Universiteit
Leiden
The Netherlands

Synthesis and application of glycans unique to S. mansoni

Harvey, M.R.

Citation
Harvey, M. R. (2020, December 1). *Synthesis and application of glycans unique to S. mansoni*. Retrieved from https://hdl.handle.net/1887/138246

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/138246

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/138246 holds various files of this Leiden University dissertation.

Author: Harvey, M.R.
Title: Synthesis and application of glycans unique to S. mansoni
Issue date: 2020-12-01

2

*Synthesis and application of α-(1-2)-fucosyl containing glycan motifs of S. mansoni**

Introduction

It has been shown that *S. mansoni* expresses a complex array of glycans and glyco-conjugates that can be targeted by both the adaptive and the innate part of the immune system (Figure 1).[1], [2] A large subgroup of these glycans is decorated with unique α-(1-2)-fucose chains.[3]–[5] These α-(1-2)-fucose chains are attached to a backbone of galactosamines and glucosamines in the native glyco-conjugates.[2], [4] It has been proven that these multi-fucosylated fragments are prime targets for host antibodies.[6], [7] A study where baboons were infected with irradiated cercariae (the larval form of *Schistosoma*) showed 80-90% reduction in worm-burden. The baboons had high levels of anti-carbohydrate antibodies against glycans carrying the fucosylated (Fuc) α-Fuc-(1-2)-α-Fuc motif.[8] In order to prepare diagnostic tools able to capture specific anti-carbohydrate antibodies or develop conjugate vaccines targeting these glycan structures, sufficient amounts of well-defined fucosylated fragments are needed. Since isolation from biological sources is impractical, chemical synthesis is a relevant alternative. This chapter describes the development of an efficient procedure towards the synthesis of the potential minimal epitopes of these complex glycan structures, the GlcNAc-Fuc-Fuc and GalNAc-Fuc-Fuc trisaccharides.

*Harvey, M.R., Hansen, T., Chiodo, F, Van der Marel, G. A., Codeé, J.D.C. have contributed to the work presented in this chapter

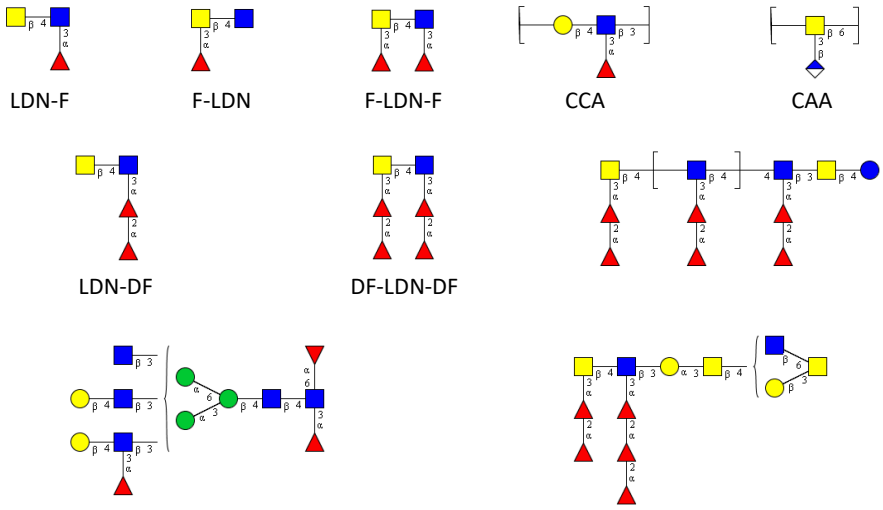


Figure 1: Overview of several observed glycans in S. mansoni. Red triangles: L-fucose, blue square: D-GlcNAc, yellow square: D-GalNAc, blue circle: D-Glucose, yellow circle: D-Galactose, green circle: D-mannose and blue/white diamond: D-Glucuronic acid. [9], [10]

The envisioned synthetic route towards the target oligosaccharides is shown in Figure 2. The chosen protecting group strategy involved the use of an azide as a precursor for the spacer amine. GlcNAc and GalNAc monosaccharides suffer from poor solubility in most common glycosylation solvents (Et_2O, DCM, ACN and toluene) and they tend to form unreactive oxazolines.[11]–[13] Therefore, the NHAc was masked as a trichloroacetamide (NHTCA) group, a versatile protecting group that can be converted back into the NHAc by a variety of methods.[14]–[16] The C6- and C4-hydroxyls of the GalNAc and GlcNAc moieties were selectively protected with a benzylidene acetal, leaving the C3-OH unprotected. It was expected that the C3-OH of building blocks **3** and **4** could be left unprotected as both the galactosamine and glucosamine donors will be condensed with the 6-azidohexan-1-ol, which should be sufficiently more nucleophilic than the secondary C3-OH of the building blocks.[17] Previous work of Van Roon *et al.* used an *iso*-propylidene group to protect the fucosyl C3- and C4-OH's to induce α-selectivity.[18] However, α-fucosides have been shown to be acid labile and deprotection of the *iso*-propylidene ketals resulted in concomitant cleavage of the fucosyl linkages.[18], [19] Taking this into account it was decided to use either benzoyl or benzyl groups on the C3- and C4-hydroxyls of the fucose moieties. The C2-OH was protected with the non-participating naphthyl group, which can be removed selectively by DDQ oxidation.[20]

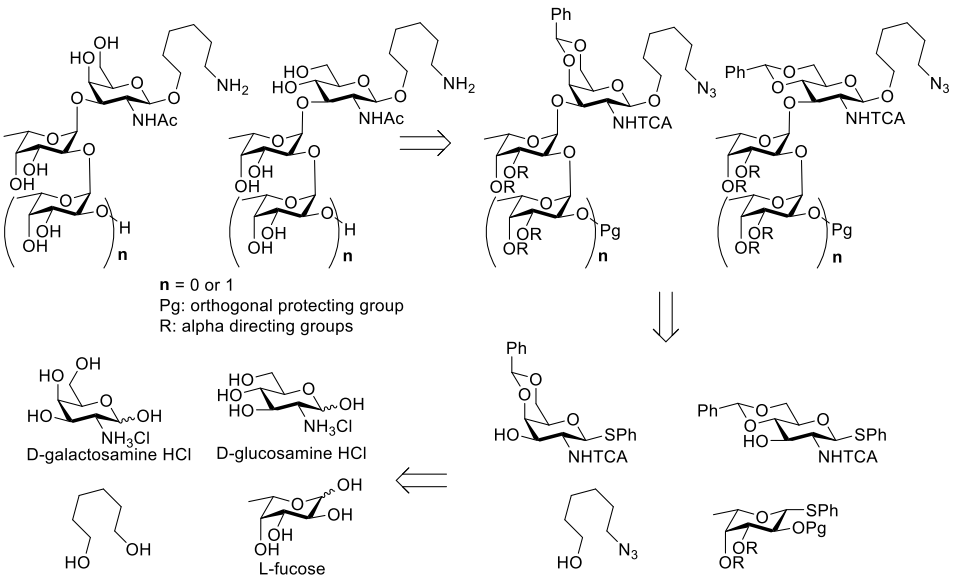


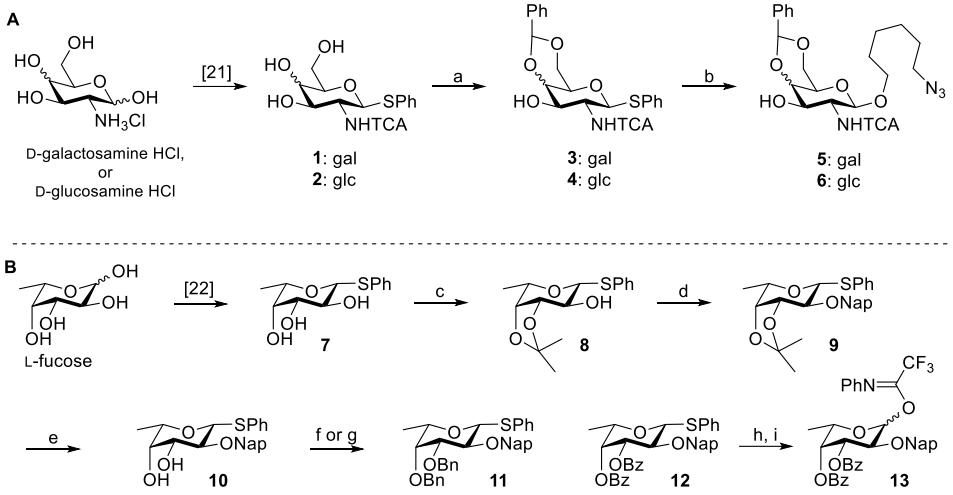
Figure 2: Retrosynthesis of unique glycan trisaccharides of S. mansoni functionalized with an amine linker for further conjugation.

Results and discussion

The synthetic route of the required synthons is depicted in Scheme 1. The galactosamine and glucosamine building blocks **5** and **6** were accessible from triols **1** and **2**, which were synthesized by following the protocol of Mulard and co-workers.[21] A benzylidene group was selectively installed on the C4- and C6-OH, leaving the C3-OH functionalities on **3** and **4** unprotected. The 6-azidohexan-1-ol was introduced by condensing it with **3** or **4** using *N*-iodosuccinimide (NIS) and TMSOTf as the glycosylation method. Removal of excess linker by silicagel chromatography proved difficult. To alleviate this problem the primary alcohol of the excess linker was selectively tritylated, by addition of trityl chloride in DCM and pyridine. After tritylation, silicagel purification was possible and led to the isolation of both acceptors **5** and **6** in a yield of 71% or 66%, respectively. The fucosyl donors were obtained from known triol **7**, which can be prepared from L-fucose in three steps.[22] The *iso*-propylidene ketal was selectively introduced on the C3- and C4-OH groups using 2,2-dimethoxypropane in acetonitrile with *p*-toluenesulfonic acid as the catalyst in a yield of 92%. The remaining alcohol on the C2 position was naphthylated with sodium hydride and naphthyl bromide giving **9** in 94% yield. The *iso*-propylidene was removed using a catalytic amount of HCl in a mixture of dioxane and water, which gave diol **10** in a quantitative yield. The diol was benzylated using sodium hydride and benzyl bromide in DMF with a catalytic amount of TBAI, which gave donor **11** in 84% yield. Alternatively, the benzoyl groups were introduced by treating diol **10** with benzoyl chloride and DMAP in DCE at 70˚C. The unusual high temperature was needed in order

to get full benzoylation, as the C4-OH is relatively unreactive at room temperature. This procedure gave benzoyl donor **12** in a near quantitative yield. Thiofucoside **12** was converted into imidate **13** by hydrolyzing **12** with NBS in wet acetone, followed by imidoylation of the formed hemi-acetal with Cs_2CO_3 and $Cl(C{=}NPh)CF_3$ in acetone. This treatment led to imidate **13** in a yield of 85% over two steps.

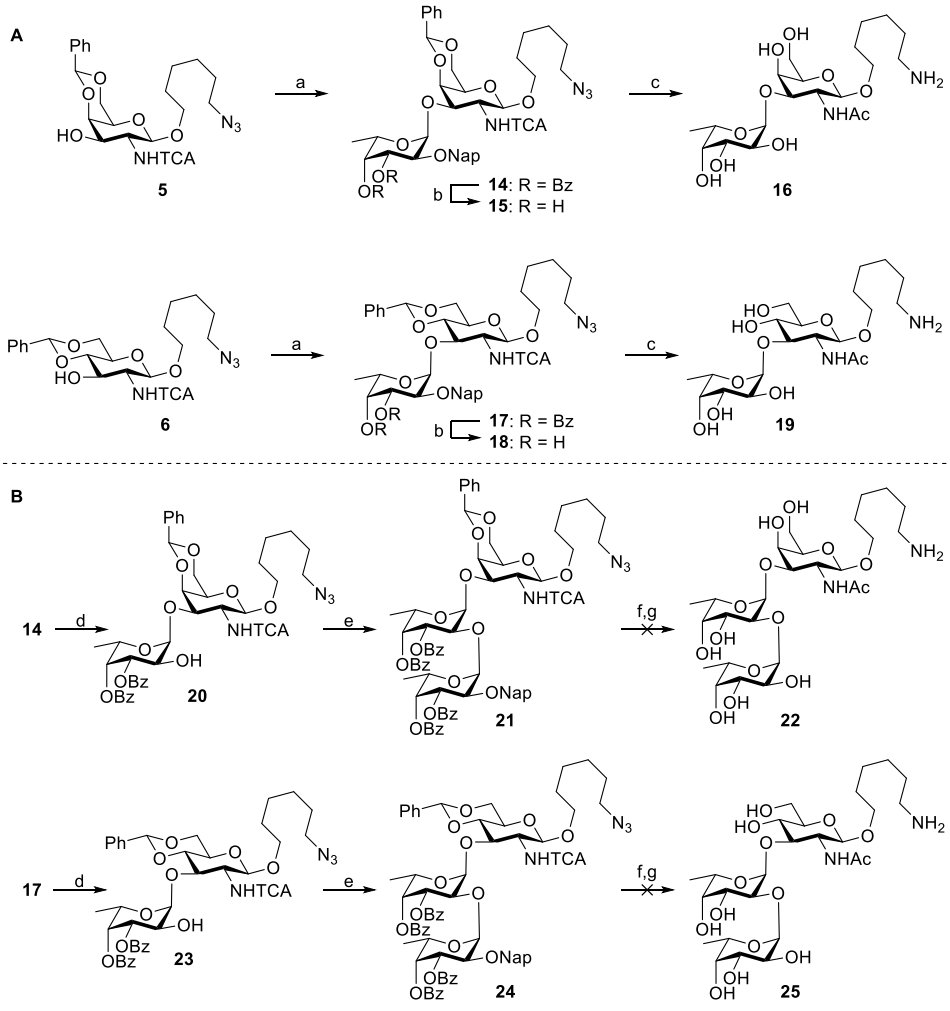
*Scheme 1: Synthesis of: **A**) GalNac and GlcNAc acceptors **5** and **6**, **B**) fucosyl donors **11-13***



Reagents and conditions: **a**: $PhCH(OMe)_2$, *p*-TsOH, ACN, 50˚C, 330 mbar, **3** 86%, **4** 85%, **b**: i) 6-azidohexan-1-ol, NIS, TMSOTf (cat.), MS (3Å), DCM -20˚C → 0˚C, ii) Trt-Cl, DMAP, DCM, 18h, **5** 72%, **6** 66%, **c**: 2,2-dimethoxypropane, *p*-TsOH, ACN, 50˚C, 330 mbar, 92%, **d**: NaH, Nap-Br, DMF, 94%, **e**: HCl (aq.), H_2O, dioxane, 99%, **f**: NaH, Bn-Br, TBAI, DMF, 84%, **g**: Bz-Cl, DMAP, DCE, 70˚C, 99%, **h**: NBS, acetone, H_2O, **i**: Cs_2CO_3, $Cl(C{=}NPh)CCl_3$, DMF, 85% (over two steps).

With the required monosaccharide synthons available, the assembly of target disaccharides **16** and **19** and trisaccharides **22** and **25** started with the fucosylation of the C3-OH of either the glucosamine building block **6** or the galactosamine building block **5** (Scheme 2). Condensation of fucosyl donor **12** with glucosamine acceptor **6** using NIS in conjunction with TMSOTf gave disaccharide **17** in 88% yield. The formation of the α-linked product was confirmed by NMR analysis ($J_{1\text{-}2}$ = 3.6 Hz and $^1J_{C\text{-}1,\ H\text{-}1}$ = 170 Hz).[23] Disaccharide **14** was synthesized in a similar manner in a yield of 76%. The benzoyl esters of **14** and **17** were cleaved using sodium methoxide in a mixture of THF and methanol to yield diols **15** and **18** in 78% and 60%, respectively. Several methods were tried to reduce the TCA group to an acetyl, such as, reduction by Zn/Cu, radical dehalogenation with Bu_3SnH and catalytic hydrogenation.[14]–[16] Unfortunately, reduction with Zn/Cu or Bu_3SnH did not give consistent results. Catalytic hydrogenation on the other hand, did give the desired products in reproducible yields. Fully deprotected disaccharides **16** and **19** were obtained in 63% and 67% yield, respectively.

*Scheme 2: Synthesis of **A**) mono-fucosylated GalNAc/GlcNAc, **B**) di-fucosylated GalNAc/GlcNAc.*

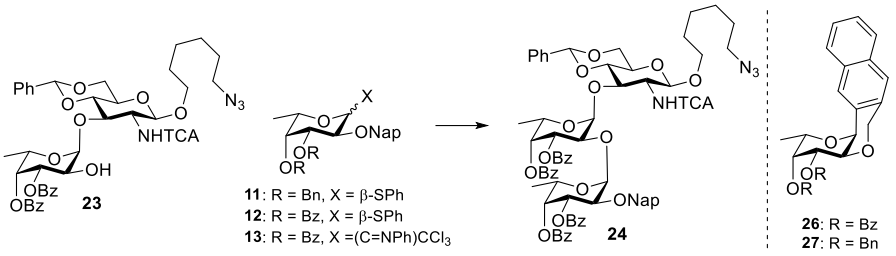


Reagents and conditions: **a**: **12**, NIS, TMSOTf, MS (3Å), DCM, -40˚C → -20˚C, **14** 76%, **17** 86%, **b**: NaOMe, MeOH, THF, **15** 60%, **18** 78% **c**: Pd/C, H_2, EtOH, **16** 67%, **19** 63%, **d**: DDQ, aqueous phosphate buffer (pH 7), DCM, 0˚C → RT, **20** 90%, **23** 88%, **e**: **12**, NIS, TMSOTf, MS (3Å), -40˚C → -20˚C, **21** 76%, **24** 47%, **f**: KOH, MeOH, THF, **g**: Pd/C, H_2, dioxane, H_2O.

Next, the assembly of the trisaccharides **22** and **25** was undertaken. To this end, the naphthyl ethers on disaccharides **14** and **17** were removed to allow for elongation of the fucoside chain with a second fucosyl residue. The use of DDQ in a mixture of DCM and water or DCM and methanol led to a substantial amount of benzylidene removal. It was reasoned that the benzylidene was cleaved by the formation of the weakly acidic quinone, and therefore an aqueous phosphate buffer (pH 7) was used instead of water or methanol to prevent this.[24], [25] Under these conditions the removal of the naphthyl

ether proceeded slower, but the formation of the de-benzylidenated byproduct was completely suppressed and the yield of disaccharides **20** and **23** was significantly increased. Subsequent fucosylation of **20** and **23** with donor **12** using the same protocol as described above gave trisaccharide **21** in 76% yield, while trisaccharide **24** was obtained in a yield of only 31%. Besides the desired trisaccharides a substantial amount of succinimidoyl fucoside was formed in these reactions. In order to increase the yield of trimer **24** several other methods were tried, which are shown in Table 1.

*Table 1: optimization of fucosylation of disaccharide **23***



entry	donor (eq.)[a]	reagents (eq.)[a]	time (h)	temp. (˚C)	yield (%)
1	**12** (1.5)	NIS (2.0), TMSOTf (0.1)	5	-40 → -20	31
2	**12** (1.5)	NIS (2.0), TMSOTf (0.1)	48	-40 → -20	7
3	**11** (1.5)	NIS (2.0), TMSOTf (0.1)	5	-40 → -20	-[b]
4	**12** (2.0)	DPS, TTBP, Tf_2O	5	-80 → -60	-[b]
5	**12** (1.5)	NIS (1.6)[c], TMSOTf (0.1)	10	-40 → -20	21
6	**12** (1.5)	NIS (1.6), TMSOTf (0.1)	5	-40 → 0	25
7	**12** (2.0)	NIS (2.2), TMSOTf (0.1)	2	-40 → -20	47
8	**13** (1.5)	TMSOTf (0.1)	3	-20	46
9	**13** (1.5)	TBSOTf (0.1)	3	-20	42

*[a] Relative to the acceptor, [b] formation of compound **26** or **27**, [c] NIS was dissolved in DCM and slowly added to the reaction mixture.*

Surprisingly, increasing the reaction time of the NIS/TMSOTf mediated glycosylation led to a severe drop in yield to 7% (entry 2). The use of armed fucosyl donor **11** resulted solely in the formation of by-product **27** (entry 3). Changing to a pre-activation method using diphenyl sulfoxide (DPS), 2,4,6-*tri-tert*-butylpyrimidine (TTBP) and triflic anhydride for the activation of donor **12** led to the formation of byproduct **26** (entry 4). Guided by the article of Marqvorsen et al., NIS was added slowly to the reaction mixture to prevent formation of the succinimidoyl fucoside (entry 5).[26] Unfortunately, this proved fruitless as the yield dropped to 21%. Increasing the temperature to 0˚C also proved detrimental to the yield (entry 6). In a final attempt to increase the yield with this activation system a shorter reaction time was employed in conjunction with an increase in the amount of donor used (entry 7). This resulted in the formation of trisaccharide **24** in a yield of 47%. Next, imidate donor **13** was used as the NIS/TMSOTf activator couple gave rise to the

formation of succinimidoyl fucoside (entries 8 and 9). Two different activators, TMSOTf and TBSOTf, were used in combination with imidate donor **13**, as it has been reported that the milder TBSOTf can give rise to higher yields.[27], [28] The use of the imidate donor **13** gave trisaccharide **24** in a yield of 46%, although the difference between the two activators was marginal. A noteworthy observation was that the acceptor was never fully consumed in any of these reactions, regardless of the reaction time.

To shed light on the unexpected difference between the reactivity of acceptors **20** and **23** , DFT calculations were performed on close analogues of these acceptors (for ease of calculations the 6-azido hexan-1-ol was replaced by a butanol moiety), to analyze their preferred geometry and flexibility.[29] Figure 3 shows the stability of different rotamers of dimers **20** and **23**, obtained by changing the angle around D1 and D2. The X-axis shows the rotation around the C3-O of glucosamine moiety and the Y-axis shows the rotation around the C1-O of the fucose residue. The plotted spheres show relevant low energy conformations. The glucosamine acceptor **23** shows only two families of local minima (Figure 3-B), which are located closely to one another. This indicates that this acceptor is relatively rigid. Upon inspection of the structure one can see that the fucosyl C2-OH group is in close proximity to the benzylidene ring, which may hamper the reaction. The galactosamine disaccharide acceptor **20** on the other hand shows several low energy structures with diverging geometries (Figure 3-A). This indicates that a larger conformational space is accessible for this acceptor, which might make it easier for this acceptor to accommodate steric interactions in the crowded glycosylation reaction transition state.

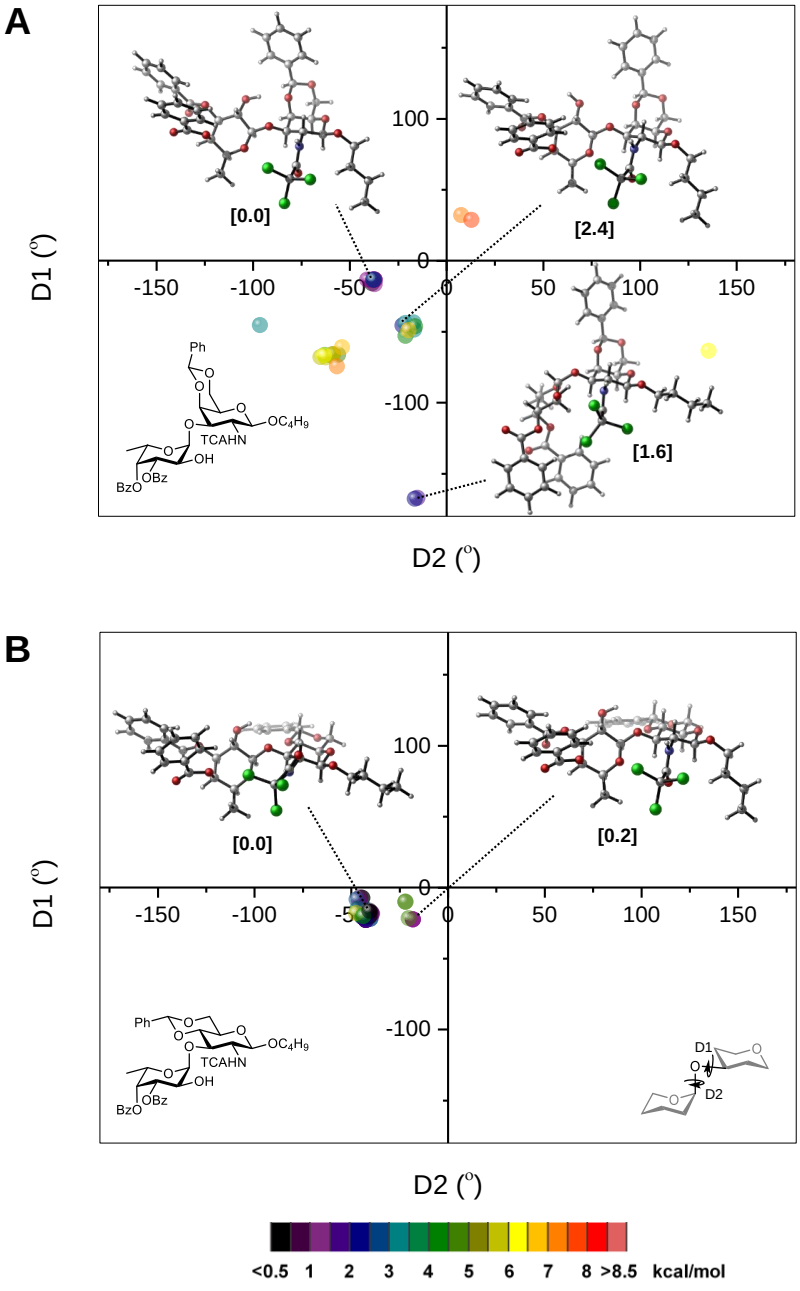


*Figure 3: Scatter plot of the computed conformational library of **20** and **23.** The X-axis shows the rotation around the C3-O3 bond of the galactosamine/glucosamine (D1) and the Y-axis shows the rotation around the C1-O1 of the fucose residue (D2).The dihedral angles D1 and D2 are plotted and the energy of the corresponding geometry is represented as the depicted color. Relevant low energy conformations are shown with their ΔG^{T}_{DCM} in brackets (kcal/mol). For ease of calculations the 6-azidohexan-1-ol linker has been replaced with a butanol moiety.*

The NMR spectra of **21** and **24** also provide an indication for the restricted rotational freedom for trisaccharide **24** (Figure 4). The proton spectrum of **21** (Figure 4-A) shows well resolved peaks, whereas the spectrum of **24** (Figure 4-B) shows three distinctly broadened peaks (marked in green). These peaks correspond to the H-3, H-5 (GlcNAc) and H-6' (Fuc), respectively. Besides these broadened signals, the peaks of the anomeric protons of the fucosyl residues (marked in blue) differ significantly as well. The two anomeric fucosyl protons of **21** have a very similar shift, while those of **24** differ by 0.8 ppm, indicating they have a different chemical environment. The ^{13}C spectra show similar features: the ^{13}C carbon spectrum of **21** shows well resolved peaks (Figure 4-C), whereas the peaks of C4, C3 and C2 (GlcNAc) and C-6' (Fuc) (marked in green) are broadened in the spectrum of **24** (Figure 4-D). The C-1 (GlcNAc) and C-1' (Fuc) anomeric carbons of **24** show distinct broadening as well. These findings can indicate restricted rotation of bonds in trisaccharide **24** as a result of a sterically demanding structure.

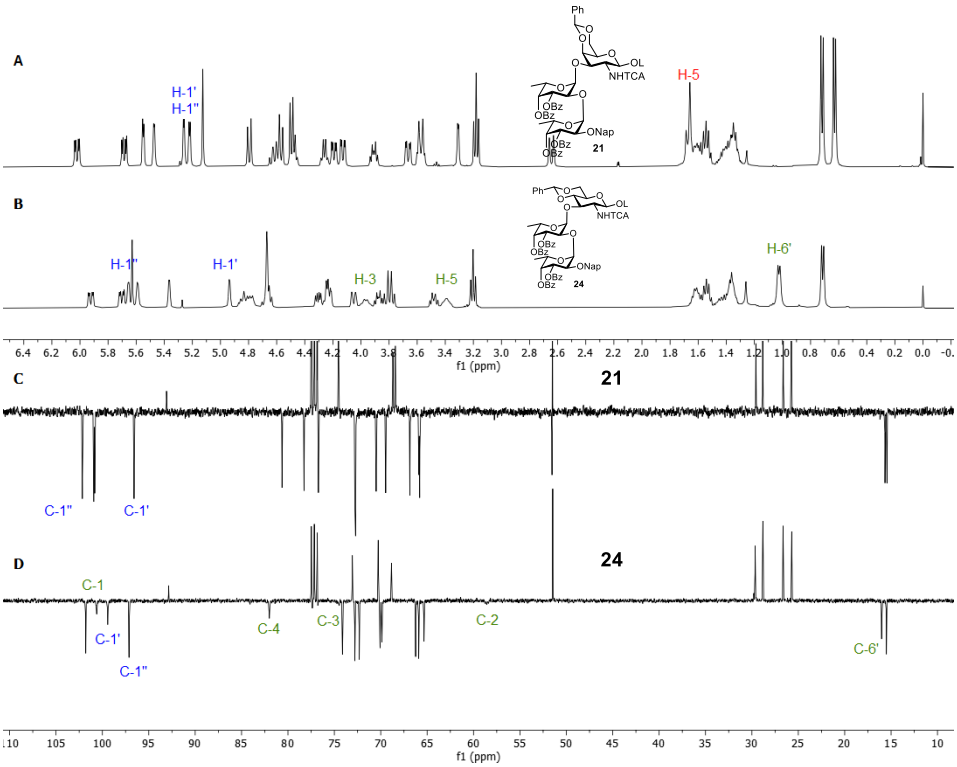


*Figure 4: Partial ^{1}H and ^{13}C-APT NMR spectra of trisaccharides **21** and **24**. Where **A** and **C** are the proton and carbon spectra of **21** and **B** and **D** are the proton and carbon spectra of **24**. The anomeric protons or carbons of the fucosyl residues are marked in blue, broadened peaks are marked in green and the H-5 of **21** is marked in red. The 6-azidohexyl linker is abbreviated as L. All spectra were recorded in $CDCl_3$ at room temperature on a 400MHz NMR.*

Although the spectra of galactosamine trisaccharide **21** do not show any peak broadening, they do have another remarkable characteristic. The peak of H-5 (GalNAc) was found at 1.7 ppm (Figure 4). Upon heating to 60˚C the H-5 proton shifts only slightly to 1.9 ppm (see supporting info), indicating that the structure adopts a relatively stable conformation. The explanation for this unusual shift is that the H-5 is positioned close to the shielding cone of the naphthyl ring system (Figure 5).[30] The GlcNAc trisaccharide **24** does not show this shift, indicating the benzylidene ring is preventing it from adopting this conformation. ^{1}H NMR spectra of the ensuing deprotection steps of both **21** and **24**, where besides the H-5 the H-1 also show this feature, are shown in the supporting information of this chapter (Figure 7-8).

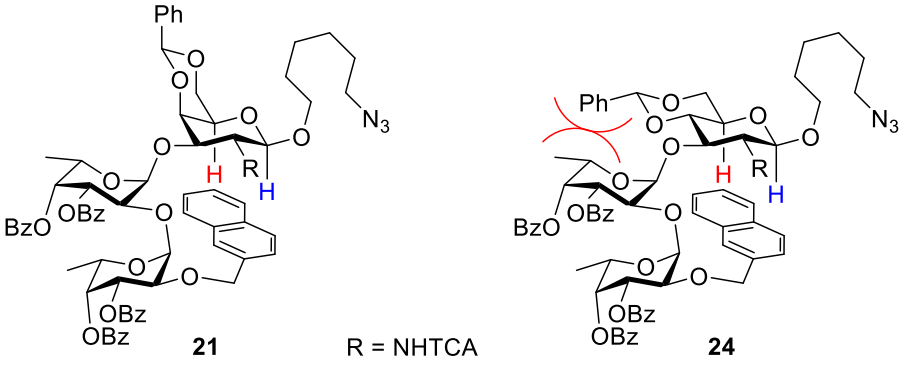


*Figure 5: Schematic representation of interaction between the naphthyl ring of trisaccharides **21** and **24** with the H-1 (blue) and H-5 (red) protons of the GalNAc or GlcNAc moiety.*

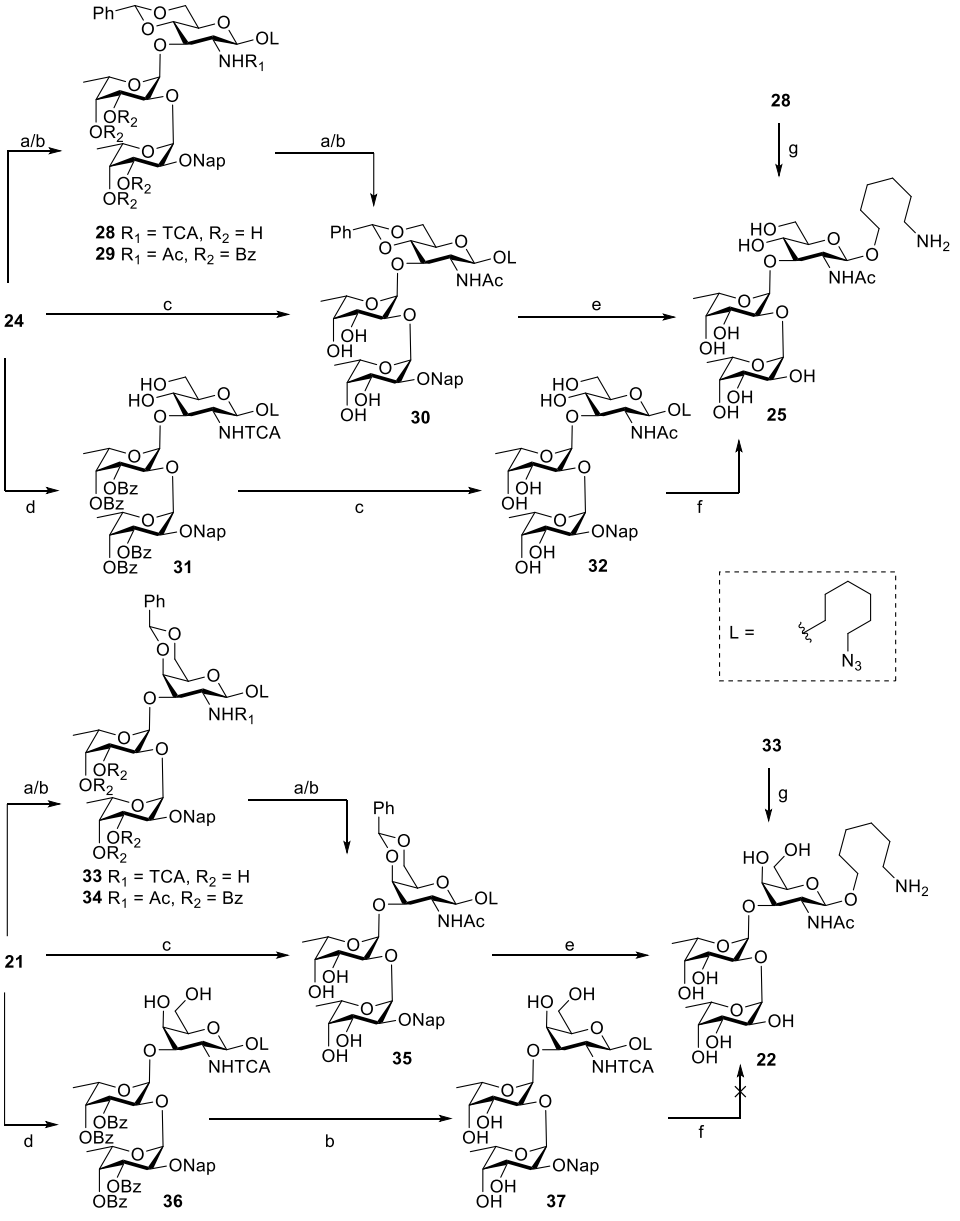
The favourable outcome of the deprotection procedure used for target disaccharides **16** and **19** was an incentive to apply the same procedures for the deprotection of trisaccharides **21** and **24** (Scheme 2). To this end the benzoyl esters were hydrolyzed using KOH in a mixture of THF and methanol, which led to the desired tetraols. A small amount (~5%) of de-TCAylated product was obtained as well. Surprisingly, the catalytic hydrogenation of these tetraols gave disaccharides **16** and **19** as the products instead of the desired trisaccharides **22** and **25**. It was hypothesized that the HCl formed by the reduction of the NHTCA induced glycosidic bond cleavage.

Several different deprotection sequences were attempted and are shown in Scheme 3. Initially, **21** and **24** were treated with sodium hydroxide to hydrolyze both the benzoyl esters and the TCA group, followed by selective acetylation of the free amine with acetic anhydride in a mixture of methanol and Et_3N, which gave **30** and **35** in 29% and 37% yield, respectively. As the purification of the free amine intermediate proved challenging a more stepwise approach was tried. Employing a protocol developed by Urabe *et al.* the TCA group was removed with Cs_2CO_3 in DMF heated to 80˚C.[31] The resulting isocyanate

was poured into saturated aqueous $NaHCO_3$, which caused decarboxylation of the amine to occur.[32] The resulting free amine was then acetylated by Ac_2O in conjunction with pyridine to give **29** and **34** in 71% and 48% yield. The benzoyl groups were cleaved as before by NaOMe in methanol/THF, resulting in compounds **30** and **35**. A reversal of these two procedures led to a significant drop in the yields. Catalytic hydrogenation of trisaccharides **30** and **35** led to the desired trisaccharides **22** and **25** in yields of 71% and 56%, respectively. Two hydrogenation cycles were required for both trisaccharides **30** and **35** as the first cycle tended to leave the benzylidene ring intact, presumably due to poisoning of the catalyst by 2-methylnaphthalene.[33], [34] In an alternative procedure the benzylidene acetals were removed first by a catalytic amount of *p*-TsOH in ACN and MeOH, which gave **31** and **36** in 48% and 69% yield. Hydrolysis of both the benzoyl esters and the NHTCA groups in **31** by NaOH, followed by selective acetylation of the free amine gave **32** in 89% yield. Trisaccharide **32** was then subjected to catalytic hydrogenation leading to the desired trisaccharide **25** in 82% yield. Unfortunately, when **36** was subjected to the same conditions the desired trisaccharide could not be isolated. Therefore milder conditions were used for the removal of the benzoyl esters. The use of NaOMe in THF/MeOH provided trisaccharide **37**, with the NHTCA intact. Compound **37** was obtained in 73% yield, but when it was subjected to catalytic hydrogenation conditions disaccharide **16** was obtained. This proved the hypothesis that reduction of the TCA group was the cause of the formation of disaccharides.

As it was shown that the acid formed by the reduction of the NHTCA group led to the glycosidic bond breakage a second strategy was attempted. It was envisioned that by neutralizing the acid during the hydrogenation of **21** and **24** the trisaccharides **22** and **25** could be isolated. Initially, a phosphate buffer (pH~7) phosphate buffer was used to counteract the acid. Although this procedure provided the desired trisaccharides, a byproduct was formed as well, hindering further purification. Addition of Et_3N led to poisoning of the Pd catalyst and was therefore ineffective. Finally, the use of two equivalents of solid $NaHCO_3$ provided an effective protocol and the desired trisaccharides **22** and **25** could be obtained 77% and 79%, respectively.

*Scheme 3: deprotection sequences of trisaccharides **22** and **25***



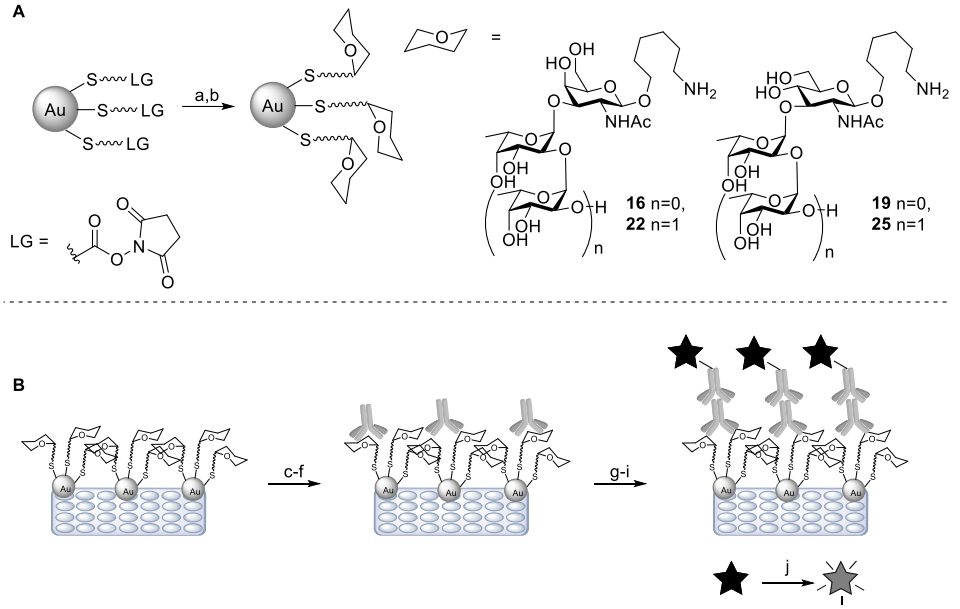
Reagents and conditions: **a**: Cs_2CO_3, DMF, 80˚C, **29** 71%, **30** 25%, **34** 48% **b**: NaOMe, THF/MeOH, **28** 81%, **30** 99%, **33** 70% **c**: i) NaOH, THF/MeOH, 60˚C, ii) Ac_2O, **30** 29%, **32** 89%, **35** 37%, **37** 73% **d**: *p*-TsOH (cat.), MeOH, ACN, **31** 48%, **36** 69% **e**: Pd/C, H_2, H_2O, THF, *t*-BuOH, **25** 71%, **22** 56% **f**: Pd/C, H_2, H_2O, **25** 82%, **g**: Pd/C, $NaHCO_3$, H_2, **25** 79%, **22** 77%.

Gold nanoparticle based ELISA's

The detection of anti-glycan antibodies in serum is highly relevant for the evaluation of carbohydrate-based vaccines and pathogen infection and for future monitoring of success of worm-elimination programs (either by vaccination or by drug treatment).[35] In order to test if the synthesized glycans can be used as a tool to determine the level of infection in infected people, several ELISA's were performed. As bonding interactions between individual glycans and antibodies tend to be relatively weak, the glycans were chemically linked to a carrier to reach a multivalent epitope presentation. To this end gold nanoparticles (AuNP's) were chosen as they are highly multivalent, immunologically inert and Glyco-AuNP's have been used in ELISA-based diagnostic systems.[36], [37] The high glycan-concentration on a small surface (2-5 nm) is responsible for their high-sensitivity. Initially, the AuNP's were screened against monoclonal antibodies (mAb's) to verify that the glycans could still be recognized. After establishing the selective interaction with these mAb's, the AuNP's were used to screen sera of infected people.

The synthesized compounds bearing the α-(1-2) linked fucosyls **16**, **19**, **22** and **25** were chemically attached to pre-formed 5 nm *N*-hydroxysuccinimide activated gold nanoparticles (Scheme 4-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 4-B).[10] Antibody 114-5B1-A (IV) was tested as it has been shown to bind di- and tri-fucosylated glucosamine structures. Antibodies 291-5D5A (II) and 291-4D10-A (V) were screened as they bind to Lewis X type structures. The mAb's 114-4D12-AA (III) and 258-3E3 (I) were screened as they have been shown to bind to glycans containing di- and trifucosyl chains. Lastly, 273-3F2-A (VI), which has been shown to recognize LDN fragments in adult worms but not in soluble egg antigen (SEA) was screened as a negative control antibody. SEA was used as the positive control in all ELISA experiments.

*Scheme 4: **A**) Functionalization of gold nanoparticles, **B**) ELISA protocol.*



Reagents and conditions: **a**: **1, 2**, **3** or **4**, AuNP, "reaction buffer", **b**: "quencher solution", **c**: AuNP, coating buffer, **d**: washing with PBS buffer, **e**: blocking with 1% BSA, **f**: monoclonal antibody or human sera, **g**: washing with PBS buffer, **h**: secondary antibody RAM/PO, **i**: washing with PBS buffer, **j**: i) TMB substrate, ii) 1M H_2SO_4 (aq.).

As can be seen from (Figure 6-A) mAb's I, II, and III were able to recognize the synthesized glycans, while IV, V and VI, did not. As expected mAb III selectively bound to the di-fucosylated structures **22** and **25**. Notably, antibody I only recognized mono- and di-fucosylated GalNac elements **16** and **22**, but not their GlcNAc counterparts. Interestingly, antibody II, known to bind LewisX type structures, bound to mono-fucosylated GalNAc, but not Fuc-GlcNAc. As can be seen in Figure 6-A the negative control particles, coated with GalNAc or bearing no glycans, were not recognized by any antibody, indicating that all recognition was due to the specific glycans on the AuNP's. The recognition of these mAb's showed that the clustered synthesized glycans can be used to probe antibody binding with the right specificity. In order to see if the functionalized AuNP's could also bind to antibodies present in human sera isolated from infected people another ELISA was performed, with non-infected human sera as the control (Figure 6-B). ELISA wells were coated with the prepared AuNP's and sera from an Ugandese infected community (Piida cohort) was added. The presence of IgG against the AuNP library was then achieved following the classic ELISA detection steps depicted in Scheme 4-B. As can be seen in Figure 6-B there is a clear difference in binding between antibodies in sera from non-infected individuals and the Piida cohort for the trisaccharides. These glycans bind more

antibodies in the sera of the infected people indicating that these two structures, bearing the distinctive di-fucosyl motif, may be used as diagnostic markers for infection.

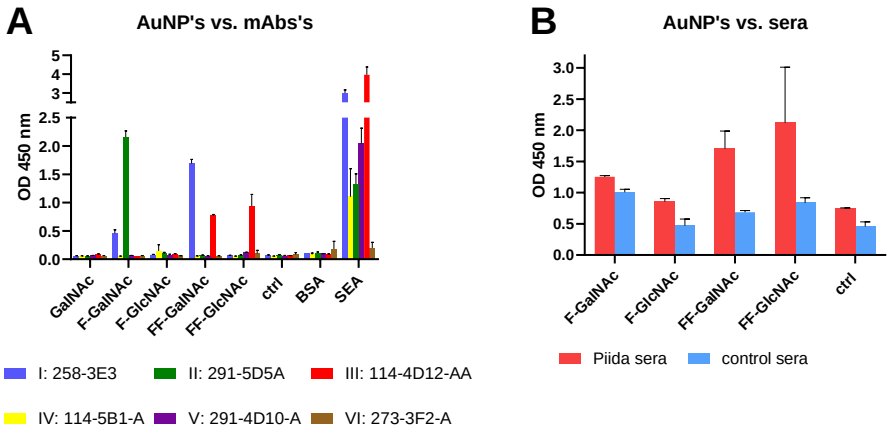


*Figure 6: Bar graph of the results of the ELISA experiment with monoclonal antibodies (**A**) and human sera (**B**) from either infected (red) or control samples (blue). AuNP: gold nanoparticle, GalNAc: 6-aminohexan-1-ol N-acetamido-β-D-galactosamine, ctrl: unfunctionalized AuNP, BSA: bovine serum albumin, SEA: soluble egg antigen, OD: optical density. Error bars indicate standard deviations of the experiment performed in duplicate measurements. The experiments were repeated three times showing similar results.*

Conclusion

This chapter dealt with the synthesis of two different epitopes of multi-fucosylated schistosomal glycans, **22** and **25**. In addition to these two trisaccharide glycans, bearing characteristic di-fucosyl chains, monofucosylated glycans **16** and **19** were synthesized. The assembly of the fucosyl chains proved challenging for the GlcNAc-trisaccharide. While fucosyl donor **12** could be successfully used to introduce the first fucosyl residue in an α-manner, extension of the α-(1-2)-fucosyl chain provided the protected Fuc-Fuc-GlcNAc trisaccharide **24** in 47% yield. The analogous fucosylation of the Fuc-GalNAc disaccharide proceeded faster and with a significantly higher yield (76%). To explain the difference between the yields of these two disaccharide acceptors, their structures were studied using DFT calculations. Conformational analysis of the GalNAc disaccharide showed this disaccharide to be more flexible than its GlcNAc counterpart. The fucosyl C2-OH in the Fuc-GlcNAc dimer was located close to the benzylidene ring of the GlcNAc moiety and the steric constraints of the relatively rigid structure impeded the glycosylation reaction. The NMR spectra of the Fuc-Fuc-GlcNAc trisaccharide showed broadened peaks, indicating restricted motion of this saccharide. The labile nature of the unique α-(1-2)-fucosyl linkage required the use of $NaHCO_3$ during the reduction of the TCA group to prevent breaking of this glycosidic linkage by the released HCl. Gold nanoparticles were successfully decorated with the generated di- and trisaccharides.

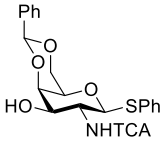
These functionalized particles were then screened against several monoclonal antibodies in an ELISA experiment, which showed that the glycans could be engaged in selective antibody binding. Further ELISA studies using sera from a schistosome infected cohort, showed that the trisaccharides, bearing the characteristic di-fucosyl chains showed selective binding of antibodies present in sera from infected people. This shows that the gold nanoparticles functionalized with the Fuc-Fuc-GlcNAc and Fuc-Fuc-GalNAc trisaccharides may be used for future diagnostic purposes to detect schistosomiasis.

Experimental

General procedures

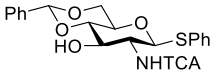
Glassware used for reactions was oven dried before use at 80°C. Anhydrous solvents were prepared by drying them over activated molecular sieves (3Å) for at least 24 hours before use. Molecular sieves were activated by flame-drying under reduced pressure. Reactions that required anhydrous conditions were 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. 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 4,6-*O*-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (3)



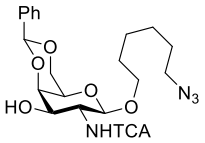
To a solution of triol **1** (15.85 mmol, 6.60 g, 1.0 eq.) dissolved in acetonitrile (120 mL, 0.15M) were added benzaldehyde dimethylacetal (17.57 mmol, 2.63 mL, 1.1 eq.) and p-toluenesulfonic acid (1.59 mmol, 0.30 g, 0.1 eq.). The solution was heated to 50 °C and stirred in a rotary evaporator at reduced pressure (350 mbar) for 2 hours. The reaction was quenched by addition of Et_3N and the reaction mixture was poured onto a cold (0 °C) mixture of 1:1 Et_2O and heptane. The solids were collected and purified by silica gel chromatography (PE: EtOAc, 9:1 → 7:3). The title compound was obtained as a white solid in a 76 % yield. (12.05 mmol, 6.08 g). Spectral data was in accordance with those reported in literature.[38]

Phenyl 4,6-*O*-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (4)



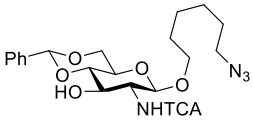
To a solution of triol **2** (10.4 g, 25.0 mmol, 1.0 eq.) dissolved in acetonitrile (150 mL, 0.15M) were added benzaldehyde dimethylacetal (60.0 mmol, 9.0 mL, 2.4 eq.) and p-toluenesulfonic acid (2.5 mmol, 0.47 g, 0.1 eq.). The solution was heated to 50 °C and stirred in a rotary evaporator at reduced pressure (350 mbar) for 3 hours. The reaction was quenched by addition of Et_3N and the reaction mixture was poured onto a mixture of 1:1 cold Et_2O and heptane. The brown solids were collected and purified by silica gel chromatography (PE: EtOAc, 9:1 → 7:3). The title compound was obtained as a white solid in 87% yield. (11.0 g, 21.8 mmol). Spectral data was in accordance with those reported in literature.[15]

6-azidohexyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (5)



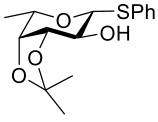
Acceptor **3** (1.53 g, 3.00 mmol, 1.0 eq.) and 6-azidohexan-1-ol (0.86 g, 6.00 mmol, 2.0 eq.) were co-evaporated trice with dry toluene and dissolved in dry DCM (20 mL, 0.15M). NIS (0.901 g, 4.00 mmol, 1.3 eq.) and MS (3Å) were added and the mixture was cooled to -20˚C and stirred at that temperature for 1 hour. TMSOTf (54 µL, 0.3 mmol, 0.1 eq.) was added and the mixture was allowed to warm up to 0˚C. When TLC analysis showed full conversion (~1 hour) Et_3N (0.5mL) was added and 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. $NaHCO_3$ (aq.) and brine, before being dried over $MgSO_4$. The $MgSO_4$ was filtered off and the volatiles were removed *in vacuo*. The crude oil was dissolved in DCM (20 mL, 0.15M) and trityl chloride (1.67 g, 6.00 mmol, 2.0 eq.) and DMAP (0.73 g, 6.00 mmol, 2.0 eq.) were added and the reaction was left to stir for 5 hours. The reaction mixture was diluted in EtOAc and washed twice with sat. $CuSO_4$ (aq.) and once with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The title compound was obtained after silicagel chromatography (PE:EtOAc, 4:1 → 1:1) as a white solid (1.15 g, 2.15 mmol, 72%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.59 – 7.43 (m, 2H, Ph), 7.43 – 7.29 (m, 3H, Ph), 7.00 (d, 1H, *J*=7.9 Hz, NH), 5.48 (s, 1H, CHPh), 4.60 (d, 1H, *J*=8.3 Hz, H-1), 4.27 (d, 1H, *J*=12.4 Hz, H-6), 4.15 (d, 1H, *J*=3.7 Hz, H-4), 4.12 – 4.01 (m, 2H, H-3, H-6), 3.95 – 3.77 (m, 2H, H-2, OCH_2), 3.48 (s, 1H, H-5), 3.42 (dt, 1H, *J*=9.5, 6.7 Hz, OCH_2), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.94 (d, 1H, *J*=9.7 Hz, 3-OH), 1.63 – 1.47 (m, 4H, CH_2, hexyl), 1.33 (td, 3H, *J*=7.1, 6.3, 3.8 Hz, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 162.6 (C=O, TCA), 137.5, 129.3, 128.3, 126.4 (Ph), 101.1 (CHPh), 100.1 (C-1), 92.7 (C_q, TCA), 75.0 (C-4), 69.6 (OCH_2), 69.4 (C-3), 69.1 (C-6), 66.6 (C-5), 56.3 (C-2), 51.4 (CH_2N_3), 29.4, 28.8, 26.5, 25.6 (CH_2, hexyl) ppm. HRMS $[M+H]^+$ calcd for $C_{21}H_{27}Cl_3N_4O_6H$: 537.10745, found 537.10681.

6-azidohexyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (6)



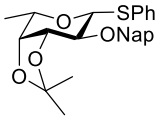
6-azidohexan-1-ol (1.07 g, 7.50 mmol, 1.5 eq.) and acceptor **4** (2.52 g, 5.00 mmol, 1.0 eq.) and were co-evaporated thrice with dry toluene and dissolved in dry DCM (40 mL, 0.15M). MS (3Å) and NIS (1.68 g, 7.50 mmol, 1.5 eq.) were added and the mixture was cooled to -20˚C and stirred at that temperature for 1 hour. TMSOTf (90 µL, 0.50 mmol, 0.1 eq.) was added and the mixture was allowed to warm up to 0˚C. When TLC analysis showed full conversion (~1 hour) Et_3N (0.5mL) was added and 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. $NaHCO_3$ (aq.) and brine, before being dried over $MgSO_4$. The $MgSO_4$ was filtered off and the volatiles were removed *in vacuo*. The crude oil was dissolved in DCM (40 mL, 0.15M) and trityl chloride (1.11 g, 4.00 mmol, 0.8 eq.) and DMAP (0.49 g, 4.00 mmol, 0.8 eq.) were added and the reaction was left to stir for 5 hours. The reaction mixture was diluted in EtOAc and washed twice with sat. $CuSO_4$ (aq.) and once with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The title compound was obtained after silicagel chromatography (PE:EtOAc, 4:1 → 1:1) as a white solid (1.79 g, 3.33 mmol, 66%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.52 – 7.45 (m, 2H, arom.), 7.42 – 7.34 (m, 3H, arom.), 6.97 (d, 1H, *J*=7.2 Hz, NH), 5.54 (s, 1H, CHPh), 4.91 (d, 1H, *J*=8.3 Hz, H-1), 4.41 – 4.26 (m, 2H, H-3, H-6), 3.89 (dt, 1H, *J*=9.6, 6.3 Hz, OCH_2), 3.79 (t, 1H, *J*=10.0 Hz, H-6), 3.59 – 3.44 (m, 4H, H-2, H-4, H-5, OCH_2), 3.25 (t, 2H, *J*=6.8 Hz, CH_2N_3), 3.02 – 2.89 (3, 1H, 3-OH), 1.66 – 1.52 (m, 4H, CH_2, hexyl), 1.43 – 1.33 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 162.3 (C=O, TCA), 137.0, 129.5, 128.5, 126.4 (arom.), 102.0 (CHPh), 100.1 (C-1), 92.6 (C_q, TCA), 81.7 (C-5), 70.4 (OCH_2), 69.7 (C-3), 68.7 (C-6), 66.3 (C-4), 59.8 (C-2), 51.5 (CH_2N_3), 29.5, 28.9, 26.6, 25.7 (CH_2-hexyl) ppm. HRMS $[M+Na]^+$ calcd for $C_{21}H_{27}Cl_3N_4O_6Na$: 559.08884, found 559.08849.

Phenyl 3,4-*O*-isopropylidene-1-thio-β-L-fucopyranoside (8)



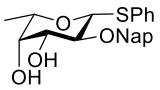
Triol **7** (18.4 g, 72 mmol, 1.0 eq.) was dissolved in dry ACN (220 mL, 0.3M). DMP (17.6 mL, 143.6 mmol, 2.0 eq.) and *p*-TsOH (1.4 g, 7.3 mmol, 0.1 eq.) were added and the flask was attached to a rotavap. The reaction was carried out at 50˚C and a pressure of 330 mbar. TLC analysis indicated full conversion after 1 hour and the reaction was stopped by addition of Et_3N. The volatiles were evaporated and the product could be obtained by crystallization from Et_2O and hexane. **8** was obtained as white needles (19.2 g, 64 mmol, 89%). Spectral data was in accordance with those reported in literature.[39]

Phenyl 3,4-*O*-isopropylidene-2-*O*-(2-naphthylmethyl)-1-thio-β-L-fucopyranoside (9)



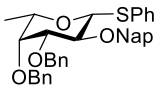
Fucoside **8** (15.3 g, 51.9 mmol, 1.0 eq.) was dissolved in dry DMF (150 mL, 0.3M) and cooled to 0˚C. After 30 min. NaH (2.5 g, 62 mmol, 1.2 eq.) was added portion-wise. Nap-Br (13.8 g, 62 mmol, 1.2 eq.) was added after the evolution of gas had stopped. Upon full conversion of the starting material (2 hours), the remaining NaH was quenched by slow addition of H_2O. The solution was transferred to a separatory funnel, were it was washed with heptane. The solution was then diluted in Et_2O and washed with brine (5x), dried over $MgSO_4$, filtered and concentrated. This gave naphthyl ether **9** as a white solid (21.4 g, 49 mmol, 94%). Spectral data was in accordance with those reported in literature.[39]

Phenyl 2-*O*-(2-naphthylmethyl)-1-thio-β-L-fucopyranoside (10)



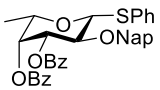
HCl (20 mL, of a 1M aqueous solution) was added to a solution of **9** (21 g, 49 mmol, 1.0 eq.) dissolved in ACN (100 mL, 0.5M). The flask was attached to a rotavap, which was then set to 50˚C at a pressure of 500 mbar. TLC analysis showed full conversion after 90 min. The reaction mixture was neutralized by addition of Et_3N and concentrated. The residue was dissolved in acetone and dry-loaded onto celite, which was then purified by silicagel chromatography (PE:EtOAc, 3:2 → 1:9) to yield **10** as a white solid (25.1 g, 48 mmol, 98%). Spectral data was in accordance with those reported in literature.[39]

Phenyl 3,4-*O*-di-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-β-L-fucopyranoside (11)



Diol **10** (0.79 g, 2.0 mmol, 1.0 eq.) was dissolved in dry DMF (20 mL, 0.1M) and cooled to 0˚C. NaH (0.19 g, 4.8 mmol, 2.4 eq.) was added and the reaction mixture was stirred until the bubbling stopped. BnBr (0.71 mL, 6.0 mmol, 3.0 eq.) was added and the ice bath was removed. When TLC analysis showed full conversion of the starting material, MeOH was added and the reaction was diluted in Et_2O. The organic phase was washed five times with brine, followed by drying over $MgSO_4$, filtered and concentrated. The residue was purified by silicagel chromatography (PE:Et_2O, 99:1 → 3:1) which gave **11** as a white solid (0.97 g, 1.68 mmol, 84%). Spectral data was in accordance with those reported in literature.[39]

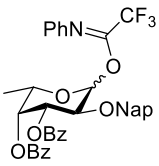
Phenyl 3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-1-thio-β-L-fucopyranoside (12)



Diol **10** (3.96 g, 10.0 mmol, 1.0 eq.) was dissolved in a mixture of DCM and pyridine (50 mL, 0.2M, 4/1, v/v) and cooled to 0˚C, before dropwise addition of Bz-Cl (3.5 mL, 30 mmol, 3.0 eq.). The solution was left to stir under inert atmosphere for 4 hours. The reaction mixture was poured into EtOAc and washed with 1M HCl (aq., 5x), sat. $NaHCO_3$ (aq., 2x) and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated. The title compound was

crystallized from ethanol (6.32 g, 9.8 mmol, 98 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.93 (d, 2H, *J*=7.6 Hz, arom.), 7.78 – 7.65 (m, 5H, arom.), 7.62 – 7.49 (m, 5H, arom.), 7.47 – 7.33 (m, 7H, arom.), 7.30 (dd, 1H, *J*=8.4, 1.6 Hz, arom.), 7.24 – 7.16 (m, 2H, arom.), 5.64 (d, 1H, *J*=3.2 Hz, H-4), 5.48 (dd, 1H, *J*=9.6, 3.3 Hz, H-3), 4.97 (d, 1H, *J*=11.0 Hz, CH_2Nap), 4.85 (d, 1H, *J*=9.6 Hz, H-1), 4.74 (d, 1H, *J*=11.0 Hz, CH_2Nap), 4.18 – 3.87 (m, 2H, H-2, H-5), 1.32 (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), 135.1, 133.4, 133.2, 133.1, 133.0, 132.8, 129.9, 129.6, 129.6, 129.4, 129.1, 128.6, 128.3, 128.1, 127.9, 127.9, 127.7, 127.0, 126.2, 126.0, 125.9 (arom.), 87.1 (C-1), 75.4 (CH_2Nap), 75.2 (C-3), 74.8 (C-2), 73.5 (C-5), 71.8 (C-4), 16.8 (C-6) ppm. HRMS $[M+Na]^+$ calcd for $C_{37}H_{32}O_6SNa$: 627.1817, found 627.1808

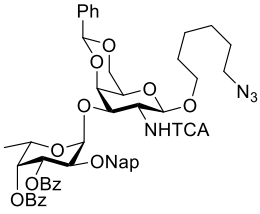
***N-phenyl-trifluoroacetimidoyl* 3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α/β-L-fucopyranoside (13)**



NBS (3.0 g, 16.7 mmol, 3.0 eq.) was added to a stirring solution of thioglycoside **12** (3.4 g, 5.6 mmol, 1.0 eq.) in a mixture of water and acetone (30 mL, 0.2M, 1/5, 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 5 min. 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 **12-OH** as a colourless foam (2.3 g, 4.5 mmol, 81%, 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. Hemi-acetal **12-OH** (2.3 g, 4.5 mmol, 1.0 eq.) was dissolved in DMF (40 mL, 0.11M) and Cs_2CO_3 (2.20 g, 6.8 mmol, 1.5 eq.) was added, followed by slow addition of $Cl(C=NPh)CF_3$ (1.63 mL, 10.4 mmol, 2.3 eq.). The mixture was stirred for 2 hours at room temperature, before being poured into Et_2O. The organic layer was washed with water, and the water layer was back extracted

with Et_2O. The combined organic layers were washed thrice with water, followed by brine, dried over $MgSO_4$, filtered and concentrated. The brown oil was purified by silicagel chromatography (PE:EtOAc:Et_3N, 19:1:0.3 → 7:3:0.3) to give the title compound as an anomeric mixture (2.44 g, 3.6 mmol, 79%). ^{1}H NMR ($CDCl_3$, 300 MHz, 323K): δ = 7.99 – 7.91 (m, 2H, arom.), 7.90 – 7.81 (m, 2H, arom.), 7.81 – 7.50 (m, 13H, arom.), 7.50 – 7.00 (m, 21H, arom.), 6.87 (d, 2H, *J*=7.8 Hz, arom.), 6.77 (d, 2H, *J*=7.8 Hz, arom.), 6.72 – 6.68 (m, 1H, H-1α), 5.80 (m, 2H, H-1β, H-3β), 5.73 (d, 1H, *J*=3.4 Hz, H-4β), 5.57 (d, 1H, *J*=3.5 Hz, H-4α), 5.43 (dd, 1H, *J*=10.0, 3.5 Hz, H-3α), 4.98 (d, 1H, *J*=11.7 Hz, CH_2Nap), 4.91 – 4.77 (m, 3H, CH_2Nap), 4.48 (q, 1H, *J*=6.5 Hz, H-5β), 4.33 (dd, 1H, *J*=10.4, 3.5 Hz, H-2β), 4.17 (dd, 1H, *J*=10.0, 7.9 Hz, H-2α), 3.91 – 3.80 (m, 1H, H-5α), 1.25 (m, 6H, H-6α, H-6β) ppm. ^{13}C-APT NMR ($CDCl_3$, 75 MHz, 323K) δ 166.0, 165.9, 165.6, 165.5 (C=O, Bz), 143.8, 143.6, 135.0, 133.4, 133.4, 133.2, 133.1, 129.9, 129.8, 129.8, 129.7, 129.6, 128.9, 128.9, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 128.1, 127.8, 127.8, 127.2, 127.0, 126.3, 126.2, 126.2, 126.1, 126.0, 125.9, 124.6, 124.5, 119.7, 119.5 (arom.), 97.8 (C-1β), 94.2 (C-1α), 75.6 (C-2α), 75.1 (CH_2Nap), 73.4 (CH_2Nap), 73.3 (C-3α), 72.8 (C-2β), 72.0 (C-4β), 71.4 (C-4α), 70.8 (C-5α), 70.5 (C-3β), 68.2 (C-5β), 16.3 (C-6α, C-6β) ppm.

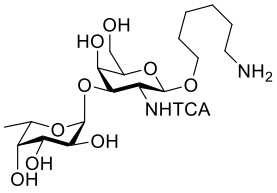
6-azidohexyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (14)



Thioglycoside **12** (0.91 g, 1.5 mmol, 1.5 eq.) and acceptor **5** (0.54 g, 1.0 mmol, 1.0 eq.) were co-evaporated together with dry toluene (3x) and dissolved in dry DCM (5.0 mL, 0.2M). NIS (0.36 g, 1.6 mmol, 1.6 eq.) and molecular sieves (3Å) were added and the flask was cooled to -40˚C and stirred at that temperature for 30 min. TMSOTf (18 µL, 0.1 mmol, 0.1 eq.) was added and the mixture was slowly warmed to -20˚C. This temperature was maintained for the duration of the reaction (2 hours). The reaction was quenched by addition of Et_3N (0.2 mL) and diluted in EtOAc. The organic layer was washed with sat. Na_2CO_3 (aq.), sat. $NaHCO_3$ (aq.) and brine, before being dried over $MgSO_4$, filtered and concentrated *in vacuo*. The yellow oil was purified by silicagel chromatography (PE:EtOAc, 9:1 → 3:2), followed by size exclusion over (LH-20, DCM/MeOH, 1/1, v/v), which gave the title compound as a yellow oil (0.78 g, 0.76 mmol, 76%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.81 – 7.63 (m, 8H, arom.), 7.57 – 7.34 (m, 10H, arom.), 7.28 – 7.18 (m, 4H, arom.), 7.15 (d, 1H, *J*=6.6 Hz, NH), 5.74 (dd, 1H, *J*=10.6, 3.4 Hz, H-3'), 5.55 (s, 1H, CHPh), 5.49 (dd, 1H, *J*=3.4, 1.3 Hz, H-4), 5.18 (d, 1H, *J*=8.2 Hz, H-1), 5.12 (d, 1H, *J*=3.6 Hz, H-1'), 4.82 – 4.68 (m, 2H, CH_2Nap), 4.66 (dd, 1H, *J*=11.0, 3.5 Hz, H-3), 4.54 (q, 1H, *J*=6.6 Hz, H-5'), 4.42 (d, 1H, *J*=3.4 Hz, H-4), 4.35 (dd, 1H, *J*=12.6, 1.5 Hz, H-6), 4.08 (m, 2H, H-6, H-2'), 3.97 (dt, 1H, *J*=9.5, 6.4 Hz, OCH_2), 3.84 (dt, 1H, *J*=10.9, 8.2, 6.6 Hz, H-2), 3.57 – 3.47 (m,

2H, H-5, OCH_2), 3.25 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.60 (tt, 4H, *J*=13.9, 5.1 Hz, CH_2, hexyl), 1.47 – 1.31 (m, 4H, CH_2, hexyl), 0.96 (d, 3H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 165.9, 165.5 (C=O, Bz), 162.2 (C=O, TCA), 137.5, 135.4, 133.2, 133.2, 133.1, 133.1, 129.8, 129.7, 129.6, 129.4, 128.5, 128.4, 128.3, 128.0, 127.8, 127.3, 126.3, 126.2, 126.1 (arom.), 101.4 (CHPh), 100.1 (C-1'), 98.6 (C-1), 92.6 (C_q, TCA), 77.1 (C-3), 75.3 (C-4), 72.8 (CH_2Nap), 72.4 (C-4'), 72.3 (C-2'), 70.6 (C-3'), 70.0 (OCH_2), 69.5 (C-6), 66.5 (C-5), 65.8 (C-5'), 55.5 (C-2), 51.5 (CH_2N_3), 29.5, 28.9, 26.6, 25.7 (CH_2, hexyl), 16.3 (C-6') ppm.

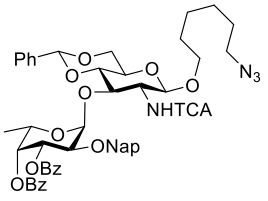
6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl) β-D-galactopyranoside (16)



Disaccharide **14** (52 mg, 0.05 mmol, 1.0 eq.) was dissolved in a mixture of DCM/MeOH (1.5 mL, 0.03M, 1/2, v/v). NaOMe (150 µL, 0.015 mmol, 0.3 eq. of a 0.1M solution in MeOH) was added and the reaction was left to stir at room temperature. TLC showed full removal of the two benzoyl groups after 4 hours. The mixture was diluted with MeOH and neutralized with Dowex H^+ resin. The resin was filtered off and washed with MeOH. The volatiles were removed *in vacuo* and the disaccharide was dry loaded unto silicagel. Silcagel chromatography (tol:EtOAc, 7:3 → 1:1) yielded diol **15** (0.027 g, 0.030 mmol, 60%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.91 – 7.70 (m, 4H, arom.), 7.57 – 7.42 (m, 5H, arom.), 7.41 – 7.30 (m, 4H, arom.), 7.05 (d, 1H, *J*=6.8 Hz, NH), 5.52 (s, 1H, CHPh), 5.05 (d, 1H, *J*=3.6 Hz, H-1'), 4.98 (d, 1H, *J*=8.3 Hz, H-1), 4.88 (d, 1H, *J*=12.7 Hz, CH_2Nap), 4.64 (d, 1H, *J*=12.7 Hz, CH_2Nap), 4.39 (dd, 1H, *J*=10.9, 3.5 Hz, H-3), 4.37 – 4.28 (m, 2H, H-4, H-6), 4.17 (q, 1H, *J*=6.6 Hz, H-5'), 4.06 (dd, 1H, *J*=12.5, 1.8 Hz, H-6), 4.02 – 3.89 (m, 2H, H-3', OCH_2), 3.83 (ddd, 1H, *J*=10.9, 8.2, 6.8 Hz, H-2), 3.68 – 3.61 (m, 2H, H-2', H-4'), 3.54 – 3.43 (m, 2H, h-5, OCH_2), 3.24 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.37 (s, 1H, 3'-OH), 2.23 (s, 1H, 4'-OH), 1.65 – 1.53 (m, 4H, CH_2, hexyl), 1.45 – 1.29 (m, 4H., CH_2, hexyl), 1.10 (d, 3H, *J*=6.6 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 162.2 (C=O, TCA), 137.8, 135.4, 133.3, 133.2, 129.2, 128.8, 128.7, 128.3, 128.1, 127.9, 127.3, 126.6, 126.4, 126.2, 125.9 (arom.), 101.1 (CHPh), 99.2 (C-1'), 98.9 (C-1), 92.6 (C_q, TCA), 76.9 (C-3), 75.6 (C-2'), 75.2 (C-4), 72.8 (CH_2Nap), 71.5 (C-4'), 69.9 (OCH_2), 69.4 (C-6), 69.4 (C-3'), 66.5 (C-5'), 66.4 (C-5), 55.2 (C-2), 51.5 (CH_2N_3), 29.5, 28.9, 26.7, 25.7 (CH_2, hexyl), 16.4 (C-6') ppm. HRMS: $[M+NH_4]^+$ calculated for $C_{38}H_{49}Cl_3N_4O_{10}$: 840.25450, found 840.25395. Diol **15** (57 mg, 0.069 mmol, 1.0 eq.) was dissolved in a mixture of dioxane and water (1.0 mL, 1/1, v/v) and degassed with argon for 15 min. Pd black (30 mg) was added and the suspension was purged with nitrogen for 5 min. The inert atmosphere was exchanged for a hydrogen atmosphere by purging with hydrogen gas. The mixture was left to stir under hydrogen atmosphere for 28 hours at room temperature. Before filtration over a Whatman filter the suspension was purged with nitrogen for 15 min. The volatiles were removed by evaporation and

the colourless film was taken up in water and purified by size exclusion LH-20 (H_2O/MeOH, 9/1). After purification the colourless film was lyophilized to give the title compound as a white solid (12.7 mg, 0.046 mmol, 67%). ^{1}H NMR (D_2O, 600 MHz): δ = 4.95 (d, 1H, *J*=4.1 Hz, H-1'), 4.46 (d, 1H, *J*=8.6 Hz, h-1), 4.09 (q, 1H, *J*=6.6 Hz, H-5'), 4.01 – 3.92 (m, 2H, H-2, H-5), 3.92 – 3.82 (m, 2H, H-3', OCH_2), 3.78 (d, 1H, *J*=3.2 Hz, H-4'), 3.77 – 3.69 (m, 3H, H-3, H-6), 3.69 – 3.61 (m, 2H, H-4, H-2'), 3.56 (dt, 1H, *J*=9.8, 6.2 Hz, OCH_2), 2.95 (t, 2H, *J*=7.6 Hz, CH_2N_3), 1.69 – 1.49 (m, 4H, CH_2, hexyl), 1.41 – 1.29 (m, 2H, CH_2, hexyl), 1.16 (d, 3H, *J*=6.6 Hz, H-6') ppm. ^{13}C-APT NMR (D_2O, 151 MHz) δ 175.7 (C=O, Ac), 102.3 (C-1, C-1'), 79.7 (C-4/C-2'), 75.9 (C-4'), 72.6 (OCH_2), 70.3 (C-3'), 69.1 (C-4/C-2'), 68.9 (C-5), 68.1 (C-5'), 61.8 (C-6), 52.5 (C-2), 40.3 (CH_2N_3), 29.3, 27.6, 26.2, 25.6 (CH_2, hexyl) 16.2 (C-6') ppm. $[M+Na]^+$ calculated for $C_{20}H_{38}N_2NaO_{10}$: 489.24187, found 489.24180.

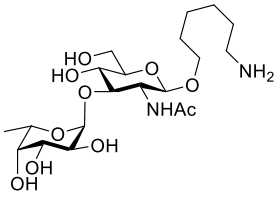
6-azidohexyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (17)



Acceptor **6** (0.54 g, 1.0 mmol, 1.0 eq.) and donor **12** (0.91 g, 1.5 mmol, 1.5 eq.) were co-evaporated with dry toluene (3x) and dissolved in dry DCM (10 mL, 0.1M). NIS (0.36 g, 1.6 mmol, 1.6 eq.) and MS (3Å) were added and the mixture was cooled to -40˚C and stirred at that temperature for 1 hour. TMSOTf (18 µL, 0.1 mmol, 0.1 eq.) was added at -40°C and the mixture was allowed to warm up to -20 °C. When TLC analysis showed complete conversion of acceptor **6** after 3 hours the reaction was quenched by addition of Et_3N (0.2 mL). The reaction mixture was diluted in EtOAc and washed with sat. $Na_2S_2O_3$ (aq.), sat. $NaHCO_3$ (aq.) and brine, before drying over $MgSO_4$ and filtration. The obtained brownish oil was purified by silica gel chromatography (PE: EtOAc, 19:1 →7:3) followed by size exclusion (LH-20, DCM/MeOH, 1/1, v/v) to give **17** as a yellow oil (0.91 mmol, 0.88 g, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.88 – 7.79 (m, 2H, arom.), 7.76 – 7.66 (m, 3H, arom.), 7.65 – 7.57 (m, 3H, arom.), 7.56 – 7.47 (m, 3H, arom.), 7.47 – 7.30 (m, 6H, arom.), 7.30 – 7.17 (m, 5H, arom.), 7.11 (d, 1H, *J*=7.2 Hz, NH), 5.80 (dd, 1H, *J*=10.5, 3.4 Hz, H-3'), 5.59 (s, 1H, CHPh), 5.48 (d, 1H, *J*=3.4 Hz, H-4), 5.32 (d, 1H, *J*=3.5 Hz, H-1'), 5.07 (d, 1H, *J*=8.2 Hz, H-1), 4.93 – 4.73 (m, 2H, CH_2Nap), 4.63 (t, 1H, *J*=9.4 Hz, H-3), 4.52 (q, 1H, *J*=6.6 Hz, H-5'), 4.40 (dd, 1H, *J*=10.5, 4.9 Hz, H-6), 4.17 (dd, 1H, *J*=10.5, 3.5 Hz, H-2'), 3.92 – 3.79 (m, 2H, H-6, OCH_2), 3.75 (t, 1H, *J*=9.2 Hz, H-4), 3.67 – 3.52 (m, 2H, H-2, H-5), 3.47 (dt, 1H, *J*=9.7, 6.6 Hz, OCH_2), 3.20 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.64 – 1.45 (m, 4H, CH_2, hexyl), 1.38 – 1.22 (m, 4H, CH_2, hexyl), 0.64 (d, 3H, *J*=6.5 Hz, h-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.0, 165.6 (C=O, Bz), 161.9 (C=O, TCA), 137.0, 135.2, 133.2, 133.1, 133.0, 132.9, 129.6, 129.6, 129.6, 129.4, 128.4, 128.3, 128.2, 127.8, 127.7, 126.5, 126.4, 126.2, 126.0, 125.6 (arom.), 102.3 (CHPh), 99.4 (C-1), 98.0 (C-1'), 92.3 (C_q, TCA), 80.5 (C-4), 74.1 (C-3),

73.7 (C-2'), 73.2 (CH_2Nap), 72.5 (C-4'), 70.7 (C-3'), 70.3 (OCH_2), 68.8 (C-6), 66.2 (C-5), 65.3 (C-5'), 60.4 (C-2), 51.3 (OCH_2), 29.4, 28.7, 26.5, 25.6 (CH_2, hexyl), 15.3 (C-6') ppm. HRMS: $[M+NH_4]^+$ calculated for $C_{52}H_{57}Cl_3N_aO_{12}$: 1048.30693, found 1048.30638.

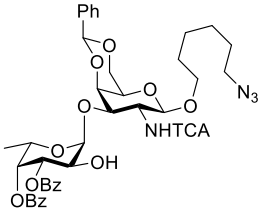
6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl) β-D-glucopyranoside (19)



Disaccharide **17** (48 mg, 0.05 mmol, 1.0 eq.) was dissolved in a mixture of THF/MeOH (1.5 mL, 0.03M, 1/2, v/v). NaOMe (150 µL, 0.015 mmol, 0.3 eq. of a 0.1M solution in MeOH) was added and the reaction was left to stir at room temperature. TLC showed full removal of the two benzoyl groups after 3 hours. The mixture was diluted with MeOH and neutralized with Dowex H^+ resin. The resin was filtered off and washed with MeOH. The volatiles were removed *in vacuo* and the disaccharide was dry loaded unto silicagel. Silcagel chromatography (tol:EtOAc, 7:3 → 1:1) yielded diol **18** (0.035 g, 0.039 mmol, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.89 – 7.73 (m, 4H, arom.), 7.57 – 7.40 (m, 5H, arom.), 7.36 (m, 3H, *arom.*), 6.85 (d, 1H, *J*=8.0 Hz, NH), 5.53 (s, 1H, CHPh), 5.30 (d, 1H, *J*=3.5 Hz, H-1'), 4.86 (d, 1H, *J*=11.8 Hz, CH_2Nap), 4.74 (d, 1H, *J*=11.9 Hz, CH_2Nap), 4.69 (d, 1H, *J*=8.2 Hz, H-1), 4.40 – 4.29 (m, 2H, H-4, H-6), 4.21 (q, 1H, *J*=6.5 Hz, H-5'), 3.99 (dd, 1H, *J*=9.8, 3.2 Hz, H-3'), 3.90 – 3.66 (m, 5H, H-2, H-3, H-6, H-2', OCH_2), 3.64 (d, 1H, *J*=3.3 Hz, H-4'), 3.48 (td, 1H, *J*=9.7, 4.9 Hz, H-5), 3.40 (dt, 1H, *J*=9.5, 6.6 Hz, OCH_2), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.32 (s, 1H, 3'-OH), 2.19 (s, 1H, 4'-OH), 1.61 – 1.46 (m, 4H, CH_2, hexyl), 1.37 – 1.24 (m, 4H, CH_2, hexyl), 0.93 (d, 3H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 161.9 (C=O, TCA), 137.2, 135.4, 133.4, 133.2, 129.4, 128.8, 128.4, 128.1, 127.9, 127.0, 126.6, 126.4, 126.3, 125.8 (arom.), 101.9 (CHPh), 100.8 (C-1), 96.6 (C-1'), 92.6 (C_q, TCA), 80.7 (C-3), 77.2 (C-2'), 73.6 (C-4), 73.3 (CH_2Nap), 71.8 (C-4'), 70.4 (OCH_2), 69.5 (C-3'), 68.8 (C-6), 66.5 (C-5), 66.2 (C-5'), 59.3 (C-2), 51.5 (CH_2N_3), 29.5, 28.8, 26.6, 25.7 (CH_2, hexyl), 15.8 (C-6') ppm. HRMS: $[M+NH_4]^+$, calculated for C38H49Cl3N5O10: 840.25450, found 840.25395. Diol **18** (35 mg, 0.039 mmol, 1.0 eq.) was dissolved in a mixture of dioxane and water (1.0 mL, 1/1, v/v) and degassed with argon for 15 min. Pd black (30 mg) was added and the suspension was purged with nitrogen for 5 min. The inert atmosphere was exchanged for a hydrogen atmosphere by purging with hydrogen gas. The mixture was left to stir under hydrogen atmosphere for 24 hours at room temperature. Before filtration over a Whatmann filter, the suspension was purged with nitrogen for 15 min. The volatiles were removed by evaporation and the colourless film was taken up in water and purified by size exclusion LH-20 (H_2O/MeOH, 9/1). After purification the colourless film was lyophilized to give the title compound as a white solid (11.4 mg, 0.024 mmol, 63%). ^{1}H NMR (D_2O, 850 MHz): δ = 4.95 (d, 1H, *J*=4.1 Hz, H-1'), 4.48 (d, 1H, *J*=8.6 Hz, H-1), 4.33 – 4.28 (q, 1H, *J*=6.8, 3.6 Hz, H-5'), 3.92 – 3.86 (m, 2H, H-6, OCH_2), 3.80 (dd, 1H, *J*=10.4, 3.4 Hz, H-3'), 3.79 – 3.76 (m, 2H, H-2, H-4'), 3.72 (dd, 1H, *J*=12.3, 5.9 Hz, H-6), 3.66

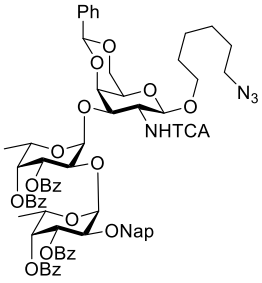
(dd, 1H, *J*=10.4, 4.1 Hz, H-2'), 3.60 (t, 1H, *J*=10.3 Hz, H-3), 3.55 (dt, 1H, *J*=10.1, 6.4 Hz, OCH_2), 3.48 (t, 1H, *J*=10.0 Hz, H-4), 3.43 (ddd, 1H, *J*=10.1, 5.9, 2.2 Hz, H-5), 1.99 (s, 3H, CH_3, Ac), 1.67 – 1.59 (m, 2H, CH_2, hexyl), 1.54 (q, 2H, *J*=6.7 Hz, CH_2, hexyl), 1.40 – 1.29 (m, 4H, CH_2, hexyl), 1.13 (d, 3H, *J*=6.6 Hz, H-6') ppm. ^{13}C-APT NMR (D_2O, 214 MHz) δ 175.4 (C=O, Ac), 101.9 (C-1), 100.9 (C-1'), 81.3 (C-3), 76.8 (C-5), 72.8 (C-4'), 71.4 (OCH_2), 70.5 (C-3'), 69.5 (C-4), 68.9 (C-2'), 67.8 (C-5'), 61.7 (C-6), 56.3 (C-2), 40.3 (CH_2N_3), 29.3, 27.6, 26.2, 25.6 (CH_2, hexyl), 23.2 (CH_3, Ac), 16.1 (C-6') ppm. HRMS: $[M+H]^+$ calculated for $C_{20}H_{39}N_2O_{10}$: 467.25992, found 467.25987.

6-azidohexyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-α-L-fucopyranosyl)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (20)



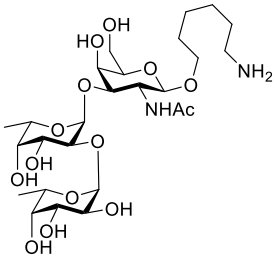
Disaccharide **14** (0.61 g, 0.58 mmol, 1.0 eq.) was dissolved in DCM (5 mL, 0.12M). Aqueous phosphate buffer (1 mL, pH 7) was added, followed by portion wise addition of DDQ (0.40 g, 1.76 mmol, 3.0 eq.). The mixture was stirred vigourously for 2.5 hours at which time TLC analysis showed full consumption of the starting material. At this point sat. $Na_2S_2O_3$ (aq., 2 mL) was added and the mixture was stirred for an additional 5min. The biphasic mixture was transferred to a separatory funnel and diluted with EtOAc. The organic phase was washed five times with sat. Na_2CO_3 (aq.), followed by brine, dried over $MgSO_4$, filtered and concentrated. The dark yellow oil was purified by silicagel chromatography (PE:EtOAc, 9:1 → 1:1) to yield the title compound as a white solid (0.46 g, 0.52 mmol, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.10 – 7.99 (m, 2H, arom.), 7.86 – 7.76 (m, 2H, arom.), 7.63 – 7.50 (m, 3H, arom.), 7.49 – 7.34 (m, 6H, arom.), 7.32 – 7.22 (m, 2H, arom.), 7.18 (d, 1H, *J*=7.0 Hz, NH), 5.58 – 5.44 (m, 3H, H-H-3', H-4', CHPh), 5.23 (d, 1H, *J*=4.0 Hz, H-1'), 5.01 (d, 1H, *J*=8.2 Hz, H-1), 4.61 (dd, 1H, *J*=11.0, 3.6 Hz, H-3), 4.52 (q, 1H, *J*=6.6 Hz, H-5'), 4.45 (d, 1H, *J*=3.5 Hz, H-4), 4.33 (dd, 1H, *J*=12.5, 1.5 Hz, H-6), 4.21 (td, 1H, *J*=10.4, 3.9 Hz, H-2'), 4.08 (dd, 1H, *J*=12.5, 1.8 Hz, H-6), 3.99 – 3.87 (m, 2H, H-2, OCH_2), 3.56 – 3.51 (s, 1H, H-5), 3.54 – 3.43 (m, 1H, OCH_2), 3.23 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.19 (d, 1H, *J*=10.8 Hz, 2'-OH), 1.68 – 1.49 (m, 4H, CH_2, hexyl), 1.45 – 1.30 (m, 4H, CH_2, hexyl), 1.09 (d, 3H, *J*=6.6 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.4, 166.0 (C=O, Bz), 162.3 (C=O, TCA), 137.4, 133.4, 133.2, 129.8, 129.8, 129.5, 129.5, 129.4, 128.6, 128.5, 128.2, 126.2 (arom.), 101.7 (C-1'), 101.3 (CHPh), 98.8 (C-1), 92.4 (C_q, TCA), 77.3 (C-3), 75.3 (C-4), 71.9 (C-4'), 71.7 (C-3'), 69.7 (OCH_2), 69.3 (C-6), 68.0 (C-2'), 66.3 (C-5), 66.1 (C-5'), 55.1 (C-2), 51.4 (CH_2N_3), 29.4, 28.8, 26.5, 25.6 (CH_2, hexyl), 16.3 (C-6') ppm.

6-azidohexyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (21)



Donor **12** (0.76 g, 1.25 mmol, 1.5 eq.) and acceptor **20** (0.74 g, 0.84 mmol, 1.0 eq.) were co-evaporated thrice with dry toluene before being dissolved in dry DCM (8 mL, 0.1M). Molecular sieves (3Å) were added and the mixture was stirred for 30 min., at which point NIS (0.32 g, 1.41 mmol, 1.7 eq.) was added and the flask was cooled to -40˚C. After 15 min. of stirring, TMSOTf (30 µL, 0.17 mmol, 0.2 eq.) was added and the reaction was allowed to warm to -20˚C and kept at that temperature. TLC analysis indicated after 2.5 hours that the donor was fully consumed and the reaction was quenched by addition of Et_3N (0.3 mL). The reaction mixture was diluted in EtOAc and washed with sat. $Na_2S_2O_3$ (aq.), $NaHCO_3$ (aq.) and brine, before drying over $MgSO_4$ and filtration. The volatiles were removed *in vacuo* and the obtained crude oil was purified by silicagel chromatography (tol:EtOAc, 1:0 → 4:1), followed by size exclusion over LH-20 (DCM/MeOH, 1/1, v/v). Trisaccharide **21** was obtained as a white foam (0.88 g, 0.63 mmol, 76%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.17 – 8.00 (m, 5H, arom.), 7.98 – 7.88 (m, 2H, arom.), 7.83 (m, 3H, arom.), 7.75 – 7.58 (m, 5H, arom.), 7.58 – 7.36 (m, 13H, arom.), 7.34 – 7.19 (m, 6H, arom.), 7.12 (dd, 1H, *J*=8.4 Hz, NH), 6.02 (dd, 1H, *J*=10.3, 3.3 Hz, H-3''), 5.69 (dd, 1H, *J*=10.2, 3.6 Hz, H-3'), 5.55 (d, 1H, *J*=3.7 Hz, H-4'), 5.47 (d, 1H, *J*=3.2 Hz, H-4''), 5.26 (d, 1H, *J*=3.0 Hz, H-1''), 5.22 (d, 1H, *J*=3.6 Hz, H-1'), 5.13 (s, 1H, CHPh), 4.79 (d, 1H, *J*=9.7 Hz, CH_2Nap), 4.67 – 4.53 (m, 2H, H-2), 4.53 – 4.43 (m, 2H, H-1, H-5'), 4.26 (q, 1H, *J*=6.5 Hz, H-5''), 4.19 (dd, 1H, *J*=10.2, 3.6 Hz, H-2'), 4.13 (dd, 1H, *J*=10.4, 3.0 Hz, H-2''), 3.91 (dt, 1H, *J*=9.5, 6.0 Hz, OCH_2), 3.66 (dd, 1H, *J*=11.0, 3.1 Hz, H-3), 3.62 – 3.52 (m, 2H, H-6, OCH_2), 3.31 (d, 1H, *J*=3.0 Hz, H-4), 3.18 (t, 2H, *J*=7.0 Hz, CH_2N_3), 2.68 – 2.61 (m, 1H, H-6), 1.73 – 1.49 (m, 5H, H-5, CH_2, hexyl), 1.48 – 1.29 (m, 4H, CH_2, hexyl), 0.72 (d, 3H, *J*=6.5 Hz, H-6'), 0.63 (d, 3H, *J*=6.5 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.3, 165.9, 165.9, 164.8 (C=O, Bz), 162.6 (C=O, TCA), 137.8, 134.3, 134.2, 133.7, 133.3, 133.1, 133.1, 133.0, 130.1, 129.9, 129.9, 129.8, 129.8, 129.7, 129.4, 129.1, 129.0, 128.8, 128.6, 128.5, 128.2, 128.2, 127.9, 127.8, 127.2, 126.7, 126.4 (arom.), 102.2 (C-1''), 100.9 (CHPh), 100.8 (C-1), 96.6 (C-1'), 93.1 (C_q, TCA), 80.6 (C-2'), 78.3 (C-3), 76.7 (C-2''), 74.6 (CH_2Nap), 72.8 (C-4''), 72.7 (C-4'), 70.5 (C-3''), 69.5 (C-3'), 68.7 (C-6), 68.4 (OCH_2), 66.9 (C-5''), 65.9 (C-5), 65.8 (C-5'), 51.5 (C-2), 51.4 (CH_2N_3), 29.6, 28.8, 26.6, 25.7 (CH_2, hexyl), 15.7 (C-6'), 15.4 (C-6'') ppm. HRMS: $[M+NH_4]^+$ calculated for $C_{72}H_{75}Cl_3N_5O_{18}$: 1402.41727, found 1402.41672.

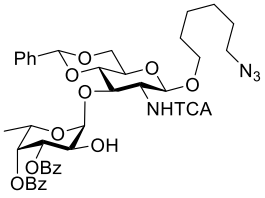
6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(2-*O*-(α-L-fucopyranosyl)-α-L-fucopyranosyl)-2-β-D-galactopyranoside (22)



Fully protected trisaccharide **21** (0.050 g, 0.036 mmol, 1.0 eq.) was dissolved in THF (0.4 mL, 0.05M). Sodium methoxide (100 µL, 0.011 mmol, 0.3 eq. of a 0.1 M solution) was added and the mixture was warmed to 50˚C and left to stir until TLC indicated full conversion (~24 hours). The mixture was diluted in MeOH (5 mL) and neutralized by addition of Dowex H^+ resin. The resin was filtered off and washed with additional methanol. The volatiles were removed *in vacuo* and the crude was dry loaded on silicagel. The silicagel was washed with toluene and ether to remove the methoxybenzoate. The product was eluted by washing with EtOAc followed by methanol. The solvents were removed by evaporation and the partially deprotected compound was obtained as a colourless film (0.025 g, 0.025 mmol, 70%). ^{1}H NMR (MeOD, 400 MHz): δ = 7.99 (s, 1H, arom.), 7.96 – 7.86 (m, 3H, arom.), 7.67 (dd, 1H, *J*=8.5, 1.7 Hz, arom.), 7.59 – 7.49 (m, 2H, arom.), 7.48 – 7.42 (m, 2H, arom.), 7.32 – 7.26 (m, 3H, arom), 5.40 (s, 1H, CHPh), 5.18 (d, 1H, *J*=3.7 Hz, H-1''), 5.05 (d, 1H, *J*=2.8 Hz, H-1'), 5.01 – 4.88 (m, 2H, CH_2Nap), 4.33 (q, 1H, *J*=6.8, 6.4 Hz, H-5''), 4.19 (dd, 1H, *J*=10.3, 3.4 Hz, H-3''), 4.13 – 4.01 (m, 3H, H2, H-3', H-5'), 3.92 – 3.75 (m, 4H, H-6, H-2', H-4', H-2''), 3.71 (m, 2H, H-4'', H-6), 3.58 – 3.44 (m, 3H, H-3, H-4, OCH_2), 3.41 (d, 1H, *J*=8.4 Hz, H-1), 3.26 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.89 – 2.73 (m, 1H, OCH_2), 2.47 (s, 1H, H-5), 1.61 – 1.50 (m, 2H, CH_2, hexyl), 1.44 – 1.26 (m, 6H, CH_2, hexyl), 1.21 (d, 3H, *J*=6.6 Hz, H-6''), 0.82 (d, 3H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR (MeOD, 101 MHz) δ 164.4 (C=O, TCA), 139.5, 137.7, 134.8, 134.7, 129.9, 129.6, 129.2, 128.9, 128.3, 127.8, 127.7, 127.5, 127.5 (arom.), 102.3 (C-1), 102.0 (CHPh), 99.4 (C-1''), 98.1 (C-1'), 79.8 (C-2''), 78.7 (C-2'), 78.4 (C-3), 75.5 (CH_2Nap), 74.2 (C-4 + C4''), 73.7 (C-4'), 71.0 (C-3''), 70.2 (OCH_2), 70.0 (C-6), 69.5 (C-3'), 68.5 (C-5'), 67.8 (C-5''), 67.3 (C-5), 53.7 (C-2), 52.4 (CH_2N_3), 30.5, 29.8, 27.6, 26.6 (CH_2, hexyl), 16.6 (C-6'+ C-6'') ppm. HRMS $[M+Na]^+$ calcd for $C_{44}H_{55}Cl_3N_4O_{14}Na$: 991.26781, found 991.26726. The partially deprotected compound (30 mg, 0.031 mmol, 1.0 eq.) was dissolved in a mixture of dioxane and water (1.0 mL, 1/1, v/v) and degassed with argon for 15 min. Pd black (30 mg) and $NaHCO_3$ (8.0 mg, 0.1 mmol, 3.0 eq.) were added and the suspension was purged with nitrogen for 5 min. The inert atmosphere was exchanged for a hydrogen atmosphere by purging with hydrogen gas. The mixture was left to stir under hydrogen atmosphere for 60 hours at room temperature. Before filtration over a Whatmann filter the suspension was purged with nitrogen for 15 min. The volatiles were removed by evaporation and the colourless film was taken up in water and purified by size exclusion LH-20 (H_2O/MeOH, 9/1). After purification the colourless film was lyophilized to give the title compound as a white solid (14.7 mg, 0.024 mmol, 77%).^{1}H NMR (D_2O, 400 MHz): δ = 5.24 (d, 1H, *J*=3.6 Hz, H-1''), 4.91 (d, 1H, *J*=3.9 Hz, H-1'), 4.49 – 4.41 (m, 1H, H-1), 4.16

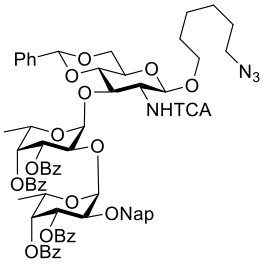
(q, 1H, *J*=6.6 Hz, H-5'), 4.08 (q, 1H, *J*=6.4 Hz, H-5''), 3.99 – 3.67 (m, 12H), 3.67 – 3.59 (m, 1H), 3.53 (dt, 1H, *J*=10.2, 6.4 Hz, OCH_2), 2.94 (t, 2H, *J*=7.6 Hz, CH_2NH_2), 2.01 (s, 3H, CH_3, Ac), 1.61 (t, 2H, *J*=7.4 Hz, CH_2, hexyl), 1.51 (t, 2H, *J*=6.7 Hz, CH_2, hexyl), 1.32 (q, 4H, *J*=4.2, 3.2 Hz, CH_2, hexyl), 1.16 (2xd, 6H, *J*=6.5 Hz) ppm. ^{13}C-APT NMR (D_2O, 101 MHz) δ 174.7 (C=O, Ac), 101.7 (C-1), 95.9, 94.9, 75.8, 74.9, 71.7, 71.6, 71.0, 70.3 (OCH_2), 69.3, 67.8, 67.6, 67.4, 67.2, 66.9, 60.8 (C-6), 51.9 (C-2), 39.4 (CH_2N_3), 28.3, 26.6, 25.2, 24.6 (CH_2, hexyl, 22.6 (CH_3, Ac), 15.3 (C-6'+ C-6'') ppm. HRMS $[M+Na]^+$ calcd for $C_{26}H_{48}N_2O_{14}H$: 613.31855, found 613.31800.

6-azidohexyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-α-L-fucopyranosyl)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (23)



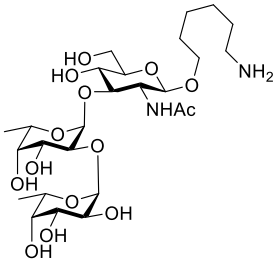
DDQ (0.96 g, 4.23 mmol, 3.0 eq.) was added portion wise to a vigorously stirring solution of **17** (1.45 g, 1.41 mmol, 1.0 eq.) in DCM (11.2 mL) and aqueous phosphate buffer (2.8 mL, pH 7). After 3 hours TLC analysis indicated full consumption of the starting material, so the reaction was quenched by addition of sat. $Na_2S_2O_3$ (aq. 6mL) and stirred until the mixture turned pale yellow. The biphasic system was transferred to a separatory funnel and the water layer was removed. The organic layer was washed repeatedly with sat. Na_2CO_3 (aq.) until the water layer remained colourless. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude oil was purified by silicagel chromatography (tol:EtOAc, 1:0 → 4:1) and product **23** was obtained as a white solid (1.12 g, 1.24 mmol, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.08 – 7.94 (m, 2H, arom.), 7.87 – 7.78 (m, 2H, arom.), 7.63 – 7.53 (m, 1H, arom.), 7.53 – 7.40 (m, 5H, arom.), 7.40 – 7.30 (m, 3H, arom.), 7.30 – 7.22 (m, 2H, arom.), 7.13 (d, 1H, *J*=8.0 Hz, NH), 5.55 (s, 1H, CHPh), 5.51 (dd, 1H, *J*=10.5, 3.4 Hz, H-3'), 5.45 (d, 1H, *J*=3.4 Hz, H-4'), 5.14 (d, 1H, *J*=3.9 Hz, H-1'), 4.97 (d, 1H, *J*=8.3 Hz, H-1), 4.46 – 4.34 (m, 3H, H-3, H-6, H-5'), 4.17 (td, 1H, *J*=9.8, 3.8 Hz, H-2'), 3.94 – 3.78 (m, 2H, H-6, OCH_2), 3.78 – 3.66 (m, 2H, H-2, H-4), 3.60 (td, 1H, *J*=9.6, 4.9 Hz, H-5), 3.51 (dt, 1H, *J*=9.6, 6.6 Hz, OCH_2), 3.24 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.24 (d, 1H, *J*=9.9 Hz, 2'-OH), 1.64 – 1.49 (m, 4H, CH_2, hexyl), 1.45 – 1.28 (m, 4H, CH_2, hexyl), 0.54 (d, 3H, *J*=6.4 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.6, 166.1 (C=O, Bz), 162.4 (C=O, TCA), 137.0, 133.4, 133.2, 129.8, 129.6, 129.5, 128.6, 128.5, 128.3, 126.6 (arom.), 102.6 (CHPh), 100.2 (C-1'), 99.9 (C-1), 92.5 (C_q, TCA), 80.1 (C-4), 75.9 (C-3), 72.2 (C-4'), 71.7 (C-3'), 70.3 (OCH_2), 68.8 (C-6), 67.6 (C-2'), 66.5 (C-5), 65.7 (C-5'), 59.6 (C-2), 51.4 (CH_2N_3), 29.5, 28.8, 26.5, 25.6 (CH_2, hexyl), 15.2 (C-6') ppm.

6-azidohexyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (24)



Disaccharide acceptor **23** (0.41 g, 0.46 mmol, 1.0 eq.) and donor **12** (0.56 g, 0.92 mmol, 2.0 eq.) were co-evaporated thrice with dry toluene and diluted in dry DCM (2.5 mL, 0.2M). Molecular sieves (3Å) and NIS (0.23 g, 1.01 mmol, 2.2 eq.) were added and the mixture was cooled to -40˚C and stirred at that temperature for 30 min. TMSOTf (17 µL, 0.091 mmol, 0.2 eq.) was added and the mixture was slowly warmed to -20˚C and kept at that temperature for 1 hour. The reaction was stopped by addition of Et_3N (0.1 mL) and the mixture was diluted with EtOAc. The organic layer was washed with sat. Na_2CO_3 (aq.), sat. $NaHCO_3$ (aq.) and brine, before being dried over $MgSO_4$ and filtered. The crude yellow oil was purified by silicagel chromatography (PE:EtOAc, 9:1 → 7:3) followed by size exclusion over LH-20 (DCM/MeOH, 1/1, v/v) to give the title compound as a white solid (0.27 g, 0.19 mmol, 47%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.17 (d, 1H, *J*=8.2 Hz, NH), 8.10 – 7.98 (m, 2H, arom.), 7.88 – 7.79 (m, 2H, arom.), 7.77 – 7.69 (m, 4H, arom.), 7.69 – 7.60 (m, 2H, arom.), 7.60 – 7.33 (m, 16H, arom.), 7.23 (m, 6H, arom.), 5.92 (dd, 1H, *J*=10.6, 3.2 Hz, H-3'), 5.70 (dd, 1H, *J*=10.3, 2.3 Hz, H-3''), 5.65 (d, 1H, *J*=3.5 Hz, H-1''), 5.63 (s, 1H, CHPh), 5.59 (d, 1H, *J*=3.5 Hz, H-4''), 5.36 (d, 1H, *J*=3.2 Hz, H-4'), 4.94 (d, 1H, *J*=2.2 Hz, H-1'), 4.90 – 4.74 (m, 2H, H-1, H-3), 4.73 – 4.59 (m, 3H, H-5', CH_2Nap), 4.30 (dd, 1H, *J*=10.6, 4.9 Hz, H-6), 4.27 – 4.18 (m, 2H, H-2'', H-5''), 4.05 (dd, 1H, *J*=10.6, 2.3 Hz, H-2'), 3.97 (p, 1H, *J*=9.3, 8.4 Hz, H-2), 3.88 (dt, 1H, *J*=9.2, 6.0 Hz, OCH_2), 3.80 (2x t, 2H, *J*=9.6, 8.8 Hz, H-4, H-6), 3.48 (dt, 1H, *J*=9.3, 6.5 Hz, OCH_2), 3.44 – 3.33 (m, 1H, H-5), 3.21 (t, 2H, *J*=6.8 Hz, CH_2N_3), 1.69 – 1.48 (m, 4H, CH_2, hexyl), 1.47 – 1.30 (m, 4H, CH_2, hexyl), 1.02 (d, 3H, *J*=6.4 Hz, H-6'), 0.71 (d, 3H, *J*=6.4 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.1, 166.0, 165.9, 165.3 (C=O, Bz), 162.8 (C=O, TCA), 137.5, 134.7, 133.6, 133.4, 133.3, 133.1, 133.0, 132.9, 130.0, 129.9, 129.9, 129.7, 129.7, 129.6, 129.5, 129.4, 129.1, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 127.9, 127.8, 127.2, 126.4, 126.4, 126.3, 126.0 (arom.), 101.8 (CHPh), 100.6 (C-1), 99.4 (C-1'), 97.1 (C-1''), 92.9 (C_q, TCA), 82.2 (C-4), 76.8 (C-2''), 74.4 (C-3, high T) 74.1 (C-2'), 73.1 (CH_2Nap), 72.8 (C-4'), 72.3 (C-4''), 70.3 (OCH_2), 70.1 (C-3''), 69.9 (C-3'), 68.8 (C-6), 66.2 (C-5''), 65.9 (C-5), 65.3 (C-5'), 58.5 (C-2, high T), 51.5 (CH_2N_3), 29.7, 28.8, 26.6, 25.7 (CH_2, hexyl), 16.0 (C-6'), 15.5 (C-6'') ppm. HRMS: $[M+NH_4]^+$ calculated for $C_{72}H_{74}Cl_3N_5O_{18}$: 1402.41727, found 1402.41672.

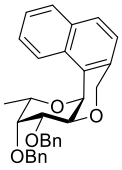
6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(2-*O*-(α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-glucopyranoside (25)



Fully protected trisaccharide **24** (0.080 g, 0.058 mmol, 1.0 eq.) was dissolved in a mixture of MeOH and THF (1 mL, 0.05M, 1/1, v/v). Sodium (1.3 mg, 0.058 mmol, 0.25 eq.) was added and the mixture was warmed to 50˚C and left to stir until TLC indicated full conversion (~24 hours). The mixture was diluted in MeOH (5 mL) and neutralized by addition of Dowex H^+ resin. The resin was filtered off and washed with additional methanol. The volatiles were removed *in vacuo* and the crude was dry loaded on silicagel. The silicagel was washed with toluene and ether to remove the methoxybenzoate. The product was eluted by washing with EtOAc followed by methanol. The solvents were removed by evaporation and the partially deprotected compound **24-SD** was obtained as a colourless film (0.045 g, 0.046 mmol, 81%). ^{1}H NMR (MeOD, 400 MHz): δ = 8.06 (s, 1H, arom.), 7.91 – 7.78 (m, 4H, arom.), 7.68 (dd, 1H, *J*=8.4, 1.7 Hz, arom.), 7.52 – 7.32 (m, 6H, arom), 5.49 (d, 1H, *J*=3.4 Hz, H-1'), 5.22 (d, 1H, *J*=3.7 Hz, H-1''), 5.15 (d, 1H, *J*=12.0 Hz, CH_2Nap), 5.05 (s, 1H, CHPh), 4.89 (d, 1H, *J*=12.0 Hz, CH_2Nap), 4.29 – 4.11 (m, 5H, H-1, H-3, H-5', H-3'', H-5''), 4.06 (dd, 1H, *J*=10.3, 4.8 Hz, H-6), 3.92 (dd, 1H, *J*=10.1, 3.2 Hz, H-3'), 3.85 (m, 3H, H-2, H-2', H-2''), 3.75 – 3.62 (m, 2H, H-4'', OCH_2), 3.53 (dd, 1H, *J*=3.3 Hz, H-4'), 3.29 – 3.27 (m, 2H, H-6, OCH_2), 3.24 (t, 3H, *J*=6.9 Hz, CH_2N_3), 3.15 (td, 1H, *J*=9.8, 4.9 Hz, H-5), 3.00 (t, 1H, *J*=9.3 Hz, H-4), 1.61 – 1.45 (m, 4H, CH_2, hexyl), 1.42 – 1.24 (m, 4H, CH_2, hexyl), 1.19 (d, 3H, *J*=6.5 Hz, H-6''), 1.04 (d, 3H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR (MeOD, 101 MHz) δ 164.2 (C=O, TCA), 139.0, 137.9, 134.8, 134.5, 129.9, 129.2, 129.2, 129.1, 129.1, 128.8, 127.7, 127.4, 127.2, 127.1, 127.0 (arom.), 102.9 (C-1), 102.1 (CHPh), 95.5 (C-1'), 94.1 (C-1''), 94.0 (C_q, TCA), 81.5 (C-4), 77.9 (C-2''), 73.9 (C-4''), 73.5 (C-4'), 73.3 (CH_2Nap), 73.2 (C-3), 72.7 (C-2'), 70.9 (OCH_2), 70.8 (C-3''), 69.7 (C-3'), 69.3 (C-6), 67.5 (C-5'), 67.4 (C-5''), 67.0 (C-5), 59.2 (C-2), 52.3 (CH_2N_3), 30.6, 29.8, 27.5, 26.7 (CH_2, hexyl), 16.6 (C-6', C-6'') ppm. HRMS: $[M+NH_4]^+$ calculated for $C_{aa}H_{59}Cl_3N_5O_{14}$: 986.31241, found 986,31186. Partially deprotected **24-SD** (23 mg, 0.023 mmol, 1.0 eq.) was dissolved in a mixture of dioxane and water (1.0 mL, 1/1, v/v) and degassed with argon for 15 min. Pd black (20 mg) and $NaHCO_3$ (6.0 mg, 0.07 mmol, 3.0 eq.) were added and the suspension was purged with nitrogen for 5 min. The inert atmosphere was exchanged for a hydrogen atmosphere by purging with hydrogen gas. The mixture was left to stir under hydrogen atmosphere for 72 hours at room temperature. Before filtration over a Whatmann filter the suspension was purged with nitrogen for 15 min. The volatiles were removed by evaporation and the colourless film was taken up in water and purified by size exclusion. After purification the colourless film was lyophilized to give the title compound as a white solid (11.2 mg, 0.018 mmol, 79%). ^{1}H NMR (D_2O, 600 MHz): δ = 5.34 (d, 1H, *J*=3.7 Hz, H-1'), 4.89 (d, 1H, *J*=4.0 Hz, H-1''), 4.52 (s, 1H, H-1), 4.34 (q, 1H, *J*=6.8, 6.1 Hz, H-5'/H-5''), 4.21 (q, 1H, *J*=6.8, 6.1 Hz, H-5'/H-5''), 3.96 – 3.75 (m, 10H), 3.75 – 3.61 (m, 1H), 3.58 – 3.47 (m, 2H), 3.43 (ddd, 1H, *J*=10.2, 5.9, 2.2 Hz), 2.95 (t, 2H, *J*=7.5 Hz, CH_2NH_2), 2.02 (s, 3H, CH_3, Ac), 1.67 – 1.58 (m, 2H, CH_2, hexyl), 1.56 – 1.47 (m, 2H, CH_2, hexyl), 1.40 – 1.26 (m, 4H, CH_2, hexyl), 1.17 (d, 3H, *J*=6.6 Hz, H-6'/H-6''), 1.14 (d, 3H, *J*=6.5 Hz, H-6'/H-6'') ppm. ^{13}C-APT NMR (D_2O, 151 MHz) δ 175.5 (C=O, Ac), 101.9 (C-1), 96.3 (C-1''), 95.6 (C-

1'), 77.9, 76.6, 72.8, 72.7, 72.3 (OCH_2), 71.4, 70.0, 69.7, 68.8, 68.6, 68.0, 67.8, 61.7 (C-6), 40.3 (CH_2NH_2), 29.3, 27.6, 26.2, 25.6 (CH_2, hexyl, 23.5 (CH_3, Ac), 16.2 (C-6', C-6'') ppm. HRMS $[M+H]^+$ calculated for $C_{26}H_{49}N_2O_{14}$: 613.31055, found 613.31783.

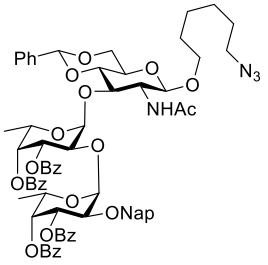
Side product (27)



^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.17 (d, 1H, *J*=8.4 Hz, arom.), 7.84 – 7.70 (m, 2H, arom.), 7.63 – 7.52 (m, 1H, arom.), 7.51 – 7.43 (m, 1H, arom.), 7.43 – 7.26 (m, 10H, arom.), 7.09 (d, 1H, *J*=8.5 Hz, arom.), 5.42 (d, 1H, *J*=1.8 Hz, H-1), 5.00 (d, 1H, *J*=15.5 Hz, CH_2arom.), 4.94 – 4.84 (m, 2H, CH_2arom.), 4.81 – 4.69 (m, 2H, CH_2arom.), 4.65 (d, 1H, *J*=11.9 Hz, CH_2arom.), 4.30 (dd, 1H, *J*=7.1, 5.7 Hz, H-5), 4.13 (t, 1H, *J*=3.5 Hz, H-4), 4.04 (dd, 1H, *J*=5.5, 3.1 Hz, H-3), 4.01 (dd, 1H, *J*=4.0, 1.9 Hz, H-2), 1.77 (d, 3H, *J*=6.9 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 133.2, 132.9, 132.5, 129.0, 128.5, 128.5, 128.5, 127.7, 127.7, 127.7, 127.6, 127.1, 126.9, 125.7, 123.5, 122.2 (arom.), 77.3 (C-4), 76.1 (C-2), 75.1 (C-3), 73.4 (CH_2Bn), 72.2 (CH_2Bn), 71.8 (C-5), 68.3 (CH_2Nap), 59.5 (C-1), 14.3 (C-6) ppm.

2

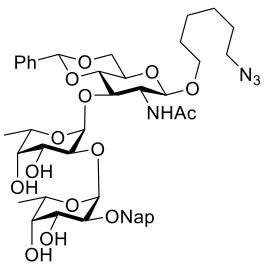
6-azidohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-glucopyranoside (29)



A mixture containing **24** (0.037 g, 0.027 mmol, 1.0 eq.) and Cs_3CO_3 (0.022 g, 0.067 mmol, 2.5 eq.) in dry DMF (0.5 mL, 0.05M) was heated to 80˚C and left to react for 15 hours under inert atmosphere. Upon completion the solution was cooled to room temperature and poured into a solution of sat. $NaHCO_3$ (aq.). The water layer was extracted with EtOAc thrice and the combined organic layers were washed thrice with brine, dried over $MgSO_4$, filtered and concentrated. The crude mixture was re-dissolved in THF (0.5 mL, 0.05M) and pyridine (6.0 µL, 0.081 mmol, 3.0 eq.) and Ac_2O (5.0 µL, 0.054 mmol, 2.0 eq.) were added and the reaction was left to stir for 18 hours. The reaction mixture was partitioned between EtOAc and H_2O. The organic layer was isolated and washed with sat. $CuSO_4$ (aq.), water, sat. $NaHCO_3$ (aq.) and brine, dried over $MgSO_4$, filtered and concentrated. The resulting pale yellow oil was purified by silicagel chromatography (tol:EtOAc, 9:1 → 7:3) which yielded the title compound as a clear oil (0.025 g, 0.019 mmol, 71%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.11 – 8.03 (m, 2H, arom.), 7.89 – 7.81 (m, 2H, arom.), 7.75 (m, 4H, arom.), 7.68 – 7.60 (m, 2H, arom.), 7.60 – 7.34 (m, 17H, NH, arom.), 7.34 – 7.13 (m, 10H, arom.), 5.88 – 5.74 (m, 2H, H-3', H-3''), 5.59 (s, 1H, CHPh), 5.48 (d, 1H, *J*=3.6 Hz, H-4''), 5.44 (d, 1H, *J*=3.6 Hz, H-1''), 5.36 (d, 1H, *J*=3.5 Hz, H-4'), 4.97 (d, 1H, *J*=3.2 Hz, H-1), 4.82 – 4.72 (m, 2H, H-1, H-3), 4.75 – 4.66 (m, 2H, CH_2Nap), 4.64 (q, 1H, *J*=6.6 Hz, H-5'), 4.33 – 4.21 (m, 3H, H-6, H-2'', H-5''), 4.06 (dd, 1H, *J*=10.5, 3.2 Hz, H-2'), 3.86 – 3.73 (m, 2H, H-6, OCH_2), 3.66 (t, 1H, *J*=9.3 Hz,

H-4), 3.52 (q, 1H, *J*=9.1, 8.3 Hz, H-2), 3.46 – 3.34 (m, 2H, H-5, OCH_2), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.92 (s, 3H, CH_3, Ac), 1.65 – 1.50 (m, 4H, CH_2, hexyl), 1.49 – 1.33 (m, 4H, CH_2, hexyl), 0.85 (d, 3H, *J*=6.4 Hz, H-6''), 0.75 (d, 3H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 171.7 (C=O, Ac), 166.1, 166.1, 166.0, 165.5 (C=O, Bz), 137.6, 134.5, 133.6, 133.4, 133.4, 133.2, 133.1, 133.0, 130.1, 130.0, 130.0, 129.8, 129.6, 129.5, 129.2, 128.8, 128.7, 128.6, 128.6, 128.4, 128.3, 127.9, 127.8, 127.3, 126.5, 126.4, 125.9 (arom.), 101.9 (CHPh), 100.7 (H-1), 98.8 (C-1'), 97.5 (C-1''), 81.5 (C-4), 76.4 (C-2''), 74.7 (C-2'), 74.5 (C-3), 73.6 (CH_2Nap), 72.8 (C-4', C-4''), 70.1 (C-3', C-3''), 69.9 OCH_2), 69.0 (C-6), 66.1 (c-5''), 66.0 (C-5), 65.3 (C-5'), 58.3 (C-2), 51.5 (CH_2N_3), 29.6, 28.9, 26.6, 25.6 (CH_2, hexyl), 23.3 (CH_3, Ac), 15.7 (C-6', C-6'') ppm. HRMS $[M+H]^+$ calculated for $C_{72}H_{74}N_4O_{18}H$: 1283.50764, found 1283.50771.

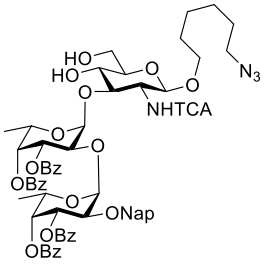
6-azidohexyl 4,6-*O*-benzylidene-2-acetamido-2-deoxy-3-*O*-(2-*O*-(2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-glucopyranoside (30)



NaOMe (60 µL, 0.006 mmol, 0.3 eq. of a 0.1M solution in MeOH) was added to a solution containing trisaccharide **29** (0.022 g, 0.017 mmol, 1.0 eq.) in THF (0.3 mL, 0.05M). The mixture was heated to 50˚C and stirred for 5 hours, when TLC analysis indicated full conversion of the starting material. The mixture was diluted with MeOH (5 mL) and neutralized with Dowex H^+ resin. The resin was filtered off and the reaction mixture was concentrated *in vacuo*. The crude trisaccharide was dry-loaded on celite and purified by silicagel chromatography (tol:EtOAc:MeOH, 2:3:0 → 0:9:1) to give **30** as a colourless film (0.015 g, 0.016 mmol, 99%). ^{1}H NMR (MeOD, 400 MHz): δ = 8.06 – 7.98 (m, 3H, arom.), 7.90 – 7.81 (m, 3H, arom.), 7.68 (dd, 1H, *J*=8.4, 1.6 Hz, arom.), 7.61 – 7.54 (m, 1H, arom.), 7.46 (m, 6H, arom.), 7.36 (dd, 3H, *J*=5.1, 1.9 Hz, arom.), 5.33 (d, 1H, *J*=2.0 Hz, H-1''), 5.25 (d, 1H, *J*=3.7 Hz, H-1'), 5.20 (s, 1H, CHPh), 5.09 (d, 1H, *J*=12.0 Hz, CH_2Nap), 4.98 (d, 1H, *J*=12.0 Hz, CH_2Nap), 4.27 (q, 1H, *J*=7.9, 7.2 Hz, H-5''), 4.21 (q, 1H, *J*=7.1, 6.4 Hz, H-5'), 4.11 (dd, 1H, *J*=10.2, 3.4 Hz, H-3''), 4.03 (dd, 1H, *J*=10.3, 4.9 Hz, H-6), 3.93 – 3.84 (m, 4H, H-3, H-2', H-3', H-2''), 3.79 (t, 1H, *J*=9.0 Hz, H-2), 3.74 – 3.65 (m, 2H, H-1, H-4''), 3.56 (d, 1H, *J*=2.0 Hz, H-4'), 3.53 – 3.47 (m, 1H, OCH_2), 3.42 (t, 1H, *J*=10.2 Hz, H-6), 3.26 (t, 2H, *J*=6.8 Hz, CH_2N_3), 3.13 (t, 1H, *J*=9.1 Hz, H-4), 3.05 – 2.92 (m, 2H, H-5, OCH_2), 2.03 (s, 3H, CH_3, Ac), 1.60 – 1.50 (m, 2H, OH), 1.46 – 1.24 (m, 8H, CH_2, hexyl), 1.22 (d, 3H, *J*=6.6 Hz, C-6''), 1.02 (d, 3H, *J*=6.5 Hz, C-6') ppm. ^{13}C-APT NMR (MeOD, 101 MHz) δ 173.7 (C=O, Ac), 139.1, 137.8, 134.8, 134.6, 133.9, 130.7, 129.8, 129.4, 129.2, 129.1, 128.8, 127.9, 127.5, 127.4, 127.2, 127.2 (arom.), 103.4 (C-1), 102.1 (CHPh), 97.0 (C-1''), 95.1 (C-1'), 82.0 (C-4), 78.9 (C-2''), 76.0 (C-3), 74.6 (CH_2Nap), 74.2 (C-4''), 73.7 (C-2', C-4'), 71.1 (C-3''), 70.5 (OCH_2), 69.8 (C-3'), 69.4 (C-6), 67.9 (C-5'), 67.7 (C-5''), 67.0 (C-5), 56.6 (C-2), 52.4 (CH_2N_3),

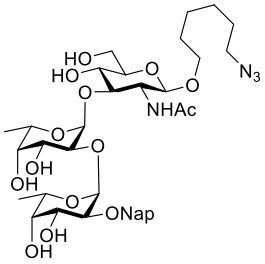
30.5, 29.9, 27.5, 26.5 (CH_2, hexyl), 23.4 (CH_3, Ac), 16.6 (C-6'), 16.5 (C-6'') ppm. HRMS $[M+H]^+$ calculated for $C_{44}H_{58}N_4O_{14}H$:867.40278, found 867.40285.

6-azidohexyl 2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (31)



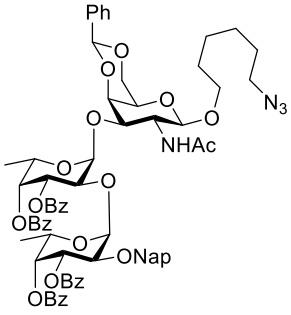
Trisaccharide **24** (0.17 g, 0.12 mmol, 1.0 eq.) was suspended in a mixture of acetic acid and water (12,5 mL, 0.01M) and heated to 80˚C for 2 hours. The mixture was poured directly into EtOAc and washed with sat. $NaHCO_3$ (aq.) until no more bubbling occurred. The organic layer was then washed with brine, dried over $MgSO_4$, filtered and concentrated. The resulting yellow oil was purified by silicagel chromatography (tol:EtOAc, 4:1 → 1:1), which yielded the title compound (0.076 g, 0.059 mmol, 48%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.09 (d, 2H, *J*=7.7 Hz, arom.), 7.92 (dd, 3H, *J*=12.4, 8.4 Hz, arom.), 7.88 – 7.75 (m, 5H, NH, arom.), 7.72 – 7.56 (m, 6H, arom.), 7.56 – 7.42 (m, 5H, arom.), 7.37 (m, 4H, arom.), 7.33 – 7.23 (m, 4H, arom.), 5.91 (dd, 1H, *J*=10.5, 3.2 Hz, H-3''), 5.79 – 5.64 (m, 3H, H-1', H-3', H-4'), 5.45 (d, 1H, *J*=3.2 Hz, H-4''), 5.17 (d, 1H, *J*=3.2 Hz, H-1''), 4.91 – 4.67 (m, 3H, H-5', CH_2Nap), 4.39 – 4.29 (m, 2H, H-1, H-2'), 4.26 (q, 1H, *J*=6.5 Hz, H-5''), 4.19 (dd, 1H, *J*=10.5, 3.1 Hz, H-2''), 4.15 – 4.03 (m, 1H, H-2), 3.96 (t, 1H, *J*=10.9 Hz, H-3), 3.84 (dt, 1H, *J*=9.5, 5.9 Hz, OCH_2), 3.67 (m, 3H, *J*=6.3 Hz, H-4, H-6), 3.39 (dt, 1H, *J*=9.6, 6.5 Hz, OCH_2), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.85 (dd, 1H, *J*=8.6, 4.2 Hz, H-5), 2.05 (d, 1H, *J*=6.2 Hz, OH), 1.59 (m, 4H, CH_2, hexyl), 1.49 – 1.25 (m, 7H, H-6', CH_2, hexyl), 0.68 (d, 3H, *J*=6.4 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.1, 165.9, 165.8, 165.0 (C=O, Bz), 162.8 (C=O, TCA), 134.2, 133.8, 133.5, 133.3, 133.2, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 128.8, 128.7, 128.7, 128.3, 128.0, 127.9, 127.9, 126.6, 126.3 (arom.), 101.2 (C-1 + C-1'') 97.9 (C-1'), 92.3 (C_q, TCA), 81.3 (C-3), 78.3 (C-2'), 75.1 (C-2''), 74.7 (C-5), 74.3 (CH_2Nap), 72.6 (C-4''), 72.3 (C-4'), 72.1 (C-4), 70.6 (C-3''), 70.0 (OCH_2), 69.0 (C-3'), 66.9 (C-5'), 66.5 (C-5''), 62.2 (C-6), 55.6 (C-2), 51.5 (CH_2N_3), 29.6, 28.8, 26.6, 25.7 (CH_2, hexyl), 16.4 (C-6'), 15.6 (C-6'') ppm. HRMS $[M+NH_4]^+$ calculated for C65H67Cl3N4O18NH_4: 1314.38596, found 1314.38633.

6-azidohexyl 2-acetamido-2-deoxy-3-*O*-(2-*O*-(2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-glucopyranoside (32)



Diol **31** (0.034 g, 0.026 mmol, 1.0 eq.) was dissolved in methanol (0.3 mL, 0.1M) and heated to 50˚C. NaOH (0.2 mL, of a 5M solution) was added and the reaction was stirred for 50 hours. The reaction mixture was neutralized (pH ~7) by addition of AcOH and concentrated *in vacuo*. The resulting white solid was suspended in a mixture of THF and water (2 mL, 0.15M, 1/1, v/v). Et_3N (0.05 mL) was added followed by Ac_2O (20 µL) and the reaction was left to stir for 4 hours. When TLC analysis showed full conversion to the desired product the reaction mixture was concentrated and purified by size exclusion chromatography (LH-20, MeOH/H_2O, 9/1, v/v). After purification trisaccharide **32** was obtained (0.018 g, 0.023 mmol, 89%). ^{1}H NMR (MeOD, 400 MHz): δ = 7.99 (d, 1H, *J*=1.6 Hz, arom.), 7.95 – 7.85 (m, 3H, arom.), 7.72 (dd, 1H, *J*=8.4, 1.7 Hz, arom.), 7.54 – 7.47 (m, 2H, arom.), 5.33 (d, 1H, *J*=3.1 Hz, H-1'), 5.26 (d, 1H, *J*=3.7 Hz, H-1''), 5.09 – 4.97 (m, 2H, CH_2Nap), 4.36 (q, 1H, *J*=7.3, 5.9 Hz, H-5''), 4.26 (q, 1H, *J*=7.2, 6.5 Hz, H-5'), 4.09 (dd, 1H, *J*=10.3, 3.4 Hz, H-3''), 3.97 – 3.82 (m, 3H, H-2', H-3', H-2''), 3.74 – 3.63 (m, 4H, H-2, H-6, H-4', H-4''), 3.56 (dd, 1H, *J*=12.0, 5.2 Hz, H-6), 3.48 – 3.37 (m, 2H, H-3, OCH_2), 3.35 (m, 1H, H-4), 3.27 (t, 2H, *J*=6.9 Hz, CH_2N_3), 3.19 (d, 1H, *J*=8.4 Hz, H-1), 2.95 – 2.87 (m, 1H, H-5), 2.70 – 2.60 (m, 1H, OCH_2), 2.02 (s, 3H, CH_3, Ac), 1.55 (m, 2H, CH_2, hexyl), 1.37 – 1.25 (m, 6H, CH_2, hexyl), 1.25 – 1.16 (2x d, 6H, H-6', H-6'') ppm. ^{13}C-APT NMR (MeOD, 101 MHz) δ 173.8 (C=O, Ac), 137.4, 134.8, 134.7, 129.7, 129.2, 128.9, 128.2, 127.6, 127.5, 127.4 (arom.), 102.9 (C-1), 98.0 (C-1'), 96.4 (C-1''), 82.0 (C-3), 79.5 (C-2''), 77.1 (C-5), 75.5 (CH_2Nap), 74.5 (C-2'), 74.4 (C-4'/4''), 73.8 (C-4'/4''), 71.5 (C-4), 71.1 (C-3''), 70.1 (OCH_2), 69.3 (C-3'), 68.7 (C-5'), 67.6 (C-5''), 62.4 (C-6), 55.5 (C-2), 52.4 (CH_2N_3), 30.4, 29.9, 27.5, 26.5 (CH_2, hexyl), 23.5 (CH_3, Ac), 16.6 (C-6', C-6'') ppm. HRMS $[M+H]^+$ calculated for $C_{37}H_{54}N_4O_{14}H$: 779.37165, found 779.37104.

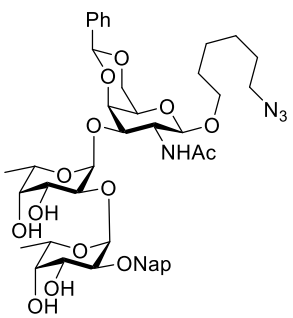
6-azidohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-galactopyranoside (34)



Cs_2CO_3 (0.092 g, 0.28 mmol, 2.5 eq.) was added to a solution of **21** (0.16 g, 0.11 mmol, 1.0 eq.) in dry DMF (1.1 mL, 0.1M). The solution was heated to 70˚C and stirred for 15 hours. Upon completion the solution was cooled to RT and poured into EtOAc. The organic phase was washed with brine (5x), dried over $MgSO_4$, filtered and concentrated. The residue was re-dissolved in THF (1.1 mL, 0.1M), pyridine (26 µL, 0.33 mmol, 3.0 eq.) and Ac_2O (21 µL, 0.22

mmol, 2.0 eq.) were added and the reaction was left to stir for 18 hours. The reaction mixture was poured into EtOAc and washed with sat. $CuSO_4$ (aq.), H_2O, sat. $NaHCO_3$ (aq.) and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by silicagel chromatography (tol:EtOAc, 1:0 → 4:1) and the title compound was obtained as a pale white solid (0.070 g, 0.054 mmol, 48%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.13 – 7.98 (m, 5H, arom.), 7.97 – 7.77 (m, 5H, arom.), 7.77 – 7.56 (m, 6H, arom.), 7.56 – 7.40 (m, 10H, arom.), 7.33 – 7.16 (m, 6H, arom.), 6.73 (d, 1H, *J*=9.5 Hz, NH), 5.84 (m, 2H, H-3', H-3''), 5.53 (d, 1H, *J*=3.6 Hz, H-4'), 5.45 (d, 1H, *J*=3.4 Hz, H-4''), 5.23 (m, 2H, H-1'', CHPh), 5.14 (d, 1H, *J*=3.3 Hz, H-1'), 4.80 (d, 1H, *J*=10.3 Hz, CH_2Nap), 4.68 (d, 1H, *J*=10.3 Hz, CH_2Nap), 4.56 – 4.43 (m, 2H, H-2, H-5'), 4.26 – 4.11 (m, 3H, H-2', H-2'', H-5''), 4.03 (d, 1H, *J*=8.6 Hz, H-1), 3.81 (dt, 1H, *J*=9.5, 6.3 Hz, OCH_2), 3.77 – 3.68 (m, 1H, H-6), 3.55 – 3.43 (m, 2H, H-4, OCH_2), 3.29 (dd, 1H, *J*=10.9, 3.3 Hz, H-3), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 3.08 (dd, 1H, *J*=12.4, 1.6 Hz, H-6), 2.12 (s, 3H, CH_3, Ac), 1.75 (s, 1H, H-5) 1.59 (m, 4H, CH_2, hexyl), 1.40 (m, 4H, CH_2, hexyl), 0.77 (d, 3H, *J*=6.5 Hz, H-6'), 0.56 (d, 3H, *J*=6.4 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 170.7 (C=O, Ac), 166.2, 165.9, 165.9, 165.0 (C=O, Bz), 137.8, 134.5, 134.0, 133.6, 133.4, 133.2, 133.1, 133.1, 130.0, 129.9, 129.9, 129.9, 129.8, 129.6, 129.3, 129.1, 128.9, 128.8, 128.7, 128.6, 128.3, 128.3, 128.2, 127.9, 127.8, 127.7, 127.1, 127.0, 126.8, 126.4, 126.2 (arom.), 101.1 (C-1, C-1'', CHPh) 97.4 (C-1'), 79.7 (C-2'), 79.4 (C-3), 76.1 (C-2''), 74.8 (CH_2Nap), 73.3 (C-4), 72.9 (C-4'), 72.5 (C-4''), 70.6 (C-3'', 69.0 (C-6), 69.0 (C-3'), 67.6 (OCH_2), 66.5 (C-5''), 66.0 (C-5'), 65.7 (C-5), 51.5 (CH_2N_3), 49.1 (C-2), 29.5, 28.9, 26.6, 25.7 (CH_2, hexyl), 23.3 (CH_3, Ac), 15.7 (C-6'), 15.5 (C-6'') ppm.

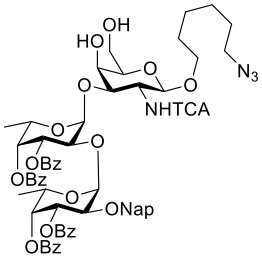
6-azidohexyl 4,6-*O*-benzylidene-2-acetamido-2-deoxy-3-*O*-(2-*O*-(2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-galactopyranoside (35)



Trisaccharide **34** (0.070 g, 0.054 mmol, 1.0 eq.) was dissolved in methanol (0.5 mL, 0.1M). NaOMe (160 µL, 0.016 mmol, 0.3 eq.) was added and the temperature was increased to 50˚C. The reaction was stirred for 20 hours, diluted in MeOH, neutralized (pH~7) by addition of Dowex H^+ resin, filtered and concentrated. The residue was purified by size exclusion on LH-20 (DCM/MeOH, 1/1, v/v) to give tetraol **35** (0.027 g, 0.031 mmol, 58%). ^{1}H NMR (MeOD, 400 MHz): δ = 8.06 – 7.90 (m, 4H, arom.), 7.80 – 7.72 (m, 1H, arom.), 7.63 – 7.52 (m, 2H, arom.), 7.50 – 7.43 (m, 2H, arom.), 7.38 – 7.28 (m, 3H, arom.), 5.53 (s, 1H, CHPh), 5.24 (d, 1H, *J*=3.6 Hz, H-1''), 5.12 – 5.06 (m, 2H, H-1', CH_2Nap), 4.98 (d, 1H, *J*=11.0 Hz, CH_2Nap), 4.47 (q, 1H, *J*=7.2, 6.5, 6.2 Hz, H-5''), 4.25 (d, 1H, *J*=3.3 Hz, H-4), 4.15 – 4.06 (m, 2H, H-5', H-3''), 4.04 – 3.92 (m, 4H, H-2', H-2'', H-6), 3.85 (dd, 1H, *J*=10.3, 3.2 Hz, H-2'), 3.79 (dd, 1H, *J*=10.3, 3.3 Hz, H-3'), 3.74 (d, 1H, *J*=3.5 Hz, H-4''), 3.54 (dd, 1H, *J*=3.3 Hz, H-4'), 3.39 – 3.28 (m, 3H, OCH_2, CH_2N_3), 3.28 – 3.22 (m,

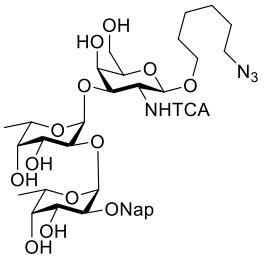
1H, H-3), 2.81 – 2.68 (m, 2H, H-1, H-5), 2.49 – 2.33 (m, 1H, OCH_2), 2.05 (s, 3H, CH_3, Ac), 1.67 – 1.51 (m, 2H, CH_2, hexyl), 1.46 – 1.11 (m, 9H, H-6'', CH_2, hexyl), 0.92 (d, 3H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR (MeOD, 101 MHz) δ 173.9 (C=O, TCA), 139.6, 137.6, 134.9, 134.8, 129.9, 129.8, 129.2, 129.1, 129.0, 129.0, 128.4, 127.8, 127.7, 127.5 (arom.), 103.4 (C-1), 102.3 (CHPh), 100.0 (C-1'), 98.5 (C-1''), 80.6 (C-2'), 80.5 (C-3), 77.0 (C-2''), 76.4 (CH_2Nap), 75.1 (C-4), 74.5 (C-4''), 73.9 (C-4'), 71.2 (C-3''), 70.1 (C-6), 70.1 (OCH_2), 69.5 (C-3'), 68.4 (C-5'), 67.7 (C-5''), 67.2 (C-5), 52.4 (CH_2N_3), 51.2 (C-2), 30.4, 29.9, 27.5, 26.4 (CH_2, hexyl), 23.6 (CH_3, Ac), 16.7 (C-6'), 16.5 (C-6'') ppm.

6-azidohexyl 2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (36)



Fully protected trisaccharide **21** (0.23 g, 0.17 mmol, 1.0 eq.) was suspended in a mixture of AcOH and water (15 mL, 4/1, v/v). The suspension was heated to 70˚C and stirred for 2 hours. The reaction mixture was poured directly into EtOAc and washed with sat. $NaHCO_3$ (aq.), until the water layer no longer discharged bubbles. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The yellow oil was purified by silicagel chromatography (tol:EtOAc, 4:1 → 1:1) to give **36** as a white foam (0.15 g, 0.11 mmol, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.12 – 7.99 (m, 5H, arom.), 7.91 – 7.79 (m, 5H, arom.), 7.71 – 7.58 (m, 5H, arom.), 7.58 – 7.38 (m, 10H, arom.), 7.27 (m, 2H, arom.), 7.12 (d, 1H, *J*=8.4 Hz, NH), 6.00 (dd, 1H, *J*=10.4, 3.3 Hz, H-3''), 5.76 – 5.60 (m, 2H, H-3', H-4'), 5.44 (d, 1H, *J*=3.3 Hz, H-4''), 5.30 – 5.15 (m, 2H, H-1', H-1''), 4.76 (d, 1H, *J*=10.2 Hz, CH_2Nap), 4.64 (q, 1H, *J*=6.4 Hz, H-5'), 4.58 (d, 1H, *J*=10.2 Hz, CH_2Nap), 4.48 (d, 1H, *J*=8.5 Hz, H-1), 4.39 (q, 1H, *J*=9.5 Hz, H-2), 4.29 – 4.18 (m, 2H, H-2', H-5''), 4.13 (dd, 1H, *J*=10.4, 3.0 Hz, H-2''), 4.02 – 3.83 (m, 2H, H-3, OCH_2), 3.55 (dt, 1H, *J*=9.7, 6.5 Hz, OCH_2), 3.34 – 3.23 (m, 2H, H-4, H-6), 3.20 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.87 – 2.73 (m, 1H, H-6), 2.50 (d, 1H, *J*=2.7 Hz, C4-OH), 2.24 (t, 1H, *J*=5.1 Hz, H-5), 1.81 – 1.71 (m, 1H, C6-OH), 1.67 – 1.48 (m, 4H, CH_2, hexyl), 1.48 – 1.29 (m, 4H, CH_2, hexyl), 1.21 (d, 3H, *J*=6.5 Hz, H-6'), 0.60 (d, 3H, *J*=6.5 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.4, 165.9, 165.8, 164.9 (C=O, Bz), 162.7 (C=O, TCA), 134.2, 134.0, 133.7, 133.5, 133.2, 133.1, 133.0, 129.9, 129.8, 129.6, 129.5, 129.2, 129.0, 128.8, 128.7, 128.3, 127.9, 127.9, 127.5, 127.1, 127.0, 126.1 (arom.), 102.1 (C-1''), 101.1 (C-1'), 95.5 (C_q, TCA), 79.8 (C-3), 79.6 (C-2'), 76.3 (C-2''), 74.5 (CH_2Nap), 73.4 (C-5), 72.7 (C-4''), 72.1 (C-4'), 70.2 (C-3''), 69.3 (C-3', OCH_2), 66.9 (C-5''), 66.5 (C-4, C-5'), 62.6 (C-6), 52.4 (C-2), 51.5 (CH_2N_3), 29.5, 28.8, 26.6, 25.7 (CH_2, hexyl), 16.5 (C-6'), 15.4 (C-6'') ppm. HRMS $[M+NH_4]^+$ calcd for $C_{65}H_{67}Cl_3N_4O_{18}NH_4$: 1314.38596, found 1314.38606.

6-azidohexyl 2-acetamido-2-deoxy-3-*O*-(2-*O*-(2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-galactopyranoside (37)



Diol **36** (0.032 g, 0.025 mmol, 1.0 eq.) was dissolved in methanol (0.5 mL, 0.05M). NaOMe (8 µL, 0.008 mmol, 0.3 eq. of a 0.1M solution in methanol) was added and the solution was stirred for 16 hours at 50˚C. The reaction mixture was diluted in methanol and the pH was adjusted to ~7, by addition of Dowex H^+ resin. The resin was filtered and the volatiles were evaporated *in vacuo*. The yellow oil was purified by LH-20 size exclusion ($MeOH/H_2O$, 9/1, v/v) to give the title compound as a colourless oil (0.016 g, 0.018 mmol, 73%). ^1H NMR (MeOD, 400 MHz): δ = 8.04 (s, 1H, arom.), 7.94 – 7.82 (m, 3H, arom.), 7.69 (dd, 1H, *J*=8.4, 1.7 Hz, arom.), 7.51 – 7.45 (m, 2H, arom.), 5.30 (d, 1H, *J*=3.4 Hz, H-1'), 5.21 (d, 1H, *J*=3.7 Hz, H-1''), 5.04 (d, 1H, *J*=11.8 Hz, CH_2Nap), 4.93 (d, 1H, *J*=11.8 Hz, CH_2Nap), 4.31 – 4.23 (m, 1H, H-5'), 4.21 – 4.04 (m, 3H, H-2, H-3'', H-5''), 3.97 (m, 2H, H-4, H-3'), 3.89 (dd, 1H, *J*=3.4, 1.8 Hz, H-3), 3.87 – 3.76 (m, 3H, H-1, H-2', H-2''), 3.68 (m, 4H, H-6, H-4', H-4''), 3.61 (dt, 1H, *J*=9.5, 5.8 Hz, OCH_2), 3.27 – 3.19 (m, 3H, H-5, CH_2N_3), 3.00 (ddd, 1H, *J*=9.6, 7.0, 5.6 Hz, OCH_2), 1.57 – 1.49 (m, 2H, CH_2, hexyl), 1.44 – 1.36 (m, 1H, CH_2, hexyl), 1.34 – 1.26 (m, 4H, CH_2, hexyl), 1.22 (d, 3H, *J*=6.6 Hz, H-6''), 1.19 (d, 3H, *J*=6.6 Hz, H-6') ppm. ^{13}C-APT NMR (MeOD, 101 MHz) δ 164.3 (C=O, TCA), 137.4, 134.8, 134.6, 129.4, 129.2, 128.8, 128.3, 127.7, 127.2, 127.1 (arom.), 102.8 (C-1), 96.4 (C-1', C-1''), 78.7 (C-2''), 76.9 (C-2'), 76.1 (C-5), 74.6 (CH_2Nap), 74.6 (C-3), 74.1 (C-4''), 73.6 (C-4'), 70.9 (C-3''), 70.3 (OCH_2), 69.4 (C-4), 68.6 (C-5''), 67.7 (C-5'), 67.6 (C-3'), 62.2 (C-6), 55.0 (C-2), 52.4 (CH_2N_3), 30.6, 29.8, 27.6, 26.7 (CH_2, hexyl), 16.6 (C-6', C-6'') ppm. HRMS $[M+NH_4]^+$ calcd for $C_{37}H_{51}Cl_3N_4O_{14}NH_4$: 898.28111, found 898.28081.

Geometry optimization

To generate a broad geometrical library, a conformer distribution search based on a Monte-Carlo algorithm included in the Spartan 14 program was performed, with the use of molecular mechanics with MMFF94 as force field.[40] This search was done from multiple starting conformers to cover a large geometrical space. All generated structures (*N*= 50−100) were further optimized with Gaussian 09 using the ωB97XD long-range corrected hybrid functional[41] and 6-31G(d) as basis set. Optimization was done in gas-phase and subsequently corrections for solvent effects were done by the use of a polarizable continuum model using dichloromethane as solvent parameter. The electronic energies ΔE_{gas} were computed by dispersion-corrected DFT given by Equation (1), in which ΔE_{DFT} is the KS-DFT SCF energy and ΔE_{disp} is the standard atom pair-wise London dispersion energy.

$$\Delta E_{gas} = \Delta E_{DFT} + \Delta E_{disp} \qquad (1)$$

The final denoted free Gibbs energy was calculated using Equation (1) in which ΔE_{gas} is the gas-phase energy (electronic energy), $\Delta G^{T}_{gas,QH}$ (T = 253.15 K and pressure = 1 atm.) is the sum of corrections from the electronic energy to free Gibbs energy in the quasi-harmonic oscillator approximation also including zero-point-vibrational energy, and ΔG_{solv} is their corresponding free solvation Gibbs energy. The $\Delta G^{T}_{gas,QH}$ were computed using the quasi-harmonic approximation in the gas phase according to the work of Truhlar.[42] The quasi-harmonic approximation is the same as the harmonic oscillator approximation except that vibrational frequencies lower than 100 cm^{-1} were raised to 100 cm^{-1} as a way to correct for the breakdown of the harmonic oscillator model for the free energies of low-frequency vibrational modes. The used free energies include unscaled zero-point vibrational energies. All found minima were checked for negative frequencies. Visualisation of the conformations of interest was done with CYLview.[43] The obtained results were visualized as a scatter plot by the Origin pro 9 software.

$$\Delta G^{T}_{in\ solution} = \Delta E_{gas} + \Delta G^{T}_{gas,QH} + \Delta G_{solv} \qquad (2)$$

$$= \Delta G^{T}_{gas} + \Delta G_{solv}$$

Synthesis of functionalized gold nanoparticles

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 protocols and reagents

Materials and reagents

Coating buffer: 50 mM Na_2CO_3, in waterpH = 9.6. Bovine serum albumin (BSA) (lyophilized powder, ≥98 %, pH 7, measured by agarose gel electrophoresis) was used (stored at 2-8 °C). The positive control used for these ELISA was Soluble Egg Antigen (SEA) (1:200 in coating buffer). BSA (1% in PBS) was used as the negative control. ELISA Nunc

MaxiSorp 96-well immunoplate (Nunc MaxiSorp® flat-bottom 96-well plate, Thermo Fisher Scientific, Roskilde, Denmark).

ELISA protocol with monoclonal antibodies

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. 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.

ELISA protocol with human sera

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. 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.

References

[1] M. H. J. Meevissen, M. Yazdanbakhsh, and C. H. Hokke, "Schistosoma mansoni egg glycoproteins and C-type lectins of host immune cells: Molecular partners that shape immune responses," *Exp. Parasitol.*, vol. 132, no. 1, pp. 14–21, 2012.

[2] A. van Diepen *et al.*, "Differential Anti-Glycan Antibody Responses in Schistosoma mansoni-Infected Children and Adults Studied by Shotgun Glycan Microarray," *PLoS Negl. Trop. Dis.*, vol. 6, no. 11, p. e1922, Nov. 2012.

[3] M. H. J. Meevissen *et al.*, "Specific glycan elements determine differential binding of individual egg glycoproteins of the human parasite Schistosoma mansoni by host C-type lectin receptors," *Int. J. Parasitol.*, vol. 42, no. 3, pp. 269–277, 2012.

[4] K. H. Khoo *et al.*, "A unique multifucosylated -3GalNAcβ1→4GlcNAcβ1→3Galα1- motif constitutes the repeating unit of the complex O-glycans derived from the cercarial glycocalyx of Schistosoma mansoni," *Journal of Biological Chemistry*, vol. 270, no. 29. pp. 17114–17123, 1995.

[5] K. H. Khoo, D. Chatterjee, J. P. Caulfield, H. R. Morris, and A. Dell, "Structural characterization of glycosphingolipids from the eggs of Schistosoma mansoni and Schistosoma japonicum," *Glycobiology*, vol. 7, no. 5, pp. 653–661, 1997.

[6] M. L. M. Robijn *et al.*, "Targeted identification of a unique glycan epitope of Schistosoma mansoni egg antigens using a diagnostic antibody," *Mol. Biochem. Parasitol.*, vol. 151, no. 2, pp. 148–161, 2007.

[7] A. van Diepen, A. J. van der Plas, R. P. Kozak, L. Royle, D. W. Dunne, and C. H. Hokke, "Development of a Schistosoma mansoni shotgun O-glycan microarray and application to the discovery of new antigenic schistosome glycan motifs," *Int. J. Parasitol.*, vol. 45, no. 7, pp. 465–475, 2015.

[8] T. M. Kariuki *et al.*, "Parameters of the attenuated schistosome vaccine evaluated in the olive baboon," *Infect. Immun.*, vol. 72, no. 9, pp. 5526–5529, 2004.

[9] C. H. Smit *et al.*, "Glycomic analysis of life stages of the human parasite schistosoma mansoni reveals developmental expression profiles of functional and antigenic Glycan motifs," *Mol. Cell. Proteomics*, vol. 14, no. 7, pp. 1750–1769, 2015.

[10] C. H. Smit *et al.*, "Surface expression patterns of defined glycan antigens change during Schistosoma mansoni cercarial transformation and development of schistosomula," *Glycobiology*, vol. 25, no. 12, pp. 1465–1479, 2015.

[11] L. Liao and F. I. Auzanneau, "The amide group in N-acetylglucosamine glycosyl acceptors affects glycosylation outcome," *J. Org. Chem.*, vol. 70, no. 16, pp. 6265–6273, 2005.

[12] R. Enugala, L. C. R. Carvalho, M. J. Dias Pires, and M. M. B. Marques, "Stereoselective glycosylation of glucosamine: The role of the N-protecting group," *Chem. - An Asian J.*, vol. 7, no. 11, pp. 2482–2501, 2012.

[13] S. S. Pertel, L. O. Kononov, A. I. Zinin, V. J. Chirva, and E. S. Kakayan, "Synthesis of some 2-alkoxy glyco-[2,1-d]-2-oxazolines and evaluation of their glycosylation reactivity," *Carbohydr. Res.*, vol. 356, pp. 172–179, 2012.

[14] S. Hou and P. Kováč, “Synthesis of the conjugation ready, downstream disaccharide fragment of the O-PS of Vibrio cholerae O:139,” *Carbohydr. Res.*, vol. 346, no. 12, pp. 1394–1397, Sep. 2011.
[15] F. Bélot and J. C. Jacquinet, “Syntheses of chondroitin 4- and 6-sulfate pentasaccharide derivatives having a methyl β-D-glucopyranosiduronic acid at the reducing end,” *Carbohydr. Res.*, vol. 326, no. 2, pp. 88–97, 2000.
[16] A. Vibert, C. Lopin-Bon, and J.-C. Jacquinet, “Efficient alternative for the reduction of N-trichloroacetyl groups in synthetic chondroitin oligosaccharide intermediates,” *Tetrahedron Lett.*, vol. 51, no. 14, pp. 1867–1869, Apr. 2010.
[17] A. M. Downey and M. Hocek, “Strategies toward protecting group-free glycosylation through selective activation of the anomeric center,” *Beilstein J. Org. Chem.*, vol. 13, pp. 1239–1279, 2017.
[18] A.-M. M. van Roon *et al.*, “Synthesis and antibody-binding studies of a series of parasite fuco-oligosaccharides.,” *Bioorg. Med. Chem.*, vol. 13, no. 10, pp. 3553–64, May 2005.
[19] N. Shao, J. Xue, and Z. Guo, “Chemical Synthesis of CD52 Glycopeptides Containing the Acid-Labile Fucosyl Linkage,” *J. Org. Chem.*, vol. 68, no. 23, pp. 9003–9011, 2003.
[20] M. Ziaco *et al.*, “Synthesis of the tetrasaccharide outer core fragment of Burkholderia multivorans lipooligosaccharide,” *Carbohydr. Res.*, vol. 403, pp. 182–191, 2015.
[21] P. Chassagne *et al.*, “Structural Studies of the O-Acetyl-Containing O-Antigen from a Shigella flexneri Serotype 6 Strain and Synthesis of Oligosaccharide Fragments Thereof,” *European J. Org. Chem.*, no. 19, pp. 4085–4106, 2013.
[22] D. Lloyd and C. S. Bennett, “Gram-scale synthesis of an armed colitose thioglycoside,” *J. Org. Chem.*, vol. 79, no. 20, pp. 9826–9829, 2014.
[23] W. A. Bubb, “NMR spectroscopy in the study of carbohydrates: Characterizing the structural complexity,” *Concepts Magn. Reson. Part A Bridg. Educ. Res.*, vol. 19, no. 1, pp. 1–19, 2003.
[24] B. M. Trost and G. Dong, “Total Synthesis of Bryostatin 16 Using a Pd-Catalyzed Diyne Coupling as Macrocyclization Method and Synthesis of C20- epi - Bryostatin 7 as a Potent Anticancer Agent,” *J. Am. Chem. Soc.*, vol. 132, no. 46, pp. 16403–16416, Nov. 2010.
[25] T. Sato, T. Oishi, and K. Torikai, “2-Naphthylmethoxymethyl as a Mildly Introducible and Oxidatively Removable Benzyloxymethyl-Type Protecting Group,” *Org. Lett.*, vol. 17, no. 12, pp. 3110–3113, 2015.
[26] M. H. S. Marqvorsen, M. J. Pedersen, M. R. Rasmussen, S. K. Kristensen, R. Dahl-Lassen, and H. H. Jensen, “Why Is Direct Glycosylation with N-Acetylglucosamine Donors Such a Poor Reaction and What Can Be Done about It?,” *J. Org. Chem.*, vol. 82, no. 1, pp. 143–156, 2017.
[27] W. Peng, J. Sun, F. Lin, X. Han, and B. Yu, “Facile Synthesis of Ginsenoside Ro,” *Synlett*, no. 2, pp. 259–262, 2004.
[28] B. Hagen, S. Ali, H. S. Overkleeft, G. A. Van der Marel, and J. D. C. Codée, “Mapping the Reactivity and Selectivity of 2-Azidofucosyl Donors for the Assembly of N-Acetylfucosamine-Containing Bacterial Oligosaccharides,” *J. Org.*

Chem., vol. 82, no. 2, pp. 848–868, 2017.
[29] J. Chen *et al.*, "1-Picolinyl-5-azido Thiosialosides: Versatile Donors for the Stereoselective Construction of Sialyl Linkages," *Angew. Chemie - Int. Ed.*, vol. 58, no. 47, pp. 17000–17008, 2019.
[30] G. Merino, T. Heine, and G. Seifert, "The induced magnetic field in cyclic molecules," *Chem. - A Eur. J.*, vol. 10, no. 17, pp. 4367–4371, 2004.
[31] D. Urabe, K. Sugino, T. Nishikawa, and M. Isobe, "A novel deprotection of trichloroacetamide," *Tetrahedron Lett.*, vol. 45, no. 51, pp. 9405–9407, 2004.
[32] J. Boutet, C. Guerreiro, and L. A. Mulard, "Efficient synthesis of six tri- to hexasaecharide fragments of shigella flexneri serotypes 3a and/or X O-antigen, including a study on acceptors containing N-trichloroacetylglucosamine versus N-acetylglucosamine," *J. Org. Chem.*, vol. 74, no. 7, pp. 2651–2670, 2009.
[33] M. J. Gaunt, J. Yu, and J. B. Spencer, "Rational Design of Benzyl-Type Protecting Groups Allows Sequential Deprotection of Hydroxyl Groups by Catalytic Hydrogenolysis," *J. Org. Chem.*, vol. 63, no. 13, pp. 4172–4173, 1998.
[34] L. Lázáar, L. Jánossy, M. Csávás, M. Herczeg, A. Borbás, and S. Antus, "Selective removal of the (2-naphthyl)methyl protecting group in the presence of p-methoxybenzyl group by catalytic hydrogenation," *Arkivoc*, vol. 2012, no. 5, pp. 312–325, 2012.
[35] O. Oyelaran, L. M. McShane, L. Dodd, and J. C. Gildersleeve, "Profiling Human Serum Antibodies with a Carbohydrate Antigen Microarray," *J. Proteome Res.*, vol. 8, no. 9, pp. 4301–4310, Sep. 2009.
[36] M. Marradi, F. Chiodo, I. García, and S. Penadés, "Glyconanoparticles as multifunctional and multimodal carbohydrate systems," *Chem. Soc. Rev.*, vol. 42, no. 11, pp. 4728–4745, 2013.
[37] F. Chiodo, M. Marradi, B. Tefsen, H. Snippe, I. van Die, and S. Penadés, "High Sensitive Detection of Carbohydrate Binding Proteins in an ELISA-Solid Phase Assay Based on Multivalent Glyconanoparticles," *PLoS One*, vol. 8, no. 8, pp. 1–11, 2013.
[38] P. Chassagne, L. Raibaut, C. Guerreiro, and L. A. Mulard, "Concise synthesis of di- and trisaccharides related to the O-antigens from Shigella flexneri serotypes 6 and 6a, based on late stage mono-O-acetylation and/or site-selective oxidation," *Tetrahedron*, vol. 69, no. 48, pp. 10337–10350, 2013.
[39] Z. B. Szabó, A. Borbás, I. Bajza, and A. Lipták, "Synthesis of fully protected α-l-fucopyranosyl-(1→2)-β-d- galactopyranosides with a single free hydroxy group at position 2', 3' or 4' using O-(2-naphthyl)methyl (NAP) ether as a temporary protecting group," *Tetrahedron Asymmetry*, vol. 16, no. 1, pp. 83–95, 2005.
[40] Y. Shao *et al.*, "Advances in methods and algorithms in a modern quantum chemistry program package," *Phys. Chem. Chem. Phys.*, vol. 8, no. 27, pp. 3172–3191, 2006.
[41] S. Grimme, "Semiempirical GGA-type density functional constructed with a long-range dispersion correction," *J. Comput. Chem.*, vol. 27, no. 15, pp. 1787–1799, Nov. 2006.
[42] R. F. Ribeiro, A. V. Marenich, C. J. Cramer, and D. G. Truhlar, "Use of solution-phase vibrational frequencies in continuum models for the free energy of

solvation," *J. Phys. Chem. B*, vol. 115, no. 49, pp. 14556–14562, 2011.
[43] Y. Garcia, F. Schoenebeck, C. Y. Legault, C. A. Merlic, and K. N. Houk, "Theoretical Bond Dissociation Energies of Halo-Heterocycles: Trends and Relationships to Regioselectivity in Palladium-Catalyzed Cross-Coupling Reactions," *J. Am. Chem. Soc.*, vol. 131, no. 18, pp. 6632–6639, May 2009.

Supporting information

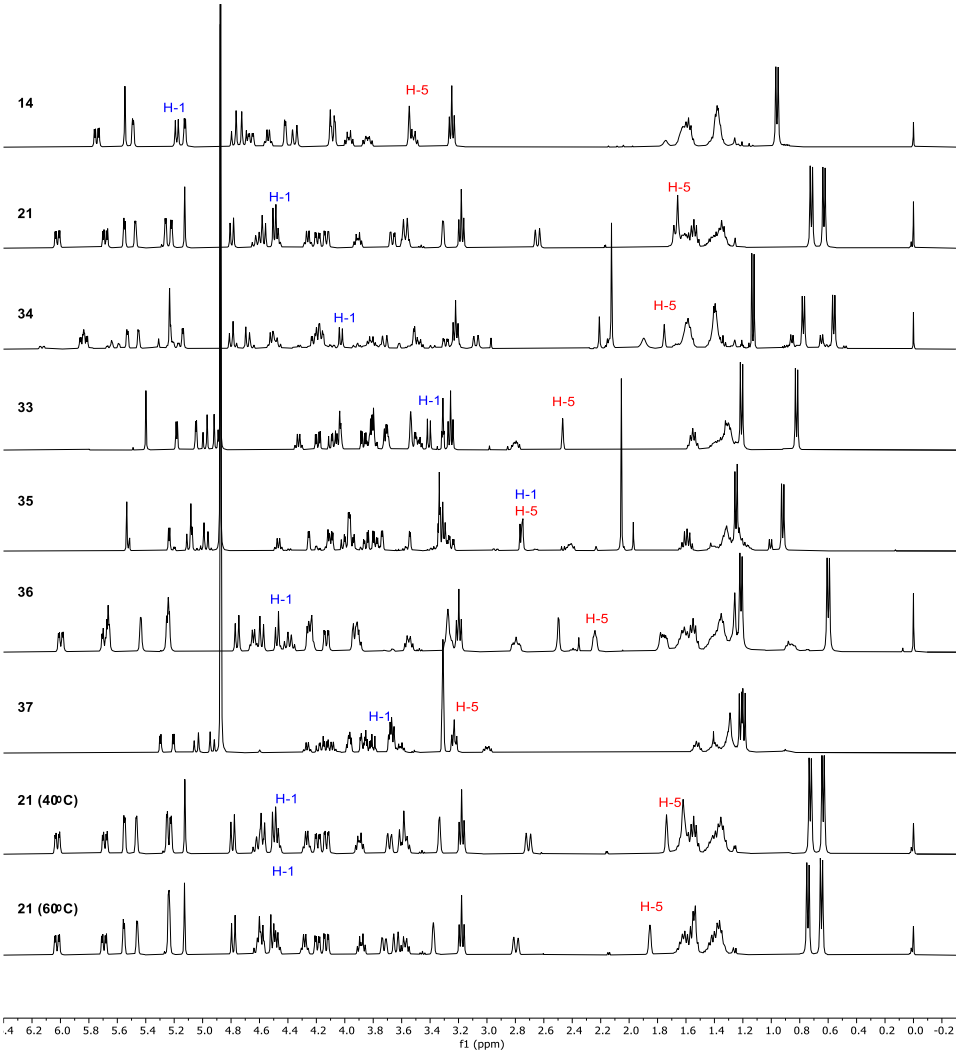
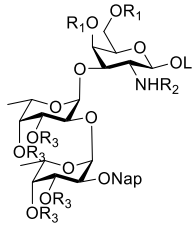


*Figure 7: Partial ^{1}H NMR spectra of various deprotecting stages of **21**. The anomeric proton of the GalNAc residue (H-1) is marked in blue and the H-5 of the GalNAc residue is marked in red. All spectra were recorded in $CDCl_3$ on a 400 MHz NMR at room temperature unless indicated otherwise.*

Table 2: Overview of protective groups present on the compounds whose ^{1}H NMRs are shown in Figure 7.



compound	R_1	R_2	R_3
21	>CHPh	TCA	Bz
34	>CHPh	Ac	Bz
33	>CHPh	TCA	H
35	>CHPh	Ac	H
36	H	TCA	Bz
37	H	H	H

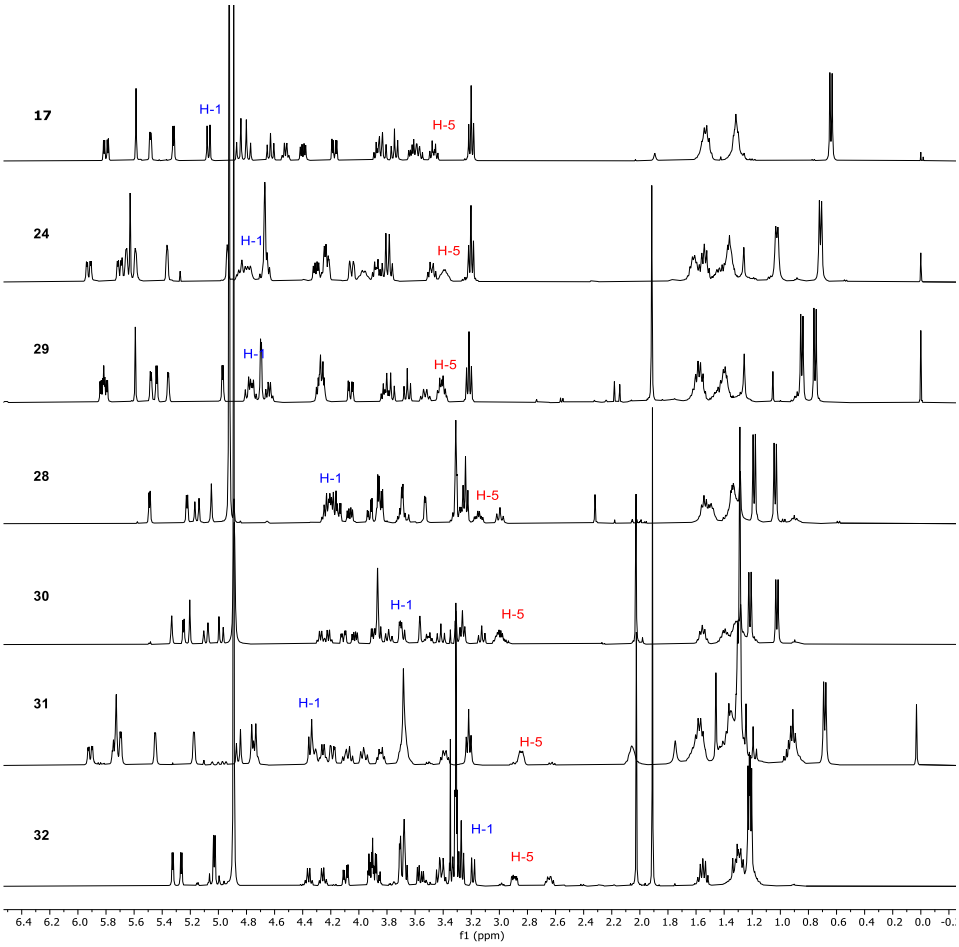
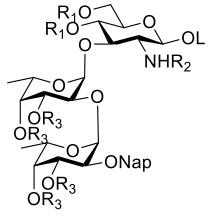


*Figure 8: Partial ^{1}H NMR spectra of various deprotecting stages of **24**. The anomeric proton of the GlcNAc residue (H-1) is marked in blue and the H-5 of the GlcNAc residue is marked in red. All spectra were recorded in $CDCl_3$ on a 400 MHz NMR at room temperature.*

Table 3: Overview of protective groups present on the compounds whose ^{1}H NMRs are shown in Figure 8.



compound	R_1	R_2	R_3
24	>CHPh	TCA	Bz
29	>CHPh	Ac	Bz
28	>CHPh	TCA	H
30	>CHPh	Ac	H
31	H	TCA	Bz
32	H	H	H