Synthesis and application of glycans unique to S. mansoni

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*Synthesis and application of Schistosoma mansoni α-(1-2)-fucosyl chains**

Introduction

It has been shown that *Schistosoma mansoni* expresses a complex array of glycans that can be targeted by both the adaptive and the innate part of the immune system.[1, 2] A large subgroup of these glycans is decorated with α-(1-2) oligofucosides.[3]–[5] It has been proven that these multi-fucosylated fragments are prime targets for generated antibodies.[6, 7] The α-(1-2)-oligofucosides are attached to a backbone of β-(1-4) linked galactosamines and/or glucosamines in the native glycans.[2, 4] Until now it is unclear whether these backbones are required for antibodies to recognize the α-(1→2) oligofucosides.To probe antibody recognition well-defined α-(1-2)-oligofucosides would be valuable tools. These would complement the set of trisaccharides described in Chapter 2 (Figure 1B). Since these α-(1-2)-oligofucosides cannot be obtained in sufficient purity from biological sources, they will have to be synthesized chemically. This chapter explores the chemistry to synthesize α-(1-2)-oligofucosides **1**-**4** (Figure 1A) in an efficient manner, followed by investigating their recognition by antibodies.

*Harvey, M. R., Noest, W, Wirkner, C, Chiodo, F, Hokke, C, Van der Marel, G.A., and Codée, J.D.C. were involved in the research described in this chapter

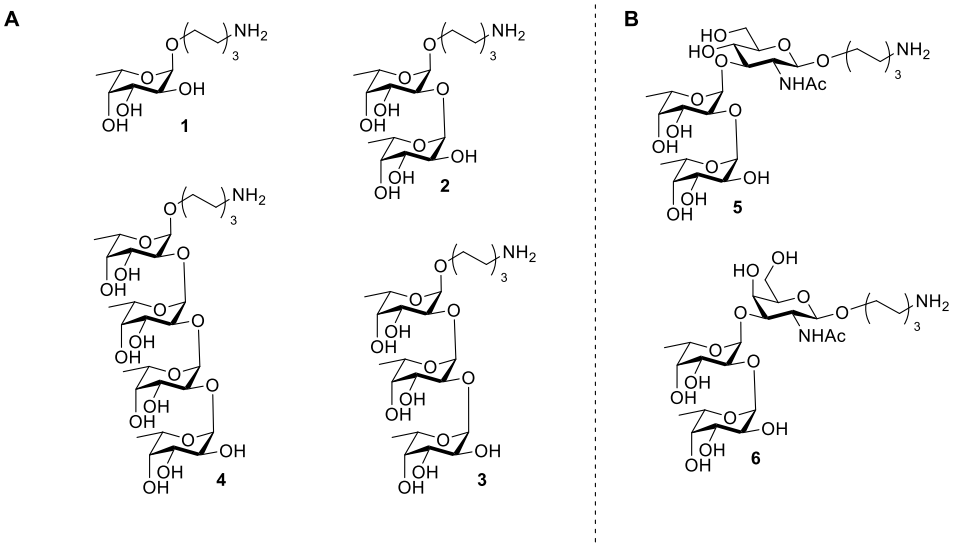


*Figure 1: **A**) Target α-(1-2) oligofucosides, **B**) Fucosylated trisaccharides described in Chapter 2.*

The synthetic routes envisioned for the α-(1-2)-oligofucosides are shown in Scheme 1. As the oligomers could be synthesized from the reducing end or from the non-reducing end with similar building blocks, synthetic routes for both approaches were developed and explored.

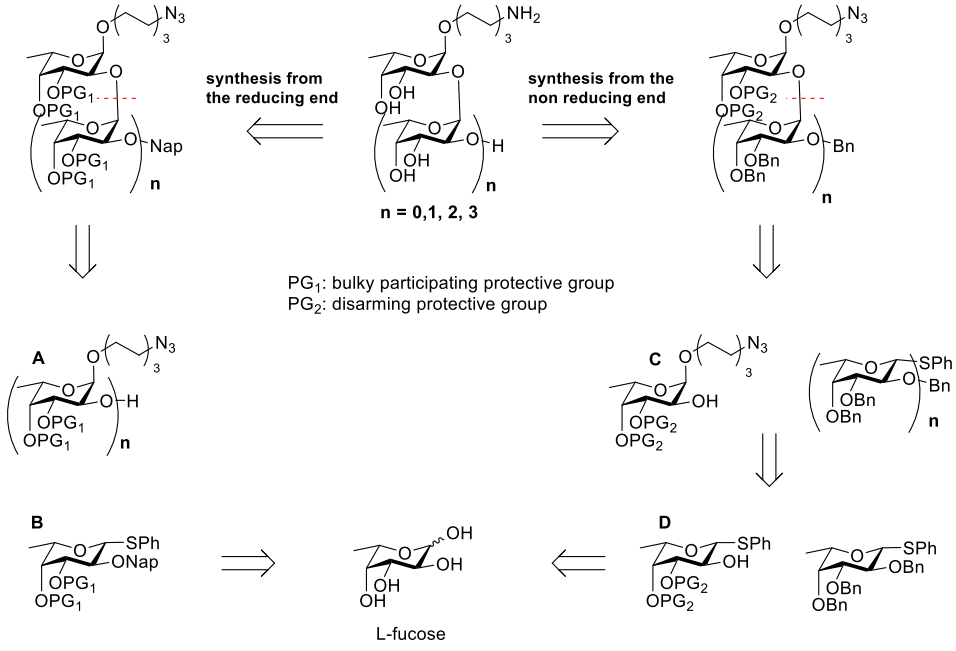
In order to synthesize the α-(1-2)-oligofucosides from the reducing end the following synthetic route was envisioned. Terminal building block **A** can be condensed with donor **B**, which bears benzoyl esters on the C3- and C4-O positions both for their steric bulk and to make use of the long range participation of the C4-O benzoyl to induce α-selectivity.[8] The naphthyl ether on the C2-O position of donor **B** was chosen as a temporary protecting group, as it is non-participating and can be orthogonally removed by either oxidative or acidic conditions.[9], [10] After removal of the naphthyl group of the newly formed glycan chain, elongation can be attained by a glycosylation with donor **B**. Repetition of these two steps would result in an oligomer of the desired chain length.

In order to synthesize the α-(1-2)-oligofucosides from the non-reducing end up, building blocks **C** and **D** were designed. It was hypothesized that an armed benzyl protected thioglycoside could be selectively activated over thioglycoside **D**, bearing disarming acyl protecting groups on the C3-O and C4-O positions. After the glycosylation reaction these disarming acyl groups could be removed selectively followed by benzylation of the free hydroxyls, thus creating a new armed donor.

Subsequently, the newly obtained armed oligosaccharide can be condensed with either **D** to allow firther elongation or terminal fucosyl **C**. This process can be repeated until the desired chain length is obtained.

In both routes the free amine on the hexane spacer is masked as an azide and the target α-(1-2) oligofucosides will be obtained by a deprotection procedure at the end of the synthesis, which comprises of treatment with a base followed by catalytic hydrogenation.

Scheme 1: Retrosynthesis of α-(1-2) linked oligofucosides.



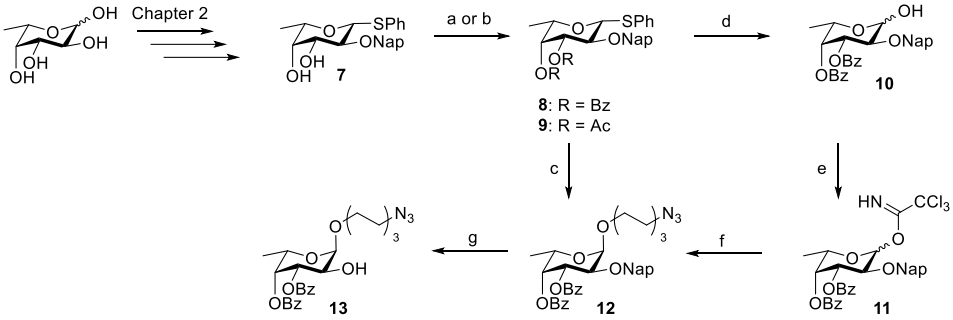
Results and discussion

Synthesis of α-(1-2) fucosyl chains from the reducing end

The syntheses of the synthons required for the synthetic route starting from the reducing end are depicted in Scheme 2. Besides benzoyl bearing donor **8** the corresponding acetyl bearing donor **9** was synthesized as well, in case the benzoyls proved to be too bulky. Diol **7** can be made from L-fucose in six steps as is described in Chapter 2. Acetylation of the hydroxyls present in compound **7** using pyridine and acetic anhydride at room temperature resulted in acetyl bearing donor **9** in a near quantitative yield. Similar benzoylation conditions required a temperature of 60˚C in order to fully benzoylate diol **7**, as the much less reactive axial C4-OH reacted sluggishly

with benzoyl chloride at room temperature. As all the glycosidic linkages in the α-(1-2) oligofucosides are *cis*-linked, it was decided to install the linker in the same configuration. To achieve this α-selective linkage with 6-azidohexan-1-ol several α-selective protocols were explored. The 6-azidohexan-1-ol was condensed with thioglycoside **8** using a protocol by Wang *et al*. that showed DMF could be used as an additive when starting from a thioglycoside based building block to induce α-selectivity.[11] This protocol, especially beneficial for secondary acceptors, did result in the formation of α-configured product **12** in 43%, but the formation of the β-side product was not fully suppressed, which greatly hindered purification. Another protocol, developed by Mukaiyama and co-workers, described a method to induce cis-linkages using imidate donors together with methyldiphenylphosphine ($MePh_2P=O$) as an additive in conjunction with TMSI.[12], [13] Thus thioglycoside **8** was transformed into imidate donor **11** by hydrolysis of the thiophenyl group using NBS in wet acetone, followed by imidoylation with trichloroacetonitrile with a catalytic amount of Cs_2CO_3 in acetonitrile.[14] Applying these conditions led to a completely α-selective introduction of the 6-azidohexan-1-ol in a 63% yield. Conversion of **12** into acceptor **13** was achieved in a yield of 85% by cleavage of the naphthyl group with DDQ in a mixture of DCM and methanol.[9]

*Scheme 2: Synthesis of building blocks **8**, **9** and **13** used in the synthesis from the reducing end.*

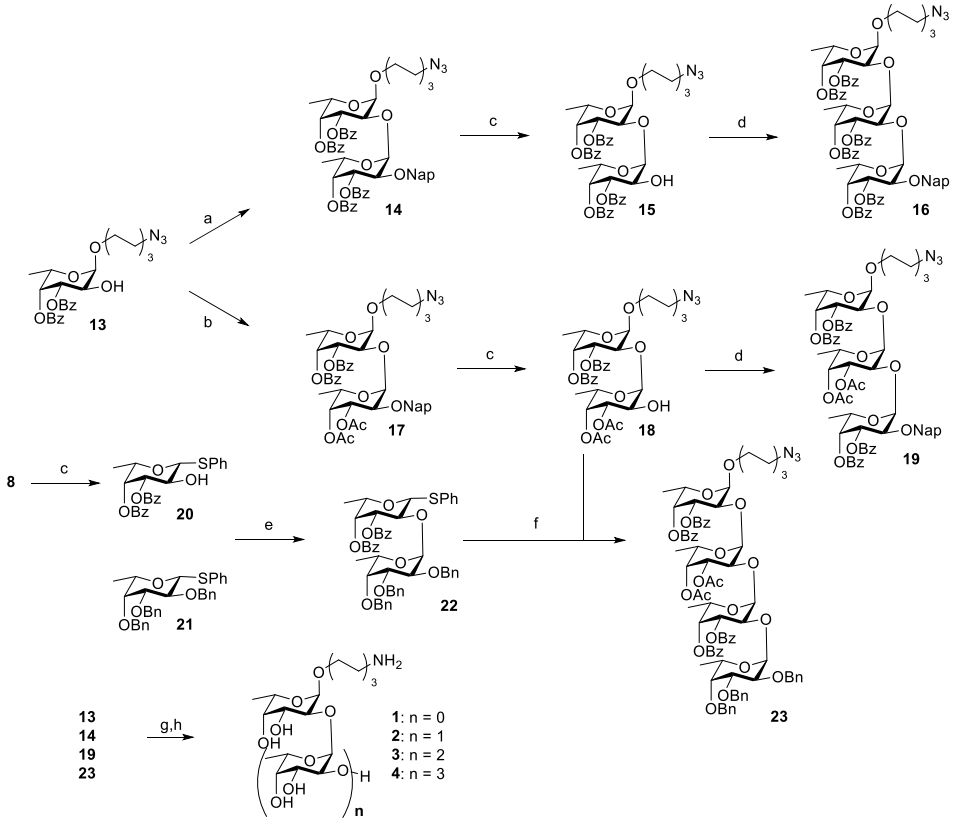


Reagents and conditions: **a**: Ac_2O, pyr, DCM, 0˚C to RT, 97% **b**: Bz-Cl, DMAP, pyr, DCE, 60˚C, 93%, **c**: DMF, NIS, TMSOTf (cat.), 6-azidohexan-1-ol, MS (3Å), DCM, 42%, **d**: NBS, acetone, H_2O, 98%, **e**: CS_2CO_3 (cat.), Cl_3CCN, DCM, 90%, α/β, 2/1, **f**: 6-azidohexan-1-ol, TMSI, $Ph_2MeP=O$, MS (3Å), DCM, 63%, **g**: DDQ, DCM, MeOH, 85%.

With the building blocks **8**, **9** and **13** in hand the oligofucosides **2**, **3** and **4** were synthesized as well as the monosaccharide **1** (Scheme 3). The stereochemistry of the newly formed α-glycosidic linkages was analyzed by ^1H- and HMBC gated NMR techniques, as these linkages have J couplings between 2 to 4 Hz and $^1J_{C\text{-}1,\ H\text{-}1}$ are 170 Hz, while those of β-linkages are between 8 to 10 Hz and 160 Hz, respectively.[15]

Acceptor **13** was condensed with benzoyl donor **8** with the NIS/TMSOTf activator couple, giving disaccharide **14** in an excellent yield of 82%. Besides the high yield the stereoselectivity was >95%, as NMR confirmed the formation of an α-glycosidic bond ($J_{1\text{-}2}$ = 3.5 Hz and $^{1}J_{C\text{-}1,\ H\text{-}1}$ = 170 Hz). This high selectivity can be attributed to both the bulky nature of the benzoyl groups as well as long range participation of the C4-O-benzoyl.[8] Removal of the naphthyl protective group by treatment with DDQ in a mixture of DCM and methanol resulted in acceptor **15** in 89% yield. Dimer acceptor **15** was then condensed with donor **8** using the same NIS/TMSOTf conditions but, unlike with dimer **14**, the yield of trimer **16** dropped to 7% (Table 1, entry 1), while the stereoselectivity remained excellent. The lower yield was attributed to the bulky nature of the benzoyl groups and the poorer accessibility of the disaccharide C2'-OH.

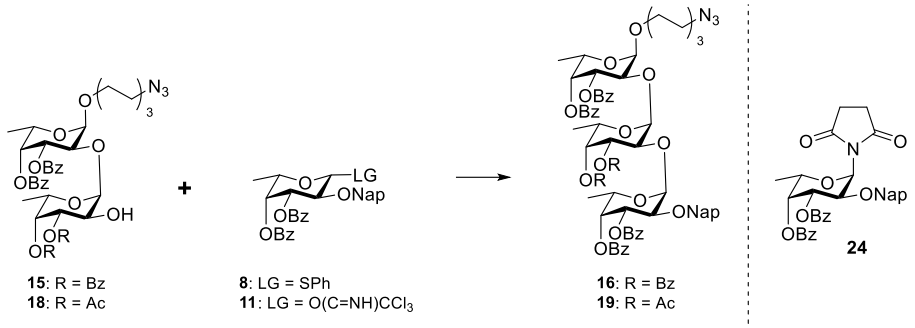
Scheme 3: Synthesis of fuc-α-(1-2)-fuc chains from the reducing end.



Reagents and conditions: **a**: **8**, NIS, TMSOTf, MS (3Å), DCM, -40˚C to -20˚C, 82%, **b**: **9**, NIS, TMSOTf (cat.), MS (3Å), DCM, -40˚C to -20˚C, 70%, **c**: DDQ, DCM, MeOH, **15** 89%, **18** 87%, **20** 99% **d**: **11**, TMSOTf, MS (3Å), DCM, -30˚C, **16** 16%, **19** 26%, **e**: IDCP, DCM, 0˚C, 73%, **f**: NIS, TMSOTf, MS (3Å), DCM, -40˚C to RT, 17%, **g**: NaOMe, MeOH/DCM, **h**: Pd/C, H_2, H_2O, **1** 25%, **2** 68%, **3** 74%, **4** 74% (over two steps).

Disaccharide **17** was prepared in order to establish whether the steric bulk of the benzoyl groups on the second fucosyl of the acceptor indeed hinder the chain elongation. Acceptor **13** was glycosylated with acetyl bearing donor **9** to give disaccharide **17** in 70% yield ($J_{1\text{-}2}$ = 3.5 Hz and $^{1}J_{C\text{-}1,\ H\text{-}1}$ = 170 Hz). The yield was slightly lower than with benzoyl donor **8** because acetyl donor **9** reacted in a somewhat less stereoselective manner (α/β = 6/1). After removal of the naphthyl ether from disaccharide **17** the obtained acceptor **18** alternative conditions to furnish a protected trifucoside were explored next (Table 1). Acceptor **18** was condensed with benzoylated donor **8**, which yielded trisaccharide **19** in 10% as a single anomer ($J_{1\text{-}2}$ = 3.5 Hz and $^{1}J_{C\text{-}1,\ H\text{-}1}$ = 170 Hz) (entry 2). Under these conditions, the formation of compound **24** was observed bearing an anomeric succinimide. Therefore, the glycosylation procedure was changed to the trichloroacetimidate method. Imidate donor **11** was condensed with both disaccharide **15** and **18**, which resulted in significant increase of yield (entries 3 and 4). The formation of the Chapman rearranged side-product at higher temperatures urged us to perform the glycosylation -30˚C (entry 5). Although this did result in a slight increase of the yield, the increase did not warrant further study.

Table 1: Optimization of chain extension by variation of the C3-O' and C4-O' substituents of the acceptor



entry	acceptor	donor	activator	T (˚C)	yield (%)[a]
1	**15**	**8** (1.5 eq.)	NIS (2 eq.), TMSOTf (0.1 eq.)	-40 → 0	7
2	**18**	**8** (1.5 eq.)	NIS (2 eq.), TMSOTf (0.1 eq.)	-40 → 0	10
3	**15**	**11** (1.5eq.)	TMSOTf (0.1 eq.)	-40 → 0	16
4	**18**	**11** (1.5 eq.)	TMSOTf (0.1 eq.)	-40 → 0	22
5	**18**	**11** (4.0 eq.)	TMSOTf (0.1 eq.)	-30	26

[a] Isolated yield of the desired trisaccharide.

Based on the low yields of the trisaccharide assembly, the stepwise elongation approach was abandoned and a more convergent [2+2] approach was employed in order to obtain tetrasaccharide **23** (Scheme 3). Thiofucose acceptor **20** was prepared by DDQ mediated cleavage of the naphthyl ether present on donor **8.** Subsequent

chemoselective condensation of perbenzylated armed thiodonor **21** with disarmed thioacceptor **20** resulted in the formation of disaccharide **22** in a yield of 74%, when IDCP was used as the activating agent. Condensation of donor **22** with disaccharide acceptor **18** applying the NIS/TMSOTf protocol yielded tetrasaccharide **23** with complete stereoselectivity albeit in a low yield of 17%.

Mono-, di-, tri-, and tetrasaccharide **13**, **14**, **19** and **23** were deprotected in two steps (Scheme 3). First the esters were hydrolyzed using Zemplén conditions. While the mono- and disaccharide were readily hydrolysed at room temperature, it was noteworthy that the tri,- and tetrasaccharide required heating to 50˚C to achieve complete saponification of the ester groups. Second concurrent removal of the naphthyl and benzyl ethers and reduction of the azide by catalytic hydrogenation was carried out, which resulted in **1**, **2**, **3** and **4**.

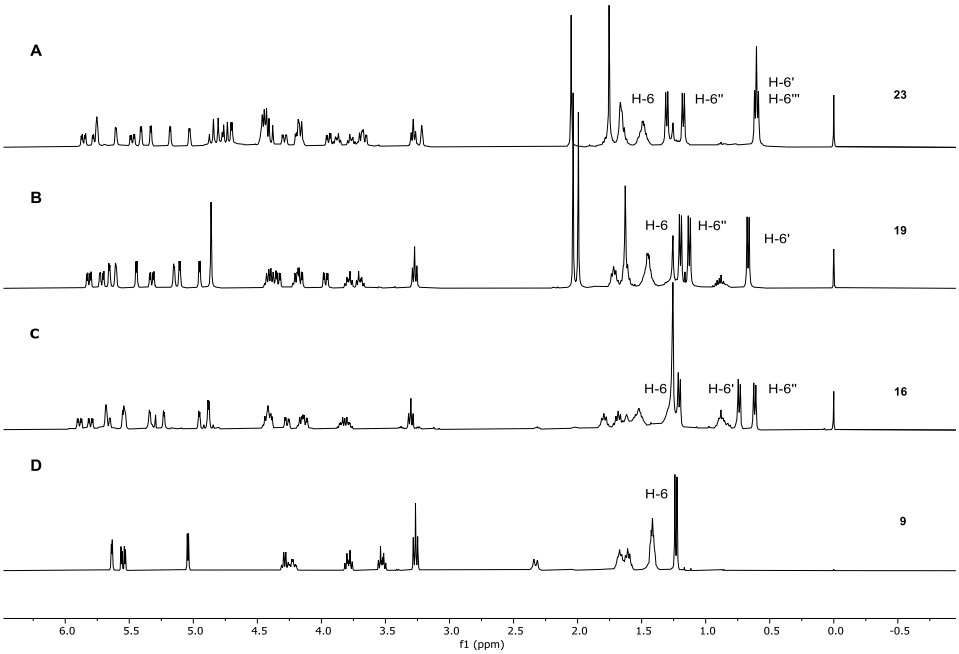


*Figure 2: Partial ^{1}H NMR spectra of **23 (A)**, **19 (B)**, **16 (C)** and **20 (D)**. The highlighted peaks represent the methyl groups (H-6) of the fucoses, where H-6 denotes the methyl group of the first fucose from the reducing end, H-6' the second and H-6'' and H-6''', the third and fourth, respectively. ^{1}H NMR were measured in $CDCl_3$ on a 400 MHz NMR at room temperature.*

It was shown in the previous chapter that the steric bulk of the benzoyl group might hinder the chain elongation. The steric bulk may induce a conformational effect in the oligosaccharide placing the acceptor OH in a less accessible position. The ^{1}H NMR spectra of the generated oligofucosides provide an indication that the nature of the protecting groups influences the shape of the oligomers (Figure 2). The CH_3 group at C-

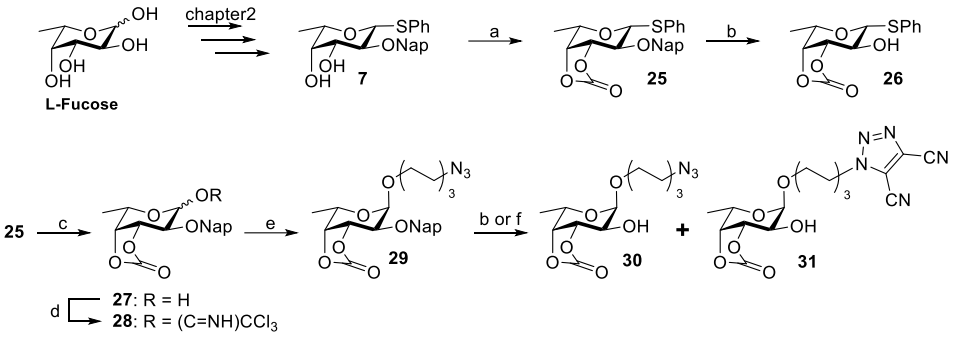
6 of a fucoside is generally located around 1.1-2 ppm as can be seen from the spectrum of monosaccharide **20** (Figure 2-D). The ^{1}H NMR of benzoylated trisaccharide **16** (Figure 2-C) two methyl groups (H-6'and H-6'') are located around 0.6 ppm, while acetyl bearing trisaccharide **19** (Figure 2-B) has only one methyl group (H-6') in this region. The same trend can be seen with the tetrasaccharide (Figure 2-A). The shifted CH_3 groups all have a benzoyl protected fucosyl attached to their reducing end, while the CH_3 groups of the fucosides bearing an acetyl protected fucosyl on their anomeric center have not. This indicates that the nature of the benzoyl groups have a profound influence on the environment of the sugar residues flanking them.

Synthesis of α-(1-2) oligofucosides from the non-reducing end

As shown in the previous synthetic route, the size of the substituent on the C4-O position unfavourably influences the yield of the glycosylation reaction. To overcome this, it was envisioned to use a less bulky group than the originally envisioned benzoyl groups. To this end the smallest protective group possible, a cyclic carbonate, was chosen as the key protecting group in the synthesis of the fucosides from the non-reducing end.

The synthesis of acceptors **26** and **30**, starting from previously described intermediate **7** (Chapter 2), is depicted in Scheme 4. En route to acceptor **26**, diol was **7** was treated with triphosgene in a mixture of DCM and pyridine to give cyclic carbonate **25** in a quantitative yield.[16] Next the naphthyl ether was removed selectively using DDQ in a mixture of DCM and methanol, giving alcohol **26** in a 78% yield over two steps. Terminal building block **30** was synthesized from **25** in four steps. Initially the thiophenyl group was hydrolysed using NBS in wet acetone, followed by imidoylation of the formed hemiacetal **27**.[17] This gave imidate donor **28** in a yield of 62% over two steps. The two anomers could be readily separated from each other by silicagel chromatography, this led to the possibility of determining if the conformation of the imidate influences the stereoselectivity of the glycosylation reaction. The condensation of 6-azidohexan-1-ol with both the α- and β-imidate **28** using the previously described $MePh_2P{=}O$/TMSI protocol by Mukaiyama and co-workers gave **29** in the same yield and selectivity.[12] As before, the naphthyl ether was removed selectively by DDQ in a mixture of DCM and methanol. However, unlike before, this reaction was extremely sluggish and a significant amount (14%) of byproduct was formed. After careful NMR, IR and HRMS analysis it was concluded that **31** was the formed byproduct.

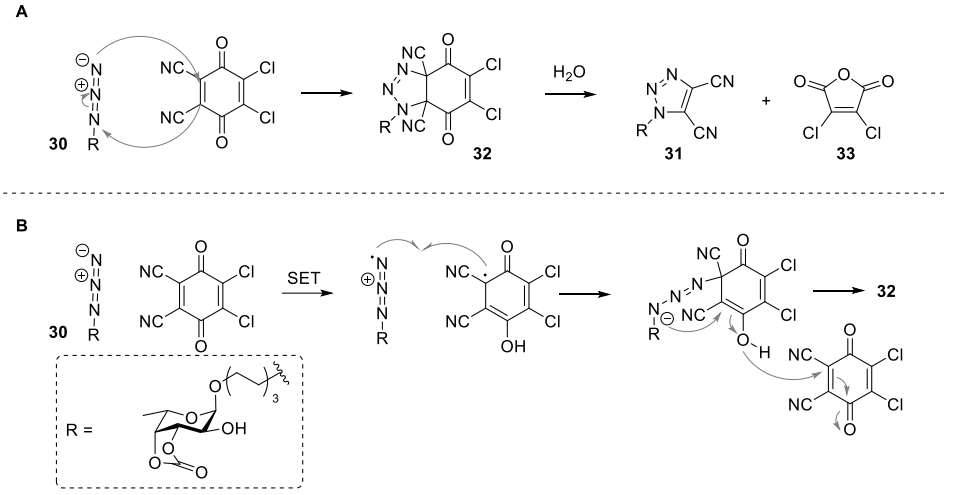
Scheme 4: Synthesis of the building blocks required for a non-reducing end up approach.



Reagents and conditions: **a**: triphosgene, pyr. DCM, 0˚C, quant., **b**: DDQ, DCM, MeOH, **26** 78%, **30** 52%, **31** 14%, **c**: NBS, acetone, H_2O, 81%, **d**: DBU (cat.), Cl_3CCN, DCM, 77%, **e**: 6-azidohexan-1-ol, TMSI, $Ph_2MeP{=}O$, MS (3Å), DCM, 70%, **f**: HCl (cat.), TES, HFIP, DCM, 65%.

Intrigued by the formation of side product **31** an in depth literature study revealed an article by Deng and coworkers, where a similar product was formed.[18] Two putative mechanisms for the formation of **31** are proposed in scheme 5. Mechanism 1 (Scheme 5A) proceeds via a cycloaddition between the azide and DDQ giving molecule **32**.[19] Subsequent hydrolysis leads to typical triazole ring, seen on the byproduct. Mechanism 2 (Scheme 5B) proceeds via a single electron transfer (SET) from DDQ to the azide, which in turn forms a C-N bond with the partially oxidized DDQ. This intermediate then cyclizes to form **32** with eviction of a hydride to another DDQ molecule. Subsequent hydrolysis then leads to the formation of the triazole ring seen in the byproduct **31**. Due to this incompatibility of DDQ with the azide present on the fucoside, a different method, involving the use of HCl in HFIP with triethylsilane as a scavenger, was applied to remove the naphthyl ether.[10] This method resulted in the successful formation of compound **30** in a yield of 65%.

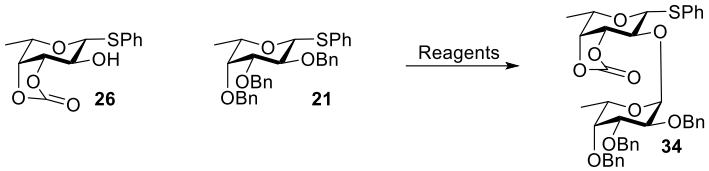
*Scheme 5: Proposed formation of byproduct **31**. **A**) by cycloaddition, **B**) by single electron transfer.*



To attain a chemoselective glycosylation procedure between **21** and **26** several activation methods were compared in order to optimize the glycosylation reaction. The challenge with this glycosylation lies in the selective activation of one of the thiophenyl groups, while leaving the other intact. Since **21** is a well-known highly reactive donor (RRV of $7.2 \cdot 10^4$) and **26** is protected with a disarming cyclic carbonate group, it was envisioned that a mild activating system, should be able to accomplish the selective activation.[20] The results of these activation methods are shown in Table 2.

Initially NIS in conjunction with a catalytic amount of TMSOTf was tried (entry 1). This method, however, was not fully selective as it also activated the disarmed acceptor **26** resulting in a complex mixture of compounds. Next, pre-activation with Ph_2SO, TTBP, Tf_2O was tried (entry 2).[21] With this method the donor is activated first and slowly added to the acceptor, lowering the risk of activating the disarmed acceptor. Although disaccharide **34** was formed using this method, the yield and selectivity were sub-optimal. Finally, IDCP, an activator that is not able to activate disarmed donors, was used (entries 3 and 4).[22] Besides a higher yield the IDCP method also has a higher stereoselectivity. Increasing the temperature from 0˚C to room temperature increased the yield slightly, but at the expense of the selectivity. With this optimized glycosylation protocol oligofucosides **2**, **3** and **4** were synthesized (Scheme 6).

Table 2: Comparison of different glycosylation methods in order to synthesize oligofucosides All reactions were performed under inert atmosphere in dry DCM with freshly flame dried molecular sieves (3Å) present. The concentration for all reactions was 0.1M.



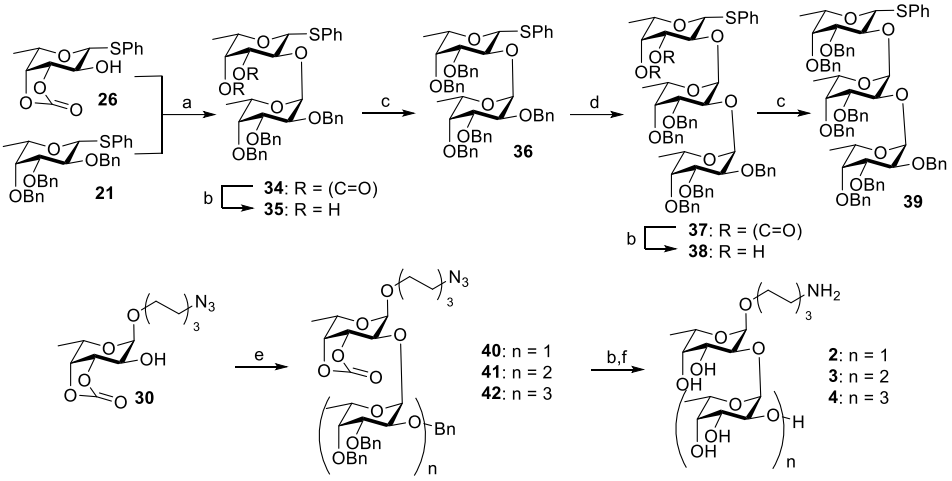
entry	reagents	T (˚C)	Yield (%)	α/β[a]
1	NIS (1.2 eq.), TMSOTf (0.1 eq.)	-40 → -20	Complex mixture	-
2	Ph_2SO, TTBP, Tf_2O[b]	-80 → -40	48%	3/1
3	IDCP (4 eq.)	0	72%	6/1
4	IDCP (4 eq.)	RT	81%	5/1

[a] Assigned by NMR, ($J_{1'-2'}$ = 4.0 Hz and $^1J_{C-1', H-1'}$ = 170 Hz).

[b] This reaction was performed with a concentration of 0.05M.

Chain elongation to provide the oligofucosides requires the conversion of disarmed disaccharide **34** into an armed disaccharide. To this end the carbonate group on disaccharide **34** was saponified using sodium hydroxide at slightly elevated temperatures (40˚C), because the reaction at room temperature proceeded slowly. Next, the obtained diol **35** was benzylated in the presence of TBAI. Similar to the hydrolysis reaction to remove the carbonate the temperature of this reaction had to be raised to 40˚C, as the reaction at room temperature didn't result in complete conversion. Presumably it is the benzylation of the C4-OH that requires the elevated temperatures. Armed disaccharide donor **36** was condensed with acceptor **26** with the IDCP conditions used earlier to synthesize **34**. Trisaccharide **37** was formed with complete α-selectivity in a yield of 78%. Disarmed trisaccharide **37** was converted into armed trisaccharide **39** by treatment with NaOH in a mixture of THF and water at 40˚C to remove the cyclic carbonate. Diol **38** was then benzylated using benzyl bromide, sodium hydride and a catalytic amount of TBAI at 40˚C, to give armed trisaccharide **39** in 70% yield. Finally, linker bearing acceptor **30** was condensed with armed donors **21**, **36** and **39** using the IDCP protocol giving disaccharide **40**, trisaccharide **41** and tetrasaccharide **42** in a yield of 60%, 70% and 61%, respectively.

Scheme 6: Synthesis of a small library of α-(1-2) linked oligofucosides from the non-reducing end.



Reagents and conditions: **a**: IDCP, MS (3Å), DCM, 0˚C, 62%, **b**: NaOH, H_2O, THF, 40˚C, **35** 98%, **38** 88%, **c**: BnBr, NaH, TBAI (cat.), DMF, 40˚C, **36** 72%, **39** 80%, **d**: **26**, IDCP, MS (3Å), DCM, 0˚C, 78%, **e**: **21**, **36** or **39**, IDCP, MS (3Å), DCM, 0˚C **40** 60%, **41** 70%, **42** 61%, **f**: Pd/C, H_2, Diox., H_2O, 40˚C, **2** 48%, **3** 18%, **4** 21%.

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The deprotection of fucosides **40**-**42** was performed by first removing the cyclic carbonate using NaOH in a mixture of THF and water, followed by hydrogenation. The hydrogenation of disaccharide **40** proceeded smoothly at room temperature, while the tri- **41** and tetrasaccharide **42** required elevated temperatures and longer reaction times for full debenzylation.

In contrast to the method in which the fucosyl chains were built up from the reducing end, the chain extension described in Scheme 6 went in significantly higher yields. It can be reasoned that this is largely due to the smaller nature of the cyclic carbonate bearing acceptor. Another factor that may have contributed to higher glycosylation efficiency is the switch from benzoyl to benzyl protecting groups. Besides being arming instead of disarming, benzyl ethers are also less rigid than benzoyl esters, thus leading to a more reactive and conformationally flexible donor. As can be seen from the ^{1}H NMR data (Figure 3) all the fucosyl methyl peaks are around 1.1-2 ppm, indicating that they are in a regular configuration.

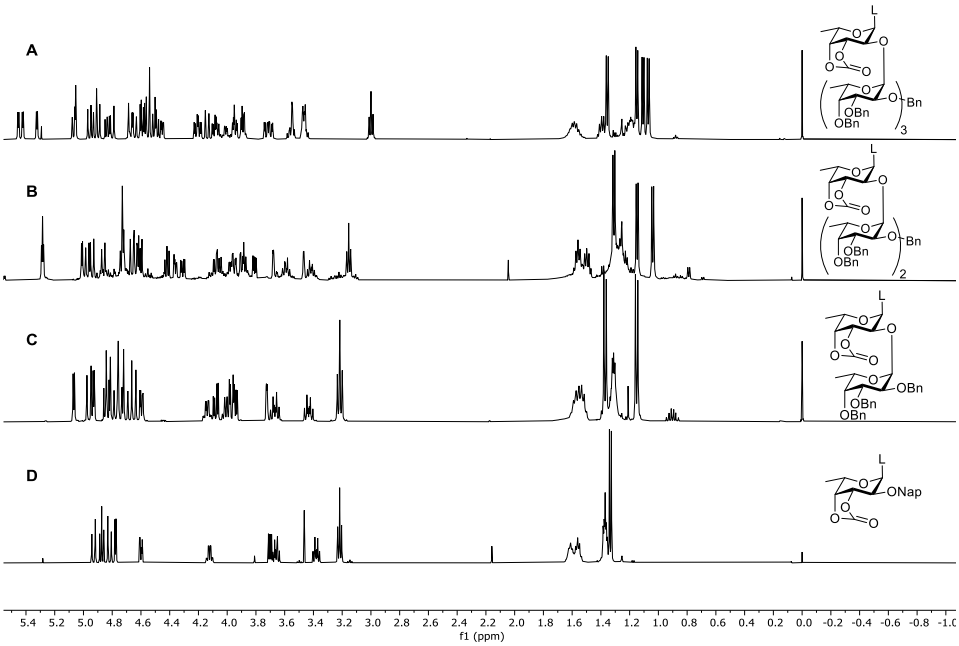


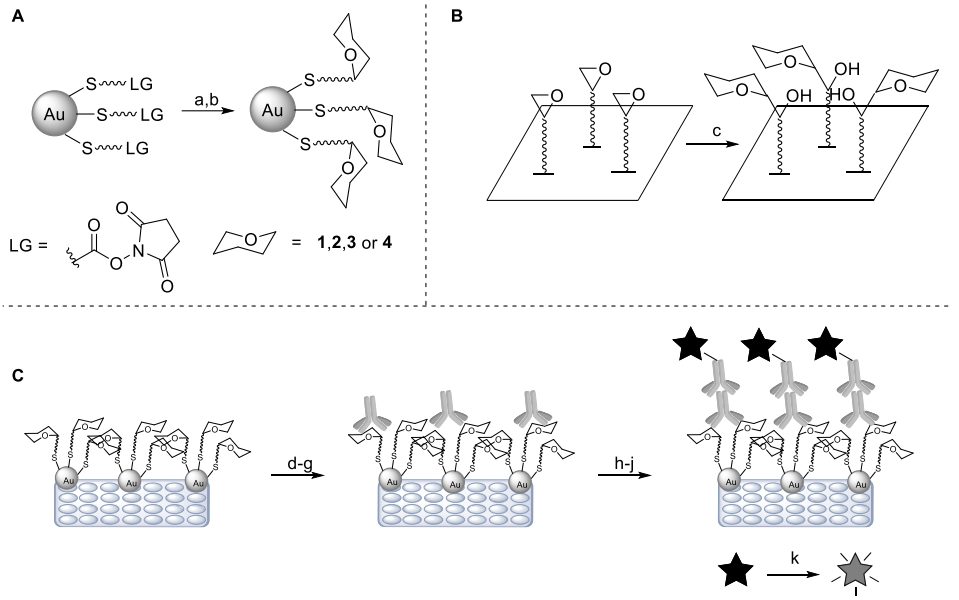
*Figure 3: Partial ^{1}H NMR spectra of **42** (A), **41** (B), **40** (C), **30** (C). The highlighted peaks represent the methyl groups (H-6) of the fucoses, where H-6 denotes the methyl group of the first fucose from the reducing end, H-6' the second and H-6'' and H-6''', the third and fourth, respectively. The 6-azidohexan-1-ol linker is denoted as L for clarity. The ^{1}H NMR spectra were measured in $CDCl_3$ at RT on a 400 MHz NMR.*

Gold nanoparticle based ELISA's

In order to test if the synthesized glycans can be used as a tool to determine the level of antibodies in infected people, several ELISA's were performed. The general procedure of these glycol-AuNP's is summarized in Scheme 7 and is described in more detail in chapter 2.[23],[24],[25] As in chapter 2, the AuNP's were screened against monoclonal antibodies (mAb's) to verify that the glycans attached to the AuNPs were properly recognized. After establishing the selective interaction with these mAb's, the AuNP's were used to screen sera of infected people.

The α-(1-2) linked oligofucosides **1-4** were covalently linked to pre-formed 5 nm *N*-hydroxysuccinimide activated gold nanoparticles (Scheme 7-A). The functionalized AuNP's were screened against a subset of monoclonal antibodies (mAb's) that show antigen recognition against several fucosylated glycans present in soluble egg antigen (SEA) by ELISA (Scheme 7-C).[26] Antibodies **II** (114-5B1-A) and **VI** (259-2A1) were tested as they have been shown to bind di- and tri-fucosylated glucosamine structures. **III (**291-5D5A) and **IV** (291-4D10-A) tend to bind to Lewis X type structures. Finally, mAb's **I** (258-3E3) and **V** (114-4D12-AA) were tested as they bound strongly to di-fucosylated trisaccharides **5** and **6** as revealed in the previous chapter.

*Scheme 7: Functionalization of **A**) gold nanoparticles, **B**: glycan array plate. **C**: ELISA protocol*



Reagents and conditions: **a**: **1, 2**, **3** or **4**, AuNP, reaction buffer, **b**: quencher solution, **c**: glycan, Et_3N, H_2O, **d**: AuNP, coating buffer (50 mM Na_2CO_3, pH = 9.6), **e**: washing with PBS buffer, **f**: blocking with 1% BSA, **g**: antibody or human sera, **h**: washing with PBS buffer, **i**: secondary antibody RAM/PO, **j**: washing with PBS buffer, **k**: i) TMB substrate, ii) H_2SO_4.

The results, depicted in Figure 4-A, clearly show that mAb's **I** and **V** recognize the α-(1-2) linked fucosyl chains present on the AuNP's. As these mAb's also recognized the fucosylated trisaccharides **5** and **6** described in the previous chapter, it can be deduced that they bind to the fucosyl chains and not the backbone of these latter glycans. The other mAb's did not show any significant binding, indicating that they do not recognize the α-(1-2) linked oligofucosides, but require other structural elements present in the backbone of the glycan structure, that structure being LeX or (Di/Tri)-fucosylated LacDiNAc oligosaccharides. The recognition by mAb's **I** and **V** proved that the synthesized fucosides could be well recognized, when bound to the AuNP's. Next, the AuNP's were screened against human sera from an infected community in Uganda (Piida co-hort) with non-infected human sera as the control (Figure 4-B). ELISA wells were coated with the prepared AuNP's and incubated with the sera. The presence of IgG bound to the AuNP library was then visualized following the classic ELISA detection steps (Scheme 7-C). As with the monoclonal antibodies, the α-(1-2) linked oligofucosides are selectively recognized by antibodies present in the sera samples of infected people, with binding increasing with increasing fucosyl chain length. The control samples did not show any significant binding in these experiments. This

indicates that the AuNP's functionalized with these oligofucosides may be used as a diagnostic tool for schistosomiasis.

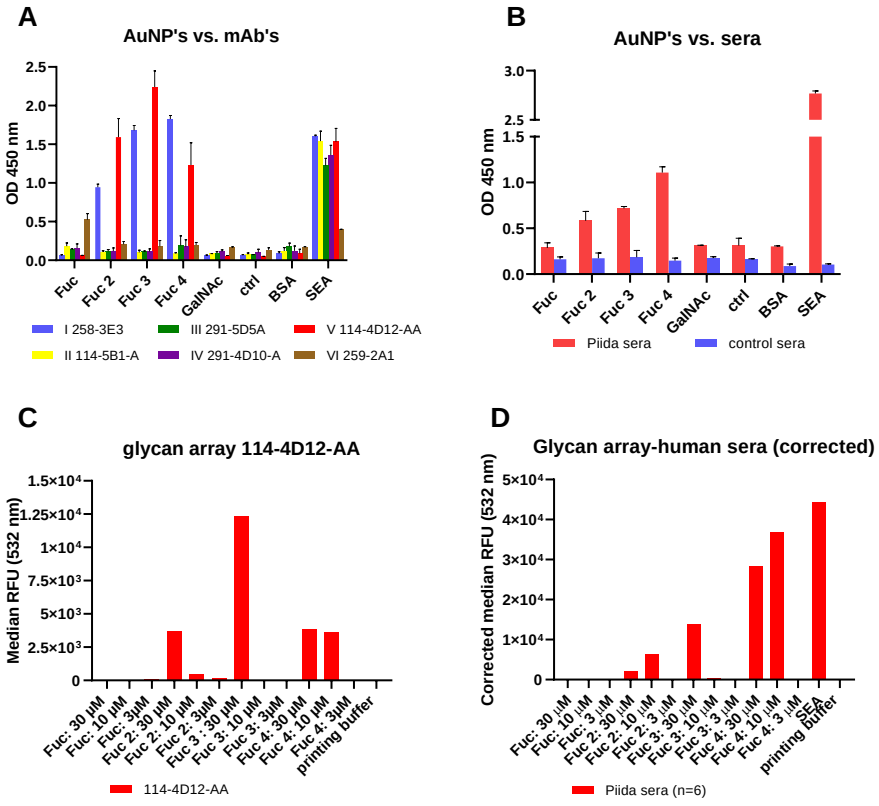


*Figure 4: Bar graph of the results of the ELISA experiment with monoclonal antibodies (**A**) and human sera (**B**) from either infected (red) or control samples (blue). Bar graph of glycan array experiment with mAb 114-4D12-AA (**C**) and with human sera (**D**). AuNP: gold nanoparticle, Fuc **x**: α-(1-2) linked fucosyl chain, where **x** denotes the number of fucoses, GalNAc: 6-aminohexan-1-ol N-acetamido-β-D-galactosamine, ctrl: unfunctionalized AuNP, BSA: bovine serum albumin, SEA: soluble egg antigen, OD: optical density, RFU: relative fluorescence units. Error bars indicate standard deviations of the experiment performed in duplicate measurements. The experiments were repeated three times showing similar results.*

Besides these ELISA's a glycan micro array was performed using the α-(1-2) linked oligofucosides as well (Scheme 7-B). Carbohydrate microarrays have become the leading edge tool for binding studies of glycans, because they have the advantage that many glycan interactions can be assessed simultaneously, while using small amounts of sample. In addition, the glycans attached to the micro array surface are presented in a multivalent manner, which, as with the case of the AuNP's, can greatly aid in the detection of weak interactions. As before, the glycans were screened against the

monoclonal antibody **V**, sera of people infected with schistosomiasis and sera of uninfected individuals, with different printing concentrations of the glycan (30 µM, 10 µM and 3 µM). The results of the microarray are shown in Figure 4. The screening against **V** (Figure 4-C), shows that the di- and trisaccharide **2** and **3** can be recognized when printed at a concentration of 30 µM. The lower concentrations did not show any significant binding. The interaction of **V** with tetramer **4** appears to be somewhat stronger as binding is also detectable at 10 µM. The binding specificity of this antibody confirms the findings of the AuNP-ELISA experiment. When essaying the array data with the Piida sera (Figure 4-D), it is again revealed that the longer fucans show stronger binding, corroborating the findings of the ELISA's experiments.

Conclusion

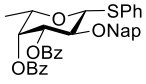
In order to answer the question if the backbone is required for recognition of α-(1-2) linked oligofucosides by antibodies a small library was synthesized consisting of a mono-, di-, tri- and tetramer. In order to synthesize these molecules, two different chemical routes were tested. Route A, consisted of synthesizing the α-(1-2) linked oligofucosides from the reducing end down. Disaccharide **14** was readily synthesized in a high yield, however, upon extending the chain to either a tri- or tetramer, the yield dropped considerably. Changing to a smaller acetyl protecting group did alleviate this problem a bit, but not enough to continue along this route. The deprotection of these molecules proceeded smoothly. Route B consisted of synthesizing the α-(1-2) linked oligofucosides from the non-reducing end up. Due to the steric hindrance of the benzoyl groups in Route A, a smaller protecting group was chosen; the carbonate group. Several activation methods were tested to see which could selectively activate the armed donor while leaving the disarmed acceptor intact. Selective activation and subsequent chain elongation was achieved by using IDCP as the activator. Although chain extension proceeded smoothly, the deprotection proved troublesome. The fucosides were coupled to 5 nm gold nanoparticles and used to analyze sera of infected people using an ELISA set-up and a glycan array. It was shown that the longest fucosyl chains were recognized selectively, revealing the tri- and tetrafucoside as potential biomarkers for schistosomiasis infection.

Experimental

General procedures

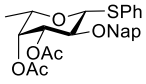
Glassware used for reactions was oven dried before use at 80°C. Anhydrous solvents were prepared by drying them over activated molecular sieves (3Å) for at least 24 hours before use. Molecular sieves were activated by flame-drying under reduced pressure. Reactions that required anhydrous conditions were co-evaporated with anhydrous toluene or anhydrous 1,4-dioxane to remove traces of water and the reactions were performed under argon or nitrogen atmosphere. EtOAc and toluene used for extractions and silica gel column chromatography were distilled before use, all other chemicals were used as received. One- and two-dimensional NMR spectra were recorded at 298 K unless stated otherwise on a Bruker AV-300 (300 MHz for ^{1}H nuclei and 75 MHz for ^{13}C nuclei), AV-400 (400 MHz for ^{1}H nuclei and 101 MHz for ^{13}C nuclei) or a Bruker AV-500 (500 MHz for ^{1}H nuclei and 126 MHz for ^{13}C nuclei). Chemical shifts (δ) are given in ppm relative to tetramethylsilane or the deuterated solvent. IR-spectra were recorded on a Shimadzu FTIT-8300. HRMS spectra were recorded on a Thermo Finnigan LTQ orbitrap mass spectrometer. Unless stated otherwise all reaction were carried out at room temperature and monitored by thin layer chromatography (TLC). TLC was carried out on Merck aluminium sheets (silica gel 60 F254). TLC analysis was performed by detecting UV adsorption (254 nm) where suitable and spraying the TLC plate with 20% H_2SO_4 in EtOH or with a solution of $(NH_4)_6Mo_7.4H_2O$ (25 g/L), KOH (1 g/L) in water or a solution of $KMnO_4$ (20 g/L) and K_2CO_3 (10 g/L) in water or an anisaldehyde solution containing H_2SO_4, glacial acetic acid and p-anisaldehyde in absolute EtOH followed by charring the TLC plate at 150°C. TLC-MS analysis was performed by extracting spots of interest off a TLC plate with a CAMAG TLC interface connected to an API 165 mass spectrometer. Silica gel column chromatography was performed on silica gel (40 - 63 µm particle size, 60 Å pore size). Size exclusion chromatography was carried out on Sephadex™ LH-20 gel.

Phenyl 3,4-di-*O*-benzoyl-2-*O*-(2-naphthylmetyl)-1-thio-α-L-fucopyranoside (8)



The synthesis and characterization of this compound are described in Chapter 2 compound **12**.

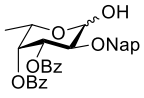
Phenyl 3,4-di-*O*-acetyl-2-*O*-(2-naphthylmetyl)-1-thio-α-L-fucopyranoside (9)



Ac_2O (0.4 mL, 4.23 mmol, 2.1 eq.) was slowly added to a solution containing diol **7** (0.79 g, 2.0 mmol, 1.0 eq.) in pyridine (4 mL, 0.5M). The solution was left to stir overnight, after which the reaction was quenched by addition of MeOH (1 mL). The mixture was diluted in EtOAc and washed

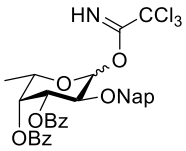
with 1M HCl (aq., 3x), sat. $NaHCO_3$ (aq., 2x) and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated. The title compound was obtained after purification by silicagel chromatography (0.95 g, 1.98 mmol, mmol, 99 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.84 – 7.74 (m, 2H, arom.), 7.71 (s, 1H, arom.), 7.65 – 7.58 (m, 2H, arom.), 7.48 – 7.42 (m, 3H, arom.), 7.34 – 7.27 (m, 3H, arom.), 5.23 (d, 1H, *J*=3.3 Hz, H-4), 5.05 (dd, 1H, *J*=9.6, 3.3 Hz, H-3), 4.99 (d, 1H, *J*=11.2 Hz, CH_2Nap), 4.73 (m, 2H, H-1, CH_2Nap), 3.78 (t, 2H, *J*=9.7 Hz, CH_2N_3), 3.70 (q, 1H, *J*=6.4, 5.9 Hz, H-5), 2.11 (s, 3H, CH_3-Ac), 1.87 (s, 3H, CH_3-Ac), 1.17 (d, 3H, *J*=6.4 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 170.5 (C=O, Ac), 170.0 (C=O, Ac), 135.5, 133.7, 133.3, 133.0, 132.0, 129.0, 128.1, 127.9, 127.7, 127.6, 126.6, 126.2, 126.0 (arom.), 87.6 (C-1), 75.5 (CH_2, Nap), 75.1 (C-2), 74.6 (C-3), 72.9 (C-5), 70.9 (C-4), 20.8 (CH_3, Ac), 20.7 (CH_3, Ac), 16.6 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{27}H_{28}O_6SNa$: 503.1504, found 503.1502.

3,4-di-*O*-benzoyl-2-*O*-(2-naphthylmetyl)-α/β-L-fucopyranoside (10)



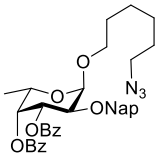
NBS (2.7 g, 15.0 mmol, 3.0 eq.) was added to a stirring solution of **8** (3.0 g, 5.0 mmol, 1.0 eq.) in a mixture of water and acetone (50 mL, 0.1M, 1/4, v/v) and left to stir for 15 min in the dark under inert atmosphere. A solution of sat. $Na_2S_2O_3$ (aq.) was added (20 mL) and after stirring for 5min. the acetone was evaporated *in vacuo*. The water layer was extracted with Et_2O (2x) and the combined organic layers were washed with sat. $NaHCO_3$ (aq., 3x) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (PE: EtOAc, 49:1 → 7:3) giving **10** as a colourless foam (2.0 g, 3.9 mmol, 78%, 1/1, α/β). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.02 – 7.94 (m, 2H, arom.), 7.94 – 7.86 (m, 2H, arom.), 7.83 – 7.78 (m, 2H, arom.), 7.78 – 7.71 (m, 3H, arom.), 7.71 – 7.61 (m, 5H, arom.), 7.61 – 7.13 (m, 23H, arom.), 5.80 (dd, 1H, *J*=10.3, 3.3 Hz, H-3α), 5.65 (d, 1H, *J*=2.4 Hz, H-4α), 5.57 (d, 1H, *J*=2.8, H-4β), 5.50 (t, 1H, *J*=3.0 Hz, H-1α), 5.42 (dd, 1H, *J*=10.1, 3.5 Hz, H-3β), 5.03 (d, 1H, *J*=11.8 Hz, CH_2Napβ), 4.97 – 4.88 (m, 2H, H-1β, CH_2Napβ), 4.88 – 4.74 (m, 2H, CH_2Napα), 4.55 (qd, 1H, *J*=6.4, 1.3 Hz, H-5α), 4.36 (dd, 1H, *J*=5.5, 2.5 Hz, 1β-OH), 4.17 (dd, 1H, *J*=10.3, 3.5 Hz, H-2α), 3.98 – 3.86 (m, 2H, H-2β, H-5β), 3.70 (t, 1H, *J*=2.1 Hz, 1α-OH), 1.25 (d, 3H, *J*=6.7 Hz, H-6β), 1.18 (d, 3H, *J*=6.6 Hz, H-6α) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 166.1, 165.8 (C=O, Bz0, 135.4, 134.8, 133.4, 133.3, 133.1, 133.0, 129.9, 129.8, 129.7, 129.6, 129.5, 128.5, 128.5, 128.4, 128.3, 128.1, 128.0, 127.8, 127.7, 127.1, 127.1, 126.3, 126.3, 126.2, 126.0, 126.0, 125.9 (arom.), 97.7 (C-1β), 91.7 (C-1α), 77.4 (C-2β), 74.7 (CH_2Napβ), 73.6 (C-2α), 73.3 (C-3β), 73.1 (CH_2Napα), 72.5 (C-4α), 71.7 (C-4β), 70.5 (C-3α), 69.6 (C-5β), 65.1 (C-5α), 16.4 (C-6β), 16.2 (C-6α) ppm.

Trichloroacetamido 3,4-*O*-carbonate-2-*O*-(2-naphthylmethyl)-α/β-L-fucopyranoside (11)



Hemi-acetal **10** (4.0 g, 7.7 mmol, 1.0 eq.) and trichloroacetonitrile (7.7 mL, 77 mmol, 10.0 eq.) were dissolved in dry DCM (77 mL, 0.1M). Cs_2CO_3 (1.3 g, 3.8 mmol, 0.5 eq.) was added and the solution was stirred under inert atmosphere for 3 hours. The orange solution was filtered over Celite® and concentrated *in vacuo*. The title compound was obtained after purification by silicagel chromatography (PE:EtOAc:Et_3N, 80:19:1) as a yellow oil (4.55 g, 6.93 mmol, 90%, 3/1, α/β). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.81 (s, 0.3H, NHβ), 8.69 (s, 1H, NHα), 7.98 – 7.92 (m, 1H, arom.), 7.89 – 7.83 (m, 2H, arom.), 7.83 – 7.74 (m, 3H, arom.), 7.74 – 7.62 (m, 4H, arom.), 7.61 – 7.31 (m, 7H, arom.), 7.31 – 7.15 (m, 7H, arom.), 6.74 (d, 1H, *J*=3.5 Hz, H-1α), 6.02 (d, 0.3H, *J*=8.1 Hz, H-1β), 5.84 (dd, 1H, *J*=10.5, 3.3 Hz, H-3α), 5.75 (dd, 1H, *J*=3.4, 1.3 Hz, H-4α), 5.63 (dd, 0.3H, *J*=3.5, 1.1 Hz, H-4β), 5.54 (dd, 0.3H, *J*=10.0, 3.6 Hz, H-3β), 5.04 (d, 0.3H, *J*=11.5 Hz, CH_2Napβ), 4.95 – 4.75 (m, 2.3H, CH_2Napα/β), 4.56 (q, 1H, *J*=6.8, 6.4 Hz, H-5α), 4.36 (dd, 1H, *J*=10.5, 3.5 Hz, H-2α), 4.23 (dd, 0.3H, *J*=10.0, 8.1 Hz, H-2β), 4.19 – 4.10 (m, 0.3H, H-5β), 1.30 (d, 0.9H, *J*=6.4 Hz, H-6β), 1.23 (d, 3H, *J*=6.5 Hz, H-6α) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 165.9, 165.6 (C=O, Bz), 161.4 (C=NH), 134.9, 133.4, 133.2, 133.1, 133.1, 129.8, 129.7, 129.7, 129.6, 129.4, 128.5, 128.4, 128.3, 128.3, 128.2, 127.9, 127.9, 127.7, 127.7, 127.1, 126.8, 126.2, 126.2, 126.1, 126.0, 125.8 (arom.), 98.7 (C-1β), 94.5 (C-1α), 75.2 (C-2β), 74.9 (CH_2Napβ), 73.1 (C-3β), 72.7 (CH_2Napα), 72.2 (C-2α), 71.8 (C-4α), 71.4 (C-4β), 70.5 (C-5β), 70.3 (C-3α), 67.9 (C-5α), 16.2 (C-6α/β) ppm.

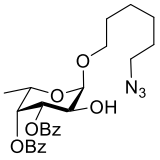
6-Azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(2-naphthylmetyl)-α-L-fucopyranoside (12)



Donor **11** (2.5 g, 3.85 mmol, 1.0 eq.), azidohexan-1-ol (0.83 g, 5.8 mmol, 1.5 eq.) and methyldiphenylphosphine oxide (5.0 g, 23.0 mmol, 6.0 eq.) were co-evaporated thrice with dry toluene and subsequently dissolved in dry DCM (38 mL, 0.1M). Molecular sieves (3Å) were added and the solution was stirred under nitrogen at RT for 1 hour. TMSI (1.0 g, 5.0 mmol, 1.3 eq.) was added and the reaction was stirred for 24 hours. Triethylamine (1 mL) was added to quench the reaction, followed by dilution in Et_2O. The organic layer was washed with sat. aq. $Na_2S_2O_3$ (2x), sat. aq. $NaHCO_3$ (2x) and brine (1x). The organic layer was dried over $MgSO_4$, filtered and concentrated. The yellow oil was purified by silicagel chromatography (PE:EtOAc 19:1 → 4:1). Compound **12** was obtained as colourless oil (1.55 g, 2.43 mmol, 63%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.95 – 7.84 (m, 2H, arom.), 7.80 (ddd, 3H, *J*=7.2, 6.0, 1.5 Hz, arom.), 7.72 – 7.64 (m, 3H, arom.), 7.59 – 7.51 (m, 1H, arom.), 7.51 – 7.41 (m, 3H, arom.), 7.38 (dd, 1H, *J*=8.5, 1.7 Hz, arom.), 7.35 – 7.20 (m, 6H, arom.), 5.77 (dd, 1H, *J*=10.5, 3.4 Hz, H-3), 5.66 (d, 1H,

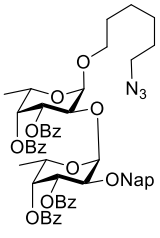
J=3.4 Hz, H-4), 4.99 (d, 1H, *J*=3.5 Hz, H-1), 4.89 – 4.76 (m, 2H, CH_2Nap), 4.31 (q, 1H, *J*=6.6 Hz. H-5), 4.17 (dd, 1H, *J*=10.5, 3.5 Hz, H-2), 3.74 (dt, 1H, *J*=9.6, 6.7 Hz, OCH_2), 3.50 (dt, 1H, *J*=9.6, 6.4 Hz, OCH_2), 3.25 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.77 – 1.55 (m, 4H, CH_2, hexyl), 1.45 (m, 4H, CH_2, hexyl), 1.18 (d, 3H, *J*=6.6 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 166.0, 165.7 (C=O, Bz), 135.5, 133.3, 133.2, 133.1, 133.0, 129.9, 129.8, 129.7, 128.6, 128.5, 128.3, 128.3, 128.0, 127.8, 127.0, 126.3, 126.1, 126.0 (arom.), 97.5 (C-1), 73.4 (C-2), 72.9 (CH_2Nap), 72.6 (C-4), 70.7 (C-3), 68.5 (OCH_2), 64.9 (C-5), 51.5 (CH_2N_3), 29.5, 28.9, 26.7, 26.0 (CH_2, hexyl), 16.2 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{37}H_{39}N_3O_7Na$: 660.2686, found 660.2674.

6-Azidohexyl 3,4-di-*O*-benzoyl-α-L-fucopyranoside (13)



Fucoside **12** (0.83 g, 1.3 mmol, 1.0 eq.) was dissolved in a 9/1 mixture of DCM/MeOH (13 mL, 0.1M). DDQ (0.89 g, 3.9 mmol, 3 eq.) was added and the reaction was stirred until completion (approx. 3 hours). The reaction mixture was taken up in EtOAc and washed with sat. aq. $Na_2S_2O_3$ (2x), sat. aq. $NaHCO_3$ until the water layer was no longer yellow and with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (PE:EtOAc, 9:1 →, 7:3) to give compound **13** as a colourless viscous oil (0.52 g, 1.05 mmol, 85%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.12 – 8.04 (m, 2H, arom.), 7.87 (d, 2H, *J*=7.3 Hz, arom.), 7.62 (t, 1H, *J*=7.4 Hz, arom.), 7.49 (t, 3H, *J*=7.8 Hz, arom.), 7.29 (dd, 2H, *J*=16.2, 8.4 Hz, arom.), 5.63 (d, 1H, *J*=3.0 Hz, H-4), 5.54 (dd, 1H, *J*=10.3, 3.3 Hz, H-3), 5.05 (d, 1H, *J*=3.8 Hz, H-1), 4.29 (q, 1H, *J*=6.5 Hz, H-5), 4.26 – 4.18 (m, 1H, H-2), 3.82 (dt, 1H, *J*=9.6, 6.7 Hz, CH_2O), 3.55 (dt, 1H, *J*=9.7, 6.5 Hz, CH_2O), 3.30 (t, 2H, *J*=6.8 Hz, CH_2N_3), 2.10 (d, 1H, *J*=11.1 Hz, OH), 1.76 – 1.58 (m, 4H, CH_2, hexyl), 1.48 – 1.41 (m, 4H, CH_2, hexyl), 1.24 (d, 3H, *J*=6.6 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 166.4 (C=O, Bz), 166.0 (C=O, Bz), 133.4, 133.1, 129.9, 129.8, 129.6, 128.6, 128.3 (arom.), 98.9 (C-1), 72.2 (C-4), 72.0 (C-3), 68.6 (CH_2O), 67.8 (C-2), 65.3 (C-5), 51.4 (CH_2N_3), 29.4, 28.8, 26.6, 25.9 (CH_2, hexyl), 16.2 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{26}H_{31}N_3O_7Na$: 520.2060, found 520.2058.

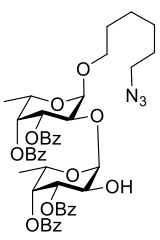
6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2-naphthylmetyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (14)



Acceptor **13** (0.48 g, 0.97 mmol, 1 eq.) and donor **8** (0.88 g, 1.45 mmol, 1.5 eq.) were coevaporated together with dry toluene (3x), after which they were dissolved in dry DCM (10 mL, 0.1M). NIS (0.65 g, 2.89 mmol, 3 eq.) and molecular sieves (3Å) were added to the reaction and the mixture was stirred under nitrogen atmosphere at RT for 30 min. After 30 min. The solution was cooled to - 40°C and after 30 min. at this

temperature TMSOTf (17 µL, 0.1 mmol, 0.1 eq.) was added. The reaction was heated to - 10°C over a period of 4 hours. When TLC analysis showed complete consumption of the acceptor, Et_3N (0.1 mL) was added to quench the reaction. The solution was poured into Et_2O, washed with sat. aq. $Na_2S_2O_3$ (1x), sat. aq. $NaHCO_3$ (2x) and with brine, dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (PE:EtOAc, 9:1 →, 4:1) followed by size exclusion (LH-20, DCM/MeOH, 1/1) to give compound **14** as a colourless oil (0.78 g, 0.79 mmol, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.08 (d, 2H, *J*=7.6 Hz, Bz), 7.81 (m, 7H, arom.), 7.74 – 7.58 (m, 4H, arom.), 7.57 – 7.41 (m, 7H, arom.), 7.41 – 7.18 (m, 7H, arom.), 5.83 (dd, 1H, *J*=10.4, 3.0 Hz, H-3), 5.75 – 5.65 (m, 2H, H-4, H-3'), 5.41 (d, 1H, *J*=2.0 Hz, H-4'), 5.18 (d, 1H, *J*=2.8 Hz, H-1), 5.15 (d, 1H, *J*=2.9 Hz, H-1'), 4.82 (m, 2H, CH_2-Nap), 4.45 – 4.36 (m, 2H, H-2, H-5), 4.26 (q, 1H, *J*=6.0 Hz, H-5'), 4.15 (dd, 1H, *J*=10.4, 3.0 Hz, H-2'), 3.68 (q, 1H, *J*=6.6 Hz, CH_2O), 3.44 (q, 1H, *J*=6.7 Hz, CH_2O), 3.15 (t, 1H, *J*=6.8 Hz, CH_2N_3), 1.63 – 1.43 (m, 4H, CH_2, hexyl), 1.35 – 1.20 (m, 7H, CH_2, hexyl, H-6), 0.74 (d, 3H, *J*=6.4 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 166.0, 165.8, 165.6, 165.3 (C=O, Bz), 135.3, 133.4, 133.2, 133.1, 133.0, 129.9, 129.8, 129.7, 129.6, 128.6, 128.5, 128.3, 127.9, 127.8, 126.9, 126.3, 126.2, 125.9 (arom.), 98.6 (C-1'), 97.6 (C-1), 74.7 (C-2), 73.5 (C-2'), 73.2 (CH_2Nap), 72.5 (C-4), 72.3 (C-4'), 70.7 (C-3), 69.9 (C-3'), 69.2 (CH_2O), 65.6 (C-5'), 64.9 (C-5), 51.5 (CH_2N_3), 29.5, 28.8, 26.6, 25.8 (CH_2, hexyl), 16.2 (C-6), 15.7 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{57}H_{57}N_3O_{13}Na$: 1014.3789, found 1014.3801.

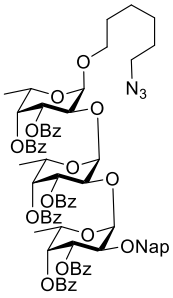
6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-benzoyl-α-L-fucopyranosyl)-α-L-fucopyranoside (15)



Disaccharide **14** (0.25 g, 0.25 mmol, 1 eq.) was dissolved in a 9/1 mixture of DCM/MeOH (2.5 mL, 0.1M). DDQ (0.23 g, 1.0 mmol, 4 eq.) was added portionwise (1 eq. per hour) and the reaction was left to stir under nitrogen atmosphere at RT. After 4 hours TLC analysis (tol:EtOAc, 4:1) showed full conversion of starting material. The reaction was poured in EtOAc and washed with sat. aq. $Na_2S_2O_3$ (2x), sat. aq. $NaHCO_3$ until the water layer was no longer yellow, followed by brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (DCM: EtOAc, 1:0 → 4:1) yielding compound **15** as a colourless oil (0.19 g, 0.22 mmol, 89%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.13 – 8.06 (m, 2H, arom.), 8.03 – 7.98 (m, 2H, arom.), 7.90 (dd, 2H, *J*=8.3, 1.2 Hz, arom.), 7.84 (dd, 2H, *J*=8.3, 1.2 Hz, arom.), 7.67 – 7.55 (m, 2H, arom.), 7.55 – 7.41 (m, 6H, arom.), 7.37 – 7.21 (m, 3H, arom.), 5.79 – 5.70 (m, 2H, H-3, H-4), 5.41 – 5.30 (m, 2H, H-3', H-4'), 5.17 (m, 2H, H-1, H-1'), 4.44 (dd, 1H, *J*=10.0, 3.8 Hz, H-2), 4.33 (q, 1H, *J*=6.6 Hz, H-5), 4.20 – 4.01 (m, 2H, H-2', H-5'), 3.79 (dt, 1H, *J*=9.4, 6.7 Hz, CH_2O), 3.50 (dt, 1H, *J*=9.5, 6.6 Hz, CH_2O), 3.25 (t, 1H, *J*=6.8 Hz, CH_2N_3), 2.62 (d, 1H, *J*=11.9 Hz, OH), 1.65 (m,

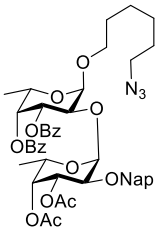
4H, CH_2, hexyl), 1.49 – 1.39 (m, 4H, CH_2, hexyl), 1.27 (d, 3H, *J*=6.8, H-6), 0.82 (d, 2H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 166.1 (C=O, Bz), 166.0 (C=O, Bz), 165.8 (C=O, Bz), 165.5 (C=O, Bz), 133.5, 133.4, 133.1, 129.9, 129.8, 129.7, 129.6, 129.5, 128.7, 128.6, 128.5, 128.3 (arom.), 98.3 (C-1'), 96.3 (C-1), 72.9 (C-2), 72.0 (C-4), 71.8 (C-4'), 71.8, C-3'), 69.5 (C-3), 68.3 (CH_2O), 67.3 (C-2'), 65.9 (C-5'), 65.0 (C-5), 51.4 (CH_2N_3), 29.4, 28.8, 26.6, 25.9 (CH_2, hexyl), 16.2 (C-6), 15.8 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{46}H_{49}N_3O_{13}Na$: 874.3163, found 874.3163.

6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2-naphthylmetyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (16)



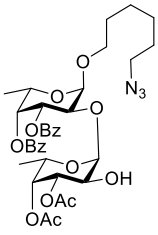
Acceptor **15** (61 mg, 70 µmol, 1 eq.) and imidate donor **11** (75 mg, 0.11 mmol, 1.5 eq.) were dissolved in dry DCM (0.7 mL, 0.1M), after being coevaporated together with toluene (3x). Molecular sieves (3Å) were added and the solution was cooled to -40°C, at which temperature it was stirred for 30 min. under nitrogen atmosphere. TMSOTf (1.3 µL, 7 µmol, 0.1 eq.) was added and the reaction was slowly warmed to -10°C over a period of 5 hours. The reaction was stopped by addition of Et_3N (0.1 mL) and diluted in DCM. The organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The yellow oil was purified using size exclusion (LH-20, DCM/MeOH, 1/1) giving trisaccharide **16** as a colourless film (15 mg, 10 µmol, 16%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.98 – 7.88 (m, 6H, arom.), 7.81 – 7.62 (m, 10H, arom.), 7.46 (m, 13H, arom.), 7.28 (m, 10H, arom.), 7.16 (t, 2H, *J*=7.8 Hz, arom.), 5.89 (dd, 1H, *J*=7.2, 3.4 Hz, H-3), 5.80 (dd, 1H, *J*=7.2, 3.4 Hz, H-3'), 5.71 – 5.64 (m, 2H, H-4, H-3''), 5.59 – 5.50 (m, 2H, H-1, H-4'), 5.34 (d, 1H, *J*=2.6 Hz, H-4''), 5.23 (d, 1H, *J*=3.1 Hz, H-1'), 4.95 (d, 1H, *J*=3.4 Hz, H-1''), 4.93 – 4.84 (m, 2H, CH_2-Nap), 4.48 – 4.37 (m, 3H, H-2, H-5, H-5'), 4.27 (dd, 1H, *J*=7.6, 3.1 Hz, H-2'), 4.19 – 4.10 (m, 2H, H-2'', H-5''), 3.92 – 3.74 (m, 2H, CH_2O), 3.30 (t, 1H, *J*=7.0 Hz, CH_2N_3), 1.84 – 1.45 (m, 8H, CH_2, hexyl), 1.21 (d, 3H, *J*=6.5 Hz, H-6), 0.74 (d, 3H, *J*=6.4 Hz, H-6'), 0.62 (d, 2H, *J*=6.5 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 166.1 (C=O, Bz), 165.9 (C=O, Bz), 165.9 (C=O, Bz), 165.8 (C=O, Bz), 165.4 (C=O, Bz), 165.2 (C=O, Bz), 134.9, 133.4, 133.3, 133.2, 133.2, 133.0, 130.2, 130.0, 129.9, 129.9, 129.8, 129.8, 129.7, 129.7, 129.6, 128.9, 128.6, 128.5, 128.3, 128.1, 127.9, 127.8, 126.5, 126.4, 126.2 (arom.), 100.6 (C-1'), 99.2 C-1''), 98.2 (C-1), 78.1 (C-2), 75.5 (C-2'), 73.4 (CH_2Nap), 72.9 (C-2''), 72.5 (C-4), 72.3 (C-4'), 72.0 (C-4''), 70.7 (C-3''), 69.4 (C-3), 69.3 (C-3'), 66.1 (C-5'), 65.7 (C-5''), 64.6 (C-5), 51.7 (CH_2N_3), 29.8, 29.0, 26.8, 26.0 (CH_2, hexyl), 16.3 (C-6), 15.7 (C-6''), 15.6 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{77}H_{75}N_3O_{19}Na$: 1368.4893, found 1368.4919.

6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-acetyl-2-*O*-(2-naphthylmetyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (17)



Donor **9** (0.77 g, 1.6 mmol, 1.5 eq.) and acceptor **13** (0.52 mmol, 1.05 mmol, 1 eq.) were coevaporated together using dry toluene (3x), after which they were dissolved in dry DCM (10 mL, 0.1M). NIS (0.71 g, 3.15 mmol, 3 eq.) and molecular sieves (3Å) were added to the reaction and the mixture was stirred under nitrogen atmosphere at RT for 30 min. After 30 min. The solution was cooled to - 40°C and after 30 min. at this temperature TMSOTf (18 µL, 0.1 mmol, 0.1 eq.) was added. The reaction was heated to - 10°C over a period of 4 hours. When TLC analysis showed complete consumption of the acceptor Et_3N (0.1 mL) was added to quench the reaction. The solution was poured into Et_2O, washed with sat. aq. $Na_2S_2O_3$ (1x), sat. aq. $NaHCO_3$ (2x) and with brine, dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (PE:EtOAc, 9:1 →, 7:3) followed by size exclusion (LH-20, DCM/MeOH, 1/1, v/v) to give compound **17** as a colourless oil (0.64 g, 0.73 mmol, 70%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.09 – 8.02 (m, 2H, arom.), 7.85 – 7.73 (m, 6H, arom.), 7.65 – 7.58 (m, 1H, arom.), 7.53 – 7.41 (m, 6H, arom.), 7.30 – 7.21 (m, 2H, arom.), 5.76 (dd, 1H, *J*=10.5, 3.5 Hz, H-3), 5.71 – 5.65 (d, 1H, *J*=3.2 Hz, H-4), 5.28 (dd, 1H, *J*=10.6, 3.3 Hz, H-3'), 5.12 (d, 1H, *J*=3.5 Hz, H-1), 5.02 (m, 2H, H-1', H-4'), 4.89 – 4.70 (m, 2H, CH_2-Nap), 4.33 (m, 2H, H-2, H-5'), 4.10 (q, 1H, *J*=4.8, 7.2 Hz, H-5), 3.85 (dd, 1H, *J*=6.8, 3.6 Hz, H-2'), 3.64 (dt, 1H, *J*=9.5, 6.7 Hz, CH_2O), 3.45 – 3.35 (m, 1H, CH_2O), 3.16 (t, 2H, *J*=7.0 Hz', CH_2N_3), 2.04 (s, 3H, CH_3-Ac), 1.94 (s, 3H, CH_3-Ac), 1.58 – 1.41 (m, 4H, CH_2, hexyl), 1.30 – 1.20 (m, 7H, H-6', CH_2, hexyl), 0.69 (d, 3H, *J*=6.5 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 170.4 (C=O, Ac), 169.8 (C=O, Ac), 165.9 (C=O, Bz), 165.5 (C=O, Bz), 135.6, 133.3, 133.2, 133.0, 133.0, 129.8, 129.7, 129.5, 128.5, 128.2, 128.2, 127.8, 127.7, 126.3, 126.3, 126.1, 125.5 (arom.), 97.9 (C-1'), 97.3 (C-1), 73.9 (C-2), 73.5 (C-2'), 73.0 (CH_2Nap), 72.3 (C-4), 71.4 (C-4'), 70.0 (C-3), 69.8 (C-3'), 68.9 (CH_2O), 64.9 (C-5), 64.7 (C-5'), 51.3 (CH_2N_3), 29.3, 28.6, 26.4, 25.6 (CH_2, hexyl), 20.8 (CH_3, Ac), 20.6 (CH_3, Ac), 16.1 (C-6'), 15.5 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{47}H_{53}N_3O_{13}Na$: 890.3476, found 890.3489.

6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-acetyl-α-L-fucopyranosyl)-α-L-fucopyranoside (18)

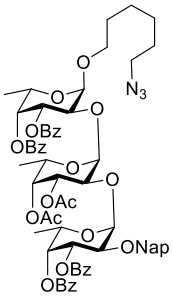


Disaccharide **17** (0.64 g, 0.73 mmol, 1 eq.) was dissolved in a 9/1 mixture of DCM/MeOH (7.5 mL, 0.1M). DDQ (0.40 g, 2.92 mmol, 3 eq.) was added portionwise (1 eq. per hour) and the reaction was left to stir under nitrogen atmosphere at RT. After 5 hours TLC analysis (PE:EtOAc, 3:2) showed full conversion of starting material. The reaction was poured in EtOAc and washed with sat. aq. $Na_2S_2O_3$ (2x),

sat. aq. $NaHCO_3$ until the water layer was no longer yellow, followed by brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (PE: EtOAc, 4:1 → 3:2) yielding compound **18** as a colourless oil (0.46 g, 0.64 mmol, 87%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.11 – 8.02 (m, 2H, arom.), 7.88 – 7.81 (m, 2H, arom.), 7.68 – 7.60 (m, 1H, arom.), 7.54 – 7.44 (m, 3H, arom.), 7.35 – 7.26 (m, 2H, arom.), 5.73 – 5.63 (m, 2H, H-3, H-4), 5.11 (d, 1H, *J*=3.7 Hz, H-1), 5.03 (d, 1H, *J*=4.0 Hz, H-1'), 4.97 – 4.89 (m, 2H, H-3', H-4'), 4.37 (dd, 1H, *J*=10.2, 3.7 Hz, H-2), 4.30 (q, 1H, *J*=6.5 Hz, H-5), 3.93 – 3.73 (m, 3H, H-2', H-5', CH_2O), 3.49 (dt, 1H, *J*=9.4, 6.5 Hz, CH_2O), 3.29 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.53 (d, 1H, *J*=11.7 Hz, OH), 2.09 (s, 3H, CH_3-Ac), 2.01 (s, 3H, CH_3 -Ac), 1.66 (dt, 5H, *J*=17.0, 6.7 Hz, CH_2, hexyl), 1.48 – 1.39 (m, 4H, CH_2, hexyl), 1.25 (d, 3H, *J*=6.5 Hz, H-6), 0.73 (d, 2H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 170.5 (C=O, Ac), 170.4 (C=O, Ac), 165.9 (C=O, Bz), 165.3 (C=O, Bz), 133.5, 133.3, 129.8, 129.6, 129.5, 129.4, 128.6, 128.4 (arom.), 97.9 (C-1), 96.1 (C-1'), 72.5 (C-2), 71.8 (C-4), 71.1 (C-3'), 70.9 (C-4'), 69.4 (C-3), 68.1 (CH_2O), 66.5 (C-2'), 65.3 (C-5'), 64.9 (C-5), 51.3 (CH_2N_3), 29.3, 28.7, 26.5, 25.8 (CH_2, hexyl), 20.9, (CH_3, Ac), 20.6 (CH_3, Ac), 16.1 (C-6), 15.6 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{36}H_{45}N_3O_{13}Na$: 750.2850, found 750.2852.

3

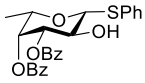
6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-acetyl-2-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2-naphthylmetyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (19)



Acceptor **18** (81 mg, 0.11 mmol, 1 eq.) and imidate donor **11** (0.26 g, 0.37 mmol, 4 eq.) were dissolved in dry DCM (1.0 mL, 0.1M), after being coevaporated together with toluene (3x). Molecular sieves (3Å) were added and the solution was cooled to -40°C, at which temperature it was stirred for 30 min. under nitrogen atmosphere. TMSOTf (1.7 µL, 10 µmol, 0.1 eq.) was added and the reaction was slowly warmed to -10°C over a period of 5 hours. The reaction was stopped by addition of Et_3N (0.1 mL) and diluted in DCM. The organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The yellow oil was purified using size exclusion (LH-20, DCM/MeOH, 1/1) followed by silicagel chromatography (PE:EtOAc,19:1, 7:3) giving trisaccharide **19** as a colourless film (35 mg, 30 µmol, 26%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.99 – 7.93 (m, 2H, arom.), 7.89 – 7.82 (m, 4H, arom.), 7.78 (d, 3H, *J*=8.0 Hz, arom.), 7.71 – 7.64 (m, 3H, arom.), 7.60 – 7.38 (m, 8H, arom.), 7.35 – 7.25 (m, 6H, arom.), 7.19 (t, 2H, *J*=7.8 Hz, arom.), 5.82 (dd, 1H, *J*=10.3, 3.5 Hz, H-3), 5.72 (dd, 1H, *J*=10.5, 3.3 Hz, H-3''), 5.66 (d, 1H, *J*=2.8 Hz, H-4), 5.61 (d, 1H, *J*=2.5 Hz, H-4''), 5.44 (d, 1H, *J*=3.5 Hz, H-1), 5.32 (dd, 1H, *J*=10.6, 3.3 Hz, H-3'), 5.15 (d, 1H, *J*=2.0 Hz, H-4'), 5.11 (d, 1H, *J*=3.3 Hz, H-1'), 4.95 (d, 1H, *J*=3.4 Hz, H-1''), 4.86 (s, 1H, CH_2-Nap), 4.48 – 4.29 (m, 3H, H-2, H-5, H-5''), 4.24 – 4.13 (m, 2H, H-5', H-2''), 3.97 (dd, 1H, *J*=10.6, 3.3 Hz, H-2'), 3.79 (dt, 1H,

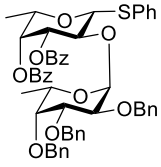
J=9.7, 6.6 Hz, CH_2O), 3.70 (dt, 1H, *J*=9.8, 6.6 Hz, CH_2O), 3.27 (t, 2H, *J*=7.0 Hz, CH_2N_3), 2.04 (s, 3H, CH_3-Ac), 1.99 (s, 3H, CH_3-Ac), 1.76 – 1.57 (m, 4H, CH_2, hexyl), 1.49 – 1.41 (m, 4H, CH_2, hexyl), 1.20 (d, 3H, *J*=6.6 Hz, H-6), 1.13 (d, 3H, *J*=6.6 Hz, H-6''), 0.67 (d, 2H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 170.5 (C=O, Ac), 169.9 (C=O, Ac), 166.1 (C=O, Bz), 165.9 (C=O, Bz), 165.8 (C=O, Bz), 165.4 (C=O, Bz), 135.0, 133.4, 133.2, 133.2, 133.0, 130.1, 129.9, 129.9, 129.8, 129.7, 129.7, 128.7, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.6, 126.5, 126.4, 126.2 (arom.), 99.9 (C-1'), 98.7 (C-1''), 98.0 (C-1), 77.3 (C-2), 74.4 (C-2'), 73.3 (CH_2, Nap), 72.7 (C-4), 72.5 (C-4''), 72.4 (C-2''), 71.7 (C-4'), 70.7 (C-3''), 70.5 (C-3), 69.3 (C-3'), 69.0 (CH_2O), 65.6 (C-5), 65.6 (C-5''), 64.6 (C-5'), 51.6 (CH_2N_3), 29.7, 28.9, 26.7, 25.9 (CH_2, hexyl), 21.0 (CH_3, Ac), 20.7 (CH_3, Ac), 16.3 (C-6), 16.2 (C-6''), 15.4 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{67}H_{71}N_3O_{19}Na$: 1244.4579, found 1244.4573.

Phenyl 3,4-di-*O*-benzoyl-1-thio-α-L-fucopyranoside (20)



Sugar **8** (3.02 g, 5.0 mmol, 1.0 eq.) was dissolved in a mixture of DCM and water (50 mL, 0.1M, 4/1, v/v). DDQ (3.41 g, 15.0 mmol, 3.0 eq.) was added portionwise to the solution over a period of 30 min. TLC analysis showed full conversion to a more polar spot after 3 hours. The reaction mixture was diluted in EtOAc and washed with sat. $Na_2S_2O_3$ (aq., 2x), sat. $NaHCO_3$ (aq., 5x) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The dark yellow residue was purified by silicagel chromatography (PE:EtOAc, 9:1 → 7:3) to give the title compound as a yellow oil (2.3 g, 4.9 mmol, 99%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.90 – 7.84 (m, 2H, arom.), 7.83 – 7.78 (m, 2H, arom.), 7.73 – 7.67 (m, 2H, arom.), 7.63 – 7.56 (m, 1H, arom.), 7.47 – 7.38 (m, 6H, arom.), 7.31 – 7.22 (m, 4H, arom.), 5.62 (dd, 1H, *J*=3.3, 1.0 Hz. H-4), 5.37 (dd, 1H, *J*=9.7, 3.3 Hz, H-3), 4.69 (d, 1H, *J*=9.5 Hz, H-1), 4.13 – 3.87 (m, 2H, H-2, H-5), 3.00 – 2.74 (m, 1H, 2-OH), 1.31 (d, 3H, *J*=6.4 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 166.1, 165.8 (C=O, Bz), 134.1, 133.5, 133.3, 130.8, 129.9, 129.9, 129.4, 129.1, 128.6, 128.5, 128.3 (arom.), 87.8 (C-1), 75.0 (C-3), 73.8 (C-5), 71.5 (C-4), 67.1 (C-2), 16.8 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{26}H_{24}O_6SNa$: 487.11913, found 487.11858.

Phenyl 3,4-di-*O*-benzoyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1-thio-β-L-fucopyranoside (22)

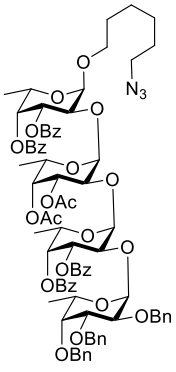


MS (3Å) were added to a solution containing donor **21** (0.32 g, 0.60 mmol, 2.0 eq.) and acceptor **20** (0.14 mmol, 0.30 mmol, 1.0 eq.) in dry DCM (3 mL, 0.1M). the solution was cooled to 0˚C and stirred at that temperature for 30 min. before addition of IDCP (0.42 g, 0.90 mmol, 3.0 eq.). The solution turned red and the ice bath was removed. After 4 hours TLC analysis showed full consumption of acceptor **20**. The reaction mixture was

diluted in EtOAc and washed with sat. $Na_2S_2O_3$ (aq., 2x), sat. $NaHCO_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The dark yellow residue was purified by silicagel chromatography (PE:EtOAc, 19:1 → 4:1) to give disaccharide **22** as a yellow oil (0.195 g, 0.22 mmol, 74%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.94 – 7.83 (m, 2H, arom.), 7.74 – 7.53 (m, 5H, arom.), 7.49 – 7.39 (m, 3H, arom.), 7.38 – 7.14 (m, 20H, arom.), 5.87 (d, 1H, *J*=3.7 Hz, H-1'), 5.63 – 5.49 (m, 2H, H-3, H-4), 4.97 – 4.72 (m, 6H, H-1, CH_2Bn), 4.59 (d, 1H, *J*=11.7 Hz, CH_2Bn), 4.44 (d, 1H, *J*=11.5 Hz, CH_2Bn), 4.28 (t, 1H, *J*=9.3 Hz, H-2), 4.06 (dd, 1H, *J*=10.3, 3.7 Hz, H-2'), 4.00 (q, 1H, *J*=6.3 Hz, H-5), 3.77 (dd, 1H, *J*=10.3, 2.8 Hz, H-3'), 3.72 (q, 1H, *J*=6.4, 1.3 Hz, H-5'), 3.19 (dd, 1H, *J*=2.8, 1.4 Hz, H-4'), 1.28 (d, 4H, *J*=6.4 Hz, H-6), 0.61 (d, 3H, *J*=6.4 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 165.9, 165.5 (C=O, Bz), 138.9, 138.5, 138.4, 134.5, 133.4, 133.2, 133.0, 131.9, 129.9, 129.6, 129.6, 129.4, 129.0, 128.6, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.5, 127.5, 127.5, 127.4, 127.4 (arom.), 96.7 (C-1'), 86.1 (C-1), 78.8 (C-3'), 77.6 (C-4'), 76.0 (C-2'), 74.8 (CH_2Bn), 74.2 (C-3), 73.5 (CH_2Bn), 73.2 (CH_2Bn), 72.9 (C-5), 71.3 (C-4), 69.8 (C-2), 66 (C-5').7, 16.7 (C-6), 16.3 (C-6') ppm. HRMS: $[M+NH_4]^+$ calcd for $C_{53}H_{52}O_{10}SNH_4$: 899.36249, found 899.29248.

3

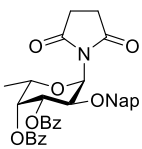
6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-acetyl-2-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (23)



Disaccharide acceptor **18** (0.12 g, 0.16 mmol, 1 eq.) and disaccharide donor **22** (0.29 g, 0.32 mmol, 2.0 eq.) were co-evaporated using toluene (3x) and subsequently dissolved in dry DCM (1.6 mL, 0.1 M). Flame dried molecular sieves (3Å) were added followed by NIS (0.15 g, 0.65 mmol, 4 eq.). The reaction was cooled to - 40°C and stirred under nitrogen at that temperature for 30 min. TMSOTf (2.9 µL, 16 µmol, 0.1 eq.) was added and the reaction was slowly warmed to -20 °C. After 3 hours the reaction was quenched by addition of Et_3N (0.1 mL) and diluted in EtOAc. The organic layer was washed with sat. aq. $Na_2S_2O_3$ (2x), sat. aq. $NaHCO_3$ (1x) and by brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (PE:EtOAc, 19:1 → 7:3) followed by size exclusion (LH-20, DCM/MeOH, 1/1) to give tetrasaccharide **23** as a colourless film (41 mg, 27 µmol, 17%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.17 – 7.99 (m, 4H, arom.), 7.81 (d, 4H, *J*=7.9 Hz, arom.), 7.67 – 7.57 (m, 1H, arom.), 7.54 – 7.43 (m, 4H, arom.), 7.43 – 6.99 (m, 34H, arom.), 5.86 (dd, 1H, *J*=10.5, 3.3 Hz, H-3), 5.81 – 5.72 (m, 2H, H-4, H-3''), 5.60 (d, 1H, *J*=2.7 Hz, H-4''), 5.48 (dd, 1H, *J*=10.5, 3.5 Hz, H-3'), 5.41 (d, 1H, *J*=3.2 Hz, H-1'), 5.33 (d, 1H, *J*=3.3 Hz, H-1), 5.18 (d, 1H, *J*=3.0 Hz, H-1''), 5.03 (d, 1H, *J*=2.5 Hz, H-4'), 4.91 – 4.73 (m, 3H, CH_2-Bn), 4.70 (d, 1H, *J*=3.6 Hz, H-1'''), 4.49 – 4.37 (m, 5H, H-2, H-5, H-5'', CH_2-Bn), 4.29 (dd, 1H,

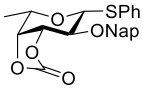
J=10.5, 3.3 Hz, H-2'), 4.22 – 4.14 (m, 3H, H-5', H-2'', CH_2-Bn), 3.94 (dd, 1H, *J*=10.2, 3.5 Hz, H-2'''), 3.88 (dt, 1H, *J*=9.3, 6.8 Hz, CH_2O), 3.77 (dt, 1H, *J*=11.4, 4.8 Hz, CH_2O), 3.74 – 3.63 (m, 2H, H-3''', H-5'''), 3.28 (t, 2H, *J*=6.6 Hz, CH_2N_3), 3.22 (s, 1H, H-4'''), 2.05 (s, 3H, CH_3-Ac), 1.75 (s, 3H, CH_3-Ac), 1.71 – 1.61 (m, 4H, CH_2, hexyl), 1.56 – 1.43 (m, 4H, CH_2, hexyl), 1.30 (d, 3H, *J*=6.5 Hz, H-6), 1.17 (d, 2H, *J*=6.5 Hz, H-6''), 0.67 – 0.52 (m, 6H, H-6', H-6''') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 170.5 (C=O, Ac), 170.0 (C=O, Ac), 166.1 (C=O, Bz), 166.1 (C=O, Bz), 166.0 (C=O, Bz), 165.4 (C=O, Bz), 138.6, 138.5, 133.5, 133.3, 133.2, 132.9, 130.0, 129.9, 129.8, 129.7, 129.6, 129.1, 128.7, 128.7, 128.6, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.6, 127.3, 126.8, 125.4 (arom.), 100.2, 100.1, 99.8, 98.1, 80.0 (C-3'''), 77.3 (C-2'), 76.8 (C-4'''), 75.6 (C-2), 75.2 (C-2''), 74.6 (CH_2, Bn), 73.4 (C-2'''), 73.3 (CH_2, Bn), 72.8 (C-4), 72.6 (C-4''), 72.1 (CH_2, Bn), 72.0 (C-4'), 70.6 (C-3), 69.6 (C-3''), 69.3 (C-3'), 69.0 (CH_2O), 67.2, 66.5 (C-5'''), 65.4 (C-5), 65.0 (C-5'), 51.5 C-5''), 29.8, 28.9, 26.8, 26.1 (CH_2, hexyl), 21.2, (CH_3, Ac), 20.5 (CH_3, Ac), 16.3 (C-6), 16.2 (C-6'), 16.1 (C-6'''), 15.6 (C-6'') ppm. HRMS $[M+Na]^+$ calcd for $C_{83}H_{91}N_3O_{23}Na$: 1520.5941, found 1520.5991.

Succinimido 3,4-di-*O*-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranoside (24)



^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.91 – 7.83 (m, 2H, arom.), 7.76 (m, 3H, arom.), 7.69 – 7.64 (m, 1H, arom.), 7.62 – 7.39 (m, 6H, arom.), 7.34 – 7.16 (m, 6H, arom.), 6.45 (dd, 1H, *J*=10.2, 3.6 Hz, H-3), 6.31 (d, 1H, *J*=7.5 Hz, H-1), 5.73 (d, 1H, *J*=3.7 Hz, H-4), 4.75 – 4.60 (m, 3H, H-5, CH_2Nap), 4.49 (dd, 1H, *J*=10.2, 7.5 Hz, H-2), 2.83 – 2.61 (m, 4H, CH_2, succinimide), 1.17 (d, 3H, *J*=6.5 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 178.1 (C=O, succinimide), 166.0, 165.4 (C=O, Bz), 134.4, 133.3, 133.1, 133.0, 129.8, 129.8, 129.7, 128.6, 128.5, 128.3, 128.0, 127.8, 127.0, 126.4, 126.2, 125.8 (arom.), 76.3 (C-1), 73.3 (CH_2Nap), 72.3 (C-4), 71.8 (C-5), 71.4 (C-3), 71.2 (C-2), 28.4 (CH_2, succinimide), 17.1 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{35}H_{31}NO_8Na$: 616.19474, found 616.19419.

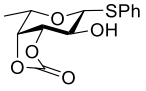
Phenyl 3,4-*O*-carbonate-2-*O*-(2-naphthylmethyl)-1-thio-α-L-fucopyranoside (25)



Fucoside **7** (0.40 g, 1.0 mmol, 1.0 eq.) was dissolved in dry DCM (10 mL, 0.1M) and cooled to 0˚C. Triphosgene (0.15 g, 0.5 mmol, 0.5 eq.) was added, followed by pyridine (0.32 mL, 4.0 mmol, 2.0 eq.). Upon addition of pyridine the reaction mixture turned from colourless to yellow. After 30 min. TLC analysis showed full conversion of the starting material into a less polar spot. The reaction was stopped by addition of sat. $NaHCO_3$ (aq., 3 mL) and transferred to a separatory funnel. The water layer was removed and the organic layer was washed with 1M HCl (aq., 3x) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound was obtained as a colourless oil (0.38 g, 0.90 mmol, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.86 – 7.75 (m, 4H, arom), 7.54 – 7.42

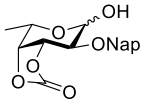
(m, 5H, arom), 7.29 – 7.22 (m, 3H, arom), 4.84 – 4.76 (m, 2H, CH_2-Nap), 4.74 (dd, 1H, *J*=7.4, 5.4 Hz, H-3), 4.71 (d, 1H, *J*=8.0 Hz, H-1), 4.43 (dd, 1H, *J*=7.4, 1.9 Hz, H-4), 3.72 (qd, 1H, *J*=6.5, 1.9 Hz, H-5), 3.65 (dd, 1H, *J*=8.0, 5.5 Hz, H-2), 1.33 (d, 2H, *J*=6.6 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 153.8 (C=O), 134.3, 133.1, 132.5, 132.4, 129.1, 128.4, 128.1, 128.0, 127.7, 127.2, 126.4, 126.3, 126.0 (arom.), 85.3 (C-1), 78.8 (C-3), 77.3 (C-4), 76.8 (C-2), 73.8 (CH_2Nap), 71.2 (C-5), 16.2 (C-6) ppm. HRMS $[M+H]^+$ calcd for $C_{24}H_{22}O_5SH$: 423.12679, found 423.12607.

Phenyl 3,4-*O*-carbonate-1-thio-α-L-fucopyranoside (26)



Fucosyl **25** (7.16 g, 16.9 mmol, 1.0 eq.) was dissolved in a 9/1 mixture of DCM/MeOH (170 mL, 0.1M). DDQ (11.51 g, 50.7 mmol, 3.0 eq.) was added portionwise over a period of 3 hours. The reaction was stirred at RT under nitrogen for 5 hours. The reaction was poured into EtOAc, washed with sat. aq. $Na_2S_2O_3$ (3x), sat. aq. Na_2CO_3 until the water layer remained colourless and brine (1x). The organic layer was concentrated *in vacuo*. The crude mix was purified using silica gel chromatography (PE: EtOAc, 9:1 →3:2) giving **26** as a white amorphous solid (3.74 g, 13.2 mmol, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.57 – 7.51 (m, 2H), 7.38 – 7.31 (m, 3H), 4.69 (t, 1H, *J*=6.8 Hz, H-3), 4.60 (dd, 1H, *J*=6.9, 2.2 Hz, H-4), 4.46 (d, 1H, *J*=9.9 Hz, H-1), 3.90 (qd, 1H, *J*=6.6, 2.2 Hz, H-5), 3.63 (dd, 1H, *J*=9.9, 6.7 Hz, H-2), 1.48 (d, 3H, *J*=6.6 Hz, H-6) ppm. ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ 154.0 (C=O), 133.5, 130.5, 129.4, 129.0 (arom.), 87.0 (C-1), 79.6 (C-3), 78.0 (C-4), 72.3 (C-5), 70.4 (C-2), 16.6 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{13}H_{14}O_5SNa$: 305.04596, found 305.04542.

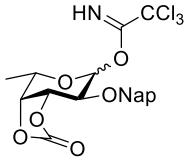
3,4-*O*-carbonate-2-*O*-(2-naphthylmethyl)-α/β-L-fucopyranoside (27)



Thioglycoside **25** (0.2 g, 0.48 mmol, 1.0 eq.) was dissolved in a 9/1 mixture of acetone/H_2O (5 mL, 0.1M). NBS (0.30 g, 1.68 mmol, 3.5 eq.) was added portionwise over 3 hours. The reaction was stirred at RT under nitrogen for 3 hours at which point sat. $Na_2S_2O_3$ (aq.) was added (2 mL). After stirring for 5min. acetone was evaporated *in vacuo*. The water layer was extracted with Et_2O (2x) and the combined organic layers were washed with sat. aq. $NaHCO_3$ (3x) and brine (1x). The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (PE: EtOAc, 4:1 →3:2) giving **27** as a white amorphous solid (0.13 g, 0.39 mmol, 81%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.90 – 7.74 (m, 6H, CH_2Nap, α,β), 7.53 – 7.39 (m, 4.5H, CH_2Nap, α,β), 5.19 (d, 1H, *J*=4.2 Hz, 1α), 4.86 – 4.81 (m, 4.5H, CH_2Nap, α,β, H-3α, H-1β), 4.74 (dd, 0.5H, *J*=7.5, 5.2 Hz, H-3β), 4.55 – 4.47 (m, 1.5H, H-4α, H-4β), 4.25 (qd, 1H, *J*=6.6, 2.0 Hz, H-5α), 3.88 (qd, 0.5H, *J*=6.6, 1.9 Hz, H-5β), 3.82 (dd, 1H, *J*=5.5, 4.2 Hz, H-2α), 3.59 (t, 0.5H, *J*=5.5 Hz, H-2β), 1.36 (d, 1.5H, *J*=6.6 Hz, H-6β), 1.28 (d, 3H, *J*=6.6 Hz, H-6α) ppm. ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ 154.1 (C=O,α,β), 134.5, 133.9, 133.3, 133.2, 128.9,

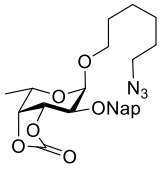
128.6, 128.1, 128.0, 127.9, 127.8, 127.6, 127.2, 126.7, 126.6, 126.4, 126.3, 125.9, 125.8 (CH, arom) , 95.3 (C-1β), 89.9 (C-1α), 78.2 (C-2β), 77.8 (C-3β), 77.5 (C-4β), 77.0 (C-4α), 75.0 (C-3α), 74.3 (CH_2Nap,α), 73.8 (CH_2Nap,β), 73.4 (C-2α), 67.7 (C-5β), 63.2 (C-5α), 16.3 (C-6β), 15.8 (C-6α) ppm. HRMS $[M+Na]^+$ calcd for $C_{18}H_{18}O_6Na$: 353.10011, found 353.09956.

Trichloroacetimido 3,4-*O*-carbonate-2-*O*-(2-naphthylmethyl)-α/β-L-fucopyranoside (28)



DBU (8 µ, 0.055 mmol, 0.2 eq.) was added to a solution of fucose **27** (0.091 g, 0.27 mmol, 1.0 eq.) and trichloroacetonitrile (0.28 mL, 2.7 mmol, 10 eq.) in dry DCM (2.7 mL, 0.1M). The reaction was stirred under inert atmosphere at RT for 1 hour. The reaction mixture was concentrated *in vacuo* followed by purification using silica gel chromatography (PE: EtOAc, 9:1 →4:1). The enantiomers eluted separately. The combined yield of both anomers was (0.10 g, 0.21 mmol, 77%). α-product: ^{1}H NMR (CD_3CN, 500 MHz): δ = 9.04 (s, 1H, NH), 7.91 – 7.81 (m, 4H. CHNap), 7.54 – 7.46 (m, 3H, CHNap), 6.40 (d, 1H, *J*=4.0 Hz, H-1), 5.02 (dd, 1H, *J*=7.3, 6.1 Hz, H-3), 4.92 (d, 1H, *J*=11.7 Hz, CH_2Nap), 4.87 – 4.79 (m, 2H, H-4, CH_2Nap), 4.42 (qd, 1H, *J*=6.6, 2.1 Hz, H-5), 4.19 (dd, 1H, *J*=6.1, 4.0 Hz, H-2), 1.29 (d, 3H, *J*=6.7 Hz, H-6) ppm. ^{13}C-APT NMR (CD_3CN, 126 MHz) δ 161.3 (C=NH), 155.3 (C=O), 136.6, 134.5, 134.4, 129.4, 129.2, 129.0, 127.9, 127.6, 127.5, 127.2 (CH, arom), 94.8 (C-1), 78.2 (C-4), 76.3 (C-3), 74.6 (C-2), 74.4 (CH_2Nap), 66.8 (C-5), 16.3 (C-6) ppm. β-product: ^{1}H NMR (CD_3CN, 500 MHz): δ = 9.08 (s, 1H, NH), 7.92 – 7.83 (m, 4H, CHNap), 7.57 – 7.46 (m, 3H, CHNap), 6.06 (d, 1H, *J*=5.6 Hz, H-1), 5.01 (dd, 1H, *J*=4.7, 3.1 Hz, H-3), 4.94 (dd, 2H, *J*=11.9, 4.9 Hz, CH_2Nap), 4.79 (dd, 1H, *J*=7.9, 1.9 Hz, H-4), 4.20 (qd, 1H, *J*=6.6, 1.9 Hz, H-5), 3.94 (dd, 1H, *J*=5.5, 5.0 Hz, H-2), 1.33 (d, 3H, *J*=6.6 Hz, H-6) ppm. ^{13}C-APT NMR (CD_3CN, 126 MHz) δ 161.4 (C=NH), 155.2 (C=O), 136.4, 134.5, 129.4, 129.1, 128.9, 128.1, 127.7, 127.6, 127.3 (C, arom), 97.6 (C-1), 78.1 (C-2), 77.9 (C-3), 76.8 (C-4), 74.5 (CH_2Nap), 69.3 (C-5), 16.8 (C-6) ppm.

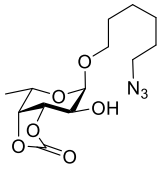
6-Azidohexyl 3,4-*O*-carbonate-2-*O*-(2-naphthylmetyl)-α-L-fucopyranoside (29)



Imidate **28** (2.62 g, 4.0 mmol, 1 eq.), azidohexan-1-ol (0.86 g, 6.0 mmol, 1.5 eq.) and methyl diphenylphosphine oxide (5.19 g, 24.0 mmol, 6 eq.) were co-evaporated together with toluene (3x) and dissolved in dry DCM (40 mL, 0.1 M). MS (4Å) were added and the reaction was stirred for 1 hour under N_2. TMSI (0.74 mL, 5.2 mmol, 1.3 eq.) and the reaction was left to stir for 36 hours. Triethylamine (1mL) was added to quench the reaction and the reaction was diluted in EtOAc, washed with sat. aq. $Na_2S_2O_3$, followed by sat. aq. $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silicagel chromatography

(tol: EtOAc, 1:0 → 4:1) gave the title compound (1.28 g, 2.8 mmol, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.88 – 7.75 (m, 4H, CH,Nap), 7.52 – 7.44 (m, 3H, CH,Nap), 4.96 – 4.80 (m, 3H, CH_2Nap, H-3), 4.78 (d, 1H, *J*=3.7 Hz, H-1), 4.60 (dd, 1H, *J*=7.0, 2.4 Hz, H-4), 4.12 (qd, 1H, *J*=6.7, 2.4 Hz, H-5), 3.73 – 3.62 (m, 2H, H-2, CH_2O), 3.38 (dt, 1H, *J*=9.8, 6.6 Hz, CH_2O), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.65 – 1.56 (m, 4H, CH_2, hexyl), 1.41 – 1.36 (m, 4H, CH_2, hexyl), 1.33 (d, 3H, *J*=6.7 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 126 MHz) δ 154.2 (C=O), 134.8, 133.3, 133.2, 128.5, 128.0, 127.8, 127.1, 126.4, 126.3, 125.8 (C, arom), 96.4 (C-1), 78.1 (C-4), 77.2 (C-3), 75.1 (C-2), 73.4 (CH_2O), 68.6 (CH_2Nap), 62.3 (C-5), 51.4 (CH_2N_3), 29.3, 28.8, 26.6, 25.8 (CH_2, hexyl), 15.8 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{24}H_{29}N_3O_6Na$: 478.49541, found 478.19486.

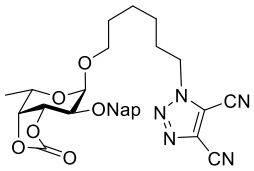
6-Azidohexyl 3,4-*O*-carbonate-2-*O*-(2-naphthylmetyl)-α-L-fucopyranoside (30)



Synthesis by HCl/HFIP: Compound **29** (0.048 g, 0.1 mmol, 1 eq.) was dissolved in a 1/1 mixture of DCM/HFIP (1 mL, 0.1M) containing triethylsilane (50 µL, 0.3 mmol, 3 eq.) and cooled to 0°C. HCl/HFIP (0.5 mL, 0.2M, 1 eq.) was added and the reaction was stirred for 3 hours. The reaction mixture was diluted in DCM and extracted with sat. aq. $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (PE: EtOAc, 4:1 → 3:2) giving **30** as a colourless oil (0.022 g, 0.065 mmol, 65%).

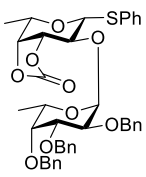
Synthesis by DDQ: Sugar **29** (1.0 g, 2.2 mmol, 1 eq.) was dissolved in a 9/1 mixture of DCM/MeOH (20 mL, 0.1M). DDQ (1.5 mmol, 6.6 mmol, 3 eq.) was added and the reaction was stirred under N_2 for 8 hours. The reaction mixture was partitioned between EtOAc and sat. aq. $Na_2S_2O_3$. The organic layer was washed with sat. aq. Na_2CO_3 until the water layer was no longer yellow and brine. Dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (tol: ACN, 95:5) giving **30** as a colourless oil (0.15 g, 0.49 mmol, 22%). ^{1}H NMR ($CDCl_3$), 500 MHz): δ = 4.93 – 4.80 (m, 2H, H-1, H-3), 4.63 (dd, 1H, *J*=7.8, 1.9 Hz, H-4), 4.21 (qd, 1H, *J*=6.6, 1.9 Hz, H-5), 4.07 (t, 1H, *J*=4.7 Hz, H-2), 3.81 (dt, 1H, *J*=9.8, 6.7 Hz, CH_2O), 3.56 (dt, 1H, *J*=9.8, 6.5 Hz, CH_2O), 3.28 (t, 2H, *J*=6.9 Hz, CH_2N_3), 3.07 (s, 1H, OH), 1.68 – 1.57 (m, 4H, CH_2, hexyl), 1.44 – 1.37 (m, 4H, CH_2, hexyl), 1.32 (d, 3H, *J*=6.7 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 126 MHz) δ 154.2 (C=O), 95.4 (C-1), 76.8 (C-4), 75.5 (C-3), 68.3 (CH_2O), 65.8 (C-2), 63.2 (C-5), 51.3 (CH_2N_3), 29.3, 28.7, 26.5, 25.6 (CH_2, hexyl), 15.5 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{13}H_{21}N_3O_6Na$: 338.13281, found 338.13226.

1,2,3-triazole-4,5-dicarbonitrilehexyl 3,4-*O*-carbonate-2-*O*-(2-naphthylmetyl)-α-L-fucopyranoside (31)



IR (cm^{-1}): 2939, 2866, 2252, 1813, 1465, 1375, 1174, 1100. ^1H NMR ($CDCl_3$, 500 MHz): δ = 8.17 – 8.13 (m, 1H, arom.), 7.87 – 7.80 (m, 2H, arom.), 7.55 – 7.47 (m, 3H, arom.), 7.45 – 7.41 (m, 1H, arom.), 5.25 (d, 1H, *J*=11.7 Hz, CH_2Nap), 5.06 (d, 1H, *J*=11.7 Hz, CH_2Nap), 4.84 (t, 1H, *J*=7.1 Hz, H-3), 4.65 (d, 1H, *J*=3.7 Hz, H-1), 4.59 (dd, 1H, *J*=7.0, 2.4 Hz, H-4), 4.39 (m, 2H, CH_2N), 4.10 (qd, 1H, *J*=6.7, 2.4 Hz, H-5), 3.76 (dd, 1H, *J*=7.2, 3.7 Hz, H-2), 3.60 (dt, 1H, *J*=9.8, 6.3 Hz, CH_2O), 3.18 (dt, 1H, *J*=9.8, 6.4 Hz, CH_2O), 1.88 (p, 2H, *J*=7.3 Hz, CH_2, hexyl), 1.56 – 1.47 (m, 2H, CH_2, hexyl), 1.33 (d, 3H, *J*= 6.7 Hz, H-6) 1.32 – 1.24 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 126 MHz) δ 154.2 (C=O), 133.9 (arom.), 133.0 (C-CN), 131.8, 129.4, 128.7, 127.4, 126.4, 126.1, 125.4, 124.3 (arom.), 108.6 (CN), 105.6 (CN), 96.5 (C-1), 78.0 (C-4), 77.1 (C-3), 75.2 (C-2), 72.5(CH_2Nap), 68.4 (CH_2O), 62.5 (C-5), 51.6 (CH_2N), 29.6, 28.9, 25.9, 25.3 (CH_2, hexyl), 15.8 (C-6) ppm. HRMS $[M+H]^+$ calcd for $C_{28}H_{29}N_5O_6H$: 532.21978, found 532.21922.

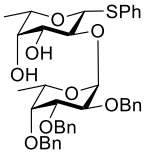
Phenyl 3,4-*O*-carbonate-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1-thio-β-L-fucopyranoside (34)



Compounds **26** (1.4 g, 5.0 mmol, 1.0 eq.) and **21** (3.2 g, 6.0 mmol, 1.2 eq.) were co-evaporated together with toluene thrice and dissolved in dry DCM (50 mL, 0.1M). Flame dried molecular sieves (3Å) were added and the mixture was cooled to 0°C and stirred at this temperature for 30 min. IDCP (4.69 g, 10.0 mmol, 2.0 eq.) was added and the reaction was stirred under nitrogen for 2h, at which point TLC analysis (PE: EtOAc 3:2) showed complete conversion. $Na_2S_2O_3$ (sat. aq. 2 mL) was added and the reaction was left to stir for 10 minutes at which point it was poured into EtOAc and washed with $CuSO_4$ (sat aq., 2x) $NaHCO_3$ (sat. aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mix was purified using silica gel chromatography (PE: EtOAc, 19:1 → 7:3) giving the title compound as a colourless oil (2.22 g, 3.1 mmol, 62%). ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.50 – 7.19 (m, 20H, arom.), 5.43 (d, 1H, J=3.9 Hz, H-1'), 4.97 (d, 1H, J=11.5 Hz, CH_2Bn), 4.89 – 4.71 (m, 6H, CH_2Bn, H-1, H-3), 4.66 (d, 1H, J=11.5 Hz, CH_2Bn), 4.55 (dd, 1H, J=7.2, 2.0 Hz, H-4), 4.14 (dd, 1H, J=10.2, 3.9 Hz, H-2'), 3.98 (dd, 2H, J=8.8, 6.0 Hz, H-2, H-5'), 3.89 (dd, 1H, J=10.2, 2.7 Hz, H-3'), 3.80 (qd, 1H, J=6.5, 1.9 Hz, H-5), 3.72 (d, 1H, J=2.0 Hz, H-4'), 1.40 (d, 3H, J=6.6 Hz, H-6), 1.19 (d, 3H, J=6.4 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 153.6 (C=O), 138.8, 138.5, 138.2, 132.7, 132.2, 129.2, 128.5, 128.4, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6, 127.5 (CH, arom), 97.7 (C-1'), 86.0 (C-1), 79.3 (C-3'), 78.5 (C-3), 77.5 (C-4'), 77.4 (C-4), 76.0 (C2'), 75.0 (CH_2Bn),

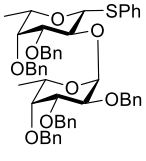
73.7 (CH_2Bn), 73.2 (CH_2Bn), 72.9 (C-2), 71.2 (C-5), 67.6 (C-5'), 16.5 (C-6), 16.4 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{40}H_{42}O_9SNa$: 721.24472, found 721.24417.

Phenyl 2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1-thio-β-L-fucopyranoside (35)



Disaccharide **34** (0.40 g, 0.57 mmol, 1 eq.) was dissolved in THF/H_2O (5:1, 5 mL, 0.1M). NaOH (5 mg, 0.11 mmol, 0.2 eq.) was added and the reaction was heated to 40°C. After 2h TLC analysis (Pe: EtOAc 3:2) showed a spot to spot conversion. The reaction was cooled to RT and NH_4Cl (0.1 mL) was added. The mixture was extracted with DCM (3x) and the combined organic layers were washed with brine (1x) and dried over $MgSO_4$ filtered and concentrated. No further purification was needed and the diol was obtained as a colourless oil (0.38 g, 0.56 mmol, 98%). ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.62 – 7.01 (m, 20H, arom.), 5.27 (d, 1H, *J*=3.8 Hz, H-1'), 4.96 (d, 1H, *J*=11.5 Hz, CH_2Bn), 4.86 (t, 2H, *J*=11.9 Hz, CH_2Bn), 4.80 – 4.67 (m, 3H, CH_2Bn, H-1), 4.63 (d, 1H, *J*=11.5 Hz, CH_2Bn), 4.30 (q, 1H, *J*=6.1 Hz, H-5'), 4.13 (dd, 1H, *J*=10.0, 3.7 Hz, H-2), 3.96 (dd, 1H, *J*=10.0, 2.7 Hz, H-3'), 3.75 (d, 1H, *J*=2.8 Hz, H-4), 3.73 – 3.57 (m, 5H, H-4', H2, H3, H-5), 2.80 (s, 2H, OH), 1.33 (d, 3H, *J*=6.5 Hz, H-6), 1.13 (d, 3H, *J*=6.5 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 138.8, 138.5, 138.3, 133.9, 131.6, 128.8, 128.4, 128.4, 128.3, 128.2, 127.7, 127.6, 127.6, 127.5, 127.3 (CH, arom.), 97.8 (C-1'), 86.3 (C-1), 79.0 (C-3'), 78.1 (C-2), 77.5 (C-4'), 76.0 (C-2'), 74.8 (CH_2Bn), 74.2 (C-5), 73.7 (C-3), 73.5 (CH_2Bn), 73.2 (CH_2Bn), 71.7 (C-4), 67.8 (C-5'), 16.7 (C-6), 16.6 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{39}H_{44}O_8SNa$: 695.26546, found 695.26491.

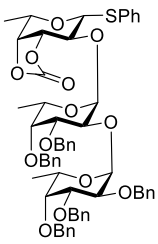
Phenyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1-thio-β-L-fucopyranoside (36)



NaH (0.16 g, 4.1 mmol, 5.0 eq.) was added to a solution of diol **35** (0.55 g, 0.82 mmol, 1 eq.) in dry DMF (20.1 ml, 0.2M) and left to stir for 15 min. BnBr (0.98 mL, 8.2 mmol, 10.0 eq.) was added and the reaction was left to stir under nitrogen atmosphere for 24h. Water was added until gas elution stopped and the mixture was poured into water. The water was extracted with EtOAc twice and the combined organic layers were washed with brine (5x), dried over $MgSO_4$ filtered and concentrated. Purification by size exclusion (LH-20, MeOH/DCM, 1/1) gave compound **36** as a colourless oil (0.51 g, 0.59 mmol, 72%). ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.63 – 6.99 (m, 30H, Ph), 5.77 (d, 1H, *J*=3.8 Hz, H-1'), 4.96 (d, 1H, *J*=11.5 Hz, CH_2Bn), 4.93 – 4.63 (m, 9H, CH_2Bn, H-1), 4.54 (d, 1H, *J*=11.7 Hz, CH_2Bn), 4.40 – 4.29 (m, 2H, CH_2Bn, H-2), 4.18 (q, 1H, *J*=6.3 Hz, H-5'), 4.04 (dd, 1H, *J*=10.2, 3.8 Hz, H-2'), 3.80 (dd, 1H, *J*=10.2, 2.8 Hz, H-3'), 3.74 (d, 1H, *J*=2.1 Hz, H-4), 3.68 (dd, 1H, *J*=9.4, 2.6 Hz, H-3), 3.60 (q, 1H, *J*=6.3 Hz, H-5), 3.17 (d, 1H, *J*=1.9 Hz, H-4'), 1.30 (d, 3H, *J*=6.3 Hz, H-6), 0.63 (d, 2H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101

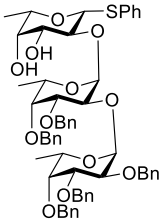
MHz): δ 139.3, 138.8, 138.8, 138.5, 137.7, 134.0, 130.9, 128.9, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.6, 126.9 (CH, arom), 96.1 (C-1'), 87.3 (C-1), 84.2 (C-3), 79.1 (C-3'), 77.6 (C-4'), 76.5 (C-2'), 76.2 (C-4), 74.8 (CH_2Bn), 74.7 (CH_2Bn), 74.5 (C-5), 73.2 (CH_2Bn), 73.1 (CH_2Bn), 72.7 (CH_2Bn), 70.0 (C-2), 66.2 (C-5'), 17.4 (C-6), 16.1 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{53}H_{56}O_8SNa$: 875.35936, found 875.35881.

Phenyl 3,4-*O*-carbonate-2-*O*-(3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-α-L-fucopyranosyl)-1-thio-β-L-fucopyranoside (37)



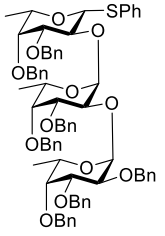
Compounds **26** (0.17 g, 0.6 mmol, 2 eq.) and **36** (0.26 g, 0.3 mmol, 1 eq.) were co-evaporated together with toluene thrice and dissolved in dry DCM (3 mL, 0.1M). Flame dried molecular sieves (3Å) were added and the mixture was cooled to 0°C and stirred at this temperature for 30 min. IDCP (0.19 g, 0.41 mmol, 1.3 eq.) was added and the reaction was stirred under nitrogen for 3h, at which point TLC analysis (tol: EtOAc 17:3) showed complete conversion. $Na_2S_2O_3$ (sat. aq. 0.5 mL) was added and the reaction was left to stir for 10 minutes after which point it was poured into EtOAc and washed with $CuSO_4$ (sat aq., 2x) $NaHCO_3$ (sat. aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude mix was purified using silica gel chromatography (PE: EtOAc, 19:1 → 7:3) giving the title compound as a colourless oil (0.24 g, 0.23 mmol, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.49 – 7.09 (m, 30H, arom.), 5.63 (d, 1H, *J*=3.8 Hz, H-1'), 5.45 (d, 1H, *J*=3.5 Hz, H-1''), 4.93 (m, 2H, CH_2Bn), 4.79 – 4.55 (m, 6H, CH_2Bn), 4.51 (dd, 1H, *J*=7.1, 5.9 Hz, H-4), 4.46 (d, 2H, *J*=5.0 Hz, CH_2Bn), 4.40 (dd, 1H, *J*=6.4, 1.6 Hz, H-2'), 4.34 (dd, 1H, *J*=7.2, 1.9 Hz, H-3), 4.19 – 4.08 (m, 2H, H-1, H-5'), 4.01 (dd, 1H, *J*=6.5, 3.5 Hz, H-2''), 3.99 – 3.84 (m, 4H, H-2, H-5, H-3', H-3''), 3.69 (d, 1H, *J*=2.1 Hz, H-4'), 3.42 (d, 1H, *J*=2.0 Hz, H-4''), 3.28 (qd, 1H, *J*=6.5, 1.8 Hz, H-5'), 1.33 (d, 3H, *J*=6.6 Hz, H-6), 1.16 (d, 3H, *J*=6.4 Hz, H6'), 1.07 (d, 3H, *J*=6.5 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 153.6 (C=O), 139.4, 138.8, 138.8, 138.7, 138.6, 132.4, 131.4, 129.3, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.0, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 127.2, 127.1 (CH, arom), 95.3 (C-1'), 93.5 (C-1''), 84.8 (C-1), 79.1 (C-3'), 78.9 (C-4), 77.8 (C-3''), 77.5 (C-3), 77.4 (C-4''), 77.3 (C-4'), 76.0 (C-2''), 75.0 (CH_2Bn), 74.9 (CH_2Bn), 72.7 (CH_2Bn), 72.6 (CH_2Bn), 71.7 (CH_2Bn), 71.4 (C-2), 70.9 (C-5'), 69.7 (C-2'), 67.3 (C-5), 66.4 (C-5''), 16.5 (C-6), 16.4 C-6'), 16.3 C-6'') ppm. HRMS $[M+Na]^+$ calcd for $C_{60}H_{64}O_{13}SNa$: 1047.39653, found 1047.39598.

Phenyl 2-*O*-(3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-α-L-fucopyranosyl)-1-thio-β-L-fucopyranoside (38)



Trisaccharide **37** (0.19 g, 0.18 mmol, 1 eq.) was dissolved in THF/H_2O (5:1, 2 mL, 0.1M). NaOH (2 mg, 0.04 mmol, 0.2 eq.) was added and the reaction was heated to 40°C. After 2h. TLC analysis (Pe: EtOAc 4:2) showed a spot to spot conversion. The reaction was cooled to RT and NH_4Cl (0.1 mL) was added. The mixture was extracted with DCM (3x) and the combined organic layers were washed with brine (1x) and dried over $MgSO_4$ filtered and concentrated. No further purification was needed and the diol was obtained as a colourless oil (0.16 g, 0.16 mmol, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.59 – 7.12 (m, 30H, arom.), 5.47 (d, 1H, *J*=3.5 Hz, H-1'), 5.35 (d, 1H, *J*=2.2 Hz, H-1''), 4.91 (m, 2H, CH_2Bn), 4.76 – 4.52 (m, 8H, CH_2Bn), 4.43 (d, 1H, *J*=9.7 Hz, H-1), 4.35 (m, 2H, H-5, H-2'), 4.09 (q, 1H, *J*=6.4 Hz, H-5''), 4.03 – 3.94 (m, 3H, H-3', H-2'', H-3''), 3.70 (t, 1H, *J*=2.1 Hz, H-4'), 3.63 (t, 1H, *J*=9.3 Hz, H-2), 3.54 (d, 1H, *J*=3.0 Hz, H-4), 3.49 – 3.41 (m, 2H, H-3, H-4''), 3.33 – 3.24 3.29 (q, 1H, *J*=6.9, 6.2 Hz, H-5'), 2.85 (bs, 2H, OH), 1.27 (d, 3H, *J*=6.5 Hz, H-6'), 1.12 (d, 3H, *J*=6.5 Hz, H-6), 0.98 (d, 3H, *J*=6.5 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 138.9, 138.8, 138.8, 138.7, 138.7, 133.6, 131.6, 129.0, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 127.7, 127.6, 127.5, 127.5, 127.4 (CH, arom), 96.5 (C-1''), 95.8 (C-1'), 85.8 (C-1), 79.4 (C-3'), 78.1 C-3''), 77.5 (C-4'), 77.4 C-4''), 76.3 (C-2''), 76.1 (C-2), 74.8 (CH_2Bn), 74.7 (CH_2Bn), 74.0 (C-5'), 73.2 (C-3), 72.9 (CH_2Bn), 72.7 (CH_2Bn), 72.5 (C-2'), 71.9 (C-4), 67.8 (C-5), 66.7 (C-5''), 16.7 (C-6), 16.5 (C-6'), 16.4 (C-6'') ppm. HRMS $[M+Na]^+$ calcd for $C_{59}H_{66}O_{12}SNa$: 1021.41727, found 1021.41672.

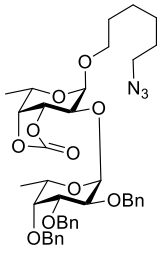
Phenyl 3,4-di-*O*-benzyl-2-*O*-(3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-α-L-fucopyranosyl)-1-thio-β-L-fucopyranoside (39)



Diol **38** (0.15 g, 0.15 mmol, 1.0 eq.) was dissolved in dry DMF (1.5 mL, 0.1M). NaH (0.018 g, 0.45 mmol, 3.0 eq.), TBAI (0.17 g, 0.45 mmol, 3.0 eq.) and BnBr (92 µL, 0.76 mmol, 5 eq.) were added at 0°C. The ice bath was removed and the reaction was stirred under nitrogen for 24 hours. The reaction was stopped by slow addition of water at 0°C. After the bubbling had stopped the mixture was poured into EtOAc. The organic layer was washed with brine (5x), dried over $MgSO_4$, filtered and concentrated. Purification by size exclusion (LH20, MeOH/DCM, 1/1) gave compound **39** as a colourless oil (0.14 g, 0.12 mmol, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.48 – 7.40 (m, 3H, Ph), 7.38 – 7.15 (m, 29H, Ph), 7.14 – 7.04 (m, 8H, Ph), 5.86 (d, 1H, *J*=3.6 Hz, H-1''), 5.55 (d, 1H, *J*=3.5 Hz, H-1'), 4.96 – 4.83 (m, 2H, CH_2Bn), 4.72 – 4.50 (m, 9H, CH_2Bn), 4.38 – 4.28 (m, 3H, H-2'', CH_2Bn), 4.26 – 4.21 (m, 2H, H-1, H-2), 4.16 – 4.08 (m, 2H, H-5', H-5''), 4.01 (dd, 1H, *J*=10.0, 3.5 Hz, H-2'), 3.92 (dd, 1H, *J*=10.0, 2.7 Hz, H-3'), 3.82 (dd, 1H, *J*=10.5, 2.8 Hz, H-3''), 3.58 (d, 1H, *J*=2.1 Hz, H-4), 3.43 (d, 1H, *J*=1.9 Hz,

H-4'), 3.34 – 3.30 (m, 1H, H-3), 3.17 (q, 1H, *J*=6.3 Hz, H-5), 3.12 (d, 1H, *J*=1.8 Hz, H-4''), 1.24 (d, 3H, *J*=6.3 Hz, H-6), 1.08 (d, 3H, *J*=6.5 Hz, H-6''), 0.65 (d, 3H, *J*=6.4 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 139.5, 139.3, 139.1, 138.9, 137.8, 134.1, 130.4, 129.0, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9, 127.9, 127.8, 127.6, 127.6, 127.5, 127.3, 127.2, 126.9 (C-arom), 94.1 (C-1''), 92.7 (C-1'), 86.8 (C-1), 84.1 (C-3), 79.1 (C-3'), 77.7 (C-4''), 77.5 (C-4'), 76.8 (C-3''), 76.3 (C-4), 76.0 (C-2'), 74.9 (CH_2Bn), 74.8 (CH_2Bn), 74.2 (C-5''), 72.8 (CH_2Bn), 72.7 (CH_2Bn), 72.6 (CH_2Bn), 71.2 (CH_2Bn), 69.8 (C-2''), 69.2 (C-2), 66.1 (C-5', C-5''), 17.4 (C-6), 16.5 (C-6''), 16.1 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{73}H_{78}O_{12}SNa$: 1201.51117, found 1201.51062.

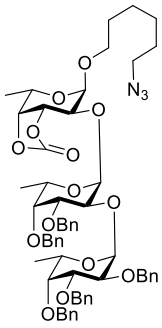
6-Azidohexyl 3,4-*O*-carbonate-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1-α-L-fucopyranoside (40)



Acceptor **30** (0.055 g, 0.174 mmol, 1.0 eq.) and donor **21** (0.138 g, 0.262 mmol, 1.5 eq.) were co-evaporated thrice with dry toluene, before being dissolved in dry DCM (1.7 mL, 0.1 M). Freshly dried MS (3Å) were added and the solution was cooled to 0˚C by an ice bath and left to stir at that temperature for 30 min. IDCP (0.163 g, 0.348 mmol, 2.0 eq.) was added and the ice bath was removed. After 2 hours TLC analysis indicated full conversion of acceptor **30**. The reaction mixture was diluted in EtOAc, transferred to a separatory funnel and washed with sat. $Na_2S_2O_3$ (aq.), sat. $CuSO_4$ (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude material was purified by silicagel chromatography (PE:EtOAc, 19:1 → 7:3), which gave title compound **40** in a 60% yield (0.076 g, 0.1 mmol) (12% yield of β-product). α-*product*: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.42 – 7.23 (m, 15H, arom.), 5.07 (d, 1H, *J*=3.8 Hz, H-1'), 4.99 – 4.91 (m, 2H, H-1, CH_2Bn), 4.87 – 4.62 (m, 6H), 4.60 (dd, 1H, *J*=6.8, 2.5 Hz, H-4), 4.14 (qd, 2H, *J*=6.7, 2.5 Hz, H-5), 4.08 (dd, 1H, *J*=10.1, 3.8 Hz, H-2'), 4.05 – 3.91 (m, 3H, H-5', H-2, H-3'), 3.72 (d, 2H, *J*=1.9 Hz, H-4'), 3.67 (dt, 1H, *J*=9.7, 7.0 Hz, CH_2O), 3.43 (dt, 1H, *J*=9.6, 6.7 Hz, CH_2O), 3.22 (t, 2H, *J*=6.8 Hz, CH_2N_3), 1.62 – 1.48 (m, 4H, CH_2, hexyl), 1.37 (d, 3H, *J*=6.7 Hz, H-6), 1.31 (m, 4H, CH_2, hexyl), 1.15 (d, 3H, *J*=6.6 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 154.1 (C=O), 138.9, 138.8, 138.5, 128.5, 128.5, 128.4, 128.3, 127.8, 127.7, 127.7, 127.6, 127.5 (arom.), 95.4 (C-1'), 95.3 (C-1), 79.2 (C-3'), 78.1 (C-4), 77.3 (C-4'), 76.0 (C-3), 75.8 (C-2'), 75.0 (CH_2Bn), 73.1 (CH_2Bn), 73.0 (CH_2Bn), 71.0 (C-2), 69.2 (CH_2O), 67.1 (C-5'), 62.5 (C-5), 51.4 (CH_2N_3), 29.4, 28.8, 26.6, 25.8 (CH_2, hexyl), 16.6 (C-6'), 15.9 (C-6) ppm. β-*product*: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.41 (m, 2H, arom.), 7.38 – 7.28 (m, 13H, arom.), 5.01 (d, 1H, *J*=3.6 Hz, H-1), 4.97 (m, 2H, CH_2Bn), 4.85 – 4.65 (m, 5H, H-3, CH_2Bn), 4.59 (dd, 1H, *J*=4.4, 2.6 Hz, H-4), 4.53 (d, 1H, *J*=7.8 Hz, H-1') 4.12 (qd, 1H, *J*=6.6, 2.6 Hz, H-5), 3.90 (dd, 1H, *J*=8.0, 3.6 Hz, H-2), 3.81 (dd, 1H, *J*=9.4, 7.7 Hz, H-2'), 3.65 (dt, 1H, *J*=9.8, 6.6 Hz, CH_2O), 3.56 – 3.43 (m, 4H, H-3', H-4', H-5', CH_2O), 3.17 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.68 – 1.46 (m, 4H, CH_2, hexyl), 1.39 (d, 2H, *J*=6.7 Hz, H-6), 1.36 – 1.29 (m, 4H, CH_2, hexyl), 1.15

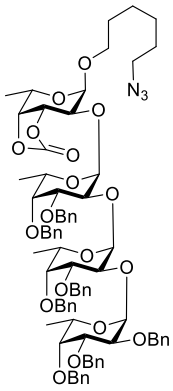
(d, 2H, *J*=6.4 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 154.2 (C=O), 138.7, 128.6, 128.5, 128.4, 128.3, 128.3, 127.8, 127.7 (arom.), 103.8 (C-1'), 97.7 (C-1), 82.3 (C-3'), 79.4 (C-2), 78.5 (C-4), 77.8 (C-4'), 76.6 (C-3), 75.8 (C-2), 75.2 (CH_2Bn), 74.9 (CH_2Bn), 73.6 (CH_2Bn), 70.8 (C-5'), 68.9 (OCH_2), 61.9 (C-5), 51.4 (CH_2N_3), 29.3, 28.8, 26.6, 25.8 (CH_2, hexyl), 16.9 (C-6), 16.0 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{40}H_{49}N_3O_{10}Na$: 754.33156, found 754.33102.

6-Azidohexyl 3,4-*O*-carbonate-2-*O*-(3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (41)



Disaccharide donor **36** (0.105 g, 0.12 mmol, 1.0 eq.) and acceptor **30** (0.058 g, 0.18 mmol, 1.3 eq.) were co-evapotared thrice with dry toluene, before dissolving in dry DCM (1.2 mL, 0.1M). Freshly dried MS (3Å) were added and the mixture was cooled to 0˚C with an ice bath. IDCP (0.092 g, 0.020 mmol, 1.5 eq.) was added after 30 min of stirring and upon addition the icebath was removed, allowing the reaction mixture to warm to RT. TLC analysis indicated full consumption of donor **36** after 2 hours, at which point the reaction mixture was diluted in EtOAc and transferred to a separatory funnel. The organic layer was washed with sat. $Na_2S_2O_3$ (aq.), sat. $CuSO_4$ (aq.) and brine, before drying over $MgSO_4$ and concentration *in vacuo*. The crude trisaccharide was purified by silicagel chromatography (PE:EtOAc, 19:1 → 4:1), yielding **41** as a colourless oil (0.088 g, 0.084 mmol, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.62 – 6.81 (m, 25H, arom.), 5.32 (2x d, 2H, *J*=3.3 Hz, H-1', H-1''), 5.04 (d, 1H, *J*=3.6 Hz, H-1), 5.03 – 4.95 (m, 2H, CH_2Bn), 4.91 – 4.87 (m, 1H, CH_2Bn), 4.79 – 4.73 (m, 3H, CH_2Bn), 4.71 – 4.62 (m, 4H, CH_2Bn), 4.45 (t, 1H, *J*=7.2 Hz, H-3), 4.39 (dd, 1H, *J*=6.9, 2.4 Hz, H-4), 4.34 (dd, 1H, *J*=10.2, 3.7 Hz, H-2''), 4.15 – 4.05 (m, 2H, H-2', H-5''), 4.05 – 3.96 (m, 2H, H-5, H-3''), 3.97 – 3.88 (m, 2H, H-3', H-5'), 3.84 (dd, 1H, *J*=7.6, 3.6 Hz, H-2), 3.74 – 3.69 (m, 1H, H-4''), 3.62 (dt, 1H, *J*=9.9, 7.4 Hz, OCH_2), 3.50 (d, 1H, *J*=3.0 Hz, H-4'), 3.48 – 3.39 (m, 1H, OCH_2), 3.18 (t, 2H, *J*=6.8 Hz, CH_2N_3), 1.65 – 1.47 (m, 4H, CH_2, hexyl), 1.36 – 1.26 (m, 7H, H-6, CH_2, hexyl), 1.18 (d, 3H, *J*=6.5 Hz, H-6'), 1.07 (d, 3H, *J*=6.5 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 126 MHz): δ 154.2 (C=O), 139.2, 138.9, 138.9, 138.7, 138.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 127.8, 127.7, 127.6, 127.6, 127.5, 127.3 (arom.), 94.8 (C-1), 93.0 (C-1''), 92.7 (C-1'), 78.9 (C-3'), 77.9 (C-4), 77.7 (C-3''), 77.6 (C-4''), 77.6 (C-4'), 76.1 (C-3, C-2'), 75.1 (CH_2Bn), 75.0 (CH_2Bn), 73.0 (CH_2Bn), 73.0 (CH_2Bn), 71.3 (CH_2Bn), 70.7 (C-2), 70.2 (C-2''), 68.8 (OCH_2), 67.3 (C-5'), 66.6 (C-5''), 62.0 (C-5), 51.3 (CH_2N_3), 29.5, 28.8, 26.6, 25.8 (CH_2, hexyl), 16.6 (C-6'), 16.5 (C-6''), 15.9 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{60}H_{71}N_3O_{14}Na$: 1080.48337, found 1080.48282.

6-Azidohexyl 3,4-*O*-carbonate-2-*O*-(3,4-di-*O*-benzyl-2-*O*-(3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (42)

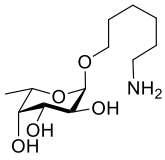


Trisaccharide **39** (0.091 g, 0.076 mmol, 1.0 eq.) and **30** (0.031 g, 0.1 mmol, 1.3 eq.) were co-evapotared thrice with dry toluene, before dissolving in dry DCM. Freshly dried MS (3Å) were added and the mixture was cooled to 0˚C with an ice bath and stirred for 30 min. IDCP (0.53 g, 0.114 mmol, 1.5 eq.) was added after 30 min of stirring and upon addition the icebath was removed, allowing the reaction mixture to warm to RT. TLC analysis indicated full consumption of donor **39** after 2 hours, at which point the reaction mixture was diluted in EtOAc and transferred to a separatory funnel. The organic layer was washed with sat. $Na_2S_2O_3$ (aq.), sat. $CuSO_4$ (aq.) and brine, before drying over $MgSO_4$ and concentration in vacuo. The crude trisaccharide was purified by silicagel chromatography (PE:EtOAc, 19:1 → 4:1), followed by size exclusion over LH-20 (DCM/MeOH, 1/1, v/v) yielding **42** as a colourless oil (0.064 g 0.0462 mmol, 61%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.46 – 7.42 (m, 2H, arom.), 7.37 – 7.17 (m, 33H, arom.), 7.15 – 7.12 (m, 2H, arom.), 5.45 (d, 1H, *J*=3.5 Hz, H-1’’’), 5.42 (d, 1H, *J*=3.5 Hz, H-1’’), 5.32 (d, 1H, *J*=3.7 Hz, H-1’), 5.10 – 5.03 (m, 2H, H-1, CH_2Bn), 4.99 – 4.88 (m, 3H, CH_2Bn), 4.86 – 4.78 (m, 2H, H-3, CH_2Bn), 4.71 – 4.48 (m, 8H, CH_2Bn), 4.45 (dd, 1H, *J*=6.6, 2.5 Hz, H-4), 4.25 – 4.17 (m, 2H, H-2’, H-2’’), 4.14 (d, 1H, *J*=12.3 Hz, CH_2Bn), 4.11 – 4.04 (m, 2H, H-2’’’, H-5’’’), 4.01 (qd, 2H, *J*=6.6, 2.5 Hz, H-5), 3.97 – 3.93 (m, 2H, H-5’’, H-3’’’), 3.92 – 3.86 (m, 2H, H-2, H-5’’), 3.76 – 3.67 (m, 2H, H-3’, H-3’’), 3.60 – 3.53 (m, 2H, H-4’, CH_2O), 3.50 – 3.43 (m, 3H, H-4’’’, H-4’’’, CH_2O), 3.00 (t, 2H, *J*=6.8 Hz, CH_2N_3), 1.65 – 1.54 (m, 3H, CH_2, hexyl), 1.42 – 1.38 (m, 3H, CH_2, hexyl), 1.36 (d, 3H, *J*=6.7 Hz, H-6), 1.24 – 1.18 (m, 2H, CH_2, hexyl), 1.15 (d, 3H, *J*=6.5 Hz, H-6’), 1.11 (d, 3H, *J*=6.5 Hz, H-6’’), 1.07 (d, 3H, *J*=6.5 Hz, H-6’’’) ppm. ^{13}C-APT NMR ($CDCl_3$, 126 MHz) δ 154.1 (C=O), 139.6, 139.5, 139.2, 139.1, 139.1, 139.0, 138.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 127.7, 127.7, 127.6, 127.5, 127.2, 127.1, 127.0, 127.0, 126.8 (arom.), 94.1 (C-1), 92.3 (C-1’), 92.0 (C-1’’’), 91.0 (C-1’’), 78.6 (c-3’’’), 78.4 (C-3’’), 78.4 (C-3’), 78.3 (C-4’), 78.2 (C-4), 78.1 (C-4’’’), 77.8 C-4’), 76.6 (C-2’’’), 76.2 (C-3), 75.2 (CH_2Bn), 75.1 (CH_2Bn), 75.0 (CH_2Bn), 73.6 (CH_2Bn), 73.2 (CH_2Bn), 73.2 (CH_2Bn), 71.1 (C-2), 70.7 (CH_2Bn), 70.3 (C-2’), 69.9 (C-2’’), 69.0 (CH_2O), 67.4 (C-5’), 67.1 (C-5’’), 66.2 (C-5’’’), 61.9 (C-5), 51.3 (CH_2N_3), 28.9, 28.7, 26.4, 25.5 (CH_2, hexyl), 16.6 (C-6’, C-6’’, C-6’’’), 15.9 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{80}H_{93}N_3O_{18}Na$: 1406.63518, found 1406.63463.

General deprotection method of oligofucosides:

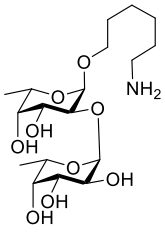
Glycan **x** (1.0 eq.) was dissolved in a mixture of THF/MeOH (0.05M, 1/1, v/v). Base (3.0 eq.) was added and the mixture was stirred at RT. After 2 hours the temperature was increased to 50˚C. TLC analysis showed full conversion of the starting material after several (3-5) hours and the reaction mixture was neutralized (pH~7) by slow addition of AcOH. The volatile components were removed *in vacuo* and the crude was purified by size exclusion over LH-20 (DCM/MeOH, 1/1, v/v). The resulting compound was dissolved in degassed H_2O (0.05M). Pd/C was added and the flask was purged with nitrogen, before introduction of the H_2 atmosphere. The reaction mixture was stirred overnight at the indicated temperature. Before filtration ove Celite®, the mixture was purged with N_2 to remove any H_2 gas. The filtrate was concentrated *in vacuo* and the colourless oil was subjected to size exclusion over LH-20 (MeOH/H_2O, 9/1, v/v). The obtained deprotected glycan was then lyophilized.

6-aminohexyl α-L-fucopyranoside (1)



Monosaccharide **13** (0.10 g, 0.20 mmol), NaOMe (30 mg, 0.6 mmol), Pd/C (20 mg). Monosaccharide **1** was obtained as a white fluffy powder (0.016 g, 0.061 mmol, 31%). ^{1}H NMR (D_2O, 500 MHz): δ = 4.87 (d, 1H, *J* = 3.8 Hz, H-1), 4.06 (q, 1H, *J*=6.4, 5.6 Hz, H-5), 3.84 (dd, 1H, *J*=10.3, 2.7 Hz, H-3), 3.81 – 3.73 (m, 2H, H-2, H-4), 3.71 – 3.57 (m, 1H, OCH_2), 3.51 (dt, 1H, *J*=9.7, 6.4 Hz, OCH_2), 2.99 (t, 2H, *J*=7.5 Hz, CH_2N_3), 1.71 – 1.51 (m, 4H, CH_2, hexyl), 1.46 – 1.28 (m, 4H, CH_2, hexyl), 1.21 (d, 3H, *J*=6.6 Hz, H-6) ppm. ^{13}C-APT NMR (D_2O, 126 MHz): δ = 98.4 (C-1), 71.9 (C-2/C-4), 69.7 (C-3), 68.3 (OCH_2), 68.0 (C-2/C-4), 66.6 (C-5), 39.5 (CH_2N_3), 28.4, 26.7, 25.4, 24.9 (CH_2, hexyl), 15.3 (C-6) ppm. HRMS $[M+H]^+$ calcd for $C_{12}H_{25}NO_5H$: 264.18127, found 264.18051.

6-aminohexyl 2-*O*-(α-L-fucopyranosyl)-α-L-fucopyranoside (2)



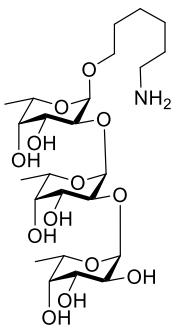
Route 1: Disaccharide **14** (42 mg, 0.04 mmol), NaOMe (6.0 mg, 0.12 mmol), Pd/C (42 mg). Disaccharide **2** was obtained as a white powder (11 mg, 0.025 mmol, 68%).

Route 2: Disaccharide **40** (35 mg, 0.05 mmol), NaOH (6.0 mg, 0.15 mmol), Pd/C (35 mg), RT. Disaccharide **2** was obtained as a white powder (9.8 mg, 0.024 mmol, 48%).

^{1}H NMR (D_2O, 400 MHz): δ = 5.06 (d, 1H, *J*=3.6 Hz, H-1'), 4.99 (d, 1H, *J*=4.0 Hz, H-1), 4.21 (q, 1H, *J*=6.6 Hz, H-5), 4.04 (q, 1H, *J*=6.4 Hz, H-5'), 3.91 (m, 2H, H-3, H-3'), 3.85 – 3.76 (m, 3H, H-2', H-4, H-4'), 3.73 (dd, 1H, *J*=10.3, 4.0 Hz, H-2), 3.70 – 3.61 (m, 1H, OCH_2), 3.51 (dt, 1H, *J*=9.7, 6.4 Hz, OCH_2), 2.83 (t, 2H, *J*=7.4 Hz, CH_2N_3), 1.58 (dt, 4H, *J*=15.3, 5.8 Hz, CH_2, hexyl), 1.42 – 1.33 (m, 4H, CH_2, hexyl), 1.18 (m, 6H, H-6, H-6') ppm. ^{13}C-APT NMR (D_2O, 101 MHz): δ 95.8 (C-1), 95.3 (C-1'), 72.0, 71.8, 71.8 (C-2', C-4,

C-4'), 69.3 (C-3), 68.1 (OCH_2), 67.9, 67.9 (C-2, C-3'), 66.9 (C-5), 66.5 (C-5'), 39.7 (CH_2N_3), 28.3, 28.1, 25.4, 24.9 (CH_2, hexyl), 15.2, 15.2 (C-6, C-6'). HRMS $[M+H]^+$ calcd for $C_{18}H_{35}NO_9H$: 410.23918, found 410.23846.

6-aminohexyl 2-*O*-(2-*O*-(α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (3)

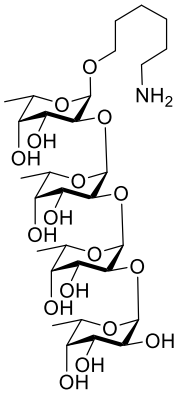


Route 1: Trisaccharide **19** (15 mg, 0.01 mmol), NaOMe (2.0 mg, 0.03 mmol), Pd/C (15 mg). Trisaccharide **3** was obatined as a white powder (4.1 mg, 0.0073 mmol, 74%).

Route 2: Trisaccharide **41** (50 mg, 0.047 mmol), NaOH (5.6 mg, 0.14 mmol), Pd/C (50 mg), 50˚C. Trisaccharide **3** was obatined as a white powder (4.6 mg, 0.0083 mmol, 18%).

^{1}H NMR (D_2O, 400 MHz): δ = 5.14 (d, 1H, *J*=3.9 Hz), 5.01 (d, 1H, *J*=3.5 Hz), 4.97 (d, 1H, *J*=3.7 Hz), 4.09 (t, 2H, *J*=6.5 Hz), 3.99 – 3.92 (m, 2H), 3.89 – 3.79 (m, 2H), 3.79 – 3.62 (m, 8H), 3.54 (dd, 1H, *J*=9.7, 6.9 Hz), 3.44 (q, 1H, *J*=8.3, 7.4 Hz), 2.90 (dt, 2H, *J*=21.0, 6.9 Hz), 1.61 – 1.48 (m, 4H), 1.29 (s, 4H), 1.10 (d, 9H, *J*=6.6 Hz) ppm. ^{13}C-APT NMR (D_2O, 101 MHz): δ 95.5, 95.3, 92.9, 72.0, 71.8, 71.7, 69.5, 68.0, 67.8, 67.1, 67.0, 66.4, 39.4, 38.6, 28.5, 26.6, 25.4, 24.9, 15.2, 15.2 ppm. HRMS $[M+H]^+$ calcd for $C_{24}H_{45}NO_{13}H$: 556.29709, found 556.29662.

6-aminohexyl 2-*O*-(2-*O*-(2-*O*-(α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (4)



Route 1: Tetrasaccharide **23** (41 mg, 0.027 mmol), NaOMe (4.0 mg, 0.081 mmol), Pd/C (41 mg). The title compound obtained as a fluffy white powder. (14.0 mg, 0.020 mmol, 74%).

Route 2: Tetrasaccharide **42** (59 mg, 0.042 mmol), NaOH (5.0 mg, 0.13 mmol), Pd/C (59 mg), 50˚C. The title compound was obtained as a fluffy white powder (6.3 mg, 0.0090 mmol, 21%).

^{1}H NMR (D_2O, 400 MHz): δ = 5.31 (d, 1H, *J*=3.6 Hz), 5.26 (d, 1H, *J*=3.7 Hz), 5.12 (t, 2H, *J*=4.3 Hz), 4.25 – 4.15 (m, 1H), 4.14 – 3.69 (m, 17H), 3.63 (dt, 1H, *J*=10.0, 7.2 Hz), 3.51 (dt, 1H, *J*=9.3, 6.5 Hz), 2.95 (t, 2H, *J*=7.6 Hz), 1.69 – 1.55 (m, 6H), 1.42 – 1.31 (m, 4H), 1.23 – 1.13 (m, 12H) ppm. ^{13}C-APT NMR (D_2O, 101 MHz): δ 95.4, 95.3, 92.8, 92.3, 72.0, 71.8, 71.6, 71.6, 71.5, 71.3, 69.4, 68.2, 68.1, 68.1, 68.0, 68.0, 67.1, 67.0, 66.7, 66.5, 39.4, 38.6, 28.7, 26.7, 25.4, 24.9, 15.4, 15.3, 15.2, 15.1 ppm. HRMS $[M+H]^+$ calcd for Chemical Formula: $C_{30}H_{55}NO_{17}H$: 702.35510, found 702.35428.

Synthesis of functionalized AuNP's

The functionalized AuNP's were synthesized with a NHS-Activated Gold Nanoparticle conjungation kit from cytodiagnostics. Sugar **x** was dissolved in HPLC grade H_2O (1M), this solution was then diluted by adding '*protein suspension buffer´* (0.5M). To a vial containing the AuNP (5nm) was added 100 µL of *'reaction buffer'*, the suspension was homogenized and divided in two equal parts of 50 µL. The sugar containing solution (3 µL) was added to the AuNP suspension giving so the final concentration of glycan is 30 mM. The vial containing the mixture was packed in aluminium foil and shaken for 2.5 hours at room temperature. After this time '*quencher solution*' (5 µL) was added and the mixture was shaken for an additional 30 min., before filtration over freshly washed 30 KDa filters (6x, 500 µL, 7000 rpm). The dark red solution (200 µL) was transferred from the filter to an Eppendorf vial and stored at 4˚C.

ELISA protocol

general

Coating buffer: 50 mM Na_2CO_3, pH = 9.6. ELISA Nunc MaxiSorp® 96-well immunoplate (Thermo Fisher Scientific, Roskilde, Denmark). Bovine serum albumin (BSA) (lyophilized powder, ≥98 %, pH 7, measured by agarose gel electrophoresis) was used. The positive control used for these ELISA were Soluble Egg Antigen (SEA) (1:200 in coating buffer). BSA (1% in PBS) was used as the negative control.

ELISA protocol

The wells of the ELISA plate were incubated with a mixture of the AuNP containing solution and coating buffer (1:150, 50 µL per well) for 3 hours. Afterwards the wells were washed with PBS (2x 200 µL) and a blocking solution was added to each well (100 µL of 1% BSA in PBS) and left at RT for 30 min. Then protocol was then followed with either monoclonal antibodies or human sera.

monoclonal antibodies: The wells were then discarded, before the addition of monoclonal antibody (50 µL of a 1:500 dilution in 0.5% BSA in PBS) at RT for 1h. After washing with PBS (3x 200 µL), secondary antibody RAM/PO (80 µL per well of a 1:200 dilution in 0.5% BSA in PBS) was added and the plate was incubated at RT for 30 min. The plate was then washed with PBS (3x200 µL), before addition of TMB substrate solution (80 µL per well) and quenched by addition of H_2SO_4 (80 µL per well in a 1M solution in H_2O) after 2 min.

human sera: The wells were then discarded, before the addition of human sera (50 µL of a 1:150 dilution in 0.5% BSA in PBS) at 37˚C for 1h. After washing with PBS (3x 200 µL), secondary antibody Polyclonal Rabbit anti-human IgG/HRP (80 µL per well of a 1:100 dilution in 0.5% BSA in PBS) was added and the plate was incubated at RT for 30 min. The plate was then washed with PBS (3x200 µL), before addition of TMB substrate solution (90 µL per well) and quenched by addition of H_2SO_4 (90 µL per well in a 1M solution in H_2O) after 2 min.

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