1.06 (s, 9H, (CH_3)$_3$CSi), 1.04 (s, 9H, (CH_3)$_3$CSi). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 137.89 (Ar-C_q), 128.69 (Ar-*C*), 128.10 (Ar-*C*), 128.07 (Ar-*C*), 92.67 (C-1), 75.84 (C-3), 70.69 (Ar-CH_2), 69.82 (C-4), 67.62 (C-5), 67.40 (C-6), 59.12 (C-2), 27.76 ((*C*H$_3$)$_3$CSi), 27.45

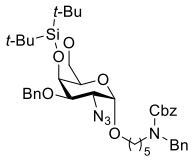
((CH_3)$_3$CSi), 23.54 ((CH_3)$_3$*C*Si), 20.82 ((CH_3)$_3$*C*Si). **HRMS**: $[M+Na]^+$ calculated for $C_{21}H_{33}N_5O_5SiNa$: 458.20872; found 458.20803

2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(di-*tert*-butylsilyene)-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-D-α-galactopyranoside (11b)



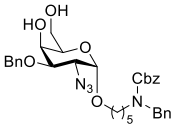
18 (3.431 g 7.859 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (40 mL, 0.2 M). K_2CO_3 (1.629 g, 11.79 mmol, 1.5 equiv.) and ClC(=NPh)CF_3 (1.9 mL, 11.79 mmol, 1.5 equiv.) and was added and the reaction was stirred at rt under N_2 overnight until TLC (pentane/EtOAc,) showed full conversion. The reaction was filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 85:15) gave **11b** in 99% yield (4.695g, 7.948 mmol). **^{1}H NMR (400 MHz, CD_3CN)** δ 7.51 – 7.38 (m, 4H), 7.42 – 7.30 (m, 4H), 7.21 – 7.13 (m, 1H), 6.90 (d, *J* = 7.8 Hz, 2H), 6.38 (s, 1H), 4.85 (s, 1H), 4.84 – 4.72 (m, 1H), 4.66 (d, *J* = 11.3 Hz, 1H), 4.33 (d, *J* = 12.9 Hz, 1H), 4.17 – 4.04 (m, 2H), 3.98 (d, *J* = 10.7 Hz, 1H), 3.89 (s, 1H), 1.12 – 1.02 (m, 18H). **^{13}C NMR (101 MHz, CD_3CN)** δ 129.00, 128.53, 128.17, 127.96, 117.42, 75.87, 70.02, 70.01, 69.89, 68.73, 66.63, 66.61, 57.84, 27.12, 26.87, 26.81, 0.65, 0.45, 0.24.

5-(Benzyl(benzyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(di-*tert*-butylsilyene)-galactopyranoside (20)



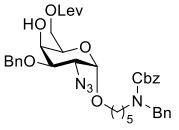
11b (1.353 mg, 2.291 mmol, 1 equiv.) and acceptor **19** (974 mg, 2.979 mmol, 1.3 equiv.) was co-evaporated with toluene (3x). The donor and acceptor was dissolved in dry DCM (23 mL, 0,1 M), added 3Å molecular sieves and stirred for 30 min. The solution was cooled to 0 ºC and added TBSOTf (0.1 mL, 0.458 mmol, 0.2 equiv.) and stirred for 30 min until TLC (pentane/EtOAc 9:1) showed full conversion. The reaction was quenched with Et_3N, dissolved in EtOAc, washed with $Na_2S_2O_3$ (sat. aq.; x1), $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 75:25 → 60:34) gave **20** in 98% yield (1.704 g, 2.852 mmol) as only the α-anomer. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.46 – 7.27 (m, 13H, Ar-*H*), 7.23 – 7.13 (m, 1H, Ar-*H*), 5.18 (d, *J* = 14.1 Hz, 2H, Ar-CH_2), 4.89 (d, *J* = 8.7 Hz, 1H, H-1), 4.76 (d, *J* = 11.5 Hz, 1H, Ar-CH_2), 4.66 (d, *J* = 11.5 Hz, 1H, Ar-CH_2), 4.63 – 4.56 (m, 1H, H-4), 4.50 (d, *J* = 7.9 Hz, 2H, Ar-CH_2), 4.30 – 4.19 (m, 1H, H-6), 4.19 – 4.09 (m, 1H, H-6), 3.91 – 3.80 (m, 1H, H-3), 3.77 (dd, *J* = 10.6, 3.5 Hz, 1H, H-2), 3.61 (d, *J* = 17.3 Hz, 2H, H-5, Linker-CH_2), 3.48 – 3.34 (m, 1H, Linker-CH_2), 3.34 – 3.17 (m, 2H, Linker-CH_2), 1.49 (s, 4H, Linker-CH_2), 1.39 – 1.21 (m, 4H, Linker-CH_2), 1.07 (s, 9H, (CH_3)$_3$CSi), 1.05 (s, 9H, (CH_3)$_3$CSi). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 137.98 (Ar-C_q), 128.67 (Ar-*C*), 128.63 (Ar-*C*), 128.06 (Ar-*C*), 128.03 (Ar-*C*), 127.96 (Ar-*C*), 127.93 (Ar-*C*), 127.44 (Ar-*C*), 127.31 (Ar-*C*), 98.43 (C-1), 75.55 (C-3), 70.51 (Ar-*C*H$_2$), 69.89 (C-4), 68.25 (Ar-*C*H$_2$), 67.50 (C-5), 67.30 (C-6), 57.99 (Linker-*C*H$_2$), 50.62 (Linker-*C*H$_2$), 50.33 (Linker-*C*H$_2$), 47.20 (Linker-*C*H$_2$), 46.23 (Linker-*C*H$_2$), 29.83 (Linker-*C*H$_2$), 29.16 (Linker-*C*H$_2$), 27.76 ((CH_3)$_3$CSi), 27.43 ((CH_3)$_3$CSi), 23.55 (Linker-*C*H$_2$), 23.44 ((CH_3)$_3$*C*Si), 20.84 ((CH_3)$_3$*C*Si). **HRMS**: $[M+Na]^+$ calculated for $C_{41}H_{56}N_4O_7SiNa$: 767.38160; found 767.38105

5-(Benzyl(benzyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-galactopyranoside (21)



20 (1.419 g, 1.903 mmol) was dissolved in THF (19 mL, 0.1 M) and cooled to 0 ºC. TBAF (1 M in THF, 4.8 mL, 4.757 mmol, 2.5 equiv.) was added and the reaction was stirred at rt under N_2 overnight until TLC (pentane/EtOAc 8:2) showed full conversion. The reaction was quenched with NH_4Cl (aq., sat.) and diluted with EtOAc. The organic phase was washed with H_2O (x3) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 6:4 → 3:7) gave **21** in 90% yield (1.041 g, 1.713 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.44 – 7.27 (m, 12H, Ar-*H*), 7.17 (d, *J* = 7.2 Hz, 1H, Ar-*H*), 5.17 (d, *J* = 16.6 Hz, 2H, Ar-CH_2), 4.94 – 4.85 (m, 1H, H-1), 4.78 – 4.65 (m, 2H, Ar-CH_2), 4.49 (d, *J* = 21.5 Hz, 2H, Ar-CH_2), 4.15 (d, *J* = 22.7 Hz, 1H, H-4), 3.96 – 3.85 (m, 2H, H-5, H-6), 3.85 – 3.71 (m, 1H, H-6), 3.71 – 3.51 (m, 2H, H-2, H-3), 3.50 – 3.27 (m, 2H, Linker-CH_2), 3.27 – 3.12 (m, 1H, Linker-CH_2), 2.83 (s, 1H, 6-OH), 2.62 (d, *J* = 22.1 Hz, 1H, 4-OH), 2.31 (s, 1H, 6-OH), 1.61 – 1.44 (m, 3H, Linker-CH_2), 1.42 – 1.19 (m, 3H, Linker-CH_2). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 137.93 (Ar-C_q), 137.22 (Ar-C_q), 128.83 (Ar-*C*), 128.69 (Ar-*C*), 128.59 (Ar-*C*), 128.43 (Ar-*C*), 128.16 (Ar-*C*), 128.10 (Ar-*C*), 127.95 (Ar-*C*), 127.47 (Ar-*C*), 127.32 (Ar-*C*), 98.09 (C-1), 76.19 (C-3), 72.15 (Ar-*C*H_2), 69.85 (C-5), 67.84 (Ar-*C*H_2), 67.40 (C-4), 67.21 (Ar-*C*H_2), 62.61 (C-6), 59.15 (C-2), 50.41 (Linker-*C*H_2), 47.09 (Linker-*C*H_2), 28.99 (Linker-*C*H_2), 27.21 (Linker-*C*H_2), 23.34 (Linker-*C*H_2). **HRMS**: $[M+Na]^+$ calculated for $C_{33}H_{40}N_4O_7Na$: 627.27947; found 627.27892

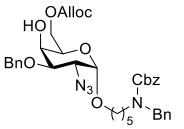
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (22)



21 (737 mg, 1.218 mmol) was co-evaporated with toluene (x3) and dissolved in dry toluene (12 mL, 0.1 M) and added Bu_2SnO (318 mg, 1.279 mmol, 1.05 equiv.) and heated to 110 ºC for 4 h under nitrogen. The reaction was cooled to rt and added Lev_2O in DCM (4.9 mL, 2.436 mmol, 0.5 M, 2 equiv.) and stirred at rt under nitrogen overnight until TLC (pentane/EtOAc, 1:1) showed full conversion. The reaction mixture was added MeOH and concentrated *in vacuo*. The residue was dissolved in EtOAc and washed with 10% KF (x1), and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 60:40 → 45:55) yielded **22** in 93% yield (792 mg, 1.133 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.45 – 7.27 (m, 12H, Ar-*H*), 7.17 (d, *J* = 7.2 Hz, 1H, Ar-*H*), 5.17 (d, *J* = 15.5 Hz, 2H, Ar-*H*), 4.88 (dd, *J* = 7.0, 3.5 Hz, 1H, H-1), 4.77 – 4.65 (m, 2H, Ar-*H*), 4.50 (d, *J* = 6.8 Hz, 2H, Ar-*H*), 4.34 (dd, *J* = 11.4, 5.5 Hz, 1H, H-6), 4.24 (dd, *J* = 11.4, 7.1 Hz, 1H, H-6), 4.06 (s, 1H, H-4), 3.96 – 3.86 (m, 2H, H-3, H-5), 3.69 – 3.59 (m, 2H. H-2, Linker-CH_2), 3.46 – 3.32 (m, 1H, Linker-CH_2), 3.32 – 3.17 (m, 2H, Linker-CH_2), 2.77 – 2.69 (m, 2H, Lev-CH_2), 2.62 – 2.52 (m, 2H, Lev-CH_2), 2.42 (t, *J* = 1.6 Hz, 1H, Linker-CH_2), 2.17 (s, 3H, Lev-CH_3), 1.60 – 1.47 (m, 3H, Linker-CH_2), 1.41 – 1.21 (m, 4H, Linker-CH_2). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 172.74 (C=O), 138.06 (Ar-C_q), 137.22 (Ar-C_q), 128.83 (Ar-*C*), 128.68 (Ar-*C*), 128.43 (Ar-*C*), 128.20 (Ar-*C*), 128.07 (Ar-*C*), 127.95 (Ar-*C*), 127.43 (Ar-*C*), 99.01 (C-1), 75.89 (C-3), 72.14 (Ar-*C*H_2), 68.30 (Ar-*C*H_2), 67.77 (C-5), 67.28 (Ar-*C*H_2), 66.17 (C-4), 63.54 (C-6), 59.01 (C-2), 50.56 (Linker-*C*H_2), 47.34 (Linker-*C*H_2), 37.96 (Lev-*C*H_2), 29.98 (Lev-*C*H_2), 29.15 (Lev-

*C*H$_3$), 27.92 (Linker-*C*H$_2$), 27.46 (Linker-*C*H$_2$), 23.53 (Linker-*C*H$_2$). **HRMS**: [M+NH$_4$]$^+$ calculated for $C_{38}H_{46}N_4O_9Na$: 720.36085; found 720.36030

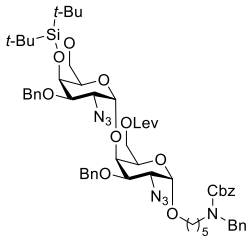
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (23)



21 (119 mg, 0.197 mmol) was dissolved in DCM (2 mL, 0.1 M) and cooled to 0 ºC. Allyl chloroformate (38 µl, 0.296 mmol, 1.5 equiv.) and pyridine (32 µL, 0.395 mmol, 2 eq) were added and the reaction was stirred for 30 min at 0 ºC under nitrogen until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction mixture was diluted in EtOAc and washed with 1 M HCl (x1), NaHCO$_3$ (x1, aq., sat.) and brine, dried over Na$_2$SO$_4$, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 9:1 → 7:3) yielded **23** in 84% yield (115 mg, 0.187 mmol). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.43 – 7.27 (m, 14H, Ar-*H*), 7.18 (d, *J* = 7.2 Hz, 1H, Ar-*H*), 5.92 (ddt, *J* = 16.5, 10.5, 5.8 Hz, 1H, C*H*=CH$_2$), 5.35 (dq, *J* = 17.2, 1.5 Hz, 1H, CH=C*H*$_2$), 5.26 (dq, *J* = 10.5, 1.3 Hz, 1H, CH=C*H*$_2$), 5.19 (d, *J* = 14.5 Hz, 2H, Ar-C*H*$_2$), 4.88 (d, *J* = 7.4 Hz, 1H, H-1), 4.75 – 4.65 (m, 2H, Ar-C*H*$_2$), 4.62 (dt, *J* = 5.8, 1.4 Hz, 2H, C*H*$_2$-allyl), 4.51 (d, *J* = 8.3 Hz, 2H, Ar-C*H*$_2$), 4.36 (d, *J* = 6.2 Hz, 2H, H-6), 4.08 (dt, *J* = 6.2, 3.5 Hz, 1H, H-4), 4.03 – 3.86 (m, 2H, H-5, H-3), 3.71 – 3.54 (m, 2H, H-2, Linker-C*H*$_2$), 3.47 – 3.34 (m, 1H, Linker-C*H*$_2$), 3.34 – 3.14 (m, 2H, Linker-C*H*$_2$), 2.52 (s, 1H, OH), 1.67 – 1.48 (m, 5H, Linker-C*H*$_2$), 1.45 – 1.22 (m, 5H, Linker-C*H*$_2$). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 154.89 (C=O), 137.99 (Ar-*C*$_q$), 137.10 (Ar-*C*$_q$), 131.48 (*C*H=CH$_2$), 128.78 (Ar-*C*), 128.62 (Ar-*C*), 128.55 (Ar-*C*), 128.39 (Ar-*C*), 128.10 (Ar-*C*), 128.01 (Ar-*C*), 127.91 (Ar-*C*), 127.38 (Ar-*C*), 127.26 (Ar-*C*), 119.18 (CH=*C*H$_2$), 97.95 (C-1), 75.85 (C-3), 72.13 (Ar-*C*H$_2$), 68.74 (*C*H$_3$-allyl), 68.29 (Ar-*C*H$_2$), 67.67 (C-5), 67.24 (Ar-*C*H$_2$), 66.70 (C-6), 66.11 (C-4), 58.96 (C-2), 50.56 (Linker-*C*H$_2$), 50.26 (Linker-*C*H$_2$), 47.15 (Linker-*C*H$_2$), 46.16 (Linker-*C*H$_2$), 29.78 (Linker-*C*H$_2$), 29.07 (Linker-*C*H$_2$), 27.91 (Linker-*C*H$_2$), 27.49 (Linker-*C*H$_2$), 23.43 (Linker-*C*H$_2$). **HRMS**: [M+Na]$^+$ calculated for $C_{37}H_{44}N_4O_9Na$: 711.30060; found 711.30005

Synthesis of the trisaccharide intermediates

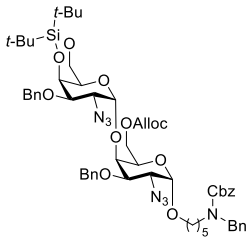
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(di-*tert*-butylsilyene)-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (24)



The reaction was carried out according to General glycosylationprocedure A using acceptor **22** (972 mg, 1.382 mmol, 1 equiv.), donor **11b** (1.225 g, 2.075 mmol, 1.5 equiv.) and TBSOTf (64 µL, 0.276 mmol, 0.2 equiv.) in DCM (14 mL, 0.1 M). The reaction was followed by TLC (pentane/EtOAc 7:3) and column chromatography (pentane/EtOAc 80:20 → 65:35) gave **24** in 82% yield (1.268 g, 1.134 mmol) as only the α-anomer. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.45 – 7.27 (m, 19H, Ar-*H*), 7.19 – 7.14 (m, 1H Ar-*H*), 5.17 (d, *J* = 15.2 Hz, 2H, C*H*$_2$-Ar), 5.00 (s, 1H, H-1'), 4.94 (d, J = 6.9 Hz, 1H, H-1), 4.74 (dd, *J* = 7.5, 4.4 Hz, 2H, C*H*$_2$-Ar), 4.64 (t, *J* = 11.3 Hz, 2H, C*H*$_2$-Ar), 4.51 (s, 2H, C*H*$_2$-Ar), 4.48 (s, 1H, H-5'), 4.47 – 4.41 (m, 1H, H-6), 4.39 – 4.30 (m, 1H, H-6), 4.26 (s, 1H, H-4'), 4.04 (s, 1H, H-4), 3.96 – 3.91 (m, 1H, H-5), 3.89 – 3.84 (m, 2H, H-2', H-3'), 3.74 (dd, J = 11.4,

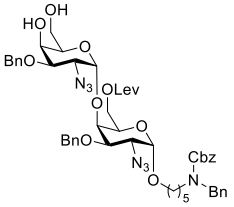
1.3 Hz, 1H, H-6'), 3.65 (dd, J = 12.7, 1.6 Hz, 2H, H-6', H-3), 3.58 (dd, J = 7.3, 3.5 Hz, 1H, H-2), 3.48 – 3.34 (m, 2H, CH_2-Linker), 3.28 – 3.16 (m, 2H, CH_2-Linker), 2.76 (q, J = 5.9 Hz, 2H, CH_2-Lev), 2.57 (t, J = 6.4 Hz, 2H, CH_2-Lev), 2.18 (s, 3H, CH_3-Lev), 1.55 – 1.50 (m, 2H, CH_2-Linker), 1.35 – 1.27 (m, 2H, CH_2-Linker), 1.11 – 1.04 (m, 2H, CH_2-Linker), 1.01 (s, 9H, *H-t*-Bu), 0.99 (s, 9H, *H-t*-Bu). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 128.72 (Ar-*C*), 128.68 (Ar-*C*), 128.63 (Ar-*C*), 128.06 (Ar-*C*), 127.96 (Ar-*C*), 127.34 (Ar-*C*), 99.18 (C-1'), 98.09 (C-1), 75.58 (C-3'), 72.23 (C-4'), 71.96 (*C*H$_2$-Ar), 70.46 (*C*H$_2$-Ar), 69.57 (C-5'), 68.36 (C-5), 67.92 (C-4), 67.82 (C-3), 67.28 (C-6'), 67.04 (*C*H$_2$-Ar), 61.82 (C-6), 59.61 (C-2), 59.11 (*C*H$_2$-Linker), 58.78 (C-2'), 50.33 (*C*H$_2$-Ar), 46.66 (*C*H$_2$-Linker), 38.08 (*C*H$_2$-Lev), 29.93 (*C*H$_3$-Lev), 29.16 (*C*H$_2$-Linker), 28.02 (*C*H$_2$-Lev), 27.73 (*C*H$_3$-*t*-Bu), 27.46 (*C*H$_3$-*t*-Bu), 23.46 (*C*H$_2$-Linker). **HRMS**: [M+Na]$^+$ calculated for $C_{59}H_{77}N_7O_{13}SiNa$: 1142.52463; found 1142.52408

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(di-*tert*-butylsilyene)-α-D-galactopyrasyl-(1→4)-6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (25)



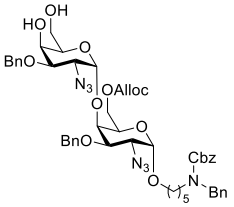
The reaction was carried out according to General glycosylationprocedure A using acceptor **23** (372 mg, 0.539 mmol, 1 equiv.), donor **11b** (478 mg, 0.809 mmol, 1.5 equiv.) and TBSOTf (25 µL, 0.108 mmol, 0.2 equiv.) in DCM (5.4 mL, 0.1 M). The reaction was followed by TLC (pentane/EtOAc 1:4) and column chromatography (pentane/EtOAc 95:5 → 80:20) gave **25** in 86% yield (514 mg, 0.464 mmol) as only the α-anomer. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.64 – 7.59 (m, 1H, Ar-*H*), 7.48 – 7.26 (m, 25H, Ar-*H*), 5.93 (ddt, J = 17.3, 10.4, 5.9 Hz, 1H, C*H*=CH$_2$), 5.37 (dq, J = 17.2, 1.5 Hz, 1H, CH=CH_2), 5.33 – 5.25 (m, 1H, CH=CH_2), 5.18 (d, J = 14.7 Hz, 3H, Ar-CH_2), 4.99 – 4.89 (m, 2H, H-1', H-1), 4.75 (dd, J = 11.5, 4.0 Hz, 3H, Ar-CH_2), 4.67 – 4.60 (m, 4H, Ar-CH_2, CH_2-allyl), 4.56 – 4.44 (m, 6H, , CH_2-linker, H-6, H-5), 4.26 (d, J = 4.8 Hz, 1H, H-4), 4.03 (s, 1H, H-4'), 3.97 (p, J = 7.2 Hz, 1H, H-5'), 3.94 – 3.83 (m, 3H, H-2', H-3, H-3'), 3.80 – 3.62 (m, H, H-6'), 3.59 (dd, J = 10.8, 3.6 Hz, 2H, H-2, CH_2-linker), 3.49 – 3.32 (m, 2H, CH_2-linker), 3.32 – 3.12 (m, 3H, CH_2-linker), 1.54 (m, 7H, CH_2-linker), 1.37 – 1.19 (m, 6H, CH_2-linker), 1.01 (d, J = 8.1 Hz, 18H, , (CH_3)$_3$CSi). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 154.70 (C=O), 137.83 (Ar-C_q), 137.18 (Ar-C_q), 131.62 (Ar-*C*), 131.39 (Ar-*C*), 129.32 (Ar-*C*), 128.71 (Ar-*C*), 128.66 (Ar-*C*), 128.60 (Ar-*C*), 128.51 (Ar-*C*), 128.04 (Ar-*C*), 127.98 (Ar-*C*), 127.94 (Ar-*C*), 127.85 (Ar-*C*), 127.41 (Ar-*C*), 127.25 (Ar-*C*), 119.48 (CH=*C*H$_2$), 99.33 (C-1, C-1'), 98.06 (C-1, C-1'), 75.62 (C-3, C-3'), 72.59 (C-4), 72.07 (Ar-*C*H$_2$), 70.43 (*C*H$_2$-allyl), 69.50 (C-5), 68.97 (C-6), 67.28 (C-5', Ar-*C*H$_2$), 66.99 (C-6), 65.27 (CH$_2$), 59.61 (C-2), 58.80 (C-2'), 50.75 (*C*H$_2$-linker), 50.33 (*C*H$_2$-linker), 47.14 (*C*H$_2$-linker), 46.22 (*C*H$_2$-linker), 27.71 ((*C*H$_3$)$_3$CSi), 27.44 ((*C*H$_3$)$_3$CSi), 23.43 (*C*H$_2$-linker). **HRMS**: [M+Na]$^+$ calculated for $C_{58}H_{75}N_7O_{13}SiNa$: 1128.50898; found 1128.50833

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (26)



24 (1.268 g, 1.132 mmol) was dissolved in THF (11 mL, 0.1 M) and cooled to 0 ºC. AcOH (0.16 mL, 2.823 mmol, 2.5 equiv.) and TBAF (1 M in THF, 2.8 mL, 2.823mmol, 2.5 equiv.) was added and the reaction was stirred at rt under N_2 overnight until TLC (pentane/EtOAc 8:2) showed full conversion. The reaction was quenched with NH_4Cl (aq., sat.) and diluted with EtOAc. The organic phase was washed with H_2O (x3) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 5:5 → 2:8) gave **26** in 86% yield (943 mg, 0.974 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.44 – 7.27 (m, 20H, Ar-*H*), 5.17 (d, *J* = 15.7 Hz, 2H, Ar-CH_2), 5.06 (d, *J* = 3.5 Hz, 1H, H-1'), 4.94 (d, *J* = 6.2 Hz, 1H, H-1), 4.85 (d, *J* = 11.7 Hz, 1H, Ar-CH_2), 4.74 – 4.65 (m, 3H, Ar-CH_2), 4.50 (d, *J* = 6.7 Hz, 2H, Ar-CH_2), 4.40 (q, *J* = 4.9 Hz, 2H, H-6), 4.26 (d, *J* = 2.7 Hz, 1H, H-4'), 4.14 (t, *J* = 1.9 Hz, 1H, H-4), 4.09 (t, *J* = 4.7 Hz, 1H, H-5'), 3.97 (d, *J* = 7.3 Hz, 1H, H-5), 3.92 (d, *J* = 3.0 Hz, 1H, H-3'), 3.90 (d, *J* = 3.0 Hz, 1H, H-2'), 3.84 (dd, *J* = 10.5, 3.5 Hz, 1H, H-3), 3.68 – 3.61 (m, 2H, H-6'), 3.60 (d, *J* = 3.6 Hz, 1H, H-2), 3.48 – 3.44 (m, 2H, CH_2-Linker), 3.30 – 3.17 (m, 2H, CH_2-Linker), 2.84 (s, 1H, O*H*), 2.76 (td, *J* = 6.0, 1.8 Hz, 2H, CH_2-Lev), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2-Lev), 2.21 (s, 1H, O*H*), 2.17 (s, 3H, CH_3-Lev), 1.58 – 1.49 (m, 3H, CH_2-Linker), 1.39 – 1.28 (m, 3H, CH_2-Linker). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 206.64 (C=O), 172.29 (C=O), 137.30 (Ar-*C*q), 137.04 (Ar-*C*q), 128.69 (Ar-*C*), 128.64 (Ar-*C*), 128.56 (Ar-*C*), 128.47 (Ar-*C*), 128.30 (Ar-*C*), 128.11 (Ar-*C*), 128.05 (Ar-*C*), 127.95 (Ar-*C*), 127.83 (Ar-*C*), 127.49 (Ar-*C*), 127.31 (Ar-*C*), 127.21 (Ar-*C*), 99.10 (C-1'), 98.02 (C-1), 76.14 (C-3), 75.51 (C-3'), 73.27 (C-4'), 72.16 (*C*H_2-Ar), 71.87 (*C*H_2-Ar), 69.34 (C-5'), 68.32 (C-6'), 68.12 (C-5), 67.68 (C-4), 67.17 (*C*H_2-Ar), 62.77 (*C*H_2-Linker), 61.86 (C-6), 59.55 (C-2'), 59.48 (C-2), 50.22 (*C*H_2-Ar), 47.12 (*C*H_2-Linker), 46.15 (*C*H_2-Linker), 37.94 (*C*H_2-Lev), 29.80 (*C*H_3-Lev), 29.04 (*C*H_2-Linker), 27.83 (*C*H_2-Lev), 23.37 (*C*H_2-Linker). **HRMS**: $[M+Na]^+$ calculated for $C_{51}H_{61}N_7O_{13}Na$: 1002.42250; found 1002.42196

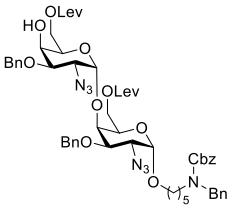
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (27)



25 (493 g, 0.446 mmol) was dissolved in THF (4.5 mL, 0.1 M) and cooled to 0 ºC. AcOH (60 µL, 1.114 mmol, 2.5 equiv.) and TBAF (1 M in THF, 1.1 mL, 1.114 mmol, 2.5 equiv.) was added and the reaction was stirred at rt under N_2 overnight until TLC (pentane/EtOAc 8:2) showed full conversion. The reaction was quenched with NH_4Cl (aq., sat.) and diluted with EtOAc. The organic phase was washed with H_2O (x3) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 7:3 → 4:6) gave **279** in 92% yield (396 mg, 0.41 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.43 – 7.26 (m, 24H, Ar-*H*), 7.18 (m, 2H, Ar-*H*), 5.93 (ddt, *J* = 17.4, 10.4, 5.8 Hz, 1H, C*H*=CH_2), 5.37 (dq, *J* = 17.2, 1.5 Hz, 1H, CH=CH_2), 5.29 (dq, *J* = 10.5, 1.2 Hz, 1H, CH=CH_2), 5.18 (d, *J* = 15.1 Hz, 2H, Ar-CH_2), 5.00 (d, *J* = 3.6 Hz, 1H, H-1'), 4.97 – 4.91 (m, 1H, H-1), 4.82 (d, *J* = 11.6 Hz, 1H, Ar-CH_2), 4.76 – 4.60 (m, 6H, Ar-CH_2), 4.48 (m, 5H, Ar-CH_2, H-6), 4.22 (s, 1H, H-4'), 4.14 (d, *J* = 2.4 Hz, 1H,

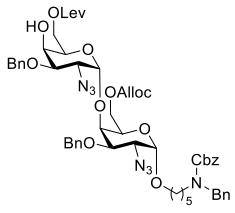
H-4), 4.09 – 4.02 (m, 1H, H-5'), 3.98 (dd, *J* = 12.9, 6.8 Hz, 1H, H-5), 3.94 – 3.81 (m, 3H, H-3, H-3', H-2'), 3.74 – 3.55 (m, 3H, CH_2-linker, H-2), 3.52 – 3.31 (m, 4H, CH_2-linker, H-6'), 3.24 (dt, *J* = 26.3, 7.6 Hz, 2H, CH_2-linker), 2.79 (s, 1H, 4-OH'), 2.11 (dd, *J* = 8.4, 4.4 Hz, 1H, 6-OH'), 1.65 – 1.44 (m, 5H, CH_2-linker), 1.40 – 1.23 (m, 5H, CH_2-linker). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 154.22 (C=O), 137.99 (Ar-C_q), 137.24 (Ar-C_q), 136.67 (Ar-C_q), 128.77 (Ar-*C*), 128.74 (Ar-*C*), 128.64 (Ar-*C*), 128.54 (Ar-*C*), 128.39 (Ar-*C*), 128.24 (Ar-*C*), 128.13 (Ar-*C*), 128.03 (Ar-*C*), 127.91 (Ar-*C*), 127.53 (Ar-*C*), 127.39 (Ar-*C*), 127.27 (Ar-*C*), 119.46 (*C*H_2-allyl), 99.36 (C-1'), 98.07 (C-1), 76.37 (C-3), 75.63 (C-3'), 73.72 (C-4'), 72.35 (Ar-*C*H_2), 71.97 (Ar-*C*H_2), 69.61 (C-5'), 68.95 (Ar-*C*H_2), 68.46 (*C*H_2-linker), 68.34 (C-5), 67.68 (C-4), 67.26 (*C*H_2), 65.07 (C-6), 62.82 (C-6'), 59.67 (C-2), 59.54 (C-2'), 50.58 (*C*H_2-linker), 50.30 (*C*H_2-linker), 47.17 (*C*H_2-linker), 46.19 (*C*H_2-linker), 29.79 (*C*H_2-linker), 28.12 (*C*H_2-linker), 27.49 (*C*H_2-linker), 23.42 (*C*H_2-linker). **HRMS**: $[M+Na]^+$ calculated for $C_{50}H_{59}N_7O_{13}Na$: 988.40685; found 988.40566

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (28)



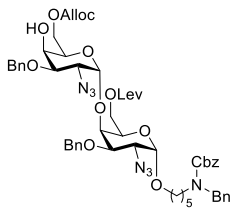
26 (558 mg, 0.569 mmol) was co-evaporated with toluene (x3) and dissolved in dry toluene (5.7 mL, 0.1 M) and added Bu_2SnO (149 mg, 0.598 mmol, 1.05 equiv.) and heated to 110 ºC for 4 h under nitrogen. The reaction was cooled to rt and added Lev_2O (0.5 M in DCM, 2.3 mL, 1.138 mmol, 2 equiv.) and stirred at rt under nitrogen overnight until TLC (pentane/EtOAc, 4:6) showed full conversion. The reaction mixture was added MeOH and concentrated *in vacuo*. The residue was dissolved in EtOAc and washed with 10% KF (x1), and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 60:40 → 40:60) yielded **28** in 92% yield (558 mg, 0.523 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.43 – 7.27 (m, 20H, Ar-*H*), 5.17 (d, *J* = 14.8 Hz, 2H, Ar-CH_2), 5.02 (d, *J* = 3.5 Hz, 1H, *H*-1'), 4.94 (d, *J* = 5.5 Hz, 1H, *H*-1), 4.85 (d, *J* = 11.9 Hz, 1H, Ar-CH_2), 4.73 (d, *J* = 11.6 Hz, 2H, Ar-CH_2), 4.68 (d, *J* = 11.9 Hz, 1H, Ar-CH_2), 4.49 (d, *J* = 6.0 Hz, 2H, Ar-CH_2), 4.39 (q, *J* = 3.9 Hz, 2H, *H*-6), 4.33 (t, *J* = 7.4 Hz, 1H. *H-4*), 4.26 – 4.19 (m, 2H, *H*-4', *H*-5'), 4.06 (s, 1H, *H*-5), 3.94 (dd, *J* = 7.6, 2.9 Hz, 2H, *H*-3', *H*-2'), 3.83 (dd, *J* = 10.2, 2.9 Hz, 2H, *H*-3, *H*-6'), 3.59 (dd, *J* = 7.3, 3.4 Hz, 2H, *H*-6', *H*-2), 3.46 – 3.34 (m, 1H, CH_2 - Linker), 3.22 (dt, *J* = 11.6, 7.1 Hz, 3H, CH_2 - Linker), 2.76 (td, *J* = 6.1, 2.8 Hz, 2H, CH_2 - Lev), 2.67 (dt, *J* = 8.7, 6.4 Hz, 2H, CH_2 - Lev), 2.56 (t, *J* = 6.2 Hz, 2H, CH_2 - Lev), 2.48 – 2.43 (m, 2H, CH_2 - Lev), 2.17 (s, 4H, CH_3 – Lev, O*H*), 2.16 (s, 3H, CH_3 - Lev), 1.57 – 1.47 (m, 3H, CH_2 - Linker), 1.34 – 1.26 (m, 3H, CH_2 - Linker). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 137.38 (Ar-C_q), 128.77 (Ar-*C*), 128.68 (Ar-*C*), 128.34 (Ar-*C*), 128.22 (Ar-*C*), 128.01 (Ar-*C*), 127.96 (Ar-*C*), 127.74 (Ar-*C*), 99.13 (*C*-1'), 98.16 (*C*-1), 76.06 (*C*-3), 75.00 (*C*-3'), 73.49 (*C*-4'), 72.05 (*C*H_2-Ar), 71.87 (*C*H_2-Ar), 68.28 (*C*-2'), 68.01 (*C*-4), 67.29 (*C*H_2-Ar), 65.25 (*C*-5), 62.57 (*C*-5'), 62.26 (*C*-6'), 62.13 (*C*H_2-Ar), 61.87 (*C*-6), 59.55 (*C*-2), 46.98 (*C*H_2-Linker), 46.49 (*C*H_2-Linker), 38.07 (*C*H_2-Lev), 37.97 (*C*H_2-Lev), 30.00 (*C*H_3-Lev), 28.43 (*C*H_2-Linker), 27.96 (*C*H_2-Lev), 27.81 (*C*H_2-Lev), 23.48 (*C*H_2-Linker). **HRMS**: $[M+NH_4]^+$ calculated for $C_{56}H_{67}N_7O_{15}Na$: 1095.50289; found 1095.50334

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (29)



27 (396 mg, 0.41 mmol) was co-evaporated with toluene (x3) and dissolved in dry toluene (4.1 mL, 0.1 M) and added Bu_2SnO (107 mg, 0.431 mmol, 1.05 equiv.) and heated to 110 ºC for 4 h under nitrogen. The reaction was cooled to rt and added Lev_2O (0.5 M in DCM, 1.6 mL, 0.821 mmol, 2 equiv.) and stirred at rt under nitrogen overnight until TLC (pentane/EtOAc,) showed full conversion. The reaction mixture was added MeOH and concentrated *in vacuo*. The residue was dissolved in EtOAc and washed with 10% KF (x1), and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, →) yielded **29** in 71% yield (308 mg, 0.291 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.44 – 7.27 (m, 19H, Ar-*H*), 7.25 – 7.14 (m, 2H, Ar-*H*), 5.92 (ddt, *J* = 17.3, 10.5, 5.9 Hz, 1H, C*H*=CH_2), 5.36 (dq, *J* = 17.2, 1.4 Hz, 1H, CH=C*H*$_2$), 5.32 – 5.24 (m, 1H, CH=C*H*$_2$), 5.17 (d, *J* = 14.6 Hz, 2H, Ar-C*H*$_2$), 4.96 (d, *J* = 3.6 Hz, 1H, H-1), 4.92 (d, *J* = 3.6 Hz, 1H, H-1'), 4.84 (d, *J* = 12.0 Hz, 1H, Ar-C*H*$_2$), 4.72 (s, 2H, Ar-C*H*$_2$), 4.68 – 4.60 (m, 3H, Ar-C*H*$_2$), 4.51 – 4.40 (m, 4H, C*H*$_2$-linker, H-6'), 4.32 – 4.25 (m, 1H, H-5), 4.25 – 4.16 (m, 2H, H-6, H-4), 4.07 (t, *J* = 2.1 Hz, 1H, H-4'), 4.04 – 3.91 (m, 2H, H-5', H-3'), 3.91 – 3.77 (m, 3H, H-3, H-6, H-2'), 3.72 – 3.54 (m, 2H, H-2', C*H*$_2$-linker), 3.52 – 3.31 (m, 1H, C*H*$_2$-linker), 3.22 (dt, *J* = 26.3, 6.9 Hz, 2H, C*H*$_2$-linker), 2.71 – 2.63 (m, 2H, CH_2-Lev), 2.48 – 2.34 (m, 2H, CH_2-Lev), 2.16 (s, 3H, CH_3-Lev), 1.67 – 1.47 (m, 5H, C*H*$_2$-linker), 1.39 – 1.16 (m, 4H, C*H*$_2$-linker).**^{13}C NMR (101 MHz, $CDCl_3$)** δ 206.75 (C=O), 172.48 (C=O), 155.42 (Ar-C_q), 137.80 (Ar-C_q), 137.28 (Ar-C_q), 136.80 (Ar-C_q), 131.38 (*C*H=CH_2), 128.73 (Ar-*C*), 128.64 (Ar-*C*), 128.30 (Ar-*C*), 128.18 (Ar-*C*), 128.00 (Ar-*C*), 127.93 (Ar-*C*), 127.61 (Ar-*C*), 127.40 (Ar-*C*), 127.28 (Ar-*C*), 119.45 (CH=*C*H_2), 99.23 (C-1'), 98.09 (C-1), 76.13 (C-3'), 75.16 (C-3), 73.70 (C-4), 72.12 (Ar-*C*H_2), 71.81 (Ar-*C*H_2), 68.94 (Ar-*C*H_2), 68.42 (Ar-*C*H_2), 68.38 (C-5), 68.10 (C-5'), 67.26 (Ar-*C*H_2), 65.23 (C-6'), 65.19 (C-4'), 62.22 (C-6), 59.64 (C-2'), 59.52 (C-2), 50.58 (CH_2-linker), 50.28 (CH_2-linker), 47.17 (CH_2-linker), 46.18 (CH_2-linker), 37.94 (CH_2-Lev), 29.9 (CH_3-Lev)5, 28.23 (CH_2-Lev), 23.40 (CH_2-linker). **HRMS**: $[M+Na]^+$ calculated for $C_{55}H_{65}N_7O_{15}Na$: 1086.44363; found 1086.44309

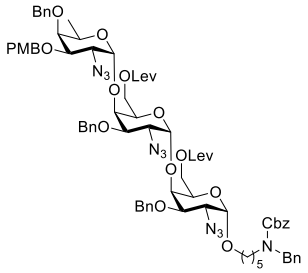
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (30)



26 (154 mg, 0.157 mmol) was dissolved in DCM (1.6 mL, 0.1 M) and cooled to 0 ºC. Allyl chloroformate (30 µl, 0.236 mmol, 1.5 equiv.) and pyridine (25 µL, 0.315 mmol, 2 eq) were added and the reaction was stirred for 1 h at 0 ºC under nitrogen until TLC (pentane/EtOAc, 1:1) showed full conversion. The reaction mixture was diluted in EtOAc and washed with 1 M HCl (x1), $NaHCO_3$ (x1, aq., sat.) and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 63:35 → 50:50) yielded **30** in 74% yield (123 mg, 0.116 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.41 – 7.27 (m, 20H, Ar-*H*), 5.94 – 5.83 (m, 1H, C*H* - Alloc), 5.33 (dd, *J* = 17.2, 1.5 Hz, 1H, C*H*$_2$ - Alloc), 5.25 (dd, *J* = 10.4, 1.4 Hz, 1H,

CH_2 - Alloc), 5.17 (d, *J* = 14.6 Hz, 2H, Ar-CH_2), 5.02 (d, *J* = 3.5 Hz, 1H, *H*-1'), 4.93 (d, *J* = 6.2 Hz, 1H, *H*-1), 4.88 (d, *J* = 12.1 Hz, 1H, Ar-CH_2), 4.70 (d, *J* = 9.0 Hz, 3H, Ar-CH_2), 4.55 (d, *J* = 5.8 Hz, 2H, CH_2 - Alloc), 4.50 (d, *J* = 6.7 Hz, 2H, Ar-CH_2), 4.40 (q, *J* = 3.7 Hz, 2H, *H*-6), 4.20 (s, 1H, *H*-4), 4.17 (t, *J* = 3.2 Hz, 1H, *H*-4'), 4.11 (t, *J* = 2.7 Hz, 1H, *H*-5'), 3.97 – 3.91 (m, 3H, *H*-5, *H*-3', *H*-2'), 3.83 (dd, *J* = 6.8, 3.5 Hz, 2H, *H*-3, *H*-6'), 3.59 (dd, *J* = 10.8, 3.6 Hz, 2H, *H*-6', *H*-2), 3.51 – 3.34 (m, 2H, CH_2 - Linker), 3.23 (dt, *J* = 25.7, 7.5 Hz, 2H, CH_2 - Linker), 2.76 (q, *J* = 5.9 Hz, 2H, CH_2 - Lev), 2.55 (t, *J* = 6.4 Hz, 2H, CH_2 - Lev), 2.48 (s, 1H, O*H*), 2.17 (s, 3H, CH_3 – Lev), 1.61 – 1.48 (m, 3H, CH_2 - Linker), 1.39 – 1.30 (m, 3H, CH_2 - Linker). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 206.56 (*C*=O), 172.29 (*C*=O), 154.43 (Ar-C_q), 138.01 (Ar-C_q), 137.37 (Ar-C_q), 137.04 (Ar-C_q), 131.51 (*C*H-Alloc), 128.75 (Ar-*C*), 128.70 (Ar-*C*), 128.62 (Ar-*C*), 128.54 (Ar-*C*), 128.36 (Ar-*C*), 128.14 (Ar-*C*), 128.01 (Ar-*C*), 127.90 (Ar-*C*), 127.68 (Ar-*C*), 127.62 (Ar-*C*), 127.38 (Ar-*C*), 127.27 (Ar-*C*), 119.07 (*C*H$_2$-Alloc), 99.00 (*C*-1'), 98.08 (*C*-1), 76.02 (*C*-3'), 74.89 (*C*-3), 73.38 (*C*-4), 71.98 (*C*H$_2$-Ar), 71.95 (*C*H$_2$-Ar), 68.62 (*C*H$_2$-Alloc), 68.32 (*C*H$_2$-Ar), 68.17 (*C*-2), 67.73 (*C*-5), 67.23 (*C*-6'), 65.52 (*C*H$_2$-Ar), 65.38 (*C*-5'), 65.18 (*C*-4') 61.95 (*C*-6), 59.57 (*C*-2), 50.27 (*C*H$_2$-Linker), 47.18 (*C*H$_2$-Linker), 46.19 (*C*H$_2$-Linker), 38.01 (*C*H$_2$-Lev), 29.84 (*C*H$_3$-Lev), 27.90 (*C*H$_2$-Linker), 27.51 (*C*H$_2$-Lev), 23.39 (*C*H$_2$-Linker). **HRMS**: $[M+Na]^+$ calculated for $C_{55}H_{65}N_7O_{15}Na$: 1086.44363; found 1086.44309

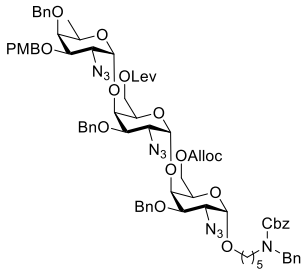
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (6)



The reaction was carried out according to General glycosylationprocedure A using acceptor **28** (523 mg, 0.485 mmol, 1 equiv.), donor **10b** (420 mg, 0.737 mmol, 1.5 equiv.) and TBSOTf (22 µL, 0.0969 mmol, 0.2 equiv.) in DCM (5 mL, 0.1 M). The reaction was followed by TLC (pentane/EtOAc 1:1) and column chromatography (pentane/EtOAc 65:35 → 50:50) gave **6** in 97% yield (687 mg, 0.470 mmol) as only the α-anomer. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.42 – 7.26 (m, 27H, Ar-*H*), 6.94 – 6.86 (m, 2H, Ar-*H*), 5.16 (d, *J* = 14.7 Hz, 2H, CH_2-Ar), 5.06 (d, *J* = 3.6 Hz, 1H, *H*-1'), 4.92 (d, *J* = 2.9 Hz, 1H, *H*-1), 4.88 (q, *J* = 5.5 Hz, 3H, *H*-1", CH_2-Ar), 4.82 (s, 1H, CH_2-Ar), 4.70 – 4.61 (m, 4H, CH_2-Ar), 4.52 (d, *J* = 11.5 Hz, 1H, CH_2-Ar), 4.49 (d, *J* = 7.0 Hz, 2H, CH_2-Ar), 4.39 (dd, *J* = 11.0, 6.9 Hz, 2H, *H*-6), 4.30 (d, *J* = 5.1 Hz, 2H, *H*-4, *H*-5'), 4.22 (s, 1H, *H*-4'), 4.20 – 4.16 (m, 2H, *H*-5, *H*-3'), 3.99 (d, *J* = 4.5 Hz, 1H, *H*-3), 3.93 – 3.89 (m, 4H, *H*-2, *H*-5", *H*-6'), 3.81 (s, 3H, CH_3-PMB), 3.75 (dd, *J* = 10.9, 3.6 Hz, 2H, *H*-2', *H*-3"), 3.62 (t, *J* = 1.9 Hz, 1H, *H*-4"), 3.52 (dd, *J* = 10.8, 3.5 Hz, 1H, *H*-2"), 3.43 – 3.32 (m, 1H, CH_2-Linker), 3.27 – 3.15 (m, 3H, CH_2-Linker), 2.75 (q, *J* = 5.4 Hz, 2H, CH_2-Lev), 2.68 (q, *J* = 6.7 Hz, 2H, CH_2-Lev), 2.57 (t, *J* = 5.5 Hz, 2H, CH_2-Lev), 2.41 (q, *J* = 7.0 Hz, 2H, CH_2-Lev), 2.17 (d, *J* = 0.7 Hz, 3H, CH_3-Lev), 2.16 (s, 3H, CH_3-Lev), 1.54 – 1.46 (m, 3H, CH_2-Linker), 1.35 – 1.26 (m, 3H, CH_2-Linker), 0.82 (d, *J* = 6.4 Hz, 3H, *H*-6"). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 172.39 (C=O), 138.44 (Ar-C_q), 137.48 (Ar-C_q), 129.61 (Ar-*C*), 128.68 (Ar-*C*), 128.64 (Ar-*C*), 128.54 (Ar-*C*), 128.36 (Ar-*C*), 127.95 (Ar-*C*), 127.59 (Ar-*C*), 127.50 (Ar-*C*), 114.04 (Ar-*C*), 99.43 (*C*-1'), 98.89 (*C*-1), 98.08 (*C*-1"), 77.71 (*C*-3'), 76.20 (*C*-4'), 74.95 (*C*H$_2$-Ar), 72.01 (*C*-4), 67.28 (*C*H$_2$-Ar), 62.14 (*C*-6), 59.62

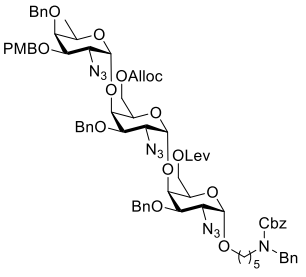
(*C*-2), 55.43(*C*H$_3$-PMB), 51.06 (*C*H$_2$-Ar), 45.97 (*C*H$_2$-Linker), 38.03 (*C*H$_2$-Lev), 37.38 (*C*H$_2$-Lev), 29.93 (*C*H$_3$-Lev), 28.41 (*C*H$_2$-Linker), 27.99 (*C*H$_2$-Lev), 27.61 (*C*H$_2$-Lev), 23.15 (*C*H$_2$-Linker), 16.72 (*C*-6''). **HRMS**: [M+Na]$^+$ calculated for $C_{77}H_{90}N_{10}O_{19}Na$: 1481.62814; found 1481.62759

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (7)



The reaction was carried out according to General glycosylationprocedure A using acceptor **29** (190 mg, 0.179 mmol, 1 equiv.), donor **10b** (153 mg, 0.268 mmol, 1.5 equiv.) and TBSOTf (8 µL, 0.0357 mmol, 0.2 equiv.) in DCM (1.8 mL, 0.1 M). The reaction was followed by TLC (pentane/EtOAc 7:3) and column chromatography (pentane/EtOAc 75:25 → 60:40) gave **7** in 92% yield (237 mg, 0.164 mmol) as only the α-anomer. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.44 – 7.12 (m, 33H, Ar-*H*), 6.94 – 6.87 (m, 2H, Ar-*H*), 5.92 (ddt, *J* = 16.5, 10.4, 5.9 Hz, 1H, C*H*=CH$_2$), 5.36 (dt, *J* = 17.2, 1.5 Hz, 1H, CH=C*H*$_2$), 5.30 – 5.25 (m, 1H, CH=C*H*$_2$), 5.17 (d, *J* = 14.2 Hz, 2H, Ar-C*H*$_2$), 5.03 (d, *J* = 3.6 Hz, 1H, H-1''), 4.92 (d, *J* = 3.3 Hz, 1H, H-1'), 4.93 – 4.84 (m, 4H, H-1, Ar-C*H*$_2$), 4.83 (d, *J* = 12.2 Hz, 1H, Ar-C*H*$_2$), 4.69 – 4.60 (m, 7H, Ar-C*H*$_2$, CH$_2$-allyl), 4.57 – 4.45 (m, 3H, Ar-C*H*$_2$, C*H*$_2$-linker), 4.41 (d, *J* = 6.9 Hz, 2H, H-6'), 4.36 – 4.25 (m, 2H, H-5', H-6), 4.23 – 4.13 (m, 3H, H-4', H-5, H-5''), 4.02 – 3.88 (m, 5H, H-6, H-2', H-3, H-3'', H-4), 3.88 – 3.77 (m, 4H, H-4'', C*H*$_3$-PMB), 3.76 (dd, *J* = 10.9, 3.5 Hz, 1H, H-2''), 3.66 – 3.59 (m, 2H, C*H*$_2$-linker, H-3), 3.54 (dd, *J* = 10.8, 3.6 Hz, 1H, H-2), 3.46 – 3.30 (m, 1H, C*H*$_2$-linker), 3.30 – 3.13 (m, 2H, C*H*$_2$-linker), 2.68 (q, *J* = 6.7 Hz, 2H, C*H*$_2$-Lev), 2.41 (q, *J* = 7.2 Hz, 2H, C*H*$_2$-Lev), 2.15 (s, 3H, C*H*$_3$-Lev), 1.63 – 1.42 (m, 5H, C*H*$_2$-linker), 1.36 – 1.12 (m, 3H, C*H*$_2$-linker), 0.82 (d, *J* = 6.4 Hz, 3H, H-6''). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 206.43 (C=O), 171.70 (C=O), 159.47 (Ar-*C*$_q$), 154.67 (Ar-*C*$_q$), 138.42 (Ar-*C*$_q$), 138.04 (Ar-*C*$_q$), 137.45 (Ar-*C*$_q$), 137.32 (Ar-*C*$_q$), 129.88 (Ar-*C*), 129.60 (Ar-*C*), 128.66 (Ar-*C*), 128.63 (Ar-*C*), 128.58 (Ar-*C*), 128.52 (Ar-*C*), 128.34 (Ar-*C*), 128.32 (Ar-*C*), 128.05 (Ar-*C*), 127.94 (Ar-*C*), 127.91 (Ar-*C*), 127.83 (Ar-*C*), 127.75 (Ar-*C*), 127.48 (Ar-*C*), 127.47 (Ar-*C*), 119.45 (CH=*C*H$_2$), 114.02 (Ar-*C*), 99.41 (C-1''), 98.99 (C-1'), 98.04 (C-1), 77.16 (C-3''/C-3), 76.16 (C-3'), 75.32 (C-3''/C-3), 74.93 (C-4'', Ar-CH$_2$), 73.02 (C-5/ C-5''), 72.21 (C-5/ C-5''), 72.06 (Ar-CH$_2$), 71.98 (Ar-CH$_2$), 71.74 (Ar-CH$_2$), 68.95 (Ar-CH$_2$), 68.90 (C-5'), 68.38 (Ar-CH$_2$), 68.33 (C-4), 67.44 (C-4'), 67.26 (Ar-CH$_2$), 65.24 (C-6'), 61.19 (C-6), 60.33 (C-2'), 60.22 (C-2''), 59.62 (C-2), 55.83 (C*H*$_3$-PMB), 50.58 (C*H*$_2$-Linker), 50.29 (C*H*$_2$-Linker), 47.08 (C*H*$_2$-Linker), 46.32 (C*H*$_2$-Linker), 38.02 (C*H*$_2$-Linker), 29.88 (C*H*$_2$-Lev), 27.78 (C*H*$_3$-Lev), 23.40 (C*H*$_2$-Lev), 16.68 (C-6''). **HRMS**: [M+Na]$^+$ calculated for $C_{76}H_{88}N_{10}O_{19}Na$: 1467.61249; found 1467.61194

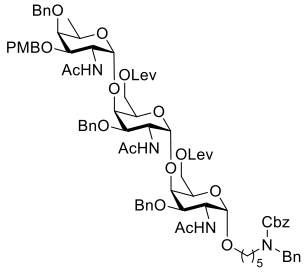
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (8)



The reaction was carried out according to General glycosylationprocedure A using acceptor **30** (301 mg, 0.283 mmol, 1 equiv.), donor **10b** (242 mg, 0.425 mmol, 1.5 equiv.) and TBSOTf (13 µL, 0.0566 mmol, 0.2 equiv.) in DCM (2.8 mL, 0.1 M). The reaction was followed by TLC (pentane/EtOAc 3:2) and column chromatography (pentane/EtOAc 75:25 → 60:40) gave **8** in 90% yield (370 mg, 0.255 mmol) as only the α-anomer. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.39 – 7.27 (m, 28H, Ar-*H*), 6.93 – 6.89 (m, 1H, Ar-*H*), 5.99 – 5.81 (m, 1H, C*H*-Alloc), 5.40 – 5.23 (m, 2H, CH_2-Alloc), 5.17 (d, *J* = 14.9 Hz, 2H, Ar-CH_2), 5.07 (d, *J* = 3.6 Hz, 1H, *H*-1'), 5.03 (d, *J* = 3.5 Hz, 1H, *H*-1), 4.97 – 4.91 (m, 3H, *H*-1", Ar-CH_2), 4.90 – 4.84 (m, 2H, Ar-CH_2), 4.68 (dd, *J* = 8.5, 4.5 Hz, 4H, Ar-CH_2), 4.55 (d, *J* = 6.0 Hz, 2H, CH_2-Alloc), 4.50 (d, *J* = 7.2 Hz, 2H, Ar-CH_2), 4.44 – 4.30 (m, 4H, *H*-6, *H*-4, *H*-5'), 4.24 – 4.14 (m, 3H, *H*-5, *H*-3', *H*-3), 4.11 (t, *J* = 1.8 Hz, 1H, *H*-4'), 3.97 – 3.88 (m, 4H, *H*-2, *H*-5", *H*-6'), 3.81 (s, 3H, CH_3-PMB), 3.63 (t, *J* = 1.7 Hz, 1H, *H*-4"), 3.59 (dd, *J* = 10.8, 3.6 Hz, 2H, *H*-2', *H*-3"), 3.53 (dd, *J* = 10.8, 3.5 Hz, 1H, *H*-2"), 3.47 – 3.17 (m, 4H, CH_2-Linker), 2.76 (q, *J* = 6.3 Hz, 2H, CH_2-Lev), 2.57 (q, *J* = 6.6 Hz, 2H, CH_2-Lev), 2.18 (s, 3H, CH_3-Lev), 1.63 – 1.48 (m, 2H, CH_2-Linker), 1.40 – 1.25 (m, 4H, CH_2-Linker), 0.82 (d, *J* = 6.4 Hz, 3H, *H*-6"). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 206.58 (*C*=O), 172.29 (*C*=*O*), 159.45 (Ar-C_q), 154.42 (Ar-C_q), 153.80 (Ar-C_q), 140.45 (Ar-C_q), 138.29 (Ar-C_q), 138.00 (Ar-C_q), 137.37 (Ar-C_q), 137.31 (Ar-C_q), 137.02 (Ar-C_q), 131.49 (*C*H-Alloc), 129.56 (Ar-*C*), 128.74 (Ar-*C*), 128.62 (Ar-*C*), 128.54 (Ar-*C*), 128.49 (Ar-*C*), 128.36 (Ar-*C*), 128.31 (Ar-*C*), 128.27 (Ar-*C*), 128.13 (Ar-*C*), 128.01 (Ar-*C*), 127.89 (Ar-*C*), 127.82 (Ar-*C*), 127.76 (Ar-*C*), 127.61 (Ar-*C*), 127.40 (Ar-*C*), 127.32 (Ar-*C*), 127.26 (Ar-*C*), 127.23 (Ar-*C*), 119.07 (*C*H2-Alloc), 113.98 (Ar-*C*), 99.40 (*C*-1'), 98.98 (*C*-1), 98.07 (*C*-1"), 77.65 (*C*-5"), 74.96 (*C*-2), 74.87 (*C*H2-Ar), 73.37 (*C*-3), 72.04 (*C*H2-Ar), 71.94 (*C*H2-Ar), 71.69 (*C*H2-Ar), 68.78 (CH_2-Linker), 68.61 (*C*-6'), 68.31 (*C*H2-Alloc), 68.16 (*C*-4"), 67.72 (*C*-5), 67.48 (*C*-4), 67.23 (*C*H2-Ar), 65.52 (*C*-5'), 65.37 (*C*-4'), 65.04 (*C*-3'), 61.96 (*C*-6), 60.19 (*C*-5"), 60.04 (*C*-3"), 59.56 (*C*-2'), 59.51 (*C*-2"), 55.36 (*C*H_3-PMB), 50.26 (*C*H_2-Ar), 47.18 (CH_2-Linker), 46.20 (CH_2-Linker), 37.96 (*C*H_2-Lev), 29.86 (*C*H_3-Lev), 29.06 (CH_2-Linker), 27.90 (*C*H_2-Lev), 27.51 (CH_2-Linker), 23.40 (CH_2-Linker), 16.60 (*C*-6"). **HRMS**: $[M+Na]^+$ calculated for $C_{76}H_{88}N_{10}O_{19}Na$: 1467.61249; found 1467.61194

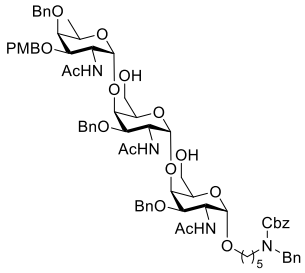
Synthesis of the trisaccharide without taurine

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (31)



The azide reduction was carried out followed the general azide reduction procedure B using **6** (152 mg, 0.104 mmol, 1 equiv.) and zinc powder (1.36 g, 20.8 mmol, 200 equiv.). Purification by column chromatography (DCM/MeOH 98:2→95:5) gave **31** in 92% yield (144 mg, 0.0918 mmol). **^{1}H NMR** (400 MHz, CD_2Cl_2) δ 7.42 – 7.23 (m, 27H), 6.95 – 6.90 (m, 2H), 5.70 (d, *J* = 9.4 Hz, 1H), 5.19 – 5.14 (m, 2H), 5.07 (d, *J* = 6.6 Hz, 1H), 4.95 (d, *J* = 3.7 Hz, 1H), 4.91 – 4.80 (m, 3H), 4.78 (s, 2H), 4.69 – 4.64 (m, 1H), 4.53 – 4.46 (m, 8H), 4.42 – 4.34 (m, 3H), 4.33 – 4.26 (m, 2H), 4.25 – 4.16 (m, 3H), 4.03 – 3.95 (m, 2H), 3.95 – 3.82 (m, 2H), 3.79 (s, 3H), 3.66 (t, *J* = 2.6 Hz, 1H), 3.62 – 3.51 (m, 2H), 3.39 – 3.30 (m, 1H), 3.28 – 3.21 (m, 3H), 2.73 (q, *J* = 6.5 Hz, 2H), 2.67 (dd, *J* = 11.9, 5.7 Hz, 2H), 2.55 (t, *J* = 6.7 Hz, 2H), 2.42 – 2.38 (m, 2H), 2.14 (d, *J* = 3.3 Hz, 6H), 1.92 – 1.84 (m, 9H), 1.54 (s, 3H), 1.37 – 1.28 (m, 3H), 0.90 (d, *J* = 6.3 Hz, 3H). **^{13}C NMR** (101 MHz, CD_2Cl_2) δ 206.96, 172.53, 170.52, 170.04, 159.66, 139.22, 138.67, 138.52, 130.88, 129.95, 129.80, 128.85, 128.82, 128.54, 128.48, 128.20, 128.03, 127.95, 127.90, 127.77, 127.58, 114.15, 113.84, 99.17, 97.91, 77.08, 76.71, 75.28, 75.09, 71.94, 71.79, 71.26, 68.80, 67.59, 67.30, 61.21, 55.59, 50.50, 49.20, 47.53, 38.13, 30.05, 29.92, 28.05, 23.46, 16.99. **HRMS**: $[M+Na]^+$ calculated for $C_{83}H_{102}N_nO_{22}Na$: 1529.68834; found 1529.68788

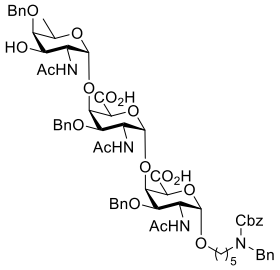
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (32)



31 (127 mg, 0.0844 mmol, 1 equiv.) was dissolved in toluene/EtOH (1:2, 0.1 M, 0.9 mL) and added hydrazine acetate (78 mg, 0.844 mmol, 10 equiv.) and stirred at rt for 45 min until TLC analysis (DCM/MeOH 95:5) showed full conversion. The solution was diluted in DCM and $NaHCO_3$ (aq., sat.) and the organic layer was dried with Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (DCM/MeOH 100:0→95:5) gave **32** in 96% yield (107 mg, 0.0814 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.36 – 7.29 (m, 27H), 6.93 – 6.89 (m, 2H), 5.61 (d, *J* = 9.5 Hz, 1H), 5.17 (s, 2H), 5.13 – 5.06 (m, 2H), 4.90 (d, *J* = 11.3 Hz, 2H), 4.86 (d, *J* = 3.5 Hz, 1H), 4.82 – 4.69 (m, 4H), 4.70 – 4.59 (m, 2H), 4.57 – 4.47 (m, 5H), 4.44 (dq, *J* = 12.0, 4.5 Hz, 6H), 4.32 – 4.23 (m, 2H), 4.18 (s, 1H), 4.09 (s, 1H), 3.78 (s, 3H), 3.74 (d, *J* = 14.4 Hz, 2H), 3.66 (d, *J* = 2.7 Hz, 1H), 3.57 (d, *J* = 6.0 Hz, 2H), 3.38 – 3.28 (m, 2H), 3.16 (d, *J* = 46.6 Hz, 2H), 2.57 (s, 1H), 2.27 (s, 1H), 1.94 (s, 3H), 1.91 (s, 3H), 1.80 (s, 3H), 1.51 – 1.44 (m, 3H), 1.35 – 1.28 (m, 3H), 0.96 (d, *J* = 6.4 Hz, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 170.68, 159.63, 139.30, 138.70, 130.95, 130.02, 128.88, 128.79, 128.76,

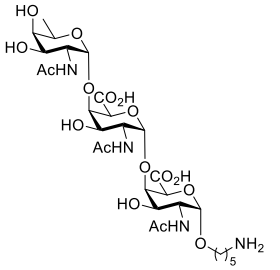
128.49, 128.30, 128.12, 127.98, 127.75, 127.65, 127.54, 114.15, 98.99, 98.21, 77.21, 76.78, 75.57, 75.09, 71.82, 71.35, 67.53, 55.61, 50.66, 49.23, 30.07, 26.42, 26.25, 23.54, 23.41, 22.92, 17.08. **HRMS**: $[M+Na]^+$ calculated for $C_{73}H_{90}N_4O_{18}Na$: 1333.61478; found 1333.61466

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-3-*O*-benzyl-2-deoxy-α-D-galacto-pyranosiduronate-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronic acid (33)



The reaction was carried out according to General oxidation procedure C using **32** (39 mg, 0.0299 mmol, 1 equiv.) in EtOAc/*t*-BuOH/H_2O (1:1:1, 0.9 mL) and TEMPO (7 mg, 0.0478 mmol, 1.6 equiv.), $NaHCO_3$ (25 mg, 0.299 mmol, 10 equiv.) and BAIB (77 mg, 0.239 mmol, 8 equiv.). The reaction was stirred for 6 days at 4 ºC and purified by column chromatography (DCM/MeOH + 1% AcOH, 97:3→90:10) to give **33** in 54% yield without the PMB (20 mg, 0.0161 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.50 – 7.08 (m, 39H), 6.94 – 6.84 (m, 1H), 6.40 – 5.49 (m, 5H), 5.26 – 4.95 (m, 5H), 4.83 (dd, *J* = 18.4, 10.4 Hz, 5H), 4.74 – 4.16 (m, 17H), 3.92 – 3.71 (m, 4H), 3.70 – 3.49 (m, 3H), 3.38 – 3.14 (m, 3H), 2.12 – 1.92 (m, 23H), 1.92 – 1.77 (m, 3H), 1.33 – 1.08 (m, 7H), 0.90 – 0.82 (m, 3H). **HRMS**: $[M+Na]^+$ calculated for $C_{65}H_{78}N_4O_{19}Na$: 1241.51580; found 1241.51548

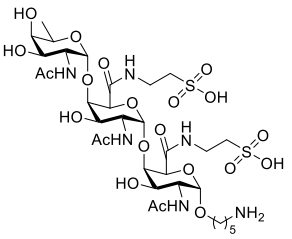
5-amino-pentyl 2-acetamide-2-deoxy-α-D-fucopyranosyl-(1→4)-2-acetamide-2-deoxy-α-D-galactopyranosiduronate-(1→4)-2-*O*-acetamide-2-deoxy-α-D-galactopyranosiduronic acid (1)



The reaction was carried out according to General hydrogenation procedure D using **33** (21 mg, 0.0169 mmol, 1 equiv.) to yield **1** in 44% yield (5.4 mg, 0.00749 mmol). **^{1}H NMR (850 MHz, D_2O)** δ 5.04 (d, *J* = 3.6 Hz, 1H, H-1), 4.96 (d, *J* = 3.7 Hz, 1H, H-1'), 4.88 (d, *J* = 3.8 Hz, 1H, H-1''), 4.75 (d, *J* = 1.3 Hz, 1H, H-5), 4.43 (d, *J* = 2.7 Hz, 1H, H-4'), 4.40 (q, *J* = 6.8 Hz, 1H, H-5''), 4.32 (s, 1H, H-4), 4.28 – 4.23 (m, 2H, H-2, H-5'), 4.18 – 4.11 (m, 3H, H-2'', H-3, H-2'), 4.09 (dd, *J* = 11.4, 3.1 Hz, 1H, H-3'), 3.97 (dd, *J* = 11.1, 3.2 Hz, 1H, H-3''), 3.83 (d, *J* = 3.2 Hz, 1H, H-4''), 3.69 (ddd, *J* = 10.2, 7.5, 5.7 Hz, 1H, C*H*$_2$-Linker), 3.54 (dt, *J* = 10.2, 6.0 Hz, 1H, C*H*$_2$-Linker), 2.98 (t, *J* = 7.7 Hz, 2H, C*H*$_2$-Linker), 2.09 (s, 6H, COC*H*$_3$), 2.03 (s, 3H, C*H*$_2$-Linker), 1.69 – 1.57 (m, 4H, C*H*$_2$-Linker), 1.44 (dhept, *J* = 13.6, 6.2 Hz, 2H, C*H*$_2$-Linker), 1.17 (d, *J* = 6.6 Hz, 3H, H-6''). **^{13}C NMR (214 MHz, D_2O)** δ 174.85 (C=O), 174.78 (C=O), 174.51 (C=O), 99.32 (C-1''), 98.33 (C-1), 96.80 (C-1'), 79.81 (C-4), 77.44 (C-4'), 71.91 (C-5), 71.20 (C-4''), 70.55 (C-5'), 67.98 (CH_2-Linker), 67.88 (C-3''), 67.63 (C-5''), 67.21 (C-3), 66.94 (C-3'), 49.81 (C-2), 49.51 (C-2'), 49.47 (C-2''), 39.33 (CH_2-Linker), 27.99 (CH_2-Linker), 26.28 (CH_2-Linker), 22.38 (CO*C*H$_3$), 22.27 (CO*C*H$_3$), 22.25 (CH_2-Linker), 21.84 (CO*C*H$_3$), 15.38 (C-6''). **HRMS**: $[M+H]^+$ calculated for $C_{29}H_{48}N_4O_{17}H$: 725.30927; found 725.30868

Synthesis of the trisaccharides with taurine on both GalNAc's

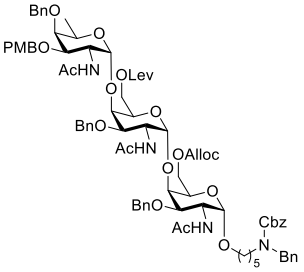
Trisaccharide with taurine on both GalNAc's (4)



33 (24 mg, 0.02 mmol, 1 eq) was dissolved in DMF (1 mL) and added HATU (23 mg, 0.0600 mmol, 2.4 equiv.) and DIPEA (26 µg, 0.150 mmol, 6 equiv.) stirred at rt for 10 min before adding taurine (13 mg, 0.100 mmol, 4 equiv.). The reaction was stirred overnight until LC-MS showed full conversion. The solution was diluted in EtOAc and washed with 1 M HCl (x1), $NaHCO_3$ (x1, aq., sat.) and brine (x1), dried with Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was used without further purification. The hydrogenation reaction was carried out according to General hydrogenation procedure D to **4** in 17% yield over two steps (3.1 mg, 0.0033 mmol). **^{1}H NMR (600 MHz, D_2O)** δ 5.00 (d, *J* = 3.9 Hz, 1H, H-1'), 4.98 (d, *J* = 3.7 Hz, 1H, H-1), 4.79 – 4.77 (m, 2H, H-1'', H-5), 4.45 (dd, *J* = 3.1, 1.0 Hz, 1H, H-4), 4.37 – 4.34 (m, 3H, H-4'. H-5, H-5''), 4.19 (dd, *J* = 11.3, 3.9 Hz, 1H, H-2'), 4.15 (dd, *J* = 11.3, 3.7 Hz, 1H, H-2), 4.08 – 4.03 (m, 2HH-3. H-3'), 4.01 (dd, *J* = 5.3, 3.4 Hz, 1H, H-2''), 3.99 (d, *J* = 3.9 Hz, 1H), 3.88 (dd, *J* = 11.2, 3.2 Hz, 1H, H-3''), 3.76 – 3.69 (m, 3H, H-4'', Taurine-CH_2), 3.64 – 3.59 (m, 2H, Linker-CH_2), 3.46 (dt, *J* = 10.1, 6.1 Hz, 2H, Linker-CH_2), 3.35 (td, *J* = 13.6, 7.4 Hz, 2H, Taurine-CH_2), 3.01 (dtt, *J* = 8.1, 4.8, 2.1 Hz, 4H. Taurine-CH_2), 2.90 (dd, *J* = 8.4, 6.9 Hz, 3H, Linker-CH_2), 2.00 (s, 6H, COOCH_3), 1.95 (s, 3H, COOCH_3), 1.62 – 1.52 (m, 6H, Linker-CH_2), 1.36 – 1.32 (m, 2H, Linker-CH_2), 1.09 (d, *J* = 6.6 Hz, 3H, H-6'').**^{13}C NMR (151 MHz, D_2O)** δ 175.54 (C=O), 175.09 (C=O), 175.04 (C=O), 171.44 (C=O), 171.18 (C=O), 97.98 (C-1), 97.96 (C-1''), 97.67 (C-1), 75.35 (C-4'), 74.98 (C-4), 72.10 (C-4''), 71.41 (C-5'), 70.70 (C-5), 69.23 (Linker-*C*H_2), 68.43 (C-3''), 68.04 (C-5''), 67.68 (C-3/C-3'), 67.52 (C-3/C-3'), 50.47 (C-2/C-2'), 50.44 (C-2/C-2'), 50.28 (C-2''), 50.20 (Taurine-*C*H_2), 50.16 (Taurine-*C*H_2), 40.29 (Linker-*C*H_2), 35.92 (Taurine-*C*H_2), 35.90 (Taurine-*C*H_2), 28.91 (Linker-*C*H_2), 27.31 (Linker-*C*H_2), 23.21 (Linker-*C*H_2), 23.08 (COO*C*H_3), 22.95 (COO*C*H_3), 22.77 (COO*C*H_3), 16.27 (C-6''). **HRMS**: $[M+H]^+$ calculated for $C_{33}H_{58}N_6O_{21}S_2H$: 939.31747; found 939.31760

Synthesis of the trisaccharides with taurine on 1st GalNAc

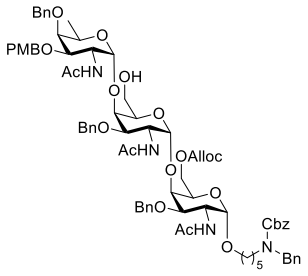
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-*O*-acetamide-6-*O*-allyloxycarbonyl-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (34)



The azide reduction was carried out followed the general azide reduction procedure B using **7** (265 mg, 0.183 mmol, 1 equiv.) and zinc powder (3.59 g, 54.89 mmol, 300 equiv.). Purification by column chromatography (DCM/MeOH 100:0→95:5) gave **34** in 80% yield (217 mg, 0.145 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.42 – 7.12 (m, 33H), 6.96 – 6.88 (m, 2H), 5.99 – 5.80 (m, 2H), 5.62 (dd, *J* = 14.2, 9.0 Hz, 1H), 5.36 (p, *J* = 1.8 Hz, 1H), 5.32 (s, 1H), 5.25 (ddd, *J*

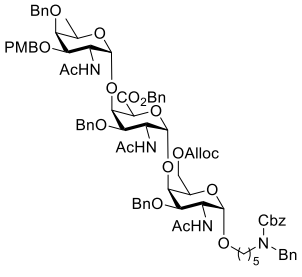
= 11.2, 3.5, 2.2 Hz, 1H), 5.17 (s, 1H), 5.05 (d, *J* = 7.5 Hz, 1H), 4.93 (d, *J* = 3.7 Hz, 1H), 4.88 (d, *J* = 11.3 Hz, 2H), 4.82 (dd, *J* = 12.3, 2.7 Hz, 2H), 4.80 – 4.74 (m, 2H), 4.69 – 4.62 (m, 1H), 4.62 – 4.57 (m, 3H), 4.56 – 4.35 (m, 12H), 4.34 – 4.24 (m, 3H), 4.17 (d, *J* = 6.7 Hz, 2H), 4.12 – 3.98 (m, 3H), 3.99 – 3.85 (m, 2H), 3.83 – 3.74 (m, 6H), 3.68 – 3.47 (m, 4H), 3.37 – 3.16 (m, 4H), 2.01 – 1.66 (m, 12H), 1.63 – 1.31 (m, 6H), 1.43 – 1.04 (m, 5H), 0.95 – 0.83 (m, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 170.63, 170.29, 170.10, 159.69, 154.87, 139.21, 138.67, 138.50, 131.92, 130.83, 130.02, 129.84, 129.81, 129.70, 129.28, 128.85, 128.83, 128.75, 128.70, 128.54, 128.49, 128.20, 128.16, 128.10, 128.01, 127.95, 127.91, 127.86, 127.78, 127.59, 127.51, 119.18, 114.16, 114.09, 114.05, 113.84, 99.23, 97.92, 76.93, 76.68, 75.25, 75.10, 71.91, 71.77, 71.59, 71.33, 69.12, 68.96, 68.54, 67.63, 67.30, 65.94, 61.21, 55.60, 55.54, 50.72, 50.51, 49.26, 49.20, 49.04, 47.52, 46.56, 38.14, 29.92, 28.02, 25.10, 23.49, 23.45, 16.99. **HRMS**: $[M+Na]^+$ calculated for $C_{82}H_{100}N_4O_{22}Na$: 1515.67269; found 1515.67424

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-3-*O*-benzyl-2-deoxy-α-D-galacto-pyrasyl-(1→4)-2-*O*-acetamide-6-*O*-allyloxycarbonyl-3-*O*-benzyl-2-deoxy-α-D-galactopy-ranoside (35)



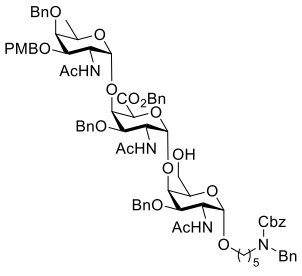
34 (217 mg, 0.145 mmol, 1 equiv.) was dissolved in toluene/EtOH (1:2, 0.1 M, 1.2 mL) and added hydrazine acetate (67 mg, 0.728 mmol, 5 equiv.) and stirred at rt for 45 min until TLC analysis (DCM/MeOH 95:5) showed full conversion. The solution was diluted in DCM and $NaHCO_3$ (aq., sat.) and the organic layer was dried with Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (DCM/MeOH 100:0→95:5) gave **35** in 89% yield (180 mg, 0.129 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.43 – 7.14 (m, 38H), 6.94 – 6.89 (m, 2H), 5.92 (ddd, *J* = 16.9, 11.1, 5.7 Hz, 2H), 5.74 (d, *J* = 9.6 Hz, 1H), 5.57 – 5.45 (m, 2H), 5.37 (s, 1H), 5.30 – 5.25 (m, 1H), 5.18 (s, 1H), 5.06 (dd, *J* = 16.9, 4.9 Hz, 2H), 4.96 – 4.87 (m, 3H), 4.87 – 4.68 (m, 5H), 4.66 – 4.56 (m, 5H), 4.56 – 4.31 (m, 13H), 4.31 – 4.18 (m, 4H), 4.12 (d, *J* = 6.8 Hz, 1H), 4.06 (ddt, *J* = 12.9, 6.5, 3.9 Hz, 2H), 3.94 (d, *J* = 7.4 Hz, 2H), 3.82 – 3.76 (m, 5H), 3.74 (dt, *J* = 11.1, 2.2 Hz, 2H), 3.69 – 3.43 (m, 5H), 3.43 – 3.13 (m, 6H), 2.00 – 1.84 (m, 13H), 1.64 – 1.42 (m, 6H), 1.37 – 1.16 (m, 4H), 0.94 (d, *J* = 6.4 Hz, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 170.59, 169.96, 167.52, 159.65, 155.51, 139.28, 138.60, 138.53, 131.93, 130.90, 129.92, 128.82, 128.72, 128.49, 128.46, 128.18, 128.07, 128.03, 127.92, 127.75, 127.59, 119.17, 114.14, 99.38, 99.03, 98.21, 77.28, 76.73, 75.33, 75.08, 71.81, 71.40, 70.73, 69.09, 67.44, 67.30, 66.26, 59.87, 55.60, 50.51, 49.19, 47.50, 26.42, 26.24, 23.54, 22.91, 17.03. **HRMS**: $[M+Na]^+$ calculated for $C_{77}H_{94}N_4O_{20}Na$: 1417.63591; found 1417.63708

Benzyl (5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronasyl)-(1→4)-2-acetamide-6-*O*-allyloxycarbonyl-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (36)



The oxidation was carried out according to General oxidation procedure C using **35** (110 mg, 0.0793 mmol, 1 equiv.) in EtOAc/*t*-BuOH/H_2O (1:1:1, 0.9 mL) and TEMPO (10 mg, 0.0635 mmol, 0.8 equiv.), $NaHCO_3$ (33 mg, 0.397 mmol, 5 equiv.) and BAIB (102 mg, 0.317 mmol, 4 equiv.). The reaction was stirred for 4 days at 4 ºC. The crude product was dissolved in DMF and cooled to 0 ºC and added Cs_2CO_3 (26 mg, 0.0793 mmol, 1 equiv.) and BnBr (19 µL, 0.159 mmol, 2 equiv.) and stirred overnight at rt until TLC analysis (DCM/MeOH, 95:5) showed full conversion. The solution was diluted in EtOAc, washed with brine (x1), dried with Na_2SO_4, filtered and concentrated i*n vacuo*. Column chromatography (DCM/MeOH, 100:0→95:5) gave **36** in 92% yield (110 mg, 0.0732 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.51 – 7.07 (m, 57H), 6.96 – 6.88 (m, 2H), 5.56 – 5.34 (m, 3H), 5.29 – 5.20 (m, 2H), 5.17 (d, *J* = 4.4 Hz, 3H), 5.08 – 4.99 (m, 2H), 4.99 – 4.70 (m, 10H), 4.68 – 4.32 (m, 23H), 4.32 – 4.11 (m, 6H), 4.10 (d, *J* = 7.1 Hz, 2H), 3.85 – 3.72 (m, 6H), 3.70 – 3.44 (m, 6H), 3.44 – 3.11 (m, 6H), 2.18 – 1.78 (m, 18H), 1.65 – 1.37 (m, 9H), 1.37 – 1.08 (m, 9H), 0.88 – 0.77 (m, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 172.53, 170.00, 169.96, 168.31, 162.35, 159.38, 154.56, 139.37, 138.26, 138.21, 134.60, 131.68, 130.62, 129.80, 129.63, 129.61, 129.43, 129.15, 129.01, 128.95, 128.89, 128.85, 128.68, 128.64, 128.58, 128.53, 128.46, 128.31, 128.24, 128.21, 128.11, 128.00, 127.92, 127.68, 127.61, 127.55, 127.50, 127.32, 126.94, 113.87, 99.80, 98.80, 98.69, 97.74, 77.75, 77.17, 76.45, 75.22, 74.95, 74.79, 74.47, 73.86, 72.10, 71.70, 70.96, 69.91, 68.89, 68.29, 67.62, 67.39, 67.02, 55.35, 53.82, 53.56, 50.97, 50.47, 50.25, 48.71, 48.47, 48.38, 47.26, 46.31, 36.28, 31.11, 29.79, 29.06, 28.00, 27.23, 26.17, 25.73, 23.57, 23.22, 16.61.

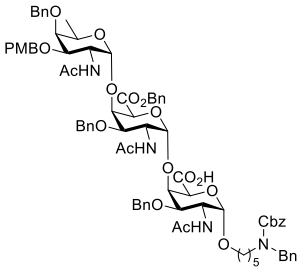
Benzyl (5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronasyl)-(1→4)-2-acetamide-2-deoxy-3-*O*-benzyl-α-D-galactopyranoside (37)



36 (121 mg, 0.0806 mmol, 1 equiv.) was dissolved in DCM (1 mL), cooled to 0 ºC and added Bu_3SnH (43 µL, 0.161 mmol, 2 equiv.) and $Pd(PPh_3)_4$ (9 mg, 0.00806 mmol, 0.1 equiv.). The reaction was stirred at 0 ºC for 1 h until TLC analysis (DCM/MeOH 95:5) showed full conversion. The solution was concentrated. Column chromatography (DCM/MeOH 98:2→95:5) gave **37** in 76% yield (87 mg, 0.0614 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.43 – 7.12 (m, 40H), 6.96 – 6.82 (m, 2H), 5.58 (dd, *J* = 39.7, 9.3 Hz, 1H), 5.25 – 4.94 (m, 5H), 4.94 – 4.69 (m, 6H), 4.69 – 4.15 (m, 19H), 3.84 – 3.71 (m, 6H), 3.71 – 3.41 (m, 7H), 3.41 – 3.09 (m, 5H), 2.05 – 1.76 (m, 14H), 1.69 – 1.36 (m, 7H), 1.36 – 1.07 (m, 7H), 0.94 – 0.78 (m, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 170.47, 170.26, 168.76, 159.63,

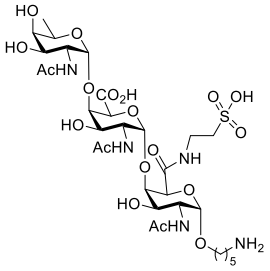
130.01, 129.32, 128.88, 128.79, 128.73, 128.50, 128.28, 128.02, 127.93, 127.78, 127.64, 114.17, 99.13, 97.95, 76.51, 74.77, 72.44, 71.28, 67.96, 67.41, 55.64, 50.72, 49.18, 48.87, 48.04, 30.06, 23.56, 16.80. **HRMS**: $[M+Na]^+$ calculated for $C_{80}H_{94}N_4O_{19}Na$: 1437.64100; found 1437.64099

Benzyl (5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronate)-(1→4)-2-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronate (38)



The oxidation was carried out according to General oxidation procedure C using **37** (53 mg, 0.0374 mmol, 1 equiv.) in EtOAc/*t*-BuOH/H_2O (1:1:1, 0.9 mL) and TEMPO (5 mg, 0.00299 mmol, 0.8 equiv.), $NaHCO_3$ (16 mg, 0.187 mmol, 5 equiv.) and BAIB (48 mg, 0.149 mmol, 4 equiv.) and stirred for 12 days at 4 C. Purification by column chromatography (DCM/MeOH 100:0→95:5) gave **38** in 65% yield (37 mg, 0.0242 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.72 – 7.62 (m, 5H), 7.62 – 7.52 (m, 3H), 7.52 – 7.42 (m, 6H), 7.46 – 7.09 (m, 64H), 6.94 – 6.87 (m, 2H), 5.75 (s, 1H), 5.20 (d, *J* = 25.9 Hz, 4H), 5.08 – 4.94 (m, 4H), 4.94 – 4.70 (m, 9H), 4.70 – 4.51 (m, 9H), 4.51 – 4.37 (m, 12H), 4.36 – 4.17 (m, 6H), 3.84 – 3.68 (m, 7H), 3.68 – 3.45 (m, 6H), 3.35 (s, 2H), 3.28 – 3.13 (m, 4H), 2.16 – 1.80 (m, 20H), 1.61 – 1.38 (m, 12H), 1.38 – 1.23 (m, 9H), 1.20 – 1.08 (m, 8H), 0.85 – 0.78 (m, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 139.00, 138.22, 132.10, 132.08, 132.00, 129.66, 129.05, 128.93, 128.69, 128.63, 128.57, 128.45, 128.24, 128.20, 127.92, 127.66, 127.50, 127.31, 125.32, 113.88, 99.72, 98.82, 97.74, 84.02, 76.60, 74.78, 71.88, 71.76, 67.59, 67.04, 55.36, 53.75, 50.77, 49.99, 48.52, 47.20, 39.82, 26.44, 23.52, 23.21, 17.50, 16.59.

Trisaccharide with taurine on 1st GalNAc deprotected (2)

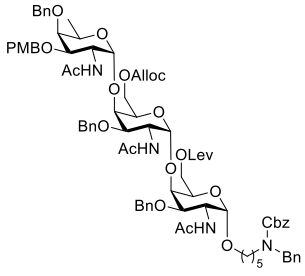


38 (37 mg, 0.0259 mmol, 1 equiv.) was dissolved in DMF (1 mL) and added HATU (12 mg, 0.0311 mmol, 1.2 equiv.) and DIPEA (14 µL, 0.0776 mmol, 3 equiv.) stirred at rt for 10 min before adding taurine (6 mg, 0.0518 mmol, 2 equiv.). The reaction was for 2 h until LC-MS showed full conversion. The solution was diluted in EtOAc and washed with 1 M HCl (x1), $NaHCO_3$ (x1, aq., sat.) and brine (x1), dried with Na_2SO_4, filtered and concentrated *in vacuo*. The crude product **39** (38 mg) was used without further purification. The hydrogenation reaction was carried out according to General hydrogenation procedure D using **39** (38 mg, crude product) to yield **2** in 7% yield over two steps (6.2 mg, 0.00743 mmol). **^{1}H NMR (850 MHz, D_2O)** δ 5.08 (d, *J* = 3.8 Hz, 1H, H-1'), 5.01 (d, *J* = 3.3 Hz, 1H, H-1), 4.90 (d, *J* = 3.8 Hz, 1H, H-1''), 4.74 (s, 1H, H-5), 4.57 (d, *J* = 3.2 Hz, 1H, H-4'), 4.42 (s, 1H, H-5'), 4.40 (q, *J* = 6.6 Hz, 1H, H-5''), 4.33 (t, *J* = 1.8 Hz, 1H, H-4), 4.27 (dd, *J* = 11.3, 3.8 Hz, 1H, H-2'), 4.20 – 4.15 (m, 3H, H-2, H-2'', H-3), 4.11 (dd, *J* = 11.3, 3.1 Hz, 1H, H-3'), 3.97 (dd, *J* = 11.1, 3.2 Hz, 1H, H-3''), 3.83 (t, *J* = 2.0 Hz, 1H, H-4''), 3.83 – 3.80 (m, 1H, Taurine-CH_2), 3.70 (dt, *J* = 10.1, 6.6 Hz, 1H, Linker-CH_2), 3.57 – 3.54 (m, 1H, Linker-CH_2), 3.45 (dt, *J* = 13.8, 6.6 Hz, 1H, Taurine-CH_2), 3.11 (t, *J* = 6.7 Hz,

2H, Taurine-CH_2), 3.00 (t, J = 7.7 Hz, 2H, Linker-CH_2), 2.10 (s, 3H, COCH_3), 2.10 (s, 3H, COCH_3), 2.05 (s, 3H, COCH_3), 1.70 – 1.61 (m, 4H, Linker-CH_2), 1.46 – 1.41 (m, 2H, Linker-CH_2), 1.17 (d, J = 6.6 Hz, 3H, H-6''). **^{13}C NMR (214 MHz, D_2O)** δ 174.88 (C=O), 174.70 (C=O), 174.56 (C=O), 174.11 (C=O), 170.41 (C=O), 99.17 (C-1''), 97.00 (C-1'), 96.24 (C-1), 79.40 (C-4), 73.19 (C-4'), 71.54 (C-5), 71.20 (C-4''), 69.66 (C-5''), 68.28 (Linker-*C*H_2), 67.87 C-3''), 67.55 (C-5'), 66.77 (C-3'/C-3), 66.74 (C-3'/C-3), 49.65 (C-2/C-2'/C-2''), 49.56 (C-2/C-2'/C-2''), 49.45 (C-2/C-2'/C-2''), 49.19 (Taurine-*C*H_2), 39.34 (Linker-*C*H_2), 34.94 (Taurine-*C*H_2), 27.94 (Linker-*C*H_2), 26.34 (Linker-*C*H_2), 22.39 (CO*C*H_3), 22.22 (CO*C*H_3), 22.03 (Linker-*C*H_2), 21.81 (CO*C*H_3), 15.35 (C-6''). **HRMS**: $[M+H]^+$ calculated for $C_{31}H_{53}N_5O_{19}SH$: 832.31337; found 832.31293

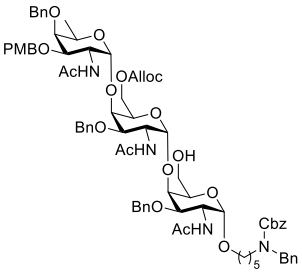
Synthesis of the trisaccharides with taurine on 2nd GalNAc

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-6-*O*-allyloxycarbonyl-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (40)



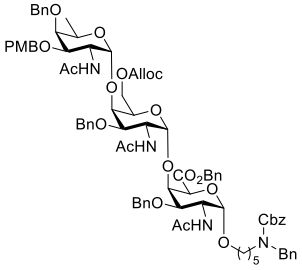
The azide reduction was carried out followed the general azide reduction procedure B using **10** (189 mg, 0.129 mmol, 1 equiv.) and zinc powder (1.687 g, 25.83 mmol, 200 equiv.). Purification by column chromatography (DCM/MeOH 100:0→95:5) gave **40** in 94% yield (181 mg, 0.121 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.42 – 7.16 (m, 27H), 6.94 – 6.86 (m, 2H), 5.96 – 5.83 (m, 1H), 5.78 – 5.53 (m, 1H), 5.25 (ddd, J = 9.0, 5.2, 2.2 Hz, 1H), 5.17 (s, 1H), 5.06 (t, J = 10.3 Hz, 1H), 4.95 (d, J = 3.7 Hz, 1H), 4.87 (dd, J = 12.0, 7.4 Hz, 2H), 4.80 (d, J = 12.4 Hz, 2H), 4.70 – 4.62 (m, 1H), 4.63 – 4.37 (m, 11H), 4.37 – 4.24 (m, 2H), 4.24 – 4.14 (m, 2H), 4.11 – 3.82 (m, 5H), 3.82 – 3.73 (m, 5H), 3.71 – 3.46 (m, 3H), 3.41 – 3.14 (m, 3H), 2.74 (t, J = 6.4 Hz, 2H), 2.58 – 2.46 (m, 2H), 2.36 (s, 1H), 2.14 (d, J = 3.2 Hz, 3H), 2.09 (s, 1H), 2.01 – 1.95 (m, 2H), 1.90 (d, J = 5.3 Hz, 7H), 1.54 (s, 4H), 1.39 – 1.21 (m, 3H), 0.90 (d, J = 6.4 Hz, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 206.91, 172.52, 159.68, 139.24, 138.53, 130.86, 129.99, 128.86, 128.79, 128.57, 128.43, 128.20, 128.12, 127.98, 127.77, 119.03, 98.99, 98.31, 97.88, 77.03, 76.60, 75.09, 71.93, 71.74, 71.21, 69.03, 68.80, 68.25, 67.68, 67.33, 62.47, 55.60, 54.38, 54.11, 53.84, 53.57, 53.30, 50.54, 49.31, 47.59, 38.13, 30.06, 29.93, 28.08, 25.10, 23.52, 16.98. **HRMS**: $[M+Na]^+$ calculated for $C_{82}H_{100}N_4O_{22}Na$: 1515.67269; found 1515.67387

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-6-*O*-allyloxycarbonyl-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (41)



40 (181 mg, 0.121 mmol, 1 equiv.) was dissolved in toluene/EtOH (1:2, 0.1 M, 1.2 mL) and added hydrazine acetate (56 mg, 0.606 mmol, 5 equiv.) and stirred at rt for 45 min until TLC analysis (DCM/MeOH 95:5) showed full conversion. The solution was diluted in DCM and $NaHCO_3$ (aq., sat.) and the organic layer was dried with Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (DCM/MeOH 100:0→95:5) gave **41** in 86% yield (145 mg, 0.104 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.43 – 7.15 (m, 29H), 6.94 – 6.87 (m, 2H), 5.95 – 5.85 (m, 1H), 5.44 (dd, *J* = 14.2, 9.5 Hz, 1H), 5.24 (ddq, *J* = 10.4, 2.6, 1.2 Hz, 1H), 5.21 – 5.02 (m, 3H), 4.94 – 4.69 (m, 5H), 4.66 (dd, *J* = 11.5, 3.1 Hz, 1H), 4.61 – 4.33 (m, 13H), 4.31 (s, 2H), 4.17 (d, *J* = 2.6 Hz, 1H), 3.78 (d, *J* = 5.4 Hz, 6H), 3.69 – 3.46 (m, 5H), 3.44 – 3.16 (m, 4H), 1.99 (s, 2H), 1.94 – 1.82 (m, 13H), 1.63 – 1.41 (m, 5H), 1.38 – 1.16 (m, 4H), 0.90 (t, *J* = 6.2 Hz, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 172.78, 170.35, 169.94, 159.69, 139.27, 138.65, 132.04, 130.86, 130.21, 129.95, 129.71, 128.90, 128.87, 128.83, 128.79, 128.76, 128.51, 128.46, 128.43, 128.30, 128.13, 128.06, 128.01, 127.93, 127.77, 127.66, 127.55, 119.00, 114.21, 99.18, 98.01, 76.68, 75.36, 75.09, 71.89, 71.78, 71.24, 69.01, 67.72, 67.32, 65.25, 55.66, 55.61, 50.68, 49.15, 49.07, 33.15, 30.58, 29.43, 26.45, 26.28, 23.56, 22.92, 16.99. **HRMS**: $[M+Na]^+$ calculated for $C_{77}H_{94}N_4O_{22}Na$: 1417.63591; found 1417.63572

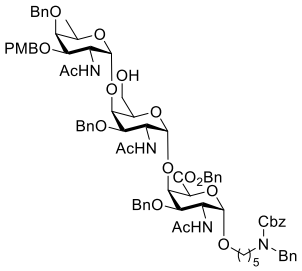
Benzyl (5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-6-*O*-allyloxycarbonyl-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronate) (42)



The oxidation was carried out according to General oxidation procedure C using **41** (37 mg, 0.0265 mmol, 1 equiv.) in EtOAc/*t*-BuOH/H_2O (1:1:1, 0.9 mL) and TEMPO (4 mg, 0.0212 mmol, 0.8 equiv.), $NaHCO_3$ (11 mg, 0.0133 mmol, 5 equiv.) and BAIB (34 mg, 0.106 mmol, 4 equiv.). The reaction was stirred for 4 days at 4 ºC. The crude product was dissolved in DMF and cooled to 0 ºC and added Cs_2CO_3 (9 mg, 0.0265 mmol, 1 equiv.) and BnBr (6 µL, 0.053 mmol, 2 equiv.) and stirred overnight at rt until TLC analysis (DCM/MeOH, 95:5) showed full conversion. The solution was diluted in EtOAc, washed with brine (x1), dried with Na_2SO_4, filtered and concentrated i*n vacuo*. Column chromatography (DCM/MeOH, 100:0 →95:5) gave **42** in 71% yield (28 mg, 0.0287 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.50 – 7.08 (m, 32H), 6.98 – 6.78 (m, 2H), 5.97 – 5.83 (m, 1H), 5.50 (dd, *J* = 36.0, 8.9 Hz, 2H), 5.27 – 5.17 (m, 2H), 5.17 – 5.00 (m, 3H), 4.91 – 4.80 (m, 3H), 4.81 – 4.71 (m, 2H), 4.71 – 4.63 (m, 1H), 4.60 – 4.21 (m, 15H), 4.21 – 4.14 (m, 1H), 4.12 – 3.86 (m, 2H), 3.81 – 3.73 (m, 4H), 3.70 – 3.48 (m, 4H), 3.36 (s, 1H), 3.21 (d, *J* = 10.2 Hz, 2H), 1.97 – 1.90

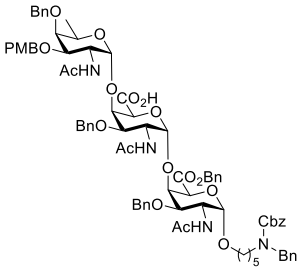
(m, 3H), 1.86 (s, 9H), 1.58 – 1.38 (m, 5H), 1.34 – 1.08 (m, 5H), 0.88 (dd, *J* = 7.9, 6.7 Hz, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 170.60, 169.83, 159.68, 139.25, 138.56, 135.46, 132.02, 129.88, 129.77, 129.23, 129.09, 129.04, 128.87, 128.80, 128.73, 128.51, 128.43, 128.20, 128.13, 127.95, 127.77, 127.60, 119.02, 114.18, 99.05, 98.20, 77.32, 76.66, 75.28, 75.09, 72.06, 71.74, 71.19, 70.13, 69.03, 67.68, 67.59, 67.32, 55.57, 54.04, 51.27, 50.53, 49.11, 48.71, 47.38, 46.71, 30.07, 29.08, 28.15, 27.57, 23.77, 23.57, 23.49, 16.97. **HRMS**: $[M+Na]^+$ calculated for $C_{84}H_{98}N_4O_{21}Na$: 1499.68018; found 1499.68118

Benzyl (5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-2-deoxy-3-*O*-benzyl-α-D-galactopyrasyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronate) (43)



42 (28 mg, 0.0185 mmol, 1 equiv.) was dissolved in DCM (1 mL), cooled to 0 ºC and added Bu_3SnH (10 µL, 0.0371 mmol, 2 equiv.) and $Pd(PPh_3)_4$ (2 mg, 0.00185 mmol, 0.1 equiv.). The reaction was stirred at 0 ºC for 30 min until TLC analysis (DCM/MeOH 95:5) showed full conversion. The solution was concentrated. Column chromatography (DCM/MeOH 100:0→95:5) gave **43** in 86% yield (145 mg, 0.104 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.42 – 7.17 (m, 51H), 6.95 – 6.81 (m, 3H), 5.67 (d, *J* = 9.6 Hz, 1H), 5.55 – 5.50 (m, 1H), 5.28 – 4.97 (m, 7H), 4.95 – 4.69 (m, 9H), 4.68 – 4.61 (m, 2H), 4.57 – 4.36 (m, 16H), 4.29 – 4.20 (m, 3H), 4.10 (dd, *J* = 8.6, 6.0 Hz, 2H), 3.78 (d, *J* = 6.6 Hz, 7H), 3.73 – 3.51 (m, 8H), 3.41 (s, 2H), 3.22 (s, 6H), 2.03 – 1.75 (m, 21H), 1.67 – 1.38 (m, 8H), 1.37 – 1.24 (m, 7H), 0.88 (d, *J* = 6.4 Hz, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 170.47, 169.83, 168.67, 159.32, 139.30, 138.79, 138.51, 130.99, 129.96, 129.80, 129.11, 128.99, 128.87, 128.77, 128.69, 128.55, 128.50, 128.47, 128.30, 128.22, 128.07, 127.94, 127.76, 127.72, 127.61, 114.13, 100.32, 99.00, 98.43, 77.40, 76.73, 76.59, 76.47, 75.54, 70.75, 67.46, 59.72, 50.54, 49.36, 49.20, 48.67, 30.07, 28.07, 23.80, 23.57, 16.99. **HRMS**: $[M+Na]^+$ calculated for $C_{80}H_{94}N_4O_{19}Na$: 1437.61400; found 1437.64042

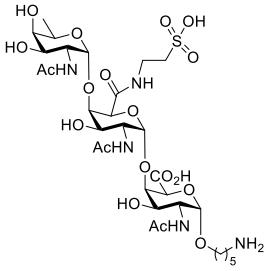
Benzyl (5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronate-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronate) (44)



The oxidation was carried out according to General oxidation procedure C using **43** (19 mg, 0.0132 mmol, 1 equiv.) in EtOAc/*t*-BuOH/H_2O (1:1:1, 0.9 mL) and TEMPO (2 mg, 0.0106 mmol, 0.8 equiv.), $NaHCO_3$ (6 mg, 0.0660 mmol, 5 equiv.) and BAIB (17 mg, 0.0528 mmol, 4 equiv.) and stirred for 12 days at 4 ºC. Purification by column chromatography (DCM/MeOH 100:0→95:5) gave **44** in 51% yield of the product with the PMB (10 mg, 0.00672 mmol) and 27% yield of the product without the PMB (5 mg, 0.00351 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.50 – 7.10 (m, 68H), 6.98 – 6.79 (m, 3H), 5.23 – 4.96 (m, 11H),

4.96 – 4.70 (m, 12H), 4.70 – 4.40 (m, 20H), 4.33 (dd, *J* = 23.7, 10.6 Hz, 8H), 3.91 – 3.73 (m, 8H), 3.71 – 3.47 (m, 7H), 3.26 – 3.15 (m, 4H), 2.03 (s, 33H), 1.58 – 1.40 (m, 9H), 1.32 – 1.16 (m, 8H), 0.88 – 0.84 (m, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 174.25, 173.76, 172.89, 171.83, 171.26, 168.18, 139.21, 138.63, 138.43, 137.91, 129.99, 129.92, 129.75, 129.63, 129.33, 129.14, 129.10, 128.87, 128.81, 128.72, 128.67, 128.60, 128.56, 128.50, 128.45, 128.36, 128.24, 128.03, 127.94, 127.84, 127.78, 127.75, 127.63, 127.54, 126.53, 121.02, 114.21, 114.16, 99.45, 99.13, 98.92, 98.45, 97.99, 91.59, 80.44, 78.01, 77.01, 76.73, 76.47, 76.26, 76.12, 75.66, 75.58, 75.32, 74.29, 73.96, 72.20, 71.97, 71.90, 71.79, 71.14, 70.31, 70.17, 69.51, 68.29, 67.63, 67.40, 59.05, 55.59, 54.04, 51.53, 50.76, 49.74, 48.70, 47.49, 46.74, 30.06, 29.27, 28.03, 26.68, 23.54, 23.39, 23.18, 23.04, 20.96, 16.93, 16.78. **HRMS**: $[M+H]^+$ calculated for $C_{80}H_{91}N_4O_{20}H$: 1429.63832; found 1429.63810

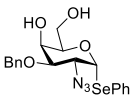
Trisaccharide with taurine on 2nd GalNAc deprotected (3)



44 (15 mg, 0.0104 mmol, 1 equiv., combined product with and without the PMB) was dissolved in DMF (1 mL) and added HATU (5 mg, 0.0124 mmol, 1.2 equiv.) and DIPEA (5 µg, 0.0311 mmol, 3 equiv.) stirred at rt for 10 min before adding taurine (3 mg, 0.0297 mmol, 2 equiv.). The reaction was stirred overnight until LC-MS showed full conversion. The solution was diluted in EtOAc and washed with 1 M HCl (x1), $NaHCO_3$ (x1, aq., sat.) and brine (x1), dried with Na_2SO_4, filtered and concentrated *in vacuo*. The crude product **45** (20 mg) was used without further purification. The hydrogenation reaction was carried out according to General hydrogenation procedure D using **45** (20 mg, crude product) to **3** in 35% yield over two steps (3 mg, 0.00362 mmol). **^{1}H NMR (600 MHz, D_2O)** δ 5.12 (d, *J* = 3.7 Hz, 1H, H-1), 4.97 (d, *J* = 2.7 Hz, 1H, H-1'), 4.88 – 4.86 (m, 2H, H-1'', H-5), 4.47 – 4.43 (m, 2H, H-4, H-5''), 4.41 (q, *J* = 1.2 Hz, 1H, H-4'), 4.34 (dd, *J* = 11.3, 3.7 Hz, 1H, H-2), 4.27 – 4.25 (m, 1H, H-5'), 4.15 (d, *J* = 2.7 Hz, 1H, H-3), 4.13 (dt, *J* = 5.4, 2.6 Hz, 2H, H-3', H-2'), 4.09 (dd, *J* = 11.2, 3.9 Hz, 1H, H-2''), 3.98 (dd, *J* = 11.2, 3.2 Hz, 1H, H-3''), 3.81 – 3.80 (m, 1H, H-4''), 3.80 – 3.75 (m, 1H, Taurine-NH-CH_2), 3.72 – 3.67 (m, 1H, Linker-CH_2), 3.54 (dt, *J* = 10.1, 3.7 Hz, 1H, Linker-CH_2), 3.46 – 3.41 (m, 1H, Taurine-NH-CH_2), 3.14 – 3.04 (m, 2H, Taurine-NH-CH$_2$-CH_2), 2.99 (t, *J* = 7.7 Hz, 2H, Linker-CH_2), 2.09 (s, 3H, COCH_3), 2.08 (s, 3H, COCH_3), 2.03 (s, 3H, COCH_3), 1.69 – 1.64 (m, 4H, Linker-CH_2), 1.46 – 1.42 (m, 2H, Linker-CH_2), 1.18 (d, *J* = 6.6 Hz, 4H, H-6''). **^{13}C NMR (151 MHz, D_2O)** δ 175.81 (C=O), 175.54 (C=O), 175.10 (C=O), 175.07 (C=O), 171.57 (C=O), 99.49 (C-1), 98.10 (C-1''), 97.78 (C-1'), 79.03 (C-4'), 75.72 (C-4), 72.12 (C-4''), 71.70 (C-5), 71.53 (C-5''), 68.96 (Linker-*C*H$_2$), 68.45 (C-3''), 68.09 (C-5''), 68.03 (C-3'), 67.72 (C-3), 50.63 (C-2'), 50.49 (C-2''), 50.34 (Taurine-NH-CH$_2$-*C*H$_2$), 50.19 (Linker-*C*H$_2$), 39.65 (Taurine-NH-*C*H$_2$), 35.83 (Linker-*C*H$_2$), 28.97 (Linker-*C*H$_2$), 27.27 (Linker-*C*H$_2$), 23.25 (CO*C*H$_3$), 23.21 (CO*C*H$_3$), 23.09 (CO*C*H$_3$), 22.83 (CO*C*H$_3$), 16.28 (C-6''). **HRMS**: $[M+H]^+$ calculated for $C_{31}H_{53}N_5O_{19}SH$: 832.31337; found 832.31278

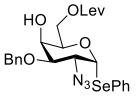
Synthesis of the hexasaccharide without taurine

Phenyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (47)



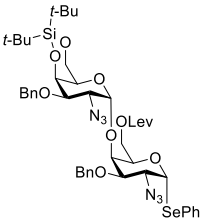
46 (2.019 g, 3.655 mmol) was dissolved in MeOH (36 mL, 0.1 M), added CSA (85 mg, 0.366 mmol, 0.1 equiv.) and stirred at rt for 1 h until TLC (pentane/EtOAc, 6:4) showed full conversion. The reaction was quenched with Et_3N and concentrated *in vacuo*. Purification by column chromatography (pentane/EtOAc, 7:3 → 4:6) yielded **47** in 82% yield (1.305 g, 3.001 mmol). **^{1}H NMR (400 MHz, CDCl3)** δ 7.62 – 7.55 (m, 2H, Ar-*H*), 7.42 – 7.32 (m, 5H, Ar-*H*), 7.32 – 7.26 (m, 3H, Ar-*H*), 5.96 (d, *J* = 5.3 Hz, 1H, H-1), 4.73 (dd, *J* = 16.9, 11.4 Hz, 2H, , Ar-CH_2), 4.25 – 4.18 (m, 2H, H-2, H-4), 4.12 (dt, *J* = 2.8, 1.3 Hz, 1H, H-3), 3.86 (ddd, *J* = 11.9, 5.9, 3.6 Hz, 1H, H-6), 3.75 – 3.65 (m, 2H, H-5, H-6), 2.75 (t, *J* = 1.4 Hz, 1H, 4-OH), 1.99 (dd, *J* = 8.7, 3.8 Hz, 1H, 6-OH). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 136.94 (Ar-C_q), 135.02 (Ar-*C*), 129.35 (Ar-*C*), 128.89 (Ar-*C*), 128.58 (Ar-*C*), 128.25 (Ar-*C*), 128.22 (Ar-*C*), 127.86 (Ar-C_q), 84.66 (C-1), 78.81 (C-5), 72.37 (Ar-*C*H_2), 72.20 (C-4), 67.33 (C-3), 62.94 (C-6), 60.31 (C-2). **HRMS**: $[M+H]^+$ calculated for $C_{19}H_{21}N_3O_4SeH$: 436.07755; found 436.07702

Phenyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (48)



47 (1.286 g, 2.960 mmol) was dissolved in dry DCM (30 mL, 0.1 M) and cooled to 0 C. LevOH (398 mg, 3.552 mmol, 1.2 equiv.), DIC (0.56 mL, 3.552 mmol, 1.2 equiv.) and DMAP (36 mg, 0.296 mmol, 0.1 equiv.) were added and the reaction was stirred at rt under N_2 for 1 h until TLC (pentane/EtOAc, 1:1) showed full conversion. The solution was filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (pentane/EtOAc, 8:2 → 5:5) yielded **48** in 92% yield (1.451 g, 2.724 mmol). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.63 – 7.57 (m, 2H, Ar-*H*), 7.44 – 7.31 (m, 5H, Ar-*H*), 7.33 – 7.24 (m, 4H, Ar-*H*), 5.94 (d, *J* = 5.3 Hz, 1H, H-1), 4.78 – 4.67 (m, 2H, Ar-CH_2), 4.42 (ddt, *J* = 7.6, 5.0, 1.4 Hz, 1H, H-5), 4.34 (dd, *J* = 11.6, 4.9 Hz, 1H, H-6), 4.24 – 4.18 (m, 2H, H-2, H-6), 4.07 (dt, *J* = 3.2, 1.6 Hz, 1H, H-4), 3.69 (dd, *J* = 10.2, 3.1 Hz, 1H, H-3), 2.70 (td, *J* = 6.4, 4.1 Hz, 2H, CH_2-Lev), 2.54 (t, *J* = 1.6 Hz, 1H, 4-OH), 2.50 (td, *J* = 6.5, 1.5 Hz, 2H, CH_2-Lev), 2.17 (s, 3H, CH_3-Lev). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 206.70 (C=O), 172.69 (C=O), 136.95 (Ar-C_q), 134.61 (Ar-*C*), 129.24 (Ar-*C*), 128.85 (Ar-*C*), 128.56 (Ar-*C*), 128.52 (Ar-*C*), 128.21 (Ar-*C*), 128.06 (Ar-*C*), 84.84 (C-1), 78.68 (C-3), 72.26 (Ar-*C*H_2), 70.40 (C-5), 65.88 (C-4), 63.25 (C-6), 60.18 (C-2), 37.96 (CH_2-Lev), 29.96 (CH_3-Lev), 27.85 (CH_2-Lev). **HRMS**: $[M+Na]^+$ calculated for $C_{24}H_{27}N_3O_6SeNa$: 556.09628; found 556.09572

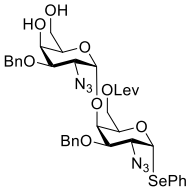
Phenyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(di-*tert*-butylsilyene)-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (49)



The reaction was carried out according to General glycosylationprocedure A using acceptor **48** (1.221 g, 2.293 mmol, 1 equiv.), donor **11b** (1.760 g, 2.980 mmol, 1.3 equiv.) and TBSOTf (120 µL, 0.459 mmol, 0.2 equiv.) in DCM (23 mL, 0.1 M). The reaction was followed by TLC (pentane/EtOAc 7:3) and column chromatography (pentane/EtOAc 85:15 → 70:30) gave **49** in 59% yield (1.296 g, 1.364 mmol) as only the α-anomer. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.73 – 7.56 (m, 2H, Ar-*H*), 7.44 – 7.25 (m, 17H, Ar-*H*), 6.00 (d, *J* = 5.3 Hz, 1H, H-1), 5.02 (d, *J* = 2.8 Hz, 1H, H-1'), 4.81 – 4.61 (m, 5H, Ar-CH_2), 4.53 (t, *J* = 1.6 Hz, 1H, H-3), 4.44 – 4.32 (m, 3H,

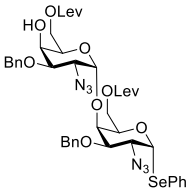
H-5, H-6), 4.28 (d, *J* = 2.8 Hz, 1Hm H-4), 4.14 (dd, *J* = 10.5, 5.3 Hz, 1H, H-2), 4.02 (s, 1H, H-4'), 3.94 – 3.84 (m, 2H, H-5', H-2'), 3.79 – 3.71 (m, 1H, H-6'), 3.71 – 3.63 (m, 2H, H-3, H-6'), 2.75 (td, *J* = 6.2, 1.8 Hz, 2H, CH_2-Lev), 2.53 (t, *J* = 6.5 Hz, 2H, CH_2-Lev), 2.19 (s, 3H, CH_3-Lev), 1.01 (d, *J* = 4.8 Hz, 18H, *H-t*-Bu). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 206.58 (C=O), 172.34 (C=O), 137.80 (Ar-C_q), 136.96 (Ar-C_q), 135.08 (Ar-*C*), 129.25 (Ar-*C*), 128.74 (Ar-*C*), 128.68 (Ar-*C*), 128.61 (Ar-*C*), 128.21 (Ar-*C*), 128.15 (Ar-*C*), 128.07 (Ar-*C*), 128.01 (Ar-*C*), 127.32 (Ar-*C*), 99.18 (C-1'), 84.93 (C-1), 78.54 (C-3), 75.44 (C-5'), 72.16 (Ar-*C*H_2), 71.82 (C-4'), 70.66 (C-5), 70.40 (Ar-*C*H_2), 69.51 (C-3), 67.90 (C-4), 66.94 (C-6'), 61.88 (C-6), 61.13 (C-2), 58.76 (C-2'), 38.10 (*C*H_2-Lev), 29.92 (*C*H_3-Lev), 27.98 (*C*H_2-Lev), 27.72 (C(*C*H_3)$_3$), 27.44 (C(*C*H_3)$_3$), 23.44 (*C*(CH$_3$)$_3$), 20.77 (*C*(CH$_3$)$_3$). **HRMS**: $[M+Na]^+$ calculated for $C_{45}H_{58}N_6O_{10}SeSiNa$: 973.30466; found 973.30430

Phenyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (50)



49 (1.263 g, 1.330 mmol) was dissolved in THF (13 mL, 0.1 M) and cooled to 0 ºC. AcOH (0.2 mL, 3.324 mmol, 2.5 equiv.) and TBAF (1 M in THF, 3.3 mL, 3.324 mmol, 2.5 equiv.) was added and the reaction was stirred at rt under N_2 overnight until TLC (pentane/EtOAc 1:1) showed full conversion. The reaction was quenched with NH_4Cl (aq., sat.) and diluted with EtOAc. The organic phase was washed with H_2O (x3) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 5:5 → 2:8) gave **50** in 86% yield (928 mg, 1.146 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.63 – 7.58 (m, 2H, Ar-*H*), 7.45 – 7.27 (m, 14H, Ar-*H*), 5.99 (d, *J* = 5.4 Hz, 1H, H-1), 5.06 (d, *J* = 3.4 Hz, 1H, H-1'), 4.85 (d, *J* = 11.7 Hz, 1H, Ar-CH_2), 4.76 – 4.65 (m, 3H, Ar-CH_2), 4.47 – 4.39 (m, 1H, H-5), 4.39 – 4.33 (m, 1H, H-6), 4.33 – 4.29 (m, 1H, H-6), 4.27 (d, *J* = 2.8 Hz, 1H, H-4), 4.21 – 4.14 (m, 2H, H-2, H-4'), 4.07 (t, *J* = 4.9 Hz, 1H, H-5'), 3.96 – 3.80 (m, 2H, H-2', H-3'), 3.67 (dd, *J* = 10.5, 2.8 Hz, 1H, H-3), 3.48 (q, *J* = 5.1, 3.5 Hz, 2H, H-6'), 2.81 (s, 1H, 4-OH'), 2.75 (dd, *J* = 7.2, 5.5 Hz, 2H, Lev-CH_2), 2.56 – 2.49 (m, 3H, Lev-CH_2, 6-OH'), 2.19 (s, 3H, Lev-CH_3). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 206.74 (C=O), 172.36 (C=O), 137.07 (Ar-C_q), 135.03 (Ar-*C*), 129.23 (Ar-*C*), 128.76 (Ar-*C*), 128.39 (Ar-*C*), 128.30 (Ar-*C*), 128.19 (Ar-*C*), 128.14 (Ar-*C*), 127.85 (Ar-C_q), 127.64 (Ar-*C*), 99.22 (C-1'), 84.89 (C-1), 78.58 (C-3), 76.22 (C-3'), 72.91 (C-4), 72.46 (Ar-*C*H_2), 71.94 (Ar-*C*H_2), 70.50 (C-5), 69.63 (C-5'), 67.62 (C-4'), 62.79 (C-6'), 61.73 (C-6), 61.00 (C-2), 59.65 (C-2'), 38.05 (Lev-*C*H_2), 29.88 (Lev-*C*H_3), 27.87 (Lev-*C*H_2). **HRMS**: $[M+Na]^+$ calculated for $C_{37}H_{42}N_6O_{10}SeNa$: 833.20253; found 833.20203

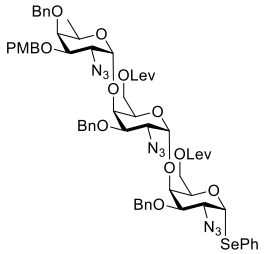
Phenyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (51)



50 (974 mg, 1.203 mmol) was dissolved in dry DCM (12 mL, 0.1 M) and cooled to 0 C. LevOH (162 mg, 1.444 mmol, 1.2 equiv.), DIC (0.23 mL, 1.444 mmol, 1.2 equiv.) and DMAP (15 mg, 0.120 mmol, 0.1 equiv.) were added and the reaction was stirred at rt under N_2 for 1 h until TLC (pentane/EtOAc, 4:6) showed full conversion. The solution was filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (pentane/EtOAc, 55:45 → 40:60) yielded **51** in 98% yield

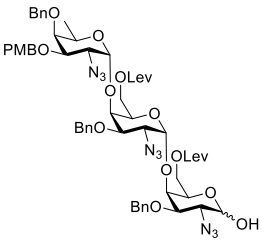
(1.073 g, 1.181 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.63 – 7.54 (m, 2H, Ar-*H*), 7.50 – 7.27 (m, 10H, Ar-*H*), 5.98 (d, *J* = 5.3 Hz, 1H, H-1), 5.03 (d, *J* = 3.6 Hz, 1H, H-1'), 4.87 (d, *J* = 12.1 Hz, 1H, Ar-CH_2), 4.78 – 4.69 (m, 3H, Ar-CH_2), 4.40 – 4.34 (m, 2H, H-5, H-6), 4.34 – 4.27 (m, 2H, H-5', H-6), 4.26 – 4.20 (m, 2H, H-4, H-6'), 4.18 (dd, *J* = 10.5, 5.3 Hz, 1H, H-2), 4.09 (dd, *J* = 3.1, 1.4 Hz, 1H, H-4'), 3.96 – 3.83 (m, 3H, H-3', H-6', H-2'), 3.64 (dd, *J* = 10.5, 2.7 Hz, 1H, H-3), 2.77 – 2.65 (m, 4H, Lev-CH_2), 2.54 – 2.42 (m, 4H, Lev-CH_2), 2.18 (s, 3H, Lev-CH_3), 2.17 (s, 3H, Lev-CH_3). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 206.75 (C=O), 172.51 (C=O), 172.36 (C=O), 137.19 (Ar-C_q), 137.10 (Ar-*C*), 134.97 (Ar-*C*), 129.23 (Ar-*C*), 128.75 (Ar-*C*), 128.70 (Ar-*C*), 128.34 (Ar-*C*), 128.22 (Ar-*C*), 128.14 (Ar-*C*), 127.81 (Ar-*C*), 99.18 (C-1'), 84.93 (C-1), 78.00 (C-3), 76.10 (C-3'), 73.07 (C-4), 72.22 (Ar-*C*H$_2$), 71.86 (Ar-*C*H$_2$), 70.60 (C-5), 68.16 (C-5'), 65.26 (C-4'), 62.28 (C-6'), 61.84 (C-6), 61.05 (C-2), 59.67 (C-2'), 38.10 (Lev-*C*H$_2$), 37.97 (Lev-*C*H$_2$), 30.08 (Lev-*C*H$_3$), 29.74 (Lev-*C*H$_3$), 27.91 (Lev-*C*H$_2$), 27.81 (Lev-*C*H$_2$). **HRMS**: $[M+Na]^+$ calculated for $C_{42}H_{46}N_6O_{12}SeNa$: 931.23931; found 931.23904

Phenyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (52)



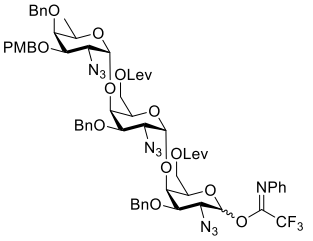
The reaction was carried out according to General glycosylationprocedure A using acceptor **51** (824 mg, 0.908 mmol, 1 equiv.), donor **10b** (777 mg, 0.1362 mmol, 1.5 equiv.) and TBSOTf (48 µL, mmol, 0.2 equiv.) in DCM (9 mL, 0.1 M). The reaction was followed by TLC (pentane/EtOAc 6:4) and column chromatography (pentane/EtOAc 70:30 → 45:55) gave **52** in 56% yield (656 mg, 0.509 mmol) as only the α-anomer. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.61 – 7.55 (m, 2H, Ar-*H*), 7.43 – 7.26 (m, 25H, Ar-*H*), 6.93 – 6.88 (m, 2H, Ar-*H*), 5.93 (d, *J* = 5.3 Hz, 1H, H-1), 5.07 (d, *J* = 3.6 Hz, 1H, H-1'), 4.95 (d, *J* = 2.7 Hz, 1H, H-1''), 4.93 – 4.83 (m, 3H, Ar-CH_2), 4.73 (d, *J* = 12.3 Hz, 1H, Ar-CH_2), 4.70 – 4.61 (m, 4H, Ar-CH_2), 4.53 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.41 – 4.33 (m, 1H, H-5'), 4.33 – 4.24 (m, 4H, H-5, H-6, H-6'), 4.23 – 4.18 (m, 3H, H-3'', H-4, H-5''), 4.13 – 4.03 (m, 2H, H-6', H-2), 3.95 – 3.86 (m, 3H, H-4', H-2'', H-3'), 3.81 (s, 3H, PMB-CH_3), 3.77 (dd, *J* = 11.0, 3.6 Hz, 1H, H-2'), 3.65 – 3.58 (m, 2H, H-4'', H-3), 2.80 – 2.68 (m, 4H, Lev-CH_2), 2.52 (t, *J* = 6.4 Hz, 2H, Lev-CH_2), 2.48 – 2.40 (m, 2H, Lev-CH_2), 2.18 (s, 3H, Lev-CH_3), 2.16 (s, 3H, Lev-CH_3), 0.85 (d, *J* = 6.4 Hz, 3H, H-6''). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 206.64 (C=O), 206.25 (C=O), 172.38 (C=O), 171.80 (C=O), 138.56 (Ar-C_q), 137.37 (Ar-C_q), 137.14 (Ar-C_q), 134.99 (Ar-*C*), 129.22 (Ar-C_q), 128.69 (Ar-*C*), 128.56 (Ar-*C*), 128.36 (Ar-*C*), 128.33 (Ar-*C*), 128.14 (Ar-*C*), 128.08 (Ar-*C*), 127.92 (Ar-*C*), 127.78 (Ar-*C*), 127.73 (Ar-*C*), 127.65 (Ar-*C*), 114.04 (Ar-*C*), 99.41 (C-1''), 98.89 (C-1'), 84.87 (C-1), 77.48 (C-4'), 76.84 (C-3), 76.20 (C-4''), 75.05 (C-3'), 74.97 (Ar-*C*H$_2$), 72.40 (C-4/ C-3''), 72.01 (Ar-*C*H$_2$), 71.69 (Ar-*C*H$_2$), 70.61 (C-5'), 68.91 (C-5), 67.48 (C-5''), 61.92 (C-6'), 61.18 (C-2), 61.11 (C-6''), 60.28 (C-2''), 60.19 (C-2'), 55.43 (PMB-*C*H$_3$), 38.07 (Lev-*C*H$_2$), 29.91 (Lev-*C*H$_3$), 27.84 (Lev-*C*H$_2$), 16.73 (C-6''). **HRMS**: $[M+Na]^+$ calculated for $C_{63}H_{71}N_9O_{16}SeNa$: 1312.40817; found 1312.40957

2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranose (53)



52 (640 mg, 0.496 mmol) was dissolved in THF/H_2O (10:1, 10 mL, 0.05 M) and cooled to 0 ºC. NIS (447 mg, 1.085 mmol, 4 equiv.) were added and the reaction was stirred for 30 min until TLC (pentane/EtOAc, 6:4) showed full conversion. The reaction mixture was quenched with $Na_2S_2O_3$ (aq., sat.) and diluted in EtOAc. The organic phases was washed with $Na_2S_2O_3$ (x1, aq., sat.), $NaHCO_3$ (xa, aq., sat.) and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 6:4 → 4:6) yielded **53** in 100% yield as a α/β=53:47 (582 g, 0.496 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.52 – 7.29 (m, 28H), 6.94 – 6.85 (m, 4H), 5.35 (t, *J* = 2.7 Hz, 1H), 5.13 (d, *J* = 3.5 Hz, 1H), 5.02 (d, *J* = 3.6 Hz, 1H), 4.97 (d, *J* = 2.0 Hz, 1H), 4.94 (d, *J* = 2.7 Hz, 1H), 4.92 – 4.85 (m, 5H), 4.82 (s, 1H), 4.74 – 4.59 (m, 9H), 4.53 (dd, *J* = 11.4, 5.3 Hz, 2H), 4.45 – 4.27 (m, 10H), 4.27 – 4.13 (m, 8H), 4.13 – 4.03 (m, 4H), 3.97 – 3.84 (m, 8H), 3.81 (s, 7H), 3.79 – 3.71 (m, 3H), 3.64 (dd, *J* = 8.9, 2.2 Hz, 3H), 3.60 – 3.50 (m, 2H), 3.26 (dd, *J* = 10.5, 2.7 Hz, 1H), 2.87 – 2.66 (m, 13H), 2.62 – 2.39 (m, 9H), 2.19 (s, 3H), 2.18 (s, 3H), 2.17 (s, 4H), 2.16 (s, 3H), 0.88 (d, *J* = 6.4 Hz, 3H), 0.84 (d, *J* = 6.4 Hz, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 207.94, 207.18, 206.86, 206.58, 172.49, 172.21, 171.79, 159.55, 138.40, 137.38, 129.63, 129.60, 128.64, 128.58, 128.54, 128.34, 128.03, 127.96, 127.88, 127.84, 127.79, 127.77, 127.70, 127.66, 127.53, 114.03, 99.42, 98.88, 98.62, 96.60, 92.37, 78.35, 77.61, 77.37, 76.17, 75.16, 75.00, 74.97, 74.95, 73.17, 72.33, 72.25, 72.16, 72.11, 71.97, 71.88, 71.69, 69.15, 68.89, 68.29, 67.46, 64.89, 62.83, 62.37, 61.65, 61.26, 60.48, 60.38, 60.26, 55.41, 38.41, 38.13, 38.04, 29.95, 29.90, 29.68, 28.24, 28.05, 27.87, 27.81, 23.58, 16.71. **HRMS**: $[M+Na]^+$ calculated for $C_{57}H_{67}N_9O_{17}Na$: 1172.45526; found 1172.45374

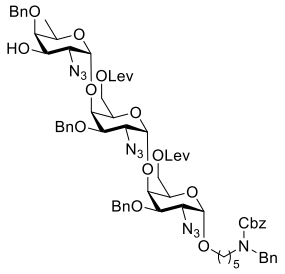
2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-1-*O*-(*N*-phenyl, 2,2,2-trifluoroacetimidoyl)-α-D-galactopyranose (54)



53 (785 mg, 0.682 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (3.4 mL, 0.2 M). K_2CO_3 (141 mg, 1.023 mmol, 1.5 equiv.) and ClC(=NPh)CF_3 (0.17 mL, 1.023 mmol, 1.5 equiv.) and was added and the reaction was stirred at rt under N_2 overnight until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction was filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 8:2 → 5:5) gave **54** in 80% yield (720 mg, 0.545 mmol). **^{1}H NMR (400 MHz, CD_3CN)** δ 7.56 – 7.26 (m, 35H), 7.24 – 7.09 (m, 2H), 6.96 – 6.77 (m, 7H), 5.45 (s, 1H), 5.07 (d, *J* = 3.4 Hz, 1H), 5.04 (d, *J* = 3.5 Hz, 1H), 4.96 – 4.84 (m, 5H), 4.82 (d, *J* = 2.9 Hz, 1H), 4.79 (d, *J* = 2.9 Hz, 1H), 4.75 (d, *J* = 3.2 Hz, 1H), 4.72 (d, *J* = 3.2 Hz, 1H), 4.69 – 4.59 (m, 4H), 4.59 – 4.52 (m, 4H), 4.43 – 4.26 (m, 8H), 4.26 – 4.19 (m, 5H), 4.11 – 3.95 (m, 6H), 3.95 – 3.83 (m, 5H), 3.81 (t, *J* = 3.7 Hz, 3H), 3.78 (s, 6H), 3.50 (dt, *J* = 8.3, 4.5 Hz, 1H), 2.81 – 2.64 (m, 7H), 2.57 – 2.33 (m, 8H), 2.16 (s, 2H), 2.12 (s, 2H), 2.09 (d, *J* = 1.3 Hz, 9H), 0.85 (t, *J* = 6.3 Hz, 6H). **^{13}C NMR (101 MHz, CD_3CN)** δ 207.97, 173.15,

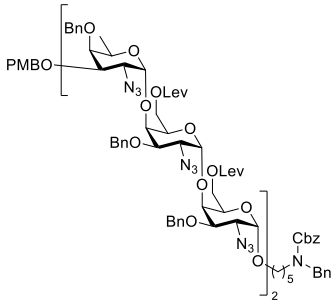
172.76, 160.42, 139.95, 139.18, 138.66, 131.20, 130.78, 129.92, 129.37, 129.35, 129.28, 129.18, 128.97, 128.72, 128.62, 128.51, 128.43, 128.36, 125.48, 120.03, 114.70, 100.29, 99.87, 79.06, 77.96, 77.58, 76.17, 75.82, 74.10, 73.76, 73.68, 73.34, 72.61, 72.55, 72.20, 72.14, 72.00, 71.85, 69.98, 69.84, 68.13, 62.91, 62.68, 62.49, 61.99, 61.56, 61.16, 61.06, 59.52, 55.88, 38.51, 29.87, 28.69, 28.56, 16.99. **HRMS**: $[M+Na]^+$ calculated for $C_{65}H_{71}F_3N_{10}O_{17}Na$: 1343.48485; found 1343.48284

5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-4-*O*-benzyl-2-deoxy-α-D-fucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (55)



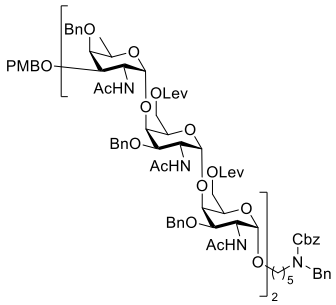
6 (560 mg, 0.383 mmol) was dissolved in DCM/H_2O (3.8 mL, 0.1 M, 20:1) and added DDQ (174 mg, 0.767 mmol, 2 equiv.). The reaction was stirred for 2 h under nitrogen until TLC (pentane/EtOAc, 6:4) showed full conversion. The reaction mixture was quenched with $Na_2S_2O_3$ (x1, aq., sat.), diluted in EtOAc and washed with, $NaHCO_3$ (x4, aq., sat.) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 7:3 → 4:6) yielded **55** in 86% yield (441 mg, 0.329 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.51 – 7.26 (m, 27H, Ar-*H*), 5.17 (d, *J* = 14.6 Hz, 2H, Linker-CH_2), 5.05 (d, *J* = 3.6 Hz, 1H, H-1'), 4.93 (d, *J* = 3.6 Hz, 1H, H-1''), 4.92 – 4.86 (m, 2H, H-1, Ar-CH_2), 4.84 (d, *J* = 12.1 Hz, 1H, Ar-CH_2), 4.74 (d, *J* = 11.5 Hz, 1H, Ar-CH_2), 4.66 (dd, *J* = 12.0, 4.3 Hz, 2H, Ar-CH_2), 4.60 (d, *J* = 11.5 Hz, 1H, Ar-CH_2), 4.49 (d, *J* = 6.6 Hz, 2H, Linker-CH_2), 4.44 – 4.24 (m, 5H, H-6, H-6', H-4'', H-5''), 4.24 – 4.18 (m, 2H, H-4, H-4'), 4.08 – 3.99 (m, 1H, H-3''), 3.98 – 3.89 (m, 2H, H-5, H-6'), 3.89 – 3.79 (m, 2H, H-5', H-3), 3.76 (dd, *J* = 10.9, 3.6 Hz, 1H, H-2'), 3.67 – 3.59 (m, 1H, Linker-CH_2), 3.58 (dd, *J* = 3.6, 1.4 Hz, 1H, H-2), 3.53 (ddd, *J* = 10.8, 3.6, 1.8 Hz, 2H, H-2'', H-3'), 3.49 – 3.31 (m, 1H, Linker-CH_2), 3.31 – 3.10 (m, 2H, Linker-CH_2), 2.80 – 2.60 (m, 4H, Lev-CH_2), 2.56 (t, *J* = 6.4 Hz, 2H, Lev-CH_2), 2.50 – 2.28 (m, 3H, Lev-CH_2), 2.17 (s, 3H, Lev-CH_3), 2.16 (s, 3H, Lev-CH_2), 1.53 (m, 4H, Linker-CH_2), 1.37 – 1.18 (m, 3H, Linker-CH_2), 0.90 (d, *J* = 6.5 Hz, 3H, H-6''). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 206.57 (C=O), 172.32 (C=O), 171.67 (C=O), 137.94 (Ar-C_q), 137.46 (Ar-C_q), 137.36 (Ar-C_q), 128.78 (Ar-*C*), 128.65 (Ar-*C*), 128.60 (Ar-*C*), 128.54 (Ar-*C*), 128.27 (Ar-*C*), 128.15 (Ar-*C*), 128.04 (Ar-*C*), 127.93 (Ar-*C*), 127.87 (Ar-*C*), 127.85 (Ar-*C*), 127.57 (Ar-*C*), 127.44 (Ar-*C*), 99.44 (C-1'), 98.87 (C-1''), 98.07 (C-1), 80.46 (C-3'), 76.22 (Ar-*C*H_2), 75.39 (C-5), 72.48 (C-4/C-4'), 71.95 (Ar-*C*H_2), 71.81 (Ar-*C*H_2), 68.87 (C-4''/C-5''), 68.71 (C-3''), 67.36 (C-4''/C-''), 67.25 (Linker-*C*H_2), 62.01 (C-6), 61.93 (C-2/C-2''), 61.22 (C-6'), 60.22 (C-2''), 59.58 (C-2/C-2''), 50.35 (Linker-*C*H_2), 50.30 (Linker-*C*H_2), 47.41 (Linker-*C*H_2), 46.32 (Lev-*C*H_2), 42.32 (Lev-*C*H_3), 38.01 (Linker-*C*H_2), 29.89 (Lev-*C*H_2), 29.10 (Linker-*C*H_2), 16.63 (C-6''). **HRMS**: $[M+Na]^+$ calculated for $C_{69}H_{82}N_{10}O_{18}Na$: 1361.57063; found 1361.56885

Hexasaccharide – Protected (56)



The reaction was carried out according to General glycosylationprocedure A using acceptor **55** (315 mg, 0.235 mmol, 1 equiv.), donor **54** (466 mg, 0.352 mmol, 1.5 equiv.) and TBSOTf (22 µL, 0.0939 mmol, 0.4 equiv.) in DCM (2.4 mL, 0.1 M). The reaction was followed by TLC (pentane/EtOAc 6:4) and column chromatography (pentane/EtOAc 65:35 → 50:50) followed by size exclusion gave **56** in 58% yield (338 mg, 0.136 mmol) as only the α-anomer. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.47 – 7.15 (m, 52H), 6.94 – 6.87 (m, 2H), 5.22 – 5.14 (m, 3H), 5.12 (d, *J* = 3.6 Hz, 1H), 5.05 (d, *J* = 3.6 Hz, 1H), 4.97 (d, *J* = 3.7 Hz, 1H), 4.96 – 4.85 (m, 7H), 4.82 (d, *J* = 12.3 Hz, 2H), 4.71 – 4.59 (m, 7H), 4.55 – 4.47 (m, 5H), 4.44 – 4.24 (m, 12H), 4.24 – 4.12 (m, 6H), 4.10 – 4.06 (m, 1H), 4.02 – 3.96 (m, 2H), 3.96 – 3.88 (m, 8H), 3.86 – 3.70 (m, 9H), 3.71 – 3.57 (m, 4H), 3.53 (dd, *J* = 10.8, 3.5 Hz, 1H), 3.46 – 3.31 (m, 1H), 3.22 (dt, *J* = 26.2, 7.8 Hz, 2H), 2.85 – 2.51 (m, 15H), 2.48 – 2.27 (m, 5H), 2.16 (s, 14H), 1.54 (t, *J* = 14.9 Hz, 5H), 1.28 (d, *J* = 11.5 Hz, 4H), 0.85 (d, *J* = 6.4 Hz, 4H), 0.83 (d, *J* = 6.4 Hz, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 206.60, 206.40, 172.39, 172.26, 171.69, 171.67, 159.45, 138.43, 138.39, 138.02, 137.46, 137.44, 137.36, 137.22, 129.86, 129.55, 128.62, 128.57, 128.52, 128.48, 128.33, 128.31, 128.29, 128.01, 127.96, 127.90, 127.83, 127.80, 127.72, 127.67, 127.53, 127.48, 127.37, 127.26, 113.99, 99.42, 98.87, 98.77, 98.01, 96.13, 77.72, 76.19, 75.43, 75.25, 75.13, 75.00, 74.91, 72.60, 72.27, 72.00, 71.82, 71.75, 71.70, 69.02, 68.81, 68.65, 68.36, 68.23, 67.53, 67.42, 67.22, 62.23, 61.68, 61.28, 61.21, 60.44, 60.30, 60.17, 60.11, 59.96, 59.51, 55.37, 50.55, 50.26, 47.18, 46.21, 38.09, 38.04, 29.85, 29.81, 27.95, 27.91, 27.76, 27.73, 16.68, 16.57. **HRMS**: $[M+Na]^+$ calculated for $C_{126}H_{147}N_{19}O_{34}Na$: 2494.02555; found 2494.02810

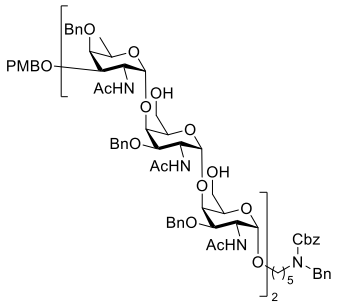
Hexasaccharide – NHAc (57)



The azide reduction was carried out followed the general azide reduction procedure B using **56** (335 mg, 0.135 mmol, 1 equiv.) and zinc powder (1.33 g, 20.32 mmol, 150 equiv.). Purification by column chromatography (DCM/MeOH 98:2→95:5) gave **57** in 100% yield (349 mg, 0.135 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.48 – 7.19 (m, 52H), 7.01 – 6.90 (m, 2H), 5.81 (s, 2H), 5.70 (s, 1H), 5.21 (s, 1H), 5.19 – 5.06 (m, 4H), 4.96 – 4.81 (m, 9H), 4.81 – 4.75 (m, 3H), 4.62 – 4.50 (m, 12H), 4.50 – 4.44 (m, 4H), 4.28 – 3.97 (m, 13H), 3.88 – 3.80 (m, 8H), 3.71 – 3.62 (m, 1H), 3.31 – 3.17 (m, 3H), 2.80 – 2.65 (m, 10H), 2.63 – 2.41 (m, 14H), 2.25 – 2.16 (m, 13H), 2.16 – 2.05 (m, 8H), 2.04 – 1.88 (m, 22H), 1.85 (s, 3H), 1.67 – 1.57 (m, 5H), 1.57 – 1.54 (m, 2H), 1.32 (d, *J* = 13.1 Hz, 3H), 1.03 – 0.88 (m, 6H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 207.52, 206.92, 173.58, 172.75, 172.39, 172.08, 170.82, 170.56, 169.40, 159.43, 138.95, 138.47, 138.23, 138.06, 129.64, 128.63, 128.57, 128.45, 128.27, 128.22, 128.19, 127.93, 127.86, 127.69, 127.51, 127.32, 113.88, 98.76, 97.64, 77.08, 76.46, 74.83, 74.59, 71.64, 71.45, 70.82, 69.18, 67.29, 67.03, 60.88, 55.34, 50.23, 48.93, 48.44, 47.57, 47.09, 37.84, 29.63,

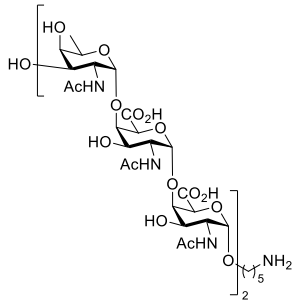
27.77, 23.21, 22.40, 20.70, 16.73. **HRMS**: $[M+Na]^+$ calculated for $C_{138}H_{171}N_7O_{40}Na$: 2589.14595; found 1294.60479

Hexasaccharide – C-6-OH (58)



57 (349 mg, 0.135 mmol, 1 equiv.) was dissolved in toluene/EtOH (1:2, 0.1 M, 1.5 mL) and added hydrazine acetate (250 mg, 2.717 mmol, 20 equiv.) and stirred at rt for 1 h until TLC analysis (DCM/MeOH 95:5) showed full conversion. The solution was diluted in DCM and $NaHCO_3$ (aq., sat.) and the organic layer was dried with Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (DCM/MeOH 96:4 →90:10) gave **58** in 96% yield (284 mg, 0.109 mmol). **^{1}H NMR (400 MHz, CD_2Cl_2)** δ 7.47 – 7.14 (m, 57H), 6.91 (dd, *J* = 8.8, 2.3 Hz, 2H), 5.69 (dd, *J* = 9.9, 5.0 Hz, 1H), 5.23 – 5.03 (m, 5H), 4.99 – 4.59 (m, 16H), 4.62 – 4.33 (m, 21H), 4.34 – 3.91 (m, 14H), 3.87 – 3.70 (m, 11H), 3.69 – 3.44 (m, 12H), 3.44 – 3.30 (m, 6H), 3.24 (d, *J* = 8.4 Hz, 4H), 2.05 – 1.76 (m, 23H), 1.57 – 1.38 (m, 7H), 1.35 – 1.14 (m, 11H), 0.96 – 0.81 (m, 6H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 171.87, 171.06, 170.62, 170.55, 170.47, 169.92, 159.66, 139.29, 138.63, 138.16, 130.91, 130.00, 128.90, 128.80, 128.50, 128.33, 128.20, 128.12, 127.96, 127.78, 127.68, 127.54, 114.15, 98.91, 98.26, 77.73, 77.18, 76.69, 75.08, 71.73, 71.31, 67.53, 67.19, 60.66, 60.04, 55.61, 50.69, 50.14, 49.20, 48.39, 47.69, 30.06, 26.42, 26.24, 23.66, 23.47, 23.22, 23.16, 22.92, 22.69, 17.07. **HRMS**: $[M+Na]^+$ calculated for $C_{118}H_{147}N_7O_{32}Na$: 2196.99884; found 1099.50702

Hexasaccharide – Deprotected (5)



The reaction was carried out according to General oxidation procedure C using **58** (45 mg, 0.0207 mmol, 1 equiv.) in EtOAc/*t*-BuOH/H_2O (1:1:1, 0.9 mL) and TEMPO (10 mg, 0.0662 mmol, 3.2 equiv.), $NaHCO_3$ (35 mg, 0.414 mmol, 20 equiv.) and BAIB (107 mg, 0.331 mmol, 18 equiv.). The reaction was stirred for 12 days at 4 ºC and purified by size exclusion chromatography to give **59** in 65% yield without the PMB (31 mg, 0.0135mmol). **59** was subjection the hydrogenation and the reaction was carried out according to General hydrogenation procedure D using **59** (30 mg, 0.0132 mmol, 1 equiv.) to yield **5** in 26% yield (7.6 mg, 0.00545 mmol) over two steps. **^{1}H NMR (600 MHz, D_2O)** δ 5.13 (d, *J* = 3.8 Hz, 1H), 5.11 – 5.05 (m, 2H), 4.98 (d, *J* = 3.6 Hz, 2H), 4.93 – 4.85 (m, 4H), 4.48 – 4.43 (m, 2H), 4.43 – 4.34 (m, 6H), 4.29 – 4.05 (m, 13H), 4.00 (ddd, *J* = 19.9, 11.3, 3.1 Hz, 2H), 3.91 (d, *J* = 3.2 Hz, 1H), 3.85 – 3.81 (m, 1H), 3.73 – 3.65 (m, 2H), 3.55 (dt, *J* = 10.1, 6.1 Hz, 1H), 2.99 (t, *J* = 7.8 Hz, 2H), 2.09 – 2.02 (m, 18H), 1.72 – 1.58 (m, 6H), 1.44 (q, *J* = 8.0 Hz, 3H), 1.19 – 1.13 (m, 6H). **^{13}C NMR (151 MHz, D_2O)** δ 175.81, 175.69, 175.51, 175.45, 174.30, 174.21, 174.08, 173.78, 99.87, 99.68, 99.59, 99.42, 97.86, 96.46, 79.44, 79.20, 78.75, 75.92, 72.13, 72.06, 71.92, 71.19, 69.41, 69.11, 68.54, 68.37, 67.87, 67.61, 67.57, 67.52, 67.33, 50.64, 50.44, 50.30, 48.56, 40.28, 28.95, 27.29, 23.24, 23.20, 23.12, 22.88, 22.79, 16.37, 16.29. **HRMS**: $[M+H]^+$ calculated for $C_{53}H_{83}N_7O_{33}H$: 1346.51100; found 1346.51311

References

(1) Visansirikul, S.; Kolodziej, S. A.; Demchenko, A. V. Staphylococcus Aureus Capsular Polysaccharides: A Structural and Synthetic Perspective. *Org. Biomol. Chem.* **2020**, *18* (5), 783–798. https://doi.org/10.1039/c9ob02546d.

(2) Scott, A. C. A Capsulate Staphylococcus Aureus. *J. Med. Microbiol.* **1969**, *2*, 253–260. https://doi.org/10.1099/00222615-2-3-253.

(3) Liau, D. F.; Melly, M. A.; Hash, J. H. Surface Polysaccharide from Staphylococcus Aureus M That Contains Taurine, D-Aminogalacturonic Acid, and D-Fucosamine. *J. Bacteriol.* **1974**, *119* (3), 913–922. https://doi.org/10.1128/jb.119.3.913-922.1974.

(4) Liau, D. F.; Hash, J. H. Structural Analysis of the Surface Polysaccharide of Staphylococcus Aureus M. *J. Bacteriol.* **1977**, *131* (1), 194–200. https://doi.org/10.1128/jb.131.1.194-200.1977.

(5) Murthy, S. V. K. N.; Ann Melly, M.; Harris, T. M.; Hellerqvist, C. G.; Hash, J. H. The Repeating Sequence of the Capsular Polysaccharide of Staphylococcus Aureus M. *Carbohydr. Res.* **1983**, *117*, 113–123. https://doi.org/10.1016/0008-6215(83)88080-X.

(6) Karakawa, W. W.; Young, D. A.; Kane, J. A. Structural Analysis of the Cellular Constituents of a Fresh Clinical Isolate of Staphylococcus Aureus, and Their Role in the Interaction between the Organisms and Polymorphonuclear Leukocytes in the Presence of Serum Factors. *Infect. Immun.* **1978**, *21* (2), 496–505. https://doi.org/10.1128/iai.21.2.496-505.1978.

(7) Karakawa, W. W.; Kane, J. A. *Seminars in Infectious Disease. Vol IV. Bacterial Vaccines*; Wienstein, L., Field, B. N., Eds.; Thieme-Stratton Inc.: Stuttgard, Germany, 1982.

(8) Melly, M. A.; Duke, L. J.; Liau, D. F.; Hash, J. H. Biological Properties of the Encapsulated Staphylococcus Aureus M. *Infect. Immun.* **1974**, *10* (2), 389–397. https://doi.org/10.1128/iai.10.2.389-397.1974.

(9) Anish, C.; Schumann, B.; Pereira, C. L.; Seeberger, P. H. Chemical Biology Approaches to Designing Defined Carbohydrate Vaccines. *Chem. Biol.* **2014**, *21* (1), 38–50. https://doi.org/10.1016/j.chembiol.2014.01.002.

(10) Hagen, B.; Van Dijk, J. H. M.; Zhang, Q.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Synthesis of the Staphylococcus Aureus Strain M Capsular Polysaccharide Repeating Unit. *Org. Lett.* **2017**, *19* (10), 2514–2517. https://doi.org/10.1021/acs.orglett.7b00747.

(11) Shirsat, A. A.; Rai, D.; Ghotekar, B. K.; Kulkarni, S. S. Total Synthesis of Trisaccharide Repeating Unit of Staphylococcus Aureus Strain M. *Org. Lett.* **2023**, *25* (16), 2913–2917. https://doi.org/10.1021/acs.orglett.3c00997.

(12) Lu, S. R.; Lai, Y. H.; Chen, J. H.; Liu, C. Y.; Mong, K. K. T. Dimethylformamide: An Unusual Glycosylation Modulator. *Angew. Chemie Int. Ed.* **2011**, *50* (32), 7315–7320. https://doi.org/10.1002/anie.201100076.

(13) Zhang, Q.; Gimeno, A.; Santana, D.; Wang, Z.; Valdes-Balbin, Y.; Rodríguez-Noda, L. M.; Hansen, T.; Kong, L.; Shen, M.; Overkleeft, H. S.; Verez-Bencomo, V.; van der Marel, G. A.; Jimenez-Barbero, Jesus Chiodo, F.; Codée, J. D. C. Synthetic, Zwitterionic Sp1 Oligosaccharides Adopt a Helical Structure Crucial for Antibody Interaction. *ACS Cent. Sci.* **2019**, *5* (8), 1407–1416. https://doi.org/10.1021/acscentsci.9b00454.

(14) Imamura, A.; Ando, H.; Korogi, S.; Tanabe, G.; Muraoka, O.; Ishida, H.; Kiso, M. Di-Tert-Butylsilylene (DTBS) Group-Directed α-Selective Galactosylation Unaffected by C-2 Participating Functionalities. *Tetrahedron Lett.* **2003**, *44* (35), 6725–6728. https://doi.org/10.1016/S0040-4039(03)01647-2.

(15) Akihiro Imamura, Hiromune Ando, Hideharu Ishida, and M. K. Di-Tert-Butylsilylene-Directed α-Selective Synthesis of 4-Methylumbelliferyl T-Antigen. *Org. Lett.* **2005**, *7*

(20), 4415–4418. https://doi.org/10.1021/ol051592z.

(16) Zhang, Y.; Gómez-Redondo, M.; Jiménez-Osés, G.; Ardá, A.; Overkleeft, H. S.; van der Marel, G. A.; Jiménez-Barbero, J.; Codée, J. D. C. Synthesis and Structural Analysis of Aspergillus Fumigatus Galactosaminogalactans Featuring α-Galactose, α-Galactosamine and α-N-Acetyl Galactosamine Linkages. *Angew. Chemie Int. Ed.* **2020**, *59* (31), 12746–12750. https://doi.org/10.1002/anie.202003951.

(17) David, S.; Hanessian, S. Regioselective Manipulation of Hydroxyl Groups via Organotin Derivatives. *Tetrahedron* **1985**, *41* (4), 643–663. https://doi.org/10.1016/S0040-4020(01)96443-9.

(18) Yu, B.; Tao, H. Glycosyl Trifluoroacetimidates. Part 1: Preparation and Application as New Glycosyl Donors. *Tetrahedron Lett.* **2001**, *42* (12), 2405–2407. https://doi.org/10.1016/S0040-4039(01)00157-5.

(19) van den Bos, L. J.; Codée, J. D. C.; Toorn, J. C. Van Der; Boltje, T. J.; Boom, J. H. Van; Overkleeft, H. S.; van der Marel, G. A. Thioglycuronides: Synthesis and Oligosaccharides in the Assembly of Acidic Oligosaccharides. *Org. Lett.* **2004**, *6* (13), 2165–2168. https://doi.org/10.1021/ol049380+.

(20) De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi, A.; Piancatelli, G. A Versatile and Highly Selective Hypervalent Iodine (III)/ 2,2,6,6-Tetraniethyl-1-Piperidinyloxyl-Mediated Oxidation of Alcohols to Carbonyl Compounds. *J. Org. Chem.* **1997**, *62* (20), 6974–6977. https://doi.org/10.1021/jo971046m.

(21) Zhang, H.; Wang, X.; Meng, Y.; Yang, X.; Zhao, Q.; Gao, J. Total Synthesis of the Tetrasaccharide Haptens of Vibrio Vulnificus MO6-24 and BO62316 and Immunological Evaluation of Their Protein Conjugates. *JACS Au* **2022**, *2* (1), 97–108. https://doi.org/10.1021/jacsau.1c00190.