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

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Chapter 2

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

Introduction

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

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

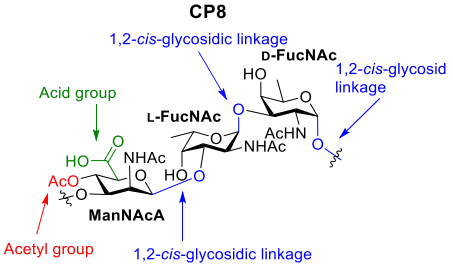


Figure 1: A schematic representation of the repeating unit of CP8.

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

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

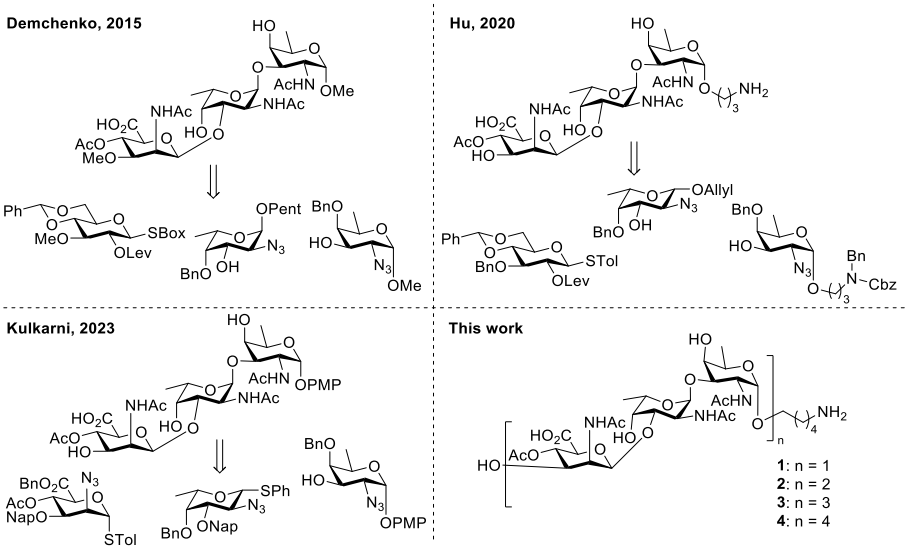


Figure 2: Previously synthesized trisaccharides and the CP8-fragments described in this thesis.

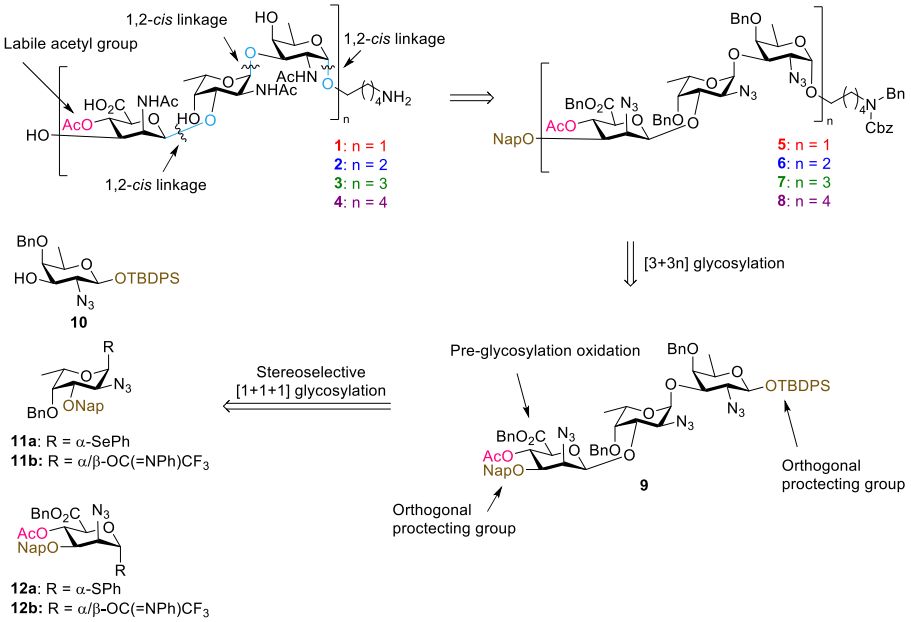
This Chapter describes the synthesis of CP8 structures carrying an *O*-acetyl on C-4 of the ManNAcA residue and their use in structural and epitope mapping studies. Specifically, this Chapter report on a set of CP8 oligosaccharides ranging in length from a trisaccharide (1 RU) to a dodecasaccharide (4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

Results and discussion

Synthesis of the CP8-fragments

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

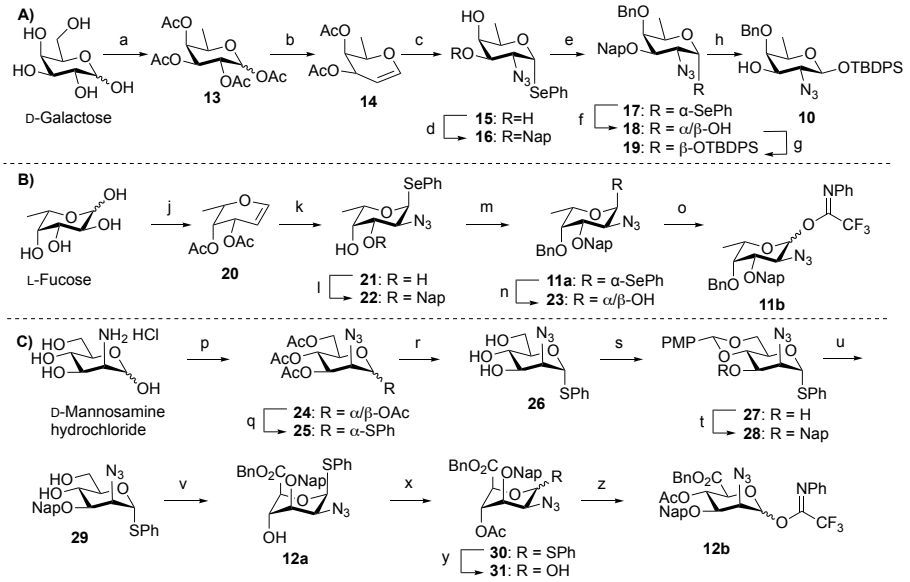
All the building blocks were synthesized from commercially available starting materials. The synthesis of the D-FucN_3 building block (Scheme 2A) commenced with D-galactose following a reported procedure.[33] In a 5-step reaction sequence in which the required galactose-to-fucose deoxygenation was achieved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



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

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

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



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

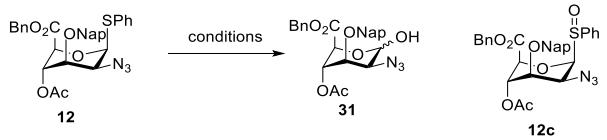
The D-ManAN_3 was obtained from D-mannosamine hydrochloride (Scheme 2C) by an azidotransfer with freshly prepared triflic azide (TfN_3) followed by an one-pot acetylation[37] giving **24** in 98% yield. Pyridine was chosen as solvent, thus enabling an in-situ acetylation to avoid formation of the glucose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

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

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

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

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



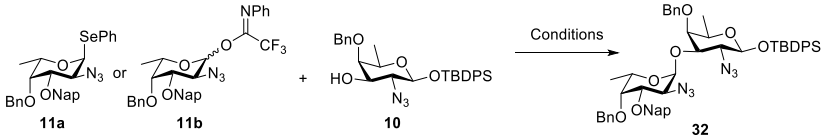
Entry	Conditions	Temp (°C)	Time (h)	Yield (%)	Notes
1	NBS (2 equiv.), acetone/H_2O 10:1	rt	1	48	33 % of **12c**
2	NBS (2 equiv.), acetone/H_2O 10:1	0 to rt	0.75	43	42 % of **12c**
3	NIS (1.1 equiv.), TFA (1.1 equiv.), DCM/H_2O 10:1	0	--	--	No reaction
4	NIS (2.5 equiv.), TFA (1.1 equiv.), DCM/H_2O 10:1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

[(a)] Impure product.

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

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

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



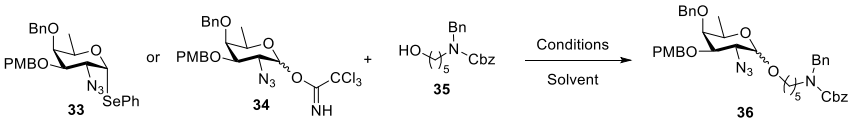
Entry	Donor	Conditions	Temp (°C)	Time (h)	Yield (%)	α/β [(a)]
1	**11a**	NIS, TMSOTf	-78 to -50	1	71	35:65
2	**11a**	NIS, TMSOTf	-80 to -60	2	51	20:80
3	**11a**	NIS, TMSOTf	rt	0.5	62	52:48
4	**11b**	TMSOTf	rt	0.5	11	99:1[(b)]
5	**11b**	TMSOTf	rt	1.5	25	99:1[(b)]
6	**11b**	TMSOTf	rt	2	36	99:1[(b)]
7	**11b**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

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

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

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

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



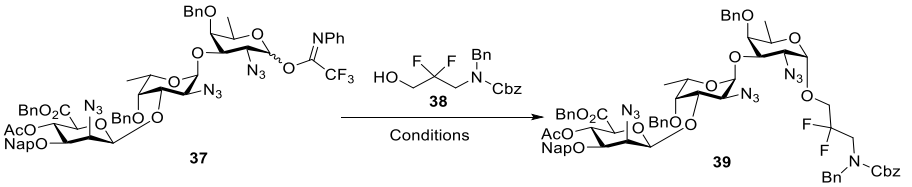
Entry	Donor	Conditions	Solvent	Temp (°C)	Time (h)	Yield (%)	α/β [(a)]
1	**33**	TMSOTf, NIS	DCM/ Et_2O 1:1	-40 to -20	1.5	78	10:90
2	**33**	TMSOTf, NIS	DCM/ Et_2O 1:1	-40 to rt	19	64	6:94
3	**33**	TMSOTf, NIS	DCM	rt	2	64	22:68
4	**34**	Ph_3PO, TMSI	DCM	rt	23	96	55:45
5	**34**	Ph_3PO, TMSI	DCM/ Et_2O 1:1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

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

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

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

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



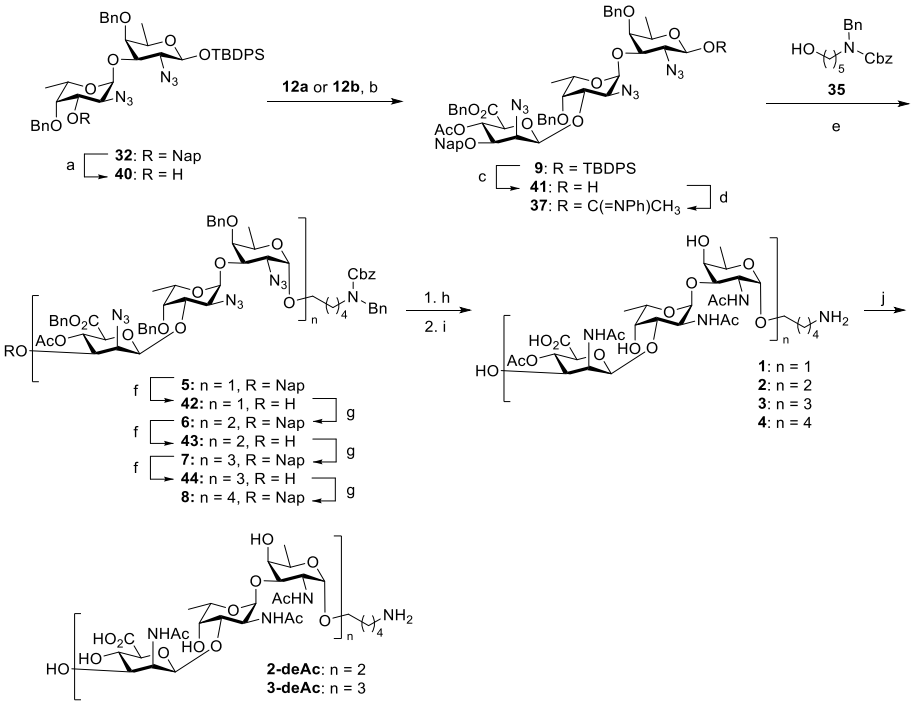
Entry	Conditions	Solvent	Time (h)	Yield (%)	α/β [(a)]
1	TMSOTf	DCM	0.5	86	64:34
2	TMSI, Ph_3PO	DCM	22	51	83:17
3	TMSI, Ph_3PO	DCM/Et_2O 1:1	22	46	87:13

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

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

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

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



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

Conjugates and antibody binding

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

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

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

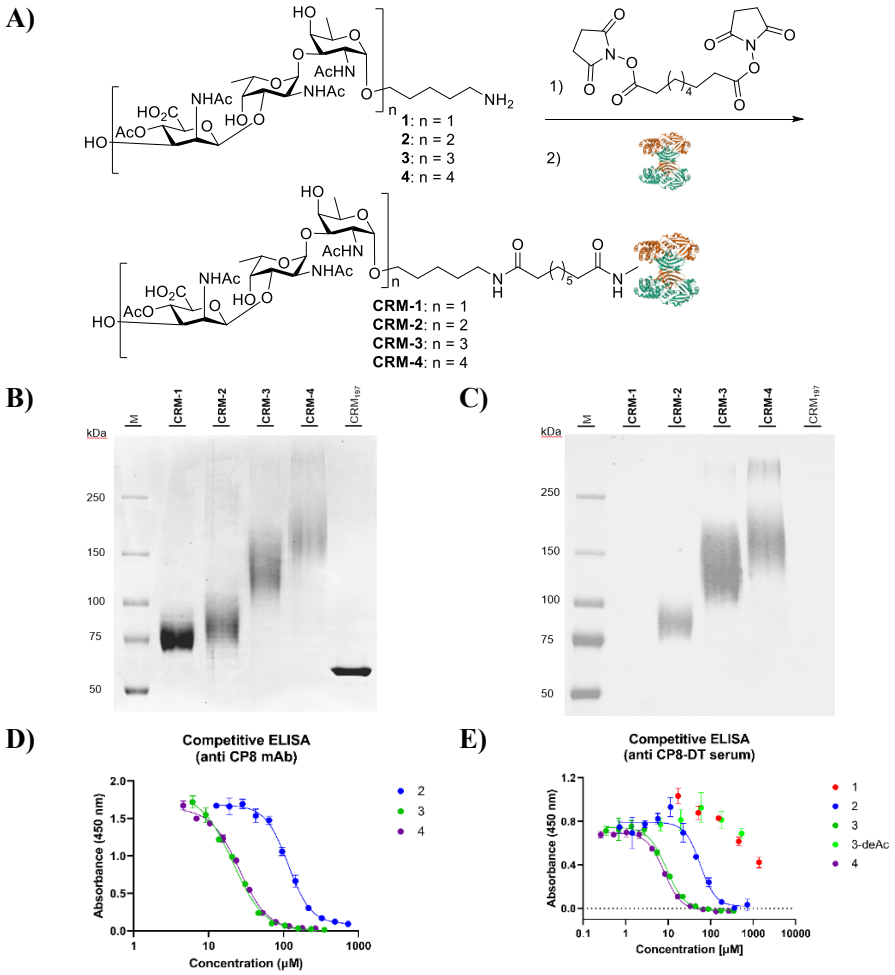


Figure 3: A) Conjugation strategy of the synthetic fragments. 1) Suberic acid bis(N-hydroxy-succinimide ester) 30 equiv. for **1** and 15 equiv. for **2-4** in DMSO/H_2O 9:1, 2) CRM_{197} in PBS or HEPES 25 mM. B) SDS-page with the conjugates **CRM1-4** created in HEPES 25 mM at pH=8. C) Western Blot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

Structural, conformational, and interaction studies

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

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

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

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

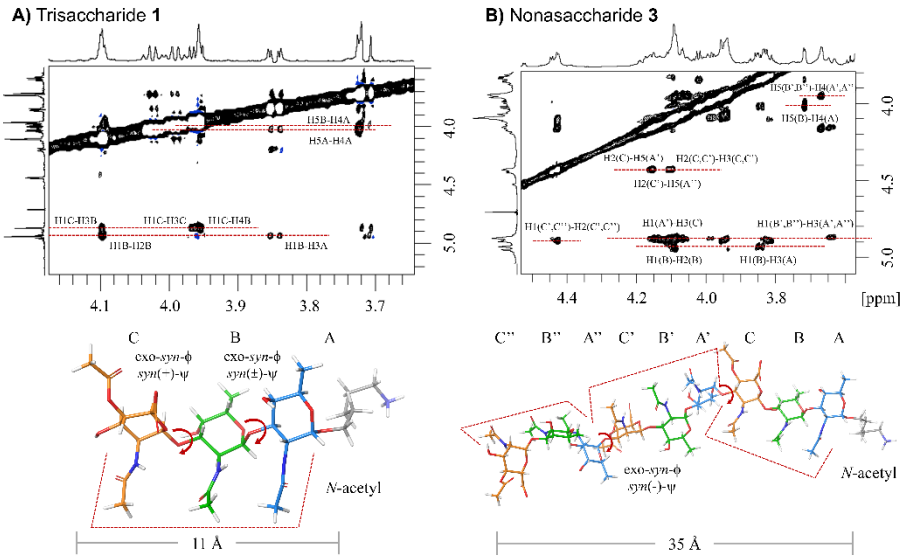


Figure 4: Conformational analysis of trisaccharide **1** and of nonasaccharide **3** as established by NMR and MM calculations. A) Zoom area of 2D NOESY spectrum of trisaccharide **1** (left) and its main conformation as defined by NOE analysis and MM calculations. B) Zoom area of 2D NOESY spectrum of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

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

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

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

***In vivo* studies**

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

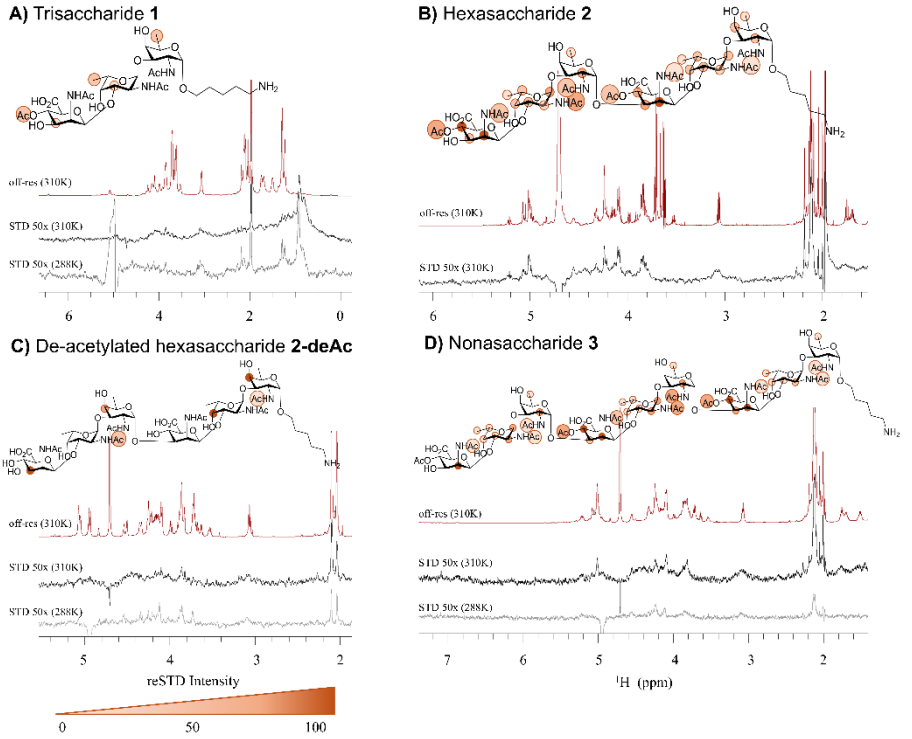


Figure 5: ^{1}H STD-NMR spectra performed for the complexes of mAb-CP8 and the trisaccharide **1**, (A) the hexasaccharide **2** (B) the de-acetylated hexasaccharide **2-deAc** (C), and the nonasaccharide **3** (D). Off-resonance spectra (in red) and corresponding STD-NMR spectra at 310 K (in black)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

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

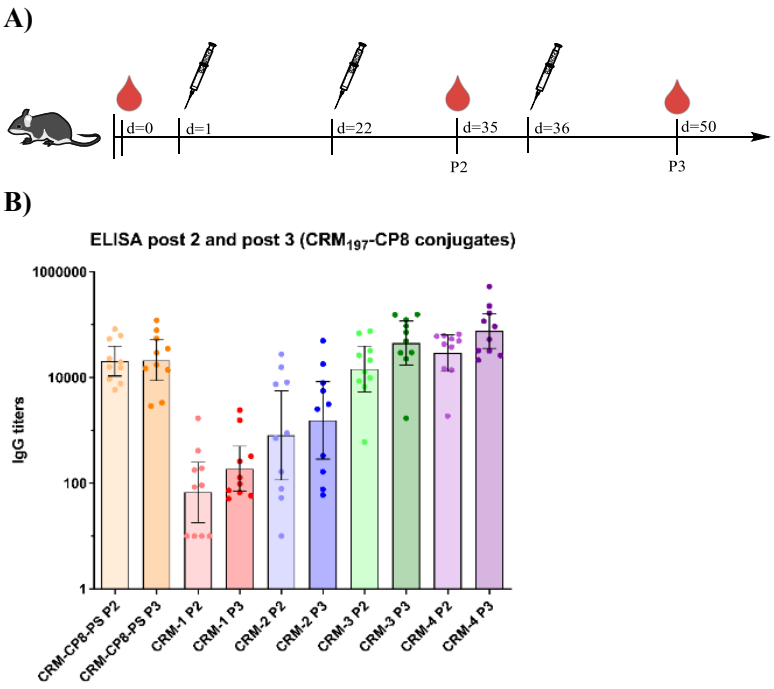


Figure 6: A) Illustration of the *in vivo* study. Injections were performed at day 1, day 22 and day 36 and blood collections were performed at day 0, day 35 (post 2) and day 50 (post 3) B) ELISA post 2 (P2) and post 3 (P3) IgG titers.

Conclusion

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

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

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

Acknowledgement

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

Experimental

General experimental procedures

All reagents were of commercial grade and used as received unless otherwise noted. All moisture sensitive reactions were performed under an argon or nitrogen (N_2) atmosphere. Dried solvents (DCM, DMF, THF, toluene, Et_2O) were stored over flame-dried 3 or 4Å molecular sieves. Reactions were monitored by thin layer chromatography (TLC) analysis conducted with Merck aluminum sheets with 0.20 mm of silica gel 60. The plates were detected by UV (254 nm) and were applicable by spraying with 20% sulfuric acid in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% sulfuric acid (aq.) followed by charring at ~150 °C. Flash column chromatography was performed with silica gel (40-63µm). Size-exclusion chromatography was carried out using SephadexTM (LH-20, GE Healthcare Life Sciences) by isocratic elution with DCM/MeOH (1:1, v:v). High-resolution mass spectra were recorded on a Thermo Finigan LTQ Orbitrap mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275 ºC) with resolution R=60.000 at m/z=400 (mass range 150-4000). ^1H and ^{13}C spectra were recorded on a Bruker AV-400 (400 and 101 MHz respectively), Bruker AV-500 (500 and 126 MHz respectively), Bruker AV-600 (600 and 151 MHz respectively), Bruker AV-850 (800 and 214 MHz respectively) or a Bruker AV-1200 (1200 and 302 MHz respectively). Chemical shifts (δ) are given in ppm relative to the residual signal of the deuterated solvent (^1H-NMR: 7.26 ppm for $CDCl_3$, 3.31 ppm for MeOD, 1.94 for $CNCD_3$ or 4.79 for D_2O. ^{13}C-NMR: 77.16 ppm for $CDCl_3$, 49.00 ppm for MeOD, 1.32 for $CNCD_3$). Coupling constants (*J*) are given in Hz. All ^{13}C spectra are proton decoupled. NMR peak assignments were made using COSY and HSQC experiments, where applicable, HMBC and GATED experiments were used to further elucidate the structure. The anomeric product ratios were analyzed through integration of proton NMR signals.

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

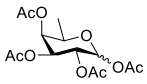
General glycosylation of [3+3], [3+6] and [3+9]
The trisaccharide donor **16** (1.3 equiv.) and the acceptor (1 equiv.) was co-evaporated with toluene (3x), dissolved in dry DCM (0.1 M), added 3Å molecular sieves at rt and stirred for 30 min at rt. TBSOTf (0.2 equiv.) was added at rt and the reaction was stirred at rt under argon until TLC showed full conversion (~30 min). The reaction was quenched with Et_3N, dissolved in EtOAc, washed with $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4 and concentrated. Purification by column chromatography and/or size exclusion gave the wanted product.

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

TLC showed full conversion. The solution was filtered, concentrated *in vacuo* and co-evaporated with toluene (x3). Column chromatography (DCM/MeOH 98:2 → 95:5) and/or size exclusion gave the wanted product. The acetamide-OS was dissolved in *t*-BuOH (1.5 mL) and added AcOH (1 mL, 0.1 mL in 100 mL MilliQ). Another 1 mL *t*-BuOH was added to dissolve the compound. The solution was birched with argon for 20 min and then added $Pd(OH)_2/C$ (catalytic amount). The reaction was again birched with argon for 5 minutes before the atmosphere was changed for H_2. The mixture was stirred for under H_2 atmosphere for three days or until completion by NMR was detected. The mixture was filtered over a Whatman filter and lyophilized. Purification by a HW40 column with NH_4OAc followed by lyophilization gave the wanted product.

Synthesis of building blocks

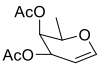
1,2,3,4-tetra-*O*-acetyl-D-fucopyranose (13)



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

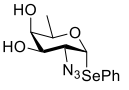
5.07 (dd, *J* = 10.4, 3.4 Hz, 1H, β-H-3), 4.30 – 4.23 (q, *J* = 6,5 Hz, 1H, α-H-5), 3.95 (*J* = 6.5 Hz, 1H, β-H-5), 2.19 (s, 3H, COC*H*$_3$), 2.18 (s, 3H, COC*H*$_3$), 2.14 (s, 3H, COC*H*$_3$), 2.11 (s, 3H, COC*H*$_3$), 2.04 (s, 3H, COC*H*$_3$), 2.01 (s, 3H, COC*H*$_3$), 2.00 (s, 3H, COC*H*$_3$), 1.99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

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



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

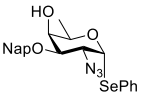
Phenyl 2-azido-2-deoxy-1-seleno-α-D-fucopyranoside (15)



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

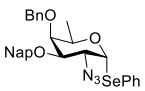
H-1), 4.29 (q, *J* = 6.5 Hz, 1H, H-5), 4.01 (dd, *J* = 9.9, 5.3 Hz, 1H, H-2), 3.76 – 3.68 (m, 2H, H-4, H-3), 1.15 (d, *J* = 6.6 Hz, 3H, H-6). **^{13}C NMR (101 MHz, MeOD)** δ 135.91 (Ar-*C*), 130.04 (Ar-*C*), 128.72 (Ar-C_q), 86.89 (C-1), 72.91 (C-3), 72.68 (C-4), 70.61 (C-5), 62.91 (C-2), 16.42 (C-6).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

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



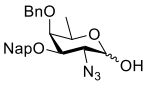
15 (3.60 g, 10.94 mmol) was co-evaporated with toluene (x3) and dissolved in dry toluene (55 ml, 0.2 M). Bu_2SnO (2.778 g, 11.16 mmol, 1.02 equiv.) was added and the flask was equipped with a Dean-Stark. The reaction was heated to 140 ºC for 3 h. The now clear solution was cooled to 60 ºC before adding Bu_4NBr (3.704 g, 11.49 mmol, 1.05 equiv.), CsF (1.965 g, 11.16 mmol, 1.02 equiv.) and NapBr (2.540 g, 11.49 mmol, 1.05 equiv.). The reaction was heated to 120 ºC for 1 h until TLC (pentane/EtOAc 3:2) showed full conversion. The reaction was allowed to cool to rt before a 10% KF solution was added and the reaction was stirred for 30 min. The aqueous phase was extracted with EtOAc (x3) and the combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 9:1 → 7:3) gave **16** in 93% yield (4.768 g, 10.18 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.92 – 7.82 (m, 4H, Ar-*H*), 7.63 – 7.45 (m, 5H, Ar-*H*), 7.33 – 7.27 (m, 3H, Ar-*H*), 5.91 (d, *J* = 5.4 Hz, 1H, H-1), 4.88 (dd, *J* = 13.6, 11.5 Hz, 2H, Ar-CH_2), 4.30 (qt, *J* = 6.6, 1.5 Hz, 1H, H-5), 4.21 (dd, *J* = 10.2, 5.3 Hz, 1H, H-2), 3.91 (dt, *J* = 3.2, 1.6 Hz, 1H, H-4), 3.76 (dd, *J* = 10.2, 3.1 Hz, 1H, H-3), 2.39 (t, *J* = 1.6 Hz, 1H, OH), 1.26 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 133.35 (Ar-*C*), 133.33 (Ar-C_q), 129.24 (Ar-C_q), 128.79 (Ar-*C*), 128.63 (Ar-C_q) 128.13 (Ar-*C*), 127.94 (Ar-*C*), 127.91 (Ar-*C*), 127.17 (Ar-*C*), 126.53 (Ar-*C*), 126.45 (Ar-*C*), 125.81 (Ar-*C*), 85.30 (C-1), 79.35 (C-3), 72.42 (Ar-CH_2), 68.71 (C-5, C-4), 60.40 (C-2), 16.83 (C-6). **HRMS**: $[M+H]^+$ calculated for $C_{23}H_{23}N_3O_3SeH$: 470.09829; found 470.09776

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



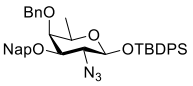
16 (3.228 g, 6.89 mmol) was dissolved in DMF (67 mL, 0.1 M) and cooled to 0 ºC. BnBr (1.06 mL, 8.96 mmol, 1.3 equiv.) and NaH (60% suspension in mineral oil, 358 mg, 8.96 mmol, 1.3 equiv.) was added and the solution was slowly allowed to warm to rt and stirred under N_2 for 18 h until TLC (pentane/EtOAc 9:1) showed full conversion. The reaction was quenched with H_2O and extracted with Et_2O (x3). The combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 85:15) gave **17** in 88% yield (3.627 g. 6.49 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.92 – 7.81 (m, 4H, Ar-*H*), 7.60 – 7.53 (m, 3H, Ar-*H*), 7.53 – 7.47 (m, 2H, Ar-*H*), 7.37 – 7.19 (m, 8H, Ar-*H*), 5.95 (d, *J* = 5.3 Hz, 1H, H-1), 5.01 – 4.87 (m, 3H, Ar-CH_2, Ar-CH_2), 4.65 (d, *J* = 11.4 Hz, 1H, Ar-CH_2), 4.40 (dd, *J* = 10.3, 5.3 Hz, 1H, H-2), 4.23 (q, *J* = 6.3 Hz, 1H, H-5), 3.79 (dd, *J* = 10.3, 2.7 Hz, 1H, H-3), 3.74 (dd, *J* = 2.8, 1.2 Hz, 1H, H-4), 1.14 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.24 (Ar-C_q), 135.09 (Ar-C_q), 134.50 (Ar-*C*), 133.42 (Ar-C_q), 133.23 (Ar-C_q), 128.54 (Ar-*C*), 128.45 (Ar-*C*), 128.29 (Ar-*C*), 128.13 (Ar-*C*), 127.92 (Ar-*C*), 127.89 (Ar-*C*), 127.80 (Ar-*C*), 126.76 (Ar-*C*), 126.41 (Ar-*C*), 126.26 (Ar-*C*), 125.81 (Ar-*C*), 85.68 (C-1), 80.79 (C-3), 75.98 (C-4), 75.16 (Ar-*C*H_2-3), 72.77 (Ar-*C*H_2), 69.55 (C-5), 61.17 (C-2), 16.69 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{30}H_{29}N_3O_3SeNa$: 582.12718; found 582.12685

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



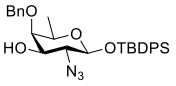
17 (3.618 g, 6.47 mmol) was dissolved in acetone/H_2O (130 mL, 10:1, 0.05 M), cooled to 0 ºC and added NIS (2.912 g, 12.94 mmol, 2 equiv.). The reaction was stirred at 0 ºC for 15 min until TLC (pentane/EtOAc 3:2) showed full conversion. The solvents were evaporated and the residue was dissolved in EtOAc,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

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



18 (2.688 g, 6.408 mmol) was co-evaporated with toluene (x3), dissolved in dry DCM 32 mL, 0.2 M) and cooled to 0 ºC. TBDPS-Cl (1.97 mL, 7.69 mmol, 1.2 equiv.), imidazole (1.091 g, 16.02 mmol, 2.5 equiv.) and DMAP (157 mg, 1.282 mmol, 0.2 equiv.) were added and the reaction was stirred at rt under N_2 for 2 h until TLC (pentane/EtOAc 95:5) showed full conversion. The solution was dissolved in EtOAc, washed with 1 M HCl (x3) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 100:0 → 90:10) gave **19** in 96% (4.067 g, 6.18 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.87 – 7.65 (m, 10H, Ar-*H*), 7.53 – 7.28 (m, 16H, Ar-*H*), 4.96 (d, *J* = 11.8 Hz, 1H, Ar-CH_2), 4.83 (d, *J* = 2.2 Hz, 2H, Ar-CH_2), 4.68 (d, *J* = 11.8 Hz, 1H, Ar-CH_2), 4.31 (d, *J* = 7.7 Hz, 1H, H-1), 3.89 (dd, *J* = 10.4, 7.7 Hz, 1H, H-2), 3.46 (dd, *J* = 3.1, 1.1 Hz, 1H, H-4), 3.25 (dd, *J* = 10.4, 2.9 Hz, 1H, H-3), 3.08 (qd, *J* = 6.4, 1.1 Hz, 1H, H-5), 1.11 (s, 9H, TBDPS-CH_3), 1.01 (d, *J* = 6.4 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.59 (Ar-C_q), 136.25 (Ar-*C*), 136.11 (Ar-*C*), 135.44 (Ar-C_q), 134.94 (Ar-C_q), 133.59 (Ar-C_q), 133.36 (Ar-C_q), 133.28 (Ar-C_q), 133.17 (Ar-C_q), 129.81 (Ar-*C*), 129.64 (Ar-*C*), 128.43 (Ar-*C*), 128.38 (Ar-*C*), 128.36 (Ar-*C*), 128.07 (Ar-*C*), 127.87 (Ar-*C*), 127.79 (Ar-*C*), 127.56 (Ar-*C*), 127.32 (Ar-*C*), 126.64 (Ar-*C*), 126.34 (Ar-*C*), 126.16 (Ar-*C*), 125.83 (Ar-*C*), 97.44 (C-1), 81.18 (C-3), 75.35 (C-4), 74.79 (Ar-*C*H2), 73.58 (Ar-*C*H$_2$), 71.28 (C-5), 66.76 (C-2), 27.01 (TBDPS-*C*H$_3$), 16.71 (C-6). **HRMS**: $[M+Na]^+$ calculated for $C_{40}H_{43}N_3O_4SiNa$: 680.29205; found 680.29150

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

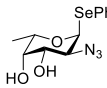


19 (4.3923 g, 5.01 mmol) was dissolved in DCM/H_2O (50 mL, 20:1, 0.1 M) and added DDQ (1.705 g, 7.51 mmol, 1.5 equiv.). The reaction was stirred at rt under N_2 for 2 h until TLC (pentane/EtOAc 9:1) showed full conversion. The solution was quenched with $Na_2S_2O_3$ (aq. sat.), dissolved and extracted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

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

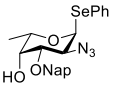
A solution of Ac_2O (80 mL, 14 equiv.) and pyridine (100 ml, 0.6 M) was cooled to 0 ºC. L-Fucose (10 g, 60.92 mmol) was added portion wise and the reaction was stirred at 4 C under N_2 for 18 h until TLC (pentane/EtOAc 3:2) showed full conversion. The solution was poured over ice and stirred until the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

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



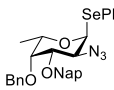
22 (6.345 g, 29.64 mmol) and $(PhSe)_2$ (9.248 g, 29.64 mmol, 1 equiv.) was dissolved in DCM (150 mL, 0.2 M) and degassed under argon at rt for 30 min. The reaction was cooled to -30 ºC and added BAIB (9.548 g, 29.64 mmol, 1 equiv.) and $TMSN_3$ (7.7 mL, 59.28 mmol, 2 equiv.). The reaction was allowed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

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



21 (6.005 g, 18.25 mmol) was co-evaporated with toluene (x3) and dissolved in toluene (91 mL, 0.2 M). Bu_2SnO (4.634 g, 18.62 mmol, 1.02 equiv.) was added and the flask was equipped with a Dean Stark. The reaction was heated to 140 ºC for 3 h and the now clear solution was cooled to 60 ºC before adding Bu_4NBr (6.178 g, 19.16 mmol, 1,05 equiv.), CsF (2.828 g, 18.62 mmol, 1.02 equiv.) and NapBr (4.235 g, 19.16 mmol, 1.05 equiv.). The reaction was heated to 120 ºC for 1 h until TLC (pentane/EtOAc 3:2) showed full consumption. The reaction was allowed to cool to rt before a 10% KF solution was added and the reaction was stirred for 30 min. The aqueous phase was extracted with EtOAc (x3) and the combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 9:1 → 7:3) gave **22** in 91% yield (7.785 g, 26.62 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.92 – 7.83 (m, 4H, Ar-*H*), 7.63 – 7.45 (m, 5H, Ar-*H*), 7.36 – 7.27 (m, 3H, Ar-*H*), 5.91 (d, *J* = 5.3 Hz, 1H, *H-1*), 4.92 (d, *J* = 11.5 Hz, 1H, Ar-C*H*$_2$), 4.86 (d, *J* = 11.5 Hz, 1H, Ar-C*H*$_2$), 4.35 – 4.26 (m, 1H, H-5), 4.21 (dd, *J* = 10.1, 5.3 Hz, 1H, H-2), 3.91 (dt, *J* = 3.1, 1.5 Hz, 1H, H-4), 3.76 (dd, *J* = 10.2, 3.1 Hz, 1H, H-3), 2.43 (t, *J* = 1.6 Hz, 1H, OH) , 1.26 (d, *J* = 6.6 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 134.55 (Ar-*C*$_q$), 134.60 (Ar-*C*), 133.33 (Ar-*C*$_q$), 133.31 (Ar-*C*$_q$), 129.23 (Ar-C), 128.76 (Ar-*C*), 127.92 (Ar-*C*), 127.89 (Ar-*C*), 127.14 (Ar-*C*), 126.51 (Ar-*C*), 126.43 (Ar-*C*), 125.80 (Ar-*C*), 85.29 (C-1), 79.35 (C-3), 72.38 (Ar-*C*H$_2$), 68.70 (C-4, C-5), 68.68 (C-4, C-5), 60.37 (C-2), 16.17 (C-6). **HRMS**: $[M+H]^+$ calculated for $C_{23}H_{23}N_3O_3SeH$: 470.09829; found 470.09776

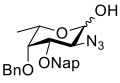
Phenyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-L-fucopyranoside (11a)



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

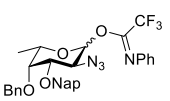
H_2O and extracted with Et_2O (x3). The combined organic phases were washed with brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 85:15) gave **11a** in 93% yield (3.743 g, 6.70 mmol). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.94 – 7.83 (m,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

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



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

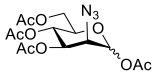
2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-L-fucopyranose (11b)



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

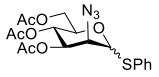
= 6.3 Hz, 3H, *H-6*). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 138.06 (Ar- C_q), 134.95 (Ar- C_q), 133.35 (Ar- C_q), 126.49 (Ar-*C*), 126.34 (Ar-*C*), 125.84 (Ar-*C*), 124.41 (Ar-*C*), 119.44 (Ar-*C*), 80.97 (C-3/C-4), 75.04 (Ar-*C*H_2), 74.74 (C-5), 73.00 (Ar-*C*H_2), 72.01 (C-3/C-4), 62.26 (C-2), 16.82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

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



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

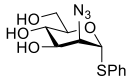
Phenyl 3,4,6 tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α/β-D-mannopyranoside[49] (25)



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

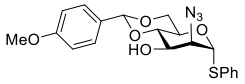
(Ar-*C*), 85.96 (C-1), 71.30 (C-4), 69.68 (C-5), 66.17 (C-3), 62.82 (C-2), 62.29 (C-6), 20.86 (CO*C*H$_3$), 20.83 (CO*C*H$_3$), 20.72 (CO*C*H$_3$). **HRMS**: [M+Na]$^+$ calculated for $C_{18}H_{21}N_3O_7SNa$: 446.09979; found 446.09924

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



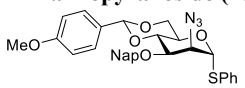
α-**25** (14.95 g, 35.30 mmol) was dissolved in MeOH (117 mL, 0.3 M) and added NaOMe (25% wt in MeOH, 0.8 mL, 3.53 mmol, 0.1 equiv.). The reaction was stirred at rt for 3 h until TLC (pentane/EtOAc 4:6) showed full conversion and then neutralized with Amberlite IR-120 H$^+$ resins, filtered and concentrated.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

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



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

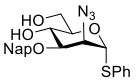
Phenyl 2-azido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-3-*O*-(2-naphthylmethyl)-1-thio-α-D- mannopyranoside (28)



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

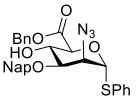
7.42 – 7.37 (m, 2H, Ar-*H*), 7.32 – 7.28 (m, 3H, Ar-*H*), 6.96 – 6.87 (m, 2H, Ar-*H*), 5.62 (s, 1H, PMP-C*H*), 5.43 (d, *J* = 1.1 Hz, 1H, H-1), 5.07 (d, *J* = 12.7 Hz, 1H, Ar-CH_2), 4.92 (d, *J* = 12.4 Hz, 1H, Ar-CH_2), 4.32 (m, 1H, H-5), 4.25 – 4.15 (m, 4H, H-2, H-3, H-4, H-6), 3.87 – 3.84 (m, 1H, H-6)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

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



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

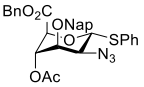
Benzyl (phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-thio-α-D-mannopyranosiduronate) (30)



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

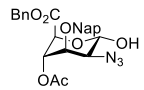
4.88 (d, *J* = 11.8 Hz, 1H, Ar-CH_2), 4.68 (d, *J* = 8.0, 1H, H-5), 4.38 (td, *J* = 7.9, 3.5 Hz, 1H, H-4), 4.00 – 3.91 (m, 2H, H-2, H-3), 2.88 (d, *J* = 3.5 Hz, 1H, C4-OH). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.80 (C-6), 135.06 (Ar-*C*), 134.83 (Ar-*C*), 133.38 (Ar-*C*), 133.28 (Ar-*C*), 132.32 (Ar-*C*),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

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



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

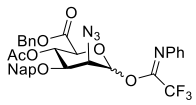
Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-D-mannopyranosiduronate) (31)



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

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

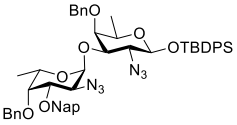
Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-mannopyranosiduronate) (12b)



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

Synthesis of the trisaccharide

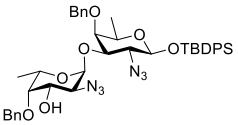
***Tert*-butyldiphenylsilyl 2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-β-D-fucopyranoside (32)**



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

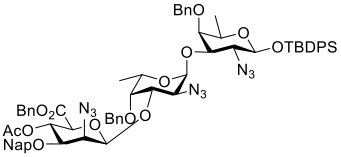
(Ar-C_q), 133.20 (Ar-C_q), 133.10 (Ar-C_q), 129.88 (Ar-*C*), 128.51 (Ar-*C*), 128.48 (Ar-*C*), 128.45 (Ar-*C*), 128.08 (Ar-*C*), 127.95 (Ar-*C*), 127.84 (Ar-*C*), 127.79 (Ar-*C*), 127.60 (Ar-*C*), 127.31 (Ar-*C*), 126.80 (Ar-*C*), 126.35 (Ar-*C*), 126.21 (Ar-*C*), 125.92 (Ar-*C*), 100.07 (C-1'), 97.35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

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



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

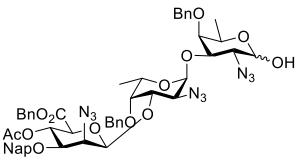
***Tert*-butyldiphenylsilyl (Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-β-D-mannopyranosiduronsyl)-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-β-D-fucopyranoside (9)**



Donor **12b** (1.02 g, 1.54 mmol, 1.5 equiv.) and acceptor **40** (780 mg, 1.00 mmol, 1 equiv.) was co-evaporated with toluene (3x), dissolved in dry DCM (10 mL, 0.1 M), added 3Å molecular sieves and stirred for 30 min. The solution was cooled to -80 ºC and TfOH (18 µL, 0.20 mmol, 0.2 equiv.) was added. The reaction was allowed to warm to -10 ºC and stirred until TLC (pentane/EtOAc 8:2) showed full conversion of the acceptor (~5 h). The reaction was quenched with Et_3N, dissolved in EtOAc, washed with $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 95:5 → 80:20) gave **9** in 68% (851 mg, 0.68 mmol) and in a α/β ratio 15:85. <u>For the β-anomer</u>: **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.83 – 7.68 (m, 8H, Ar-*H*), 7.52 – 7.27 (m, 24H, Ar-*H*), 5.46 (t, *J* = 9.2 Hz, 1H, H-4''), 5.19 (dd, *J* = 12.2, 3.7 Hz, 1H, H-1'), 5.05 (dd, *J* = 12.2, 1.6 Hz, 2H, Ar-CH_2), 4.83 – 4.71 (m, 3H, Ar-CH_2), 4.66 (d, *J* = 7.2 Hz, 1H, Ar-CH_2), 4.63 (d, *J* = 6.9 Hz, 1H, Ar-

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

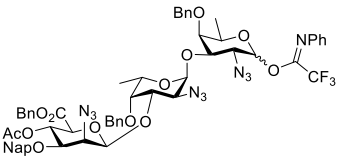
(Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-β-D-mannopyranosiduronsyl))-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α/β-D-fucopyranose (41)



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

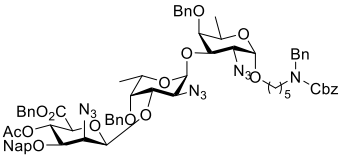
and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 7:3 → 5:5) gave **41** in 84% yield (613 mg, 0.60 mmol) as a α/β mixture. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.81 (t, *J* = 8.0 Hz, 6H), 7.74 (d, *J* = 1.7 Hz, 2H), 7.52 – 7.42 (m, 6H), 7.39 – 7.27 (m, 27H), 5.46 (td, *J* = 9.3, 1.0 Hz,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

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



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

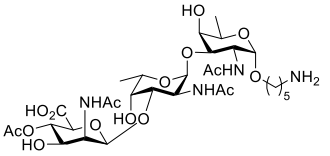
5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (4-*O*-acetyl-2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-β-D-mannopyranosiduronsyl)-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-D-fucopyranoside (5)



Donor **37** (202 mg, 0.17 mmol, 1 equiv.) and *N*-(Benzyl)-benzyloxycarbonyl-5-aminopentan-1-ol[50] **35** (72 mg, 0.22 mmol, 1.3 equiv.) was co-evaporated with toluene (3x). The donor, acceptor and Ph_3P=O (284 mg, 1.021 mmol, 6 equiv.) was dissolved in dry DCM/Et_2O (1.7 mL, 1:1, 0,1 M), added 3Å molecular sieves and stirred for 1 h. The solution was added TMSI (24 µL, 0;17 mmol, 1 equiv.) and stirred for 24 h until TLC (pentane/EtOAc 3:2) showed full conversion. The reaction was quenched with Et_3N, dissolved in EtOAc, washed with $Na_2S_2O_3$ (sat. aq.; x1), $NaHCO_3$ (sat. aq.; x1) and brine (x1), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography (pentane/EtOAc 75:25 → 55:45) gave **5** in 93% yield (209 mg, 0.158 mmol) and in a α/β ratio 75:25. <u>For the α-anomer:</u> **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.84 – 7.71 (m, 4H, Ar-*H*), 7.53 – 7.41 (m, 3H, Ar-*H*), 7.39 – 7.26 (m, 26H, Ar-*H*), 7.25 – 7.11 (m, 2H, Ar-*H*), 5.46 (t, *J* = 9.1 Hz, 1H, H-4''), 5.23 – 5.13 (m, 3H, H-1', Ar-CH_2), 5.03 (d, *J* = 2.6 Hz, 2H, Ar-CH_2), 4.90 (d, *J* = 7.7 Hz, 1H, H-1), 4.82 – 4.72 (m, 3H, Ar-CH_2), 4.69 – 4.63 (m, 2H, Ar-CH_2), 4.59 – 4.55 (m, 2H, H-1'', Ar-CH_2), 4.54 – 4.45 (m, 3H, CH_2-Linker), 4.21 (dd, *J* = 10.6, 2.8 Hz, 1H, H-3'), 4.03 (d, *J* = 10.8 Hz, 1H, H-3), 3.96 – 3.87 (m, 1H, H-5), 3.87 – 3.77 (m, 4H, H-5', H-2, H-5'', H-2'), 3.67 – 3.61 (m, 1H, H-4), 3.58 (dd, *J* = 9.1, 3.5 Hz, 1H, H-2''), 3.54 (d, *J* = 4.9 Hz, 2H), 3.47 – 3.32 (m, 2H, H-4', H-3''), 3.31 – 3.14 (m, 2H, CH_2-Linker), 1.84 (s, 3H, COCH_3), 1.73 – 1.46 (m, 4H, CH_2-Linker), 1.41 – 1.24 (m, 4H, CH_2-Linker), 1.21 (d, *J* = 6.5 Hz, 3H, H-6), 1.14 (d, *J* = 6.5 Hz, 3H, H-6). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.34 (C=O), 166.57 (C=O), 138.61 (Ar-C_q), 138.03 (Ar-C_q), 135.09 (Ar-C_q), 134.66 (Ar-C_q), 133.22 (Ar-C_q), 128.82 (Ar-*C*), 128.67 (Ar-*C*), 128.61 (Ar-*C*), 128.55 (Ar-*C*), 128.51 (Ar-*C*), 128.48 (Ar-*C*), 128.27 (Ar-*C*), 128.06 (Ar-*C*), 127.96 (Ar-*C*), 127.94 (Ar-*C*), 127.90 (Ar-*C*), 127.77 (Ar-*C*), 127.72 (Ar-*C*), 126.91 (Ar-*C*), 126.55 (Ar-*C*), 126.40 (Ar-*C*), 125.70 (Ar-*C*), 99.89 (C-1'), 98.17 (C-1), 97.61 (C-1''), 80.05 (C-4'), 77.48 (C-3''), 76.84 (C-3'), 76.11 (C-3), 75.50 (Ar-*C*H$_2$), 75.25 (Ar-*C*H$_2$), 73.76 (C-5'), 72.43 (Ar-*C*H$_2$), 68.45 (C-4''), 68.05 (Ar-*C*H$_2$), 67.73 (C-5), 67.46 (Ar-*C*H$_2$), 66.87 (C-4), 61.40 (C-2''), 60.25 (C-2'), 58.91 (C-2), 29.25 (*C*H$_2$-Linker), 23.50 (*C*H$_2$-Linker), 20.76 (CO*C*H$_3$), 16.96 (C-6, C-6'), 16.80 (C-6, C-6'). <u>For the β-anomer:</u> **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.85 – 7.78 (m, 3H, Ar-*H*), 7.74 (d, *J* = 1.7 Hz, 1H, Ar-*H*), 7.51 – 7.41 (m, 3H, Ar-*H*), 7.37 – 7.26 (m, 24H, Ar-*H*), 7.17 (s, 1H, Ar-*H*), 5.46 (t, *J* = 9.2 Hz, 1H, H-4''), 5.25 (d, *J* = 3.8 Hz, 1H, H-1'), 5.21 – 5.13 (m, 3H, Ar-*H*), 5.04 (d, *J* = 2.5 Hz, 2H, Ar-*H*), 4.83 – 4.68 (m, 4H, Ar-*H*), 4.58 – 4.52 (m, 2H, Ar-*H*, H-1''), 4.49 (d, *J* = 6.9 Hz, 2H, CH_2-Linker), 4.17 (m, 2H, H-1, H-3'), 3.87 – 3.80 (m, 2H, H-2, H-5''), 3.70 (td, *J* = 10.9, 5.2 Hz, 2H, H-2', H-5'), 3.64 (dt, *J* = 3.6, 1.6 Hz, 1H, H-2''), 3.59 (ddd, *J* = 9.0, 3.5, 1.8 Hz, 1H, H-3''), 3.57 – 3.47 (m, 2H, H-4', H-5), 3.44 (dd, *J* = 10.5, 2.8 Hz, 1H, H-4), 3.40 – 3.36 (m, 1H, CH_2-Linker), 3.33 – 3.15 (m, 3H, CH_2-Linker), 1.85 (d, *J* = 2.4 Hz, 3H, COCH_3), 1.27 (d, *J* = 2.9 Hz, 3H, H-6, H-6'), 1.12 (d, *J* = 6.7 Hz, 3H, H-6, H-6'). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.32 (C=O), 166.48 (C=O), 138.61 (Ar-C_q), 138.05 (Ar-C_q), 135.05 (Ar-C_q), 134.65 (Ar-C_q), 133.23 (Ar-C_q), 128.83 (Ar-*C*), 128.80 (Ar-*C*), 128.66 (Ar-*C*), 128.63 (Ar-*C*), 128.61 (Ar-*C*), 128.54 (Ar-*C*), 128.47 (Ar-*C*), 128.39 (Ar-*C*), 128.31 (Ar-*C*), 128.26 (Ar-*C*), 128.07 (Ar-*C*), 127.94 (Ar-*C*), 127.89 (Ar-*C*), 127.76 (Ar-*C*), 127.63 (Ar-*C*), 127.37 (Ar-*C*), 126.88 (Ar-*C*), 126.56 (Ar-*C*), 126.41 (Ar-*C*), 125.68 (Ar-*C*), 102.72 (C-1), 100.21 (C-

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

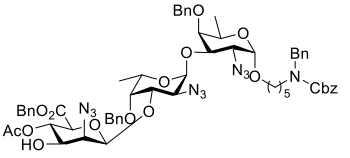
5-aminopentyl 2-acetamide-4-*O*-acetyl-2-deoxy-β-D-mannopyranosiduronsyl-(1→3)-2-acetamide-2-deoxy-α-L-fucopyranosyl-(1→3)-2-acetamide-2-deoxy-α-D-fucopyranoside (1)



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

Synthesis of longer fragments

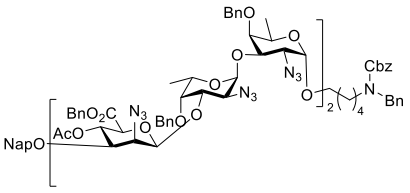
5-(Benzyl(benzyloxycarbonyl)amino)pentyl (Benzyl (4-*O*-acetyl-2-azido-2-deoxy-β-D-mannopyranosiduronsyl)-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-α-D-fucopyranoside (42)



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

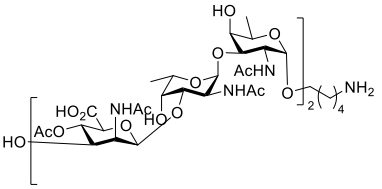
Hz, 1H), 4.05 (d, *J* = 11.0 Hz, 1H), 3.93 (m, 1H), 3.90 – 3.80 (m, 3H), 3.75 (dd, *J* = 10.6, 3.6 Hz, 1H), 3.68 – 3.51 (m, 6H), 3.49 – 3.32 (m, 1H), 3.23 (m, 4H), 2.63 (d, *J* = 9.9 Hz, 1H), 1.86 (s, 3H), 1.64 – 1.45 (m, 5H), 1.40 – 1.25 (m, 4H), 1.21 (t, *J* = 6.1 Hz, 8H). **^{13}C NMR (101 MHz, $CDCl_3$)**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

Hexasaccharide protected (6)



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

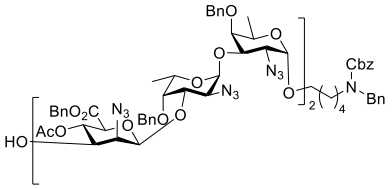
CP8-hexasaccahride (2)



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

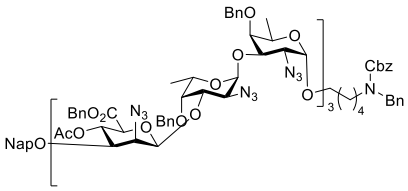
3.73 (d, *J* = 2.6 Hz, 1H, H^4-4), 3.70 (dd, *J* = 10.9, 3.1 Hz, 1H H^1-4), 3.67 – 3.59 (m,1H, C*H*$_2$-Linker), 3.43 (dt, *J* = 10.0, 6.2 Hz, 1H, C*H*$_2$-Linker), 3.00 – 2.93 (m, 2H, C*H*$_2$-Linker), 2.09 (s, 3H, COC*H*$_3$), 2.06 – 1.99 (m, 12H, COC*H*$_3$), 1.95 (s, 3H, COC*H*$_3$), 1.91 (s, 3H,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

Hexasaccharide-acceptor (43)



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

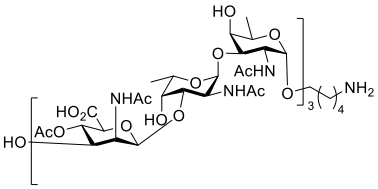
Nonasaccharide protected (7)



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

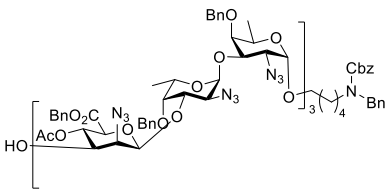
4H), 7.40 – 7.27 (m, 52H), 7.22 – 7.13 (m, 2H), 5.44 (t, *J* = 9.2 Hz, 1H), 5.35 – 5.28 (m, 3H), 5.24 (d, *J* = 3.7 Hz, 1H), 5.21 – 5.12 (m, 4H), 5.03 – 4.99 (m, 4H), 4.98 (s, 2H), 4.97 – 4.92 (m, 2H), 4.90 (d, *J* = 8.6 Hz, 1H), 4.84 – 4.77 (m, 4H), 4.75 – 4.67 (m, 5H), 4.67 – 4.53 (m, 9H), 4.49 (d,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

CP8-Nonasaccharide (3)



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

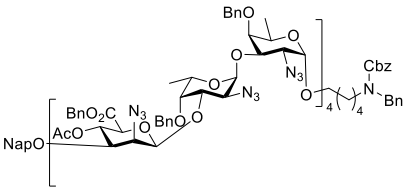
Nona-acceptor (44)



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

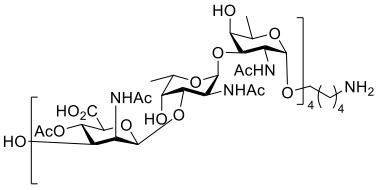
– 3.85 (m, 5H), 3.81 (d, *J* = 3.2 Hz, 1H), 3.78 – 3.71 (m, 3H), 3.67 (dt, *J* = 10.5, 6.5 Hz, 1H), 3.47 (dt, *J* = 12.1, 6.3 Hz, 1H), 3.00 (t, *J* = 7.7 Hz, 2H), 2.14 (s, 3H), 2.09 – 2.05 (m, 20H), 2.04 (d, *J* = 3.4 Hz, 6H), 1.99 (s, 3H), 1.95 (s, 5H), 1.66 (ddq, *J* = 43.9, 14.0, 7.4 Hz, 4H), 1.44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

Dodeca-saccharide (8)



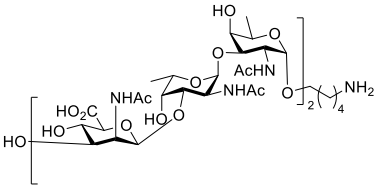
The glycosylation was performed using the general glycosylation procedure with donor **35** (13 mg, 0.0104 mmol, 1.5 equiv.) and acceptor **44** (20 mg, 0.00691 mmol, 1 equiv.) dissolved in dry DCM (1 mL, 0.007 M) and added (TBSOTf 0.3 µL, 0.00138 mmol, 0.2 equiv.) The reaction was followed with TLC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

CP8-Dodeca (4)



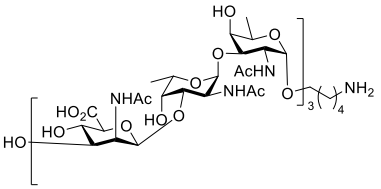
8 (12.2 mg, 0.00299 mmol, 1 equiv.) was deprotected following the general experimental for the deprotection yielding **4** in 33% yield over two steps (2.65 mg, 0.0010 mmol). **^{1}H NMR (850 MHz, D_2O)** δ 5.26 (s, 2H), 5.17 (d, *J* = 10.2 Hz, 3H), 5.08 (s, 2H), 5.07 – 4.89 (m, 10H), 4.60 (s, 1H), 4.50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

CP8-deAc-Hexasaccharide (2-deAc)



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

CP8-deAc-Nonasaccharide (3-deAc)



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

Supporting information

Preparation of *S. aureus* type 8 conjugates

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

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

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

Evaluation by SDS-PAGE:

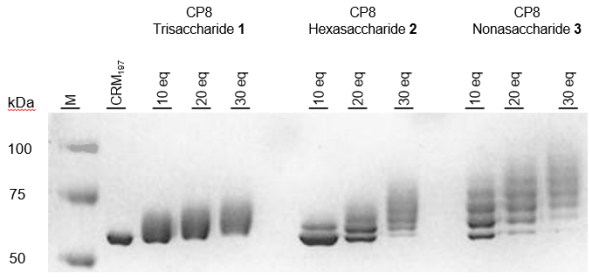


Figure S1: Evaluation of the CP8-conjugates performed in PBS

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

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

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

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

Evaluation by SDS-PAGE:

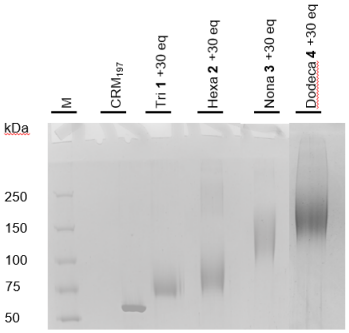


Figure S2: Evaluation of the CP8-conjugates performed in HEPES 25 mM

Evaluation of the conjugates can be found in Table S1.

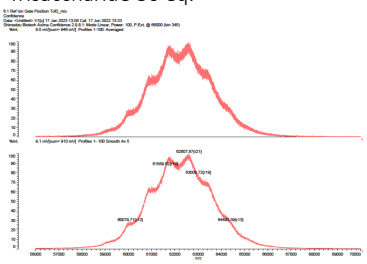
	1	2	3	4
Average number of conjugated sugar chains (MALDI)	11	8	13	14
Protein quantification (BCA) [mg/mL]	1.68	1.19	0.92	0.75
Average protein [µM]	19.0	10.3	9.2	12.8
Average sugar [µM]	316	163	206	187
Average sugar [µg/mL]	228	224	409	490
Saccharide/protein w/w	0.14	0.19	0.44	0.65

Table S1: Evaluation of the conjugates

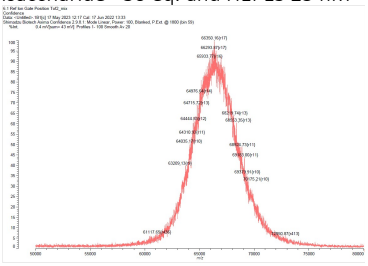
MALDI-TOF MS

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

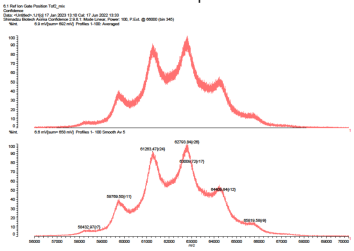
CP8 – Trisaccharide 30 eq.



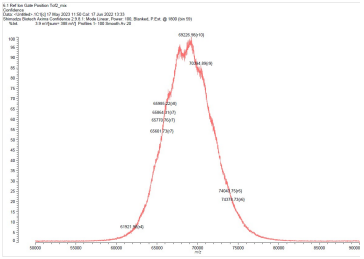
CP8 – Trisaccharide +30 eq. and HEPES 25 nM



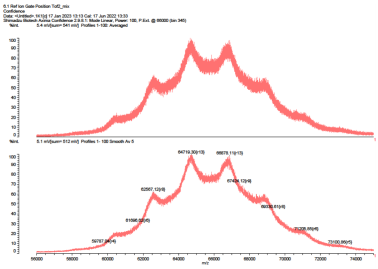
CP8 – Hexasaccharide 30 eq.



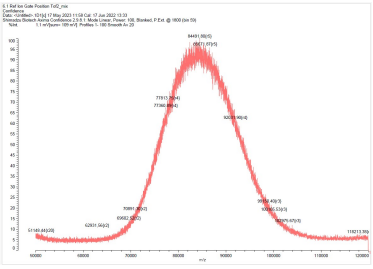
CP8 – Hexasaccharide +30 eq. and HEPES 25 nM



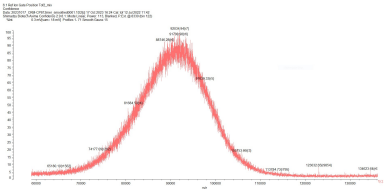
CP8 – Nonasaccharide 30 eq



CP8 – Nonasaccharide +30 eq. and HEPES 25 nM



CP8 – Dodecasaccharide +30 eq. and HEPES 25 nM



Protocol for Western Blot (using mAb and pAb)

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

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

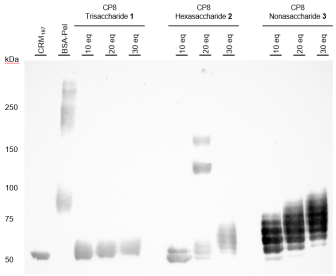


Figure S3: Western Blot with the CP8-Crm_{197} conjugates in PBS with pAb

Protocol for competitive ELISA with mAb

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

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

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

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

ELISA titers (synthetic fragments and mAb)

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

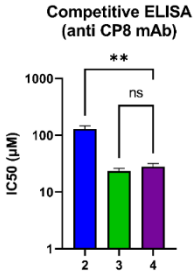


Figure S4: ELISA titers with synthetic fragments and mAb

Protocol for competitive ELISA with pAb

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

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

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

ELISA titers (synthetic fragments and pAb)

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

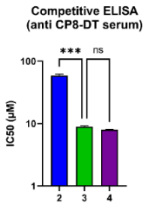


Figure S5. ELISA titers with synthetic fragments and pAb

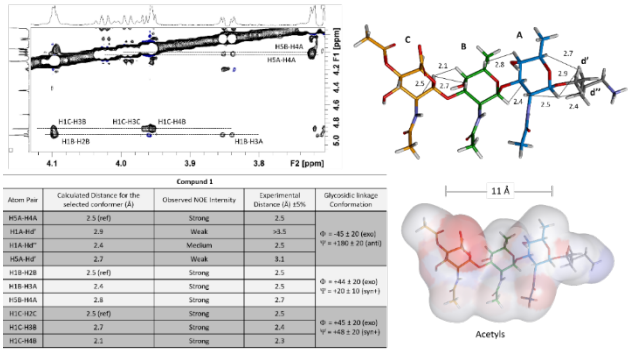
Structural conformation

Structure and conformational studies

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

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

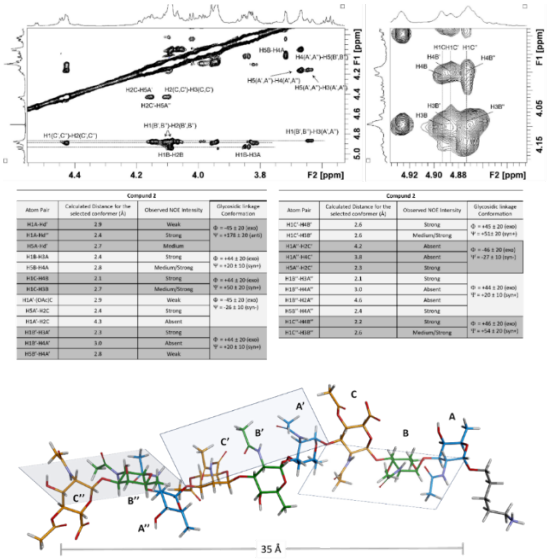
Molecular Mechanics Calculations. The geometry optimization was performed by using the Jaguar/Schroedinger package (version 13.5) and the AMBER* force field, with the GB/SA continuum solvent model for water. The glycosidic torsion angles were defined as ϕ (H1'-C1'-Ox-Cx) and ψ (C1'-Ox-Cx-Hx). Extended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



Compound 1

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

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



Compound 2

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

Compound 2

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

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

Ligands-Antibody interaction studies

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

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

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

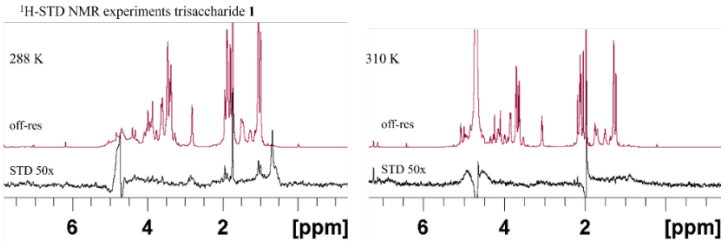


Figure S8: ^{1}H-STD NMR experiments of the trisaccharide **1** in presence on the mAb CP8.

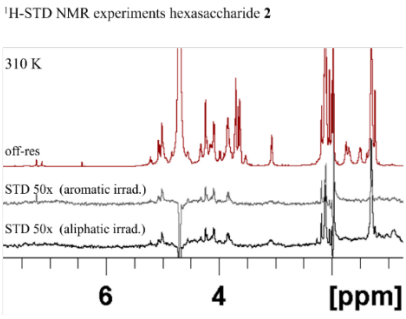


Figure S9: ^{1}H-STD NMR experiments of the hexasaccharide **2** in presence on the mAb CP8.

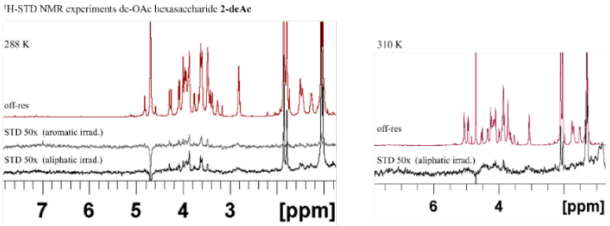


Figure S10: [1]H-STD NMR experiments of the de *O*-acetylated hexasaccharide **2-deAc** in presence on the mAb CP8.

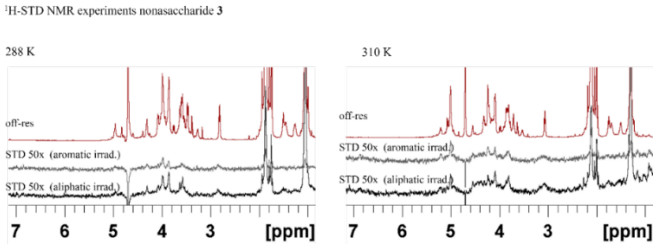


Figure S11: 1H-STD NMR experiments of the nonasaccharide **3** in presence on the mAb CP8.

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

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

Immunizations

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

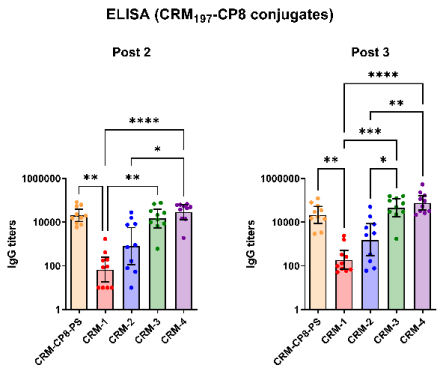
Five groups of 10 CD1 mice (5-week-old, female) were injected subcutaneous on day 1, 22 and 36 with 1.0 µg (saccharide titer) of conjugated carbohydrate antigen formulated with aluminum hydroxide as adjuvant. Sera were collected at day 0 (before first injection), 35 (32 days after first immunization)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

ELISA protocol for *in vivo* studies

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

ELISA titers from the in vivo studies

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



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