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

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List of Abbreviations

Å	Ångström
Ac	acetyl
Alloc	*N*-allyloxycarbonyl
AMR	antimicrobial resistance
Aq.	aqueous
Ar	aryl
BAIB	(diacetoxyiodo)benzene
BCR	B-cell receptor
Bn	benzyl
BSP	1-benzenesulfinyl piperidine
Bu	butyl
Bz	benzoyl
C	chair
cat.	catalytic
Cbz	benzoyloxycarbonyl
COSY	homonuclear correlation spectroscopy
CP	capsular polysaccharide
C_q	quaternary carbon atom
CRM_{197}	cross-reactive material 197
CSA	camphore-10-sulfonic acid
d	doublet
DCE	1,2-dichloroethane
DCM	dichloromethane
dd	double of doublet
ddd	doublet of double doublets
dddd	double doublet of double doublets
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
ddt	doublet of double triplets
DIC	*N*,*N*′-diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DP	degree of polymerization
DPS	diphenylsulfoxide
dq	double quartet
dt	double triplets
DTBS	di-*tert*-butylsilylidene
dtd	double of triple doublets
ELISA	enzyme-linked immunosorbent assay
equiv.	equivalent
Et	ethyl
Fuc	fucose
Gal	galactose
GalA	galacturonic acid
Glc	glucose
GlcA	glucuronic acid
h	hour(s)
HATU	hexafluorophosphate azabenzotriazole tetramethyl uronium
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HFIP	hexafluoroisopropa-1-ol
HMBC	heteronuclear multiple bond correlation
HPLC	high-performance liquid chromatography
HSQC	heteronuclear single quantum coherence
Hz	hertz
J	coupling constant
K	kelvin
Lev	levulinoyl
LTA	lipoteichoic acid
M	molar or mega
m	multiplet
m/z	mass over charge ratio
mAb	monoclonal antibody
MALDI	matrix assisted laser desorption/ionization
Man	mannose
ManA	mannuronic acid
Me	methyl
MHC II	major histocompatibility complex II
min	minute(s)
MM	molecular mechanics
MRSA	methicillin-resistant *Staphylococcus aureus*

MurA	muramic Acid
Nap	2-methylnaphthyl
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser enhancement spectroscopy
OS	oligosaccharide
p	para
p	pentet
pAb	polyclonal antibody
PBS	phosphate-buffered saline
Ph	phenyl
Pico	picoloyl
PMB	4-methoxybenzyl
PMP	4-methoxyphenyl
ppm	parts per million
Pr	propyl
PS	polysaccharide
q	quartet
qd	quartet of doublets
ROESY	rotating frame Overhauser effect spectroscopy
rt	room temperature
RU	repeating Unit
s	singlet
S. aureus	*Staphylococcus aureus*
sat.	saturated
STD	saturation transfer difference
t	triplet
TBAF	tetra-butylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TCA	trichloroacetyl
td	triple doublet
Temp	temperature
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
TES	triethylsilane
Tf	trifyl: trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TOCSY	total correlation spectroscopy
Troc	trichloroethyl carbamate
tt	triple triplet
TTBP	2,4,6-tri-*tert*-butylpyrimidine
UDP	uridine diphosphate
UV	ultraviolet
VA-044	2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride
VRSA	vancomycin-resistant *Staphylococcus aureus*
WTA	wall teichoic acid
δ	chemical shift

Chapter 1

General introduction and outline

In the modern world, the rise of antibiotic-resistant bacteria has become a major threat to the health care system. A large percentage of the infections caused by multi-drug resistant bacteria today arises from the ESKAPE pathogens,[1] which have been marked by WHO as high priority pathogens. The ESKAPE pathogens include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. All these bacteria have learned to adapt to the modern health care environment. In the recent years much effort has been invested in finding new treatment regiments for the ESKAPE pathaogens,[2] including therapeutic strategies using different antibiotic combinations, bacteriophages and photodynamic therapies.[3,4] *Staphylococcus aureus* (*S. aureus*), is one of the ESKAPE pathogens causing hospital-acquired infections, and with the rise of antibiotic-resistant strains,[5] such as methicillin-resistant *S. aureus* (MRSA)[6] and vancomycin-resistant *S. aureus* (VRSA)[7] the development of new therapeutic strategies is urging. To face the problems associated with antimicrobial resistance (AMR), efforts have been directed on improving clinical management, increasing the speed of diagnosis, developing (new) antimicrobial treatment,[8,9] but also on active or passive vaccination strategies.[4,10] *S. aureus* is a Gram-positive human pathogen and it is found in the environment, but is also part of the normal human microbiome[11] and it is one of the most common opportunistic pathogens. It is found in human mucous membranes and skin, and does normally not cause infections, but when the bacterium penetrates the protective skin and mucous barriers, it can cause various infections, ranging from minor skin abscesses to deadly bloodstream infections (bacteremia), heart valve infections (endocarditis), bone infections (osteomyelitis), lung infections (pneumonia), meningitis and septic shock.[11,12] It especially poses a threat to newborns and immunocompromised patients, such as, elderly, post-surgical and dialysis patients.

The cell wall of *S. aureus* (Figure 1) is complex and is composed of a peptidoglycan layer with different characteristic glycopolymers,[13–15] and cell wall proteins. The cell wall glycopolymers are involved in several physiological processes and play a key role in staphylococcal virulence. The major classes of cell wall glycopolymers include capsular polysaccharides (CPs), wall teichoic acids

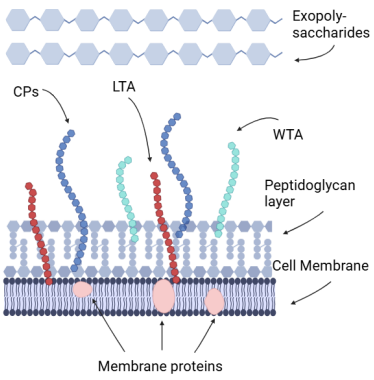


Figure 1: A schematic representation of the cell wall of *S. aureus*.

(WTAs) and lipoteichoic acids (LTAs). Exopolysaccharides can be generated by the bacterium as part of a protective biofilm. All these glycopolymer structures may be used as targets for a protective immune response.[16,17]

To date 13 different *S. aureus* CP serotypes have been identified from clinical isolates, but only four of the 13 serotypes have been structural characterized: serotype 1, 2, 5 and 8 as depicted in Table 1. Serotype 3, 4, 6, 7, 9, 10 and 11 have only been identified by immunological methods and no complete structural characterization has been reported yet.[18] The serotypes can be classified into two main groups depending on colony morphology. Serotype 1 and 2 belong to the mucoid-type species as they are heavily encapsulated forming mucoid colonies, while type 3-11 form non-mucoid colonies, generating only thin layer capsules.[19,20] Serotype 1 and 2 are rarely encountered among clinical isolates while serotype 5 and 8 are amongst the most abundant clinical isolates, comprising more than 80% of all isolates.[20–23] CP type 5 and 8 can serve to target protective antibodies and are widely investigated as vaccine candidates for protection against *S. aureus*.

Table 1: The serotypes of *S. aureus* capsular polysaccharides.

Type	Strain	Final chemical structure of the repeating unit	Established
1	D	→4)-α-D-GalNAcA-(1→4)-α-D-GalNAcA-(1→3)-α-D-FucNAc-(1→	Karakawa, 1982[24]
	M	→4)-α-D-GalNAcA-(1→4)-α-D-GalNAcA-(1→3)-α-D-FucNAc-(1→ [(a)]	Murthy, 1983[25]
2	Smith or K-93M	→4)-β-D-GlcNAcA-(1→4)-β-D-GlcNAcA-(L-alanyl)	Hanessian, 1964[26,27]
3	Mardi	Unknown	NA
4	T	D-ManNAcA-(1→3)-D-FucNAc [(b)]	Wu, 1971[28]
	7007	ManNAcA-(1→3)-FucNAc [(b)]	Karakawa, 1974 [29]
5	Reynold	→4)-β-D-ManNAcA-(1→4)-α-L-FucNAc(3-OAc)-(1→3)-α-D-FucNAc-(1→	Jones, 2005[30]
6	C	Unknown	NA
7	207	Unknown	NA
8	Becker	→3)-β-D-ManNAcA(4-OAc)-(1→4)-α-L-FucNAc-(1→3)-α-D-FucNAc-(1→	Jones, 2005[30]
9	91	Unknown	NA
10	537	Unknown	NA
11	797	Unknown	NA

[(a)] A taurine is found on every fourth α-D-GalNAcA connected through an amide bond with the carboxylic acid. [(b)] No complete structure has been published yet.

Biosynthesis of CP5 and CP8

The structures of CP5 and CP8 are very similar, and they consist of the same three monomeric sugar residues: *N*-acetyl-D-fucosamine (D-FucNAc), *N*-acetyl-L-fucosamine (L-FucNAc) and *N*-acetyl-D-mannosaminuronic acid (D-ManNAcA), but differ in glycosylic linkages and *O*-acetylation pattern as depicted in Figure 2.

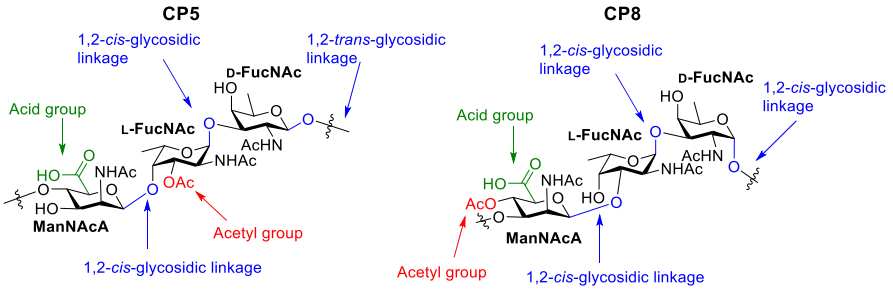


Figure 2: A schematic representation of the common repeating units of CP5 and CP8.

The biosynthetic pathways by which CP type 5 and 8 are assembled are also very similar. Several of the same genes are involved in the biosynthesis of both polysaccharides, encoding the proteins required for polymer synthesis,[31–33] *O*-acetylation,[34] transport and regulation of CP production.[35,36] The proposed pathway for the biosynthesis of the CPs is depicted in Figure 3 with CP5 on the left and CP8 on the right. The synthesis of the soluble uridine diphosphate (UDP) building blocks begins in the cytoplasm via three reaction cascades, wherein the universal precursor UDP-D-*N*-acetyl-glucosamine (UDP-D-*N*-GlcNAc) is converted into the three different nucleotide-coupled sugars, UDP-D-FucNAc, UDP-L-FucNAc and UDP-D-ManNAcA. The biosynthesis of the universal UDP-D-FucNAc (pathway I) starts with the enzymes CapD (a dehydratase) and CapN (a reductase) to transform D-GlcNAc into D-FucNAc. This is followed by transfer to the phosphosugar onto the membrane lipid carrier undecaprenol phosphate ($C_{55}P$) catalyzed by CapM, generating membrane-bound D-FucNAc (Lipid I_{cap}). From here, both CP5 and CP8 can be generated. The CP5 pathway relies on catalysis by Cap5I, whereas CP8 depends on catalysis by Cap8H.[18] UDP-L-FucNAc (pathway II for both CP5 and CP8) is formed from UDP-D-*N*-GlcNAc by the action of the trifunctional enzyme CapE (having dehydratase, 3-epimerase and 5-epimerase activity) followed by CapG (an epimerase) and CapF (a reductase).[32,37] For CP5, the acetyl transferase Cap5H catalyzes the *O*-acetylation of the UDP-L-FucNAc C-3-OH. The L-FucNAc is then transferred to the lipid carrier by the transferase CapL to give the disaccharide Lipid II_{cap}. For the final building block, UDP-D-

ManNAcA (pathway III), CapP epimerizes the C-2 of D-GlcNAc and the dehydrogenase CapO subsequently oxidizes the C-6-OH. In the CP8 biosynthesis route, Cap8J acetylates the C-4-OH of UDP-D-*N*-ManNAcA.[31,33] CapI then transfers the monosaccharide to the lipid carrier to complete the trisaccharide precursor Lipid III_{cap}.[34] The modified trisaccharides are transferred to the outer surface of the cell membrane by the flippase CapK, after which the polymerase Cap5J (for CP5) or Cap8J (for CP8) generates the longer fragments.[20,38] Finally, the attachment of the CP precursor to the *N*-acetylmuramic acid of the peptidoglycan is achieved by an yet unknown pathway.[37]

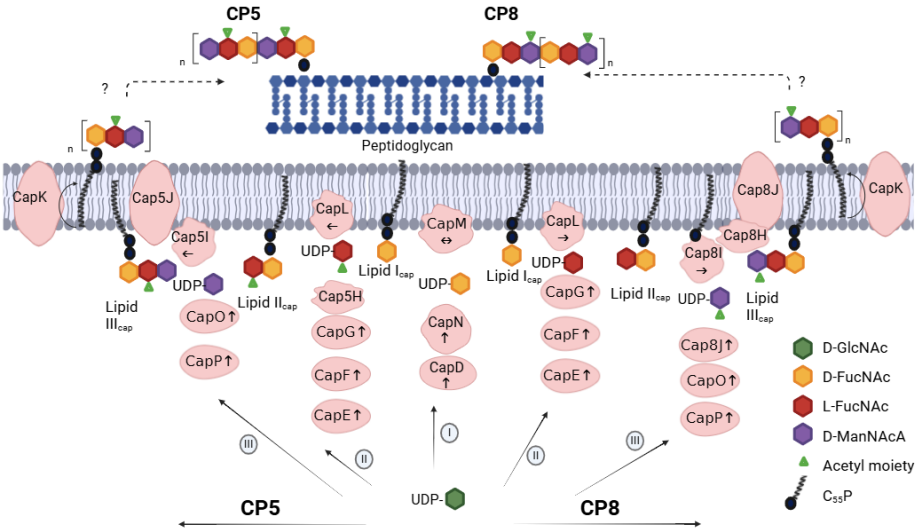


Figure 3: Model for the proposed biosynthetic pathway for CP5 (left) and CP8 (right) in *S. aureus*.

Glycoconjugate vaccines

As mentioned above, the cell wall components of bacteria can be used as targets to direct an immune response, and various bacterial CPs have been used to develop anti-bacterial vaccines.[39] Glycoconjugate vaccines have become one of the most effective and safe preventive treatments to combat bacterial infections with successful vaccines against several bacterial pathogens, including *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and *Neisseria meningitidis* serogroups A, C, W and Y.[40–42] With promising results for other pathogens, and the high demand for new treatment strategies, developing a vaccine against *S.*

aureus is of high interest.[43] When designing a vaccine using polysaccharides (PS), it is necessary to conjugate these to a carrier protein as the PS itself is not sufficiently immunogenic and cannot elicit a strong and lasting immune response. This is because polysaccharides by themselves cannot engage in T-cells activation and are therefore generally referred to as T cell independent antigens.[44] When the PSs are conjugated to a carrier protein, the immunogenic properties are characterized by the generation of high affinity IgG antibodies and the development of immunological memory.[39] The generally accepted immunological mechanism of a glycoconjugate vaccine depends on the PS and the carrier protein as illustrated in Figure 4. First, the conjugated polysaccharide is taken up by the B-cell receptor (BCR), and after internalization the glycoconjugate is processed during which the protein is degraded to form smaller peptides that can bind to the major histocompatibility complex II (MHC II) and can subsequently be presented to the T-cell. Cross talk between the B and T cells then leads to T cell activation and B cell maturation. The T cell stimulates the B cell to develop into polysaccharide specific memory B cells and plasma cells, and the development of high affinity antibodies by IgM to IgG class switching.[45,46] The carrier protein is especially import for a response in infants and toddlers, as no response against a stand-alone polysaccharide vaccine can be elicited. For adults, stand-alone polysaccharides can induce a short-term antibody response, but, because of the lack of T cell help, no memory effect or booster response can be achieved.[44]

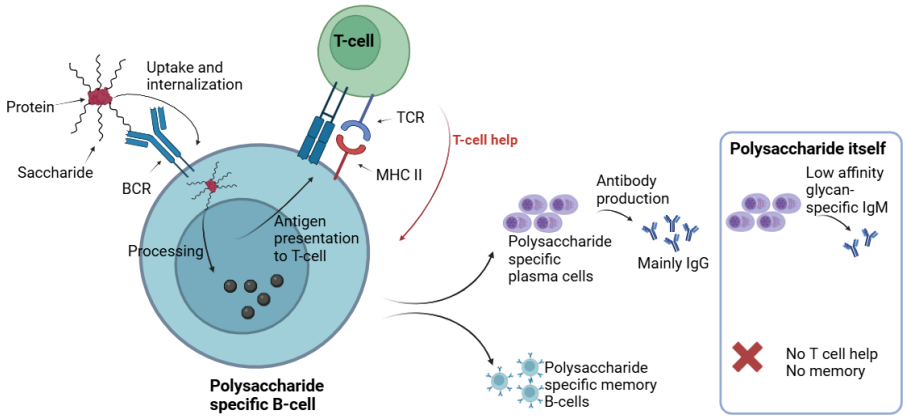


Figure 4: Schematic representation of the generally accepted immunological mechanism of a glycoconjugate vaccine.

Several glycoconjugate vaccine candidates using capsular polysaccharides and teichoic acids of *S. aureus* have been developed.[47] The polysaccharides used in these formulations are normally produced by isolation and purification from

bacterial sources. Generally, this leads to relatively heterogeneous material, varying in length and differences in substitution pattern of the polysaccharides, especially when labile substituents such as *O*-acetyl esters are present (Figure 5A).[48] When using isolated polysaccharides in combination with random conjugation chemistry, the resulting vaccine modalities are high molecular weight, crosslinked, heterogeneous and ill-defined structures.[46,49] Through the use of chemical polysaccharide sizing, often using acidic or oxidative conditions or enzymatic fragmentation and size exclusion chromatography, smaller polysaccharide fragments with a more defined degree of polymerization (DP) can be obtained.[50–52] In addition, the controlled site selective cleavage of the PS, often at the anomeric center of one of the constituting monosaccharides, can enable site selective conjugation chemistry to generate conjugates that are better defined. Even more control over glycoconjugate structure and composition can be achieved using minimal oligosaccharide (OS), generated through chemical or enzymatic synthesis (Figure 5B), which also enables standardized and reproducible vaccine production.[48] While the length can impact the effectiveness of a glycoconjugate vaccine as the oligosaccharide has to present the active epitope and allow for B cell receptor crosslinking, also the protein, the linker and type of conjugation pattern in the design of a vaccine candidate can make a difference.[48]

Various glycoconjugate vaccines comprising isolated *S. aureus* CPs and different carrier proteins have been reported. The most notable has been the StaphVAX in the 1990's, which was explored up to a phase III trial. StaphVAX, a bivalent conjugate vaccine generated using isolated *S. aureus* CP5 and CP8 polysaccharides conjugated to the nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A, was developed by Nabi Pharmaceuticals.[53] StaphVAX did show prevention of infections up to 10 month after one injection, however when explored in a phase III trial, it showed limited efficacy,[54] as the vaccine was found to fail to reduce incidence of invasive infections in hemodialysis patients.[55] StaphV, developed by GSK, combines conjugates of CP5 and CP8 polysaccharides to tetanus toxid (TT) carrier protein mixed with detoxified α-toxin (Hla H35L) and clumping factor A and was evaluated with and without adjuvant AS03B. The phase I trial showed to induce adequate antibody responses and no safety concerns.[56] Another tetravalent antigen *S. aureus* vaccine SA4G was investigated by Pfizer using CP5 and CP8 polysaccharides conjugated to cross-reacting material 197 (CRM_{197}) with two additional protein antigens. It was shown to be safe and well tolerated and to induce antibody responses in healthy older adults in a phase II clinical trial.[43,57] However SA4G failed in the phase IIb trial, conducted with recipients

that underwent spinal surgery as no reduction in the incidence of *S. aureus* bloodstream infections were detected (NCT02388165).

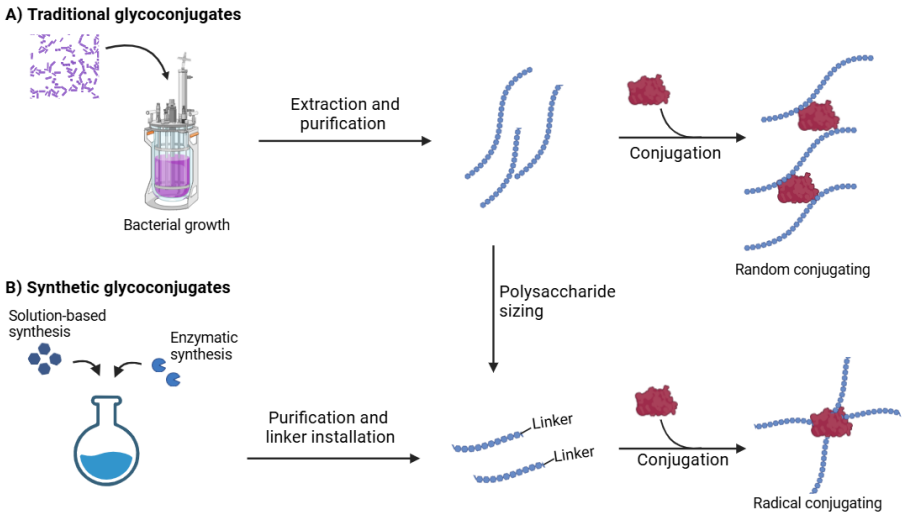


Figure 5: Different approaches to generate glycoconjugate vaccines. A) Traditional approach towards formation of glycoconjugate vaccines by bacterial fermentation, followed by extraction, purification and conjugation of the CP to a carrier protein. B) Synthetic approach towards glycoconjugates by chemical or enzymatic synthesis followed by purification, linker installation and then conjugation to a carrier protein.

The vaccine candidates tested so far have not led to any successful vaccine yet, emphasizing the challenges associated with the development, which includes lability of the linker and side-effects from impurities and non-protective epitopes that may hinder eliciting an adequate immune response against heterogeneous polysaccharides.[54,58] Another reason for the lack of a vaccine has been reasoned to be due to the lack of successful translation of vaccine protectivity, that is observed in the pre-clinical trials performed on animal subject, to human subjects.[43] The solution could include different animal models, representative *in vitro* models and *ex vivo* human tissue to study the pathogenicity. Currently different vaccine candidates that do not contain CPs are being tested in various stages of clinical trials and using either cell-wall proteins,[59,60] antigen proteins[61] or toxoids.[62] Preclinical research using CP5 and CP8 is still ongoing, implementing bioconjugation strategies and semisynthetic glycoconjugates.[63,64] Success of vaccination has been hindered by lack of established correlates of protection in humans and the complexity of the staphylococcal pathogenesis. Vaccines against different infections using synthetic material have been already developed, including the *H. influenzae* type b (Hib) vaccine using a synthetic capsular polysaccharide, which

has been licensed in Cuba[65] and a vaccine against *Shigella flexneri* 2a using a synthetic O-specific polysaccharide, which has progressed to phase II trial.[66] The synthetic oligosaccharides have thus attracted attention as antigen candidates and could therefore be considered as antigen candidates in a vaccine against *S. aureus*.

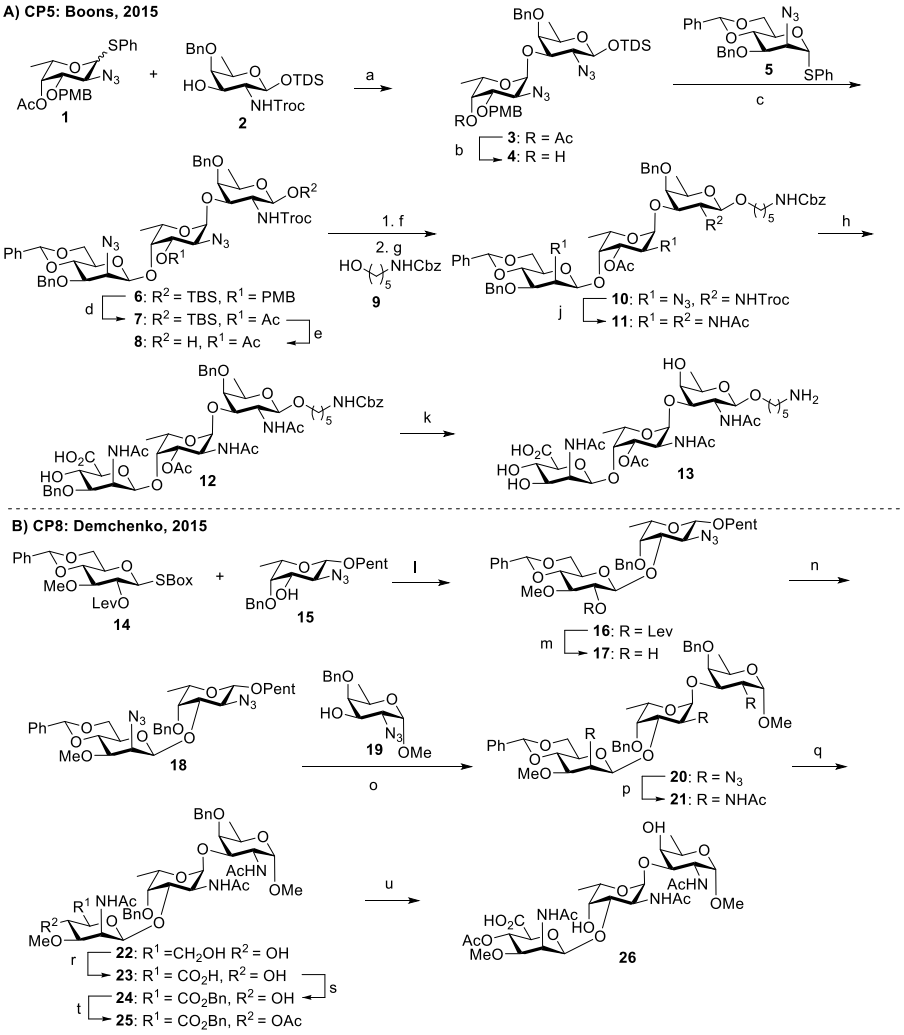
Synthetic capsular polysaccharides

Because of their biological relevance, significant efforts have been devoted to the synthesis of different fragments of the capsular polysaccharides of *S. aureus*. These CPs are complex molecules consisting of different rare monosaccharides, that are interconnected through linkages that are difficult to construct, and they carry different functional groups including a varying *N*- and *O*-acetylation pattern, which makes their synthesis a challenging task.[67] Out of the 13 different serotypes only four of them (serotype 1, 2, 5 and 8, see Table 1) have been structurally characterized and only the synthesis of serotype 1, 5 and 8 has been reported to date. Only small fragments (trisaccharide units) or protected larger fragments of CP1,[68,69] CP5[71–75] and CP8[64,70,76,77] have been published, indicating the difficulty of the synthesis.

In 2015, the group of Boons described the synthesis of a conjugation ready CP5 trisaccharide, shown in Scheme 1A.[72] Their synthesis relied on the use of the monosaccharide building blocks **1**, **2** and **5** as precursors for the L-fucosamine, D-fucosamine and D-mannosaminuronic acid residues, respectively and the trisaccharide was built from the reducing end towards the non-reducing end. Glycosylation between donor **1** and acceptor **2** in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave disaccharide **3** in a yield of 73% and with an α/β ratio of 4:1. The promoter was found to influence the yield and stereoselectivity of the reaction as the promoter systems 1-benzenesulfinyl piperidine (BSP) or diphenylsulfoxide (DPS) with trifluoromethanesulfonic anhydride (Tf_2O) and 2,4,6-tri-*tert*-butylpyrimidine (TTBP) gave the α-product in only 30 % yield. Removal of the *O*-acetyl ester gave acceptor **8**. The protecting group on the C-3-OH of the L-FucN_3 building block was found to be important in the glycosylation reaction towards the trisaccharide, as an electron-withdrawing acetyl group at this position lowers the reactivity of the axial fucosyl C-4-OH.[78,79] With the *p*-methoxybenzyl (PMB)-acceptor **8**, the glycosylation to give trisaccharide **6** proceeded in 73% yield and with excellent β-selectivity, as a result of the stereoselective nucleophilic displacement of the *in situ* formed mannosazide α-triflate. Next, the PMB ether was exchanged for an *O*-acetyl ester,

which was followed by installation of a 5-(benzyloxycarbonyl)aminopentanol linker (**9**) using the generated imidate donor. Trisaccharide **10** was formed as the sole β-anomer arising from effective neighboring group participation by the trichloroethyl carbamate (Troc) on the C-2-nitrogen. Next, the azides and the Troc carbamate were transformed into the corresponding acetamides, after which the benzylidene on C-4 and C-6 of the mannose residue was hydrolyzed to set the stage for the regioselective oxidation of the C-6-OH which gave **12** in 61% yield.[80] This protecting/functional group manipulation scheme was implemented to avoid possible intramolecular lactam formation of the mannosaminuronic acid residue. Lastly, hydrogenation afforded trisaccharide **13**. While this strategy could allow both for the generation of longer oligomers, and the conjugation to a carrier protein, no investigation towards these goals has been published.

Demchenko and co-workers synthesized the CP8 trisaccharide **26**, shown in Scheme 1B, having methyl groups at both the reducing and non-reducing end, in 2015.[76] They built the trisaccharide from the non-reducing end to the reducing end relying on building blocks **14**, **15** and **19**. The chemoselective glycosylation between donor **14** and acceptor **15** in the presence of AgOTf in 1,2-dichloroethane (1,2-DCE) proceeded in 73% yield and delivered the β-glucosyl product due to neighboring group participation. They chose to use a glucosyl precursor to generate the β-mannosamine residue, as direct mannosylation failed. Next, the C-2 position of the glucosyl residue was epimerized by first removal of the levulinoyl (Lev) group in 72% yield followed by installation of the triflate and azide displacement[81] to give disaccharide **16** in 90% over two steps. The so-formed **16** was used directly as a donor in the glycosylation with acceptor **19** in the presence of NIS and triflic acid (TfOH) in 1,2-DCE to give trisaccharide **20** in 87% yield and with excellent α-selectivity. Now the intermediate trisaccharide **16** was deprotected by reduction of the azide with propane-1,3-dithiol followed by acetylation to give the acetamide in 94% over two steps. The benzylidene was hydrolyzed and the liberated C-6-OH was subsequently selectively oxidized and esterified. The free C-4-OH was acetylated and finally the benzyl protecting groups were removed by hydrogenation yielding trisaccharide **26**. Because both capping ends are equipped with methyl groups the trisaccharide cannot be elongated or conjugated.



Scheme 1: Literature synthetic approaches towards a trisaccharide repeating unit of CP5 from Boons (A) and a CP8 trisaccharide from Demchenko (B). *Reaction conditions*: A) a) NIS, TMSOTf, DCM, Et_2O 4:1, -60 ºC, 72%, α/β = 4:1, b) Na (cat), MeOH, guanidine·HCl, rt, quant, c) DPS, Tf_2O, DCM, -60 ºC to -30 ºC, 72%, d) i) DDQ, DCM/H_2O 9:1, rt, ii) Ac_2O, pyridine, DCMAP, rt, 85% over two steps, e) HF/pyridine, THF, rt, 90%, f) $CF_3(NPh)CCl$, Cs_2CO_3, DCM, g) TMSOTf, DCM/MeCN 1:1, -78 ºC, 72% over two steps, j) zinc, THF:AcOH:Ac_2O, 63%, h) i) 80% aq. AcOH, 90%, ii) TEMPO, BAIB, DCM/H_2O then $NaClO_2$, 2-methyl-2-butene, *t*-BuOH, 61% over two steps, k) $Pd(OH)_2$, MeOH, AcOH, quant. B) l) AgOTf, 1,2-DCE, rt, 73%, m) hydrazine acetate, DCM/MeOH 20:1, 0 ºC, 72%, n) i)Tf_2O, pyridine, DCM, 0 ºC, ii) NaN_3, DMF 60 ºC, 90%, o) NIS, TfOH, 1,2-DCE, 0 ºC, 87%, p) i) $HS(CH_2)_3SH$, Et_3N, pyridine, H_2, rt, ii) Ac_2O, MeOH, rt, 94%, q) TFA aq., DCM, 92%, r) TEMPO, BAIB, aq. DCM, rt, s) BnBr, $NaHCO_3$, DMF, rt, 61% over two steps, t) Ac_2O, pyridine, rt, 99%, u) H_2, Pd/C, aq. EtOH, rt, 97%.

Both the shown synthetic procedures,[72,76] and most of the published synthetic strategies to date to generate CP5 and CP8 oligosaccharides,[64,73,75,77,82] either use a glucosyl synthon or a mannosamine building block to construct the *N*-acetyl β-mannosaminuronic acid, implementing a late-stage modification on larger saccharides. While these procedures can be efficient on relatively small saccharides, implementing them on oligomers comprising several repeating units can be more challenging, as this requires multiple transformations to be executed on the same molecule. Especially the late-stage oxidation of multiple alcohol into the corresponding carboxylates, requiring two oxidation steps for each transformation, has been found difficult.[77,83,84] In 2023, Kulkarni and co-workers implemented a pre-glycosylation oxidation strategy using a $ManN_3A$ donor for the synthesis of a CP8-trisaccharide. However, because of the low reactivity of the donor, a large excess was needed for the glycosylation reaction. With this procedure late-stage modifications were minimized and the deprotection strategy only involved three steps.[70]

Neither of the described molecules above have been used for biological evaluation. Adamo and co-workers reported a synthetic protocol towards a CP5 trisaccharide, bearing a linker for conjugation purposes in 2012.[71] However, during the deprotection steps the amino group in the linker, intended for conjugation, was transformed into an acetamide, rendering the linker unsuitable for functionalization. Therefore, the trisaccharide was only evaluated in competitive ELISA and dot blot studies using murine anti-CP5 serum, generated against a CP5-polysaccharide conjugate. Recognition of the synthetic structure could be shown, but the interaction was significantly weaker than the natural polysaccharide indicating that larger structures are likely needed as potential vaccine candidates. Hu *et al*. reached the same conclusion in their report on a synthetic CP8 trisaccharide protein conjugate.[64] Mice immunization with their CP8-trisaccharide conjugate did lead to the production of anti-CP8 antibodies as revealed by glycan micro array, but longer structures were deemed necessary to generate a more potent vaccine candidate.

Outline of the thesis

In this Thesis synthetic chemistry has been developed to generate well-defined fragments of three different *S. aureus* serotype capsular polysaccharides. All of the synthetic saccharides are equipped with an aminoalkyl linker to conjugate them to a carrier protein to generate synthetic vaccine modalities. **Chapter 2** describes the synthesis of fragments of the capsular polysaccharide type 8, with the oligosaccharides ranging in length from a trisaccharide to a dodecasaccharide. The synthetic approach developed relies on a [3+3n] strategy and a pre-glycosylation oxidation and *O*-acetylation strategy has been implemented to simplify the deprotection at the end of the synthesis. The four generated saccharides are conjugated to a carrier protein, and their binding to monoclonal and polyclonal antibodies is described using Western Blot and ELISA. Conformational investigations and NMR interaction studies have revealed the epitopes recognized by the antibodies. Immunization studies are reported showing the longer synthetic fragments to serve as potent analogues of the natural polysaccharide. In **Chapter 3** the synthesis of fragments of capsular polysaccharide type 5 are described. The assembly of tri- hexa- and nonasaccharides is presented and again a pre-glycosylation oxidation and [3+3n] glycosylation strategy is implemented to obtain the saccharides. The synthetic saccharides were conjugated to a carrier protein and tested for binding with monoclonal and polyclonal antibodies using Western Blot and Surface plasmon Resonance (SPR). Conformational studies have revealed the three-dimensional structure of the oligosaccharides. In **Chapter 4** the synthesis of four frameshifted CP5 and CP8 trisaccharides is presented, complementing the trisaccharides generated in Chapter 2 and Chapter 3. Again, the saccharides are investigated for interaction with monoclonal antibodies raised against native CP5 and CP8, and quite surprisingly one of the CP5 trisaccharides was revealed to be a potent binder for anti-CP5 antibodies. **Chapter 5** describes the synthesis of oligosaccharides derived from CP type 1. Four different trisaccharides with a different taurine substitution pattern is presented together with a non-taurinated hexasaccharide. In contrast to the work of CP5 and CP8, now post-glycosylation modifications are implemented to be able to tune the taurination pattern. In **Chapter 6** the work in Chapter 2-5 is summarized and an outline for potential future directions is presented.

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Chapter 2

Long, synthetic *Staphylococcus aureus* type 8 capsular oligosaccharides reveal structural epitopes for effective immune recognition

Introduction

Staphylococcus aureus (*S. aureus*), a Gram-positive bacterium that is part of the human microbiome, is one of the most common opportunistic pathogens. It is found in human mucous membranes and skin, and when these barriers are breached can cause various diseases, ranging from minor skin abscesses to deadly bloodstream infections (bacteremia), heart valve infections (endocarditis), bone infections (osteomyelitis), lung infections (pneumonia), meningitis and septic shock.[1,2] It especially poses a threat to newborns and immunocompromised patients, such as elderly, post-surgical and dialysis patients. *S. aureus* is one of the ESKAPE bacteria and a WHO high priority pathogen with the rise of antibiotic-resistant strains,[3] like methicillin-resistant *S. aureus* (MRSA)[4] and vancomycin-resistant *S. aureus* (VRSA).[5] This urges the development of new therapeutic strategies, such as active or passive vaccination strategies.[6,7] The complex cell wall of *S. aureus* features several characteristic glycopolymers,[8–10] including capsular polysaccharides (CPs), wall teichoic acids (WTA) and lipoteichoic acids (LTA) that may be used as targets for agents eliciting a protective immune response.[11,12] Various bacterial CPs have been used to develop anti-bacterial vaccines, and glycoconjugate vaccines have become one of the most effective and safe preventive treatments to combat bacterial infections. To date 13 different *S. aureus* CP serotypes have been identified from clinical isolates with CP type 5 (CP5) and type 8 (CP8) being the most abundant, comprising more than 80% of the clinical isolates.[13–16] Conjugate vaccines, generated using isolated CP5 and CP8 *S. aureus* polysaccharides, have been explored up to phase III trials, where they unfortunately and surprisingly showed limited efficacy.[17–20] Suboptimal epitope presentation may hinder eliciting a sufficient immune response against conjugated heterogeneous polysaccharides, and therefore synthetic oligosaccharides have attracted attention.[17,21]

The structure of *S. aureus* CP5 and CP8 share the same three constituting rare monosaccharides: *N*-acetyl D-mannosaminuronic acid (ManNAcA), *N*-acetyl L-fucosamine (L-FucNAc) and *N*-acetyl D-fucosamine (D-FucNAc), as depicted in Figure 1.[22–24] They differ in glycosidic linkages and *O*-acetylation pattern. CP8 was first

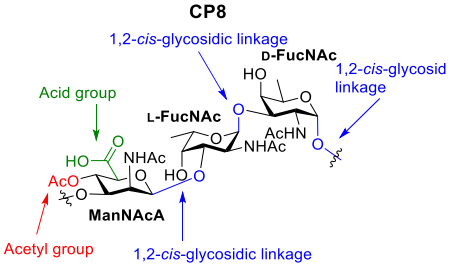


Figure 1: A schematic representation of the repeating unit of CP8.

isolated in 1984 by Founier[25] and originally thought to consist of *N*-acetyl fucosamine and *N*-acetyl galactosaminuronic acid. This was revised in 1988 when the chemical structure was found to be similar to CP5[22] and in 2005 Jones established this structure to have the repeating unit (RU) →3-β-D-ManNAcA(4-*O*Ac)-(1→3)-α-L-FucNAc-(1→3)-α-D-FucNAc-(1→.[24] CP8 is *O*-acetylated at the C-4 position of the ManNAcA residue and this acetylation has been found to be important for the induction of protective anti-CP antibody responses upon vaccination.[26]

Due to their biological importance, several attempts to synthesize CP8 fragments have been reported over the past years as summarized in Figure 2. The synthesis of CP8 oligosaccharides is challenging because of the 1,2-*cis* glycosylic linkages, the rare monosaccharides and many types of functional groups (carboxylates, acetamides and *O*-acetyl esters). The first synthesis of a CP8 oligosaccharide was reported by Demchenko and co-workers in 2015,[27] who prepared a trisaccharide with methyl groups on both capping ends, which made conjugation and elongation impossible. They synthesized the trisaccharide starting from the non-reducing end and their approach involved a post-glycosylation-oxidation of the mannose residue at the trisaccharide level, a post-glycosylation inversion of C-2 to generate the mannosamine stereochemistry on a disaccharide and installation of the *O*-acetyl on the trisaccharide. The glucose donor building block, used as precursor for the ManNAcA, carried an orthogonal C-2-levulinoyl participating group to guarantee the formation of the desired β-linkage. Later, Demchenko and co-workers presented the synthesis of a protected hexasaccharide, that was assembled in a [2+4] glycosylation strategy, because attempts at a [3+3] strategy failed.[28] The final protecting group manipulations however proved ineffective and the target deprotected hexasaccharide could not be obtained, highlighting the difficulty in synthesizing these complex bacterial glycans. In 2020 Hu and co-workers reported a similar route towards the trisaccharide repeating unit, using similar post-glycosylation modulations to create the β-mannosamine linkage, but they chose to perform the oxidation, inversion at C-2 and *O*-acetylation on the trisaccharide stage. They installed a linker on the reducing end of the trisaccharide and showed conjugation to a carrier protein was possible.[29] In 2023 Kulkarni and co-workers reported the first synthesis of CP8 trisaccharide RU that relied on the use of a mannosaminuronic acid building block. They built the CP8 trisaccharide from the reducing to the non-reducing end and installed orthogonal protecting groups on the capping ends of the trisaccharide to allow for elongation in either direction in the future.[28]

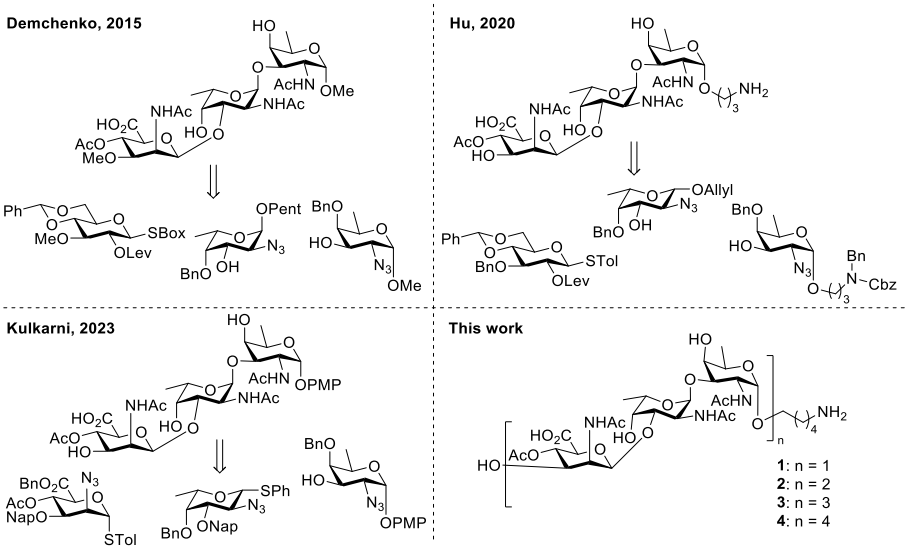


Figure 2: Previously synthesized trisaccharides and the CP8-fragments described in this thesis.

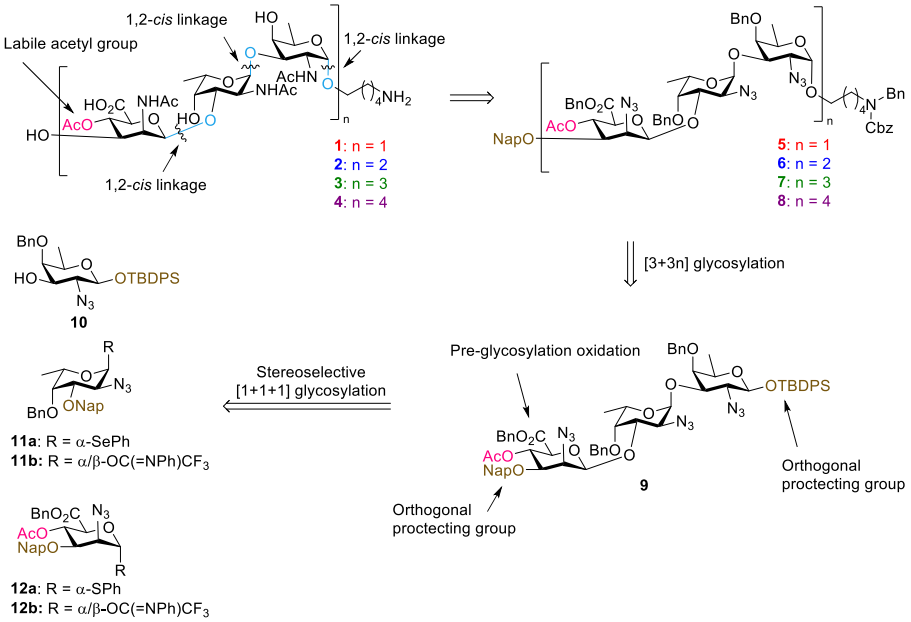
This Chapter describes the synthesis of CP8 structures carrying an *O*-acetyl on C-4 of the ManNAcA residue and their use in structural and epitope mapping studies. Specifically, this Chapter report on a set of CP8 oligosaccharides ranging in length from a trisaccharide (1 RU) to a dodecasaccharide (4 RUs). The developed synthetic strategy is designed such, that manipulations on large oligosaccharides are minimized. For this purpose, pre-glycosylation oxidation strategy has been employed in which the mannosaminuronic acid carboxylic acid and C-4-*O*-acetyl ester were installed on the monosaccharide level. The fragments are equipped with an orthogonal amine linker which has enabled the conjugation to a carrier protein, to generate glycoconjugate vaccine modalities for immunological studies. Structural studies on the well-defined oligomers provided insight into the 3D-structure of the CP8 fragments and revealed that ManNAcA C-4-*O*-acetyl groups play a crucial role in constraining the conformational freedom of the longer fragments, leading to extended structures in which the *O*-acetyl esters and acetamide groups form hydrophobic patches along the axis of the oligosaccharides. Epitope mapping studies have revealed monoclonal antibodies to bind to an extended epitope and the longer fragments not only bound better to antibodies raised against native CP8, but also raised higher IgG titers following immunization of mice with the glycoconjugates.

Results and discussion

Synthesis of the CP8-fragments

The retrosynthetic analysis of the CP8 fragments is shown in Scheme 1 and comprises the assembly of the oligosaccharides, each equipped with an amino linker for site-selective conjugation purposes, using the central trisaccharide building block **9**. This key synthon incorporates the mannuronic acid's carboxylic acid functionality and the C-4-*O*-acetyl ester, which precludes challenging modifications later in the synthesis scheme. Especially the execution of multiple oxidations on a complex, partially protected glycan can be arduous.[28,30,31] The protecting group strategy was designed such that only two steps are required post-glycosylation to unmask all functional groups: transformation of the azides – required as non-participating groups in the *cis*-glycosylation reactions – into the corresponding acetamides, and hydrogenation of all benzyl-type groups. The trisaccharide building block **9** further carries a *tert*-butyldiphenylsilyl (TBDPS) on the anomeric position and a 2-methylnaphthyl (Nap) ether on the mannosaminuronic acid C-3-OH to allow for orthogonal deprotection and selective elongation at either the reducing or nonreducing end. The larger glycans are build exploiting the reliable stereoselectivity of the 2-azidofucose donor, as it has previously been established that 2-azidofucose donors, carrying ether type protecting groups, in combination with weakly nucleophilic alcohol acceptors, such as the 2-azidomannuronic acid C-3-OH, can provide the desired 1,2-*cis*-2-azidofucose linkages with excellent stereoselectivity.[32] Trisaccharide **9** was aimed to be prepared from three monosaccharide building blocks: the D-2-azidofucose (D-FucN_3) building block **10**, L-FucN_3 building block **11** and D-2-azidomannuronic acid (ManN_3A) building block **12**, in a [1+1+1] glycosylation approach. Mannuronic acid building blocks are amongst the most effective donors to install the challenging 1,2-*cis*-mannose type glycosidic linkages and the use of the ManN_3A building block thus not only obviates the need for late-stage oxidation reactions but should also streamline the stereoselective assembly of the central trisaccharide **9**.

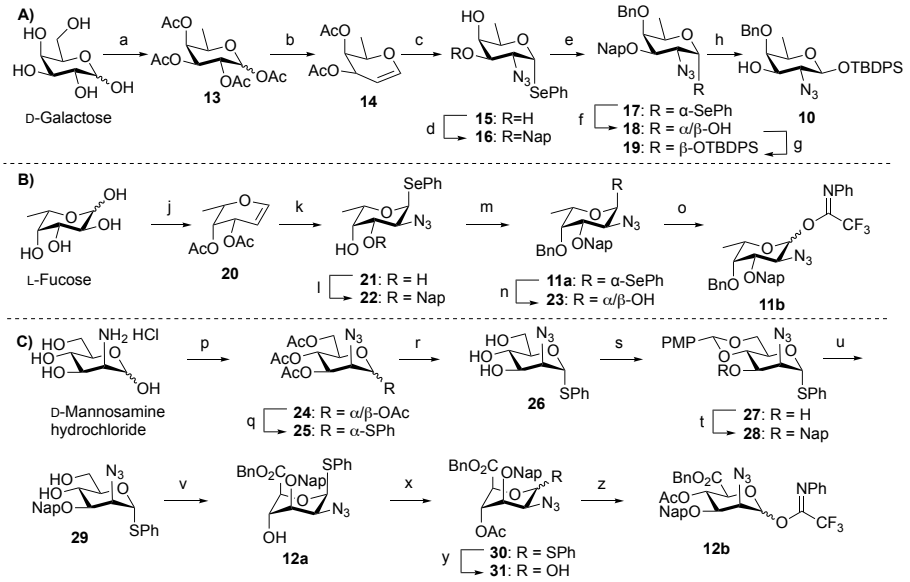
All the building blocks were synthesized from commercially available starting materials. The synthesis of the D-FucN_3 building block (Scheme 2A) commenced with D-galactose following a reported procedure.[33] In a 5-step reaction sequence in which the required galactose-to-fucose deoxygenation was achieved by iodination of the C-6 position and radical reduction of the primary iodide, the acetylated D-fucose **13** was obtained in 54% yield from D-galactose on large scale.



Scheme 1: Retrosynthetic analysis of the set of target CP8 oligosaccharides.

Next, anomeric bromination followed by elimination using zinc and NH_4Cl gave fucal **14** in 48% yield. A regio- and stereoselective azidophenylselenation using the more soluble azidotrimethylsilane ($TMSN_3$) instead of NaN_3 together with (diacetoxyiodo)benzene (BAIB) and diphenyldiselenide ($(SePh)_2$) by a procedure develop by Nifantiev and co-workers[34] followed by saponification afforded **15** in 67% yield. Now, the C-3-OH was selectively naphthylated via the intermediate tin-acetal,[35] allowing for benzylation of the free C-4-OH giving **17**. The anomeric phenylselenyl group was hydrolyzed using *N*-iodosuccinimide (NIS) in acetone/water and the lactol, was then silylated using *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) providing **19** in 96% yield. Lastly, the Nap ether was oxidative cleaved with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in 90% yield to give acceptor **10**.

The same approach was implemented for the L-FucN_3 building block (Scheme 2B), however now starting from commercially available L-fucose. Peracetylation followed by bromination and elimination gave fucal **20** in 47% yield. Azidoselenation followed by saponification gave **21** in 64%. Selective naphthylation of the C-3-OH followed by benzylation of C-4-OH gave **11a** in 86% yield. Hydrolysis of the anomeric phenylselenyl and installation of the *N*-phenyl trifluoroacetimidate[36] functionality delivered donor **11b** in excellent yield.



Scheme 2: Synthesis of the building blocks **10** (A), **11** (B) and **12** (C). *Reaction conditions:* A) a) i) conc. H_2SO_4, acetone, ii) PPh_3, I_2, imidazole, toluene/MeCN, 90 °C, iii) VA-044, aq. H_3PO_2, Et_3N, i-PrOH, 60 °C, iv) 80% aq. AcOH, 90 °C, v)Ac_2O, pyridine, 0 °C to rt, 5 steps 54%, b) i) HBr in AcOH 33%, DMC, 0 °C to rt, ii) zinc, NH_4Cl, EtOAc, 60 °C, 48% over two steps, c) i) $(PhSe)_2$, BAIB, $TMSN_3$, DCM, -20 to -30 °C, ii) NaOMe, MeOH, 67% over two steps, d) Bu_2SnO, toluene, 140 °C then Bu_4NBr, CsF, NapBr, 120 °C, 93%, e) BnBr, NaH, DMF, 0 °C to rt, 88%, f) NIS, acetone/H_2O, 0 °C, 99%, g) TBDPS-Cl, imidazole, DMAP, DCM, 0 °C to rt, 96%, h) DDQ, DCM/H_2O, 90%, B) j) i) Ac_2O, pyridine, 0 °C to rt, ii) HBr in AcOH 33%, DMC, 0 °C, iii) zinc, NH_4Cl, EtOAc, 60 °C, 3 steps 47%, k) i) $(PhSe)_2$, BAIB, $TMSN_3$, DCM, -20 to -30 °C, ii) NaOMe, MeOH, 64% over two steps, l) Bu_2SnO, toluene, 140 °C then Bu_4NBr, CsF, NapBr, 120 °C, 89%, m) BnBr, NaH, DMF, 0 °C to rt, 86%, n) NIS, acetone/H_2O, 0 °C, 93%, *o*) $ClC(=NPh)CF_3$, K_2CO_3, acetone, 95%, C) p) i) Tf_2O, NaN_3, $CuSO_4{\cdot}5\ H_2O$, pyridine, 0 °C ii) Ac_2O, 0 °C to rt, 98% over two steps, q) PhSH, $BF_3{\cdot}Et_2O$, DCM, 0 °C to rt, 88%, r) NaOMe, MeOH, 90%, s) *p*-MeO-PhCH$(OMe)_2$, CSA, MeCN, 300 mbar, 50 °C, 88%, t) NapBr, NaH, DMF, 0 °C to rt, 93%, u) CSA, MeOH, 87%, v) i) TEMPO, BAIB, AcOH, DCM/*t*-BuOH/H_2O, 4 °C, ii) BnBr, K_2CO_3, DMF, 74% over two steps, x) Ac_2O, DMAP, pyridine, 0 °C, 90%, y) NIS, TFA, DCM, 0 °C then Et_3N, 80%, *z*) $ClC(=NPh)CF_3$, K_2CO_3, acetone, 95%.

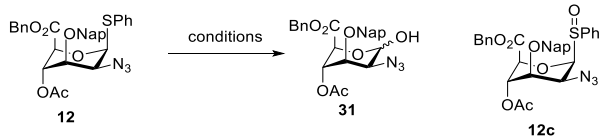
The D-ManAN_3 was obtained from D-mannosamine hydrochloride (Scheme 2C) by an azidotransfer with freshly prepared triflic azide (TfN_3) followed by an one-pot acetylation[37] giving **24** in 98% yield. Pyridine was chosen as solvent, thus enabling an in-situ acetylation to avoid formation of the glucose epimer side-product, which have been reported previously.[32] Next, synthesis to compound **25** followed a literature procedure,[38] by first installation of a thiophenyl group to provide **25** in 88% yield. Saponification of the remaining three acetyl esters was

followed by the installation of a *p*-methoxybenzylidene to mask the C-4 and C-6-hydroxyl groups. Protection of the remaining C-3-OH as the Nap ether delivered **28**. The *p*-methoxybenzylidene was removed with camphorsulfonic acid (CSA) to enable the regio- and chemoselective oxidation of the primary alcohol using 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and BAIB.[39,40] Alkylation of the newly formed carboxylic acid as the corresponding benzyl ester delivered **30** in 74% overall yield. The remaining C-4-OH was acetylated in 90% yield giving **12a**. NMR analysis of this building block revealed a ring flip from a $^4C_1$ to a $^1C_4$ conformation.[41,42]

The removal of thiophenyl from **12a** proved to be much more difficult than first anticipated and different attempts were investigated to optimize this transformation. First, hydrolysis using *N*-bromosuccinimide (NBS) in acetone/water resulted in low yields due to oxidation of the thiophenyl group to give **12c** (Table 1, Entry 1-2). A procedure using NIS and trifluoroacetic acid (TFA) in dichloromethane (DMC) and water did not lead to any reaction (Entry 3), even when an excess of the reagents was used (Entry 4). By performing this reaction under anhydrous conditions, the desired product was obtained after quenching with sat. aq. $Na_2S_2O_3$ (Entry 5), but an unknown impurity was formed which could not be removed during purification. Unfortunately, the nature of the impurity, revealed in the NMR spectrum, could not be identified. Neither prolonging the reaction time (Entry 6) or quenching with piperidine before adding sat. aq. $Na_2S_2O_3$ (Entry 7) improved the outcome. Using NBS and trimethylsilyl trifluoromethanesulfonate (TMSOTf) in DCM/water (Entry 8) also provided an impure product. Finally, it was found that using 1.5 equiv. NIS and 1 equiv. TFA in DCM (Entry 9) under anhydrous conditions and quenching with Et_3N before adding sat. aq. $Na_2S_2O_3$ yielded the desired product in 80% yield, however several column chromatography purifications were needed. After obtaining the hemiacetal **31** the *N*-phenyl trifluoroacetimidate donor was installed yielding **12b** in 95% yield and the ring was found to flip back to a $^4C_1$ conformation as judged by NMR.

The construction of the central trisaccharide **9** was started with the synthesis of the L-FucN_3-D-FucN_3 disaccharide **32**. First, the L-FucN_3 selenophenyl donor **11a** was investigated using NIS and TMSOTf as promoter in DCM. Surprisingly, a moderately β-selective glycosylation was found (Table 2, Entry 1). While lowering the temperature gave even more of the β-product (Entry 2), increasing the temperature led to formation of more of the desired α-product (Entry 3). Next, the use of imidate donor **11b** was explored with TMSOTf as promoter in DCM at rt.

Table 1: Optimization of the thiophenyl removal of compound **12**.



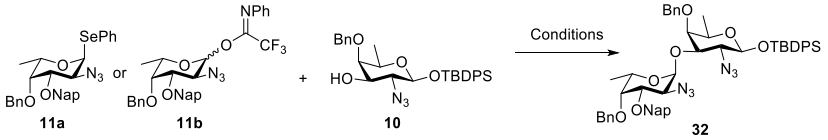
Entry	Conditions	Temp (°C)	Time (h)	Yield (%)	Notes
1	NBS (2 equiv.), acetone/H_2O 10:1	rt	1	48	33 % of **12c**
2	NBS (2 equiv.), acetone/H_2O 10:1	0 to rt	0.75	43	42 % of **12c**
3	NIS (1.1 equiv.), TFA (1.1 equiv.), DCM/H_2O 10:1	0	--	--	No reaction
4	NIS (2.5 equiv.), TFA (1.1 equiv.), DCM/H_2O 10:1	0	--	--	No reaction
5	NIS (2 equiv.), TFA (2 equiv.), DCM	0	2	77 [(a)]	Unknown impurity
6	NIS (2 equiv.), TFA (2 equiv.), DCM	0	3	90 [(a)]	Unknown impurity
7	NIS (1.1 equiv.), TFA (1.1 equiv.), DCM, then piperidine	0	4	59 [(a)]	Unknown impurity
8	NBS (1.5 equiv.), TMSOTf (1 equiv.), DCM/H_2O 20:1	0	0.75	87 [(a)]	Unknown impurity
9	NIS (1.5 equiv.), TFA (1.1 equiv.), DCM, then Et_3N	0	1	80	

[(a)] Impure product.

Using these conditions, a highly α-selective glycosylation was achieved, but the disaccharide **32** was formed in low yield (Entry 4). Increasing the reaction time (Entries 5 and 6) improved the yield only moderately. Then, switching the promoter from TMSOTf to *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (Entry 7) led to an improved yield of 98%, while the excellent α-

selectivity was maintained.[43] The markedly different outcome of these latter glycosylations may be explained by the fact that TMSOTf can lead to silylation of the acceptor alcohol, hampering the glycosylation. The α-linkage was confirmed by ^{1}H-NMR, with the anomeric proton of the newly formed acetal appearing as a doublet at 5.24 ppm with a coupling constant $J_{H1\text{-}H2}$ of 2.4 Hz.

Table 2: Optimization of disaccharide **32** glycosylation.



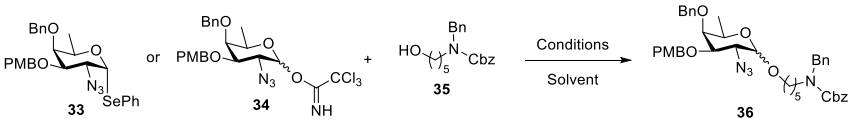
Entry	Donor	Conditions	Temp (°C)	Time (h)	Yield (%)	α/β [(a)]
1	**11a**	NIS, TMSOTf	-78 to -50	1	71	35:65
2	**11a**	NIS, TMSOTf	-80 to -60	2	51	20:80
3	**11a**	NIS, TMSOTf	rt	0.5	62	52:48
4	**11b**	TMSOTf	rt	0.5	11	99:1[(b)]
5	**11b**	TMSOTf	rt	1.5	25	99:1[(b)]
6	**11b**	TMSOTf	rt	2	36	99:1[(b)]
7	**11b**	TBSOTf	rt	0.5	98	95:5

General conditions: 3 Å molecular sieves, 0.1 M DCM, 0.2 equiv. promoter, 1.5 equiv. NIS, 1.2 equiv. of **11a** or 1.3 equiv. of **11b**. [(a)] The α/β ratio was determined by NMR of the purified products. [(b)] No β-product was isolated.

Next the Nap ether in disaccharide **32** was oxidatively cleaved by DDQ in DCM/water yielding the disaccharide acceptor **40** in 86% yield as shown in Scheme 3, setting the stage for the [1+2] glycosylation to form the central trisaccharide **9**. Using the ManN_3A thioglycoside **12a** in combination with NIS and triflic acid (TfOH) as promoter delivered the target compound **9** in relatively poor yield but decent selectivity (44%, α/β = 25:75). Switching to the corresponding imidate donor **12b** the yield was improved to 66% and the α/β ratio increased to 14:86, as shown in Scheme 3. The β-linked trisaccharide **9** could easily be purified by column chromatography and the β-linkage was confirmed by ^{1}H-NMR and ^{13}C-NMR with the β-ManN_3A anomeric proton and carbon having a CH-coupling constant of $J_{C1\text{-}H1}$ = 158.8 Hz. The TBDPS group on the anomeric position of the D-FucN_3 in **9** was removed using *tetra*-butylammonium fluoride (TBAF) buffered by acetic acid (AcOH) to give hemiacetal **41** in 84% yield and this was followed by installation of the *N*-phenyl trifluoroacetimidate to give key trisaccharide **37** in

93% yield. The stereoselective installation of the linker proved challenging because of the relatively high reactivity of the primary alcohol of the alkane linker **35**.[44] First, installation of the linker was investigated using monosaccharide donor **33**. It was found that use of the phenylselenyl donor in combination with NIS and TMSOTf mainly gave the β-product (Table 3, Entry 1-3). Gratifyingly, activation of the corresponding imidate donor **34** using trimethylsilyl iodide (TMSI) and triphenylphosphine oxide (Ph_3PO) did lead to the desired α-linked product (entry 5).[45] These conditions were transferred to the trisaccharide imidate donor **37** to give **5** in 93% yield and a α/β ratio of 75:25.

Table 3: Investigation of the linker installation on the monosaccharide level.



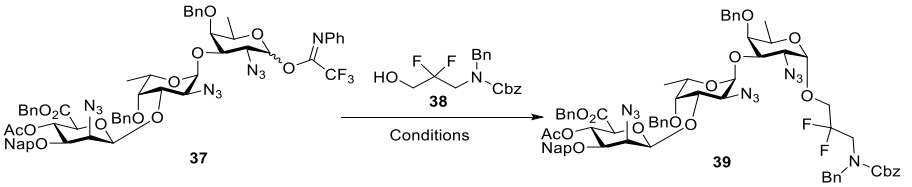
Entry	Donor	Conditions	Solvent	Temp (°C)	Time (h)	Yield (%)	α/β [(a)]
1	**33**	TMSOTf, NIS	DCM/ Et_2O 1:1	-40 to -20	1.5	78	10:90
2	**33**	TMSOTf, NIS	DCM/ Et_2O 1:1	-40 to rt	19	64	6:94
3	**33**	TMSOTf, NIS	DCM	rt	2	64	22:68
4	**34**	Ph_3PO, TMSI	DCM	rt	23	96	55:45
5	**34**	Ph_3PO, TMSI	DCM/ Et_2O 1:1	rt	20	98	81:19

General conditions: 3 Å molecular sieves, 0.1 M solvent, either 0.2 equiv. promoter and 1.5 equiv. NIS or 1 eq TMSI and 6 equiv. PhP_3O. [(a)] The α/β ratio was determined by NMR of the purified products.

In another attempt to obtain better α-selectivity the reactivity of the linker alcohol was modified by use of difluorinated alcohol **38** as seen in Table 4. Placing two fluorine atoms close to the hydroxy group of the linker precursor lowers the nucleophilicity of the alcohol group, further improving the stereoselectivity. The linker was synthesized following a procedure of Seeberger and co-workers.[46] Using **38** as a nucleophile, high α-selectivity was found (Table 4, Entry 1), especially with the TMSI/Ph_3PO system (Entry 2-3), although the yields of these glycosylations diminished. Overall, the use of the non-fluorinated linker appeared to be

more effective and therefore the synthesis was continued with non-fluorinated linker **35**, also because it was cheaper and easier to prepare.

Table 4: Investigation of the fluorinated linker **38** installation on **37**.



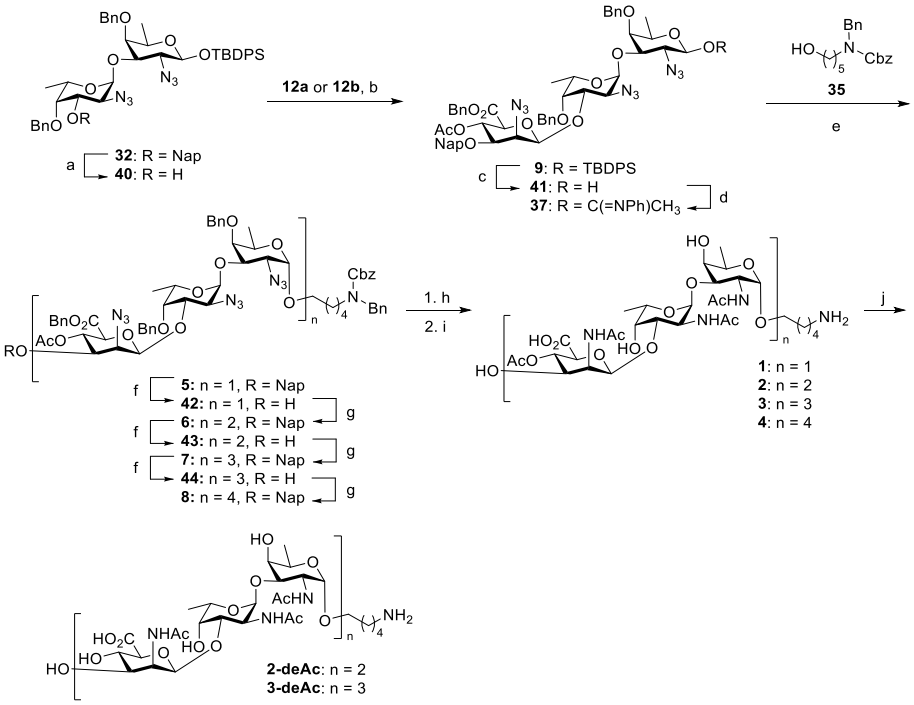
Entry	Conditions	Solvent	Time (h)	Yield (%)	α/β [(a)]
1	TMSOTf	DCM	0.5	86	64:34
2	TMSI, Ph_3PO	DCM	22	51	83:17
3	TMSI, Ph_3PO	DCM/Et_2O 1:1	22	46	87:13

General conditions: 3 Å molecular sieves, 0.1 M solvent, rt, either 1 equiv. TMSI and 6 equiv. PhP_3O or 0.2 equiv. TMSOTf. [(a)]The α/β ratio was determined by NMR of the purified products.

Now the stage was set for the assembly of the larger fragments using the projected [3+3n] glycosylation strategy. Thus, hexasaccharide **6** was synthesized by unmasking the $ManN_3A$ C-3-OH by oxidative denaphthylation of **5** with DDQ giving acceptor **42** in 80% yield. The [3+3] glycosylation of acceptor **42** and donor **35** using TBSOTf as promoter at room temperature yielded the target hexamer **6** as a single anomer in 87% yield. The nonasaccharide **7** was synthesized using similar steps and the nonasaccharide **7** was obtained in the [3+6] glycosylation in 78% yield in a highly stereoselective manner. Finally, the dodecasaccharide was synthesized by first transforming nonamer **7** into the corresponding acceptor **44** in 57% yield. The [3+9] glycosylation solely afforded the α-anomer of the dodecasaccharide **8** in 68% yield. All the newly formed α-linkages were confirmed by ^{1}H-NMR and ^{13}C-NMR. Overall, the assembly strategy proved to be very effective, providing the protected CP8 oligomers of unprecedented length, in a highly stereoselective manner.

Then, turning to the deprotection of the synthetic CP8 oligosaccharides. First, one-pot azide reduction and acetylation using zinc, AcOH and acetic anhydride (Ac_2O) afforded the acetamides in yields ranging from 77% to 98%. Previously, lactamization of the mannosaminuronic acid residue upon reduction of the azide has been observed,[32] but by reducing the azide in the presence of AcOH, lactam formation was effectively prevented. Final and global hydrogenation with $Pd(OH)_2/C$ in *t*-BuOH/H_2O with AcOH gave the target compounds **1-4** in yields

ranging from 34 to 53% after gel filtration purification, completing the assembly of the set of CP8 oligosaccharides.



Scheme 3: Synthesis of the target oligosaccharides **1**, **2**, **3** and **4**. *Reaction conditions*: a) DDQ, DCM/H_2O, 86%, b) TfOH, DCM, -78 to -10 °C, 66%, α/β = 14:86, c) TBAF, AcOH, THF, 0 °C to rt °C, 84%, d) ClC(=NPh)CF_3, K_2CO_3, acetone, 93%, e) TMSI, Ph_3P=O, DCM/Et_2O, 83%, α/β = 75:25, f) DDQ, DCM/H_2O, **42**=80%, **43**=54%, **44**=57%, g) **37**, TBSOTf, DCM, **6**=87%, **7**=77%, **8**=68%, h) zinc, AcOH, Ac_2O, THF, 50 °C, i) $Pd(OH)_2$/C, AcOH, H_2, *t*-BuOH/H_2O, yield over two steps **1**=45%, **2**=37%, **3**=57%, **4**=33%, j) 1 M NaOH in H_2O, **2-deAc**=41%, **3-deAc** =46%.

Conjugates and antibody binding

Having the synthetic fragments in hand, next step was to map their binding to monoclonal antibodies, as well as polyclonal serum raised against native CP8, and to generate semi-synthetic model vaccines to explore their immunogenic properties. To do so, first a set of conjugates were generated in which the synthetic oligomers were conjugated to Cross-Reactive Material 197 (CRM_{197}), which is an oft-used, non-toxic carrier protein, that has been found to adequately raise a T-cell based immune response and to be safe and efficient in children.[47] It can be readily

modified, exploiting the surface exposed lysine residues and therefore first the synthetic CP8 fragments were functionalized with a suberic acid cross-linker on the reducing end aminopentyl group (Figure 3A). To optimize loading on the protein the amount of the oligomers was varied (using 10, 20 and 30 equivalents) as well as the constitution of the buffer. The generated conjugates were analyzed by SDS-PAGE and MALDI-TOF, to reveal that the amount of oligosaccharide used had a large impact on the loading of the carrier protein and that a HEPES buffer (25 mM) gave superior results with respect to PBS (See SI for details). Using 30 equivalents of the synthetic fragments carrying the activated succinimide suberic acid esters, **CRM-1**, **CRM-2**, **CRM-3** and **CRM-4** were assembled, having an average of 11 trisaccharide, 8 hexasaccharide, 13 nonasaccharide and 14 dodecasaccharide moieties per protein, respectively (See Figure 3B).

With the **CRM1-4** conjugates, first the recognition by monoclonal anti-CP8 antibodies (mAb-CP8) was investigated using a Western Blot experiment. As the Western Blot in Figure 3C shows, the trisaccharide conjugate **CRM-1** was poorly recognized, while conjugates **CRM2-4** all bound well to the mAb-CP8, providing a first indication that larger fragments are required to present an effective epitope. To provide more quantitative insight into the binding affinity, a competitive ELISA was performed, using ELISA plates pre-coated with isolated, natural CP8 polysaccharide (CP8-PS). These showed a clear concentration-dependent competition for hexasaccharide **2**, nonasaccharide **3** and dodecasaccharide **4**, with no binding being detected for trisaccharide **1** (Figure 3D). Also, the nonasaccharide lacking the ManNAcA-C4-*O*-acetyl esters, **3-deAc**, generated by saponification of nonamer **3** (Scheme 3), could not compete for binding. Binding to the nonasaccharide was significantly stronger than binding to the hexasaccharide and on par with binding to dodecasaccharide **4**. Apparently, nonamer **9** is large enough to harbor the epitope for the mAb-CP8, while hexamer **2** is too short. A competitive ELISA with polyclonal anti-CP8 serum (pAb-CP8), provided a similar picture, with stronger competition being observed in comparison to the competition for binding with the monoclonal antibody, for all fragments (Figure 3E). Also in this experiment, trisaccharide **1** and de-acetylated **3-deAc** showed relatively poor binding, and nonamer **3** and dodecasaccharide **4** surfaced as the best binders.

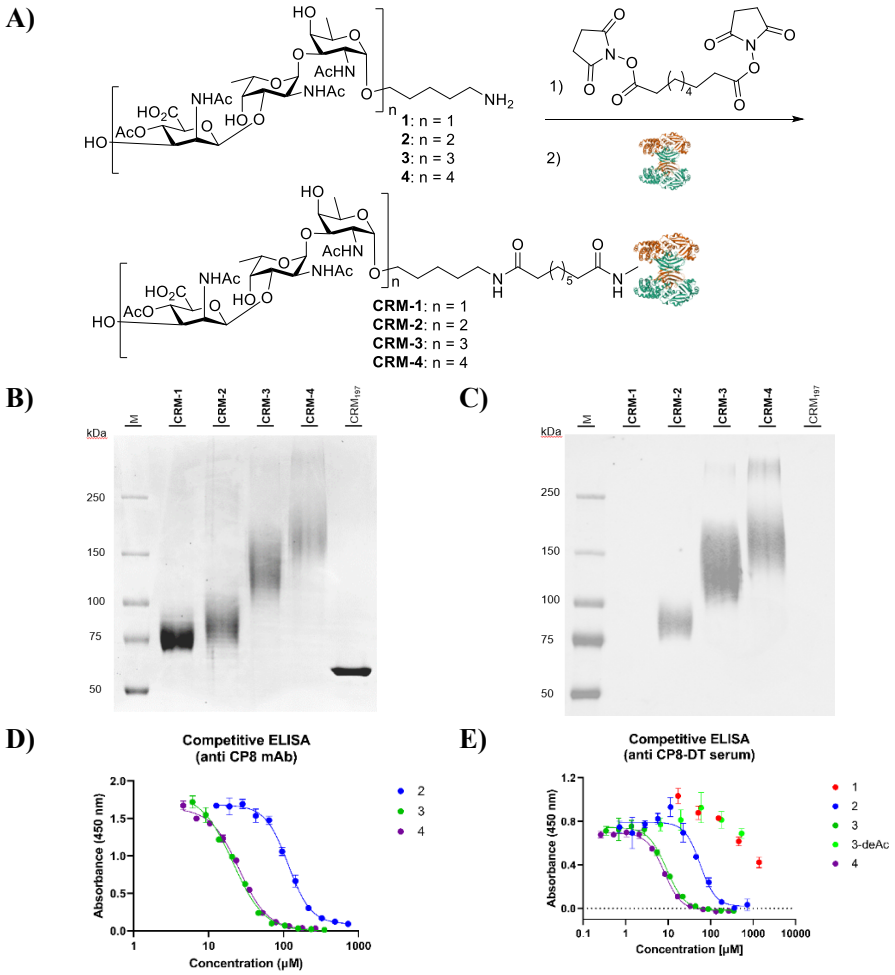


Figure 3: A) Conjugation strategy of the synthetic fragments. 1) Suberic acid bis(N-hydroxy-succinimide ester) 30 equiv. for **1** and 15 equiv. for **2-4** in DMSO/H_2O 9:1, 2) CRM_{197} in PBS or HEPES 25 mM. B) SDS-page with the conjugates **CRM1-4** created in HEPES 25 mM at pH=8. C) Western Blot performed with anti mAb-CP8 showed only recognition of the **CRM2-4**. No recognition of the CRM_{197} itself was not observed. D) Competitive ELISA with anti mAb-CP8 showed that longer fragments granted a better immune response. E) Competitive ELISA with anti pAb-CP8 showed the same pattern as for mAb, however with better response. Also recognition of **1** and **3-deOAc** was observed.

Structural, conformational, and interaction studies

To account for the established structure-activity relationships in the above ELISA experiments, next it was set out to probe the structure of the synthetic fragments and map the epitopes present in the oligosaccharides using saturation transfer difference (STD) NMR spectroscopy (STD-NMR). The conformation and dynamics of the synthetic oligomers in solution were investigated using a combination of NMR methodologies (using *J*-couplings and NOE-interactions), assisted by computational protocols (MM).[48] First, trisaccharide **1** was investigated. The resulting intra- and inter-residue NOE cross-peaks allowed to unequivocally define its major conformation in solution (Figure 4A). Specifically, the analysis of the intra-residual NOE and *J*-couplings established that the three pyranoside residues (A: α-D-FucNAc; B: α-L-FucNAc; C: β-D-ManNAcA) adopt the expected chair conformations ($^4C_1$ for residues A and C, and $^1C_4$ for residue B). Next, the conformation around the glycosidic linkages was analyzed. The simultaneous observation of strong NOEs for the H1(B)- and H3(A) and H5(B)-H4(A) proton pairs were indicative for the presence of a major, well-defined exo-*syn*-ϕ/*syn*(±)-ψ conformation around the B-A glycosidic linkage (Figure 4A, left). Fittingly, MM calculations also predicted the predominance of the exo-syn-ϕ/syn(±)-ψ conformation for this glycosidic linkage. Integration of the observed NOEs cross peaks was used to estimate the ensemble average proton-proton distances (Å), which resulted in the definition of the ψ angle value of ca. ψ= +20±10. In contrast, for the B-C glycosidic linkage, the MM calculations predicted the existence of an equilibrium between a major exo-syn-ϕ/syn(+)-ψ and a minor exo-syn-ϕ/anti-ψ geometry. Fittingly, the strong NOEs observed for the H1(C)-H4(B) and H1(C)-H3(B) proton pairs, together with the very low intensity for H1(C) and H2(B) NOE, assessed that the exo-*syn*-ϕ/*syn*(+)-ψ conformation around the C-B linkage is the most populated one in solution. Overall, this leads to the major conformation for the trisaccharide **1** shown in Figure 4A (Figure 4A, right). Inspection of this 3D structure revealed that alternative conformations around the two glycosidic linkages are prevented because of steric clashes of the acetamide groups of residues A and B with the methyl and carboxylate moieties of residues B and C, respectively. For this major conformation, the average length of the trisaccharide is ~11 Å, while the three acetamide groups are oriented in the same spatial direction with respect to the plane defined by the sugar rings.

A similar analysis was performed on nonasaccharide **3**. NOE cross peaks could be identified that were in full agreement with those observed for trimer **1** (See Figure 4B, left). Nonetheless, the severe NMR signal overlap precluded the

quantitative integration of the cross-peaks. Thus, a qualitative characterization in terms of weak, medium, and strong intensity signals was used to define the conformations (See SI Figure S6-S7). In line with the structure for trimer **1**, the exo-*syn*-ϕ/*syn*(±)-ψ conformation around the B-A linkage was found to be most populated. Similarly, the exo-*syn*-ϕ/*syn*(+)-ψ conformation dominates the C-B glycosidic linkage. The additional A-C glycosidic linkages populate exclusively the exo-*syn*-ϕ/*syn*(-)-ψ conformation, as deduced from the exclusive presence of strong H1(A')-H3(C) and H5(A')-H2(C) NOEs. Interestingly, the spatial orientation of the ManNAcA C-4-*O*-acetyl and C-2-acetamide groups provides an energy barrier for rotation around the A'-C and A"-C' glycosidic linkages. As a result, nonasaccharide **3** adopts an extended conformation of ~35 Å average length, which roughly corresponds to three times the length of the trisaccharide. The negative charges of the carboxylate moieties are at a distance of 15-16 Å of each other. Furthermore, in this structure, the three acetamide groups of each repeating unit (RU), and the ManNAcA *O*-acetyl ester of the RU at the reducing end, are presented in the same spatial direction, with the trisaccharide RUs being tilted by a dihedral angle close to 90° between two consecutive RUs (Figure 4B, right). The proximity of the *N*- and *O*-acetyl methyl groups creates hydrophobic patches that may be important in binding to antibodies.

To reveal the structural elements that define the optimal binding epitope, the interaction of the saccharides to the mAb-CP8 was explored by ^{1}H STD-NMR experiments. In particular, the tri-, hexa- and nonasaccharide **1**, **2** and **3**, as well as the deacetylated hexasaccharide (**2-deAc**, generated by saponification of hexamer **2**, Scheme 3) were tested. For trisaccharide **1**, the resulting NMR spectrum acquired at the physiological temperature (310 K) showed no significant STD-NMR signals. At lower temperature (288 K) the STD-NMR signals slightly increased, suggesting the existence of a very weak interaction (Figure 5A). In contrast, the STD-NMR spectrum of the hexasaccharide **2** at 310 K revealed clear STD signals (Figure 5B). The de-acetylated hexamer **2-deAc** showed only marginal STD-NMR signals, the intensity of which again enhanced upon lowering the temperature (Figure 5C). This result indicates a weak interaction between the mAb and the deacetylated hexasaccharide, and thus suggests a key role of the *O*-acetyl group of the mannuronic residue for mAb binding. Consistently, in the absence of the ManNAcA *O*-acetyl, the ManNAcA residues did not significantly contribute to the binding, as deduced from the negligible intensities found for the signals of this residue (comparison Figure 5B-Figure 5C). Interestingly, the STD-NMR spectrum for nonasaccharide **3** showed less intense STD signals than the spectrum of hexasaccharide **2** (compare Figure 5B and Figure 5D).

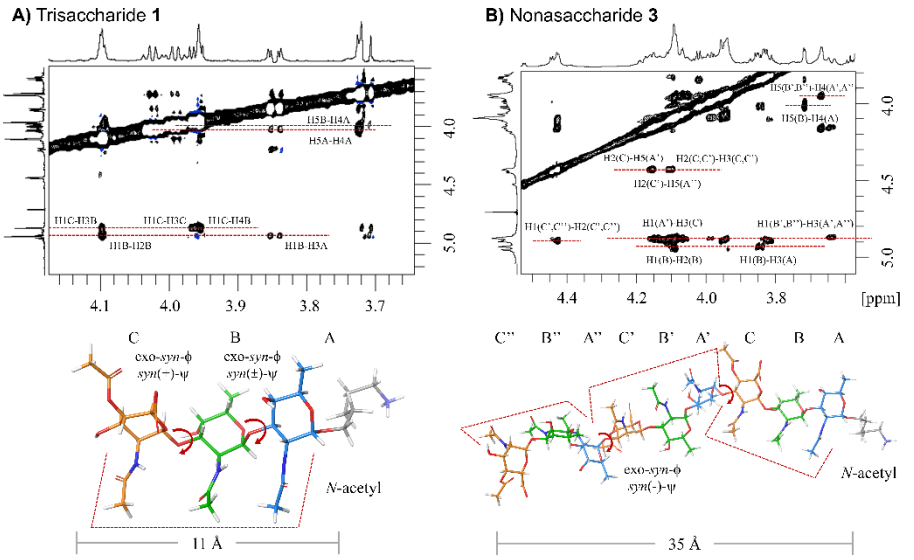


Figure 4: Conformational analysis of trisaccharide **1** and of nonasaccharide **3** as established by NMR and MM calculations. A) Zoom area of 2D NOESY spectrum of trisaccharide **1** (left) and its main conformation as defined by NOE analysis and MM calculations. B) Zoom area of 2D NOESY spectrum of the nonasaccharide **3** (left) and its main conformation as defined by NOE analysis and MM calculations. Monosaccharide residues are labeled with a letter code. The main conformation at each glycosidic linkage, the spatial orientation of the acetyl groups, and the average length are reported.

Since the success of a STD NMR experiment depends on a fast dissociation rate of the ligand-mAb complex on the NMR relaxation time scale, the observed low intensities of the STD signals found for the nonamer may be explained by the too strong binding for this molecule, as revealed in the ELISA assays. Consistent with this hypothesis, the STD-NMR signals became clearer at higher temperature, where dissociation of the ligand from the antibody becomes faster (Figure 5D). Next, the relative STD-NMR signal intensities were used to define the corresponding binding epitopes. In general, a similar STD profile was observed for hexasaccharide **2** and nonasaccharide **3**. The strongest STD-NMR signals were observed for the mannuronic acid, the α-L-FucNAc residues, and the corresponding methyl groups of the *O*-acetyl esters and acetamide moieties. In particular, the H2 and H4 protons of the ManNAcA residues displayed the strongest STD effects, ranging between 75 and 100% of the maximum STD relative intensity. The *O*-acetyl at the mannuronic residue, the *N*-acetyl and the H1-H2 of the L-FucNAc, together with D-FucNAc H2 displayed relative STD intensities ranging between 50 and 74%. Weaker STD signals were recorded for the H3 and the *N*-acetyl

moiety of the ManNAcA, the H3-H5 of the L-FucNAc and for the *N*-acetyl group of the L-FucNAc. Interestingly, marginal STD signals (below 25%) were measured for the methyl groups of the L- and D-FucNAc residues, all along the saccharide chain, as well as for the *N*-acetyl moieties of the reducing end terminal saccharides. Yet, comparison of the STD results of the hexa- and nonasaccharide reveals a shift in the main epitope (comparison Figure 5B and Figure 5D). For the longer oligosaccharide, the strongest STD signals arose from the central RU, while for the hexasaccharide, the main epitope is formed by the terminal repeating unit at the nonreducing end.

Overall, these data clearly indicate that the mAb recognizes the CP8 oligosaccharides through an extended binding epitope that spans over 2 RUs, and that is mainly defined by the interaction of the *O*-acetylated ManNAcA and L-Fuc residues. For the longer nonasaccharide (3 RUs) the central region of the oligosaccharide chain is in close contact with the antibody binding site, while in the shorter hexasaccharide engages mostly in binding with the non-reducing end terminal part.

***In vivo* studies**

Finally, the immunological properties of the CRM-CP8 conjugates were investigated in a mouse immunization study, in which the conjugates (with a dose of 1 µg carbohydrate per immunization) were injected together with aluminum hydroxide (AlOH, 3 mg/mL) as an adjuvant. Besides the four synthetic CP8-conjugates also a CP8-PS-CRM conjugate was used for comparison. Five groups of 10 mice (5 weeks old, female) were injected subcutaneous three times, at day 1, 22 and 36, taking a blood sample at day 35 (post 2) and day 50 (post 3, the final bleed). The anti-CP8 IgG titers in the collected sera were measured using ELISAs. As shown in Figure 6, a clear oligosaccharide length-dependent immune response was observed for the conjugates of the synthetic oligosaccharides. The immunization with the trisaccharide conjugate **CRM-1**, led to the lowest anti-CP8 titers, while slightly higher titers were found for the hexasaccharide **CRM-2**. Antibody levels elicited by the conjugate of the shortest oligosaccharides appeared more scattered as opposed to the longest structures. For the nona- and dodecasaccharide high titers were found with only a small difference between the two fragments in favor of the dodecasaccharide **CRM-4**. The titers from the nona- and dodecasaccharide conjugates compared well with the titers found in the immunization with

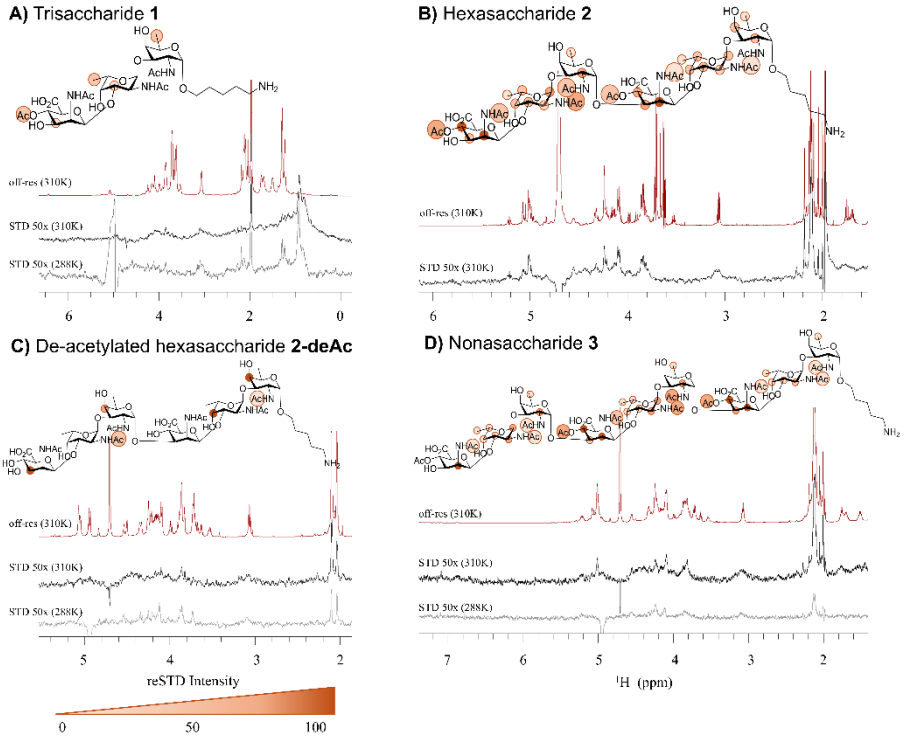


Figure 5: ^{1}H STD-NMR spectra performed for the complexes of mAb-CP8 and the trisaccharide **1**, (A) the hexasaccharide **2** (B) the de-acetylated hexasaccharide **2-deAc** (C), and the nonasaccharide **3** (D). Off-resonance spectra (in red) and corresponding STD-NMR spectra at 310 K (in black) and at 288 K (in gray). The representation of the epitope map disclosed by the analysis of the relative STD-NMR signal intensities for each oligosaccharide is reported as color legend associated with the STD% values.

the natural CP8-PS conjugate. After injection two and three a small boost was observed for the synthetic conjugates, with the boosting effect being strongest for the shortest, weakest antigens (trisaccharide **1** and hexasaccharide **2**). No boost effect was observed for the CP8-PS conjugate. Overall, these results show that the synthetic oligosaccharides mimic the antigenicity of the full polysaccharide well, if sufficiently long (*i.e.*, three RUs or more) saccharides are used.

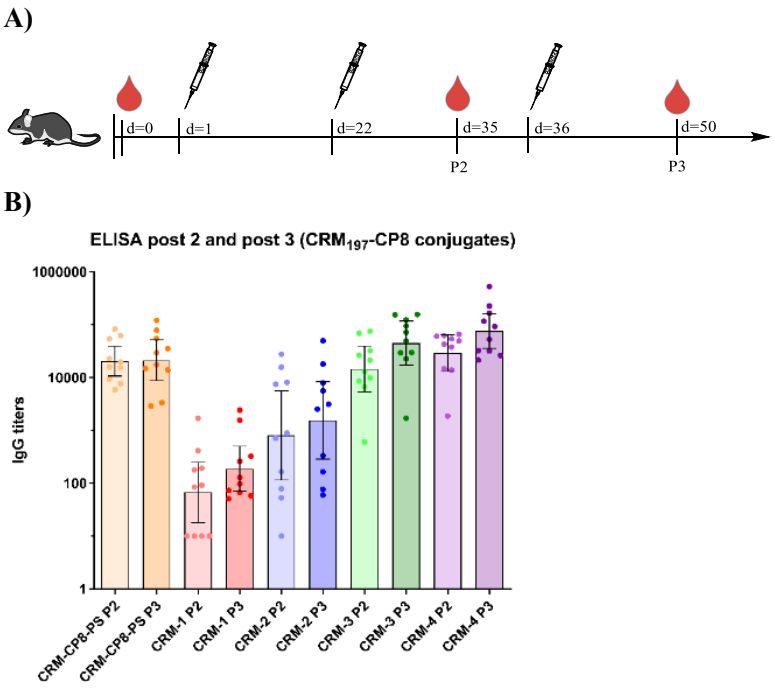


Figure 6: A) Illustration of the *in vivo* study. Injections were performed at day 1, day 22 and day 36 and blood collections were performed at day 0, day 35 (post 2) and day 50 (post 3) B) ELISA post 2 (P2) and post 3 (P3) IgG titers.

Conclusion

In this work, a convergent strategy for the assembly of synthetic, conjugation-ready *S. aureus* CP8 oligosaccharides comprising multiple repeating units, has been developed. By using a pre-glycosylation oxidation strategy to introduce the mannosaminuronic acids, in combination with two 2-fucoseamine synthons an effective route to generate the required trisaccharide building block is disclosed. Using an orthogonally protected trisaccharide, a set of CP8 oligosaccharides has been assembled, ranging in length from a tri- to a dodecasaccharide, carrying *O*-acetyl esters at the ManNAcA C-4-OH. The developed protecting group strategy has enabled high yielding and stereoselective glycosylation reactions to construct all required 1,2-*cis* linkages. It also allowed for a highly efficient global deprotection scheme, requiring only two transformations and leaving the *O*-acetyl ester unscathed. An aminopentyl linker was installed which allowed for conjugation to CRM_{197} to construct a set of model conjugate vaccines. The

glycoconjugates were evaluated for their binding to mono- and polyclonal antibodies and used in immunization experiments. These revealed a clear length-dependent immune response. While the trisaccharide was found too short to bind the antibodies or raise an immune response capable of adequately recognizing the natural polysaccharide, the hexasaccharide bound the antibodies better and the nona- and dodecasaccharide provided optimal epitopes for recognition. The conjugates of the latter oligomers raised a high titer of antibodies recognizing the natural polysaccharide well. Detailed structural studies revealed that the oligosaccharides adopt an extended, almost linear structure, in which all acetyl groups of each trisaccharide repeating unit point in the same direction, generating hydrophobic patches along the periphery of the oligosaccharide chain. These formed important recognition elements in the epitope for the monoclonal antibody. The interaction and immunization studies have revealed the requirements for at least three repeating units to deliver a strong binding epitope.

This study has highlighted the advantages of larger synthetic oligosaccharides for immunological studies at the molecular level. Because of the challenges associated with the assembly of bacterial oligosaccharides often oligosaccharides, comprising only a single repeating unit, are reported. This obviously simplifies the synthesis campaign, but it does bring about the risk of synthesizing a suboptimal frameshift of the repeating unit, and it fails to capture epitopes spanning multiple repeating units. The work illustrates how progressing insight into glycosylation chemistry, which enables the effective stereoselective construction of difficult glycosidic linkages, alongside the development of even more effective protecting and functional group manipulations, required to install all the different functionalities present in bacterial glycans, opens the way to construct longer, fully functional oligosaccharides. These not only enable the conception of synthetic vaccines, but they can also be used as high value tool compounds to probe bacterial biosynthesis enzymes and investigate (multivalent) interactions with host (immune cell) receptors.

Acknowledgement

Luca Unione and Cristian García-Sepúlveda from CIC BioGune are acknowledged for their help and contribution to the conformational analysis and STD NMR experiments. Filippo Carboni and Linda Del Bino from GSK vaccines are acknowledged for their help and contribution with the *in vivo* studies.

Conflict of interest: Kitt Østerlid has participated in a post graduate studentship program at GSK. This work was sponsored by GlaxoSmithKline Biologicals SA.

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 experimental for deprotection of the 2-methylnaphthyl ether
The fully protected CP8-OS (1 equiv.) was dissolved in DCM/H_2O (0.1 M) and added DDQ (2 equiv.). The reaction was stirred under N_2 at rt until TLC showed full conversion (~4-6 h). The reaction was quenched with $Na_2S_2O_3$ (aq., sat.) and diluted in EtOAc and extracted (x3). The combined organic layers were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography gave the wanted product.

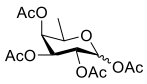
General glycosylation of [3+3], [3+6] and [3+9]
The trisaccharide donor **16** (1.3 equiv.) and the acceptor (1 equiv.) was co-evaporated with toluene (3x), dissolved in dry DCM (0.1 M), added 3Å molecular sieves at rt and stirred for 30 min at rt. TBSOTf (0.2 equiv.) was added at rt and the reaction was stirred at rt under argon until TLC showed full conversion (~30 min). 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 and concentrated. Purification by column chromatography and/or size exclusion gave the wanted product.

General deprotection of 5, 6, 7 and 8
Protected CP8-OS was dissolved in dry, distilled THF (3 mL) and added zinc powder (300 equiv.), AcOH (1 mL) and Ac_2O (0.5 mL). The reaction was stirred at 50 ºC overnight until

TLC showed full conversion. The solution was filtered, concentrated *in vacuo* and co-evaporated with toluene (x3). Column chromatography (DCM/MeOH 98:2 → 95:5) and/or size exclusion gave the wanted product. The acetamide-OS 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 then 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 for under H_2 atmosphere for three days or until completion by NMR was detected. 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 building blocks

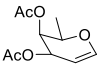
1,2,3,4-tetra-*O*-acetyl-D-fucopyranose (13)



Acetone (1200 mL) was cooled to 0 ºC and slowly dropwise added conc. H_2SO_4 (40 mL). D-Galactose (50 g, 277.5 mmol) was added portion wise and the reaction was allowed to warm to rt and stirred for 7 h until TLC (pentane/EtOAc, 1:1) showed full conversion. The now yellow solution was cooled to 0 ºC and neutralized with $NaHCO_3$ (sat. aq.) until pH~8-9. The acetone was evaporated and the aqueous phase was extracted with EtOAc (x3). The combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product (62.04 g, 238.3 mmol) was dissolved in toluene/MeCN (2:1, 700 mL). First PPh_3 (118.66 g, 524.4 mmol, 2.2 equiv.) and imidazole (71.39 g, 1050 mmol, 4.4 equiv.) were added followed by portion wise addition of I_2 (90.74 g, 357.5 mmol, 1.5 equiv.). The reaction was heated to 90 ºC and stirred for 24 h until TLC (pentane/EtOAc 1:1) showed full conversion. After cooling to rt, the solvents were evaporated and the residue was dissolved in EtOAc, washed with $Na_2S_2O_3$ (aq., sat., x2), H_2O (x2) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. To the crude product in *i*-PrOH (700 mL) were added Et_3N (199 mL, 1450 mmol, 6 equiv.) and aq. H_3PO_2 (50%, 84 mL, 953.2 mmol, 4 equiv.) and the mixture was stirred under N_2 for 30 min. 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044, 23.11 g, 71.49 mmol, 0.3 equiv.) was added at rt and the reaction was heated to 80 ºC and stirred under N_2 for 1 h until TLC (pentane/EtOAc 4:1) showed full conversion. The solvent was evaporated and residue was dissolved in EtOAc, washed with NH_4Cl (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was dissolved in 80% aq. AcOH (600 mL) and stirred at 90 ºC for 18 h until TLC (pentane/EtOAc 4:1) showed full conversion. The solvents were evaporated and the residue was co-evaporated with toluene (x4). The residue was dissolved in pyridine (700 mL) and cooled to 0 ºC. Ac_2O (400 mL) was added and the reaction was slowly allowed to warm to rt and stirred for 18 h until TLC (pentane/EtOAc 4:1) showed full conversion. The solvents were evaporated and the residue wash dissolved in EtOAc, washed with 1 M HCl (x2), sat. aq. $NaHCO_3$ (sat. aq.; x3) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 85:15 → 70:30) gave **13** in 54% yield (49.42 g, 149 mmol) in a α/β ratio = 0.8:1. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 6.34 (s, 1H, α-H-1), 5.68 (d, *J* = 8.3 Hz, 1H, *β-H-1*), 5.33 (t, *J* = 1.4 Hz, 2H, α-H-4, α-H-3), 5.33 – 5.32 (m, 1H, α-H-2), 5.32 – 5.29 (m, 1H, β-H-4), 5.27 (dd, *J* = 3.5, 1.1 Hz, 1H, β-H-2),

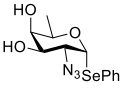
5.07 (dd, *J* = 10.4, 3.4 Hz, 1H, β-H-3), 4.30 – 4.23 (q, *J* = 6,5 Hz, 1H, α-H-5), 3.95 (*J* = 6.5 Hz, 1H, β-H-5), 2.19 (s, 3H, COC*H*$_3$), 2.18 (s, 3H, COC*H*$_3$), 2.14 (s, 3H, COC*H*$_3$), 2.11 (s, 3H, COC*H*$_3$), 2.04 (s, 3H, COC*H*$_3$), 2.01 (s, 3H, COC*H*$_3$), 2.00 (s, 3H, COC*H*$_3$), 1.99 (s, 3H, COC*H*$_3$), 1.22 (d, *J* = 6.4 Hz, 3H, *β-H-6*), 1.15 (d, *J* = 6.5 Hz, 3H, *α-H-6*). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 170.70 (C=O), 170.68 (C=O), 170.36 (C=O), 170.20 (C=O), 170.11 (C=O), 169.63 (C=O), 169.33 (C=O), 169.32 (C=O), 92.31 (β-C-1), 90.09 (α-C-1), 71.39 (β-C-3), 70.71 (α-C-3/ β-C-4/ α-C-4), 70.39 (β-C-5), 70.05 (β-C-2), 68.03 (α-C-3/ β-C-4/ α-C-4), 67.96 (α-C-2), 67.42 (α-C-5), 66.59 (α-C-3/ β-C-4/ α-C-4), 21.08 (CO*C*H$_3$), 21.00 (CO*C*H$_3$), 20.83 (CO*C*H$_3$), 20.80 (CO*C*H$_3$), 20.77 (CO*C*H$_3$), 20.73 (CO*C*H$_3$), 16.07 (C-6), 16.06 (C-6). **HRMS**: [M+Na]$^+$ calculated for $C_{14}H_{20}O_9Na$: 355.10050; found 355.09974

3,4-di-*O*-acetyl-D-fucal (14)



13 (17.83 g, 53.7 mmol) was dissolved in DCM (215 mL, 0.25 M), cooled to 0 ºC and added HBr in AcOH (33 wt%, 14.6 mL, 80.55 mmol, 1.5 equiv.) using a dropping funnel. The reaction was stirred at 0 ºC under N_2 for 2 h until TLC (pentane/EtOAc 4:1) showed full conversion. The solution was poured over ice and stirred until the ice was molten. The aqueous phase was extracted with DCM (x3) and the combined organic phases were washed with H_2O (x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene (x3) and used immediately without any further purification. The crude product (17.51 g, 49.74 mmol) was dissolved in EtOAc (166 mL, 0.3 M) and zinc powder (22.77 g, 348.2 mmol, 7 equiv.) and NH_4Cl (18.62 g, 348.2 mmol, 7 equiv.) were added portion wise. The reaction was stirred at 60 ºC under N_2 for 1 h until TLC (pentane/EtOAc 4:1) showed full conversion. The mixture was cooled to rt, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc + 1% Et_3N 9:1 → 7:3) gave **14** in 44% yield (5.11 g, 23.9 mmol) (49% brsm). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 6.46 (dd, *J* = 6.4, 2.0 Hz, 1H, H-1), 5.60 – 5.52 (m, 1H, H-3), 5.28 (dq, *J* = 4.7, 1.8 Hz, 1H, H-4), 4.63 (dt, *J* = 6.3, 1.9 Hz, 1H, H-2), 4.21 (q, *J* = 6.5 Hz, 1H, H-5), 2.15 (s, 3H, COC*H*$_3$), 2.01 (s, 3H, COC*H*$_3$), 1.27 (d, *J* = 6.6 Hz, 3H, H-6). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 170.86 (C=O), 170.56 (C=O), 146.25 (C-1), 98.39 (C-2), 71.65 (C-3), 66.37 (C-4), 65.17 (C-5), 21.00 (CO*C*H$_3$), 20.85 (CO*C*H$_3$), 16.66 (C-6). **HRMS**: [M+Na]$^+$ calculated for $C_{10}H_{14}O_5Na$: 237.07389; found 237.07422

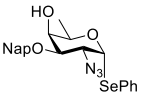
Phenyl 2-azido-2-deoxy-1-seleno-α-D-fucopyranoside (15)



14 (6.562 g, 30.66 mmol) and $(PhSe)_2$ (9.57 g, 30.66 mmol, 1 equiv.) was dissolved in DCM (155 mL, 0.2 M) and degassed under argon at rt for 30 min. The reaction was cooled to -30 ºC, added BAIB (9.87 g, 30.66 mmol, 1 equiv.) and $TMSN_3$ (8.1 mL, 61.31 mmol, 2 equiv.) and stirred at -20 ºC overnight until TLC (toluene/EtOAc 9:1) showed full conversion. Cyclohexene (12 mL) was added, and the reaction was stirred at rt for 30 min before being concentrated *in vacuo*. The lipophilic byproducts were removed by column chromatography (pentane/EtOAc 10:0 → 7:3) were all the carbohydrate positive fractions were collected. The crude residue (14.46 g, 35 mmol, impure) was dissolved in MeOH (120 mL, 0.3 M) and added NaOMe (1.6 mL, 7 mmol, 0.2 equiv.). The reaction was stirred at rt for 2 h until TLC (pentane/EtOAc 1:1) showed full conversion and then neutralized with Amberlite IR-120 H^+ resins, filtered and concentrated *in vacuo*. The crude product was recrystallized in hot toluene to give **15** in 67% yield (6.78 g, 20.7 mmol). **^{1}H NMR (400 MHz, MeOD)** δ 7.60 – 7.55 (m, 2H, Ar-*H*), 7.34 – 7.22 (m, 3H, Ar-*H*), 5.91 (dd, *J* = 5.2, 0.6 Hz, 1H,

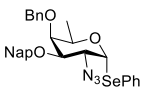
H-1), 4.29 (q, *J* = 6.5 Hz, 1H, H-5), 4.01 (dd, *J* = 9.9, 5.3 Hz, 1H, H-2), 3.76 – 3.68 (m, 2H, H-4, H-3), 1.15 (d, *J* = 6.6 Hz, 3H, H-6). **^{13}C NMR (101 MHz, MeOD)** δ 135.91 (Ar-*C*), 130.04 (Ar-*C*), 128.72 (Ar-C_q), 86.89 (C-1), 72.91 (C-3), 72.68 (C-4), 70.61 (C-5), 62.91 (C-2), 16.42 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{12}H_{15}N_3O_3SeNa$: 352.01763; found 352.01709

Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-seleno-α-D-fucopyranoside (16)



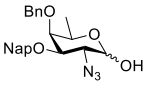
15 (3.60 g, 10.94 mmol) was co-evaporated with toluene (x3) and dissolved in dry toluene (55 ml, 0.2 M). Bu_2SnO (2.778 g, 11.16 mmol, 1.02 equiv.) was added and the flask was equipped with a Dean-Stark. The reaction was heated to 140 ºC for 3 h. The now clear solution was cooled to 60 ºC before adding Bu_4NBr (3.704 g, 11.49 mmol, 1.05 equiv.), CsF (1.965 g, 11.16 mmol, 1.02 equiv.) and NapBr (2.540 g, 11.49 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 *in vacuo*. Column chromatography (pentane/EtOAc 9:1 → 7:3) gave **16** in 93% yield (4.768 g, 10.18 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.92 – 7.82 (m, 4H, Ar-*H*), 7.63 – 7.45 (m, 5H, Ar-*H*), 7.33 – 7.27 (m, 3H, Ar-*H*), 5.91 (d, *J* = 5.4 Hz, 1H, H-1), 4.88 (dd, *J* = 13.6, 11.5 Hz, 2H, Ar-CH_2), 4.30 (qt, *J* = 6.6, 1.5 Hz, 1H, H-5), 4.21 (dd, *J* = 10.2, 5.3 Hz, 1H, H-2), 3.91 (dt, *J* = 3.2, 1.6 Hz, 1H, H-4), 3.76 (dd, *J* = 10.2, 3.1 Hz, 1H, H-3), 2.39 (t, *J* = 1.6 Hz, 1H, OH), 1.26 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 133.35 (Ar-*C*), 133.33 (Ar-C_q), 129.24 (Ar-C_q), 128.79 (Ar-*C*), 128.63 (Ar-C_q) 128.13 (Ar-*C*), 127.94 (Ar-*C*), 127.91 (Ar-*C*), 127.17 (Ar-*C*), 126.53 (Ar-*C*), 126.45 (Ar-*C*), 125.81 (Ar-*C*), 85.30 (C-1), 79.35 (C-3), 72.42 (Ar-CH_2), 68.71 (C-5, C-4), 60.40 (C-2), 16.83 (C-6). **HRMS**: $[M+H]^+$ calculated for $C_{23}H_{23}N_3O_3SeH$: 470.09829; found 470.09776

Phenyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-1-seleno-α-D-fucopyranoside (17)



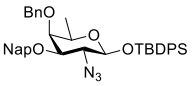
16 (3.228 g, 6.89 mmol) was dissolved in DMF (67 mL, 0.1 M) and cooled to 0 ºC. BnBr (1.06 mL, 8.96 mmol, 1.3 equiv.) and NaH (60% suspension in mineral oil, 358 mg, 8.96 mmol, 1.3 equiv.) was added and the solution was slowly allowed to warm to rt and stirred under N_2 for 18 h until TLC (pentane/EtOAc 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 **17** in 88% yield (3.627 g. 6.49 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.92 – 7.81 (m, 4H, Ar-*H*), 7.60 – 7.53 (m, 3H, Ar-*H*), 7.53 – 7.47 (m, 2H, Ar-*H*), 7.37 – 7.19 (m, 8H, Ar-*H*), 5.95 (d, *J* = 5.3 Hz, 1H, H-1), 5.01 – 4.87 (m, 3H, Ar-CH_2, Ar-CH_2), 4.65 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.40 (dd, *J* = 10.3, 5.3 Hz, 1H, H-2), 4.23 (q, *J* = 6.3 Hz, 1H, H-5), 3.79 (dd, *J* = 10.3, 2.7 Hz, 1H, H-3), 3.74 (dd, *J* = 2.8, 1.2 Hz, 1H, H-4), 1.14 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.24 (Ar-C_q), 135.09 (Ar-C_q), 134.50 (Ar-*C*), 133.42 (Ar-C_q), 133.23 (Ar-C_q), 128.54 (Ar-*C*), 128.45 (Ar-*C*), 128.29 (Ar-*C*), 128.13 (Ar-*C*), 127.92 (Ar-*C*), 127.89 (Ar-*C*), 127.80 (Ar-*C*), 126.76 (Ar-*C*), 126.41 (Ar-*C*), 126.26 (Ar-*C*), 125.81 (Ar-*C*), 85.68 (C-1), 80.79 (C-3), 75.98 (C-4), 75.16 (Ar-*C*H_2-3), 72.77 (Ar-*C*H_2), 69.55 (C-5), 61.17 (C-2), 16.69 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{30}H_{29}N_3O_3SeNa$: 582.12718; found 582.12685

2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α/β-D-fucopyranose (18)



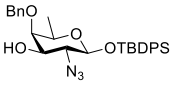
17 (3.618 g, 6.47 mmol) was dissolved in acetone/H_2O (130 mL, 10:1, 0.05 M), cooled to 0 ºC and added NIS (2.912 g, 12.94 mmol, 2 equiv.). The reaction was stirred at 0 ºC for 15 min until TLC (pentane/EtOAc 3:2) showed full conversion. The solvents were evaporated and the residue was dissolved in EtOAc, washed with sat. aq. $Na_2S_2O_3$ (sat. aq.; x1), sat. aq. $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 8:2 → 6:4) gave **S6** in 99% yield in a α/β ratio 1:0.9 (2.687 g, 6.405 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.91 – 7.79 (m, 7H, Ar-*H*), 7.59 – 7.45 (m, 6H, Ar-*H*), 7.41 – 7.26 (m, 9H, Ar-*H*), 5.33 (t, *J* = 2.9 Hz, 1H, α-H-1), 4.97 (dd, *J* = 11.5, 4.5 Hz, 2H, Ar-CH_2), 4.89 (d, *J* = 10.1 Hz, 4H, Ar-CH_2), 4.68 (dd, *J* = 16.6, 11.5 Hz, 2H, Ar-CH_2), 4.47 (t, *J* = 7.5 Hz, 1H, β-H-1), 4.15 – 4.07 (m, 1H, β-H-5), 4.06 – 3.95 (m, 2H, αH-3, α-H-2), 3.80 (dd, *J* = 10.3, 7.9 Hz, 1H, β-H-2), 3.76 – 3.71 (m, 1H, β-H-4), 3.58 (dd, *J* = 2.8, 1.0 Hz, 1H, α-H-4), 3.48 (qd, *J* = 6.4, 1.1 Hz, 1H, α-H-5), 3.41 (dd, *J* = 10.3, 2.8 Hz, 1H, β-H-3), 3.29 (d, *J* = 7.1 Hz, 1H, β-OH), 2.77 (dd, *J* = 3.0, 0.9 Hz, 1H, α-OH), 1.21 (d, *J* = 6.4 Hz, 2H, α-H-6), 1.17 (d, *J* = 6.5 Hz, 3H, β-H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.29 (Ar-C_q), 138.16 (Ar-C_q), 135.21 (Ar-C_q), 135.14 (Ar-C_q), 133.23 (Ar-C_q), 128.55 (Ar-*C*), 128.52 (Ar-*C*), 128.46 (Ar-*C*), 128.11 (Ar-*C*), 128.07 (Ar-*C*), 127.98 (Ar-*C*), 127.94 (Ar-*C*), 127.90 (Ar-*C*), 127.88 (Ar-*C*), 96.58 (β-C-1), 92.58 (α-C-1), 81.05 (β-C-3), 77.88 (α-C-3), 76.22 (β-C-4), 75.03 (Ar-*C*H$_2$), 74.95 (Ar-*C*H$_2$), 72.86 (Ar-*C*H$_2$), 72.57 (Ar-*C*H$_2$), 71.20 (α-C-5), 67.01 (β-C-5), 64.96 (β-C-2), 60.46 (α-C-2), 17.05 (C-6), 16.97 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{24}H_{25}N_3O_4Na$: 442.17428; found 442.17373

***Tert*-butyldiphenylsilyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-β-D-fucopyranoside (19)**



18 (2.688 g, 6.408 mmol) was co-evaporated with toluene (x3), dissolved in dry DCM 32 mL, 0.2 M) and cooled to 0 ºC. TBDPS-Cl (1.97 mL, 7.69 mmol, 1.2 equiv.), imidazole (1.091 g, 16.02 mmol, 2.5 equiv.) and DMAP (157 mg, 1.282 mmol, 0.2 equiv.) 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 solution was dissolved in EtOAc, washed with 1 M HCl (x3) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 100:0 → 90:10) gave **19** in 96% (4.067 g, 6.18 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.87 – 7.65 (m, 10H, Ar-*H*), 7.53 – 7.28 (m, 16H, Ar-*H*), 4.96 (d, *J* = 11.8 Hz, 1H, Ar-CH_2), 4.83 (d, *J* = 2.2 Hz, 2H, Ar-CH_2), 4.68 (d, *J* = 11.8 Hz, 1H, Ar-CH_2), 4.31 (d, *J* = 7.7 Hz, 1H, H-1), 3.89 (dd, *J* = 10.4, 7.7 Hz, 1H, H-2), 3.46 (dd, *J* = 3.1, 1.1 Hz, 1H, H-4), 3.25 (dd, *J* = 10.4, 2.9 Hz, 1H, H-3), 3.08 (qd, *J* = 6.4, 1.1 Hz, 1H, H-5), 1.11 (s, 9H, TBDPS-CH_3), 1.01 (d, *J* = 6.4 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.59 (Ar-C_q), 136.25 (Ar-*C*), 136.11 (Ar-*C*), 135.44 (Ar-C_q), 134.94 (Ar-C_q), 133.59 (Ar-C_q), 133.36 (Ar-C_q), 133.28 (Ar-C_q), 133.17 (Ar-C_q), 129.81 (Ar-*C*), 129.64 (Ar-*C*), 128.43 (Ar-*C*), 128.38 (Ar-*C*), 128.36 (Ar-*C*), 128.07 (Ar-*C*), 127.87 (Ar-*C*), 127.79 (Ar-*C*), 127.56 (Ar-*C*), 127.32 (Ar-*C*), 126.64 (Ar-*C*), 126.34 (Ar-*C*), 126.16 (Ar-*C*), 125.83 (Ar-*C*), 97.44 (C-1), 81.18 (C-3), 75.35 (C-4), 74.79 (Ar-*C*H2), 73.58 (Ar-*C*H$_2$), 71.28 (C-5), 66.76 (C-2), 27.01 (TBDPS-*C*H$_3$), 16.71 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{40}H_{43}N_3O_4SiNa$: 680.29205; found 680.29150

***Tert*-butyldiphenylsilyl 2-azido-4-*O*-benzyl-2-deoxy-β-D-fucopyranoside (10)**

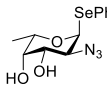


19 (4.3923 g, 5.01 mmol) was dissolved in DCM/H_2O (50 mL, 20:1, 0.1 M) and added DDQ (1.705 g, 7.51 mmol, 1.5 equiv.). The reaction was stirred at rt under N_2 for 2 h until TLC (pentane/EtOAc 9:1) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (aq. sat.), dissolved and extracted with EtOAc x3. The combined organic phases were washed with sat. aq. $NaHCO_3$ (sat. aq.; x4, until the yellow color disappeared) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 80:20) gave **10** in 84% yield (2.89 g, 5.58 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.82 – 7.69 (m, 4H, Ar-*H*), 7.51 – 7.33 (m, 11H, Ar-*H*), 4.82 (d, *J* = 11.6 Hz, 1H, Ar-CH_2), 4.71 (d, *J* = 11.6 Hz, 1H, Ar-CH_2), 4.36 (d, *J* = 7.7 Hz, 1H, H-1), 3.58 (dd, *J* = 10.3, 7.7 Hz, 1H, H-2), 3.47 (dd, *J* = 3.6, 1.2 Hz, 1H, H-4), 3.39-3.33 (m, 1H, H-3), 3.21 (qd, *J* = 6.5, 1.2 Hz, 1H, H-5), 2.24 (d, *J* = 7.4 Hz, 1H, OH), 1.15 (s, 9H, TBDPS-CH_3), 1.12 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.10 (Ar-C_q), 136.21 (Ar-*C*), 136.05 (Ar-*C*), 133.56 (Ar-C_q), 133.17 (Ar-C_q), 129.88 (Ar-*C*), 129.71 (Ar-*C*), 128.76 (Ar-*C*), 128.25 (Ar-*C*), 128.22 (Ar-*C*), 127.60 (Ar-*C*), 127.35 (Ar-*C*), 96.99 (C-1), 78.84 (C-4), 76.04 (Ar-*C*H_2), 72.93 (C-3), 70.87 (C-5), 67.53 (C-2), 27.00 (TBDPS-*C*H_3), 16.69 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{29}H_{35}N_3O_4SiNa$: 540.22945; found 540.22890

3,4-di-*O*-acetyl-L-fucal (20)

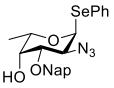
A solution of Ac_2O (80 mL, 14 equiv.) and pyridine (100 ml, 0.6 M) was cooled to 0 ºC. L-Fucose (10 g, 60.92 mmol) was added portion wise and the reaction was stirred at 4 C under N_2 for 18 h until TLC (pentane/EtOAc 3:2) showed full conversion. The solution was poured over ice and stirred until the ice was molten. The aqueous phase was extracted with DCM (x3) and the combined organic phases were washed with 1 M HCl (x3), H_2O (x2) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene (x3) and used without any further purification. The crude product (18.9 g, 56.91 mmol) was dissolved in DCM (230 mL, 0.25 M) and cooled to 0 ºC. HBr in AcOH (33%, 15.5 mL, 85.36 mmol, 1.5 equiv.) was added using a dropping funnel and the reaction was stirred at 0 ºC under N_2 for 2 h until TLC (pentane/EtOAc 4:1) showed full conversion. The solution was poured over ice and stirred until the ice was molten. The aqueous phase was extracted with DCM (x3) and the combined organic phases were washed with aq. sat. $NaHCO_3$ (sat. aq.; x1), H_2O (x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene (x3) and used immediately without any further purification. The crude product (18. g, 53.69 mmol) was dissolved in EtOAc (180 mL, 0.3 M) and zinc powder (24.58 g, 375.8 mmol, 7 equiv.) and NH_4Cl (20.10 g, 375.8 mmol, 7 equiv.) were added portion wise. The reaction was stirred at 60 ºC under N_2 for 1 h until TLC (pentane/EtOAc 4:1) showed full conversion, cooled to rt, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc + 1% Et_3N 9:1 → 7:3) gave **20** in 48% yield (6.35 g, 29.6 mmol) over 3 steps. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 6.46 (dd, *J* = 6.3, 2.0 Hz, 1H, H-1), 5.57 (dtd, *J* = 4.9, 2.0, 1.1 Hz, 1H, H-3), 5.31 – 5.25 (m, 1H, H-4), 4.63 (dt, *J* = 6.4, 2.0 Hz, 1H, H-2), 4.20 (q, *J* = 6.6 Hz, 1H, H-5), 2.15 (s, 3H, COC*H*3), 2.01 (s, 3H, COC*H*3), 1.27 (d, *J* = 6.6 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.85 (C=O), 170.55 (C=O), 146.24 (C-1), 98.39 (C-2), 72.16 (C-5), 66.37 (C-4), 65.17 (C-3), 21.00 (CO*C*H_3), 20.85 (CO*C*H_3), 16.66 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{10}H_{14}O_5Na$: 237.07389; found 237.07334

Phenyl 2-azido-2-deoxy-1-seleno-α-L-fucopyranoside (21)



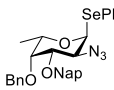
22 (6.345 g, 29.64 mmol) and $(PhSe)_2$ (9.248 g, 29.64 mmol, 1 equiv.) was dissolved in DCM (150 mL, 0.2 M) and degassed under argon at rt for 30 min. The reaction was cooled to -30 ºC and added BAIB (9.548 g, 29.64 mmol, 1 equiv.) and $TMSN_3$ (7.7 mL, 59.28 mmol, 2 equiv.). The reaction was allowed to warm to -20 ºC and stirred overnight until TLC (toluene/EtOAc 10:1) showed full conversion. Cyclohexene (10 mL) was added and the reaction was stirred at rt for 30 min before concentration *in vacuo*. The lipophilic by products were removed by column chromatography (pentane/EtOAc 10:0 → 7:3) were all the carbohydrate positive fraction were collected. The crude residue (12.399 g, 30.01 mmol) was dissolved in MeOH (100 mL, 0.3 M) and added NaOMe (1.4 mL, 6.004 mmol, 0.2 equiv.). The reaction was stirred at rt for 2 h until TLC (pentane/EtOAc 1:1) showed full conversion. The solution was neutralized with Amberlite IR-120 H^+ resins, filtered and concentrated *in vacuo*. The crude product was recrystallized in hot toluene to give **21** in 64% yield (4.296 g. 13.09 mmol) over two steps. **^{1}H NMR (400 MHz, MeOD)** δ 7.63 – 7.53 (m, 2H, Ar-*H*), 7.31 – 7.27 (m, 3H, Ar-*H*), 5.91 (d, *J* = 5.4 Hz, 1H, H-1), 4.30 (q, *J* = 6.5 Hz, 1H, H-5), 4.01 (dd, *J* = 9.9, 5.3 Hz, 1H, H-2), 3.76 – 3.67 (m, 2H, H-3, H-4), 1.15 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, MeOD)** δ 135.91 (Ar-*C*), 130.04 (Ar-*C*), 128.73 (Ar-*C*), 86.90 (C-1), 72.92 (C-4), 72.68 (C-3), 70.62 (C-5), 62.91 (C-2), 16.42 (C-6). **HRMS**: $[M+H]^+$ calculated for $C_{12}H_{15}N_3O_3SeH$: 330.03569; found 330.03514

Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-seleno-α-L-fucopyranoside (22)



21 (6.005 g, 18.25 mmol) was co-evaporated with toluene (x3) and dissolved in toluene (91 mL, 0.2 M). Bu_2SnO (4.634 g, 18.62 mmol, 1.02 equiv.) was added and the flask was equipped with a Dean Stark. The reaction was heated to 140 ºC for 3 h and the now clear solution was cooled to 60 ºC before adding Bu_4NBr (6.178 g, 19.16 mmol, 1,05 equiv.), CsF (2.828 g, 18.62 mmol, 1.02 equiv.) and NapBr (4.235 g, 19.16 mmol, 1.05 equiv.). The reaction was heated to 120 ºC for 1 h until TLC (pentane/EtOAc 3:2) showed full consumption. 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 *in vacuo*. Column chromatography (pentane/EtOAc 9:1 → 7:3) gave **22** in 91% yield (7.785 g, 26.62 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.92 – 7.83 (m, 4H, Ar-*H*), 7.63 – 7.45 (m, 5H, Ar-*H*), 7.36 – 7.27 (m, 3H, Ar-*H*), 5.91 (d, *J* = 5.3 Hz, 1H, *H-1*), 4.92 (d, *J* = 11.5 Hz, 1H, Ar-C*H*$_2$), 4.86 (d, *J* = 11.5 Hz, 1H, Ar-C*H*$_2$), 4.35 – 4.26 (m, 1H, H-5), 4.21 (dd, *J* = 10.1, 5.3 Hz, 1H, H-2), 3.91 (dt, *J* = 3.1, 1.5 Hz, 1H, H-4), 3.76 (dd, *J* = 10.2, 3.1 Hz, 1H, H-3), 2.43 (t, *J* = 1.6 Hz, 1H, OH) , 1.26 (d, *J* = 6.6 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 134.55 (Ar-*C*$_q$), 134.60 (Ar-*C*), 133.33 (Ar-*C*$_q$), 133.31 (Ar-*C*$_q$), 129.23 (Ar-C), 128.76 (Ar-*C*), 127.92 (Ar-*C*), 127.89 (Ar-*C*), 127.14 (Ar-*C*), 126.51 (Ar-*C*), 126.43 (Ar-*C*), 125.80 (Ar-*C*), 85.29 (C-1), 79.35 (C-3), 72.38 (Ar-*C*H$_2$), 68.70 (C-4, C-5), 68.68 (C-4, C-5), 60.37 (C-2), 16.17 (C-6). **HRMS**: $[M+H]^+$ calculated for $C_{23}H_{23}N_3O_3SeH$: 470.09829; found 470.09776

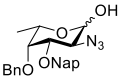
Phenyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-L-fucopyranoside (11a)



22 (3.371 g, 7.20 mmol) was dissolved in DMF (72 mL, 0.1 M) and cooled to 0 ºC. BnBr (1.1 mL, 9.35 mmol, 1.3 equiv.) and NaH (374 mg, 9.36 mmol, 1.3 equiv.) was added and the solution was stirred under N_2 at rt for 16 h until TLC (pentane/EtOAc 9:1) showed full conversion. The reaction was quenched with

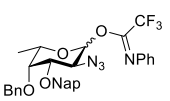
H_2O and extracted with Et_2O (x3). The combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 85:15) gave **11a** in 93% yield (3.743 g, 6.70 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.94 – 7.83 (m, 4H, Ar-*H*), 7.64 – 7.53 (m, 4H, Ar-H), 7.41 – 7.21 (m, 9H, Ar-H), 5.98 (d, *J* = 5.3 Hz, 1H, H-1), 4.99 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.97 – 4.88 (m, 2H, Ar-CH_2), 4.67 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.43 (dd, *J* = 10.3, 5.3 Hz, 1H, H-2), 4.25 (q, *J* = 6.1 Hz, 1H, H-5), 3.81 (dd, *J* = 10.3, 2.7 Hz, 1H, H-3), 3.76 (m, 1H, H-4), 1.16 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.21 (Ar-C_q), 135.06 (Ar-C_q), 133.39 (Ar-*C*), 133.19 (Ar-C_q), 129.14 (Ar-C_q), 128.80 (Ar-*C*), 128.50 (Ar-C_q), 128.42 (Ar-*C*), 128.25 (Ar-*C*), 128.10 (Ar-*C*), 127.89 (Ar-*C*), 127.86 (Ar-*C*), 127.77 (Ar-*C*), 126.72 (Ar-*C*), 126.38 (Ar-*C*), 126.22 (Ar-*C*), 125.77 (Ar-*C*), 85.64 (C-1), 80.76 (C-3), 75.94 (C-4), 72.72 (Ar-CH_2), 69.52 (Ar-CH_2), 61.13 (C-5), 16.66 (C-6). **HRMS**: $[M+NH_4]^+$ calculated for $C_{30}H_{29}N_3O_3SeNH_4$: 577.17179; found 577.17128

2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α/β-L-fucopyranose (23)



11a (3.71 g, 6.673 mmol) was dissolved in acetone/H_2O (133 mL, 10:1, 0.05 M), cooled to 0 ºC and added NIS (3 g, 13.32 mmol, 2 equiv.). The reaction was stirred at 0 ºC for 15 min. The solvents were evaporated and the residue was dissolved in EtOAc, washed with sat. aq. $Na_2S_2O_3$ (sat. aq.; x1), sat. aq. $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 8:2 → 6:4) gave **23** in 89% yield (2.497 g, 5.95 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.90 – 7.78 (m, 8H, Ar-*H*), 7.59 – 7.44 (m, 6H, Ar-*H*), 7.39 – 7.26 (m, 10H, Ar-*H*), 5.33 (t, *J* = 2.3 Hz, 1H, α-*H-1*), 4.97 (dd, *J* = 11.5, 4.4 Hz, 2H, Ar-CH_2), 4.89 (d, *J* = 9.5 Hz, 4H, Ar-CH_2), 4.68 (dd, *J* = 15.1, 11.5 Hz, 2H, Ar-CH_2), 4.47 (t, *J* = 6.9 Hz, 1H, β-*H-1*), 4.12 (q, *J* = 6.3 Hz, 1H, α-*H-5*), 4.01 (m, 2H, , α-*H-3*, α-*H-2*), 3.79 (dd, *J* = 10.3, 7.9 Hz, 1H, β-*H-2*), 3.73 (dd, *J* = 2.1, 1.2 Hz, 1H, α-*H-4*), 3.58 (dd, *J* = 2.9, 1.1 Hz, 1H, β-*H-3*), 3.48 (q, *J* = 6.4, 5.9 Hz, 1H, β-*H-5*), 3.41 (dd, *J* = 10.3, 2.8 Hz, 1H, β-*H-4*), 3.19 (d, *J* = 6.7 Hz, 1H, β-O*H*), 2.71 (d, *J* = 2.9 Hz, 1H, α-O*H*), 1.21 (d, *J* = 6.4 Hz, 2H, β-*H-6*), 1.17 (d, *J* = 6.5 Hz, 3H, α-*H-6*). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 135.42 (Ar-C_q), 133.32 (Ar-C_q), 128.54 (Ar-*C*), 128.46 (Ar-*C*), 128.11 (Ar-*C*), 128.07 (Ar-*C*), 127.94 (Ar-*C*), 127.88 (Ar-*C*), 96.56 (β-C-1), 92.60 (α-C-1), 81.05 (β-C-4), 77.87 (α-C-3), 76.21 (α-C-4), 75.03 (Ar-*C*H_2), 74.97 (Ar-*C*H_2), 72.86 (Ar-*C*H_2), 72.56 (Ar-*C*H_2), 71.19 (β-C-5), 67.02 (α-C-5), 64.95 (β-C-2), 61.08 (α-C-2), 17.06 (C-6), 16.98 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{24}H_{25}N_3O_4Na$: 442.17428; found 442.17373

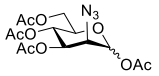
2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-L-fucopyranose (11b)



23 (1.0951 g, 2.61 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (19 mL, 0.2 M). K_2CO_3 (722 mg, 5.22 mmol, 2 equiv.) and ClC(=NPh)CF_3 (0.85 mL, 5.22 mmol, 2 equiv.) and was added and the reaction was stirred at rt under N_2 overnight until TLC (pentane/EtOAc 4:1) showed full conversion. The reaction was filtered on Celite and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 85:15) gave **11b** in 97% yield (1.492 g, 2.526 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.96 – 7.79 (m, 4H, Ar-*H*), 7.59 – 7.47 (m, 3H, Ar-*H*), 7.43 – 7.28 (m, 6H, Ar-*H*), 7.09 (td, *J* = 7.5, 1.1 Hz, 1H, Ar-*H*), 6.83 (d, *J* = 7.8 Hz, 2H, Ar-*H*), 5.47 (bs, 1H, *H-1*), 4.99 (d, *J* = 11.6 Hz, 1H, Ar-CH_2), 4.89 (s, 2H, Ar-CH_2), 4.71 (d, *J* = 11.6 Hz, 1H, Ar-CH_2), 4.09 (t, *J* = 9.3 Hz, 1H, *H-2*), 3.59 (s, 1H, *H-5*), 3.44 (s, 2H, *H-3*, *H-4*), 1.21 (d, *J*

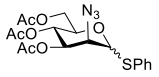
= 6.3 Hz, 3H, *H-6*). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.06 (Ar- C_q), 134.95 (Ar- C_q), 133.35 (Ar- C_q), 126.49 (Ar-*C*), 126.34 (Ar-*C*), 125.84 (Ar-*C*), 124.41 (Ar-*C*), 119.44 (Ar-*C*), 80.97 (C-3/C-4), 75.04 (Ar-*C*H_2), 74.74 (C-5), 73.00 (Ar-*C*H_2), 72.01 (C-3/C-4), 62.26 (C-2), 16.82 (C-6). **HRMS** found for the hydrolyzed donor: $[M+Na]^+$ calculated for $C_{24}H_{25}N_3O_7Na$: 442.17428; found 442.17327

1,3,4,6 Tetra-*O*-acetyl-α/β-D-mannopyranose (24)



To an ice-cool solution of NaN_3 (4.522 g, 69.55 mmol, 1.5 equiv.) in pyridine (80 mL) was slowly added Tf_2O (9.3 mL, 55.65 mmol, 1.2 equiv.) and the resulting orange mixture was stirred at 0 ºC for 2 h. Mannosamine hydrochloride (10 g, 46.37 mmol) was dissolved in pyridine (47 mL) and added Et_3N (12.9 mL, 92.74 mmol, 2 equiv.) and $CuSO_4 \cdot 5\ H_2O$ (116 mg, 0.46 mmol, 0.01 equiv.) dissolved in as little H_2O as possible. The resulting blue mixture was cooled to 0 ºC and the freshly made TfN_3 solution was added dropwise via a dropping funnel. The resulting green mixture was stirred at 0 ºC for 4 h until TLC (DCM/MeOH/Et_3N 20:75:5) showed full conversion of the starting material. The solution turned yellow. Ac_2O (48.2 mL) was added and the reaction was stirred overnight after which TLC (pentane/EtOAc 3:2) showed full conversion. The reaction was dissolved in EtOAc and the organic phase was washed with 1 M HCl aq. (x3), sat. aq. $NaHCO_3$ (sat. aq.; x3), H_2O (x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo* and co-evaporated with toluene x2 to remove pyridine and **24** was obtained in an quant. yield and a α/β ratio on 5:2. Used without further purification. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 6.12 (d, *J* = 1.9 Hz, 1H, α-H-1), 5.84 (d, *J* = 1.4 Hz, 1H, β-H-1), 5.43 – 5.34 (m, 2H, α-H-4, α-H-3), 5.30 (t, *J* = 9.9 Hz, 1H, β-H-4), 5.07 (dd, *J* = 9.8, 3.7 Hz, 1H, β-H-3), 4.27 (m, 2H, α/β-H-6), 4.18 – 4.12 (m, 1H, β-H-2), 4.09 (dd, *J* = 12.4, 2.4 Hz, 2H, α/β-H-6), 4.06 – 3.98 (m, 2H, α-H-2, α-H-5), 3.74 (ddd, *J* = 9.9, 4.8, 2.3 Hz, 1H, β-H-5), 2.19 (s, 3H, β-COC*H*$_3$), 2.17 (s, 3H, α-COC*H*$_3$), 2.12 (d, *J* = 1.1 Hz, 6H, α/β-COC*H*$_3$), 2.10 (s, 3H, α-COC*H*$_3$), 2.09 (s, 3H, β-COC*H*$_3$), 2.06 (s, 3H, α-COC*H*$_3$), 2.05 (s, 3H, β-COC*H*$_3$). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.93 (C=O), 170.23 (C=O), 169.53 (C=O), 168.39 (C=O), 91.52 (α -C-1), 91.33 (β-C-1), 73.45 (β-C-5), 72.02 (β-C-3), 70.89 (α -C-3), 70.70 (α-C-5), 65.43 (α-C-4), 65.01 (β-C-4), 61.90 (α-C-6), 61.84 (β-C-6), 61.20 (β-C-2), 60.65 (α-C-2), 21.05 (CO*C*H$_3$), 20.88 (CO*C*H$_3$), 20.77 (CO*C*H$_3$), 20.68 (CO*C*H$_3$). **HRMS**: $[M+Na]^+$ calculated for $C_{14}H_{19}N_3O_9Na$: 396.10190; found 396.10135

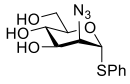
Phenyl 3,4,6 tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α/β-D-mannopyranoside[49] (25)



To an ice-cooled solution of **24** (17.09 g, 45.78 mmol) in dry DCM (230 mL, 0.2 M) was slowly added PhSH (4.7 mL, 45.78 mmol, 1 equiv.) and BF_3OEt_2 (11.3 mL, 91.56 mmol, 2 equiv.) the resulting mixture was allowed to warm to rt and stirred under N_2 until TLC (pentane/EtOAc 3:2) showed full composition of the starting material (3 days). The reaction was quenched with Et_3N, diluted in DCM and the organic phase was washed with sat. aq. $NaHCO_3$ (sat. aq.; x1), 1 M NaOH (x3), H_2O (x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 90:10 → 70:30) gave **25** in 88% yield (16.99 g, 40.12 mmol) with a α/β ratio on 89:11. NMR reported for the α-anomer. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.54 – 7.44 (m, 2H, Ar-*H*), 7.37 – 7.29 (m, 3H, Ar-*H*), 5.53 (d, *J* = 1.0 Hz, 1H, *H-1*), 5.39 – 5.33 (m, 2H, H-3, H-*4*), 4.52 – 4.46 (m, 1H, *H-5*), 4.31 – 4.24 (m, 2H, H-2, H-6), 4.08 (dd, *J* = 12.3, 2.4 Hz, 1H, H-6), 2.12 (s, 3H, COC*H*$_3$), 2.08 (s, 3H, COC*H*$_3$), 2.06 (s, 3H, COC*H*$_3$). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.84 (C=O), 170.11 (C=O), 169.63 (C=O), 132.08 (Ar-*C*), 129.45 (Ar-*C*), 128.40

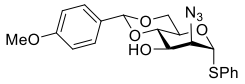
(Ar-*C*), 85.96 (C-1), 71.30 (C-4), 69.68 (C-5), 66.17 (C-3), 62.82 (C-2), 62.29 (C-6), 20.86 (CO*C*H$_3$), 20.83 (CO*C*H$_3$), 20.72 (CO*C*H$_3$). **HRMS**: [M+Na]$^+$ calculated for $C_{18}H_{21}N_3O_7SNa$: 446.09979; found 446.09924

Phenyl 2-azido-2-deoxy-1-thio-α-D-mannopyranoside (26)



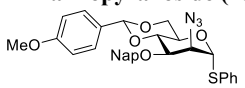
α-**25** (14.95 g, 35.30 mmol) was dissolved in MeOH (117 mL, 0.3 M) and added NaOMe (25% wt in MeOH, 0.8 mL, 3.53 mmol, 0.1 equiv.). The reaction was stirred at rt for 3 h until TLC (pentane/EtOAc 4:6) showed full conversion and then neutralized with Amberlite IR-120 H$^+$ resins, filtered and concentrated. The crude **26** was isolated in 90% (9.42 g, 31.69 mmol) and used without further purification. **^{1}H NMR (400 MHz, MeOD)** δ 7.57 – 7.50 (m, 2H, Ar-*H*), 7.36 – 7.26 (m, 3H, Ar-*H*), 5.48 (d, *J* = 1.4 Hz, 1H, H-1), 4.14 (dd, *J* = 3.8, 1.5 Hz, 1H, H-2), 4.06 – 4.01 (m, 1H, H-5), 3.94 (dd, *J* = 9.3, 3.8 Hz, 1H, H-3), 3.81 (dd, *J* = 12.1, 2.4 Hz, 1H, H-6), 3.72 (dd, *J* = 12.1, 5.7 Hz, 1H, H-6), 3.67 (t, *J* = 9.5 Hz, 1H, H-4). **^{13}C NMR (101 MHz, MeOD)** δ 135.99 (Ar- *C$_q$*), 133.73 (Ar-*C*), 130.16 (Ar-*C*), 128.31 (Ar-*C*), 87.29 (C-1), 75.82 (C-5), 73.09 (C-3), 69.30 (C-4), 67.10 (C-2), 60.72 (C-6). **HRMS**: [M+Na]$^+$ calculated for $C_{12}H_{15}N_3O_4SNa$: 320.06810; found 320.06755

Phenyl 2-azido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-1-thio-α-D-mannopyranoside (27)



26 (9.47 g, 31.85 mmol) was co-evaporated with toluene (x3) and dissolved in dry MeCN (160 mL, 0.2 M). Anisaldehyde dimethyl acetal (7 mL, 41.41 mmol, 2 equiv.) and camphorsulfonic acid (370 mg, 1.59 mmol, 5 mol%) was added sequentially and the reaction was stirred on the rotary evaporator (300 mbar at 50 ºC) until TLC (pentane/EtOAc 7:3) showed full conversion (~1 h). The reaction was quenched with Et$_3$N and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 75:25) gave **27** in 90% (11.93 g, 28.72 mmol). **^{1}H NMR (500 MHz, CDCl$_3$)** δ 7.50 – 7.39 (m, 4H, Ar-*H*), 7.39 – 7.29 (m, 3H, Ar-*H*), 6.95 – 6.88 (m, 2H, Ar-*H*), 5.55 (s, 1H, PMP-C*H*), 5.47 (d, *J* = 1.2 Hz, 1H, H-1), 4.33-4.28 (td, *J* = 9.7, 4.9 Hz, 1H, H-5), 4.26-4.23 (dt, *J* = 9.7, 3.9 Hz, 1H, H-3), 4.22 – 4.20 (m, 1H, H-2), 4.19 (d, *J* = 5.0 Hz, 1H, H-6), 3.81 (s, 3H, OC*H*$_3$), 3.79 (d, *J* = 10.3 Hz, 1H, H-6), 2.82 (d, *J* = 3.9 Hz, 1H, OH). **^{13}C NMR (126 MHz, CDCl$_3$)** δ 160.48 (Ar-*C$_q$*), 133.11 (Ar-*C$_q$*), 132.06 (Ar-*C*), 130.4 (Ar-*C$_q$*), 129.42 (Ar-*C*), 128.25 (Ar-*C*), 127.77 (Ar-*C*) 113.92 (Ar-*C*), 102.43 (PMB-*C*H), 87.65 (C-1), 79.16 (C-4), 69.39 (C-3), 68.43 (C-6), 65.20 (C-2), 64.73 (C-5), 55.46 (O*C*H$_3$). **HRMS**: [M+H]$^+$ calculated for $C_{20}H_{21}N_3O_5SH$: 416.12802; found 416.12876

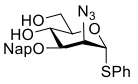
Phenyl 2-azido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-3-*O*-(2-naphthylmethyl)-1-thio-α-D- mannopyranoside (28)



27 (11.07 g, 26.65 mmol) was co-evaporated with toluene (x3), dissolved in DMF (266 mL, 0.1 M) and cooled to 0 ºC. NaH (60% in mineral oil, 1.386 g, 34.64 mmol, 1.3 equiv.) was added and the mixture was stirred for 20 min. Then NapBr (7.656 g, 34.64 mmol, 1.3 equiv.) was added and the reaction was slowly allowed to warm to rt and stirred for 22 h (overnight) under N$_2$ after which TLC (pentane/EtOAc 4:1) showed full conversion. The reaction was quenched with H$_2$O and extracted with Et$_2$O (x3). The combined organic phases was washed with brine (x1) and dried over Na$_2$SO$_4$, filtered and concentrated *in vacuo.* Column chromatography (pentane/EtOAc 95:5 → 80:20) gave **28** in 98% yield (14.46 g, 2602 mmol). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.88 – 7.81 (m, 3H, Ar-*H*), 7.54 – 7.46 (m, 3H, Ar-*H*), 7.47 – 7.43 (m, 2H, Ar-*H*),

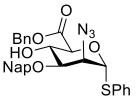
7.42 – 7.37 (m, 2H, Ar-*H*), 7.32 – 7.28 (m, 3H, Ar-*H*), 6.96 – 6.87 (m, 2H, Ar-*H*), 5.62 (s, 1H, PMP-C*H*), 5.43 (d, *J* = 1.1 Hz, 1H, H-1), 5.07 (d, *J* = 12.7 Hz, 1H, Ar-CH_2), 4.92 (d, *J* = 12.4 Hz, 1H, Ar-CH_2), 4.32 (m, 1H, H-5), 4.25 – 4.15 (m, 4H, H-2, H-3, H-4, H-6), 3.87 – 3.84 (m, 1H, H-6) 3.84 (s, 3H, OCH_3). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 160.23 (Ar-C_q), 135.35 (Ar-C_q), 133.41 (Ar-C_q), 133.17 (Ar-C_q), 132.91 (Ar-C_q), 132.14 (Ar-*C*), 129.95 (Ar-C_q), 129.39 (Ar-*C*), 128.43 (Ar-*C*), 128.25 (Ar-*C*), 128.17 (Ar-*C*), 127.83 (Ar-*C*), 127.59 (Ar-*C*), 126.58 (Ar-*C*), 126.27 (Ar-*C*), 126.13 (Ar-*C*), 125.64 (Ar-*C*), 113.75 (Ar-*C*), 101.87 (PMP-*C*H), 87.34 (C-1), 79.19 (C-3/C-4), 75.95 (C-3/C-4), 73.55 (Ar-*C*H_2), 68.46 (C-6), 65.33 (C-5), 64.27 (C-2), 55.45 (O*C*H_3). **HRMS**: $[M+H]^+$ calculated for $C_{31}H_{29}N_3O_5SH$: 556.19062; found 556.19007

Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-thio-α-D-mannopyranoside (29)



28 (14.46 g, 26.05 mmol) was co-evaporated with toluene (x2) and dissolved in MeOH (0.1 M). CSA (605 mg, 2.61 mmol, 0.1 equiv.) was added and the reaction was stirred for 1 h at rt until TLC (pentane/EtOAc 4:1) showed full conversion. The reaction was quenched with Et_3N and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 80:20 → 50:50) gave **29** in 88% yield (9.97 g, 22.79 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.92 – 7.81 (m, 4H, Ar-*H*), 7.58 – 7.47 (m, 3H, Ar-*H*), 7.46 – 7.36 (m, 2H, Ar-*H*), 7.33 – 7.28 (m, 3H, Ar- *H*), 5.42 (d, *J* = 1.5 Hz, 1H, H-1), 4.92 (d, *J* = 11.7 Hz, 1H, Ar-CH_2), 4.84 (d, *J* = 11.7 Hz, 1H, Ar-CH_2), 4.18 – 4.10 (m, 2H, H-2, H-5), 4.05 (td, *J* = 9.3, 2.6 Hz, 1H, H-4), 3.94 (dd, *J* = 9.1, 3.5 Hz, 1H, H-3), 3.83 (dt, *J* = 4.9, 2.8 Hz, 2H, H-6), 2.80 (d, *J* = 3.0 Hz, 1H, C4-OH), 2.10 – 1.97 (m, 1H, C6-OH). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 134.63 (Ar-C_q), 133.38 (Ar-C_q), 133.33 (Ar-*C*), 132.98 (Ar-*C*), 132.35 (Ar-*C*), 129.39 (Ar-*C*), 128.84 (Ar-*C*), 128.31 (Ar-*C*), 128.16 (Ar-*C*), 127.91 (Ar-*C*), 127.39 (Ar-*C*), 126.53 (Ar-*C*), 126.45 (Ar-*C*), 125.95 (Ar-*C*), 86.63 (C-1), 79.67 (C-3), 73.41 (C-5), 72.56 (*C*H_2-Ar), 67.22 (C-4), 62.36 (C-6), 62.09 (C-2). **HRMS**: $[M+Na]^+$ calculated for $C_{23}H_{23}N_3O_4SNa$: 460.13073; found 460.13015

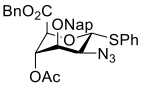
Benzyl (phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-thio-α-D-mannopyranosiduronate) (30)



29 (9.95 g, 22.76 mmol) was dissolved in DCM/H_2O/*t*-BuOH (8:4:1, 114 mL, 0.2 M) and under vigorous stirring added AcOH (0.26 mL, 4.55 mmol, 0.2 equiv.), TEMPO (711 mg, 4.55 mmol, 0.2 equiv.) and $PhI(AcO)_2$ (BAIB, 18.32 g, 56.89 mmol, 2.5 equiv.). The reaction was stirred at 4 ºC overnight until full consumption on TLC (pentane/EtOAc 1:1) was observed. The reaction was quenched with $Na_2S_2O_3$ (aq., sat.) and the aqueous phase was extracted with EtOAc (x3). The combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene (x3) and used without any further purifications. The crude product (22.76 mmol) was dissolved in DMF (230 mL, 0.1 M) and cooled to 0 ºC. K_2CO_3 (6.291 g, 45.55 mmol, 1.5 equiv.) and BnBr (5.4 mL, 45.52 mmol, 1.5 equiv.) were added and the reaction was stirred overnight until TLC (pentane/EtOAc 7:3) showed full conversion. The reaction was quenched with H_2O and extracted with Et_2O (x3). The combined organic phase was washed with H_2O (x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 90:10 → 70:30) gave **30** in 75% yield (9.30 g, 17.17 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.90 – 7.80 (m, 4H, Ar-*H*), 7.52 – 7.43 (m, 5H, Ar-*H*), 7.36 – 7.32 (m, 3H, Ar-*H*), 7.30 – 7.26 (m, 2H, Ar-*H*), 7.25 – 7.16 (m, 3H, Ar-*H*), 5.52 (d, *J* = 2.9 Hz, 1H, H-1), 5.16 (s, 2H, Ar-CH_2), 4.95 (d, *J* = 11.9 Hz, 1H, Ar-CH_2),

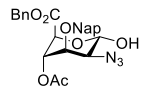
4.88 (d, *J* = 11.8 Hz, 1H, Ar-CH_2), 4.68 (d, *J* = 8.0, 1H, H-5), 4.38 (td, *J* = 7.9, 3.5 Hz, 1H, H-4), 4.00 – 3.91 (m, 2H, H-2, H-3), 2.88 (d, *J* = 3.5 Hz, 1H, C4-OH). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.80 (C-6), 135.06 (Ar-*C*), 134.83 (Ar-*C*), 133.38 (Ar-*C*), 133.28 (Ar-*C*), 132.32 (Ar-*C*), 129.25 (Ar-*C*), 128.75 (Ar-*C*), 128.63 (Ar-*C*), 128.61 (Ar-*C*), 128.31 (Ar-*C*), 128.25 (Ar-*C*), 128.16 (Ar-*C*), 127.88 (Ar-*C*), 127.10 (Ar-*C*), 126.41 (Ar-*C*), 126.31 (Ar-*C*), 125.89 (Ar-*C*), 85.70 (C-1), 78.10 (C-2), 73.63 (Ar-CH_2), 72.95 (C-5), 68.64 (C-4), 67.53 (Ar-CH_2), 61.38 (C-3). **HRMS**: $[M+Na]^+$ calculated for $C_{30}H_{27}N_3O_5SNa$: 564.15691; found 564.15636

Benzyl (phenyl 4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-thio-α-D-mannopyranosiduronate) (12a)



30 (1.662 g, 3.05 mmol) was dissolved in pyridine (15 mL, 0.2 M) and cooled to 0 ºC. Ac_2O (0.57 mL, 6.10 mmol, 2 equiv.) and DMAP (74 mg, 0.61 mmol, 0.2 equiv.) was added and the reaction was stirred under N_2 for 30 min until TLC (pentane/EtOAc 3:1) showed full conversion. The reaction was quenched with MeOH, diluted in EtOAc and washed with 1 M HCl (x3), sat. $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 9:1 → 6:4) gave **12a** in 95% yield (1.70 g, 2.91 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.84 – 7.77 (m, 3H, Ar-*H*), 7.75 (d, *J* = 1.7 Hz, 1H, Ar-*H*), 7.65 – 7.55 (m, 2H, Ar-*H*), 7.52 – 7.39 (m, 3H, Ar-*H*), 7.24 (dt, *J* = 5.1, 2.5 Hz, 6H, Ar-*H)*, 7.15 – 7.07 (m, 2H, Ar-*H*), 5.78 (d, *J* = 9.3 Hz, 1H, H-1), 5.62 (dd, *J* = 4.8, 2.9 Hz, 1H, H-4), 5.01 (d, *J* = 12.1 Hz, 1H, Ar-CH_2), 4.82 (d, *J* = 12.2 Hz, 1H, Ar-CH_2), 4.67 (s, 2H, Ar-CH_2), 4.62 (d, *J* = 2.9 Hz, 1H, H-5), 3.98 (dd, *J* = 4.7, 3.0 Hz, 1H, H-3), 3.45 (dd, *J* = 9.5, 2.9 Hz, 1H, H-2), 2.02 (s, 3H, COC*H*$_3$). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.74 (C-6), 167.86 (C=O), 134.84 (Ar-C_q), 133.96 (Ar-C_q), 133.25 (Ar-C_q), 133.21 (Ar-*C*), 132.45 (Ar-C_q), 131.91 (Ar-*C*), 128.99 (Ar-*C*), 128.65 (Ar-*C*), 128.58 (Ar-*C*), 128.48 (Ar-*C*), 128.11 (Ar-*C*), 128.02 (Ar-*C*), 127.82 (Ar-*C*), 127.36 (Ar-*C*), 126.38 (Ar-*C*), 126.34 (Ar-*C*), 125.93 (Ar-*C*), 81.09 (C-1), 74.62 (C-3), 73.66 (C-5), 73.02 (Ar-*C*H$_2$), 68.47 (C-4), 67.53 (Ar-*C*H$_2$), 57.90 (C-2), 21.00 (CO*C*H$_3$). **HRMS**: $[M+Na]^+$ calculated for $C_{32}H_{29}N_3O_6SNa$: 606.16743; found 606.16693

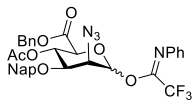
Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-D-mannopyranosiduronate) (31)



12a (1.327 g, 2.27 mmol) was co-evaporated with toluene (x3), dissolved in dry DCM (23 mL, 0.1 M) and cooled to 0 ºC. NIS (767 mg, 3.41 mmol, 1.5 equiv.) and TFA (0.17 mL, 2.27 mmol, 1 equiv.) was added and the reaction was stirred at 0 ºC under N_2 until TLC (pentane/EtOAc 7:3) showed full conversion (~4 h). The reaction was quenched with Et_3N (1 equiv.) and $NaHCO_3$ (sat. aq.) was added and the solution was stirred vigorously. The solution was diluted in EtOAc, washed with $Na_2S_2O_3$ (sat. aq.; x1), sat. $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated. Column chromatography (pentane/EtOAc 8:2 → 6:4) gave **31** in 75% yield (833 mg, 1.69 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.81 (dd, *J* = 8.3, 2.7 Hz, 3H, Ar-*H*), 7.73 (d, *J* = 1.6 Hz, 1H, Ar-*H*), 7.54 – 7.43 (m, 2H, Ar-*H*), 7.40 (dd, *J* = 8.5, 1.7 Hz, 1H, Ar-*H*), 7.35 – 7.21 (m, 4H, Ar-*H*), 7.18 (dd, *J* = 6.7, 3.0 Hz, 2H, Ar-*H*), 5.66 (dd, *J* = 6.6, 4.4 Hz, 1H, H-1), 5.55 (dd, *J* = 5.3, 3.9 Hz, 1H, H-4), 5.05 (d, *J* = 12.1 Hz, 1H, Ar-C*H*$_2$), 4.86 (d, *J* = 12.2 Hz, 1H, Ar-C*H*$_2$), 4.74 – 4.65 (dd, 2H, *J* = 11.5, 5.5 Hz, , Ar-C*H*$_2$), 4.57 (d, *J* = 3.9 Hz, 1H, H-5), 3.99 (dd, *J* = 5.4, 3.1 Hz, 1H, H-3), 3.94 (d, *J* = 4.8 Hz, 1H, OH), 3.62 (dd, *J* = 6.7, 3.1 Hz, 1H, H-2), 2.01 (s, 3H, COC*H*$_3$). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.91 (C=O), 168.34 (C=O), 134.84 (Ar-C_q), 133.25

(Ar-C_q), 133.20 (Ar-C_q), 128.69 (Ar-*C*), 128.66 (Ar-*C*), 128.40 (Ar-*C*), 128.09 (Ar-*C*), 127.83 (Ar-*C*), 126.81 (Ar-*C*), 126.38 (Ar-*C*), 126.26 (Ar-*C*), 125.67 (Ar-*C*), 91.65 (C-1), 75.09 (C-3), 72.98 (C-5), 72.37 (Ar-*C*H_2), 68.88 (C-4), 67.73 (Ar-*C*H_2), 60.60 (C-2), 21.01 (CO*C*H_3). **HRMS**: $[M+Na]^+$ calculated for $C_{26}H_{25}N_3O_7Na$: 514.15902; found 514.15847

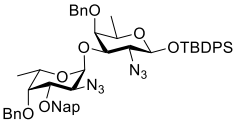
Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-mannopyranosiduronate) (12b)



31 (1.656 g, 2.37 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (12 mL, 0.2 M). K_2CO_3 (656 mg, 4.74 mmol, 2 equiv.) and ClC(=NPh)CF_3 (0.77 mL, 4.74 mmol, 2 equiv.) were added and the reaction was stirred overnight at rt under N_2 until TLC (pentane/EtOAc 4:1) showed full conversion. The reaction was filtered on Celite and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 9:1 → 6:4) gave **12b** in 95% yield (1.49 g, 2.249 mmol). **^{1}H NMR (400 MHz, CD_3CN)** δ 7.93 – 7.77 (m, 6H, Ar-*H*), 7.56 – 7.41 (m, 4H, Ar-*H*), 7.43 – 7.19 (m, 10H, Ar-*H*), 7.19 – 7.07 (m, 2H, Ar-*H*), 6.79 (d, *J* = 8.1 Hz, 3H, Ar-*H*), 6.40 (bs, 1H, H-1), 5.41 (t, *J* = 6.8 Hz, 1H, H-4), 5.07 (d, *J* = 12.1 Hz, 1H, Ar-C*H*$_2$), 4.96 (d, *J* = 12.2 Hz, 1H, Ar-C*H*$_2$), 4.79 (d, *J* = 2.7 Hz, 3H, Ar-C*H*$_2$), 4.48 (d, *J* = 6.2 Hz, 1H, H-5), 4.19 – 4.10 (m, 2H, H-2/H-3), 2.12 (s, 3H, CO*C*H_3). **^{13}C NMR (101 MHz, CD_3CN)** δ 135.88 (Ar-C_q), 134.08 (Ar-C_q), 129.81 (Ar-*C*), 129.45 (Ar-*C*), 129.43 (Ar-*C*), 129.36 (Ar-*C*), 129.10 (Ar-*C*), 128.74 (Ar-*C*), 128.54 (Ar-*C*), 127.96 (Ar-*C*), 127.29 (Ar-*C*), 127.20 (Ar-*C*), 127.03 (Ar-*C*), 75.77 (C-3), 73.61 (C-5/ Ar-*C*H_2), 68.35 (C-4), 68.28 (Ar-*C*H_2), 60.04 (C-2), 29.62 (C-O*C*H_3). **HRMS**: $[M+Na]^+$ calculated for $C_{34}H_{39}F_3N_4O_7Na$: 685.1886; found 685.18778

Synthesis of the trisaccharide

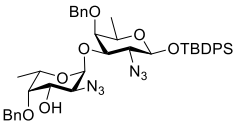
***Tert*-butyldiphenylsilyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-β-D-fucopyranoside (32)**



Donor **11b** (2.46 g, 4.171 mmol, 1.3 equiv.) and acceptor **10** (1.661 g, 3.21 mmol, 1 equiv.) was co-evaporated with toluene (3x), dissolved in dry DCM (32 mL, 0.1 M), added 3Å molecular sieves at rt and stirred for 30 min. TBSOTf (0.15 mL, 0.64 mmol, 0.2 equiv.) was added at rt and the reaction was stirred at rt until TLC (pentane/EtOAc 9:1) showed full conversion of the acceptor (~30 min). The reaction was quenched with Et_3N, dissolved in EtOAc, washed with $NaHCO_3$ (sat. aq.; x1), brine (x1), dried over Na_2SO_4 and concentrated. Column chromatography (pentane/EtOAc 95:5 → 80:20) gave **32** in 86% yield (2.55 g, 2.77 mmol) and in a α/β ratio 95:5. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.85 – 7.80 (m, 2H, Ar-*H*), 7.80 – 7.70 (m, 6H, Ar-*H*), 7.53 – 7.27 (m, 19H, Ar-*H*), 5.24 (d, *J* = 2.3 Hz, 1H, H-1'), 4.95 (d, *J* = 11.5 Hz, 1H, Ar-C*H*$_2$), 4.88 (d, *J* = 11.4 Hz, 1H, Ar-C*H*$_2$), 4.80 (d, *J* = 12.0 Hz, 1H, Ar-C*H*$_2$), 4.72 (d, *J* = 11.7 Hz, 1H, Ar-C*H*$_2$), 4.61 (dd, *J* = 11.6, 5.5 Hz, 2H, Ar-C*H*$_2$), 4.34 (d, *J* = 7.7 Hz, 1H, H-1), 3.93 – 3.89 (m, 1H, H-2), 3.87 (t, *J* = 1.3 Hz, 2H, H-3', H-2'), 3.78 (q, *J* = 6.4 Hz, 1H, H-5'), 3.55 (d, *J* = 1.5 Hz, 1H, H-4'), 3.37 (dd, *J* = 10.6, 2.9 Hz, 1H, H-4), 3.29 (dd, *J* = 3.0, 1.0 Hz, 1H, H-5), 3.17 (q, *J* = 6.9 Hz, 1H, H-5), 1.12 (s, 9H, TBDPS-C*H*$_3$), 1.06 (d, *J* = 6.5 Hz, 3H, H-6'), 1.04 (d, *J* = 6.4 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.61 (Ar-C_q), 138.22 (Ar-C_q), 136.23 (Ar-*C*), 136.06 (Ar-*C*), 135.21 (Ar-C_q), 133.53 (Ar-C_q), 133.38

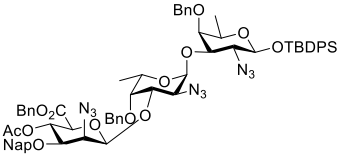
(Ar-C_q), 133.20 (Ar-C_q), 133.10 (Ar-C_q), 129.88 (Ar-*C*), 128.51 (Ar-*C*), 128.48 (Ar-*C*), 128.45 (Ar-*C*), 128.08 (Ar-*C*), 127.95 (Ar-*C*), 127.84 (Ar-*C*), 127.79 (Ar-*C*), 127.60 (Ar-*C*), 127.31 (Ar-*C*), 126.80 (Ar-*C*), 126.35 (Ar-*C*), 126.21 (Ar-*C*), 125.92 (Ar-*C*), 100.07 (C-1'), 97.35 (C-1), 79.30 (C-4), 78.90 (C-3), 77.16 (C-3'), 76.42 (C-4'), 75.41 (Ar-*C*H_2), 75.06 (Ar-*C*H_2), 72.75 (Ar-*C*H_2), 70.73 (C-5), 67.51 (C-5'), 66.54 (C-2'), 59.70 (C-2), 26.99 (TBDPS-*C*H_3), 16.86 (C-6), 16.67 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{53}H_{58}N_6O_7SiNa$: 941.40339; found 941.40285

***Tert*-butyldiphenylsilyl 2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-β-D-fucopyranoside (40)**



32 (1.34 g, 1.45 mmol) was dissolved in DCM/H_2O (14.5 mL, 20:1, 0.1 M), added DDQ (660 mg, 2.91 mmol, 2 equiv.) stirred at rt under N_2 for 1.5 h until TLC (pentane/EtOAc 9:1) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (aq., sat.), dissolved in EtOAc and extracted (x3), and the combined organic phases were washed with sat. aq. $NaHCO_3$ (sat. aq.; x4, until the yellow color disappeared) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 80:20) gave **40** in 86% yield (976 mg, 1.25 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.83 – 7.69 (m, 4H, Ar-*H*), 7.48 – 7.29 (m, 16H, Ar-*H*), 5.23 (d, *J* = 3.7 Hz, 1H, H-1'), 4.77 (d, *J* = 11.6 Hz, 2H, Ar-CH_2), 4.69 (dd, *J* = 11.7, 8.5 Hz, 2H, Ar-CH_2), 4.36 (d, *J* = 7.7 Hz, 1H, H-1), 3.91 (dd, *J* = 10.5, 7.7 Hz, 1H, H-2), 3.86 (dd, *J* = 10.9, 3.4 Hz, 1H, H-4'), 3.83 – 3.77 (m, 1H, H-5'), 3.51 (dd, *J* = 3.4, 1.3 Hz, 1H, H-3'), 3.44 – 3.31 (m, 3H, H-2', H-3, H-4), 3.19 (q, *J* = 7.1, 6.5 Hz, 1H, H-5), 1.17 (d, *J* = 6.6 Hz, 3H, H-6'), 1.13 (s, 9H, TBDPS-CH_3), 1.08 (d, *J* = 6.4 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.55 (Ar-C_q), 137.82 (Ar-C_q), 136.22 (Ar-*C*), 136.03 (Ar-*C*), 133.52 (Ar-C_q), 133.10 (Ar-C_q), 129.87 (Ar-*C*), 129.66 (Ar-*C*), 128.80 (Ar-*C*), 128.50 (Ar-*C*), 128.36 (Ar-*C*), 128.32 (Ar-*C*), 127.85 (Ar-*C*), 127.58 (Ar-*C*), 127.30 (Ar-*C*), 100.01 (C-1'), 97.45 (C-1), 79.80 (C-3'), 79.38 (C-4), 78.66 (C-3), 76.20 (Ar-*C*H_2), 75.45 (Ar-*C*H_2), 70.81 (C-5), 68.45 (C-4'), 67.27 (C-5'), 66.49 (C-2), 60.74 (C-2'), 26.99 (TBDPS-*C*H_2), 16.86 (C-6), 16.70 (C-6').**HRMS**: $[M+Na]^+$ calculated for $C_{42}H_{50}N_6O_7SiNa$: 801.34079; found 801.34025

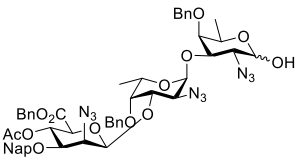
***Tert*-butyldiphenylsilyl (Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-β-D-mannopyranosiduronsyl)-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-β-D-fucopyranoside (9)**



Donor **12b** (1.02 g, 1.54 mmol, 1.5 equiv.) and acceptor **40** (780 mg, 1.00 mmol, 1 equiv.) was co-evaporated with toluene (3x), dissolved in dry DCM (10 mL, 0.1 M), added 3Å molecular sieves and stirred for 30 min. The solution was cooled to -80 ºC and TfOH (18 µL, 0.20 mmol, 0.2 equiv.) was added. The reaction was allowed to warm to -10 ºC and stirred until TLC (pentane/EtOAc 8:2) showed full conversion of the acceptor (~5 h). 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 (pentane/EtOAc 95:5 → 80:20) gave **9** in 68% (851 mg, 0.68 mmol) and in a α/β ratio 15:85. <u>For the β-anomer</u>: **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.83 – 7.68 (m, 8H, Ar-*H*), 7.52 – 7.27 (m, 24H, Ar-*H*), 5.46 (t, *J* = 9.2 Hz, 1H, H-4''), 5.19 (dd, *J* = 12.2, 3.7 Hz, 1H, H-1'), 5.05 (dd, *J* = 12.2, 1.6 Hz, 2H, Ar-CH_2), 4.83 – 4.71 (m, 3H, Ar-CH_2), 4.66 (d, *J* = 7.2 Hz, 1H, Ar-CH_2), 4.63 (d, *J* = 6.9 Hz, 1H, Ar-

CH_2), 4.55 (d, *J* = 1.5 Hz, 1H, H-1''), 4.52 (d, *J* = 11.7 Hz, 1H, Ar-CH_2), 4.33 (d, *J* = 7.7 Hz, 1H, H-1), 4.16 (dd, *J* = 10.7, 2.9 Hz, 1H, H-3'), 3.91 – 3.86 (m, 1H, H-2), 3.84 (d, *J* = 9.3 Hz, 1H, H-5''), 3.75 – 3.65 (m, 2H, H-5', H-2'), 3.63 (dd, *J* = 3.6, 1.4 Hz, 1H, H-2''), 3.59 (dd, *J* = 9.1, 3.5 Hz, 1H, H-3''), 3.49 (dd, *J* = 3.0, 1.3 Hz, 1H, H-4'), 3.33 (dd, *J* = 10.5, 3.0 Hz, 1H, H-3), 3.15 (q, *J* = 6.3 Hz, 1H, H-4), 3.20 – 3.11 (q, *J* = 6.3 Hz, 1H, H-4), 1.86 (s, 3H, , COCH_3), 1.11 (s, 9H, TBDPS-CH_3), 1.07 (d, *J* = 6.6 Hz, 3H, H-6'), 1.05 (d, *J* = 6.3 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.32 (*C*=O), 166.55 (*C*=O), 138.81 (Ar-C_q), 138.11 (Ar-C_q), 136.24 (Ar-*C*), 136.06 (Ar-*C*), 134.67 (Ar-C_q), 133.80 (Ar-C_q), 133.59 (Ar-C_q), 133.26 (Ar-C_q), 133.08 (Ar-C_q), 129.88 (Ar-*C*), 129.65 (Ar-*C*), 128.88 (Ar-*C*), 128.68 (Ar-*C*), 128.64 (Ar-*C*), 128.55 (Ar-*C*), 128.45 (Ar-*C*), 128.32 (Ar-*C*), 128.07 (Ar-*C*), 127.95 (Ar-*C*), 127.92 (Ar-*C*), 127.77 (Ar-*C*), 127.60 (Ar-*C*), 127.30 (Ar-*C*), 126.91 (Ar-*C*), 126.58 (Ar-*C*), 126.42 (Ar-*C*), 125.71 (Ar-*C*), 100.14 (C-1'), 97.61 (C-1''), 97.38 (C-1), 79.42 (C-4), 78.99 (C-3), 77.48 (C-4'), 77.16 (C-4''), 75.57 (Ar-*C*H_2), 75.47 (C-3'), 75.05 (Ar-*C*H_2), 73.85 (C-5''), 72.45 (Ar-*C*H_2), 70.75 (C-5), 68.09 (C-4''), 67.81 (Ar-*C*H_2), 67.20 (C-5'), 66.30 (C-2), 61.47 (C-2''), 58.55 (C-2'), 27.00 (TBDPS-*C*H_3), 20.79 (CO*C*H_3), 16.77 (C-6', C-6). <u>For the α-anomer</u>: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.87 – 7.69 (m, 13H, Ar-*H*), 7.53 – 7.27 (m, 32H, Ar-*H*), 7.25 – 7.13 (m, 7H, Ar-*H*), 7.07 – 6.98 (m, 2H, Ar-*H*), 5.70 (d, *J* = 7.5 Hz, 1H, H-1''), 5.54 (dd, *J* = 4.6, 2.8 Hz, 1H, H-4''), 5.36 (d, *J* = 3.8 Hz, 1H, H-1'), 5.05 (d, *J* = 11.7 Hz, 2H, Ar-CH_2), 4.94 (d, *J* = 11.6 Hz, 1H, Ar-CH_2), 4.82 – 4.59 (m, 9H, Ar-CH_2), 4.59 – 4.49 (m, 2H, Ar-CH_2, H-5''), 4.40 – 4.33 (m, 2H, H-1, H-3'), 4.02 – 3.95 (m, 2H, H-2, H-3'), 3.95 – 3.77 (m, 2H, H-2', H-5'), 3.67 (dd, *J* = 7.6, 2.9 Hz, 1H, H-2''), 3.45 – 3.31 (m, 3H, H-3, H-4, H-4'), 3.19 (q, *J* = 6.4 Hz, 2H, H-5), 2.06 (s, 3H, CO*C*H_3), 1.14 (s, 9H, TBDPS-CH_3), 1.08 – 1.02 (m, 6H, H-6, H-6'). **^{13}C NMR** ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.81 (*C*=O), 167.38 (*C*=O), 138.73 (Ar-C_q), 138.52 (Ar-C_q), 136.24 (Ar-*C*), 136.22 (Ar-*C*), 136.05 (Ar-C_q), 136.03 (Ar-C_q), 134.91 (Ar-C_q), 134.39 (Ar-C_q), 133.60 (Ar-C_q), 133.26 (Ar-C_q), 133.19 (Ar-C_q), 133.15 (Ar-C_q), 129.85 (Ar-*C*), 129.63 (Ar-*C*), 128.80 (Ar-*C*), 128.60 (Ar-*C*), 128.56 (Ar-*C*), 128.50 (Ar-*C*), 128.46 (Ar-*C*), 128.41 (Ar-*C*), 128.35 (Ar-*C*), 128.32 (Ar-*C*), 128.30 (Ar-*C*), 128.14 (Ar-*C*), 127.85 (Ar-*C*), 127.82 (Ar-*C*), 127.66 (Ar-*C*), 127.58 (Ar-*C*), 127.29 (Ar-*C*), 126.53 (Ar-*C*), 126.38 (Ar-*C*), 126.21 (Ar-*C*), 125.52 (Ar-*C*), 100.00 (C-1'), 98.91 (C-1''), 97.51 (C-1), 78.77 (C-3, C4, C-4', C-5'), 78.70 (C-3, C4, C-4', C-5'), 78.65 (C-3, C4, C-4', C-5'), 78.32 (C-3'), 75.19 (Ar-*C*H_2), 75.09 (C-3''), 75.00 (Ar-*C*H_2), 73.05 (Ar-*C*H_2), 70.81 (C-5), 68.73 (C-4''), 67.34 (Ar-*C*H_2), 67.30 (C-5''), 66.45 (C-2), 60.18 (C-2'), 59.40 (C-2''), 27.01 (TBDPS-*C*H_3), 21.06 (CO*C*H_3), 16.74 (C-6', C-6), 16.69 (C-6', C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{68}H_{73}N_9O_{13}SiNa$: 1274.49948; found 1274.49893.

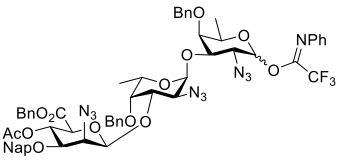
(Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-β-D-mannopyranosiduronsyl))-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α/β-D-fucopyranose (41)



9 (906 mg, 0.72 mmol) was dissolved THF (7.2 mL, 0.1 M) and cooled to 0 ºC. AcOH (80 µL, 1.45 mmol, 2 equiv.) and TBAF (1 M in THF, 1.44 mL, 1.45 mmol, 2 equiv.) was added and the reaction was allowed to warm to rt under N_2 and stirred overnight (~18 h) until TLC (pentane/EtOAc 3:2) showed full conversion. The reaction was quenched with NH_4Cl (aq. sat.) and dissolved in EtOAc. The organic layer was washed with H_2O (x3) and brine (x1), dried over Na_2SO_4, filtered

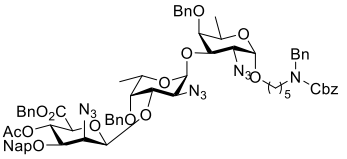
and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 7:3 → 5:5) gave **41** in 84% yield (613 mg, 0.60 mmol) as a α/β mixture. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.81 (t, *J* = 8.0 Hz, 6H), 7.74 (d, *J* = 1.7 Hz, 2H), 7.52 – 7.42 (m, 6H), 7.39 – 7.27 (m, 27H), 5.46 (td, *J* = 9.3, 1.0 Hz, 2H), 5.34 (t, *J* = 2.8 Hz, 1H), 5.25 (d, *J* = 3.7 Hz, 1H), 5.21 (d, *J* = 3.6 Hz, 1H), 5.03 (dd, *J* = 3.4, 2.3 Hz, 4H), 4.84 – 4.74 (m, 5H), 4.73 – 4.60 (m, 5H), 4.60 – 4.53 (m, 4H), 4.49 (t, *J* = 6.9 Hz, 1H), 4.28 – 4.21 (m, 1H), 4.20 – 4.13 (m, 1H), 4.06 (dd, *J* = 10.6, 2.7 Hz, 1H), 3.94 (dd, *J* = 10.6, 3.4 Hz, 1H), 3.85 (ddt, *J* = 9.4, 6.2, 3.4 Hz, 4H), 3.77 (tt, *J* = 10.6, 3.4 Hz, 3H), 3.68 – 3.61 (m, 3H), 3.60 (dd, *J* = 3.6, 1.8 Hz, 1H), 3.57 (dq, *J* = 2.5, 1.5 Hz, 3H), 3.55 – 3.51 (m, 2H), 3.48 (dd, *J* = 10.5, 2.8 Hz, 1H), 3.41 (d, *J* = 2.8 Hz, 1H), 3.01 (d, *J* = 2.6 Hz, 1H), 1.84 (s, 6H), 1.27 – 1.20 (m, 6H), 1.14 (dd, *J* = 6.6, 2.5 Hz, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.00, 166.59, 166.53, 138.47, 138.39, 138.05, 138.01, 135.06, 135.02, 134.63, 133.21, 133.19, 128.81, 128.77, 128.65, 128.60, 128.54, 128.50, 128.47, 128.35, 128.33, 128.09, 128.08, 127.98, 127.92, 127.87, 127.77, 126.88, 126.56, 126.54, 126.40, 125.67, 99.94, 99.56, 97.61, 97.53, 96.84, 92.41, 79.37, 79.34, 78.50, 77.27, 76.99, 76.95, 76.80, 75.80, 75.56, 75.48, 75.07, 73.73, 72.44, 72.42, 71.13, 68.04, 67.76, 67.71, 67.58, 67.38, 67.18, 65.20, 61.46, 61.40, 61.17, 59.05, 58.59, 20.74, 17.11, 16.95, 16.77, 16.74. **HRMS**: $[M+Na]^+$ calculated for $C_{52}H_{55}N_9O_{13}Na$: 1036.38170; found 1036.38115

(Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-β-D-mannopyranosiduronsyl))-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-fucopyranose (37)



41 (576 g, 0.57 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (2.8 mL, 0.2 M). K_2CO_3 (157 mg, 1.14 mmol, 2 equiv.) and $ClC(=NPh)CF_3$ (0.18 mL, 1.14 mmol, 2 equiv.) was added and the reaction was stirred overnight at rt under N_2 until TLC (pentane/EtOAc 7:3) showed full conversion. The reaction was filtered on Celite and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 8:2 → 6:4) gave **37** in 93% yield (627 mg, 0.529 mmol). **^{1}H NMR (400 MHz, CD_3CN)** δ 7.92 – 7.78 (m, 4H), 7.56 – 7.42 (m, 4H), 7.44 – 7.27 (m, 20H), 7.19 – 7.09 (m, 1H), 6.94 – 6.82 (m, 2H), 5.55 (bs, 1H), 5.24 (d, *J* = 3.7 Hz, 1H), 5.20 – 5.09 (m, 1H), 5.02 – 4.96 (m, 2H), 4.96 – 4.89 (m, 1H), 4.88 – 4.81 (m, 2H), 4.80 – 4.68 (m, 2H), 4.62 – 4.53 (m, 2H), 4.20 (dd, *J* = 11.2, 2.8 Hz, 1H), 4.11 (d, *J* = 1.4 Hz, 1H), 4.01 – 3.82 (m, 5H), 3.78 – 3.74 (m, 1H), 3.58 (m, 2H), 1.82 (s, 3H), 1.23 – 1.15 (m, 6H). **^{13}C NMR (101 MHz, CD_3CN)** δ 170.44, 167.84, 139.65, 139.59, 136.36, 136.23, 134.06, 133.92, 129.80, 129.49, 129.35, 129.29, 129.26, 129.12, 129.05, 128.70, 128.65, 128.56, 127.59, 127.27, 127.10, 126.88, 125.42, 120.03, 101.13, 97.93, 79.13, 78.83, 78.27, 77.87, 76.64, 76.24, 76.18, 74.17, 72.73, 72.69, 68.93, 68.16, 68.03, 64.34, 62.58, 59.00, 20.88, 16.87, 16.72. **HRMS**: $[M+H]^+$ calculated for $C_{60}H_{59}F_3N_{10}O_{13}H$: 1185.42934; found 1185.42829

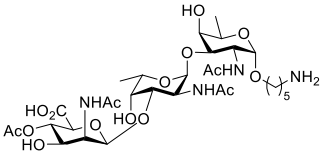
5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-β-D-mannopyranosiduronsyl)-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-D-fucopyranoside (5)



Donor **37** (202 mg, 0.17 mmol, 1 equiv.) and *N*-(Benzyl)-benzyloxycarbonyl-5-aminopentan-1-ol[50] **35** (72 mg, 0.22 mmol, 1.3 equiv.) was co-evaporated with toluene (3x). The donor, acceptor and Ph_3P=O (284 mg, 1.021 mmol, 6 equiv.) was dissolved in dry DCM/Et_2O (1.7 mL, 1:1, 0,1 M), added 3Å molecular sieves and stirred for 1 h. The solution was added TMSI (24 µL, 0;17 mmol, 1 equiv.) and stirred for 24 h until TLC (pentane/EtOAc 3:2) 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 → 55:45) gave **5** in 93% yield (209 mg, 0.158 mmol) and in a α/β ratio 75:25. <u>For the α-anomer:</u> **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.84 – 7.71 (m, 4H, Ar-*H*), 7.53 – 7.41 (m, 3H, Ar-*H*), 7.39 – 7.26 (m, 26H, Ar-*H*), 7.25 – 7.11 (m, 2H, Ar-*H*), 5.46 (t, *J* = 9.1 Hz, 1H, H-4''), 5.23 – 5.13 (m, 3H, H-1', Ar-CH_2), 5.03 (d, *J* = 2.6 Hz, 2H, Ar-CH_2), 4.90 (d, *J* = 7.7 Hz, 1H, H-1), 4.82 – 4.72 (m, 3H, Ar-CH_2), 4.69 – 4.63 (m, 2H, Ar-CH_2), 4.59 – 4.55 (m, 2H, H-1'', Ar-CH_2), 4.54 – 4.45 (m, 3H, CH_2-Linker), 4.21 (dd, *J* = 10.6, 2.8 Hz, 1H, H-3'), 4.03 (d, *J* = 10.8 Hz, 1H, H-3), 3.96 – 3.87 (m, 1H, H-5), 3.87 – 3.77 (m, 4H, H-5', H-2, H-5'', H-2'), 3.67 – 3.61 (m, 1H, H-4), 3.58 (dd, *J* = 9.1, 3.5 Hz, 1H, H-2''), 3.54 (d, *J* = 4.9 Hz, 2H), 3.47 – 3.32 (m, 2H, H-4', H-3''), 3.31 – 3.14 (m, 2H, CH_2-Linker), 1.84 (s, 3H, COCH_3), 1.73 – 1.46 (m, 4H, CH_2-Linker), 1.41 – 1.24 (m, 4H, CH_2-Linker), 1.21 (d, *J* = 6.5 Hz, 3H, H-6), 1.14 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.34 (C=O), 166.57 (C=O), 138.61 (Ar-C_q), 138.03 (Ar-C_q), 135.09 (Ar-C_q), 134.66 (Ar-C_q), 133.22 (Ar-C_q), 128.82 (Ar-*C*), 128.67 (Ar-*C*), 128.61 (Ar-*C*), 128.55 (Ar-*C*), 128.51 (Ar-*C*), 128.48 (Ar-*C*), 128.27 (Ar-*C*), 128.06 (Ar-*C*), 127.96 (Ar-*C*), 127.94 (Ar-*C*), 127.90 (Ar-*C*), 127.77 (Ar-*C*), 127.72 (Ar-*C*), 126.91 (Ar-*C*), 126.55 (Ar-*C*), 126.40 (Ar-*C*), 125.70 (Ar-*C*), 99.89 (C-1'), 98.17 (C-1), 97.61 (C-1''), 80.05 (C-4'), 77.48 (C-3''), 76.84 (C-3'), 76.11 (C-3), 75.50 (Ar-*C*H$_2$), 75.25 (Ar-*C*H$_2$), 73.76 (C-5'), 72.43 (Ar-*C*H$_2$), 68.45 (C-4''), 68.05 (Ar-*C*H$_2$), 67.73 (C-5), 67.46 (Ar-*C*H$_2$), 66.87 (C-4), 61.40 (C-2''), 60.25 (C-2'), 58.91 (C-2), 29.25 (*C*H$_2$-Linker), 23.50 (*C*H$_2$-Linker), 20.76 (CO*C*H$_3$), 16.96 (C-6, C-6'), 16.80 (C-6, C-6'). <u>For the β-anomer:</u> **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.85 – 7.78 (m, 3H, Ar-*H*), 7.74 (d, *J* = 1.7 Hz, 1H, Ar-*H*), 7.51 – 7.41 (m, 3H, Ar-*H*), 7.37 – 7.26 (m, 24H, Ar-*H*), 7.17 (s, 1H, Ar-*H*), 5.46 (t, *J* = 9.2 Hz, 1H, H-4''), 5.25 (d, *J* = 3.8 Hz, 1H, H-1'), 5.21 – 5.13 (m, 3H, Ar-*H*), 5.04 (d, *J* = 2.5 Hz, 2H, Ar-*H*), 4.83 – 4.68 (m, 4H, Ar-*H*), 4.58 – 4.52 (m, 2H, Ar-*H*, H-1''), 4.49 (d, *J* = 6.9 Hz, 2H, CH_2-Linker), 4.17 (m, 2H, H-1, H-3'), 3.87 – 3.80 (m, 2H, H-2, H-5''), 3.70 (td, *J* = 10.9, 5.2 Hz, 2H, H-2', H-5'), 3.64 (dt, *J* = 3.6, 1.6 Hz, 1H, H-2''), 3.59 (ddd, *J* = 9.0, 3.5, 1.8 Hz, 1H, H-3''), 3.57 – 3.47 (m, 2H, H-4', H-5), 3.44 (dd, *J* = 10.5, 2.8 Hz, 1H, H-4), 3.40 – 3.36 (m, 1H, CH_2-Linker), 3.33 – 3.15 (m, 3H, CH_2-Linker), 1.85 (d, *J* = 2.4 Hz, 3H, COCH_3), 1.27 (d, *J* = 2.9 Hz, 3H, H-6, H-6'), 1.12 (d, *J* = 6.7 Hz, 3H, H-6, H-6'). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.32 (C=O), 166.48 (C=O), 138.61 (Ar-C_q), 138.05 (Ar-C_q), 135.05 (Ar-C_q), 134.65 (Ar-C_q), 133.23 (Ar-C_q), 128.83 (Ar-*C*), 128.80 (Ar-*C*), 128.66 (Ar-*C*), 128.63 (Ar-*C*), 128.61 (Ar-*C*), 128.54 (Ar-*C*), 128.47 (Ar-*C*), 128.39 (Ar-*C*), 128.31 (Ar-*C*), 128.26 (Ar-*C*), 128.07 (Ar-*C*), 127.94 (Ar-*C*), 127.89 (Ar-*C*), 127.76 (Ar-*C*), 127.63 (Ar-*C*), 127.37 (Ar-*C*), 126.88 (Ar-*C*), 126.56 (Ar-*C*), 126.41 (Ar-*C*), 125.68 (Ar-*C*), 102.72 (C-1), 100.21 (C-

1'), 97.56 (C-1''), 79.35 (C-3), 78.85 (C-4), 77.39 (C-4', C-5), 77.01 (C-3''), 75.59 (C-Ar-*C*H$_2$), 75.33 (C-3'), 75.14 (Ar-*C*H$_2$), 73.80 (C-5''), 72.44 (Ar-*C*H$_2$), 70.78 (C-4', C-5), 68.06 (C-5'), 67.76 (Ar-*C*H$_2$), 67.25 (Ar-*C*H$_2$), 67.20 (C-2), 63.65 (C-2''), 61.47 (C-2'), 58.43 (*C*H$_2$-Linker), 50.66 (*C*H$_2$-Linker), 50.26 (*C*H$_2$-Linker), 29.88 (*C*H$_2$-Linker), 29.22 (*C*H$_2$-Linker), 27.92, (*C*H$_2$-Linker) 27.47 (*C*H$_2$-Linker), 20.75 (CO*C*H$_3$), 17.13 (C-6, C-6'), 16.78 (C-6, C-6'). **HRMS**: $[M+Na]^+$ calculated for $C_{72}H_{76}N_{10}O_{15}Na$: 1345.55458; found 1345.55403

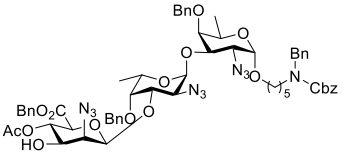
5-aminopentyl 2-acetamide-4-*O*-acetyl-2-deoxy-β-D-mannopyranosiduronsyl-(1→3)-2-acetamide-2-deoxy-α-L-fucopyranosyl-(1→3)-2-acetamide-2-deoxy-α-D-fucopyranoside (1)



5 (59 mg, 0.0445 mmol) was deprotected following the general experimental for the deprotection yielding **1** in 45% yield over two steps (14.3 mg, 0.0198 mol). **^{1}H NMR (600 MHz, D$_2$O)** δ 5.00 – 4.95 (m, 2H, H-1', H-4''), 4.91 (d, *J* = 1.4 Hz, 1H, H-1''), 4.74 (d, *J* = 3.8 Hz, 1H, H-1), 4.47 (dd, *J* = 4.3, 1.4 Hz, 1H, H-2''), 4.23 (dd, *J* = 11.1, 3.8 Hz, 1H, H-2), 4.18 – 4.09 (m, 2H, H-3', H-2'), 4.07 (q, *J* = 7.7, 7.1 Hz, 1H, H-5), 4.05 – 4.01 (m, 1H, H-5'), 4.01 – 3.97 (m, 2H, H-4', H-3''), 3.89 (dd, *J* = 11.1, 3.2 Hz, 1H, H-3), 3.77 (d, *J* = 3.3 Hz, 1H, H-4), 3.75 (d, *J* = 10.1 Hz, 1H, H-5''), 3.62 (dt, *J* = 9.9, 6.5 Hz, 1H, C*H*$_2$-linker), 3.41 (dt, *J* = 10.0, 6.2 Hz, 1H, C*H*$_2$-linker), 2.95 (dd, *J* = 8.7, 6.7 Hz, 2H, C*H*$_2$-linker), 2.08 (s, 3H, COCH$_3$), 2.03 (s, 3H, COCH$_3$), 2.02 (s, 3H, COCH$_3$), 1.94 (s, 3H, COC*H*$_3$), 1.68 – 1.55 (m, 4H, C*H*$_2$-linker), 1.40 (tq, *J* = 14.4, 7.4, 6.5 Hz, 2H, C*H*$_2$-linker), 1.24 – 1.15 (m, 6H, H-6, H-6'). **^{13}C NMR (151 MHz, D$_2$O)** δ 176.54 (C=O), 175.73 (C=O), 174.93 (C=O), 174.67 (C=O), 173.87 (C=O), 99.91 (C-1'), 97.89 (C-1), 95.50 (C-1''), 75.30 (C-3'), 75.20 (C-3), 73.53 (C-5''), 72.14 (C-4), 71.31 (C-4''), 70.70 (C-3''), 68.64 (*C*H$_2$-linker), 68.38 (C-4'), 67.74 (C-5), 67.28 (C-5'), 54.12 (C-2''), 49.61 (C-2), 48.54 (C-2'), 40.27 (*C*H$_2$-linker), 28.95 (*C*H$_2$-linker), 27.41 (*C*H$_2$-linker), 23.23 (*C*H$_2$-linker), 23.17 (CO*C*H$_3$), 22.85 (CO*C*H$_3$), 22.79 (CO*C*H$_3$), 21.25(CO*C*H$_3$), 16.42 (C-6), 16.23 (C-6'). **HRMS**: $[M+H]^+$ calculated for $C_{31}H_{52}N_4O_{16}H$: 737.34566; found 737.34407

Synthesis of longer fragments

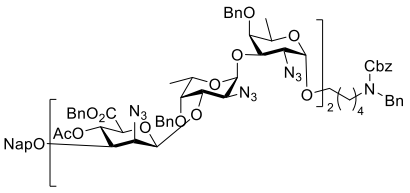
5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (4-*O*-acetyl-2-azido-2-deoxy-β-D-mannopyranosiduronsyl)-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-D-fucopyranoside (42)



The 2-methylnaphthyl was cleaved from **5** (170 mg, 0.128 mmol, 1 equiv.) using the general experimental procedure for deprotection of the 2-methylnaphthyl ether in DCM/H$_2$O (1.3 mL, 20:1, 0.1 M) with DDQ (58 mg, 0.256 mmol, 2 equiv.). The reaction was followed by TLC (pentane/EtOAc 3:2) and purification by column chromatography (pentane/EtOAc 6:4 → 5:5) gave **42** in 80% yield (121 mg, 0.102 mmol). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.42 – 7.26 (m, 25H), 5.23 (d, *J* = 3.7 Hz, 1H), 5.21 – 5.10 (m, 4H), 5.06 (s, 2H), 4.90 (d, *J* = 7.0 Hz, 1H), 4.76 (t, *J* = 12.5 Hz, 1H), 4.72 – 4.61 (m, 4H), 4.49 (d, *J* = 7.2 Hz, 2H), 4.21 (dd, *J* = 10.6, 2.9

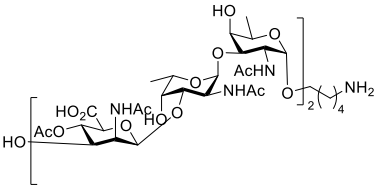
Hz, 1H), 4.05 (d, *J* = 11.0 Hz, 1H), 3.93 (m, 1H), 3.90 – 3.80 (m, 3H), 3.75 (dd, *J* = 10.6, 3.6 Hz, 1H), 3.68 – 3.51 (m, 6H), 3.49 – 3.32 (m, 1H), 3.23 (m, 4H), 2.63 (d, *J* = 9.9 Hz, 1H), 1.86 (s, 3H), 1.64 – 1.45 (m, 5H), 1.40 – 1.25 (m, 4H), 1.21 (t, *J* = 6.1 Hz, 8H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.59, 166.29, 138.66, 138.02, 137.89, 135.14, 128.79, 128.69, 128.67, 128.59, 128.51, 128.34, 128.29, 128.06, 127.96, 127.93, 127.87, 127.73, 127.38, 99.90, 98.20, 98.00, 80.09, 76.84, 75.79, 75.33, 73.75, 71.12, 69.80, 68.31, 67.69, 67.43, 67.28, 66.88, 64.02, 60.20, 58.58, 50.76, 50.44, 47.09, 29.73, 29.23, 23.47, 20.65, 16.98, 16.86. **HRMS**: $[M+Na]^+$ calculated for $C_{61}H_{70}N_{10}O_{15}Na$: 1205.49198; found 1205.49143

Hexasaccharide protected (6)



The glycosylation was performed using the general glycosylation procedure with donor **35** (142 mg, 0.120 mmol, 1.3 equiv.) and acceptor **42** (109 mg, 0.0921 mmol, 1 equiv.) dissolved in dry DCM (4.6 mL, 0.02 M) and added TBSOTf (4 µL, 0.0184 mmol, 0.2 equiv.). The reaction was followed with TLC (pentane/EtOAc 1:1) and purification by column chromatography (pentane/EtOAc 7:3 → 5:5) and size exclusion gave **6** in 87% yield (175 mg, 0.0803 mmol). **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.85 – 7.70 (m, 5H), 7.51 – 7.41 (m, 4H), 7.39 – 7.26 (m, 45H), 7.21 – 7.14 (m, 2H), 5.45 (t, *J* = 9.2 Hz, 1H), 5.33 (t, *J* = 9.9 Hz, 1H), 5.24 (d, *J* = 3.7 Hz, 1H), 5.22 – 5.14 (m, 4H), 5.03 – 4.99 (m, 4H), 4.95 (d, *J* = 2.5 Hz, 1H), 4.94 – 4.87 (m, 2H), 4.84 – 4.77 (m, 4H), 4.75 – 4.72 (m, 1H), 4.71 – 4.64 (m, 3H), 4.64 – 4.54 (m, 6H), 4.53 – 4.45 (m, 3H), 4.26 (td, *J* = 10.6, 2.9 Hz, 2H), 4.20 (q, *J* = 5.9, 5.3 Hz, 1H), 4.10 – 4.01 (m, 1H), 3.96 (t, *J* = 1.9 Hz, 2H), 3.95 – 3.89 (m, 2H), 3.89 – 3.79 (m, 8H), 3.79 – 3.74 (m, 1H), 3.64 (dd, *J* = 3.5, 1.4 Hz, 1H), 3.62 – 3.51 (m, 8H), 3.49 – 3.35 (m, 1H), 3.23 (m, 2H), 1.87 (s, 3H), 1.83 (s, 4H), 1.70 – 1.47 (m, 6H), 1.40 – 1.24 (m, 5H), 1.24 – 1.11 (m, 14H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 169.31, 169.17, 166.52, 166.14, 138.57, 138.37, 138.12, 138.03, 137.92, 135.06, 135.03, 134.64, 133.24, 133.22, 128.84, 128.77, 128.66, 128.63, 128.61, 128.55, 128.53, 128.51, 128.41, 128.27, 128.23, 128.19, 128.05, 127.95, 127.92, 127.89, 127.87, 127.77, 127.38, 126.87, 126.54, 126.39, 125.67, 100.76, 99.87, 99.59, 98.18, 97.39, 97.35, 79.96, 79.64, 79.47, 76.77, 76.35, 75.50, 75.33, 75.29, 74.88, 73.97, 73.73, 72.61, 72.36, 68.52, 68.06, 67.74, 67.67, 67.64, 67.51, 66.89, 63.70, 61.27, 61.01, 60.28, 58.98, 58.54, 50.59, 50.26, 47.22, 46.26, 29.22, 23.46, 20.72, 20.48, 17.05, 16.95, 16.91, 16.74. **HRMS**: $[M+Na]^+$ calculated for $C_{113}H_{123}N_{19}O_{27}Na$: 2201.87670; found 2201.87586

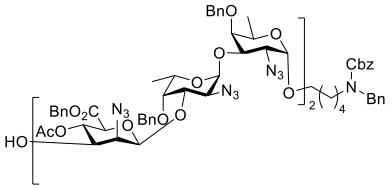
CP8-hexasaccahride (2)



6 (60 mg, 0.0275 mmol) was deprotected following the general experimental for the deprotection yielding the **2** in 37% yield over two steps (8.2 mg, 0.00598 mg). **^{1}H NMR (600 MHz, D_2O)** δ 5.13 (t, *J* = 10.0 Hz, 1H, H^3-4), 5.03 – 4.95 (m, 2H, H^6-4, H^2-1), 4.95 – 4.87 (m, 4H, H^3-1, H^6-1, H^4-1,H H^5-1,1), 4.75 (d, *J* = 3.9 Hz, 1H, H^1-1), 4.50 – 4.44 (m, 2H, H^6-2, H^3-2), 4.27 – 4.21 (m, 2H, H^5-2, H^4-5), 4.24 – 4.17 (m, 1H, H^1-2), 4.18 – 4.09 (m, 4H, H^1-2, H^5-4, H^2-4, H^3-3), 4.07 (ddd, *J* = 20.1, 13.9, 7.2 Hz, 2H, H^1-5, H^5-5), 4.04 – 3.98 (m, 4H, H^3-5, H^5-3, H^2-3, H^6-3), 3.90 (dd, *J* = 10.8, 3.4 Hz, 1H, H^1-3), 3.78 (d, *J* = 3.8 Hz, 1H, H^1-4), 3.75 (dd, *J* = 10.1, 1.4 Hz, 2H, H^6-5, H^3-5),

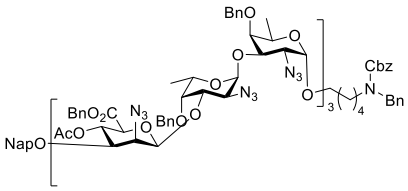
3.73 (d, *J* = 2.6 Hz, 1H, H^4-4), 3.70 (dd, *J* = 10.9, 3.1 Hz, 1H H^1-4), 3.67 – 3.59 (m,1H, C*H*$_2$-Linker), 3.43 (dt, *J* = 10.0, 6.2 Hz, 1H, C*H*$_2$-Linker), 3.00 – 2.93 (m, 2H, C*H*$_2$-Linker), 2.09 (s, 3H, COC*H*$_3$), 2.06 – 1.99 (m, 12H, COC*H*$_3$), 1.95 (s, 3H, COC*H*$_3$), 1.91 (s, 3H, COC*H*$_3$), 1.70 – 1.55 (m, 4H, C*H*$_2$-Linker), 1.45 – 1.36 (m, 2H, C*H*$_2$-Linker), 1.25 – 1.16 (m, 12H, H^1-6, H^2-6, H^4-6, H^5-6). **^{13}C NMR (151 MHz, D$_2$O)** δ 176.49 (C=O), 175.92 (C=O), 175.71 (C=O), 175.44 (C=O), 174.90 (C=O), 174.87 (C=O), 174.64 (C=O), 174.49 (C=O), 173.86 (C=O), 173.32 (C=O), 99.92 (C^2-1), 99.70 (C^4-1/ C^5-1), 99.43 (C^4-1/ C^5-1), 97.88 (C^1-1), 95.37 (C^3-1/ C^6-1), 95.24 (C^3-1/ C^6-1), 75.28 (C^3-5/ C^6-5), 75.19 (C^3-5/ C^6-5), 74.93, 74.87, 74.56, 73.48, 73.38, 72.13, 71.91, 71.30, 71.19, 70.71, 68.62, 68.38, 68.30, 67.73, 67.69, 67.58, 67.27, 54.10, 53.42, 49.59, 49.38, 48.48, 48.40, 40.26, 28.93, 27.39, 23.21, 23.13, 22.92, 22.82, 22.77, 22.70, 21.24, 21.11, 16.44, 16.40, 16.22, 16.17. **HRMS**: [M+H]$^+$ calculated for $C_{57}H_{92}N_7O_{31}H$: 1370.58377; found 1370.58302

Hexasaccharide-acceptor (43)



The 2-methylnaphthyl was cleaved from **6** (179 mg, 0.0820 mmol) using the general experimental procedure for deprotection of the 2-methylnaphthyl ether in DCM/H$_2$O (4.1 mL, 20:1, 0.02 M) with DDQ (37 mg, 0.164 mmol, 2 equiv.). The reaction was followed by TLC (pentane/EtOAc 3:2) and purification by column chromatography (pentane/EtOAc 6:4 → 5:5) gave **43** in 54% yield (90 mg, 0.0441 mmol). **^{1}H NMR (500 MHz, CDCl$_3$)** δ 7.40 – 7.26 (m, 39H), 7.22 – 7.14 (m, 2H), 5.33 (t, *J* = 9.8 Hz, 2H), 5.24 (d, *J* = 3.7 Hz, 1H), 5.22 – 5.11 (m, 4H), 5.02 (d, *J* = 5.6 Hz, 4H), 4.96 (d, *J* = 3.1 Hz, 1H), 4.90 (d, *J* = 9.8 Hz, 1H), 4.80 (dd, *J* = 11.6, 9.5 Hz, 2H), 4.75 – 4.61 (m, 7H), 4.59 – 4.55 (m, 1H), 4.49 (d, *J* = 9.1 Hz, 2H), 4.30 – 4.17 (m, 3H), 4.05 (d, *J* = 11.1 Hz, 1H), 3.98 (dd, *J* = 4.3, 2.6 Hz, 2H), 3.95 – 3.89 (m, 1H), 3.89 – 3.82 (m, 5H), 3.80 (d, *J* = 6.5 Hz, 1H), 3.80 – 3.74 (m, 2H), 3.65 – 3.53 (m, 8H), 3.48 – 3.34 (m, 1H), 3.34 – 3.16 (m, 2H), 2.58 (d, *J* = 9.9 Hz, 1H), 1.87 (s, 3H), 1.84 (s, 3H), 1.59 (m, 10H), 1.36 – 1.25 (m, 5H), 1.21 (td, *J* = 10.9, 10.4, 5.4 Hz, 12H). **^{13}C NMR (126 MHz, CDCl$_3$)** δ 170.52, 169.18, 166.29, 166.15, 138.58, 138.43, 138.04, 137.92, 135.14, 135.04, 128.78, 128.72, 128.70, 128.67, 128.66, 128.63, 128.60, 128.55, 128.54, 128.51, 128.30, 128.29, 128.19, 128.03, 127.96, 127.93, 127.87, 127.77, 127.39, 100.76, 99.86, 99.66, 98.19, 97.80, 97.39, 79.96, 79.64, 79.58, 76.91, 76.58, 76.39, 75.64, 75.51, 75.38, 75.29, 75.01, 74.90, 73.98, 73.72, 71.09, 69.82, 68.54, 68.31, 67.75, 67.63, 67.60, 67.52, 67.27, 66.99, 66.90, 63.92, 63.71, 60.99, 60.29, 58.70, 58.57, 50.68, 50.34, 29.83, 29.23, 28.08, 27.43, 22.79, 20.61, 20.01, 17.07, 16.95, 16.91, 16.82. **HRMS**: [M+Na]$^+$ calculated for $C_{102}H_{115}N_{19}O_{27}Na$: 2061.81410; found 2061.81322

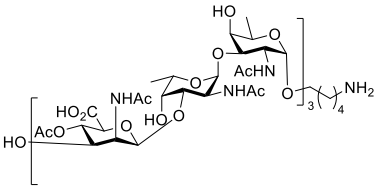
Nonasaccharide protected (7)



The glycosylation was performed using the general glycosylation procedure with donor **35** (66 mg, 0.0564 mmol, 1.3 equiv.) and acceptor **43** (91 mg, 0.0446 mmol, 1 equiv.) dissolved in dry DCM (2.2 mL, 0.02 M) and added TBSOTf ((2 µL, 0.00892 mmol, 0.2 equiv.) The reaction was followed with TLC (pentane/EtOAc 1:1) and purification by size exclusion to give **7** in 77% yield (104 mg, 0.0342 mmol). **^{1}H NMR (500 MHz, CDCl$_3$)** δ 7.83 – 7.71 (m, 5H), 7.50 – 7.41 (m,

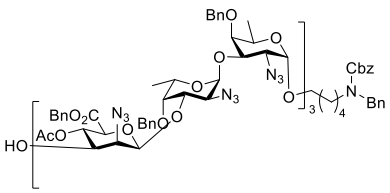
4H), 7.40 – 7.27 (m, 52H), 7.22 – 7.13 (m, 2H), 5.44 (t, *J* = 9.2 Hz, 1H), 5.35 – 5.28 (m, 3H), 5.24 (d, *J* = 3.7 Hz, 1H), 5.21 – 5.12 (m, 4H), 5.03 – 4.99 (m, 4H), 4.98 (s, 2H), 4.97 – 4.92 (m, 2H), 4.90 (d, *J* = 8.6 Hz, 1H), 4.84 – 4.77 (m, 4H), 4.75 – 4.67 (m, 5H), 4.67 – 4.53 (m, 9H), 4.49 (d, *J* = 8.9 Hz, 2H), 4.30 – 4.22 (m, 3H), 4.22 – 4.16 (m, 2H), 4.05 (d, *J* = 10.9 Hz, 1H), 4.00 – 3.94 (m, 4H), 3.93 – 3.71 (m, 14H), 3.66 – 3.50 (m, 12H), 3.48 – 3.33 (m, 2H), 3.33 – 3.14 (m, 2H), 1.87 (s, 5H), 1.85 (s, 3H), 1.82 (s, 4H), 1.66 – 1.43 (m, 9H), 1.27 – 1.12 (m, 21H). **^{13}C NMR (126 MHz, CDCl$_3$)** δ 169.31, 169.20, 166.54, 166.17, 166.14, 138.59, 138.39, 138.37, 138.14, 137.93, 135.08, 135.05, 135.03, 134.66, 133.25, 133.24, 128.78, 128.74, 128.69, 128.67, 128.64, 128.62, 128.56, 128.53, 128.51, 128.31, 128.28, 128.24, 128.20, 128.19, 128.06, 128.04, 127.96, 127.94, 127.92, 127.90, 127.87, 127.77, 127.38, 126.88, 126.55, 126.40, 125.68, 100.78, 99.87, 99.60, 98.20, 97.43, 97.38, 97.25, 79.98, 79.64, 79.59, 79.51, 79.44, 77.16, 76.37, 75.52, 75.35, 75.30, 73.99, 73.92, 73.75, 72.38, 68.54, 68.09, 67.75, 67.68, 67.65, 67.53, 67.02, 66.91, 63.73, 63.63, 61.31, 61.00, 60.30, 59.01, 58.74, 58.58, 29.24, 23.56, 23.44, 20.73, 20.49, 20.46, 17.07, 17.05, 16.96, 16.91, 16.84, 16.74. **HRMS**: $[M+Na]^+$ calculated for $C_{154}H_{168}N_{28}O_{39}Na$: 3056.19212; found 1528.12675

CP8-Nonasaccharide (3)



7 (26 mg, 0.0084 mmol, 1 equiv.) was deprotected following the general experimental for the deprotection yielding **3** in 57% yield over two steps (9.7 mg,0.0048 mmol). **^{1}H NMR (850 MHz, D$_2$O)** δ 5.21 – 5.12 (m, 2H), 5.06 – 4.99 (m, 3H), 4.97 (dt, *J* = 11.1, 6.4 Hz, 5H), 4.54 – 4.49 (m, 2H), 4.30 – 4.21 (m, 5H), 4.21 – 4.12 (m, 7H), 4.11 (t, *J* = 6.4 Hz, 2H), 4.10 – 4.00 (m, 7H), 3.96 – 3.85 (m, 5H), 3.81 (d, *J* = 3.2 Hz, 1H), 3.80 – 3.70 (m, 3H), 3.67 (dt, *J* = 10.5, 6.5 Hz, 1H), 3.47 (dt, *J* = 12.1, 6.3 Hz, 1H), 3.00 (t, *J* = 7.7 Hz, 2H), 2.14 (s, 3H), 2.09 – 2.05 (m, 20H), 2.04 (t, *J* = 2.8 Hz, 4H), 1.99 (s, 4H), 1.95 (d, *J* = 5.8 Hz, 5H), 1.66 (ddq, *J* = 43.9, 14.0, 7.4 Hz, 4H), 1.44 (dt, *J* = 16.6, 7.6 Hz, 2H), 1.28 – 1.21 (m, 18H). **^{13}C NMR (214 MHz, D$_2$O)** δ 176.48, 175.91, 174.82, 174.66, 174.48, 174.07, 173.96, 173.41, 99.90, 99.70, 99.64, 99.48, 97.87, 95.83, 95.72, 95.53, 95.49, 76.79, 75.19, 74.76, 74.60, 74.54, 74.48, 74.33, 74.20, 73.84, 73.68, 72.11, 71.87, 71.07, 70.98, 70.53, 68.63, 68.46, 68.42, 68.39, 67.80, 67.74, 67.69, 67.61, 67.27, 53.99, 53.32, 51.46, 49.56, 49.33, 48.48, 48.44, 48.41, 48.37, 40.25, 28.91, 27.37, 23.19, 23.17, 23.13, 22.90, 22.82, 22.78, 22.72, 22.53, 21.17, 21.12, 21.05, 16.40, 16.36, 16.33, 16.19, 16.14. **HRMS**: $[M+H]^+$ calculated for $C_{83}H_{130}N_{10}O_{46}H$: 2003.82189; found 2003.82134

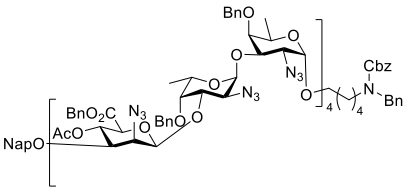
Nona-acceptor (44)



The 2-methylnaphthyl was cleaved from **7** (38.3 mg, 0.0126 mmol) using the general experimental procedure for deprotection of the 2-methylnaphthyl ether in DCM/H$_2$O (1.3 mL, 10:1, 0.01 M) with DDQ (6 mg, 0.0252 mmol, 2 equiv.). The reaction was followed by TLC (pentane/EtOAc 1:1) and purification by column chromatography (pentane/EtOAc 60:40 → 45:55) gave **44** in 57% yield (20.9 mg, 0.0072 mmol). **^{1}H NMR (850 MHz, CDCl$_3$)** δ 5.21 – 5.14 (m, 2H), 5.05 – 5.01 (m, 2H), 4.99 – 4.93 (m, 5H), 4.54 – 4.49 (m, 2H), 4.30 – 4.21 (m, 5H), 4.21 – 4.15 (m, 7H), 4.14 – 4.09 (m, 2H), 4.09 – 4.01 (m, 6H), 3.96

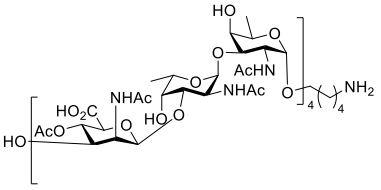
– 3.85 (m, 5H), 3.81 (d, *J* = 3.2 Hz, 1H), 3.78 – 3.71 (m, 3H), 3.67 (dt, *J* = 10.5, 6.5 Hz, 1H), 3.47 (dt, *J* = 12.1, 6.3 Hz, 1H), 3.00 (t, *J* = 7.7 Hz, 2H), 2.14 (s, 3H), 2.09 – 2.05 (m, 20H), 2.04 (d, *J* = 3.4 Hz, 6H), 1.99 (s, 3H), 1.95 (s, 5H), 1.66 (ddq, *J* = 43.9, 14.0, 7.4 Hz, 4H), 1.44 (dt, *J* = 16.6, 7.6 Hz, 2H), 1.29 – 1.20 (m, 20H). **^{13}C NMR (214 MHz, D_2O)** δ 176.48, 175.91, 174.83, 174.66, 174.48, 174.07, 173.96, 173.41, 99.90, 99.70, 99.64, 99.56, 99.48, 97.87, 95.83, 95.72, 95.53, 95.49, 76.79, 75.19, 74.76, 74.60, 74.54, 74.32, 74.20, 73.84, 73.68, 72.11, 71.87, 71.07, 70.98, 70.53, 68.63, 68.46, 68.42, 68.39, 67.80, 67.74, 67.69, 67.61, 67.27, 53.99, 53.32, 51.46, 49.56, 49.33, 48.48, 48.44, 48.41, 48.37, 40.25, 28.91, 27.37, 23.19, 23.17, 23.13, 22.90, 22.82, 22.78, 22.72, 22.53, 21.17, 21.12, 21.05, 16.40, 16.36, 16.33, 16.19, 16.14. **HRMS:** [M+Na]+ calculated for C143H160N28O39Na: 2916.12952; found 1458.09829

Dodeca-saccharide (8)



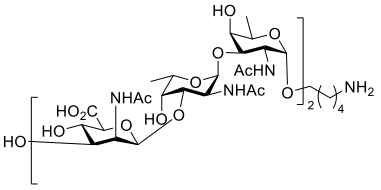
The glycosylation was performed using the general glycosylation procedure with donor **35** (13 mg, 0.0104 mmol, 1.5 equiv.) and acceptor **44** (20 mg, 0.00691 mmol, 1 equiv.) dissolved in dry DCM (1 mL, 0.007 M) and added (TBSOTf 0.3 µL, 0.00138 mmol, 0.2 equiv.) The reaction was followed with TLC (pentane/EtOAc 6:4) and purification by size exclusion to give **8** in 68% yield (18.3 mg, 0.0047 mmol). **^{1}H NMR (850 MHz, $CDCl_3$)** δ 7.83 – 7.77 (m, 3H), 7.72 (s, 1H), 7.50 – 7.40 (m, 4H), 7.40 – 7.27 (m, 61H), 7.23 – 7.13 (m, 3H), 5.43 (t, *J* = 9.1 Hz, 1H), 5.34 – 5.27 (m, 3H), 5.23 (t, *J* = 4.5 Hz, 1H), 5.19 – 5.11 (m, 5H), 5.02 – 4.87 (m, 12H), 4.82 – 4.75 (m, 5H), 4.75 – 4.62 (m, 9H), 4.62 – 4.55 (m, 5H), 4.55 – 4.44 (m, 6H), 4.28 – 4.13 (m, 7H), 4.04 (t, *J* = 10.9 Hz, 1H), 4.01 – 3.90 (m, 7H), 3.90 – 3.66 (m, 18H), 3.66 – 3.48 (m, 15H), 3.47 – 3.32 (m, 2H), 3.25 (dt, *J* = 27.9, 7.2 Hz, 1H), 3.21 – 3.10 (m, 1H), 1.85 (d, *J* = 6.9 Hz, 3H), 1.82 (d, *J* = 2.3 Hz, 6H), 1.81 (d, *J* = 4.7 Hz, 3H), 1.72 – 1.43 (m, 12H), 1.40 – 1.02 (m, 40H). **^{13}C NMR (214 MHz, $CDCl_3$)** δ 170.59, 169.41, 169.25, 166.51, 166.37, 166.24, 166.13, 166.10, 166.09, 156.83, 156.28, 138.56, 138.53, 138.47, 138.43, 138.35, 138.28, 138.23, 138.21, 138.00, 137.96, 137.91, 137.79, 137.78, 137.72, 136.94, 136.91, 136.78, 135.11, 135.01, 134.92, 134.89, 134.86, 134.53, 133.15, 133.09, 129.53, 128.94, 128.86, 128.84, 128.79, 128.71, 128.67, 128.64, 128.62, 128.59, 128.57, 128.55, 128.53, 128.51, 128.44, 128.41, 128.36, 128.29, 128.26, 128.10, 128.08, 128.02, 127.93, 127.91, 127.89, 127.80, 127.78, 127.69, 127.61, 127.42, 127.36, 127.28, 126.93, 126.56, 126.42, 125.69, 100.76, 99.96, 99.91, 99.68, 98.08, 97.32, 97.24, 97.22, 97.19, 96.99, 79.86, 79.56, 79.50, 79.37, 79.30, 79.29, 76.82, 76.69, 76.59, 76.55, 76.29, 76.07, 75.67, 75.58, 75.50, 75.35, 75.33, 75.30, 74.75, 74.71, 74.38, 74.33, 73.82, 73.74, 73.55, 72.26, 68.25, 68.20, 67.82, 67.77, 67.73, 67.70, 67.69, 67.61, 67.55, 67.28, 67.24, 67.22, 67.19, 66.80, 66.70, 63.59, 63.48, 60.90, 60.87, 60.21, 60.18, 58.73, 58.43, 58.33, 57.89, 50.51, 50.20, 47.11, 46.54, 46.23, 46.17, 32.06, 29.84, 29.80, 29.52, 29.23, 29.16, 27.94, 27.53, 23.46, 23.38, 23.27, 23.20, 22.85, 20.78, 20.51, 20.48, 17.13, 17.06, 17.05, 16.95, 16.93, 16.83, 16.79.

CP8-Dodeca (4)



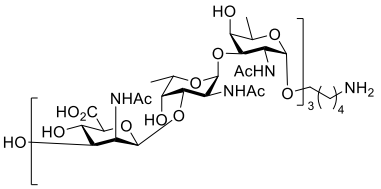
8 (12.2 mg, 0.00299 mmol, 1 equiv.) was deprotected following the general experimental for the deprotection yielding **4** in 33% yield over two steps (2.65 mg, 0.0010 mmol). **^{1}H NMR (850 MHz, D_2O)** δ 5.26 (s, 2H), 5.17 (d, *J* = 10.2 Hz, 3H), 5.08 (s, 2H), 5.07 – 4.89 (m, 10H), 4.60 (s, 1H), 4.50 (t, *J* = 20.3 Hz, 4H), 4.35 – 3.96 (m, 28H), 3.86 (d, *J* = 58.5 Hz, 10H), 3.76 (s, 4H), 3.70 – 3.59 (m, 2H), 3.46 (t, *J* = 5.2 Hz, 1H), 3.00 (t, *J* = 7.7 Hz, 2H), 2.15 – 1.92 (m, 49H), 1.66 (dq, *J* = 39.1, 7.6 Hz, 5H), 1.50 – 1.36 (m, 2H), 1.28 – 1.20 (m, 25H). **^{13}C NMR (302 MHz, D_2O)** δ 99.28, 99.00, 98.59, 98.58, 96.98, 74.47, 73.94, 73.50, 72.64, 71.64, 70.99, 70.22, 69.76, 69.16, 68.82, 67.52, 67.32, 66.86, 66.80, 66.74, 66.71, 66.37, 54.30, 53.16, 52.49, 50.62, 48.69, 48.45, 48.17, 47.55, 39.37, 28.01, 26.47, 22.37, 22.24, 22.03, 21.94, 21.89, 21.83, 21.63, 20.32, 20.25, 20.19, 20.14, 15.51, 15.47, 15.42, 15.35, 15.31, 15.25. **HRMS**: $[M+2H]^+$ calculated for $C_{109}H_{170}N_{13}O_{61}H_2$: 2638.06783; found 1329.03337

CP8-deAc-Hexasaccharide (2-deAc)



2 (1.76 mg, 0.00128 mmol) was dissolved in 1 M NaOH (0.5 mL) and stirred overnight at rt. The solution was diluted with H_2O and neutralized with Amberlite IR-120 H^+ resins, filtered and lyophilized. Purification by HW-40 with NH_4OAc gave **2-deAc** in 41% yield (0.68 mg, 0.000529 mmol). **^{1}H NMR (850 MHz, D_2O)** δ 4.91 (t, *J* = 3.6 Hz, 2H), 4.88 (d, *J* = 4.0 Hz, 1H), 4.80 (d, *J* = 1.4 Hz, 1H), 4.78 (d, *J* = 1.3 Hz, 1H), 4.38 (d, *J* = 3.7 Hz, 1H), 4.34 (dd, *J* = 4.4, 1.4 Hz, 1H), 4.19 – 4.16 (m, 2H), 4.12 – 4.07 (m, 3H), 4.08 – 4.00 (m, 3H), 3.98 (dt, *J* = 11.8, 6.6 Hz, 2H), 3.94 (dd, *J* = 12.5, 2.7 Hz, 2H), 3.83 (dd, *J* = 11.0, 3.1 Hz, 2H), 3.79 – 3.63 (m, 10H), 3.60 – 3.54 (m, 4H), 3.51 (t, *J* = 9.8 Hz, 1H), 3.36 (dt, *J* = 10.0, 6.2 Hz, 1H), 2.90 (t, *J* = 7.7 Hz, 2H), 1.95 – 1.93 (m, 14H), 1.89 – 1.86 (m, 7H), 1.58 (q, *J* = 7.7 Hz, 2H), 1.54 (q, *J* = 8.2, 7.4 Hz, 2H), 1.35 (dq, *J* = 16.2, 7.7 Hz, 2H), 1.18 – 1.08 (m, 20H). **HRMS**: $[M+H]^+$ calculated for $C_{53}H_{82}N_7O_{29}H$: 1286.56264; found 1286.56234

CP8-deAc-Nonasaccharide (3-deAc)



2 (1.4 mg, 0.000699 mmol) was dissolved in 1 M NaOH (0.5 mL) and stirred overnight at rt. The solution was diluted with H_2O and neutralized AcOH and lyophilized. Purification by HW-40 with NH_4OAc gave **2-deAc** in 46% yield (0.6 mg, 0.000319 mmol). **^{1}H NMR (850 MHz, D_2O)** δ 4.93 – 4.89 (m, 4H), 4.80 (d, *J* = 16.8 Hz, 3H), 4.39 (d, *J* = 4.2 Hz, 1H), 4.36 (d, *J* = 4.3 Hz, 1H), 4.19 (dd, *J* = 11.0, 3.8 Hz, 3H), 4.13 – 3.92 (m, 18H), 3.87 – 3.83 (m, 1H), 3.77 – 3.67 (m, 10H), 3.62 – 3.50 (m, 6H), 3.37 (p, *J* = 6.1 Hz, 1H), 2.91 (t, *J* = 7.9 Hz, 2H), 1.99 – 1.93 (m, 19H), 1.90 – 1.87 (m, 9H), 1.57 (dp, *J* = 45.1, 7.3 Hz, 6H), 1.35 (dt, *J* = 16.1, 8.1 Hz, 2H), 1.16 – 1.13 (m, 12H), 1.12 (d, *J* = 7.1 Hz, 7H). **HRMS**: $[M+H]^+$ calculated for $C_{77}H_{124}N_{10}O_{43}H$: 1877.79020; found 1877.79204

Supporting information

Preparation of *S. aureus* type 8 conjugates

Preparation of S. aureus type 8 conjugates (CRM_{197} in PBS x1)

The CP8-OS were solubilized in 350 µL of a 9:1 DMSO:H_2O solution with either 30 equiv. (for **1**) or 15 equiv. (for **2** and **3**) of linker (suberic acid bis(*N*-hydroxysuccinimide ester)) and stirred for 2 h at rt. The derivatized CP8-OS were purified by EtOAc precipitation. The solution was first incubated with 5 mL cold EtOAc and 250 µL NaCl (3 M, aq.) for 1 h at 4 °C. The EtOAc layer was discarded and the bottom phase was washed with cold EtOAc (3 mL) 10-15 times. The resulting solids were lyophilized overnight. The mass after linker installation was measured and a 90% recovery was predicted.

For conjugation, a 20 mg/mL CRM_{197} solution in phosphate-buffered saline (PBS) was used with estimated 10, 20 and 30 eq of weighed derivatized CP8-OS.

Evaluation by SDS-PAGE:

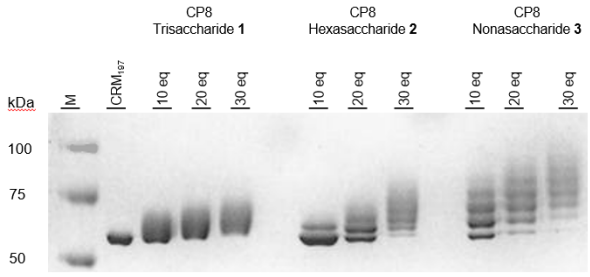


Figure S1: Evaluation of the CP8-conjugates performed in PBS

Preparation of S. aureus type 8 conjugates (CRM_{197} in HEPES 25 mM)

A CRM_{197} stock solution was buffer-exchanged to 25 mM HEPES pH 8.0 through Zeba™ Spin Desalting Column 7K MWCO. The derivatized **1**, **2** and **3** from earlier were used. 1.24 mg of 12-mer were derivatized following the same procedure as for the others.

To avoid weighing or not fully solubilizing the sugar in DMSO due to the remaining NaCl, the whole derivatized sample of **4** was used for conjugation, corresponding to approx. 35 equiv. For the **1**, **2** and **3**, the conjugation was made at 10.8 mg/mL of CRM_{197} with an estimated 30 equiv. of sugar. However, due to the initial low loading, the rest of the derivatized sugar solubilized in DMSO was added, for an estimated 60 equiv. of sugar (at approx. 8.8, 6.3 and 4.4 mg/mL of CRM_{197} for the **1**, **2** and **3**, respectively).

The conjugates were filtered (ø 0.2 µm) under sterile conditions. The protein content was quantified in triplicates with the Qubit™ Protein Assay (Invitrogen).

Evaluation by SDS-PAGE:

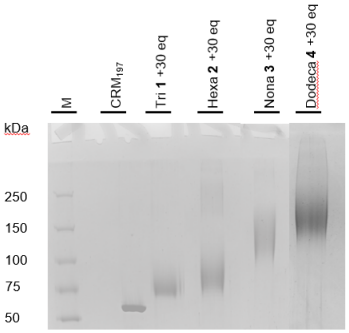


Figure S2: Evaluation of the CP8-conjugates performed in HEPES 25 mM

Evaluation of the conjugates can be found in Table S1.

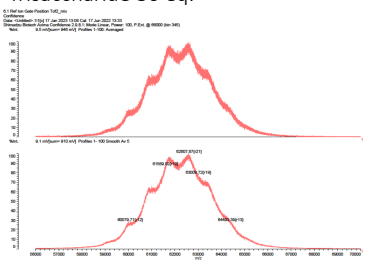
	1	2	3	4
Average number of conjugated sugar chains (MALDI)	11	8	13	14
Protein quantification (BCA) [mg/mL]	1.68	1.19	0.92	0.75
Average protein [µM]	19.0	10.3	9.2	12.8
Average sugar [µM]	316	163	206	187
Average sugar [µg/mL]	228	224	409	490
Saccharide/protein w/w	0.14	0.19	0.44	0.65

Table S1: Evaluation of the conjugates

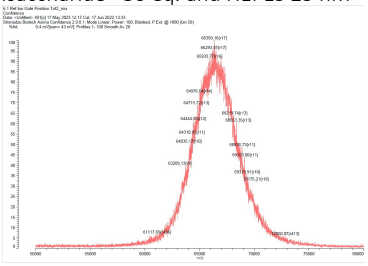
MALDI-TOF MS

For MALDI analysis (MALDI-TOF MS, AXIMA Performance, Shimadzu), 40 µL of conjugate samples (1 mg/mL), and CRM_{197} (0.5 mg/mL) were desalted in Amicon 0.5 mL MWCO 3K and exchanged to 1% TFA in MilliQ. For adding the sample to the matrix, a saturated solution of sinapic acid in 70% 1%TFA in MilliQ and 30% MeCN was used while a saturated solution of sinapic acid in absolute EtOH was used for priming the matrix.

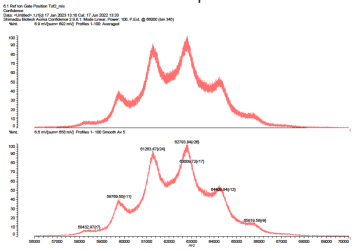
CP8 – Trisaccharide 30 eq.



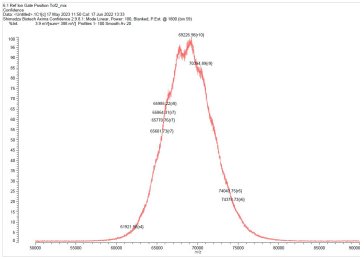
CP8 – Trisaccharide +30 eq. and HEPES 25 nM



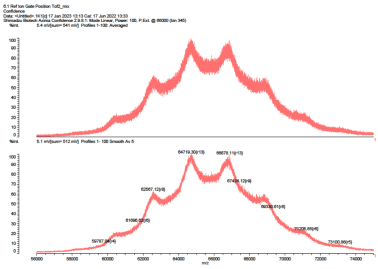
CP8 – Hexasaccharide 30 eq.



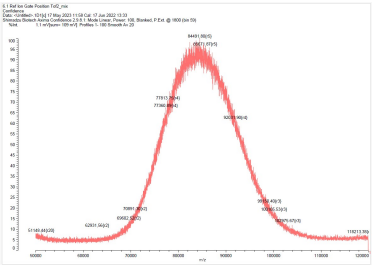
CP8 – Hexasaccharide +30 eq. and HEPES 25 nM



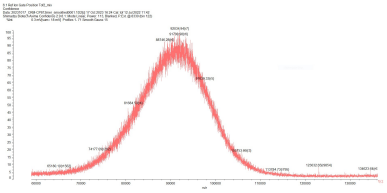
CP8 – Nonasaccharide 30 eq



CP8 – Nonasaccharide +30 eq. and HEPES 25 nM



CP8 – Dodecasaccharide +30 eq. and HEPES 25 nM



Protocol for Western Blot (using mAb and pAb)

SDS-PAGE were run the **1**-, **2**-, **3**- and **4** conjugates and a BSA-Pel-CRM_{197} conjugate (negative control), with a 7.5% acrylamide gel. The gel was transferred to a membrane for 30 min, which was blocked in 5% w/v milk in PBST (PBS supplemented with 0.1% Tween20) blocking solution for 1 h at rt. The membrane was then incubated for 1 h at rt with 1:1000 anti-CP8 mAb or 1:1000 anti-CP8 pAb (in blocking solution) followed by washing with PBST three times. Next, the membrane was incubated for 30 min at rt with 1:2000 IgGκ (m-IgGκ BP-HRP: sc-516102, Santa Cruz Biotechnology, in blocking solution) and again washed with PBST three times. The membrane was detected with Clarity Max Western ECL Substrate (Bio-Rad).

Western Blot performed with pAb and 1, 2 and 3:

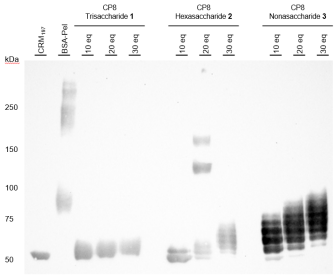


Figure S3: Western Blot with the CP8-Crm_{197} conjugates in PBS with pAb

Protocol for competitive ELISA with mAb

A 96-well plate was coated with 50 µL CP8 10 µg/mL in PBS, incubated at 4 °C overnight then washed with PBST (0.1% Tween-20 in PBS pH 7.4). The plate was blocked with 3% milk in PBST 0.1%) at 37 °C for 1.5 h. A 1.5-fold serial dilution of the CP8-OS competitors was prepared, followed by a pre-incubation with anti-CP8 mAb (final 0.63 µg/mL) at 37 °C for 30 min, after which 50 µL of each competitor sample were pipetted into their corresponding wells. The final competitor concentrations can be found in Table S2.

The plate was incubated at 37 °C for 1 h, then washed with PBST. 50 µL of anti-mouse IgG (secondary antibody, m-IgGκ BP-HRP: sc-516102, Santa Cruz Biotechnology) diluted 1:1000 in PBST 0.3% milk were pipetted into each well. The plate was incubated at 37 °C for 1.5 h, washed with PBST, then developed with 50 µL of coloring solution (Invitrogen, 1X TMB Substrate Solution) for 30 min at rt. The reaction was stopped with 25 µL of 0.16 M H_2SO_4 after which it was read at 450 nm.

	Competitor concentrations (µg/mL)											
1	1000	667	444	296	198	132	88	59	39	26	17	0
2	1000	667	444	296	198	132	88	59	39	26	17	0
3	700	467	311	207	138	92	61	41	27	18	12	0
4	300	200	133	89	59	40	26	18	12	8	5	0
CP8 PS	7	4.67	3.11	2.07	1.38	0.92	0.61	0.41	0.27	0.18	0.12	0

Table S2: The final competitor concentrations for competitive ELISA with mAb.

ELISA titers (synthetic fragments and mAb)

The calculation of IC50 values were performed with GraphPad Prism software using the variable slope model (GraphPad Prism Inc.). The means of each group were compared with a one-way ANOVA analysis; "**" denotes the significant result within $p < 0.01$, "ns" means not significant.

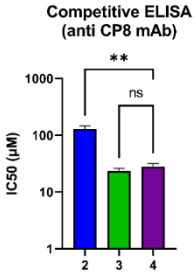


Figure S4: ELISA titers with synthetic fragments and mAb

Protocol for competitive ELISA with pAb

The competitive ELISA with a polyclonal serum against CP8-DT conjugate was run in the same fashion as for the mAb one (see above). A 2-fold (**2**, **3**, **4**) or 3-fold (**1** and **3**-deAc) serial dilution of the CP8-OS competitors was prepared, followed by a pre-incubation with anti-CP8 pAb (final dilution 1:500) at 37 °C for 30 min, after which 50 µL of each competitor sample were pipetted into their corresponding wells. The final competitor concentrations can be found in Table S3.

	Competitor concentrations (µg/mL)											
2	1000	500	250	125	63	31	16	8	4	2	1	0
3	700	350	175	88	44	22	11	5	3	1	1	0
4	700	350	175	88	44	22	11	5	3	1	1	0
1 / 3-deAc	1000	333	111	37	12	-	-	-	-	-	-	-

Table S3: The final competitor concentrations for competitive ELISA with pAb.

ELISA titers (synthetic fragments and pAb)

The calculation of IC50 values were performed with GraphPad Prism software using the variable slope model (GraphPad Prism Inc.). The means of each group were compared with a one-way ANOVA analysis; "***" denotes the significant result within $p < 0.001$, "ns" means not significant.

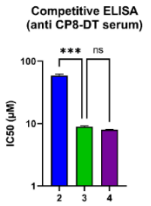


Figure S5. ELISA titers with synthetic fragments and pAb

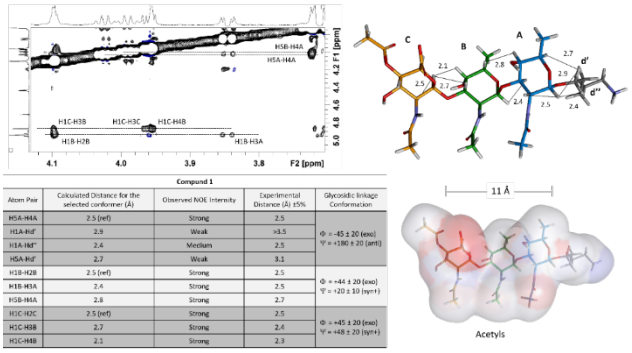
Structural conformation

Structure and conformational studies

NMR methods. NMR experiments were performed in a Bruker Avance III 800 MHz spectrometer equipped with a TCI cryoprobe. Samples were dissolved in D_2O at 1.0 mM concentration. Experiments were acquired at the temperature of 298 K.

^{1}H and ^{13}C NMR resonances of the molecules **1**, **2**, **2-deAc**, and **3** were assigned through standard 2D-TOCSY, 2D-ROESY, 2D-NOESY, 2D ^{1}H-^{13}C-HSQC. 2D-TOCSY experiments were acquired with 30 ms mixing time, 1.0 s of relaxation delay, 4 scans, and 4096x256 (F2xF1) points with a spectral width of 6556.0 Hz. 2D-ROESY experiment was acquired with mixing time of 200 ms, 1.0 s of relaxation delay, 48 scans, and 4096x256 (F2xF1) points with a spectral width of 6880.7 Hz. 2D-NOESY experiment was acquired with mixing time of 200 ms, 1.5 s of relaxation delay, 32 scans, and 4096x256 (F2xF1) points with a spectral width of 6242.2 Hz. 2D ^{1}H,^{13}C-HSQC experiments were acquired with 1.0 s of relaxation delay, 48 scans, and 4096x220 (F2xF1) points with a spectral width of 6250.0 Hz (F2) and 24144.6 Hz (F1). The data were processed with Topspin 4.2 (Bruker Biospin) using a 90° shifted qsine window function to a total of 16K × 2K data points (F2 × F1), followed by automated baseline- and phase correction.

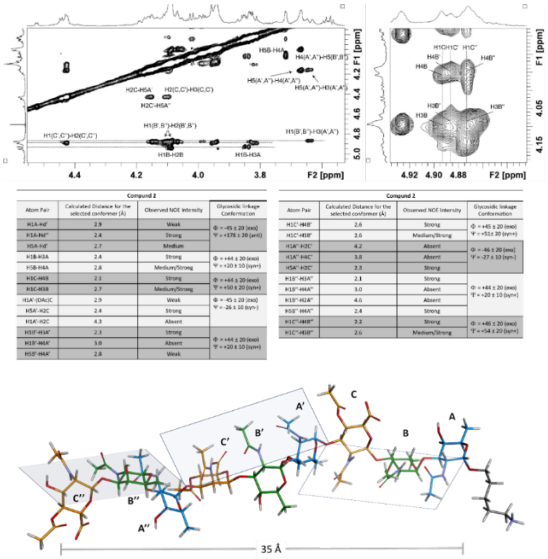
Molecular Mechanics Calculations. The geometry optimization was performed by using the Jaguar/Schroedinger package (version 13.5) and the AMBER* force field, with the GB/SA continuum solvent model for water. The glycosidic torsion angles were defined as ϕ (H1'-C1'-Ox-Cx) and ψ (C1'-Ox-Cx-Hx). Extended nonbonded cut-off distances (van der Waals cut-off of 8.0 Å and electrostatic cut-off of 20.0 Å) were used. The conformers for the tri- and nonasaccharide molecules **1** and **3** were generated employing geometric restrictions to respect the *exo*-anomeric effect. The possible staggered rotamers around ψ were selected and minimized. The coordinates of the obtained local minima were employed to measure the key inter-proton distances that were then compared to those obtained experimentally by the ROESY and NOESY NMR experiments through integration of the observed NOEs cross peaks using the ISPA approximation. The resulting conformations and NOE distances analysis are reported in Table S4 and Figure S9, for the trisaccharide **1**, and in Table S8 and Figure S18 for the nonasachharide **3**.



Compound 1

Atom Pair	Calculated Distance for the selected conformer (Å)	Observed NOE Intensity	Experimental Distance (Å) ±5%	Glycosidic linkage Conformation
H5A-H4A	2.5 (ref)	Strong	2.5	
H1A-H4'	2.9	Weak	>3.5	Φ = -45 ± 20 (exo) Ψ = +180 ± 20 (anti)
H1A-H4''	2.4	Medium	2.5	
H5A-H4'	2.7	Weak	3.1	
H1B-H2B	2.5 (ref)	Strong	2.5	
H1B-H3A	2.4	Strong	2.5	Φ = +44 ± 20 (exo) Ψ = +20 ± 10 (syn+)
H5B-H4A	2.8	Strong	2.7	
H1C-H2C	2.5 (ref)	Strong	2.5	
H1C-H3B	2.7	Strong	2.4	Φ = +45 ± 20 (exo) Ψ = +48 ± 20 (syn+)
H1C-H4B	2.1	Strong	2.3	

Figure S6 and Table S4: Expansion of NOESY spectrum of trimer **1**. Key cross-peaks defining the conformation around the glycosidic linkages are indicated. Table reporting the analysis of the experimental NMR-NOEs data and the derived conformation. Main conformation of **1** as determined by NOEs based calculated interatomic distances. Stick and surface representation of 3D structure of **1** in solution. The oligosaccharide length and orientation of the hydrophobic acetyl groups is represented.



Compound 2

Atom Pair	Calculated Distance for the selected conformer (Å)	Observed NOE Intensity	Glycosidic linkage Conformation
H1A-H4'	2.9	Weak	Φ = -45 ± 20 (exo) Ψ = +178 ± 20 (anti)
H1A-H4''	2.4	Strong	
H5A-H4'	2.7	Medium	
H1B-H3A	2.4	Strong	Φ = +44 ± 20 (exo) Ψ = +20 ± 10 (syn+)
H5B-H4A	2.8	Medium/Strong	
H3C-H4B	2.1	Strong	Φ = +44 ± 20 (exo) Ψ = +50 ± 20 (syn+)
H3C-H3B	2.7	Medium/Strong	
H1A'-(OAc)C	2.9	Weak	Φ = -45 ± 20 (exo) Ψ = -26 ± 10 (syn-)
H5A'-H2C	2.4	Strong	
H1A'-H2C	4.3	Absent	
H3F'-H4A'	2.3	Strong	
H3F'-H4A'	3.0	Absent	Φ = +44 ± 20 (exo) Ψ = +20 ± 10 (syn+)
H2F'-H4A'	2.8	Weak	

Compound 2

Atom Pair	Calculated Distance for the selected conformer (Å)	Observed NOE Intensity	Glycosidic linkage Conformation
H1C'-H4B'	2.0	Strong	Φ = +45 ± 20 (exo) Ψ = +51 ± 20 (syn+)
H1C'-H3B'	2.6	Medium/Strong	
H1A''-H2C'	4.2	Absent	Φ = -46 ± 20 (exo) Ψ = -27 ± 10 (syn-)
H1A''-H4C'	3.8	Absent	
H5A''-H2C'	3.3	Strong	
H1B''-H5A''	2.3	Strong	
H1B''-H4A''	3.0	Absent	Φ = +44 ± 20 (exo) Ψ = +20 ± 10 (syn+)
H1B''-H2A''	4.6	Absent	
H5B''-H4A''	2.4	Strong	
H1C''-H4B''	3.2	Strong	Φ = +46 ± 20 (exo) Ψ = +54 ± 20 (syn+)
H1C''-H3B''	2.6	Medium/Strong	

Figure S7 and Table S5: Expansion of NOESY spectrum of nonasaccharide **3**. Key cross-peaks defining the conformation around the glycosidic linkages are indicated, together with some intra-residue NOE as reference. Table reporting the qualitative analysis of the experimental NMR-NOEs data and the derived conformation. The major conformation of **3** as determined by the NOE analysis, assisted by MM calculations. The relative orientation of the imaginary planes containing the sugar's rings of each RU are represented.

Ligands-Antibody interaction studies

^{1}H-STD NMR experiments & methods. For the acquisition of the ^{1}H-STD-NMR experiments the mAb-CP8 antibody was buffer exchanged to deuterated PBS 1X pD 7.8 using centrifuge filters (Sartorius Vivaspin 6 50000 MWCO) up to an antibody concentrated of 2 µM. 100 equivalents of ligands (**1**-**3**) were added, which resulted into a solution of 2 µM of mAb and 200 µM of the ligand.

The STD experiments were recorded using Bruker AVANCE II 800 MHz NMR spectrometer equipped with cryo-probe (Bruker Inc.; Billerica, MA, US) at different temperatures that ranged between 288 and 310 K. The used ^{1}H-STD pulse sequence includes T2 filter, for protein NMR signal suppression, and excitation sculpting, for residual water NMR signal suppression. The STD NMR spectra were acquired with 2880 scans and 5 s of relaxation delay. Different conditions were screened for STD experiments. All the STD experiments were performed at both on-resonances, at the aliphatic (0.8 ppm) and aromatic (7.0 ppm) regions. The resulting STD spectra provided similar results (Figure S9). The on- and off-resonance spectra were registered in the interleaved mode with the same number of scans. The on-resonance protein saturation was obtained using a Gaussian shape pulse of 50 ms with a total saturation time of 2 s at a frequency of δ 0.8 ppm (aliphatic region). The off-resonance frequency was always set at δ 100 ppm.

The analysis was carried out using the ^{1}H NMR signals of the STD spectrum and from their comparison with the off-resonance spectrum, the STD-AF (Average Factor) was obtained. The strongest STD intensity was used as reference (100% of STD effect). On this basis, the relative STD intensities for the other protons were estimated from the comparison of the corresponding integrals. These relative STD intensities (STD%) were used to map the ligand-binding epitope.

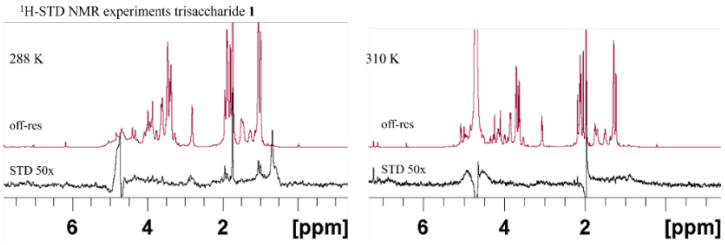


Figure S8: ^{1}H-STD NMR experiments of the trisaccharide **1** in presence on the mAb CP8.

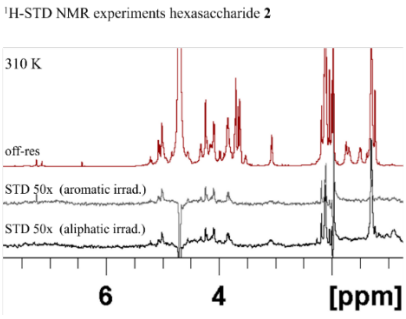


Figure S9: ^{1}H-STD NMR experiments of the hexasaccharide **2** in presence on the mAb CP8.

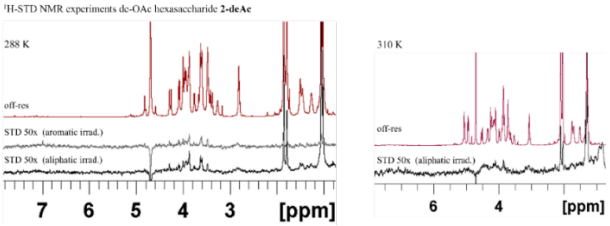


Figure S10: [1]H-STD NMR experiments of the de *O*-acetylated hexasaccharide **2-deAc** in presence on the mAb CP8.

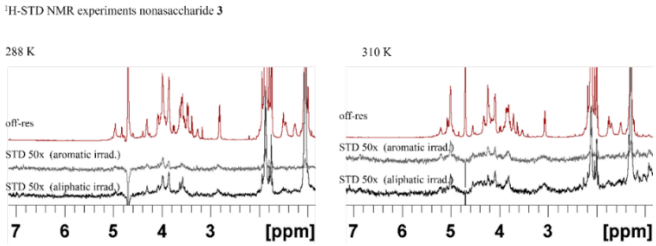


Figure S11: 1H-STD NMR experiments of the nonasaccharide **3** in presence on the mAb CP8.

Characterization of CP8-conjugated used for *in vivo* studies

Sample	Saccharide (µg/ml)	Protein (µg/ml)	Sacch/Prot (w/w)	LAL test (EU/ug)	Buffer
CP8 PS 100% OAc-CRM Lot FC09Ago21	121.5	145.7	0.8	0.40	PBS 1x pH 7.2
CRM197-CP8 TRI (lot KE230525-TRI)	228.1	1680.0	0.14	0.29	PBS 1x pH 7.2
CRM197-CP8 HEXA (lot KE230525-HEXA)	223.7	1191.8	0.19	<0.02	PBS 1x pH 7.2
CRM197-CP8 NONA (lot KE230525-NONA)	409.2	924.6	0.44	0.02	PBS 1x pH 7.2
CRM197-CP8 12-MER (lot KE230623)	491.1	749.4	0.65	0.07	PBS 1x pH 7.2

Immunizations

Animal studies were ethically reviewed by the local AWB and carried out at a GSK Animal Facility in Siena in accordance with Italian legislation law D.Lgs. 26/2014, and the GSK Policies on the Care, Welfare and Treatment of Animals. The welfare of the animals was maintained in accordance with the general principles of the Association for Assessment and Accreditation of Laboratory Animal Care.

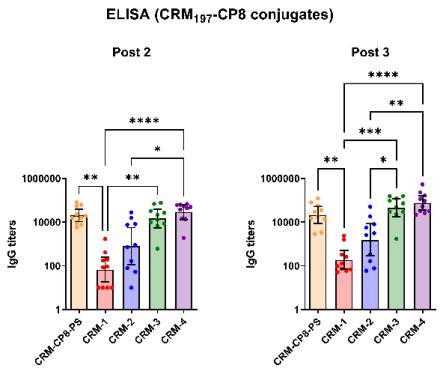
Five groups of 10 CD1 mice (5-week-old, female) were injected subcutaneous on day 1, 22 and 36 with 1.0 µg (saccharide titer) of conjugated carbohydrate antigen formulated with aluminum hydroxide as adjuvant. Sera were collected at day 0 (before first injection), 35 (32 days after first immunization) and 50 (14 days after second injection). The samples were collected from the retromandibular plexus.

ELISA protocol for *in vivo* studies

IgG titers in collected sera were estimated by ELISA. 96-well microtiter plates were coated with 0.1 µg of CP8 polysaccharide. The plates were incubated overnight at 2-8°C, then washed three times with PBST (0.05% Tween-20 in PBS pH 7.4). The wells were saturated with 250 µL/well of blocking buffer (2% Bovine Serum Albumin in PBST) for 90 min at 37°C. Two-fold serial dilutions of sera in blocking buffer were added to each well. The plates were then incubated at 37°C for 1h, washed with PBST, and then incubated for 90 min at 37°C with anti-mouse (whole molecule) IgG-alkaline phosphatase (Sigma-Aldrich) diluted 1:2000 in blocking buffer. The plates were washed with PBST and developed with a 4 mg/mL solution of p-Nitrophenyl Phosphate (pNPP) in 1 M diethanolamine (DEA) pH 9.8, at rt for 30 min. The absorbance was measured using a SPECTRAmax plate reader 405 nm. IgG titers were calculated by the reciprocal serum dilution giving an Optical Density (OD) of 1.

ELISA titers from the in vivo studies

The calculation of IC50 values were performed with GraphPad Prism software using Kruskal-Wallis with Dunn's multiple comparisons; "***" denotes the significant result within $p < 0.001$, "ns" means not significant.



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Chapter 3

Synthesis, conformational analysis and antibody binding of *Staphylococcus aureus* capsular polysaccharide type 5 oligosaccharides

Introduction

Capsular polysaccharides (CPs) can be found at the outer layer of encapsulated bacteria. They are anchored to the cell wall by covalent attachment to the peptidoglycan layer and can be built up of various monosaccharides to form long linear or branched compounds. The diversity of these compounds is enormous as they can be composed of different monosaccharides that are interconnected through different glycosidic linkages. They can feature varying *N*- and *O*-acetylation patterns, and be further modified with pyruvic acid ketals, phosphor mono- or diesters and lactic acids among others.[1] *Staphylococcus aureus* (*S. aureus*) is a pathogenic Gram-positive bacterium and 13 different serotypes have been identified to date based on different capsular polysaccharides. CP type 5 (CP5), CP type 1 (CP1) and CP type 8 (CP8) represent the most studied strains,[2,3] and CP5 and CP8 comprise the majority of the clinical isolates.[3] CP5 and CP8 have been found to be very similar in chemical structure, as they consist of the same three rare monosaccharides, but differ in glycosidic linkages and *O*-acetyl pattern. CP5 was first isolated in 1987 for the *S. aureus* Reynolds strain,[4] and its structure was elucidated through efforts of Vann,[5] Moreau[6] and Jones.[7] The structure has been established to be →4)-*O*-(2-acetamido-2-deoxy-β-D-mannopyranosyl uronic acid)-(1→4)-*O*-(2-acetamido-3-*O*-acetyl-2-deoxy-α-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-fucopyranosyl-(1→. The *O*-acetyl was found on the C-3-OH of L-FucNAc residue instead of the D-ManNAcA as shown in Figure 1, differing from CP8, which has the *O*-acetyl on the D-ManNAcA. As serotype 5 is one the most abundant *S. aureus* strains, its CP has been proposed to be a promising antigen, and therefore CP5 has been tested in different conjugate vaccine candidates.[8–10] Unfortunately clinical trials using a CP5 conjugate has not passed further than Phase I, in which limited efficacy was shown.[11,12] The reasons for the limited efficacy remain unknown, and in light of many other successful glycoconjugate vaccines it is quite surprising. The conjugates tested so far were relatively ill-defined as they were generated by random conjugation of fragmented polysaccharides to a carrier protein. To overcome some of the problems that can occur with isolated material, much attention has been directed towards the generation of well-defined synthetic fragments, which

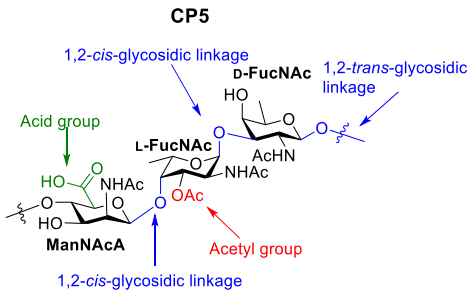


Figure 1: A schematic representation of the repeating unit of CP5.

allows the definition of the length of the saccharides, *O*-acetylation pattern and conjugation site.

Several synthetic CP5 trisaccharides have been synthesized using different strategies to install the 1,2-*cis* linkages, and incorporate the *O*-acetyl esters and carboxylic acid moieties, as summarized in Figure 2. To date only CP5-trisaccharides have been assembled, attesting to the challenges associated with the synthesis of these complex glycans. The first synthetic trisaccharide was reported by Adamo and co-workers back in 2012.[13] This trisaccharide was constructed from the non-reducing end and the strategy relied on a post-glycosylation epimerization of C-2 of a glucuronic acid derivative to generate the β-ManNAcA residue. The [2+1] glycosylation did not proceed in a stereospecific manner (α/β=2.3:1), showcasing the challenges associated with the synthesis of these glycans. The generated trisaccharide was evaluated in a competitive ELISA together with the isolated *S. aureus* CP5 polysaccharide, but no signification inhibition of binding to murine anti-CP5 serum was found. A dot blot assay did show weak recognition of the trisaccharide by the anti-CP5 serum, indicating that larger fragments of CP5 are probably needed for adequate recognition. In 2015, Boons and co-coworkers reported a synthetic strategy, relying on late-stage oxidation of the C-6-OH of a ManNAc residue to avoid lactam formation, which may form when mannuronic acid building blocks are used.[14] The trisaccharide was synthesized from the reducing end with late-stage *O*-acetylation of the L-FucN_3 residue. Stereoselectivity in the glycosylations were ensured using a pre-activation glycosylation strategy for the α-fucosylation and β-mannosylation reaction, while the use of the trichloroethyl chloroformate (Troc) protection of the amine in the D-FucN residue ensured β-selectivity in the glycosylation reaction with the linker. In 2016 Demchenko and co-workers reported a synthetic trisaccharide with methyl groups on both capping ends.[15] The trisaccharide was constructed from the non-reducing end and the synthetic plan relied on post-glycosylation epimerization and oxidation on a disaccharide and installation of the *O*-acetyl group prior to the final [2+1] glycosylation. The oxidation was executed after azide reduction to avoid lactamization. In 2017, Hagen *et al.* synthesized the trisaccharide repeating unit bearing a linker for conjugation purposes.[16] The required α-selectivity in the glycosylation delivering the L-Fuc-D-Fuc disaccharide was obtained by using a reactive fucose donor and a weak fucose acceptor nucleophile. For the β-mannosylation a ManN_3A donor was used to avoid the post-glycosylation oxidation. It was described however that a large excess of donor and nearly equimolar amounts of Lewis acid were required to obtain a good yield in the ManN_3A glycosylation reaction. The *O*-acetyl of the L-Fuc moiety was installed on the trisaccharide and

it was found necessary to install the *O*-acetyl before azide reduction and *N*-acetylation to avoid lactamization. In 2020, Kulkarni and co-workers published a synthetic route towards the CP5 repeating unit,[17] where the L-Fuc-D-Fuc disaccharide intermediate could be synthesized in good yields using a L-FucN_3 donor equipped with electron-withdrawing groups on both C-3 and C-4. For the [1+2] glycosylation a benzylidene glucose donor was used, and the efficiency of this glycosylation depended on the L-Fuc C-3-*O*-protecting group, with higher yields being obtained with the 2-methylnapthyl protected acceptor than with the C-3-*O*-acetyl acceptor.

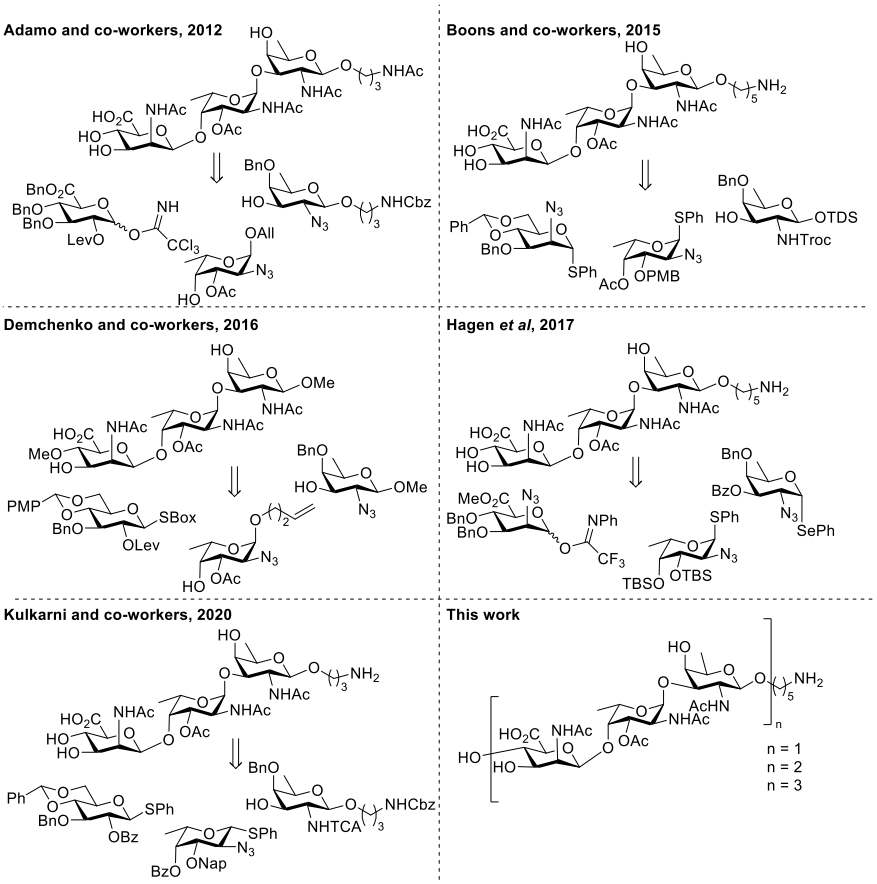


Figure 2: Previously reported synthesis of CP5 trisaccharides and the set of CP5 oligosaccharides reported in this Chapter.

This Chapter reports on the synthesis of conjugation ready CP5 fragments ranging from a trisaccharide to a nonasaccharide, having the *O*-acetyl on the C-3-OH of the L-FucNAc monosaccharides. In line with the work described in Chapter

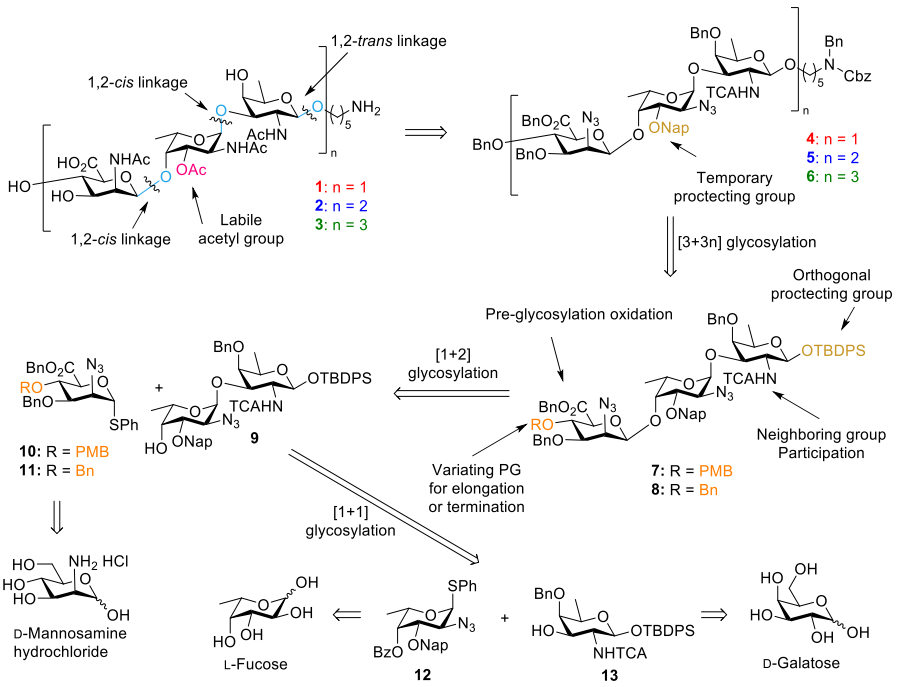
2, by implementing a $ManAN_3$ donor, the oxidation of multiple primary alcohols is prevented, as this has previously been found difficult.[18–20] However, in contrast to the syntheses described in Chapter 2, the *O*-acetyl esters will be installed at a late-stage to ensure higher reactivity in the L-Fuc-D-Fuc disaccharide glycosylation. The strategy relies on construction of the trisaccharide building block in a [1+2] manner and elongation of this building block to deliver larger saccharides in a [3+3n] manner. The structural studies of the well-defined structures show the adoption of extended conformations as it is revealed that the acetyl groups point in the opposite directions of two constructive trisaccharide units. SPR-experiments uncovered that the trisaccharide binds very weakly, but that both the hexa- and nonasaccharide are long enough to bound better to the antibodies raised against native CP5.

Results and discussion

Synthesis of the CP5-fragments

The retrosynthetic scheme of the target *S. aureus* CP5 oligosaccharides is shown in Scheme 1. A similar strategy as used for the assembly of the CP8-oligosaccharides descripted in Chapter 2, was implemented for the CP5-oligosaccharides, however now relying on neighboring group participation to ensure the stereoselective construction of the β-D-fucosamine linkages connecting the trisaccharides in the [3+3n] glycosylations. For the CP5-fragments, the *O*-acetyl at the C-3-OH position of the L-Fuc moiety would be installed at a late-stage as the electron withdrawing group lowers the α/β selectivity of the L-Fuc-D-Fuc glycosylation as found by Hagen *et al.*[16] Instead, a temporary 2-methylnaphthyl (Nap) ether is introduced as a masked precursor for the C-3-*O*-acetate. The anomeric position of the D-Fuc moiety in the key trisaccharide was protected with a temporary *tert*-butyldiphenylsilyl (TBDPS) group. To ensure the β-selective construction of the D-FucN linkages, the D-FucN acetamide was masked as a trichloroacetyl (TCA) to enable neighboring group participation, while the rest of the acetamides were masked as azides to ensure 1,2-*cis* selectivity. Both types of amine-protecting group can be reduced and acetylated in a one-pot reaction with zinc, acetic acid (AcOH) and acetic anhydride (Ac_2O) to deliver the required acetamides. For the D-ManA building block either a *p*-methoxybenzyl (PMB) or a benzyl were installed on the C-4-OH, with the PMB ether enabling orthogonal deprotection and the benzyl ether being installed for permanent protection in the terminating building block. In line with the CP8 assembly strategy, the use of

mannosaminuronic acid building blocks prevents challenging modification reactions on larger oligosaccharides. Global deprotection should be facilitated by the use of hydrogenation-labile permanent protecting groups.

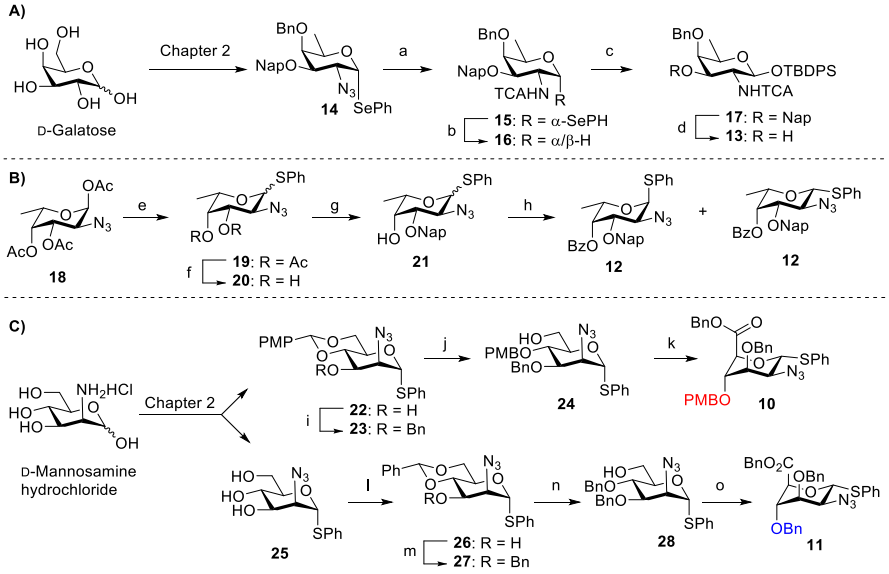


Scheme 1: Retrosynthetic analysis of the set of target CP5 oligosaccharides.

The D-FucNAc building block was generated from a D-FucN_3 intermediate, which was made using the same protocol as described in Chapter 2 (Scheme 2A). The azide in **14** was exchanged for a trichloroacetamide by reduction of the azide and subsequent acetylation with trichloroacetyl chloride (TCA-Cl)[17] giving **15** in 93% over two steps.[i] Hydrolysis of the anomeric phenylselenyl with *N*-iodosuccinimide (NIS) followed by silylation of the so-formed lactol with TBDSPS-Cl yielded **17** in good yields. Finally, acceptor **13** was achieved in respectable yields by oxidatively cleaving the Nap ether with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in wet dichloromethane (DCM).

[i] Trichloro acetylation of a building block in which the *O*-TBDPS had already been installed or on a building block with a free C-3-OH and C-1-*O*-TBDPS resulted in lower yields of 66% and 36%, respectively.

Synthesis of the L-FucN_3 building block started from **18** (Scheme 2B), where first a thiophenyl group was introduced on the anomeric position using boron trifluoride etherate ($BF_3{\cdot}Et_2O$) as a Lewis acid delivering **19** in 84% yield as an inseparable 2:1 α/β-mixture. Thiofucoside **19** was saponified under Zemplén conditions, followed by selective protection of the C-3-OH via a tin-acetal intermediate[21] to give **21**. The remaining C-4-OH was benzoylated to give donor **12** in 95% yield. At this stage the α- and β-products were separable by column chromatography.



Scheme 2: Synthesis of the building blocks **13** (A), **12** (B), **10** (C top) and **11** (C bottom). *Reaction conditions*: A) a) i) zinc, AcOH, THF, ii) TCA-Cl, THF, 0 °C, 93% over two steps, b) NIS, acetone/H_2O, 0 °C, 91%, c) TBDPS-Cl, imidazole, DMAP, DCM, 0 °C to rt, 92%, d) DDQ, DCM/H2O, 92%, B) e) PhSH, $BF_3{\cdot}Et_2O$, DCM, 84%, α/β=2:1, f) NaOMe, MeOH, 99%, g) Bu_2SnO, toluene, 140 °C then Bu_4NBr, CsF, NapBr, 120 °C, 99%, h) BzCl, DMAP, DCM/pyridine, 0 °C to rt, 95%, α/β = 56:44, C) i) BnBr, NaH, DMF, 0 °C to rt, 88%, j) $BH_3{\cdot}THF$, TMSOTf, DCM, 0 °C, 89%, k) i) TEMPO, BAIB, AcOH, DCM/*t*-BuOH/H_2O, 0 °C to rt °C, ii) BnBr, K_2CO_3, DMF, 91% over two steps, l) $PhCH(OMe)_2$, CSA, MeCN, 50 °C, 300 mbar, 79%, m) BnBr, NaH, DMF, 0 °C to rt, 75%, n) $BH_3{\cdot}THF$, TMSOTf, DCM, 0 °C, 96%, o) i) TEMPO, BAIB, AcOH, DCM/*t*-BuOH/H_2O, 0 °C to rt, ii) BnBr, K_2CO_3, DMF, 79% over two steps.

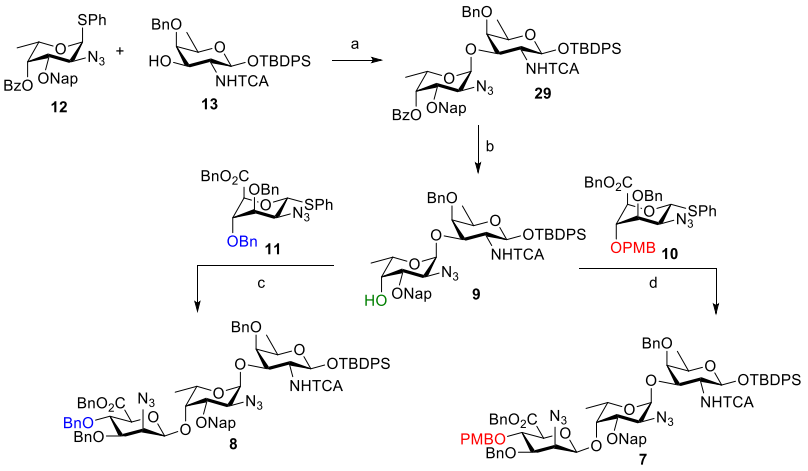
Two different D-ManN_3A building blocks were needed: one for elongation – equipped with a PMB ether, and one for the terminal end – equipped with a benzyl ether (Scheme 2C). For the PMB-building block **10** a route starting from **22**, previously described in Chapter 2, was implemented. The free C-3-OH in **22** was benzylated to give **23** which was followed by regioselective opening of the *p*-

methoxybenzylidene using borane tetrahydrofuran complex (BH_3·THF) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)[22] to give the C-4-*O*-PMB **24** in 89% yield. Dry conditions and molecular sieves were found important to avoid complete cleavage of the *p*-methoxybenzylidene acetal. The liberated C-6-OH was oxidized with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and (bisacetoxyiodo)benzene (BAIB)[23,24] followed by alkylation of the crude carboxylic acid with benzyl bromide to provide the first mannoazide donor **10** in good yields. ^{1}H-NMR analysis revealed the ManN_3A ring to flip from a $^4C_1$ to a $^1C_4$ -conformation. For the assembly of the Bn-protected **11** a route starting from **25**, described in Chapter 2, was pursued, by first introducing a 4,6-benzylidene acetal followed by benzylation of the free C-3-OH to give **27**. Regioselective opening using conditions described above now provided the C-4-*O*-Bn mannosazide **28** in 96% yield, in which the free C-6-OH was oxidized and alkylated as described above to give the second mannosazide donor **11**, which also was found to flip from a $^4C_1$ to a $^1C_4$ -conformation as judged by ^{1}H-NMR.

Now, the stage was set to investigate the synthesis of two trisaccharides – one for elongation from the nonreducing end equipped with the PMB ether on the C-4-Man$NAcN_3$ and one for the terminal end equipped with the benzyl ether on the C-4-Man$NAcN_3$. A [1+2] glycosylation strategy was implemented (Scheme 3), by first synthesizing the required L-FucN_3-D-FucN_3 disaccharide by glycosylation of the α-thiodonor **12** and acceptor **13** in the presence of NIS and TMSOTf to deliver the disaccharide **29** in 91% yield and an excellent α/β ratio on 95:5.[ii] The newly formed α-bond was confirmed by ^{1}H-NMR where the α-L-FucN_3 anomeric proton appeared as a doublet at 5.01 ppm with a coupling constant $J_{H1,H2}$ of 3.7 Hz. Acceptor **9** was obtained by saponification of the benzoyl ester under Zemplén conditions in 92% yield. It was observed that the cleavage required 10 days when using a catalytic amount (0.2 equivalents) of NaOMe, however the reaction time was reduced to two days upon use of a stoichiometric amount of NaOMe. The [1+2] glycosylation with benzyl donor **11** or PMB-donor **10** in the presence of NIS and TMSOTf proceeded in 91% and 86% yield, with an α/β ratio of 21:79 and 30:70, respectively. Both anomeric product mixtures were readily separated by column chromatography and the β-linkage in **8** and **7** was confirmed by ^{1}H-NMR and ^{13}C-NMR, with the β-ManN_3A anomeric proton and carbon

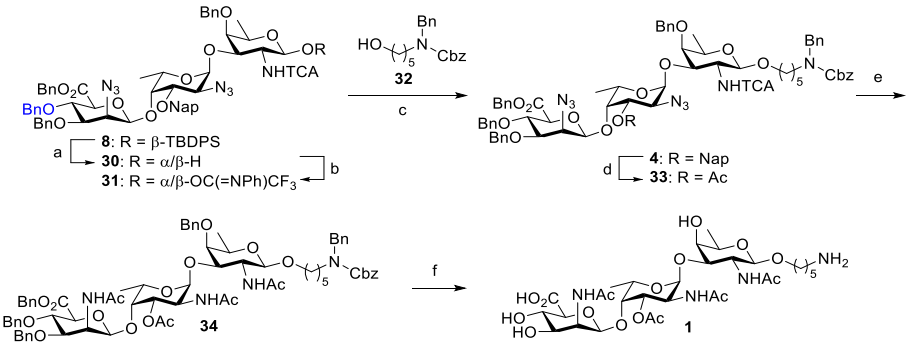
[ii] When using the β-thiophenyl donor the outcome of the reaction depending strongly on the temperature and reaction time, and the C-1-OTBDPS group was found to epimerize. See Appendix for detailed information.

having a CH-coupling constant of $J_{C1,H1}$ = 161.2 Hz and $J_{C1,H1}$ = 160.0 respectively for **8** and **7**.



Scheme 3: Synthesis of the two trisaccharides **8** and **7**. *Reaction conditions:* a) NIS, TMSOTf, DCM, -60 to -30 °C, 91%, α/β=95:5, b) NaOMe, MeOH, 92%, c) NIS, TMSOTf, DCM, -60 to -10 °C, 89%, α/β=21:79, d) NIS, TMSOTf, DCM, -60 to -30 °C, 86%, α/β=30:70.

With trisaccharide **8** in hand, assembly of first target trisaccharide **1** was undertaken as depicted in Scheme 4. First the TBDPS group was removed with tetrabutylammonium fluoride (TBAF) buffered by AcOH yielding hemiacetal **30** in 81% followed by installation of the *N*-phenyl trifluoroacetimidate[25] to give donor **31**. Glycosylation between donor **31** and the 5-amino-*N*-benzyl-*N*-benzyloxycarbonylpentanol[26] linker **32** in the presence of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) gave **4** in 73%, with the β-product being the only isolated product due to neighboring group participation. The newly formed β-linkage was confirmed by ^{1}H-NMR and ^{13}C-NMR with a clear doublet in the ^{1}H-NMR at 4.85 ppm with a $J_{H1,H2}$ = 7.8 Hz and a CH-coupling constant of $J_{C1,H1}$ = 160.7 Hz.

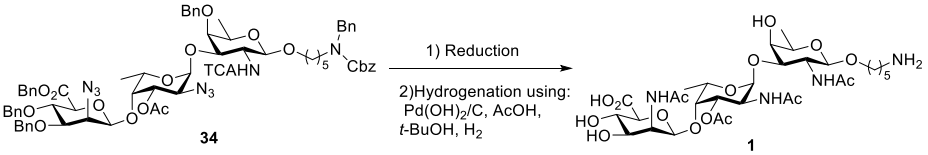


Scheme 4: Deprotection of the trisaccharide to form target **1**. *Reaction conditions:* a) TBAF, AcOH, THF, 0 °C to rt, 81%, b) $ClC(=NPh)CF_3$, K_2CO_3, acetone, 92%, c) TBSOTf, DCM/MeCN, -50 °C, 73%, d) i) DDQ, DCM/H_2O, ii) Ac_2O, DMAP, pyridine, 89 % over two steps, e) zinc, AcOH, Ac_2O, THF, 50 °C, 18%, f) $Pd(OH)_2$/C, AcOH, H_2, *t*-BuOH/H_2O, 56%.

Next, the L-Fuc C-3-*O*-Nap ether was oxidatively cleaved with DDQ, and the required *O*-acetyl ester was installed to give **33**. Subsequently, the azides and the TCA group were reduced with zinc and AcOH and acetylated using Ac_2O in one pot (Table 1, Entry 1). Unfortunately, after hydrogenation of the so-formed product, the product appeared to be impure (purity 60-75% by NMR), which was reasoned to be a consequence of incomplete reduction of the TCA group. Therefore, different conditions were investigated to optimize the reduction reaction (see Table 2). The use of activated zinc (Entry 2) or performing the reduction twice (Entry 3) did not lead to more pure material after hydrogenation. Chemoselective reduction of the azides using either Adams catalyst (PtO_2)[27,28] (Entry 4) or Ru/Al_2O_3[29], (Entry 5 and 6) did not lead to any reduced product at all, and employing a chemoselective Staudinger reduction of the azides did not improve the purity either (Entry 7). Finally, the pure product was obtained by implementation of a HPLC-purification step after the zinc reduction leading to 96% pure material based on the NMR after hydrogenation. Unfortunately, this did lead to a significant loss of yield and trisaccharide **34** was obtained in 18% yield.[iii] Hydrogenation of this trimer provided **1** in 56% after gel-filtration.

[iii] A ^{1}H-NMR spectrum comparing the impure and pure trisaccharide **1** can be found in the Appendix.

Table 1: Deprotection optimization towards trisaccharide **1**.

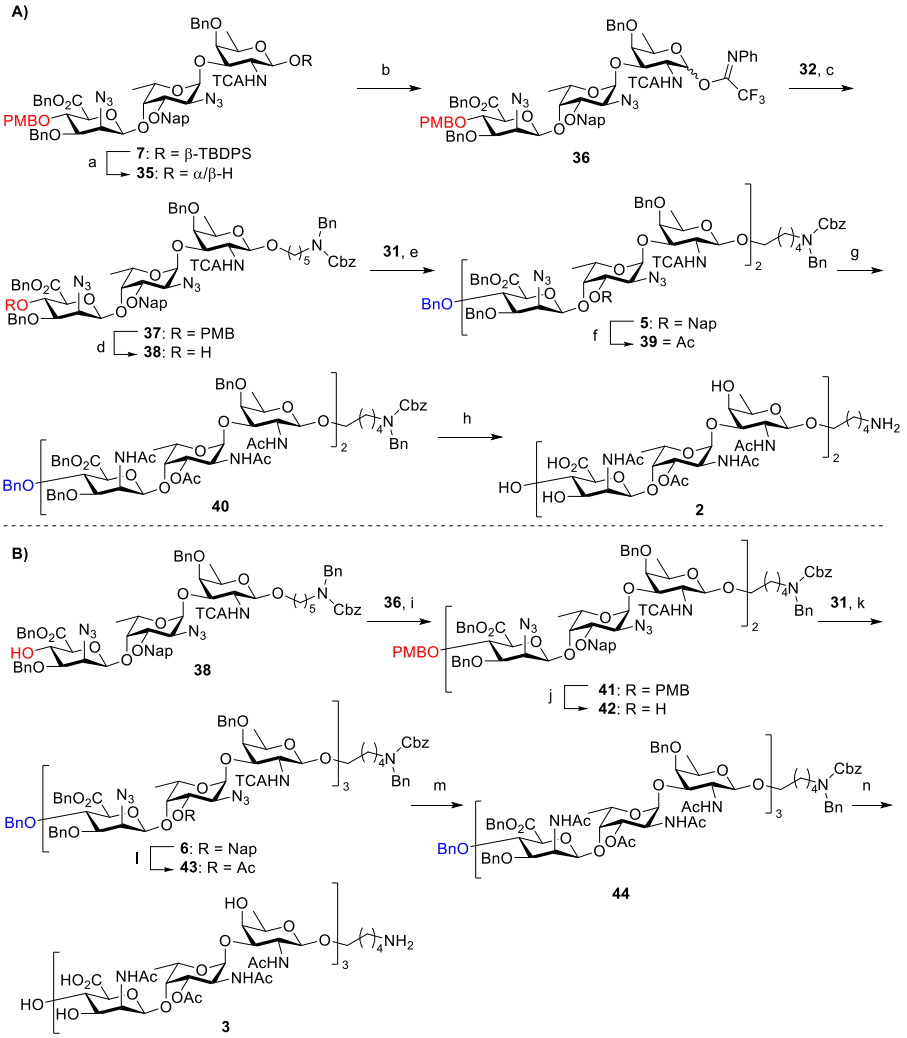


Entry	Conditions	Time	Yield (%)	Purity after hydrogenation (%) [a]
1	1) Zinc, AcOH, Ac_2O, THF, silica gel column 2) Hydrogenation	1) 22 h 2) 3 days	1) 78 2) 43	75
2	1) activated zinc, AcOH, Ac_2O, THF, silica gel column 2) Hydrogenation	1) 22 h 2) 3 days	1) 49 2) 79	60
3	1) Zinc, AcOH, Ac_2O, THF, silica gel column; put up twice 2) Hydrogenation	1) 22 h + 22 h 2) 3 days	1) 72 2) 81	75
4	1) PtO_2, EtOAc, H_2 then Ac_2O, MeOH 2) --	1) 3 days then 1 day 2) --	1) -- 	No reaction
5	1) Ru/Al_2O3, AcOH, EtOAc/ toluene, H_2 then Ac_2O, MeOH 2) --	1) 1 day then 1 day 2) --	1) -- 	No reaction
6	1) Ru/Al_2O3, AcOH, MeOH, H_2 then Ac_2O, MeOH 2) --	1) 1 day then 1 day 2) --	1) -- 	No reaction
7	1) PPh_3, H_2O, THF then H_2O, $NaHCO_3$, Ac_2O 2) Hydrogenation	1) 1 day then 2 h 2) 3 days	1) 83 [b] 2) --	Complex mixture [C]
8	1) Zinc, AcOH, Ac_2O, THF, silica gel column, followed by HPLC 2) Hydrogenation	1) 22 h 2) 3 days	1) 18 2) 56	96

[a] Purification calculated with H-NMR. [b] The NMR was a mess, thus the yield could be lower.
[c] Complex mixture according to NMR with impure the product being present.

The longer saccharides were synthesized by extending trisaccharide **7**. This was done by implementing the [3+3n] glycosylation strategy by turning trisaccharide **7** and **8** into suitable donors and acceptors. For the synthesis of hexasaccharide **2**, trisaccharide **7** (Scheme 5A) was first desilylated followed by installation of the *N*-phenyl trifluoroacetimidate giving donor **36**. Glycosylation of linker **32** with this donor delivered **37** in good yields and excellent β-selectivity again

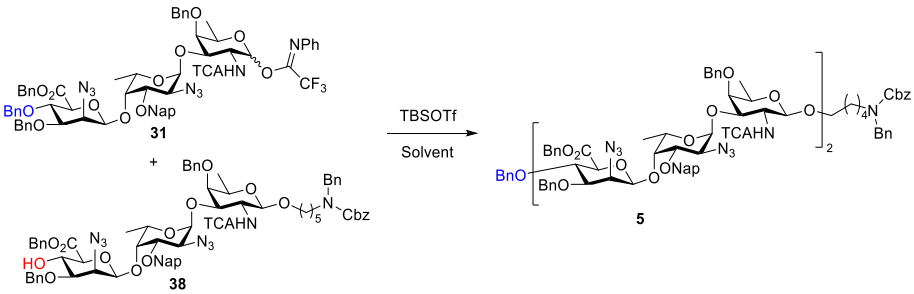
confirmed by ^{1}H-NMR and ^{13}C-NMR, with the D-FucN_3 proton and carbon having a CH-coupling constant of $J_{C1,H1}$ = 162.2. The PMB ether was removed using HCl in CH_2Cl_2/hexafluoroisopropanol (HFIP)[30] to provide acceptor **38**.



Scheme 5: Synthesis of hexasaccharide **2** (A) and nonasaccharide **3** (B). *Reaction conditions*: A) a) TBAF, AcOH, THF, 0 °C to rt °C, 87%, b) ClC(=NPh)CF_3, K_2CO_3, acetone, 96%, c) X, TBSOTf, DCM/MeCN, -50 °C, 88%, d) HCl in HFIP, TES, DCM, 0 °C, 76%, e) TBSOTf, DCM/MeCN, -78 °C, 70%, f) i) DDQ, DCM/H_2O, ii) Ac_2O, DMAP, pyridine, 82% over two steps, g) zinc, AcOH, Ac_2O, THF, 50 °C, 11%, h) $Pd(OH)_2$/C, AcOH, H_2, *t*-BuOH/H_2O, 47%, B) i) TBSOTf, DCM/MeCN, -78 °C, 80%, j) HCl in HFIP, TES, DCM, 0 °C, 76%, k) TBSOTf, DCM/MeCN, -78 °C, 79%, l) i) DDQ, DCM/H_2O, ii) Ac_2O, DMAP, pyridine, 74% over two steps, m) zinc, AcOH, Ac_2O, THF, 50 °C, 16%, n) $Pd(OH)_2$/C, AcOH, H_2, *t*-BuOH/H_2O, 49%.

The hexasaccharide was generated by glycosylation of acceptor **38** and donor **31** at -78 °C with TBSOTf as promoter. For this glycosylation, the temperature and solvent system were found to be important for the β-selectivity. Surprisingly, the use of a 1:1 DCM/ acetonitrile (MeCN) solvent system at -30 °C delivered the product as an inseparable α/β mixture (Table 3, Entry 1). The use of a higher reaction temperature did not improve the stereoselectivity (Entry 2), while lowering the temperature to -78 °C led to a frozen reaction mixture (Enry 3). By increasing the amount of DCM in the solvent system (to a 2:1 DCM/MeCN ratio) the reaction temperature could be lowered to -78 °C, and excellent β-selectivity was obtained, providing hexasaccharide **5** in 70% yield. Hexasaccharide **5** was deprotected by oxidative cleavage of the Nap ethers and acetylation giving **39** in 82% yield. Next, the azides and TCA groups were reduced and acetylated to provide the acetamides in **40**. Again, a purification method employing silica gel column chromatography and subsequent HPLC purification was found necessary to obtain pure **40**, which was isolated in 11%. Omission of the HPLC purification did lead to a significantly higher yield, but material that was ~75% pure after hydrogenation (as estimated from ^{1}H-NMR). Hydrogenation then gave hexasaccharide **2** in 47% yield.

Table 2: Optimization of the [3+3] glycosylation to form hexasaccharide **5**.



Entry	Solvent	Temp (°C)	Yield (%)	α/β	Notes
1	DCM/MeCN 1:1	-30	18	Inseparable [(a)]	
2	DCM/MeCN 1:1	0	38	Inseparable [(a)]	
3	DCM/MeCN 1:1	-78	--	--	Reaction froze – no reaction
4	DCM/MeCN 2:1	-78	70	1:99	

[(a)] The α/β ratio was impossible to identify from ^{1}H-NMR and ^{13}C-NMR

For the synthesis of nonasaccharide **3** (Scheme 5B), first hexasaccharide **41** was assembled from the trisaccharides **38** and **36**. The use of the optimized glycosylation conditions described above, stereoselectively delivered the hexamer in 80% yield. The PMB ether was removed to set the stage for the [3+6]

glycosylation and the union of acceptor **42** and donor **31** gave nonasaccharide **6** in 70% yield. The established deprotection scheme was again implemented by first transforming the Nap ethers into *O*-acetyl esters and ensuing reduction followed by the two-step purification gave **44** in 16% yield. Finally, hydrogenation afforded pure nonasaccharide **3** in 49% yield.

Conjugation and antibody binding

With the three synthetic fragments in hand, their interaction with anti-CP5 antibodies was investigated. To this end, the three synthetic fragments were first equipped with a suberic ester linker for conjugation to CRM_{197} as illustrated in Figure 3A. The derivatized sugars were conjugated to the carrier protein in 50 mM HEPES and pH 8.0 using 30 equivalents of sugar. The conjugates were evaluated by SDS-PAGE, and as shown in Figure 3B on the left no free protein was present and the conjugation was clearly visible, with every separate band representing an extra added oligosaccharide to the protein. The conjugates were tested for antibody recognition using Western Blot and two different anti-CP5 antibodies (anti-CP5 mAb) were investigated – the two mAbs were generated in both mice and rats (via Hybridoma Monoclonal Antibody Production). The mouse anti-CP5 mAb showed no recognition of the trisaccharide conjugate, but clearly bound to the hexa- and nonasaccharide equipped proteins. The rat anti-CP5-mAb also strongly recognized the hexa- and nonasaccharides and delivered a very faint band with the trisaccharide conjugate as seen in Figure 3B on the right. Both antibodies did not bind to CRM_{197} excluding the possibility that recognition of the protein led to visualization of the bands. Next, binding of the synthetic fragments to the rat mAb was tested by competitive surface plasmon resonance (SPR) using the natural CP5 polysaccharide (CP5-PS) as the immobilized component. For the trisaccharide, in line with the Western Blot experiments, very poor inhibition was found. In contrast, the hexa- and nonasaccharide bound the rat mAb well leading to inhibition by **2** and **3** with similar IC50 values as seen in Figure 3C. It can also be observed that binding of the natural PS is stronger than binding of the oligomers, which can be accounted for by multivalent binding of the antibodies because of the presence of multiple epitopes within the same polysaccharide chain. The binding of the hexa- and nonasaccharides indicates that the epitopes that are recognized likely span more than one repeating unit, or that the preferred epitope is constituted by a different frameshift of the repeating unit.

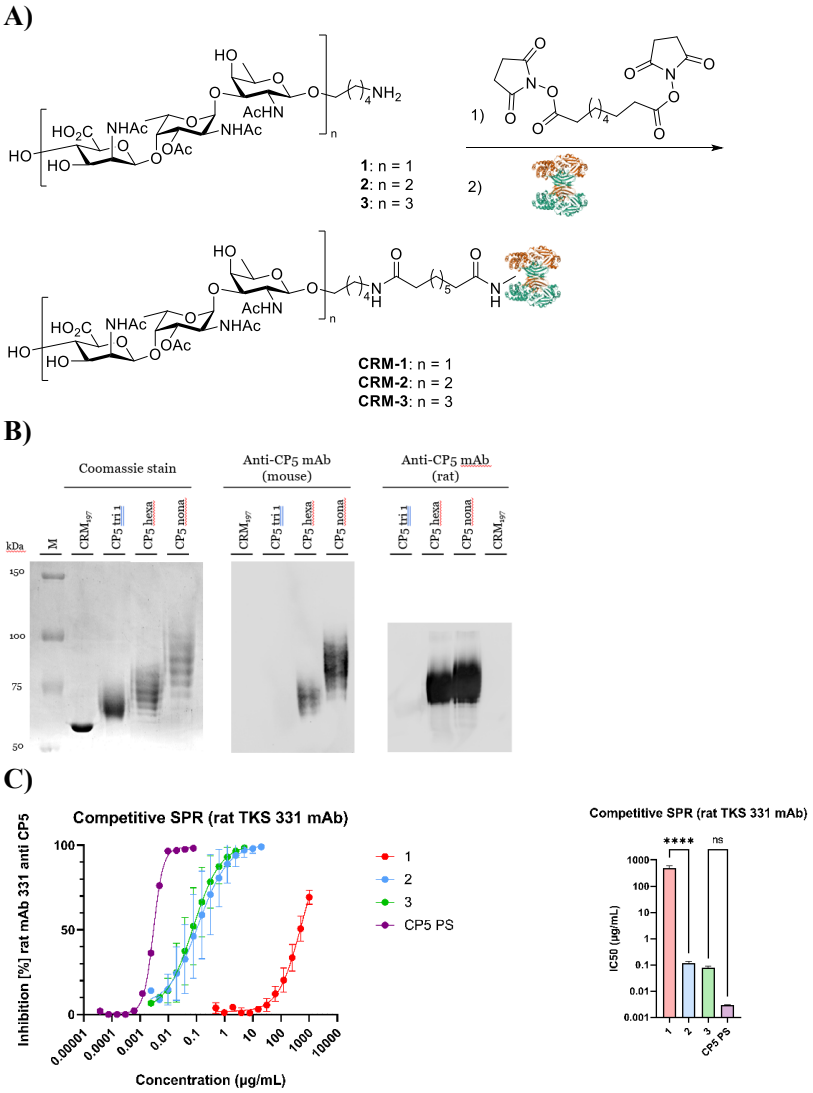
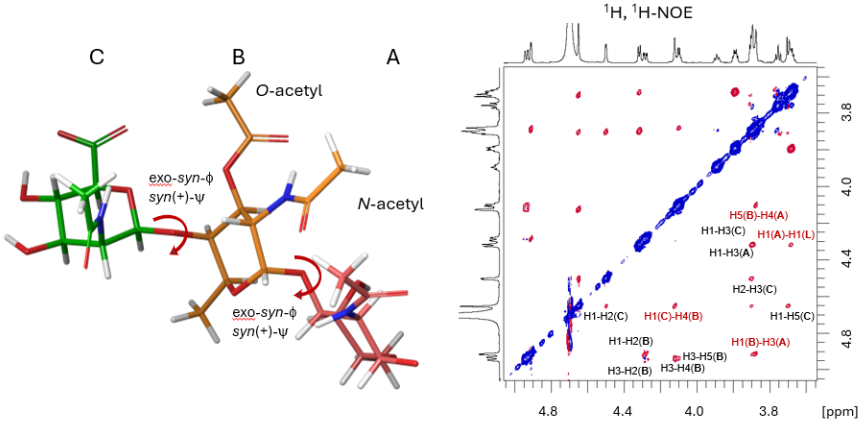


Figure 3: A) Conjugation of the synthetic fragments, 1) Suberic acid bis(N-hydroxysuccinimide ester) 15 equiv. in DMSO/H_2O 9:1, 2) CRM_{197} in 50 mM HEPES. B) SDS-PAGE of the conjugates CRM**1-3** (left) and Western Blots performed with either the mouse (middle) or rat (right) anti-mAb-CP5, which showed only weak recognition of **CRM-1** with the rat anti-mAb-CP5 and not the mouse version and both antibodies showed strong recognition of both the **CRM-2** and **CRM-3**. C) Competitive SPR results using the synthetic oligosaccharides and rat TKS 331 mAb showed that **1** was barely recognized by the antibody, while the recognition of **2** and **3** are similar. **** identify a significant difference, ns identify no significant difference.

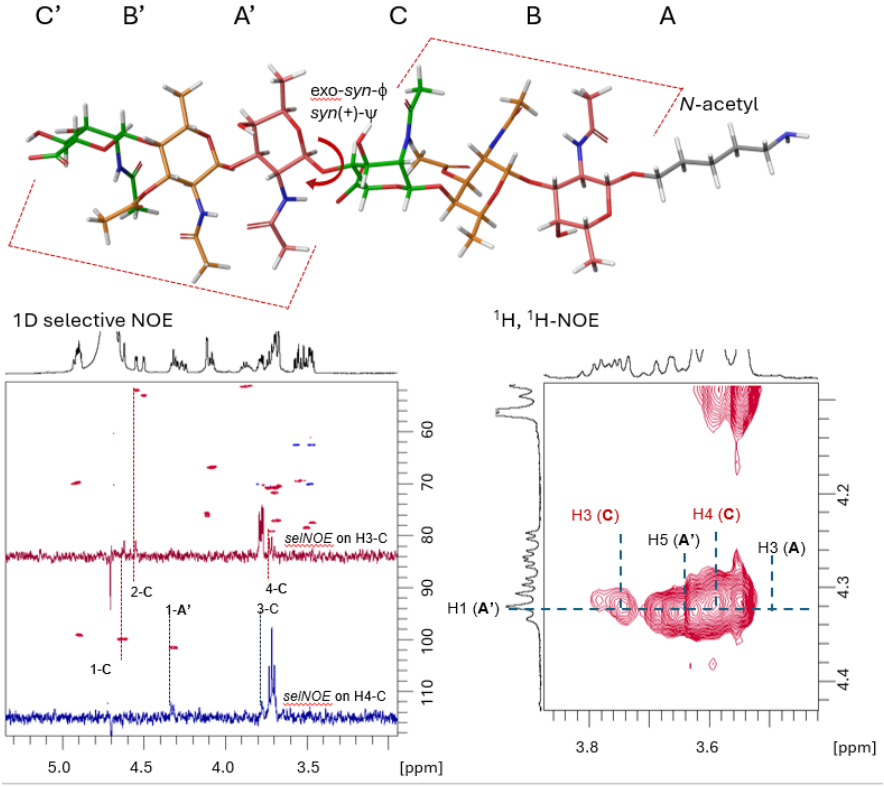
Structural and conformational studies

The next step was to determine the structures of the synthetic fragments by solving the conformation and dynamics of the synthetic fragments in solution. The spatial structures were determined by using a combination of NMR-methodologies (*J*-couplings and NOE-interactions) and computational protocols (MM). First, trisaccharide **1** was investigated as depicted in Figure 4A. By analyzing the intra-residual NOE and *J*-couplings the three pyranosides (C: D-ManNAcA, B: L-FucNAc and A: D-FucNAc) were found to adopt the predicted chair conformations ($^4C_1$ for the D-ManNAcA and D-FucNAc and $^1C_4$ for L-FucNAc). The conformation around the glycosidic linkages was investigated by comparing the NMR based derived structure with the MM calculation based one. For the C-B bond, strong NOEs of the H1(C)-H4(B) and H1(C)-H6(B) proton pairs suggest an equilibrium between the exo-*syn*-ϕ/*syn*(+)-ψ and the exo-*syn*-ϕ/*syn*(-)-ψ conformations with the exo-*syn*-ϕ/*syn*(+)-ψ being the major populated on. For the B-A linkage, strong NOE between the H1(B)-H3(A) and H5(B)-H4(A) proton pairs suggested an equilibrium between the exo-*syn*-ϕ/*syn*(+)-ψ and the exo-*syn*-ϕ/*syn*(-)-ψ conformations, with the exo-*syn*-ϕ/*syn*(+)-ψ being the most populated one. By MM calculation exo-*syn*-ϕ/*syn*(-)-ψ was found to be 1 Kcal/mol higher in energy. When expanding to hexasaccharide **2** the same configurations within the trisaccharide units were found, while the conformation around the glycosidic linkage between the two trisaccharidic units was found to be exo-*syn*-ϕ/*syn*(+)-ψ as seen in Figure 4B. The 2D ^{1}H-^{1}H-NOE spectrum showed correlation between the H1 from the A' with both the H3 and H4 of C. To determine the conformation, a 1D selective NOE experiment revealed strong NOE correlation for the H1(A') with H4(C) and not H3(C), demonstrating the main conformation to be exo-*syn*-ϕ/*syn*(+)-ψ. For nonasaccharide **3** the same observations as described for **1** and **2** could be established (Figure 4C), but now the 2D NOE experiments revealed **3** to adopt an extended conformation with the acetyl groups pointing in the same direction within the trisaccharidic units and in opposite direction (approximately 180 degree) between two constructive trisaccharidic units.

A) Trisaccharide **1**



B) Hexasaccharide **2**



C) Nonasaccharide **3**

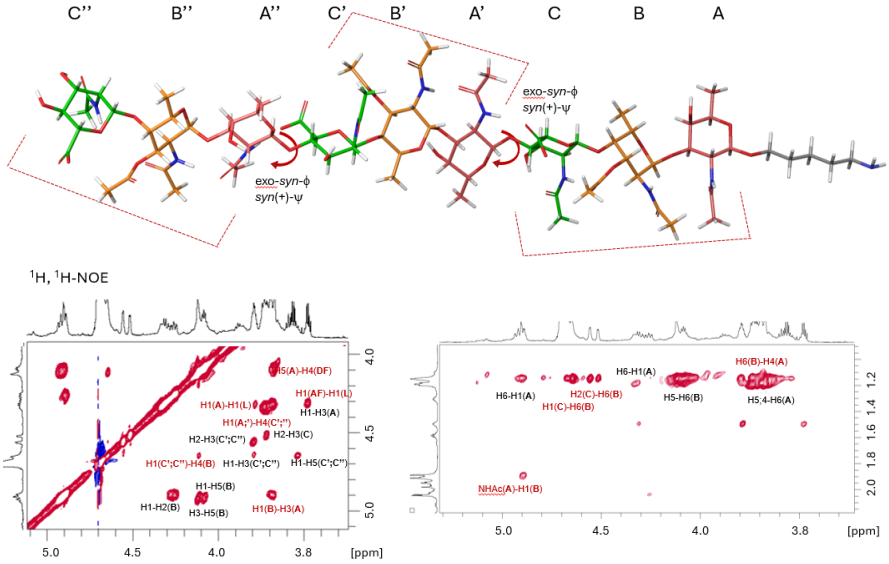


Figure 4: Conformational analysis of trisaccharide **1** (A), hexasaccharide **2** (B) and of nonasaccharide **3** (C) as established by NMR and MM calculations. Monosaccharidic residues are labeled with a letter code. The main conformation at each glycosidic linkage, the spatial orientation of the acetyl groups, and the average length are reported. All the main conformations are defined by NOE analysis and MM calculations A) Zoom area of 2D NOESY spectrum of trisaccharide **1** and its main conformation. B) Zoom area of 2D NOESY spectrum and the 1D selective NOE spectrum of hexasaccharide **2** and its main conformation. C) Zoom area of 2D NOESY spectrum of nonasaccharide **3** and its main conformation.

Conclusion

In this Chapter, a synthetic protocol for conjugation-ready CP5 oligosaccharides has been developed, and immunological experiments highlight the need for longer synthetic fragments for effective antibody responses, with the hexasaccharide identified as the minimal binding epitope. The synthetic strategy employs a pre-glycosylation oxidation method to introduce mannosaminuronic acids and two fucosazide synthons, allowing for the assembly of two key intermediate trisaccharides – one for elongation and one for the terminal end. Larger structures, ranging from a trimer to a nonamer, were generated using a [3+3n] glycosylation method. Deprotection of the fragments proved challenging, as obtaining fully reduced and acetylated TCAs was difficult. After extensive investigation, it was found that implementing silica gel chromatography followed by HPLC purification after the reduction step yielded pure material, albeit in extremely low yields.

Each fragment was equipped with an aminopentyl linker, facilitating conjugation to CRM_{197} to create a set of model conjugate vaccines. The glycoconjugates were evaluated for their binding to monoclonal antibodies. It was found that fragment length significantly impacted binding, with the trisaccharide being too short to effectively bind the antibodies or elicit an immune response capable of recognizing the natural polysaccharide. In contrast, the hexasaccharide and nonasaccharide exhibited comparable binding levels, indicating that the minimal binding epitope is present in the hexasaccharide. These results were confirmed by SPR experiments, which showed similar binding levels for the hexasaccharide and nonasaccharide. The structural conformation of the well-defined fragments revealed a linear formation, with the acetyl groups in each trisaccharide unit pointing in opposite directions in two consecutive trisaccharide units.

Acknowledgement

Luca Unione from CIC BioGune is acknowledged for his contribution to the conformational analysis. Filippo Carboni and Linda Del Bino from GSK vaccines are acknowledged for their help with the SPR-experiments.

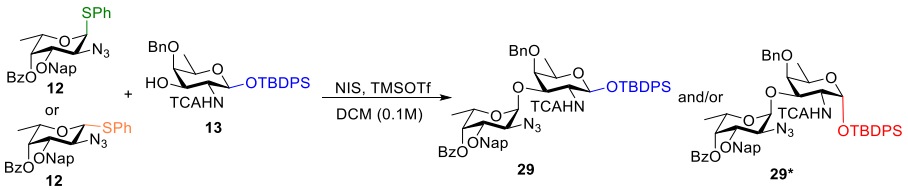
Conflict of interest: Kitt Østerlid has participated in a post graduate studentship program at GSK. This work was sponsored by GlaxoSmithKline Biologicals SA.

Appendix

Glycosylation of the disaccharide 29 using the β-thiophenyl donor

For the disaccharide glycosylation the reactivity and outcome of the glycosylation were found to be depending on thiophenyl-donor. A slower and warmer glycosylation was found to affect the anomeric TBDPS group on the D-Fuc residue. The TBDPS group was found to epimerize giving the α-TBDPS (**29***) if the β-donor-**12** was used at higher temperatures.[31] When preforming the glycosylation with the β-donor at -60 to -30 °C (Table S1, Entry 2), as was performed for the α-donor (Entry 1) a yield on 48% was obtained of **29**, however a quite long reaction time (3.5 h) was needed for conversion. The lower yields could be explained by long-range participation, where maybe a dioxolenium ion is formed directly from the α- or β-thiophenyl donor and the benzoyl in the α-donor is better positioned for this formation, thus the α-donor is more reactive. An attempt to improve the yield was investigated by raising the temperature from -60 to -10 °C and a reaction time on 4 h (Entry 3). Now 63% of only **29*** was obtained. By raising the temperature and lowering the reaction time (Entry 4 and 5) a higher ratio of product **29** was obtained. When prolonging the reaction time and/or raising the temperature the TBDPS group was found epimerizing to the α-TBDPS, which is normally not obtained due to steric. Obtaining only **29** using the β-thio donor has not been achieved, however **29*** can easily be used for further reactions.

Table S1: Glycosylation between two different thiophenyl donors and the D-Fuc acceptor resulting in different isomerized disaccharide products.



Entry	Donor	Temp (°C)	Time (h)	Product	Yield (%)	α/β [a]
1	**12α** (1.3 equiv.)	-60 to -30	0.75	**29**	89	91:8
2	**12β** (1.4 equiv.)	-60 to -30	3.35	**29**	48	99:1[b]
3	**12β** (1.5 equiv.)	-60 to -10	4	**29***	63	99:1[b]
4	**12β** (1.5 equiv.)	-30 to -10	2	**29**:**29***=1:3.5	74	99:1[b]
5	**12β** (1.5 equiv.)	-10	1	**29**:**29***=1:1	65	99:1[b]

[a]) The α/β ratio was determined from NMR of the purified product. [b] No β-product was found.

Purity of trisaccharide 1

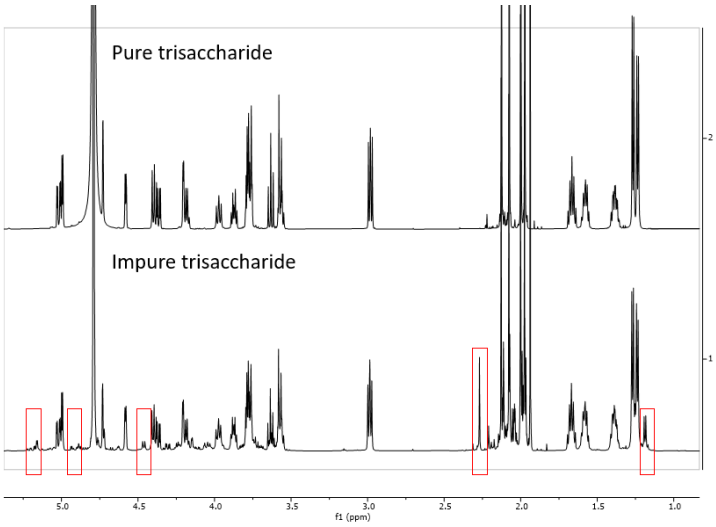


Figure S1: Difference between the pure and impure trisaccharide, obtained with and without HPLC purification, respectively.

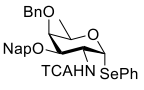
Experimental

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 °C) 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.

Synthesis of the building blocks

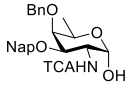
Phenyl 4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-2-*N*-trichloroacetamide-1-seleno-α-D-fucopyranoside (15)



14 (3.471 g, 6.214mmol) was dissolved in distilled THF (62 mL, 0.1 M). zinc powder (4.47 g, 68.36 mmol, 11 equiv.) was gently added to the solution followed by AcOH (3.2 mL, 55.92 mmol, 9 equiv.). The reaction was stirred under nitrogen at rt overnight until TLC (pentane/EtOAc, 95:5) showed full conversion. The reaction mixture was filtered over a path of celite and concentrated *in vacuo*. The crude was co-evaporated with toluene (x3) before being dissolved in distilled THF (41 mL, 0.15 M). Flamed dried 3Å molecular sieves were added to the solution and the mixture was stirred under nitrogen for 30 min. Then. The solution was cooled to 0 °C and trichloroacetyl chloride (1.4 mL, 12.43 mmol, 2 equiv.) was added. The reaction mixture was stirred for 30 min at 0 °C under nitrogen until TLC (pentane/EtOAc, 8:2) showed full conversion. The reaction mixture was diluted in DCM, washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 95:15 → 85:15) yielded **15** in 76% yield (3.212 g, 4.598

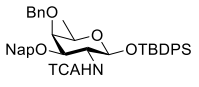
mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.90 – 7.78 (m, 4H, *Ar-H*), 7.55 – 7.44 (m, 5H, Ar-H), 7.41 – 7.20 (m, 8H, Ar-H), 6.86 (d, *J* = 7.5 Hz, 1H, *H*N(CO)CCl_3), 6.04 (d, *J* = 4.7 Hz, 1H, H-1), 5.01 (d, *J* = 11.5 Hz, 1H, ArC*H*$_2$), 4.90 (d, *J* = 12.2, 0.8 Hz, 1H, ArC*H*$_2$), 4.84 – 4.74 (m, 1H, H-2), 4.74 – 4.67 (m, 2H, ArC*H*$_2$), 4.27 – 4.17 (m, 1H, H-5), 3.86 (dd, *J* = 2.6, 1.3 Hz, 1H, H-4), 3.65 (dd, *J* = 11.0, 2.5 Hz, 1H, H-3), 1.28 (d, *J* = 6.5 Hz, 3H, *H*-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 161.68 (*C*=O), 138.13 (Ar-C_q), 134.66 (Ar-C_q), 134.18 (Ar-C_q), 133.39 (Ar-C_q), 133.25 (Ar-C_q), 129.41 (Ar-*C*), 128.93 (Ar-*C*$_q$), 128.84 (Ar-*C*), 128.50 (Ar-*C*), 128.39 (Ar-*C*), 128.09 (Ar-*C*), 128.06 (Ar-*C*), 127.96 (Ar-*C*), 127.92 (Ar-*C*), 126.77 (Ar-*C*), 126.60 (Ar-*C*), 126.41 (Ar-*C*), 125.57 (Ar-*C*), 89.27 (C-1), 78.81 (C-3), 74.93 (Ar-*C*H$_2$), 74.60 (C-4), 71.70 (Ar-*C*H$_2$), 70.62 (C-5), 52.07 (C-2), 16.97 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{32}H_{30}Cl_3NO_4SeNa$: 700.03033; found 700.0293

4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-2-*N*-trichloroacetamide-α-D-fucopyranose (16)



15 (3.212 g, 4.738 mmol) was dissolved in acetone/ H_2O (9:1; 95 mL, 0.05 M). The reaction was cooled to 0 °C, followed by addition of NIS (2.132 g, 9.475 mmol, 2 equiv.). The reaction mixture was stirred 15 min at 0 °C until TLC (pentane/EtOAc, 8:2) showed full conversion. The reaction was quenched with $Na_2S_2O_3$ (sat., aq.) and the solvent was evaporated *in vacuo*. The crude residue was diluted in EtOAc and the organic phase was 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, 80:20 → 60:40) yielded hemiacetal **16** in 95% (2.431 g, 4.512 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.86 – 7.77 (m, 4H, Ar-*H*), 7.56 – 7.43 (m, 3H, Ar- *H*), 7.41 – 7.35 (m, 2H, Ar- *H*), 7.34 – 7.26 (m, 3H, Ar- *H*), 6.81 (d, *J* = 8.9 Hz, 1H, *H*N(CO)CCl_3), 5.38 (t, *J* = 3.6 Hz, 1H, H-1), 5.03 (d, *J* = 11.6 Hz, 1H, ArC*H*$_2$), 4.87 (d, *J* = 12.2 Hz, 1H, ArC*H*$_2$), 4.81 – 4.61 (m, 3H, H-2, ArC*H*$_2$), 4.14 – 4.06 (m, 1H, H-5), 3.85 (dd, *J* = 10.9, 2.5 Hz, 1H, *H*-3), 3.81 – 3.73 (m, 1H, H-4), 2.94 (dd, *J* = 3.5, 1.5 Hz, 1H, O*H*), 1.20 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 161.97 (*C*=O), 138.29 (Ar-*C*$_q$), 135.13 (Ar-*C*$_q$), 133.35 (Ar-*C*$_q$), 133.18 (Ar-*C*$_q$), 128.63 (Ar-*C*), 128.56 (Ar-*C*), 128.51 (Ar-*C*), 128.46 (Ar-*C*), 128.07 (Ar-*C*), 127.91 (Ar-*C*), 127.87 (Ar-*C*), 126.63 (Ar-*C*), 126.43 (Ar-*C*), 126.23 (Ar-*C*), 125.70 (Ar-*C*), 91.78 (C-1), 77.03 (C-3), 75.01 (C-4), 74.74 (Ar*C*H$_2$), 71.84 (Ar*C*H$_2$), 67.09 (C-5), 51.25 (C-2), 17.11 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{26}H_{26}Cl_3NO_5Na$: 560.07743; found 560.07688

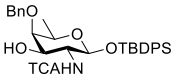
***Tert*-butyldiphenylsilyl 4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-2-*N*-trichloroacetamide-β-D-fucopyranoside (17)**



16 (2.417 g, 4.486 mmol) was co-evaporated with toluene (x3) before being dissolved in dry DCM (22 mL, 0.2 M). The reaction was cooled to 0 °C followed by addition of DMAP (110 mg, 0.897 mmol, 0.2 equiv.) and imidazole (764 mg, 11.216 mmol, 2.5 equiv.) and TBDPS-Cl (1.4 mL, 5.383 mmol, 1.5 equiv.). The reaction mixture was stirred under nitrogen at rt overnight until TLC analysis (pentane/EtOAc, 7:3) showed full conversion. The reaction mixture was diluted in EtOAc, washed with 1 M HCl (aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 98:5 → 90:10) furnished **17** in 86% yield (2.99 g, 3.846 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.80 (m, 3H, Ar-*H*), 7.76 – 7.69 (m, 3H, Ar-*H*), 7.67 – 7.59 (m, 2H, Ar-*H*), 7.52 – 7.27 (m, 14H, Ar-*H*), 6.80 (d, *J* = 7.1 Hz, 1H, *H*N(CO)CCl_3),

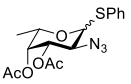
4.96 (d, *J* = 11.8 Hz, 1H, Ar-C*H*$_2$), 4.88 (d, *J* = 7.3 Hz, 1H, H-1), 4.78 (d, *J* = 11.7 Hz, 1H, Ar-C*H*$_2$), 4.68 (d, *J* = 11.7 Hz, 1H, Ar-C*H*$_2$), 4.67 (d, *J* = 11.8 Hz, 1H, ArC*H*$_2$), 4.12 – 3.97 (m, 2H, H-2, H-3), 3.59 (dd, *J* = 2.6, 1.1 Hz, 1H, H-4), 3.30 – 3.20 (m, 1H, H-5), 1.09 – 1.01 (m, 12H, H-6, (C*H*$_3$)$_3$). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 138.55 (Ar-*C*$_q$), 136.24 (Ar-*C*), 135.98 (Ar-*C*), 135.19 (Ar-*C*$_q$), 133.55 (Ar-*C*$_q$), 133.42 (Ar-*C*$_q$), 133.32 (Ar-*C*$_q$), 133.16 (Ar-*C*$_q$), 129.76 (Ar-*C*), 129.65 (Ar-*C*), 128.49 (Ar-*C*), 128.39 (Ar-*C*), 128.18 (Ar-*C*), 128.04 (Ar-*C*), 127.85 (Ar-*C*), 127.75 (Ar-*C*), 127.60 (Ar-*C*), 127.34 (Ar-*C*), 126.87 (Ar-*C*), 126.38 (Ar-*C*), 126.21 (Ar-*C*), 125.97 (Ar-*C*), 94.84 (C-1), 77.95 (C-3), 75.14 (C-4), 74.74 (Ar-*C*H$_2$), 72.28 (Ar-*C*H$_2$), 70.60 (C-5), 57.66 (C-2), 27.11 ((*C*H$_3$)$_3$), 16.82 (*C*-6). **HRMS**: [M+Na]$^+$ calculated for $C_{42}H_{44}Cl_3NO_5SiNa$: 798.19520; found 798.19465

***Tert*-butyldiphenylsilyl 4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-β-D-fucopyranoside (13)**



17 (2.99 g, 3.846 mmol) was dissolved in DCM/H$_2$O (20:1, 38 mL, 0.1 M) followed by addition of DDQ (1.309 g, 5.769 mmol, 1.5 equiv.). The reaction mixture was stirred under nitrogen for 2 h at rt until TLC (pentane/EtOAc, 9:1) showed full conversion. The reaction mixture was subsequently quenched with $Na_2S_2O_3$ (sat. aq.) and diluted in EtOAc. The organic phase was washed with $NaHCO_3$ (sat. aq.; x5) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 85:15 → 80:20) furnished acceptor **13** in 85% yield (2.088g, 3.278 mmol). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.76 – 7.68 (m, 2H, Ar-*H*), 7.68 – 7.61 (m, 2H, Ar-*H*), 7.46 – 7.28 (m, 11H, Ar-*H*), 6.61 (d, *J* = 7.9 Hz, 1H, *H*N(CO)CCl$_3$), 4.80 (d, *J* = 11.6 Hz, 1H, ArC*H*$_2$), 4.71 (d, *J* = 11.6 Hz, 1H, ArC*H*$_2$), 4.56 (d, *J* = 7.9 Hz, 1H, H-1), 4.00 (dt, *J* = 10.8, 7.9 Hz, 1H, H-2), 3.66 (td, *J* = 10.7, 9.8, 3.5 Hz, 1H, H-3), 3.50 (dd, *J* = 3.6, 1.1 Hz, 1H, H-4), 3.34 – 3.24 (m, 1H, H-5), 2.56 (d, *J* = 9.8 Hz, 1H, O*H*), 1.14 (d, J = 6.4 Hz, 3H, H-6), 1.07 (s, 9H, (C*H*$_3$)$_3$). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 136.24 (Ar-*C*), 135.96 (Ar-*C*), 133.19 (Ar-*C*$_q$), 129.95 (Ar-*C*), 129.85 (Ar-*C*), 128.74 (Ar-*C*), 128.18 (Ar-*C*), 127.74 (Ar-*C*), 127.46 (Ar-*C*), 95.46 (C-1), 79.26 (C-4), 76.17 (ArCH$_2$), 72.70 (C-3), 70.99 (C-5), 58.70 (C-2), 27.05 ((*C*H$_3$)$_3$), 19.34 (*C*(CH$_3$)$_3$), 16.74 (C-6). **HRMS**: [M+Na]$^+$ calculated for $C_{31}H_{36}Cl_3NO_5SiNa$: 658.13260; found 658.13205

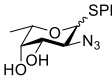
Phenyl 3,4-di-*O*-acetyl-2-azido-2-deoxy-1-thio-α/β-L-fucopyranoside (19)



2-azido-2-deoxy-1,3,4-tri-*O*-acetyl-α-L-fucopyranoside **18** (3.155 g, 10 mmol) was co-evaporated with toluene (x3) and dissolved in dry DCM (50 mL, 0.2 M). The solution was cooled to 0°C followed by addition of PhSH (1 mL, 10 mmol, 1 equiv.) and $BF_3{\cdot}OEt_2$ (2.5 mL, 20 mmol, 2 equiv.). The reaction mixture was stirred for 6 days under nitrogen at rt until TLC (pentane/EtOAc, 9:1) showed full conversion after which the mixture was quenched with Et_3N and diluted in DCM. The organic phase was washed with $NaHCO_3$ (sat. aq.; x1), 1 M NaOH (aq.; x3) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 95:5 → 85:15) furnished **19** in 90% yield. (3.289 g, 9 mmol) in a α/β ratio = 1.5: 1. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.67 – 7.60 (m, 1H, Ar-*H*), 7.52 – 7.44 (m, 2H, Ar-*H*), 7.41 – 7.25 (m, 7H, Ar-*H*), 5.65 (d, *J* = 5.5 Hz, 1H, H-1α), 5.33 (dd, *J* = 3.3, 1.3 Hz, 1H, H-4α), 5.22 – 5.19 (m, 1H, H-3α), 5.17 (d, *J* = 3.2 Hz, 1H, H-4β), 4.86 (dd, *J* = 10.2, 3.2 Hz, 1H, *H*-3β), 4.67 – 4.58 (m, 1H, H-5α), 4.50 (d, *J* = 10.1 Hz, 1H, H-1β), 4.29 (dd, *J* = 11.1, 5.5 Hz, 1H, H-2α), 3.78 (qd, *J* = 6.4, 1.1 Hz, 1H, H-5β),

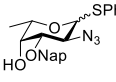
3.64 (t, *J* = 10.2 Hz, 1H, H-2β), 2.19 (s, 3H, COC*H*$_3$α), 2.12 (s, 3H, COC*H*$_3$β), 2.07 (s, 3H, COC*H*$_3$α), 2.04 (s, 3H, COC*H*$_3$β), 1.24 (d, *J* = 6.4 Hz, 3H, *H*-6β), 1.14 (d, *J* = 6.5 Hz, 3H, *H*-6α). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 170.48 (*C*=O), 169.81 (*C*=O), 133.47 (Ar-*C*), 133.29 (Ar-*C*$_q$), 132.20 (Ar-*C*), 129.27 (Ar-*C*), 129.09 (Ar-*C*), 128.54 (Ar-*C*), 127.90 (Ar-*C*), 87.26 (C-1α), 86.57 (C-1β), 73.52 (C-3β), 73.23 (C-5β), 70.73 (C-4α), 70.50 (C-4β), 69.76 (C-3α), 65.99 (C-5α), 59.43 (C-2β), 58.28 (C-2α), 20.81 (CO*C*H$_3$), 20.77 (CO*C*H$_3$), 16.74 (C-6β), 16.00 (C-6α). **HRMS**: [M+Na]$^+$ calculated for $C_{16}H_{19}N_3O_5SNa$: 388.09431; found 388.09376

Phenyl 2-azido-2-deoxy-1-thio-α/β-L-fucopyranoside (20)



19 (3.273 g, 8.957 mmol) was dissolved in MeOH (30 mL, 0.3 M) followed by addition of NaOMe (25 wt.% in MeOH, 0.2 mL, mmol, 0.1 equiv.). The resulting solution was stirred for 3 h at rt until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction was quenched with Amberlite (IR-120, H$^+$ form), filtered and concentrated *in vacuo* to yield diol **20** in 92% (2.318 g, 8.24 mmol) in a α/β = 1:1.7. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.64 – 7.54 (m, 2H, Ar-*H*), 7.53 – 7.46 (m, 1H, Ar-*H*), 7.40 – 7.28 (m, 7H, Ar-*H*), 5.61 (d, *J* = 5.5 Hz, 1H, H-1α), 4.54 – 4.47 (m, 1H, *H*-5α), 4.42 (d, *J* = 10.0 Hz, 1H, H-1β), 4.16 – 4.06 (m, 1H, H-2α), 3.90 – 3.84 (m, 2H, H-3α, H-4α), 3.74 (dd, *J* = 3.2, 1.1 Hz, 1H, H-4β), 3.63 (qd, *J* = 6.5, 1.1 Hz, 1H, H-5β), 3.54 (dd, *J* = 9.5, 3.2 Hz, 1H, H-3β), 3.50 – 3.41 (m, 1H, H-2β), 2.29 (s, 4H, 3-O*H*, 4-O*H*), 1.36 (d, *J* = 6.5 Hz, 3H, H-6β), 1.30 (d, *J* = 6.6 Hz, 3H, H-6α). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 133.79 (Ar-*C*$_q$), 133.16 (Ar-*C*), 132.10 (Ar-*C*), 131.97 (Ar-*C*$_q$), 129.23 (Ar-*C*), 129.18 (Ar-*C*), 128.42 (Ar-*C*), 127.76 (Ar-*C*), 87.31 (C-1α), 86.67 (C-1β), 74.82 (C-5β), 74.53 (C-3β), 71.63 (C-3α), 71.12 (C-4β), 70.59 (C-4α), 67.16 (C-5α), 63.00 (C-2β), 61.25 (C-2α), 16.81 (C-6β), 16.18 (C-6α). **HRMS**: [M+Na]$^+$ calculated for $C_{12}H_{15}N_3O_3SNa$: 304.07318; found 304.07263

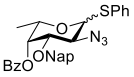
Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-thio-α/β-L-fucopyranoside (21)



20 (2.314 g, 8.226 mmol) in dry toluene (40 mL, 0.2 M) was added Bu$_2$SnO (2.089 g, 8.39 mmol, 1.02 equiv.) and the flask was equipped with a Dean-Stark apparatus. The reaction mixture was stirred at 140 °C for 3 h under nitrogen after which it was cooled to 60 °C followed by addition of CsF (1.274 g, 8.39 mmol, 1.02 equiv.), Bu$_4$NBr (2.784 g, 8.637 mmol, 1.05 equiv.) and NapBr (1.909 g, 8.637 mmol, 1.05 equiv.). The mixture was heated to 120 °C for 1 h until TLC (pentane/EtOAc, 6:4) showed full conversion. The reaction mixture was cooled to rt and quenched with 10% KF (aq.). After stirring for 30 minutes, the aqueous phase was extracted with EtOAc (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 → 80:20) yielded alcohol **21** in 100% (3.467 g, 8.22 mmol) in a α/β = 1:1.7. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.91 – 7.77 (m, 7H, Ar-*H*), 7.65 – 7.54 (m, 3H, Ar-*H*), 7.54 – 7.44 (m, 7H, Ar-*H*), 7.36 – 7.22 (m, 7H, Ar-*H*), 5.59 (d, *J* = 5.5 Hz, 1H, H-1α), 4.96 – 4.79 (m, 4H, ArC*H*$_2$α/β), 4.45 – 4.37 (m, 1H, H-5α), 4.34 (d, *J* = 10.2 Hz, 1H, H-1β), 4.27 (dd, *J* = 10.4, 5.5 Hz, 1H, H-2α), 3.94 – 3.88 (m, 1H, H-4α), 3.84 – 3.75 (m, 2H, H-3α, H-4β), 3.60 (t, *J* = 9.8 Hz, 1H, H-2β), 3.51 (qt, *J* = 6.5, 1.1 Hz, 1H, H-5β), 3.44 (dd, *J* = 9.5, 3.2 Hz, 1H, H-3β), 2.41 (t, *J* = 1.6 Hz, 1H, O*H*α), 2.19 (dd, *J* = 3.2, 1.1 Hz, 1H, O*H*β), 1.36 (d, J = 6.5 Hz, 3H, H-6β), 1.29 (d, J = 6.5 Hz, 3H, H-6α)**. ^{13}C NMR (101 MHz, CDCl$_3$)** δ 134.54 (Ar-*C*$_q$), 134.51 (Ar-*C*$_q$), 133.83 (Ar-*C*$_q$), 133.40 (Ar-*C*), 133.31 (Ar-*C*$_q$), 133.28 (Ar-*C*$_q$), 131.92 (Ar-*C*), 131.77 (Ar-*C*$_q$), 129.19 (Ar-*C*), 129.10 (Ar-*C*), 128.78 (Ar-*C*),

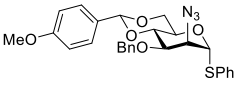
128.72 (Ar-*C*), 128.34 (Ar-*C*), 128.12 (Ar-*C*), 128.06 (Ar-*C*), 127.89 (Ar-*C*), 127.62 (Ar-*C*), 127.21 (Ar-*C*), 127.17 (Ar-*C*), 126.52 (Ar-*C*), 126.49 (Ar-*C*), 126.44 (Ar-*C*), 126.41 (Ar-*C*), 125.87 (Ar-*C*), 125.83 (Ar-*C*), 87.38 (C-1α), 86.22 (C-1β), 81.34 (C-3β), 78.34 (C-3α), 74.54 (C-5β), 72.42 (Ar-*C*H_2α), 72.23 (Ar-*C*H_2β), 68.95 (C-4α), 68.26 (C-4β), 67.03 (C-5α), 61.04 (C-2β), 59.74 (C-2α), 16.93 (C-6β), 16.25 (C-6α). **HRMS**: $[M+Na]^+$ calculated for $C_{23}H_{23}N_3O_3SNa$: 444.13578; found 444.13523

Phenyl 2-azido-4-*O*-benzoyl-2-deoxy-3-*O*-(2-naphthylmethyl)-1-thio-α/β-L-fucopyranoside (12)



21 (3.553 g, 8.43 mmol) was co-evaporated with toluene (x3) before dissolving in DCM/pyridine (42 mL, 4:1, 0.2 M). The reaction mixture was cooled to 0 °C followed by addition of DMAP (103 mg, 0.843 mmol, 0.1 equiv.) and BzCl (1.2 mL, 10.12 mmol, 1.2 equiv.). The reaction mixture was allowed to warm to rt and stirred overnight under nitrogen. When TLC (pentane/EtOAc, 8:2) showed full conversion, the reaction mixture was quenched by the addition of H_2O, diluted in EtOAc, washed with HCl (1 M, x3), $NaHCO_3$ (sat. aq.; x3) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc; 95:5 → 90:10) furnished **12** in 88% (α: 2.184 g, 4.15 mmol; β: 1.712 g, 3.26 mmol) in a α/β = 56:44. NMR reported for the α-anomer: **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.15 – 8.08 (m, 2H, Ar-*H*), 7.78 (m, *J* = 12.5, 6.1, 3.1 Hz, 4H, Ar-*H*), 7.63 – 7.54 (m, 1H, Ar-*H*), 7.53 – 7.41 (m, 8H, Ar-*H*), 7.36 – 7.26 (m, 2H, Ar-*H*), 5.76 (dd, *J* = 3.3, 1.3 Hz, 1H, H-4), 5.70 (d, *J* = 5.5 Hz, 1H, H-1), 5.01 (d, *J* = 11.1 Hz, 1H, Ar-CH_2), 4.76 (d, *J* = 11.1 Hz, 1H, Ar-CH_2), 4.71 – 4.61 (m, 1H, H-5), 4.34 (dd, *J* = 10.5, 5.5 Hz, 1H, H-2), 3.98 (dd, *J* = 10.6, 3.2 Hz, 1H, H-3), 1.23 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 166.20 (*C*=O), 134.55 (Ar-C_q), 133.58 (Ar-C_q), 133.53 (Ar-*C*), 133.35 (Ar-C_q), 133.22 (Ar-C_q), 132.18 (Ar-*C*), 130.04 (Ar-*C*), 129.68 (Ar-C_q), 129.25 (Ar-*C*), 128.67 (Ar-*C*), 128.35 (Ar-*C*), 128.13 (Ar-*C*), 127.81 (Ar-*C*), 127.79 (Ar-*C*), 127.32 (Ar-*C*), 126.16 (Ar-*C*), 126.09 (Ar-*C*), 87.58 (C-1), 76.45 (C-3), 71.78 (Ar-CH_2), 69.86 (C-4), 66.56 (C-5), 60.14 (C-2), 16.41 (C-6). NMR reported for the β-anomer**: ^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.93 – 7.86 (m, 2H, Ar-*H*), 7.84 – 7.72 (m, 4H, Ar-*H*), 7.76 – 7.65 (m, 2H, Ar-*H*), 7.64 – 7.55 (m, 1H, Ar-*H*), 7.52 – 7.38 (m, 8H, Ar-*H*), 5.60 (d, *J* = 1.1 Hz, 1H, H-4), 4.92 (d, *J* = 11.5 Hz, 1H, Ar-CH_2), 4.70 (d, *J* = 11.5 Hz, 1H, Ar-CH_2), 4.42 – 4.35 (m, 1H, H-1), 3.83 – 3.73 (m, 1H, H-5), 3.66 – 3.56 (m, 2H, H-2, H-3), 1.30 (d, *J* = 6.4 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 166.13 (*C*=O), 135.00 (Ar-*C*), 134.49 (Ar-C_q), 133.50 (Ar-*C*), 133.28 (Ar-C_q), 130.38 (Ar-C_q), 130.13 (Ar-*C*), 129.50 (Ar-C_q), 129.10 (Ar-*C*), 128.71 (Ar-*C*), 128.55 (Ar-*C*), 128.44 (Ar-*C*), 128.08 (Ar-*C*), 127.81 (Ar-*C*), 127.42 (Ar-*C*), 126.23 (Ar-*C*), 126.17 (Ar-*C*), 126.15 (Ar-*C*), 85.32 (C-1), 79.39 (C-3), 73.73 (C-5), 71.71 (Ar-*C*H_2), 68.95 (C-4), 60.81 (C-2), 17.09 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{30}H_{27}N_3O_4SNa$: 548.16200; found 548.16145

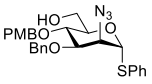
Phenyl 2-azido-2-deoxy-4,6-*O*-*p*-methoxybenzylidene-3-*O*-benzyl-1-thio-α-D-mannopyranoside (23)



22 (1.826 g, 4.395 mmol) was co-evaporated with toluene (x3) and dissolved in DMF (44 mL, 0.1 M). The mixture was cooled to 0°C followed by addition of NaH (229 mg, 5.173 mmol, 1.3 equiv.) and BnBr (0.68 mL, 5.713 mmol, 1.3 equiv.). The reaction mixture was allowed to warm to rt and stirred under nitrogen overnight until TLC (pentane/EtOAc, 9:1) showed full conversion. The

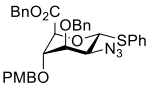
reaction was quenched by the addition of H_2O and diluted in Et_2O. The aqueous phase was extracted with Et_2O (x3) and the combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 100:0 → 90:10) provided thioglycoside **23** in 98% yield (2.177g, 4.31 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.47 – 7.25 (m, 12H, Ar-*H*), 6.95 – 6.87 (m, 2H, Ar-*H*), 5.59 (s, 1H, H-1), 5.43 (d, *J* = 1.3 Hz, 1H, PMPC*H*), 4.92 (d, *J* = 12.1 Hz, 1H, H-5, Ar-CH_2), 4.75 (d, *J* = 12.1 Hz, Ar-CH_2), 4.36 – 4.26 (m, 1H, H-5), 4.23 – 4.06 (m, 4H, H-2, H-3, H-4, H-6), 3.82 (t, 4H, OCH_3, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 160.19 (Ar-C_q), 137.90 (Ar-C_q), 132.97 (Ar-C_q), 132.13 (Ar-*C*), 129.93 (Ar-C_q), 129.41 (Ar-*C*), 128.65 (Ar-*C*), 128.25 (Ar-*C*), 128.05 (Ar-*C*), 127.82 (Ar-*C*), 127.52 (Ar-*C*), 113.73 (Ar-*C*), 101.79 (C-1), 87.35 (Ar-*C*H), 79.23 9 (C-2, C-3, C-4), 75.86 (C-2, C-3, C-4), 73.63 (Ar-*C*H_2), 68.44 (C-6), 65.28 (C-5), 64.29 (C-2, C-3, C-4), 55.45 (O*C*H_3). **HRMS**: $[M+Na]^+$ calculated for $C_{27}H_{27}N_3O_5SNa$: 528.15691; found 528.15636

Phenyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (24)



23 (1.637 g, 3.238 mmol) was co-evaporated with toluene (x3) before being dissolved in dry DCM (32 mL, 0.1 M). 3Å molecular sieves was added and stirred for 30 min at rt. The solution cooled to 0 °C and added BH_3·THF (1 M in THF; 16 mL, mmol, 5 equiv.) and TMSOTf (0.09 mL, 0.486 mmol, 0.15 equiv.). The reaction was stirred for 3 h at rt under argon until TLC (pentane/EtOAc, 9:1) showed full conversion. The reaction mixture was quenched by the addition of Et_3N and MeOH. The resulting solution was concentrated *in vacuo* and co-evaporated with MeOH (x3). Column chromatography (pentane/EtOAc, 9:1 → 8:2) yielded alcohol **24** in 82% yield (1.343 g, 2.65 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.47 – 7.25 (m, 10H, Ar-*H*), 7.29 – 7.20 (m, 2H, Ar-*H*), 6.92 – 6.84 (m, 2H, Ar-*H*) 5.41 (d, *J* = 1.5 Hz, 1H, H-1), 4.82 (d, *J* = 10.5 Hz, 1H, Ar-CH_2), 4.77 (s, 2H, Ar-CH_2), 4.60 (d, *J* = 10.5 Hz, 1H, Ar-CH_2), 4.16 – 4.07 (m, 2H, H-2, H-5), 4.04 (dd, *J* = 9.1, 3.5 Hz, 1H, H-3), 3.90 (t, *J* = 9.4 Hz, 1H, H-4), 3.81 (s, 3H, OCH_3), 3.79 – 3.73 (m, 2H, H-6), 1.72 (dd, *J* = 7.4, 5.9 Hz, 1H, O*H*). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 159.57 (Ar-C_q), 137.59 (Ar-C_q), 133.09 (Ar-C_q), 132.34 (Ar-*C*), 130.24 (Ar-C_q), 129.99 (Ar-*C*), 129.38 (Ar-*C*), 128.79 (Ar-*C*), 128.29 (Ar-*C*), 128.26 (Ar-*C*), 128.23 (Ar-*C*), 114.07 (Ar-*C*), 86.47 (C-1), 79.98 (C-3), 75.25 (Ar-*C*H_2), 74.14 (C-4), 73.34 (C-5), 72.96 (Ar-*C*H_2), 62.90 (C-2), 62.02 (C-6), 55.44 (O*C*H_3). **HRMS**: $[M+Na]^+$ calculated for $C_{27}H_{29}N_3O_5SNa$: 530.17256; found 530.17201

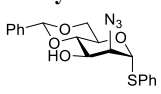
Benzyl (phenyl 2-azido-3-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranosiduronate) (10)



Alcohol **24** (1.678 g, 3.51 mmol) was dissolved in DCM/*t*-BuOH/H_2O (18 mL, 8:4:1, 0.2 M). The mixture was cooled to 0 °C, followed by addition of TEMPO (110 mg, 0.703 mmol, 0.2 equiv.), BAIB (2.829 g, 8.785 mmol, 2.5 equiv.) and AcOH (0.02 mL, 0.351 mmol, 0.2 equiv.). The reaction mixture was stirred for 2h at rt until TLC analysis (pentane/EtOAc, 7:3) showed full conversion. The reaction mixture was quenched with $Na_2S_2O_3$ (sat. aq.) and the aqueous phase was extracted with EtOAc (x3). The organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was co-evaporated with toluene (x3) before dissolving in DMF (35 mL, 0.1 M). The reaction mixture was cooled to 0 °C, followed by addition of K_2CO_3 (971 mg, 7.028 mmol, 2 equiv.) and BnBr (0.8 mL, 7.028 mmol, 2 eq). The mixture was allowed to warm to rt

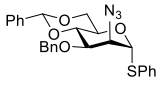
and stirred overnight under nitrogen until TLC analysis (pentane/EtOAc, 7:3) showed full conversion. The reaction mixture was quenched with H_2O. The aqueous phase was extracted with Et_2O (x3), and the combined the organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 95:5 → 85:15) furnished donor **10** in 78% yield (1.493 g, 2.44 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.57 – 7.50 (m, 2H, Ar-*H*), 7.40 – 7.12 (m, 15H, Ar-*H*), 6.88 – 6.80 (m, 2H, Ar-*H*), 5.65 (d, *J* = 7.8 Hz, 1H, H-1), 5.02 (d, *J* = 12.2 Hz, 1H Ar-CH_2), 4.91 (d, *J* = 12.2 Hz, 1H, Ar-CH_2), 4.64 (d, *J* = 4.2 Hz, 1H, Ar-CH_2), 4.56 (d, *J* = 11.1 Hz, 1H, H-5), 4.52 – 4.46 (m, 3H, Ar-CH_2), 4.20 (dd, *J* = 5.6, 4.3 Hz, 1H, H-4), 3.88 (dd, *J* = 5.6, 3.0 Hz, 1H, H-3), 3.81 (s, 3H, OCH_3), 3.68 (dd, *J* = 7.9, 2.9 Hz, 1H, H-2). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.04 (*C*=O), 159.59 (Ar-C_q), 136.95 (Ar-C_q), 135.17 (Ar-C_q), 132.37 (Ar-C_q), 132.28 (Ar-*C*), 129.70 (Ar-*C*), 129.49 (Ar-C_q), 129.01 (Ar-*C*), 128.68 (Ar-*C*), 128.62 (Ar-*C*), 128.55 (Ar-*C*), 128.53 (Ar-*C*), 128.32 (Ar-*C*), 128.30 (Ar-*C*), 127.84 (Ar-*C*), 114.02 (Ar-*C*), 77.00 (C-3), 74.38 (C-4), 73.23 (C-5), 73.06 (Ar-*C*H_2), 72.83 (Ar-*C*H_2), 67.32 (Ar-*C*H_2), 55.44 (O*C*H_3). **HRMS**: $[M+Na]^+$ calculated for $C_{34}H_{33}N_3O_6SNa$: 634.19878; found 634.19823

Phenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-mannopyranoside (26)



Triol **25** (2.11 g, 7.1 mmol) was dissolved in MeCN (23 mL, 0.3 M) followed by addition of benzaldehyde dimethyl acetal (1.3 mL, 8.52 mmol, 1.2 equiv.) and CSA (165 mg, 0.71 mmol, 0.1 equiv.). The reaction mixture was stirred on the rotary evaporator at 50 °C under reduced pressure (300 mbar) for 2 h until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction was quenched by the addition of Et_3N and concentrated *in vacuo*. Purification by column chromatography (pentane/EtOAc, 90:10 → 80:20) yielded **26** in 80% (2.00 g, 5.71 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.54 – 7.44 (m, 4H, Ar-*H*), 7.44 – 7.38 (m, 3H, Ar-*H*), 7.38 – 7.30 (m, 3H, Ar-*H*), 5.60 (s, 1H, Ph-C*H*), 5.49 (d, *J* = 1.2 Hz, 1H, H-1), 4.37 – 4.19 (m, 4H, H-3, H-4, H-2, H-6a), 3.98 (t, *J* = 9.5 Hz, 1H, H-5), 3.83 (t, *J* = 10.3 Hz, 1H, H-6b), 2.72 (d, *J* = 3.8 Hz, 1H, O*H*). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 137.05 (Ar-C_q), 133.07 (Ar-C_q), 132.09 (Ar-*C*), 129.56 (Ar-*C*), 129.44 (Ar-*C*), 128.58 (Ar-*C*), 128.29 (Ar-*C*), 126.41 (Ar-*C*), 102.48 (Ph-*C*H), 87.68 (C-1), 78.86 (C-5), 69.43 (C-3/ C-4), 68.46 (C-6), 65.18 (C-2), 64.71 (C-3/ C-4). **HRMS**: $[M+Na]^+$ calculated for $C_{19}H_{19}N_3O_4SNa$: 408.09940; found 408.09885

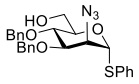
Phenyl 2-azido-4,6-*O*-benzylidene-3-*O*-benzyl-2-deoxy-1-thio-α-D-mannopyranoside (27)



26 (2.194 g, 5.69 mmol) was co-evaporated with toluene (x3) before being dissolved in DMF (57 mL, 0.1 M). The reaction mixture was cooled to 0 °C followed by addition of NaH (60% suspension in mineral oil, 341 mg, 8.45 mmol, 1.5 equiv.) and BnBr (1 mL, 8.54 mmol, 1.5 equiv.). The reaction was stirred overnight at rt under nitrogen until TLC (pentane/EtOAc, 8:2) showed full conversion. The reaction mixture was quenched by addition of H_2O and diluted in Et_2O. The aqueous phase was extracted with Et_2O (x3) and the combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 98:2 → 90:10) provided **27** in 100 % yield (2.75 g, 5.69 mmol) **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.54 – 7.49 (m, 2H, Ar-*H*), 7.45 – 7.29 (m, 14H, Ar-*H*), 5.64 (s, 1H, Ph-C*H*-1), 5.44 (d, *J* = 1.2 Hz, 1H, *H*-1), 4.94 (d, *J* = 12.1 Hz, 1H, Ar-CH_2), 4.76 (d, *J* = 12.1 Hz, 1H, Ar-CH_2), 4.33 (ddd, *J* = 9.9, 8.9, 4.7 Hz, 1H, H-5), 4.25 – 4.16 (m, 3H, H-6, H-4, H-2), 4.16 – 4.12 (m, 1H, H-3), 3.85

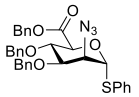
(t, *J* = 10.2 Hz, 1H, *H*-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 137.88 (Ar-C_q), 137.42 (Ar-C_q), 132.14 (Ar-C_q), 129.42 (Ar-*C*), 129.16, 128.66 (Ar-*C*), 128.40 (Ar-*C*), 128.27 (Ar-*C*), 128.07 (Ar-*C*), 127.83 (Ar-*C*), 126.20 (Ar-*C*), 101.79 (Ph-*C*H), 87.36 (C-1), 79.30 (C-4), 75.86 (C-3), 73.64 (Ar-*C*H_2), 68.49 (C-6), 65.26 (C-5), 63.77 (C-2). **HRMS**: $[M+Na]^+$ calculated for $C_{26}H_{25}N_3O_4SNa$: 498.14635; found 498.14580

Phenyl 2-azido-2-deoxy-3,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (28)



27 (2.356 g, 4.659 mmol) was co-evaporated with toluene (x3) and dissolved in dry DCM (46 mL, 0.1 M). 3Å molecular sieves was added and stirred for 30 min and rt. The solution cooled to 0 °C and added BH_3·THF (1 M in THF; 23 mL, 23.29 mmol, 5 equiv.) and TMSOTf (0.13 mL, 0.699 mmol, 0.15 equiv.). The reaction was stirred for 4 h at rt under argon until TLC (pentane/EtOAc, 9:1) showed full conversion. The reaction was quenched with Et_3N and MeOH. The mixture was concentrated *in vacuo* and co-evaporated with MeOH (x3). Column chromatography (pentane/EtOAc, 9:1 → 8:2) gave **28** in 100 % yield (2.252 g, 4.659 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.45 – 7.28 (m, 15H, Ar-*H*), 5.42 (d, *J* = 1.5 Hz, 1H, *H*-1), 4.92 (d, *J* = 10.9 Hz, 1H, Ar-CH_2), 4.77 (s, 2H, Ar-CH_2), 4.67 (d, *J* = 10.9 Hz, 1H, Ar-CH_2), 4.17 – 4.11 (m, 1H, H-5), 4.12 – 4.09 (m, 1H, H-2), 4.06 (dd, *J* = 9.0, 3.6 Hz, 1H, H-3), 3.93 (dd, *J* = 9.7, 9.1 Hz, 1H, H-4), 3.84 – 3.74 (m, 2H, H-6), 1.75 (dd, *J* = 7.4, 6.0 Hz, 1H, O*H*). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.09 (Ar-C_q), 137.53 (Ar-C_q), 133.07 (Ar-C_q), 132.34 (Ar-*C*), 129.39 (Ar-*C*), 128.79 (Ar-*C*), 128.66 (Ar-*C*), 128.30 (Ar-*C*), 128.10 (Ar-*C*), 86.47 (C-1), 79.95 (C-3), 75.5 (Ar-*C*H_2), 74.42 (C-4), 73.34 (C-5), 72.96 (Ar-*C*H_2), 63.36 (C-2), 61.97 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{26}H_{27}N_3O_4SNa$: 500.16200; found 500.16145

Benzyl (phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-thio-α-D-mannopyranosiduronate) (11)

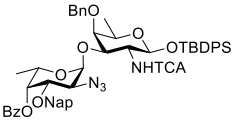


Alcohol **28** (2.473 g, 5.178 mmol) was dissolved in DCM/*t*-BuOH/H_2O (26 mL, 8:4:1, 0.2 M). TEMPO (162 mg, 1.036 mmol, 0.2 equiv.), BAIB (4.169 g, 12.944 mmol, 2.5 equiv.) and AcOH (30 µL, 0.518 mmol, 0.1 equiv.) were added and the reaction mixture was stirred at rt for 2 h until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction was quenched with $Na_2S_2O_3$ (sat. aq.) and the aqueous phase was extracted with DCM (x3). The combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was co-evaporated with toluene (x3) before being dissolved in DMF (52 mL, 0.1 M). The solution was cooled to 0 °C before K_2CO_3 (1.431 g, 10.356 mmol, 2 equiv.) and BnBr (1.2 mL, 10.356 mmol, 2 equiv.) were added. The reaction was stirred at rt overnight under nitrogen until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction was quenched by the addition of H_2O and the mixture was diluted in Et_2O. The aqueous phase was extracted with Et_2O (x3) and the combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 95:15 → 85:15) furnished donor **11** in 79% yield (2.393 g, 4.11 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.57 – 7.52 (m, 2H, Ar-*H*), 7.40 – 7.27 (m, 13H, Ar-*H*), 7.24 – 7.15 (m, 7H, Ar-*H*), 5.66 (d, *J* = 7.8 Hz, 1H, H-1), 5.03 (d, *J* = 12.1 Hz, 1H, Ar-CH_2), 4.91 (d, *J* = 12.2 Hz, 1H, Ar-CH_2), 4.67 (d, *J* = 4.2 Hz, 1H, H-5), 4.61 (q, *J* = 11.4 Hz, 2H, Ar-CH_2), 4.51 (d, *J* = 2.3 Hz, 2H, Ar-CH_2), 4.22 (dd, *J* = 5.7, 4.2 Hz, 1H, H-4), 3.92 (dd, *J* = 5.6, 3.0 Hz, 1H, H-3), 3.70 (dd, *J* = 7.8, 2.9 Hz, 1H, H-2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.98 (*C*=O), 137.41 (Ar-C_q), 136.90 (Ar-C_q), 135.13 (Ar-C_q), 132.37 (Ar-*C*), 132.24 (Ar-C_q), 129.01 (Ar-*C*), 128.67 (Ar-*C*), 128.62 (Ar-*C*), 128.54 (Ar-*C*), 128.52 (Ar-*C*), 128.33 (Ar-*C*), 128.31 (Ar-*C*), 128.13 (Ar-*C*), 127.95 (Ar-*C*), 127.88 (Ar-*C*), 82.55 (C-1), 76.84 (C-3), 74.74 (C-4), 73.17 (C-5), 73.08 (Ar-*C*H_2), 67.33 (Ar-*C*H_2), 58.97 (C-2). **HRMS**: $[M+Na]^+$ calculated for $C_{33}H_{31}N_3O_5SNa$: 604.18821; found 604.18766

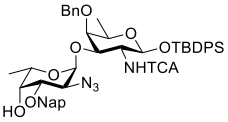
Synthesis of longer fragments

***Tert*-butyldiphenylsilyl 2-azido-4-*O*-benzoyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-deoxy-2-*N*-trichloroacetamide-4-*O*-benzyl-β-D-fucopyranoside (29)**



Acceptor **13** (1.605 g, 2.52 mmol, 1 equiv.) and donor **12α** (1.721 g, 3.275 mmol, 1.3 equiv.) were co-evaporated with toluene (x3) before being dissolved in dry DCM (25 mL, 0.1 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The reaction was cooled to -60 °C followed by addition of NIS (850 mg, 3.779 mmol, 1.5 equiv.) and TMSOTf (91 µL, 0.504 mmol, 0.2 equiv.). The reaction was allowed to warm to -30 °C and stirred for 1 h under argon until TLC (pentane/EtOAc, 8:2) showed full conversion. The reaction was quenched with Et_3N at -30 °C and diluted in EtOAc. The organic phase was washed $Na_2S_2O_3$ (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, 95:5 → 80:20) yielded the α-1,3-linked disaccharide **29** in 98% yield (α: 2.331 g, 2.21 mmol; β: 263 mg, 0.25 mmol) in a α/β = 9:1. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.08 – 8.01 (m, 2H, Ar-*H*), 7.81 – 7.71 (m, 6H, Ar-*H*), 7.71 – 7.62 (m, 2H, Ar-*H*), 7.61 – 7.52 (m, 1H, Ar-*H*), 7.48 – 7.25 (m, 15H, Ar-*H*), 7.20 (d, *J* = 6.9 Hz, 1H, *H*N(CO)CCl_3), 5.49 (dd, J = 3.3, 1.3 Hz, 1H, H-4'), 5.01 (d, *J* = 3.7 Hz, 1H, H-1'), 4.97 (d, *J* = 7.3 Hz, 1H, H-1), 4.90 (d, *J* = 11.0 Hz, 1H, Ar-CH_2), 4.78 (d, *J* = 12.2 Hz, 1H, Ar-CH_2), 4.69 (d, *J* = 12.2 Hz, 1H, Ar-CH_2), 4.59 (d, *J* = 11.0 Hz, 1H, Ar-CH_2), 4.17 – 4.01 (m, 3H, H-2, H-3, H-5'), 3.96 (dd, *J* = 10.5, 3.2 Hz, 1H, H-3'), 3.80 (dd, *J* = 10.5, 3.6 Hz, 1H, H-2'), 3.49 (d, *J* = 1.9 Hz, 1H, *H*-4), 3.33 (q, *J* = 6.3 Hz, 1H, H-5), 1.07 (t, 15H, (CH_3)$_3$), H-6, H-6'). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 166.18 (*C*=O), 161.93 (*C*=O), 138.83 (Ar-C_q), 136.21 (Ar-*C*), 135.98 (*C*H_{Ar}), 134.73 (Ar-C_{qr}), 133.71 (Ar-C_q), 133.51 (Ar-C_q), 133.47 (Ar-*C*), 133.35 (Ar-C_q), 133.14 (Ar-C_q), 129.98 (Ar-*C*), 129.73 (Ar-*C*), 129.63 (Ar-*C*), 129.62 (Ar-*C*), 128.63 (Ar-C_q), 128.55 (Ar-*C*), 128.25 (Ar-*C*), 128.10 (Ar-*C*), 127.75 (Ar-*C*), 127.55 (Ar-*C*), 127.32 (Ar-*C*), 127.09 (Ar-*C*), 127.01 (Ar-*C*), 126.10 (Ar-*C*), 126.02 (Ar-*C*), 99.45 (C-1'), 94.78 (C-1), 79.55 (C-4), 78.54 (C-3), 75.22 (Ar-*C*H_2), 75.18 (C-3'), 71.51 (Ar-*C*H_2), 70.70 (C-5), 69.64 (C-5'), 66.17 (C-4'), 60.15 (C-2'), 57.63 (C-2), 27.16 ((CH_3)$_3$), 19.40 (C(CH_3)$_3$), 16.93 (C-6'), 16.48 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{55}H_{57}Cl_3N_4O_9SiNa$: 1073.28581; found 1073.28526

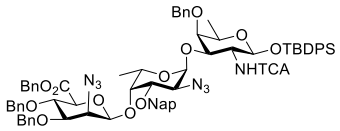
***Tert*-butyldiphenylsilyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-deoxy-2-*N*-trichloroacetamide-4-*O*-benzyl-β-D-fucopyranoside (9)**



Disaccharide **29** (2.296 g, 2.182 mmol) was dissolved in MeOH (11 mL, 0.2 M), followed by addition of NaOMe (25% wt. in MeOH, 0.5 mL, 2.182 mmol, 1 equiv.). The reaction mixture was stirred for 2 days at rt until TLC analysis (pentane/EtOAc, 8:2) showed full

conversion. The reaction mixture was neutralized by the addition of Amberlite (IR-120, H^+ form) until pH≈8-9, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 90:10 → 70:30) provided acceptor **9** in 90% yield (1.852 g, 1.95 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.88 – 7.69 (m, 6H, Ar-*H*), 7.69 – 7.61 (m, 2H, Ar-*H*), 7.52 – 7.44 (m, 3H, Ar-*H*), 7.43 – 7.22 (m, 11H, Ar-*H*), 7.13 (d, *J* = 7.5 Hz, 1H, *H*N(CO)CCl_3), 4.94 – 4.87 (m, 2H, H-1, H-1'), 4.82 – 4.67 (m, 4H, Ar-CH_2), 4.11 (m, 1H, H-2), 4.02 (dd, *J* = 11.1, 2.8 Hz, 1H, H-3'), 3.86 (q, *J* = 6.8 Hz, 1H, H-5'), 3.81 (dd, *J* = 10.3, 3.0 Hz, 1H, H-3), 3.73 (dd, *J* = 10.4, 3.6 Hz, 1H, H-2'), 3.68 (d, *J* = 3.0 Hz, 1H, H-4'), 3.47 (d, *J* = 3.1 Hz, 1H, H-4), 3.34 – 3.24 (m, 1H, H-5), 2.27 (s, 1H, O*H*), 1.16 (d, *J* = 6.6 Hz, 3H, H-6'), 1.05 (m, 12H, H-6, (CH_3)$_3$). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 161.83 (*C*=O), 138.89 (Ar-C_q), 136.21 (Ar-*C*), 135.98 (Ar-*C*), 134.62 (Ar-C_q), 133.68 (Ar-C_q), 133.49 (Ar-C_q), 133.35 (Ar-C_q), 133.28 (Ar-C_q), 129.72 (Ar-*C*), 129.62 (Ar-*C*), 128.75 (Ar-*C*), 128.46 (Ar-*C*), 128.11 (Ar-*C*), 127.88 (Ar-*C*), 127.64 (Ar-*C*), 127.54 (Ar-*C*), 127.31 (Ar-*C*), 127.18 (Ar-*C*), 126.95 (Ar-*C*), 126.50 (Ar-*C*), 126.41 (Ar-*C*), 125.70 (Ar-*C*), 99.32 (C-1'), 94.98 (C-1), 79.25 (C-4), 78.61 (C-3'), 77.28 (C-3), 75.13 (Ar-*C*H$_2$), 72.05 (Ar-*C*H$_2$), 70.67 (C-5), 68.58 (C-4'), 66.58 (C-5'), 59.67 (C-2'), 57.43 (C-2), 27.15 ((*C*H$_3$)$_3$), 19.39 (*C*(CH$_3$)$_3$), 16.84 (C-6), 16.33 (C-6'). **HRMS**: $[M+Na]^+$ calculated for $C_{48}H_{53}Cl_3N_4O_8SiNa$: 969.25959; found 969.25905

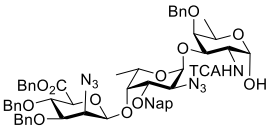
***Tert*-butyldiphenylsilyl (Benzyl (2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-β-D-fucopyranoside (8)**



Acceptor **9** (500 mg, 0.527 mmol, 1 equiv.) and thio-donor **11** (460 mg, 0.791 mmol, 1.5 equiv.) were co-evaporated with toluene (x3) before being dissolved in dry DCM (3.5 mL, 0.15 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The reaction mixture was cooled to -78 °C, followed by addition of NIS (237 mg, 1.054 mmol, 2 equiv.) and TBSOTf (24 µL, 0.105 mmol, 0.2 equiv.). The reaction was stirred for 4 h and allowed to warm to -20 °C. When TLC (pentane/EtOAc, 8:2) showed full conversion of the acceptor, the reaction mixture was quenched with Et_3N and diluted in EtOAc. The organic phase was washed with $Na_2S_2O_3$ (sat. aq.; x1), $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 90:10 → 75:25) furnished trisaccharide **8** in 91% yield (α: 145.5 mg, 0.102 mmol; β: 537 g, 0.377 mmol) in a α/β = 21:79**. ^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.82 – 7.74 (m, 3H, Ar-*H*), 7.75 – 7.68 (m, 1H, Ar-*H*), 7.67 – 7.57 (m, 3H, Ar-*H*), 7.54 – 7.49 (m, 1H, Ar-*H*), 7.45 – 7.42 (m, 2H, Ar-*H*), 7.38 – 7.23 (m, 22H, Ar-*H*), 7.21 – 7.10 (m, 5H, Ar-*H*), 7.08 – 7.01 (m, 4H, Ar-*H*), 4.91 (d, *J* = 11.3 Hz, 1H, Ar-CH_2), 4.89 (d, *J* = 3.7 Hz, 1H, H-1'), 4.85 (d, *J* = 7.8 Hz, 1H, H-1), 4.78 – 4.62 (m, 7H, Ar-CH_2), 4.61 (d, *J* = 1.1 Hz, 1H, H-1''), 4.41 (t, *J* = 10.8 Hz, 2H, Ar-CH_2), 4.15 – 4.08 (m, 1H, H-2), 4.06 (t, *J* = 9.5 Hz, 1H, H-4''), 4.03 – 4.00 (m, 2H, H-2'', H-4'), 3.96 (dd, *J* = 10.5, 3.6 Hz, 2H, H-2', H-3), 3.90 (q, *J* = 6.6 Hz, 1H, H-5'), 3.79 (dd, *J* = 10.5, 2.9 Hz, 1H, H-3'), 3.74 (d, *J* = 9.7 Hz, 1H, H-5''), 3.53 (dd, *J* = 9.2, 3.6 Hz, 1H, H-3''), 3.44 (d, *J* = 2.2 Hz, 1H, H-4), 3.24 (q, *J* = 6.8 Hz, 1H, H-5), 1.09 (d, *J* = 6.6 Hz, 3H, H-6'), 1.06 (s, 9H, (CH_3)$_3$)), 1.01 (d, *J* = 6.4 Hz, 3H, H-6). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 167.19 (*C*=O), 161.87 (*C*=O), 138.70 (Ar-C_q), 137.91 (Ar-C_q), 137.32 (Ar-C_q), 136.14 (Ar-*C*), 135.92 (*C*H$_{Ar}$), 135.47 (Ar-C_q), 134.84 (Ar-C_q), 133.66 (Ar-C_q), 133.43 (Ar-C_q), 133.35 (Ar-C_q), 133.02 (Ar-*C*), 132.33

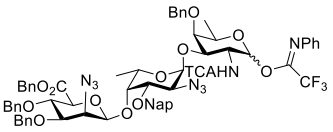
(Ar-*C*), 130.01 (Ar-*C*), 129.64 (Ar-*C*), 129.53 (Ar-*C*), 129.45 (Ar-*C*), 128.74 (Ar-*C*), 128.70 (Ar-*C*), 128.50 (Ar-*C*), 128.40 (Ar-*C*), 128.33 (Ar-*C*), 128.23 (Ar-*C*), 128.14 (Ar-*C*), 128.08 (Ar-*C*), 127.93 (Ar-*C*), 127.90 (Ar-*C*), 127.77 (Ar-*C*), 127.75 (Ar-*C*), 127.60 (Ar-*C*), 127.47 (Ar-*C*), 127.23 (Ar-*C*), 126.45 (Ar-*C*), 126.02 (Ar-*C*), 125.98 (Ar-*C*), 125.82 (Ar-*C*), 100.97 (C-1''), 98.91 (C-1'), 95.19 (C-1), 79.76 (C-3''), 78.89 (C-3), 78.58 (C-4), 75.69 (C-3'), 75.34 (C-5''), 75.25 (Ar-CH_2), 75.13 (C-4''), 74.91 (Ar-CH_2), 74.89 (C-4'), 72.25 (Ar-CH_2), 70.76 (Ar-CH_2), 70.52 (C-5), 67.34 (Ar-CH_2), 67.11 (C-5'), 61.33 (C-2''), 59.50 (C-2'), 57.07 (C-2), 27.07 ((CH_3)$_3$), 19.30 (C(CH_3)$_3$), 17.08 (C-6'), 16.72 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{75}H_{78}Cl_3N_7O_{13}SiNa$: 1440.43901; found 1440.43847

(Benzyl (2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy 3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-α-D-fucopyranose (30)



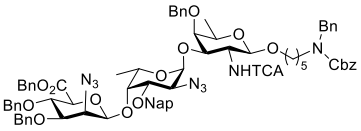
Trisaccharide **8** (529 mg, 0.372 mmol) was dissolved in THF (3.7 mL, 0.1 M) and cooled to 0 °C. AcOH (0.03 mL, 0.558 mmol, 1.5 equiv.) and TBAF (1 M in THF; 0.6 mL, 0.558 mmol, 1.5 equiv.) were added. The reaction mixture was stirred overnight at rt under nitrogen until TLC (pentane/EtOAc, 6:4) showed full conversion. The reaction was quenched by the addition of NH_4Cl (sat. aq.) and diluted in EtOAc. The organic phase was washed with H_2O (x3) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 80:20 → 60:40) furnished hemiacetal **30** in 86% yield (377 mg, 0.319 mmol). **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.86 – 7.76 (m, 5H, Ar-*H*), 7.69 (d, *J* = 6.4 Hz, 1H, Ar-*H*), 7.55 – 7.42 (m, 3H, Ar-*H*), 7.41 – 7.21 (m, 17H, Ar-*H*), 7.21 – 7.10 (m, 3H, Ar-*H*), 7.09 – 7.03 (m, 2H, Ar-*H*), 7.03 – 6.96 (m, 2H, Ar-*H*), 5.59 (t, *J* = 3.5 Hz, 1H, H-1), 4.99 (d, *J* = 3.6 Hz, 1H, H-1'), 4.97 (d, *J* = 10.9 Hz, 1H, Ar-CH_2), 4.85 (d, *J* = 11.7 Hz, 1H, Ar-CH_2), 4.75 – 4.65 (m, 6H, Ar-CH_2), 4.64 (s, 1H, H-1''), 4.61 (d, *J* = 12.2 Hz, 1H, Ar-CH_2), 4.47 – 4.39 (m, 3H, *H*-2, ArCH_2), 4.20 – 4.01 (m, 7H, H-2', H-4', H-5, H-3, H-4'', H-5', H-2''), 3.89 (dd, *J* = 10.5, 2.9 Hz, 1H, H-3'), 3.73 (d, *J* = 9.8 Hz, 1H, H-5''), 3.69 (d, *J* = 1.5 Hz, 1H, H-4), 3.53 (dd, *J* = 9.2, 3.6 Hz, 1H, H-3''), 2.81 (dd, *J* = 3.7, 1.4 Hz, 1H, O*H*), 1.16 (d, *J* = 6.5 Hz, 3H, H-6), 1.11 (d, *J* = 6.5 Hz, 3H, H-6'). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 167.21 (*C*=O), 162.35 (*C*=O), 138.37 (Ar-C_q), 137.97 (Ar-C_q), 137.41 (Ar-C_q), 135.36 (Ar-C_q), 134.90 (Ar-C_q), 133.43 (Ar-C_q), 133.12 (Ar-C_q), 128.80 (Ar-*C*), 128.77 (Ar-*C*), 128.55 (Ar-*C*), 128.53 (Ar-*C*), 128.40 (Ar-*C*), 128.30 (Ar-*C*), 128.23 (Ar-*C*), 128.17 (Ar-*C*), 128.01 (Ar-*C*), 127.96 (Ar-*C*), 127.85 (Ar-*C*), 127.83 (Ar-*C*), 127.76 (Ar-*C*), 126.52 (Ar-*C*), 126.10 (Ar-*C*), 125.99 (Ar-*C*), 125.91 (Ar-*C*), 101.07 (C-1''), 98.81 (C-1'), 90.82 (C-1), 79.79 (C-3''), 77.99 (C-4'/C-5/C-3/C-4''/C-5'), 77.41 (C-4), 76.91 (C-3'), 75.41 (C-5''), 75.33 (Ar-CH_2), 75.22 (C-4'/C-5/C-3/C-4''/C-5'), 74.98 (C-4'/C-5/C-3/C-4''/C-5'), 74.87 (Ar-CH_2), 72.41 (Ar-CH_2), 71.13 (Ar-CH_2), 67.83 (C-4'/C-5/C-3/C-4''/C-5'), 67.41 (Ar-CH_2), 66.75 (C-4'/C-5/C-3/C-4''/C-5'), 61.59 (C-2''), 60.28 (C-2'), 51.85 (C-2), 17.16 (C-6'), 16.99 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{59}H_{60}Cl_3N_7O_{13}Na$: 1202.32124; found 1202.32069

(Benzyl (2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy 3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α-D-fucopyranose (31)



Hemiacetal **30** (352 mg, 0.298 mmol,) was co-evaporated with toluene (x3) before being dissolved in dry acetone (1.5 mL, 0.2 M). K_2CO_3 (82 mg, 0.596 mmol, 2 equiv.) was added to the solution followed by $CF_3C(NPh)Cl$ (0.1 mL, 0.596 mmol, 2 equiv.). The reaction mixture was stirred overnight under nitrogen until TLC (pentane/EtOAc, 7:3) showed full conversion. The mixture was filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 9:1 → 7:3) yielded imidate donor **31** in 96% yield (387 mg, 0.286 mmol). **^{1}H NMR (500 MHz, CD_3CN)** δ 7.90 – 7.72 (m, 7H), 7.53 – 7.39 (m, 8H), 7.39 – 7.24 (m, 19H), 7.24 – 7.21 (m, 1H), 7.20 – 7.04 (m, 11H), 6.81 (s, 2H), 5.23 (s, 1H), 4.96 – 4.87 (m, 2H), 4.87 – 4.69 (m, 8H), 4.69 – 4.61 (m, 3H), 4.46 – 4.33 (m, 5H), 4.30 – 4.20 (m, 3H), 4.10 – 4.03 (m, 2H), 4.00 (q, *J* = 6.9 Hz, 1H), 3.94 – 3.86 (m, 3H), 3.86 – 3.82 (m, 3H), 3.81 – 3.73 (m, 3H), 1.25 – 1.21 (m, 6H). **^{13}C NMR (126 MHz, CD_3CN)** δ 168.73, 163.36, 138.68, 136.89, 134.40, 133.53, 129.89, 129.41, 129.36, 129.18, 129.03, 128.85, 128.82, 128.71, 128.60, 127.15, 126.87, 118.32, 101.79, 99.54, 80.61, 79.00, 76.69, 76.42, 75.80, 75.67, 72.34, 71.00, 70.79, 68.44, 67.88, 62.29, 60.95, 52.15, 29.71, 17.11, 16.90. **HRMS**: $[M+Na]^+$ calculated for $C_{67}H_{64}Cl_3F_3N_8O_{13}Na$: 1375.34787; found 1375.34717

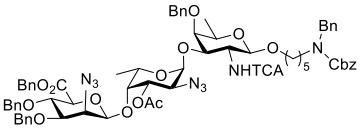
5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)- 4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-α-D-fucopyranoside (4)



Donor **31** (169 mg, 0.125 mmol, 1 equiv.) and acceptor **32** (53 mg, 0.163 mmol, 1.3 equiv.) were co-evaporated with toluene (x3) before being dissolved in DCM/MeCN (1:1, 1.3 mL, 0.1 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The reaction mixture was cooled to -50 °C, followed by addition of TBSOTf (6 µL, 0.025 mmol, 0.2 equiv.). The mixture was stirred for 1 h while warming to -40 °C until TLC (pentane/EtOAc, 7:3) showed full conversion of the donor. The reaction was quenched with Et_3N and diluted in EtOAc. The organic phase was washed with H_2O (x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 7:3 → 6:4) and size exclusion chromatography yielded trisaccharide **4** in 73% yield (136 mg, 0.91 mmol) as the sole β anomer. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.84 – 7.76 (m, 4H), 7.53 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.46 (dd, *J* = 6.3, 3.3 Hz, 2H), 7.41 – 7.27 (m, 19H), 7.24 – 7.11 (m, 6H), 7.13 – 7.02 (m, 4H), 5.18 (d, *J* = 9.5 Hz, 2H), 4.96 (d, *J* = 3.6 Hz, 1H), 4.90 (d, *J* = 11.4 Hz, 1H), 4.87 – 4.78 (m, 2H), 4.75 (d, *J* = 3.9 Hz, 1H), 4.73 – 4.66 (m, 5H), 4.63 (d, *J* = 1.1 Hz, 1H), 4.52 – 4.40 (m, 4H), 4.35 – 4.26 (m, 1H), 4.10 (t, *J* = 9.4 Hz, 1H), 4.04 (d, *J* = 3.2 Hz, 1H), 3.98 (s, 1H), 3.93 (dd, *J* = 10.6, 3.7 Hz, 1H), 3.92 – 3.77 (m, 4H), 3.77 (d, *J* = 9.7 Hz, 2H), 3.75 – 3.71 (m, 1H), 3.62 (q, *J* = 6.3 Hz, 1H), 3.59 – 3.52 (m, 2H), 3.45 – 3.31 (m, 1H), 3.20 (dt, *J* = 30.7, 7.8 Hz, 2H), 1.60 – 1.42 (m, 4H), 1.33 – 1.25 (m, 5H), 1.14 (d, *J* = 6.5 Hz, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 167.22, 162.08, 138.51, 137.99, 137.94, 137.34, 135.43, 134.86, 133.35, 133.06, 128.80, 128.73, 128.62, 128.54, 128.39, 128.36, 128.26, 128.15, 128.12, 127.96, 127.94,

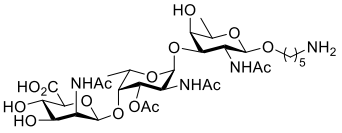
127.81, 127.78, 127.71, 127.50, 127.34, 127.25, 100.99, 99.29, 99.22, 79.83, 79.30, 78.00, 77.48, 77.16, 76.84, 75.40, 75.30, 75.15, 74.99, 72.29, 70.84, 70.65, 69.76, 67.40, 67.22, 66.94, 61.34, 59.25, 56.82, 50.57, 50.31, 47.21, 46.24, 29.26, 28.00, 27.48, 17.20, 17.13. **HRMS**: $[M+Na]^+$ calculated for $C_{79}H_{83}Cl_3N_8O_{15}Na$: 1511.49412; found 1511.49357

5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosiduronsyl)-(1→4)-3-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranosyl-(1→3)- 4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-α-D-fucopyranoside (33)



4 (138 mg, 0.0926 mmol) was dissolved in DCM/H_2O (4:1, 1.85 mL, 0.05 M) and added DDQ (42 mg, 0.185 mmol, 2 equiv.). The reaction was stirred at rt under nitrogen for 6 h until TLC (pentane, EtOAc, 6:4) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (sat. aq.) and diluted/extracted with EtOAc (x3). The combined organic phases were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was used without further purification. The residue was dissolved in pyridine (2 mL) and cooled to 0 °C and added Ac_2O (0.3 mL) and DMAP (catalytic amount) and stirred at rt under nitrogen overnight until TLC (pentane/acetone, 7:3) showed full conversion. The mixture was dissolved in EtOAc, washed with 1 M HCl (x1), $NaHCO_3$ (sat. aq.; x1) and brin (x1), dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 7:3 → 6:4) yielded trisaccharide **33** in 92% yield (119 mg, 0.0856 mmol).**^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.40 – 7.27 (m, 27H), 7.26 – 7.07 (m, 9H), 5.24 – 5.10 (m, 5H), 5.03 – 4.94 (m, 3H), 4.91 – 4.80 (m, 1H), 4.80 – 4.75 (m, 3H), 4.75 – 4.62 (m, 3H), 4.50 – 4.39 (m, 5H), 4.39 – 4.31 (m, 1H), 4.08 – 4.00 (m, 3H), 4.00 (dd, *J* = 3.7, 1.0 Hz, 2H), 3.98 – 3.88 (m, 2H), 3.82 (q, *J* = 11.3, 9.6 Hz, 3H), 3.72 (dd, *J* = 9.8, 6.0 Hz, 1H), 3.63 (q, *J* = 6.4 Hz, 1H), 3.58 – 3.49 (m, 2H), 3.46 – 3.31 (m, 1H), 3.28 – 3.04 (m, 2H), 2.04 (s, 4H), 1.62 – 1.40 (m, 5H), 1.33 – 1.28 (m, 5H), 1.26 (d, *J* = 1.8 Hz, 4H), 1.05 (d, *J* = 6.5 Hz, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.62, 167.58, 138.22, 137.99, 137.82, 137.29, 134.85, 128.87, 128.74, 128.62, 128.52, 128.42, 128.29, 127.96, 127.94, 127.90, 127.82, 127.66, 127.33, 101.05, 99.79, 99.01, 79.54, 78.59, 78.07, 75.65, 75.50, 75.30, 75.12, 72.30, 70.73, 70.28, 69.75, 69.61, 67.71, 67.23, 66.28, 61.06, 57.66, 55.97, 53.88, 31.03, 29.37, 29.25, 23.51, 23.15, 20.86, 17.29, 16.52. **HRMS**: $[M+NH_4]^+$ calculated for $C_{70}H_{77}Cl_3N_8O_{16}NH_4$:1408.48669; found 1408.48614

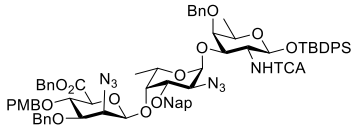
5-aminopentyl 2-*N*-acetamide-2-deoxy-β-D-mannopyranosiduronsyl-(1→4)-2-*N*-acetamide-3-*O*-acetyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-*N*-acetamide-2-deoxy-β-D-fucopyranoside (1)



33 (106 mg, 0.0762 mmol) was dissolved in THF (distilled, 3 mL) and added zinc powder (1.49 g, 22.86 mmol, 300 equiv.), AcOH (1 mL) and Ac_2O (0.5 mL). The resulting mixture was stirred at 50 °C overnight until TLC (DCM/MeOH, 95:5) showed full conversion. The cooled mixture was filtered through Celite, evaporated *in vacuo* and co-evaporated with toluene (x3). The crude product was first purified by column chromatography (DCM/MeOH, 98:2 → 90:10) followed by HPLC given **34** in 18% yield (18 mg, 0.0136 mmol). The product **34** (13 mg, 0.00976 mmol) 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 then $Pd(OH)_2/C$ (catalytic amount) was added. The reaction was again birched with argon for 5 minutes before the atmosphere was changed for H_2. The mixture was stirred for 3 days under H_2 atmosphere, after which it was filtered over a Whatman filter and lyophilized. Purification by a HW40 column with NH_4OAc followed by lyophilization gave **1** in 56% yield (4 mg, 0.0054 mmol). **^{1}H NMR (600 MHz, D2O)** δ 5.02 (dd, *J* = 11.6, 3.0 Hz, 1H, H'-3), 5.00 (d, *J* = 3.9 Hz, 1H, H'-1), 4.74 (d, *J* = 1.4 Hz, 1H, H''-1), 4.59 (dd, *J* = 4.3, 1.4 Hz, 1H, H-1), 4.40 (d, *J* = 8.6 Hz, 1H, H'-2), 4.37 (dd, *J* = 11.6, 3.9 Hz, 1H, H'-4), 4.21 (d, *J* = 3.1 Hz, 1H, H'-5), 4.18 (q, *J* = 6.4 Hz, 1H, H-2), 3.98 (dd, *J* = 10.3, 8.6 Hz, 1H, C*H*$_2$-Linker), 3.88 (dt, *J* = 10.1, 6.0 Hz, 1H), 3.82 – 3.74 (m, 4H, H''-3, H-3, H'-4, H-5), 3.64 (t, *J* = 9.7 Hz, 1H, H''-4), 3.61 – 3.54 (m, 2H, H''-5, C*H*$_2$-Linker), 2.99 (t, *J* = 7.7 Hz, 2H, C*H*$_2$-Linker), 2.13 (s, 3H, COC*H*$_3$), 2.08 (s, 3H, COC*H*$_3$), 2.00 (s, 3H, COC*H*$_3$), 1.97 (s, 3H, COC*H*$_3$), 1.67 (p, *J* = 7.7 Hz, 2H, C*H*$_2$-Linker), 1.63 – 1.55 (m, 2H, C*H*$_2$-Linker), 1.39 (pd, *J* = 7.1, 2.0 Hz, 2H, C*H*$_2$-Linker), 1.27 (d, *J* = 6.4 Hz, 3H, H-6, H'-6), 1.24 (d, *J* = 6.5 Hz, 3H, H-6/ H'-6). **^{13}C NMR (151 MHz, D2O)** δ 176.53 (C=O), 176.15 (C=O), 175.18 (C=O), 175.02 (C=O), 174.73 (C=O), 102.44, 100.75, 99.94, 79.26, 78.03, 76.95, 72.54, 71.62, 71.35, 70.98, 70.74, 70.34, 67.74, 53.93, 52.23, 48.05, 40.24, 29.08, 27.32, 23.10, 23.06, 22.95, 22.89, 21.24, 16.27 (C-6/ C'-6), 16.18 (C-6/ C'-6). **HRMS**: $[M+H]^+$ calculated for $C_{31}H_{52}N_4O_{16}H$: 737.34566; found 737.34497

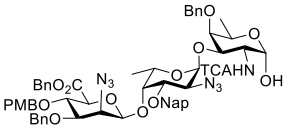
***Tert*-butyldiphenylsilyl (Benzyl (2-azido-2-deoxy-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-β-D-fucopyranoside (7)**



Acceptor **9** (417 mg, 0.44 mmol, 1 equiv.) and donor **10** (404 mg, 0.66 mmol, 1.5 equiv.) were co-evaporated trice with toluene before being dissolved in dry DCM 3 (mL, 0.15 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The reaction mixture was cooled to -78 °C followed by addition of NIS (198 mg, 0.88 mmol, 2 equiv.) and TBSOTf (20 µL, 0.088 mmol, 0.2 equiv.). The reaction mixture was allowed to warm to -10 °C and stirred for 4 h under argon until TLC (toluene/EtOAc, 8:2) showed full conversion. The reaction was quenched with Et_3N and diluted in EtOAc. The organic phase was washed with $Na_2S_2O_3$ (sat. aq.; x1), $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 90:10 → 75:25) yielded 82% of trisaccharide **7** (α: 155 mg, 0.107 mmol; β: 367 mg, 0.253 mmol) in a α/β = 30:70. NMR reported for the β-anomer. **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.82 – 7.75 (m, 3H, Ar-*H*), 7.73 – 7.68 (m, 2H, Ar-*H*), 7.65 – 7.61 (m, 2H, Ar-*H*), 7.50 (dd, *J* = 8.5, 1.6 Hz, 1H, Ar-*H*), 7.47 – 7.43 (m, 2H, Ar-*H*), 7.38 – 7.26 (m, 17H, Ar-*H*), 7.19 – 7.13 (m, 4H, Ar-*H*, *H*N(CO)CCl_3), 7.07 – 7.03 (m, 2H, Ar-*H*), 6.99 – 6.94 (m, 1H, Ar-*H*), 6.78 – 6.73 (m, 2H, Ar-*H*), 4.92 – 4.84 (m, 3H, H-1', H-1, Ar-C*H*$_2$), 4.77 (d, *J* = 12.0 Hz, 1H, Ar-C*H*$_2$), 4.73 – 4.63 (m, 6H, Ar-C*H*$_2$), 4.59 (d, *J* = 1.1 Hz, 1H, *H*-1''), 4.41 (d, *J* = 11.2 Hz, 1H, Ar-C*H*$_2$), 4.34 (d, *J* = 10.2 Hz, 1H, Ar-C*H*$_2$), 4.13 – 4.07 (m, 1H, H-2), 4.04 (t, *J* = 9.5 Hz, 1H, H-4''), 4.01 – 3.96 (m, 3H, H-2'', H-4', H-3), 3.94 (dd, *J* = 10.6, 3.6 Hz, 1H, H-2'), 3.89 (q, *J* = 6.6 Hz, 1H, H-5'), 3.80 – 3.74 (m, 4H, H-3', OC*H*$_3$), 3.71 (d, *J* = 9.8 Hz, 1H, H-5''), 3.50 (dd, *J* = 9.2, 3.6 Hz, 1H, H-3''), 3.44 (d, *J* = 2.9 Hz, 1H, H-4), 1.08 (d, *J* = 6.7 Hz, 3H, H-6'), 1.05 (s, 9H, (C*H*$_3$)$_3$), 1.00 (d, *J* = 6.4 Hz, 3H, *H*-6). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 167.26 (*C*=O), 161.93 (*C*=O), 138.80

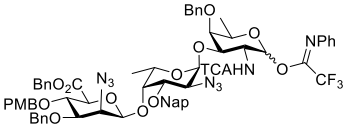
(Ar-C_q), 137.47 (Ar-C_q), 136.21 (Ar-*C*), 135.98 (Ar-*C*), 135.54 (Ar-C_q), 134.98 (Ar-C_q), 133.77 (Ar-C_q), 133.53 (Ar-C_q), 133.44 (Ar-C_q), 133.11 (Ar-C_q), 130.17 (Ar-C_q), 129.72 (Ar-*C*), 129.69 (Ar-*C*), 129.57 (Ar-*C*), 128.82 (Ar-*C*), 128.77 (Ar-*C*), 128.58 (Ar-*C*), 128.46 (Ar-*C*), 128.28 (Ar-*C*), 128.21 (Ar-*C*), 128.14 (Ar-*C*), 127.98 (Ar-*C*), 127.82 (Ar-*C*), 127.65 (Ar-*C*), 127.52 (Ar-*C*), 127.28 (Ar-*C*), 126.53 (Ar-*C*), 126.06 (Ar-*C*), 126.05 (Ar-*C*), 113.82 (Ar-*C*), 101.06 (*C*-1''), 99.00 (C-1'), 95.22 (C-1), 79.89 (C-3''), 78.93 (C-3), 78.71 (C-4), 75.74 (C-3'), 75.49 (C-4'), 75.04 (Ar-*C*H_2), 74.98 (Ar-*C*H_2), 74.95 (C-4''), 74.95 (C-5''), 72.37 (Ar-*C*H_2), 70.84 (Ar-*C*H_2), 70.60 (C-5), 67.39 (Ar-*C*H_2), 67.17 (C-5'), 61.46 (C-2''), 59.58 (C-2'), 57.21 (C-2), 55.39 (O*C*H_3), 27.14 ((*C*H_3)$_3$), 19.36 (*C*(CH$_3$)$_3$), 17.15 (C-6'), 16.78 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{76}H_{80}Cl_3N_7O_{14}Na$:1470.44958; found 1470.44903

(Benzyl (2-azido-3-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-β-D-fucopyranose (35)



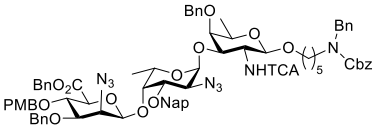
Trisaccharide **7** (297 mg, 0.205 mmol) was dissolved in THF (2 mL, 0.1 M) and cooled to 0 °C. Following, AcOH (24 µL, 0.41 mmol, 2 equiv.) and TBAF (1 M in THF; 0.4 mL, 0.41 mmol, 2 equiv.) were added. The reaction mixture was stirred overnight at rt under nitrogen atmosphere until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction was quenched by the addition of NH_4Cl (sat. aq.) and diluted in EtOAc. The organic phase was washed with H_2O (x3) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 80:20 → 60:40) furnished hemiacetal **35** in 83% yield (205 mg, 0.17 mmol). **^{1}H NMR (500 MHz, CDCl$_3$)** δ 7.86 – 7.76 (m, 4H, Ar-*H*), 7.70 (d, *J* = 6.4 Hz, 1H, *H*N(CO)CCl$_3$), 7.51 (dd, *J* = 8.3, 1.7 Hz, 1H, Ar-*H*), 7.48 – 7.42 (m, 2H, Ar-*H*), 7.39 – 7.27 (m, 9H, Ar-*H*), 7.22 – 7.13 (m, 3H, Ar-*H*), 7.04 – 7.00 (m, 2H, Ar-*H*), 6.98 – 6.93 (m, 2H, Ar-*H*), 6.78 – 6.73 (m, 2H, Ar-*H*), 5.60 (t, *J* = 3.6 Hz, 1H, H-1), 5.03 – 4.93 (m, 2H, *H*-1', Ar-C*H*$_2$), 4.85 (d, *J* = 11.7 Hz, 1H, Ar-C*H*$_2$), 4.74 – 4.60 (m, 7H, H-1'', Ar-C*H*$_2$), 4.48 – 4.43 (m, 1H, H-2), 4.41 (d, *J* = 10.9 Hz, 1H, Ar-C*H*$_2$), 4.33 (d, *J* = 10.1 Hz, 1H, Ar-C*H*$_2$), 4.17 – 4.00 (m, 7H, H-2', H-5, H-3, H-4', H-5', H-4'', H-2''), 3.88 (dd, *J* = 10.5, 2.9 Hz, 1H, H-3'), 3.77 (s, 3H, OC*H*$_3$), 3.72 (d, *J* = 9.7 Hz, 1H, H-5''), 3.69 (d, *J* = 2.6 Hz, 1H, H-4), 3.51 (dd, *J* = 9.2, 3.6 Hz, 1H, H-3''), 2.73 (dd, *J* = 3.6, 1.4 Hz, 1H, O*H*), 1.15 (d, *J* = 6.5 Hz, 3H, H-6), 1.10 (d, *J* = 6.6 Hz, 3H, H-6'). **^{13}C NMR (126 MHz, CDCl$_3$)** δ 167.23 (*C*=O), 162.35 (*C*=O), 138.35 (Ar-C_q), 137.46 (Ar-C_q), 135.37 (Ar-C_q), 134.91 (Ar-C_q), 133.40 (Ar-C_q), 133.05 (Ar-C_q), 130.12 (Ar-C_q), 129.73 (Ar-*C*), 128.82 (Ar-*C*), 128.78 (Ar-*C*), 128.57 (Ar-*C*), 128.53 (Ar-*C*), 128.30 (Ar-*C*), 128.23 (Ar-*C*), 128.17 (Ar-*C*), 127.98 (Ar-*C*), 127.86 (Ar-*C*), 127.83 (Ar-*C*), 127.77 (Ar-*C*), 126.52 (Ar-*C*), 126.09 (Ar-*C*), 125.99 (Ar-*C*), 125.91 (Ar-*C*), 113.81 (Ar-*C*), 101.09 (C-1''), 98.81 (C-1'), 90.82 (C-1), 79.80 (C-3''), 77.98 (C-3/C-4'/C-4''/C-5'), 77.51 (C-4), 76.54 (Ar-*C*H_2), 75.44 (C-3'), 75.06 (C-5''), 74.96 (C-3/C-4'/C-4''/C-5'), 74.96 (C-3/C-4'/C-4''/C-5'), 74.86 (Ar-*C*H_2), 72.44 (Ar-*C*H_2), 71.10 (Ar-*C*H_2), 67.83 (Ar-*C*H_2), 67.39 (Ar-*C*H_2), 66.75 (C-5'), 61.61 (C-2''), 60.28 (C-2'), 55.40 (O*C*H_3), 51.84 (C-2), 17.17 (C-6'), 16.99 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{60}H_{62}Cl_3N_7O_{14}Na$: 1232.33180; found 1232.33125

(Benzyl (2-azido-3-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-β-D-fucopyranose (36)



Hemiacetal **35** (205 mg, 0.17 mmol) was co-evaporated with toluene (x3) before being dissolved in dry acetone (1.7 mL, 0.1 M). K_2CO_3 (47 g, 0.339 mmol, 2 equiv.) and $CF_3C(NPh)Cl$ (0.06 mL, 0.339 mmol, 2 equiv.) were added and the reaction mixture was stirred overnight under nitrogen until TLC (pentane/EtOAc, 7:3) showed full conversion. The mixture was filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 9:1 → 7:3) yielded imidate donor **36** in 97% yield (227 mg, 0.164 mmol). **^{1}H NMR (500 MHz, CD_3CN)** δ 7.89 – 7.76 (m, 6H), 7.53 – 7.46 (m, 3H), 7.46 – 7.41 (m, 3H), 7.40 – 7.27 (m, 13H), 7.26 – 7.16 (m, 5H), 7.15 – 7.06 (m, 4H), 7.03 – 6.97 (m, 2H), 6.86 – 6.76 (m, 5H), 5.24 (s, 1H), 4.96 – 4.88 (m, 1H), 4.87 – 4.75 (m, 6H), 4.70 – 4.59 (m, 4H), 4.48 – 4.40 (m, 2H), 4.35 (dd, *J* = 3.6, 1.2 Hz, 1H), 4.33 – 4.28 (m, 2H), 4.27 – 4.22 (m, 2H), 4.09 – 4.03 (m, 2H), 3.95 – 3.91 (m, 1H), 3.90 – 3.86 (m, 2H), 3.85 – 3.81 (m, 3H), 3.77 – 3.75 (m, 2H), 3.74 (s, 3H), 1.23 (d, *J* = 6.3 Hz, 6H). **^{13}C NMR (126 MHz, CD_3CN)** δ 168.93, 163.36, 160.28, 144.65, 139.61, 139.16, 136.90, 136.29, 134.24, 133.90, 131.24, 130.60, 129.91, 129.44, 129.39, 129.37, 129.31, 129.25, 129.16, 129.03, 128.87, 128.84, 128.71, 128.63, 127.17, 127.06, 126.91, 126.87, 118.34, 114.51, 101.80, 99.57, 80.68, 79.02, 78.01, 76.71, 76.50, 76.35, 76.11, 75.89, 75.34, 72.38, 71.05, 70.81, 68.47, 67.88, 62.37, 60.99, 55.86, 52.18, 29.73, 17.13, 16.94. **HRMS** (found for the hemiacetal): $[M+Na]^+$ calculated for $C_{60}H_{62}Cl_3N_7O_{14}Na$: 1234.32885; found 1234.32680.

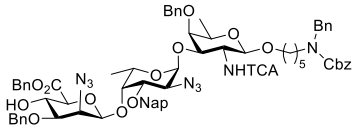
5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (2-azido-3-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-α-D-fucopyranoside (37)



Donor **36** (256 mg, 0.185 mmol, 1 equiv.) and acceptor **32** (79 mg, 0.241 mmol, 1.3 equiv.) were co-evaporated with toluene (x3) before being dissolved in DCM/MeCN (1.9 mL, 1:1; 0.1 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The reaction mixture was cooled to -50 °C, followed by addition of TBSOTf (8.5 µL, 0.037 mmol, 0.2 equiv.). The mixture was allowed to warm to -30 °C and stirred for 1 h until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction was quenched with Et_3N and diluted in EtOAc, washed with H_2O (x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 7:3 → 6:4) and size exclusion chromatography yielded trisaccharide **37** in 80% yield (225 mg, 0.148 mmol) as the sole β-anomer. **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.78 – 7.74 (m, 3H, Ar-*H*), 7.50 (dd, *J* = 8.5, 1.6 Hz, 1H, Ar-*H*), 7.45 – 7.42 (m, 2H, Ar-*H*), 7.36 – 7.12 (m, 25H, Ar-*H*), 7.08 – 7.06 (m, 1H, Ar-*H*), 7.00 – 6.96 (m, 2H, Ar-*H*), 6.79 – 6.74 (m, 2H, Ar-*H*), 5.15 (d, *J* = 10.9 Hz, 2H, C*H*$_2$Ph-linker), 4.94 (d, *J* = 3.8 Hz, 1H, H-1'), 4.87 (d, *J* = 11.5 Hz, 1H, Ar-C*H*$_2$), 4.85 – 4.78 (m, 2H, *H*-1, Ar-C*H*$_2$), 4.75 – 4.62 (m, 6H, Ar-C*H*$_2$), 4.59 (d, *J* = 1.1 Hz, 1H, H-1''), 4.46 (d, *J* = 7.9 Hz, 2H, C*H*$_2$Ph-linker), 4.42 (d, *J* = 11.4 Hz, 1H, Ar-C*H*$_2$), 4.35 (d, *J* = 10.2 Hz, 1H,

Ar-CH_2), 4.28 (s, 1H, H-3), 4.05 (t, J = 9.5 Hz, 1H, H-4''), 4.01 (d, J = 3.2 Hz, 1H, H-2''), 3.95 (d, J = 2.9 Hz, 1H, H-4'), 3.90 (dd, J = 10.5, 3.8 Hz, 1H, H-2'), 3.82 (q, J = 6.7 Hz, 2H, H-5, H-2), 3.76 (s, 3H, OCH_3), 3.74 – 3.68 (m, 2H, H-5'', H-3'), 3.60 (q, J = 6.4 Hz, 1H, H-5'), 3.55 – 3.49 (m, 2H, H-3'', H-4), 3.37 (d, J = 21.3 Hz, 1H, CH_2-linker), 3.18 (d, J = 35.4 Hz, 2H, CH_2-linker), 1.58 – 1.40 (m, 4H, CH_2-linker), 1.26 (q, J = 14.5, 10.5 Hz, 12H, CH_2-linker, H-6'), 1.11 (d, J = 6.6 Hz, 3H, H-6). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 167.25 (*C*=O), 162.09 (*C*=O), 159.36 (*C*=O), 138.53 (Ar-C_q), 138.02 (Ar-C_q), 137.43 (Ar-C_q), 135.45 (Ar-C_q), 134.94 (Ar-C_q), 133.37 (Ar-C_q), 133.08 (Ar-C_q), 130.13 (Ar-C_q), 129.70 (Ar-*C*), 128.80 (Ar-*C*), 128.74 (Ar-*C*), 128.62 (Ar-*C*), 128.55 (Ar-*C*), 128.40 (Ar-*C*), 128.25 (Ar-*C*), 128.15 (Ar-*C*), 128.13 (Ar-*C*), 127.95 (Ar-*C*), 127.79 (Ar-*C*), 127.71 (Ar-*C*), 127.51 (Ar-*C*), 126.57 (Ar-*C*), 126.07 (Ar-*C*), 126.02 (Ar-*C*), 125.88 (Ar-*C*), 113.79 (Ar-*C*), 101.01 (C-1''), 99.31 (C-1'), 99.24 (C-1), 79.90 (C-3''/C-4), 79.34 (C-3''/C-4), 78.02 (C-3), 75.47 (C-5''/C-3'), 75.31 (C-5''/C-3'), 75.28 (Ar-*C*H$_2$), 75.01 (Ar-*C*H$_2$), 74.91 (C-4''/C-4'), 74.91 (C-4''/C-4'), 72.34 (Ar-*C*H$_2$), 70.86 (Ar-*C*H$_2$), 70.67 (C-5'), 67.38 (Ar-*C*H$_2$), 67.23 (*C*H$_2$Ph-linker), 66.97 (C-5), 61.42 (C-2''), 59.28 (C-2'), 55.98 (C-2), 55.36 (O*C*H$_3$), 50.61 (*C*H$_2$Ph-linker), 50.34 (*C*H$_2$Ph-linker), 48.21 (CH_2-linker), 46.44 (CH_2-linker), 29.28 (*C*H$_2$-linker), 27.51 (*C*H$_2$-linker), 23.44 (*C*H$_2$-linker), 23.35 (*C*H$_2$-linker), 17.21 (C-6'), 17.14 (C-6). **HRMS**: $[M+NH_4]^+$ calculated for $C_{80}H_{85}Cl_3N_8O_{16}NH_4$:1536.54929; found 1536.54874

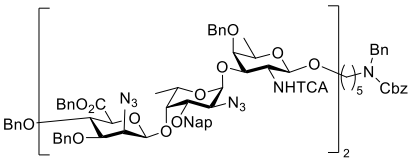
5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-α-D-fucopyranoside (38)



Trisaccharide **37** (268 mg, 0.176 mmol) was dissolved in DCM (1.8 mL, 0.1 M) and cooled to 0 °C after which TES (0.14 mL, 0.881 mmol, 5 equiv.) and HCl in HFIP (0.2 M, 0.26 mL, 0.3 equiv.) were added to the solution. The reaction was stirred for 1 h at 0 °C until TLC (pentane/EtOAc, 6:4) showed full conversion. The reaction mixture was quenched by the addition of $NaHCO_3$ (sat. aq.) and diluted in EtOAc. The organic phase was washed with $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 7:3 → 5:5) afforded acceptor **38** in 78% yield (192 mg, 0.137 mmol). **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.83 – 7.77 (m, 1H, Ar-*H*), 7.77 – 7.71 (m, 3H, Ar-*H*), 7.50 (dd, J = 8.4, 1.7 Hz, 1H, Ar-*H*), 7.42 – 7.26 (m, 17H, Ar-*H*), 7.26 – 7.14 (m, 6H, Ar-*H*), 7.13 – 7.08 (m, 2H, Ar-*H*), 5.17 (d, J = 11.5 Hz, 2H, CH_2Ph-linker), 4.95 (s, 1H, H-1'), 4.93 – 4.88 (m, 2H, Ar-CH_2), 4.83 (d, J = 8.3 Hz, 1H, H-1), 4.79 – 4.68 (m, 4H, Ar-CH_2), 4.66 (s, 1H, H-1''), 4.64 (s, 1H, Ar-CH_2), 4.48 (d, J = 6.6 Hz, 2H, CH_2Ph-linker), 4.43 (d, J = 11.4 Hz, 1H, Ar-CH_2), 4.28 (d, J = 10.7 Hz, 1H, H-3), 4.18 (t, J = 9.4 Hz, 1H, H-4''), 4.03 (d, J = 3.6 Hz, 1H, H-2''), 4.00 (d, J = 2.9 Hz, 1H, H-4'), 3.91 (dd, J = 10.6, 3.7 Hz, 1H, H-2'), 3.87 – 3.78 (m, 2H, H-2, H-5), 3.74 – 3.69 (m, 2H, H-3', H-5''), 3.61 (q, J = 6.6 Hz, 1H, H-5'), 3.53 (d, J = 2.8 Hz, 1H, H-4), 3.45 (s, 1H, H-3''), 3.41 – 3.32 (m, 1H, CH_2-linker), 3.28 – 3.12 (m, 2H, CH_2-linker), 1.59 – 1.43 (m, 4H, CH_2-linker), 1.35 – 1.22 (m, 6H, CH_2-linker), H-6'), 1.14 (d, J = 6.6 Hz, 3H, H-6). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 168.34 (*C*=O), 162.09 (*C*=O), 138.52 (Ar-C_q), 137.97 (Ar-C_q), 137.53 (Ar-C_q), 135.40 (Ar-C_q), 134.64 (Ar-C_q), 133.30 (Ar-C_q), 133.04 (Ar-C_q), 128.88 (Ar-*C*), 128.82 (Ar-*C*), 128.72 (Ar-*C*), 128.61 (Ar-*C*), 128.51 (Ar-*C*), 128.37 (Ar-*C*), 128.23 (Ar-*C*), 128.10 (Ar-*C*), 128.04 (Ar-*C*), 127.95 (Ar-*C*), 127.92 (Ar-*C*),

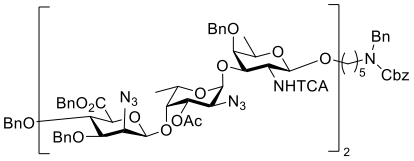
127.77 (Ar-*C*), 127.69 (Ar-*C*), 127.55 (Ar-*C*), 127.52 (Ar-*C*), 126.68 (Ar-*C*), 126.09 (Ar-*C*), 126.03 (Ar-*C*), 125.90 (Ar-*C*), 100.82 (C-1''), 99.24 (C-1'), 99.24 (C-1''), 79.22 (C-4), 78.64 (C-3''), 78.02 (C-3), 75.19 (Ar-*C*H_2), 75.12 (C-3'/C-5''), 75.03 (C-3'/C-5''), 74.69 (C-4'), 72.63 (Ar-*C*H_2), 70.72 (Ar-*C*H_2), 70.65 (C-5'), 69.86 (*C*H_2Ph-linker), 69.73 (*C*H_2Ph-linker), 68.01 (C-4''), 67.61 (N Ar-*C*H_2), 67.22 (*C*H_2Ph-linker), 66.91 (C-5), 61.33 (C-2''), 59.22 (C-2'), 55.88 (C-2), 50.58 (*C*H_2Ph-linker), 50.31 (*C*H_2-linker), 47.20 (*C*H_2-linker), 46.24 (*C*H_2-linker), 29.77 (*C*H_2-linker), 29.25 (*C*H_2-linker), 27.46 (*C*H_2-linker), 23.39 (*C*H_2-linker), 17.19 (C-6'), 17.13 (C-6). **HRMS**: $[M+NH_4]^+$ calculated for $C_{72}H_{77}Cl_3N_8O_{15}NH_4$:1415.49177; found 1416.49122

Hexasaccharide-protected with ONap on L-Fuc and Bn on D-Man (5)



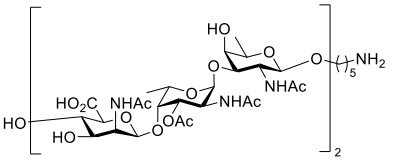
Acceptor **38** (57 mg, 0.041 mmol, 1 equiv.) and donor **31** (74 mg, 0.054 mmol, 1.3 equiv.) were co-evaporated with toluene (x3) before being dissolved in dry DCM/MeCN (1 mL, 2:1; 0.04 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The mixture was cooled to -78 °C, after which TBSOTf (2 µL, mmol, 0.2 equiv.) was added. The reaction mixture was stirred at -78 °C for 1 h until TLC (pentane/EtOAc, 6.5:3.5) showed full conversion. The reaction mixture was quenched with Et_3N and diluted in EtOAc. The organic phase was washed with $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Size exclusion column chromatography furnished hexamer **5** in 84% yield (88 mg, 0.0342 mmol) as the sole β-anomer. **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.78 (tdd, *J* = 14.4, 5.1, 3.2 Hz, 8H), 7.55 – 7.39 (m, 7H), 7.39 – 7.26 (m, 26H), 7.25 – 7.12 (m, 16H), 7.07 (dddd, *J* = 20.2, 8.5, 6.4, 2.0 Hz, 7H), 6.78 (d, *J* = 8.6 Hz, 1H), 5.17 (d, *J* = 13.1 Hz, 2H), 4.92 (dd, *J* = 11.9, 2.9 Hz, 3H), 4.84 (ddd, *J* = 12.1, 8.7, 5.9 Hz, 3H), 4.80 – 4.71 (m, 5H), 4.71 – 4.60 (m, 9H), 4.57 (d, *J* = 11.9 Hz, 1H), 4.51 – 4.45 (m, 3H), 4.42 (dd, *J* = 10.7, 3.5 Hz, 2H), 4.39 – 4.21 (m, 4H), 4.17 (ddd, *J* = 11.8, 9.4, 7.5 Hz, 1H), 4.12 – 4.03 (m, 2H), 4.01 (td, *J* = 8.3, 3.7 Hz, 3H), 3.97 (d, *J* = 3.1 Hz, 1H), 3.94 (t, *J* = 6.6 Hz, 1H), 3.88 (tdd, *J* = 10.3, 7.0, 3.7 Hz, 3H), 3.81 (dt, *J* = 10.4, 3.4 Hz, 3H), 3.77 – 3.72 (m, 2H), 3.72 – 3.66 (m, 1H), 3.65 – 3.58 (m, 1H), 3.57 – 3.50 (m, 2H), 3.50 – 3.44 (m, 1H), 3.43 (s, 2H), 3.39 – 3.31 (m, 1H), 3.23 (s, 1H), 3.16 (q, *J* = 6.4 Hz, 1H), 1.50 (d, *J* = 29.3 Hz, 3H), 1.30 – 1.25 (m, 5H), 1.18 (d, *J* = 6.4 Hz, 3H), 1.11 (td, *J* = 11.2, 10.1, 6.4 Hz, 6H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 167.96, 167.24, 162.07, 161.92, 138.63, 138.53, 138.31, 138.02, 137.96, 137.40, 135.49, 135.47, 134.92, 134.83, 133.39, 133.37, 133.06, 129.31, 128.85, 128.81, 128.78, 128.75, 128.73, 128.65, 128.52, 128.50, 128.42, 128.40, 128.35, 128.25, 128.21, 128.17, 128.15, 128.08, 128.06, 127.96, 127.94, 127.90, 127.85, 127.82, 127.79, 127.77, 127.72, 127.59, 127.54, 127.48, 127.36, 127.25, 127.21, 127.16, 126.72, 126.49, 126.05, 126.02, 126.00, 125.89, 125.83, 100.97, 100.71, 99.94, 99.24, 99.02, 80.21, 79.87, 79.24, 78.05, 77.16, 76.91, 76.03, 75.38, 75.31, 75.27, 75.23, 75.12, 74.93, 74.48, 74.11, 73.84, 72.38, 71.17, 70.90, 70.68, 70.66, 69.81, 69.79, 67.64, 67.36, 67.23, 66.94, 62.56, 61.58, 59.55, 59.20, 55.94, 54.05, 50.65, 50.38, 29.80, 29.26, 23.38, 17.20, 17.16, 17.09, 17.01. **HRMS**: $[M+NH_4]^+$ calculated for $C_{113}H_{123}Cl_6N_{15}O_{29}NH_4$:2384.70901; found 2385.70722

Hexasaccharide-protected with OAc on L-Fuc and Bn on D-Man (39)



5 (129 mg, 0.051 mmol) was dissolved in DCM/H_2O (4:1, 5 mL, 0.01 M) and added DDQ (46 mg, 0.202 mmol, 4 equiv.). The reaction was stirred at rt under nitrogen for 5 h until TLC (pentane, EtOAc, 6:4) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (sat. aq.) and diluted/extracted with EtOAc (x3). The combined organic phases were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was used without further purification. The residue was dissolved in pyridine (2 mL) and cooled to 0 °C and added Ac_2O (0.3 mL) and DMAP (catalytic amount) and stirred at rt under nitrogen overnight until TLC (pentane/acetone, 7:3) showed full conversion. The mixture was dissolved in EtOAc, washed with 1 M HCl (x1), $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (pentane/acetone, 7:3) yielded **39** in 73% yield (87 mg, 0.037 mmol). **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.51 – 7.28 (m, 44H), 7.24 – 7.04 (m, 13H), 6.77 (dd, *J* = 17.0, 8.2 Hz, 1H), 5.27 – 5.21 (m, 2H), 5.20 – 5.11 (m, 6H), 5.04 – 4.94 (m, 4H), 4.93 – 4.73 (m, 10H), 4.72 – 4.65 (m, 5H), 4.52 (d, *J* = 8.1 Hz, 1H), 4.50 – 4.40 (m, 7H), 4.37 – 4.21 (m, 4H), 4.20 – 3.93 (m, 12H), 3.91 – 3.76 (m, 6H), 3.75 – 3.59 (m, 6H), 3.60 – 3.40 (m, 7H), 3.28 – 3.09 (m, 4H), 2.04 – 2.01 (m, 6H), 1.78 – 1.38 (m, 10H), 1.31 (d, *J* = 6.4 Hz, 4H), 1.27 (dd, *J* = 7.8, 2.6 Hz, 4H), 1.19 (dd, *J* = 8.6, 6.3 Hz, 5H), 1.07 – 1.02 (m, 6H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 170.60, 170.34, 167.81, 167.60, 162.03, 138.28, 138.06, 137.81, 137.32, 135.08, 134.86, 133.00, 129.51, 128.97, 128.90, 128.86, 128.77, 128.64, 128.56, 128.53, 128.46, 128.44, 128.39, 128.32, 127.99, 127.96, 127.85, 127.75, 127.70, 127.56, 127.35, 101.12, 100.77, 99.10, 99.02, 98.99, 98.87, 79.57, 79.17, 78.62, 78.05, 77.94, 77.41, 75.75, 75.33, 75.27, 75.14, 74.99, 73.40, 72.39, 70.78, 70.60, 70.22, 67.94, 67.76, 67.26, 66.53, 62.00, 61.19, 57.77, 57.67, 56.05, 54.84, 50.60, 50.34, 47.17, 46.49, 29.24, 23.48, 20.88, 17.32, 17.18, 16.54.

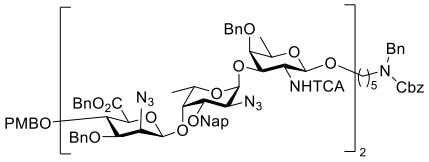
CP5-Hexasaccharide (2)



39 (62 mg, 0.026 mmol) was dissolved in THF (distilled, 3 mL) and added zinc powder (510 mg, 7.804 mmol, 300 equiv.), AcOH (1 mL) and Ac_2O (0.5 mL). The resulting mixture was stirred at 50 °C overnight until TLC (DCM/MeOH, 95:5) showed full conversion. The cooled mixture was filtered through Celite, evaporated *in vacuo* and co-evaporated with toluene (x3). The crude product was first purified by column chromatography (DCM/MeOH, 98:2 → 90:10) followed by HPLC given **40** in 11% yield (6.2 mg, 0.00279 mmol). The product **40** (5.2 mg, 0.00234 mmol) 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 then $Pd(OH)_2$/C (catalytic amount) was added. The reaction was again birched with argon for 5 minutes before the atmosphere was changed for H_2. The mixture was stirred for 3 days under H_2 atmosphere, after which it was filtered over a Whatman filter and lyophilized until NMR showed full conversion. Purification by a HW40 column with NH_4OAc followed by lyophilization gave **2** in 47% yield (1.5 mg, 0.0011 mmol). **^{1}H NMR (600 MHz, D_2O)** δ

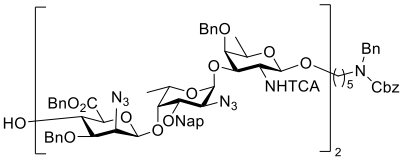
5.03 – 4.96 (m, 4H), 4.74 (d, *J* = 1.4 Hz, 1H), 4.71 (s, 1H), 4.63 (dd, *J* = 4.4, 1.2 Hz, 1H), 4.59 (dd, *J* = 4.3, 1.4 Hz, 1H), 4.43 – 4.32 (m, 4H), 4.20 (d, *J* = 3.1 Hz, 2H), 4.16 (q, *J* = 6.5 Hz, 2H), 4.01 – 3.91 (m, 2H), 3.91 – 3.84 (m, 2H), 3.85 – 3.74 (m, 8H), 3.67 – 3.59 (m, 2H), 3.59 – 3.53 (m, 2H), 2.98 (t, *J* = 7.7 Hz, 2H), 2.13 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.99 (s, 6H), 1.98 (s, 3H), 1.97 (s, 3H), 1.67 (p, *J* = 7.7 Hz, 2H), 1.61 – 1.54 (m, 2H), 1.42 – 1.34 (m, 2H), 1.27 (dd, *J* = 8.2, 6.4 Hz, 6H), 1.23 (dd, *J* = 6.6, 3.2 Hz, 6H). **^{13}C NMR (151 MHz, D_2O)** δ 176.53, 176.45, 175.94, 175.47, 175.20, 175.16, 174.99, 174.72, 174.61, 174.39, 102.43, 102.34, 100.81, 100.75, 100.01, 99.93, 79.96, 79.05, 78.11, 77.96, 77.90, 76.99, 76.52, 72.54, 71.66, 71.63, 71.32, 71.24, 71.03, 70.96, 70.80, 70.61, 70.27, 67.71, 53.91, 52.89, 52.22, 47.98, 40.24, 29.07, 27.31, 23.37, 23.09, 23.05, 22.97, 22.94, 22.89, 21.23, 21.15, 16.26, 16.22, 16.16, 16.09. **HRMS**: $[M+H]^+$ calculated for $C_{57}H_{91}N_7O_{31}H$: 1370.58377; found 1370.58355

Hexasaccharide-protected with ONap on L-Fuc and PMB on D-Man (41)



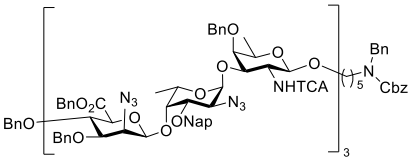
Acceptor **38** (61 mg, 0.0435 mmol) and donor **36** (91 mg, 0.0655 mmol, 1.5 equiv.) were co-evaporated with toluene (x3) before being dissolved in dry DCM/ MeCN (1 mL, 2:1; 0.04 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The mixture was cooled to -78 °C, after which TBSOTf (2 µL, 0.0087 mmol, 0.2 equiv.) was added. The reaction mixture was stirred at -78 °C for 1 h until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction mixture was quenched with Et_3N and diluted in EtOAc. The organic phase was washed with $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Size exclusion column chromatography furnished hexamer **41** in 80% yield (85 mg, 0.0329 mmol) as the sole β-anomer. **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.78 (ddd, *J* = 17.7, 8.9, 3.8 Hz, 10H), 7.54 – 7.39 (m, 9H), 7.41 – 7.12 (m, 52H), 7.13 – 7.03 (m, 6H), 7.00 – 6.93 (m, 3H), 6.77 (dd, *J* = 8.5, 5.3 Hz, 4H), 5.16 (d, *J* = 12.1 Hz, 2H), 4.91 (dd, *J* = 11.6, 3.0 Hz, 4H), 4.87 – 4.80 (m, 4H), 4.76 (dq, *J* = 15.6, 9.0, 7.9 Hz, 5H), 4.72 – 4.59 (m, 13H), 4.56 (d, *J* = 11.9 Hz, 1H), 4.47 (t, *J* = 8.8 Hz, 4H), 4.39 – 4.29 (m, 4H), 4.26 (dt, *J* = 14.1, 8.7 Hz, 3H), 4.20 – 4.13 (m, 1H), 4.08 – 3.97 (m, 7H), 3.96 (s, 1H), 3.92 (t, *J* = 6.5 Hz, 1H), 3.90 – 3.79 (m, 6H), 3.77 (s, 5H), 3.74 – 3.65 (m, 5H), 3.61 (t, *J* = 6.4 Hz, 1H), 3.55 – 3.49 (m, 3H), 3.48 – 3.32 (m, 5H), 3.25 – 3.21 (m, 1H), 3.15 (q, *J* = 6.4 Hz, 2H), 1.58 – 1.42 (m, 4H), 1.28 (d, *J* = 6.2 Hz, 4H), 1.17 (d, *J* = 6.4 Hz, 4H), 1.15 – 1.06 (m, 9H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 167.96, 167.26, 161.93, 159.74, 138.63, 138.32, 138.01, 137.46, 135.49, 134.96, 134.81, 133.39, 133.06, 130.12, 129.69, 129.33, 128.78, 128.74, 128.66, 128.63, 128.54, 128.52, 128.42, 128.41, 128.36, 128.26, 128.18, 128.08, 127.95, 127.86, 127.77, 127.73, 127.60, 127.55, 127.16, 126.73, 126.49, 126.01, 125.90, 125.83, 113.78, 100.99, 100.72, 99.97, 99.25, 99.02, 80.23, 79.88, 78.04, 77.41, 76.02, 75.42, 75.30, 75.25, 75.10, 74.45, 74.14, 73.86, 72.40, 71.15, 70.68, 70.64, 67.66, 67.39, 67.35, 66.93, 62.56, 61.62, 59.55, 59.19, 55.95, 55.37, 54.03, 17.21, 17.10, 17.02 **HRMS**: $[M+NH_4]^+$ calculated for $C_{132}H_{137}Cl_6N_{15}O_{28}NH_4$:2610.82364; found 2611.82279

Hexasaccharide-protected with ONap on L-Fuc as acceptor (42)



Hexasaccharide **41** (179 mg, 0.0692 mmol) was dissolved in DCM (1.4 mL, 0.05 M) and cooled to 0 °C after which TES (0.06 mL, 0.346 mmol, 5 equiv.) and HCl in HFIP (0.2 M, 0.1 mL, 0.2 equiv.) were added to the solution. The reaction was stirred for 1 h at 0 °C until TLC (pentane/EtOAc, 6:4) showed full conversion. The reaction mixture was quenched by the addition of $NaHCO_3$ (sat. aq.) and diluted in EtOAc. The organic phase was washed with $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 6:4 → 5:5) afforded acceptor **42** in 78% yield (133 mg, 0.054 mmol). **^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.84 – 7.68 (m, 9H), 7.50 – 7.42 (m, 7H), 7.40 – 7.28 (m, 22H), 7.26 – 7.10 (m, 20H), 7.07 (ddd, *J* = 7.8, 6.0, 2.3 Hz, 3H), 6.76 (dd, *J* = 8.7, 4.3 Hz, 1H), 5.16 (d, *J* = 12.6 Hz, 2H), 4.97 – 4.89 (m, 4H), 4.88 – 4.78 (m, 5H), 4.78 – 4.71 (m, 5H), 4.71 – 4.58 (m, 9H), 4.54 (d, *J* = 11.8 Hz, 1H), 4.45 (dd, *J* = 19.2, 10.1 Hz, 4H), 4.33 – 4.18 (m, 4H), 4.18 – 4.08 (m, 3H), 4.08 – 3.97 (m, 5H), 3.95 (d, *J* = 3.6 Hz, 1H), 3.93 – 3.87 (m, 2H), 3.87 – 3.74 (m, 6H), 3.71 (dd, *J* = 9.5, 2.5 Hz, 1H), 3.69 – 3.62 (m, 3H), 3.60 (q, *J* = 6.2 Hz, 1H), 3.51 (d, *J* = 2.8 Hz, 1H), 3.47 – 3.31 (m, 5H), 3.22 (s, 1H), 3.14 (q, *J* = 6.4 Hz, 2H), 2.90 (d, *J* = 2.8 Hz, 1H), 1.43 (q, *J* = 5.3, 4.4 Hz, 5H), 1.31 – 1.24 (m, 8H), 1.15 (dd, *J* = 10.6, 6.3 Hz, 5H), 1.10 (dd, *J* = 6.6, 3.8 Hz, 6H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 168.31, 167.99, 161.94, 138.65, 138.32, 138.02, 137.58, 135.51, 134.80, 134.69, 133.34, 133.04, 129.34, 128.77, 128.67, 128.64, 128.59, 128.51, 128.42, 128.38, 128.27, 128.17, 128.11, 128.05, 127.99, 127.97, 127.88, 127.79, 127.62, 127.59, 127.57, 127.34, 127.20, 126.74, 126.60, 126.07, 126.04, 125.88, 100.81, 100.72, 99.99, 99.25, 98.97, 80.24, 79.27, 78.56, 78.05, 75.81, 75.29, 75.08, 74.89, 74.72, 74.42, 74.18, 73.87, 72.72, 71.00, 68.11, 67.60, 67.28, 66.94, 62.55, 61.53, 59.49, 59.18, 55.95, 54.01, 29.83, 17.20, 17.04. **HRMS**: $[M+NH_4]^+$ calculated for $C_{124}H_{129}Cl_6N_{15}O_{27}NH_4$: 2490.76613; found 2491.76488

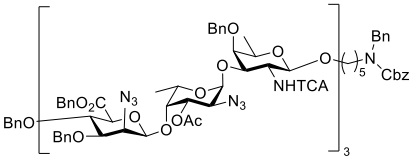
Nonasaccharide-protected with ONap on L-Fuc and Bn on D-Man (6)



Acceptor **42** (129 mg, 0.0524 mmol) and donor **31** (106 mg, 0.0787 mmol, 1.5 equiv.) were co-evaporated with toluene (x3) before being dissolved in dry DCM/ MeCN (0.7 mL, 2:1; 0.075 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The mixture was cooled to -78 °C, after which TBSOTf (2.4 µL, 0.0105 mmol, 0.2 equiv.) was added. The reaction mixture was stirred at -78 °C for 4 h until TLC (pentane/acetone, 6.5:3.5) showed full conversion. The reaction mixture was quenched with Et_3N and diluted in EtOAc. The organic phase was washed with $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Size exclusion column chromatography furnished nonamer **6** in 74% yield (141 mg, 0.0388 mmol) as the sole β-anomer. **^{1}H NMR (600 MHz, $CDCl_3$)** δ 7.85 – 7.71 (m, 11H), 7.56 – 7.42 (m, 9H), 7.42 – 7.28 (m, 23H), 7.24 – 7.11 (m, 19H), 7.11 – 6.99 (m, 8H), 6.80 – 6.73 (m, 2H), 5.17 (d, *J* = 15.4 Hz, 2H), 4.96 – 4.87 (m, 5H), 4.88 – 4.79 (m, 5H), 4.79 – 4.58 (m, 18H), 4.55 (d, *J* = 11.7 Hz, 1H), 4.48 (td, *J* = 7.9, 3.6 Hz, 4H), 4.41 (d, *J* = 10.7 Hz, 1H), 4.35 (t, *J* = 11.1 Hz, 2H), 4.31 – 4.20 (m, 5H), 4.20 – 4.12

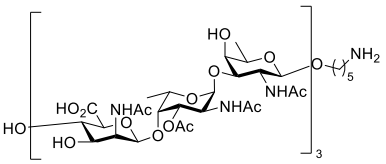
(m, 2H), 4.11 – 3.95 (m, 9H), 3.95 – 3.66 (m, 13H), 3.61 (q, *J* = 6.3 Hz, 1H), 3.53 (dd, *J* = 8.9, 3.4 Hz, 2H), 3.46 (dd, *J* = 7.7, 4.4 Hz, 2H), 3.43 – 3.31 (m, 5H), 3.25 – 3.10 (m, 4H), 1.50 (ddd, *J* = 39.9, 14.5, 7.3 Hz, 4H), 1.28 (d, *J* = 5.6 Hz, 6H), 1.20 – 1.07 (m, 13H). **^{13}C NMR (151 MHz, $CDCl_3$)** δ 167.95, 167.91, 167.24, 161.96, 161.93, 138.60, 138.57, 138.50, 138.29, 138.28, 138.00, 137.93, 137.37, 135.54, 135.44, 134.88, 134.81, 134.78, 133.37, 133.34, 133.05, 133.01, 129.31, 129.29, 128.76, 128.73, 128.64, 128.62, 128.52, 128.44, 128.42, 128.40, 128.35, 128.26, 128.20, 128.16, 128.13, 128.08, 128.05, 128.04, 127.96, 127.94, 127.91, 127.85, 127.80, 127.76, 127.60, 127.55, 127.19, 127.15, 126.71, 126.63, 126.48, 126.05, 126.02, 125.98, 125.89, 125.83, 100.96, 100.70, 100.66, 99.99, 99.94, 99.29, 99.22, 99.00, 98.90, 80.19, 79.83, 79.18, 78.02, 77.91, 77.37, 76.02, 75.71, 75.35, 75.28, 75.19, 75.09, 74.92, 74.89, 74.44, 74.12, 73.88, 73.84, 72.37, 71.16, 70.82, 70.67, 70.63, 69.88, 69.74, 67.64, 67.58, 67.38, 67.34, 67.26, 67.21, 66.93, 62.64, 62.53, 61.56, 59.52, 59.40, 59.17, 55.90, 53.99, 50.60, 50.32, 47.22, 46.26, 29.80, 29.23, 23.32, 17.20, 17.16, 17.13, 17.08, 17.00. **HRMS**: $[M+2H]^+$ calculated for $C_{183}H_{187}Cl_9N_{22}O_{39}H_2$: 3639.06871.76613 (1819.53435); found 1819.53301

Nonasaccharide-protected with OAc on L-Fuc and Bn on D-Man (43)



6 (91 mg, 0.025 mmol) was dissolved in DCM/H_2O (4:1, 2.5 mL, 0.01 M) and added DDQ (34 mg, 0.151 mmol, 6 equiv.). The reaction was stirred at rt under nitrogen for 6 h until TLC (pentane, EtOAc, 6.5:3.5) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (sat. aq.) and extracted with EtOAc (x3). The combined organic phases were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was used without further purification. The residue was dissolved in pyridine (2 mL) and cooled to 0 °C and added Ac_2O (0.3 mL) and DMAP (catalytic amount) and stirred at rt under nitrogen overnight until TLC (pentane/acetone, 7:3) showed full conversion. The mixture was dissolved in EtOAc, washed with 1 M HCl (x1), $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (pentane/acetone, 8:2 → 5:5) yielded **42** in 74% yield (62 mg, 0.0186 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.53 – 7.28 (m, 31H), 7.24 – 7.05 (m, 8H), 5.23 (q, *J* = 5.0, 3.7 Hz, 2H), 5.20 – 5.10 (m, 3H), 5.03 – 4.92 (m, 3H), 4.92 – 4.74 (m, 7H), 4.74 – 4.57 (m, 6H), 4.57 – 4.38 (m, 6H), 4.29 (ddd, *J* = 17.5, 12.6, 8.2 Hz, 3H), 4.17 – 3.88 (m, 11H), 3.88 – 3.37 (m, 13H), 3.29 – 3.10 (m, 3H), 2.11 – 1.92 (m, 9H), 1.34 – 1.13 (m, 16H), 1.13 – 0.98 (m, 8H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.59, 170.34, 167.58, 162.00, 138.28, 138.05, 137.80, 137.41, 135.08, 134.86, 129.53, 128.97, 128.90, 128.86, 128.76, 128.64, 128.55, 128.53, 128.45, 128.32, 127.99, 127.96, 127.84, 127.74, 127.69, 127.35, 101.11, 100.77, 98.95, 79.55, 78.70, 78.04, 77.68, 75.31, 74.97, 73.42, 73.10, 72.39, 70.79, 70.23, 68.38, 67.75, 67.25, 66.53, 62.16, 61.18, 57.74, 55.79, 54.85, 29.82, 20.88, 17.33, 17.19, 16.56.

CP5-Nonasaccharide (3)



43 (62 mg, 0.0186 mmol) was dissolved in THF (distilled, 3 mL) and added zinc powder (366 mg, 5.59 mmol, 300 equiv.), AcOH (1 mL) and Ac_2O (0.5 mL). The resulting mixture was stirred at 50 °C overnight until TLC (DCM/MeOH, 95:5) showed full conversion. The cooled mixture was filtered through Celite, evaporated *in vacuo* and co-evaporated with toluene (x3). The crude product was first purified by column chromatography (DCM/MeOH, 98:2 → 90:10) followed by HPLC given **44** in 16% yield (9.6 mg, 0.0031 mmol). The product **44** (9 mg, 0.00288 mmol) 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 then $Pd(OH)_2/C$ (catalytic amount) was added. The reaction was again birched with argon for 5 minutes before the atmosphere was changed for H_2. The mixture was stirred for 3 days under H_2 atmosphere, after which it was filtered over a Whatman filter and lyophilized until NMR showed full conversion. Purification by a HW40 column with NH_4OAc followed by lyophilization gave **3** in 49% yield (2.8 mg, 0.0014 mmol). **^{1}H NMR (850 MHz, D_2O)** δ 5.18 (d, *J* = 17.4 Hz, 1H), 4.99 (td, *J* = 16.8, 14.2, 7.5 Hz, 5H), 4.72 (d, *J* = 28.1 Hz, 3H), 4.65 – 4.61 (m, 1H), 4.59 (d, *J* = 4.4 Hz, 1H), 4.46 – 4.31 (m, 5H), 4.24 – 4.09 (m, 7H), 4.09 – 3.92 (m, 6H), 3.92 – 3.67 (m, 17H), 3.66 – 3.55 (m, 5H), 2.99 (t, *J* = 7.8 Hz, 2H), 2.17 – 1.94 (m, 56H), 1.67 (q, *J* = 7.8 Hz, 2H), 1.58 (d, *J* = 8.7 Hz, 2H), 1.41 – 1.36 (m, 2H), 1.29 – 1.20 (m, 19H). **^{13}C NMR (214 MHz, D_2O)** δ 177.69, 175.21, 174.20, 173.67, 101.48, 101.37, 99.85, 99.80, 99.06, 98.97, 79.03, 78.30, 77.32, 77.00, 76.06, 75.57, 71.62, 70.71, 70.67, 70.36, 70.30, 70.11, 70.01, 69.86, 69.64, 69.38, 66.76, 52.98, 51.94, 51.27, 47.01, 39.29, 28.11, 26.35, 22.42, 22.10, 22.02, 21.94, 20.96, 20.28, 20.19, 15.26, 15.20, 15.13. **HRMS**: $[M+2H]^+$ calculated for $C_{83}H_{130}N_{10}O_{46}H_2$: 2004.82189; found 1002.41545

Preparation of *S. aureus* type 5 conjugates

The CP5-OS were solubilized in 350 µL of a 9:1 DMSO: H_2O solution with 15 equiv. of linker (suberic acid bis(*N*-hydroxysuccinimide ester)) and stirred for 2 h at rt. The derivatized CP5-OS were purified by EtOAc precipitation. The solution was first incubated with 5 mL cold EtOAc and 250 µL NaCl (3 M, aq.) for 1 h at 4 °C. The EtOAc layer was discarded and the bottom phase was washed with cold EtOAc (3 mL) 10-15 times. The resulting solids were lyophilized overnight. The mass after linker installation was measured and a 90% recovery was predicted.

A CRM_{197} stock solution was buffer-exchanged to 50 mM HEPES pH 8.0 through Zeba™ Spin Desalting Column 7K MWCO to a final concentration of 20 mg/mL CRM197 solution. The reaction is incubated overnight at 4 °C.

Protocol for Western Blot

SDS-PAGE were run the **1**-, **2**-, and **3** conjugates with a 7.5% acrylamide gel. The gel was transferred to a membrane for 30 min, which was blocked in 5% w/v milk in PBST (PBS supplemented with 0.1% Tween20) blocking solution overnight at 4 °C. The membrane was then incubated for 4 days at 4 °C with 1:1000 anti-CP5 mAb (in PBST) followed by washing with PBST three times. Next, the membrane was incubated for 1 h at rt with 1:1000 anti-rat IgG (in PBST), and again washed with PBST three times. The membrane was detected with Clarity Max Western ECL Substrate (Bio-Rad).

SPR experiments

The SPR experiments were conducted using a Surface Plasmon Resonance Biacore X100 from GE Healthcare Biacore Life Science. CP5-biotin (CP5-biotin, lot EB23GIU16, M=351.6 µg/mL) was immobilized on a SA-chip using a 20 µg/mL solution. After the run 311.7 AU was immobilized on the chip. For the SPR-experiments a 20 nM rat anti-CP5 mAb 331 concentration was used together with the CP5-OS concentrations as summarized in Tabel X. From the Biacore X100 control software the binding levels were collected and used for calculation of the inhibition percentage.

Competitor concentrations (µg/mL)	**1**	**2**	**3**	**CP5-PS**
	1000	20	5	0.0781
	500	10	2.5	0.0391
	250	5	1.25	0.0195
	125	2.5	0.625	0.00977
	62.5	1.25	0.313	0.00488
	31.25	0.625	0.156	0.00244
	15.63	0.313	0.0781	0.00122
	7.81	0.156	0.0391	0.000610
	3.91	0.0781	0.0195	0.000305
	1.95	0.0391	0.00977	0.000153
	0.977	0.0195	0.00488	7.629E-05
	0.488	0.00977	0.00244	3.815E-05
	0	0	0	0

SPR IC50 values

The calculation of IC50 values were performed with GraphPad Prism software using Kruskal-Wallis with Dunn's multiple comparisons; "***" denotes the significant result within $p < 0.001$, "ns" means not significant.

Structural conformation

NMR methods. NMR experiments were performed in a Bruker Avance III 800 MHz spectrometer equipped with a TCI cryoprobe. Samples were dissolved in D_2O at 1.0 mM concentration. Experiments were acquired at the temperature of 298 K.

^{1}H and ^{13}C NMR resonances of the molecules **1**, **2** and **3** were assigned through standard 2D-TOCSY, 2D-ROESY, 2D-NOESY, 2D ^{1}H-^{13}C-HSQC. 2D-TOCSY experiments were acquired with 30 ms mixing time, 1.0 s of relaxation delay, 4 scans, and 4096x256 (F2xF1) points with a spectral width of 6556.0 Hz. 2D-ROESY experiment was acquired with mixing time of 200 ms, 1.0 s of relaxation delay, 48 scans, and 4096x256 (F2xF1) points with a spectral width of 6880.7 Hz. 2D-NOESY experiment was acquired with mixing time of 200 ms, 1.5 s of relaxation delay, 32 scans, and 4096x256 (F2xF1) points with a spectral width of 6242.2 Hz. 2D ^{1}H,^{13}C-HSQC experiments were acquired with 1.0 s of relaxation delay, 48 scans, and 4096x220 (F2xF1) points with a spectral width of 6250.0 Hz (F2) and 24144.6 Hz (F1). The data were processed with Topspin 4.2 (Bruker Biospin) using a 90° shifted qsine window function to a total of 16K × 2K data points (F2 × F1), followed by automated baseline- and phase correction.

Molecular Mechanics Calculations. The geometry optimization was performed by using the Jaguar/Schroedinger package (version 13.5) and the AMBER* force field, with the GB/SA continuum solvent model for water. The glycosidic torsion angles were defined as ϕ (H1'-C1'-Ox-Cx) and ψ (C1'-Ox-Cx-Hx). Extended nonbonded cut-off distances (van der Waals cut-off of 8.0 Å and electrostatic cut-off of 20.0 Å) were used. The conformers for the tri- hexa- and nonasaccharide molecules **1**, **2** and **3** were generated employing geometric restrictions to respect the *exo*-anomeric effect. The possible staggered rotamers around ψ were selected and minimized. The coordinates of the obtained local minima were employed to measure the key inter-proton distances that were then compared to those obtained experimentally by the ROESY and NOESY NMR experiments through integration of the observed NOEs cross peaks using the ISPA approximation.

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Chapter 4

Investigation of trisaccharide repeating unit frameshifts of *Staphylococcus aureus* capsular polysaccharide type 5 and 8 to define the minimal binding epitope for antibody recognition

Introduction

The bacterial cell-wall of both Gram-positive and Gram-negative bacteria can consist of capsular polysaccharides (CPs), which generally are long, complex molecules built up of different monosaccharides, interconnected through various types of stereoisomeric and regioisomeric linkages, carrying different functional groups.[1] The repeating units (RUs), that make up the polymers, can contain several different monosaccharides. Because of the complexity of bacterial glycans that often contain rare monosaccharides, carrying multiple functional groups (uronic acids, free amines, acetamides), their synthesis presents a significant challenge. In the search for active epitopes for vaccine development, pressed by the synthetic hurdles, often the synthesis of a single RU is undertaken. However, if a CP is built up from RUs that contain multiple different monosaccharides, different frameshifted RUs may be defined. To ensure coverage of all possible RUs in a single molecule, larger molecules spanning at least 2 RU would have to be synthesized. Alternatively, different frameshifts of the minimal RU can be synthesized. For example, for a trisaccharide repeating unit, three different repeats "ABC", "BCA" and "CAB" can be defined.

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium, which can be encapsulated by 13 different types of CPs, thereby defining their serotype. Of these, serotypes 5 and 8 are amongst the most isolated strains from clinical sources.[2–5] The *S. aureus* CP type 5 (CP5) and type 8 (CP8) are both built up from a D-ManNAcA – L-FucNAc – D-FucNAc (DM-LF-DF) trisaccharide repeating unit, as depicted in Figure 1A. Several syntheses have been reported of these trisaccharide repeats,[6–14] of which all have the same monosaccharide order (DM-LF-DF), which perhaps can be explained to originate from the biosynthesis route.[15] As described in Chapter 1, the polysaccharide is built up from DM-LF-DF trisaccharides, that are first generated on a phospholipid anchor in the cytoplasm lipid membrane, and then polymerized on the outer cell membrane in a [3+3n] matter. So far, the DM-LF-DF trisaccharides of CP5 and CP8 have shown limited antibody recognition, as demonstrated by the work of the groups of Adamo[6] and Hu,[13] which could indicate that these are too short to present the minimal binding epitope. However, it could also be that the wrong frameshifts have been investigated.

To date, only limited work has been published regarding the synthesis of frameshifted CP5/8 trisaccharides, other than the DM-LF-DF frameshift. In 2022 Demchenko and co-workers reported the assembly of the protected CP8 DF-DM-LF frameshift (Figure 1B).[16] By installing a picoloyl ester (Pico) group on the C-

3-OH of a $ManN_3A$ donor building block they took advantage of the H-bond-mediated aglycone delivery methodology they previously introduced,[17–19] to form the desired β-product in good yield and selectivity. The generated trisaccharide has not been deprotected and with the chosen protecting group pattern, conjugation to a carrier protein would be impossible.

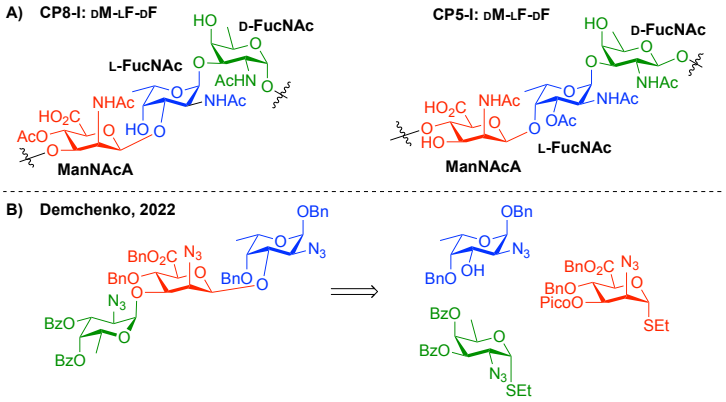
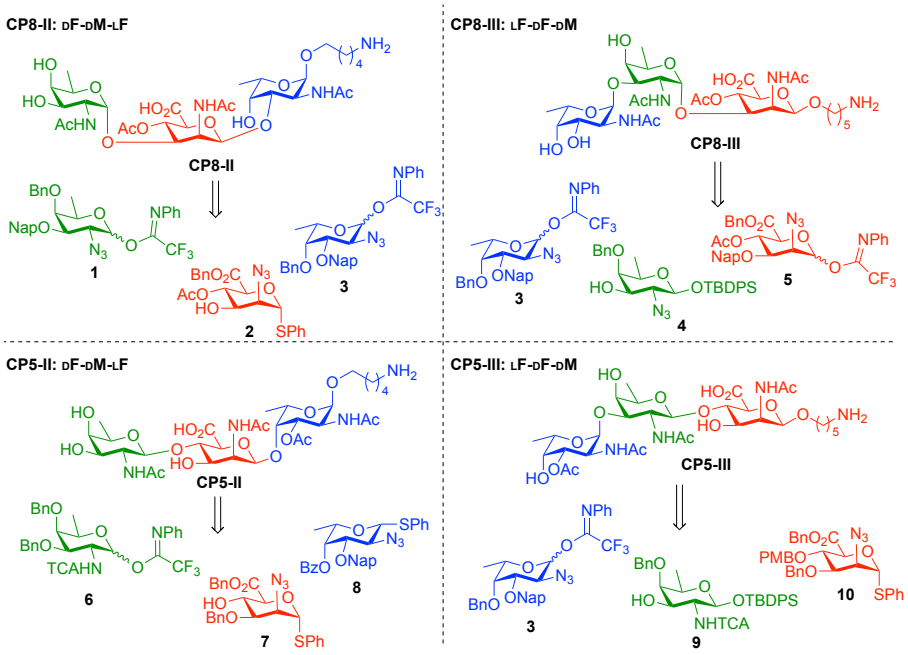


Figure 1: A) A schematic representation of the repeating unit of CP8 (left) and CP5 (right), B) Previously synthesis of a frameshifted CP8 trisaccharide.

In Chapter 2 and 3 the DM-LF-DF trisaccharides of CP5 and CP8 have been synthesized together with longer saccharides, and these were evaluated for antibody recognition. For both CP types, the DM-LF-DF trisaccharide did not show any or limited antibody recognition, where the longer oligosaccharides showed adequate binding, pointing to the oligosaccharide length as a crucial factor for binding. However, different trisaccharide frameshifts have not been investigated. Therefore, this Chapter presents the synthesis of all possible CP5 and CP8 RUs and their binding to anti-CP5 and anti-CP8 monoclonal antibodies. Besides the previously generated trisaccharides, two other CP5 and CP8 frameshifts are possible, one with the LF-DF-DM sequence and one having the DF-DM-LF structure. The same synthetic principles as implemented for the CP5 and CP8 oligosaccharides, *i.e.*, the use of a pre-glycosylation oxidation strategy and *O*-acetylation of the ManNAcA residue, the use of azides for the construction of the 1,2-*cis* linkages and trichloroacetamide protection for the β-D-FucNAc residue and the use of permanent benzyl-type protecting groups.

Results and discussion

The retrosynthesis of the two CP5/CP8-trisaccharides frameshifts is shown in Scheme 1. The same building blocks synthesized for the assembly of CP5 and CP8 oligosaccharides in Chapter 2 and 3 were applied, sometimes with minor modifications. As mentioned above, the same protection strategies were implemented for the acetamides and the installation of the *O*-acetyl esters. Different from the previous syntheses was the timing of the installation of the linker, as it will be installed on the monosaccharide level, to save steps at the more precious trisaccharide stage. Global deprotection should be facilitated by hydrogenation of the permanent benzyl-type protecting groups.

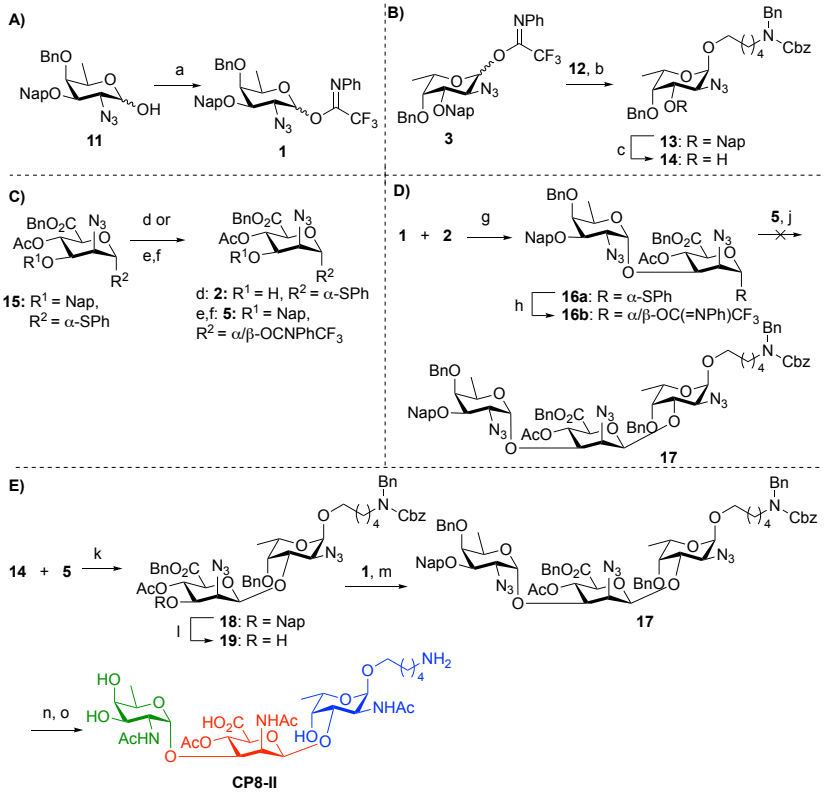


Scheme 1: Retrosynthetic analysis of the four different frameshifted trisaccharide of CP5/8

Synthesis of the frameshifted CP8 trisaccharides

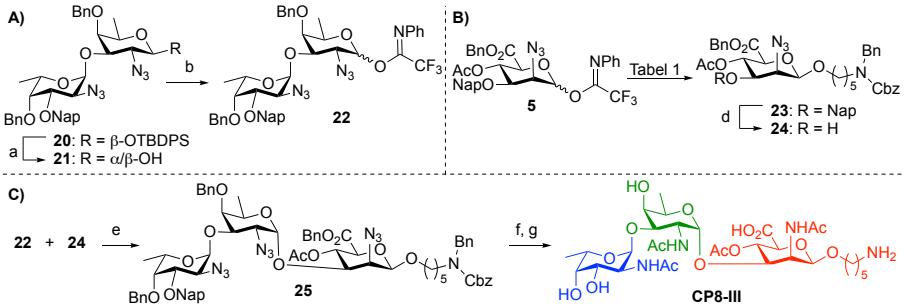
First, the CP8 DF-DM-LF trisaccharide (**CP8-II**) was constructed, and the synthesis of the required building blocks **1**, **2** and **3** (see retrosynthesis in Scheme 1) started from building blocks **11**, **3** and **15**, respectively, prepared in Chapter 2. The D-FucN_3 building block **11** was transformed into donor **1**, by installation of the *N*-phenyl trifluoroacetimidate leaving group (Scheme 2A). Next, the L-FucN_3

building block **3** was equipped with linker **12** to obtain **13** in 90% yield. Triphenylphosphine oxide (Ph_3PO) and trimethylsilyl iodide (TMSI)[20] were used to ensure the α-selectivity of the glycosylation delivering the product as a 70:30 α/β-mixture, from which the α-product could be purified by column chromatography (Scheme 2B). The newly formed α-bond was confirmed by ^{1}H-NMR with a doublet at 4.87 ppm with a coupling constant $J_{H1,H2}$ of 3.0 Hz. Acceptor **14** was then obtained by oxidative cleavage of the 2-methylnaphthyl (Nap) ether in good yields. For the mannosazide building block, **15** was converted into an acceptor by oxidative cleavage of the Nap ether giving **2** in 65% yield (Scheme 2C). With the three building blocks in hand, the construction of the trisaccharide started with investigation of a [2+1] strategy (Scheme 2D). Glycosylation between donor **1** and acceptor **2** in the presence of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) afforded **16a** with excellent α-selectivity. Disaccharide **16a** was used directly as a donor in the glycosylation with acceptor **5**. Unfortunately, the [2+1] glycosylation did not yield the desired trisaccharide **17**. Neither using the thiophenyl donor **16a** or the imidate donor **16b** gave the desired glycosylation product, likely as the result of the difficult activation of the donor.[21] Therefore, a [1+2] glycosylation strategy was explored, where first glycosylation of acceptor **14** and imidate donor **5** in the presence of TBSOTf produced disaccharide **18**. Formation of the new β-linkage was confirmed by ^{1}H-NMR and ^{13}C-NMR, with the β-ManN_3A anomeric proton and carbon having a CH-coupling constant of $J_{C1,H1}$ = 160.5 Hz. Disaccharide **18** was converted into acceptor **19** by removal of the Nap ether and the ensuing glycosylation with imidate donor **1** with TBSOTf now afforded trisaccharide **17** in 57% yield with excellent α-selectivity. The stereochemistry of the newly formed glycosidic bond was again confirmed by the CH-coupling of $J_{C1,H1}$ = 170.1 Hz, as the new anomeric proton appeared in a multiplet. The same deprotection strategy, devised in Chapter 2 was implemented. Thus, reduction of the azides and one pot acetylation followed by hydrogenation gave trisaccharide **CP8-II** in 77% yield.



Scheme 2: Synthesis of the **CP8-II** trisaccharide. *Reaction conditions:* A) a) $ClC(=NPh)CF_3$, K_2CO_3, acetone, 81%, B) b) Ph_3PO, TMSI, DCM/Et_2O, 90%, α/β=70:30, c) DDQ, DCM/H_2O, 93%, C) d) DDQ, DCM/H_2O, 65%, e) NIS, TFA, DCM, 0 °C then Et_3N, 80%, f) $ClC(=NPh)CF_3$, K_2CO_3, acetone, 95%, D) g) TBSOTf, DCM, rt, 84%, h) i) NIS, TFA, DCM the Et_3N, 0 ºC, 44%, ii) $ClC(=NPh)CF_3$, K_2CO_3, acetone, 75%, j) For 1**6a**: TfOH, NIS, DCM, -78 to -10°C, 0%, for **16b**: TBSOTf, DCM, -78 to -40°C, 0%, E) k) TBSOTf, DCM, 0 °C, 53%, l) DDQ, DCM/H_2O, 53%, m) TBSOTf, DCM, rt, 57%, n) zinc, AcOH, Ac_2O, THF, 50 °C, quant., o) $Pd(OH)_2$/C, AcOH, H_2, *t*-BuOH/H_2O, 77%.

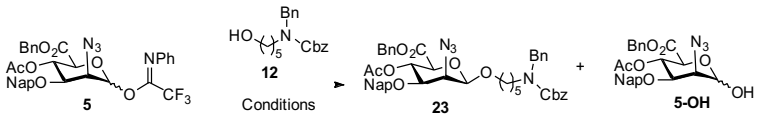
The construction of the CP8 LF-DF-DM (**CP8-III**) trisaccharide started with formation of disaccharide donor **22**, by removal of the *tert*-butyldiphenylsilyl (TBDPS) group in **20** followed by *N*-phenyl trifluoroacetimidate installation on the newly formed lactol giving donor **22**.



Scheme 3: Synthesis of the **CP8-III** trisaccharide. *Reaction conditions:* A) a) TBAF, AcOH, THF, 0 °C to rt, 65%, b) $ClC(=NPh)CF_3$, K_2CO_3, acetone, 70%, B) c) TBSOTf, DCM, -78 to -30 °C, 24%, d) DDQ, DCM/H_2O, 51%, C) e) TBSOTf, DCM, rt, 65%, f) zinc, AcOH, Ac_2O, THF, 50 °C, 78%, g) $Pd(OH)_2$/C, AcOH, H_2, *t*-BuOH/H_2O, 60%.

Next, the mannosaziduronic acid **5** was equipped with the linker by glycosylation between donor **5** and linker **12**. Unfortunately, the yield was quite low, and while solely the β-anomer was formed (as confirmed by the CH-coupling constant of $J_{C1,H1}$ = 161.6 Hz), product **23** was isolated in only 28% yield. Different attempts to improve the low yields were investigated (see Table 1). Switching the promoter from triflic acid (TfOH, Entry 1) to TBSOTf (Entry 2) only afforded **23** in 20% yield together with 6% of lactol **5-OH**, and neither changing the concentration to 0.3 M (Entry 3), prolonging the reaction time (Entry 4) or using stoichiometric amount of promoter were found to improve the yield of **23**, and only more lactol **5-OH** was formed. As enough material could be procured, the synthesis was continued without further optimization. Cleavage of the Nap ether afforded acceptor **24**, which was glycosylated with disaccharide donor **22** to give trisaccharide **25** in 65% yield and excellent α-selectivity (again confirmed by the CH-coupling constant of $J_{C1,H1}$ = 170.4 Hz, as the new anomeric proton appeared in a multiplet). Deprotection by reduction of the azides and concomitant acetylation followed by hydrogenation then gave trisaccharide **CP8-III** in 60% yield.

Table 1: Optimization of the linker installation using donor **5**.

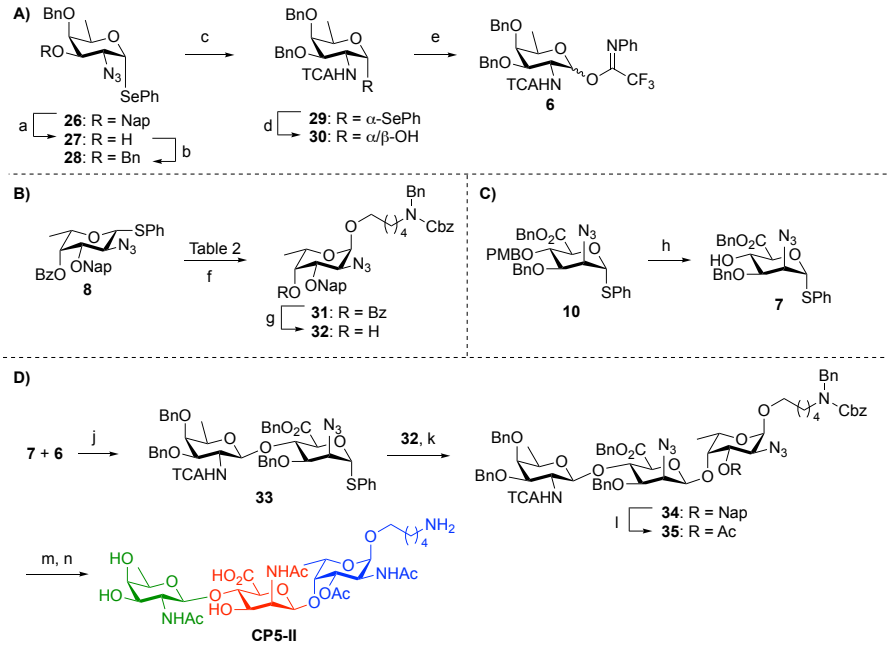


Entry	Promoter	Concentration (M)	Time (h)	Yield 23 (%)	Yield 5-OH (%)	α/β[a]
1	TfOH (0.2 equiv.)	0.1	1.5	27[b]	0	1:99[c]
2	TBSOTf (0.2 equiv.)	0.1	3	20[b]	6	1:99[c]
3	TBSOTf (0.2 equiv.)	0.3	1.5	28	33	1:99[c]
4	TBSOTf (0.2 equiv.)	0.1	19	28	45	1:99[c]
5	TBSOTf (1 equiv.)	0.1	2	25	43	1:99[c]

General conditions: 3 Å molecular sieves, -78 to -40 ºC, 1.3 equiv. acceptor for entry 1-3, 3 equiv. acceptor for entry 4-5, in DCM. [a] The α/β ratio was determined by NMR after purification. [b] Based on recovered donor. [c] No α-product was isolated.

Synthesis of the frameshifted CP5 trisaccharides

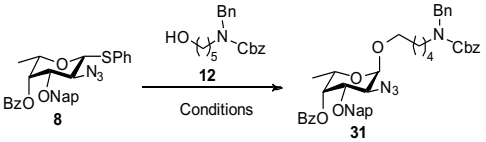
Turning to the CP5 trisaccharide frameshifts, the first trisaccharide to be investigated was the CP5 DF-DM-LF (**CP5-II**), which was synthesized from building blocks **26**, **8** and **10** (see Scheme 4) that were generated as described in Chapter 2 and 3. For the D-FucN_3 synthon, it was necessary to exchange the Nap ether with a permanent benzyl protection group to ensure orthogonality with respect to the Nap ether on the L-FucN_3 building block, that was used as precursor for the *O*-acetyl ester. Therefore, oxidative cleavage of the Nap ether yielded **26** and subsequent benzylation of the newly released alcohol gave **28** (Scheme 4A). To ensure the 1,2-*trans* linkages in the upcoming glycosylation reactions, the azide was changed to a trichloroacetamide by reduction and trichloroacetyl (TCA) acetylation to give **29** in excellent yield. Hydrolysis of the phenylselenyl group and installation of an *N*-phenyl trifluoroacetimidate on the newly formed lactol gave donor **6**.



Scheme 4: Synthesis of the **CP5-II** trisaccharide. *Reaction conditions:* A) a) DDQ, DCM/H_2O, 91%, b) BnBr, NaH, DMF, 0 °C to rt, 99%, c) i) zinc, AcOH, THF, ii) TCA-Cl, THF, 0 °C, 93% over two steps, d) NIS, acetone/H_2O, 0 °C, 75%, e) ClC(=NPh)CF_3, K_2CO_3, acetone, 99%, B) f) NIS, TBSOTf, DCM, 99%, α/β=43:57, g) NaOMe, MeOH, 87%, C) h) DDQ, DCM/H_2O, 95%, D) j) TMSOTf, DCM/MeCN, -78 °C, 51%, α/β=29:71, k) NIS, TBSOTf, DCM, -30 to 10 °C, 52%, α/β=15:85, %, l) i) DDQ, DCM/H_2O, ii) Ac_2O, DMAP, pyridine, 81% over two steps, m) zinc, AcOH, Ac_2O, THF, 50 °C, 21%, n) $Pd(OH)_2$/C, AcOH, H_2, *t*-BuOH/H_2O, 64%.

The L-FucN_3 was equipped with a linker by glycosylation of β-thiophenyl-donor **8** and linker **12** to give **31** (Scheme 4B). However, it was found difficult to obtain good α-selectivity, due to the high reactivity of the acceptor alcohol. When using *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promoter at low temperature (-60 to -30 °C), only β-product was obtained (Table 2, Entry 1). Increasing the temperature to 0 °C (Entry 2) or room temperature (Entry 3) influenced the stereoselectivity, but still favored the β-product (α/β=35:65 and α/β=34:66, respectively). By switching the promoter to NIS and TBSOTf the α/β-selectivity increased to 43:57. Also the use of NIS in combination with TMSI and Ph_3PO was investigated, as similar conditions have shown to provide α-selective glycosylation reactions with reactive primary alcohol acceptors previously,[22] but these conditions did not work with the thiophenyl donor **8**. As sufficient material was available, no further optimization has been undertaken. Acceptor **32** was obtained by saponification of the benzoyl ester under Zemplén condition.

Table 2: Optimization of the linker installation using donor **8**.



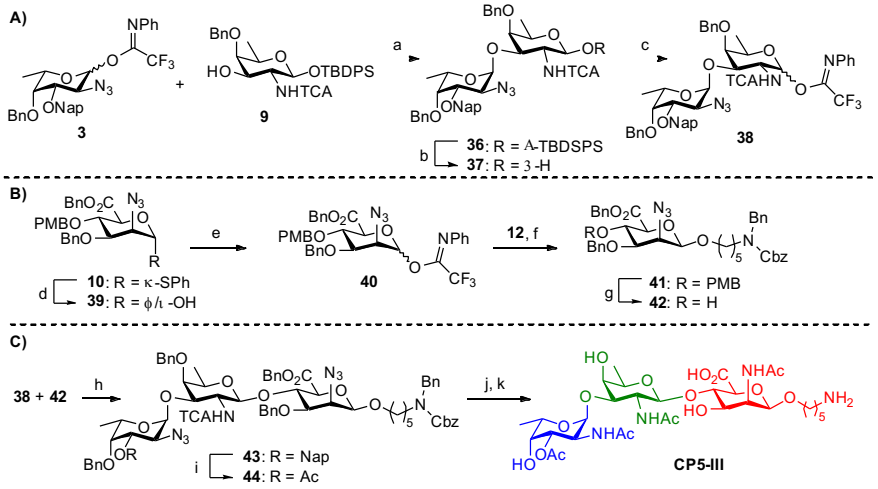
Entry	Conditions	Temp (°C)	Yield (%)	α/β [(a)]
1	NIS, TMSOTf	-60 to -30	77	0:100
2	NIS, TMSOTf	0	94	35:65
3	NIS, TMSOTf	rt	94	34:66
4	NIS, TBSOTf	rt	99	43:57
5	NIS, Ph_3PO, TMSI	rt	--	--

General conditions: 3 Å molecular sieves, 1.3 equiv. acceptor, 1.5 equiv. NIS and 0.2 equiv. TMSOTf /TBSOTf or 6 equiv. Ph_3PO and 1 equiv. TMSI, 0.1 M DCM. [(a)] The α/β ratio was determined by NMR after purification.

The D-ManAN_3 acceptor **7** was synthesized by cleavage of the *p*-methoxybenzyl (PMB) ether from **10**, to set the stage for the assembly trisaccharide (Scheme 4C). First, the glycosylation of donor **6** and acceptor **7** afforded disaccharide **33** in 51% yield with an α/β-ratio of 29:71 (Scheme 4D). The β-product could be readily purified by column chromatography and the stereochemistry was confirmed by ^{1}H-NMR with the D-FucN anomeric proton appeared as a doublet at 5.67 ppm with a coupling constant $J_{H1,H2}$ of 10.3 Hz. Both the use of low temperature and acetonitrile (MeCN) as a co-solvent were found to be necessary to obtain sufficient β-selectivity. Disaccharide **33** was used directly as donor in a glycosylation with acceptor **32** to give trisaccharide **34** in 52% yield (Scheme 4D). The trisaccharide was obtained as an inseparable α/β-mixture (α/β = 15:85), and the desired β-product was separated after the introduction of the *O*-acetyl. The structure of the main β-product was confirmed by the C1'-H1'-coupling constant of 159.6 Hz. The deprotection strategy for trisaccharide **34** followed the same strategy as described in Chapter 3. First the Nap ether was exchanged for an *O*-acetyl ester. Next, the azides and the TCA group were reduced and acetylated, which was followed by a purification method employing silica gel column chromatography and subsequent HPLC purification to obtain the pure product in 21% yield. Lastly, hydrogenation afforded the wanted trisaccharide **CP5-II** in 64% yield.

Finally, the last CP5 LF-DF-DM (**CP5-III**) trisaccharide was created by a glycosylation of acceptor **9** and donor **3** to provide disaccharide **36** in 87% yield and excellent α-selectivity, as confirmed by ^{1}H-NMR with the L-FucN H1 doublet

resonating at 4.98 ppm with a coupling constant $J_{H1,H2}$ of 3.7 Hz (Scheme 5A). Disaccharide **36** was converted into a donor by removal of the anomeric TBDPS group followed by installation of a *N*-phenyl trifluoroacetimidate on the newly formed lactol giving disaccharide donor **38**. The linker was installed on the D-ManAN$_3$ building block **10**, by first hydrolysis of the thiophenyl group providing lactol **36** followed by installation of a *N*-phenyl trifluoroacetimidate to give donor **40**. Glycosylation with linker acceptor **12** gave **41** in 65% yield and an α/β ratio of 24:76. The β-product was separable by column chromatography and the newly formed β-linkage was confirmed by the CH-coupling constant $J_{C1,H1}$ of 161.3 Hz. The use of low temperature (-78 °C) was found to increase the β-selectivity of the reaction, and it was noted that the glycosylation of thio-donor **10** did not lead to any glycosylation product. Continuing the assembly, the PMB ether in **41** was cleaved yielding acceptor **42**. Trisaccharide **43** was then obtained in 81% yield by glycosylation of donor **38** and acceptor **42** at -78 °C (Scheme 3) as an inseparable 10:90 α/β mixture. The newly generated β-linkage was again confirmed by the CH-coupling constant $J_{C1,H1}$ of 159.2 Hz. The desired stereoisomer was separated on a later stage, namely after silica gel column and the reduction from azides/TCA to acetamides, however before the HPLC purification.



Scheme 5: Synthesis of the **CP5-III** trisaccharide. *Reaction conditions*: A) a) TBSOTf, DCM, 87%, α/β=99:1, b) TBAF, AcOH, THF, 0 °C to rt, 90%, c) ClC(=NPh)CF$_3$, K$_2$CO$_3$, acetone, 99%, B) d) NIS, TFA, DCM, 0 °C, 93% yield, e) ClC(=NPh)CF$_3$, K$_2$CO$_3$, acetone, 87%, f) TBSOTf, DCM, -78 °C, 65%, α/β=24:76, g) DDQ, DCM/H$_2$O, 92%, C) h) TBSOTf, DCM, -78 °C, 81%, α/β=10:90, i) i) DDQ, DCM/H$_2$O, ii) Ac$_2$O, DMAP, pyridine, 81% over two steps, j) zinc, AcOH, Ac$_2$O, THF, 50 °C, 35%, k) Pd(OH)$_2$/C, AcOH, H$_2$, *t*-BuOH/H$_2$O, 57%.

The final steps involved exchanging the Nap ether to an *O*-acetyl and reduction of the azides and TCA group and concomitant acetylation, which was followed by silica gel column chromatography and subsequent HPLC purification. Hydrogenation afforded trisaccharide **CP5-III**. It was observed however that migration of the C''-3-*O*-acetyl to the neighboring C''-4-OH took place after purification, leading to a mixture of the two regioisomeric acetylated trisaccharides.

Antibody binding

Having the CP5/8 trisaccharide frameshifts in hand, the binding properties to monoclonal antibodies (mAb) and polyclonal antibodies (pAb) was investigated. First the binding properties of the CP8-fragments was explored. **CP8-II** and **CP8-III** were included in a competitive ELISA experiment using both anti-CP8-mAb and anti-CP8-pAb sera, however no inhibition was observed for either of the trisaccharides even at high concentrations. These findings were also confirmed by Saturation-Transfer Difference (STD) NMR, were low to no STD was found for either of the trisaccharides. These findings are in line with the results from Chapter 2, where the length of the CP8 oligosaccharides was found to be important for antibody recognition, and the binding epitope was found to consist of at least 3 RUs.

For the CP5 trisaccharide frameshifts a different picture emerged. Only **CP5-II** was assessed together with **CP5-I**, as **CP5-III** showed *O*-acetyl migration, leading to a non-natural trisaccharide. The two trisaccharides together with the hexa- and nonasaccharide from Chapter 3 were evaluated for binding to a rat anti-CP5 mAb using competitive surface plasmon resonance (SPR). To this end, the natural CP5 polysaccharide (CP5 PS) was immobilized on the SA-chip. A large difference in binding was discovered between the **CP5-II** trisaccharide and the **CP5-I** fragment as shown in Figure 2. No significant inhibition for **CP5-I** was observed, but **CP5-II** was found to inhibit binding of the rat anti-CP5 mAb at low concentration with an IC50 value close to the IC50 value obtained for the hexasaccharide, although the latter bound slightly better. In 2012 Adamo and co-workers tested **CP5-I** for its ability to induce an antibody response, but no to minimal binding levels were found. They concluded that longer fragments were needed for a model vaccine candidate,[6] however the findings in this Chapter indicate that the LF-DM-DF trisaccharide **CP5-II** could be used as a minimal epitope in future conjugate vaccine design, as it seems to hold the minimal binding epitope for antibody binding.

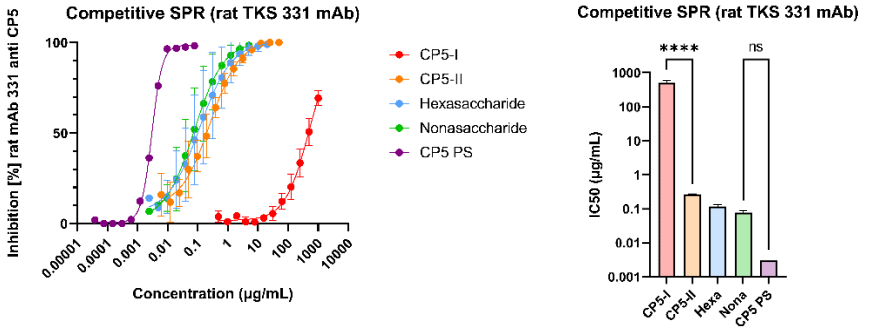


Figure 2: Competitive SPR results using the synthetic oligosaccharides and rat TKS 331 mAb showed that **CP5-I** is barely recognized while the recognition of **CP5-II** is similar to the hexasaccharide and nonasaccharide. **** Identifies a significant difference; ns identifies no significant difference.

Conclusion

In this Chapter, the frameshifted trisaccharides of CP5 and CP8 have been synthesized. The syntheses were found to be more complicated than the syntheses used in the previous chapters to access the other repeating units, as lower yields and poorer anomeric selectivity were obtained. For CP8, two frameshifted trisaccharides were synthesized and tested for their ability to recognize mAb and pAb. Both trisaccharides failed to show binding indicating that longer fragments of CP8 are necessary for good interaction. Also, two CP5 frameshift trisaccharides were generated, but only one was useful for biological evaluation as *O*-acetyl migration from the C''-3-*O*-acetyl to the C''-4-OH occurred in the **CP5-III** trisaccharide. Interestingly, the generated **CP5-II** frameshift showed good binding of the rat-anti-CP5 mAb, with binding levels being comparable to the hexasaccharide.

Overall, this Chapter has shown that in the search for an optimal minimal epitope, it may be required to generate longer oligosaccharides encompassing multiple RUs. At the same time, scanning different frameshift RUs may reveal shorter oligosaccharides to be adequate antigens. It is difficult to predict upfront what the optimal synthetic antigen will be for any given bacterial polysaccharide. Binding studies using systematic sets of oligosaccharides, differing in length and nature of the RU, in combination with structural studies such as reported in the preceding chapters are imperative to deepen the insights into which structural features are most important in shaping oligosaccharide-antibody interactions.

Acknowledgement

Filippo Carboni and Linda Del Bino from GSK vaccines are acknowledged for their help with the SPR-experiments. Luca Unione from CIC BioGune is acknowledged for his contribution to the conformational analysis (see supporting information).

Conflict of interest: Kitt Østerlid has participated in a post graduate studentship program at GSK. This work was sponsored by GlaxoSmithKline Biologicals SA.

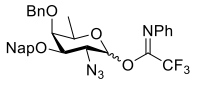
Experimental

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). ^{1}H 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 (^{1}H-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.

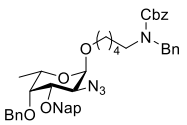
CP8-II: DF–DM–LF

2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphtylmethyl)-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-fucopyranoside (1)



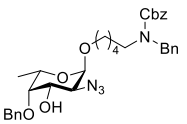
11 (300 mg, 0.72 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (4 mL, 0.2 M). K_2CO_3 (200 mg, 1.44 mmol, 2 equiv.) and $ClC(=NPh)CF_3$ (0.24 mL, 1.44 mmol, 2 equiv.) were added and the mixture was stirred under N_2 for 18 h until TLC analysis (pentane/EtOAc, 7:3) showed full conversion of starting material. The mixture was filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 99:1 → 85:15) to give **1** in 98% yield (416.7 mg, 0.70 mmol) **^{1}H NMR (400 MHz; CD_3CN)** δ : 7.96 – 7.78 (4H, m), 7.69 – 7.05 (11H, m), 6.91 – 6.81 (2H, m), 6.30 (1H, s), 5.07 – 4.78 (3H, m), 4.65 (1H, dd, J = 11.1, 7.2 Hz), 4.16 – 3.70 (4H, m), 1.98 (3H, s). **^{13}C NMR (100.65 MHz; CD_3CN)** δ : 139.69, 139.66, 136.56, 134.25, 134.21, 134.00, 130.09, 129.88, 129.85, 129.26, 129.08, 128.81, 128.68, 128.62, 127.74, 127.64, 127.28, 127.13, 127.08, 125.46, 122.06, 120.12, 118.30, 81.28, 78.13, 76.71, 75.49, 72.81, 72.64, 72.40, 70.21, 63.44, 59.82, 16.98, 16.89.

5-(Benzyl(benzyloxycarbonyl)amino)pentyl 2-azido-2-deoxy-4-*O*-benzyl-3-*O*-(2-naphtylmethyl)-α-L-fucopyranoside (13)



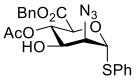
Donor **3** (59 mg, 0.10 mmol, 1 equiv.) and acceptor **12** (42 mg, 0.13 mmol, 1.3 equiv.) were co-evaporated with toluene (x3) and dissolved in dry DCM/Et_2O (1 mL, 1:1, 0.1 M). Triphenylphosphine oxide (169 mg, 0.6 mmol, 6 equiv.) was added and the mixture was stirred with 3 Å molecular sieve under argon for 1 h before TMSI (14 µL, 0.10 mmol, 1 equiv.) was added. The mixture was stirred for 22 h at rt until TLC analysis (pentane/EtOAc, 8:2) showed full conversion of the donor. The reaction was quenched with Et_3N, dissolved in EtOAc, washed with $Na_2S_2O_3$ (sat. aq.; 1x), $NaHCO_3$ (sat. aq.; 1x) and brine (1x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 9:1 → 8:2) to give **13** in 90% yield and with α/β = 70:30 (65.3 mg, 0.09 mmol). NMR data are reported for the pure α-isomer. **^{1}H NMR (400 MHz; $CDCl_3$)** δ : 7.88 – 7.78 (m, 4H), 7.56 – 7.45 (m, 3H), 7.39 – 7.24 (m, 15H), 5.18 (2H, d, J = 13.3 Hz, Ar-CH_2), 4.96 (1H, d, J = 11.5 Hz, Ar-CH_2), 4.88 (3H, m, H-1, Ar-CH_2), 4.66 (1H, d, J = 11.5 Hz, Ar-CH_2), 4.50 (2H, d, J = 8.8 Hz, Ar-CH_2), 3.99 (1H, q, J = 12.6, 11.6 Hz, H-3), 3.87 (2H, dd, J = 10.7, 3.6 Hz, H-2, H-5), 3.74 (1H, s, H-4), 3.68 – 3.14 (4H, m, CH_2-Linker), 1.66 – 1.47 (6H, m, CH_2-Linker), 1.17 (3H, d, J = 6.5 Hz, CH_3). **^{1}H NMR (400 MHz; $CDCl_3$)** δ : 138.39 (Ar-C_q), 137.83 (Ar-C_q), 135.09 (Ar-C_q), 133.42 (Ar-C_q), 133.18 (Ar-C_q), 128.67 (Ar-*C*), 128.58 (Ar-*C*), 128.46 (Ar-*C*), 128.42 (Ar-*C*), 128.09 (Ar-*C*), 127.94 (Ar-*C*), 127.88 (Ar-*C*), 127.85 (Ar-*C*), 127.41 (Ar-*C*), 126.65 (Ar-*C*), 126.31 (Ar-*C*), 126.14 (Ar-*C*), 125.83 (Ar-*C*), 97.37 (C-1), 77.89 (C-3), 76.34 (CH_2), 75.67 (CH_2), 72.52 (CH_2), 68.16, 67.29 (C-4), 66.69 (C-2, C-5), 59.82 (C-2, C-5), 29.84 (*C*H_2-Linker), 16.91(CH_3). **HRMS**: $[M+Na]^+$ calculated for $C_{44}H_{46}N_4O_6Na$: 751.34715; found 751.34661

5-(Benzyl(benzyloxycarbonyl)amino)pentyl 2-azido-2-deoxy-4-*O*-benzyl-α-L-fucopyranoside (14)



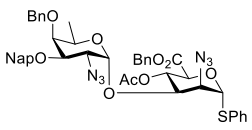
13 (91 mg, 0.14 mmol, 1 equiv.) was dissolved in DCM (1.3 mL , 0.1 M). DDQ (64 mg, 0.28 mmol, 2 equiv.) and H_2O (0.1 mL) were added, and the mixture was stirred for 3 h until TLC analysis (pentane/EtOAc, 8:2) showed full conversion of starting material. The reaction was quenched with $Na_2S_2O_3$ (sat. aq) and extracted with EtOAc (3x). The combined organic layers were washed with $NaHCO_3$ (sat. aq.; 4x) and brine (1x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 9:1 → 7:3) to give **14** in 50% yield (39 mg, 0.07 mmol). **^{1}H NMR (400 MHz; $CDCl_3$)** δ : 7.39 – 7.24 (m, 15H), 4.96 (1H, d, J = 11.5 Hz, CH_2), 4.88 (3H, m, H-1, Ar-CH_2), 4.66 (1H, d, J = 11.5 Hz, Ar-CH_2), 4.51 (2H, d, J = 8.8 Hz, Ar-CH_2), 3.99 (1H, q, J = 12.6, 11.6 Hz, H-3), 3.87 (2H, dd, J = 10.7, 3.6 Hz, H-2, H-5), 3.74 (1H, s, H-4), 3.68 – 3.14 (4H, m, CH_2-Linker), 1.66 – 1.47 (6H, m, CH_2-Linker), 1.17 (3H, d, J = 6.5 Hz, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 137.95 (Ar-C_q), 128.74 (Ar-*C*), 128.62 (Ar-*C*), 128.54 (Ar-*C*), 128.24 (Ar-*C*), 128.01 (Ar-*C*), 127.89 (Ar-*C*), 127.37 (Ar-*C*), 127.28 (Ar-*C*), 98.22 (C-1), 80.22 (C-4), 76.21 (Ar-*C*H_2), 68.78 (C-3), 68.15 (Ar-*C*H_2), 67.24 (Ar-*C*H_2), 66.52 (C-5), 60.98 (C-2), 50.57 (*C*H_2-Linker), 50.29 (*C*H_2-Linker), 47.16 (*C*H_2-Linker), 46.21 (*C*H_2-Linker), 29.18 (*C*H_2-Linker), 28.03 (*C*H_2-Linker), 27.94 (*C*H_2-Linker), 27.52 (*C*H_2-Linker), 23.46 (*C*H_2-Linker), 16.87 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{33}H_{40}N_4O_6Na$: 611.28455; found 611.28401

Benzyl 4-*O*-acetyl-2-azido-2-deoxy-1-thio-α-D-mannopyranosiduronate (2)



15 (500 mg, 0.86 mmol, 1 equiv.) was dissolved in DCM (9.50 mL). DDQ (778 mg, 3.43 mmol, 4 equiv.) and H_2O (0.5 mL) were added, and the mixture was stirred for 6 h until TLC analysis (pentane/EtOAc, 8:2) showed full conversion of starting material. The reaction was quenched with $Na_2S_2O_3$ (sat. aq.) and extracted with EtOAc (x3). The combined organic layers were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 95:5 → 80:20) to give **2** in 65% yield (248 mg, 0.56 mmol). **^{1}H NMR (400 MHz; $CDCl_3$)** δ : 7.84 – 7.79 (3H, m, Ar-*H*), 7.76 (1H, s, Ar-*H*), 7.62 – 7.58 (2H, m, Ar-*H*), 7.50 – 7.46 (2H, m, Ar-*H*), 7.43 (1H, dd, *J* = 8.5, 1.7 Hz, Ar-*H*), 7.27 – 7.22 (6H, m, Ar-*H*), 7.13 – 7.09 (2H, m, Ar-*H*), 5.78 (1H, d, *J* = 9.3 Hz, H-1), 5.62 (1H, dd, *J* = 4.8, 2.9 Hz, H-4), 5.02 (1H, d, *J* = 12.1 Hz, Ar-CH_2), 4.83 (1H, d, *J* = 12.2 Hz, Ar-CH_2), 4.68 (2H, s, Ar-CH_2), 4.63 (1H, d, *J* = 2.9 Hz, H-5), 3.98 (1H, dd, *J* = 4.7, 2.9 Hz, H-3), 3.45 (1H, dd, *J* = 9.4, 2.9 Hz, H-2), 2.03 (3H, s, COCH_3). **^{13}C NMR (100.65 MHz; $CDCl_3$)** δ : 169.75 (C=O), 167.88 (C=O), 134.86 (Ar-C_q), 133.98 (Ar-C_q), 133.28 (Ar-C_q), 133.23 (Ar-C_q), 132.47 (Ar-*C*), 131.93 (Ar-C_q), 129.01 (Ar-*C*), 128.67 (Ar-*C*), 128.60 (Ar-*C*), 128.58 (Ar-*C*), 128.49 (Ar-*C*), 128.13 (Ar-*C*), 128.03 (Ar-*C*), 127.84 (Ar-*C*), 127.38 (Ar-*C*), 126.40 (Ar-*C*), 126.36 (Ar-*C*), 125.95 (Ar-*C*), 81.06 (C-1), 74.64 (C-3), 73.69 (C-5), 73.04 (Ar-CH_2), 68.50 (C-4), 67.56 (Ar-CH_2), 57.93 (CH-2), 21.02 (CO*C*H_3). **HRMS**: $[M+Na]^+$ calculated for $C_{21}H_{21}N_3O_6Na$: 466.10488; found 466.10433

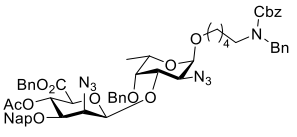
Phenyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphtylmethyl)-α-D-fucopyranosyl-(1→3)-benzyl(4-*O*-acetyl-2-azido-2-deoxy-1-thio)-α-D-mannopyranosiduronate (16)



Acceptor **2** (120 mg, 0.27 mmol, 1 equiv.) and donor **1** (245 mg, 0.41 mmol, 1.5 equiv.) were co-evaporated with toluene (3x), dissolved in dry DCM (3 mL), added 3 Å molecular sieve and stirred under argon for 30 min. TBSOTf (12 µL, 0.05 mmol, 0.2 equiv.) was added at rt and the mixture was stirred at rt under argon for 30 min until TLC analysis (pentane/EtOAc, 8:2) showed full conversion of the acceptor. The reaction was quenched with Et_3N, dissolved in EtOAc, washed with $NaHCO_3$ (sat. aq.; 1x) and brine (1x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 9:1 → 8:2) to give **16** in 84% yield (191.1 mg, 0.23 mmol). **^{1}H NMR (400 MHz; $CDCl_3$)** δ : 7.88 – 7.81 (5H, m, Ar-*H*), 7.55 – 7.46 (6H, m, Ar-*H*), 7.40 – 7.24 (11H, m, Ar-*H*), 5.66 (1H, d, *J* = 4.1 Hz, H-1'), 5.53 (1H, t, *J* = 7.8 Hz, H-4'), 5.24 – 5.13 (2H, m, Ar-CH_2), 5.07 (1H, d, *J* = 3.6 Hz, H-1), 4.94 (1H, d, *J* = 11.4 Hz, Ar-CH_2), 4.90 – 4.85 (2H, m, Ar-CH_2), 4.72 (1H, d, *J* = 7.6 Hz, H-3), 4.62 (1H, d, *J* = 11.5 Hz, Ar-CH_2), 4.17 (1H, dd, *J* = 7.9, 3.5 Hz, H-5'), 4.08 – 3.99 (3H, m, H-2', H-3, H-5), 3.94 (1H, dd, *J* = 10.8, 3.6 Hz, H-2), 3.72 (1H, m, H-4), 1.93 (3H, s, COCH_3), 1.20 (3H, d, *J* = 6.5 Hz, CH_3). **^{13}C NMR (100.65 MHz; $CDCl_3$)** δ : 170.04 (C=O), 167.30 (C=O), 138.69 (Ar-C_q), 135.98 (Ar-C_q), 134.94 (Ar-C_q), 133.40 (Ar-C_q), 133.19 (Ar-C_q), 129.36 (Ar-C_q), 128.74 (Ar-*C*), 128.66 (Ar-*C*), 128.45 (Ar-*C*), 128.35 (Ar-*C*), 128.31 (Ar-*C*), 128.15 (Ar-*C*), 127.94 (Ar-*C*), 127.81 (Ar-*C*), 126.96 (Ar-*C*), 126.31 (Ar-*C*), 126.18 (Ar-*C*), 125.97 (Ar-*C*), 99.69 (C-1'), 84.68 (C-1) , 77.50 (C-2', C-3, C-5), 76.09 (C-4, C-5'), 75.06 (Ar-*C*H_2), 72.73 (Ar-*C*H_2), 71.55 (C-3'), 68.10 (C-2', C-3, C-5), 67.90 (C-4'), 67.87 (Ar-*C*H_2), 59.49 (C-2, C-2', C-3, C-5), 20.71

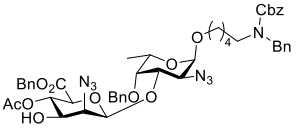
(COCH_3), 16.96 (CH_3). **HRMS**: $[M+Na]^+$ calculated for $C_{45}H_{44}N_6O_9Na$: 867.27036; found 867.27827

5-(Benzyl(benzyloxycarbonyl)amino)pentyl benzyl(4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl))-β-D-mannopyranosiduronsyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranoside (18)



Donor **5** (106 mg, 0.16 mmol, 2 equiv.) and acceptor **14** (50 mg, 0.08 mmol, 1 equiv.) were co-evaporated with toluene (3x), dissolved in dry DCM (1 mL, 0.1 M), added 3 Å molecular sieve and stirred under argon for 30 min. The mixture was cooled to 0°C and added TBSOTf (3.7 µL, 0.016 mmol, 0.2 equiv.). The mixture was stirred for 4 h until TLC analysis (pentane/EtOAc, 8:2) showed full conversion of the acceptor. The reaction was quenched with Et_3N at 0°C, warmed to rt, dissolved in EtOAc, washed with $NaHCO_3$ (sat. aq.; 1x) and brine (1x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 9:1 → 6:4) to give **18** in 53% yield (45.3 mg, 0.043 mmol). **^{1}H NMR (400 MHz; $CDCl_3$)** δ : 7.85 – 7.75 (4H, m, Ar-*H*), 7.75 – 7.71 (1H, m, Ar-*H*), 7.51 – 7.40 (4H, m, Ar-*H*), 7.37 – 7.11 (18H, m, Ar-*H*), 5.44 (1H, t, *J* = 9.4 Hz, H-4), 5.15 (3H, d, *J* = 11.6 Hz, Ar-CH_2), 5.08 (2H, d, *J* = 3.9 Hz, Ar-CH_2), 4.86 (1H, s, H-1'), 4.82 – 4.69 (3H, m, Ar-CH_2), 4.59 (2H, d, *J* = 3.6 Hz, H-5), 4.54 (1H, s, H-1'), 4.47 (2H, d, *J* = 7.6 Hz, Ar-CH_2), 4.26 (1H, d, *J* = 11.1 Hz, H-3'), 3.89 – 3.77 (2H, m, H-3', H-3), 3.70 – 3.66 (6H, m, H-2', H-5', H-2, CH_2-Linker), 3.48 (3H, t, *J* = 6.7 Hz, H-4', CH_2-Linker), 3.20 (2H, m, CH_2-Linker), 1.83 (3H, s, COCH_3), 1.62 – 1.47 (6H, m, CH_2-Linker), 1.29 – 1.21 (3H, m, CH_3). **^{13}C NMR (100.65 MHz; $CDCl_3$)** δ : 169.89 (C=O), 168.69 (C=O), 166.50 (C=O), 134.81 (Ar-C_q), 132.83 (Ar-C_q), 128.68 (Ar-*C*), 128.61 (Ar-*C*), 128.57 (Ar-*C*), 128.49 (Ar-*C*), 128.28 (Ar-*C*), 128.07 (Ar-*C*), 127.94 (Ar-*C*), 126.85 (Ar-*C*), 126.57 (Ar-*C*), 126.42 (Ar-*C*), 125.66 (Ar-*C*), 99.17 (C-1'), 96.97 (C-1), 75.75 (CH_2), 73.84 (CH-3), 72.63 (CH_2), 72.23 (CH_2), 71.43 (CH_2), 70.64 (CH_2), 70.32 (CH_2), 68.76 (CH_2), 68.14 (C-4), 67.70 (CH_2), 67.16 (C-4'), 66.43 (C-3'), 62.02 (CH_2), 61.47 (C-2, C-5'), 58.27 (C-2', C-5), 31.78 (CH_2-Linker), 29.84 (CH_2-Linker), 27.44 (CH_2-Linker), 20.43 (COCH_3), 18.96 (CH_2-Linker), 16.60 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{59}H_{63}N_7O_{12}Na$: 775.27036; found 775.26981

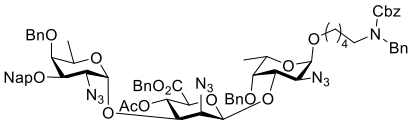
5-(Benzyl(benzyloxycarbonyl)amino)pentyl benzyl(4-*O*-acetyl-2-azido-2-deoxy)-β-D-mannopyranosiduronsyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranoside (19)



18 (58 mg, 0.0546 mmol) was dissolved in DCM/H_2O (20:1, 1 mL, 0.05 M). DDQ (25 mg, 0.109 mmol, 2 equiv.) was added and the mixture was stirred for 4 h at rt until TLC analysis (pentane/EtOAc, 6:4) showed full conversion of starting material. The reaction was neutralized with $Na_2S_2O_3$ (sat. aq.) and extracted with EtOAc (x3). The combined organic layers were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 8:2 → 5:5) to give **19** in 53% yield (27 mg, 0.0289 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.41 – 7.27 (m, 15H, Ar-*H*), 7.26 – 7.13 (m, 3H, Ar-*H*), 5.22 – 5.04 (m, 5H, Ar-CH_2, H-4'), 4.88 (d, *J* = 5.1 Hz, 1H, H-1), 4.71 (s, 2H, Ar-CH_2), 4.67 (s, 1H, H-1'), 4.53 – 4.44 (m, 2H, CH_2-Linker), 4.37 – 4.26 (m, 1H, H-

3), 3.92 (q, *J* = 9.1, 7.8 Hz, 1H, H-5), 3.85 (d, *J* = 9.7 Hz, 1H, H-5'), 3.71 – 3.68 (m, 1H, H-4), 3.62 – 3.55 (m, 4H, H-2, H-3', H-2', CH_2-Linker), 3.45 – 3.34 (m, 1H, CH_2-Linker), 3.34 – 3.11 (m, 2H, CH_2-Linker), 2.69 (s, 1H, OH), 1.86 (s, 3H, COCH_3), 1.65 – 1.46 (m, 6H, CH_2-Linker), 1.46 – 1.21 (m, 12H, H-6, CH_2-Linker). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 170.62 (C=O), 166.40 (C=O), 138.00 (Ar-C_q), 135.16 (Ar-C_q), 128.67 (Ar-*C*), 128.57 (Ar-*C*), 128.33 (Ar-*C*), 128.26 (Ar-*C*), 128.18 (Ar-*C*), 128.06 (Ar-*C*), 127.92 (Ar-*C*), 127.31 (Ar-*C*), 98.69 (C-1), 97.76 (C-1'), 77.48 (C-4), 76.84 (C-3), 75.12 (Ar-*C*H$_2$), 73.71 (C-5'), 71.17 (C-3'), 69.79 (C-4'), 68.32 (*C*H$_2$-Linker), 67.65 (Ar-*C*H$_2$), 67.27 (Ar-*C*H$_2$), 66.43 (C-5), 64.42 (C-2/C-2'), 58.09 (C-2/C-2'), 50.36 (*C*H$_2$-Linker), 47.51 (*C*H$_2$-Linker), 46.27 (*C*H$_2$-Linker), 31.75 (*C*H$_2$-Linker), 29.82 (*C*H$_2$-Linker), 29.16 (*C*H$_2$-Linker), 20.66 (CO*C*H$_3$), 16.95 (C-6). **HRMS:** [M+Na]$^+$ calculated for $C_{48}H_{55}N_7O_{12}Na$: 944.38064; found 944.38009

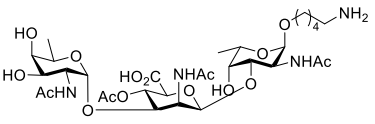
5-(Benzyl(benzyloxycarbonyl)amino)pentyl 2-azido -4-*O*-benzyl-2-deoxy -3-*O*-(2-naphtylmethyl)- α-D-fucopyranosyl-(1→3)-benzyl(4-*O*-acetyl-2-azido-2-deoxy)-β-D-mannopyranosiduronsyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranoside (17)



Donor **1** (26 mg, 0.0446 mmol, 1.5 equiv.) and Acceptor **19** (27 mg, 0.0297 mmol, 1 equiv.) were co-evaporated with toluene (3x), dissolved in dry DCM (1 mL, 0.03 M), added 3 Å molecular sieve and stirred under argon for 30 min. The mixture was added TBSOTf (1.3 µL, 0.0059 mmol, 0.2 equiv.) at rt and stirred for 25 min until TLC analysis (pentane/EtOAc, 7:3) showed full conversion of the acceptor. The reaction was quenched with Et_3N dissolved in EtOAc, washed with $NaHCO_3$ (sat. aq.; 1x) and brine (1x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 8:2 → 6:4) to give **17** in 57% yield (22.5 mg, 0.017 mmol). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.88 – 7.80 (m, 4H, Ar-*H*), 7.55 – 7.46 (m, 3H, Ar-*H*), 7.39 – 7.27 (m, 22H, Ar-*H*), 7.17 (d, *J* = 7.3 Hz, 1H, Ar-*H*), 5.38 (t, *J* = 9.8 Hz, 1H, H-4'), 5.17 (d, *J* = 12.8 Hz, 2H, Ar-CH_2), 5.11 (d, *J* = 4.2 Hz, 2H, Ar-CH_2), 4.96 – 4.87 (m, 4H, Ar-CH_2, H-1''), 4.92 – 4.82 (m, 4H, Ar-CH_2, H-1), 4.71 (s, 2H, Ar-CH_2), 4.60 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.57 (d, *J* = 1.5 Hz, 1H, H-1'), 4.49 (d, *J* = 7.9 Hz, 2H, CH_2-Linker), 4.34 (m, 1H, H-3), 4.10 (q, *J* = 6.3 Hz, 1H, H-5''), 4.03 (dd, *J* = 10.8, 2.6 Hz, 1H, H-3), 3.98 – 3.87 (m, 2H, H-2'', H-5), 3.87 – 3.78 (m, 2H, H-5', H-2'), 3.72 (dd, *J* = 2.7, 1.2 Hz, 1H, H-4''), 3.71 – 3.67 (m, 1H, H-4), 3.65 – 3.56 (m, 3H, H-3', H-2, CH_2-Linker), 3.49 – 3.32 (m, 1H, CH_2-Linker), 3.32 – 3.11 (m, 2H, CH_2-Linker), 1.88 (s, 3H, COCH_3), 1.60 – 1.45 (m, 4H, CH$_2$-Linker), 1.29 – 1.23 (m, 7H, CH$_2$-Linker, H-6), 1.12 (d, *J* = 6.5 Hz, 3H, H-6''). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 169.36 (C=O), 166.38 (C=O), 138.12 (Ar-C_q), 137.96 (Ar-C_q), 135.06 (Ar-C_q), 133.42 (Ar-C_q), 133.19 (Ar-C_q), 128.73 (Ar-*C*), 128.67 (Ar-*C*), 128.65 (Ar-*C*), 128.63 (Ar-*C*), 128.58 (Ar-*C*), 128.52 (Ar-*C*), 128.46 (Ar-*C*), 128.26 (Ar-*C*), 128.18 (Ar-*C*), 128.06 (Ar-*C*), 127.99 (Ar-*C*), 127.93 (Ar-*C*), 127.81 (Ar-*C*), 127.33 (Ar-*C*), 126.90 (Ar-*C*), 126.31 (Ar-*C*), 126.17 (Ar-*C*), 125.95 (Ar-*C*), 101.12 (C-1''), 98.70 (C-1), 97.03 (C-1'), 79.32 (C-3'), 77.78 (C-3''), 77.16 (C-4), 76.14 (C-4''), 75.65 (Ar-*C*H$_2$), 75.17 (Ar-*C*H$_2$), 74.92 (C-3), 73.87 (C-5'), 72.79 (Ar-*C*H$_2$), 68.33 (Ar-*C*H$_2$), 68.18 (C-5''), 67.76 (Ar-*C*H$_2$), 67.34 (C-4'), 67.26 (Ar-*C*H$_2$), 66.48 (C-5), 63.80 (C-2'), 59.84 (C-2''), 58.09 (C-2), 50.62 (*C*H$_2$-Linker), 50.37 (*C*H$_2$-Linker), 47.27 (*C*H$_2$-Linker), 46.23 (*C*H$_2$-Linker), 29.83 (*C*H$_2$-Linker), 29.16 (*C*H$_2$-Linker), 20.60 (CO*C*H$_3$), 17.02

(C-6''/C-6), 16.98 (C-6''/C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{72}H_{78}N_{10}O_{15}Na$: 1345.55458; found 1345.55403

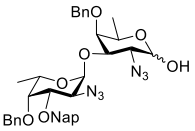
5-aminopentyl 2-*N*-acetamide-2-deoxy-α-D-fucopyranosyl-(1→3)-4-*O*-acetyl-2-*N*-acetamide-2-deoxy-β-D-mannopyranosiduronsyl-(1→3)-2-*N*-acetamide-2-deoxy-α-L-fucopyranoside (CP8-II)



17 (17 mg, 0.0128 mmol) was dissolved in THF (3 mL) and added zinc powder (252 mg, 3.85 mmol, 300 equiv.), AcOH (1 mL) and Ac_2O (0.5 mL). The mixture was heated to 50 °C and stirred for 18 h until TLC analysis (DCM/MeOH, 95:5) showed full conversion of the starting material. The solution was cooled to rt, filtered over a path of Celite and concentrated *in vacuo*. The crude product was purified by column chromatography (DCM/MeOH, 98:1 → 95:5). The product (18 mg, 0.0135 mmol) was dissolved in *t*-Bu-OH (2.5 mL). AcOH (0.1 mL in 100 mL MilliQ, 1 mL) was added and the mixture was birched under argon for 20 min. $Pd(OH)_2$ (catalytic amount) was added and the mixture was birched under argon for 5 min, then with H_2 for 2 min, before to be stirred for 3 days. The mixture was birched with argon for 20 min, filtered over a Whatman filter and lyophilized. Purification by a HW40 column with NH_4OAc followed by lyophilization gave **CP8-II** in 77% yield (7.6 mg, 0.0104 mmol). **^{1}H NMR (600 MHz, D_2O)** δ 5.18 (t, *J* = 9.9 Hz, 1H, H'-4), 5.00 (d, *J* = 3.9 Hz, 1H, H''-1), 4.98 (d, *J* = 1.5 Hz, 1H, H'-1), 4.89 (d, *J* = 1.8 Hz, 1H, H-1), 4.51 (dd, *J* = 4.5, 1.4 Hz, 1H, H'-2), 4.26 (q, *J* = 7.2, 6.7 Hz, 1H, H-5), 4.15 (d, *J* = 4.5 Hz, 1H, H-2), 4.15 – 4.11 (m, 2H, H-4, H'-3), 4.08 – 4.05 (m, 1H, H''5), 4.06 – 4.04 (m, 1H, H''-2), 4.03 – 4.00 (m, 1H,H-3), 3.81 (d, *J* = 10.1 Hz, 1H, H'-5), 3.77 (dd, *J* = 3.3, 1.1 Hz, 1H, H''-4), 3.75 – 3.71 (m, 1H, H''-3), 3.69 (dt, *J* = 10.2, 6.3 Hz, 1H, CH_2-Linker), 3.45 (dt, *J* = 10.1, 6.2 Hz, 1H, CH_2-Linker), 2.99 (dd, *J* = 8.6, 6.8 Hz, 2H, CH_2-Linker), 2.08 (s, 3H, COCH_3), 2.07 (s, 6H, COCH_3), 2.01 (s, 3H, COCH_3), 1.72 – 1.58 (m, 4H, CH_2-Linker), 1.48 – 1.36 (m, 2H, CH_2-Linker), 1.25 (dd, *J* = 6.7, 3.0 Hz, 6H, H-6, H''-6). **^{13}C NMR (151 MHz, D_2O)** δ 175.97 (C=O), 175.48 (C=O), 175.27 (C=O), 175.03 (C=O), 173.46 (C=O), 99.49 (C''-1), 97.78 (C'-1), 95.60 (C-1), 75.08 (C-4), 74.98 (C'-5), 73.88 (C'-3), 71.95 (C''-4), 71.17 (C'-4), 68.77 (C-3), 68.76 (CH_2-Linker), 68.40 (C''-3), 67.93 (C-5), 67.30 (C''-5), 53.44 (C'-2), 50.35 (C''-2), 48.91 (C-2), 40.28 (CH_2-Linker), 28.83 (CH_2-Linker), 27.38 (CH_2-Linker), 23.24 (CH_2-Linker), 22.96 (COCH_3), 22.92 (COCH_3), 22.67 (COCH_3), 21.20 (COCH_3), 16.42 (C''-6/ C-6), 16.30 (C''-6/ C-6). **HRMS**: $[M+H]^+$ calculated for $C_{31}H_{52}N_4O_{16}H$: 737.34566; found 737.34510

CP8-III: LF–DF–DM

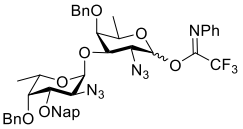
2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α/β-D-fucopyranose (21)



20 (279 mg, 0.304 mmol) was dissolved in dry THF (2.5 mL, 0.1 M) and cooled to 0°C. AcOH (26 µL, 0.456 mmol, 1.5 equiv.) and TBAF (1M in THF, 0.46 mL, 0.456 mmol, 1.5 equiv.) were added subsequently and the mixture was stirred for 19 h until TLC analysis (pentane/EtOAc, 8:2) showed full conversion of starting material. The reaction was

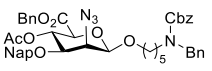
quenched with NH_4Cl (sat. aq.). The mixture was extracted with EtOAc (x3), and the combined organic phases were wash with H_2O (x3), brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 9:1 → 7:3) to give **21** in 65% yield (135 mg, 0.198 mmol). **^{1}H NMR (400 MHz; $CDCl_3$)** δ : 7.89 – 7.78 (8H, m), 7.76 – 7.69 (1H, m), 7.57 – 7.51 (3H, m), 7.50 – 7.42 (4H, m), 7.38 – 7.27 (21H, m), 5.36 (1H, s), 5.30 (1H, d, J = 3.3 Hz), 5.24 (1H, d, J = 3.5 Hz), 4.97 (2H, d, J = 11.5 Hz), 4.93 – 4.73 (6H, m), 4.67 – 4.60 (4H, m), 4.53 – 4.47 (1H, m), 4.19 – 4.12 (1H, m), 4.09 (1H, dd, J = 10.6, 2.7 Hz), 4.04 – 3.86 (8H, m), 3.84 – 3.76 (2H, m), 3.61 – 3.47 (5H, m), 3.40 (1H, dd, J = 2.8, 1.0 Hz), 3.25 (1H, s), 1.12 (6H, d, J = 6.4 Hz). **^{13}C NMR (100.65 MHz; $CDCl_3$)** δ : 138.35, 138.17, 138.13, 135.19, 135.13, 133.34, 133.16, 128.53, 128.50, 128.48, 128.44, 128.05, 128.03, 127.97, 127.94, 127.92, 127.82, 127.80, 126.81, 126.78, 126.34, 126.31, 126.19, 126.17, 125.90, 125.88, 99.96, 99.80, 96.87, 92.44, 79.86, 78.88, 78.82, 77.52, 76.41, 76.35, 75.43, 75.39, 75.09, 75.05, 72.75, 72.70, 71.09, 67.75, 67.63, 67.10, 65.39, 61.22, 59.98, 59.71, 17.01, 16.85. **HRMS**: $[M+Na]^+$ calculated for $C_{37}H_{40}N_6O_7Na$: 703.28562; found 703.28507

2-Azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-fucopyranose (22)



21 (135 mg, 0.198 mmol, 1 equiv.) was co-evaporated with toluene (x3) and dissolved in dry acetone (1 mL, 0.2 M). K_2CO_3 (54 mg, 0.396 mmol, 2 equiv.) and ClC(=NPh)CF_3 (64 µL, 0.396 mmol, 2 equiv.) were added and the mixture was stirred under N_2 for 18 h until TLC analysis (pentane/EtOAc, 8:2) showed full conversion of starting material. The mixture was filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 99:1 → 85:15) to give **22** in 70% yield (116 mg, 0.136 mmol) **^{1}H NMR (400 MHz; CD_3CN)** δ : 7.93 – 7.83 (4H, m), 7.79 – 7.72 (1H, m), 7.65 – 7.53 (1H, m), 7.39 – 7.27 (11H, m), 7.19 – 7.09 (1H, m), 6.88 (2H, d, J = 7.7 Hz), 5.25 (1H, d, J = 3.7 Hz), 4.91 (2H, dd, J = 11.5, 9.1 Hz), 4.86 – 4.73 (2H, m), 4.62 (2H, dd, J = 17.6, 11.1 Hz), 4.01 – 3.81 (4H, m), 3.77 (1H, dd, J = 10.9, 3.7 Hz), 3.63 (1H, d, J = 24.0 Hz), 2.16 (1H, s), 1.97 – 1.93 (2H, m), 1.24 – 1.14 (8H, m). **^{13}C NMR (100.65 MHz; CD_3CN)** δ : 139.83, 139.68, 136.74, 134.69, 134.23, 129.86, 129.35, 129.24, 129.09, 129.03, 128.79, 128.62, 128.61, 127.49, 127.23, 127.07, 127.03, 125.48, 120.09, 118.30, 100.69, 79.42, 78.45, 77.49, 76.49, 75.58, 73.24, 72.81, 72.31, 71.66, 68.48, 67.84, 60.42, 60.40, 17.01, 16.72.

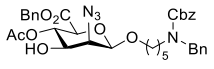
5-(Benzyl(benzyloxycarbonyl)amino)pentyl benzyl(4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphtylmethyl))-β-D-mannopyranoside (23)



Donor **5** (472 mg, 0.71 mmol, 1 equiv.) and acceptor **12** (278 mg, 0.93 mmol, 1.3 equiv.) were co-evaporated with toluene (3x), dissolved in dry DCM (2.5 mL, 0.3 M) added 3 Å molecular sieve and stirred under argon for 30 min. The mixture was cooled to -78°C and added TBSOTf (34 µL, 0.15 mmol, 0.2 equiv.). The mixture was stirred for 2 h during which it was allowed to warm to -30°C until TLC analysis (pentane/EtOAc, 7:3) showed full conversion of the donor. The reaction was quenched with Et_3N and warmed to rt. The mixture was dissolved in EtOAc, washed with Na-HCO_3 (sat. aq.; 1x) and brine (1x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 8:2 → 7:3) to give **23**

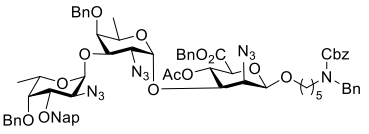
in 24% yield (139 mg, 0.17 mmol). **^{1}H NMR (400 MHz; $CDCl_3$)** δ : 7.86 – 7.79 (3H, m, Ar-*H*), 7.78 – 7.74 (1H, m, Ar-*H*), 7.52 – 7.47 (2H, m, Ar-*H*), 7.46 – 7.43 (1H, m, Ar-*H*), 7.37 – 7.24 (15H, m, Ar-*H*), 5.43 (1H, t, *J* = 9.0 Hz, H-4), 5.21 – 5.02 (2H, m, Ar-CH_2), 4.91 – 4.67 (2H, m, Ar-CH_2), 4.60 – 4.44 (4H, m, CH_2, H-1, H-2), 3.87 (1H, d, *J* = 9.0 Hz, H-3), 3.72 – 3.63 (1H, m, H-5), 3.47 – 3.12 (4H, m, CH_2-Linker), 1.83 (3H, s, COCH_3), 1.66 – 1.45 (4H, m, CH_2-Linker), 1.37 – 1.27 (2H, m, CH_2-Linker). **^{13}C NMR (100.65 MHz; $CDCl_3$)** δ : 169.47 (C=O), 168.24 (C=O), 167.11 (C=O), 137.99 (Ar-C_q), 135.02 (Ar-C_q), 134.88 (Ar-C_q), 134.72 (Ar-C_q), 134.48 (Ar-C_q), 133.20 (Ar-C_q), 128.76 (Ar-*C*), 128.63 (Ar-*C*), 128.58 (Ar-*C*), 128.56 (Ar-*C*), 128.36 (Ar-*C*), 128.05 (Ar-*C*), 128.01 (Ar-*C*), 127.96 (Ar-*C*), 127.91 (Ar-*C*), 127.86 (Ar-*C*), 127.80 (Ar-*C*), 127.41 (Ar-*C*), 127.34 (Ar-*C*), 126.74 (Ar-*C*), 126.47 (Ar-*C*), 126.32 (Ar-*C*), 126.22 (Ar-*C*), 125.65 (Ar-*C*), 125.60 (Ar-*C*), 99.81 (C-1), 76.72 (C-5), 73.08 (C-3), 72.92 (Ar-*C*H_2), 72.16 (Ar-*C*H_2), 70.12 (Ar-*C*H_2), 70.07 (Ar-*C*H_2), 68.54 (C-2), 68.22 (C-4), 67.71 (*C*H_2-Linker), 67.59 (*C*H_2-Linker), 67.24 (*C*H_2-Linker), 50.58 (*C*H_2-Linker), 50.27 (*C*H_2-Linker), 47.12 (*C*H_2-Linker), 46.20 (*C*H_2-Linker), 29.80 (*C*H_2-Linker), 29.12 (*C*H_2-Linker), 27.89 (*C*H_2-Linker), 27.42 (*C*H_2-Linker), 23.13 (*C*H_2-Linker), 20.74 (CO*C*H_3). **HRMS**: $[M+Na]^+$ calculated for $C_{46}H_{48}N_4O_9Na$: 823.33190; found 823.33135

5-(Benzyl(benzyloxycarbonyl)amino)pentyl benzyl(4-*O*-acetyl-2-azido-2-deoxy)-β-D-mannopyranoside (24)



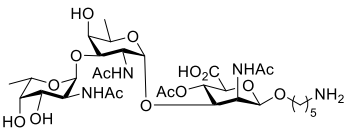
23 (94 mg, 0.117 mmol) was dissolved in DCM (1.1 mL, 0.1 M). DDQ (53 mg, 0.234 mmol, 2 equiv.) and H_2O (50 µL) were added and the mixture was stirred for 3 h until TLC analysis (pentane/EtOAc, 1:1) showed full conversion of the starting material. The reaction was quenched with $Na_2S_2O_3$ (sat. aq.) and extracted with EtOAc (x3). The combined organic layers were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 7:3 → 5:5) to give **24** in 51% yield (39 mg, 0.06 mmol). **^{1}H NMR (400 MHz; $CDCl_3$)** δ : 7.37 – 7.24 (15H, m, Ar-*H*), 5.43 (1H, t, *J* = 9.0 Hz, H-4), 5.21 – 5.02 (3H, m, CH_2), 4.91 – 4.67 (3H, m, CH_2), 4.60 – 4.44 (4H, m, CH_2, H-1, H-2), 3.87 (1H, d, *J* = 9.0 Hz, H-3), 3.72 – 3.63 (1H, m, H-5), 3.47 – 3.12 (4H, m, CH_2-Linker), 1.83 (3H, s, COCH_3), 1.66 – 1.45 (4H, m, CH_2-Linker), 1.37 – 1.27 (2H, m, CH_2-Linker). **^{13}C NMR (100.65 MHz; $CDCl_3$)** δ : 169.47 (C=O), 168.24 (C=O), 167.11 (C=O), 137.99 (Ar-C_q), , 134.88 (Ar-C_q), 134.48 (Ar-C_q), 128.76 (Ar-*C*), 128.63 (Ar-*C*), 128.58 (Ar-*C*), 128.56 (Ar-*C*), 128.36 (Ar-*C*), 128.05 (Ar-*C*), 128.01 (Ar-*C*), 127.96 (Ar-*C*), 127.91 (Ar-*C*), 127.86 (Ar-*C*), 127.80 (Ar-*C*), 127.41 (Ar-*C*), 126.47 (Ar-*C*), 125.65 (Ar-*C*), 125.60 (Ar-*C*), 99.81 (C-1), 76.72 (C-5), 73.08 (C-3), 72.92 (CH_2), 72.16 (CH_2), 70.12 (CH_2), 70.07 (CH_2), 68.54 (C-2), 68.22 (C-4), 67.59 (CH_2), 67.24 (CH_2), 50.58 (*C*H_2-Linker), 47.12 (*C*H_2-Linker), 46.20 (*C*H_2-Linker), 29.80 (*C*H_2-Linker), 29.12 (*C*H_2-Linker), 27.89 (*C*H_2-Linker), 27.42 (*C*H_2-Linker), 23.13 (*C*H_2-Linker), 20.74 (CO*C*H_3). **HRMS**: $[M+Na]^+$ calculated for $C_{35}H_{40}N_4O_9Na$: 683.26930; found 683.26875

5-(Benzyl(benzyloxycarbonyl)amino)pentyl 2-Azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-D-fucopyranosyl-(1→3)-benzyl(4-*O*-acetyl-2-azido-2-deoxy)-β-D-mannopyranoside (25)



Acceptor **24** (30 mg, 0.045 mmol, 1 equiv.) and Donor **22** (58 mg, 0.068 mmol, 1.5 equiv.) were co-evaporated with toluene (x3), dissolved in DCM (1 mL), added 3 Å molecular sieve and stirred under argon for 30 min. TBSOTf (3.1 µL, 0.014 mmol, 0.2 equiv.) was added and the mixture was stirred at rt for 30 min until TLC analysis (pentane/EtOAc, 7:3) showed full conversion of the acceptor. The reaction was quenched with Et_3N, dissolved in EtOAc, washed with $NaHCO_3$ (sat. aq.; 1x) and brine (1x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (pentane/EtOAc 8:2 → 7:3) to give **25** in 65% yield (38 mg, 0.029 mmol). **^{1}H NMR (400 MHz; $CDCl_3$)** δ : 7.85 – 7.77 (4H, m, Ar-*H*), 7.75 – 7.70 (1H, m, Ar-*H*), 7.54 – 7.50 (1H, m, Ar-*H*), 7.48 – 7.44 (3H, m, Ar-*H*), 7.38 – 7.26 (23H, m, Ar-*H*), 5.31 (1H, t, *J* = 9.8 Hz, H-4''), 5.22 – 5.11 (5H, m, H-1, H-3', CH_2), 5.01 – 4.72 (5H, m, H-1', CH_2), 4.66 – 4.58 (3H, m, H-2'', H-1'', CH_2), 4.50 (2H, d, *J* = 6.8 Hz, CH_2), 4.06 – 3.84 (8H, m, H-2, H-2', H-3, H-3'', H-4, H-5', C*H*$_2$-Linker), 3.76 – 3.68 (1H, m, H-5''), 3.58 (2H, s, H-5, H-4'), 3.50 – 3.15 (4H, m, C*H*$_2$-Linker), 1.85 (3H, s, COC*H*$_3$), 1.71 – 1.45 (6H, m, C*H*$_2$-Linker), 1.25 – 1.10 (6H, m, H-6, H-6'). **^{13}C NMR (100.65 MHz; $CDCl_3$)** δ : 169.30 (C=O), 166.81 (C=O), 138.29 (Ar-C_q), 138.23 (Ar-C_q), 138.17 (Ar-C_q), 138.03 (Ar-C_q), 135.22 (Ar-C_q), 135.00 (Ar-C_q), 133.36 (Ar-C_q), 133.16 (Ar-C_q), 128.88 (Ar-*C*), 128.70 (Ar-*C*), 128.67 (Ar-*C*), 128.65 (Ar-*C*), 128.56 (Ar-*C*), 128.53 (Ar-*C*), 128.49 (Ar-*C*), 128.43 (Ar-*C*), 128.38 (Ar-*C*), 128.05 (Ar-*C*), 127.98 (Ar-*C*), 127.93 (Ar-*C*), 127.90 (Ar-*C*), 127.81 (Ar-*C*), 127.42 (Ar-*C*), 127.37 (Ar-*C*), 127.34 (Ar-*C*), 126.73 (Ar-*C*), 126.32 (Ar-*C*), 126.16 (Ar-*C*), 125.87 (Ar-*C*), 100.91 (C-1'), 100.07 (C-1''), 99.80 (C-1), 79.81 (CH), 79.48 (CH), 77.58 (CH), 76.28 (CH), 76.20 (CH), 75.57 (CH_2), 74.99 (CH_2), 73.37 (CH), 72.61 (CH_2), 70.08 (CH_2), 70.06 (CH_2), 68.44 (CH), 67.84 (CH_2), 67.80 (CH), 67.25 (CH_2), 63.55 (CH), 61.05 (CH), 59.98 (CH), 29.82 (*C*H_2-Linker), 29.18 (*C*H_2-Linker), 23.09 (*C*H_2-Linker), 20.47 (CO*C*H_3), 17.07 (CH_3), 16.89 (CH_3). **HRMS:** $[M+Na]^+$ calculated for $C_{72}H_{78}N_{10}O_{15}Na$: 1345.55458; found 1345.55403

5-aminopentyl 2-*N*-acetamide-2-deoxy-α-L-fucopyranosyl-(1→3)-2-*N*-acetamide-2-deoxy-α-D-fucopyranosyl-(1→3)- 4-*O*-acetyl-2-*N*-acetamide-2-deoxy-β-D-mannopyranoside (CP8-III)

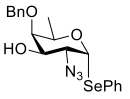


25 (38 mg, 0.029 mmol, 1 equiv.) was dissolved in THF (3 mL) and added zinc powder (0.575 g, 8.73 mmol, 300 equiv.), AcOH (1 mL) and Ac_2O (0.5 mL). The mixture was heated to 50°C and stirred for 18 h until TLC analysis (DCM/MeOH, 95:5) showed full conversion of the starting material. The solution was cooled to rt, filtered over a path of Celite and concentrated *in vacuo*. The crude product was purified by column chromatography (DCM/MeOH, 99:1 → 95:5) to give the acetamide intermediate (31 mg, 0.0227 mmol) in 78%. The product was dissolved in *t*-BuOH (2.5 mL). AcOH (0.1 mL in 100 mL MilliQ, 1 mL) was added and the mixture was birched under argon for 20 min. $Pd(OH)_2$/C (catalytic amount) was added and the mixture was birched under argon for 5 min, then with H_2 for 2 min, before to be stirred for 3 days. The

mixture was birched with argon for 20 min, filtered over a Whatman filter and lyophilized. Purification by a HW40 column with NH_4OAc followed by lyophilization gave **CP8-III** in 60% yield (3 mg, 0.00421 mmol). **^{1}H NMR (600 MHz, D_2O)** δ 5.14 (t, *J* = 10.0 Hz, 1H, H-4), 4.97 (d, *J* = 4.1 Hz, 1H, H''-1), 4.95 (d, *J* = 4.0 Hz, 1H, H'-1), 4.86 (d, *J* = 1.5 Hz, 1H, H-1), 4.50 (dd, *J* = 4.6, 1.5 Hz, 1H, H-2), 4.36 (q, *J* = 6.5 Hz, 1H, H''-5), 4.24 (dd, *J* = 10.9, 4.0 Hz, 1H, H''-2), 4.16 (dd, *J* = 9.8, 4.6 Hz, 1H, H-3), 4.12 (dd, *J* = 11.1, 4.0 Hz, 1H, H'-2), 4.08 (q, *J* = 6.5 Hz, 1H, H'-5), 3.92 (dd, *J* = 11.1, 3.2 Hz, 1H, H'-3), 3.86 (dt, *J* = 10.2, 6.4 Hz, 1H, CH_2-Linker), 3.83 – 3.82 (m, 1H, H'-4), 3.80 (d, *J* = 10.2 Hz, 1H, H-5), 3.79 – 3.78 (m, 1H, H''-4), 3.74 (dd, *J* = 11.0, 3.2 Hz, 1H, H''3), 3.67 (dt, *J* = 10.2, 6.5 Hz, 1H, CH_2-Linker), 3.00 (t, *J* = 7.5 Hz, 2H, CH_2-Linker), 2.11 (s, 3H, COCH_3), 2.05 (s, 3H, COCH_3), 2.03 (s, 3H, COCH_3), 1.96 (s, 3H, COCH_3), 1.72 – 1.60 (m, 4H, CH_2-Linker), 1.48 – 1.38 (m, 2H, CH_2-Linker), 1.25 (dd, *J* = 8.7, 6.6 Hz, 6H, H'-6, H''-6). **^{13}C NMR (151 MHz, D_2O)** δ 175.78 (C=O), 175.47 (C=O), 175.18 (C=O), 174.62 (C=O), 173.34 (C=O), 99.53 (C'-1), 99.51 (C-1), 99.25 (C''-1), 75.17 (C''-5), 74.82 (C-3), 74.11 (C''-3), 71.91 (C'-5/C''-5), 71.88 (C'-5/C''-5), 71.39 (C-4), 70.78 (*C*H_2-Linker), 68.59 (C'-3), 68.06 (C'-5), 67.68 (C''-5), 53.37 (C-2), 50.38 (C'-2), 49.44 (C''-2), 40.30 (*C*H_2-Linker), 28.92 (*C*H_2-Linker), 27.22 (*C*H_2-Linker), 23.18 (CO*C*H_3), 22.98 (CO*C*H_3), 22.95 (*C*H_2-Linker), 21.13 (CO*C*H_3), 16.34 (C'-6/ C''-6), 16.20 (C'-6/ C''-6). **HRMS**: $[M+H]^+$ calculated for $C_{31}H_{52}N_4O_{16}H$: 737.34566; found 737.34511

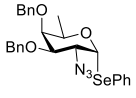
CP5-II: DF–DM–LF

Phenyl 2-azido-4-*O*-benzyl-2-deoxy-1-seleno-α-D-fucopyranoside (27)



26 (727 mg, 1.3 mmol) was dissolved in DCM/H_2O (20:1, 13 mL, 0.1 M) and added DDQ (590 mg, 2.6 mmol, 2 equiv.). The reaction was stirred at rt under N_2 for 2 h until TLC (pentane, EtOAc, 9:1) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (sat. aq.) and diluted/extracted with EtOAc (x3). The combined organic phases were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 95:5 → 80:20) yielded **27** in 89% (483 mg, 1.15 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.61 – 7.54 (m, 2H, Ar-*H*), 7.41 – 7.31 (m, 5H, Ar-*H*), 7.31 – 7.26 (m, 3H, Ar-*H*), 5.91 (d, *J* = 5.2 Hz, 1H, H-1), 4.82 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.71 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.34 (q, *J* = 6.5 Hz, 1H, H-4), 4.03 (dd, *J* = 10.3, 5.2 Hz, 1H, H-2), 3.82 (ddd, *J* = 10.3, 8.7, 3.4 Hz, 1H, H-3), 3.70 (dd, *J* = 3.5, 1.3 Hz, 1H, H-5), 2.23 (d, *J* = 8.7 Hz, 1H, O*H*), 1.26 (d, *J* = 6.6 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.07 (Ar-C_q), 134.51 (Ar-*C*), 129.23 (Ar-*C*), 128.86 (Ar-*C*), 128.42 (Ar-*C*), 128.24 (Ar-*C*), 127.90 (Ar-*C*), 85.33 (C-1), 79.89 (C-5), 76.32 (Ar-*C*H_2), 72.07 (C-3), 69.49 (C-4), 62.72 (C-2), 16.09 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{19}H_{21}N_3O_3SeNa$: 442.06458; found 442.06405

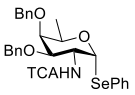
Phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-seleno-α-D-fucopyranoside (28)



27 (475 mg, 1.14 mmol) was co-evaporated with toluene (x3), dissolved in DMF (11 mL, 0.1 M) and cooled to 0 °C. NaH (60% dispersion in mineral oil, 59 mg, 1.48 mmol, 1.3 equiv.) and BnBr (0.17 mL, 1.48 mmol, 1.3 equiv.) were added and the reaction was allowed to warm to rt and stirred under N_2 overnight until TLC (pentane/EtOAc, 9:1) showed full conversion. The reaction was quenched

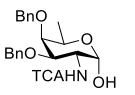
with H_2O and extracted with Et_2O (x3). The combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 100:0 → 90:10) yielded **28** in 99 % (577 mg, 1.13 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.60 – 7.54 (m, 2H, Ar-*H*), 7.47 – 7.27 (m, 12H, Ar-*H*), 7.26 – 7.22 (m, 1H, Ar-*H*), 5.93 (d, *J* = 5.3 Hz, 1H, H-1), 4.94 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.77 (d, *J* = 2.2 Hz, 2H, Ar-CH_2), 4.61 (d, *J* = 11.5 Hz, 1H, Ar-CH_2), 4.36 (dd, *J* = 9.7, 5.3 Hz, 1H, H-2), 4.23 (q, *J* = 6.5 Hz, 1H, H-5), 3.76 – 3.70 (m, 2H, *H*-4, H-3), 1.13 (d, *J* = 6.4 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.25 (Ar-C_q), 137.57 (Ar-C_q), 134.48 (Ar-*C*), 129.17 (Ar-*C*), 128.84 (Ar-C_q), 128.74 (Ar-*C*), 128.45 (Ar-*C*), 128.31 (Ar-*C*), 128.18 (Ar-*C*), 127.99 (Ar-*C*), 127.92 (Ar-*C*), 127.79 (Ar-*C*), 85.71 (C-1), 80.78 (C-4), 75.87 (C-3), 75.12 (Ar-*C*H$_2$), 72.66 (Ar-*C*H$_2$), 69.53 (C-5), 61.05 (C-2), 16.24 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{26}H_{27}N_3O_3SeNa$: 532.11153; found 532.11115

Phenyl 3,4-di-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-1-seleno-α-D-fucopyranoside (29)



28 (566 mg, 1.11 mmol) was dissolved in distilled, dry THF (11 mL, 0.1 M) and added zinc powder (800 mg, 12.24 mmol, 11 equiv.) and AcOH (0.6 mL, 10.02 mmol, 9 equiv.). The reaction was stirred under N_2 at rt overnight until TLC (pentane/EtOAc, 90:10) showed full conversion. The solution was filtered over a path of Celite and concentrated *in vacuo*. The crude was co-evaporated with toluene (x3) and dissolved in distilled, dry THF (7.5 mL, 0.15 M). Activated 3Å molecular sieves were added to the solution and the mixture was stirred under N_2 for 30 min. The solution was cooled to 0 °C and trichloroacetyl chloride (0.25 mL, 2.26 mmol, 2 equiv.) was added and stirred for 30 min at 0 °C under N_2 until TLC (pentane/EtOAc, 9:1) showed full conversion. The reaction mixture was diluted in DCM, washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 95:15 → 80:20) yielded **29** in 93% yield (657 mg, 1.05 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.54 – 7.47 (m, 2H, Ar-*H*), 7.42 – 7.26 (m, 11H, Ar-*H*), 6.84 (d, *J* = 7.5 Hz, 1H, N*H*), 6.04 (d, *J* = 4.7 Hz, 1H, H-1), 4.99 (d, *J* = 11.6 Hz, 1H, Ar-CH_2), 4.79 – 4.70 (m, 2H, Ar-CH_2, H-2), 4.67 (d, *J* = 11.5 Hz, 1H, Ar-CH_2), 4.54 (d, *J* = 11.9 Hz, 1H, Ar-CH_2), 4.21 (q, *J* = 6.3 Hz, 1H, H-5), 3.82 (dd, *J* = 2.7, 1.3 Hz, 1H, H-4), 3.58 (dd, *J* = 11.0, 2.5 Hz, 1H, H-3), 1.29 – 1.22 (m, 5H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 134.16 (Ar-*C*), 129.38 (Ar-*C*), 128.91 (Ar-*C*), 128.46 (Ar-*C*), 128.38 (Ar-*C*), 128.05 (Ar-*C*), 127.95 (Ar-*C*), 88.93 (C-1), 78.57 (C-3), 74.88 (Ar-*C*H$_2$), 74.48 (C-4), 71.54 (Ar-*C*H$_2$), 70.56 (C-5), 51.97 (C-2), 16.75 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{28}H_{28}Cl_3NO_4SeNa$: 650.01468; found 650.01367

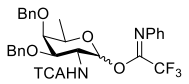
3,4-di-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-α-D-fucopyranose (30)



29 (586 mg, 0.934 mmol) was dissolved in acetone/H_2O (10:1, 18 mL, 0.05 M) and cooled to 0 °C. NIS (420 mg, 1.87 mmol, 2 equiv.) was added and the reaction was stirred at 0 °C for 20 min until TLC (pentane/EtOAc, 9:1) showed full conversion. The reaction was quenched with $Na_2S_2O_3$ and the acetone was evaporated. The residue was dissolved in EtOAc and 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, 80:20 → 60:40) yielded **30** in 75% (340 mg, 0.695 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.41 – 7.25 (m, 13H, Ar-*H*), 6.79 (d, *J* = 8.9 Hz, 1H, N*H*), 5.38

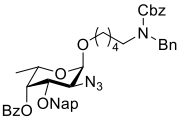
(t, *J* = 3.6 Hz, 1H, H-1), 5.00 (d, *J* = 11.6 Hz, 1H, Ar-C*H*$_2$), 4.76 – 4.67 (m, 1H, Ar-C*H*$_2$), 4.70 – 4.61 (m, Ar-C*H*$_2$, 2H, H-2), 4.56 (d, *J* = 12.0 Hz, 1H, Ar-C*H*$_2$), 4.10 (q, *J* = 6.3 Hz, 1H, H-5), 3.86 – 3.77 (m, 1H, H-3), 3.78 – 3.71 (m, 1H, H-4), 2.88 (dd, *J* = 3.5, 1.5 Hz, 1H, O*H*), 1.20 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 138.28 (Ar-*C*$_q$), 137.76 (Ar-*C*$_q$), 128.99 (Ar-*C*), 128.75 (Ar-*C*), 128.57 (Ar-*C*), 128.52 (Ar-*C*), 128.45 (Ar-*C*), 128.32 (Ar-*C*), 128.08 (Ar-*C*), 127.90 (Ar-*C*), 127.81 (Ar-*C*), 91.80 (C-1), 77.16 (C-3), 75.02 (C-6), 74.73 (Ar-*C*H$_2$), 71.80 (Ar-*C*H$_2$), 67.10 (C-5), 51.22 (C-2), 17.11 (C-6). **HRMS**: [M+Na]$^+$ calculated for $C_{22}H_{24}Cl_3NO_5Na$: 510.01678; found 510.016123

3,4-di-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-fucopyranose (6)



30 (340 mg, 0.695 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (3.3 mL, 0.2 M). K_2CO_3 (183 mg, 1.324 mmol, 2 equiv.) and ClC(=NPh)CF_3 (0.2 mL, 1.324 mmol, 2 equiv.) were added to the reaction and it was stirred overnight under N_2 at rt until TLC (pentane/EtOAc, 8:2) showed full conversion. The mixture was filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 95:5 → 80:20) furnished imidate donor **6** in 97% (446 mg, 0.676 mmol). **^{1}H NMR (400 MHz, CD$_3$CN)** δ 7.68 – 7.22 (m, 38H), 7.17 – 7.08 (m, 2H), 6.82 (d, *J* = 7.8 Hz, 3H), 6.26 (d, *J* = 6.8 Hz, 1H), 4.92 (d, *J* = 11.2 Hz, 1H), 4.84 (dd, *J* = 24.1, 11.1 Hz, 3H), 4.76 (s, 2H), 4.70 – 4.59 (m, 4H), 4.44 (m, 1H), 4.26 (dd, *J* = 7.9, 6.8 Hz, 1H), 4.20 – 4.02 (m, 4H), 4.02 – 3.95 (m, 1H), 3.82 (dd, *J* = 2.9, 1.6 Hz, 1H), 3.51 (dd, *J* = 7.9, 2.7 Hz, 1H), 1.25 (s, 3H). **^{13}C NMR (101 MHz, CD$_3$CN)** δ 139.76, 139.64, 139.13, 130.09, 129.91, 129.40, 129.34, 129.30, 129.27, 129.11, 129.04, 129.01, 128.78, 128.71, 128.64, 128.59, 128.55, 126.98, 122.08, 108.93, 81.30, 76.61, 76.20, 75.97, 75.36, 74.78, 72.09, 72.05, 71.69, 70.66, 66.45, 51.69, 29.72, 17.33, 17.10.

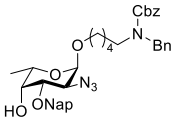
5-(Benzyl(benzyloxycarbonyl)amino)pentyl 2-azido-4-*O*-benzoyl-2-deoxy-3-*O*-(2-naphthylmethyl)-L-fucopyranoside (31)



Donor **8** (201 mg, 0.383 mmol, 1 equiv.) and acceptor **12** (181 mg, 0.575 mmol, 1.5 equiv.) were co-evaporated with toluene (x3) before being dissolved in DCM (3.8 mL, 0.1 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. NIS (129 mg, 0.575 mmol, 1.5 equiv.) and TBSOTf (18 µL, 0.0766 mmol, 0.2 equiv.) were added at rt and the reaction was stirred at rt for 15 min until TLC (pentane/EtOAc, 8:2) showed full conversion. The reaction was quenched with Et_3N, diluted 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, 90:10 → 70:30) yielded **31** in 98% (α: 103 mg, 0.139 mmol; β: 175 mg, 0.235 mmol) in a α/β = 37:63. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 8.13 – 8.06 (m, 2H, Ar-*H*), 7.83 – 7.64 (m, 6H, Ar-*H*), 7.64 – 7.26 (m, 22H, Ar-*H*), 5.77 – 5.65 (m, 1H, H-4), 5.24 – 5.12 (m, 2H, Ar-C*H*$_2$), 4.99 (d, *J* = 11.0 Hz, 1H, Ar-C*H*$_2$), 4.94 (d, *J* = 8.9 Hz, 1H, H-1), 4.71 (d, *J* = 11.1 Hz, 1H, Ar-C*H*$_2$), 4.49 (t, *J* = 9.4 Hz, 4H, C*H*$_2$-Linker), 4.18 – 4.03 (m, 2H, H-3, H-5), 4.06 – 3.90 (m, 1H, C*H*$_2$-Linker), 3.75 (dd, *J* = 10.6, 3.5 Hz, 1H, H-2), 3.71 – 3.36 (m, 2H, C*H*$_2$-Linker), 3.32 – 3.08 (m, 2H, C*H*$_2$-Linker), 1.68 – 1.42 (m, 3H, C*H*$_2$-Linker), 1.40 – 1.13 (m, 6H, C*H*$_2$-Linker, H-6). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 166.32 (C=O), 134.84 (Ar-*C*$_q$), 133.42 (Ar-*C*), 133.34 (Ar-*C*$_q$), 133.16 (Ar-*C*$_q$),

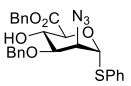
132.21 (Ar-*C*), 130.02 (Ar-*C*), 129.17 (Ar-*C*), 128.67 (Ar-*C*), 128.61 (Ar-*C*), 128.27 (Ar-*C*), 128.08 (Ar-*C*), 127.98 (Ar-*C*), 127.96 (Ar-*C*), 127.74 (Ar-*C*), 127.44 (Ar-*C*), 127.31 (Ar-*C*), 127.14 (Ar-*C*), 126.14 (Ar-*C*), 126.06 (Ar-*C*), 125.98 (Ar-*C*), 125.35 (Ar-*C*), 98.30 (C-1), 74.40 (C-5/C-3), 71.59 (Ar-*C*H_2), 70.12 (C-4), 68.45 (*C*H_2-Linker), 67.30 (Ar-*C*H_2), 65.29 (C-3/C-5), 59.52 (C-2), 50.33 (*C*H_2-Linker), 47.22 (*C*H_2-Linker), 29.47 (*C*H_2-Linker), 23.09 (*C*H_2-Linker), 16.50 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{44}H_{46}N_4O_7Na$: 765.32642; found 765.32587

5-(Benzyl(benzyloxycarbonyl)amino)pentyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-L-fucopyranoside (32)



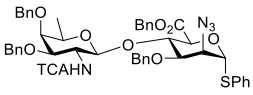
31 (264 mg, 0.355 mmol) was dissolved in MeOH (1.8 mL, 0.2 M) and added NaOMe (0.08 mL, 0.355 mmol, 1 equiv.) and stirred at rt for 2 days until TLC (pentane/EtOAc, 8:2) showed full conversion. The reaction was neutralized with Amberlite IR-120 H^+ until pH≈8-9, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 80:20 → 60:40) yielded **32** in 87% (201 mg, 0.315 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.91 – 7.78 (m, 4H, Ar-*H*), 7.53 – 7.41 (m, 3H, Ar-*H*), 7.40 – 7.26 (m, 12H, Ar-*H*), 7.17 (d, *J* = 7.2 Hz, 2H, Ar-*H*), 5.18 (d, *J* = 12.5 Hz, 2H, Ar-CH_2), 4.94 – 4.78 (m, 3H, H-1, Ar-CH_2), 4.50 (d, *J* = 6.9 Hz, 2H, Ar-CH_2), 3.99 – 3.80 (m, 3H, H-3, H-4, H-5), 3.64 (dd, *J* = 10.4, 3.6 Hz, 1H, H-2), 3.59 (m, 1H, CH_2-Linker), 3.61 – 3.11 (m, 3H, CH_2-Linker), 2.37 (s, 1H, OH), 1.61 – 1.45 (m, 5H, CH_2-Linker), 1.46 – 1.18 (m, 5H, H-6, CH_2-Linker). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.09 (Ar-C_q), 134.72 (Ar-C_q), 133.39 (Ar-C_q), 128.73 (Ar-*C*), 128.68 (Ar-*C*), 128.10 (Ar-*C*), 127.96 (Ar-*C*), 127.89 (Ar-*C*), 127.43 (Ar-*C*), 126.46 (Ar-*C*), 126.36 (Ar-*C*), 125.84 (Ar-*C*), 99.01 (C-1), 76.48 (C-3), 72.19 (Ar-*C*H_2), 69.06 (C-4/C-5), 68.21 (*C*H_2-Linker), 67.31 (Ar-*C*H_2), 65.60 (C-4/C-5), 59.07 (C-2), 50.24 (*C*H_2-Linker), 46.14 (*C*H_2-Linker), 29.21 (*C*H_2-Linker), 22.93 (*C*H_2-Linker), 16.37 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{37}H_{42}N_4O_6Na$: 661.30020; found 661.2996

Benzyl (2-azido-3-*O*-benzyl-2-deoxy-1-thio)-α-D-mannopyranosiduronate (7)



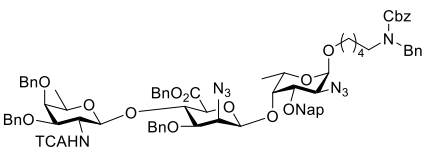
10 (405 mg, 0.663 mmol) was dissolved in DCM/H_2O (20:1, 6.6 mL, 0.1 M), added DDQ (451 mg, 1.98 mmol, 2 equiv.) and stirred at rt under N_2 for 2 h until TLC (pentane/EtOAc, 8:2) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (sat. aq.) and diluted/extracted with EtOAc (x3). The combined organic phases were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 90:10 → 75:25) yielded **7** in 93% (304 mg, 0.618 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.53 – 7.48 (m, 2H, Ar-*H*), 7.44 – 7.30 (m, 10H, Ar-*H*), 7.29 – 7.21 (m, 3H, Ar-*H*), 5.56 (d, *J* = 3.4 Hz, 1H, H-1), 5.18 (s, 2H, Ar-CH_2), 4.76 (dd, *J* = 11.6, 10.1 Hz, 2H, Ar-CH_2), 4.70 (d, *J* = 8.1 Hz, 1H, H-5), 4.39 (td, *J* = 8.0, 3.5 Hz, 1H, H-4), 3.98 (t, *J* = 3.5 Hz, 1H, H-2), 3.92 (dd, *J* = 8.0, 3.4 Hz, 1H, H-3), 2.90 (t, *J* = 3.0 Hz, 1H, O*H*). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.39 (C=*O*), 137.35 (Ar-C_q), 135.07 (Ar-C_q), 132.50 (Ar-C_q), 132.28 (Ar-*C*), 129.25 (Ar-*C*), 128.76 (Ar-*C*), 128.61 (Ar-*C*), 128.33 (Ar-*C*), 128.23 (Ar-*C*), 128.18 (Ar-*C*), 85.55 (C-1), 78.08 (C-3), 73.45 (Ar-*C*H_2), 72.98 (C-5), 68.53 (C-4), 67.52 (Ar-*C*H_2), 61.20 (C-2). **HRMS**: $[M+Na]^+$ calculated for $C_{19}H_{19}N_3O_5SNa$: 492.15932; found 492.15877

Phenyl 3,4-di-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-β-D-fucopyranosyl-(1→4) benzyl (2-azido-3-*O*-benzyl-2-deoxy-1-thio)-α-D-mannopyranosiduronate (33)



Acceptor **7** (195 mg, 0.396 mmol, 1 equiv.) and donor **6** (339 mg, 0.514 mmol, 1.3 equiv.) were co-evaporated with toluene (x3), dissolved in dry DCM/MeCN (2:1, 4 mL, 0.1 M), added activated 3Å molecular sieves and stirred for 30 min under argon at rt. The mixture was cooled to -78 °C, after which TBSOTf (18 µL, 0.0791 mmol, 0.2 equiv.) was added. The reaction mixture was stirred at -78 °C for 3 h until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction mixture was quenched with Et_3N and diluted in EtOAc. The organic phase was washed with $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 85:15 → 70:30) and size exclusion chromatography furnished **33** in 51% yield (α: 56 mg, 0.058 mmol; β: 140 mg, 0.146 mmol) in a α/β = 29:71. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.58 – 7.46 (m, 2H, Ar-*H*), 7.43 – 7.23 (m, 22H, Ar-*H*), 7.22 – 7.03 (m, 6H, Ar-*H*), 6.88 (d, *J* = 7.2 Hz, 1H, , N*H*), 5.67 (d, *J* = 10.3 Hz, 1H, H-1'), 5.02 (d, *J* = 8.3 Hz, 1H, Ar-CH_2), 4.94 (d, *J* = 11.9 Hz, 2H, Ar-CH_2), 4.79 (d, *J* = 12.1 Hz, 1H, Ar-CH_2), 4.70 – 4.63 (m, 2H, Ar-CH_2), 4.55 (d, *J* = 11.3 Hz, 1H, Ar-CH_2), 4.52 – 4.42 (m, 4H, Ar-CH_2, H-4, H-5), 4.21 (dd, *J* = 11.0, 2.8 Hz, 1H, H-3), 4.07 (t, *J* = 3.4 Hz, 1H, H-3'), 3.74 (dt, *J* = 11.1, 7.7 Hz, 1H, H-2), 3.66 (d, *J* = 2.7 Hz, 1H, H-4'), 3.60 – 3.46 (m, 2H, H-5', H-2'), 1.15 (d, *J* = 6.3 Hz, 3H, H-6'). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 168.84 (*C*=O), 162.34 (*C*=O), 138.05 (Ar-C_q), 137.40 (Ar-C_q), 136.92 (Ar-C_q), 135.00 (Ar-C_q), 132.39 (Ar-C_q), 132.16 (Ar-*C*), 128.80 (Ar-*C*), 128.71 (Ar-*C*), 128.66 (Ar-*C*), 128.54 (Ar-*C*), 128.50 (Ar-*C*), 128.42 (Ar-*C*), 128.36 (Ar-*C*), 128.31 (Ar-*C*), 128.22 (Ar-*C*), 128.08 (Ar-*C*), 127.62 (Ar-*C*), 98.73 (C-1'), 80.56 (C-1), 77.59 (C-3), 75.95 (C-3'), 74.89 (Ar-*C*H$_2$), 74.68 (C-4, C-4', C-5), 74.56 (C-4, C-4', C-5), 74.41 (C-4, C-4', C-5), 73.05 (Ar-*C*H$_2$), 72.77 (Ar-*C*H$_2$), 71.12 (C-5'), 67.39 (Ar-*C*H$_2$), 57.39 (C-2), 55.71 (C-2'), 17.06 (C-6'). **HRMS**: $[M+Na]^+$ calculated for $C_{48}H_{47}Cl_3N_4O_9SNa$: 983.20270; found 983.20215

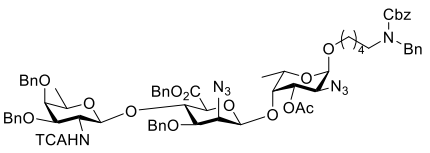
5-(Benzyl(benzyloxycarbonyl)amino)pentyl 3,4-di-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-β-D-fucopyranosyl-(1→4) benzyl(2-azido-3-*O*-benzyl-2-deoxy-1-thio)-α-D-mannopyranosiduronsyl-(1→4)- 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-L-fucopyranoside (34)



Acceptor **32** (68 mg, 0.107 mmol, 1 equiv.) and donor **33** (154 mg, 0.16 mmol, 1.5 equiv.) were co-evaporated with toluene (x3), dissolved in dry DCM (1 mL, 0.1 M), added activated 3Å molecular sieves and stirred for 30 min under argon at rt. The mixture was cooled to -30 °C and added NIS (48 mg, 0.213 mmol, 2 equiv.) and TBSOTf (5 µL, 0.0213 mmol, 0.2 equiv.). The reaction was allowed to warm to 10 °C and stirred for 5 h until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction mixture was quenched with Et_3N, diluted in EtOAc, washed with NaS_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, 80:20 → 65:35) furnished **34** in 52% yield (83 mg, 0.0555 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.90 – 7.68 (m, 5H, Ar-*H*), 7.65 – 7.27 (m, 34H, Ar-*H*), 7.26 – 6.89 (m, 19H, Ar-*H*), 6.56 (d, *J* = 8.6 Hz, 1H, Ar-*H*), 5.17 (d, *J* = 10.1 Hz, 2H, Ar-CH_2), 5.00 – 4.87 (m, 5H, Ar-CH_2), 4.78 (d, *J* = 11.9 Hz, 2H, H-1, Ar-CH_2), 4.72 – 4.64 (m, 4H, H-1', Ar-

CH_2), 4.59 (dd, *J* = 11.8, 6.8 Hz, 3H, Ar-CH_2), 4.52 – 4.43 (m, 5H, Ar-CH_2), 4.35 (d, *J* = 8.3 Hz, 1H, H-1''), 4.25 (t, *J* = 9.2 Hz, 1H, H-4'), 4.16 – 4.07 (m, 2H, H-3, H-2''), 4.03 (d, *J* = 3.8 Hz, 1H, H-2'), 3.86 – 3.79 (m, 2H, H-4, H-5), 3.76 (dd, *J* = 10.1, 4.3 Hz, 2H, H-2, H-5''), 3.55 (d, *J* = 2.8 Hz, 1H, H-4''), 3.49 (dd, *J* = 8.9, 3.7 Hz, 1H, H-3'), 3.39 (dt, *J* = 12.2, 4.0 Hz, 2H, H-3'', CH_2-Linker), 3.33 – 3.13 (m, 3H, CH_2-Linker), 3.10 (q, *J* = 6.3 Hz, 1H, H-5''), 1.62 – 1.41 (m, 6H, CH_2-Linker), 1.33 – 1.09 (m, 15H, CH_2-Linker, H-6'', H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 167.99 (C=O), 161.90 (C=O), 138.63 (Ar-C_q), 138.23 (Ar-C_q), 137.77 (Ar-C_q), 135.65 (Ar-C_q), 134.85 (Ar-C_q), 133.36 (Ar-C_q), 133.08 (Ar-C_q), 129.26 (Ar-*C*), 128.77 (Ar-*C*), 128.74 (Ar-*C*), 128.69 (Ar-*C*), 128.65 (Ar-*C*), 128.59 (Ar-*C*), 128.56 (Ar-*C*), 128.51 (Ar-*C*), 128.45 (Ar-*C*), 128.42 (Ar-*C*), 128.35 (Ar-*C*), 128.32 (Ar-*C*), 128.26 (Ar-*C*), 128.21 (Ar-*C*), 128.16 (Ar-*C*), 128.07 (Ar-*C*), 127.95 (Ar-*C*), 127.92 (Ar-*C*), 127.84 (Ar-*C*), 127.82 (Ar-*C*), 127.74 (Ar-*C*), 127.68 (Ar-*C*), 127.64 (Ar-*C*), 127.37 (Ar-*C*), 126.90 (Ar-*C*), 126.53 (Ar-*C*), 126.23 (Ar-*C*), 125.99 (Ar-*C*), 125.83 (Ar-*C*), 100.92 (C-1'), 99.53 (C-1''), 98.04 (C-1), 79.29 (C-3''), 77.89 (C-3'), 75.36 (C-5'), 74.98 (C-3), 74.71 (C-5), 74.68 (Ar-*C*H_2), 74.00 (C-4''), 73.62 (C-4'), 70.60 (Ar-*C*H_2), 71.72 (Ar-*C*H_2), 70.60 (C-5''), 68.25 (Ar-*C*H_2), 67.56 (Ar-*C*H_2), 67.26 (Ar-*C*H_2), 66.10 (Ar-*C*H_2), 65.89 (C-4), 62.36 (C-2'), 58.61 (C-2), 54.63 (C-2''), 50.60 (CH_2-Linker), 50.31 (CH_2-Linker), 47.20 (CH_2-Linker), 46.21 (CH_2-Linker), 29.82 (CH_2-Linker), 29.13 (CH_2-Linker), 27.95 (CH_2-Linker), 27.52 (CH_2-Linker), 23.40 (CH_2-Linker), 17.21 (C-6''/C-6), 17.07 (C-6''/C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{79}H_{83}Cl_3N_8O_{15}Na$: 1511.49412; found 1511.49357

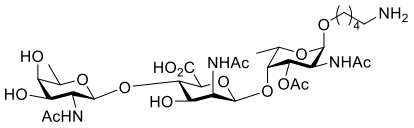
5-(Benzyl(benzyloxycarbonyl)amino)pentyl 3,4-di-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-β-D-fucopyranosyl-(1→4) benzyl(2-azido-3-*O*-benzyl-2-deoxy-1-thio)-α-D-mannopyranosiduronsyl-(1→4)-2-*O*-acetyl-2-azido-2-deoxy-L-fucopyranoside (35)



34 (81 mg, 0.0545 mmol) was dissolved in DCM/H_2O (4:1, 2.7 mL, 0.02 M) and added DDQ (25 mg, 0.109 mmol, 2 equiv.). The reaction was stirred at rt under N_2 for 5 h until TLC (pentane, EtOAc, 7:3) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (sat. aq.) and diluted/extracted with EtOAc (x3). The combined organic phases were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was used without further purification. The residue was dissolved in pyridine (2 mL) and cooled to 0 °C and added Ac_2O (0.3 mL) and DMAP (catalytic amount) and stirred overnight at rt under N_2 until TLC (pentane/EtOAc, 7:3) showed full conversion. The mixture was dissolved in EtOAc, washed with 1 M HCl (x1), $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 8:2 → 6:4) yielded **35** in 57% (44 mg, 0.0312 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.52 – 7.26 (m, 27H), 7.26 – 7.12 (m, 8H), 6.57 (d, *J* = 8.1 Hz, 1H), 5.28 – 5.14 (m, 4H), 5.09 – 5.03 (m, 1H), 4.94 (d, *J* = 11.5 Hz, 2H), 4.90 – 4.74 (m, 2H), 4.74 – 4.55 (m, 4H), 4.57 – 4.42 (m, 5H), 4.26 (t, *J* = 9.2 Hz, 1H), 4.18 – 4.08 (m, 1H), 4.04 – 3.88 (m, 3H), 3.88 – 3.72 (m, 2H), 3.72 – 3.63 (m, 2H), 3.57 (d, *J* = 2.7 Hz, 2H), 3.51 (dp, *J* = 7.5, 3.6 Hz, 1H), 3.46 – 3.13 (m, 4H), 2.02 (s, 3H), 1.72 – 1.47 (m, 6H), 1.41 – 1.21 (m, 5H), 1.16 (d, *J* = 6.6 Hz, 7H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.77, 167.78, 162.03, 138.52, 137.98, 137.74, 135.15, 129.32, 129.27, 128.90, 128.81, 128.77, 128.73, 128.68, 128.66, 128.59, 128.49, 128.42, 128.33, 128.25, 128.11,

128.07, 128.04, 127.94, 127.89, 127.83, 127.77, 127.70, 127.67, 127.37, 100.94, 98.74, 97.94, 78.50, 78.01, 75.68, 75.35, 74.75, 74.72, 73.15, 73.11, 71.95, 70.66, 69.68, 68.39, 67.76, 67.27, 65.30, 61.79, 56.99, 55.27, 50.65, 50.35, 47.19, 46.26, 29.82, 29.17, 23.92, 20.98, 17.13, 16.56.

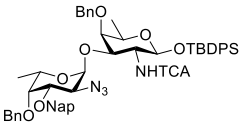
5-aminopentyl 2-acetamide-2-deoxy-β-D-fucopyranosyl-(1→4) 2-acetamide-2-deoxy-α-D-mannopyranosiduronsyl-(1→4)-2-*O*-acetyl-2-acetamide-2-deoxy-L-fucopyranoside (CP5-II)



35 (42 mg, 0.0302 mmol) was dissolved in THF (distilled, 3 mL) and added zinc powder (592 mg, 9.047 mmol, 300 equiv.), AcOH (1 mL) and Ac_2O (0.5 mL). The resulting mixture was stirred at 50 °C overnight until TLC (DCM/MeOH, 95:5) showed full conversion. The cooled mixture was filtered through Celite, evaporated *in vacuo* and co-evaporated with toluene (x3). The crude product was first purified by column chromatography (DCM/MeOH, 98:2 → 90:10) followed by HPLC given the acetamide intermediate in 21% yield (8 mg, 0.00605 mmol). The product (8 mg, 0.00605 mmol) was dissolved in *t*-BuOH (2.5 mL) and added AcOH (1 mL, 0.1 mL in 100 mL MilliQ). The solution was birched with argon for 20 min and then $Pd(OH)_2/C$ (catalytic amount) was added. The reaction was again birched with argon for 5 minutes before the atmosphere was changed for H_2. The mixture was stirred for 3 days under H_2 atmosphere, after which it was birched with argon for 20 min, filtered over a Whatman filter and lyophilized. Purification by a HW40 column with NH_4OAc followed by lyophilization gave **CP5-II** in 64% yield (3.1 mg, 0.00423 mmol). **^{1}H NMR (600 MHz, D_2O)** δ 4.94 (dd, *J* = 11.6, 3.0 Hz, 1H, H-3), 4.85 (d, *J* = 3.7 Hz, 1H, H-1), 4.71 (d, *J* = 1.4 Hz, 1H, H-1'), 4.64 (dd, *J* = 4.5, 1.4 Hz, 1H, H-2'), 4.39 (d, *J* = 8.4 Hz, 1H, H-1), 4.34 (dd, *J* = 11.6, 3.7 Hz, 1H, H-2), 4.18 – 4.12 (m, 2H, H-4, H-5), 3.86 (dd, *J* = 9.6, 4.4 Hz, 1H, H-3'), 3.85 – 3.77 (m, 4H, H-4'', H-2'', H-5'', H-4'), 3.75 (d, *J* = 3.8 Hz, 1H, H-5'), 3.72 – 3.66 (m, 2H, H-3'', CH_2-Linker), 3.58 (d, *J* = 9.5 Hz, 1H, H-5'), 3.50 (dt, *J* = 10.1, 6.2 Hz, 1H, CH_2-Linker), 3.00 (t, *J* = 7.7 Hz, 2H, CH_2-Linker), 2.13 (s, 3H, COCH_3), 2.05 (s, 3H, COCH_3), 2.04 (s, 3H, COCH_3), 1.99 (s, 3H, COCH_3), 1.71 – 1.60 (m, 4H, CH_2-Linker), 1.50 – 1.40 (m, 2H, CH_2-Linker), 1.28 (d, *J* = 6.4 Hz, 3H, H-6''), 1.24 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (151 MHz, D_2O)** δ 176.43 (C=O), 176.01 (C=O), 175.37 (C=O), 174.78 (C=O), 174.72 (C=O), 102.56 (C-1''), 100.99 (C-1'), 97.90 (C-1), 80.19 (C-4'), 78.32 (C-5'), 77.10 (C-4), 71.9 (C-5''/ C-3''), 71.92 (C-5''/ C-3''), 71.26 (C-5), 71.13 (C-3'), 70.95 (C-3), 68.87 (CH_2-Linker), 67.15 (C-5), 52.87 (C-2'/ C-2''), 48.17 (C-2), 40.28 (CH_2-Linker), 28.92 (CH_2-Linker), 27.38 (CH_2-Linker), 23.42 (COCH_3), 23.22 (CH_2-Linker), 22.91 (COCH_3), 22.72 (COCH_3), 21.22 (COCH_3), 16.33 (C-6''), 16.12 (C-6). **HRMS**: $[M+H]^+$ calculated for $C_{31}H_{52}N_4O_{16}H$: 737.34566; found 737.34526

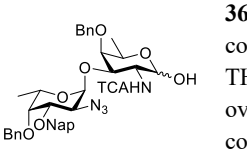
CP5-III: LF–DF–DM

***Tert*-butyldiphenylsilyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-deoxy-2-*N*-trichloroacetamide-4-*O*-benzyl-β-D-fucopyranoside (36)**



Acceptor **9** (160 mg, 0.251 mmol, 1 2 equiv.) and donor **3** (222 mg, 0.376 mmol, 1.5 equiv.) were co-evaporated with toluene (x3), dissolved in dry DCM (2.5 mL, 0.1 M) and added activated 3Å molecular sieves and stirred for 30 min under argon at rt. TBSOTf (12 µL, 0.0501 mmol, 0.2 equiv.) was added at rt and the reaction was stirred for 30 min under argon until TLC (pentane/EtOAc, 8:2) showed full conversion. The reaction was quenched with Et_3N, diluted in EtOAc, washed $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 95:5 → 80:20) yielded the α-1,3-linked disaccharide **36** in 89% yield (231 mg, 0.222 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.90 – 7.79 (m, 4H, Ar-*H*), 7.79 – 7.74 (m, 2H, Ar-*H*), 7.71 – 7.64 (m, 3H, Ar-*H*), 7.55 – 7.46 (m, 4H, Ar-*H*), 7.44 – 7.27 (m, 20H, Ar-*H*), 4.98 (d, *J* = 3.7 Hz, 1H, H-1'), 4.95 (d, *J* = 7.6 Hz, 1H, H-1), 4.91 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.83 (s, 2H, Ar-CH_2), 4.75 (d, *J* = 8.2 Hz, 2H, Ar-CH_2), 4.71 (d, *J* = 4.2 Hz, 1H, Ar-CH_2), 4.60 (d, *J* = 11.5 Hz, 1H, Ar-CH_2), 4.20 – 4.12 (m, 1H, H-2), 4.06 (dd, *J* = 11.0, 2.8 Hz, 1H, H-3), 4.00 (dd, *J* = 10.6, 3.6 Hz, 1H, H-2'), 3.94 – 3.84 (m, 2H, H-5, H-3'), 3.60 (dd, *J* = 2.7, 1.2 Hz, 1H, H-4), 3.48 (d, *J* = 2.7 Hz, 1H, H-4'), 3.31 (q, *J* = 6.5 Hz, 1H, H-5'), 1.10 (s, 9H, (CH_3)$_3$), 1.08 (d, , *J* = 6.4 Hz, 3H, H-6), 1.06 (d, *J* = 6.4 Hz, 3H, H-6'). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 161.82 (Ar-C_q), 138.86 (Ar-C_q), 138.09 (Ar-C_q), 136.33 (Ar-*C*), 136.18 (Ar-*C*), 136.10 (Ar-*C*), 135.95 (Ar-*C*), 135.08 (Ar-C_q), 133.68 (Ar-C_q), 133.46 (Ar-C_q), 133.38 (Ar-C_q), 133.13 (Ar-C_q), 129.68 (Ar-*C*), 129.58 (Ar-*C*), 128.65 (Ar-*C*), 128.45 (Ar-*C*), 128.42 (Ar-*C*), 128.38 (Ar-*C*), 128.07 (Ar-*C*), 127.94 (Ar-*C*), 127.82 (Ar-*C*), 127.63 (Ar-*C*), 127.51 (Ar-*C*), 127.29 (Ar-*C*), 127.28 (Ar-*C*), 126.48 (Ar-*C*), 126.34 (Ar-*C*), 126.17 (Ar-*C*), 125.66 (Ar-*C*), 99.52 (C-1'), 94.97 (C-1), 79.19 (C-4'), 78.48 (C-3), 78.44 (C-3'), 75.81 (C-4), 75.11 (Ar-*C*H_2), 75.01 (Ar-*C*H_2), 72.32 (Ar-*C*H_2), 70.60 (C-5'), 67.68 (C-5), 60.37 (C-2'), 57.45 (C-2), 27.12((*C*H_3)$_3$), 16.88(C-6), 16.76(C-6'). **HRMS**: $[M+Na]^+$ calculated for $C_{55}H_{59}Cl_3N_4O_8SiNa$: 1059.30654; found 1059.30600

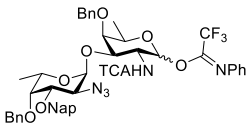
2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-deoxy-2-*N*-trichloroacetamide-4-*O*-benzyl-α/β-D-fucopyranose (37)



36 (196 mg, 0.189 mmol) was dissolved in THF (1.9 mL, 0.1 M) and cooled to 0 °C. AcOH (16 µL, mmol, 1.5 equiv.) and TBAF (1 M in THF; 0.3 mL, mmol, 1.5 equiv.) were added and the reaction was stirred overnight at rt under N_2 until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction was quenched with NH_4Cl (sat. aq.), diluted in EtOAc, washed with H_2O (x3) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 80:20 → 50:50) furnished hemiacetal **37** in 96% (145 mg, 0.181 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.35 – 7.28 (m, 8H, Ar-*H*), 7.24 – 7.19 (m, 2H, Ar-*H*), 7.15 – 7.11 (m, 2H, Ar-*H*), 6.86 – 6.77 (m, 2H, Ar-*H*), 5.47 (t, *J* = 5.3 Hz, 1H, H-1), 5.07 (d, *J* = 12.2 Hz, 1H, Ar-CH_2), 4.96 (d, *J* = 12.2 Hz, 1H, Ar-CH_2), 4.58 – 4.49 (m, 5H, H-5, Ar-CH_2), 4.13 (dd, *J* = 6.2, 5.2 Hz, 1H, H-4), 3.92 (dd, *J* = 6.2, 3.1 Hz, 1H, H-3), 3.80 (s, 3H, Ar-CH_3), 3.73 (dd, *J* = 5.6, 3.1 Hz, 1H, H-2). **^{13}C NMR (101 MHz, $CDCl_3$)** δ

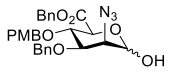
169.33 (*C*=O), 159.51 (Ar-C_q), 137.33 (Ar-C_q), 135.16 (Ar-C_q), 129.76 (Ar-*C*), 129.49 (Ar-C_q), 128.76 (Ar-*C*), 128.72 (Ar-*C*), 128.67 (Ar-*C*), 128.59 (Ar-*C*), 128.55 (Ar-*C*), 128.08 (Ar-*C*), 128.06 (Ar-*C*), 127.94 (Ar-*C*), 113.96 (Ar-*C*), 92.18 (C-1), 77.26 (C-3), 74.48 (C-4), 72.90 (Ar-*C*H_2), 72.73 (C-5), 67.38 (Ar-*C*H_2), 60.97 (C-2), 55.39 (PMB-*C*H_3). **HRMS**: $[M+Na]^+$ calculated for $C_{39}H_{41}Cl_3N_4O_8Na$: 821.18877; found 821.18822

2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-deoxy-2-*N*-trichloroacetamide-4-*O*-benzyl-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-fucopyranose (38)



37 (183 mg, 0.228 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (1.2 mL, 0.2 M). K_2CO_3 (63 mg, 0.456 mmol, 2 equiv.) and ClC(=NPh)CF_3 (0.075 mL, 0.456 mmol, 2 equiv.) were added and the reaction was stirred overnight under N_2 at rt until TLC (pentane/EtOAc, 7:3) showed full conversion. The mixture was filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 90:10 → 70:30) furnished imidate donor **38** in 89% (186 mg, 0.203 mmol). **^{1}H NMR (400 MHz, CD_3CN)** δ 7.94 – 7.67 (m, 7H), 7.64 – 7.40 (m, 6H), 7.42 – 7.19 (m, 16H), 7.12 (t, *J* = 7.5 Hz, 1H), 6.81 (d, *J* = 7.6 Hz, 2H), 6.56 – 6.17 (m, 1H), 5.23 (s, 1H), 4.98 – 4.85 (m, 3H), 4.85 – 4.78 (m, 1H), 4.74 (d, *J* = 11.6 Hz, 1H), 4.68 (d, *J* = 2.8 Hz, 1H), 4.64 (d, *J* = 11.1 Hz, 2H), 4.52 (d, *J* = 8.7 Hz, 1H), 4.27 (t, *J* = 7.3 Hz, 1H), 4.17 – 4.00 (m, 3H), 4.00 – 3.88 (m, 4H), 1.18 (s, 17H). **^{13}C NMR (101 MHz, CD_3CN)** δ 163.97, 139.76, 139.69, 136.61, 129.84, 129.33, 129.24, 129.18, 129.12, 129.05, 129.01, 128.97, 128.93, 128.87, 128.71, 128.68, 128.62, 128.56, 127.58, 127.33, 127.20, 127.16, 127.02, 126.89, 100.41, 98.47, 79.22, 78.53, 78.20, 77.39, 77.05, 76.37, 76.29, 75.89, 75.57, 72.27, 72.10, 71.66, 70.77, 69.28, 68.22, 66.88, 61.26, 55.13, 52.77, 29.26, 17.14, 17.06, 16.85. **HRMS**: $[M+Na]^+$ calculated for $C_{47}H_{45}Cl_3F_3N_3O_8Na$: 992.21835; found 992.21808

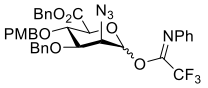
Benzyl (2-azido-2-deoxy-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl)-α/β-D-mannopyranosiduronate (39)



10 (249 mg, 0.407 mmol) was co-evaporated with toluene (x3) and dissolved in dry DCM (4 mL, 0.1 M) and cooled to 0 °C. NIS (137 mg, 0.610 mmol, 1.5 equiv.) and TFA (0.03 mL, 0.407 mmol, 1 equiv.) were added and the reaction stirred at 0 °C under N_2 for 1 h until TLC (pentane/EtOAc, 75:25) showed full conversion. The reaction was quenched with Et_3N and added $NaHCO_3$ (sat. aq.) and stirred vigorously. The mixture was diluted in EtOAc and 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, 80:20 → 65:35) furnished hemiacetal **39** in 91% (192 mg, 03369 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.35 – 7.28 (m, 8H, Ar-*H*), 7.24 – 7.19 (m, 2H, Ar-*H*), 7.15 – 7.11 (m, 2H, Ar-*H*), 6.86 – 6.77 (m, 2H, Ar-*H*), 5.47 (t, *J* = 5.3 Hz, 1H, H-1), 5.07 (d, *J* = 12.2 Hz, 1H, Ar-CH_2), 4.96 (d, *J* = 12.2 Hz, 1H, Ar-CH_2), 4.58 – 4.49 (m, 5H, Ar-CH_2, H-5), 4.13 (dd, *J* = 6.2, 5.2 Hz, 1H, H-4), 3.92 (dd, *J* = 6.2, 3.1 Hz, 1H, H-3), 3.80 (m, 3H, CH_3-PMB), 3.73 (dd, *J* = 5.6, 3.1 Hz, 1H, H-2). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.33 (*C*=O), 159.51 (Ar-C_q), 137.33 (Ar-C_q), 135.16 (Ar-C_q), 129.76 (Ar-*C*), 129.49 (Ar-C_q), 128.76 (Ar-*C*), 128.72 (Ar-*C*), 128.67 (Ar-*C*), 128.59 (Ar-*C*), 128.55 (Ar-*C*), 128.08 (Ar-*C*), 128.06 (Ar-*C*), 127.94 (Ar-*C*), 113.96 (Ar-*C*), 92.18 (C-1), 77.26 (C-3), 74.48 (C-4), 73.19 (Ar-*C*H_2), 72.90

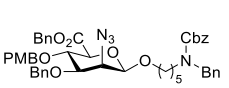
(Ar-*C*H$_2$), 72.73 (C-5), 67.38 (Ar-*C*H$_2$), 60.97 (C-2), 55.39 (*C*H$_3$-PMB). **HRMS**: [M+Na]$^+$ calculated for $C_{28}H_{29}N_3O_7Na$: 542.19032; found 543.18942

Benzyl (2-azido-2-deoxy-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl))-α/β-D-mannopyranosiduronate (40)



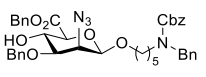
39 (291 mg, 0.559 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (2.8 mL, 0.2 M). K_2CO_3 (155 mg, 1.12 mmol, 2 equiv.) and ClC(=NPh)CF_3 (0.18 mL, 1.12 mmol, 2 equiv.) were added to the reaction and it was stirred overnight under N_2 at rt until TLC (pentane/EtOAc, 7:3) showed full conversion. The mixture was filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 90:10 → 70:30) furnished imidate donor **40** in 87% (334 mg, 0.484 mmol**). ^{1}H NMR (400 MHz, CD$_3$CN)** δ 7.40 – 7.25 (m, 18H), 7.21 – 7.11 (m, 4H), 6.88 – 6.80 (m, 5H), 5.19 – 5.09 (m, 1H), 5.09 – 5.00 (m, 2H), 4.69 – 4.59 (m, 4H), 4.50 (d, *J* = 10.9 Hz, 1H), 4.35 (m, 2H), 4.14 – 4.03 (m, 3H), 3.78 (s, 3H), 3.76 (s, 1H). **^{13}C NMR (101 MHz, CD$_3$CN)** δ 130.71, 129.84, 129.53, 129.45, 129.37, 129.33, 129.29, 129.11, 128.95, 114.60, 78.09, 74.84, 74.78, 73.35, 68.02, 59.99, 55.82, 29.65. **HRMS**: [M+Na]$^+$ calculated for $C_{36}H_{33}F_3N_4O_7Na$: 713.21990; found 713.21935

5-(Benzyl(benzyloxycarbonyl)amino)pentyl benzyl(2-azido-2-deoxy-4-*O*-*p*-methoxybenzyl-3-*O*-benzyl)-β-D-mannopyranosiduronate (41)



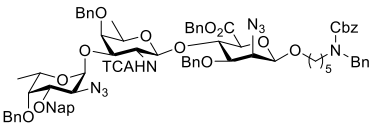
Donor **40** (244 mg, 0.353 mmol, 1 equiv.) and acceptor **12** (150 mg, 0.459 mmol, 1.3 equiv.) were co-evaporated with toluene (x3), dissolved in dry DCM (2.4 mL, 0.1 M), added activated 3Å molecular sieves and stirred for 30 min under argon at rt. The reaction mixture was cooled to -78 °C, followed by addition of TBSOTf (16 µL, 0.0706 mmol, 0.2 equiv.). The mixture was allowed to warm to -30 °C and stirred for 1 h until TLC (pentane/EtOAc, 8:2) showed full conversion. The reaction was quenched with Et_3N and diluted in EtOAc, washed with NaHCO3 (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 85:15 → 70:30) yielded **41** in 69% (α: 42 mg, 0.0511 mmol; β: 157 mg, 0.192 mmol) in a α/β = 21:79. **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.41 – 7.27 (m, 20H), 7.06 – 7.02 (m, 2H, Ar-*H*), 6.81 – 6.76 (m, 2H, Ar-*H*), 5.28 – 5.14 (m, 4H, Ar-C*H*$_2$), 4.77 – 4.64 (m, 3H, Ar-C*H*$_2$), 4.50 (d, *J* = 6.9 Hz, 3H, Ar-C*H*$_2$, H-1), 4.40 (d, *J* = 10.2 Hz, 1H, Ar-C*H*$_2$), 4.05 (t, *J* = 9.3 Hz, 1H, H-4), 3.95 – 3.86 (m, 2H, H-3, H-2), 3.86 – 3.81 (m, 1H, C*H*$_2$-Linker), 3.78 (s, 4H, C*H*$_3$-PMB), 3.65 – 3.59 (m, 1H, H-5), 3.45 – 3.15 (m, 3H, C*H*$_2$-Linker), 1.59 – 1.49 (m, 3H, C*H*$_2$-Linker), 1.37 – 1.27 (m, 3H, C*H*$_2$-Linker). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 167.93 (C=O), 159.39 (C=O), 138.02 (Ar-*C*$_q$), 137.49 (Ar-*C*$_q$), 135.27 (Ar-*C*$_q$), 129.78 (Ar-*C*), 128.73 (Ar-*C*), 128.69 (Ar-*C*), 128.65 (Ar-*C*), 128.58 (Ar-*C*), 128.55 (Ar-*C*), 128.21 (Ar-*C*), 128.02 (Ar-*C*), 127.96 (Ar-*C*), 127.94 (Ar-*C*), 127.37 (Ar-*C*), 113.83 (Ar-*C*), 100.31 (C-1), 79.99 (C-5), 75.37 (C-4), 74.70 (Ar-*C*H$_2$), 72.37 (Ar-*C*H$_2$), 70.06 (*C*H$_2$-Linker), 67.41 (Ar-*C*H$_2$), 61.64 (C-3), 55.38 (C-2), 50.60 (*C*H$_2$-Linker), 29.81 (*C*H$_2$-Linker), 29.18 (*C*H$_2$-Linker), 23.17 (*C*H$_2$-Linker). **HRMS**: [M+Na]$^+$ calculated for $C_{48}H_{52}N_4O_9Na$: 851.36320; found 851.36265

5-(Benzyl(benzyloxycarbonyl)amino)pentyl benzyl(2-azido-2-deoxy-3-*O*-benzyl)-β-D-mannopyranosiduronate (42)



41 (121 mg, 0.148 mmol) was dissolved in DCM/H_2O (20:1, 1.5 mL, 0.1 M) and added DDQ (67 mg, 0.296 mmol, 2 equiv.) and stirred at rt under N_2 for 2 h until TLC (pentane/EtOAc, 7:3) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (sat. aq.) and diluted/extracted with EtOAc (x3). The combined organic phases were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 80:20 → 50:50) yielded **42** in 92% (96 mg, 0.136 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.42 – 7.27 (m, 17H, Ar-*H*), 7.20 (m, 2H, Ar-*H*), 5.23 (d, *J* = 8.1 Hz, 2H, Ar-CH_2), 5.17 (d, *J* = 11.2 Hz, 2H, Ar-CH_2), 4.76 (d, *J* = 2.0 Hz, 2H, Ar-CH_2), 4.51 – 4.41 (m, 3H, H-1, Ar-CH_2), 4.14 (td, *J* = 9.4, 2.6 Hz, 1H, H-4), 3.95 – 3.79 (m, 2H, H-2, CH_2-Linker), 3.79 – 3.69 (m, 1H, H-5), 3.53 – 3.32 (m, 2H, H-3, CH_2-Linker), 3.30 – 3.11 (m, 2H, CH_2-Linker), 2.92 (dt, *J* = 2.6, 1.1 Hz, 1H, OH), 1.64 – 1.44 (m, 3H, CH_2-Linker), 1.40 – 1.19 (m, 3H, CH_2-Linker). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.02 (Ar-C_q), 137.50 (Ar-C_q), 128.80 (Ar-*C*), 128.75 (Ar-*C*), 128.67 (Ar-*C*), 128.41 (Ar-*C*), 128.30 (Ar-*C*), 128.04 (Ar-*C*), 127.95 (Ar-*C*), 127.39 (Ar-*C*), 101.82 (C-1), 79.82 (C-3), 75.60 (C-5), 72.15 (Ar-*C*H_2), 70.12 (*C*H_2-Linker), 68.36 (C-4), 67.54 (Ar-*C*H_2), 67.28 (Ar-*C*H_2), 61.56 (C-2), 50.70 (*C*H_2-Linker), 29.22 (*C*H_2-Linker), 23.20 (*C*H_2-Linker). **HRMS**: $[M+Na]^+$ calculated for $C_{40}H_{44}N_4O_8Na$: 731.30568; found 731.30514

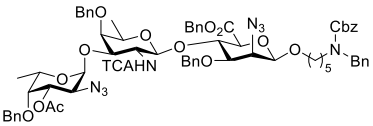
5-(Benzyl(benzyloxycarbonyl)amino)pentyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-deoxy-2-*N*-trichloroacetamide-4-*O*-benzyl-β-D-fucopyranosyl-(1→4) benzyl(2-azido-2-deoxy-3-*O*-benzyl)-β-D-mannopyranosiduronate (43)



Acceptor **38** (88 mg, 0.125 mmol, 1 equiv.) and donor **42** (186 mg, 0.203 mmol, 1.6 equiv.) were co-evaporated with toluene (x3), dissolved in dry DCM (1.2 mL, 0.1 M), added activated 3Å molecular sieves and stirred for 30 min under argon at rt. The mixture was cooled to -78 °C, after which TBSOTf (6 µL, 0.0244 mmol, 0.2 equiv.) was added. The reaction mixture was stirred for 1 h until TLC (pentane/EtOAc, 7:3) showed full conversion. The reaction mixture was quenched with Et_3N, diluted in EtOAc, washed with $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 75:25 → 65:35) furnished **43** in 81% yield (153 mg, 0.101 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.88 – 7.71 (m, 5H, Ar-*H*), 7.53 – 7.43 (m, 4H, Ar-*H*), 7.43 – 7.26 (m, 31H, Ar-*H*), 7.25 – 7.12 (m, 6H, Ar-*H*), 6.93 (d, *J* = 8.5 Hz, 1H, N*H*), 5.18 (d, *J* = 10.7 Hz, 2H, Ar-CH_2), 4.96 (d, *J* = 3.7 Hz, 1H, H-1''), 4.95 – 4.89 (m, 2H, Ar-CH_2), 4.88 – 4.76 (m, 5H, Ar-CH_2), 4.72 – 4.63 (m, 3H, Ar-CH_2), 4.60 (dd, *J* = 11.5, 4.6 Hz, 2H, Ar-CH_2), 4.49 (d, *J* = 8.4 Hz, 4H, CH_2-Linker, H-1', H-1), 4.28 (t, *J* = 8.2 Hz, 1H, H-4), 4.20 (dt, *J* = 11.0, 8.4 Hz, 1H, H-2'), 4.04 (dd, *J* = 10.6, 3.6 Hz, 1H, H-2''), 3.93 – 3.90 (m, 1H, H-5''), 3.89 (d, *J* = 2.1 Hz, 1H, H-3''), 3.85 (d, *J* = 8.3 Hz, 1H, H-5), 3.82 – 3.71 (m, 3H, H-2), 3.69 – 3.57 (m, 3H, H-3', H-4'', H-3), 3.50 – 3.39 (m, 2H, H-4', CH_2-Linker), 3.34 (d, *J* = 7.2 Hz, 1H, H-5'), 3.31 – 3.14 (m, 3H, CH_2-Linker), 1.62 – 1.46 (m, 2H, CH_2-Linker), 1.39 – 1.21 (m, 6H, CH_2-Linker), 1.17 (d, *J* = 6.3 Hz, 3H, H-6''), 1.10 (d, *J* = 6.5 Hz, 3H, H-6'). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 168.82 (C=O), 161.94 (C=O), 138.67 (Ar-C_q), 138.28 (Ar-C_q), 138.14 (Ar-C_q), 138.03 (Ar-C_q),

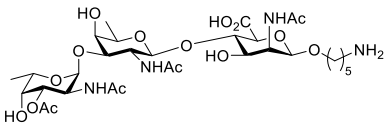
135.35 (Ar-C_q), 135.10 (Ar-C_q), 133.37 (Ar-C_q), 133.13 (Ar-C_q), 128.96 (Ar-*C*), 128.77 (Ar-*C*), 128.73 (Ar-*C*), 128.68 (Ar-*C*), 128.63 (Ar-*C*), 128.49 (Ar-*C*), 128.43 (Ar-*C*), 128.39 (Ar-*C*), 128.31 (Ar-*C*), 128.29 (Ar-*C*), 128.09 (Ar-*C*), 128.02 (Ar-*C*), 127.95 (Ar-*C*), 127.93 (Ar-*C*), 127.81 (Ar-*C*), 127.77 (Ar-*C*), 127.72 (Ar-*C*), 127.63 (Ar-*C*), 127.53 (Ar-*C*), 127.34 (Ar-*C*), 126.57 (Ar-*C*), 126.45 (Ar-*C*), 126.32 (Ar-*C*), 126.16 (Ar-*C*), 125.73 (Ar-*C*), 125.57 (Ar-*C*), 99.84 (C-1', C-1), 99.69 (C-1''), 79.60 (C-3'), 78.66 (C-5''), 78.42 (C-4'), 75.98 (C-3), 75.69 (C-4''), 75.08 (Ar-*C*H_2), 74.45 (C-4, C-5), 73.43 (Ar-*C*H_2), 72.50 (Ar-*C*H_2), 70.85 (C-5'), 70.04 (C-3''), 67.86 (Ar-*C*H_2), 67.50 (Ar-*C*H_2), 67.24 (Ar-*C*H_2), 6-.96 (C-2), 60.32 (C-2''), 54.45 (C-2'), 50.59 (CH_2-Linker), 50.27 (CH_2-Linker), 46.95 (CH_2-Linker), 29.82 (CH_2-Linker), 29.15 (CH_2-Linker), 23.19 (CH_2-Linker), 16.99 (C-6', C-6''), 16.91 (C-6', C-6''). **HRMS**: $[M+Na]^+$ calculated for $C_{79}H_{83}Cl_3N_8O_{15}Na$: 1511.49412; found 1511.49357

5-(Benzyl(benzyloxycarbonyl)amino)pentyl 3-*O*-Acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-deoxy-2-*N*-trichloroacetamide-4-*O*-benzyl-β-D-fucopyranosyl-(1→4) benzyl(2-azido-2-deoxy-3-*O*-benzyl)-β-D-mannopyranosiduronate (44)



43 (143 mg, 0.095 mmol) was dissolved in DCM/H_2O (4:1, 2 mL, 0.05 M) and added DDQ (43 mg, 0.19 mmol, 2 equiv.). The reaction was stirred at rt under N_2 for 3 h until TLC (pentane, EtOAc, 7:3) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (sat. aq.) and extracted with EtOAc (x3). The combined organic phases were washed with $NaHCO_3$ (sat. aq.; x4) and brine (x1), dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was used without further purification. The residue was dissolved in pyridine (2 mL) and cooled to 0 °C and added Ac_2O (0.3 mL) and DMAP (catalytic amount) and stirred at rt under N_2 overnight until TLC (pentane/EtOAc, 7:3) showed full conversion. The mixture was dissolved in EtOAc, washed with 1 M HCl (x1), $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 8:2 → 6:4) yielded **44** in 92% yield (123 mg, 0.087 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.44 – 7.27 (m, 36H), 7.25 – 7.10 (m, 8H), 6.88 (d, *J* = 8.3 Hz, 1H), 5.25 (d, *J* = 12.2 Hz, 1H), 5.23 – 5.12 (m, 5H), 5.13 – 5.00 (m, 2H), 4.98 (d, *J* = 3.7 Hz, 1H), 4.90 – 4.83 (m, 3H), 4.71 – 4.63 (m, 4H), 4.62 – 4.55 (m, 2H), 4.56 – 4.53 (m, 2H), 4.52 – 4.45 (m, 4H), 4.31 – 4.23 (m, 1H), 4.16 – 4.04 (m, 2H), 3.98 – 3.88 (m, 3H), 3.88 – 3.80 (m, 3H), 3.79 – 3.70 (m, 3H), 3.62 (dd, *J* = 7.9, 3.6 Hz, 2H), 3.51 – 3.38 (m, 2H), 3.33 – 3.12 (m, 4H), 2.06 (d, *J* = 6.3 Hz, 4H), 1.39 – 1.19 (m, 10H), 1.19 (d, *J* = 6.3 Hz, 4H), 1.11 (d, *J* = 6.5 Hz, 5H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.27, 161.92, 138.39, 138.21, 138.03, 137.62, 135.38, 128.91, 128.76, 128.72, 128.67, 128.64, 128.61, 128.56, 128.51, 128.40, 128.34, 128.32, 128.24, 128.02, 127.94, 127.68, 127.58, 127.36, 99.85, 99.51, 78.84, 78.28, 75.82, 74.94, 74.37, 73.28, 72.28, 70.94, 70.05, 67.47, 67.32, 67.25, 58.18, 54.78, 50.52, 50.07, 46.82, 46.26, 29.15, 23.19, 20.98, 17.11, 16.59. **HRMS**: $[M+Na]^+$ calculated for $C_{70}H_{77}Cl_3N_8O_{16}Na$: 1413.44308; found 1413.48622

5-aminopentyl 3-*O*-Acetyl-2-acetamide-deoxy-α-L-fucopyranosyl-(1→3)-2-deoxy-2-acetamide-β-D-fucopyranosyl-(1→4) 2-acetamide-2-deoxy-β-D-mannopyranosiduronate (CP5-III)



44 (36 mg, mmol) was dissolved in THF (distilled, 3 mL) and added zinc powder (500 mg, 7.69 mmol, 300 equiv.), AcOH (1 mL) and Ac_2O (0.5 mL). The resulting mixture was stirred at 50 °C overnight until TLC (DCM/MeOH, 95:5) showed full conversion. The cooled mixture was filtered through Celite, evaporated *in vacuo* and co-evaporated with toluene (x3). The crude product was first purified by column chromatography (DCM/MeOH, 98:2 → 90:10) followed by HPLC given the acetamide product in 35 % yield (12 mg, 0.0091 mmol). SM (12 mg, 0.0091 mmol) was dissolved in *t*-BuOH (2.5 mL) and added AcOH (1 mL, 0.1 mL in 100 mL MilliQ). 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 for 3 days under H_2 atmosphere until NMR showed full conversion, after which it was burched with argon for 20 min, filtered over a Whatman filter and lyophilized. Purification by a HW40 column with NH_4OAc followed by lyophilization gave **CP5-III** in 57% yield (4.3 mg, 0.0058 mmol). However migration of the 3-*O*-acetyl on the L-Fuc residue to the C-4 of the L-Fuc was observed. NMR reported for the migrated product. **^{1}H NMR (600 MHz, D_2O)** δ 5.25 – 5.23 (m, 0H), 5.17 (dd, *J* = 11.4, 3.1 Hz, 0H), 5.02 (dd, *J* = 7.8, 3.8 Hz, 1H), 4.96 (d, *J* = 4.0 Hz, 0H), 4.75 (s, 2H), 4.49 (dd, *J* = 4.3, 1.4 Hz, 1H), 4.45 – 4.35 (m, 1H), 4.25 (q, *J* = 6.7 Hz, 1H), 4.20 – 4.13 (m, 1H), 4.13 – 4.07 (m, 1H), 4.00 – 3.90 (m, 2H), 3.88 – 3.73 (m, 7H), 3.69 – 3.62 (m, 2H), 2.99 – 2.95 (m, 2H), 2.22 (s, 2H), 2.09 (s, 1H), 2.06 (s, 3H), 2.03 (s, 2H), 2.02 – 1.97 (m, 4H), 1.90 (s, 1H), 1.69 – 1.56 (m, 4H), 1.44 – 1.35 (m, 2H), 1.29 – 1.24 (m, 3H), 1.21 (dd, *J* = 6.6, 4.5 Hz, 2H), 1.12 (d, *J* = 6.6 Hz, 2H). **^{13}C NMR (151 MHz, D_2O)** δ 176.12, 175.52, 175.45, 175.17, 175.12, 175.07, 174.84, 174.11, 102.03, 100.31, 99.99, 79.28, 79.19, 78.38, 78.31, 78.29, 77.86, 77.71, 77.54, 74.34, 71.94, 71.88, 71.67, 71.29, 71.23, 70.72, 69.70, 68.51, 68.05, 67.77, 66.98, 66.87, 54.61, 53.02, 52.12, 50.76, 50.40, 48.04, 40.30, 28.87, 27.22, 23.43, 23.39, 23.15, 23.09, 22.97, 22.95, 22.94, 21.12, 16.29, 16.22, 16.16, 16.13. **HRMS**: $[M+H]^+$ calculated for $C_{31}H_{52}N_4O_{16}H$: 737.34566; found 737.34511

Supporting information

SPR experiments

The SPR experiments were conducted using a Surface Plasmon Resonance Biacore X100 from GE Healthcare Biacore Life Science. CP5-biotin (CP5-biotin, lot EB23GIU16, M=351.6 µg/mL) was immobilized on a SA-chip using a 20 µg/mL solution. After the run 311.7 AU was immobilized on the chip. For the SPR-experiments a 20 nM rat anti-CP5 mAb 331 concentration was used together with the CP5-OS concentrations as summarized in Table S1. From the Biacore X100 control software the binding levels were collected and used for calculation of the inhibition percentage.

Competitor concentrations (µg/mL)	CP5-I	CP5-II	Hexasaccharide	Nonasaccharide	CP5-PS
	1000	50	20	5	0.0781
	500	25	10	2.5	0.0391
	250	12.5	5	1.25	0.0195
	125	6.25	2.5	0.625	0.00977
	62.5	3.125	1.25	0.313	0.00488
	31.25	1.563	0.625	0.156	0.00244
	15.63	0.781	0.313	0.0781	0.00122
	7.81	0.391	0.156	0.0391	0.000610
	3.91	0.195	0.0781	0.0195	0.000305
	1.95	0.0977	0.0391	0.00977	0.000153
	0.977	0.0488	0.0195	0.00488	7.629E-05
	0.488	0.0244	0.00977	0.00244	3.815E-05
	0	0	0	0	0

Table S1: CP5-OS concentrations for the SPR-experiments

SPR IC50 values

The calculation of IC50 values were performed with GraphPad Prism software using Kruskal-Wallis with Dunn's multiple comparisons; "***" denotes the significant result within $p < 0.001$, "ns" means not significant.

Structural studies

Acknowledgements: Luca Unione from CIC BioGune is acknowledged for his contribution to the conformational analysis.

To gain insight into the structure of the trisaccharide that showed best binding, **CP5-II** was investigated using a combination of NMR-methodologies (*J*-couplings and NOE-interactions) and computational protocols (MM). As depicted in Figure 3 and as analyzed by the intra-residual NOE and *J*-couplings, the three pyranosides (C: D-ManNAcA, B: L-FucNAc and A: D-FucNAc) were found to adopt the common chair conformations ($^4C_1$ for the D-ManNAcA and D-FucNAc and $^1C_4$ for L-FucNAc), in line with the structural findings in Chapter 3 for the **CP5-I** trisaccharide. For the conformation around the C-A bond, the strong NOE between the H1(A)-H4(C)/ H6(C) proton pair and the H2(A)-H6(C) proton pair, suggested, together with the predicted MM calculations, a conformational equilibrium between the exo-*syn*-ϕ/*syn*(+)-ψ and the exo-*syn*-ϕ/*syn*(-)-ψ conformations with the exo-*syn*-ϕ/*syn*(+)-ψ being the major populated on. For the C-B glycosidic linkage the strong NOE between the H1(B)-H4(C) proton pair and the

weaker NOE between the H1(B)-H3(C) and H1(B)- H5(C) proton pairs suggested an exo-*syn*-ϕ/*syn*(-)-ψ conformation.

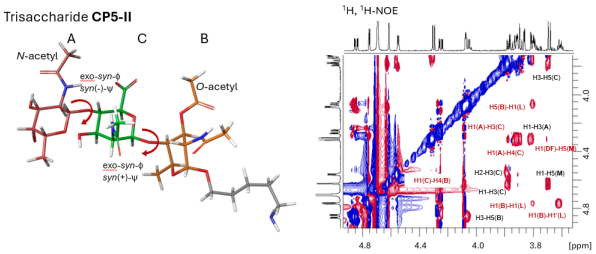


Figure 3: Conformational analysis of **CP5-II** as established by NMR and MM calculations. Zoom area of 2D NOESY spectrum of **CP5-II** and its main conformation as defined by NOE analysis and MM calculations. Monosaccharidic residues are labeled with a letter code. The main conformation at each glycosidic linkage and the spatial orientation of the acetyl are reported.

Structure and conformational studies

NMR methods. NMR experiments were performed in a Bruker Avance III 800 MHz spectrometer equipped with a TCI cryoprobe. Samples were dissolved in D_2O at 1.0 mM concentration. Experiments were acquired at the temperature of 298 K.

^{1}H and ^{13}C NMR resonances of the molecules **CP5-II** were assigned through standard 2D-TOCSY, 2D-ROESY, 2D-NOESY, 2D ^{1}H-^{13}C-HSQC. 2D-TOCSY experiments were acquired with 30 ms mixing time, 1.0 s of relaxation delay, 4 scans, and 4096x256 (F2xF1) points with a spectral width of 6556.0 Hz. 2D-ROESY experiment was acquired with mixing time of 200 ms, 1.0 s of relaxation delay, 48 scans, and 4096x256 (F2xF1) points with a spectral width of 6880.7 Hz. 2D-NOESY experiment was acquired with mixing time of 200 ms, 1.5 s of relaxation delay, 32 scans, and 4096x256 (F2xF1) points with a spectral width of 6242.2 Hz. 2D ^{1}H,^{13}C-HSQC experiments were acquired with 1.0 s of relaxation delay, 48 scans, and 4096x220 (F2xF1) points with a spectral width of 6250.0 Hz (F2) and 24144.6 Hz (F1). The data were processed with Topspin 4.2 (Bruker Biospin) using a 90° shifted qsine window function to a total of 16K × 2K data points (F2 × F1), followed by automated baseline- and phase correction.

Molecular Mechanics Calculations. The geometry optimization was performed by using the Jaguar/Schroedinger package (version 13.5) and the AMBER* force field, with the GB/SA continuum solvent model for water. The glycosidic torsion angles were defined as ϕ (H1'-C1'-Ox-Cx) and ψ (C1'-Ox-Cx-Hx). Extended nonbonded cut-off distances (van der Waals cut-off of 8.0 Å and electrostatic cut-off of 20.0 Å) were used. The conformers for **CP5-II** were generated employing geometric restrictions to respect the *exo*-anomeric effect. The possible staggered rotamers around ψ were selected and minimized. The coordinates of the obtained local minima were employed to measure the key inter-proton distances that were then compared to those obtained experimentally by the ROESY and NOESY NMR experiments through integration of the observed NOEs cross peaks using the ISPA approximation.

Reference

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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.

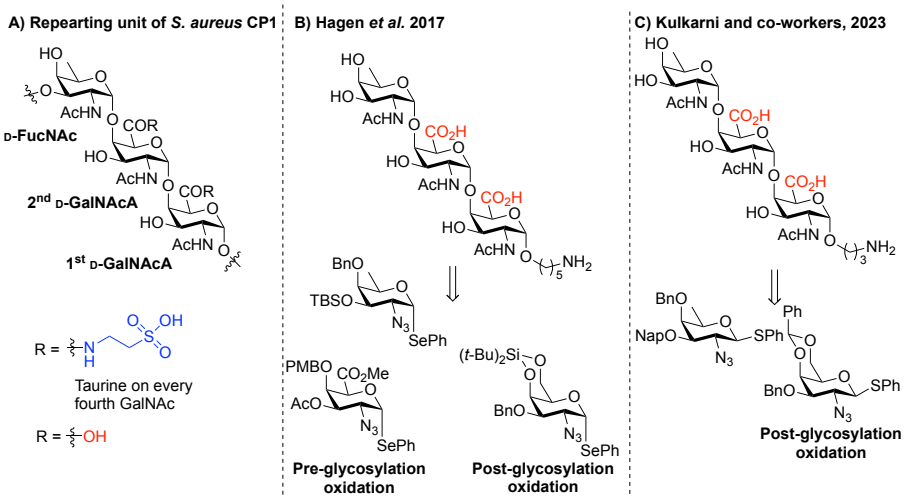


Figure 1: A) A schematic representation of the repeating unit of CP1 and the possible taurine pattern. B) Previous synthetic work of the CP1-trisaccharide by Hagen *et al.* C) Previous synthetic work of the CP1-trisaccharide by Kulkarni and co-workers.

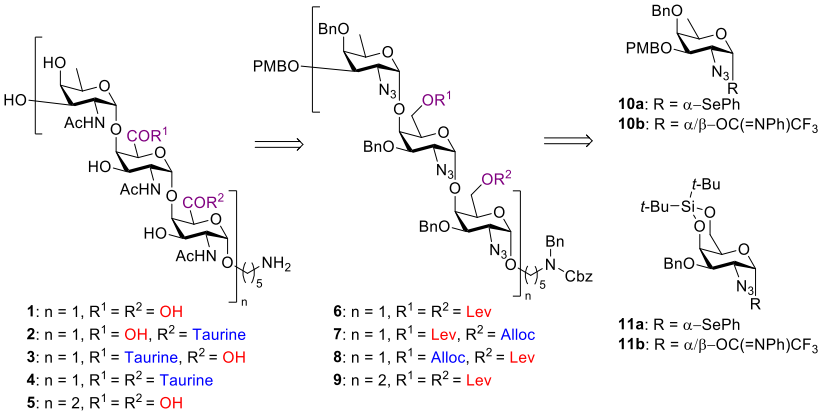
In 2017, the first reported synthesis of CP1 strain M was reported by Hagen *et al.* (Figure 1B).[10] The synthesis of a trisaccharide repeating unit (RU) relied on a post glycosylation oxidation strategy to ensure the 1,2-*cis* glycosylic linkages, through the use of silylene-protected galactosazide ($GalN_3$) 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.

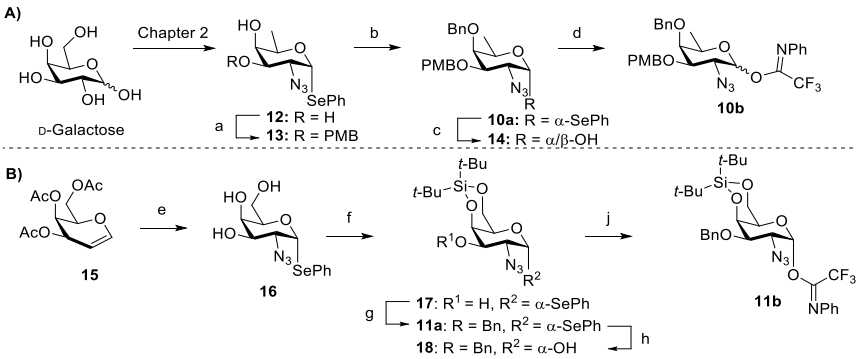


Scheme 1: Retrosynthetic analysis of the four different trisaccharides and one hexasaccharide.

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.

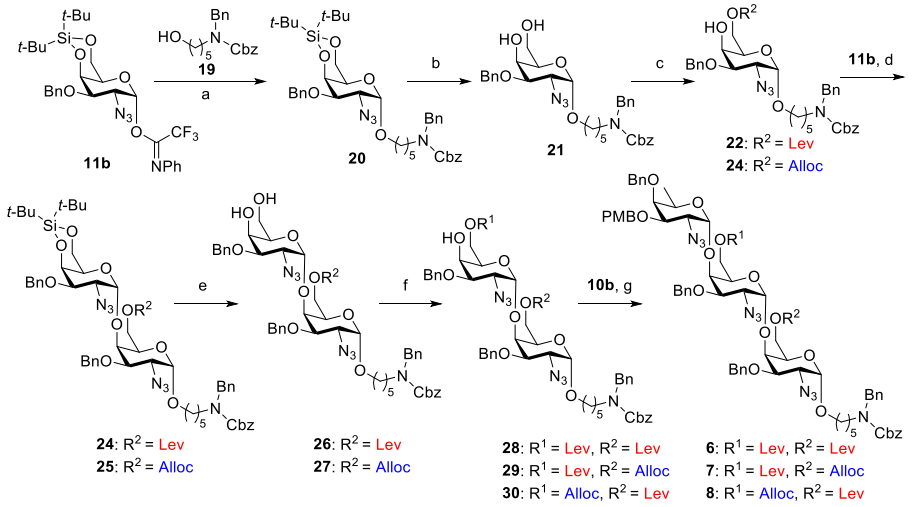
[iv] It was found that shorter reaction times (from overnight to 4 h) improved the yield from 56% to 78% of the benzylation due to hydrolysis of the anomeric seleno acetal.



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**).



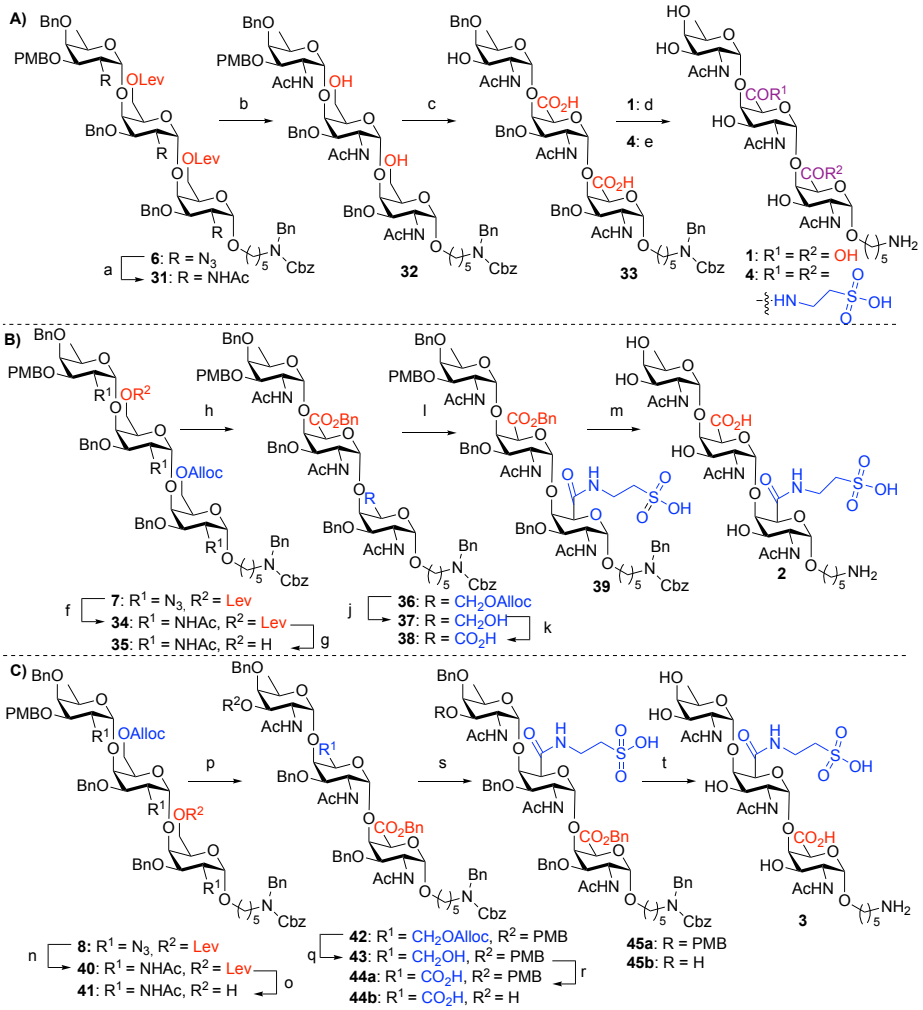
Scheme 3: Synthesis of the three different trisaccharide intermediates **6**, **7** and **8**. *Reaction conditions*: a) TBSOTf, DCM, 0 ºC, 98%, b) TBAF, THF, 91%, c) **22**: Bu_2SnO, toluene, Lev_2O, DCM, 93%; **23**: Alloc-Cl, pyridine, DCM, 84%. d) TBSOTf, DCM, 0 ºC, **24**: 82%, **25**: 86%, e) TBAF, 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.

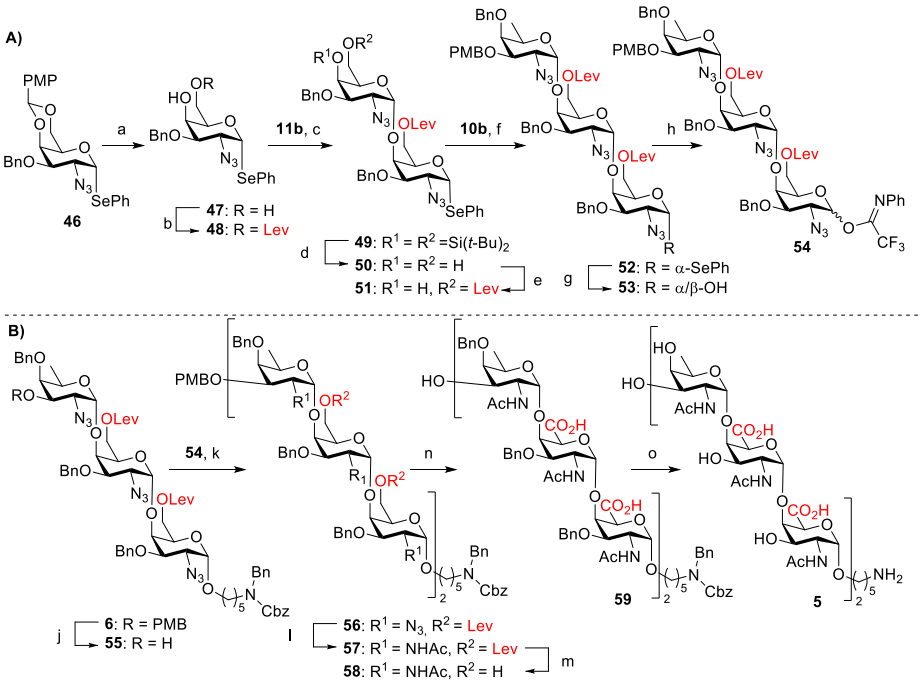
The second trisaccharide with taurines on both GalNAcA residues was obtained from intermediate **33** by installation of the taurine amide on both carboxylic acids using taurine in the presence of hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) and *N*,*N*-diisopropylethylamine (DIPEA). 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 $NaHCO_3$

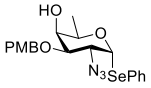
The starting material was dissolved in EtOAc/*t*-BuOH/H_2O (1:1:1), cooled to 0 ºC, added TEMPO (0.8 equiv. pr. hydroxy group) and $NaHCO_3$ (5 equiv. pr. hydroxy group) and stirred at 0 ºC for 10 min before adding BAIB (4 equiv. pr. hydroxy group). The reaction was stirred at 4 ºC until LC-MS showed 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.

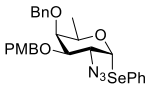
Synthesis of the building blocks

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



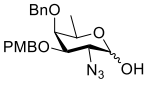
Phenyl 2-azido-2-deoxy-1-seleno-α-D-fucopyranoside **12** (3.61 g, 10.96 mmol) was co-evaporated with toluene (x3) and dissolved in dry toluene (50 ml, 0.2 M). Bu_2SnO (2.784 g, 11.18 mmol, 1.02 equiv.) was added. The flask was equipped with a Dean-Stark and the reaction was heated to 140 ºC for 3 h. 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)



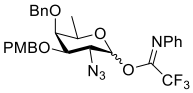
13 (4.278 g, 9.525 mmol) was dissolved in DMF (95 mL, 0.1 M) and cooled to 0 ºC. NaH (60% suspension in mineral oil, 495 mg, 12.38 mmol, 1.3 equiv.) and BnBr (1.5 mL, 12.38 mmol, 1.3 equiv.) were added and the solution was warmed to rt and stirred under N_2 for 4 h until TLC (pentane/EtOAc 9:1) showed 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-C*H*$_2$), 4.70 (d, *J* = 1.6 Hz, 2H, Ar-C*H*$_2$), 4.60 (d, *J* = 11.4 Hz, 1H, Ar-C*H*$_2$), 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-OC*H*$_3$), 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

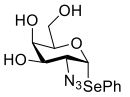
2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-fucopyranose (10b)



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.

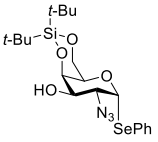
Phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-seleno-α-D-galactopyranoside (16)



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

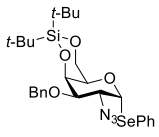
Phenyl 2-azido-2-deoxy-4,6-O-(di-*tert*-butylsilyene)-1-seleno-D-α-galactopyranoside (17)



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

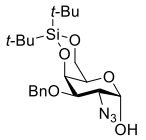
(pentane/EtOAc 100:0 → 85:15) gave **17** in 96% yield (8.665 g, 17.85 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.58 – 7.52 (m, 2H, Ar-*H*), 7.32 – 7.27 (m, 3H, Ar-*H*), 5.93 (d, *J* = 5.2 Hz, 1H, H-1), 4.49 (dd, *J* = 3.4, 1.2 Hz, 1H, H-4), 4.30 (dd, *J* = 12.7, 2.3 Hz, 1H, H-6), 4.20 (q, *J* = 1.8 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

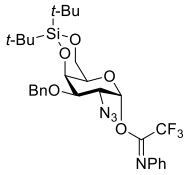
2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(di-*tert*-butylsilyene)-D-α-galactopyranoside (18)



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

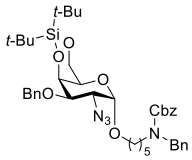
((CH_3)$_3$CSi), 23.54 ((CH_3)$_3$*C*Si), 20.82 ((CH_3)$_3$*C*Si). **HRMS**: $[M+Na]^+$ calculated for $C_{21}H_{33}N_5O_5SiNa$: 458.20872; found 458.20803

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)



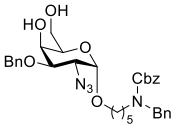
18 (3.431 g 7.859 mmol) was co-evaporated with toluene (x3) and dissolved in dry acetone (40 mL, 0.2 M). K_2CO_3 (1.629 g, 11.79 mmol, 1.5 equiv.) and ClC(=NPh)CF_3 (1.9 mL, 11.79 mmol, 1.5 equiv.) and was added and the reaction was stirred at rt under N_2 overnight until TLC (pentane/EtOAc,) showed 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)



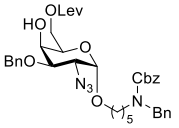
11b (1.353 mg, 2.291 mmol, 1 equiv.) and acceptor **19** (974 mg, 2.979 mmol, 1.3 equiv.) was co-evaporated with toluene (3x). The donor and acceptor was dissolved in dry DCM (23 mL, 0,1 M), added 3Å molecular sieves and stirred for 30 min. The solution was cooled to 0 ºC and added TBSOTf (0.1 mL, 0.458 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

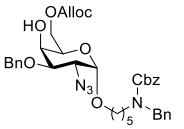
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (22)



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

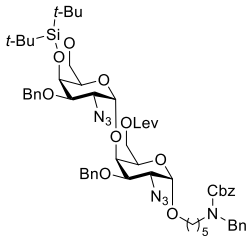
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (23)



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

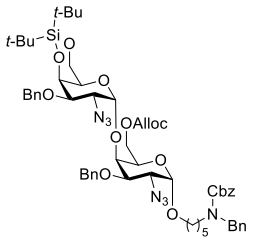
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(di-*tert*-butylsilyene)-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (24)



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,

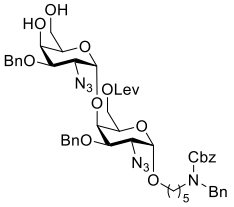
1.3 Hz, 1H, H-6'), 3.65 (dd, J = 12.7, 1.6 Hz, 2H, H-6', H-3), 3.58 (dd, J = 7.3, 3.5 Hz, 1H, H-2), 3.48 – 3.34 (m, 2H, CH_2-Linker), 3.28 – 3.16 (m, 2H, CH_2-Linker), 2.76 (q, J = 5.9 Hz, 2H, CH_2-Lev), 2.57 (t, J = 6.4 Hz, 2H, CH_2-Lev), 2.18 (s, 3H, CH_3-Lev), 1.55 – 1.50 (m, 2H, CH_2-Linker), 1.35 – 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)



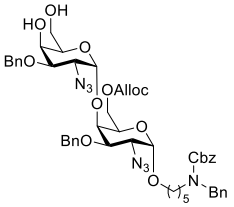
The reaction was carried out according to General glycosylationprocedure A using acceptor **23** (372 mg, 0.539 mmol, 1 equiv.), donor **11b** (478 mg, 0.809 mmol, 1.5 equiv.) and TBSOTf (25 µL, 0.108 mmol, 0.2 equiv.) in DCM (5.4 mL, 0.1 M). The reaction was followed by TLC (pentane/EtOAc 1:4) and column 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=C*H*$_2$), 5.33 – 5.25 (m, 1H, CH=C*H*$_2$), 5.18 (d, J = 14.7 Hz, 3H, Ar-C*H*$_2$), 4.99 – 4.89 (m, 2H, H-1', H-1), 4.75 (dd, J = 11.5, 4.0 Hz, 3H, Ar-C*H*$_2$), 4.67 – 4.60 (m, 4H, Ar-C*H*$_2$, C*H*$_2$-allyl), 4.56 – 4.44 (m, 6H, , C*H*$_2$-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, C*H*$_2$-linker), 3.49 – 3.32 (m, 2H, C*H*$_2$-linker), 3.32 – 3.12 (m, 3H, C*H*$_2$-linker), 1.54 (m, 7H, C*H*$_2$-linker), 1.37 – 1.19 (m, 6H, C*H*$_2$-linker), 1.01 (d, J = 8.1 Hz, 18H, , (C*H*$_3$)$_3$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

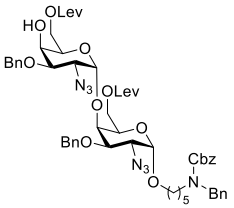
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (27)



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,

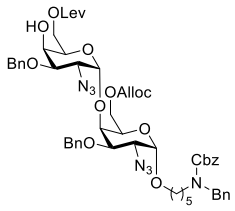
H-4), 4.09 – 4.02 (m, 1H, H-5'), 3.98 (dd, *J* = 12.9, 6.8 Hz, 1H, H-5), 3.94 – 3.81 (m, 3H, H-3, H-3', H-2'), 3.74 – 3.55 (m, 3H, CH_2-linker, H-2), 3.52 – 3.31 (m, 4H, CH_2-linker, H-6'), 3.24 (dt, *J* = 26.3, 7.6 Hz, 2H, CH_2-linker), 2.79 (s, 1H, 4-OH'), 2.11 (dd, *J* = 8.4, 4.4 Hz, 1H, 6-OH'), 1.65 – 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)



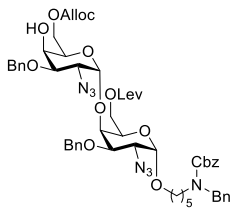
26 (558 mg, 0.569 mmol) was co-evaporated with toluene (x3) and dissolved in dry toluene (5.7 mL, 0.1 M) and added Bu_2SnO (149 mg, 0.598 mmol, 1.05 equiv.) and heated to 110 ºC for 4 h under nitrogen. The reaction was cooled to rt and added Lev_2O (0.5 M in DCM, 2.3 mL, 1.138 mmol, 2 equiv.) and stirred 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-C*H*$_2$), 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-C*H*$_2$), 4.73 (d, *J* = 11.6 Hz, 2H, Ar-C*H*$_2$), 4.68 (d, *J* = 11.9 Hz, 1H, Ar-C*H*$_2$), 4.49 (d, *J* = 6.0 Hz, 2H, Ar-C*H*$_2$), 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, C*H*$_2$ - Linker), 3.22 (dt, *J* = 11.6, 7.1 Hz, 3H, C*H*$_2$ - Linker), 2.76 (td, *J* = 6.1, 2.8 Hz, 2H, C*H*$_2$ - Lev), 2.67 (dt, *J* = 8.7, 6.4 Hz, 2H, C*H*$_2$ - Lev), 2.56 (t, *J* = 6.2 Hz, 2H, C*H*$_2$ - Lev), 2.48 – 2.43 (m, 2H, C*H*$_2$ - Lev), 2.17 (s, 4H, C*H*$_3$ – Lev, O*H*), 2.16 (s, 3H, C*H*$_3$ - Lev), 1.57 – 1.47 (m, 3H, C*H*$_2$ - Linker), 1.34 – 1.26 (m, 3H, C*H*$_2$ - 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

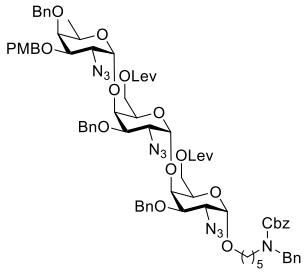
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (30)



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, OH), 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 (CH-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 (CH$_2$-Alloc), 99.00 (C-1'), 98.08 (C-1), 76.02 (C-3'), 74.89 (C-3), 73.38 (C-4), 71.98 (CH$_2$-Ar), 71.95 (CH$_2$-Ar), 68.62 (CH$_2$-Alloc), 68.32 (CH$_2$-Ar), 68.17 (C-2), 67.73 (C-5), 67.23 (C-6'), 65.52 (CH$_2$-Ar), 65.38 (C-5'), 65.18 (C-4') 61.95 (C-6), 59.57 (C-2), 50.27 (CH$_2$-Linker), 47.18 (CH$_2$-Linker), 46.19 (CH$_2$-Linker), 38.01 (CH$_2$-Lev), 29.84 (CH$_3$-Lev), 27.90 (CH$_2$-Linker), 27.51 (CH$_2$-Lev), 23.39 (CH$_2$-Linker). **HRMS**: $[M+Na]^+$ calculated for $C_{55}H_{65}N_7O_{15}Na$: 1086.44363; found 1086.44309

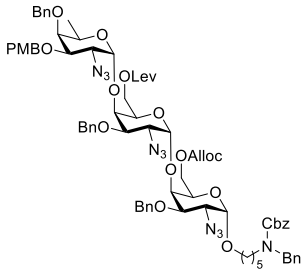
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (6)



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 (CH$_2$-Ar), 72.01 (C-4), 67.28 (CH$_2$-Ar), 62.14 (C-6), 59.62

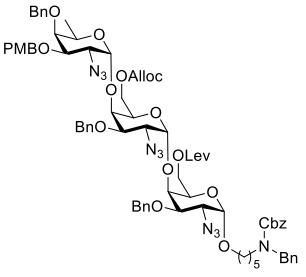
(*C*-2), 55.43(*C*H$_3$-PMB), 51.06 (*C*H$_2$-Ar), 45.97 (*C*H$_2$-Linker), 38.03 (*C*H$_2$-Lev), 37.38 (*C*H$_2$-Lev), 29.93 (*C*H$_3$-Lev), 28.41 (*C*H$_2$-Linker), 27.99 (*C*H$_2$-Lev), 27.61 (*C*H$_2$-Lev), 23.15 (*C*H$_2$-Linker), 16.72 (*C*-6''). **HRMS**: [M+Na]$^+$ calculated for $C_{77}H_{90}N_{10}O_{19}Na$: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

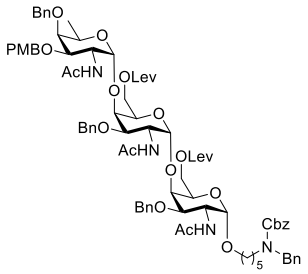
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-6-*O*-allyloxycarbonyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (8)



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

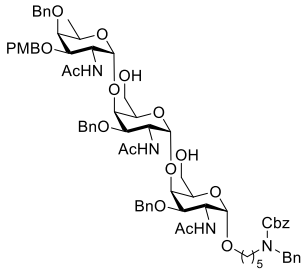
Synthesis of the trisaccharide without taurine

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

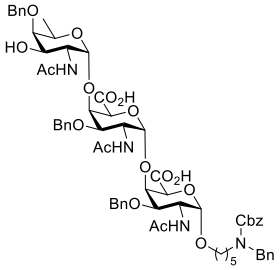
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (32)



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,

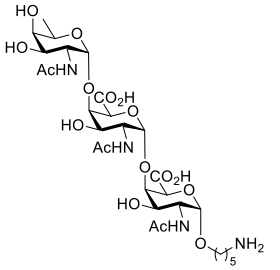
128.49, 128.30, 128.12, 127.98, 127.75, 127.65, 127.54, 114.15, 98.99, 98.21, 77.21, 76.78, 75.57, 75.09, 71.82, 71.35, 67.53, 55.61, 50.66, 49.23, 30.07, 26.42, 26.25, 23.54, 23.41, 22.92, 17.08. **HRMS**: $[M+Na]^+$ calculated for $C_{73}H_{90}N_4O_{18}Na$: 1333.61478; found 1333.61466

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

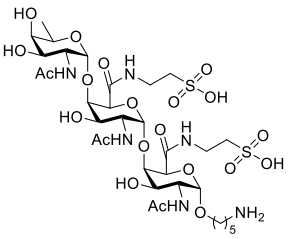
5-amino-pentyl 2-acetamide-2-deoxy-α-D-fucopyranosyl-(1→4)-2-acetamide-2-deoxy-α-D-galactopyranosiduronate-(1→4)-2-*O*-acetamide-2-deoxy-α-D-galactopyranosiduronic acid (1)



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

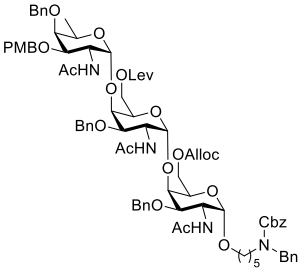
Trisaccharide with taurine on both GalNAc's (4)



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

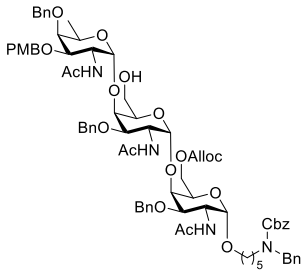
5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-*O*-acetamide-6-*O*-allyloxycarbonyl-3-*O*-benzyl-2-deoxy-α-D-galactopyranoside (34)



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*

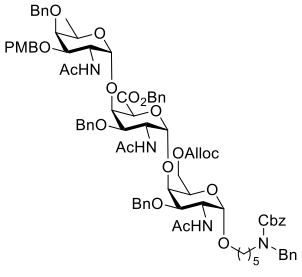
= 11.2, 3.5, 2.2 Hz, 1H), 5.17 (s, 1H), 5.05 (d, *J* = 7.5 Hz, 1H), 4.93 (d, *J* = 3.7 Hz, 1H), 4.88 (d, *J* = 11.3 Hz, 2H), 4.82 (dd, *J* = 12.3, 2.7 Hz, 2H), 4.80 – 4.74 (m, 2H), 4.69 – 4.62 (m, 1H), 4.62 – 4.57 (m, 3H), 4.56 – 4.35 (m, 12H), 4.34 – 4.24 (m, 3H), 4.17 (d, *J* = 6.7 Hz, 2H), 4.12 – 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)



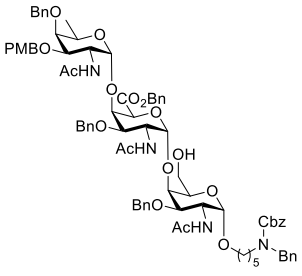
34 (217 mg, 0.145 mmol, 1 equiv.) was dissolved in toluene/EtOH (1:2, 0.1 M, 1.2 mL) and added hydrazine acetate (67 mg, 0.728 mmol, 5 equiv.) and stirred at rt for 45 min until TLC analysis (DCM/MeOH 95:5) showed full conversion. The solution was diluted in DCM and $NaHCO_3$ (aq., sat.) and the organic 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.

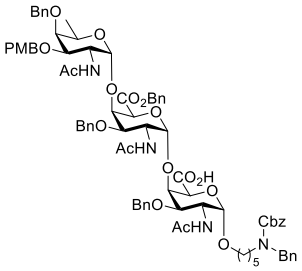
Benzyl (5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronasyl)-(1→4)-2-acetamide-2-deoxy-3-*O*-benzyl-α-D-galactopyranoside (37)



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,

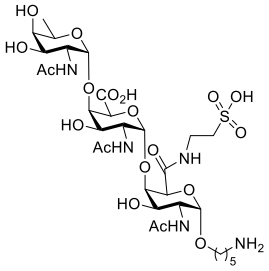
130.01, 129.32, 128.88, 128.79, 128.73, 128.50, 128.28, 128.02, 127.93, 127.78, 127.64, 114.17, 99.13, 97.95, 76.51, 74.77, 72.44, 71.28, 67.96, 67.41, 55.64, 50.72, 49.18, 48.87, 48.04, 30.06, 23.56, 16.80. **HRMS**: $[M+Na]^+$ calculated for $C_{80}H_{94}N_4O_{19}Na$: 1437.64100; found 1437.64099

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)

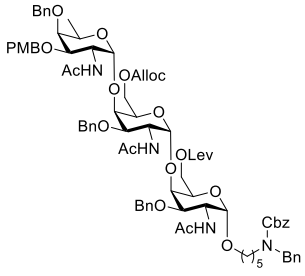


38 (37 mg, 0.0259 mmol, 1 equiv.) was dissolved in DMF (1 mL) and added HATU (12 mg, 0.0311 mmol, 1.2 equiv.) and DIPEA (14 µL, 0.0776 mmol, 3 equiv.) stirred at rt for 10 min before adding taurine (6 mg, 0.0518 mmol, 2 equiv.). The reaction was for 2 h until LC-MS showed full conversion. The solution 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

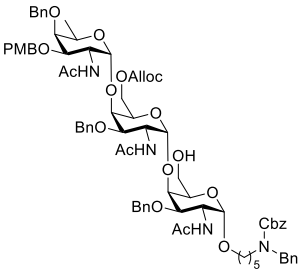
Synthesis of the trisaccharides with taurine on 2nd GalNAc

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)



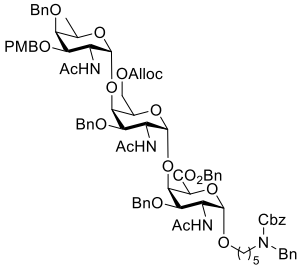
The azide reduction was carried out followed the general azide reduction procedure B using **10** (189 mg, 0.129 mmol, 1 equiv.) and zinc powder (1.687 g, 25.83 mmol, 200 equiv.). Purification by column chromatography (DCM/MeOH 100:0→95:5) gave **40** in 94% yield (181 mg, 0.121 mmol). **^{1}H NMR (400 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

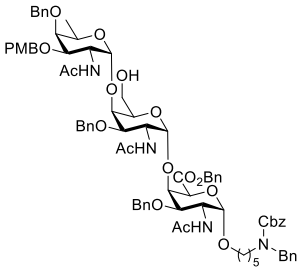
Benzyl (5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-6-*O*-allyloxycarbonyl-3-*O*-benzyl-2-deoxy-α-D-galactopyrasyl-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronate) (42)



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

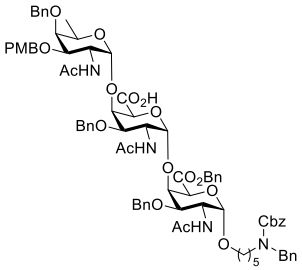
(m, 3H), 1.86 (s, 9H), 1.58 – 1.38 (m, 5H), 1.34 – 1.08 (m, 5H), 0.88 (dd, *J* = 7.9, 6.7 Hz, 3H). **^{13}C NMR (101 MHz, CD_2Cl_2)** δ 170.60, 169.83, 159.68, 139.25, 138.56, 135.46, 132.02, 129.88, 129.77, 129.23, 129.09, 129.04, 128.87, 128.80, 128.73, 128.51, 128.43, 128.20, 128.13, 127.95, 127.77, 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

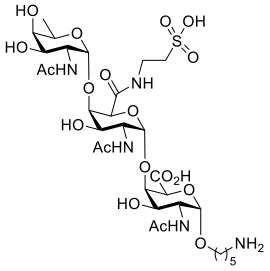
Benzyl (5-(benzyl(benzoyloxycarbonyl)amino)pentyl 2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronate-(1→4)-2-*O*-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosiduronate) (44)



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

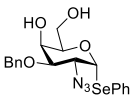
Trisaccharide with taurine on 2nd GalNAc deprotected (3)



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

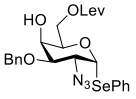
Synthesis of the hexasaccharide without taurine

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



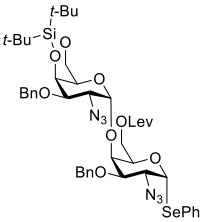
46 (2.019 g, 3.655 mmol) was dissolved in MeOH (36 mL, 0.1 M), added CSA (85 mg, 0.366 mmol, 0.1 equiv.) and stirred at rt for 1 h until TLC (pentane/EtOAc, 6:4) showed full conversion. The reaction was quenched with Et_3N and concentrated *in vacuo*. Purification by column chromatography (pentane/EtOAc, 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

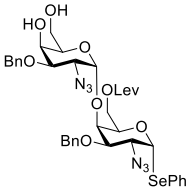
Phenyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(di-*tert*-butylsilyene)-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (49)



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,

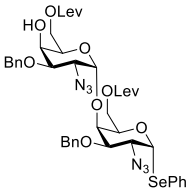
H-5, H-6), 4.28 (d, *J* = 2.8 Hz, 1Hm H-4), 4.14 (dd, *J* = 10.5, 5.3 Hz, 1H, H-2), 4.02 (s, 1H, H-4'), 3.94 – 3.84 (m, 2H, H-5', H-2'), 3.79 – 3.71 (m, 1H, H-6'), 3.71 – 3.63 (m, 2H, H-3, H-6'), 2.75 (td, *J* = 6.2, 1.8 Hz, 2H, CH_2-Lev), 2.53 (t, *J* = 6.5 Hz, 2H, CH_2-Lev), 2.19 (s, 3H, CH_3-Lev), 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

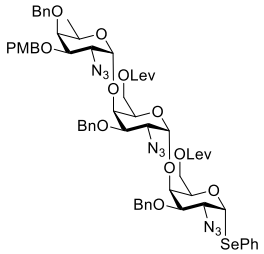
Phenyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyranoside (51)



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

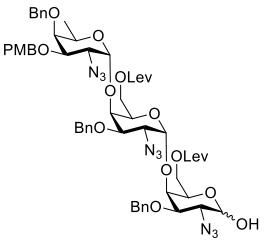
(1.073 g, 1.181 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.63 – 7.54 (m, 2H, Ar-*H*), 7.50 – 7.27 (m, 10H, Ar-*H*), 5.98 (d, *J* = 5.3 Hz, 1H, H-1), 5.03 (d, *J* = 3.6 Hz, 1H, H-1'), 4.87 (d, *J* = 12.1 Hz, 1H, Ar-CH_2), 4.78 – 4.69 (m, 3H, Ar-CH_2), 4.40 – 4.34 (m, 2H, H-5, H-6), 4.34 – 4.27 (m, 2H, 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)



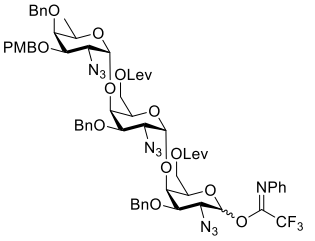
The reaction was carried out according to General glycosylationprocedure A using acceptor **51** (824 mg, 0.908 mmol, 1 equiv.), donor **10b** (777 mg, 0.1362 mmol, 1.5 equiv.) and TBSOTf (48 µL, mmol, 0.2 equiv.) in DCM (9 mL, 0.1 M). The reaction was followed by TLC (pentane/EtOAc 6:4) and column chromatography (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

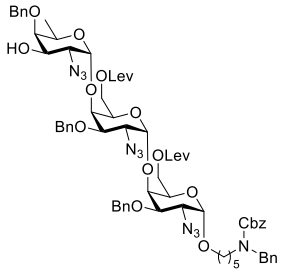
2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(*p*-methoxybenzyl)-α-D-fucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-galactopyrasyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-1-*O*-(*N*-phenyl, 2,2,2-trifluoroacetimidoyl)-α-D-galactopyranose (54)



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,

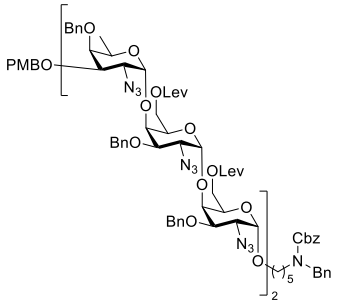
172.76, 160.42, 139.95, 139.18, 138.66, 131.20, 130.78, 129.92, 129.37, 129.35, 129.28, 129.18, 128.97, 128.72, 128.62, 128.51, 128.43, 128.36, 125.48, 120.03, 114.70, 100.29, 99.87, 79.06, 77.96, 77.58, 76.17, 75.82, 74.10, 73.76, 73.68, 73.34, 72.61, 72.55, 72.20, 72.14, 72.00, 71.85, 69.98, 69.84, 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)



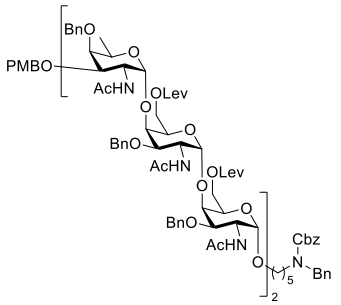
6 (560 mg, 0.383 mmol) was dissolved in DCM/H_2O (3.8 mL, 0.1 M, 20:1) and added DDQ (174 mg, 0.767 mmol, 2 equiv.). The reaction was stirred for 2 h under nitrogen until TLC (pentane/EtOAc, 6:4) showed full conversion. The reaction mixture was quenched with $Na_2S_2O_3$ (x1, aq., sat.), diluted in EtOAc 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

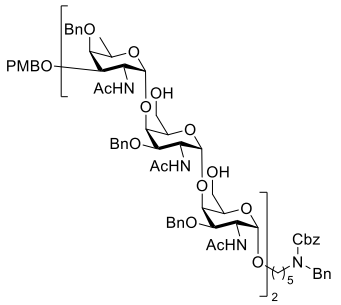
Hexasaccharide – NHAc (57)



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,

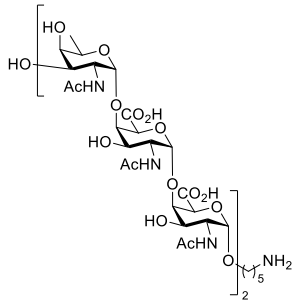
27.77, 23.21, 22.40, 20.70, 16.73. **HRMS**: $[M+Na]^+$ calculated for $C_{138}H_{171}N_7O_{40}Na$: 2589.14595; found 1294.60479

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

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Chapter 6

Summary and future prospects

Summary

This Thesis presents the synthesis and evaluation of antibody recognition for various capsular polysaccharide (CP) fragments of *Staphylococcus aureus* (*S. aureus*). Previous glycoconjugate vaccine candidates to combat *S. aureus* infections, that made use of isolated CP5 and CP8, have all failed in late-stage clinical trials,[1–3] prompting the focus in this Thesis on well-defined synthetic materials. The synthesized CP fragments that have been studied include type 8, type 5, and type 1 (see Figure 1). To facilitate conjugation, all these saccharides were equipped with an amino-functionalized linker. All these CP-fragments are built up from rare monosaccharides, that were synthesized in effective multi-step routes from commercially available materials. The saccharides feature various functional groups, including carboxylic acids, acetamides, acetyl esters, and taurine amides, and are linked together in diverse configurations, through 1,2-*cis* and 1,2-*trans* linkages. The synthetic pathways were designed to produce these fragments as effective as possible, incorporating as little as possible modifications at the oligosaccharide stage and enabling the introduction of desired functionalities. For the 1,2-*trans* linkages, neighboring group participation was utilized, while for the 1,2-*cis* linkages, different factors such as donor protecting groups, donor/acceptor reactivity matching and solvent were employed to achieve the desired stereoselectivity in the formation of the linkages.

Chapter 1 introduces the capsular polysaccharides of *S. aureus* to provide the context for the research described in this Thesis. The biosynthesis routes of the two most clinically prevalent strains, CP5 and CP8 are presented and the working principles of glycoconjugate vaccines are introduced. Different modes for the generation of glycoconjugate vaccines are presented and synthetic precedents for the assembly of well-defined CP5 and CP8 trisaccharide fragments are described.

CP5 and 8 have previously been used as antigen candidates. Synthetic approaches in the past have so far only delivered the trisaccharide repeating unit[4–6] and a protected hexasaccharide,[7] highlighting the difficulties in the synthesis of these complex glycans. In **Chapter 2** the synthesis of a set of CP8-oligosaccharides, varying in length from a trisaccharide to a dodecasaccharide, is presented (**1-4** in Figure 1A). The oligosaccharides were synthesized in a [3+3n] matter employing a key trisaccharide intermediate, which was transformed into the required acceptor and donor synthons. The synthetic plan relied on introducing the acid functionality and the *O*-acetylation in the $ManN_3A$ building prior to assembly of the oligosaccharides, to minimize the post-glycosylation modification steps, in

contrast to the majority of reported synthetic methods.[4,5,7] Deprotection could be achieved in only two steps to yield the pure target compounds. The synthetic fragments were conjugated to CRM_{197} and evaluated for their ability to act as an antigen in interaction studies with both monoclonal antibodies (mAb) and polyclonal antibodies (pAb). Western Blot and ELISA experiments showed that the trisaccharide was poorly recognized and did not bind to either the mAb or pAb, while a clear concentration-dependent competition for hexasaccharide **2**, nonasaccharide **3** and dodecasaccharide **4** was detected. Binding to **3** and **4** was comparable and significantly stronger than binding to **2**, indicating that **3** holds the minimal binding epitope for the mAb/pAb-CP8. Structural studies revealed that the CP8 oligosaccharides adopt a linear structure with the acetamides of the repeating units oriented in the same direction with respect to the oligosaccharide backbone and forming extended hydrophobic anchor points for binding. The spatial orientation of the *O*-acetyl and acetamide groups provided an energy barrier for the rotation of the FucNAc-ManNAcA linkage, which locked the saccharide in a linear conformation. STD-NMR revealed the binding epitope to span over two repeating units (RUs), in which the ManNAcA and L-FucNAc residues provided key interactions. Immunization studies confirmed the length-dependent recognition, and the long synthetic oligosaccharides, having a minimum or 3 RUs, mimicked the antigenicity of the natural polysaccharide well.

In **Chapter 3**, a set of CP5-oligosaccharide ranging in length from a trisaccharide to a nonasaccharide is presented (**7**-**9** in Figure 1B). Also for this CP, previously published synthetic approaches have only delivered a trisaccharide unit,[8–12] where the work of Adamo and co-workers[8] highlighted the need for longer saccharides to induce a useful antibody response. The focus in this Chapter therefore relied on producing longer fragments and for this, the same synthetic principals as implemented in Chapter 2 were applied, with the minor difference that now the *O*-acetyl was installed on larger fragments to ensure the selectivity in the glycosylations. Instead, a temporary 2-methylnapthyl (Nap) ether was installed, leading to the need for two trisaccharide building blocks to keep the orthogonality – one for elongation and one for the terminal end. Deprotection was found to be more difficult than anticipated, as complete reduction of the TCA groups was difficult to obtain. A two-step purification after the reduction using both silica-gel column chromatography and HPLC were utilized to provide pure oligosaccharides, unfortunately leading to loss of material. The synthetic oligosaccharides were conjugated to CRM_{197} and Western Blots and SPR experiments showed trisaccharide **7** to be too short to bind to antibodies, in line with the findings of Adamo and coworkers. The longer hexasaccharide **8** and nonasaccharide **9** showed equal

antibody binding and similar IC50 values. Conformational analysis revealed a linear conformation with the acetyls within a RU pointing in the same direction and the RUs being flipped ~180º with respect to the flanking RUs.

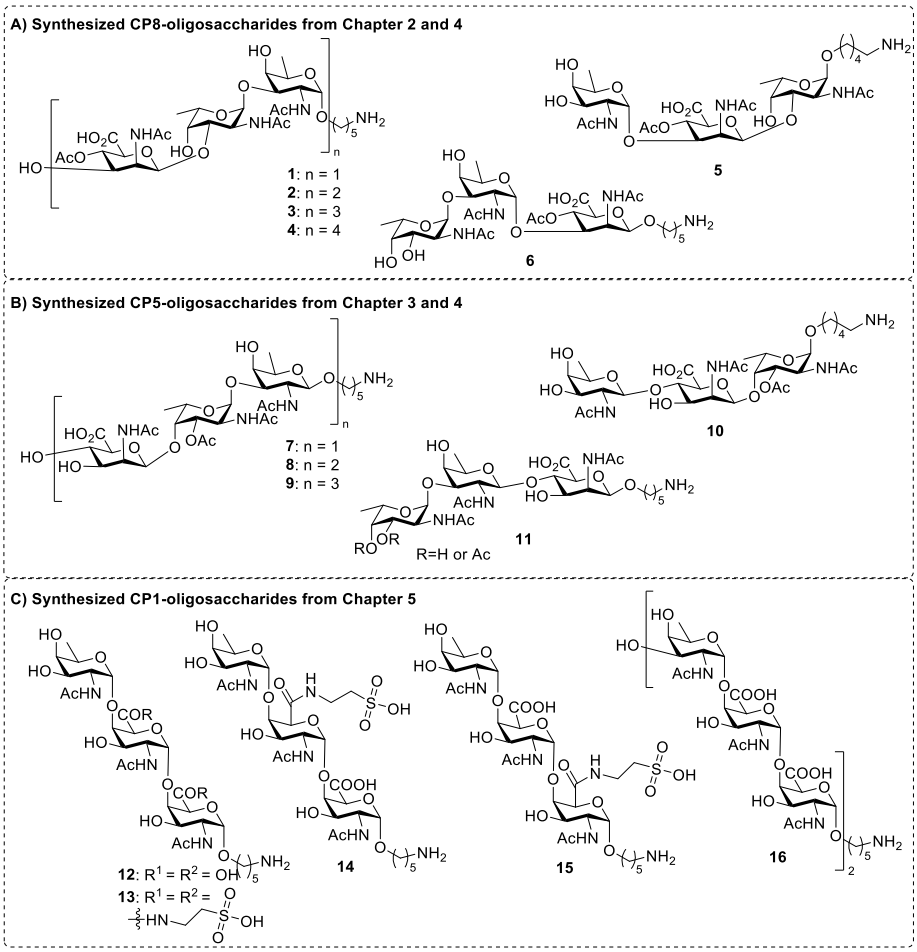


Figure 1: The synthesized oligosaccharides in this Thesis. A) A set of CP8-oligosaccharides ranging from a trisaccharide to a dodecasaccharide and two frameshifted trisaccharides synthesized in Chapter 2 and 4. B) A set of CP5-oligosaccharides ranging from a trisaccharide to a nonasaccharide and two frameshifted trisaccharides synthesized in Chapter 3 and 4. C) A set of CP1-trisaccharides with varying taurine pattern and a hexasaccharide synthesized in Chapter 5.

Chapter 2 and 3 only report on the synthesis and evaluation of a single trisaccharide, where three different frameshift trisaccharides can be defined, and therefore the question remains whether the length of the fragments or the exact frameshift is important for antibody recognition. In **Chapter 4** the other frameshifted CP5 and CP8 trisaccharides were investigated by synthesizing the

remaining frameshifted trisaccharides **5**, **6**, **10** and **11** (Figure 1A and B). The synthetic principals from Chapter 2 and 3 were implemented with the difference that the linker was installed prior to elongation to spare valuable trisaccharide material. In general, the yields where lower and the stereoselectivity in the used glycosylation reactions was worse than those reported in Chapter 2 and 3. Nonetheless, the set of four frameshifted trisaccharides was obtained. For **11**, *O*-acetyl migration from C-3 in the L-FucNAc to the C-4 in the L-FucNAc was observed due to the *cis*-configuration of the hydroxy groups, making this trisaccharide unfit for binding studies. The CP8 frameshift trisaccharides **5** and **6**, were found not to bind to the anti-CP8 antibodies, in either ELISA experiments or STD-NMR. This is in line with the finding in Chapter 2, where the minimal binding epitope was determined to span at least 3 RU. On the other hand, CP5 trisaccharide **10** was found to bind to anti-CP5 antibodies, and binding was comparable to binding of hexasaccharide **8** in the SPR experiments. This indicated that the binding epitope likely consists of the D-FucNAc–D-ManNAcA–L-FucNAc trisaccharide, which is present in both **10** and **8**.

Besides CP5 and CP8, several other *S. aureus* CP types have been found, including CP1. In the past, two synthetic approaches towards a CP1 trisaccharide unit without the characteristic taurine substitution have been published,[13,14] and **Chapter 5** was therefore focused on the synthesis of the four possible CP1 trisaccharides (*i.e.* none, one or two taurines per repeating unit) with a different taurine substitution pattern and a non-taurinated hexasaccharide (**12**-**16** in Figure 1C). In contrast to the work in Chapter 2-4, now major modifications were performed on the larger saccharides, because of the following reasons. Firstly, to obtain α-selectivity in the glycosylation reactions, a di-*tert*-butylsilylidene protected galactosyl donor was used because previous work found galacturonic acid donors to give poor selectivity.[13] Secondly, this allowed to steer the position of the taurine substitution pattern by using orthogonal C-6-OH protecting groups. A four-step modification/deprotection sequence gave the non-taurinated trisaccharide **12** and a five-step sequence gave double-taurinated trisaccharide **13**, while a seven-step modification/deprotection sequence gave taurinated trisaccharide **14** and **15**. For the assembly of hexasaccharide **16** a [3+3] strategy was implemented yielding the hexasaccharide in fine yield. Again, a four-step modification and deprotection sequence gave non-taurinated hexasaccharide **16**.

Potential antibody interaction with the CP5 oligosaccharides

To investigate the interaction between synthetic oligosaccharides and monoclonal antibodies, saturation transfer difference nuclear magnetic resonance (^{1}H STD-NMR) experiments have been conducted where the STD-NMR results revealed the structural elements that defined the binding epitopes. In Chapter 2, clear binding epitopes were identified for oligosaccharides **2** and **3**, with the epitope for **2** originating from the terminal end and that for **3** from the middle of the fragments. In contrast, no binding was observed for trisaccharides **1**, **5**, and **6**. Initial STD-NMR studies for the CP5 oligosaccharides showed different results as illustrated in Figure 2. For trisaccharide **7**, which was found to give very low binding in the SPR experiment, a binding epitope was found by STD-NMR. The STD signals were found to arise from the central L-FucNAc unit and the terminal D-ManNAcA unit with the strongest STD signals arising from the *O*-acetyl moiety and the *N*-acetyl groups of the L-FucNAc and D-ManNAcA units. The *N*-acetyl of the D-FucNAc unit provided lower STD signals. On the other end, STD-NMR analysis of hexasaccharide **8** and nonasaccharide **9** provided no STD signals. The absence of signals can be caused by either too weak binding or very strong binding. Given the clear STD binding epitope identified for trisaccharide **7** and the strong binding observed for both hexasaccharide **8** and nonasaccharide **9** in the SPR experiments, it is most likely that the lack of signals originates from too strong binding with the antibody. To further investigate whether these results originate from weak or strong binding, competitive STD-NMR experiments can be conducted by including trisaccharide **7**, which shows binding. The appearance of STD NMR signals will indicate weak binding of the longer saccharides, while absence of STD-NMR signals will be an indication for binding of the antibody with the longer fragments and not to the competing trisaccharide.

STD-NMR analysis using trisaccharide **10** also showed no STD, as illustrated in Figure 2B, consistent with the findings for hexasaccharides **8** and nonasaccharide **9**. Again, the absence of signals may indicate very weak or very strong binding. Considering the similar IC50 values for **10** and **8** and **9**, along with the binding epitope identified for trisaccharide **7**, it is likely that the lack of STD signals reflects strong binding. Again, conducting competition NMR experiments with trisaccharide **7** could further validate these results.

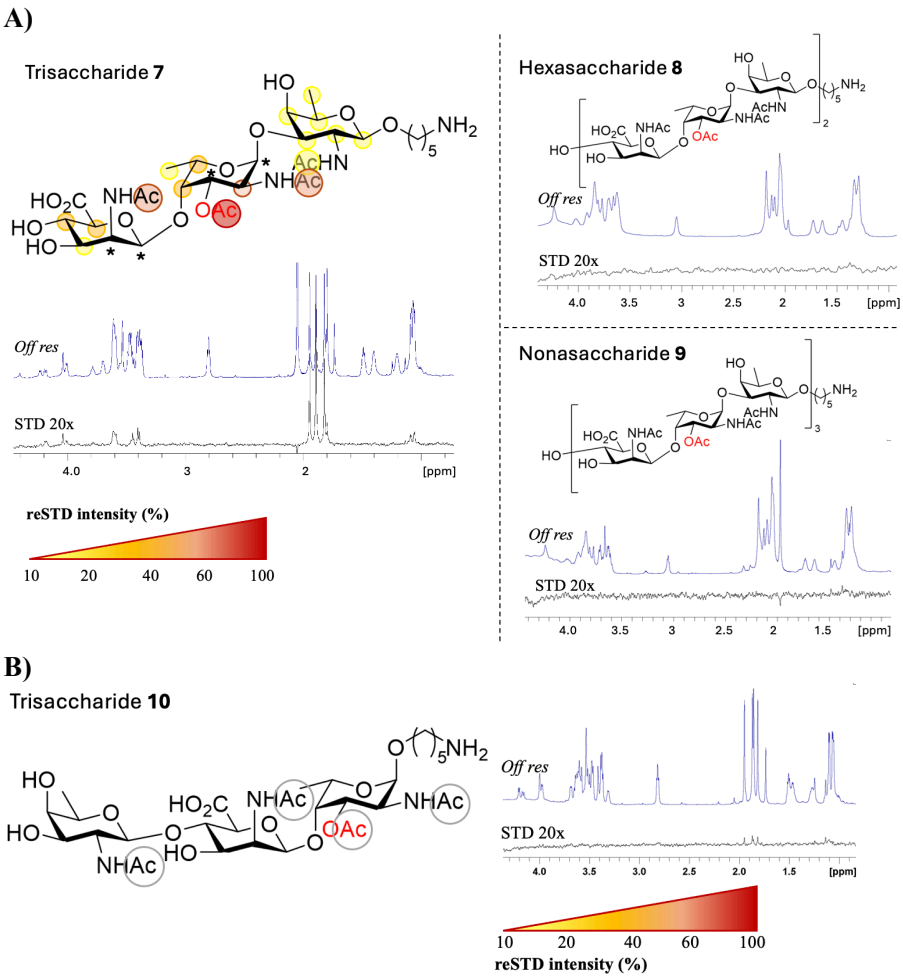


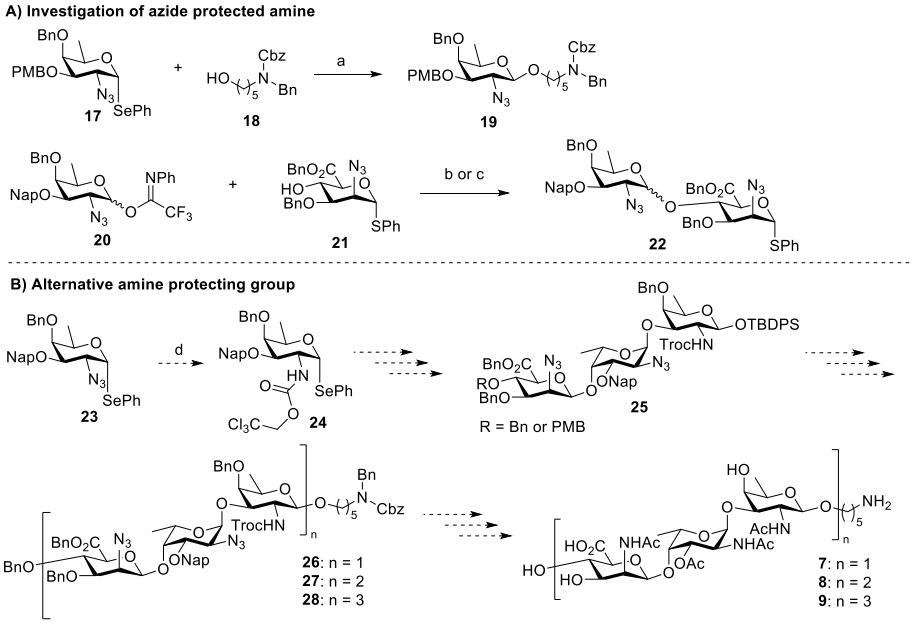
Figure 2: A) ^{1}H STD-NMR spectra performed for the complexes of mAb-CP5 and the trisaccharide **7**, the hexasaccharide **8**, and the nonasaccharide **9**. B) ^{1}H STD-NMR spectra performed for the complexes of mAb-CP5 and **10**. Off-resonance spectra (in blue) and corresponding STD-NMR spectra at 310 K (in black). The representation of the epitope map disclosed by the analysis of the relative STD-NMR signal intensities for each oligosaccharide is reported as color legend associated with the STD% value. *Cannot be estimated due to water suppression.

Potential new amine protecting group for the CP5 synthesis

In all Chapters of this Thesis, an azide moiety was implemented in the building blocks as a precursor for the acetamides, if the neighboring glycosylic linkage was of a 1,2-*cis* nature. The azides could easily be reduced to the corresponding amines and acetylated in a one-pot fashion to obtain the acetamides. For the CP5

fragments, described in Chapter 3 and 4, a D-FucNAc 1,2-*trans* linkage had to be installed and to obtain the β-selective glycosylations, a protecting group enabling neighboring group participation was implemented. The trichloroacetyl (TCA) group was chosen as it can enable neighboring group participation and is stable under various conditions. In addition, the TCA group can be transformed into the corresponding acetamide under the same conditions used for the transformation of the azides, reducing the amount of deprotection steps on the larger saccharides. Unfortunately, partial reduction of the TCA group(s) led to product mixtures, that were difficult to purify, which led to low yields in the reduction reaction and the generation of only small amounts of pure material.

To overcome these problems, an alternative will have to be found to replace the TCA groups. By only using azides as precursors for the acetamides, the reduction may be achieved without problems. However, as a D-FucNAc 1,2-*trans* linkages is needed, alternative glycosylation conditions will have to be developed to install this linkage stereoselectively. In Chapter 2, linker **18** was installed on the D-FucN_3 donor **17** in a highly β-selective manner, which can be explained to arise from the high reactivity from the primary alcohol, being capable of directly displacing the anomeric α-triflate (Scheme 1A). However, when connecting the trisaccharides in a [3+3n] manner, a bond between the D-FucN_3 and the ManN_3A is required, and the glycosylation between monosaccharide D-FucN_3 donor **20** and monosaccharide ManN_3A acceptor **21** provided mainly α-disaccharide **22**. Unfortunately, also the use of participating nitrile solvents proved unsuccessful in promoting the formation of the β-linked product (Scheme 1A). Alternatively, a different acetamide protecting group, capable of neighboring group participation can be studied. To this end the trichloroethyl carbamate (Troc) group can be explored, which has been found to ensure 1,2-*trans* selectivity in glycosylation reactions.[15] The Troc group can be installed on the FucN building block, in a similar fashion as for the TCA, by reduction of the azide in **23** with zinc and acetic acid followed by treatment with Troc-Cl and $NaHsCO_3$ (Scheme 1B). Just like TCA, the Troc group is stable under hydrolytic, strongly acidic, nucleophilic, and mild reductive conditions, and it can be reduced using zinc and acetic acid, after which acetylated in a one-pot reaction can provide the required acetamides. Substituting the TCA for a Troc should thus allow the same synthetic strategy to obtain the CP5-fragments.



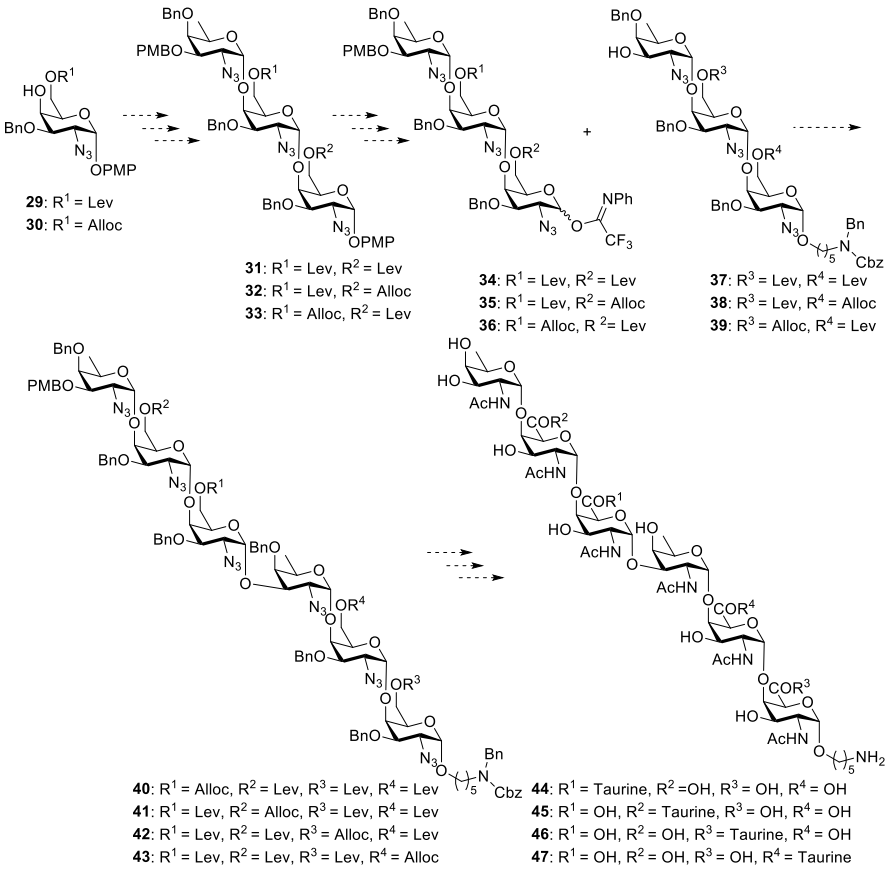
Scheme 1: Alternative amine protecting group for the D-Fuc-N in CP5. A) The investigation of using only azides a) TMSOTf, NIS, DCM/Et_2O 1:1, 78%, α/β=10:90, b) TBSOTf, DCM/MeCN 2:1, -78 ºC, 98% α/β=78:22, c) TBSOTf, MeCN, -40 ºC, 98% α/β=75:25. B) The proposed route using Troc protection of the amine d) i) zinc, AcOH, THF, ii) Troc-Cl, $NaHCO_3$, THF.

No immunization studies have yet been performed with the generated CP5 oligomers. It would thus be of interest to test the glycoconjugates generated with the synthetic oligosaccharides and especially trisaccharide **10** is of interest, as it represents a relatively small saccharide, but was shown to be capable of binding anti-CP5 antibodies, raised against the native polysaccharide, in contrast to the trisaccharide frame shift reported by Adamo and co-workers.[8]

Potential synthesis of CP1 strain M hexasaccharides with a various taurine pattern

In Chapter 5, CP1 trisaccharides with different taurine pattern were synthesized. To investigate the synthesis of longer oligomers, a synthetic method for a non-taurinated hexasaccharide was developed. The glycosylation reactions to generate the trisaccharide donor went in moderate yield (56-65%), which may be improved by replacing the anomeric phenylselenyl group in the acceptor building blocks, as this moiety may react with electrophilic species, generated during the glycosylation reactions. For example, a *p*-methoxyphenyl (PMP) protecting group

may be used, as it can selectively be removed under mild conditions, in the presence of both levulinoyl (Lev), allyloxycarbonyl (Alloc), *p*-methoxybenzyl (PMB) and sillyl groups, with Ag(II)(hydrogen dipicolinate)$_2$ and sodium acetate.[16]



Scheme 2: Global overview of the proposed synthesis of CP1 hexasaccharides bearing taurine residues.

With the [3+3n] methodology confirmed to give the α-linked product, the next step would be to investigate the synthesis of the hexasaccharides bearing taurine amides. Three trisaccharides, bearing orthogonal protecting groups on the reducing and non-reducing end, as well as on the GalN_3 C-6-hydroxy groups, can be synthesized and used to provide different taurine patterns. The linker would be installed on the trisaccharides and through [3+3n] couplings longer oligomers may be attained. Scheme 2 shows a global overview of the proposed synthetic pathway.

Potential multivalent conjugate vaccines using synthetic material

The anti-*S. aureus* CP8 model vaccine candidates described in this Thesis were shown to be capable of inducing an effective antibody response, comparable to the response induced by a conjugate vaccine carrying the natural polysaccharide (Chapter 2). Previously, vaccine candidates using isolated material have been shown to induce an immune response in healthy adults, but these failed in late-stage clinical trials when moving to immunocompromised subjects (see Chapter 1),[17] for yet unknown reasons. The complexity of the isolated polysaccharides and heterogenicity of the conjugates can be prevented by using synthetic material.

The model vaccines described in this Thesis incorporated a single type of antigen, either CP5 or CP8. Previously, vaccine candidates have reported incorporating of isolated material of both CP5/8 as well as other antigens and these have been shown to induce an immunogenic response in healthy subjects. Instead of conjugating the carrier protein with one type of synthetic antigen, different synthetic antigens can be introduced to obtain a more effective vaccine candidate, which can target several *S. aureus* strains. Different types of synthetic CPs can be used, which in this case would be CP1, CP5 and CP8. A different approach would be to include other types of antigens, such as well-defined synthetic wall teichoic acid (WTA) fragments. This approach is not unprecedented; various classes of cell wall glycopolymers have been utilized previously in vaccine candidates. For instance, the pentavalent vaccine PentaStaph, which incorporates two detoxified toxin components alongside conjugates of isolated CP5, CP8, as well as β-1,3-GlcNAc-modified WTA, was patented in 2005 by Nabi Biopharmaceuticals[18] and later acquired by GlaxoSmithKline in 2009. This vaccine has demonstrated promising results and has undergone phase I/II clinical development.[19,20] The advantages of using synthetic material over isolated fragments, include improved definition and homogeneity of the glycoconjugates, and better control of the relative composition of the different incorporated antigens, which may enhance their efficacy. When working with multiple antigens of varying size and carrying different (labile) functionalities, controlling and quantifying the sugar content in glycoconjugate vaccines can become increasingly complex and the availability of well-defined fragments will aid in the generation of better defined and controlled conjugates.

Acknowledgement

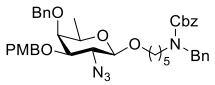
Luca Unione from CIC BioGune is acknowledged for his contribution to the STD-NMR.

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.

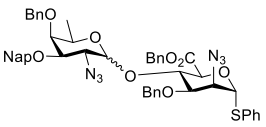
5-(Benzyl(benzyloxycarbonyl)amino)pentyl 2-azide-4-*O*-benzyl-2-deoxy-3-*O*-*p*-methoxybenzyl-β-D-fucopyranoside (18)



Donor **17** (82 mg, 0.151 mmol, 1 equiv.) and acceptor **16** (99 mg, 0.308 mmol, 2 equiv.) and were co-evaporated with toluene (x3) before being dissolved in dry DCM/Et_2O (1:1, 1.5 mL, 0.1 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The reaction was cooled to -40 °C followed by addition of NIS (44 mg, 0.198 mmol, 1.3 equiv.) and TMSOTf (3 µL, 0.0303 mmol, 0.2 equiv.). The reaction was allowed to warm to -20 °C and stirred for 1 h under argon until TLC (pentane/EtOAc, 8:2) showed full conversion. The reaction was quenched with Et_3N and diluted with EtOAc. The organic phase was washed $Na_2S_2O_3$ (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, 95:5 → 80:20) yielded **18** in 78% yield (83 mg, 0.117 mmol) in a α/β = 1:9. NMR reported for the β-anomer. ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.08 (m, 17H, Ar-*H*), 6.93 – 6.87 (m, 2H, Ar-*H*), 5.17 (d, *J* = 11.8 Hz, 2H, CH_2-Linker), 4.92 (d, *J* = 11.6 Hz, 1H, CH_2-Ar), 4.65 (d, *J* = 11.5 Hz, 3H, CH_2-Ar), 4.49 (d, *J* = 7.2 Hz, 2H, CH_2-Linker), 4.12 (t, *J* = 8.8 Hz, 1H, H-1), 3.81 (s, 3H, CH_3-PMB),

3.79 – 3.71 (m, 1H, H-2), 3.49 (dd, *J* = 2.9, 1.0 Hz, 1H, H-4), 3.45 – 3.30 (m, 2H, H-5, CH_2-Linker), 3.28 – 3.24 (m, 2H, H-3, CH_2-Linker), 3.19 (t, *J* = 7.4 Hz, 1H, CH_2-Linker), 1.65 – 1.44 (m, 4H, CH_2-Linker), 1.39 – 1.22 (m, 3H, CH_2-Linker), 1.17 (d, *J* = 6.4 Hz, 3H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.50 (C=O), 138.38 (C_q-Ar), 138.07 (C_q-Ar), 129.91 (C_q-Ar), 129.60 (*C*-Ar), 128.62 (*C*-Ar), 128.60 (*C*-Ar), 128.47 (*C*-Ar), 128.31 (*C*-Ar), 127.99 (*C*-Ar), 127.93 (*C*-Ar), 127.79 (*C*-Ar), 127.39 (*C*-Ar), 127.33 (*C*-Ar), 114.00 (*C*-Ar), 102.34 (C-1), 80.66 (C-3), 75.01 (C-4), 74.72 (CH_2-Ar), 72.39 (CH_2-Ar), 70.61 (C-5), 67.22 (CH_2-Linker), 63.20 (C-2), 55.40 (CH_3-PMB), 50.60 (CH_2-Linker), 50.29 (CH_2-Linker), 47.16 (CH_2-Linker), 46.30 (CH_2-Linker), 29.29 (CH_2-Linker), 27.99 (CH_2-Linker), 27.68 (CH_2-Linker), 23.31 (CH_2-Linker), 17.00 (C-6).

Phenyl 2-azide-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-β/α-D-fucopyranosyl (1→4)-(Benzyl (2-azido-3-*O*-benzyl-2-deoxy-1-thio-α-D-mannopyranosiduronate)) (21)



Acceptor **20** (66 mg, 0.134 mmol, 1 equiv.) and donor **19** (118 mg, 0.200 mmol, 1.5 equiv.) were co-evaporated with toluene (x3) before being dissolved in dry DCM/MeCN (2:1, 1.5 mL, 0.1 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The reaction was cooled to -78 °C followed by addition of TBSOTf (6 µL, 0.0267 mmol, 0.2 equiv.). The reaction was stirred at -78 °C and stirred for 1 h under argon until TLC (pentane/EtOAc, 8:2) showed full conversion. The reaction was quenched with Et_3N and diluted in EtOAc. The organic phase was washed $Na_2S_2O_3$ (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, 95:5 → 75:25) yielded **21** in 98% yield (117 mg, 0.131 mmol) in a α/β = 78:22. NMR reported for the major α-anomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 – 7.84 (m, 6H), 7.67 – 7.47 (m, 7H), 7.40 – 7.25 (m, 18H), 7.24 – 7.10 (m, 5H), 5.71 (d, *J* = 9.6 Hz, 1H), 5.06 (d, *J* = 3.6 Hz, 1H), 5.02 – 4.91 (m, 3H), 4.91 – 4.76 (m, 4H), 4.72 (d, *J* = 2.7 Hz, 1H), 4.66 (d, *J* = 11.5 Hz, 1H), 4.56 (q, *J* = 11.2 Hz, 2H), 4.44 (dd, *J* = 4.5, 2.6 Hz, 1H), 4.04 (dd, *J* = 4.5, 2.8 Hz, 1H), 3.90 – 3.78 (m, 3H), 3.73 – 3.65 (m, 2H), 3.58 – 3.44 (m, 3H), 1.17 (d, *J* = 6.4 Hz, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.49, 138.11, 136.58, 134.98, 133.69, 133.41, 131.20, 128.79, 128.65, 128.59, 128.52, 128.49, 128.44, 128.38, 128.33, 128.31, 128.26, 128.12, 128.08, 128.02, 127.97, 127.85, 127.78, 126.52, 126.39, 126.21, 125.87, 125.62, 100.08, 77.73, 76.53, 75.75, 75.13, 75.04, 74.93, 73.25, 72.52, 67.72, 67.30, 63.38, 59.58, 57.58, 31.86, 29.80, 19.40, 16.82.

Ligand-antibody interaction studies

^{1}H-STD NMR experiments & methods. For the acquisition of the ^{1}H-STD-NMR experiments the mAb-CP8 antibody was buffer exchanged to deuterated PBS 1X pD 7.8 using centrifuge filters (Sartorius Vivaspin 6 50000 MWCO) up to an antibody concentrated of 2 µM. 100 equivalents of ligands (**1**-**3**) were added, which resulted into a solution of 2 µM of mAb and 200 µM of the ligand.

The STD experiments were recorded using Bruker AVANCE II 800 MHz NMR spectrometer equipped with cryo-probe (Bruker Inc.; Billerica, MA, US) at different temperatures that ranged

between 288 and 310 K. The used ^{1}H-STD pulse sequence includes T2 filter, for protein NMR signal suppression, and excitation sculpting, for residual water NMR signal suppression. The STD NMR spectra were acquired with 2880 scans and 5 s of relaxation delay. Different conditions were screened for STD experiments. All the STD experiments were performed at both on-resonances, at the aliphatic (0.8 ppm) and aromatic (7.0 ppm) regions. The resulting STD spectra provided similar results. The on- and off-resonance spectra were registered in the interleaved mode with the same number of scans. The on-resonance protein saturation was obtained using a Gaussian shape pulse of 50 ms with a total saturation time of 2 s at a frequency of δ 0.8 ppm (aliphatic region). The off-resonance frequency was always set at δ 100 ppm.

The analysis was carried out using the ^{1}H NMR signals of the STD spectrum and from their comparison with the off-resonance spectrum, the STD-AF (Average Factor) was obtained. The strongest STD intensity was used as reference (100% of STD effect). On this basis, the relative STD intensities for the other protons were estimated from the comparison of the corresponding integrals. These relative STD intensities (STD%) were used to map the ligand-binding epitope.

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Samenvatting in het Nederlands

Synthese, structuur en epitoop studies van goed-gedefinieerde *Staphylococcus aureus* capsulaire polysacchariden

Staphylococcus aureus (*S. aureus*) is een grampositieve bacterie die deel uitmaakt van het menselijke microbioom en ernstige infecties kan veroorzaken bij immuun gecompromitteerde personen, zoals gehospitaliseerde patiënten, ouderen en pasgeborenen. De opkomst van antibioticaresistente stammen dringt aan op de ontwikkeling van nieuwe therapeutische strategieën, waarbij vaccinatie een belangrijke methode is. Eerdere glycoconjugaat vaccins om *S. aureus*-infecties te bestrijden, die gebruikmaakten van de geïsoleerde capsulaire polysachariden CP5 en CP8, voldeden niet tijdens de eindfase van klinische proeven, wat de focus in dit proefschrift op goed gedefinieerde synthetische materialen heeft doen ontstaan. De celwand van *S. aureus* bestaat uit verschillende glycopolymeren welke allemaal als antigenen kunnen worden beschouwd, waaronder de capsulaire polysacharide (CP's). Tot op heden zijn alleen trisacharide-eenheden van CP1, CP5 en CP8, samen met een beschermd hexasaccharide van CP8, gesynthetiseerd, wat de synthetische uitdagingen van deze moleculen onderschrijft. Dit proefschrift beschrijft de synthese en evaluatie van verschillende typen capsulaire polysachariden van verschillende lengtes en substitutiepatronen.

Hoofdstuk 1 introduceert de capsulaire polysachariden van *S. aureus* om de context voor het onderzoek te bieden welke in dit proefschrift wordt beschreven. De biologische syntheseroutes van de twee meest klinisch voorkomende stammen, CP5 en CP8, worden gepresenteerd en de werkprincipes van glycoconjugaat vaccins worden geïntroduceerd. Verschillende modi voor de generatie van glycoconjugaat vaccins worden gepresenteerd en synthetische precedenten voor de assemblage van goed gedefinieerde CP5- en CP8-trisaccharidefragmenten worden beschreven.

Hoofdstuk 2 beschrijft de synthese van een set CP8-oligosachariden die in lengte variëren van een trisacharide tot een dodecasaccharide. De oligosachariden werden gesynthetiseerd in een [3+3n]-structuur die voortkomt uit een belangrijk trisacharide-intermediair. Het synthetische plan was gebaseerd op het introduceren van de zuur functionaliteit en de *O*-acetylering van de $ManN_3A$-bouwsteen voorafgaand aan de assemblage van de trisacharide-herhalende eenheid om de post-glycosyleringsmodificatiestappen te minimaliseren. Deprotectie werd in slechts twee stappen gefaciliteerd om zuiver materiaal te verkrijgen. De

gesynthetiseerde fragmenten werden geconjugeerd aan het CRM_{197}-dragereiwit en geëvalueerd op diens vermogen om een antilichaamrespons te induceren. Western Blot- en ELISA-experimenten toonden aan dat de trisacharide slecht werd herkend door anti-CP8-antilichamen, terwijl een duidelijke concentratieafhankelijke competitie voor de hexa-, nona- en dodecasaccharide werd gedetecteerd. Structurele en conformationele studies onthulden een lineaire conformatie met de acetamiden van de herhalende eenheden georiënteerd in dezelfde richting. STD-NMR onthulde dat het bindende epitoop zich uitstrekt over twee herhalende eenheden (HE's). Ten slotte bevestigden immunisatiestudies de lengteafhankelijke herkenning waarbij de synthetische oligosachariden (met een minimum van drie HE's) de antigeniciteit van de natuurlijke polysacharide goed nabootsten.

Hoofdstuk 3 beschrijft een set CP5-oligosaccharide variërend in lengte van een trisacharide tot een nonasaccharide. Dezelfde synthetische principes als geïmplementeerd in Hoofdstuk 2 werden toegepast, met het kleine verschil dat de *O*-acetylgroepen in een later stadium op grotere fragmenten werden geïnstalleerd om de selectiviteit in de glycosyleringen te beïnvloeden. De synthetische oligosachariden werden geëvalueerd door Western Blot-analyse en SPR-experimenten, en opnieuw werd een oligosacharide lengte-activiteitsrelatie gevonden, hoewel deze minder uitgesproken was dan deze voor CP8 was. De trisacharide bleek te kort om een antilichaamrespons te induceren, en de langere hexasaccharide en nonasaccharide vertoonden een gelijk antilichaambindend vermogen en vergelijkbare IC50-waarden. Conformatie-analyse onthulde een lineaire conformatie voor de oligomeren, waarbij de acetylen binnen een HE dezelfde richting aanhielden en de HE's ~180º werden omgedraaid ten opzichte van de flankerende HE's.

Hoofdstuk 4 beschrijft de synthese van alle mogelijke geframeshifte trisachariden van CP5 en CP8. De synthetische methodes uit Hoofdstuk 2 en 3 werden geïmplementeerd met het verschil dat de linker voorafgaand aan de glycosyleringwerd geïnstalleerd om waardevol trisacharide materiaal te sparen. Over het algemeen waren de opbrengsten lager en was de selectiviteit van de gebruikte glycosyleringsreacties slechter dan in Hoofdstuk 2 en 3, maar de set van vier geframeshifte trisachariden kon uiteindelijk worden gesynthetiseerd. De CP8 geframeshifte bonden niet aan anti-CP 8 antilichamen in Western Blot, ELISA of STD-NMR experimenten, terwijl een van de CP5 trisachariden relatief goed bleek te binden aan een anti-CP5 antilichaam met een binding die vergelijkbaar was met de hexasaccharide in de gebruikte SPR experimenten. Dit suggereert dat een minimaal bindend epitoop zou kunnen bestaan uit het D-Fuc – D-Man – L-Fuc frame.

Hoofdstuk 5 beschrijft drie verschillende CP1 trisachariden met een verschillend taurine-amidepatroon en een CP1 hexasaccharide zonder taurine. In tegenstelling tot het werk in hoofdstukken 2-4 werden grote modificaties uitgevoerd op de grotere sachariden om verschillende redenen. Ten eerste werden 2-azidogalactose bouwstenen gebruikt die beschermd waren met een 4,6-di-*tert*-butyl silyleengroep om α-selectiviteit te verkrijgen in de glycosyleringsreacties. Ten tweede kon het taurine substitutiepatroon gecontroleerd worden door gebruik te maken van orthogonale C-6-OH beschermende groepen. Een vierstaps modificatie/deprotectie sequentie gaf het niet-getaurineerde trisacharide, terwijl een vijfstaps sequentie leidde tot het dubbelgetaurineerde trimeer, en een zevenstaps modificatie/deprotectie sequentie twee trisachariden opleverde welke een enkel taurine amide op een van de galactosaminuronic zuren gekoppeld hadden. Voor de assemblage van de hexasaccharide werd een [3+3]-strategie geïmplementeerd, wat de hexasaccharide in goede opbrengst en stereoselectiviteit opleverde. Implementatie van een vierstaps deprotectiesequentie resulteerde in de taurine-vrije hexasaccharide.

Resume på dansk

Syntese, struktur og epitop studier af veldefinerede *Staphylococcus aureus* kapselpolysakkarider

Staphylococcus aureus (*S. aureus*) er en Gram-positiv bakterie, der er en del af menneskets mikrobiom, som kan forårsage alvorlige infektioner hos immunkompromitterede personer, som for eksempel indlagte patienter, ældre og nyfødte. Stigningen af antibiotikaresistente varianter fremmer udviklingen af nye terapeutiske strategier, hvor vaccination er en vigtig tilgang. Tidligere glycokonjugatvaccinekandidater til bekæmpelse af *S. aureus*-infektioner, med brug af isolerede kapselpolysakkarider (capsular polysakkarides (CP'er)) type 5 og type 8, har alle fejlet i sene kliniske forsøg. Derfor fokuserer denne afhandling på at syntetisere og evaluere veldefineret syntetisk materiale som grundstenen til en vaccinekandidat. Cellevæggen hos *S. aureus* består af forskellige glycopolymerer, som alle kan betragtes som antigener, blandt andet kapselpolysakkarider. Til dags dato er kun trisakkarid-enheder af CP1, CP5 og CP8 sammen med et beskyttet hexasakkarid af CP8 blevet syntetiseret, hvilket viser de syntetiske udfordringer. Denne afhandling beskriver syntesen og evalueringen af forskellige typer kapselpolysakkarider af varierende længde og substitutionsmønster.

I **Kapitel 1** introduceres kapselpolysakkarider af *S. aureus* for at give konteksten for den forskning, der er beskrevet i denne afhandling. Biosyntesevejene for de to mest klinisk udbredte viranter, CP5 og CP8, præsenteres, og principperne for glycokonjugatvacciner introduceres. Forskellige metoder til produktion af glycokonjugatvacciner præsenteres, og syntetisk præcedens for samling af veldefinerede CP5- og CP8-trisakkaridfragmenter beskrives.

Kapitel 2 beskriver syntesen af et sæt CP8-oligosakkarider, der varierer i længde fra et trisakkarid til et dodecasakkarid. Oligosakkariderne blev syntetiseret ud fra en [3+3n]-strategi, med udgangspunkt i et trisakkarid-mellemprodukt som nøgle-element. Det syntetiske arbejde afhang af introduktionen af syrefunktionaliteten og *O*-acetyleringen af $ManN_3A$-byggeblokken før samling af trisakkaridgentagelsesenheden for at minimere post-glykosylerings-modifikationstrinene. Afbeskyttelse blev udført i kun to trin for at give rent materiale. De syntetiske fragmenter blev konjugeret til et CRM_{197}-bærerprotein og deres evne til at inducere antistofrespons evalueret. Western Blot og ELISA eksperimenter viste, at trisakkaridet var dårligt genkendt af CP8-antistoffer, mens en klar koncentrationsafhængig konkurrence for hexa-, nona- og dodecasakkaridet blev påvist. Strukturelle og konformationelle undersøgelser afslørede en lineær konformation med

acetamiderne af de gentagne enheder orienteret i samme retning. STD-NMR afslørede, at bindingsepitopen spænder over 2 gentagne enheder (GE'er). Endelig bekræftede immuniserings-undersøgelser den længdeafhængige genkendelse, hvor de syntetiske oligosakkarider (med et minimum af 3 GE 'er) på tilfredsstillende vis efterlignede antigeniciteten af det naturlige polysakkarid.

Kapitel 3 beskriver et sæt CP5-oligosakkarider, der varierer i længde fra et trisakkarid til et nonasakkarid. De samme syntetiske principper som implementeret i kapitel 2 blev anvendt, dog med den forskel, at *O*-acetylerne blev installeret på et senere tidspunkt på større fragmenter for at sikre selektiviteten i glykosyleringerne. De syntetiske oligosakkarider blev evalueret med Western Blot og SPR-eksperimenter, og igen blev det fundet at et oligosakkarid længdeafhængige genkendelse, selvom det var mindre udtalt end for CP8. Trisakkaridet var for kort til at inducere et antistofrespons, og det længere hexasakkarid og nonasakkarid viste ens antistofbindingsevne og lignende IC50-værdier. Konformationel analyse afslørede en lineær konformation for oligomererne, hvor acetylerne inden for en GE pegede i samme retning, og GE'erne blev vendt ~180º i forhold til de flankerende GE'er.

Kapitel 4 beskriver syntesen af alle mulige trisakkarid-rammeforskydninger (såkaldte frameshifts) af CP5 og CP8. De syntetiske principper fra kapitel 2 og 3 blev implementeret med den forskel, at linkeren blev installeret før forlængelse for at spare værdifuldt trisakkaridmateriale. Generelt var udbytterne lavere, og selektiviteten af de anvendte glycosyleringsreaktioner værre end i kapitel 2 og 3, men sættet af fire rammeforskudte trisakkarider kunne til sidst syntetiseres. CP8-rammeforskydningerne bandt ikke til CP8-antistoffer i hverken Western Blot, ELISA- eller STD-NMR-eksperimenter, mens et af CP5-trisakkariderne blev fundet til at binde relativt godt til CP5-antistof med binding svarende til hexasakkaridet i SPR-forsøgene. Dette indikerer, at en minimal bindingsepitope kunne bestå af en D-Fuc – D-Man – L-Fuc ramme.

Kapitel 5 beskriver tre forskellige CP1-trisakkarider med et forskelligt taurinamidmønster og et ikke-taurineret CP1-hexasakkarid. I modsætning til arbejdet i kapitel 2-4 blev større modifikationer udført på de store sakkarider af følgende årsager. For det første, for at opnå α-selektivitet i glykosyleringsreaktionerne, blev der brugt 2-azidogalactose-byggeblokke, der var beskyttet med en 4,6-di-*tert*-butylsilylengruppe. For det andet kunne taurin-substitutionsmønsteret kontrolleres ved brug af ortogonale C-6-OH-beskyttelsesgrupper. En fire-trins modifikation/afbeskyttelsessekvens gav det ikke-taurinerede trisakkarid, mens en

fem-trins sekvens førte til den dobbelt taurinerede trimer, og en syv-trins modifikation/afbeskyttelsessekvens gav to trisakkarider, der bar et enkelt taurinamid på en af galactosaminuronsyrerne. Til samlingen af hexasakkaridet blev der implementeret en [3+3]-strategi, der gav hexasakkaridet i godt udbytte og stereoselektivitet. Implementering af en fire-trins afbeskyttelsessekvens gav det ikke-taurinerede hexasakkarid.

List of publications

Østerlid, K.E., Del Bino, L., Ettelbruck, C., Unione, L., Carboni, F., Arda, A., Overkleeft, H.S., van der Marel, G.A., Romano, M.R., Jiménez-Barbero, J., Adamo, R., Codée. J.D.C., Staphylococcus aureus capsular polysaccharide type 5 and 8 trisaccharide repeating unit frameshifts to define the minimal binding epitope for antibody recognition. *Manuscript in preparation.*

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Curriculum Vitae

Kitt Emilie Østerlid was born on the 2nd of January 1994 in Svendborg, Denmark. She attended high school at Midtfyns Gymnasium in Ringe and graduated in 2014 with a specialization in mathematics, physics and chemistry. She then moved to Copenhagen to study chemistry at Copenhagen University and after finishing her bachelor project in the group of associate professor Christian Marcus Pedersen working on catalytic activation of trichloroacetyl glucuronic acid donors, she obtained her bachelor's degree in 2018. She continued her master studies in organic chemistry at Copenhagen University and obtained her master's degree in 2020 after finishing an internship in the group of Christian Marcus Pedersen working on the synthesis of well-defined oncofetal chondroitin sulfate oligomers.

In 2020, Kitt moved to Leiden, The Netherlands, to start her PhD research under the supervision of Prof. Dr. Jeroen Codée in the Bio-organic Synthesis group. The work in the Thesis describes the synthesis and evaluation of different capsular polysaccharides of *Staphylococcus aureus*. Parts of the research presented in this Thesis have been conducted in the group of Jesús Jiménez-Barbero at BioGune in Bilbao, Spain and in the group of Maria Romano at GSK in Siena, Italy, where she has been stationed for shorter internships. Parts of this Thesis have been presented as a poster at the GSK PhD and PostDoc student workshop and as a presentation at Eurocarb 2023 in Paris. During her PhD she has attended the following courses and workshops: PAVax first workshop: Glycoconjugate vaccines: Targeting infectious agents with well-defined vaccines, PAVax second workshop: Carbohydrate chemistry, Project Management Course and Transferable skill courses (Science communication, Management of intellectual property in chemical field, Ethical issues in biomedical research, Bio-entrepreneurship and venture capital and Pitch your project.)

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Kitt Emilie Retoft Østerlid