Synthesis and applications of cell wall glycopolymer fragments from Staphilococci and Enterococci

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Chapter 2

Chemical synthesis of fragments of the capsular polysaccharide of Staphylococcus aureus serotype 5

INTRODUCTION

Capsular polysaccharides (CPs) are found as the protective outer layer of many bacterial species. They are built up from monosaccharides leading to linear or branched, homo- or heteropolymers, that are covalently attached to the cell membrane. Interfacing with their surroundings, their main biological roles are communication with and protection from the environment. The diversity of CPs structures is driven not only by the nature of the constituting monosaccharides and the way these are interconnected, but also by the presence of cationic or anionic charges, substituents such *N*- or *O*- acetyls, pyruvic acid ketals, lactic acids among the most abundant.[1]

The first use of CP as immunogenic component in vaccines were reported at the beginning of the last century,[2] but it is only in the last decades that glycoconjugate based vaccines have been effectively applied for controlling and preventing infectious diseases caused by for example *H. influenzae* type b (Hib), *N. meningitidis*, *S. pneumoniae* or group *B Streptococcus*.[3] However, their potential has not yet been fully exploited and better understanding of their molecular composition can help to design more effective glycoconjugate vaccines in the future.[4]

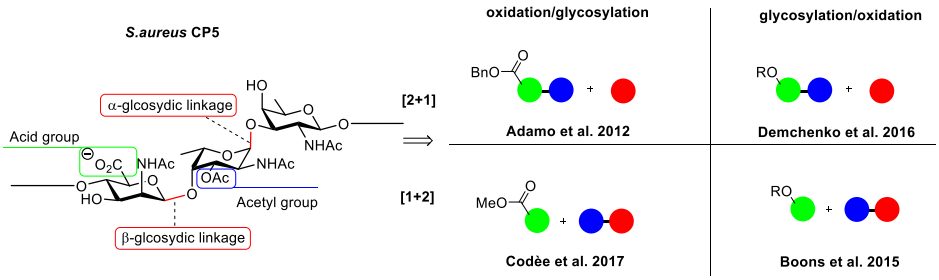
Methicillin resistant *S. aureus* (MRSA) strains have been appointed by the WHO as one of those pathogens (ESKAPE), for which new treatments are urgently needed.[5] Serotype 5 and 8 are the most abundant among the clinical isolates and they are characterized by the presence of a capsular polysaccharide referred to as respectively CP5 and CP8. Vaccine candidates based on both native CP serotypes have shown promising results in preclinical trial settings, but discordancy in efficacy has been observed during advanced clinical phase studies.[6] The negative outcome has been related to various strategies of the bacterium to evade the innate and adaptive host immune system,[7] but the failure has also been attributed to possible manufacturing inconsistencies.

The use of native extracted glycopolymers, and the method for protein-conjugation, can lead to heterogeneous molecular composition and variability of the glycoconjugate vaccines.[8] Organic chemistry can deliver structurally defined glycopolymer fragments, equipped with a conjugation handle, which can be used to generate more homogeneous vaccine modalities, and these well-defined molecules can be employed for the determination of structure-immunogenicity relationships. In addition, they can be used as tools for analytical and diagnostic assessment.[9] Therefore several syntheses of *S. aureus* CP-fragments have been reported as described previously in Chapter 1.

As depicted in Figure 1, the repeating unit of CP5 is a trisaccharide unit, consisting of *N*-acetyl-D-mannuronic acid, *N*-acetyl-L-fucose and *N*-acetyl-D-fucose. Several challenging structural features have to be overcome in order to achieve the synthesis of well-defined fragments such as the introduction of 1,2 *cis*-glycosidic bonds, the presence of the carboxylic acids and the regioselective installation of the *O*-acetyl groups. Moreover, a zwitterionic charge motif can be present due to partial *N*-deacetylation of the fucosyl residues, the position and the degree of which can vary depending on the strain.[10] The synthesis of a trisaccharide repeating unit has been achieved by several groups via different routes, but longer fragments have not been assembled to date.[11-15] A schematic overview is depicted in Figure 1, with four strategically different pathways, based on the

order of glycosylation [2+1] or [1+2] and the pre- or post-oxidation of the primary alcohol of the mannose residue.

Figure 1: *Chemical structure of CP5 and schematic overview of the synthetic strategies for the trisaccharide unit.*

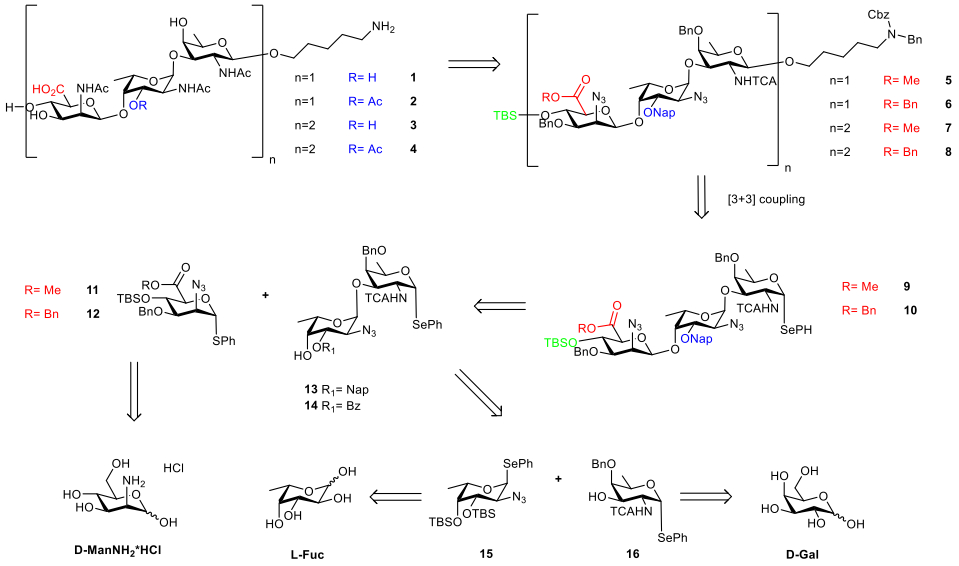


The only immunological evaluation of a synthetic CP5-fragment was reported by Adamo and co-workers, who used the synthetic trisaccharide fragment in a competitive ELISA and immunodot blot assay. The inefficient binding of this trimer by IgG antibodies raised against the native polysaccharide suggests that longer oligomers are needed.[11] Therefore, this chapter is directed to the development a convergent synthetic route to generate well-defined CP5 oligosaccharide fragments longer than a trimer. Because the importance of the presence of *O*-acetyl substituent for the immunogenicity has been assessed using only native CP sources,[16] fragments with and without *O*-acetyl substituents have been targeted as well.

RESULTS AND DISCUSSION

To develop a convergent synthetic route to fragments of CP5, two trimers (**1** and **2**) and two hexamers (**3** and **4**) were selected as targets. Both the trimers and the hexamers differ in the presence or absence of an acetyl substituent on C-3 of the L-fucosyl moiety. As shown in the retrosynthetic analysis (Figure 2) the feasibility of a 3+3 block coupling strategy was investigated.

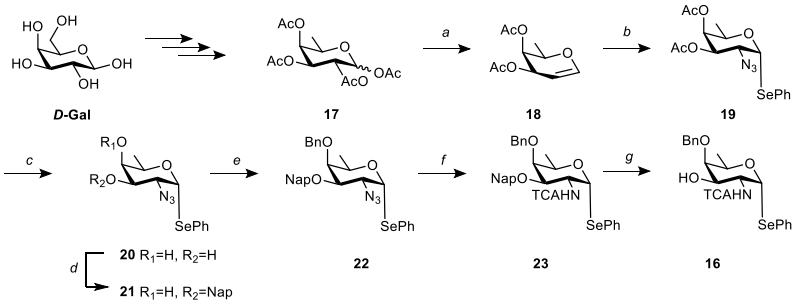
Figure 2: *Retrosynthetic analysis of S. aureus CP5*



Before the removal of all protecting groups, the final operation to obtain target oligomers **2** and **4** is the introduction of the acetyl group in fully protected oligomers (**5**-**8**) that without this operation also can lead to targets **1**, **3**. Oligomers **5**-**8** are derived from trisaccharide donors **9** or **10** having three orthogonally protecting groups: a phenylselenyl group on the anomeric position, a TBS masking the hydroxy group that is to be elongated and an oxidatively cleavable Naphthyl group for the installation of the *O*-acetyl substituent. The *N*-acetyl groups on both D-mannose and L-fucose residues were masked as azides, while an *N*-trichloroacetyl protecting group on the D-fucosyl moiety allows a 1,2-*trans* glycosylation. Two groups were explored for the protection of the mannuronate ester, a methyl and a benzyl ester. While the first one has been employed by Hagen *et al.*[14] in a previous synthesis of the CP5 trimer, the second one would reduce the number of final deprotection steps. Although different synthetic strategies are feasible for the synthesis of trisaccharide donors **9** and **10** (see Figure 1) a [1+2] pre-oxidation glycosylation strategy was chosen, since this requires the least number of reaction steps. Moreover, the *cis*-stereoselectivity of mannuronate donors is usually very high.[17,18] Disaccharide acceptors **13** and **14** can be obtained by glycosylation of an L-fucosyl donor **15** and D-fucosyl acceptor **16**, the protecting groups of which were chosen based on a previous extensive study on the reactivity of fucosazide building blocks.[14]

The synthesis of D-fucosyl acceptor **16** commenced with D-galactose as starting compound (Scheme 1). Peracetylated fucal intermediate **17** was obtained in five steps

Scheme 1: *Synthesis of D-FucNTCA acceptor **16***

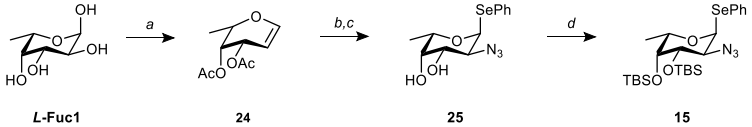


Reagents and conditions: *a) (i) HBr 33% in AcOH, DCM, 0° C to r.t.; (ii) Zn, NH_4Cl, EtOAc, 70° C, 77% (over two steps); b) $(PhSe)_2$, Me_3SiN_3, DCM, -8°C (±2°C), 70%; c) $Na_{(s)}$, MeOH, quant.; d) (i) Bu_2SnO, Toluene, 120° C; (ii) NapBr, CsF, Bu_4NBr, 120° C, 77%; e) BnBr, NaH, DMF, 0° C to r.t., 88%; f) (i) PPh_3, THF, 40° C (3h), H_2O, 60° C (o.n.); (ii) trichloroacetyl chloride, Pyridine, quant (over two steps) or (i) Pd/C, THF, tBuOH, AcOH; (ii) trichloroacetyl chloride, Pyridine, 96% (over two steps); g) DDQ, DCM:H_2O (4:1), 72%.*

following a reported procedure.[19] After bromination and zinc mediated elimination, fucal derivative **18** was obtained in 77% yield over the two steps. A regio- and stereoselective azidophenylselenation (APS) was performed using a protocol developed by Nifantiev and co-workers,[20] in which the more soluble $TMSN_3$ is used as azide source instead of NaN_3.[21] Keeping the temperature constant at -8°C (±2°C), the desired product **19** was isolated in 70%. Deacetylation under Zémplen conditions was followed by regioselective introduction of a naphthyl protecting group via the formation of a tin acetal intermediate. After benzylation of the remaining free hydroxyl group, the azide was converted into a trichloroacetimidoyl group by reduction and subsequent acetylation using trichloroacetyl chloride in pyridine. Both Staudinger conditions and hydrogenolysis were employed for the reduction step, affording compound **23** in similar yields. For the latter procedure, it might be speculated that the phenylselenyl group is responsible for the chemoselective reduction of the azide in the presence of the benzyl and the naphthyl group, since selenium containing compounds can deactivate the Pd/C catalyst in hydrogenation reaction.[23] Indeed, no formation of any related byproduct was observed by TLC even after 24h of stirring at room temperature.[24] Finally, acceptor **16** was obtained in good yield by DDQ treatment to remove the naphthyl group.

Synthesis of L-fucosyl donor **15** was accomplished by following a similar procedure to the one of D-fucosyl acceptor **16** (Scheme 2). Donor **15** was obtained in quantitative yield by silylation of intermediate **25**, using TBSOTf and catalytic amount of DMAP in pyridine at 70° C.

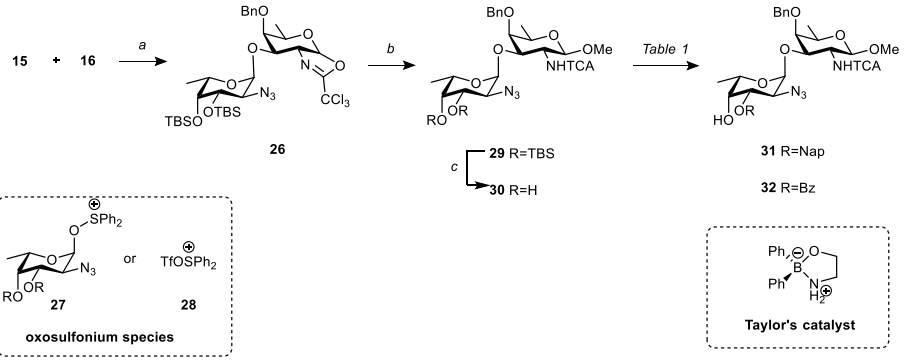
Scheme 2: *Synthesis of L-FucN_3 donor* ***15***



Reagents and conditions: *a) (i) Ac_2O, Pyridine, 4°C; (ii) HBr 33% in AcOH, DCM, 0° C to r.t.; (ii) Zn, NH_4Cl, EtOAc, 70° C, 79% (over three steps); b) $(PhSe)_2$, Me_3SiN_3, DCM, -8°C (±2°C), 73%; c) $Na_{(s)}$, MeOH, quant.; d) TBSOTf, DMAP (cat.), Pyridine, 70° C quant.*

Next, the coupling of D-fucosyl donor **15** with L-fucosyl acceptor **16** and the ensuing processing of the disaccharide product was investigated (Scheme 3). The Ph_2SO/Tf_2O mediated preactivation protocol was chosen as it was observed that highly reactive L-FucN_3 donors favourably form 1,2-*cis*-glycosidic linkages.[14] Moreover, this glycosylation procedure, in combination with anomeric phenylselenyl group in acceptor **16** has the potential for an iterative one-pot glycosylation event.[26]

Scheme 3*: Glycosylation reaction between donor* ***15*** *and acceptor* ***16*** *under preactivation conditions*



Reagents and conditions: *a) Ph_2SO, TTBP, 3Å MS, DCM; Tf_2O, -80° C to -70° C;* ***16****, -80° C to -50° C, 71% (****26****); b) after purification by size exclusion gel chromatography (DCM:MeOH); c) TBAF, THF, quant.*

A first attempt was carried out using 1.3 equivalents of donor **15**, Ph_2SO and Tf_2O with respect to acceptor **16** (Scheme 3). After a reaction time of 30 minutes at -50°C and purification by column chromatography, oxazoline **26** was isolated in 71% yield. The formation of the oxazoline moiety in **26** likely originates from intramolecular cyclization of the trichloroacetoimidoyl group, upon activation of the selenylphenyl group by oxosulfonium species **27** or **28**, as the presence of the former has been previously observed by NMR studies.[14] When oxazoline dimer **26** was purified by size exclusion chromatography (DCM:MeOH=1:1), β-O-methyl difucoside **29** was isolated. This made it possible to use this dimer as model for the intended functionalization. For the removal of the silyl groups, an excess of 8 equivalents of TBAF was required, while the regioselective protection of the 3'-OH needed optimization, the results of which are summarized in Table 1. Tin ketal mediated installation of the naphthyl group afforded

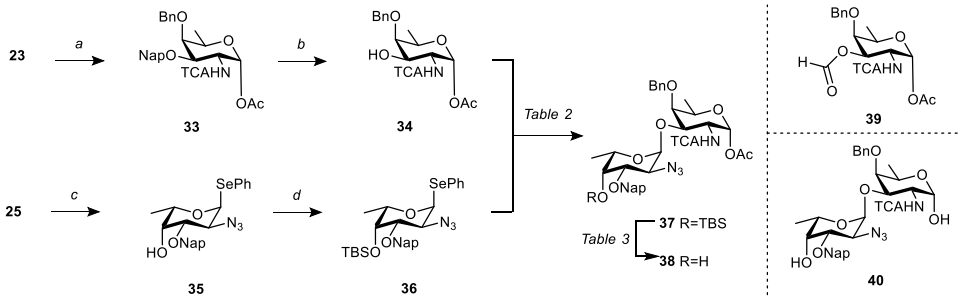
compound **31** in 22% (entry 1) or 35% (entry 2) yield. In both cases no starting material could be recovered but several side products were detected by TLC analysis. The introduction of the benzoyl group using Taylor's catalyst (B cat.) in acetonitrile failed, even when the reaction was run for 24 hours at 60° C (entry 3 and 4).[27] Solubility issues of the starting material **30** were reduced by the addition of dichloromethane and after 3 days of stirring compound **32** was isolated in 41% yield.

Table 1**: Regioselective protection of C-3' from disaccharide **30

Entry	Conditions	Time	Yield	30
1	(i) Bu_2SnO, Toluene, 110° C (ii) NapBr, DMF, Bu_4NBr, CeF, 90° C	(i) 2 h (ii) 5 h	22% (**31**)	-
2	(i) Bu_2SnO, Toluene, 60° C, 100 mbar (ii) NapBr, DMF, Bu_4NBr, CeF, 90° C	(i) 30 min. (ii) 3 h	35% (**31**)	-
3	B cat., DIPEA, BzCl, ACN	24 h	0% (**32**)	94%
4	B cat., DIPEA, BzCl, ACN, 60° C	24 h	0% (**32**	87%
5	B cat., DIPEA, BzCl, ACN:DCM (2:1), 60° C	3 days	41% (**32**)	55%

Based on the outcome of the study above, the protecting group strategy was adapted and **38** was chosen as new target dimer, requiring the syntheses of D-fucosyl acceptor **34** and L-fucosyl donor **36** (Scheme 4). To achieve this, phenylselenyl fucoside **23** was hydrolyzed and subsequently acetylated, leading to the predominant formation of α-acetyl fucoside **33**. Oxidative cleavage of the napthyl group in **33** gave D-fucosyl acceptor **34** in 69% yield. Donor **36** was prepared by a tin-mediated regioselective introduction of the naphthyl group, followed by protection of the hydroxyl group at C-3 with a TBS group using the procedure described above. Testing of the glycosylation methods started with the exploration of preactivation conditions (Table 2 entry 1). Acceptor **34** proved to be poorly soluble in dichloromethane, explaining the isolation of dimer **37** in 47% yield. The yield was slightly improved using the NIS/TBSOTf activator combination but still acceptor was recovered in 38% (entry 2). Improvement of the solubility by the use of a mixture of DCM and DMF (8:2) (entry 3) led to the isolation of target **37** and by-product **39** in a yield of 33% and 62%, respectively. Fortunately, the yield towards **37** could be improved considerably (entry 4) when a more diluted (0.05 M) mixture of donor and acceptor in dichloromethane was sonicated for 10 minutes before cooling the temperature to -78° C.

Scheme 4**: Synthesis of disaccharides **37** and **38



***Reagents and conditions:** a) (i) NIS, THF:H_2O (4:1); (ii) Ac_2O, Pyridine, 92% over two steps; b) DDQ, DCM:H_2O (4:1), 71%; c) (i) Bu_2SnO, Toluene, 120° C; (ii) NapBr, CsF, Bu_4NBr, 120° C, 82%; d) TBSOTf, DMAP (cat.), Pyridine, 70° C quant.*

Table 2:** Glycosylation conditions to achieve disaccharide **37

Entry	Conditions	Temp (° C)	Yield	Notes
1	(i) Ph_2S, TTBP, 3Å MS, DCM (ii) Tf_2O (iii) **34**	(i) r.t. (ii) -80 to -60 (iii) -80 to -40	47%	41% **34**
2	NIS, TBSOTf, DCM	-78° C to r.t.	56%	38% **34**
3	NIS, TBSOTf, DCM:DMF (8:2)	-80 to-30	33%	62% **39**
4	NIS, TBSOTf, DCM (0,05 M)	-80 to -50	96%	-

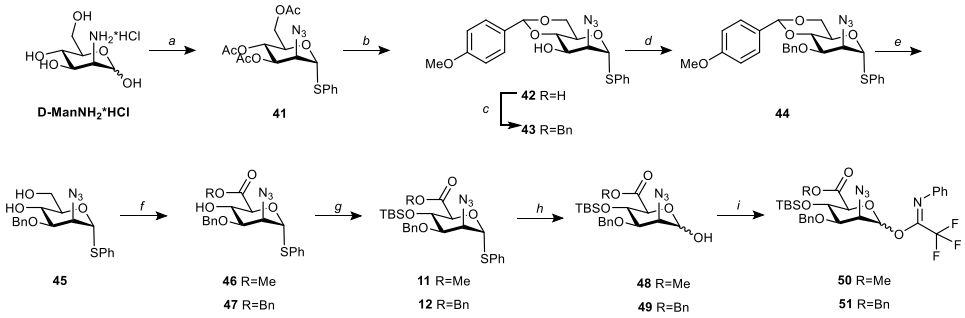
The removal of the TBS group in **37** to give target dimer **38** proved to be challenging and several conditions were probed (Table 3). Using 2 equivalents of TBAF, either at room temperature or at 66°C (entry 1 and 2), no product formation was observed and the starting material was fully recovered. Compound **38** was achieved by increasing the number of equivalents of the fluorine reagent. However, under these conditions the acetyl group was also cleaved (**40**) and the best yield was obtained with a large excess of TBAF and a relatively short reaction time (45 minutes, entry 3). Unfortunately, these conditions led to inconsistent yields when the reaction was performed on a larger scale. Addition of AcOH (entry 4) or the use of HF* pyridine (entry 5) didn't lead to any desired product. Finally, a quantitative yield was achieved when a large excess of 3HF*TEA was used in boiling THF (entry 6).

Table 3:** Attempts towards disaccharide acceptor **38

Entry	Conditions	Temp (° C)	Time	38 (%)	37 (%)
1	TBAF (2 eq), THF	r.t.	24 h	0%	quant
2	TBAF (2 eq), THF	66	24 h	0%	quant
3	TBAF (15 eq), THF	r.t.	45 min	75%	-
4	TBAF (5 eq), AcOH (5 eq), THF	66	24 h	0%	quant
5	HF*Py (20 eq), THF	66	24 h	0%	quant
6	3HF*TEA (20 eq), THF	66	24 h	quant	-

For the synthesis of mannuronate donors **11**, **12**, **50** and **51**, mannosamine hydrochloride was chosen as starting material. First a diazo transfer reaction was performed using freshly prepared TfN_3 in pyridine and $CuSO_4$ as catalyst. The choice of pyridine allowed the subsequent *in situ* acetylation, to avoid the formation of the glucose epimer by-product as previously observed.[14] The crude product thus obtained was used directly for the introduction of the anomeric thiophenyl group, achieving intermediate **41** over three steps in 76% yield.[28] The subsequent protective group manipulation involved deacetylation via Zèmplen conditions, introduction of the 4,6-*O*-pOMe-benzylidene group, benzylation of the remaining free hydroxyl and removal of the benzylidene group with catalytic amounts of TfOH in MeOH, delivering diol **45** in excellent yield. The primary alcohol in **45** was selectively oxidized using TEMPO/BAIB system and the crude carboxylic acid was alkylated to give methyl and benzyl mannuronate esters (**46** and **47**). Silylation of the hydroxyl at C-4 using TBSOTf in pyridine and catalytic amount of DMAP proceeded quantitatively to furnish donors **11** and **12.** Finally, the corresponding imidoyl donors **50** and **51** were obtained in high yields after NBS-mediated hydrolysis of the thiophenyl functionality, followed by treatment with *N*-phenyl-2,2,2-trifluoroimidoyl chloride and Cs_2CO_3.

Scheme 5: *Synthesis of mannuronate donors **11/12** and **50/51***

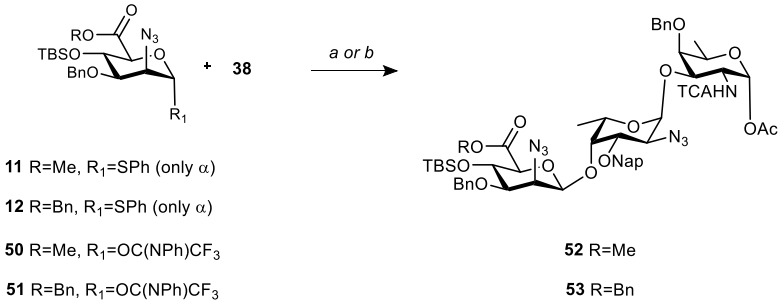


Reagents and conditions: *a) (i) TfN_3, $CuSO_4$, TEA, Pyridine, H_2O; (ii) Ac_2O; (iii) PhSH, BF_3*OEt_2, DCM 76% (over 3 steps); b) $Na_{(s)}$, MeOH, quant.; c) p-MeO-PhCH(OMe)$_2$, Cu(OTf), ACN, 60° C, 82%; d) BnBr, NaH, DMF, 0° C to r.t., 88%; e) TfOH, MeOH, quant.; f) (i) TEMPO, BAIB, AcOH, tBuOH, H_2O, DCM; (ii) BnBr or MeI, K_2CO_3, DMF, 72% (**46**), 75% (**47**); g) TBSOTf, DMAP (cat.), Pyridine, quant.(**11** and **12**); h) NBS, acetone, H_2O, quant (**48** and **49**); i) CF_3(Cl)CNPh, Cs_2CO_3, DCM, 88% (**50**), 84% (**51**).*

Now the stage was set to investigate the assembly of orthogonally protected trisaccharides **52** and **53**, using the prepared thiophenyl donors **11** and **12** and *N*-phenyl trifluoroacetimidate donors **50** and **51** in combination with dimer acceptor **38**. With donor **50,** three different promoters (TfOH, TMSOTf and TBSOTf) were tested. A molar ratio of 0.1:2.5:1 between these promoters, the donor and acceptor was chosen since mannuronate donors have relatively low reactivity. The addition of promoters happened at -78° C, after which the temperature was raised to -40° C and the reaction was left stirring at this temperature for 12 hours. After quenching with TEA and aqueous work

up, the trisaccharide **52** was isolated in respectively 15%, 38% and 45% yield. Therefore, TBSOTf was chosen as promoter while the number of equivalents was increased, keeping the temperature of reaction at -40° C, since decomposition of the α-triflate-mannuronate intermediate at higher temperature has been reported.[29] TBSOTf also proved to be a suitable promotor for the glycosylations not only with donor **51**, but also in combination with NIS to activate thiodonors **11** and **12,** producing the trisaccharides **52** and **53** in around 70% yield (Scheme 6).

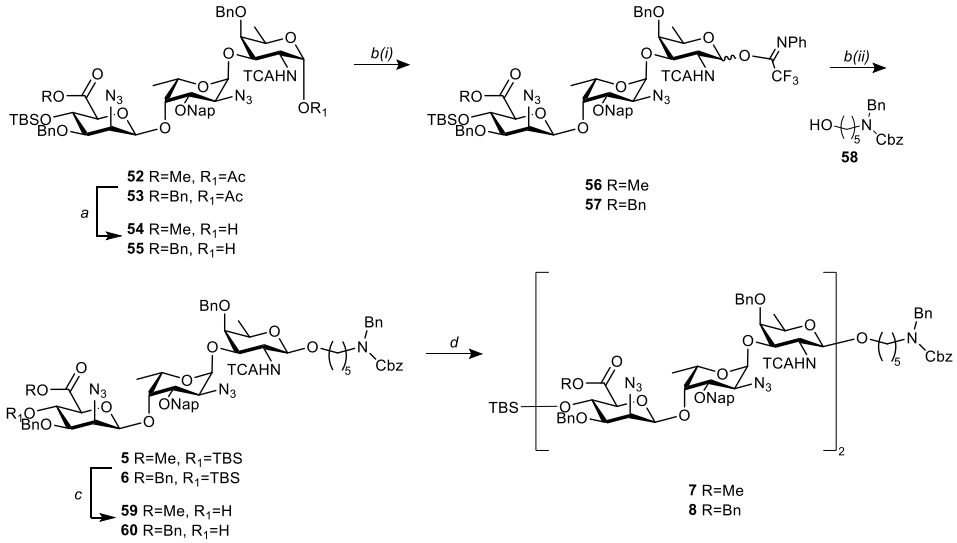
Scheme 6: *Synthesis of orthogonally protected trisaccharides **52** and **53***



Reagents and conditions: *a) Donor **11** or **12**, **38**, NIS, TBSOTf, DCM, -78° C to -40° C, 67% (**52**), 70% (**53**); b) Donor **50** or **51**, TBSOTf, DCM, -78° C to -40° C, 70% (**52**), 69% (**53**).*

A prerequisite to construct a hexamer by a 3+3 block coupling is the conversion of trimer **52** or **53** in a suitable donor (Scheme 7). The first step to provide imidates **56** and **57** is anomeric deacetylation, a conversion for which different conditions were tested. With one equivalent hydrazine acetate in DMF or ACN the reaction proceeded very slowly but with ten equivalents completion was reached within 18 h to give **54** in 82% yield. Alternatively sub stoichiometric amounts of Bu_3SnOMe (0.5 equivalents) in MeOH at reflux could deliver **54** in 81% yield. It was later noticed that when a new fresh pot of hydrazine acetate was employed, the desired products **54** and **55** could be obtained in quantitively yields in less than one hour. Treatment of **54** and **55** with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride in the presence of Cs_2CO_3 led to the formation of **56** and **57**. Trisaccharide donors **56** and **57** could not withstand purification with column chromatography and were directly used in the ensuing glycosylation. Coupling of donor **56** or **57** with pentenyl acceptor **58** (2 eq) in DCM under influence of TBSOTf (0.2 equivalents) with increasing the temperature from -78° C to -35° C, resulted in complete consumption of the donors after one hour and trimers **5** and **6** were isolated in 68% and 71% yield, respectively. Removal of the TBS group in **5** and **6**, using the aforementioned conditions, gave the spacer containing acceptors **59** and **60** for the final key [3+3] coupling toward hexamers **7** and **8**. TBSOTf (0.02 eq) mediated coupling of donor **56** with 1.3 equivalent acceptor **59** (and similarly donor **57** with **60**) in DCM with a temperature increase from -78°C to -50°C resulted after one hour in the formation of hexasaccharides **7** and **8** that were isolated in excellent yields.

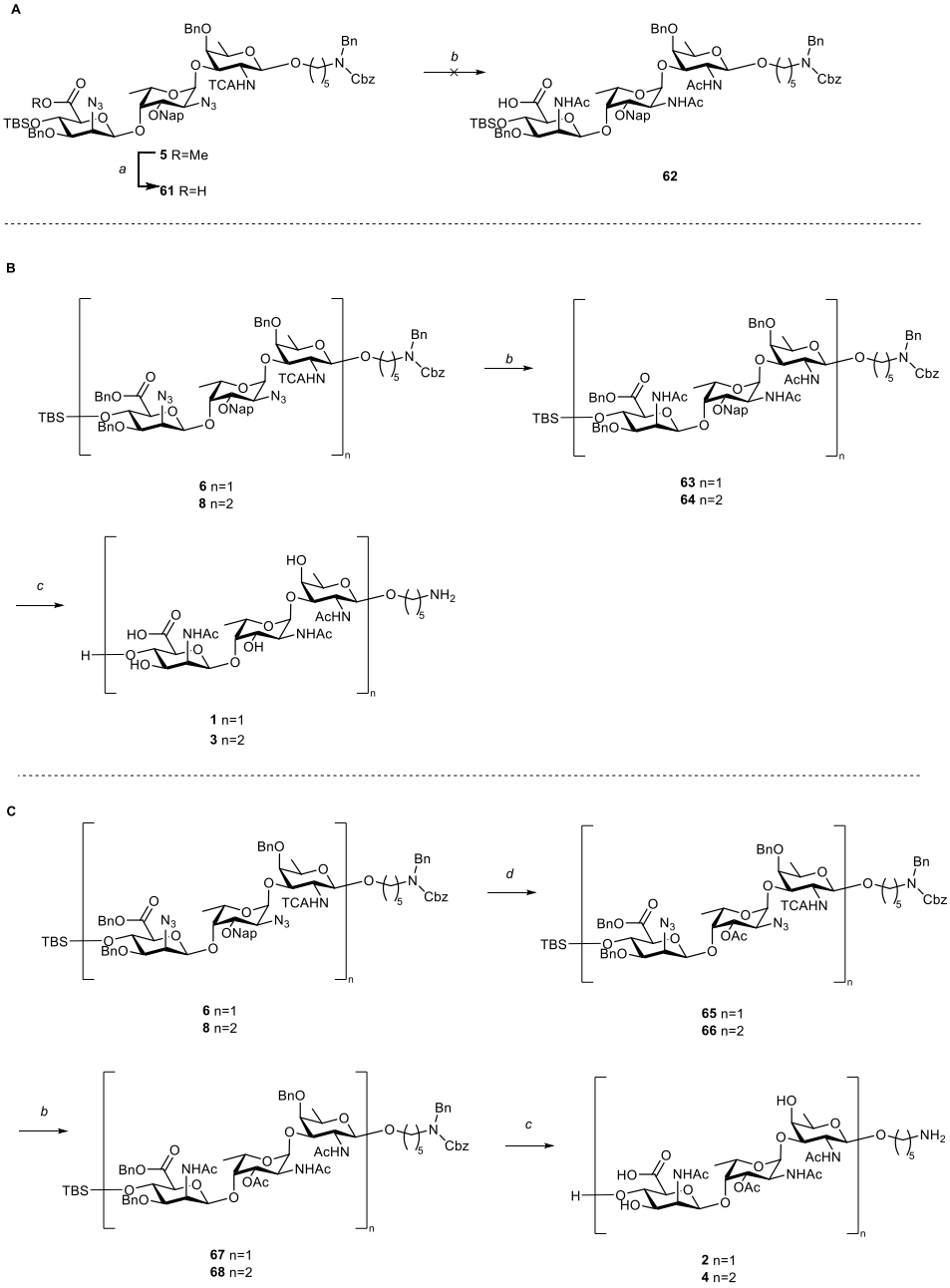
Scheme 7: *Synthesis of fully protected trimers **5** and **6** and hexamers **7** and **8***



Reagents and conditions: *a) NH_2NH_2*AcOH, DMF, quant. (**54** and **55**); b) (i) $CF_3(Cl)CNPh$, Cs_2CO_3, DCM, 88%; (ii) TBSOTf, DCM, -78° C to -35° C, 68% (**5**), 71% (**6**); c) TEA*HF, THF, 66° C, quant (**59** and **60**); d) TBSOTf, DCM, -78° C to -50° C, 88% (**7**), 96% (**8**)*

With the availability of the protected progenitors of the target tri- and hexamers, attention was directed to explore the most efficient deprotection strategy. To obtain trisaccharide **1**, lacking the acetyl ester, protected trimer **5** was subjected to $LiOH/H_2O_2$ mediated saponification of the methyl mannuronate ester in THF/H_2O (4:1) (Scheme 8). However, after 2 days 93% of starting material **5** was recovered. Observed solubility issues were an incentive to replace LiOH by TBAOH and together with raising the temperature to 40° C, intermediate **61** could be isolated in quantitative yield. The subsequent simultaneous conversion of azide and TCA groups in to acetamido groups, with Zn in $THF/AcOH/Ac_2O$ [12] led to a complex mixture, in which a lactamized by-product prevailed. Subjection of benzyl ester **6** to the same reaction conditions afforded trimer **63** in 67% yield. Complete removal of the TBS group in **63** and hydrogenolysis of the crude product, using Pearlman's catalyst in a mixture of tBuOH and H_2O (4:1) and purification by size exclusion chromatography (HW40) afforded target trimer **1** in 96% yield. Application of the same deprotection protocol to protected progenitor **8** afforded target hexamer **3** in 55% overall yield. To obtain the acetyl substituted trimer **2** and hexamer **4**, the naphthyl group in **6** and **8** was removed using DDQ, followed by acetylation to give trimer **65** and hexamer **66** in 98% and 88% yield, respectively. The acetamido derivatives **67** and **68** were obtained in similar yield using the aforementioned procedure, followed by removal of TBS group and hydrogenolysis. Final size exclusion chromatography delivered final targets **2** and **4** in 88% and 62% yield.

Scheme 8: *Final deprotections towards final trimer **1/2** and hexamer **3/4** targets; (**A**) starting from compound **54**; (**B**) non acetylated fragments and (**C**) acetylated fragments from compounds **55** and **59***



Reagents and conditions*: a) TBAOH (40% w in H_2O), H_2O_2, THF, quant.; b) Zn, AcOH, Ac_2O, THF, 40° C, 67% (**63**), 62% (**64**), 65% (**67**), 64% (**68**); c) (i) TEA*HF, THF, 40° C, (ii) Pd/C, AcOH, H_2, tBuOH:H_2O*

*(4:1), 96% (**1**), 55% (**3**), 88% (**2**), 62% (**4**); d) (i) DDQ, DCM:H_2O (4:1); (ii) Ac_2O, Pyridine, 98% (**65**), 88% (**66**).*

CONCLUSION

This chapter deals with the development of an efficient synthetic route to fragments of the capsular polysaccharide of *S. aureus* type 5, with and without a C3 acetyl ester in the *N*-acetyl-L-fucose residue. A fully protected trisaccharide building block, corresponding to the repeating unit of the capsular polysaccharide was constructed that gave access not only to an acetylated and a non-acetylated trimer with an anomeric amino spacer but also, by means of a [3+3] block coupling, towards the corresponding hexamers. Two trisaccharide building blocks with either a methyl or benzyl ester mannuronate residue were synthesized using a [1+2] glycosylation assembly. Although no differences were found at the coupling step, the benzyl protection proved to be superior during the deprotection procedure towards the target oligomers. Other notable findings concern the replacement of the anomeric selenophenyl by an acetyl group in the D-FucNHTCA acceptor to prevent oxazoline formation during the glycosylation toward the difucoside intermediate and the favorable use of the naphthyl protecting group to allow the selective introduction of the acetyl substituent in the final stage of the assembly. This chapter has reported the first successful synthesis of CP5 fragments longer that the trimer repeating unit. The fragments will be evaluated for their capacity to bind to antibodies raised against the native CP5 polysaccharide and in the generation of conjugate vaccine modalities.

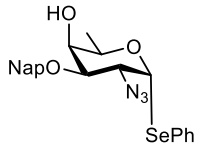
EXPERIMENTAL SECTION

General procedures

All reactions were carried out in oven-dried glassware (85 °C). Prior to reactions, traces of water and solvent were removed by co-evaporation with toluene where appropriate. Reactions sensitive to air or moisture were carried out under an atmosphere of argon (balloon). Solvents for reactions were of reagent grade and stored over 4Å molecular sieves (3Å for DCM, MeOH and ACN), except pyridine and DMF. TEA was stored over KOH pellets. Tf_2O used in glycosylations was dried over P_2O_5 (~3 hours), followed by distillation, and stored in a Schlenk flask at -20 °C. All other chemicals were used as received. Reaction progress was monitored using aluminium-supported silica gel TLC plates (Merck, Kieselgel 60, with fluorescent indicator); visualization was carried out by irradiation with UV light (λ: 254 nm), followed by spraying with 20% H_2SO_4 in EtOH (w/v) or Hanessian's stain ($(NH_4)_6Mo_7O_2$*$4H_2O$, 25 g/L; $(NH_4)_4Ce(SO_4)$*$2H_2O$, 10 g/L; in 10% aq. H_2SO_4). Column chromatography was carried out using silica gel (Screening Devices, 0.040-0.063 mm). Size-exclusion chromatography was carried out using Sephadex WH-40 (GE Healthcare). NMR spectra were recorded on Bruker AV-400, AV-500 or AV 850 instruments. Chemical shifts (δ) are reported in ppm relative to Me_4Si (δ: 0.00 ppm) or residual solvent signals. NMR spectra were recorded at ambient temperature, and samples were prepared in $CDCl_3$ unless noted otherwise. ^{13}C-APT spectra are ^{1}H

decoupled. Structural assignment was achieved using HH-COSY and HSQC 2D experiments. Coupling constants of anomeric carbon atoms (JH_1,C_1) were determined using HMBC-GATED experiments. LC-MS analyses were performed on a Thermo Finnigan Surveyor HPLC system equipped with a Gemini C-18 column (250 x 10 mm), connected to a Thermo Finnigan LCQ Advantage Max Ion-trap mass spectrometer with (ESI+). Eluents used were ACN, H_2O with addition of TFA (0.1%). HRMS spectra were recorded on a Thermo Finnigan LCQ Orbitrap instrument.

Phenyl 2-azido-2-deoxy-3-*O*-naphtyl-1-seleno-α-D-fucopyranoside (21)



Diol **20** (40 mmol) was dissolved in toluene (200 mL, 0.2 M) in a two-necked flask, equipped with a stopcock and a Dean-Stark trap. Bu_2SnO (41 mmol, 1.02 eq) was added to the mixture, which was heated to 120°C in an oil bath for 2 hours. After cooling to 60°C, Bu_4NBr (41 mmol, 1.02 eq), CeF (41 mmol, 1.02 eq) and NapBr (41 mmol, 1.02 eq) were added and the mixture was heated to 120°C and stirred for 1 hour after which TLC (Pentane:EtOAc, 6:4) indicated complete conversion of the starting material. The mixture was cooled to room temperature and a 10% aq solution of KF was added. After 30 minutes of vigorous stirring, the layers were separated, the aqueous phase extracted with EtOAc and the combined organic fractions were washed with Brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The desired product **21** was isolated by column chromatography (Pentane:EtOAc, 9:1 → 7:3) in 77% yield as yellow oil.

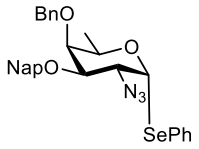
TLC analysis: R_f=0.32 (Pentane:EtOAc; 8:2)

^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.91-7.79 (4H, H_{arom}, m), 7.60-7.45 (5H, H_{arom}, m), 7.32-7.20 (3H, H_{arom}, m), 5.89 (1H, H_1, $J_{1\text{-}2}$=5.4 Hz, d), 4.90 (1H, CH*H*$_{Nap}$, J=11.5 Hz, d), 4.85 (1H, CH*H*$_{Nap}$, d), 4.28 (1H, H_5, $J_{5\text{-}6}$=6.6 Hz, q), 4.20 (1H, H_2, $J_{2\text{-}3}$=10.2 Hz, dd), 3.89 (1H, H_4, $J_{4\text{-}3}$=3.2 Hz, d), 3.74 (1H, H_3, dd), 1.24 (3H, 3 x H_6, d).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 134.6 (CH_{arom}), 134.5, 133.3 x2 (C_q), 129.2, 128.8 (CH_{arom}), 128.6 (C_q), 128.1, 127.9 x 2, 127.1, 126.5, 126.4, 125.8 (CH_{arom}), 85.3 (C_1), 79.4 (C_3), 72.4 (CH_{2_Nap}), 68.7 (C_5, C_4), 60.4 (C_2), 16.2 (C_6)

HRMS: $C_{23}H_{23}N_3O_3Se$ + H^+ required 469.0905, found 470.0978

Phenyl 2-azido-2-deoxy-3-*O*-naphtyl-4-*O*-benzyl-1-seleno-α-D-fucopyranoside (22)

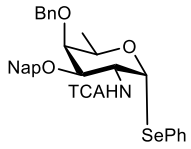


Compound **21** (21 mmol) was dissolved in DMF. At 0°C NaH (23 mmol, 1.1 eq, as 60% dispersion in mineral oil) and BnBr (23 mmol, 1.1 eq) were added slowly to the reaction mixture. The solution was allowed to warm to room temperature and after 2 hours TLC analysis (Pentane:EtOAc, 8:2) showed complete consumption of starting material. The reaction was quenched by slow addition of cold MeOH, followed by partitioning of the mixture between Et_2O and water. After extraction of the aqueous phase (Et_2O x 2), the combined organic phases were washed with brine, dried over $Na_2S_2O_4$, filtered and concentrated *in vacuo*. The final product was isolated by column chromatography (Pentane:EtOAc, 97:3 → 8:2) yielding 88% of **22**.

TLC analysis: R_f= 0.48 (Pentane:EtOAc; 9:1)

^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.91-7.80 (4H, H_{arom}, m), 7.60-7.45 (5H, H_{arom}, m), 7.35-7.20 (8H, H_{arom}, m), 5.94 (1H, H_1, J_{1-2}=5.3 Hz, d), 5.00-4.86 (3H,3 x CH*H*, m), 4.65 (1H, CH*H*, J=11.4 Hz, d), 4.39 (1H, H_2, J_{3-2}=10.3 Hz, dd), 4.22 (1H, H_5, J_{5-6}=6.7 Hz, q), 3.78 (1H, H_3, J_{4-3}=2.7 Hz, dd), 3.73 (1H, H_4, d), 1.13 (3H, 3 x H_6, d).
^{13}C-NMR (400 MHz, $CDCl_3$), δ: 138.2, 135.1 (C_q) 134.5 (CH_{arom}), 133.4, 133.2 (C_q), 129.2 (CH_{arom}), 128.8 (C_q), 128.5 x 2, 128.3, 128.1, 128.0, 127.9, 127.8, 126.8, 126.4, 126.3, 125.8 (CH_{arom}), 85.7 (C_1), 80.8 (C_3), 76.0 (C_4), 75.2 (CH_2), 72.8 (CH_2), 69.6 (C_5), 61.2 (C_2), 16.7 (C_6)
HRMS: $C_{30}H_{29}N_3O_3Se$ + Na^+ required 582.1266, found 582.1269

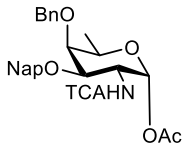
Phenyl 2-trichloroacetylamido-2-deoxy-3-*O*-naphtyl-4-*O*-benzyl-1-seleno-α-D-fucopyranoside (23)



Method a: Compound **22** (1 mmol) was dissolved in a mixture of THF:*t*BuOH (4:1, 5 mL, 0.2 M) and 1 drop of acetic acid was added. After purging $Ar_{(g)}$ for 20 minutes, Pd/C was added and the reaction was stirred overnight under $H_{2(g)}$ atmosphere. The reaction mixture was filtered through Celite® and concentrated *in vacuo*. After co-evaporation with toluene (x3), the residual crude was dissolved in pyridine (10 mL, 0.1 M) and at 0°C trichloroacetylchloride (1.1 mmol, 1.1 eq) was added dropwise. The reaction was let to warm up to room temperature and after 1 hour TLC analysis showed complete consumption of starting material (DCM:MeOH:AcOH, 95:4.5:0.5). After quenching with cold water, the reaction mixture was diluted with EtOAc, washed (HCl 1M, H_2O, $NaHCO_{3(aq)}$, H_2O, brine), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The final compound **23** was isolated by column chromatography in 96% yield as white foam.
Method b: Compound **22** (1 mmol) was dissolved in THF (10 mL, 0.1M) and upon addition of PPh_3 the resulting mixture was heated at 45°C for 4 hours. H_2O was added and the reaction was stirred at reflux overnight. The solution was concentrated *in vacuo*, co-evaporated with toluene (x3) and the residual crude was dissolved in pyridine (10 mL, 0.1 M) and at 0°C trichloroacetylchloride (1.1 mmol, 1.1 eq) was added dropwise. The reaction was let to warm up to room temperature and after 1 hour TLC analysis (DCM:MeOH:AcOH, 95:4.5:0.5) showed complete consumption of starting material. After quenching with cold water, the reaction mixture was diluted with EtOAc, washed (HCl 1M, H_2O, $NaHCO_{3(aq)}$, H_2O, brine), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The final compound **23** was isolated by column chromatography (Pentane:EtOAc, 95:5 → 8:2) in 96% yield as white foam.
TLC analysis: R_f= 0.31 (Pentane:EtOAc; 9:1)
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.90-7.76 (4H, H_{arom}, m), 7.55-7.43 (5H, H_{arom}, m), 7.41-7.18 (8H, H_{arom}, m), 6.86 (1H, NH, J=7.5 Hz, d), 6.04 (1H, H_1, J_{1-2}=4.8 Hz, d), 5.01 (1H, CH*H*, J=11.6 Hz, d), 4.90 (1H, CH*H*, J=12.1 Hz, d), 4.83-4.75 (1H, H_2, m), 4.74-4.65 (2H,2 x CH*H*, m), 4.22 (1H, H_5, J_{5-6}=6.5 Hz, q), 3.86 (1H, H_4, J_{4-3}=2.6 Hz, d), 3.65 (1H, H_3, J_{3-2}=11 Hz, dd), 1.28 (3H, 3 x H_6, d).
^{13}C-NMR (400 MHz, $CDCl_3$), δ: 161.6, 138.1, 134.6 (C_q) 134.2 (CH_{arom}), 133.4, 133.2 (C_q), 129.4 (CH_{arom}), 128.9 (C_q), 128.8, 128.5, 128.3, 128.0 x 2, 127.9 x 2, 126.7, 126.5, 126.4,

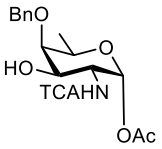
125.5 (CH_{arom}), 89.2 (C_1), 78.8 (C_3), 74.9 (CH_2), 74.6 (C_4), 71.7 (CH_2), 70.6 (C_5), 52.0 (C_2), 16.9 (C_6)
HRMS: $C_{32}H_{30}Cl_3NO_4Se + NH_4^+$ required 695.0744, found 695.0740

2-trichloroacetylamido-2-deoxy-3-*O*-naphtyl-4-*O*-benzyl-1-*O*-acetyl-α-D-fucopyranoside (33)



Compound **23** (12 mmol) was dissolved in THF (120 mL, 0.1 M) and NIS (14.4 mmol, 1.2 eq) and H_2O (2.2 mL, 10 eq) were added. After 1 hour TLC analysis (Pentane:EtOAc, 9:1) showed complete consumption of starting material. The reaction mixture was diluted with DCM and washed with $Na_2S_2O_{3(sat.aq.)}$ (x2), H_2O and brine. After drying over $MgSO_4$ and filtration, the organic phase was concentrated *in vacuo*. The residual crude was dissolved in pyridine (120 mL, 0.1 M) and at 0°C Ac_2O (3.4 mL, 3 eq) and DMAP (0.02 mmol, 2%) were added. The reaction was let to warm up to room temperature and after 1 hour TLC analysis (Pentane:EtOAc, 1:1) showed complete consumption of starting material. After quenching with cold water, the reaction mixture was diluted with EtOAc, washed (HCl 1M, H_2O, $NaHCO_{3(aq)}$, H_2O, brine), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The final compound **33** was isolated by column chromatography (Pentane:EtOAc, 8:2→6:4) in 92% yield as white foam and as a mixture of α/β 5.3:1.
TLC analysis: R_f= 0.41 (Pentane:EtOAc; 7:3)
Only the signals relative to the α anomer are reported
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.90-7.76 (4H, H_{arom}, m), 7.55-7.43 (3H, H_{arom}, m), 7.41-7.26 (5H, H_{arom}, m), 6.41 (1H, NH_{TCA}, J=8.1 Hz, d), 6.33 (1H, H_1, J_{1-2}=3.8 Hz, d), 5.00 (1H, CH*H*, J=11.6 Hz, d), 4.90 (1H, CH*H*, J=12.1 Hz, d), 4.79-4.65 (3H,2 x CH*H*, H_2, m), 3.95 (1H, H_5, J_{5-6}=6.5 Hz, q), 3.88-3.81 (2H, H_4, H_3, m), 2.02 (3H, CH_{3_OAc}, s), 1.28-1.19 (3H, 3 x H_6, m).
^{13}C-NMR (400 MHz, $CDCl_3$), δ: 169.0, 161.8, 138.0, 134.8, 133.4, 133.3 (C_q), 128.8, 128.6, 128.5 x 3, 128.4, 128.0 x 2, 127.9, 126.7, 126.6, 126.4, 125.6 (CH_{arom}), 91.2 (C_1), 76.7 (C_3), 74.9 (CH_2), 74.5 (C_4), 71.5 (CH_2), 69.4 (C_5), 50.2 (C_2), 21.0 (CH_{3_OAc}), 17.1 (C_6).
HRMS: $C_{28}H_{22}Cl_3NO_6 + NH_4^+$ required 597.1315, found 597.1321

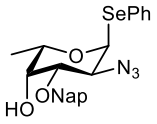
2-trichloroacetylamido-2-deoxy-4-*O*-benzyl-1-*O*-acetyl-α-D-fucopyranoside (34)



Compound **33** (10 mmol) was dissolved in a mixture of DCM:H_2O (4:1, 100 mL, 0.1 M). DDQ (20 mmol, 2 eq) was added and after 2.5 hours of vigorous stirring TLC analysis (Pentane:EtOAc, 7:3) showed complete consumption of starting material. After dilution with DCM, the reaction mixture was washed with a 10% aq solution of $Na_2S_2O_3$. The aqueous layer was extracted with DCM and the combined organic phases were washed with saturated aq solution of $NaHCO_3$, H_2O and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound **34** was isolated by column chromatography (Pentane:EtOAc, 75:25→1:1) in 71% yield as a white foam.
TLC analysis: R_f= 0.29 (Pentane:EtOAc; 7:3)
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.44-7.29 (5H, H_{arom}, m), 6.62 (1H, NH_{TCA}, J=8.3 Hz, d), 6.27 (1H, H_1, J_{1-2}=3.6 Hz, d), 4.87 (1H, CH*H*, J=11.4 Hz, d), 4.67 (1H, CH*H*, d), 4.44-3.31 (1H, H_2,

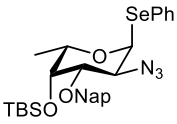
m), 4.03 (1H, H_5, $J_{5\text{-}6}$=6.6 Hz, q), 3.73 (1H, H_3, $J_{3\text{-}2}$=10.6, $J_{3\text{-}4}$=3.4, dd), 1.32 (1H, H_4, d), 2.12 (3H, CH_{3_OAc}, s), 1.32 (3H, 3 x H_6, d).
^{13}C-NMR (400 MHz, $CDCl_3$), δ: 169.1, 162.8, 137.6 (C_q), 128.9, 128.4, 128.1 (CH_{arom}), 114.2 (C_q), 91.1 (C_1), 79.6 (C_3), 76.4 (CH_2), 69.4 (C_4), 69.3 (CH_2), 52.0 (C_2), 20.9 (CH_{3_OAc}), 17.0 (C_6).
HRMS: $C_{17}H_{20}Cl_3NO_6$ + NH_4^+ required 457.0654, found 457.0636

Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-1-seleno-α-L-fucopyranoside (35)



Diol **25** (22 mmol) was dissolved in toluene (110 mL, 0.2 M) in a two-necked flask, equipped with a stopcock and a Dean-Stark trap. Bu_2SnO (22 mmol, 1eq) was added to the mixture, which was heated to 140°C in an oil bath for 3 hours. After cooling to 60°C, Bu_4NBr (23 mmol, 1.05 eq), CeF (23 mmol, 1.05 eq) and NapBr (23 mmol, 1.05 eq) were added and the mixture was heated to 120°C and stirred for 4 hours after which TLC (Pentane:EtOAc, 3:2) indicated complete conversion of the starting material. The mixture was cooled to room temperature and a 10% aq solution of KF was added. After 30 minutes of vigorous stirring, the layers were separated, the aqueous phase extracted with EtOAc and the combined organic fractions were washed with Brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The desired product **35** was isolated by column chromatography in 82% yield as yellow oil.
TLC analysis: R_f=0.32 (Pentane:EtOAc; 8:2)
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.91-7.79 (4H, H_{arom}, m), 7.60-7.45 (5H, H_{arom}, m), 7.32-7.20 (3H, H_{arom}, m), 5.90 (1H, H_1, $J_{1\text{-}2}$=5.4 Hz, d), 4.92 (1H, CH*H*$_{Nap}$, J=11.5 Hz, d), 4.86 (1H, CH*H*$_{Nap}$, d), 4.29 (1H, H_5, $J_{5\text{-}6}$=6.6 Hz, q), 4.20 (1H, H_2, $J_{2\text{-}3}$=10.2 Hz, dd), 3.90 (1H, H_4, $J_{4\text{-}3}$=3.2 Hz, d), 3.75 (1H, H_3, dd), 1.24 (3H, 3 x H_6, d).
^{13}C-NMR (400 MHz, $CDCl_3$), δ: 134.6 (CH_{arom}), 134.5, 133.3 x2 (C_q), 129.2, 128.8 (CH_{arom}), 128.6 (C_q), 128.1, 127.9 x 2, 127.1, 126.5, 126.4, 125.8 (CH_{arom}), 85.3 (C_1), 79.4 (C_3), 72.4 (CH_{2_Nap}), 68.7 (C_5, C_4), 60.4 (C_2), 16.2 (C_6)
HRMS: $C_{23}H_{23}N_3O_3Se$ + H^+ required 469.0905, found 470.0978

Phenyl 2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(*tert*butyldimethyl)silyl-1-seleno-α-L-fucopyranoside (36)

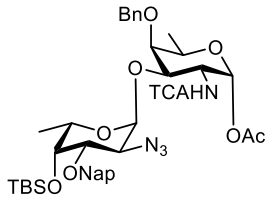


Compound **35** (10 mmol) was dissolved in pyridine (50 mL, 0.2 M). TBSOTf (11 mmol, 1.1 eq) and DMAP (0.05 mmol, 0.5%) were added at 0°C and after 10 minutes stirring the reaction mixture was heated to 70°C. After 16 hours TLC analysis (Pentane:EtOAc, 8:2) showed complete consumption of starting material and the reaction mixture was cooled to room temperature. After quenching with cold MeOH and diluting with EtOAc, the mixture was washed with 10% aq solution of $CuSO_4$ (x2), H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography afforded the title compound **36** in quantitative yield as a light-yellow oil.
TLC analysis: R_f=0.30 (Pentane:EtOAc; 95:5)
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.91-7.79 (4H, H_{arom}, m), 7.63-7.43 (5H, H_{arom}, m), 7.32-7.20 (3H, H_{arom}, m), 5.93 (1H, H_1, $J_{1\text{-}2}$=5.4 Hz, d), 4.94-4.82 (2H, CH_{2_Nap}, m), 4.28 (1H, H_2, $J_{2\text{-}3}$=10.4 Hz, dd), 4.22 (1H, H_5, $J_{5\text{-}6}$=6.5 Hz, q), 3.98-3.89 (1H, H_4, m), 3.66 (1H, H_3, $J_{3\text{-}4}$=2.5 Hz,

dd), 1.16 (3H, 3 x H_6, d), 0.88 (9H, 3 x CH_{3_tBu}, m), 0.06 (3H, CH_{3_Me}, s), 0.05 (3H, CH_{3_Me}, s).
^{13}C-NMR (400 MHz, $CDCl_3$), δ: 135.0 (C_q), 134.9 (CH_{arom}), 133.4, 133.2 (C_q), 129.2 (CH_{arom}), 128.8 (C_q), 128.3, 128.1, 127.9 x 2, 126.9, 126.3, 126.1, 126.0 (CH_{arom}), 85.9 (C_1), 80.2 (C_3), 73.1 (CH_{2_Nap}), 71.2 (C_4), 70.2 (C_5), 61.1 (C_2), 26.2 (CH_{3_tBu}), 18.7 (C_q), 17.1 (C_6), -3.8, -4.5 (CH_{3_Me}).

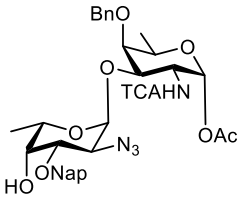
HRMS: $C_{17}H_{20}Cl_3NO_6$ + Na^+ required 606.1662, found 606.1664

2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(*tert*butyldimethyl)silyl-α-L-fucopyranosyl)-4-*O*-benzyl-1-*O*-acetyl-α-D-fucopyranoside (37)



Donor **36** (1.3 mmol, 1.3 eq) and acceptor **34** (1 mmol) were co-evaporated together with toluene. After dissolving in DCM (20 mL, 0.05 M), freshly activated 4 Å MS were added and the reaction mixture was cooled to -78°C. NIS (1.3 mmol, 1.3 eq) ad TBSOTf (0.3 mmol, 0.3 eq) were added and the reaction mixture was left to stir at -55°C. After 1 hour TLC analysis (Pentane:EtOAc 7:3) showed complete consumption of acceptor **34** and the reaction was quenched by addition of TEA (0.3 mmol, 0.3 eq). The reaction mixture was diluted in EtOAc and washed with a saturated aq. solution of $Na_2S_2O_3$, H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (Pentane:EtOAc, 8:2→7:3) afforded **37** in quantitative yield.
TLC analysis: R_f= 0.43 (Pentane:EtOAc; 7:3)
^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.78-7.42 (4H, H_{arom}, m), 7.59-7.41 (4H, H_{arom}, NH_{TCA}, m), 7.40-7.24 (5H, H_{arom}, m), 6.53 (1H, H_{1D}, J_{1-2}=3.6 Hz, d), 5.06 (1H, H_{1L}, J=3.6 Hz, d), 4.94-4.78 (2H, CH_2, m), 4.78-4.63 (2H, CH_2, m), 4.63-4.52 (1H, H_{2D}, m), 4.10 (1H, H_{2L}, J_{2-3}=10.4 Hz, dd), 4.05-3.90 (4H, H_{5L}, H_{5D}, H_{3D}, H_{4L}, m), 3.85 (1H, H_{3L}, J_{3-4}=2.4 Hz, dd), 3.75 (1H, H_{4D}, J_{3-4}=2.8 Hz, d), 2.11 (3H, CH_{3_OAc}, s), 1.19 (3H, 3 x H_6, J_{6-5}=6.5 Hz, d), 1.14 (3H, 3 x H_6, J_{6-5}=6.5, d) 0.88 (9H, 3 x CH_{3_tBu}, m), 0.03 (3H, CH_{3_Me}, s), 0.02 (3H, CH_{3_Me}, s).
^{13}C-NMR (400 MHz, $CDCl_3$), δ: 169.0, 162.3, 138, 134.7, 133.4, 133.1 (C_q), 128.6, 128.5, 128.3, 128.1, 127.9 x 2, 127.6, 126.4, 126.3, 126.1, 125.4 (CH_{arom}), 99.6 (C_{1L}) , 92.7 (C_q), 90.3 (C_{1D}), 78.9 (C_{3L}), 78.3 (C_{3D}), 77.4 (C_{4L}), 75.1 (CH_2), 72.8 (CH_2), 70.8 (C_{4D}), 69.5 (C_5), 69.3 (C_5), 61.2 (C_{2L}), 50.7 (C_{2D}), 26.1 (CH_{3_tBu}), 21.0 (CH_{3_OAc}), 18.7 (C_q), 17.5 (C_6),17.0 (C_6), -3.8, -4.5 (CH_{3_Me}).
HRMS: $C_{40}H_{51}Cl_3N_4O_9Si$ + Na^+ required 887.2383, found 887.2383

2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-4-*O*-benzyl-1-*O*-acetyl-α-D-fucopyranoside (38)



Compound **37** (1 mmol) was dissolved in dry THF (10 mL, 0.1 M) and TEA*HF (20 mmol, 20 eq) was added. The reaction mixture was stirred at reflux 3 days. After reaching room temperature, the reaction mixture was diluted with EtOAc and transfer in a beaker containing a stirring cold solution of saturated aq. solution of $NaHCO_3$. Once the ice melted, the organic layer was separated and the aqueous phase was reextracted (x2). The combined EtOAc solutions were washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound **38** was isolated by column chromatography in 92% yield as white solid.

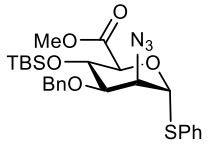
TLC analysis: R_f= 0.35 (Pentane:EtOAc; 1:1)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.91-7.75 (4H, H_{arom}, m), 7.58-7.42 (4H, H_{arom}, NH_{TCA}, m), 7.40-7.24 (5H, H_{arom}, m), 6.52 (1H, H_{1D}, J_{1-2}=3.6 Hz, d), 5.04 (1H, H_{1L}, J=3.4 Hz, d), 4.90-4.67 (4H, 2 x CH_2, m), 4.63-4.55 (1H, H_{2D}, m), 4.10-3.92 (5H, H_{2L}, H_{5L}, H_{5D}, H_{3D}, H_{3L}, m), 3.85 (1H, H_{4D}, J_{3-4}=2.9 Hz, dd), 3.75 (1H, H_{4L}, J_{3-4}=2.8 Hz, d), 2.11 (3H, CH_{3_OAc}, s), 1.22 (3H, 3 x H_6, J_{6-5}=6.5 Hz, d), 1.18 (3H, 3 x H_6, J_{6-5}=6.5, d).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 169.0, 162.2, 138.1, 134.3, 133.3 (C_q), 128.9, 128.6, 128.1, 127.9 x 2, 127.8, 127.7, 127.1, 126.6, 126.5, 125.7 (CH_{arom}), 99.0 (C_{1L}) , 92.7 (C_q), 90.4 (C_{1D}), 78.3 (C_{3L}, C_{3D}), 76.8 (C_{4L}), 75.0 (CH_2), 72.3 (CH_2), 69.2 (C_5), 68.3 (C_{4D}), 67.5 (C_5), 60.7 (C_{2L}), 50.7 (C_{2D}), 21.0 (CH_{3_OAc}), 17.0 (C_q), 16.4 (C_6).

HRMS: $C_{35}H_{37}Cl_3N_4O_9$ + Na^+ required 773.1518, found 773.1518

Methyl (phenyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-1-thio-α-D-mannopyranosiduronate) (11)



Compound **46** (6.6 mmol) was dissolved in pyridine (33 mL, 0.2 M). TBSOTf (19.8 mmol, 3 eq) and DMAP (0.06 mmol, 1%) were added at 0°C and after 10 minutes stirring the reaction mixture was heated to 70°C. After 5 hours TLC analysis (Pentane:EtOAc, 8:2) showed complete consumption of starting material and the reaction mixture was cooled to room temperature. After quenching with cold MeOH and diluting with EtOAc, the mixture was washed with 10% aq solution of $CuSO_4$ (x2), H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (Pentane:EtOAc, 98:2→8:2) afforded the title compound **11** in quantitative yield as a light-yellow oil.

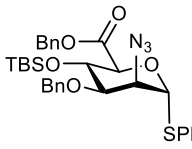
TLC analysis: R_f= 0.55 (Pentane:EtOAc; 9:1)

^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.68-7.58 (2H, H_{arom}, m), 7.39-7.23 (8H, H_{arom}, m), 7.24-7.14 (5H, H_{arom}), 5.61 (1H, H_1, J_{1-2}=8.9 Hz, d), 4.76-4.52 (2H, 2 x $HCHBn$, m) 4.89 (1H, $HCHBn$, d), 4.50 (1H, $HCHBn$, J=11.4 Hz, d), 4.47-4.37 (3H, $HCHBn$, H_4, H_5, m), 3.70 (1H, H_3, J_{3-4}=4.6 Hz, J=2.9 Hz, dd), 3.49 (1H, H_2, dd), 0.81 (9H, 3 x CH_{3_tBu}, s), 0.03, -0.02 (3H, CH_{3_Me}, s).4.48-4.26 (2H, H_5, H_4, m), 3.73 (1H, H_3, J_{3-2}=4.9 Hz, J_{3-4}=2.8 Hz, dd), 3.59-3.48 (4H, CH_{3_Me}, H_2, m), 0.82 (9H, 3 x CH_{3_tBu}, s), 0.06 (3H, CH_{3_Me}, s), 0.01 (3H, CH_{3_Me}, s).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 169.7, 137.2, 135.2 (C_q), 128.9, 128.8, 128.6 x 2, 128.4, 128.3 x 2, 128.1, 128.0 (CH_{arom}), 92.0 (C_1), 78.6 (C_3), 76.2 (C_4), 73.1 (CH_2), 68.6 (C_5), 60.4 (C_2), 52.4 (CH_3), 25.8 (CH_{3_tBu}), 18.1 (C_q), -4.5 (CH_3), -5.02 (CH_3).

HRMS: $C_{26}H_{35}N_3O_5SSi$ + Na^+ required 552.1959, found 552.1959

Benzyl (phenyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-1-thio-α-D-mannopyranosiduronate) (12)



Compound **47** (4.3 mmol) was dissolved in pyridine (21 mL, 0.2 M). TBSOTf (13 mmol, 3 eq) and DMAP (0.04 mmol, 1%) were added at 0°C and after 10 minutes stirring the reaction mixture was heated to 70°C. After 16 hours TLC analysis (Pentane:EtOAc, 8:2) showed complete consumption of starting material and the reaction mixture was cooled to room temperature. After quenching with cold MeOH and diluting with EtOAc, the mixture was washed with 10% aq. solution of CuSO (x2), H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (Pentane:EtOAc, 98:2→8:2) afforded the title compound in quantitative yield as a light yellow oil.

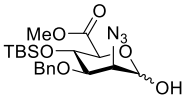
TLC analysis: R_f= 0.57 (Pentane:EtOAc; 9:1)

^{1}H-NMR (400 MHz, $CDCl_3$), δ: δ: 7.63-7.54 (2H, H_{arom}, m), 7.35-7.24 (8H, H_{arom}, m), 7.24-7.14 (5H, H_{arom}), 5.66 (1H, H_1, $J_{1\text{-}2}$=9.4 Hz, d), 5.03 (1H, $HC\mathit{H}HBn$, J=12.1 Hz, d), 4.89 (1H, $HC\mathit{H}HBn$, d), 4.50 (1H, $HC\mathit{H}HBn$, J=11.4 Hz, d), 4.47-4.37 (3H, $HC\mathit{H}HBn$, H_4, H_5, m), 3.70 (1H, H_3, $J_{3\text{-}4}$=4.6 Hz, J=2.9 Hz, dd), 3.49 (1H, H_2, dd), 0.81 (9H, 3 x CH_{3_tBu}, s), 0.03, -0.02 (3H, CH_{3_Me}, s).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 169.0, 136.8, 135.2 (C_q), 132.8 (CH_{arom}), 131.6 (C_q), 128.9, 128.6 x 2, 128.4, 128.3, 127.9 (CH_{arom}), 80.9 (C_1), 78.0 (C_3), 77.1 (C_4), 73.1 (CH_2), 68.6 (C_5), 68.6 (C_5), 67.2 (CH_2), 57.3 (C_2), 25.7 (CH_{3_tBu}), 17.9 (C_q), -4.3 (CH_3), -5.6 (CH_3).

HRMS: $C_{32}H_{39}N_3O_5SSi$ + Na^+ required 628.2272, found 628.2272

Methyl (2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-α/β-D-mannopyranosiduronate) (48)



To a solution of compound **11** (1 mmol) in acetone:H_2O (10 mL, 0.1M, 4:1), NBS (3 mmol, 3 eq) was added at 0°C. The reaction mixture assumed an orange-brown colour and after 1.5 hour TLC analysis (Pentane:EtOAc, 9:1) showed complete consumption of starting material. The reaction mixture was diluted with EtOAc and washed with saturated aq. solution of $Na_2S_2O_3$ (x2), H_2O and brine. After drying over $MgSO_4$ and filtration, the organic phase was concentrated *in vacuo*. The final product was isolated by column chromatography (Pentane:EtOAc, 9:1→7:3) in quantitative yield as a white foam and as a mixture of α/β mixture 1:4.

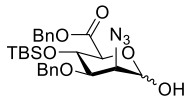
TLC analysis: R_f= 0.33 (Pentane:EtOAc; 8:2)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.43-7.26 (5H, H_{arom}, m), 5.57-5.49 (1H, H_1, m), 4.69-4.54 (2H, CH_{2_Bn}, m), 4.40-4.34 (2H, H_5, H_4, m), 3.82-3.75 (1H, H_3, m), 3.68 (1H, H_2, $J_{2\text{-}1}$=6.5 Hz, $J_{2\text{-}3}$=2.9 Hz, dd), 3.60 (3H, CH_{3_OMe}, s), 3.58-3.50 (1H, OH, b), 0.9 (9H, 3 x CH_{3_tBu}, s), 0.08 (3H, CH_3, s), 0.06 (3H, CH_3, s).

^{13}C-NMR (500 MHz, $CDCl_3$), δ: 169.7, 137.2 (C_q), 128.9, 128.8, 128.6 x 2, 128.4, 128.3 x 2, 128.1, 128.0 (CH_{arom}), 92.0 (C_1), 78.6 (C_3), 76.2 (C_4), 73.1 (CH_2), 68.6 (C_5), 60.4 (C_2), 52.4 (CH_3), 25.8 (CH_{3_tBu}), 18.1 (C_q), -4.5 (CH_3), -5.02 (CH_3).

HRMS: $C_{20}H_{31}N_3O_6Si$ + Na^+ required 460.1874, found 460.1874

Benzyl (2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-α/β-D-mannopyranosiduronate) (49)



To a solution of compound **12** (1 mmol) in acetone:H_2O (10 mL, 0.1 M, 4:1) NBS (3 mmol, 3 eq) was added at 0°C. The reaction mixture assumed an orange-brown colour and after 1.5 hour TLC analysis (Pentane:EtOAc, 9:1) showed complete consumption of starting material. The reaction mixture was diluted with EtOAc and washed with a saturated aq. solution of $Na_2S_2O_3$ (x2), H_2O and brine. After drying over $MgSO_4$ and filtration, the organic phase was concentrated *in vacuo*. The final product was isolated by column chromatography (Pentane:EtOAc, 9:1→7:3) in quantitative yield as a white foam and as a mixture of α/β mixture 1:6.7.

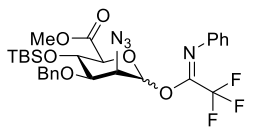
TLC analysis: R_f= 0.35 (Pentane:EtOAc; 8:2)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.40-7.23 (10H, H_{arom}, m), 5.62-5.54 (1H, H_1, m), 5.08 (1H, CH*H*, J=12.2 Hz, d), 4.95 (1H, CH*H*, J=12.2 Hz, d), 4.55 (1H, CH*H*, J=11.6 Hz, d), 4.49 (1H, CH*H*, J=11.6 Hz, d), 4.42-4.36 (2H, H_5, H_4, m), 3.78-3.72 (1H, H_3, m), 3.64 (1H, H_2, $J_{2\text{-}1}$=7.0 Hz, $J_{2\text{-}3}$=2.9 Hz, dd), 3.31 (1H, OH, d), 0.9 (9H, 3 x CH_{3_tBu}, s), 0.06 (3H, CH_3, s), 0.04 (3H, CH_3, s).

^{13}C-NMR (500 MHz, $CDCl_3$), δ: 169.2, 137.1, 135.2 (C_q), 128.8, 128.7 x 2, 128.6, 128.5 x 2, 128.4, 128.1 x 2 (CH_{arom}), 91.7 (C_1), 78.2 (C_3), 76.5 (C_4), 73.0 (CH_2), 68.7 (C_5), 67.2 (CH_2), 60.3 (C_2), 25.8 (CH_{3_tBu}), 18.1 (C_q), -4.7 (CH_3), -5.06 (CH_3).

HRMS: $C_{26}H_{35}N_3O_6Si$ + Na^+ required 536.2187, found 536.2187

Methyl (2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-mannopyranosiduronate) (50)



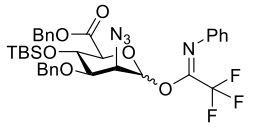
Compound **48** (0.5 mmol) was dissolved in dry acetone (1.5 mL, 0.33 M) and *N*-phenyl-2,2,2-trifluoroimidoyl chloride (0.65 mmol, 1.5 eq) and Cs_2CO_3 (0.6 mmol, 1.2 eq) were added. After1.5 hour, TLC analysis (Pentane:EtOAc, 9:1) showed complete consumption of the starting material. The reaction mixture was diluted with acetone, filtered and concentrated *in vacuo*. Compound **50** was isolated by column chromatography (Pentane:EtOAc, 95:5, 1% TEA) in 74% yield as transparent oil.

TLC analysis: R_f= 0.7 (Pentane:EtOAc; 9:1)

^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.47-7.28 (7H, H_{arom}, m), 7.20-7.10 (1H, H_{arom}, m), 6.88 (2H, H_{arom}, J=7.7 Hz, d), 6.42-6.20 (1H, H_1, bs), 4.71 (1H, $HC\mathit{H}HBn$, J=11.6 Hz, d), 4.65 (1h, $HC\mathit{H}HBn$, d), 4.36-4.17 (2H, H_5, H_4, m), 4.12-4.01 (1H, H_3, m), 3.92-3.83 (1H, H_2, m), 3.65 (3H, CH_3, s), 0.87 (9H, 3 x CH_{3_tBu}, s), 0.07 (3H, CH_{3_Me}, s), 0.05 (3H, CH_{3_Me}, s).

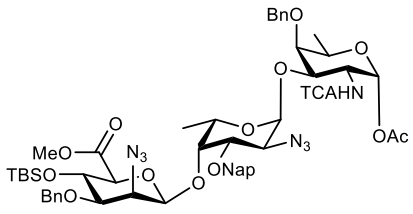
^{13}C-NMR (500 MHz, $CDCl_3$), δ: 169.7, 137.2 (C_q), 128.9, 128.8, 128.6 x 2, 128.4, 128.3 x 2, 128.1, 128.0 (CH_{arom}), 92.0 (C_1), 78.6 (C_3), 76.2 (C_4), 73.1 (CH_2), 68.6 (C_5), 60.4 (C_2), 52.4 (CH_3), 25.8 (CH_{3_tBu}), 18.1 (C_q), -4.5 (CH_3), -5.02 (CH_3).

Benzyl (2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-mannopyranosiduronate) (51)



Compound **49** (0.5 mmol) was dissolved in dry acetone (1.5 mL, 0.33 M) and *N*-phenyl-2,2,2-trifluoroimidoyl chloride (0.65 mmol, 1.2 eq) and Cs_2CO_3 (0.6 mmol, 1.2 eq) were added. After1.5 hour, TLC analysis (Pentane:EtOAc, 9:1) showed complete consumption of the starting material. The reaction mixture was diluted with acetone, filtered and concentrated *in vacuo*. Compound **51** was isolated by column chromatography (Pentane:EtOAc, 95:5, 1% TEA) in 77% yield as transparent oil.
TLC analysis: R_f= 0.7 (Pentane:EtOAc; 9:1)
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.51-7.21 (12H, H_{arom}, m), 7.18-7.09 (1H, H_{arom}, m), 6.83-6.75 (2H, H_{arom}, m), 6.50-6.21 (1H, H_1, bs), 5.11 (1H, $HCHBn$, J=12.4 Hz, d), 5.00 (1H, $HCHBn$, d), 4.64 (1H, $HCHBn$, J=11.6 Hz, d), 4.58 (1H, $HCHBn$, d), 4.38-4.28 (2H, $H_{5,\ H}4$, m), 4.06-3.97 (1H, H_3, m), 3.91-3.82 (1H, H_2, m), 0.85 (9H, 3 x CH_{3_tBu}, s), 0.05 (6H, 2 x CH_3, s).
^{13}C-NMR (500 MHz, $CDCl_3$), δ: 169.2, 137.1, 135.2 (C_q), 128.8, 128.7 x 2, 128.6, 128.5 x 2, 128.4, 128.1 x 2 (CH_{arom}), 91.7 (C_1), 78.2 (C_3), 76.5 (C_4), 73.0 (CH_2), 68.7 (C_5), 67.2 (CH_2), 60.3 (C_2), 25.8 (CH_{3_tBu}), 18.1 (C_q), -4.7 (CH_3), -5.06 (CH_3).

2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(methyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-β-D-mannopyranosiduronate)-α-L-fucopyranosyl)-4-*O*-benzyl-1-*O*-acetyl-α-D-fucopyranoside (52)

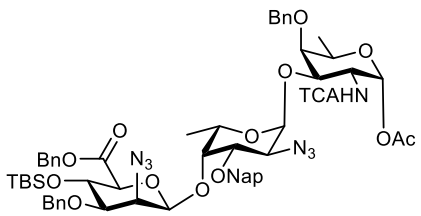


Method A: Donor **11** (0.2 mmol, 2.5 eq) and acceptor **38** (0.08 mmol) were co-evaporated with toluene three times and dissolved in DCM (0.8 mL, 0.1 M). Freshly activated 4 Å MS were added to the reaction mixture, which was then cooled to -60°C. NIS (0.2 mmol, 2.5 eq) and TBSOTf (0.024 mmol, 0.3 eq) were added and the reaction was kept at -40°C for 8 hours, after which TLC analysis (Pentane:EtOAc, 1:1) showed complete consumption of acceptor. The reaction mixture was quenched by addition of TEA (0.024 mmol, 0.3 eq) and let to warm up to room temperature. The reaction was diluted with DCM and washed with saturated aq. solution of $Na_2S_2O_4$, H_2O and brine. Purification by column chromatography (Pentane:EtOAc, 8:2→6:4) afforded the title compound **52** in 67% yield as white solid.

Method B: Donor **50** (0.242 mmol, 2.5 eq) and acceptor **38** (0.097 mmol) were co-evaporated with toluene three times and dissolved in dry DCM (1 mL, 0.1 M). Freshly activated 4 Å MS were added to the reaction mixture, which was then cooled to -60°C. TBSOTf (0.03 mmol, 0.3 eq) was added dropwise and the reaction was kept at -40°C for 10 hours, after which TLC analysis (Pentane:EtOAc, 1:1) showed complete consumption of acceptor. The reaction mixture was quenched by addition of TEA (0.03 mmol, 0.3 eq) and let to warm up to room temperature. The reaction was diluted with DCM and washed with H_2O and brine. Purification by column chromatography (Pentane:EtOAc, 8:2→6:4) afforded the title compound **52** in 70% yield as white solid.
TLC analysis: R_f= 0.35 (DCM:Acetone; 98:2)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.90-7.75 (4H, H_{arom}, m), 7.73 (1H, NH_{TCA}, J_{NH-H2}=5.4 Hz, d), 7.53-7.43 (3H, H_{arom}, m), 7.40-7.24 (10H, H_{arom}, m), 6.53 (1H, H_{1D}, J_{1-2}=3.6 Hz, d), 5.02 (1H, H_{1L}, J_{1-2}=3.6 Hz, d), 4.95 (1H, CH*H*, J=11.1 Hz, d), 4.85 (1H, CH*H*, J=11.6 Hz, d), 4.72-4.42 (6H, 4 x CH*H*, H_{1M}, H_{2D}, m), 4.22 (1H, H_{2L}, J_{2-3}=10.4 Hz, dd), 4.12-3.99 (4H, H_{4M}, H_{5L}, H_{3D}, H_{4L}, m), 3.97-3.91 (1H, H_{5D}, m), 3.89 (1H, H_{3L}, J_{3-4}=2.8 Hz, dd), 3.85 (1H, H_{2M}, J_{2-1}=1.1 Hz, J_{2-3}=3.6 Hz, dd), 3.73 (1H, H_{4D}, J_{3-4}=2.8 Hz, J_{4-5}=1.2 Hz, dd), 3.64 (1H, H_{5M}, J_{5-4}=9.4 Hz, d), 3.33-3.24 (4H, H_{3M}, OMe, m), 2.07 (3H, CH_{3_OAc}, s), 1.15 (3H, 3 x H_{6D}, J_{6-5}=6.4 Hz, d), 1.09 (3H, 3 x H_6, J_{6-5}=6.6, d), 0.78 (9H, *t*Bu, s), 0.02 (3H, Me, s), -0.06 (3H, Me, s).
^{13}C-NMR (500 MHz, $CDCl_3$), δ: 169.0, 167.8, 162.3, 138.1, 137.4, 135.1, 133.4, 133.1 (C_q), 128.7, 128.5, 128.5, 128.3, 128.2, 128.1 x 2, 127.9, 127.8 x 2, 127.7 x 2, 126.4, 126.1 x 2, 126.0, 125.8 (CH_{arom}), 101.0 (C_{1M}), 98.6 (C_{1L}), 92.6 (C_q), 90.0 (C_{1D}), 80.2 (C_{3M}), 78.2 (C_{3D}), 74.5 (H_{5M}), 77.0 (C_{3L}), 75.3 (C_{4L}), 74.9, 72.3, 71.3 (CH_2), 69.1 (C_{5D}), 68.4 (C_{4D}), 68.1 (C_5), 61.4 (C_{2M}), 60.5 (C_{2L}), 52.2 (CH_{3_OMe}), 50.7 (C_{2D}), 25.8 (CH_{3_tBu}), 20.9 (CH_{3_OAc}), 18.0 (C_q), 17.1 (C_{6L}), 16.9 (C_{6D}), -3.8 (CH_3), -5.4 (CH_3).
HRMS: $C_{54}H_{66}Cl_3N_7O_{14}Si$ + Na^+ required 1192.3395, found 1192.3395

2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(benzyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-β-D-mannopyranosiduronate)-α-L-fucopyranosyl)-4-*O*-benzyl-1-*O*-acetyl-α-D-fucopyranoside (53)



Method A: Donor: Donor **12** (0.125 mmol, 2.5 eq) and acceptor **38** (0.05 mmol) were co-evaporated with toluene three times and dissolved in DCM (0.5 mL, 0.1 M). Freshly activated 4 Å MS were added to the reaction mixture, which was then cooled to -60°C. NIS (0.125 mmol, 2.5 eq) and TBSOTf (0.015 mmol, 0.3 eq) were added and the reaction was kept at -40°C for 7 hours, after which TLC analysis (Pentane:EtOAc, 1:1) showed complete consumption of acceptor. The reaction mixture was quenched by addition of TEA (0.04 mmol, 0.5 eq) and let to warm up to room temperature. The reaction was diluted with DCM and washed with saturated aq. solution of $Na_2S_2O_4$, H_2O and brine. Purification by column chromatography (Pentane:EtOAc, 8:2→6:4) afforded the title compound **53** in 70% yield as white solid.

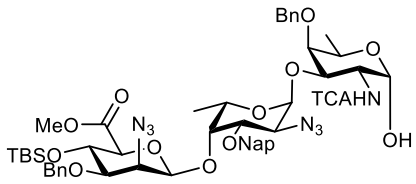
Method B: Donor **51** (0.195 mmol, 2.4 eq) and acceptor **38** (0.08 mmol) were co-evaporated with toluene three times and dissolved in DCM (0.8 mL, 0.1 M). Freshly activated 4 Å MS were added to the reaction mixture, which was then cooled to -60°C. TBSOTf (0.024 mmol, 0.3 eq) was added dropwise and the reaction was kept at -40°C for 10 hours, after which TLC analysis (Pentane:EtOAc, 1:1) showed complete consumption of acceptor. The reaction mixture was quenched by addition of TEA (0.02 mmol, 0.2 eq) and let to warm up to room temperature. The reaction was diluted with DCM and washed with H_2O and brine. Purification by column chromatography (Pentane:EtOAc, 8:2→6:4) afforded the title compound **53** in 69% yield as white solid.
TLC analysis: R_f= 0.36 (DCM:Acetone; 98:2)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: δ: 7.91-7.75 (5H, H_{arom}, NH, m), 7.60-7.00 (4H, H_{arom}, NH_{TCA}, m), 6.57 (1H, H_{1D}, J_{1-2}=3.6 Hz, d), 5.04 (1H, H_{1L}, J=3.6 Hz, d), 4.95-4.81 (4H, 4 x CH*H*, m), 4.74-4.58 (4H, 3 x CH*H*, H_{1M}, m), 4.57-4.59 (1H, H_{2D}, m), 4.38 (1H, CH*H*, J=11.1 Hz, d),4.62-4.14 (2H, H_{2L}, H_{4M}, m), 4.12-4.00 (3H, H_{3D}, H_{5L}, H_{4D}, m), 4.00-3.84 (3H, H_{5D}, H_{2M}, H_{3L}, m), 3.78-3.68 (2H, H_{5M}, H_{4L}, m), 3.35 (1H, H_{3M}, J_{2-3}=3.6, J_{3-4}=8.9 Hz, dd), 2.11 (3H, CH_{3_OAc}, s), 1.18 (3H, 3 x H_6, J_{6-5}=6.4 Hz, d), 1.12 (3H, 3 x H_6, J_{6-5}=6.5, d), 0.81 (9H, *t*Bu, s), 0.05 (3H, CH_3, s), -0.02 (3H, CH_3, s).

^{13}C-NMR (500 MHz, $CDCl_3$), δ: 169.0, 167.4, 162.3, 138.1, 137.4, 135.0, 134.7, 133.3, 133.1 (C_q), 128.7 x 2, 128.5 x 3, 128.4, 128.3, 128.2 x 2, 128.1, 127.9, 127.8, 127.7, 126.8, 126.1, 126.0 (CH_{arom}), 101.1 (C_{1M}), 98.7 (C_{1L}), 92.6 (C_q), 90.0 (C_{1D}), 80.3 (C_{3M}), 78.2 (C_{3D}), 77.3 (H_{5M}), 76.6 (C_{3L}, C_{4L}), 74.9 (C_{4D}), 74.9, 72.1, 71.1 (CH_2), 69.1 (C_{5D}), 68.4 (C_{4M}), 68.0 (C_5), 67.5 (CH_2), 61.1 (C_{2M}), 60.4 (C_{2L}), 50.7 (C_{2D}), 25.9 (CH_{3_tBu}), 21.0 (CH_{3_OAc}), 18.1 (C_q), 17.1 (C_{6L}), 16.9 (C_{6D}), -3.8 (CH_3), -5.3 (CH_3).

HRMS: $C_{60}H_{70}Cl_3N_7O_{14}Si$ + Na^+ required 1268.3708, found 1268.3708

2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(methyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-β-D-mannopyranosiduronate)-α-L-fucopyranosyl)-4-*O*-benzyl-α-D-fucopyranoside (54)



52 (0.160 mmol) was dissolved in dry MeOH (1.6 mL, 0.1M) and under inert atmosphere Bu_3SnOMe (0.16 mmol, 1 eq) was added. The reaction mixture was stirred 6 hours at 50°C, after which TLC analysis (DCM:Acetone, 98:2) showed complete consumption of starting material. After cooling at room temperature, the reaction mixture was diluted with EtOAc and washed with H_2O. The aqueous phase was extracted with EtOAc and the combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. Product **54** was isolated in 81% by column chromatography (DCM:Acetone, 99:1→90:10) as white solid.

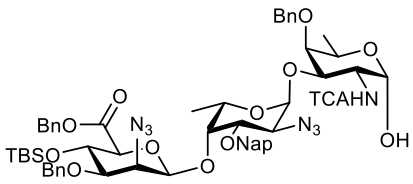
TLC analysis: Rf= 0.33 (DCM:Acetone; 9:1)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.91-7.75 (4H, H_{arom}, m), 7.58-7.42 (4H, H_{arom}, NH_{TCA}, m), 7.40-7.24 (5H, H_{arom}, m), 6.52 (1H, H_{1D}, J_{1-2}=3.6 Hz, d), 5.04 (1H, H_{1L}, J=3.4 Hz, d), 4.90-4.67 (4H, 2 x CH_2, m), 4.63-4.55 (1H, H_{2D}, m), 4.10-3.92 (5H, H_{2L}, H_{5L}, H_{5D}, H_{3D}, H_{3L}, m), 3.85 (1H, H_{4D}, J_{3-4}=2.9 Hz, dd), 3.75 (1H, H_{4L}, J_{3-4}=2.8 Hz, d), 2.11 (3H, CH_{3_OAc}, s), 1.22 (3H, 3 x H_6, J_{6-5}=6.5 Hz, d), 1.18 (3H, 3 x H_6, J_{6-5}=6.5, d).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 169.0, 162.2, 138.1, 134.3, 133.3 (C_q), 128.9, 128.6, 128.1, 127.9 x 2, 127.8, 127.7, 127.1, 126.6, 126.5, 125.7 (CH_{arom}), 99.0 (C_{1L}), 92.7 (C_q), 90.4 (C_{1D}), 78.3 (C_{3L}, C_{3D}), 76.8 (C_{4L}), 75.0 (CH_2), 72.3 (CH_2), 69.2 (C_5), 68.3 (C_{4D}), 67.5 (C_5), 60.7 (C_{2L}), 50.7 (C_{2D}), 21.0 (CH_{3_OAc}), 17.0 (C_q), 16.4 (C_6).

HRMS: $C_{52}H_{64}Cl_3N_7O_{13}Si$ + Na^+ required 1150.3289, found 1150.3289

2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(benzyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-β-D-mannopyranosiduronate)-α-L-fucopyranosyl)-4-*O*-benzyl-α-D-fucopyranoside (55)



53 (0.3 mmol) was dissolved in DMF (10 mL, 0.03 M) and under inert atmosphere NH_2NH_2OAc (1.5 mmol, 5 eq) was added. The reaction mixture was stirred for one hour, after which TLC analysis (DCM:Acetone, 98:2) showed complete consumption of starting material. After cooling at room temperature, the reaction mixture was diluted with EtOAc and washed with H_2O. The aqueous phase was extracted with EtOAc and the combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. Product **55** was quantitatively isolated by column chromatography (DCM:Acetone, 99:1→90:10) as white solid.

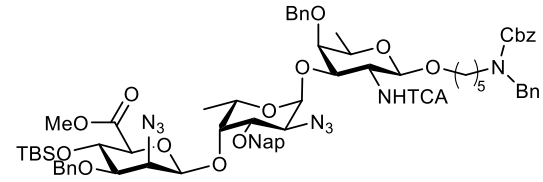
TLC analysis: Rf= 0.33 (DCM:Acetone; 9:1)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.91-7.75 (4H, H_{arom}, m), 7.58-7.42 (4H, H_{arom}, NH_{TCA}, m), 7.40-7.24 (5H, H_{arom}, m), 6.52 (1H, H_{1D}, J_{1-2}=3.6 Hz, d), 5.04 (1H, H_{1L}, J=3.4 Hz, d), 4.90-4.67 (4H, 2 x CH_2, m), 4.63-4.55 (1H, H_{2D}, m), 4.10-3.92 (5H, H_{2L}, H_{5L}, H_{5D}, H_{3D}, H_{3L}, m), 3.85 (1H, H_{4D}, J_{3-4}=2.9 Hz, dd), 3.75 (1H, H_{4L}, J_{3-4}=2.8 Hz, d), 2.11 (3H, CH_{3_OAc}, s), 1.22 (3H, 3 x H_6, J_{6-5}=6.5 Hz, d), 1.18 (3H, 3 x H_6, J_{6-5}=6.5, d).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 169.0, 162.2, 138.1, 134.3, 133.3 (C_q), 128.9, 128.6, 128.1, 127.9 x 2, 127.8, 127.7, 127.1, 126.6, 126.5, 125.7 (CH_{arom}), 99.0 (C_{1L}), 92.7 (C_q), 90.4 (C_{1D}), 78.3 (C_{3L}, C_{3D}), 76.8 (C_{4L}), 75.0 (CH_2), 72.3 (CH_2), 69.2 (C_5), 68.3 (C_{4D}), 67.5 (C_5), 60.7 (C_{2L}), 50.7 (C_{2D}), 21.0 (CH_{3_OAc}), 17.0 (C_q), 16.4 (C_6).

HRMS: $C_{58}H_{68}Cl_3N_7O_{13}Si + Na^+$ required 1226.3602, found 1226.3602

5-(benzyl(benzyloxicarbonyl)amino)pentyl 2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(methyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-β-D-mannopyranosiduronate)-α-L-fucopyranosyl)-4-*O*-benzyl-β-D-fucopyranoside (5)



54 (0.060 mmol) was dissolved in dry acetone (0.3 mL, 0.2 M) and under inert atmosphere $CsCO_3$ (0.066 mmol, 1.1 eq) was added. The reaction mixture was cooled to 0°C and $CF_3CN(Ph)Cl$ (0.072 mmol, 1.2 eq) was added. After 1 hour, TLC analysis (Pentane:EtOAc, 7:3) showed complete consumption of starting material. The reaction was diluted in acetone, filtered over Celite® and concentrated *in vacuo*. Acceptor **58** (0.120 mmol, 2 eq) was added and the two compounds were coevaporated three times with toluene. The resulting mixture was dissolved in dry DCM (0.6 mL, 0.1 M) and 3Å MS were added. After10 minutes the reaction was cooled to -78°C and TBSOTf (0.02 mmol, 0.3 eq) was added. The reaction mixture was allowed to warm up and after 1 hour at -33 °C, TLC analysis (Pentane:EtOAc, 9:1) showed complete consumption of donor. The reaction mixture was diluted with DCM, washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound was purified by column chromatography (Pentane:EtOAc, 8:2→65:25) in 68% yield as white foam.

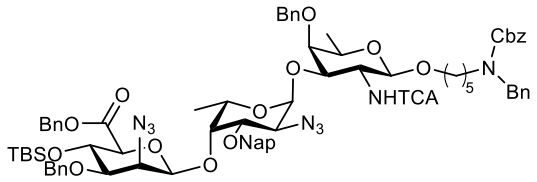
TLC analysis: R_f= 0.38 (Pentane:EtOAc; 7:3)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.91-7.75 (4H, H_{arom}, m), 7.58-7.46 (3H, H_{arom}, m), 7.44-7.13 (23H, H_{arom}, NH_{TCA}, m), 5.25-5.14 (2H, H_{arom}, m), 4.98 (1H, H_{1L}, J=3.7 Hz, d), 4.94-4.82 (2H, $H_{CH}HBn$, H_{1D}, m), 4.79-4.45 (8H, 7 x $H_{CH}HBn$, H_{1M}, m), 4.35-4.25 (1H, H_{3D}, m), 4.15 (1H, H_{4M}, $J_{4\text{-}3}$=$J_{4\text{-}5}$=9.1 Hz, t), 4.01-3.80 (6H, H_{2L}, H_{2L}, H_{2M}, $H_{CH2OLinker}$, H_{4L}, H_{5L}, m), 3.77 (1H, H_{3L}, $J_{3\text{-}2}$=10.6 Hz, $J_{3\text{-}4}$=2.8Hz, dd), 3.70 (1H, H_{5M}, d), 3.64 (1H, 3.67-3.60 (1H, H_{5D}, m), 3.56 (1H, H_{4D}, $J_{4\text{-}3}$=2.8 Hz, d), 3.46-3.30 (5H, $H_{CH2OLinker}$, OMe, H_{3M}, m), 3.30-3.13 (2H, $CH_{2\_NLinker}$, m), 1.65-1.42 (4H, 2 x CH_{2_Linker}, m), 1.39-1.22 (5H, $CH_{2\_NLinker}$, H_{6D}, m), 1.12 (3H, H_{6L}, $J_{6\text{-}5}$=6.5 Hz, d), 0.84 (9H, 3 x CH_{3_tBu}, s), 0.06 (3H, CH_3, s), -0.01 (3H, CH_3, s).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 167.86, 162.0, 138.5, 138.0, 135.4, 133.4, 133.0 (C_q), 128.6 x 2, 128.5, 128.4, 128.2, 128.1 x 2, 128.0, 127.9 x 2, 127.8, 127.7 x 2, 127.5, 127.3, 126.4, 126.1, 125.9 x 2 (CH_{arom}), 100.1 (C_{1M}), 99.3 (C_{1L}, C_{1D}) , 92.6 (C_q), 80.3 (C_{3M}), 79.3 (C_{4D}), 78.1 (C_{3D}), 77.5 (C_{5M}), 75.4 (C_{3M}), 75.3 (CH_{4L}), 72.2, 71.1, 70.6 (CH_2), 70.4 (C_{5D}), 69.7 ($CH_{2OLinker}$), 68.3 (C_{4M}), 67.1 (CH_2), 67.0 (C_{5L}), 61.1 (C_{2D}), 59.3 (C_{2L}), 55.9 (C_{2M}), 52.2 (CH_{3OMe}), 50.3 (CH_2), 47.1, 46.2 ($CH_{2NLinker}$), 28.7 (CH_2), 25.9 (3 x CH_{3tBu}), 17.9 (C_q), 17.1 (C_{6L}, C_{6D}), -3.9, -5.4 (CH_{3Me}).

HRMS: $C_{71}H_{85}Cl_3N_8O_{15}Si$ + Na^+ required 1445.4861, found 1445.4861

5-(benzyl(benzyloxicarbonyl)amino)pentyl 2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(benzyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-β-D-mannopyranosiduronate)-α-L-fucopyranosyl)-4-*O*-benzyl-β-D-fucopyranoside (6)



55 (0.060 mmol) was dissolved in dry acetone (0.3 mL, 0.2 M) and under inert atmosphere $CsCO_3$ (0.066 mmol, 1.1 eq) was added. The reaction mixture was cooled to 0°C and $CF_3CN(Ph)Cl$ (0.072 mmol, 1.2 eq) was added. After 1 hour, TLC analysis (Pentane:EtOAc, 7:3) showed complete consumption of starting material. The reaction was diluted in acetone, filtered over Celite® and concentrated *in vacuo*. Acceptor **58** (0.120 mmol, 2 eq) was added and the two compounds were coevaporated three times with toluene. The resulting mixture was dissolved in dry DCM (0.6 mL, 0.1 M) and 3Å MS were added. After10 minutes the reaction was cooled to -78°C and TBSOTf (0.02 mmol, 0.3 eq) was added. The reaction mixture was allowed to warm up and after 1 hour at -33 °C, TLC analysis (Pentane:EtOAc, 9:1) showed complete consumption of donor. The reaction mixture was diluted with DCM, washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound was purified by column chromatography (Pentane:EtOAc, 8:2→65:25) in 71% yield as white foam.

TLC analysis: R_f= 0.38 (Pentane:EtOAc; 7:3)

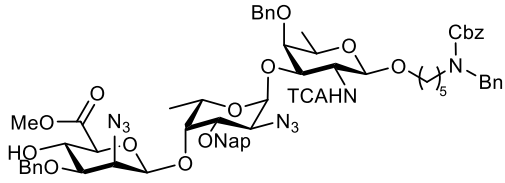
^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.89-7.75 (4H, H_{arom}, m), 7.51-7.05 (29H, H_{arom}, NH, m), 5.22-5.10 (2H, 2 x $H_{CH}H$, m), 4.96-4.88 (3H, H_{1L}, 2 x $H_{CH}H$, m), 4.87-4.73 (2H, H_{1D}, $H_{CH}H$, m), 4.73-4.54 (5H, 4 x $H_{CH}H$, H_{1M}, m), 4.54-4.36 (3H, $H_{CH}H$, m), 4.32-4.22 (1H, H_{3D}, m), 4.19 (1H, H_{4M}, $J_{4\text{-}5}$=$J_{4\text{-}3}$=9.1 Hz, pt), 3.91-3.74 (5H, H_{2L}, H_{2D}, $H_{CH}HOLinker$, H_{5L}, H_{4D}, m) 3.75-3.64 (2H, H_{5M}, H_{3M}, m), 3.60 (1H, H_{5D}, $J_{5\text{-}6}$=6.4 Hz, q), 3.51 (1H, H_{4L}, $J_{4\text{-}3}$=3.51 Hz, d), 3.45-3.29 (2H, $H_{CH}HOLinker$, H_{3M}, m), 3.28-3.09 (2H, $H_{CH}HNLinker$), 1.62-1.39 (4H, 4 x $H_{CH}HLinker$, m), 1.39-1.12 (5H, 2 x

$H_{CHHLinker}$, H_{6D}, m),1.10 (3H, H_{6L}, $J_{6\text{-}5}$=6.4 Hz, d), 0.81 (9H, H_{tBu}, s), 0.03 (3H, CH_3, s), -0.03 (3H, CH_3, s).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 167.4, 162.0, 138.5, 137.9, 137.3 x 2, 134.7, 133.3, 133.0 (C_q), 128.9, 128.8, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4 x 2, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.5, 127.3, 126.7, 126.4, 126.1, 126.0 x 2, 125.8 (CH_{arom}), 101.0 (C_{1M}), 99.3 (C_{1L}), 99.1 (C_{1D}), 92.6 (C_q), 80.3 (C_{3M}), 79.2 (C_{4D}), 77.9 (C_{4L}), 77.4 (C_{5M},), 75.1 x 2 (CH_2, C_{5D}), 72.0, 70.8 (CH_2), 70.6 (C_{5L}), 69.8 (CH_2), 69.7 ($CH_{2OLinker}$), 68.3 (C_{4M}), 67.4 (CH_2), 67.2 (C_{6L}), 60.8 (C_{2M}), 59.2 (C_{2L}), 56.0 (C_{2D}), 50.5 (CH_2), 47.2, 46.2 (CH_{2NLink}), 29.2, 28.0 ($CH_{2Linker}$), 27.4 (CH_{3tBu}), 25.8 ($CH_{2Linker}$), 17.2 (CH_{3_6D}), 17.0 (CH_{3_6L}), -3.9, -5.3 (CH_{3_Me}).

HRMS: $C_{77}H_{89}Cl_3N_8O_{15}Si$ + H^+ required 1521.5174, found 1521.9714

5-(benzyl(benzyloxicarbonyl)amino)pentyl 2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(methyl 2-azido-2-deoxy-3-*O*-benzyl-β-D-mannopyranosiduronate)-α-L-fucopyranosyl)-4-*O*-benzyl-β-D-fucopyranoside (59)



5 (0.035 mmol) was dissolved in THF (0.350 mL, 0.1 M) and subsequently TEA*3HF (0.35 mmol, 10 eq) was added. The reaction was stirred for 24 hours at reflux, after which TLC analysis (Pentane:EtOAc, 7:3) showed complete consumption of starting material. The reaction was diluted in EtOAc and transfer in a beaker containing a cold saturated aq. solution of $NaHCO_3$. Once the ice melted, the organic layer was separated and the aqueous phase was reextracted (x2). The combined EtOAc solutions were washed with water and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound **59** was isolated by column chromatography in quantitative yield as white solid.

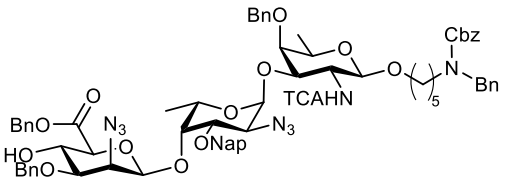
TLC analysis: R_f= 0.31 (DCM:Acetone; 95:5)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.91-7.75 (4H, H_{arom}, m), 7.58-7.46 (3H, H_{arom}, m), 7.44-7.13 (23H, H_{arom}, NH_{TCA}, m), 5.25-5.14 (2H, H_{arom}, m), 4.98 (1H, H_{1L}, J=3.7 Hz, d), 4.94-4.82 (2H, H_{CHHBn}, H_{1D}, m), 4.79-4.45 (8H, 7 x H_{CHHBn}, H_{1M}, m), 4.35-4.25 (1H, H_{3D}, m), 4.15 (1H, H_{4M}, $J_{4\text{-}3}$=$J_{4\text{-}5}$=9.1 Hz, t), 4.01-3.80 (6H, H_{2L}, H_{2L}, H_{2M}, $H_{CH2OLinker}$, H_{4L}, H_{5L}, m), 3.77 (1H, H_{3L}, $J_{3\text{-}2}$=10.6 Hz, $J_{3\text{-}4}$=2.8Hz, dd), 3.70 (1H, H_{5M}, d), 3.64 (1H, 3.67-3.60 (1H, H5D, m), 3.56 (1H, H_{4D}, $J_{4\text{-}3}$=2.8 Hz, d), 3.46-3.30 (5H, $H_{CH2OLinker}$, OMe, H_{3M}, m), 3.30-3.13 (2H, $CH_{2\_NLinker}$, m), 1.65-1.42 (4H, 2 x CH_{2_Linker}, m), 1.39-1.22 (5H, $CH_{2\_NLinker}$, H_{6D}, m), 1.12 (3H, H_{6L}, $J_{6\text{-}5}$=6.5 Hz, d), 0.84 (9H, 3 x CH_{3_tBu}, s), 0.06 (3H, CH_3, s), -0.01 (3H, CH_3, s).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 167.86, 162.0, 138.5, 138.0, 135.4, 133.4, 133.0 (C_q), 128.6 x 2, 128.5, 128.4, 128.2, 128.1 x 2, 128.0, 127.9 x 2, 127.8, 127.7 x 2, 127.5, 127.3, 126.4, 126.1, 125.9 x 2 (CH_{arom}), 100.1 (C_{1M}), 99.3 (C_{1L}, C_{1D}) , 92.6 (C_q), 80.3 (C_{3M}), 79.3 (C_{4D}), 78.1 (C_{3D}), 77.5 (C_{5M}), 75.4 (C_{3M}), 75.3 (CH_{4L}), 72.2, 71.1, 70.6 (CH_2), 70.4 (C_{5D}), 69.7 ($CH_{2OLinker}$), 68.3 (C_{4M}), 67.1 (CH_2), 67.0 (C_{5L}), 61.1 (C_{2D}), 59.3 (C_{2L}), 55.9 (C_{2M}), 52.2 (CH_{3OMe}), 50.3 (CH_2), 47.1, 46.2 ($CH_{2NLinker}$), 28.7 (CH_2), 25.9 (3 x CH_{3tBu}), 17.9 (C_q), 17.1 (C_{6L}, C_{6D}), -3.9, -5.4 (CH_{3Me}).

HRMS: $C_{65}H_{71}Cl_3N_8O_{15}$ + Na^+ required 1331.3997, found 1331.3997

5-(benzyl(benzyloxicarbonyl)amino)pentyl 2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(benzyl 2-azido-2-deoxy-3-*O*-benzyl-β-D-mannopyranosiduronate)-α-L-fucopyranosyl)-4-*O*-benzyl-β-D-fucopyranoside (60)



6 (0.035 mmol) was dissolved in THF (0.350 mL, 0.1 M) and subsequently TEA*3HF (0.35 mmol, 10 eq) was added. The reaction was stirred for 24 hours at reflux, after which TLC analysis (Pentane:EtOAc, X:X) showed complete consumption of starting material. The reaction was diluted in EtOAc and transfer in a beaker containing a cold saturated aq. solution of $NaHCO_3$. Once the ice melted, the organic layer was separated and the aqueous phase was reextracted (x2). The combined EtOAc solutions were washed with water and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound **60** was isolated by column chromatography in quantitative yield as white solid.

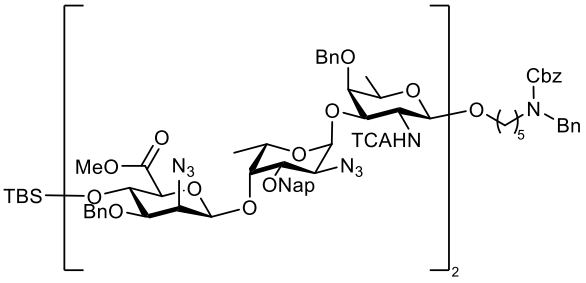
TLC analysis: R_f= 0.31 (DCM:Acetone; 95:5)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.80-7.67 (4H, H_{arom}, m), 7.51-7.11 (27H, H_{arom}, NH, m), 7.11-7.05 (4H, H_{arom}, m), 5.19-5.09 (2H, 2 x H_{CHH}, m), 4.95-4.82 (3H, H_{1L}, 2 x H_{CHH}, m), 4.81 (1H, H_{1D}, $J_{1\text{-}2}$=7.89 Hz, d), 4.77-4.55 (6H, 5 x H_{CHH}, H_{1M}, m), 4.49-4.36 (3H, H_{CHH}, m), 4.30-4.22 (1H, H_{3D}, m), 4.16 (1H, H_{4M}, m), 4.03-3.94 (2H, H_{2M}, H_{4L}, m), 3.91-3.73 (4H, H_{2L}, H_{5L}, H_{2D}, $H_{CHHOLinker}$), 3.71-3.62 (2H, H_{5M}, H_{3L}), 3.59 (1H, H_{5D}, $J_{5\text{-}6}$=6.4 Hz, q), 3.51 (1H, H_{4D}, $J_{4\text{-}3}$=2.88 Hz, d), 3.43-3.29 (2H, $H_{CHHOLinker}$, H_{3M}, m), 3.25-3.08 (2H, $H_{CHHNLinker}$), 1.62-1.39 (4H, 4 x $H_{CHHLinker}$, m), 1.39-1.12 (5H, 2 x $H_{CHHLinker}$, H_{6D}, m),1.10 (3H, H_{6L}, $J_{6\text{-}5}$=6.4 Hz, d).

^{13}C-NMR (400 MHz, $CDCl_3$), δ:

HRMS: $C_{71}H_{75}Cl_3N_8O_{15}$ + Na^+ required 1407.4310, found 1407.4310

Fully protected hexasaccharide (7)



54 (0.030 mmol) was dissolved in dry acetone (0.150 mL, 0.2 M) and under inert atmosphere $CsCO_3$ (0.033 mmol, 1.1 eq) was added. The reaction mixture was cooled to 0°C and $CF_3CN(Ph)Cl$ (0.035 mmol, 1.2 eq) was added. After 1 hour, TLC analysis (Pentane:EtOAc, 7:3) showed complete consumption of starting material. The reaction was diluted in acetone, filtered over Celite® and concentrated *in vacuo*. Acceptor **59** (0.040 mmol, 1.3 eq) was added and the two compounds were coevaporated three times with toluene. The resulting mixture was dissolved in dry DCM (0.300 mL, 0.1 M) and 3Å MS were added. After 10 minutes the reaction was cooled to -78°C and a freshly prepared 0.6 M solution of TBSOTf in dry DCM (0.006 mmol, 0.02 eq) was added. The reaction mixture was allowed to warm up and after 1 hour at -50 °C, TLC analysis (Pentane:EtOAc, 7:3) showed

complete consumption of donor. The reaction mixture was diluted with DCM, washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound was purified by column chromatography (Pentane:EtOAc, 9:1→6:4) in 88% yield as white foam.

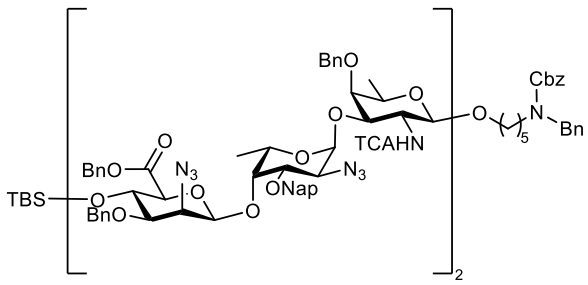
TLC analysis: R_f= 0.31 (Pentane:EtOAc; 7:3)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.90-7.72 (8H, H_{arom}, m), 7.56-7.08 (38H, 46 x H_{arom}, 2 x NH, m), 5.20-5.10 (2H, 2 x H_{CHH}, m), 5.00-4.73 (7H, 2 x H_{1L}, H_{1D}, 2 x H_{CHH}, m), 4.72-4.41 (15H, 2 x H_{1M}, H_{1D}, 6 x CH_2), 4.31-4.16 (3H, H_{3D}, H_{4M}, H_{2D}, m), 4.16-4.05 (2H, H_{4M}, H_{3D}, m), 4.05-3.88 (6H, 2 x H_{2M}, 2 x H_{2L}, H_{2D}, H_{5L}, m), 3.88-3.25 (19H, 2 x H_{3L}, H_{5L}, 2 x H_{4L}, 2 x H_{5D}, 2 x H_{4D}, 2 x $H_{CHHOLinker}$, 2 x H_{5M}, 2 x H_{3M}, 2 x CH_3, m), 3.25-3.09 (2H, 2 x $H_{CHHNLinker,}$ m), 1.58-1.39 (4H, 4 x $H_{CHHLinker}$, m), 1.39-1.00 (5H, 2 x $H_{CHHLinker}$, 2 x H_{6D}, 2 x H_{6L}, m), 0.81 (9H, H_{tBu}, s), 0.03 (3H, CH_3, s), -0.03 (3H, CH_3, s).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 168.8, 167.9, 167.7, 162.1, 161.8, 139.4, 138.7, 138.6, 138.5, 138.4, 138.0, 137.6, 137.4, 137.0, 136.0, 135.5, 133.4, 133.4, 133.3, 133.0, 133.0 (C_q), 128.9, 128.8, 128.7 x 2, 128.6, 128.5 x 2, 128.4 x 2, 128.3 x 2, 128.2 x 3, 128.1, 128.1, 128.0 x 5, 127.9, 127.8 x 2, 127.7, 127.6 x 3, 127.5 x 3, 127.3, 127.2, 126.6, 126.4, 126.3 x 2, 126.2, 126.2, 126.1, 126.0, 126.0 x 2, 125.9, 125.8, 125.7, 125.7, 124.9 (CH_{arom}), 100.9 (C_{1M}), 100.8 (C_{1D}), 100.7 (C_{1M}), 99.2 x 2 (C_{1L}), 99.0 (C_{1D}), 93.1 (C_q), 92.7 (C_q), 80.6 (C_{4D}), 80.3 x 2 (C_{3M}), 79.3 (C_{4D}), 78.6 (C_{4L}), 78.1 (C_{3D}), 77.8 (C_{4L}), 77.7 (C_{5M},), 77.5 (C_{5M}), 75.7, 75.6 (C_{5L}), 75.3 (CH_2), 75.2 (C_{4D}), 75.0 (CH_2), 73.8 (CH_2), 72.6 (CH_2), 72.0 (CH_2), 71.3 (CH_2), 70.6 x 2 (C_{5D}), 70.0 ($CH_{2OLinker}$), 68.7 x 2 (C_{4M}), 67.7 (CH_2), 62.6 (C_{2D}), 61.7 x 2 (C_{2L}, C_{2D}), 59.6 (C_{2L}) 56.3 x 2(C_{2M}), 52.6 x 2 (CH_{3OMe}), 50.7 (CH_2), 47.5, 46.7 (CH_{2NLink}), 29.7, 28.0 ($CH_{2Linker}$), 25.9 (CH_{3tBu}), 23.9 ($CH_{2Linker}$), 17.1 x 4 (CH_{3_6L}, CH_{3_6D}), -3.7, -5.3 (CH_{3_Me}).

HRMS: $C_{118}H_{135}Cl_3N_{15}O_{27}Si + NH_4^+$ required 2449.7891, found 2453.7888.

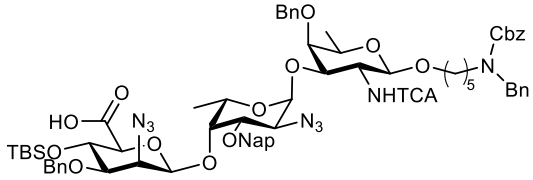
Fully protected hexasaccharide (8)



55 (0.18mmol) was dissolved in dry acetone (0.900 mL, 0.2 M) and under inert atmosphere $CsCO_3$ (0.19 mmol, 1.1 eq) was added. The reaction mixture was cooled to 0°C and $CF_3CN(Ph)Cl$ (0.23 mmol, 1.2 eq) was added. After 1 hour, TLC analysis (Pentane:EtOAc, 7:3) showed complete consumption of starting material. The reaction was diluted in acetone, filtered over Celite® and concentrated *in vacuo*. Acceptor **60** (0.23 mmol, 1.3 eq) was added and the two compounds were coevaporated three times with toluene. The resulting mixture was dissolved in dry DCM (1.8 mL, 0.1 M) and 3Å MS were added. After 10 minutes the reaction was cooled to -78°C and a freshly prepared 0.6 M solution of TBSOTf in dry DCM (0.036 mmol, 0.02 eq) was added. The reaction mixture was allowed to warm up and after 45 min at -50 °C, TLC analysis (Pentane:EtOAc, 7:3) showed complete consumption of donor. The reaction mixture was diluted with DCM, washed with H_2O and brine, dried

over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound was purified by column chromatography (Pentane:EtOAc, 9:1→6:4) in 96% yield as white foam.
TLC analysis: R_f= 0.33 (Pentane:EtOAc; 65:35)
^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.89-7.75 (8H, H_{arom}, m), 7.51-6.98 (48H, 46 x H_{arom}, 2 x NH, m), 5.21-5.09 (2H, 2 x $HCHH$, m), 4.96-4.51 (19H, 2 x H_{1L}, H_{1D}, 14 x $HCHH$, H_{1M}, m), 4.51-4.10 (11H, H_{1D}, 6 x $HCHH$, 2 x H_{3D}, 2 x H_{4M}, m), 4.01-3.90 (3H, 2 x H_{2M}, H_{4D}, m), 3.90-3.63 (9H, 2 x H_{2L}, 2 x H_{2D}, 2 x H_{5L}, 2 x H_{5M}, $HCHHOLinker$, m), 3.25-3.09 (3H, 2 x $HCHHNLinker$, H_{5D}, m), 1.58-1.39 (4H, 4 x $HCHHLinker$, m), 1.39-1.00 (5H, 2 x $HCHHLinker$, 2 x H_{6D}, 2 x H_{6L}, m), 0.81 (9H, H_{tBu}, s), 0.03 (3H, CH_3, s), -0.03 (3H, CH_3, s).
^{13}C-NMR (400 MHz, $CDCl_3$), δ: 168.0, 167.4, 167.2, 162.1, 161.9, 138.7, 138.6, 138.3, 138.0, 137.5, 137.4, 137.0, 135.5, 135.3, 134.9, 134.8, 133.4, 133.1 x 2 (C_q), 129.3, 129.1, 128.9, 128.8, 128.7 x 2, 128.6 x 3, 128.5 x 3, 128.4 x 2, 128.2 x 2, 128.1 x 3, 128.0, 127.9, 127.8 x 2, 127.7 x 2, 127.6 x 4, 127.5 x 2, 127.4, 127.2 x 2, 126.7 x 2, 126.6, 126.2, 126.1 x 3, 126.0 x 2, 125.9 2 (CH_{arom}), 101.0 (C_{1M}), 100.7 (C_{1M}), 99.9 (C_{1D}), 99.3 x 2 (C_{1L}), 99.1 (C_{1D}), 93.2 (C_q), 92.7 (C_q), 80.4 x 2 (C_{3M}), 79.3 (C_{4D}), 78.3 (C_{4L}), 78.2 (C_{3D}), 77.8 (C_{4L}), 77.5 (C_{5M},), 75.4 x 3 (2 x C_{5L}, C_{5M}), 75.2 (CH_2), 75.1 (C_{4D}), 74.9 (CH_2), 73.8 (CH_2), 72.1 (CH_2), 71.2 (CH_2), 71.0 (CH_2), 70.7 x 2 (C_{5D}), 69.8 ($CH_{2OLinker}$), 68.6 x 2 (C_{4M}), 67.4 (CH_2), 67.2 (CH_2), 63.0 (C_{2M}), 61.1 x 2 (C_{2L}), 59.7 (C_{2M}), 59.3 (C_{2D}), 56.2 (C_{2D}), 50.6 (CH_2), 47.4, 46.5 (CH_{2NLink}), 29.7, 28.0 ($CH_{2Linker}$), 25.9 (CH_{3tBu}), 23.9 ($CH_{2Linker}$), 17.1 x 4 (CH_{3_6L}, CH_{3_6D}), -3.7, -5.3 (CH_{3_Me}).
HRMS: $C_{118}H_{135}Cl_3N_{15}O_{27}Si + NH_4^+$ required 2601.8517, found 2605.8520

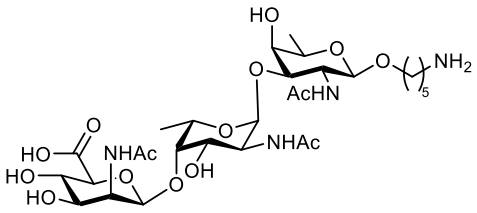
5-(benzyl(benzyloxicarbonyl)amino)pentyl 2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-(2-naphthylmethyl)-4-*O*-(2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-β-D-mannopyranosiduronic)-α-L-fucopyranosyl)-4-*O*-benzyl-β-D-fucopyranoside (61)



Compound **5** (0.013 mmol) was dissolved in THF. After cooling to 0°C, 50 µL of a solution containing H_2O_2 (0.195 mmol, 15 eq) and TBAOH (40%w in H_2O, 0.065 mmol, 5 eq) were slowly added. The temperature was raised at 40°C and after 1 hour TLC analysis (Pentane:EtOAc, 7:3) showed complete consumption of starting material. The solution was acidified with 1M HCl (pH ≈ 3), diluted with DCM and washed one with water. The aqueous layer was reextracted 5 times with DCM, the organic phase dried, filtered and concentrated *in vacuo*. The desired product was isolated by column chromatography (DCM:MeOH, 99:1→97:3→95:5) in quantitative yield.
TLC analysis: R_f= 0.34 (DCM:MeOH; 9:1 + 1%AcOH)
^{1}H-NMR (500 MHz, $CDCl_3$) δ: 4.94 (1H, H_{1L}, J_{1-2}=3.9 Hz, d), 4.90 (1H, H_{1M}, J_{1-2}=1.6 Hz, d), 4.64 (1H, H_{2M}, J_{2-3}=4.3 Hz, dd), 4.40 (1H, H_{1D}, J_{1-2}=8.4 Hz, d), 4.16 (1H, H_{5D}, J_{5-6}=8.3 Hz, d), 4.38 (1H, H_{2L}, dd), 4.22 (1H, H_{4L}, d), 4.19 (1H, H_{5L}, J_{5-6}=6.6 Hz, q), 4.01-3.96 (1H, H_{2D}, m), 3.91-3.86 (1H, H $CHHOlinker$, m), 3.82-3.75 (4H, H_{4D}, H_{5L}, H_{3D}, H_{3M}, m), 3.65 (1H, H_{4M}, J_{4-3}=J_{4-5}=9.7 Hz, t), 3.61-3.55 (2H, H_{5M}, H $CHHOlinker$, m), 3.03-2.96 (2H, $HCHHNlinker$, m), 2.14 (3H, CH_{3_NHAcM}, s), 2.08 (3H, CH_{3_OAc}, s), 2.01 (3H, CH_{3_NHAcL}, s), 1.99 (3H, CH_{3_NHAcD}, s), 1.71- 1.64

(2H, $H_{CH2Linker}$, m), 1.63-1.55 (2H, $H_{CH2Linker}$, m), 1.43-1.33 (2H, $H_{CH2Linker}$, m), 1.28 (3H, H_{6D}, $J_{6\text{-}5}$=6.5 Hz, d), 1.25 (3H, H_{6L}, d).
^{13}C NMR (214 MHz, $CDCl_3$) δ: 176.5, 176.3, 175.2, 175.0, 174.7 (C_q), 102.4 (C_{1D}), 100.7 (C_{1M}), 99.9 (C_{1L}), 79.4 (C_{5M}), 78.0 (C_{4D}), 76.9 (C_{4L}), 72.5, 71.6, 71.3 (C_{5D}, C_{3D}, C_{3M}), 71.0 ($CH_{2OLinker}$), 70.7 (C_{3L}), 70.4 (C_{4M}), 67.7 (C_{4L}), 53.9 (C_{2M}), 52.2 (C_{2D}), 48.0 (C_{2L}), 40.2 ($CH_{2NLinker}$), 29.1, 27.3 ($CH_{2Linker}$), 23.1 (CH_{3NAcD}), 23.0 ($CH_{2Linker}$), 22.9 (CH_{3NAcL}, CH_{3NAcM}), 21.2 (CH_{3OAc}), 16.3 (CH_{3_6D}), 16.2 (CH_{3_6L}).
HRMS: $C_{118}H_{135}Cl_3N_{15}O_{27}Si + Na^+$ required 1431.4705, found 1431.4705

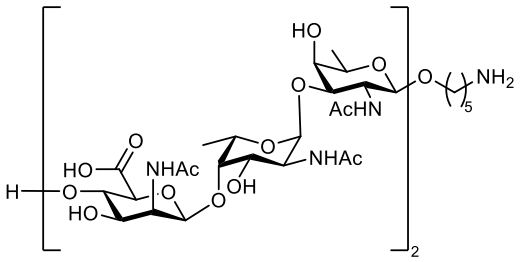
5-amino-pentyl 2-acetylamido-2-deoxy-3-*O*-(2-acetylamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-β-D-mannopyranosiduronyl)-α-L-fucopyranosyl)-β-D-fucopyranoside (1)



Compound **6** (0.025 mmol) was dissolved in THF (0.25 mL, 0.1M) and under inert atmosphere AcOH (1.75 mmol, 70 eq), Ac_2O (2.11 mmol, 84 eq) and Zn dust (1,22 mmol, 50 eq) were added. The reaction mixture was heated at 50°C and left stirring overnight. After TLC analysis (DCM:MeOH, 95:5) showed complete consumption of the starting material, the reaction was diluted with DCM, filtered and concentrated *in vacuo*. The desired product was isolated after column chromatography (DCM:MeOH, 99:1→97:3→95:5→9:1) in 67% yield as white solid. Intermediate **63** was dissolved in dry THF (1mL, 0.01M) and under inert atmosphere TEA*HF (0.25 mmol, 10 eq) was added. The reaction was stirred overnight at 50°C, after which TLC analysis (DCM:MeOH, 9:1) showed complete consumption of starting material. The reaction was diluted in EtOAc and washed with a saturated aq. solution of $NaHCO_3$ (x3), water and brine. The aqueous phases were reextracted and the combined organic phases dried over $MgSO_4$, filtered and concentrated *in vacuo*. The removal of the TBS group was confirmed by ^{1}H-NMR. The resulting crude was dissolved in a mixture of *t*Bu:H_2O (4:1, 0.01M) and one drop of AcOH was added. After purging Ar for 30 min, $Pd(OH)_2$ (20 weight% on carbon, 50% water) was added and the mixture was purged with Ar for 15 min, followed by H_2 (30s). The reaction was stirred under H_2 for three days. After filtration over Celite® and concentration *in vacuo*, the product was purified by size exclusion chromatography (HW40, 10 mM NH_4OAc in water). After repeated lyophilization to remove NH_4OAc, the final trisaccharide was isolated in 96% yield.
^{1}H-NMR (500 MHz, D_2O) δ: 4.94 (1H, H_{1L}, $J_{1\text{-}2}$=3.9 Hz, d), 4.90 (1H, H_{1M}, $J_{1\text{-}2}$=1.6 Hz, d), 4.64 (1H, H_{2M}, $J_{2\text{-}3}$=4.3 Hz, dd), 4.40 (1H, H_{1D}, $J_{1\text{-}2}$=8.4 Hz, d), 4.16 (1H, H_{5D}, $J_{5\text{-}6}$=8.3 Hz, d), 4.38 (1H, H_{2L}, dd), 4.22 (1H, H_{4L}, d), 4.19 (1H, H_{5L}, $J_{5\text{-}6}$=6.6 Hz, q), 4.01-3.96 (1H, H_{2D}, m), 3.91-3.86 (1H, H *CH*OlinkerH, m), 3.82-3.75 (4H, H_{4D}, H_{5L}, H_{3D}, H_{3M}, m), 3.65 (1H, H_{4M}, $J_{4\text{-}3}$=$J_{4\text{-}5}$=9.7 Hz, t), 3.61-3.55 (2H, H_{5M}, H *CH*OlinkerH, m), 3.03-2.96 (2H, H*CH*HNlinkerH, m), 2.14 (3H, CH_{3_NHAcM}, s), 2.08 (3H, CH_{3_OAc}, s), 2.01 (3H, CH_{3_NHAcL}, s), 1.99 (3H, CH_{3_NHAcD}, s), 1.71- 1.64 (2H, $H_{CH2Linker}$, m), 1.63-1.55 (2H, $H_{CH2Linker}$, m), 1.43-1.33 (2H, $H_{CH2Linker}$, m), 1.28 (3H, H_{6D}, $J_{6\text{-}5}$=6.5 Hz, d), 1.25 (3H, H_{6L}, d).

^{13}C NMR (214 MHz, D_2O) δ: 176.5, 176.3, 175.2, 175.0, 174.7 (C_q), 102.4 (C_{1D}), 100.7 (C_{1M}), 99.9 (C_{1L}), 79.4 (C_{5M}), 78.0 (C_{4D}), 76.9 (C_{4L}), 72.5, 71.6, 71.3 (C_{5D}, C_{3D}, C_{3M}), 71.0 ($CH_{2OLinker}$), 70.7 (C_{3L}), 70.4 (C_{4M}), 67.7 (C_{4L}), 53.9 (C_{2M}), 52.2 (C_{2D}), 48.0 (C_{2L}), 40.2 ($CH_{2NLinker}$), 29.1, 27.3 ($CH_{2Linker}$), 23.1 (CH_{3NAcD}), 23.0 ($CH_{2Linker}$), 22.9 (CH_{3NAcL}, CH_{3NAcM}), 21.2 (CH_{3OAc}), 16.3 (CH_{3_6D}), 16.2 (CH_{3_6L}).
HRMS: $C_{28}H_{48}N_4O_{15}$ + Na^+ required 703.3008, found 703.3008

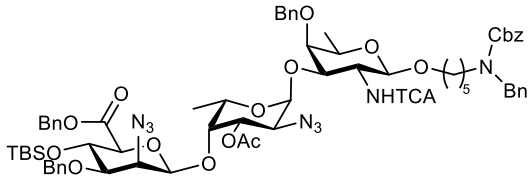
Hexasaccharide without 3-*O*-acetyl (3)



Compound **8** (0.014 mmol) was dissolved in THF (0.60 mL, 0.03M) and under inert atmosphere AcOH (0.98 mmol, 70 eq), Ac_2O (1.18 mmol, 84 eq) and Zn dust (0.70 mmol, 50 eq) were added. The reaction mixture was heated at 50°C and left stirring overnight. After TLC analysis (DCM:MeOH, 95:5) showed complete consumption of the starting material, the reaction was diluted with DCM, filtered and concentrated *in vacuo*. The desired product was isolated after column chromatography (DCM:MeOH, 99:1→97:3→95:5→9:1) in 62% yield as white solid. Intermediate **64** was dissolved in dry THF (0.860 mL, 0.01M) and under inert atmosphere TEA*HF (0.086 mmol, 10 eq) was added. The reaction was stirred overnight at 50°C, after which TLC analysis (DCM:MeOH, 9:1) showed complete consumption of starting material. The reaction was diluted in EtOAc and washed with a saturated aq. solution of $NaHCO_3$ (x3), water and brine. The aqueous phases were reextracted and the combined organic phases dried over $MgSO_4$, filtered and concentrated *in vacuo*. The removal of the TBS group was confirmed by ^{1}H-NMR. The resulting crude was dissolved in a mixture of *t*Bu:H_2O (4:1, 0.01M) and one drop of AcOH was added. After purging Ar for 30 min, $Pd(OH)_2$ (20 weight% on carbon, 50% water) was added and the mixture was purged with Ar for 15 min, followed by H_2 (30s). The reaction was stirred under H_2 for three days. After filtration over Celite® and concentration *in vacuo*, the product was purified by size exclusion chromatography (HW40, 10 mM NH_4OAc in water). After repeated lyophilization to remove NH_4OAc, the final trisaccharide was isolated in 55% yield.
^{1}H-NMR (500 MHz, D_2O) δ: 4.93-4.88 (4H, 2 x H_{1L}, 2 x H_{1M}, m), 4.64 (1H, H_{2MB}, s), 4.59 (1H, H_{2MA}, s), 4.40-4.32 (4H, 2 x H_{1D}, 2x H_{2L}, m), 4.22-4.18 (4H, 2 x H_{4L}, 2 x H_{5L}, d), 4.16-3.96 (4H, H_{5D}, 2 x H_{2D}, m), 3.91-3.86 (1H, H *CH*HOlinker, m), 3.82-3.61 (8H, 2 x H_{4D}, 2 x H_{5L}, 2 x H_{3D}, 2 x H_{3M}, m), 3.65-3.52 (5H, 2 x H_{4M}, 2 x H_{5M}, H *CH*HOlinker, m), 3.03-2.96 (2H, H*CH*HNlinker, m), 2.14-1.96 (18H, 2 x CH_{3_NHAcM}, 2 x CH_{3_OAc}, 2 x CH_{3_NHAcL}, 2 x CH_{3_NHAcD}, m), 1.73- 1.65 (2H, $H_{CH2Linker}$, m), 1.62-1.57 (2H, $H_{CH2Linker}$, m), 1.44-1.33 (2H, $H_{CH2Linker}$, m), 1.29 (3H, H_{6D}, J_{6-5}=6.5 Hz, d), 1.27 (3H, H_{6L}, d).
^{13}C NMR (214 MHz, D_2O) δ: 176.5, 176.2, 175.2, 175.1, 174.9, 173.8, 173.5 (C_q), 102.4 (2 x C_{1D}), 100.5 (2 x C_{1M}), 99.3 (2 x C_{1L}), 79.4 (2 x C_{5M}), 77.9 (2 x C_{4D}), 76.9 (2 x C_{4L}), 72.5, 71.6, 71.3 (2 x C_{5D}, 2 x C_{3D}, 2 x C_{3M}), 71.0 ($CH_{2OLinker}$), 70.5 (2 x C_{3L}), 70.2 (2 x C_{4M}), 67.8 (2 x C_{4L}), 53.9 (2 x C_{2M}), 52.2 (2 x C_{2D}), 48.0 (2 x C_{2L}), 41.1 (2 x $CH_{2NLinker}$), 29.7, 27.2 (2 x $CH_{2Linker}$),

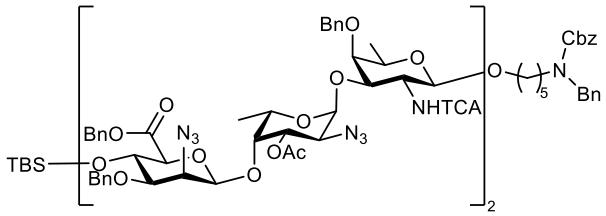
23.1 (2 x CH_{3NAcD}), 23.0 ($CH_{2Linker}$, 2 x CH_{3NAcL}, 2 x CH_{3NAcM}), 21.1 (CH_{3OAc}), 17.1 (CH_{3_6D}), 15.9 (CH_{3_6L}).
HRMS: $C_{53}H_{87}N_7O_{29}$ + 2xH^+ required 643.7847, found 643.7847

5-(benzyl(benzyloxicarbonyl)amino)pentyl 2-trichloroacetylamido-2-deoxy-3-*O*-(2-azido-2-deoxy-3-*O*-acetyl-4-*O*-(benzyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(*tert*butyldimethyl)silyl-β-D-mannopyranosiduronate)-α-L-fucopyranosyl)-4-*O*-benzyl-β-D-fucopyranoside (65)



6 (0.021 mmol) was dissolved in a mixture of DCM/H_2O (8:2, 0.1M) and DDQ (0.053 mmol, 2.5 eq) was added. The reaction mixture was stirred at room temperature for 2 hours, after which TLC analysis (Pent:EtOAc, 7:3) showed complete consumption of starting material. The reaction was diluted with DCM and washed with a 10% aq. solution of $Na_2S_2O_3$, a saturated aq. solution of $NaHCO_3$, H_2O and brine subsequently. The organic phase was dried over $MgSO_4$ and concentrated in vacuo. The crude mixture was then dissolved in a mixture of DCM/Pyridine (1:1, 0.05M) and acetic anhydride (0.196 mmol, 10 eq) was added. The reaction mixture was stirred overnight, after which TLC analysis (DCM:Acetone, 96:4) showed complete consumption of starting material. The reaction mixture was diluted with DCM and washed with HCl 1M solution, saturated $NaHCO_3$ and water. After reextraction of the aqueous layers, the combined organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo*. After purification by column chromatography (Pent:EtOAc, 8:2→7:3→6:4), compound **65** was obtained in 98% yield as white solid over the two steps.
TLC analysis: R_f= 0.33 (Pentane:EtOAc; 7:3)
^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.41-7.08 (21H, H_{arom}, NH_{TCA}, m), 5.26-5.04 (4H, 4 x H_{CHHBn}, m), 5.00-4.91 (2H, H_{1L}, H_{3L}, m), 5.00-4.92 (2H, H_{1L}, H_{3L}, m), 4.90-4.83 (1H, H_{1D}, m), 4.80 (1H, H_{CHHBn}, J_{1-2}=11.8 Hz, d),4.74 (1H, H_{CHHBn}, d), 4.64-4.54 (2H, 2 x H_{CHHBn}, m), 4.52-4.40 (3H, H_{CHHBn}, H_{1M}, m), 4.37-4.28 (1H, H_3, m), 4.14 (1H, H_{4M}, J_{4M-5M}= J_{4M-3M}=9.1 Hz, pt), 4.03 (1H, H_{4L}, J_{4-3}=3.3 Hz, d), 3.94-3.73 (5H, H_{2L}, H_{2M}, H_{2D}, $H_{CHHOlinker}$, H_{5L}, m), 3.67 (1H, H_{5M}, d), 3.62 (1H, H_{5D}, J=6.5 Hz, q), 3.56 (1H, H_{4D}, J_{4-3}=2.8 Hz, d), 3.45-3.29 (2H, $H_{CHHOlinker}$, H_{3D}, m),3.27-3.10 (2H, $H_{CHHNlinker}$, m), 1.97 (3H, CH_{3_OAc}, s), 1.60-1.40 (4H, $H_{CH2Linker}$, m), 1.33-1.24 (5H, $H_{CH2Linker}$, H_{6D}, m), 1.00 (3H, 3 x H_{6L}, d), 0.78 (9H, H_{tBu}, s), 0.02 (3H, H_{CH3_Me}, s), -0.01 (3H, H_{CH3_Me}).
^{13}C-NMR (400 MHz, $CDCl_3$), δ: 170.7 (C_q), 167.8 (C_q), 162.1 (C_q), 138.3 (C_q), 138.0(C_q), 137.3 (C_q), 134.8 (2 x C_q), 129.2, 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.5, 127.3 (CH_{arom}), 101.2 (C_{1M}), 99.1 (C_{1D}), 99.04 (C_{1D}), 92.6 (C_q), 80.2 (C_{3M}), 78.6 (C_{4D}), 78.1 (C_{5M}), 77.23 (C_{3D}), 75.5 (C_{4L}), 75.1 (CH_{2Bn}), 72.1 (CH_{2Bn}), 70.8 (C_{5D}), 70.3 (C_{3L}), 70.0 ($CH_{2OLinker}$), 68.6 (C_{4M}), 67.8 (CH_{2Bn}), 67.7 (CH_{2Bn}), 66.3 (C_{5L}), 60.5 (C_{2M}), 57.7 (C_{2L}), 57.3 (C_{2D}), 50.6 (CH_{2Bn}), 47.8, 46.7 ($CH_{2NLinker}$), 29.8, 29.5, 29.3 ($CH_{2Linker}$), 25.9 (CH_{3tBu}), 23.5 ($CH_{2Linker}$),20.9 (CH_{3OAc}), 17.7 (CH_{3_6D}), 17.3 (CH_{3_6L}), -3.8, -5.2 (CH_{3_Me}).
HRMS: $C_{68}H_{83}Cl_3N_8O_{16}Si$ + Na^+ required 1425.4625, found 1425.4648

Fully protected (3-*O*-acetyl) hexasaccharide (66)



8 (0.017 mmol) was dissolved in a mixture of DCM/H_2O (8:2, 0.1M) and DDQ (0.088 mmol, 5 eq) was added. The reaction mixture was stirred at room temperature for 2 hours, after which TLC analysis (Pent:EtOAc, 7:3) showed complete consumption of starting material. The reaction was diluted with DCM and washed with a 10% aq. solution of $Na_2S_2O_3$, a saturated aq. solution of $NaHCO_3$, H_2O and brine subsequently. The organic phase was dried over $MgSO_4$ and concentrated in vacuo. The crude mixture was then dissolved in a mixture of DCM/Pyridine (1:1, 0.05M) and acetic anhydride (0.196 mmol, 10 eq) was added. The reaction mixture was stirred overnight, after which TLC analysis (DCM:Acetone, 96:4) showed complete consumption of starting material. The reaction mixture was diluted with DCM and washed with HCl 1M solution, saturated $NaHCO_3$ and water. After reextraction of the aqueous layers, the combined organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo*. After purification by column chromatography (Pent:EtOAc, 8:2→7:3→6:4), compound **66** was obtained in 88% yield as white solid over the two steps.

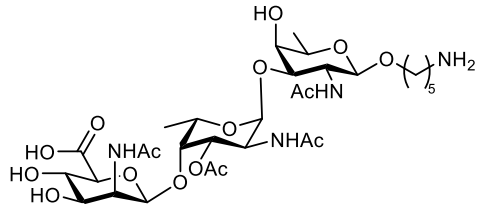
TLC analysis: R_f= 0.33 (Pentane:EtOAc; 7:3)

^{1}H-NMR (500 MHz, $CDCl_3$), δ: 7.46-7.11 (42H, H_{arom}, NH_{TCA}, m), 5.26-5.04 (4H, 4 x H_{CHHBn}, m), 5.00-4.42 (16H, 2 x H_{1L}, 2 x H_{3L}, 2 x H_{1D}, 8 x H_{CHHBn}, 2 x H_{1M}, m), 4.36-4.11 (1H,2 x H_3, 2 x H_{4M}, m), 4.03-3.31 (1H, 2 x H_{4L}, 2 x H_{2L}, 2 x H_{2M}, 2 x H_{2D}, 2 x $H_{CHHOlinker}$, 2 x H_{5L}, 2 x H_{5M}, 2 x H_{5D}, 2 x H_{4D}, 2 x H_{3D}, m), 3.27-3.10 (2H, $H_{CHHNlinker}$, m), 2.01 (3H, CH_{3_OAc}, s), 1.97 (3H, CH_{3_OAc}, s), 1.60-1.40 (4H, $H_{CH2Linker}$, m), 1.33-1.24 (5H, $H_{CH2Linker}$, 2 x CH_{3_6D}, m), 1.00 (3H, 2 x CH_{3_6L}, d), 0.78 (9H, H_{tBu}, s), 0.01 (3H, H_{CH3_Me}, s), -0.02 (3H, H_{CH3_Me}).

^{13}C-NMR (400 MHz, $CDCl_3$), δ: 170.7, 169.6, 167.7, 162.1, 138.3, 138.0, 137.9, 137.3, 134.8 (C_q), 129.2, 129.0, 128.8, 128.7, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, (CH_{arom}), 101.1 (2 x C_{1M}), 98.9 (2 x C_{1D}), 99.04 (2 x C_{1D}), 79.8 (2 x C_{3M}), 78.6 (2 x C_{4D}), 78.2 (2 x C_{5M}), 77.3 (2 x C_{3D}), 75.5 (2 x C_{4L}), 75.3 (CH_{2Bn}), 72.4 (CH_{2Bn}), 70.4 (2 x C_{5D}), 70.3 (2 x C_{3L}), 69.7 ($CH_{2OLinker}$), 68.7 (C_{4M}), 67.8 (CH_{2Bn}), 67.5 (CH_{2Bn}), 66.3 (2 x C_{5L}), 60.5 (2 x C_{2M}), 58.2 (2 x C_{2L}), 57.4 (2 x C_{2D}), 51.0 (CH_{2Bn}), 47.8, 46.7 ($CH_{2NLinker}$), 29.8, 29.5, 29.3 ($CH_{2Linker}$), 25.9 (CH_{3tBu}), 23.5 ($CH_{2Linker}$), 20.9 (2 x CH_{3OAc}), 17.5 (2 x CH_{3_6D}), 17.2 (2 x CH_{3_6L}), -3.8, -5.2 (CH_{3_Me}).

HRMS: $C_{68}H_{83}Cl_3N_8O_{16}Si + Na^+$ required 2412.7001, found 2412.7094

5-amino-pentyl 2-acetylamido-2-deoxy-3-*O*-(2-acetylamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-β-D-mannopyranosiduronyl)-α-L-fucopyranosyl)-β-D-fucopyranoside (2)



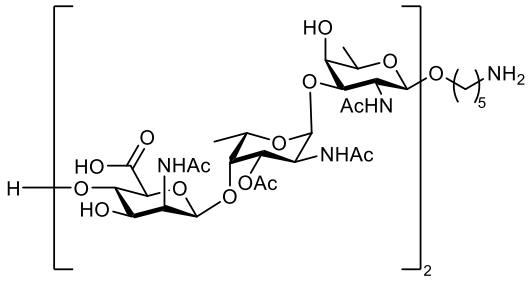
Compound **65** (0.023 mmol) was dissolved in THF (0.23 mL, 0.1M) and under inert atmosphere AcOH (1.6 mmol, 70 eq), Ac_2O (1.93 mmol, 84 eq) and Zn dust (1.15 mmol, 50 eq) were added. The reaction mixture was heated at 50°C and left stirring overnight. After TLC analysis (DCM:MeOH, 95:5) showed complete consumption of the starting material, the reaction was diluted with DCM, filtered and concentrated *in vacuo*. A column chromatography (DCM:MeOH, 99:1→97:3→95:5→9:1) was performed to remove minor impurities. Intermediate **67** was dissolved in dry THF (1mL, 0.01M) and under inert atmosphere TEA*HF (0.25 mmol, 10 eq) was added. The reaction was stirred overnight at 50°C, after which TLC analysis (DCM:MeOH, 9:1) showed complete consumption of starting material. The reaction was diluted in EtOAc and washed with a saturated solution of $NaHCO_3$ (x3), water and brine. The aqueous phases were reextracted and the combined organic phases dried over $MgSO_4$, filtered and concentrated *in vacuo*. The removal of the TBS group was confirmed by ^{1}H-NMR. The resulting crude was dissolved in a mixture of *t*Bu:H_2O (4:1, 0.01M) and one drop of AcOH was added. After purging Ar for 30 min, $Pd(OH)_2$ (20 weight% on carbon, 50% water) was added and the mixture was purged with Ar for 15 min, followed by H_2 (30s). The reaction was stirred under H_2 for three days. After filtration over Celite® and concentration *in vacuo*, the product was purified by size exclusion chromatography (HW40, 10 mM NH_4OAc in water). After repeated lyophilization to remove NH_4OAc, the final trisaccharide was isolated in 88% yield.

^{1}H-NMR (850 MHz, D_2O) δ: 5.03 (1H, H_{3L}, $J_{3\text{-}2}$=11.6 Hz, $J_{3\text{-}4}$=3.0 Hz, dd), 5.01 (1H, H_{1L}, $J_{1\text{-}2}$=4.0 Hz, d), 4.74 (1H, H_{1M}, m), 4.59 (1H, H_{2M}, $J_{2\text{-}3}$=4.3 Hz, d), 4.41 (1H, H_{1D}, $J_{1\text{-}2}$=8.3 Hz, d), 4.38 (1H, H_{2L}, dd), 4.22 (1H, H_{4L}, d), 4.19 (1H, H_{5L}, $J_{5\text{-}6}$=6.6 Hz, q), 4.01-3.96 (1H, H_{2D}, m), 3.91-3.86 (1H, H *CH*HOlinker, m), 3.82-3.75 (4H, H_{4D}, H_{5L}, H_{3D}, H_{3M}, m), 3.65 (1H, H_{4M}, $J_{4\text{-}3}$=$J_{4\text{-}5}$=9.7 Hz, t), 3.61-3.55 (2H, H_{5M}, H *CH*HOlinker, m), 3.03-2.96 (2H, H*CH*HNlinker, m), 2.14 (3H, CH_{3_NHAcM}, s), 2.08 (3H, CH_{3_OAc}, s), 2.01 (3H, CH_{3_NHAcL}, s), 1.99 (3H, CH_{3_NHAcD}, s), 1.71- 1.64 (2H, $H_{CH2Linker}$, m), 1.63-1.55 (2H, $H_{CH2Linker}$, m), 1.43-1.33 (2H, $H_{CH2Linker}$, m), 1.28 (3H, H_{6D}, $J_{6\text{-}5}$=6.5 Hz, d), 1.25 (3H, H_{6L}, d).

^{13}C NMR (214 MHz, D_2O) δ: 176.5, 176.3, 175.2, 175.0, 174.7 (C_q), 102.4 (C_{1D}), 100.7 (C_{1M}), 99.9 (C_{1L}), 79.4 (C_{5M}), 78.0 (C_{4D}), 76.9 (C_{4L}), 72.5, 71.6, 71.3 (C_{5D}, C_{3D}, C_{3M}), 71.0 ($CH_{2OLinker}$), 70.7 (C_{3L}), 70.4 (C_{4M}), 67.7 (C_{4L}), 53.9 (C_{2M}), 52.2 (C_{2D}), 48.0 (C_{2L}), 40.2 ($CH_{2NLinker}$), 29.1, 27.3 ($CH_{2Linker}$), 23.1 (CH_{3NAcD}), 23.0 ($CH_{2Linker}$), 22.9 (CH_{3NAcL}, CH_{3NAcM}), 21.2 (CH_{3OAc}), 16.3 (CH_{3_6D}), 16.2 (CH_{3_6L}).

HRMS: $C_{30}H_{50}N_4O_{16}$ + H^+ required 723.3295, found 723.3295

3-O-acetyl Hexasaccharide (4)



Compound **66** (0.012 mmol) was dissolved in THF (0.12 mL, 0.1M) and under inert atmosphere AcOH (0.84 mmol, 70 eq), Ac_2O (1.00 mmol, 84 eq) and Zn dust (0.6 mmol, 50 eq) were added. The reaction mixture was heated at 50°C and left stirring overnight. After TLC analysis (DCM:MeOH, 95:5) showed complete consumption of the starting material, the reaction was diluted with DCM, filtered and concentrated *in vacuo*. A column chromatography (DCM:MeOH, 99:1→97:3→95:5→9:1) was performed to remove minor impurities. Intermediate **68** was dissolved in dry THF (1mL, 0.01M) and under inert atmosphere TEA*HF (0.12 mmol, 10 eq) was added. The reaction was stirred overnight at 50°C, after which TLC analysis (DCM:MeOH, 9:1) showed complete consumption of starting material. The reaction was diluted in EtOAc and washed with a saturated solution of $NaHCO_3$ (x3), water and brine. The aqueous phases were reextracted and the combined organic phases dried over $MgSO_4$, filtered and concentrated *in vacuo*. The removal of the TBS group was confirmed by ^{1}H-NMR. The resulting crude was dissolved in a mixture of *t*Bu:H_2O (4:1, 0.01M) and one drop of AcOH was added. After purging Ar for 30 min, $Pd(OH)_2$ (20 weight% on carbon, 50% water) was added and the mixture was purged with Ar for 15 min, followed by H_2 (30s). The reaction was stirred under H_2 for three days. After filtration over Celite® and concentration *in vacuo*, the product was purified by size exclusion chromatography (HW40, 10 mM NH_4OAc in water). After repeated lyophilization to remove NH_4OAc, the final trisaccharide was isolated in 62% yield.

^{1}H-NMR (850 MHz, D_2O) δ: 4.94-4.90 (4H, 2 x H_{3L}, 2 x H_{1L}, m), 4.70-4.63 (2H, 2 x H_{1M}, n.d.), 4.57 (1H, H_{2MB}, s), 4.52 (1H, H_{2MA}, s), 4.34-4.23 (4H, 2 x H_{1D}, 2 x H_{2L}, m), 4.15-4.06 (4H, 2 x H_{4L}, 2 x H_{5L}, m), 3.91-3.85 (2H, 2 x H_{2D}, m), 3.83-3.77 (2H, H $_{CH}$Olinker, H_{3MB}, m), 3.77-3.60 (9H, 2 x H_{4D}, 2 x H_{5D}, 2 x H_{3D}, H_{3MA}, 2 x H_{4M}, m), 3.59-3.55 (2H, 2 x H_{5M}, m), 3.51-3.46 (1H, H $_{CH}$Olinker, m), 2.93-2.88 (2H, H_{CH}HNlinker, m), 2.06-1.84 (24H, 2 x CH_{3_NHAcM}, 2 x CH_{3_OAc}, 2 x CH_{3_NHAcL}, 2 x CH_{3_NHAcD}, m), 1.71- 1.64 (2H, $H_{CH2Linker}$, m), 1.63-1.55 (2H, $H_{CH2Linker}$, m), 1.43-1.33 (2H, $H_{CH2Linker}$, m), 1.23-1.10 (12H, 2 x H_{6D}, 2 x H_{6L}, m).

^{13}C NMR (214 MHz, D_2O) δ: 175.6, 175.5, 174.3, 174.2, 174.1, 173.7, 173.6 (C_q), 101.5 (2 x C_{1D}), 99.9 (2 x C_{1M}), 99.0 (2 x C_{1L}), 79.0 (2 x C_{4M}), 77.2 (2 x C_{5M}), 76.1 (2 x C_{4L}), 71.7 (2 x C_{4D}) 70.8 (2 x C_{5D}, 2 x C_{3D}), 70.3 (2 x C_{3M}), 70.1 ($CH_{2OLinker}$), 69.1 (2 x C_{3L}), 66.7 (2 x C_{5L}), 52.9 (2 x C_{2MA}), 51.9 (2 x C_{2MB}), 51.3 (2 x C_{2D}), 47.1 (2 x C_{2L}), 39.5 ($CH_{2NLinker}$), 28.2, 26.6, 22.0 ($CH_{2Linker}$), 21.9 (6 x CH_{3NAc}), 20.3 (CH_{3OAc}), 15.4-15.2 (CH_{3_6D}, CH_{3_6L}).

HRMS: $C_{57}H_{91}N_7O_{31}$ + 2xH^+ required 685.7963, found 685.7953

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