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

Østerlid, K.E.

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

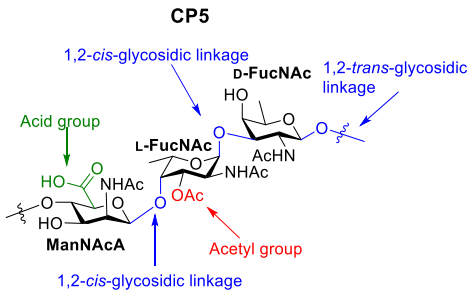


Figure 1: A schematic representation of the repeating unit of CP5.

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.

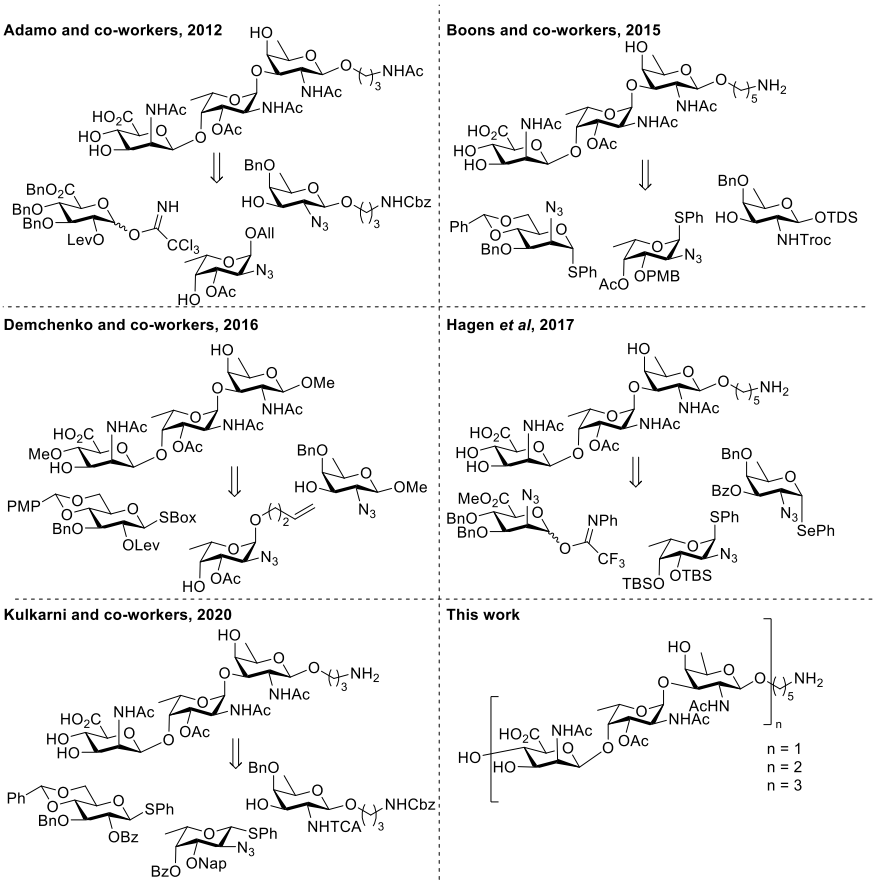


Figure 2: Previously reported synthesis of CP5 trisaccharides and the set of CP5 oligosaccharides reported in this Chapter.

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

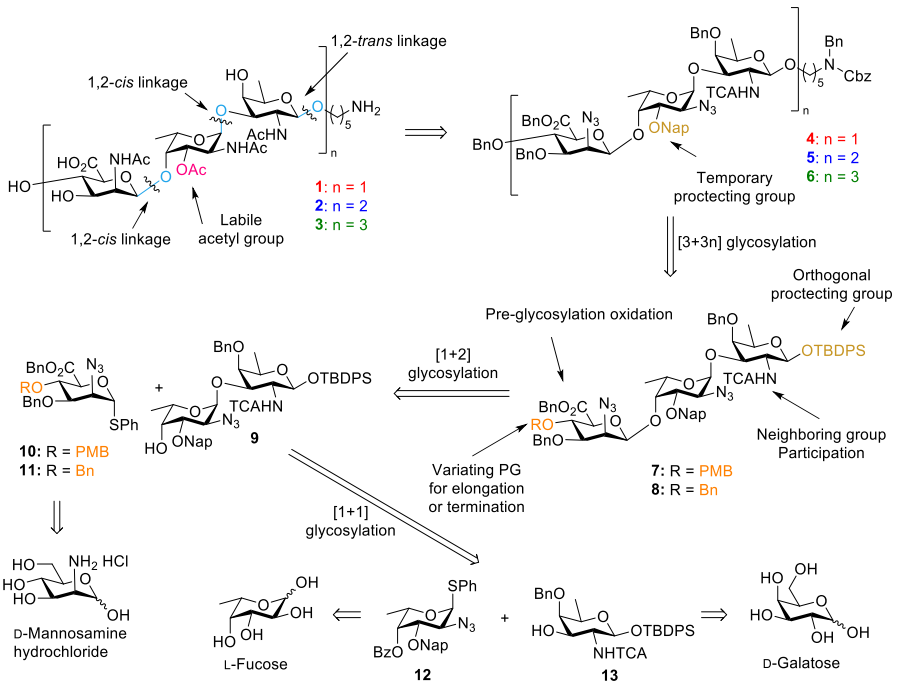
2, by implementing a $ManAN_3$ donor, the oxidation of multiple primary alcohols is prevented, as this has previously been found difficult.[18–20] However, in contrast to the syntheses described in Chapter 2, the *O*-acetyl esters will be installed at a late-stage to ensure higher reactivity in the L-Fuc-D-Fuc 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.

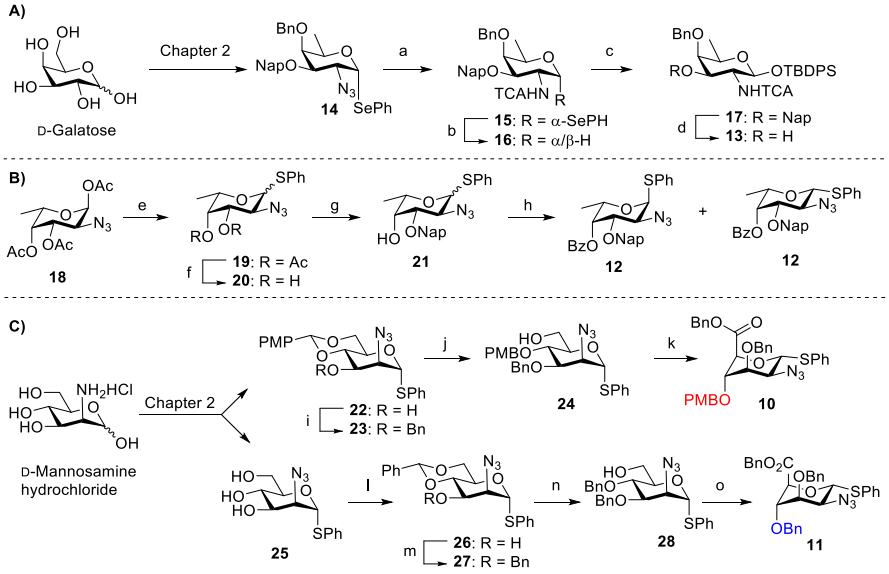


Scheme 1: Retrosynthetic analysis of the set of target CP5 oligosaccharides.

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.

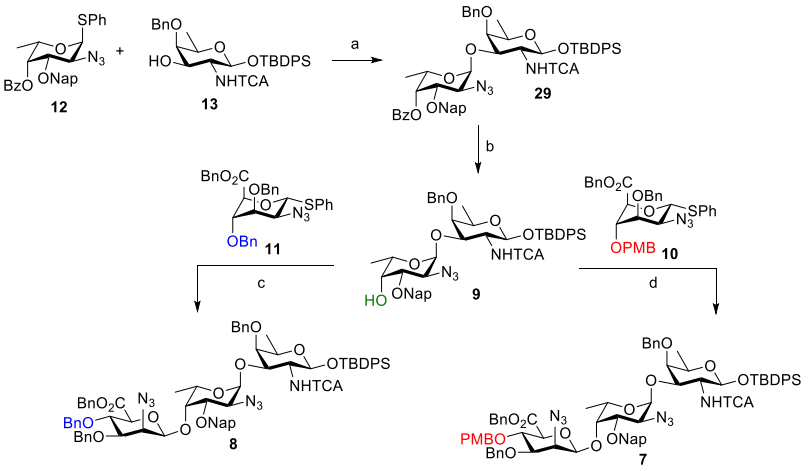
Two different D-ManN_3A building blocks were needed: one for elongation – equipped with a PMB ether, and one for the terminal end – equipped with a benzyl ether (Scheme 2C). For the PMB-building block **10** a route starting from **22**, previously described in Chapter 2, was implemented. The free C-3-OH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

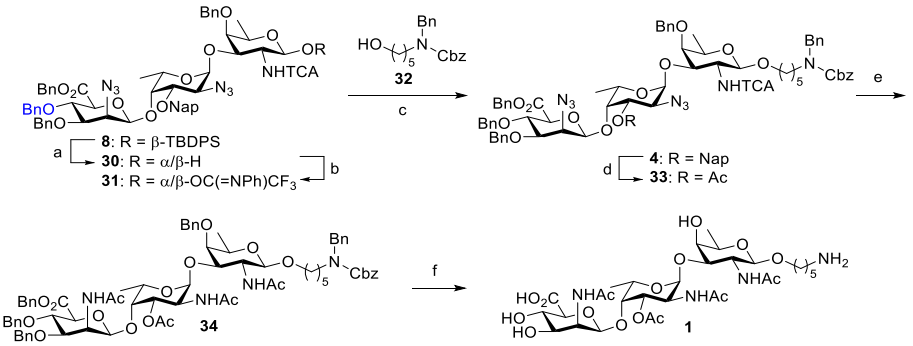
[ii] When using the β-thiophenyl donor the outcome of the reaction depending strongly on the temperature and reaction time, and the C-1-OTBDPS group was found to epimerize. See Appendix for detailed information.

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.

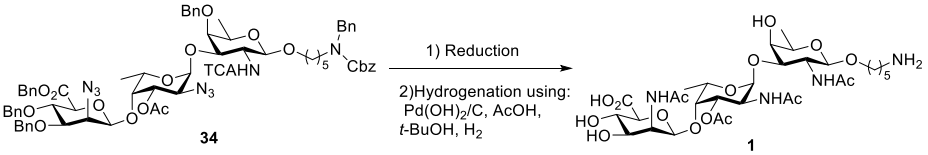


Scheme 4: Deprotection of the trisaccharide to form target **1**. *Reaction conditions:* a) TBAF, AcOH, THF, 0 °C to rt, 81%, b) $ClC(=NPh)CF_3$, K_2CO_3, acetone, 92%, c) TBSOTf, DCM/MeCN, -50 °C, 73%, d) i) DDQ, DCM/H_2O, ii) Ac_2O, DMAP, pyridine, 89 % over two steps, e) zinc, AcOH, Ac_2O, THF, 50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

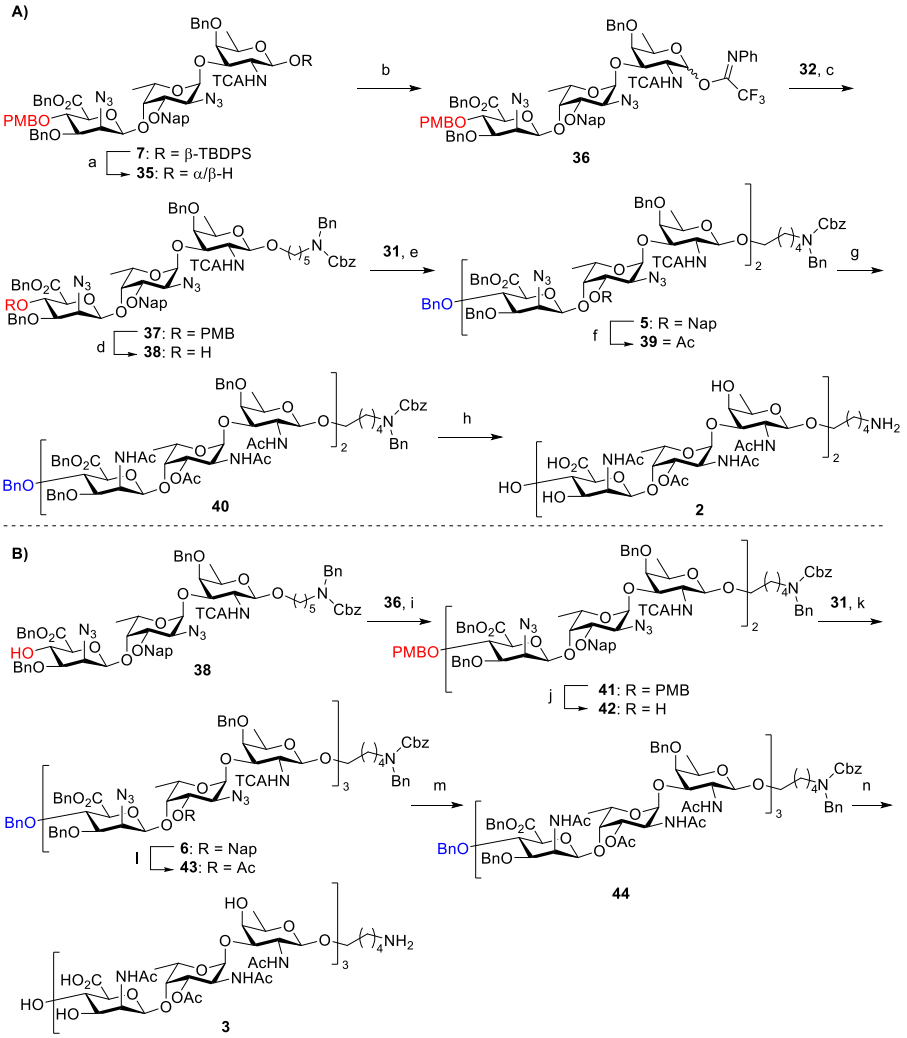


Entry	Conditions	Time	Yield (%)	Purity after hydrogenation (%) [a]
1	1) Zinc, AcOH, Ac_2O, THF, silica gel column 2) Hydrogenation	1) 22 h 2) 3 days	1) 78 2) 43	75
2	1) activated zinc, AcOH, Ac_2O, THF, silica gel column 2) Hydrogenation	1) 22 h 2) 3 days	1) 49 2) 79	60
3	1) Zinc, AcOH, Ac_2O, THF, silica 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

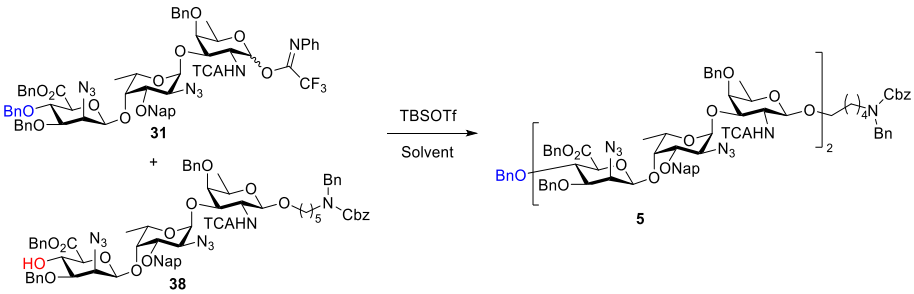
confirmed by ^{1}H-NMR and ^{13}C-NMR, with the D-FucN_3 proton and carbon having a CH-coupling constant of $J_{C1,H1}$ = 162.2. The PMB ether was removed using HCl in CH_2Cl_2/hexafluoroisopropanol (HFIP)[30] to provide acceptor **38**.



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.

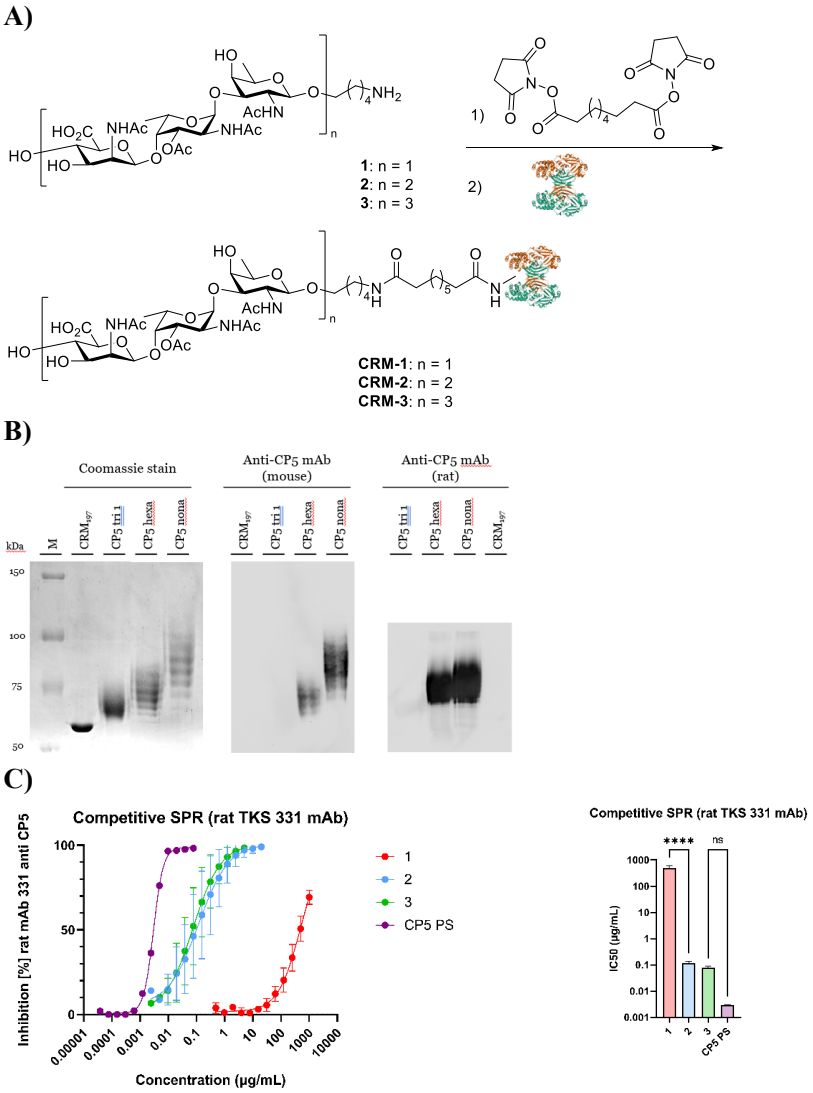
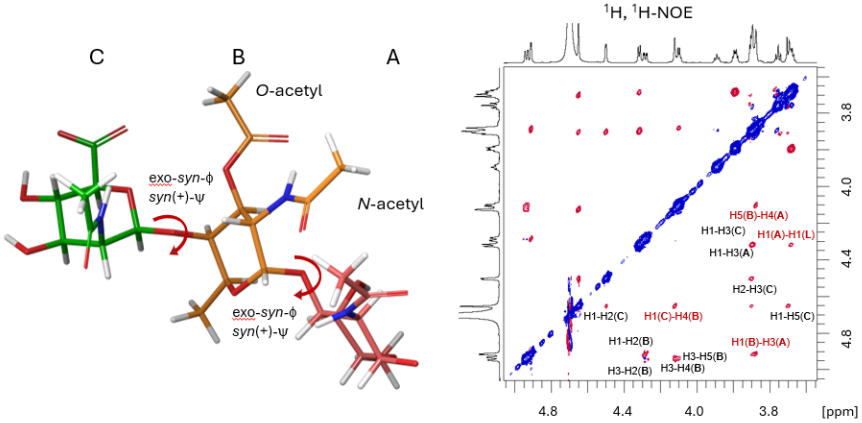


Figure 3: A) Conjugation of the synthetic fragments, 1) Suberic acid bis(N-hydroxysuccinimide ester) 15 equiv. in DMSO/H_2O 9:1, 2) CRM_{197} in 50 mM HEPES. B) SDS-PAGE of the conjugates CRM**1-3** (left) and Western Blots performed with either the mouse (middle) or rat (right) anti-mAb-CP5, which showed 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.

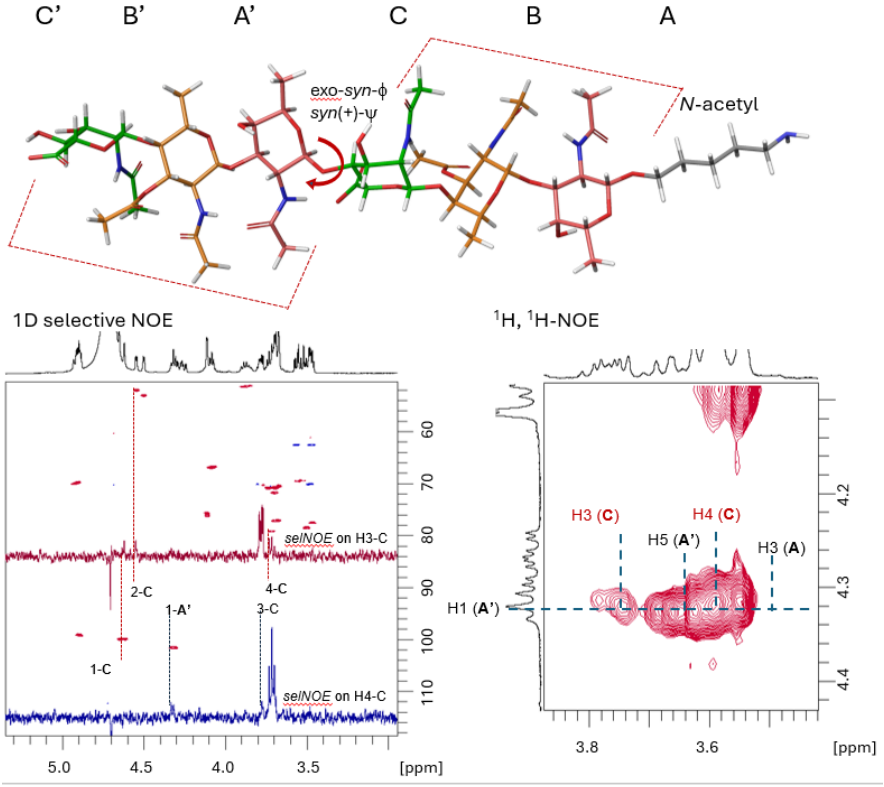
Structural and conformational studies

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**

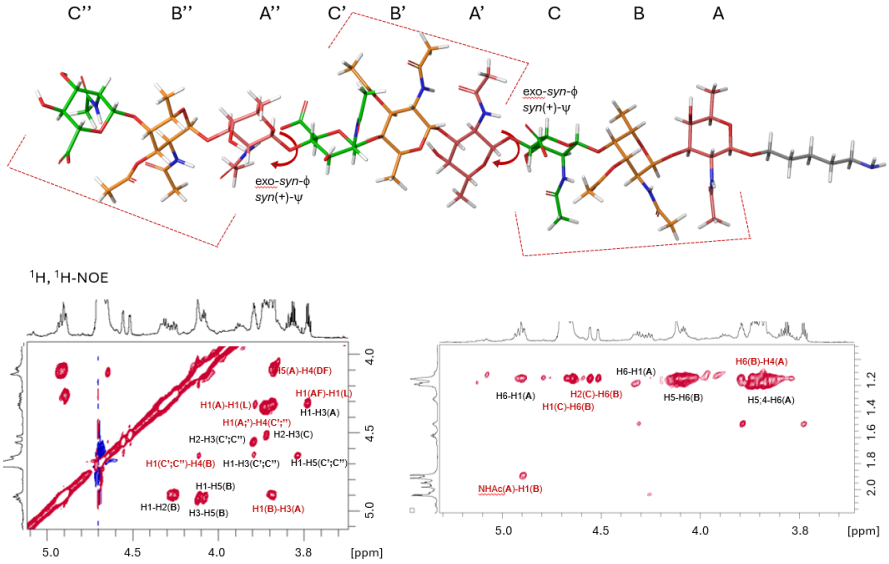


Figure 4: Conformational analysis of trisaccharide **1** (A), hexasaccharide **2** (B) and of nonasaccharide **3** (C) as established by NMR and MM calculations. Monosaccharidic residues are labeled with a letter code. The main conformation at each glycosidic linkage, the spatial orientation of the acetyl 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.

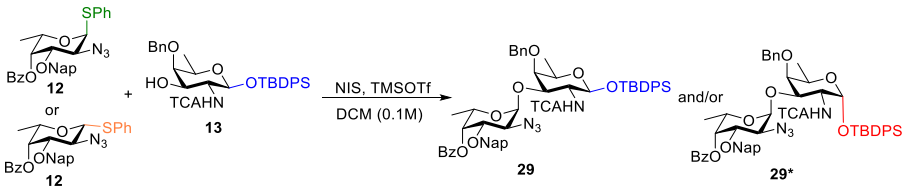
Conflict of interest: Kitt Østerlid has participated in a post graduate studentship program at GSK. This work was sponsored by GlaxoSmithKline Biologicals SA.

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

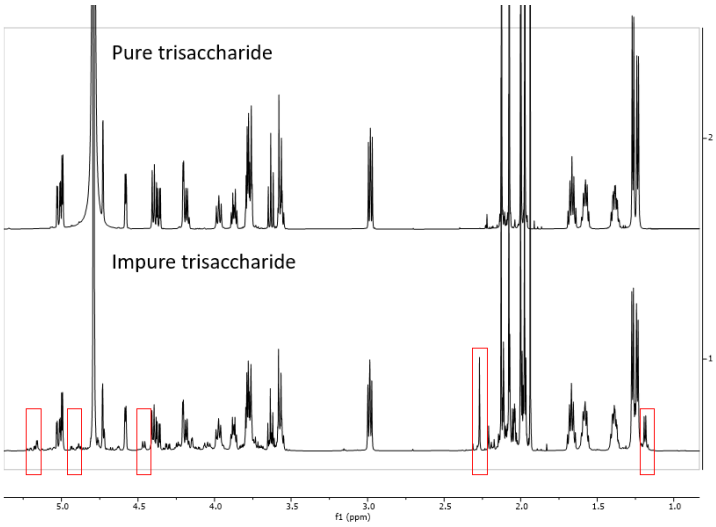


Figure S1: Difference between the pure and impure trisaccharide, obtained with and without HPLC purification, respectively.

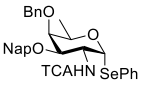
Experimental

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

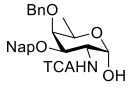
Phenyl 4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-2-*N*-trichloroacetamide-1-seleno-α-D-fucopyranoside (15)



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

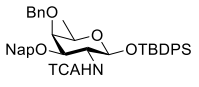
mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.90 – 7.78 (m, 4H, *Ar-H*), 7.55 – 7.44 (m, 5H, Ar-H), 7.41 – 7.20 (m, 8H, Ar-H), 6.86 (d, *J* = 7.5 Hz, 1H, *H*N(CO)CCl_3), 6.04 (d, *J* = 4.7 Hz, 1H, H-1), 5.01 (d, *J* = 11.5 Hz, 1H, ArC*H*$_2$), 4.90 (d, *J* = 12.2, 0.8 Hz, 1H, ArC*H*$_2$), 4.84 – 4.74 (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

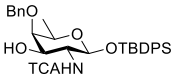
***Tert*-butyldiphenylsilyl 4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-2-*N*-trichloroacetamide-β-D-fucopyranoside (17)**



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),

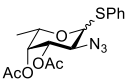
4.96 (d, *J* = 11.8 Hz, 1H, Ar-C*H*$_2$), 4.88 (d, *J* = 7.3 Hz, 1H, H-1), 4.78 (d, *J* = 11.7 Hz, 1H, Ar-C*H*$_2$), 4.68 (d, *J* = 11.7 Hz, 1H, Ar-C*H*$_2$), 4.67 (d, *J* = 11.8 Hz, 1H, ArC*H*$_2$), 4.12 – 3.97 (m, 2H, H-2, H-3), 3.59 (dd, *J* = 2.6, 1.1 Hz, 1H, H-4), 3.30 – 3.20 (m, 1H, H-5), 1.09 – 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

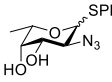
Phenyl 3,4-di-*O*-acetyl-2-azido-2-deoxy-1-thio-α/β-L-fucopyranoside (19)



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),

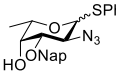
3.64 (t, *J* = 10.2 Hz, 1H, H-2β), 2.19 (s, 3H, COC*H*$_3$α), 2.12 (s, 3H, COC*H*$_3$β), 2.07 (s, 3H, COC*H*$_3$α), 2.04 (s, 3H, COC*H*$_3$β), 1.24 (d, *J* = 6.4 Hz, 3H, *H*-6β), 1.14 (d, *J* = 6.5 Hz, 3H, *H*-6α). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 170.48 (*C*=O), 169.81 (*C*=O), 133.47 (Ar-*C*), 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

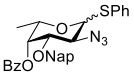
Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-thio-α/β-L-fucopyranoside (21)



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*),

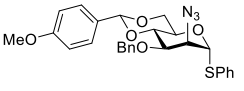
128.72 (Ar-*C*), 128.34 (Ar-*C*), 128.12 (Ar-*C*), 128.06 (Ar-*C*), 127.89 (Ar-*C*), 127.62 (Ar-*C*), 127.21 (Ar-*C*), 127.17 (Ar-*C*), 126.52 (Ar-*C*), 126.49 (Ar-*C*), 126.44 (Ar-*C*), 126.41 (Ar-*C*), 125.87 (Ar-*C*), 125.83 (Ar-*C*), 87.38 (C-1α), 86.22 (C-1β), 81.34 (C-3β), 78.34 (C-3α), 74.54 (C-5β), 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

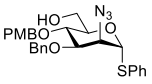
Phenyl 2-azido-2-deoxy-4,6-*O*-*p*-methoxybenzylidene-3-*O*-benzyl-1-thio-α-D-mannopyranoside (23)



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

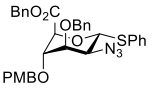
reaction was quenched by the addition of H_2O and diluted in Et_2O. The aqueous phase was extracted with Et_2O (x3) and the combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc, 100:0 → 90:10) provided thioglycoside **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

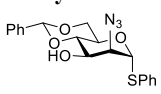
Benzyl (phenyl 2-azido-3-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranosiduronate) (10)



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

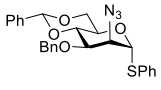
and stirred overnight under nitrogen until TLC analysis (pentane/EtOAc, 7:3) showed full conversion. The reaction mixture was quenched with H_2O. The aqueous phase was extracted with Et_2O (x3), and the combined the organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *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

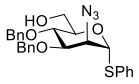
Phenyl 2-azido-4,6-*O*-benzylidene-3-*O*-benzyl-2-deoxy-1-thio-α-D-mannopyranoside (27)



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

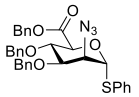
(t, *J* = 10.2 Hz, 1H, *H*-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 137.88 (Ar-C_q), 137.42 (Ar-C_q), 132.14 (Ar-C_q), 129.42 (Ar-*C*), 129.16, 128.66 (Ar-*C*), 128.40 (Ar-*C*), 128.27 (Ar-*C*), 128.07 (Ar-*C*), 127.83 (Ar-*C*), 126.20 (Ar-*C*), 101.79 (Ph-*C*H), 87.36 (C-1), 79.30 (C-4), 75.86 (C-3), 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)

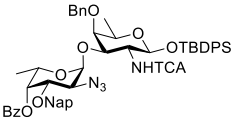


Alcohol **28** (2.473 g, 5.178 mmol) was dissolved in DCM/*t*-BuOH/H_2O (26 mL, 8:4:1, 0.2 M). TEMPO (162 mg, 1.036 mmol, 0.2 equiv.), BAIB (4.169 g, 12.944 mmol, 2.5 equiv.) and AcOH (30 µL, 0.518 mmol, 0.1 equiv.) were added and the reaction mixture was stirred at rt for 2 h until TLC (pentane/EtOAc, 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

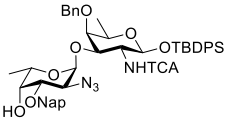
Synthesis of longer fragments

***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

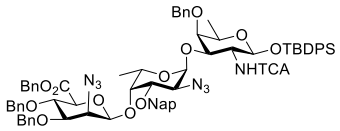
***Tert*-butyldiphenylsilyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-deoxy-2-*N*-trichloroacetamide-4-*O*-benzyl-β-D-fucopyranoside (9)**



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

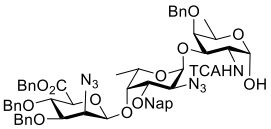
***Tert*-butyldiphenylsilyl (Benzyl (2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-β-D-fucopyranoside (8)**



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

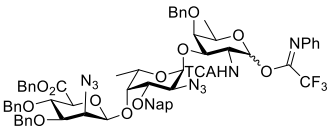
(Ar-*C*), 130.01 (Ar-*C*), 129.64 (Ar-*C*), 129.53 (Ar-*C*), 129.45 (Ar-*C*), 128.74 (Ar-*C*), 128.70 (Ar-*C*), 128.50 (Ar-*C*), 128.40 (Ar-*C*), 128.33 (Ar-*C*), 128.23 (Ar-*C*), 128.14 (Ar-*C*), 128.08 (Ar-*C*), 127.93 (Ar-*C*), 127.90 (Ar-*C*), 127.77 (Ar-*C*), 127.75 (Ar-*C*), 127.60 (Ar-*C*), 127.47 (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)



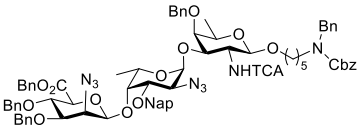
Trisaccharide **8** (529 mg, 0.372 mmol) was dissolved in THF (3.7 mL, 0.1 M) and cooled to 0 °C. AcOH (0.03 mL, 0.558 mmol, 1.5 equiv.) and TBAF (1 M in THF; 0.6 mL, 0.558 mmol, 1.5 equiv.) were added. The reaction mixture was stirred overnight at rt under nitrogen until TLC (pentane/EtOAc, 6:4) showed 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

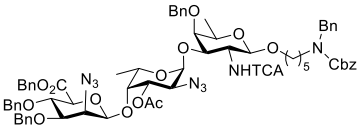
5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)- 4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-α-D-fucopyranoside (4)



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,

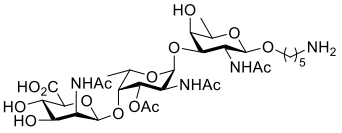
127.81, 127.78, 127.71, 127.50, 127.34, 127.25, 100.99, 99.29, 99.22, 79.83, 79.30, 78.00, 77.48, 77.16, 76.84, 75.40, 75.30, 75.15, 74.99, 72.29, 70.84, 70.65, 69.76, 67.40, 67.22, 66.94, 61.34, 59.25, 56.82, 50.57, 50.31, 47.21, 46.24, 29.26, 28.00, 27.48, 17.20, 17.13. **HRMS**: $[M+Na]^+$ calculated 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

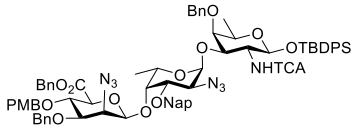
5-aminopentyl 2-*N*-acetamide-2-deoxy-β-D-mannopyranosiduronsyl-(1→4)-2-*N*-acetamide-3-*O*-acetyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-*N*-acetamide-2-deoxy-β-D-fucopyranoside (1)



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

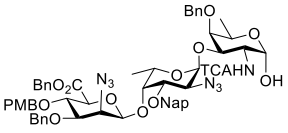
***Tert*-butyldiphenylsilyl (Benzyl (2-azido-2-deoxy-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-β-D-fucopyranoside (7)**



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

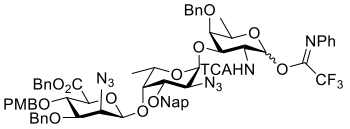
(Ar-C_q), 137.47 (Ar-C_q), 136.21 (Ar-*C*), 135.98 (Ar-*C*), 135.54 (Ar-C_q), 134.98 (Ar-C_q), 133.77 (Ar-C_q), 133.53 (Ar-C_q), 133.44 (Ar-C_q), 133.11 (Ar-C_q), 130.17 (Ar-C_q), 129.72 (Ar-*C*), 129.69 (Ar-*C*), 129.57 (Ar-*C*), 128.82 (Ar-*C*), 128.77 (Ar-*C*), 128.58 (Ar-*C*), 128.46 (Ar-*C*), 128.28 (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)



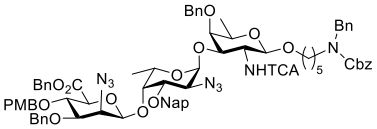
Trisaccharide **7** (297 mg, 0.205 mmol) was dissolved in THF (2 mL, 0.1 M) and cooled to 0 °C. Following, AcOH (24 µL, 0.41 mmol, 2 equiv.) and TBAF (1 M in THF; 0.4 mL, 0.41 mmol, 2 equiv.) were added. The reaction mixture was stirred overnight at rt under nitrogen atmosphere until TLC (pentane/EtOAc, 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.

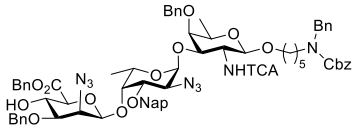
5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (2-azido-3-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-α-D-fucopyranoside (37)



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

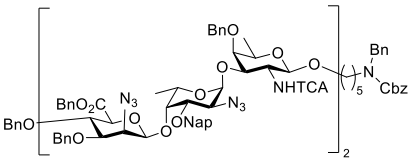
5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosiduronsyl)-(1→4)-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamide-α-D-fucopyranoside (38)



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*),

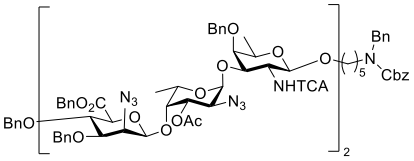
127.77 (Ar-*C*), 127.69 (Ar-*C*), 127.55 (Ar-*C*), 127.52 (Ar-*C*), 126.68 (Ar-*C*), 126.09 (Ar-*C*), 126.03 (Ar-*C*), 125.90 (Ar-*C*), 100.82 (C-1''), 99.24 (C-1'), 99.24 (C-1''), 79.22 (C-4), 78.64 (C-3''), 78.02 (C-3), 75.19 (Ar-*C*H_2), 75.12 (C-3'/C-5''), 75.03 (C-3'/C-5''), 74.69 (C-4'), 72.63 (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)



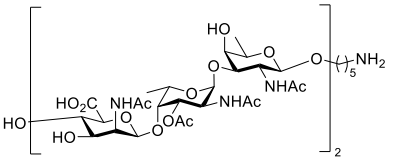
Acceptor **38** (57 mg, 0.041 mmol, 1 equiv.) and donor **31** (74 mg, 0.054 mmol, 1.3 equiv.) were co-evaporated with toluene (x3) before being dissolved in dry DCM/MeCN (1 mL, 2:1; 0.04 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The mixture 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.

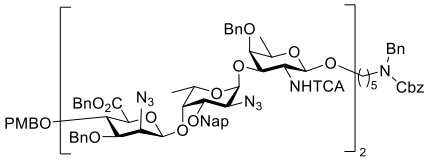
CP5-Hexasaccharide (2)



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

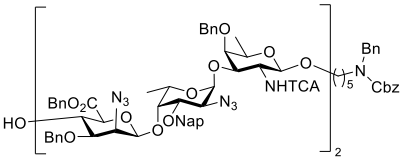
5.03 – 4.96 (m, 4H), 4.74 (d, *J* = 1.4 Hz, 1H), 4.71 (s, 1H), 4.63 (dd, *J* = 4.4, 1.2 Hz, 1H), 4.59 (dd, *J* = 4.3, 1.4 Hz, 1H), 4.43 – 4.32 (m, 4H), 4.20 (d, *J* = 3.1 Hz, 2H), 4.16 (q, *J* = 6.5 Hz, 2H), 4.01 – 3.91 (m, 2H), 3.91 – 3.84 (m, 2H), 3.85 – 3.74 (m, 8H), 3.67 – 3.59 (m, 2H), 3.59 – 3.53 (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)



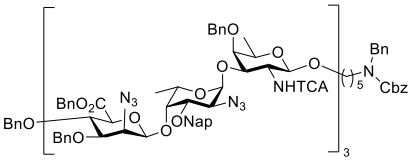
Acceptor **38** (61 mg, 0.0435 mmol) and donor **36** (91 mg, 0.0655 mmol, 1.5 equiv.) were co-evaporated with toluene (x3) before being dissolved in dry DCM/ MeCN (1 mL, 2:1; 0.04 M). Activated 3Å molecular sieves were added and the solution was stirred for 30 min under argon at rt. The mixture was 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

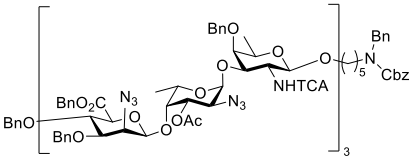
Nonasaccharide-protected with ONap on L-Fuc and Bn on D-Man (6)



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

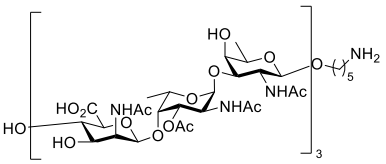
(m, 2H), 4.11 – 3.95 (m, 9H), 3.95 – 3.66 (m, 13H), 3.61 (q, *J* = 6.3 Hz, 1H), 3.53 (dd, *J* = 8.9, 3.4 Hz, 2H), 3.46 (dd, *J* = 7.7, 4.4 Hz, 2H), 3.43 – 3.31 (m, 5H), 3.25 – 3.10 (m, 4H), 1.50 (ddd, *J* = 39.9, 14.5, 7.3 Hz, 4H), 1.28 (d, *J* = 5.6 Hz, 6H), 1.20 – 1.07 (m, 13H). **^{13}C NMR (151 MHz, $CDCl_3$)** 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.

References

(1) Cescutti, P. *Microbial Glycobiology - Chapter 6 - Bacterial Capsular Polysaccharides and Exopolysaccharides*; Academic Press, 2010.

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

(3) O'Riordan, K.; Lee, J. C. Staphylococcus Aureus Capsular Polysaccharides. *Clin. Microbiol. Rev.* **2004**, *17* (1), 218–234. https://doi.org/10.1128/CMR.17.1.218-234.2004.

(4) Fournier, J. M.; Hannon, K.; Moreau, M.; Karakawa, W. W.; Vann, W. F. Isolation of Type 5 Capsular Polysaccharide from Staphylococcus Aureus. *Ann. Inst. Pasteur. Microbiol.* **1987**, *138* (5), 561–567. https://doi.org/10.1016/0769-2609(87)90041-X.

(5) Vann, W. F.; Moreau, M.; Sutton, A.; Byrd, R. A.; Karakawa, W. W. Structure and Immunochemistry of Staphylococcus Aureus Capsular Polysaccharides. *ICN-UCLA Symp. Mol. Cell. Biol.* **1988**, *64*, 187–198.

(6) Moreau, M.; Richards, J. C.; Fournier, J. M.; Byrd, R. A.; Karakawa, W. W.; Vann, W. F. Structure of the Type 5 Capsular Polysaccharide of Staphylococcus Aureus. *Carbohydr. Res.* **1990**, *201* (2), 285–297. https://doi.org/10.1016/0008-6215(90)84244-o.

(7) Jones, C. Revised Structures for the Capsular Polysaccharides from Staphylococcus Aureus Types 5 and 8, Components of Novel Glycoconjugate Vaccines. *Carbohydr. Res.* **2005**, *340* (6), 1097–1106. https://doi.org/10.1016/j.carres.2005.02.001.

(8) Fattom, A. I.; Schneerson, R.; Watson, D. C.; Karakawa, W. W.; Fitzgerald, D.; Pastan, I.; Li, X.; Shiloach, J.; Bryla, D. A.; Robbins, J. B. Laboratory and Clinical Evaluation of Conjugate Vaccines Composed of Staphylococcus Aureus Type 5 and Type 8 Capsular Polysaccharides Bound to Pseudomonas Aeruginosa Recombinant Exoprotein A. *Infect. Immun.* **1993**, *61* (3), 1023–1032. https://doi.org/10.1128/iai.61.3.1023-1032.1993.

(9) Levy, J.; Licini, L.; Haelterman, E.; Moris, P.; Lestrate, P.; Damaso, S.; Van Belle, P.; Boutriau, D. Safety and Immunogenicity of an Investigational 4-Component Staphylococcus Aureus Vaccine with or without AS03B Adjuvant: Results of a Randomized Phase I Trial. *Hum. Vaccines Immunother.* **2015**, *11* (3), 620–631. https://doi.org/10.1080/21645515.2015.1011021.

(10) Creech, C. B.; Frenck, R. W.; Sheldon, E. A.; Seiden, D. J.; Kankam, M. K.; Zito, E. T.; Girgenti, D.; Severs, J. M.; Immermann, F. W.; McNeil, L. K.; Cooper, D.; Jansen, K. U.; Gruber, W. C.; Eiden, J.; Anderson, A. S.; Baber, J. Safety, Tolerability, and Immunogenicity of a Single Dose 4-Antigen or 3-Antigen Staphylococcus Aureus Vaccine in Healthy Older Adults: Results of a Randomised Trial. *Vaccine* **2017**, *35* (2), 385–394. https://doi.org/10.1016/j.vaccine.2016.11.032.

(11) Fattom, A. I.; Horwith, G.; Fuller, S.; Propst, M.; Naso, R. Development of StaphVAX TM , a Polysaccharide Conjugate Vaccine against S . Aureus Infection: From the Lab Bench to Phase III Clinical Trials. *Vaccine* **2004**, *22* (7), 880–887. https://doi.org/10.1016/j.vaccine.2003.11.034.

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

(13) Danieli, E.; Proietti, D.; Brogioni, G.; Romano, M. R.; Cappelletti, E.; Tontini, M.; Berti, F.; Lay, L.; Costantino, P.; Adamo, R. Synthesis of Staphylococcus Aureus Type 5 Capsular Polysaccharide Repeating Unit Using Novel L-FucNAc Synthons and Immunochemical Evaluation. *Bioorganic Med. Chem.* **2012**, *20* (21), 6403–6415. https://doi.org/10.1016/j.bmc.2012.08.048.

(14) Gagarinov, I. A.; Fang, T.; Liu, L.; Srivastava, A. D.; Boons, G.-J. Synthesis of Staphylococcus Aureus Type 5 Trisaccharide Repeating Unit: Solving the Problem of Lactamization. *Org. Lett.* **2015**, *17* (4), 928–931. https://doi.org/10.1021/acs.orglett.5b00031.

(15) Yasomanee, J. P.; Visansirikul, S.; Papapida, P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V. Synthesis of the Repeating Unit of Capsular Polysaccharide Staphylococcus Aureus Type 5 To Study Chemical Activation and Conjugation of Native CP5. *J. Org. Chem.* **2016**, *81* (14), 5981–5987. https://doi.org/10.1021/acs.joc.6b00910.

(16) Hagen, B.; Ali, S.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Mapping the Reactivity and Selectivity of 2-Azidofucosyl Donors for the Assembly of N-Acetylfucosamine-Containing Bacterial Oligosaccharides. *J. Org. Chem.* **2017**, *82* (2), 848–868. https://doi.org/10.1021/acs.joc.6b02593.

(17) Behera, A.; Rai, D.; Kulkarni, S. S. Total Syntheses of Conjugation-Ready Trisaccharide Repeating Units of Pseudomonas Aeruginosa O11 and Staphylococcus Aureus Type 5 Capsular Polysaccharide for Vaccine Development. *J. Am. Chem. Soc.* **2020**, *142* (1), 456–467. https://doi.org/10.1021/jacs.9b11309.

(18) Visansirikul, S.; Kolodziej, S. A.; Demchenko, A. V. Synthesis of Oligosaccharide Fragments of Capsular Polysaccharide Staphylococcus Aureus Type 8. *J. Carbohydr. Chem.* **2020**, *39* (7), 301–333. https://doi.org/10.1080/07328303.2020.1821042.

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

(20) Wang, Z.; Gimeno, A.; Lete, M. G.; Overkleeft, H. S.; van der Marel, G. A.; Chiodo, F.; Jiménez-Barbero, J.; Codée, J. D. C. Synthetic Zwitterionic Streptococcus Pneumoniae Type 1 Oligosaccharides Carrying Labile O-Acetyl Esters. *Angew. Chemie Int. Ed.* **2023**, *62* (1), e202211940. https://doi.org/10.1002/anie.202211940.

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

(22) Ohlin, M.; Johnsson, R.; Ellervik, U. Regioselective Reductive Openings of 4,6-Benzylidene Acetals: Synthetic and Mechanistic Aspects. *Carbohydr. Res.* **2011**, *346* (12), 1358–1370. https://doi.org/10.1016/j.carres.2011.03.032.

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

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

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

(26) Noti, C.; Paz, J. L. De; Polito, L.; Seeberger, P. H. Preparation and Use of Microarrays Containing Synthetic Heparin Oligosaccharides for the Rapid Analysis of Heparin – Protein Interactions. *Chem. - A Eur. J.* **2006**, *12*, 8664–8686. https://doi.org/10.1002/chem.200601103.

(27) Walker, E.; Jeanloz, R. W. The Synthesis of Oligosaccharide-L-Asperagine

Compounds. Part VI. Di-N-Acetylisochitobiose-Lasparagine, 2-Acetamido-6-O-(2-Acetamido-2-Deoxy-β-D-Glucopyranosyl)-1-N-(L-Aspart-4-Oyl-2-Deoxy-β-D-Glycopyranosylamine. *Carbohydr. Res.* **1974**, *32* (613), 145–154. https://doi.org/10.1016/S0008-6215(00)82471-4.

(28) Gaitonde, V.; Sucheck, S. J. Synthesis of β-Glycosyl Amides from N-Glycosyl Dinitrobenzenesulfonamides. *J. Carbohydr. Chem.* **2012**, *31* (4–6), 353–370. https://doi.org/10.1080/07328303.2012.663431.

(29) Ghirardello, M.; Ledru, H.; Sau, A.; Galan, M. C. Chemo-Selective Rh-Catalysed Hydrogenation of Azides into Amines. *Carbohydr. Res.* **2020**, *489* (February), 107948. https://doi.org/10.1016/j.carres.2020.107948.

(30) Volbeda, A. G.; Kistemaker, H. A. V.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V.; Codée, J. D. C. Chemoselective Cleavage of P-Methoxybenzyl and 2-Naphthylmethyl Ethers Using a Catalytic Amount of HCl in Hexafluoro-2-Propanol. *J. Org. Chem.* **2015**, *80* (17), 8796–8806. https://doi.org/10.1021/acs.joc.5b01030.

(31) Doyle, L. M.; Meany, F. B.; Murphy, P. V. Lewis Acid Promoted Anomerisation of Alkyl O- and S-Xylo-, Arabino- and Fucopyranosides. *Carbohydr. Res.* **2019**, *471* (October 2018), 85–94. https://doi.org/10.1016/j.carres.2018.11.010.