Synthesis and application of glycans unique to S. mansoni

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Synthesis and application of glycans unique to *S. Mansoni*

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 1 december 2020
klokke 11:15 uur

door

Michael Rene Harvey

geboren te Leeds in 1991

Promotiecomissie

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Printed by: GVO drukkers & vormgevers B.V.

ISBN: 978-94-6332-705-3

This is 10% luck, 20% skill,

15% concentrated power of will.

5% pleasure and 50% pain

-Fort Minor-

Table of contents

List of abbreviations

Ac	acetyl
ACN	acetonitrile
All	allyl
APT	attached proton test
aq.	aqueous
arom.	aromatic
AuNP	gold nanoparticle
BAIB	bis(acetoxy)iodobenzene
Bn	benzyl
BSA	bovine serum albumin
Bz	benzoyl
cat.	catalyst
CAA	circulating anodic antigen
CCA	circulating cathodic antigen
Cbz	benzyloxycarbamate
ClAc	2-chloroacetyl
C_q	quaternary carbon
CSA	camphorsulphonic acid
δ	chemical shift
d	doublet
DBU	1,8-diazabicyclo-[5.4.0]-undec-7-ene
DCE	1,2-dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-benzoquinone
DFT	density functional theory
DMAP	4-(N,N-dimethylamino) pyridine
DMF	N,N-dimethylformamide
DMP	2,2 dimethoxypropane
DMTST	dimethyl(methylthio) sulfonium triflate
DPS	diphenyl sulfoxide
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
DTBS	di-*tert*-butylsilyl
ELISA	enzyme-linked immunosorbent assay
eq.	equivalent(s)
ESI	electrospray ionization
Et	ethyl
et al.	*et alia* ‘and others’
Fuc	L-fucose

Fmoc	(9*H*-fluoren-9-yl) methoxycarbonyl
GalNAc	D-N-acetyl galactosamine
GlcA	D-glucuronic acid
GlcNAc	D-N-acetyl glucosamine
h	hour(s)
HFIP	1,1,1,3,3,3-hexafluoro-*iso*-propanol
HMBC	heteronuclear multiple bond correlation
HRMS	high resolution mass spectroscopy
HRP	horseradish peroxidase
J	*J*-coupling
KK	Kato-Katz
LC-MS	liquid chromatography mass spectrometry
LDN	GalNAc-β-(1-4)-GlcNAc
Lev	levulinoyl
m	multiplet
M	molar
mAb	monoclonal antibody
MBz	4-methylbenzoyl
Me	methyl
MS	molecular sieves
Nap	2-methylnaphthyl
NBS	N-bromosuccinimide
NIS	N-iodosuccinimide
NMR	nuclear magnetic resonance
OD	optical density
OTf	triflate
PDC	pyridinium dichromate
PE	petroleum ether
PG	Protective group
Ph	phenyl
Phth	phthalimide
ppm	parts per million
p-TSOH	*para*-toluenesulphonic acid
PZQ	Praziquantel
q	quartet
quant.	quantitative
RAM/PO	Rabbit Anti-Mouse IgG Peroxidase Conjugated
RFU	relative fluorescence units
RRV	relative reactivity value
RT	room temperature
s	singlet
S.	Schistosoma

SE	2-silanoethyl
sat.	saturated
t	triplet
TBAA	tetrabutylammonium acetate
TBABr	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
t-Bu	*tert*-butyl
TBDMS	*tert*-butyldimethylsiliyl
TCA	2,2,2-trichloro acetyl
TCE	2,2,2-trichloroethyl
TEMPO	2,2,6,6-tetramethylpiperidinyl-oxyl
TES	triethylsilane
TFA	trifluoro acetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMB	3,3',5,5'-tetramethylbenzidine
TMS	trimethylsilyl
Tol	toluene
Troc	trichloroethyl carbamate
TTBP	2,4,6-tri-*tert*-butylpyrimidine
UV	ultraviolet
WHO	World Health Organization

1

Introduction and outline

General

Schistosomiasis is an acute and chronic disease caused by blood dwelling parasitic trematodes of the genus *Schistosoma*.[1] The World Health Organization (WHO) classified it as the second most socioeconomically devastating parasitic disease, second only to malaria. The number of infections is estimated at 249 million people per year, of which 200.000 end in loss of life.[2]–[5] Schistosomiasis mainly occurs in developing countries especially in areas without access to clean drinking water and inadequate sanitation (Figure 1).[6] The disease is contracted by exposure to schistosome infected water.

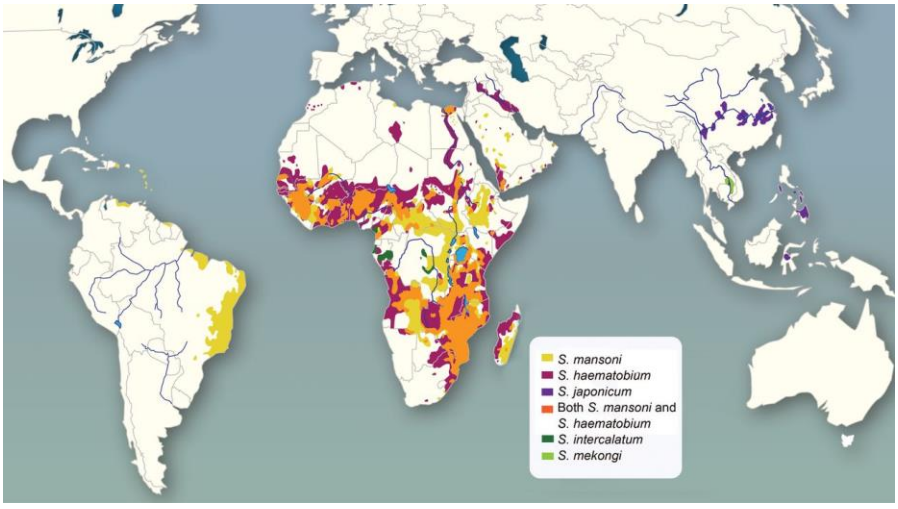


Figure 1: Global distribution of schistosomiasis[7]

Life cycle of schistosoma

The life cycle of *S. mansoni*, depicted in Figure 2, consists of two main stages: the water stage (1-5) and the host stage (6-10). Eggs are secreted by the host and hatch when they come into contact with fresh water.[8] The eggs release miracidia, which in turn infect fresh water snails. The miracidia reproduce asexually forming sporocysts in the snail. When these sporocysts burst they release cerceriae in the water.[3] These cerceriae are able to penetrate the skin of a host by releasing secretion vessels from their acetabular glands. These vessels contain a variety of proteins such as serpins, elastases, paramyosin and glutathione-S-transferases.[9]–[11] While entering the host, the cerceriae undergo a variety of changes. They lose their tail and become schistosomulae, that are able to circulate in the bloodstream and migrate to the lung capillaries.[12] In particular at this stage they shed their thick carbohydrate based layer (glycocalyx), which seems to be crucial for the evasion of the hosts' immune system.[9] After nine days of incubation in the lung capillaries, the schistosomulae migrate to the hepatic portal system, where they mature into schistosomes, while feeding on red blood cells. After they have fully matured a male and female schistosome permanently pair up and migrate to the mesenteric venules of the bowels. The paired schistosomes release approximately 200 eggs per day, most of which are excreted via the stool, some however, get trapped into various host organs causing inflammation, granuloma and fibrosis.[13]

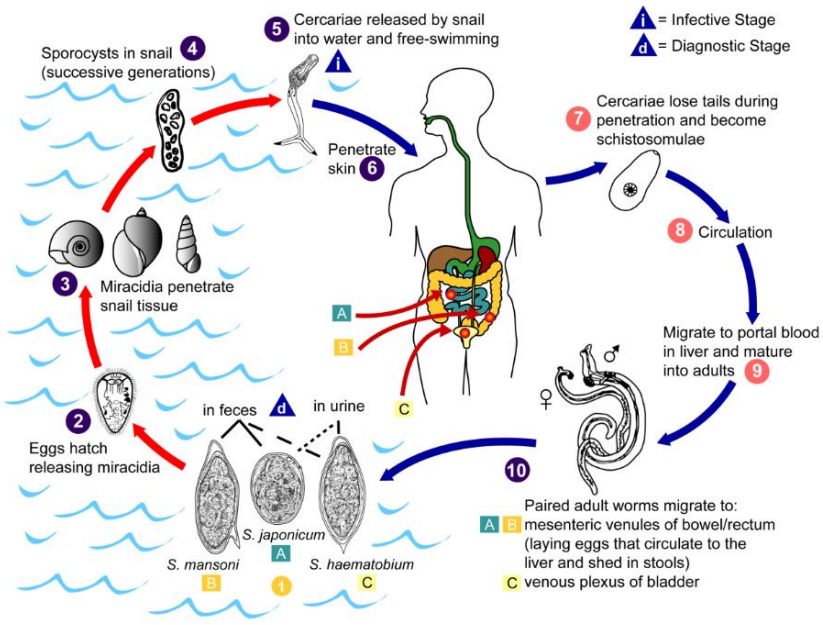


Figure 2: The life cycle of S. Mansoni.[14]

Schistosomiasis

Schistosomiasis is divided into two major classes: acute and chronic schistosomiasis. Acute schistosomiasis causes Katayama fever and occurs between six and eight weeks after the initial infection. When infected for the first time symptoms include fever, headache, abdominal pain and eosinophilia, while for people from endemic areas this stage can be asymptomatic.[15], [16] This phenomenon is explained by prior exposure *in utero* in schistosoma infected mothers. [17], [18]

Chronic schistosomiasis is caused by granulomatous inflammation against schistosome eggs that have become lodged in various organs and tissues. This inflammatory response can then cause tissue lesions in those organs. The symptoms of this stage of the disease are ulceration, abscess formation, chronical diarrhea and per rectal bleeding.[19]

Present treatment

The common treatment for schistosomiasis is Praziquantel (PZQ), which has been in use since the 1980's. It is sold in tablet form as a racemic mixture of which only the R enantiomer (see Figure 3-A) is active against schistosomiasis. The S enantiomer has little to no anti helminth activity, but instead is the cause of many of the side effects of PZQ.[20]

Although it has been used for over thirty years its precise mode of action is still not fully understood. It has been observed that PZQ causes a rapid intake of Ca^{2+}, which in turn causes paralytic muscular contraction, which dislodges the schistosome from the host.[21] Angelucci *et al.* proposed that PZQ acts as an adenosine analogue (see Figure 3-B and C), since they observed that PZQ blocked the uptake of purines, which in some vertebrates modulate the uptake of Ca^{2+} ions.[22] Besides these Ca^{2+} induced contractions, the outer protective layer of the worm, the tegument, is disrupted as well by PZQ. As a result of this the nematode is no longer able to resist digestion by the host.[23], [24] Notably, despite being used for well over thirty years very few PZQ-resistant strains have emerged.[25], [26]

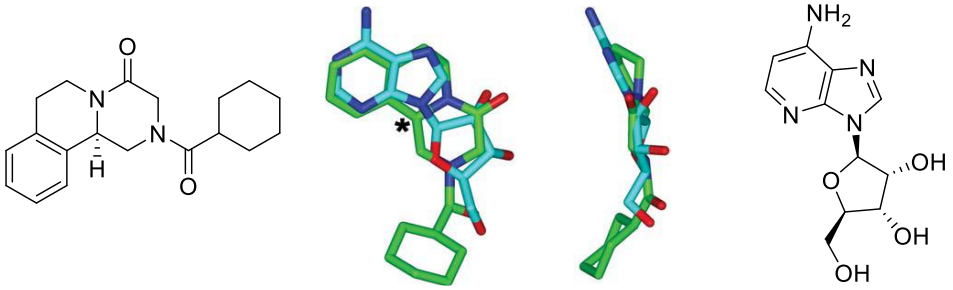


Figure 3: A: Structure of R-praziquantel, B: superimposition of praziquantel and adenosine, the asterisk marks the chiral centre of PZQ, C; structure of adenosine[22]

Despite its effectiveness, the use of PZQ is not without problems, as the S-enantiomer causes side effects such as diarrhea, vomiting, dizziness, vertigo, and headaches. The severity of these symptoms depends on the severity of the infection.[27] Unfortunately, there is no cost effective synthesis for enantiomerically pure PZQ at this time. Besides these negative side effects the drug has two more drawbacks. The first being that PZQ only targets adult worms, so often a second treatment is needed.[28] The second drawback is that PZQ does not offer immunity to protect against a future infection, making vaccination an attractive option. At the time of writing two possible vaccine candidates are being tested in clinical trials, however no results have been made available about these trials.[29], [30]

Diagnosis

There are various tools used for the diagnosis of schistosomiasis. The most frequently used and easiest to perform test is the WHO recommended Kato-Katz (KK) thick stool smear technique, which is based on the egg count in a stool sample.[31] Although the KK test is easy to perform it lacks in its accuracy. The accuracy of the KK test is good when the worm load in the host is high, but in case of a low worm load the accuracy drops as the egg secretion is not as high.[32] Since the schistosome egg excretion varies on a day

to day basis, the sensitivity of the KK test drops even further.[33], [34] The low accuracy at low worm load makes it difficult to assess if another PZQ treatment is required.[35] Another problem is that the eggs tend to clump together and therefore may not be present in the sample used for the KK test.

While the previous method describes a direct parasitological approach, much research has been conducted to develop an immunological diagnosis. Schistosomes, like all organisms, express a large range of complex glycans, some of which are unique to these species and these can therefore be used as an analytical marker and possibly as a target for a cure. Some examples of glycans of the glycocalyx of *S. mansoni* that so far appear unique to the *Schistosoma* genus are shown in Figure 4.[36]

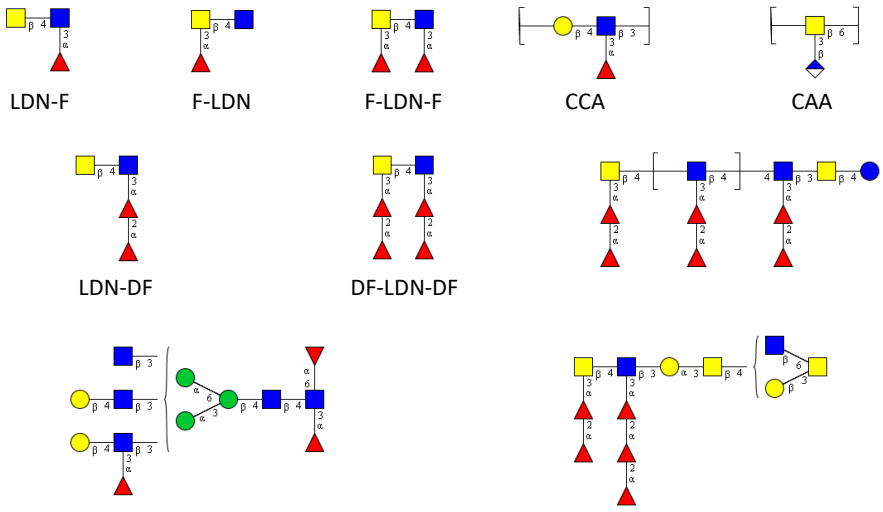


Figure 4: biological representation of glycans present in the glycocalys of S. Mansoni. Yellow square: GalNAc, yellow circle: Gal, blue square: GlcNAc, blue circle: Glc, green circle: Man, red triangle: Fuc, blue and white diamond: GlcA. LDN: LacDiNAc, F: fucosyl, DF: di-fucosyl, CCA: circulating cathodic antigen, CAA: circulating anodic antigen[37], [38]

The two most important glycans that are used for diagnosing schistosomiasis are the circulating anodic antigen (CAA), a polymer of 6-[GlcA-β-(1-3)-GalNAc-β-(1-] and the circulating cathodic antigen (CCA), a polymer of Lewis X.[39], [40] Another unique structural element are the L-fucose α-(1→2)-L-fucose chains present in many antigens. These fucose chains are present in every developmental stage of *S. mansoni*, unlike CAA and CCA, which are only present in adult worms. Several antibodies against multi-fucosylated glycans have been discovered, but the specific target of most of them is as of yet unknown. [41]

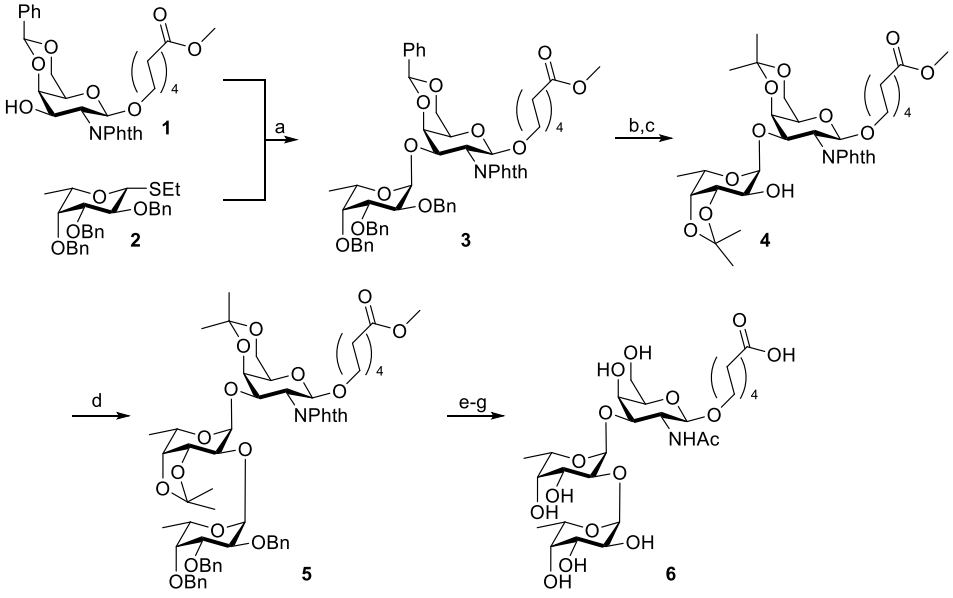
Diagnostic tests have been designed to detect anti-schistosomal antibodies or circulating schistosomal antigens in plasma, urine, serum or sputum.[7] Generally the antigens used in antibody detection assays are cercarial, egg or worm extracts containing multiple components, resulting in a lower specificity. To generate more specific diagnostic tools pure antigen could be used.[42], [43] As most of the biosynthesis enzymes that make these specific antigens are as of yet unknown, synthetic chemistry can provide the right tools to achieve this. Below, the syntheses of schistosome antigens reported to date are described.

Chemically synthesized antigens of *S. mansoni*

Several sugars of the *S. mansoni* glycocalyx have been synthesized by different groups. Most of them focused on the introduction of one or more of the α-(1-2)-fucosyl moieties. Short multi-fucosylated sugars have been synthesized by Hindsgaul and coworkers and Van Roon *et al*.[44], [45] Their syntheses are described in Schemes 1-3. Hindsgaul and co-workers generated a di-fucosyl galactosamine trisaccharide, while van Roon *et al.* assembled oligofucose chains appended to a glucosamine moiety. Larger multi-fucosylated glycans have been synthesized by Kanaya *et al*.[46] These glycans have a GalNAc-β-(1→4)-GlcNAc moiety (LacdiNAc or LDN) as the backbone. Vliegenthart and coworkers focused on a set of CAA fragments instead of the α-(1-2)-fucosides and synthesized up to the pentamer level of CAA (Scheme 6).[47]–[49] The key elements of these syntheses are discussed below in Schemes 1-6.

Hindsgaul and coworkers synthesized GalNAc-Fuc-Fuc trisaccharide **6** from appropriately protected building blocks **1** and **2** (Scheme 1).[44] Galactosamine **1** was fucosylated with donor **2** in presence of dimethyl(methylthio) sulfonium triflate (DMTST) and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) giving disaccharide **3** in 80% yield.[50], [51] Disaccharide **3** was then subjected to palladium catalyzed hydrogenation to remove all benzyl ethers as well as the benzylidene acetal. The generated pentol was then protected with two *iso*-propylidene groups, leaving the fucosyl C2 hydroxyl unprotected (**4**). The characteristic α-(1-2)-fucose was introduced by reacting donor **2** with disaccharide **4** using AgOTf/bromine as the activator system.[52] This furnished trisaccharide **5** in 61% yield. Trisaccharide **5** was deprotected by first removing the *iso*-propylidene groups and hydrolysis of the methyl ester by acid catalyzed hydrolysis using 60% aqueous acetic acid at 50°C for 30 minutes. Prolonged exposure to these conditions led to decomposition of the formed trisaccharide. Next, the amine was liberated by treatment with hydrazine, after which the amine was selectively acetylated with Ac_2O in methanol. Finally, the benzyls were removed by catalytic hydrogenation giving **6** in 42% yield over three steps. The linker was equipped with a carboxylate functionality to enable conjugation to carrier proteins, by using peptide coupling reagents.

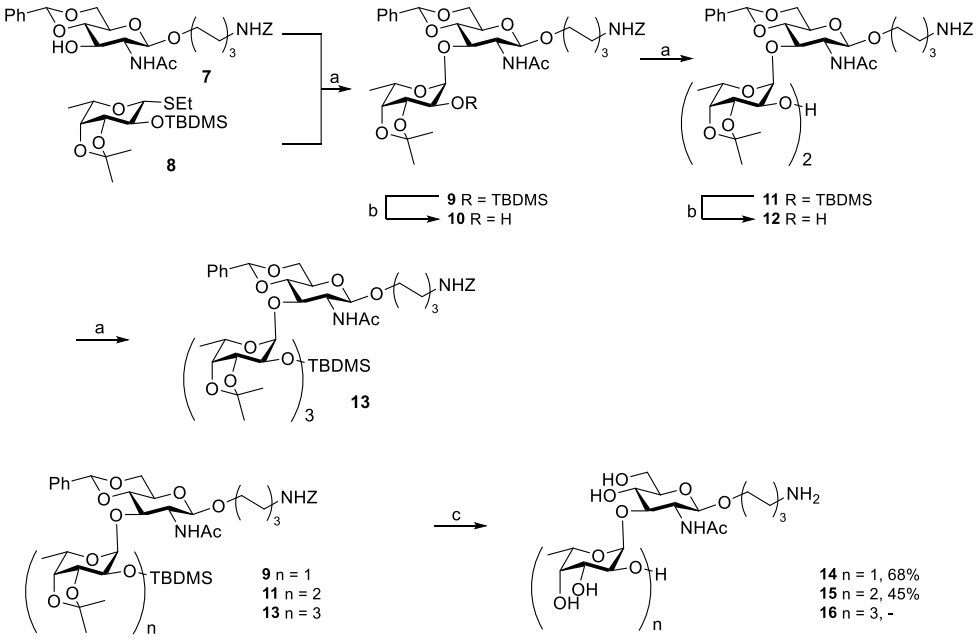
*Scheme 1: Synthesis of GalNAc-Fuc-Fuc **6** antigen by Hindsgaul and coworkers.[44]*



Reagents and conditions: **a**: DMTST, DTBMP, MS (4Å), DCM, 80%, **b**: Pd/C, H_2, EtOH, **c**: *p*-TsOH (cat.), DMP, ACN/DMF, **d**: AgOTf, Br_2, DTBMP, MS (4Å), DCM, 61%, **e**: 60% AcOH, 50˚C, **f**: i) N_2H_4 ·AcOH, MeOH, 65˚C, ii) Ac_2O, MeOH, **g**: Pd/C, H_2, MeOH, 42%.

Van Roon *et al.* synthesized a small set of fucosylated GlcNAc oligosaccharides (Scheme 2).[45] In these syntheses building block **8** was used as their fucosyl donor, which was orthogonally protected at the C2-O with a *tert*-butyldimethylsilyl (TBDMS) ether. In order to obtain disaccharide **9**, fucosyl donor **8** and glucosamine acceptor **7** were condensed using $CuBr_2$ with tetrabutylammonium bromide (TBABr) in a 1:1 mixture of DCM/DMF to obtain α-selectivity (α/β = 12/1).[53]. Disaccharide **9** was obtained in an excellent yield of 85% in a fully stereoselective manner. The TBDMS group was removed selectively by tetrabutylammonium fluoride (TBAF) in THF, which gave rise to disaccharide acceptor **10**. The fucosyl chain was elongated using the same coupling procedure as before resulting in trisaccharide **11** in 52% yield. The TBDMS ether on **11** was removed and the resulting acceptor **12** was extended in a similar manner as for **7** and **10**, giving tetrasaccharide **13** in 17% yield. The substantial difference in yield was attributed to steric hindrance of the bulky *iso*-propylidene groups on the acceptor. Besides the yield, the stereoselectivity dropped as well (α/β = 5/2). The protected glycans **9**, **11** and **13** were deprotected by treatment with 60% aqueous acetic acid, removing all the acetals, followed by catalytic hydrogenation. The di- and trisaccharide **14** and **15** were obtained in 68% and 45%, respectively. Unfortunately, tetrasaccharide **16** could not be obtained, as the terminal fucose was cleaved during hydrolysis of the *iso*-propylidene and benzylidene acetals.

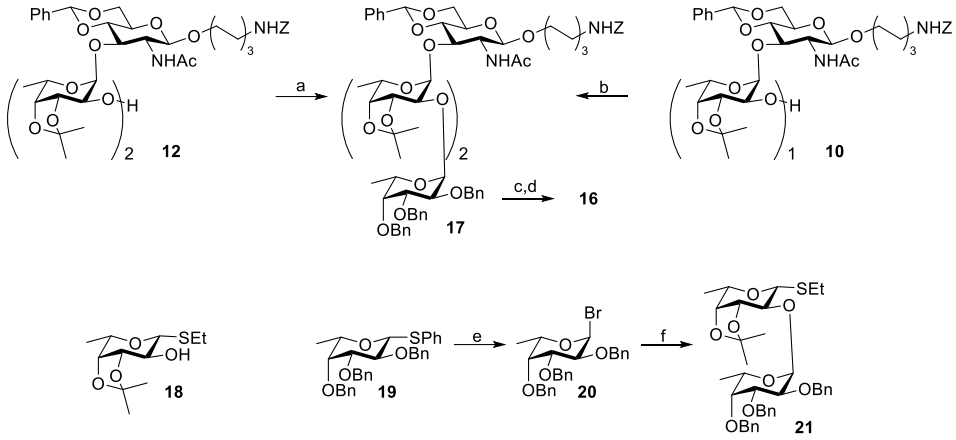
*Scheme 2: Synthesis of GlcNAc-Fuc-Fuc antigen **15** by van Roon et al.[45]*



Reagents and conditions: **a**: $CuBr_2$, TBABr, DMF:DCM (1:1), **9** 85%, **11** 52% (α/β, 12:1), **13** 17% (α/β, 5:2), **b**: TBAF, THF, 88-93%, **c**: i) 60% AcOH, 50 °C, ii) H_2, Pd/C, EtOH, H_2O, HCl, **14** 68%, **15** 45%, **15** 0%.

To prevent decomposition of the tetrasaccharide, Van Roon *et al.* turned to benzylated fucosyl **19** as a donor (Scheme 3). Donor **19** was successfully condensed with trisaccharide acceptor **12** using the same protocol as used for the synthesis of **9**, **11** and **13**, resulting in tetrasaccharide **17** in 17% yield. Although this time tetrasaccharide **17** could be obtained after acidic hydrolysis and catalytic hydrogenation (40%, over two steps), the yield of the glycosylation reaction was still poor. In order to increase the yield, they resorted to a [2 + 2] block coupling. To this end donor **19** was converted to the anomeric bromide **20** using bromine in DCM and then condensed with acceptor **18**, to give disaccharide donor **21** in 68% yield. Acceptor **10** and donor **21** were coupled under the activation of $CuBr_2$ as described above, which gave tetrasaccharide **17** in 36% yield. Notably, the stereoselectivity of this glycosylation dropped significantly, as 16% of undesired β-linked product was isolated as well.

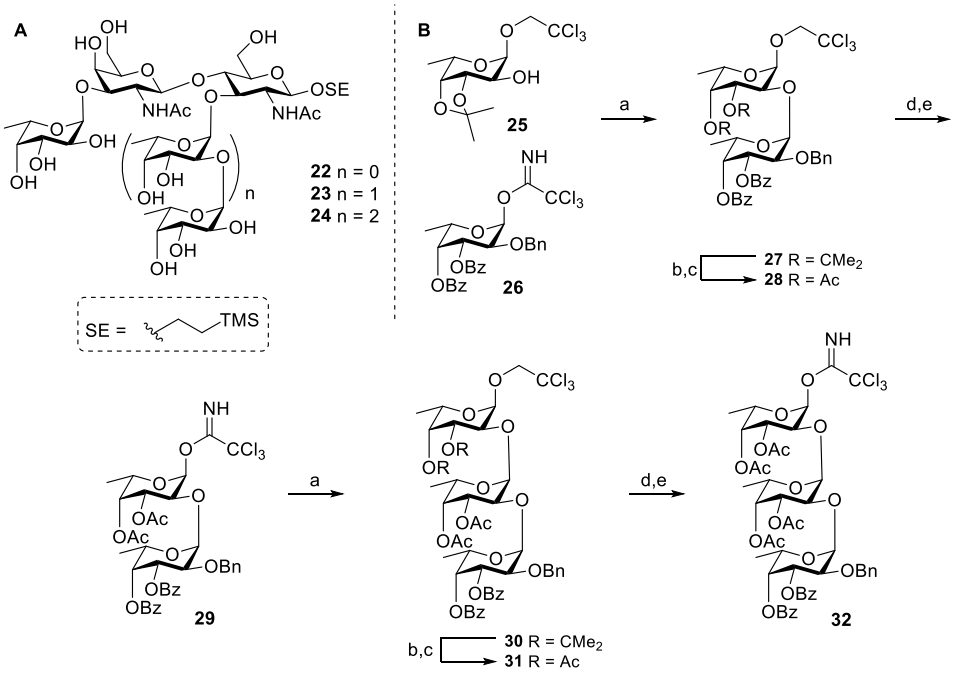
*Scheme 3: Alternative synthesis of GlcNAc-Fuc-Fuc-Fuc antigen **16** by van Roon et al.*[45]



Reagents and conditions: **a**: **19**, $CuBr_2$, TBABr, DMF:DCM (1:1), 16%, **b**: **21**,$CuBr_2$, TBABr, DMF:DCM (1:1), 36% (16% of the β-isomer), **c**: 60% AcOH, 50 °C, 57% **d**: H_2, Pd/C, EtOH, H_2O, HCl, 70% **e**: Br_2, DCM, 0°C, **f**: **18,** TBABr, DMF:DCM (1:1), 68%.

Kanaya *et al*. have synthesized several fucosylated *N*-acetylgalactosamine-β-(1-4)-*N*-acetylglucosamine (LDN) disaccharide structures, examples of which are highlighted in Scheme 4Scheme 5.[46] The α-(1-2)-fucosyl chains were introduced *en block* to the LDN backbone using donors **26**, **29** or **32**, which were synthesized from the non-reducing end (Scheme 4B). Fucosyl acceptor **25** was orthogonally protected with a trichloroethyl (TCE) group on the anomeric center and an *iso*-propylidene on the C3- and C4-hydroxyls. [54] Imidate donor **26** was chosen over thiophenyl donor **19** as that was prone to form a fucose-succinimide complex upon activation with NIS/TfOH.[55] Imidate donor **26** was condensed with *iso*-propylidene bearing fucosyl acceptor **25** to give disaccharide **27** in 75% yield. The *iso*-propylidene group was removed selectively by hydrolysis in 80% acetic acid, followed by acetylation of the obtained diol with Ac_2O and pyridine, which gave disaccharide **28** in 86% yield. The TCE group was then removed selectively with Zinc in AcOH, and the resulting hemi-acetal was treated with a catalytic amount of DBU and trichloroacetonitrile in DCM to give α-imidate disaccharide **29** in 77% yield over two steps. This disaccharide donor was in turn condensed with acceptor **25** giving trisaccharide **30** in 80% yield. The *iso*-propylidene in **30** was removed and exchanged for two acetyl esters as described above giving **31**. Trisaccharide **31** was transformed into imidate donor **32** by the same procedure as described for the conversion of **28** into donor **29**.

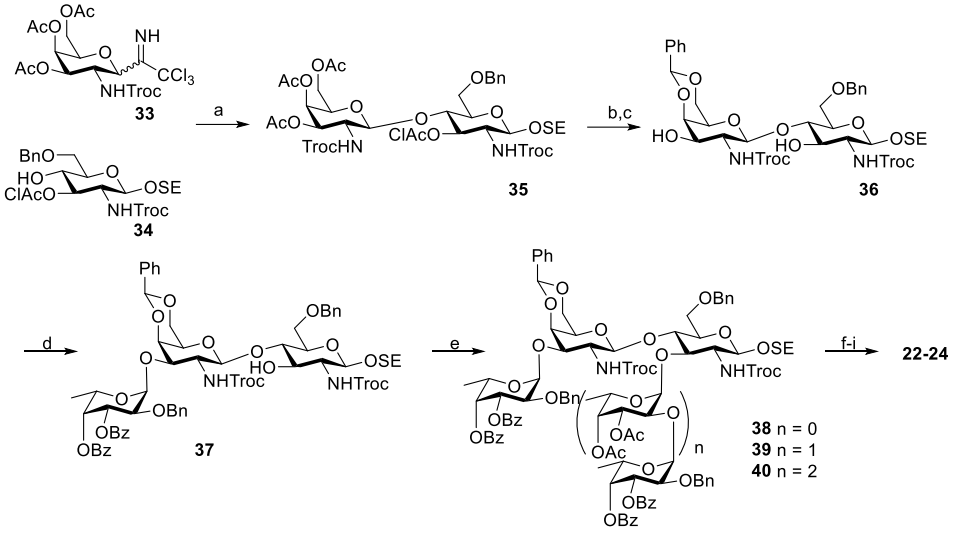
*Scheme 4: **A**: Synthesized structures, **B**: Synthesis of fucosyl chain donors **29** and **32**.*[46]



Reagents and conditions: **a**: TMSOTf (cat.), MS (4Å), DCM, -40˚C, **27** 75%, **30** 80%, **b**: 80% AcOH, 50˚C, **c**: Ac_2O, pyr., **28** 86%, **31** 63%, **d**: Zn, AcOH, 55 ˚C, **e**: DBU, CCl_3CN, DCM, -20 ˚C, **29** 77%, **32** 78%.

The LDN backbone was synthesized by condensing galactosamine imidate donor **33** with glucosamine acceptor **34**, which gave disaccharide **35** in 79% (*Scheme **5***). The acetyl and chloroacetyl groups were hydrolyzed by guanidinium nitrate and sodium methoxide in a mixture of methanol and chloroform. Subsequently, the C'6- and C'4-OH were selectively protected with a benzylidene group to give **36** with both the C3- and C'3-hydroxyls free.[56] The C'3-OH was selectively fucosylated using donor **26**, which gave rise to trisaccharide **37**. Next, the α-(1-2)-fucosyl chain on the C3-OH was introduced by condensing **37** with either **26**, **29** or **32**, which resulted in the isolation of **38**, **39** and **40** in yields of 78%, 67% and 43%, respectively. The LDN fragments **38**-**40** were deprotected in three steps. First the NHTroc group was changed into an acetamide by liberating the amines with zinc, followed by acetylation of the freed amines. Next, the benzylidene acetal and benzyl ethers were removed by catalytic hydrogenation. For ease of purification the glycans were fully acetylated, purified and de-acetylated using NaOMe in methanol, giving **22**, **23** and **24** in a yield of 23%, 32% and 35%, respectively.

*Scheme 5: Synthesis of fucosylated fragments **22**-**24**.[46]*

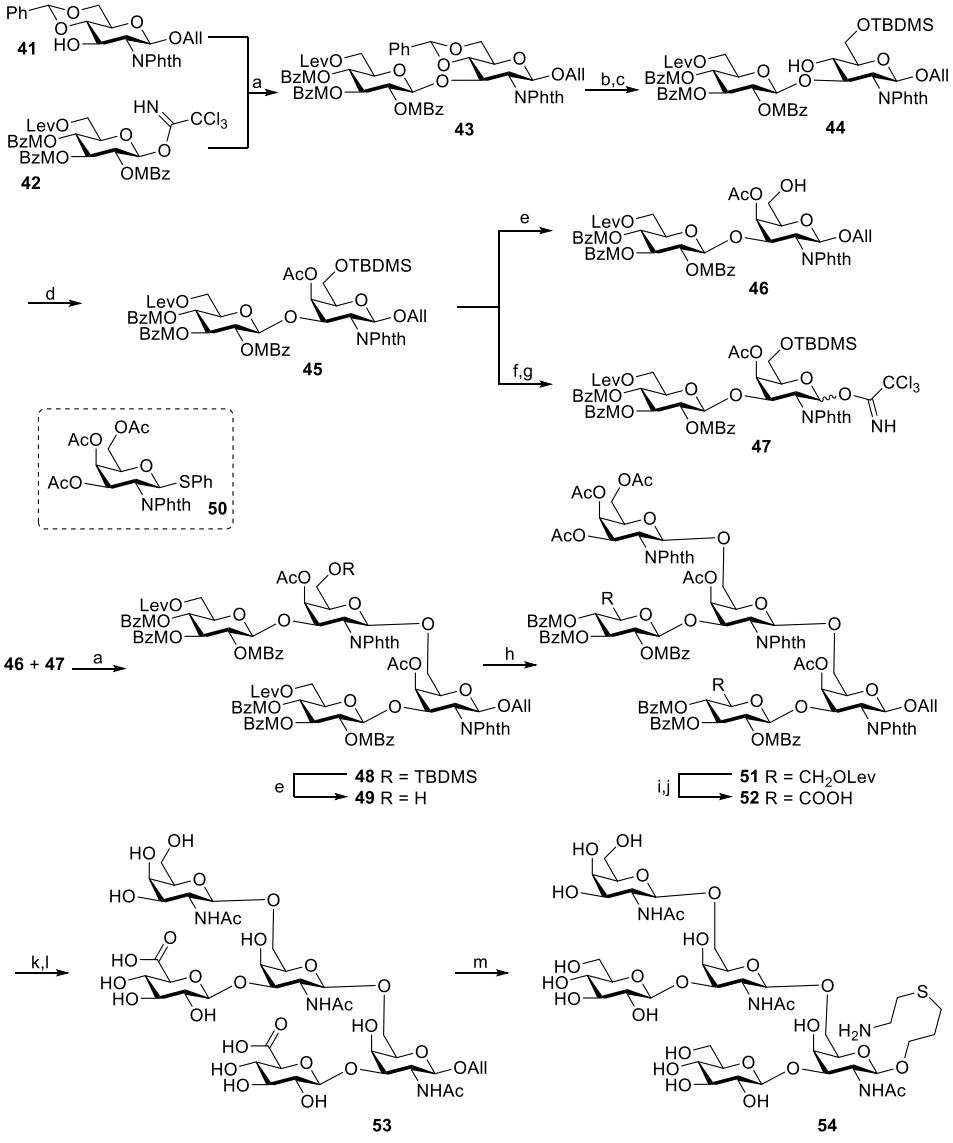


Reagents and conditions: **a**: TMSOTf (cat.), MS (4Å), DCM, 79%, **b**: guanidinium nitrate, NaOMe, MeOH/$CHCl_3$, **c**: benzaldehyde dimethyl acetal, $NaHSO_4 \cdot SiO_2$, ACN, 58% (2 steps), **d**: **37**, TMSOTf (cat.), MS (4Å), DCM, -40 ˚C, 66%, **e**: TMSOTf (cat.), MS (4Å), DCM, -40 ˚C , **38** 78%, **39** 67%, **40** 43%, **f** Zn, Ac_2O, AcOH, **g**: Pd/C, H_2, MeOH, **h**: Ac_2O, pyr., **i**: NaOMe, MeOH, **22** 23%, **23** 32%, **24** 35%.

The structure of CAA was first determined in 1994 by Vliegenthart and coworkers. Using NMR spectroscopy they proved that CAA consisted of a polymer composed of (→6)-[glcA-β-(1-3)]galNAc-(1→) repeats.[39] The same group synthesized a small set of CAA fragments.[47]–[49] These CAA molecules were conjugated to bovine serum albumin (BSA) in order to discover the optimal antigen. Their studies consisting of ELISA's and SPR's showed that the di- and tetrasaccharide CAA fragments were recognized best by several anti CAA monoclonal antibodies. There were, however, a lot of CAA antibodies that did bind with the native polymer but did not bind to any of the synthesized fragments, indicating that synthesis of bigger fragments was required.[57]–[59]

A brief overview of the synthesis of the CAA fragments by Vliegenthart and coworkers is shown in Scheme 6. They started from D-glucose and D-glucosamine, to synthesize both acceptor **41** and donor **42**, respectively. D-glucosamine was chosen over D-galactosamine as the starting material as it was significantly cheaper. The glucosamine moiety could be inverted to the corresponding galactosamine at the disaccharide stage. Their synthesis uses a late state oxidation, as a glucuronic acid donor was considered to be too unreactive for usage in glycosylation reactions.[60]

*Scheme 6: Synthesis of CAA pentamer **53** by Vliegenthart and co-workers.[47]–[49]*



Reagents and conditions: **a**: TMSOTf (cat.), MS (4Å), DCM, **43** 89%, **52** 73% **b**:TFA, DCM/H_2O, 98%, **c**: TBDMSCl, pyr, 96%, **d**: i) Tf_2O, pyr, DCM, 0 ˚C, ii) TBAA, DMF, 78%, **e**:*p*-TsOH (cat.), ACN, H_2O, **46** 91%, **49** 82% **f**:i) $(PPH_3)_4RhCl$, EtOH, ii) NIS, DCM, H_2O, **g**: CCl_3CN, DBU, DCM, 71% (two steps), **h**: **50**, NIS, AgOTf, MS (4Å), tol, 66%, **i**: N_2H_4·OAc, EtOH, Tol, 92%, **j**: PDC, MS (4Å), DCM, 73%, **k**: $MeNH_2$, EtOH, **l**: Ac_2O, MeOH, 0 ˚C, 65%, **m**: cysteamine hydrochloride, H_2O, UV, 80%.

Disaccharide **43** was synthesized by condensing donor **42** and acceptor **41** under influence of a catalytic amount of TMSOTf, which afforded disaccharide **43** in an excellent 89% yield. The benzylidene acetal in **43** was hydrolyzed under acidic conditions, followed

by selective protection of the released primary hydroxyl group with a TBDMS group, giving **44** in 95% yield. The free hydroxyl on the C4 position of glucosamine was inverted by triflation, followed by treatment with tetrabutylammonium acetate (TBAA) to give compound **45** in a 78% yield.[61], [62] Disaccharide **45** was then turned into either acceptor **46** or donor **47**. Acceptor **46** was obtained by selective removal of the TBDMS under mild acidic conditions to prevent any migration of the C4-acetyl to the C6-OH position. Donor **47** was synthesized by removal of the anomeric allyl group and subsequent imidoylation of the anomeric alcohol.[63]–[65] With both donor **47** and acceptor **46** in hand, tetrasaccharide **48** could be synthesized by utilizing the same coupling conditions as described for the formation of disaccharide **43**, which gave **48** in 73% yield. The TBDMS ether of **48** was removed under the same mild acidic conditions, as described for the transformation of **45** into **46**, resulting in tetrasaccharide **49**. Acceptor **49** and galactosamine donor **50** were condensed with the NIS/AgOTf activator couple to give pentasaccharide **51** in 66% yield. The primary hydroxyls on the glucose moieties were orthogonally deprotected by removing the levulinoyl (Lev) groups using hydrazine acetate, thus liberating them for oxidation. Initially, a Swern oxidation was performed followed by further oxidation by $NaClO_2$.[66] Unfortunately, this method did not give full conversion to the two carboxylic acids even after 96 hours of stirring. A different oxidation method using pyridinium dichromate (PDC) was explored next.[67], [68] PDC was able to oxidize both primary alcohols to the carboxylic acids in 4.5 hours in 73% yield. After successful oxidation **52** was deprotected with methylamine and subsequent acetylation of the free amines giving **53**.[69] The allyl was then functionalized by treatment with cysteamine hydrochloride in water under UV-light to obtain amine spacer containing pentasaccharide **54**.[70]

Outline of this thesis

The research in this Thesis describes the development of synthetic routes towards several glycans that are unique to *S. mansoni* and their use in diagnostic tools. **Chapter 2** describes the synthesis of Fuc-α-(1-2)-Fuc-α-(1-3)-GlcNAc and Fuc-α-(1-2)-Fuc-α-(1-3)-GalNAc, the shortest unique repeating units present in many glycans of the glycocalix of *S. mansoni*. A novel approach to the installment of the Fuc-α-(1-2)-Fuc was explored, together with an in depth NMR analysis and conformational analysis of the fully protected trisaccharides. The synthesized glycans were tested for biological activity by ELISA against monoclonal antibodies and sera of (un)-infected people. **Chapter 3** dives deeper into the synthesis of the α-(1-2)-Fuc linked oligofucosides, which are not anchored to any backbone. Two synthetic approaches toward these oligofucosides, comprising either elongation from the reducing end or from the non-reducing end, were explored and compared. Valuable insights on the applicability of the protective groups on the C3- and C4-hydroxyls of the fucosides, in terms of size and rigidity, were

discovered. Oligofucosides up to the tetramer were prepared and all glycans obtained were screened against monoclonal antibodies and sera of (un)-infected people. The synthesis of (di)-fucosylated GalNAc-β-(1-4)-GlcNAc disaccharides (GalNAc-β-(1-4)-GlcNAc, LacdiNAc or LDN), is described in **chapter 4**. A wide variety of orthogonal protective groups for both acceptor and donor glycosides were screened to attain the most favorable glycosylation reactions. Although F-LDN, LDN-F and F-LDN-F oligosaccharides were assembled successfully, the subsequent introduction of di-fucosyl side chains could not be achieved. **Chapter 5** describes the synthesis of CAA fragments. Two synthetic routes have been tested, one based on the use of monosaccharide glucose and galactose synthons, the other on isolating the desired disaccharide from Chondroitin A, a cheap and readily available biopolymer. A summary of this thesis and several future prospects are outlined in **chapter 6**.

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1

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

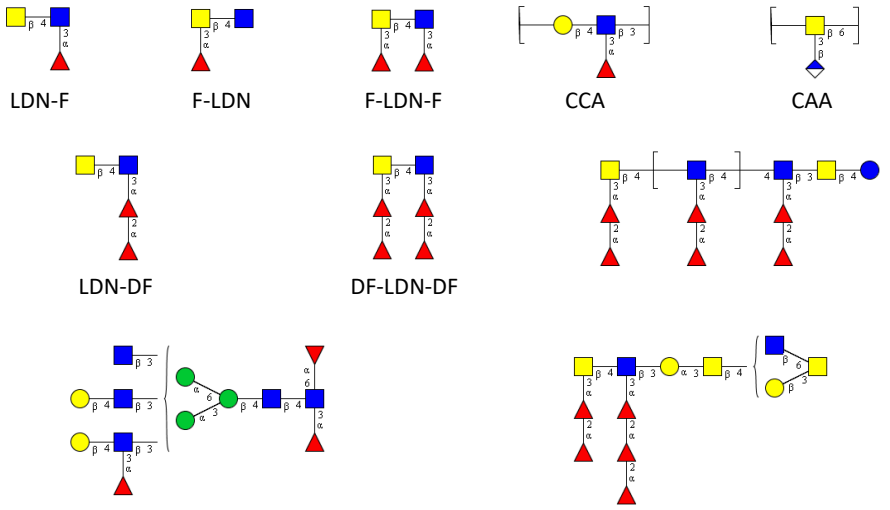


Figure 1: Overview of several observed glycans in S. mansoni. Red triangles: L-fucose, blue square: D-GlcNAc, yellow square: D-GalNAc, blue circle: D-Glucose, yellow circle: D-Galactose, green circle: D-mannose and blue/white diamond: D-Glucuronic acid. [9], [10]

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]

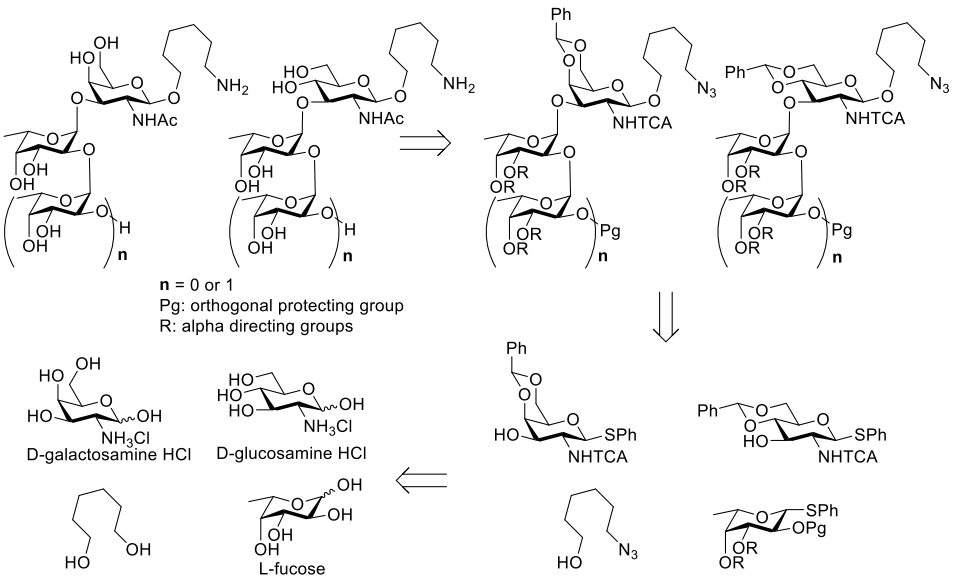


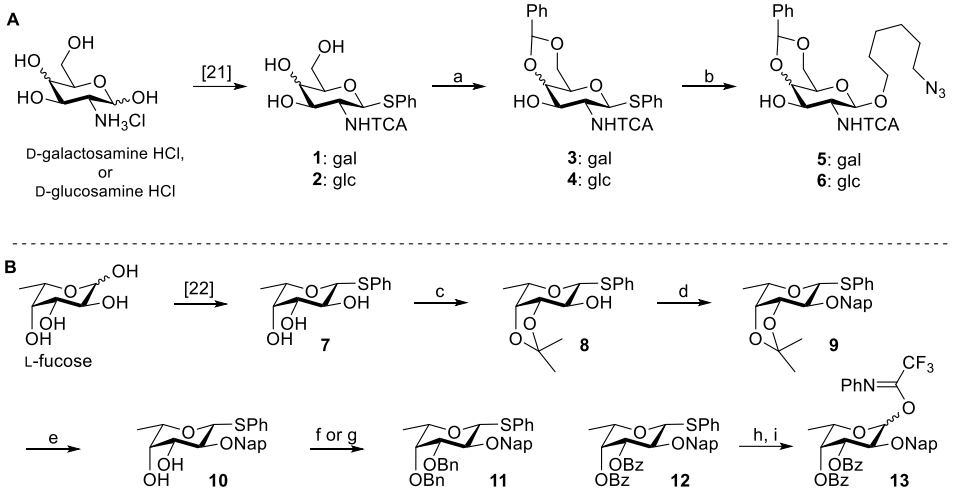
Figure 2: Retrosynthesis of unique glycan trisaccharides of S. mansoni functionalized with an amine linker for further conjugation.

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.

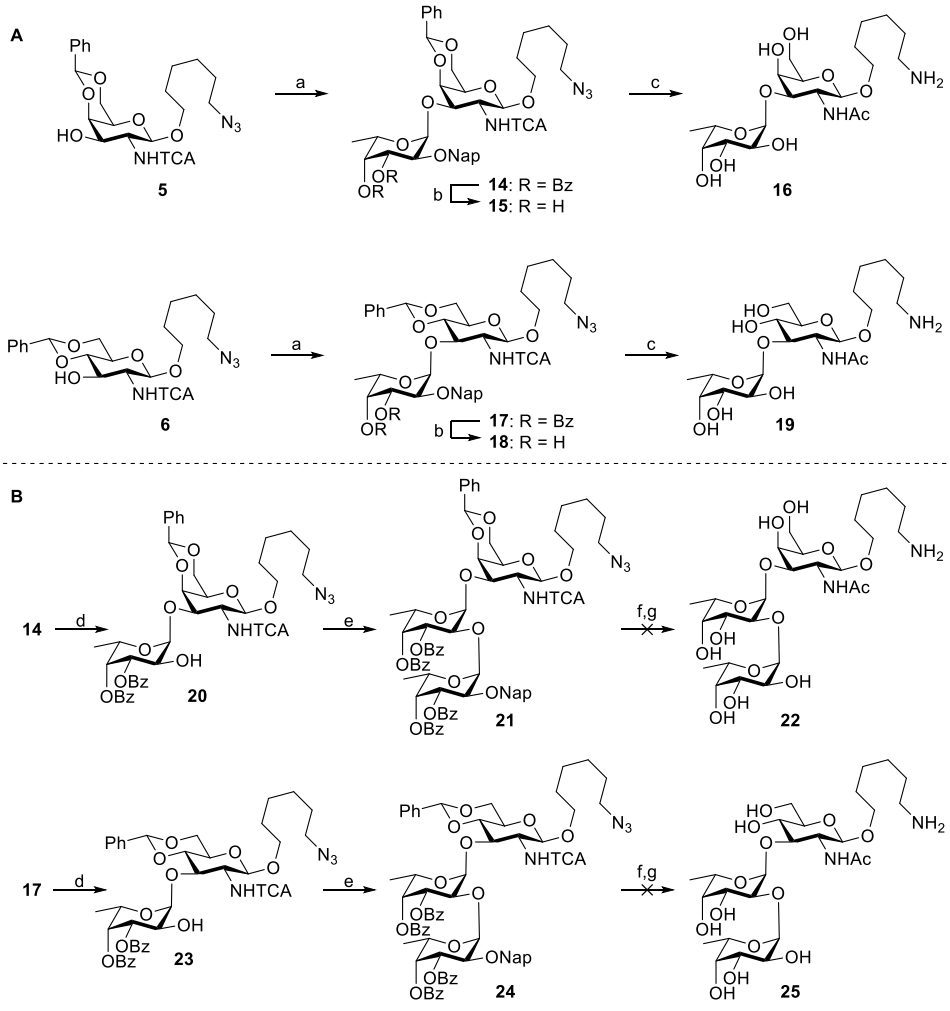
*Scheme 1: Synthesis of: **A**) GalNac and GlcNAc acceptors **5** and **6**, **B**) fucosyl donors **11-13***



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.*

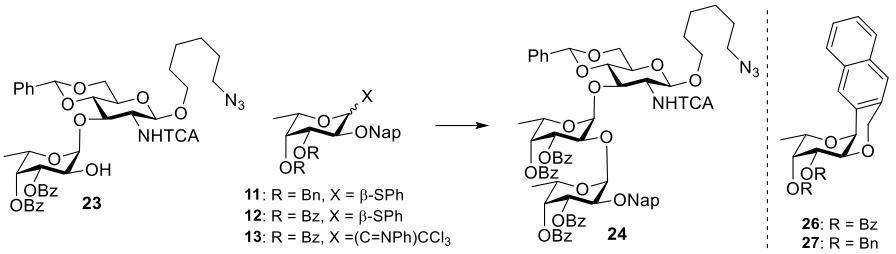


Reagents and conditions: **a**: **12**, NIS, TMSOTf, MS (3Å), DCM, -40˚C → -20˚C, **14** 76%, **17** 86%, **b**: NaOMe, MeOH, THF, **15** 60%, **18** 78% **c**: Pd/C, H_2, EtOH, **16** 67%, **19** 63%, **d**: DDQ, aqueous phosphate buffer (pH 7), DCM, 0˚C → RT, **20** 90%, **23** 88%, **e**: **12**,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

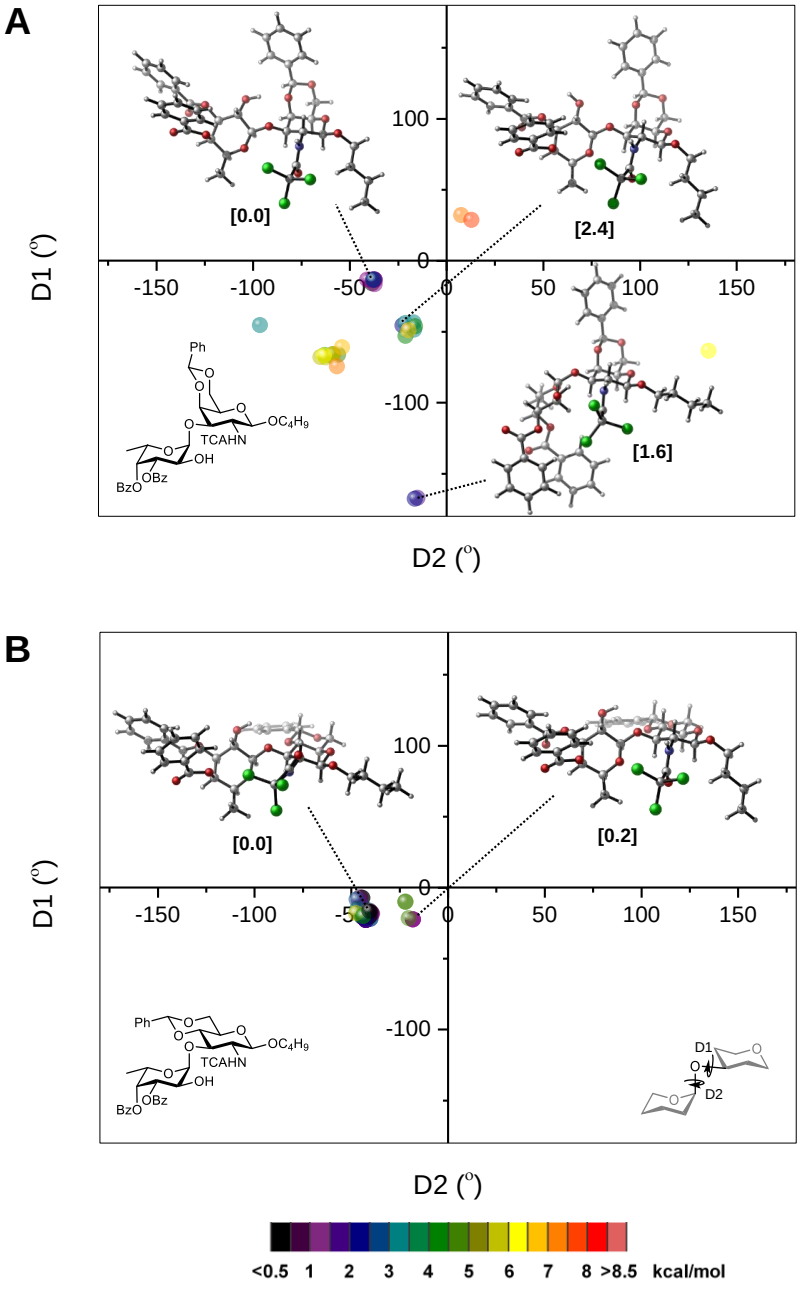


*Figure 3: Scatter plot of the computed conformational library of **20** and **23.** The X-axis shows the rotation around the C3-O3 bond of the galactosamine/glucosamine (D1) and the Y-axis shows the rotation around the C1-O1 of the fucose residue (D2).The dihedral angles D1 and D2 are plotted and the 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.

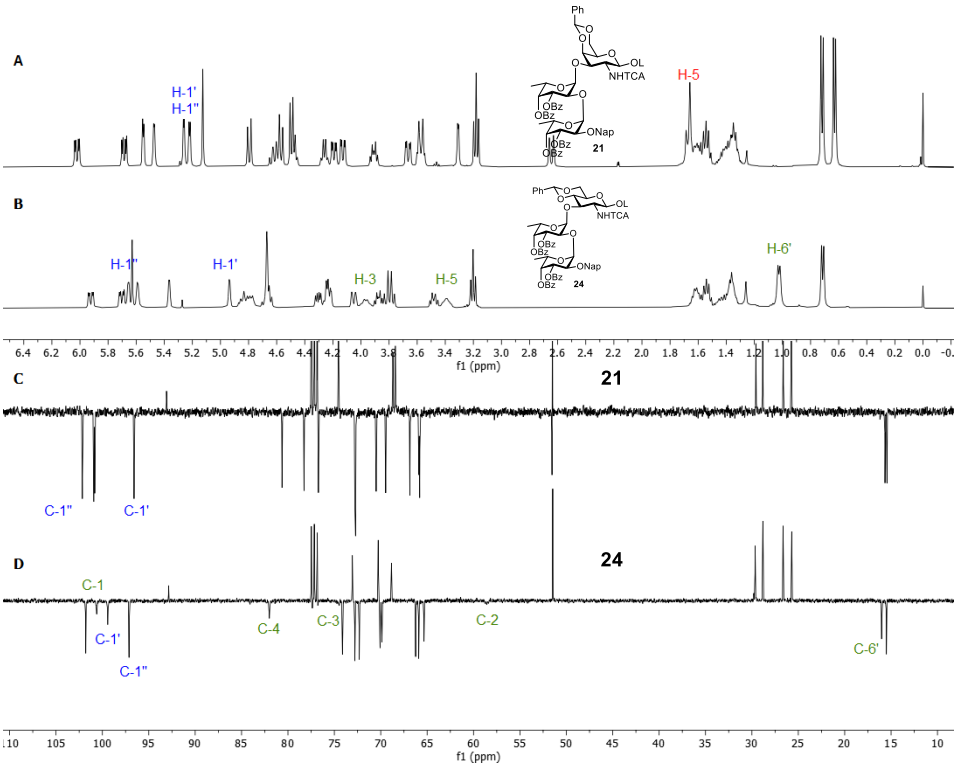


*Figure 4: Partial ^{1}H and ^{13}C-APT NMR spectra of trisaccharides **21** and **24**. Where **A** and **C** are the proton and carbon spectra of **21** and **B** and **D** are the proton and carbon spectra of **24**. The anomeric protons or carbons of the fucosyl residues are marked in blue, broadened 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).

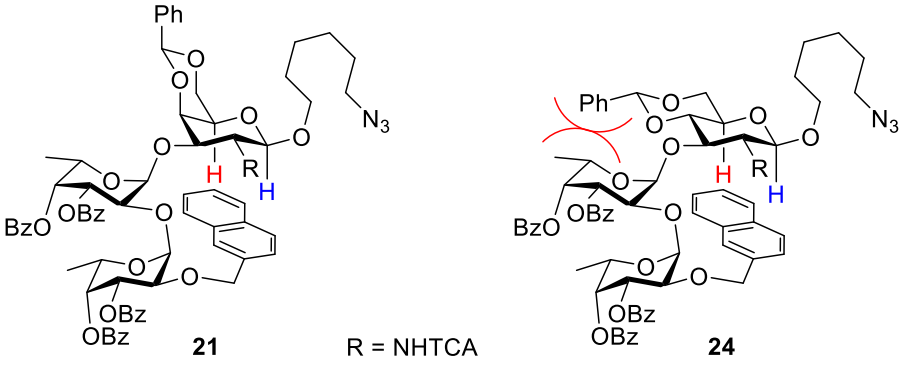


*Figure 5: Schematic representation of interaction between the naphthyl ring of trisaccharides **21** and **24** with the H-1 (blue) and H-5 (red) protons of the GalNAc or GlcNAc moiety.*

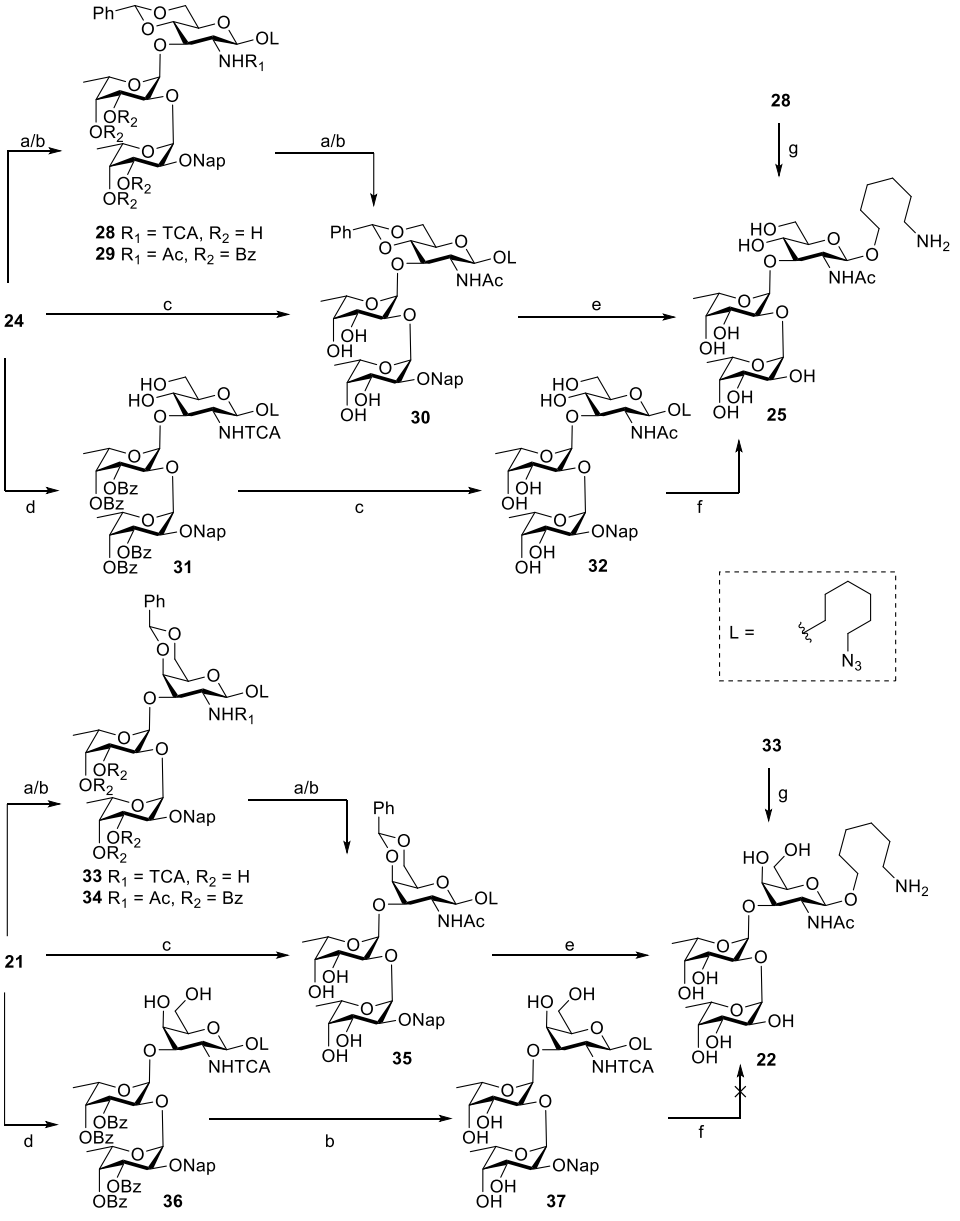
The favourable outcome of the deprotection procedure used for target disaccharides **16** and **19** was an incentive to apply the same procedures for the deprotection of trisaccharides **21** and **24** (Scheme 2). To this end the benzoyl esters were hydrolyzed using KOH in a mixture of THF and methanol, 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***



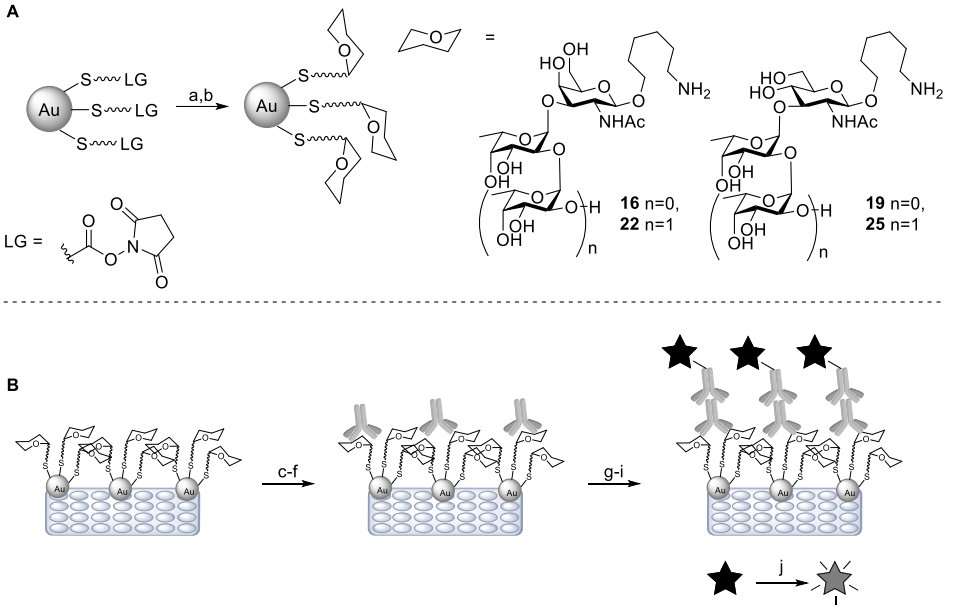
Reagents and conditions: **a**: Cs_2CO_3, DMF, 80˚C, **29** 71%, **30** 25%, **34** 48% **b**: NaOMe, THF/MeOH, **28** 81%, **30** 99%, **33** 70% **c**: i) NaOH, THF/MeOH, 60˚C, ii) Ac_2O, **30** 29%, **32** 89%, **35** 37%, **37** 73% **d**: *p*-TsOH (cat.), MeOH, ACN, **31** 48%, **36** 69% **e**: 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.

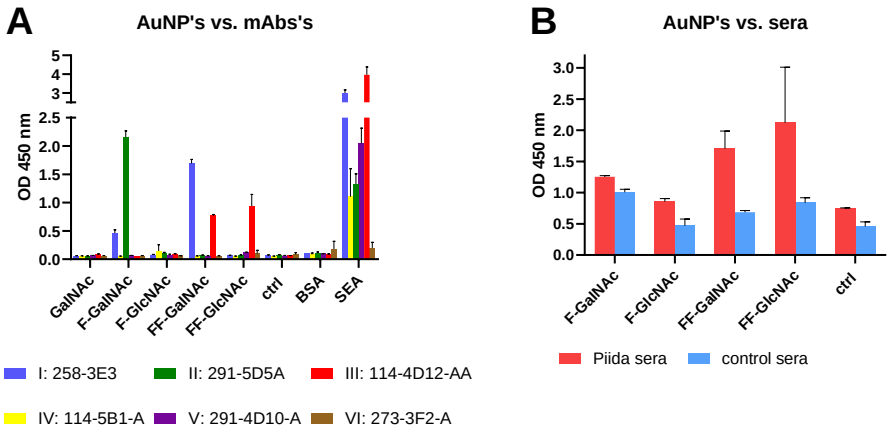


*Figure 6: Bar graph of the results of the ELISA experiment with monoclonal antibodies (**A**) and human sera (**B**) from either infected (red) or control samples (blue). AuNP: gold nanoparticle, GalNAc: 6-aminohexan-1-ol N-acetamido-β-D-galactosamine, ctrl: unfunctionalized AuNP, BSA: bovine serum 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.

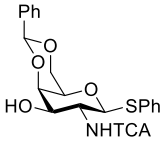
These functionalized particles were then screened against several monoclonal antibodies in an ELISA experiment, which showed that the glycans could be engaged in selective antibody binding. Further ELISA studies using sera from a schistosome infected cohort, showed that the trisaccharides, bearing the 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

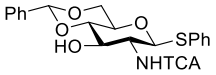
Glassware used for reactions was oven dried before use at 80°C. Anhydrous solvents were prepared by drying them over activated molecular sieves (3Å) for at least 24 hours before use. Molecular sieves were activated by flame-drying under reduced pressure. Reactions that required anhydrous conditions were 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)



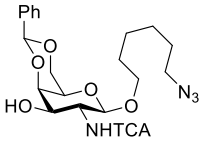
To a solution of triol **1** (15.85 mmol, 6.60 g, 1.0 eq.) dissolved in acetonitrile (120 mL, 0.15M) were added benzaldehyde dimethylacetal (17.57 mmol, 2.63 mL, 1.1 eq.) and p-toluenesulfonic acid (1.59 mmol, 0.30 g, 0.1 eq.). The solution was heated to 50 °C and stirred in a rotary evaporator at reduced 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)



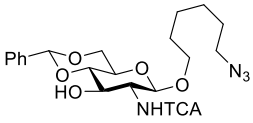
To a solution of triol **2** (10.4 g, 25.0 mmol, 1.0 eq.) dissolved in acetonitrile (150 mL, 0.15M) were added benzaldehyde dimethylacetal (60.0 mmol, 9.0 mL, 2.4 eq.) and p-toluenesulfonic acid (2.5 mmol, 0.47 g, 0.1 eq.). The solution was heated to 50 °C and stirred in a rotary evaporator at reduced 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)



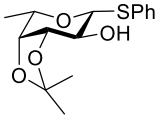
Acceptor **3** (1.53 g, 3.00 mmol, 1.0 eq.) and 6-azidohexan-1-ol (0.86 g, 6.00 mmol, 2.0 eq.) were co-evaporated trice with dry toluene and dissolved in dry DCM (20 mL, 0.15M). NIS (0.901 g, 4.00 mmol, 1.3 eq.) and MS (3Å) were added and the mixture was cooled to -20˚C and stirred at that temperature 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)



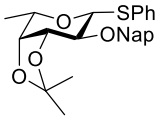
6-azidohexan-1-ol (1.07 g, 7.50 mmol, 1.5 eq.) and acceptor **4** (2.52 g, 5.00 mmol, 1.0 eq.) and were co-evaporated thrice with dry toluene and dissolved in dry DCM (40 mL, 0.15M). MS (3Å) and NIS (1.68 g, 7.50 mmol, 1.5 eq.) were added and the mixture was cooled to -20˚C and stirred at that temperature 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)



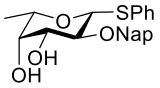
Triol **7** (18.4 g, 72 mmol, 1.0 eq.) was dissolved in dry ACN (220 mL, 0.3M). DMP (17.6 mL, 143.6 mmol, 2.0 eq.) and *p*-TsOH (1.4 g, 7.3 mmol, 0.1 eq.) were added and the flask was attached to a rotavap. The reaction was carried out at 50˚C and a pressure of 330 mbar. TLC analysis indicated full conversion 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)



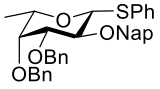
Fucoside **8** (15.3 g, 51.9 mmol, 1.0 eq.) was dissolved in dry DMF (150 mL, 0.3M) and cooled to 0˚C. After 30 min. NaH (2.5 g, 62 mmol, 1.2 eq.) was added portion-wise. Nap-Br (13.8 g, 62 mmol, 1.2 eq.) was added after the evolution of gas had stopped. Upon full conversion of the starting material (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)



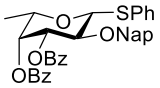
HCl (20 mL, of a 1M aqueous solution) was added to a solution of **9** (21 g, 49 mmol, 1.0 eq.) dissolved in ACN (100 mL, 0.5M). The flask was attached to a rotavap, which was then set to 50˚C at a pressure of 500 mbar. TLC analysis showed full conversion after 90 min. The reaction mixture was neutralized 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]

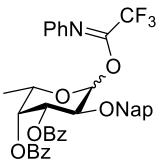
Phenyl 3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-1-thio-β-L-fucopyranoside (12)



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

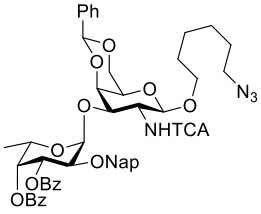
***N-phenyl-trifluoroacetimidoyl* 3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α/β-L-fucopyranoside (13)**



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.

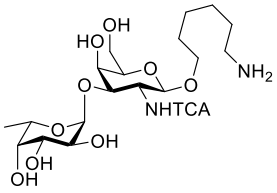
6-azidohexyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (14)



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.

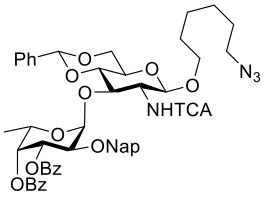
6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl) β-D-galactopyranoside (16)



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.

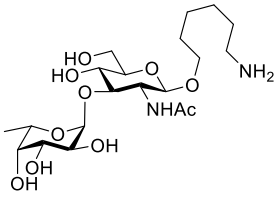
6-azidohexyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (17)



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.

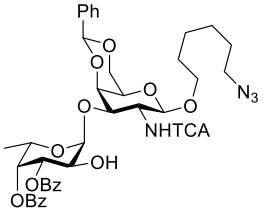
6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl) β-D-glucopyranoside (19)



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

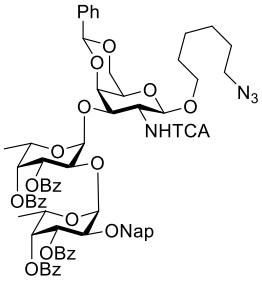
(dd, 1H, *J*=10.4, 4.1 Hz, H-2'), 3.60 (t, 1H, *J*=10.3 Hz, H-3), 3.55 (dt, 1H, *J*=10.1, 6.4 Hz, OCH_2), 3.48 (t, 1H, *J*=10.0 Hz, H-4), 3.43 (ddd, 1H, *J*=10.1, 5.9, 2.2 Hz, H-5), 1.99 (s, 3H, CH_3, Ac), 1.67 – 1.59 (m, 2H, CH_2, hexyl), 1.54 (q, 2H, *J*=6.7 Hz, CH_2, hexyl), 1.40 – 1.29 (m, 4H, CH_2, 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)



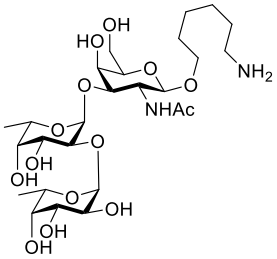
Disaccharide **14** (0.61 g, 0.58 mmol, 1.0 eq.) was dissolved in DCM (5 mL, 0.12M). Aqueous phosphate buffer (1 mL, pH 7) was added, followed by portion wise addition of DDQ (0.40 g, 1.76 mmol, 3.0 eq.). The mixture was stirred vigourously for 2.5 hours at which time TLC analysis showed full consumption 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.

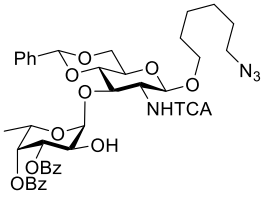
6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(2-*O*-(α-L-fucopyranosyl)-α-L-fucopyranosyl)-2-β-D-galactopyranoside (22)



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

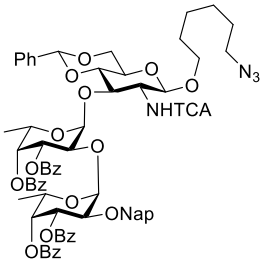
(q, 1H, *J*=6.6 Hz, H-5'), 4.08 (q, 1H, *J*=6.4 Hz, H-5''), 3.99 – 3.67 (m, 12H), 3.67 – 3.59 (m, 1H), 3.53 (dt, 1H, *J*=10.2, 6.4 Hz, OCH_2), 2.94 (t, 2H, *J*=7.6 Hz, CH_2NH_2), 2.01 (s, 3H, CH_3, Ac), 1.61 (t, 2H, *J*=7.4 Hz, CH_2, hexyl), 1.51 (t, 2H, *J*=6.7 Hz, CH_2, hexyl), 1.32 (q, 4H, *J*=4.2, 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)



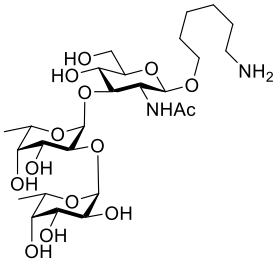
DDQ (0.96 g, 4.23 mmol, 3.0 eq.) was added portion wise to a vigorously stirring solution of **17** (1.45 g, 1.41 mmol, 1.0 eq.) in DCM (11.2 mL) and aqueous phosphate buffer (2.8 mL, pH 7). After 3 hours TLC analysis indicated full consumption of the starting material, so the reaction was quenched by 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.

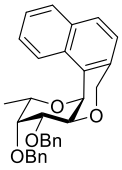
6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(2-*O*-(α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-glucopyranoside (25)



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.

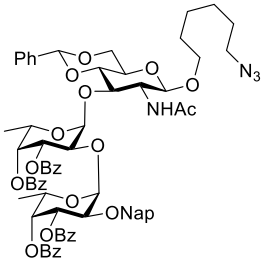
Side product (27)



^{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

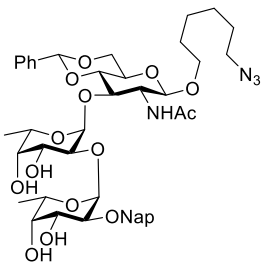
6-azidohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-glucopyranoside (29)



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.

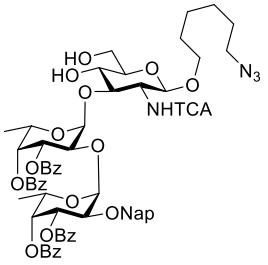
6-azidohexyl 4,6-*O*-benzylidene-2-acetamido-2-deoxy-3-*O*-(2-*O*-(2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-glucopyranoside (30)



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),

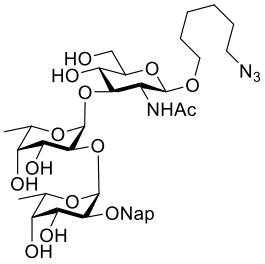
30.5, 29.9, 27.5, 26.5 (CH_2, hexyl), 23.4 (CH_3, Ac), 16.6 (C-6'), 16.5 (C-6'') ppm. HRMS $[M+H]^+$ calculated for $C_{44}H_{58}N_4O_{14}H$:867.40278, found 867.40285.

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)



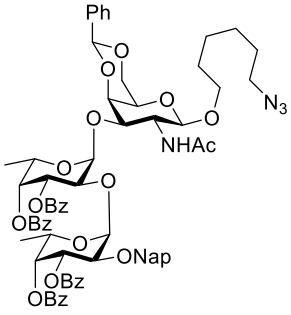
Trisaccharide **24** (0.17 g, 0.12 mmol, 1.0 eq.) was suspended in a mixture of acetic acid and water (12,5 mL, 0.01M) and heated to 80˚C for 2 hours. The mixture was poured directly into EtOAc and washed with sat. $NaHCO_3$ (aq.) until no more bubbling occurred. The organic layer was then washed with 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.

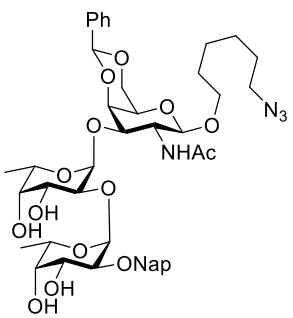
6-azidohexyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4-*O*-di-benzoyl-2-*O*-(3,4-*O*-di-benzoyl-2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-galactopyranoside (34)



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.

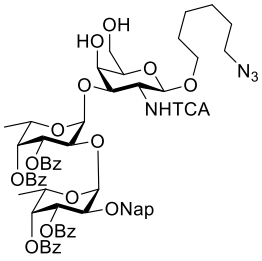
6-azidohexyl 4,6-*O*-benzylidene-2-acetamido-2-deoxy-3-*O*-(2-*O*-(2-*O*-(2-naphthylmethyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-β-D-galactopyranoside (35)



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,

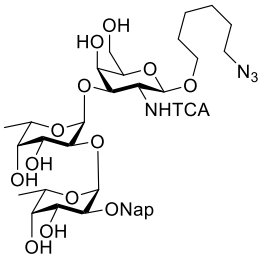
1H, H-3), 2.81 – 2.68 (m, 2H, H-1, H-5), 2.49 – 2.33 (m, 1H, OCH_2), 2.05 (s, 3H, CH_3, Ac), 1.67 – 1.51 (m, 2H, CH_2, hexyl), 1.46 – 1.11 (m, 9H, H-6'', CH_2, hexyl), 0.92 (d, 3H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR (MeOD, 101 MHz) δ 173.9 (C=O, TCA), 139.6, 137.6, 134.9, 134.8, 129.9, 129.8, 129.2, 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

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Supporting information

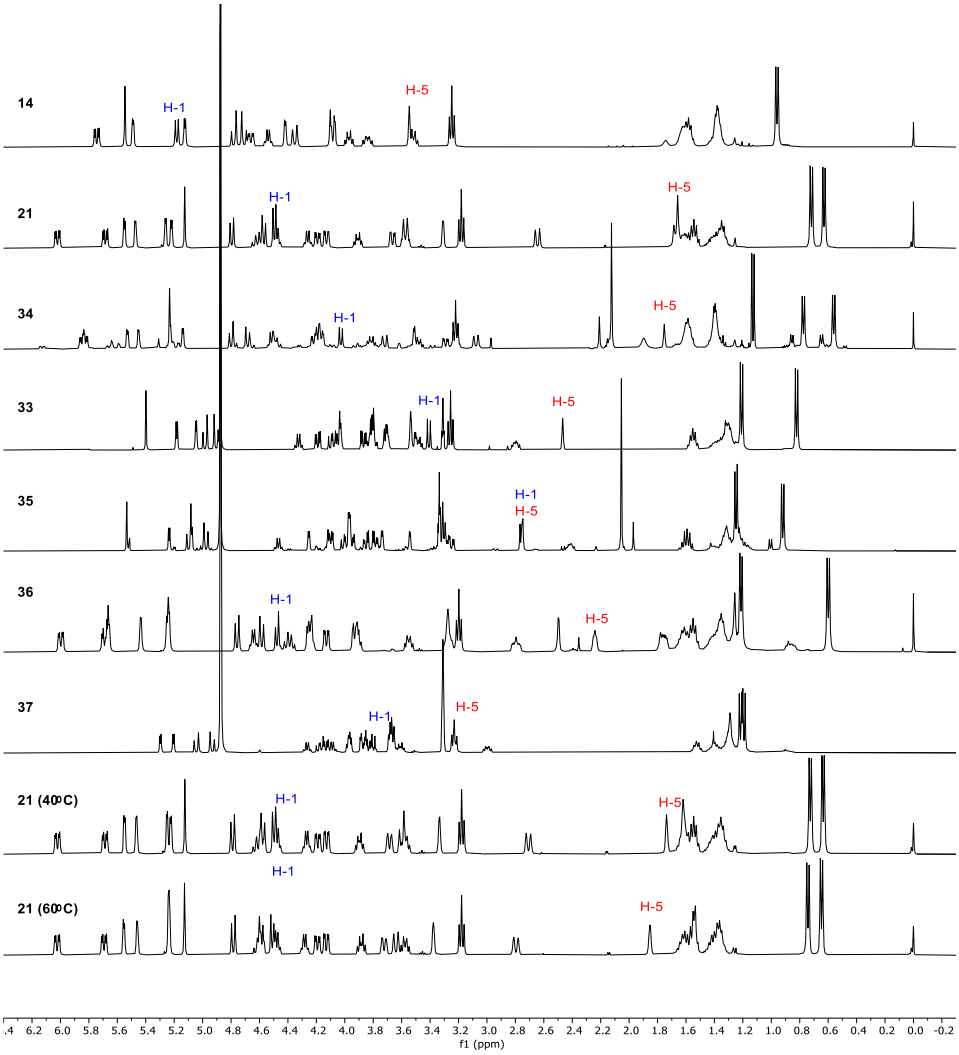
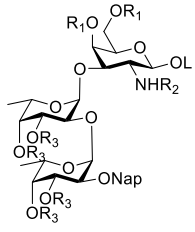


*Figure 7: Partial ^{1}H NMR spectra of various deprotecting stages of **21**. The anomeric proton of the GalNAc residue (H-1) is marked in blue and the H-5 of the GalNAc residue is marked in red. All spectra were recorded in $CDCl_3$ on a 400 MHz NMR at room temperature unless indicated otherwise.*

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

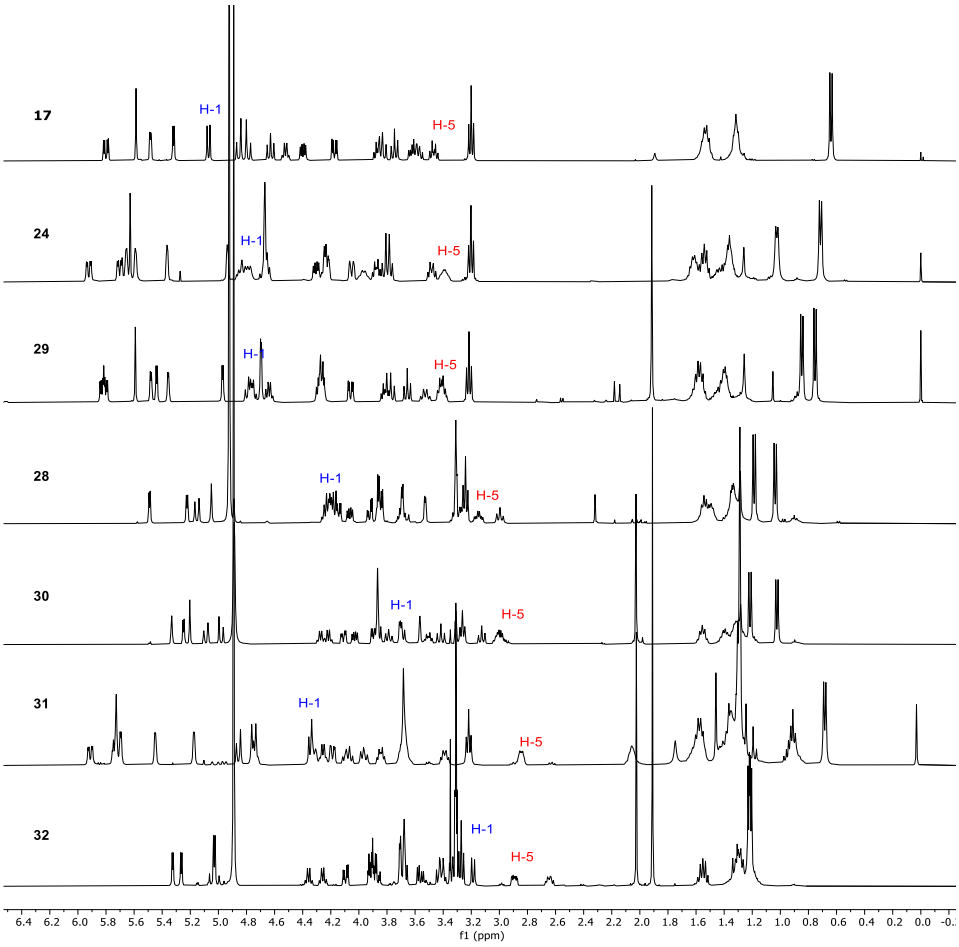
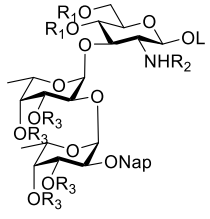


*Figure 8: Partial ^{1}H NMR spectra of various deprotecting stages of **24**. The anomeric proton of the GlcNAc residue (H-1) is marked in blue and the H-5 of the GlcNAc residue is marked in red. All spectra were recorded in $CDCl_3$ on a 400 MHz NMR at room temperature.*

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

3

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

Introduction

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

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

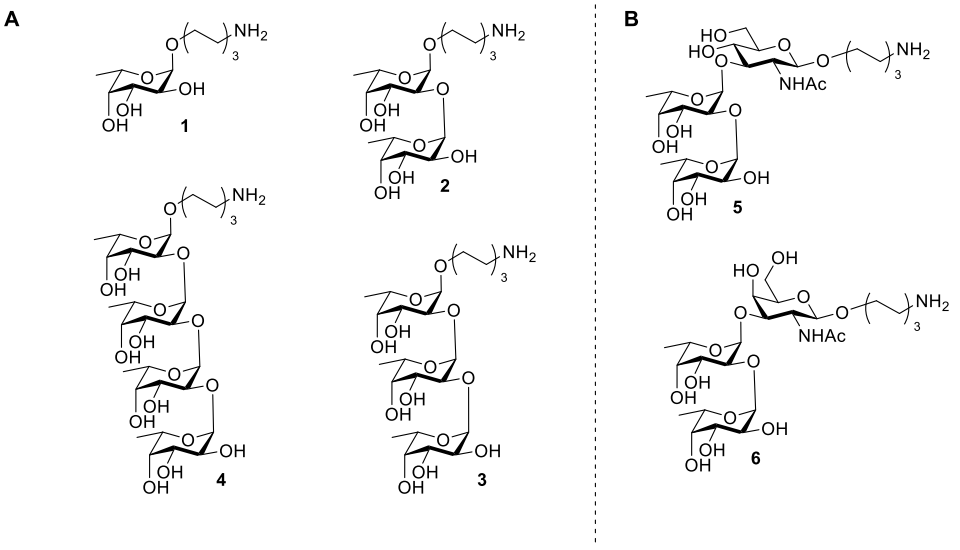


*Figure 1: **A**) Target α-(1-2) oligofucosides, **B**) Fucosylated trisaccharides described in Chapter 2.*

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

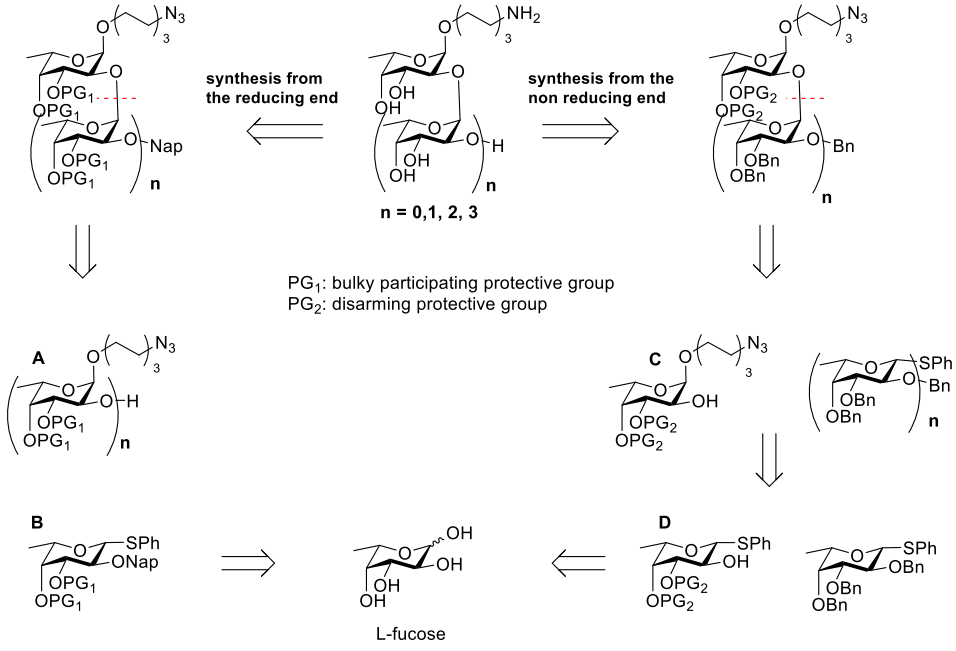
In order to synthesize the α-(1-2)-oligofucosides from the reducing end the following synthetic route was envisioned. Terminal building block **A** can be condensed with donor **B**, which bears benzoyl esters on the C3- and C4-O positions both for their steric bulk and to make use of the long range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

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

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

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

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



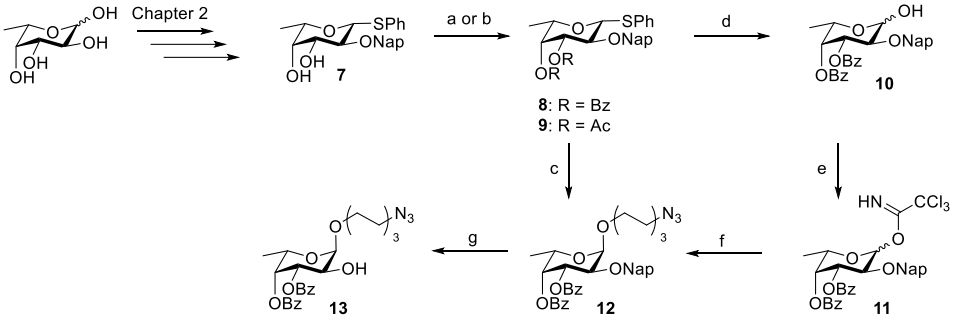
Results and discussion

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

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

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

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

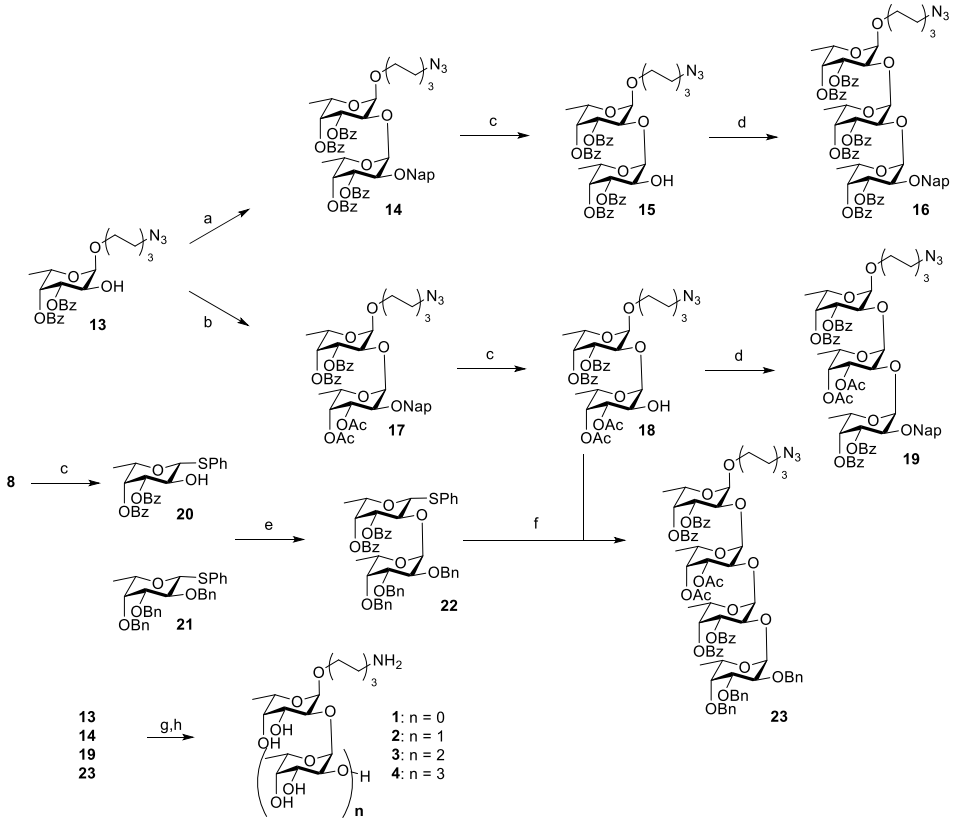


Reagents and conditions: **a**: Ac_2O, pyr, DCM, 0˚C to RT, 97% **b**: Bz-Cl, DMAP, pyr, DCE, 60˚C, 93%, **c**: DMF, NIS, TMSOTf (cat.), 6-azidohexan-1-ol, MS (3Å), DCM, 42%, **d**: NBS, acetone, H_2O, 98%, **e**: CS_2CO_3 (cat.), Cl_3CCN, DCM, 90%, α/β, 2/1, **f**: 6-azidohexan-1-ol, TMSI, $Ph_2MeP=O$,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

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

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

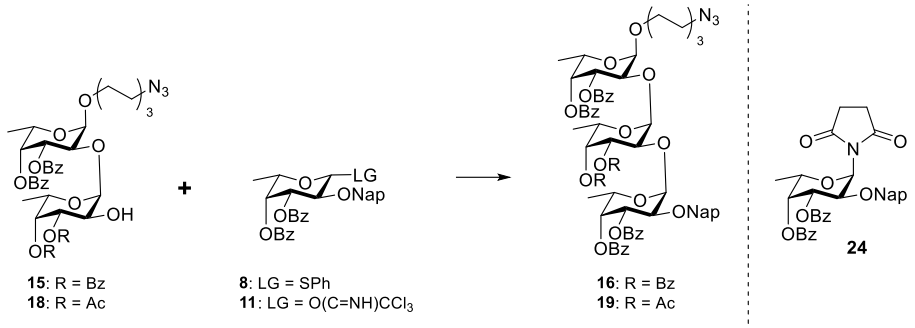
Scheme 3: Synthesis of fuc-α-(1-2)-fuc chains from the reducing end.



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

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

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



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

[a] Isolated yield of the desired trisaccharide.

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

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

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

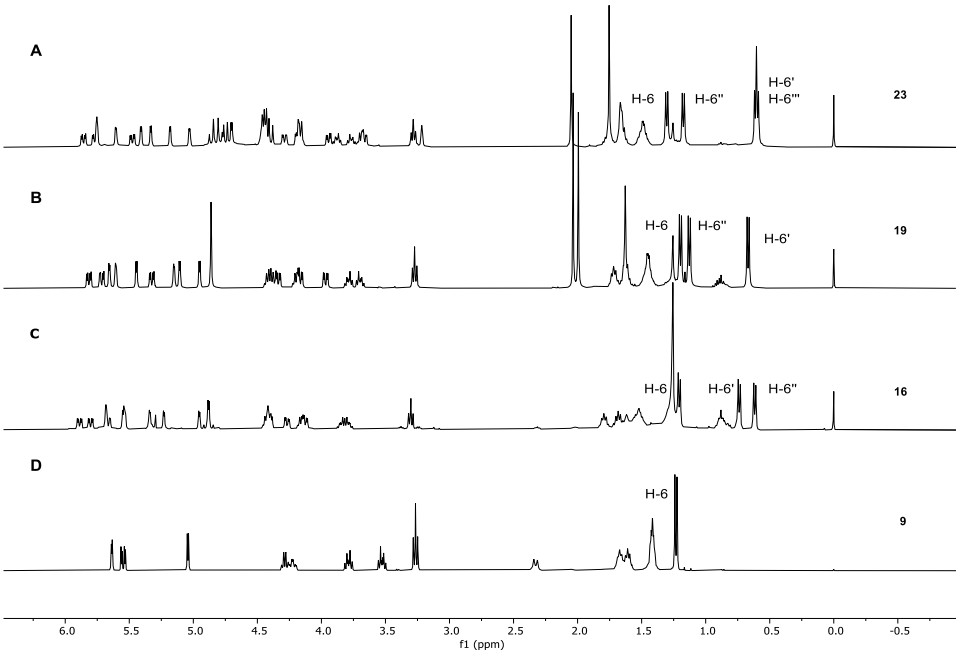


*Figure 2: Partial ^{1}H NMR spectra of **23 (A)**, **19 (B)**, **16 (C)** and **20 (D)**. The highlighted peaks represent the methyl groups (H-6) of the fucoses, where H-6 denotes the methyl group of the first fucose from the reducing end, H-6' the second and H-6'' and H-6''', the third and fourth,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

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

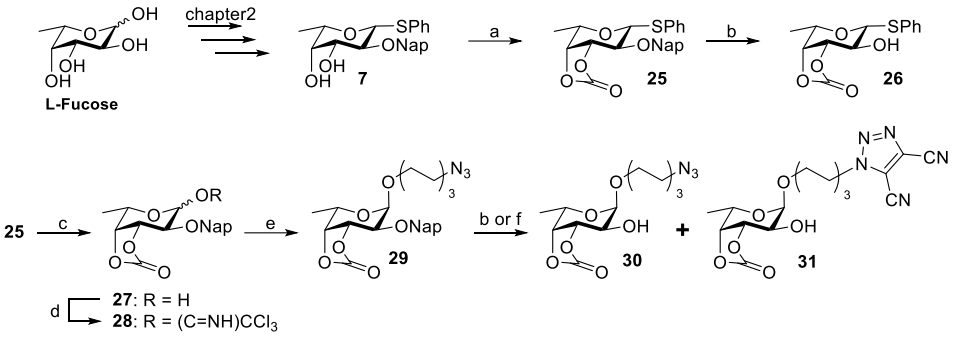
6 of a fucoside is generally located around 1.1-2 ppm as can be seen from the spectrum of monosaccharide **20** (Figure 2-D). The ^{1}H NMR of benzoylated trisaccharide **16** (Figure 2-C) two methyl groups (H-6'and H-6'') are located around 0.6 ppm, while acetyl bearing trisaccharide **19** (Figure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

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

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

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

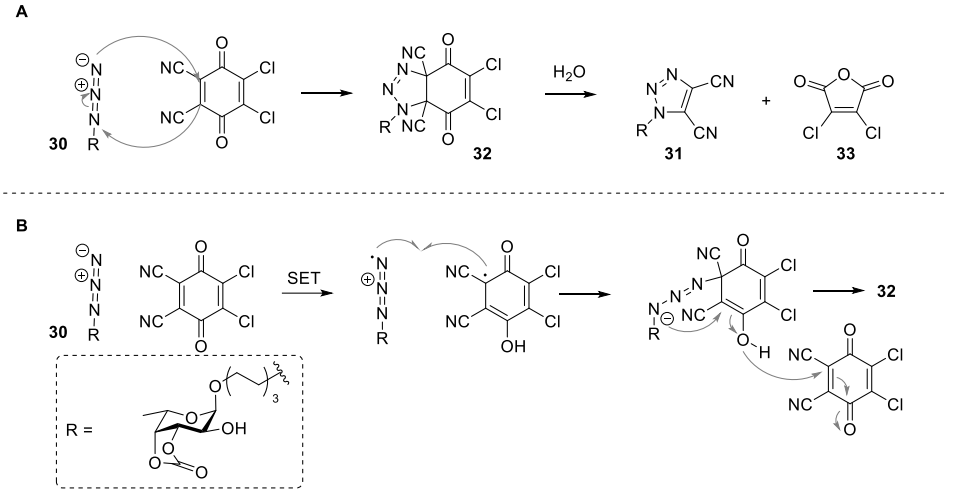
Scheme 4: Synthesis of the building blocks required for a non-reducing end up approach.



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

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

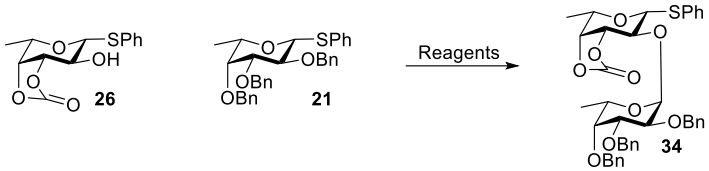
*Scheme 5: Proposed formation of byproduct **31**. **A**) by cycloaddition, **B**) by single electron transfer.*



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

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

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



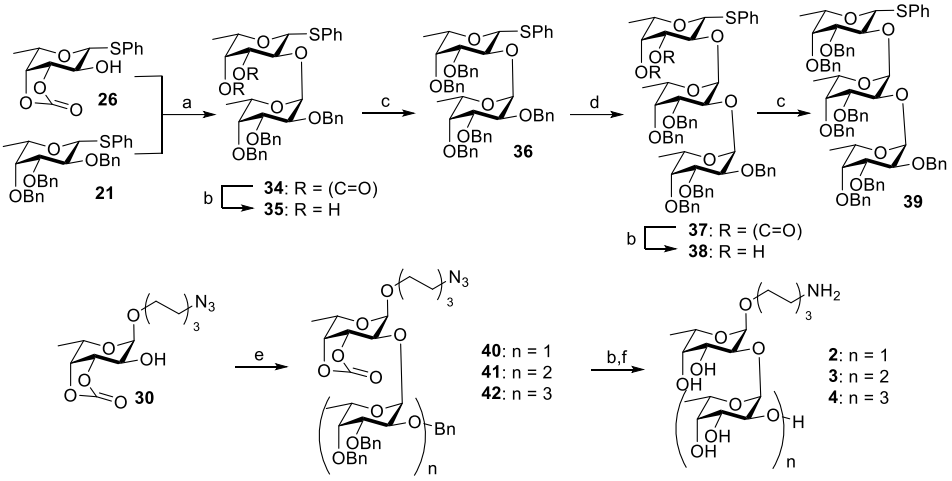
entry	reagents	T (˚C)	Yield (%)	α/β[a]
1	NIS (1.2 eq.), TMSOTf (0.1 eq.)	-40 → -20	Complex mixture	-
2	Ph_2SO, TTBP, Tf_2O[b]	-80 → -40	48%	3/1
3	IDCP (4 eq.)	0	72%	6/1
4	IDCP (4 eq.)	RT	81%	5/1

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

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

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

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



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

3

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

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

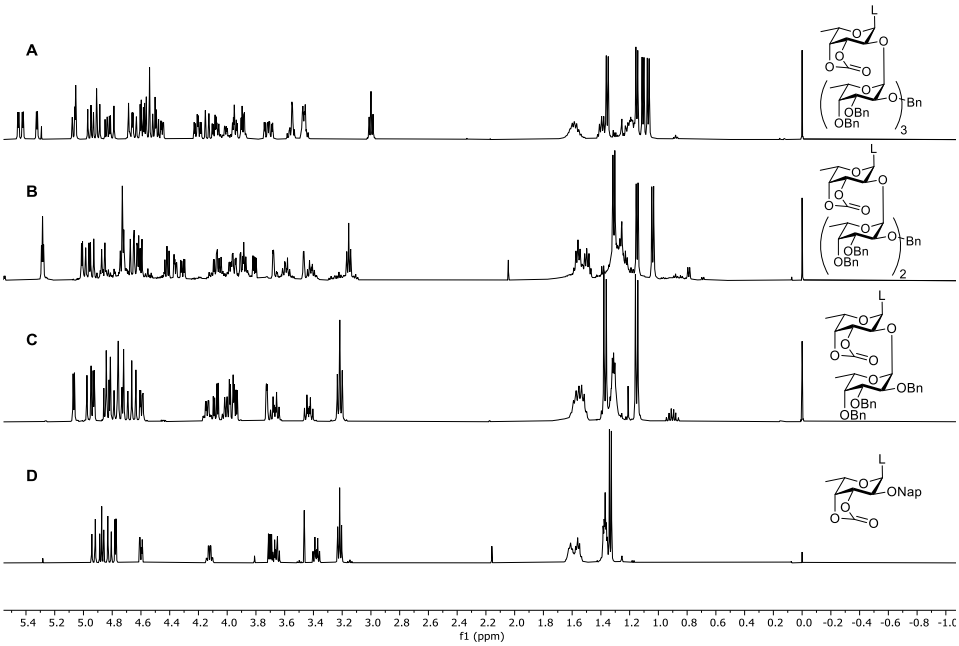


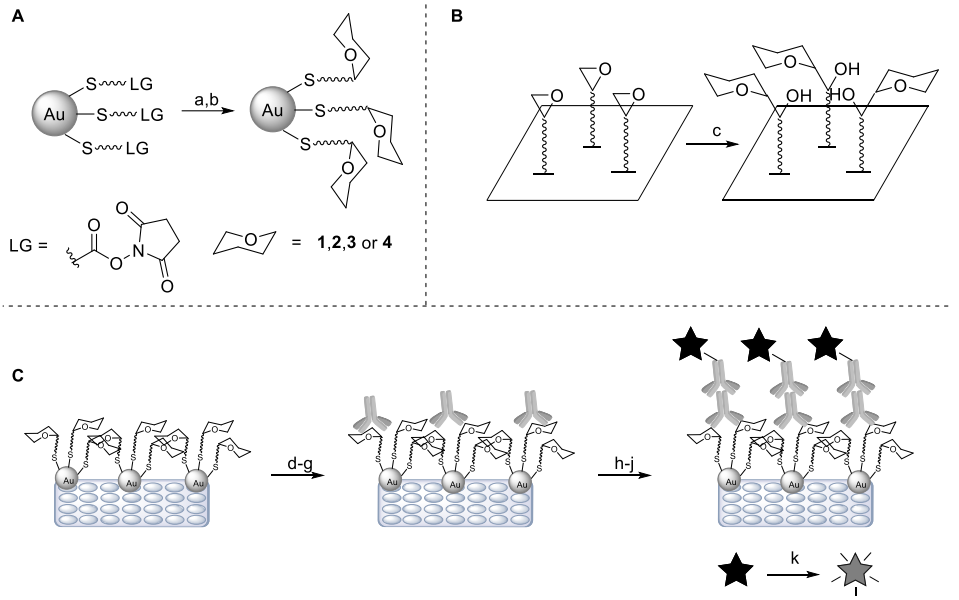
*Figure 3: Partial ^{1}H NMR spectra of **42** (A), **41** (B), **40** (C), **30** (C). The highlighted peaks represent the methyl groups (H-6) of the fucoses, where H-6 denotes the methyl group of the first fucose from the reducing end, H-6' the second and H-6'' and H-6''', the third and fourth, respectively.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

Gold nanoparticle based ELISA's

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

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

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



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

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

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

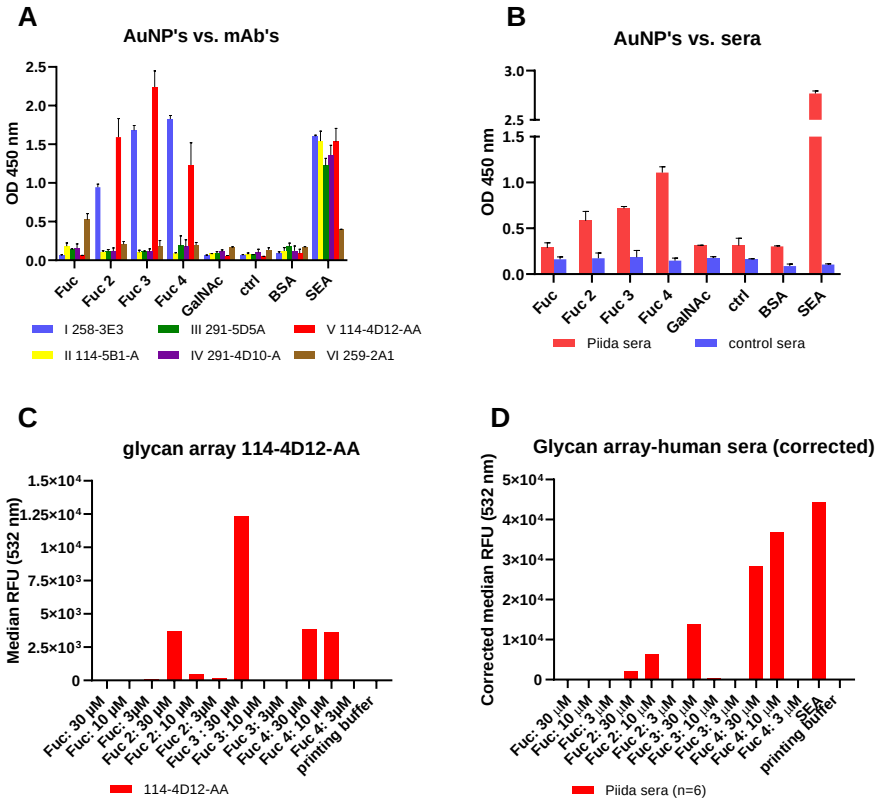


*Figure 4: Bar graph of the results of the ELISA experiment with monoclonal antibodies (**A**) and human sera (**B**) from either infected (red) or control samples (blue). Bar graph of glycan array experiment with mAb 114-4D12-AA (**C**) and with human sera (**D**). AuNP: gold nanoparticle, Fuc **x**: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

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

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

Conclusion

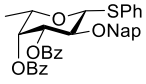
In order to answer the question if the backbone is required for recognition of α-(1-2) linked oligofucosides by antibodies a small library was synthesized consisting of a mono-, di-, tri- and tetramer. In order to synthesize these molecules, two different chemical routes were tested. Route A, consisted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

Experimental

General procedures

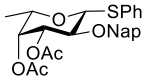
Glassware used for reactions was oven dried before use at 80°C. Anhydrous solvents were prepared by drying them over activated molecular sieves (3Å) for at least 24 hours before use. Molecular sieves were activated by flame-drying under reduced pressure. Reactions that required anhydrous conditions were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

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



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

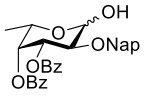
Phenyl 3,4-di-*O*-acetyl-2-*O*-(2-naphthylmetyl)-1-thio-α-L-fucopyranoside (9)



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

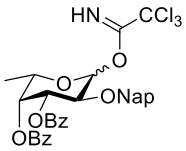
with 1M HCl (aq., 3x), sat. $NaHCO_3$ (aq., 2x) and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated. The title compound was obtained after purification by silicagel chromatography (0.95 g, 1.98 mmol, mmol, 99 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.84 – 7.74 (m, 2H, arom.),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

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



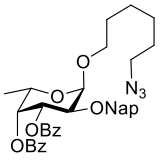
NBS (2.7 g, 15.0 mmol, 3.0 eq.) was added to a stirring solution of **8** (3.0 g, 5.0 mmol, 1.0 eq.) in a mixture of water and acetone (50 mL, 0.1M, 1/4, v/v) and left to stir for 15 min in the dark under inert atmosphere. A solution of sat. $Na_2S_2O_3$ (aq.) was added (20 mL) and after stirring for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

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



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

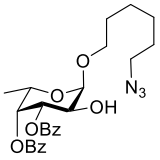
6-Azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(2-naphthylmetyl)-α-L-fucopyranoside (12)



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

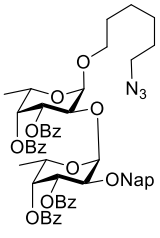
J=3.4 Hz, H-4), 4.99 (d, 1H, *J*=3.5 Hz, H-1), 4.89 – 4.76 (m, 2H, CH_2Nap), 4.31 (q, 1H, *J*=6.6 Hz. H-5), 4.17 (dd, 1H, *J*=10.5, 3.5 Hz, H-2), 3.74 (dt, 1H, *J*=9.6, 6.7 Hz, OCH_2), 3.50 (dt, 1H, *J*=9.6, 6.4 Hz, OCH_2), 3.25 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.77 – 1.55 (m, 4H, CH_2, hexyl), 1.45 (m,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

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



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

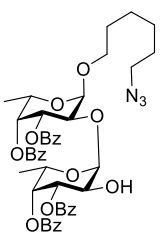
6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2-naphthylmetyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (14)



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

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

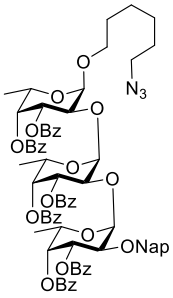
6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-benzoyl-α-L-fucopyranosyl)-α-L-fucopyranoside (15)



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

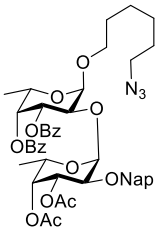
4H, CH_2, hexyl), 1.49 – 1.39 (m, 4H, CH_2, hexyl), 1.27 (d, 3H, *J*=6.8, H-6), 0.82 (d, 2H, *J*=6.5 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 166.1 (C=O, Bz), 166.0 (C=O, Bz), 165.8 (C=O, Bz), 165.5 (C=O, Bz), 133.5, 133.4, 133.1, 129.9, 129.8, 129.7, 129.6, 129.5, 128.7, 128.6, 128.5, 128.3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

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



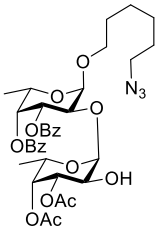
Acceptor **15** (61 mg, 70 µmol, 1 eq.) and imidate donor **11** (75 mg, 0.11 mmol, 1.5 eq.) were dissolved in dry DCM (0.7 mL, 0.1M), after being coevaporated together with toluene (3x). Molecular sieves (3Å) were added and the solution was cooled to -40°C, at which temperature it was stirred for 30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

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



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

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

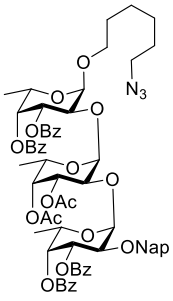


Disaccharide **17** (0.64 g, 0.73 mmol, 1 eq.) was dissolved in a 9/1 mixture of DCM/MeOH (7.5 mL, 0.1M). DDQ (0.40 g, 2.92 mmol, 3 eq.) was added portionwise (1 eq. per hour) and the reaction was left to stir under nitrogen atmosphere at RT. After 5 hours TLC analysis (PE:EtOAc, 3:2) showed full conversion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

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

3

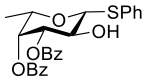
6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-acetyl-2-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2-naphthylmetyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (19)



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

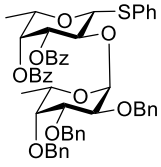
J=9.7, 6.6 Hz, CH_2O), 3.70 (dt, 1H, *J*=9.8, 6.6 Hz, CH_2O), 3.27 (t, 2H, *J*=7.0 Hz, CH_2N_3), 2.04 (s, 3H, CH_3-Ac), 1.99 (s, 3H, CH_3-Ac), 1.76 – 1.57 (m, 4H, CH_2, hexyl), 1.49 – 1.41 (m, 4H, CH_2, hexyl), 1.20 (d, 3H, *J*=6.6 Hz, H-6), 1.13 (d, 3H, *J*=6.6 Hz, H-6''), 0.67 (d, 2H, *J*=6.5 Hz, H-6')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

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



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

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

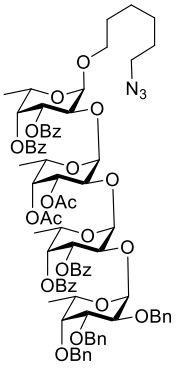


MS (3Å) were added to a solution containing donor **21** (0.32 g, 0.60 mmol, 2.0 eq.) and acceptor **20** (0.14 mmol, 0.30 mmol, 1.0 eq.) in dry DCM (3 mL, 0.1M). the solution was cooled to 0˚C and stirred at that temperature for 30 min. before addition of IDCP (0.42 g, 0.90 mmol, 3.0 eq.). The solution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

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

3

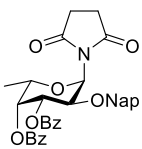
6-azidohexyl 3,4-di-*O*-benzoyl-2-*O*-(3,4-di-*O*-acetyl-2-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranosyl)-α-L-fucopyranoside (23)



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

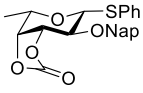
J=10.5, 3.3 Hz, H-2'), 4.22 – 4.14 (m, 3H, H-5', H-2'', CH_2-Bn), 3.94 (dd, 1H, *J*=10.2, 3.5 Hz, H-2'''), 3.88 (dt, 1H, *J*=9.3, 6.8 Hz, CH_2O), 3.77 (dt, 1H, *J*=11.4, 4.8 Hz, CH_2O), 3.74 – 3.63 (m, 2H, H-3''', H-5'''), 3.28 (t, 2H, *J*=6.6 Hz, CH_2N_3), 3.22 (s, 1H, H-4'''), 2.05 (s, 3H, CH_3-Ac),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

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



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

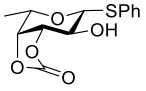
Phenyl 3,4-*O*-carbonate-2-*O*-(2-naphthylmethyl)-1-thio-α-L-fucopyranoside (25)



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

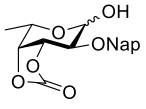
(m, 5H, arom), 7.29 – 7.22 (m, 3H, arom), 4.84 – 4.76 (m, 2H, CH_2-Nap), 4.74 (dd, 1H, *J*=7.4, 5.4 Hz, H-3), 4.71 (d, 1H, *J*=8.0 Hz, H-1), 4.43 (dd, 1H, *J*=7.4, 1.9 Hz, H-4), 3.72 (qd, 1H, *J*=6.5, 1.9 Hz, H-5), 3.65 (dd, 1H, *J*=8.0, 5.5 Hz, H-2), 1.33 (d, 2H, *J*=6.6 Hz, H-6) ppm. ^{13}C-APT NMR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

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



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

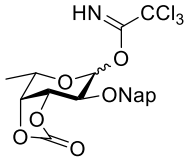
3,4-*O*-carbonate-2-*O*-(2-naphthylmethyl)-α/β-L-fucopyranoside (27)



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

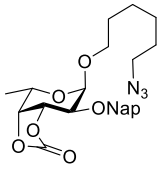
128.6, 128.1, 128.0, 127.9, 127.8, 127.6, 127.2, 126.7, 126.6, 126.4, 126.3, 125.9, 125.8 (CH, arom) , 95.3 (C-1β), 89.9 (C-1α), 78.2 (C-2β), 77.8 (C-3β), 77.5 (C-4β), 77.0 (C-4α), 75.0 (C-3α), 74.3 (CH_2Nap,α), 73.8 (CH_2Nap,β), 73.4 (C-2α), 67.7 (C-5β), 63.2 (C-5α), 16.3 (C-6β), 15.8 (C-6α) ppm. HRMS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

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



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

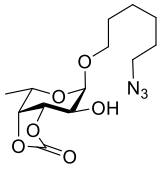
6-Azidohexyl 3,4-*O*-carbonate-2-*O*-(2-naphthylmetyl)-α-L-fucopyranoside (29)



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

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

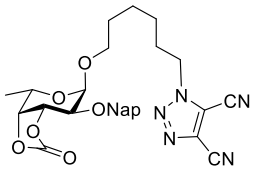
6-Azidohexyl 3,4-*O*-carbonate-2-*O*-(2-naphthylmetyl)-α-L-fucopyranoside (30)



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

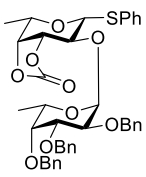
Synthesis by DDQ: Sugar **29** (1.0 g, 2.2 mmol, 1 eq.) was dissolved in a 9/1 mixture of DCM/MeOH (20 mL, 0.1M). DDQ (1.5 mmol, 6.6 mmol, 3 eq.) was added and the reaction was stirred under N_2 for 8 hours. The reaction mixture was partitioned between EtOAc and sat. aq. $Na_2S_2O_3$. The organic layer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

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



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

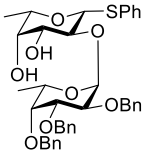
Phenyl 3,4-*O*-carbonate-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1-thio-β-L-fucopyranoside (34)



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

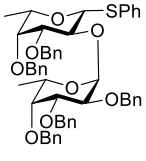
73.7 (CH_2Bn), 73.2 (CH_2Bn), 72.9 (C-2), 71.2 (C-5), 67.6 (C-5'), 16.5 (C-6), 16.4 (C-6') ppm. HRMS $[M+Na]^+$ calcd for $C_{40}H_{42}O_9SNa$: 721.24472, found 721.24417.

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



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

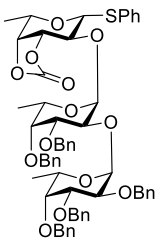
Phenyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1-thio-β-L-fucopyranoside (36)



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

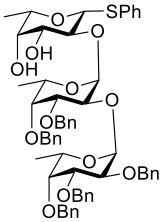
MHz): δ 139.3, 138.8, 138.8, 138.5, 137.7, 134.0, 130.9, 128.9, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.6, 126.9 (CH, arom), 96.1 (C-1'), 87.3 (C-1), 84.2 (C-3), 79.1 (C-3'), 77.6 (C-4'), 76.5 (C-2'), 76.2 (C-4), 74.8 (CH_2Bn), 74.7 (CH_2Bn), 74.5 (C-5), 73.2 (CH_2Bn), 73.1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

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



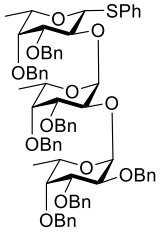
Compounds **26** (0.17 g, 0.6 mmol, 2 eq.) and **36** (0.26 g, 0.3 mmol, 1 eq.) were co-evaporated together with toluene thrice and dissolved in dry DCM (3 mL, 0.1M). Flame dried molecular sieves (3Å) were added and the mixture was cooled to 0°C and stirred at this temperature for 30 min. IDCP (0.19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

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



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

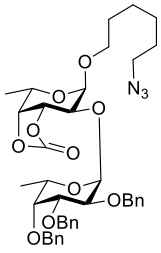
Phenyl 3,4-di-*O*-benzyl-2-*O*-(3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-α-L-fucopyranosyl)-1-thio-β-L-fucopyranoside (39)



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

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

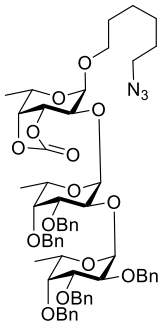
6-Azidohexyl 3,4-*O*-carbonate-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1-α-L-fucopyranoside (40)



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

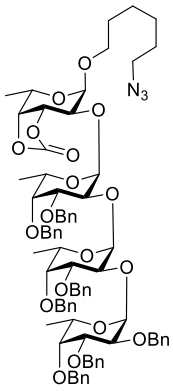
(d, 2H, *J*=6.4 Hz, H-6') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 154.2 (C=O), 138.7, 128.6, 128.5, 128.4, 128.3, 128.3, 127.8, 127.7 (arom.), 103.8 (C-1'), 97.7 (C-1), 82.3 (C-3'), 79.4 (C-2), 78.5 (C-4), 77.8 (C-4'), 76.6 (C-3), 75.8 (C-2), 75.2 (CH_2Bn), 74.9 (CH_2Bn), 73.6 (CH_2Bn), 70.8 (C-5'),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

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



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

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

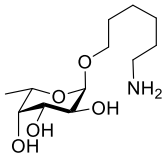


Trisaccharide **39** (0.091 g, 0.076 mmol, 1.0 eq.) and **30** (0.031 g, 0.1 mmol, 1.3 eq.) were co-evapotared thrice with dry toluene, before dissolving in dry DCM. Freshly dried MS (3Å) were added and the mixture was cooled to 0˚C with an ice bath and stirred for 30 min. IDCP (0.53 g, 0.114 mmol, 1.5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

General deprotection method of oligofucosides:

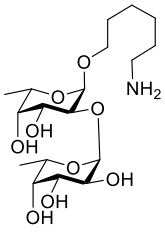
Glycan **x** (1.0 eq.) was dissolved in a mixture of THF/MeOH (0.05M, 1/1, v/v). Base (3.0 eq.) was added and the mixture was stirred at RT. After 2 hours the temperature was increased to 50˚C. TLC analysis showed full conversion of the starting material after several (3-5) hours and the reaction mixture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

6-aminohexyl α-L-fucopyranoside (1)



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

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



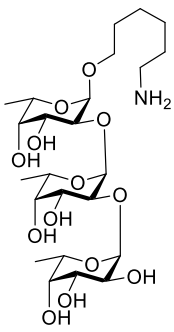
Route 1: Disaccharide **14** (42 mg, 0.04 mmol), NaOMe (6.0 mg, 0.12 mmol), Pd/C (42 mg). Disaccharide **2** was obtained as a white powder (11 mg, 0.025 mmol, 68%).

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

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

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

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

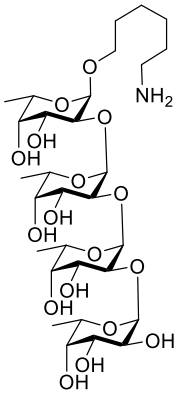


Route 1: Trisaccharide **19** (15 mg, 0.01 mmol), NaOMe (2.0 mg, 0.03 mmol), Pd/C (15 mg). Trisaccharide **3** was obatined as a white powder (4.1 mg, 0.0073 mmol, 74%).

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

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

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



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

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

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

Synthesis of functionalized AuNP's

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

ELISA protocol

general

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

ELISA protocol

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

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

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

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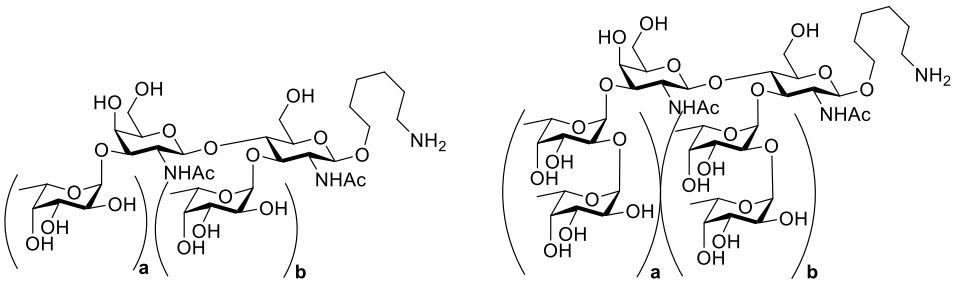
4

*Synthesis of fucosylated S. Mansoni LDN fragments**

Introduction

The *N*-acetylgalactosamine-β-(1-4)-*N*-acetylglucosamine disaccharide (GalNAc-β-(1-4)-GlcNAc, LacdiNAc or LDN), is a common backbone motif in Schistosoma glycans in all stages of its life cycle.[1] It is frequently fucosylated on either the C3-OH of the GlcNAc (LDN-F) or the C3-OH of the GalNAc (F-LDN) residue, but can also be fucosylated on both positions (F-LDN-F).[2], [3] Although the LDN-F motif is also present in humans and therefore not that useful as a biomarker, both F-LDN and F-LDN-F are unique to Schistosomes making them good candidates as biomarkers.[4], [5] F-LDN and F-LDN-F and difucosylated LDN fragments (F2)-LDN-(F2) are secreted in the urine of infected individuals. The monoclonal antibody 114-4D12 (See Chapter 2 and 3 for more detail) can then be used to detect these multi-fucosylated oligosaccharides in urine, which would allow for the development of an easy method to determine the level of infection, similar to that of the circulating cathodic antigen (CCA) dipstick test.[6]

*Harvey, M.R., Van der Marel, G.A., and Codée, J.D.C. have contributed to the work described in this chapter



a = 1, **b** = 0: F-LDN
a = 0, **b** = 1: LDN-F
a = **b** = 1: F-LDN-F

a = 1, **b** = 0: F2-LDN
a = 0, **b** = 1: LDN-F2
a = **b** = 1: F2-LDN-F2

Figure 1: Fucosylated LDN fragments, left: mono-fucosylated LDN fragments, right: di-fucosylated LDN fragments.

Besides being potential biomarkers of schistosomiasis these fucosylated LDN fragments can be used as tools to probe biosynthesis enzymes. These fucosylated LDN fragments can be used to find both the enzymes that extend the α-fucosyl chains and the enzymes that hydrolyse the α-fucosyl chains, which are as of yet unknown.[7] The (F)-LDN-(F) fragments can be used to identify the former, while the (F2)-LDN-(F2) fragments can be used to identify the latter. This chapter describes a method to synthesize the fucosylated LDN fragments depicted in Figure 1.

Results and discussion

The synthetic route to the small set of fucosylated LDN fragments is retrosynthetically explained in Figure 2. The NHAc functionality in the target oligosaccharides is unsuitable for use in glycosylation reactions as the NHAc is known to form stable oxazolines and reduce the reactivity of the acceptor hydroxyl groups.[8]–[10] In order to avoid this the NHAc was masked as a trichloro acetamide (TCA) group. The amine of the linker was masked as an azide. Both the TCA and the azide can be converted into the NHAc and the amine, respectively, by a variety of methods enabling a flexible deprotection strategy at the end of the synthesis.[11]–[13] The LDN disaccharide backbone is decorated with two orthogonal protecting groups (R_1 and R_2) on the C3-O positions of both the GlcNAc and the GalNAc residues in order to install a mono- or di-fucosyl residue on this disaccharide selectively. The disaccharide can be synthesized from appropriately protected D-glucosamine and D-galactosamine building blocks. The galactosamine building block can also be synthesized from glucosamine, which is a significantly cheaper starting material. The NHTCA groups on these building blocks should induce the required β-selective linkages. Both these building blocks will be synthesized as thiophenyl glycosides, as thioglycosides can withstand a wide variety of protective group manipulations and can be used as glycosylating agents, or they can be transformed into other anomeric leaving

groups (such as imidates). The mono-fucosylated fragments will be synthesized by condensing the intrinsically α-selective fucosyl building block, whose preparation and use are described in Chapter 2, with the appropriate disaccharide. The di-fucosylated fragments will be synthesized using di-fucosyl building blocks, the synthesis of which is described in Chapter 3.

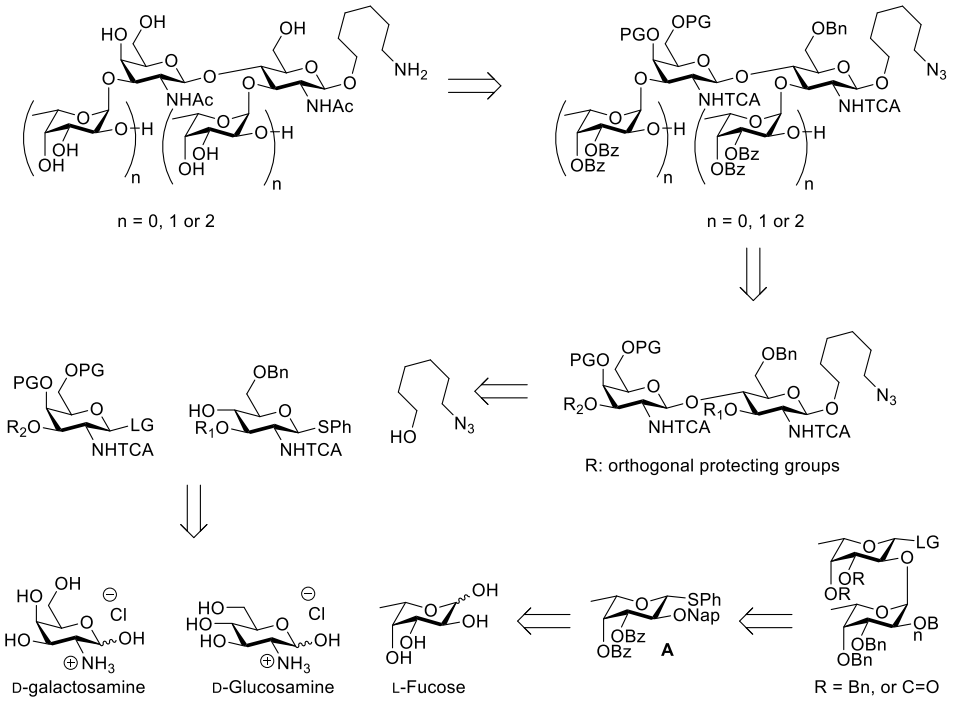


Figure 2: Retrosynthesis of fucosylated LDN fragments.

The synthesis of the glucosamine synthon is depicted in Scheme 1. Several different orthogonal protecting groups, naphthyl, benzoyl, levulinoyl and acetyl were explored at the C3-O position in order to optimize the backbone coupling. The naphthyl ether can be removed selectively using DDQ, the levulinoyl can be removed selectively using hydrazine and the acetyl and benzoyl esters can be removed by sodium methoxide. All final building blocks could be synthesized from known partially protected glucosamine **1**.

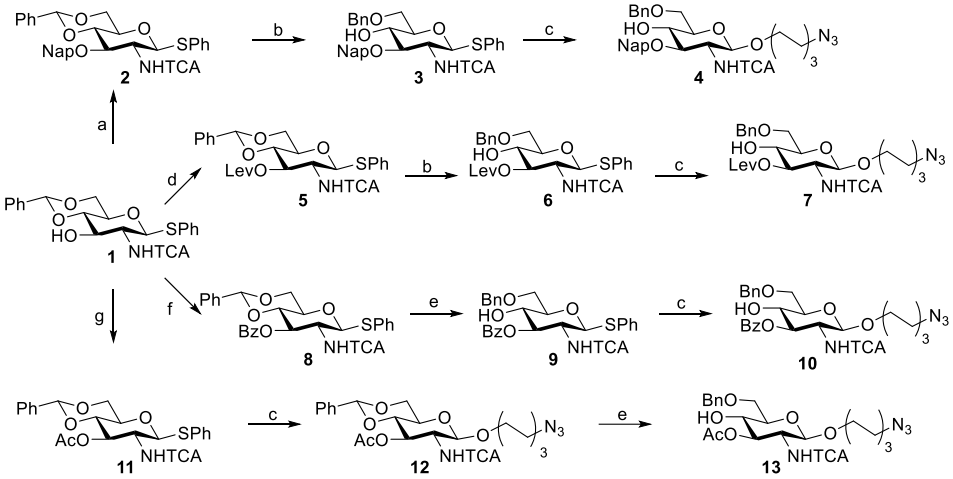
The naphthyl group was installed by treating **1** with an excess of three equivalents of sodium hydride and one equivalent of naphthyl bromide. This protocol reduced the unwanted alkylation of the amide, presumably due to formation of both the deprotonated amide and the C3-O alkoxide, the latter of is more nucleophilic, thus leading to the desired compound. The low solubility of compound **2** in DCM prevented

the introduction of the 6-azidohexan-1-ol using NIS/TMSOTf as the activator couple at this stage. Therefore, the benzylidene ring was opened prior to glycosylation. Several ring opening protocols were tested, including TfOH and TES in DCM at -78˚C, TFA with TES in DCM and iodine with TES in acetonitrile.[14]–[16] Although both the TfOH and TFA methods did result in the desired product, the yields were low. The solubility of compound **2** proved to be a severe limitation for these methods as the reactions had to be very dilute in order to dissolve it. However, when $BF_3{\cdot}OEt_2$ was used as the Lewis acid together with twelve equivalents of TES, the suspension of **2** immediately turned into a clear yellow solution, allowing it to react.[17] Compound **3** could be obtained in a yield of 61% and the location of the benzyl ether was verified after acetylation of the remaining alcohol and subsequent NMR analysis. Compound **3** was much more soluble in DCM than its precursor and the 6-azidohexan-1-ol linker could be installed selectively in a β-fashion using NIS/TMSOTf as the activator couple to give acceptor **4** with a C3-O naphthyl group in 64% yield.

En route to the corresponding levulinoyl ester protected acceptor **7**, compound **1** was subjected to a Steglich esterification using levulinic acid, DIC and DMAP. The same benzylidene ring opening methods as described above were screened and, like before, the use of $BF_3{\cdot}OEt_2$ as the lewis acid gave **6** in the highest yield (~60%).[17] Reduction of the ketone functionality in the levulinoyl ester, proved to be a major side reaction in these Lewis acid catalyzed reductive reactions. The 6-azidohexan-1-ol linker was introduced selectively in a β-fashion using NIS/TMSOTf as the activator couple resulting in acceptor **7** in 55% yield. A drawback in the synthesis of the levulinoyl protected compounds **5**, **6** and **7** was the similar polarity of these compounds, which hindered purification.

The synthesis of the C3-O benzoyl protected acceptor **10**, started by benzoylation of **1** using benzoyl chloride and DMAP in pyridine, which gave **8** in 81% yield. The benzylidene ketal in **8** was opened regioselectively and this time the TFA and TES couple gave the highest yield of **9** (69%).[15] The 6-azidohexan-1-ol linker could be installed using NIS/TMSOTf as the activator couple in 73% yield. Reversing the order of the ring-opening reaction and condensation reactions, led to a lower overall yield. The C3-O acetyl bearing acceptor **13** was synthesized by first acetylating the free alcohol in **1** using Ac_2O in pyridine and DCM, followed by introduction of the 6-azidohexan-1-ol linker using NIS/TMSOTf as the activator couple. The benzylidene acetal in to so-formed glucosamine (**12**) was opened regioselectively using the TFA/TES method.[15] Unlike the syntheses of the naphthyl, levulinoyl and benzoyl bearing acceptors the efficiency of the acetyl bearing acceptor was highest when the linker was installed prior to the reductive opening of the benzylidene ring.

*Scheme 1: Synthesis of glucosamine acceptors **4, 7, 10** and **13.***



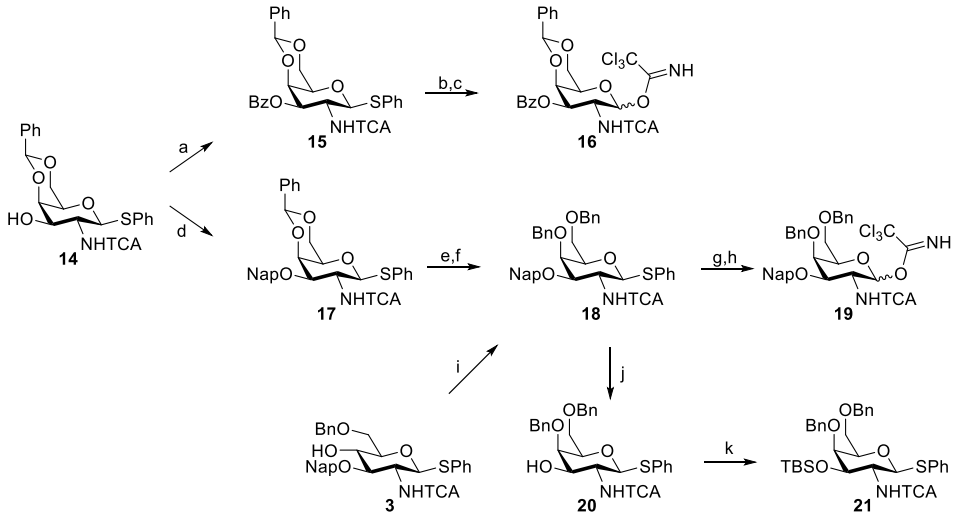
Reagents and conditions: **a**: NaH, Nap-Br, DMF, -20˚C to RT, 84%, **b**: $BF_3{\cdot}OEt_2$, TES, DCM, 0˚C, **3** 61%, **6** 60% **c**: 6-azidohexan-1-ol, NIS, TMSOTf (cat.), DCM, -20˚C to 0˚C, **4** 64%, **7** 55%, **10** 73%, **12** 59%, **d**: Lev-OH, DIC, DMAP, 0˚C to RT, 99%, **e**: TFA, TES, DCM, **9** 69%, **13** 96%, **f**: Bz-Cl, DMAP, DCM, pyr., 81%, **g**: Ac_2O, pyr., DCM, 95%.

In order to arrive at the backbone disaccharide a variety of galactosamine donors was synthesized from known partially protected galactosamine **14** (Scheme 2). Initially, the benzylidene protected donors were explored, but the benzylidene on these donors, however, reduced the reactivity of the donor and therefore the acetal was replaced by two benzyl ethers. Similar to the glucosamine synthon a variety of orthogonal groups were probed for protection of the galactosamine synthon as well. The C3-OH of these synthons were protected with either a naphthyl, benzoyl or tert-butyldimethylsilyl (TBDMS) group.

Benzoylation of the C3-OH in **14** using benzoyl chloride in conjunction with a catalytic amount of DMAP in a mixture of pyridine and DCM resulted in thioglycoside **15**. This thioglycoside donor was turned into imidate donor **16** by hydrolysis of the thiophenyl group with NBS in acetone followed by imidoylation of the formed hemi-acetal.[18] In order to increase the reactivity of the donor it was decided to exchange the benzylidene ring for two benzyl groups.[19] The naphthyl ether at the C3-OH was introduced by treatment of **14** with three equivalents of sodium hydride, followed by addition of naphthyl bromide, which gave compound **17** as in 85% yield. Any attempts to selectively open the benzylidene ring resulted in failure due to the low solubility of **17**. Therefore, the benzylidene was removed using *p*-TsOH in a mixture of dichloroethane and methanol at 50˚C to provide the diol in 80% yield. The subsequent benzylation using benzyl bromide with five equivalents of sodium hydride in DMF also required an elevated temperature of 50˚C , which resulted in donor **18** in 84% yield.

An alternative route to obtain donor **18** starting from the much cheaper glucosamine was investigated as well. The C4-OH of previously synthesized compound **3** was inverted in two steps. First the C4-OH was triflated by treating **3** with triflic anhydride in a mixture of DCM and pyridine.[20] Second the formed triflate was substituted using benzyl alcohol and triethylamine. To this end the mixture was heated to 50˚C and left for 18 hours. Although this method did lead to compound **18**, the yield (40%) was not competitive enough to make this a viable route. The thiophenyl group in fully protected naphthyl donor **18** was hydrolysed using NBS in aqueous acetone. The formed hemiacetal was treated with trichloroacetonitrile and a catalytic amount of DBU in dry DCM, which gave imidate donor **19** in 78% yield over two steps. In order to further increase the reactivity of the donor, the naphthyl group was exchanged for a TBDMS group. The selective removal of the naphthyl ether using DDQ in a mixture of DCM and methanol afforded compound **20** in a 85% yield.[21] The C3-OH of **20** was silylated by treating it with TBDMS-Cl and DMAP in DMF.[22] As the reaction proceeded slowly at room temperature, the temperature was increased to 80˚C, which swiftly led to complete silylation giving donor **21** in 86% yield.

*Scheme 2: Synthesis of galactosamine donors **15**, **16**, **18**, **19** and **21**.*

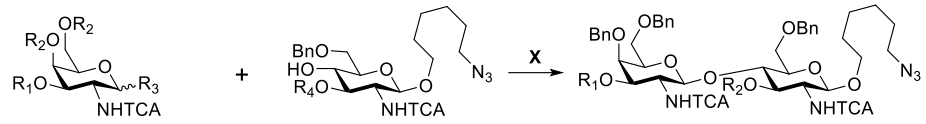


Reagents and conditions: **a**: Bz-Cl, DMAP, pyr., DCM, 99%, **b**: NBS, acetone, H_2O, 92%, **c**: CCl_3CN, DBU (cat.), ACN, 71%, **d**: NaH, Nap-Br, DMF, 85%, **e**: *p*-TsOH, MeOH, DCE, 80%, **f**: NaH, Bn-Br, DMF, 0˚C to 50˚C, 84%, **g**: NBS, acetone, H_2O, 98%, **h**: CCl_3CN, DBU (cat.), ACN, 78%, **i**: 1) Tf_2O, pyr., DCM, 0˚C, 2) Bn-OH, Et_3N, DMF, 50˚C, 40%, **j**: DDQ, DCM, MeOH, 85%, **k**: TBDMS-Cl, pyr, DMAP, DMF, 80˚C, 86%.

An overview of all attempted glycosylations to obtain the LDN backbone is shown in Table 1. Initially, thioglycoside **15** and acceptor **4** were condensed using NIS/TMSOTf as the activator couple (entries 1) but in this reaction the donor formed a stable oxazoline,

which did not react any further. Boutet *et al.* decribed a method to activate TCA oxazoline donors with $CuCl_2$, but this method failed.[23] The combination of donor **17** with acceptor **10** also provided an unproductive glycosylation (entry 2). The TCA oxazoline has been shown to be a good donor to use in β-selective glycosylations.[24] The reason for its apparent stability in this case is probably due the low reactivity of the C4-OH on the acceptor. When imidate donor **16** was combined with **4**, the desired dimer was formed, but separation from formed side products proved impossible (entry 3). Next the more reactive di-benzyl donor **18** was used in a glycosylation with acceptor **10** using NIS/TfOH. Unexpectedly, the β-(1-3) linked product **26** instead of the desired β-(1-4) linked product **22** was obtained (entry 4). Apparently, the C3-O-benzoyl group migrates to the C4-O position under these conditions, liberating the more reactive C3-OH, which in turn reacted with the activated donor.[25], [26] The thioglycoside was exchanged for imidate donor **19**, which reacted within 1 hour, opposed to the >18 hours of the previous entry, and gave the desired β-(1-4) linked product **22** in 45% yield (entry 5). Since the benzoyl proved difficult to remove in a later stage, the other donors were explored next. Condensation of TBDMS bearing donor **21** with naphthyl bearing acceptor **18** using the NIS/TfOH activator couple resulted in the formation of product **23** in 16% and 47% of oxazoline (**18BP**) (entry 6).[27] Several attempts to improve the yield turned out to be unsuccessful (entries 7 and 8). Next, attention was directed to the levulinoyl protected coupling partners. Naphthyl bearing donor **19** and levulinoyl bearing acceptor **7** were condensed using TfOH to give the dimer **24**, which -surprisingly- was formed as a mixture of α/β-anomers with the undesired α-isomer prevailing (α/β = 9/1, entry 9). To prevent the formation of the α-anomer, the solvent was changed from dichloromethane to the more β-directing solvent acetonitrile.[28] The change of solvent did improve the β-selectivity, but, not enough to render these building blocks useful for the construction of the backbone dimer (entries 10 and 11). The last reaction pair tried was naphthyl donor **19** and acetyl acceptor **13**. Like with levulinoyl protected acceptor **7**, acceptor **13** showed a lower β-selectivity, but less than the former (entry 12). The β-selectivity was improved by using a combination of DCM and acetonitrile as the solvent (entries 13-15). Employing this solvent mixture to condense donor **19** and acceptor **13** with TfOH as the activator resulted in the formation of disaccharide **25** in 82% yield (entry 15).

Table 1: Overview of glycosylations to form the LDN backbone.



15 R_1 = Bz, R_2 = CHPh, R_3 = SPh
16 R_1 = Bz, R_2 = CHPh, R_3 =O(N=H)CCl_3
17 R_1 = Nap, R_2 = CHPh, R_3 = SPh
18 R_1 = Nap, R_2 = Bn, R_3 = SPh
19 R_1 = Nap, R_2 = Bn, R_3 = O(N=H)CCl_3
21 R_1 = TBDMS, R_2 = Bn, R_3 = SPh

4 R_4 = Nap
7 R_4 = Lev
10 R_4 = Bz
13 R_4 = Ac

22 R_1 = Nap, R_2 = Bz
23 R_1 = TBDMS, R_2 = Nap
24 R_3 = Nap, R_2 = Lev
25 R_3 = Nap, R_2 = Ac
26 β-(1-3) product

entry	Don.	Acc.	T (˚C)	X[a]	prod.	yield	α/β
1	**15**	**4**	-40 → 0	NIS, TMSOTf	-	-	-
2	**17**	**10**	-40 → 0	NIS, TMSOTf	-	-	-
3	**16**	**4**	-40 → -20	TMSOTf	-	-[b]	-
4	**18**	**10**	-20	NIS, TfOH	**26**	47	0/1
5	**19**	**10**	-20 → 0	TfOH	**22**	45	0/1
6	**21**	**4**	-40 → -20	NIS, TfOH	**23**	16	0/1
7	**21**	**4**	-20	NIS, TfOH	**23**	17	0/1
8	**21**	**4**	0	NIS, TfOH	**23**	5	0/1
9	**19**	**7**	-20	TfOH	**24**	43	9/1
10	**19**	**7**	-20	TfOH[c]	**24**	44	1/9
11	**19**	**7**	0	TfOH[d]	**24**	37	1/3
12	**19**	**13**	-20 → 0	TfOH	**25**	35	1/8
13	**19**	**13**	-40 → -30	TfOH	**25**	62	1/8
14	**19**	**13**	-40 → -30	TfOH[d]	**25**	65	1/20
15	**19**	**13**	-40 → -30	TfOH[c]	**25**	82	1/20

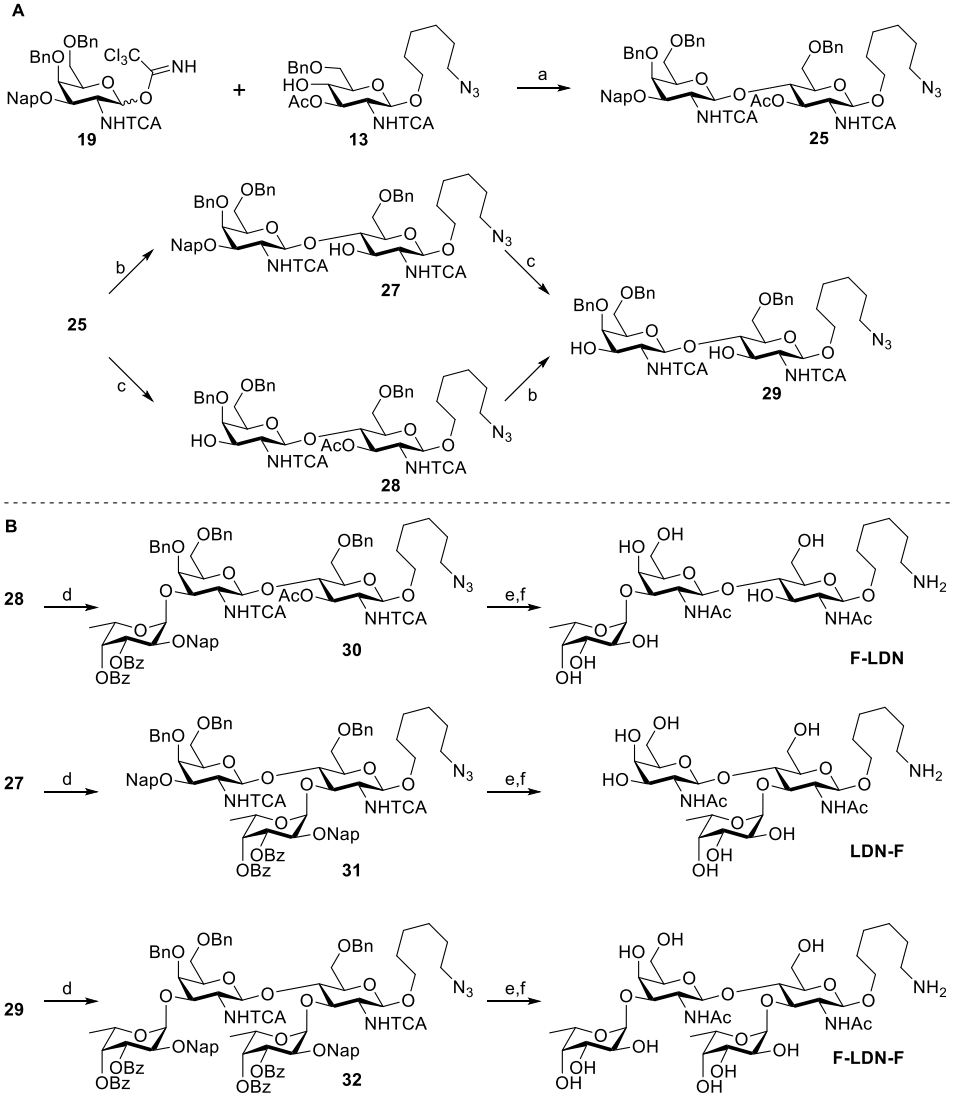
[a] All reactions were performed in presence of freshly dried molecular sieves (3Å) at a concentration of 0.1M under inert atmosphere in DCM unwise indicated otherwise
[b] obtained as an inseparable mixture.
[c] reaction was performed in a mixture of DCM/ACN, 4/1, v/v.
[d] reaction was performed in ACN

In order to obtain the three desired backbone acceptors, the orthogonal protective groups on either the C3-O, or C'3-O or both had to be removed (Scheme 3A). Disaccharide **22** was subjected to Zemplén conditions to hydrolyze the benzoyl group. Unfortunately, the benzoyl group proved to be very stable, presumably due to steric hindrance. When the temperature was increased, the TCA groups were hydrolyzed as well, reducing the usefulness of the benzoyl group. The hydrolysis of the more labile acetyl group on **25** under Zemplén conditions proceeded sluggishly as well. Therefore, sodium hydroxide, a smaller nucleophile was used. Although this did increase both the

reaction speed and the yield of compound **27** (58%), a significant amount of TCA removal was still observed. In order to obtain disaccharide **28** the naphthyl group was selectively removed by DDQ oxidation. This reaction proceeded very sluggishly, and the desired disaccharide was obtained in a low yield (16%). Addition of β-pinene did not significantly improve the yield, and therefore a different protocol was employed, which involves the use of HCl in HFIP with triethylsilane as a scavenger.[29] These conditions led to the selective removal of the naphthyl group giving disaccharide **28** in a 55% yield.[30] Approximately 40% of unreacted starting material was recovered and this could be retreated with the same conditions. Leaving the reaction for longer than one hour led to significant byproduct formation. Compound **28** had to be stored at -20˚C in order to prevent degradation. Diol acceptor **29** was synthesized from either **27** or **28**, by either hydrolyzing the ester or removing the naphthyl ether. Both reactions proceeded in similar yields as before.

With the properly protected dimers **27**-**29** available the fucosyl residues were introduced using the optimized protocol that is described in Chapter 2. The disaccharide backbones were condensed using two equivalents of fucosyl donor **A** (Figure 2) per hydroxyl present on the dimer acceptor, and NIS/TMSOTf as the activator couple (Scheme 3B). This resulted in trisaccharides **30** and **31** and tetrasaccharide **32** in yields ranging between 65% and 69%. NMR analysis confirmed the selective formation of *cis* linkages ($^{3}J_{1,2}$ = 3.6 Hz and $^{1}J_{C\text{-}1,\,H\text{-}1}$ = 170 Hz).[31] Of note, the C-2 peak of trisaccharide **31** and tetrasaccharide **32** was barely visible on their ^{13}C-APT NMR at room temperature.

Scheme 3: A) Synthesis of LDN acceptors, B) Synthesis of (F)-LDN-(F) fragments.



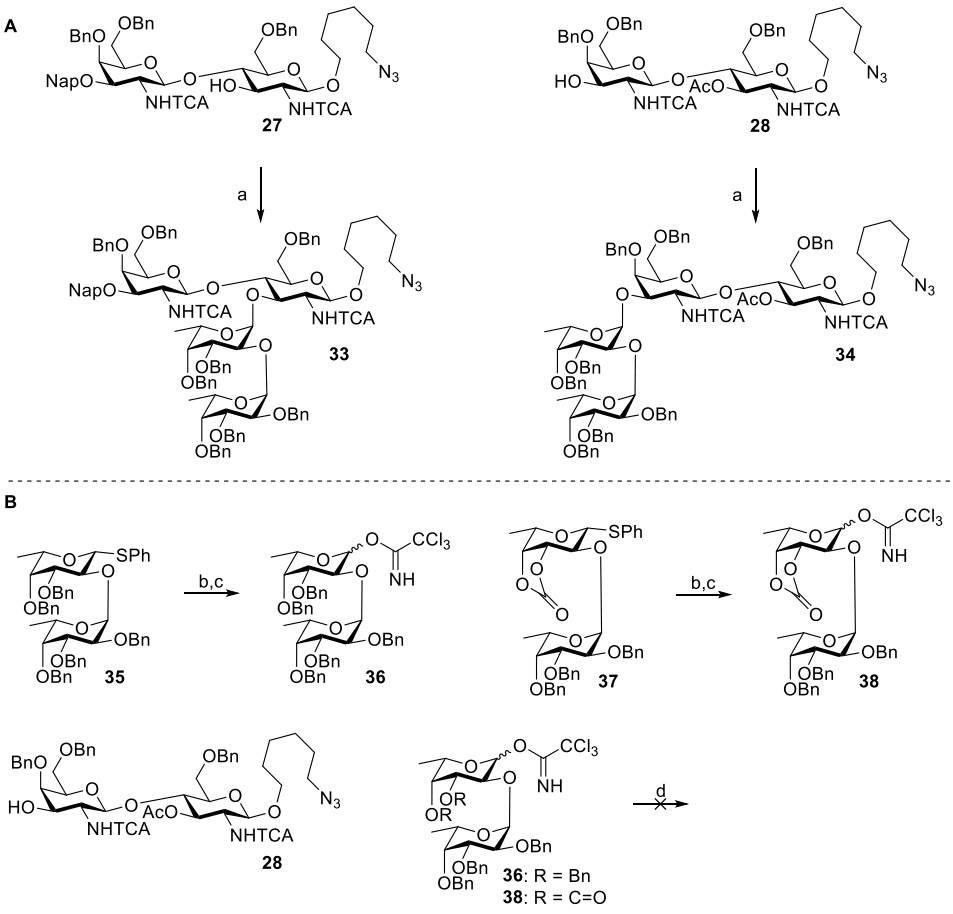
Reagents and conditions: **a**: **13**, TfOH, MS (3Å), DCM, ACN, -40 ˚C to -20˚C, 82% , **b**: NaOH, dioxane, H_2O, **27** 58%, **29** 47% **c**: HCl, HFIP, DCM, **28** 55%, **29** 61%, **d**: **A**, NIS, TMSOTf, MS (3Å), DCM, -40˚C→ -20˚C, **30** 65%, **31** 69%, **32** 69%, **e**: NaOMe (cat.), THF/MeOH, **30** 64%, **31** 65%, **32** 63%, **f**: Pd/C (cat.) H_2, dioxane, H_2O, **F-LDN** 38%, **LDN-F** 24%, **F-LDN-F** 24%.

Finally, the protective groups on **30-32** were removed in two steps. First, the esters were hydrolysed by sodium methoxide in a mixture of THF and MeOH. Although the benzoyl esters could all be removed by stirring overnight at room temperature, the removal of the acetyl group on the C3-O position required the temperature to be increased to 40˚C. As benzoyl groups are generally less susceptible to hydrolysis than acetyl groups, this

indicates that the C3-O is in a difficult position to react because of steric congestion, as also found in the deprotection reactions described above. Second, hydrolysed compounds **30-32** were subjected to catalytic hydrogenation. This converted the azide into a free amine, the TCA into an acetyl and removed the benzyl and naphthyl ethers. **F-LDN**, **LDN-F** and **F-LDN-F** were obtained in yields of 38%, 25% and 24%. Besides the desired products significant amounts of de-fucosylated products were observed, which were probably formed due to acidic hydrolysis of the fucosyl linkages as a result of the HCl that was released by the reduction of the TCA groups.

Lastly, the synthesis of LDN bearing di-fucosyl side chains was undertaken. To this end, the fucosyl dimers were attached to the C3-OH positions on the LDN backbones employing a [2+2] block coupling approach (Scheme 4). The optimized coupling method, described in Chapter 3, using two equivalents of fucosyl donor **35** with IDCP as the activator, was applied. Unfortunately, the condensations with acceptors **27** and **28** proceeded with poor yields (7% for **33** and 14% for **34**).

Scheme 4: A) Synthesis of (F2)-LDN-(F2) fragments, B) synthesis of imidate di-fucosyl donors and attempted glycosylations.

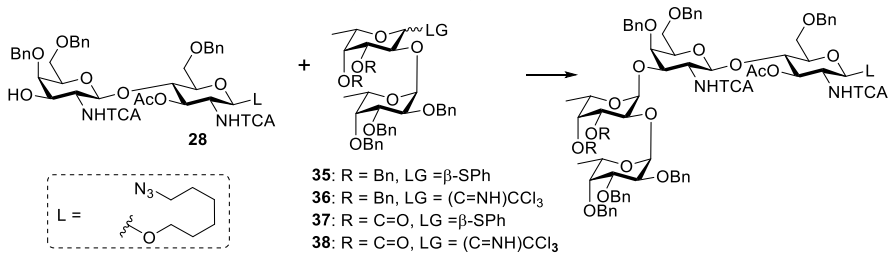


Reagents and conditions: **a**: **35**, IDCP, MS (3Å), DCM, 0˚C → RT, **33** 7%, **34** 14%, **b**: NBS, H_2O, acetone, 71% (from **35**), 64% (from **37**), **c**: CCl_3CN, DCM, DBU (cat.) **36** 97%, **38** 99%, **d**: **36** or **38**, TMSOTf (cat.), MS (3Å), DCM, -40˚C → -20˚C.

To improve the yield of the difucosylated tetrasaccharides, several glycosylation procedures were evaluated, the results of which are summarized in Table 2. In this study, acceptor **28** was used for optimization as it was reasoned that the C3'-OH is more accessible than the C3-OH. Initially the mild IDCP activation method was replaced by the pre-activation method using diphenylsulfoxide (DPS), 2,4,6-tri-*tert*-butylpyrimidine (TTBP) and triflic anhydride (entry 2).[32] Although this did result in a net higher yield of tetrasaccharide **34**, the stereoselectivity of the reaction was lower and the tetrasaccharide was isolated as an anomeric mixture. Bennet and co-workers showed that the α-selectivity of the pre-activation protocol could be increased by addition of TBAI (entry 3).[33] Unfortunately, when these glycosylation conditions were used no

product formation was observed. Next, thioglycoside **35** was turned into imidate **36**, by first hydrolyzing the thiophenyl group, followed by imidoylation of the formed hemi-acetal, to give imidate donor **36** in 71% over two steps.[34] Condensation of donor **36** with acceptor **28** by addition of a catalytic amount of TMSOTf was disappointing, as no significant quantity of the target compound could be isolated (entry 4). Besides activated donor **35**, disarmed di-fucosyl donor **37** was explored. The condensation of thioglycoside **37** with acceptor **28** using the NIS/TMSOTf activator couple (entry 5) resulted in the formation of the succinimide adduct of **37** (not isolated). In order to prevent the formation of this adduct, thioglycoside **37** was converted to an imidate. A similar method was used by hydrolyzing the thiophenyl group using NBS in aqueous acetone. The formed hemi-acetal was then imidoylated using trichloroacetonitrile with a catalytic amount of DBU to give imidate **38** in a yield of 64% over two steps.[34] Acceptor **28** was condensed with imidate donor **38**, and although LC-MS indicated product formation, the product could not be isolated in sufficient quantities for NMR analysis (entry 6).

Table 2: Optimization of the introduction of the di-fucosyl chains



entry	donor	reagents	Conditions[a]	product	Yield (%)
1	**35**	IDCP	0.2M, 0˚C → RT	**34**	14
2	**35**	DPS, TTBP, Tf_2O	0.05M, -80˚C → 0˚C	**34**[b]	20
3	**35**	DPS, TTBP, Tf_2O, TBAI	0.05M, -80˚C → RT	-	-
4	**36**	TMSOTf	0.1M, -40˚C → 0˚C	-	trace[c]
5	**37**	NIS, TMSOTf	0.1M, -20˚C → RT	-	-
6	**38**	TMSOTf	0.1M, -40˚C → 0˚C	-	trace[c]

[a] all reactions were performed under inert atmosphere in dry DCM, with MS (3Å) present using 0.05 mmol of acceptor **28**.

[b] Obtained as an anomeric mixture (α/β, 1/1).

[c] the mass was observed on LC-MS but not enough could be isolated for NMR.

As both armed and disarmed donors were used, it is assumed that the reactivity of the acceptor is the leading cause of the low yields. It has been documented that the reactivity of C3-OH glucosamine derivatives, with an amide on the C2 position is significantly lower due to both inter- and intra- molecular hydrogen bonding and similar effects may play a role in the galactosamine system studied here.[10], [35], [36] Of note, Kanaya *et al.* managed to install a di-fucosyl moiety on a similar backbone, but they used Troc groups instead of a TCA groups to mask the amines.[37]

Conclusion

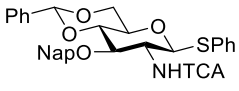
In order to synthesize a small library of fucosylated LDN fragments depicted in Figure 1, five different GlcNAc acceptors and five different GalNAc donors were synthesized, both bearing an orthogonal protecting group on the C3-O position. The synthesis of the LDN backbone proved to be troublesome, requiring reactive benzyl ether prptected donors to avoid oxaziline formation. Of the different protecting groups explored on the GlcNAc building block (naphthyl, benzoyl, levulinoyl and acetyl), the benzoyl showed the best stereoselectivity but this group proved difficult to remove at a later stage. The levulinoyl protected acceptor surprisingly led to the selective formation of the α-linked products, in spite of the TCA group in the donor glycosides. The acetyl protected acceptor showed a bit lower β-selectivity in comparison to its benzoyl counterpart, but the lower stereoselectivity could be remedied by using acetonitrile as a co-solvent. Mono-fucosides could be introduced on the GalN-GlcN backbone using NIS/TMSOTf mediated to provide tri- and tetrasaccharides that were successfully deprotected to obtain F-LDN, LDN-F, and F-LDN-F. The di-fucosyl chains could not be installed and the exact reason for this is currently attributed to steric hindrance.

Experimental

General

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

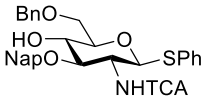
Phenyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(2-methylnaphthyl)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (2)



NaH (0.12 g, 3.0 mmol, 3.0 eq.) was added portionwise to a solution of **1** (0.50 g, 1.0 mmol, 1.0 eq.) in dry DMF (10 mL, 0.1M) at -20˚C. The reaction was left to stir until the evolution of gas stopped, at which point the Nap-Br (0.24 g, 1.1 mmol, 1.1 eq.) was added. The solution was allowed to warm up to RT. Upon completion (~3 hours) the reaction mixture was slowly poured into H_2O. The white precipitate was filtered over a glass filter, washed with H_2O (3x) and cold Et_2O (3x). This gave the title compound as a white solid (0.54 g, 0.84 mmol, 84%). ^{1}H NMR (Acetone-d_6, 400 MHz): δ = 8.59 (d, 1H, *J*=8.4 Hz, NH), 7.93 – 7.64 (m, 4H, arom.), 7.61 – 7.45 (m, 7H, arom.), 7.45 – 7.22 (m, 6H, arom.), 5.78 (s, 1H,

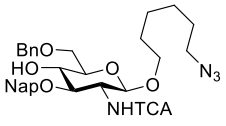
CHPh), 5.24 (d, 1H, *J*=9.8 Hz, H-1), 5.05 (d, 1H, *J*=12.1 Hz, CH_2Nap), 4.95 (d, 1H, *J*=11.5 Hz, CH_2Nap), 4.33 (dd, 1H, *J*=10.3, 5.0 Hz, H-6), 4.24 – 4.04 (m, 2H, H-2, H-3), 4.02 – 3.84 (m, 2H, H-4, H-6), 3.60 (td, 1H, *J*=9.8, 5.0 Hz, H-5) ppm. ^{13}C-APT NMR (Acetone-d_6, 101 MHz): δ 161.5 (C=O, TCA), 138.0, 136.1, 133.3, 133.1, 131.7, 129.0, 128.7, 128.1, 127.8, 127.6, 127.6, 126.3, 126.2, 126.1, 126.0, 125.7 (arom.), 101.0 (CHPh), 87.2 (C-1), 81.8 (C-3), 79.2 (C-4), 74.2 (CH_2Nap), 70.4 (C-5), 68.1 (C-6), 56.0 (C-2) ppm. HRMS $[M+NH_4]^+$ calcd for $C_{32}H_{28}Cl_3O_5SNH_4$: 661.10920, found 661.11008.

Phenyl 6-*O*-benzyl-2-deoxy-3-*O*-(2-methylnaphthyl)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (3)



Compound **2** (3.03 g, 4.7 mmol, 1.0 eq.) was suspended in DCM (47 mL, 0.1M) and the flask was cooled to 0˚C. TES (9.0 mL, 56.4 mmol, 12 eq.) was added followed by dropwise addition of $BF_3{\cdot}OEt_2$ (1.16 mL, 9.40 mmol, 2.0 eq.). The suspension was stirred at 0˚C until all was dissolved, at which point TLC analysis showed full conversion of the starting material. The solution was diluted with EtOAc and washed with sat. $NaHCO_3$ (aq.) and brine, followed by drying over $MgSO_4$, filtered and concentrated. Pure **3** was obtained after purification by silicagel chromatography (tol:EtOAc, 1:0 → 4:1) as a white solid (1.84 g, 2.84 mmol, 61%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.99 – 7.68 (m, 4H, arom.), 7.62 – 7.42 (m, 5H, arom.), 7.42 – 7.13 (m, 8H, arom.), 6.87 (d, 1H, *J*=8.0 Hz, NH), 5.15 (d, 1H, *J*=10.3 Hz, H-1), 4.92 (q, 2H, *J*=11.3 Hz, CH_2arom), 4.74 – 4.45 (m, 2H, CH_2arom), 4.01 (dd, 1H, *J*=10.0, 8.5 Hz, H-3), 3.92 – 3.69 (m, 3H, H-4, H-6), 3.66 – 3.45 (m, 2H, H-2, H-5), 2.84 (d, 1H, *J*=2.6 Hz, 3-OH) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 161.7 (C=O, TCA), 137.7, 135.3, 133.4, 133.3, 133.2, 132.0, 129.2, 128.7, 128.5, 128.1, 128.1, 127.9, 127.8, 127.2, 126.3, 126.2, 126.1 (arom.), 85.0 (C-1), 81.3 (C-3), 78.0 (C-5), 75.2 (CH_2arom), 73.9 (CH_2arom), 73.5 (C-4), 70.7 (C-6), 56.7 (C-2) ppm.

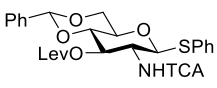
6-azidohexyl 6-*O*-benzyl-2-deoxy-3-*O*-(2-methylnaphthyl)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (4)



Donor **3** (0.97 g, 1.61 mmol, 1.0 eq.) and 6-azidohexan-1-ol (0.3 g, 2.1 mmol, 1.3 eq.) were co-evaporated together thrice with dry toluene, before dissolving them in dry DCM (16 mL, 0.1M). Freshly dried MS (3Å) were added and the mixture was stirred for 15 min. at room temperature. Next NIS (0.43 g, 1.93 mmol, 1.2 eq.) was added and the mixture was cooled to -20˚C, at which temperature it was stirred for an additional 30 min. TMSOTf (58 µL, 0.32 mmol, 0.2 eq.) was added and the mixture was allowed to warm up to 0˚C and kept at that temperature. After 2 hours the reaction was stopped by addition of Et_3N (0.5 mL). The reaction mixture was diluted in EtOAc, washed twice with sat. $Na_2S_2O_3$ (aq.), followed by sat. $NaHCO_3$ (aq.) and brine. After drying over $MgSO_4$ and

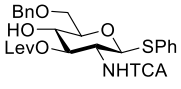
filtration the solvents were removed by evaporation. The title compound was separated from byproducts by silicagel chromatography (tol:EtOAc, 1:0 → 4:1) and isolated as a white solid (1.03 g, 0.64 mmol, 64%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.87 – 7.66 (m, 4H, arom.), 7.53 – 7.42 (m, 3H, arom.), 7.42 – 7.22 (m, 5H, arom.), 6.98 (d, 1H, *J*=7.7 Hz, NH), 5.07 – 4.85 (m, 2H, CH_2arom), 4.83 (d, 1H, *J*=8.2 Hz, H-1), 4.57 (q, 2H, *J*=12.0 Hz, CH_2arom), 4.04 (dd, 1H, *J*=10.5, 8.5 Hz, H-3), 3.84 (dt, 1H, *J*=9.6, 6.2 Hz, OCH_2), 3.79 – 3.62 (m, 3H, H-4, H-6), 3.59 – 3.48 (m, 2H, H-2, H-5), 3.43 (dt, 1H, *J*=9.6, 6.7 Hz, OCH_2), 3.21 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.93 (s, 1H, 4-OH), 1.62 – 1.46 (m, 4H, CH_2, hexyl), 1.39 – 1.26 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 162.0 (C=O, TCA), 137.6, 135.5, 133.3, 133.1, 128.6, 128.5, 128.1, 128.0, 127.9, 127.8, 127.0, 126.3, 126.1, 126.1 (arom.), 99.5 (C-1), 92.6 (C_q, TCA), 79.6 (C-3), 74.8 (CH_2arom), 73.8 (CH_2arom), 73.7 (C-5), 73.6 (C-4), 70.6 (C-6), 69.9 (OCH_2), 58.3 (C-2), 51.4 (CH_2N_3), 29.5, 28.8, 26.5, 25.6 (CH_2, hexyl) ppm. HRMS $[M+H]^+$ calcd for $C_{32}H_{37}Cl_3N_4O_6H$: 679.18587, found 679.18846.

Phenyl 3-*O*-levulinoyl-4,6-*O*-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (5)



Sugar **1** (8.6 g, 17,1 mmol, 1.0 eq.) was dissolved in DCM (60 mL, 0.3M) and cooled to 0˚C. Levulinic acid (5.9 g, 51.3 mmol, 3.0 eq.) was added to this solution followed by DIC (5.0 g, 24 mmol, 1.4 eq.) and DMAP (0.22 g, 1.71 mmol, 0.1 eq.). The solution was left to stir at RT for 1 hour, when TLC analysis showed complete conversion. The reaction mixture was diluted in EtOAc and washed thrice with sat. $CuSO_4$ (aq.) , thrice with sat. $NaHCO_3$ (aq.) and once with brine. The organic layer was dried over $MgSO_4$, filtered and the solvents were removed under reduced pressure. The obtained brown oil was purified via column chromatography (PE:EtOAc, 9:1 → 6:4) to afford compound as a white solid (10.3 g, 17.1 mmol, 99 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.54 – 7.38 (m, 3H, arom.), 7.38 – 7.22 (m, 9H, NH, arom.), 5.57 – 5.42 (m, 2H, H-3, CHPh), 4.95 (d, 1H, *J*=10.4 Hz, H-1), 4.17 (dd, 1H, *J*=10.4, 4.9 Hz, H-6), 4.04 (q, 1H, *J*=10.2, 9.3 Hz, H-2), 3.86 – 3.66 (m, 3H, H-4, H-6), 3.55 (dt, 1H, *J*=9.5, 4.8 Hz, H-5), 2.81 – 2.47 (m, 4H, CH_2, Lev), 2.09 (s, 3H, CH_3, Lev) ppm. ^{13}C-APT NMR ($CDCl_3$, 75 MHz) δ 133.0, 129.3, 129.2, 128.5, 128.3, 126.3, 126.2 (arom.), 101.4 (CHPh), 87.3 (H-1), 78.5 (C-3), 72.5 (C-5), 70.8 (C-4), 68.4 (C-6), 55.2 (C-2), 38.1 (CH_2, Lev), 28.2 (CH_2, Lev), 23.5 (CH_3, Lev). HRMS $[M+H]^+$ calcd for $C_{26}H_{30}Cl_3NO_7SNa$: 624.03932, found 624.04485.

Phenyl 3-*O*-levulinoyl-6-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (6)

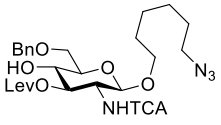


Sugar **5** (1 mmol, 0.6 g, 1.0 eq.) was co-evaporated thrice with toluene before dissolving it in dry DCM (10 mL, 0.1M). Molecular sieves (3Å) were added and the solution was left to stir at RT under inert

atmosphere for 30 min. TES (1.93 mL, 12 mmol, 12 eq.) was added and the solution was again left to stir for 10 minutes under inert atmosphere. $BF_3{\cdot}OEt_2$ (0.24 mL, 2 mmol, 2.0 eq.) was added and the solution was left to stir for 30 minutes. When TLC analysis showed complete conversion, the reaction mixture was diluted in EtOAc and washed with 1M HCl (aq.), thrice with sat. $NaHCO_3$ (aq.) and once with brine. The organic layer was dried over $MgSO_4$, filtered and the solvents were removed under reduced pressure. The obtained liquid was dissolved in 10 mL DCM (0,1M), to this Trt-Cl (0.31 g, 1.1 mmol, 1.1 eq.) and triethylamine (0.21 mL, 1.5 mmol, 1.5 eq.) were added and the mixture was left to stir overnight. The solvents were removed under reduced pressure and the obtained liquid was purified by column chromatography (tol:EtOAc, 1:0 → 6:4) to afford compound as a white solid (0.36 g, 0.6 mmol, 60%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.61 – 7.41 (m, 2H, arom.), 7.43 – 7.08 (m, 9H, NH, arom.), 5.36 (t, 1H, *J*=10.0, 8.5 Hz, H-3), 4.84 (d, 1H, *J*=10.3 Hz, H-1), 4.64 – 4.48 (m, 2H, CH_2Bn), 4.01 (q, 1H, *J*=10.1 Hz, H-2), 3.88 – 3.65 (m, 4H, H-4, H-5, H-6), 3.55 (s, 1H, 4-OH), 2.68 (t, 2H, *J*=6.5 Hz, CH_2, Lev), 2.59 – 2.31 (m, 2H, CH_2, Lev), 2.05 (s, 3H, CH_3, Lev) ppm. ^{13}C-APT NMR ($CDCl_3$, 75 MHz) δ 207.9 (CH_3C=O, Lev), 173.7 (C=O, Lev), 161.9 (C=O, TCA), 138.1, 132.5, 132.5, 132.5, 129.0, 128.5, 128.0, 127.8, 127.7 (arom.), 92.5 (C_q, TCA), 85.9 (C-1), 78.7 (C-5), 76.8 (C-3), 73.6 (CH_2Bn), 69.8 (C-6), 69.7 (C-4), 54.2 (C-2), 38.3 (CH_2, Lev), 29.8 (CH_3, Lev), 28.3 (CH_2, Lev) ppm. HRMS $[M+H]^+$ calcd for $C_{26}H_{28}Cl_3NO_7SNa$: 626.05498, found 626.05394.

4

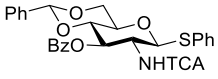
6-azidohexyl 3-*O*-levulinoyl-6-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (7)



Donor **6** (0.16 g, 0.26 mmol, 1.0 eq.) and 6-azidohexan-1-ol (0.074 g, 0.52 mmol, 2.0 eq.) were co-evaporated together thrice with dry toluene, before dissolving them in dry DCM (2.5 mL, 0.1M). Freshly dried MS (4Å) were added and the mixture was stirred for 15 min. at room temperature. Next NIS (0.071 g, 0.31 mmol, 1.2 eq.) was added and the mixture was cooled to -20˚C, at which temperature it was stirred for an additional 30 min. TMSOTf (52 µL, 0.55 mmol, 0.2 eq., of a 0.1M in DCM) was added and the mixture was allowed to warm up to 0˚C. After 2 hours the reaction was stopped by addition of Et_3N (0.1 mL). The reaction mixture was diluted in EtOAc, washed twice with sat. $Na_2S_2O_3$ (aq.), followed by sat. $NaHCO_3$ (aq.) and brine. After drying over $MgSO_4$ and filtration the solvents were removed by evaporation. The title compound was separated from byproducts by silicagel chromatography (PE:EtOAc, 9:1 → 1:1) and isolated (0.092 g, 0.14 mmol, 55%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.37 – 7.26 (m, 5H, arom.), 7.13 (d, 1H, *J*=9.1 Hz, arom.), 5.27 (dd, 1H, *J*=10.8, 8.8 Hz, H-3), 4.67 – 4.49 (m, 3H, H-1, CH_2Bn), 4.03 – 3.69 (m, 5H, H-2, H-4, H-6, OCH_2), 3.69 – 3.53 (m, 1H, H-5), 3.46 (m, 2H, OCH_2, 4-OH), 3.23 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.76 (m, 2H, CH_2, Lev), 2.63 – 2.44 (m, 2H, CH_2, Lev), 2.15 (s, 3H, CH_3, Lev), 1.63 – 1.49 (m, 4H, CH_2, hexyl), 1.41 – 1.30 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT

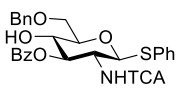
NMR ($CDCl_3$, 75 MHz): δ 207.8 (CH_3C=O, Lev), 173.5 (C=O, Lev), 162.1 (C=O, TCA), 128.5, 127.9, 127.8 (Ph), 100.7 (C-1), 75.4 (C-3), 74.5 (C-5), 73.7 (CH_2Bn), 70.2 (C-4), 69.8 (C-6, OCH_2), 55.7 (C-2), 51.4 (OCH_2N_3), 38.4 (CH_2, Lev), 29.5 (CH_2, Lev), 28.8, 28.3, 26.5, 25.6 (CH_2, hexyl) ppm.

Phenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (8)



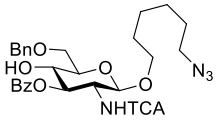
Benzoyl chloride (1.4 mL, 12.0 mmol, 2 eq.) was slowly added to a solution of **1** (3.0 g, 5.95 mmol, 1.0 eq.) in DCM and pyridine (60 mL, 0.1M, 3/1, v/v). The reaction was stirred for 1 hour at room temperature. After this time MeOH (2 mL) was added and the reaction was diluted in EtOAc. The organic layer was washed with sat. $CuSO_4$ (aq., 3x), sat. $NaHCO_3$ (aq., 1x) and brine, before drying over $MgSO_4$, filtration and concentration *in vacuo*. The crude mixture was purified by silicagel chromatography (PE:EtOAc, 99:1 → 4:1) and isolated as a white solid (2.94 g, 4.8 mmol, 81%). Spectral data was in accordance with those reported previously.[38]

Phenyl 3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (9)



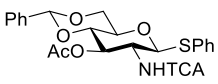
Compound **8** (0.065 g, 0.10 mmol, 1.0 eq.) was dissolved in dry DCM (1.1 mL, 0.1M) and the solution was cooled to 0˚C. TES (68 µL, 0.42 mmol, 4.0 eq.) and TFA (32 µL, 0.42 mmol, 4.0 eq.) were added after 3 hours of stirring at 0˚C. TLC analysis showed complete consumption of the starting material. The reaction mixture was transferred to a separatory funnel, diluted with EtOAc, washed thrice with sat. $NaHCO_3$ (aq.) and once with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Silicagel chromatography (PE:EtOAc, 99:1 → 7:3) gave the title compound as a colourless oil (0.045 g, 0.074 mmol, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.05 – 7.72 (m, 2H, arom.), 7.66 – 7.46 (m, 4H, NH, arom.), 7.46 – 7.04 (m, 10H, arom.), 5.78 (t, 1H, *J*=10.2 Hz, H-3), 4.99 (d, 1H, *J*=10.3 Hz, H-1), 4.67 – 4.43 (m, 2H, CH_2Bn), 4.27 (q, 1H, *J*=10.1 Hz, H-2), 3.92 (m, 1H, H-4), 3.88 – 3.70 (m, 3H, H-5, H-6), 3.23 (s, 1H, 4-OH) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 167.9 (C=O, Bz), 162.1 (C=O, TCA), 137.7, 133.9, 132.8, 132.4, 130.0, 129.1, 128.8, 128.7, 128.6, 128.2, 128.0, 127.8 (arom.), 92.3 (C_q, TCA), 86.7 (C-1), 78.3 (C-5), 77.5, 77.2, 76.9, 76.8 (C-3), 73.8 (CH_2Benzyl), 70.8 (C-4), 70.3 (C-6), 54.4 (C-2) ppm. HRMS $[M+Na]^+$ calcd for $C_{28}H_{26}Cl_3NO_6SNa$: 632.04441, found 632.04386.

6-azidohexyl 3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (10)



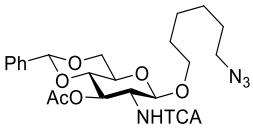
Donor **9** (1.68 g, 2.75 mmol, 1.0 eq.) and 6-azidohexan-1-ol (0.59 g, 4.12 mmol, 1.5 eq.) were co-evaporated together thrice with dry toluene, before dissolving them in dry DCM (27 mL, 0.1M). Freshly dried MS (3Å) were added and the mixture was stirred for 15 min. at room temperature. Next NIS (0.93 g, 4.12 mmol, 1.5 eq.) was added and the mixture was cooled to 0˚C, at which temperature it was stirred for an additional 30 min. TMSOTf (100 µL, 0.55 mmol, 0.2 eq.) was added and the mixture was stirred at 0˚C. After 2 hours the reaction was stopped by addition of Et_3N (0.5 mL). The reaction mixture was diluted in EtOAc, washed twice with sat. $Na_2S_2O_3$ (aq.), followed by sat. $NaHCO_3$ (aq.) and brine. After drying over $MgSO_4$ and filtration the solvents were removed by evaporation. The title compound was separated from byproducts by silicagel chromatography (PE:EtOAc, 19:1 → 4:1) and isolated (1.28 g, 2.0 mmol, 73%). Spectral data was in accordance with those reported previously.[38]

Phenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (11)



Acetic anhydride (0.94 mL, 10 mmol, 2.0 eq.) was added to a solution containing sugar **1** (2.52 g, 5.0 mmol, 1.0 eq.) and pyridine (1.2 mL, 15 mmol, 3eq.). The mixture was left to stir at room temperature for 2 hours, diluted in EtOAc and transferred to a separatory funnel. The organic layer was washed with sat. $CuSO_4$ (aq., 3x), sat. $NaHCO_3$ (aq.) and brine, before being dried over $MgSO_4$, filtered and concentrated. Compound **11** was obtained after purification by silicagel chromatography (PE:EtOAc, 19:1 → 4:1) as a white solid (2.60 g, 4.74 mmol, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.53 (d, 1H, *J*=9.7 Hz, NH), 7.49 – 7.42 (m, 2H, arom.), 7.42 – 7.35 (m, 2H, arom.), 7.35 – 7.27 (m, 5H, arom.), 7.27 – 7.20 (m, 3H, arom), 5.55 (t, 1H, *J*=9.8 Hz, H-3), 5.47 (s, 1H, CHPh), 4.79 (d, 1H, *J*=10.4 Hz, H-1), 4.17 (q, 1H, *J*=10.0 Hz, H-2), 3.98 (dd, 1H, *J*=10.5, 4.7 Hz, H-6), 3.67 (t, 2H, *J*=9.8 Hz., H-4, H-6), 3.50 (td, 1H, *J*=9.6, 4.8 Hz, H-5), 2.05 (s, 3H, CH_3, Ac) ppm ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 171.9 (C=O, Ac), 162.1 (C-O, TCA), 136.9, 133.1, 132.2, 129.2, 128.5, 128.3, 126.0 (arom.), 101.1 (ChPh), 87.8 (C-1), 78.4 (C-3), 72.9 (C-5), 70.7 (C-4), 68.3 (C-6), 54.8 (C-2), 21.0 (CH_3) ppm.

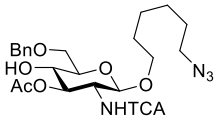
6-azidohexyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (12)



Compound **11** (3.03 g, 6.0 mmol, 1.0 eq.) was dissolved in EtOAc (20 mL, 0.3M) and cooled to 0˚C. Pyridine (1.5 mL, 18 mmol, 3.0 eq.) and Ac_2O (1.13 mL, 12.0 mmol, 2.0 eq.) were added and the ice bath was removed. TLC analysis showed full conversion after 2 hours and the reaction was diluted in EtOAc and transferred to a separatory funnel. The organic layer was washed with sat. $CuSO_4$ (aq., 3x), sat. $NaHCO_3$ (aq., 3x) and brine, followed by drying over $MgSO_4$, filtration and concentration. The yellow solid was purified by silicagel chromatography (tol:ACN, 1:0 → 9:1) to give **12** as a white solid (3.32 g, 5.73 mmol, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.86 (d, 1H, *J*=9.7 Hz, NH), 7.43 (dd, 2H, *J*=7.1, 2.6 Hz, arom.), 7.33 (dd, 3H, *J*=5.2, 2.1 Hz, arom.), 5.57 (t, 1H, *J*=10.1 Hz, H-3), 5.49 (s, 1H, CHPh), 4.33 (d, 1H, *J*=8.3 Hz, H-1), 4.28 – 4.02 (m, 2H, H-2, H-5), 3.84 – 3.66 (m, 2H, H-4, H-6), 3.66 – 3.46 (m, 2H, H-6, OCH_2), 3.23 (t, 2H, *J*=6.9 Hz, CH_2N_3), 3.14 (dt, 1H, *J*=9.7, 6.5 Hz, OCH_2), 2.11 (s, 3H, Ac), 1.69 – 1.41 (m, 3H, CH_2, hexyl), 1.41 – 1.20 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 171.9 (C=O), 162.4 (C=O, TCA), 137.2, 128.8, 128.1, 125.7 (arom.), 101.5 (C-1), 100.8 (CHPh), 92.8 (C_q, TCA), 78.8 (C-4), 72.0 (C-3), 70.1 (OCH_2), 68.3 (C-6), 65.7 (C-5), 55.4 (C-2), 51.3 (CH_2N_3), 29.2, 28.7, 26.4, 25.4 (CH_2-hexyl), 20.8 (CH_3, Ac) ppm. HRMS $[M+Na]^+$ calcd for $C_{23}H_{29}Cl_3N_4O_7Na$: 601.09995, found 601.09940.

4

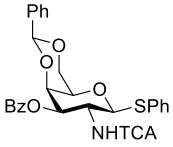
6-azidohexyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (13)



Compound **12** (3.3 g, 5.7 mmol, 1.0 eq.) was dissolved in dry DCM (60 mL, 0.1M). Freshly dried molecular sieves (3Å) were added and the solution was cooled to 0˚C. TES (4.6 mL, 28.7 mmol, 5.0 eq.) and TFA (2.2 mL, 28.7 mmol, 5.0 eq.) were added after 1 hour of stirring at 0˚C. After 2.5 hours TLC analysis showed spot to spot conversion to a more polar compound. The reaction mixture was transferred to a separatory funnel, diluted with DCM, washed thrice with sat. $NaHCO_3$ (aq.) and once with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Silicagel chromatography (PE:EtOAc, 4:1 → 1:1) gave the title compound as a colourless oil (3.2 g, 5.5 mmol, 96%).^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.44 – 7.28 (m, 5H, arom.), 7.02 (d, 1H, *J*=9.2 Hz, NH), 5.25 (dd, 1H, *J*=10.9, 9.0 Hz, H-3), 4.73 – 4.41 (m, 3H, H-1, CH_2Bn), 3.95 (dt, 1H, *J*=10.8, 8.7 Hz, H-2), 3.87 (dt, 1H, *J*=9.5, 6.1 Hz, OCH_2), 3.83 – 3.71 (m, 3H, H-6, H-4), 3.59 (dt, 1H, *J*=9.7, 4.8 Hz, H-5), 3.44 (dt, 1H, *J*=9.6, 6.7 Hz, OCH_2), 3.23 (t, 2H, *J*=6.9 Hz, CH_2N_3), 3.13 (s, 1H, OH), 2.09 (s, 3H, Ac), 1.70 – 1.44 (m, 4H, CH_2, hexyl), 1.34 (td, 3H, *J*=6.2, 4.7, 2.6 Hz, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 172.0 (C=O, Ac), 162.2 (C=O, TCA), 128.7, 128.1, 127.9 (arom.), 100.9 (C-1), 74.6 (C-3), 74.0 (C-5), 73.9 (CH_2Bn), 71.1 (C-4),

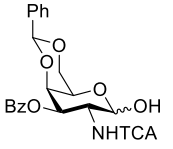
70.4 (C-6), 69.9 (OCH_2), 55.8 (C-2), 51.5 (CH_2N_3), 29.4, 28.8, 26.6, 25.6 (CH_2-hexyl), 21.0 (CH_3, Ac) ppm. HRMS $[M+Na]^+$ calcd for $C_{23}H_{31}Cl_3N_4O_7Na$: 603.11560, found 603.11505.

Phenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (15)



Compound **14** (2.52 g, 5.0 mmol, 1.0 eq.) was dissolved in a mixture of DCM and pyridine (50 mL, 0.1M, 2/1, 1/1) and cooled to 0˚C with an ice bath. Bz-Cl (1.2 mL, 10.0 mmol, 2.0 eq.) was added slowly and the ice bath was removed. After 45min. TLC analysis showed full consumption of the starting material and the reaction mixture was poured into H_2O (50 mL). The layers were separated and the water layer was extracted twice with DCM. The organic layers were combined and washed with sat. $CuSO_4$ (aq. 4x), sat. $NaHCO_3$ (aq., 2x) and brine, followed by drying over $MgSO_4$, filtration and concentration *in vacuo*. Compound **15** was obtained after purification by silicagel chromatography (PE:EtOAc, 4:1 → 3:2) as a white solid (3.0 g, 4.9 mmol, 99%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.24 – 8.02 (m, 2H, arom.), 8.01 – 7.85 (m, 2H, arom.), 7.73 – 7.61 (m, 3H, arom.), 7.59 – 7.47 (m, 4H, arom.), 7.47 – 7.40 (m, 2H, arom.), 7.40 – 7.19 (m, 4H, arom.), 6.95 (d, 1H, *J*=8.8 Hz, NH), 5.65 (dd, 1H, *J*=10.8, 3.2 Hz, H-3), 5.52 (s, 1H, CHPh), 5.20 (d, 1H, *J*=10.1 Hz, H-1), 4.58 – 4.30 (m, 3H, H-2, H-4, H-6), 4.07 (dd, 1H, *J*=12.5, 1.7 Hz, H-6), 3.76 (q, 1H, *J*=1.5 Hz, H-5) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.4 (C=O, Bz), 161.6 (C=O, TCA), 137.7, 134.7, 133.8, 133.6, 131.0, 130.6, 130.1, 129.2, 129.1, 129.0, 128.5, 128.5, 128.2, 126.5 (arom.), 100.8 (CHPh), 84.7 (C-1), 73.5 (C-4), 72.2 (C-3), 70.0 (C-5), 69.3 (C-6), 50.7 (C-2) ppm. HRMS [M+Na] calcd for $C_{28}H_{24}Cl_3NO_6SNa$: 630.02876, found 630.0291.

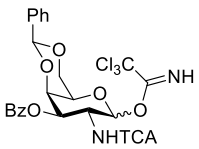
3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroacetamido)-α/β-D-galactopyranoside (15a)



Thioglycoside **15** (1.33 g, 2.2 mmol, 1.0 eq.) was dissolved in a mixture of acetone and water (22 mL, 0.1M, 9/1, v/v). NBS (1.96 g, 11 mmol, 5 eq.) was added and the reaction was stirred in the dark under inert atmosphere for 30 min., before addition of sat. $Na_2S_2O_3$ (aq., 10 mL). The reaction mixture was stirred until colourless and afterwards reduced *in vacuo* to approximately 10 ml. The concentrated solution was extracted with Et_2O thrice. The combined organic layers were then washed with sat. $NaHCO_3$ (aq.) and brine, followed by drying over $MgSO_4$, filtration and concentration. The resulting yellow oil was purified by silicagel chromatography (PE: EtOAc, 9:1 → 4:1), which gave **15a** as a single isomer (1.04 g, 2.02, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.09 – 7.98 (m, 2H, arom.), 7.53 (m, 3H, arom.), 7.46 – 7.30 (m, 5H, arom.), 7.22 (d, 1H, *J*=9.4 Hz, NH), 5.57 – 5.50 (m, 2H, H-3, CHPh), 5.46 (d, 1H, *J*=3.4 Hz, H-1), 5.10 – 5.00 (s, 1H, 1-OH), 4.81 (ddd, 1H, *J*=11.1, 9.3,

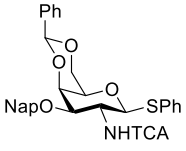
3.4 Hz, H-2), 4.35 (d, 1H, *J*=3.3 Hz, H-4), 4.26 (dd, 1H, *J*=12.7, 1.5 Hz, H-6), 4.03 (dd, 1H, *J*=12.7, 1.7 Hz, H-6), 3.97 (s, 1H, H-5) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.8 (C=O, Bz), 162.3 (C=O, TCA), 137.4, 133.6, 130.1, 130.0, 129.1, 129.0, 128.5, 128.4, 128.3, 126.0 (arom.), 100.4 (CHPh), 92.1 (C_q, TCA), 91.7 (C-1), 73.6 (C-4), 69.6 (C-3), 69.3 (C-6), 62.4 (C-5), 50.1 (C-2) ppm. HRMS [M+Na] calcd for $C_{22}H_{20}Cl_3NO_7Na$: 538.02031, found 538.0204.

3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroacetamido)-α/β-D-galactopyranoside (16)



Hemi-acetal **15a** (0.52 g, 1.0 mmol, 1.0 eq.) and trichloro acetonitrile (1.0 mL, 10 mmol, 10 eq.) were dissolved in dry DCM (10 mL, 0.1M). DBU (0.050 mL, 0.1 mmol, 0.1 eq.) was added and after 15 min. The reaction mixture was concentrated. The black residue was purified by silicagel chromatography over neutralized silica (PE:EtOAc, 9:1 → 3:2) to yield **16** as a yellow oil (0.47 g, 0.71 mmol, 71%). ^{1}H NMR (Acetone-d_6, 400 MHz): δ = 9.51 (s, 1H, C=NH), 8.19 (d, 1H, *J*=7.9 Hz, NH), 8.14 – 7.95 (m, 3H, arom.), 7.71 – 7.57 (m, 1H, arom.), 7.57 – 7.41 (m, 6H, arom.), 7.41 – 7.28 (m, 4H, arom.), 6.76 (d, 1H, *J*=3.3 Hz, H-1), 5.90 – 5.67 (m, 2H, H-3, CHPh), 5.00 (ddd, 1H, *J*=11.4, 7.9, 3.4 Hz, H-2), 4.95 (dd, 1H, *J*=3.2, 1.2 Hz, H-4), 4.39 – 4.16 (m, 3H, H-5, H-6) ppm. ^{13}C-APT NMR (Acetone-d_6, 101 MHz,) δ 166.1 (C=O, Bz), 162.2 (C=O, TCA), 159.7 (C=NH), 138.5, 133.7, 129.7, 129.6, 128.9, 128.7, 128.6, 128.1, 126.3 arom., 100.4 (CHPh), 95.0 (C-1), 72.9 (C-4), 69.3 (C-3), 68.6 (C-6), 65.3 (C-5), 50.1 (C-2) ppm.

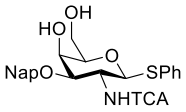
Phenyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(2-methylnaphthyl)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (17)



Galactopyranoside **14** (8.5 g, 16.8 mmol, 1.0 eq.) was dissolved in dry DMF (170 mL, 0.1 mL) and cooled to 0˚C. NaH (2.7 g, 67.3 mmol, 3.0 eq.) was added portionwise over a period of 30 min., followed by Nap-Br (4.1 g, 18.5 mmol, 1.1 eq.). The ice bath was removed and the reaction was left to stir for 3 hours. Upon completion the reaction mixture was slowly poured into H_2O. The white precipitate was filtered over a glass filter, washed with H_2O (3x) and cold Et_2O (3x). This gave the title compound as a white solid (9.3 g, 14.3 mmol, 85%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.85 – 7.65 (m, 6H, arom.), 7.53 – 7.33 (m, 8H, arom), 7.33 – 7.15 (m, 3H, arom.), 6.85 (d, 1H, *J*=7.0 Hz, NH), 5.50 – 5.41 (m, 2H, H-1, CHPh), 4.83 – 4.68 (m, 2H, CH_2Nap), 4.49 (dd, 1H, *J*=10.5, 3.3 Hz, H-3), 4.38 (dd, 1H, *J*=12.4, 1.7 Hz, H-6), 4.20 (d, 1H, *J*=3.3 Hz, H-4), 3.98 (dd, 1H, *J*=12.4, 1.7 Hz, H-6), 3.78 (td, 1H, *J*=10.3, 7.0 Hz, H-2), 3.54 (s, 1H, H-5) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 161.7 (C=O, TCA), 137.8, 135.0, 134.1, 133.2, 129.2, 128.5, 128.4, 128.2, 127.9, 127.7, 126.9, 126.5, 126.3, 126.2, 125.8 (arom.), 100.9 (CHPh), 82.5 (C-1), 75.1 (C-3), 72.8

(C-4), 71.9 (CH_2Nap), 70.1 (C-5), 69.5 (C-6), 52.9 (C-2) ppm. HRMS $[M+Na]^+$ calcd for $C_{32}H_{28}Cl_3NO_5SNa$: 666.06515, found 666.06460.

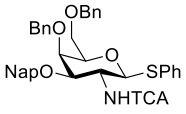
Phenyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2-methylnaphthyl)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (17a)



Thioglycoside **17** (9.3 g, 14.3 mmol, 1.0 eq.) was suspended in a mixture of DCE and methanol (170 mL, 0.1 M, 3/1, v/v). *p*-TsOH (0.27 g, 1.43 mmol, 0.1 eq.) and the mixture was heated to 50˚C. When TLC analysis showed full consumption of the starting material, Et_3N was added and the mixture was concentrated *in vacuo*. The resulting pale yellow solid was washed with heptane (2x), water (2x) and dried *in vacuo* to obtain the title compound as a pure white solid (6.3 g, 11.4 mmol, 80%). ^{1}H NMR (Acetone-d_6, 400 MHz): δ = 8.37 (d, 1H, *J*=9.5 Hz, NH), 7.94 – 7.76 (m, 4H, arom.), 7.59 – 7.42 (m, 5H, arom.), 7.40 – 7.17 (m, 3H, arom.), 5.07 (d, 1H, *J*=10.6 Hz, H-1), 4.93 (d, 1H, *J*=12.0 Hz, CH_2Nap), 4.76 (d, 1H, *J*=12.0 Hz, CH_2Nap), 4.48 – 4.33 (m, 2H, H-2, H-4), 4.22 (d, 1H, *J*=3.8 Hz, 4-OH), 4.02 – 3.89 (m, 2H, H-3, 6-OH), 3.86 – 3.79 (m, 2H, H-6), 3.61 (t, 1H, *J*=6.4, Hz, H-5) ppm. ^{13}C-APT NMR (101 MHz, Acetone): δ 162.3 (C=O, TCA), 136.9, 135.9, 134.2, 133.9, 131.7, 129.7, 128.7, 128.6, 128.5, 127.8, 127.1, 126.9, 126.8, 126.6 (arom.), 87.9 (C-1), 80.4 (C-3), 80.2 (C-5), 71.4 (CH_2Nap), 65.7 (C-4), 62.4 (C-6), 52.9 (C-2) ppm. HRMS $[M+Na]^+$ calcd for $C_{25}H_{24}Cl_3NO_5SNa$: 578.03385, found 578.03330.

4

Phenyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2-methylnaphthyl)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (18)



From **17a:**

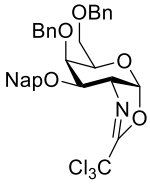
Diol **17a** (6.3 g, 11.4 mmol, 1.0 eq.) was dissolved in dry DMF (110 mL, 0.1M) and cooled to 0˚C, before portionwise addition of NaH (2.3 g, 57 mmol, 5.0 eq.). Bn-Br (3.0 mL, 25.1, 2.2 eq.) was added after 30 min. of stirring and the ice bath was removed. After 1 hour the temperature was increased to 50˚C and stirred at that temperature for an additional hour. When TLC analysis showed full consumption of the starting material, the reaction was quenched by slow addition of methanol. Once cooled the mixture was transferred to a separatory funnel, diluted with EtOAc, washed with H_2O (3x) and brine (2x). The organic layer was dried over $MgSO_4$, filtered and concentrated. The title compound was obtained by silicagel chromatography (tol:ACN, 1:0 → 19:1) as a white amorphous solid (7.1 g, 9.6 mmol, 84%).

From **3:**

Glucopyranoside **3** (2.4 g, 3.72 mmol, 1.0 eq.) was dissolved in a mixture of DCM and pyridine (37 mL, 0.1M, 3/1, v/v) and cooled to 0˚C. Tf_2O (0.81 mL, 4.84 mmol, 1.3 eq.) was added slowly. The colourless solution turned orange after addition of Tf_2O. After 30 min. the mixture was diluted in DCM and transferred to a separatory funnel. The organic

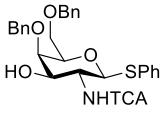
layer was washed with sat. $CuSO_4$ (aq., 5x) and brine. Before being dried over $MgSO_4$, filtered and concentrated at room temperature. The orange oil was redissolved in dry DMF (40 mL, 0.1M) and benzyl alcohol (1.9 mL, 18.6 mmol, 5.0 eq.) and Et_3N (1.0 mL, 7.44 mmol, 2.0 eq.) were added. The solution was heated to 50˚C and left to stir overnight. The reaction mixture was poured into EtOAc and washed with H_2O and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The brown oil was purified by silicagel chromatography (PE:EtOAc, 19:1 → 7:3) to give the title compound as a white amorphous solid (1.1 g, 1.49 mmol, 40%).
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.87 – 7.69 (m, 4H, arom.), 7.58 – 7.38 (m, 5H, arom.), 7.38 – 7.11 (m, 13H, arom.), 6.82 (d, 1H, *J*=7.5 Hz, NH), 5.27 (d, 1H, *J*=10.2 Hz, H-1), 4.91 (d, 1H, *J*=11.4 Hz, CH_2arom.), 4.80 (d, 1H, *J*=11.4 Hz, CH_2arom.), 4.67 (d, 1H, *J*=11.4 Hz, CH_2arom.), 4.60 (d, 1H, *J*=11.4 Hz, CH_2arom.), 4.56 – 4.42 (m, 2H, CH_2arom.), 4.31 (dd, 1H, *J*=10.5, 2.7 Hz, H-3), 4.10 (d, 1H, *J*=2.6 Hz, H-4), 3.96 (td, 1H, *J*=10.4, 7.4 Hz, H-2), 3.81 – 3.72 (m, 1H, H-5), 3.72 – 3.60 (m, 2H, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 161.7 (C=O, TCA), 138.5, 137.8, 134.7, 133.2, 133.1, 132.8, 132.2, 129.0, 128.5, 128.5, 128.2, 128.0, 127.9, 127.9, 127.7, 127.6, 127.0, 126.3, 126.2, 125.9 (arom.), 84.4 (C-1), 78.3 (C-3), 77.5 (C-5), 74.6 (CH_2arom.), 73.6 (CH_2arom.), 72.5 (CH_2arom.), 72.4 (C-4), 68.4 (C-6), 53.8 (C-2) ppm. HRMS: $[M+Na]^+$ calcd for $C_{39}H_{36}Cl_3NO_5SNa$: 758.12775, found 758.12720.

3,4-di-*O*-benzyl-2-deoxy-3-*O*-(2-methylnaphthyl)-1,2-trichlorooxazolino-α-D-galactopyranoside (18BP)



^{1}H NMR (CD_3CN, 400 MHz): δ 7.96 – 7.83 (m, 4H, arom.), 7.62 – 7.48 (m, 3H, arom.), 7.41 – 7.25 (m, 10H, arom.), 6.30 (d, 1H, *J*=6.7 Hz, H-1), 5.01 – 4.83 (m, 3H, CH_2arom.), 4.63 (d, 1H, *J*=11.4 Hz, CH_2arom.), 4.58 – 4.47 (m, 2H, CH_2arom), 4.39 (dd, 1H, *J*=7.8, 6.7 Hz, H-2), 4.10 – 3.97 (m, 2H, H-4, H-5), 3.73 – 3.54 (m, 3H, H-3, H-6) ppm. ^{13}C-APT NMR (CD_3CN, 101 MHz): δ 162.3 (C=N), 139.2, 138.9, 136.5, 133.9, 133.6, 129.0, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 127.0, 126.9, 126.7, 126.5, 107.7 (C-1), 80.2 (C-3), 74.9 (CH_2arom.), 74.3 (C-5), 73.6 (CH_2arom.), 72.3 (C-4), 71.9 (CH_2arom), 69.4 (C-6), 67.4 (C-2) ppm.

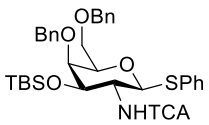
Phenyl 4,6-di-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (20)



Glycan **18** (1.90 g, 2.57 mmol, 1.0 eq.) was dissolved in a mixture of methylene chloride and methanol (25 mL, 0.1M, 9/1, 1/1). DDQ (1.75 g, 7.72 mmol, 3.0 eq.) was added portionwise (1.0 eq. per 30 min.) and the reaction was left to stir under inert atmosphere. When TLC analysis showed full conversion to a single more polar spot, 20 mL of sat. $Na_2S_2O_3$ (aq.) was added. The mixture was stirred until the solution turned colourless. The colourless mixture was

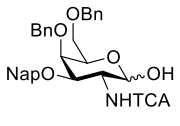
transferred to a separatory funnel, the water layer was removed and the organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. After purification by silicagel chromatography the title compound was obtained as a white amorphous solid (1.31 g, 2.2 mmol, 85%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.56 – 7.46 (m, 2H, arom.), 7.39 – 7.16 (m, 13H, arom.), 6.88 (d, 1H, *J*=7.4 Hz, NH), 4.86 (d, 1H, *J*=9.8 Hz, H-1), 4.65 (d, 2H, *J*=1.4 Hz, CH_2Bn), 4.56 – 4.39 (m, 2H, CH_2Bn), 3.90 (m, 3H, H-2, H-3, H-4), 3.78 – 3.56 (m, 3H, H-5, H-6), 2.85 – 2.31 (m, 1H, 3-OH) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 162.4 (C=O, TCA), 138.1, 137.6, 132.5, 132.4, 129.0, 128.6, 128.5, 128.0, 128.0, 127.7 (arom.), 92.5 (C_q, TCA), 85.7 (C-1), 77.5 (C-5), 75.9 (C-4), 75.2 (CH_2Bn), 73.6 (CH_2Bn), 72.5 (C-3), 68.3 (C-6), 54.8 (C-2) ppm. HRMS $[M+Na]^+$ calcd for $C_{28}H_{28}Cl_3NO_5SNa$: 618.06515, found 618.06460.

Phenyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-(*tert*-butyldimethylsilyl)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside (21)



Thioglycoside **20** (1.31 g, 2.2 mmol, 1.0 eq.) was dissolved in DMF (22 mL, 0.1M) together with DMAP (2.68 g, 22 mmol, 10 eq.). TBDMS-Cl (2.3 mL of a 50% solution in toluene) was added and the reaction was heated to 80˚C and left to stir overnight. The mixture was cooled and subsequently diluted with EtOAc. The mixture was then transferred to a separatory funnel and the organic layer was washed with 1M HCl (aq., 2x), sat. $NaHCO_3$ (aq., 1x) and brine (4x), before being dried over $MgSO_4$, filtered and concentrated. The crude mixture was purified by silicagel chromatography (PE:EtOAc, 99:1 → 9:1) to give the title compound as a white solid (1.35 g, 1.90 mmol, 86%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.61 – 7.42 (m, 2H, arom.), 7.42 – 7.08 (m, 13H, arom.), 6.79 (d, 1H, *J*=8.0 Hz, NH), 5.25 (d, 1H, *J*=10.3 Hz, H-1), 4.98 (d, 1H, *J*=11.1 Hz, CH_2Bn), 4.54 – 4.40 (m, 3H, CH_2Bn), 4.29 (d, 1H, *J*=10.0 Hz, H-3), 3.97 (d, 1H, *J*=9.2 Hz, H-2), 3.83 (d, 1H, *J*=2.7 Hz, H-4), 3.75 (t, 1H, *J*=6.5 Hz, H-5), 3.66 (d, 2H, *J*=6.4 Hz, H-6), 0.89 (s, 9H, *t*-Bu, TBDMS), 0.17 (s, 3H, CH_3, TBDMS), 0.10 (s, 3H, CH_3, TBDMS) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 161.4 (C=O, TCA), 138.7, 138.0, 133.0, 132.4, 129.0, 128.5, 128.3, 128.0, 127.9, 127.8, 127.6, 127.5 (arom.), 92.6 (C_q, TCA), 84.8 (C-1), 77.4 (C-5), 76.8 (C-4), 75.2 (CH_2Bn), 73.6 (CH_2Bn), 73.2 (C-3), 68.6 (C-6), 55.0 (C-2), 25.9 (*t*-Bu, TBDMS) 18.0 (C_q, TBDMS), -3.4 (CH_3, TBDMS), -4.9 (CH_3, TBDMS) ppm.

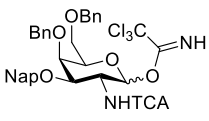
4,6-di-*O*-benzyl-3-*O*-(2-methylnaphthyl)-2-deoxy-2-(2,2,2-trichloroacetamido)-α/β-D-galactopyranoside (18a)



Thioglycoside **18** (7.1 g, 9.6 mmol, 1.0 eq.) was dissolved in a mixture of water in acetone (1/9, v/v, 100 mL, 0.1M). NBS (5.1 g, 28.7 mmol, 3.0 eq.) was added and the mixture was stirred in the dark for 30 min. under inert atmosphere. A solution of sat. $Na_2S_2O_3$ (aq.) (30 mL) was

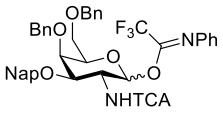
added and the mixture was stirred until the solution turned colourless. The acetone was removed in vacuo and the water layer was extracted thrice with Et_2O. The combined organic layers were washed with sat. $NaHCO_3$ (aq.) followed by brine. The organic layer was dreid over $MgSO_4$, filtered and concentrated. The crude compound was purified by silicagel chromatography (tol: ACN, 1:0 → 9:1) to give **18a** as a pale white solid (6.1 g, 9.46 mmol, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.86 – 7.67 (m, 4H, arom.), 7.51 – 7.38 (m, 3H, arom), 7.33 – 7.19 (m, 11H, arom), 6.83 (d, 1H, *J*=9.1 Hz, NH), 5.29 (t, 1H, *J*=3.6 Hz, H-1), 4.93 (d, 1H, *J*=11.6 Hz, CH_2arom), 4.79 (d, 1H, *J*=12.3 Hz, CH_2arom), 4.70 – 4.50 (m, 4H, CH_2arom), 4.45 (d, 1H, *J*=11.9 Hz, CH_2arom), 4.35 (d, 1H, *J*=11.9 Hz), 4.10 (t, 1H, *J*=6.2 Hz, H-5), 3.91 (d, 1H, *J*=2.5 Hz, H-4), 3.77 (dd, 1H, *J*=10.7, 2.5 Hz, H-3), 3.57 (dd, 1H, *J*=9.8, 7.2 Hz, H-6), 3.36 (dd, 1H, *J*=9.6, 5.2 Hz, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 161.4 (C=O, TCA), 137.7, 137.0, 134.7, 132.9, 132.7, 128.1, 128.1, 128.0, 128.0, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 126.1, 125.9, 125.7, 125.2 (arom.), 92.4 (C_q, TCA), 91.2 (C-1), 76.5 (C-3), 74.1 (CH_2arom.), 73.2 (CH_2arom.), 72.0 (C-4), 71.5 (CH_2arom.), 69.3 (C-5), 69.2 (C-6), 51.1 (C-2) ppm. HRMS $[M+Na]^+$ calcd for $C_{33}H_{32}Cl_3NO_6Na$: 666.11929, found 666.11874.

Trichloroacetimidoyl 4,6-di-*O*-benzyl-3-*O*-(2-methylnaphthyl)-2-deoxy-2-(2,2,2-trichloroacetamido)-α/β-D-galactopyranoside (19)



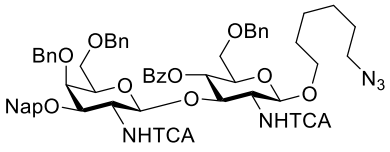
DBU (0.28 mL, 1.9 mmol, 0.2 eq.) was added to a solution of hemi-acetal **18a** (6.1 g, 9.5 mmol, 1.0 eq.) and trichloro acetonitrile (9.5 mL, 94.6 mmol, 10 eq.) in dry DCM (95 mL, 0.1M). Upon stirring for 15 min. TLC indicated full conversion and the mixture was concentrated in vacuo. The brown oil was purified over neutralized silica (tol:ACN, 1:0 → 19:1) to give imidate **19** as a yellow oil (5.9 g, 7.5 mmol, 78%, α/β, 5/1). NMR of the α-anomer: ^{1}H NMR ($CDCl_3$, 400 MHz, 400 MHz): δ 8.57 (s, 1H, C=NH), 7.94 – 7.70 (m, 6H, arom.), 7.60 – 7.40 (m, 5H, arom.), 7.40 – 7.22 (m, 12H, arom.), 6.46 (d, 1H, *J*=3.4 Hz, H-1), 6.38 (d, 1H, *J*=8.1 Hz, NH), 5.06 – 4.75 (m, 3H, H-2, CH_2arom), 4.72 – 4.56 (m, 2H, CH_2arom), 4.54 – 4.35 (m, 2H, CH_2arom), 4.24 (d, 1H, *J*=2.4 Hz, H-4), 4.20 – 4.07 (m, 1H, H-5), 3.94 (dd, 1H, *J*=8.5, 2.4 Hz, H-3), 3.76 – 3.46 (m, 2H, H-6) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 162.0 (C=NH), 160.1 (C=O, TCA), 138.1, 138.0, 137.6, 134.8, 134.3, 133.2, 133.1, 129.0, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.7, 127.4, 127.0, 126.8, 126.6, 126.5, 126.3, 126.2, 126.0, 125.9, 125.8 (arom.), 95.1 (C-1), 92.3 (C_q, TCA), 90.8 (C_q, imidate), 74.8 (CH_2arom), 74.7 (C-3), 73.6 (CH_2arom), 72.5 (C-5), 71.4 (C-4), 71.1 (CH_2arom), 68.0 (C-6), 50.8 (C-2) ppm.

N-Phenyl-trifluoroacetimidoyl 4,6-di-*O*-benzyl-3-*O*-(2-methylnaphthyl)-2-deoxy-2-(2,2,2-trichloroacetamido)-α/β-D-galactopyranoside (19a)



Hemiacetal **18a** (3.1 g, 4.7 mmol, 1.0 eq.) was dissolved in acetone (47 mL, 0.1M) and cooled to 0˚C. Cs_2CO_3 (4.59 g, 14.1 mmol, 3.0 eq.) was added, followed by $CF_3(C{=}NPh)Cl$ (1.52 mL, 9.4 mmol, 2.0 eq.). The reaction mixture was left to stir overnight under inert atmosphere. After filtration over celite the volatiles were evaporated *in vacuo*. The residue was purified by silicagel chromatography using neutralized silica (tol:Et_2O, 1:0 → 9:1) to give **19a** as a yellow oil (3.65 g, 4.47 mmol, 95%). ^{1}H NMR (CD_3CN, 300 MHz, 323K): δ = 7.94 – 7.77 (m, 4H, arom.), 7.64 – 7.45 (m, 3H, arom.), 7.45 – 7.19 (m, 10H, arom.), 7.18 – 7.05 (m, 1H, arom.), 6.88 – 6.70 (m, 2H, arom.), 6.31 (s, 1H, H-1), 5.01 – 4.74 (m, 2H, CH_2arom), 4.70 – 4.44 (m, 3H, H-2, CH_2arom), 4.36 – 4.10 (m, 3H, H-3, H-4, H-5), 3.76 – 3.52 (m, 2H, H-6) ppm. ^{13}C-APT NMR (CD_3CN, 75 MHz, 323 K) δ 163.4 (C=O, TCA), 144.7, 139.7, 139.6, 136.9, 134.5, 134.2, 130.0, 129.5, 129.4, 129.2, 129.1, 128.9, 128.9, 128.8, 128.8, 127.7, 127.4, 127.2, 125.6, 120.4 (arom.), 96.0 (C-1), 76.8 (C-3), 75.9 (CH_2arom), 74.1 (C-4 + CH_2arom), 73.7 (C-5), 72.5 (CH_2arom), 70.0 (C-6), 52.3 (C-2) ppm.

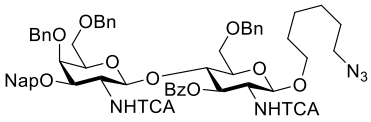
6-azidohexyl 4-*O*-benzoyl-6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2-methylnaphthyl)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (26)



Acceptor **10** (0.037 g, 0.058 mmol, 1.0 eq.) and donor **18** (0.065 g, 0.088 mmol, 1.5 eq.) were co-evaporated thrice with dry toluene, before dissolving in dry DCM (0.6 mL, 0.1M). NIS (0.022 g, 0.098 mmol, 1.7 eq.) and freshly dried molecular sieves (4Å) were added and the reaction mixture was cooled to -20˚C and left to stir for 1 hour at that temperature. TfOH (100 µL, 0.011 mmol, 0.2 eq. of a 0.1M solution in DCM) was added and the reaction kept at -20˚C for 8 hours, after which it was left overnight at 0˚C. TLC analysis showed full consumption of donor **18** and Et_3N (0.05 mL) was added. The reaction mixture was diluted in EtOAc and transferred to a separatory funnel. The organic layer was washed with HCl (1M, aq.), sat. $NaHCO_3$ (aq.) and brine. Any traces of water were removed by drying over $MgSO_4$ followed by filtration and concentration *in vacuo*. The title compound **26** was obtained by silicagel chromatography (tol:ACN, 1:0 → 19:1) followed by size exclusion (LH-20, DCM/MeOH, 1/1, v/v) as a viscous colourless oil (0.035 g, 0.027 mmol, 47%) ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.95 – 7.87 (m, 2H, arom.), 7.85 – 7.66 (m, 4H, arom.), 7.51 – 7.38 (m, 4H, arom.), 7.38 – 7.28 (m, 7H, arom.), 7.28 – 7.08 (m, 14H, arom.), 7.02 (d, 1H, *J*=9.1 Hz, NH), 6.84 (d, 1H, *J*=7.3 Hz, NH'), 5.43 (dd, 1H, *J*=10.5, 8.7 Hz, H-4), 4.93 (d, 1H, *J*=8.2 Hz, H-1'), 4.78 – 4.68 (m, 2H, CH_2arom), 4.68 – 4.51 (m, 4H, H-1, CH_2arom), 4.41 (d, 1H,

J=11.5 Hz, CH_2arom), 4.26 – 4.09 (m, 4H, H-2, H-3, CH_2arom), 4.06 (dd, 1H, *J*=11.0, 2.8 Hz, H-3'), 3.92 (d, 1H, *J*=2.7 Hz, H-4'), 3.90 – 3.84 (m, 1H, OCH_2), 3.78 – 3.57 (m, 4H, H-5, H-6, H-2'), 3.46 (dt, 1H, *J*=9.5, 6.6 Hz, OCH_2), 3.30 (dd, 1H, *J*=8.8, 5.3 Hz, H-5'), 3.21 (t, 2H, *J*=6.9 Hz, CH_2N_3), 3.17 – 3.04 (m, 2H, H-6'), 1.62 – 1.48 (m, 4H, CH_2, hexyl), 1.40 – 1.27 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.9 (C=O, Bz), 162.1 (C=O, TCA), 138.6, 138.2, 137.8, 135.0, 133.3, 133.2, 133.1, 130.1, 129.8, 128.6, 128.5, 128.4, 128.3, 128.3, 128.0, 128.0, 128.0, 127.8, 127.8, 127.8, 127.6, 126.7, 126.4, 126.2, 125.8 (arom.), 100.9 (C-1), 98.5 (C-1'), 92.6, 92.5 (C_q, TCA), 76.8 (C-3'), 75.0 (C-5), 74.7 (CH_2arom), 73.8 (C-3), 73.4 (CH_2arom), 73.2 (CH_2arom), 73.1 (C-4), 73.0 (C-5'), 72.1 (CH_2arom), 71.9 (C-4'), 69.7 (OCH_2), 68.2 (C-6), 67.3 (C-6'), 56.3 (C-2'), 55.7 (C-2), 51.5 (CH_2N_3), 29.5, 28.9, 26.6, 25.7 (CH_2, hexyl) ppm.

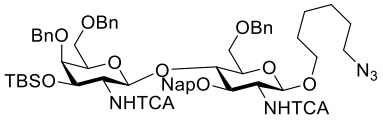
6-azidohexyl 3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2-methylnaphthyl)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (22)



Acceptor **10** (1.0 g, 1.5 mmol, 1.0 eq.) and donor **19** (1.32 g, 1.6 mmol, 1.1 eq.) were co-evaporated separately with dry toluene and dissolved in DCM (7.5 mL, 0.2M each). Molecular sieves (3Å) were added to both flasks and the solutions were stirred for 1 hour at RT. Both solutions were cooled to -20˚C and stirred for 15 min. TfOH (40 µL, 0.45 mmol, 0.3 eq.) was added to the acceptor and the solution containing donor **19** was slowly added to the solution containing acceptor **10** and TfOH. The mixture was kept at -20˚C and for the duration of the reaction. After 2 hours TLC indicated full conversion of the donor. The reaction was quenched by addition of Et_3N (0.5 mL), diluted and transferred to a separatory funnel. The organic layer was washed with sat. $NaHCO_3$ (aq.) and brine, followed by drying over $MgSO_4$, filtration and concentration. The brown mixture was purified by size exclusion (LH-20, DCM/MeOH, 1/1, v/v) to give compound **22** (0.76 g, 0.6 mmol, 40%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.98 – 7.89 (m, 2H, arom.), 7.83 – 7.62 (m, 4H, arom.), 7.50 – 7.40 (m, 3H, arom.), 7.39 – 7.09 (m, 21H, NH, arom.), 7.04 (d, 1H, *J*=7.3 Hz, NH'), 5.53 (dd, 1H, *J*=10.5, 8.8 Hz, H-3), 4.97 (d, 1H, *J*=8.2 Hz, H-1), 4.76 – 4.50 (m, 6H, H-1, CH_2Bn), 4.41 (d, 1H, *J*=11.7 Hz, CH_2Bn), 4.22 (ddd, 1H, *J*=10.5, 9.3, 8.1 Hz, H-2), 4.18 – 4.11 (m, 3H, H-4, H-3', CH_2Bn), 4.08 (dd, 1H, *J*=10.9, 2.8 Hz), 3.91 (d, 1H, *J*=2.8 Hz, H-4'), 3.84 (dd, 1H, *J*=10.8, 4.7 Hz, OCH_2), 3.81 – 3.60 (m, 4H, H-5, H-6, H-2'), 3.47 (dt, 1H, *J*=9.5, 6.4 Hz, OCH_2), 3.29 (dd, 1H, *J*=8.3, 6.0 Hz, H-5'), 3.16 (t, 2H, *J*=6.9 Hz, CH_2N_3), 3.10 – 2.97 (m, 2H, H-6'), 1.60 – 1.43 (m, 4H, CH_2, hexyl), 1.40 – 1.20 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.9 (C=O, Bz), 162.2, 162.0 (C=O, TCA), 138.6, 138.2, 137.8, 135.0, 133.3, 133.2, 133.0, 130.1, 129.7, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 126.6, 126.3, 126.1, 125.7 (arom.), 100.5 (C-1),

98.9 (C-1'), 92.6, 92.5 (C_q, TCA), 76.9 (C-3'), 75.0 (C-5), 74.6 (CH_2arom), 74.5 (C-4), 73.4 (C-3), 73.3 (CH_2arom), 73.1 (CH_2arom), 72.9 (C-5'), 72.1 (CH_2arom), 71.8 (C-4'), 69.2 (OCH_2), 68.0 (C-6), 67.2 (C-6'), 56.3 (C-2'), 55.4 (C-2), 51.4 (CH_2N_3), 29.5, 28.8, 26.5, 25.7 (CH_2, hexyl) ppm. HRMS $[M+Na]^+$ calcd for $C_{61}H_{63}Cl_6N_5O_{12}Na$: 1290.25021, found 1290. 24966.

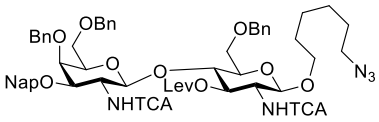
6-azidohexyl 6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-(*tert*-butyldimethylsilyl)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-3-*O*-(2-methylnaphthyl)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (23)



Acceptor **4** (0.068 g, 0.10 mmol, 1.0 eq.) and donor **21** (0.14 g, 0.2 mmol, 2.0 eq.) were co-evaporated separately with dry toluene and dissolved in DCM (0.5 mL, 0.2M each). Molecular sieves (3Å) were added to both flasks and the solutions were stirred for 1 hour at RT. NIS (0.050 g, 0.22 mmol, 2.2 eq.) was added to the solution containing acceptor **4**. Both solutions were cooled to -40˚C and stirred for 15 min. TfOH (200 µL, 0.02 mmol, 0.2 eq. of a 0.1M solution in DCM) was added to the acceptor and the solution containing donor **21** was slowly added to the solution containing acceptor **4**, NIS and TfOH. The mixture was allowed to warm up to -20˚C and kept at that temperature for the duration of the reaction. After 8 hours TLC indicated full conversion of the donor. The reaction was quenched by addition of Et_3N (0.05 mL), diluted and transferred to a separatory funnel. The organic layer was washed with sat. $Na_2S_2O_3$ (aq.), sat. $NaHCO_3$ (aq.) and brine, followed by drying over $MgSO_4$, filtration and concentration. The brown mixture was purified by silicagel chromatography (PE:EtOAc, 99:1 → 4:1), followed by size exclusion (LH-20, DCM/MeOH, 1/1, v/v) to give compound **23** as a pale yellow oil (0.021 g, 0.016 mmol, 16%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.82 – 7.55 (m, 4H, arom.), 7.53 – 6.95 (m, 23H, NH, arom.), 6.63 (d, 1H, *J*=7.9 Hz, NH'), 5.01 (dd, 2H, *J*=23.8, 11.0 Hz, CH_2arom.), 4.81 (d, 1H, *J*=7.3 Hz, H-1), 4.79 (d, 1H, *J*=7.8 Hz, H-1'), 4.73 – 4.63 (m, 2H, CH_2arom), 4.51 (d, 1H, *J*=12.1 Hz, CH_2arom), 4.43 (d, 1H, *J*=11.1 Hz, CH_2arom), 4.33 – 4.10 (m, 4H, H-4, CH_2arom), 4.08 – 3.91 (m, 3H, H-3, H-2', H-3'), 3.89 – 3.68 (m, 4H, H-6, H-4', OCH_2), 3.67 – 3.53 (m, 2H, H-2, H-5), 3.50 – 3.28 (m, 4H, H-5', H-6', OCH_2), 3.20 (t, 3H, *J*=6.9 Hz, CH_2N_3), 1.60 – 1.48 (m, 4H, CH_2, hexyl), 1.37 – 1.28 (m, 4H, CH_2, hexyl), 0.90 (s, 9H, *t*-Bu, TBDMS), 0.17 (s, 3H, CH_3, TBDMS), 0.11 (s, 3H, CH_3, TBDMS) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 161.8, 161.6 (C=O, TCA), 138.7, 138.3, 138.0, 135.8, 133.3, 133.0, 129.0, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 126.8, 126.5, 126.4, 125.9, 125.7 (arom.), 99.4 (C-1'), 98.8 (C-1), 92.7, 92.6 (C_q, TCA), 77.4 (C-3), 76.5 (C-4'), 75.6 (CH_2arom), 75.2 (C-4), 75.1 (C-5), 74.2 (CH_2arom), 73.5 (CH_2arom), 73.3 (CH_2arom), 73.2 (C-5'), 72.1 (C-3'), 69.7 (OCH_2), 68.8 (C-

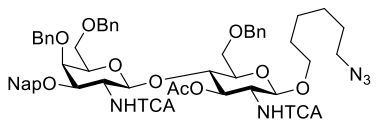
6), 68.0 (C-6'), 57.2 (C-2'), 56.9 (C-2), 51.5 (CH_2N_3), 29.4, 28.8, 26.6 (CH_2, hexyl), 25.9 (*t*-Bu, TBDMS), 25.7 (CH_2, hexyl), 18.0 (C_q, TBDMS), -3.3, -4.8 (CH_3, TBDMS) ppm.

6-azidohexyl 6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2-methylnaphthyl)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-3-*O*-levulinoyl-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (24)



Donor **19** (0.050 g, 0.065 mmol, 1.3 eq.) and acceptor **10** (0.032 g, 0.050 mmol, 1.0 eq.) were co-evaporated together with dry toluene (3x) and dissolved in a mixture of dry DCM and ACN (0.5 mL, 0.1M, 4/1, v/v). Freshly dried molecular sieves (3Å) were added and the mixture was cooled to -20˚C and stirred for 30 min. before TfOH (100 µL, 0.2 eq. of a 0.1M solution in DCM) was added. Upon completion the reaction was stopped by addition of Et_3N (50 µL), diluted in EtOAc, washed with sat $NaHCO_3$ (aq.) and brine, followed by drying over $MgSO_4$, filtering and concentration *in vacuo*. The resulting yellow oil was purified by silicagel chromatography (tol:ACN, 19:1 → 9:1) to give the title compound (0.028 g, 0.022 mmol, 44%) ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.86 – 7.71 (m, 4H, arom.), 7.47 (m, 2H, arom.), 7.44 – 7.12 (m, 21H, arom.), 7.01 (d, 1H, *J*=7.7 Hz, NH'), 6.73 (d, 1H, *J*=8.8 Hz, NH), 5.17 (dd, 1H, *J*=10.5, 9.1 Hz, H-3), 4.88 (d, 1H, *J*=11.1 Hz, CH_2arom.), 4.85 – 4.76 (m, 2H, H-1', CH_2arom.), 4.70 – 4.48 (m, 7H, H-1, CH_2arom.), 4.13 – 3.98 (m, 3H, H-4, H-3', H-4'), 3.90 – 3.69 (m, 4H, H-2, H-2', H-6, OCH_2), 3.69 – 3.46 (m, 5H, H-5, H-6, H-5', H-6'), 3.44 – 3.32 (m, 1H, OCH_2), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.49 – 2.21 (m, 4H, CH_2Lev)), 1.91 (s, 3H, CH_3, Lev), 1.63 – 1.47 (m, 4H, CH_2, hexyl), 1.41 – 1.26 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 206.9 (CH_3C=O, Lev), 173.0 (OC=O, Lev), 162.0, 162.0 (C=O, TCA), 138.6, 138.3, 137.8, 135.1, 133.3, 133.2, 129.2, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 126.8, 126.4, 126.2, 125.8, 125.4 (arom.), 100.8 (C-1), 97.5 (C-1'), 92.7, 92.5 (C_q, TCA), 77.3 (C-3'), 75.0 (CH_2arom.), 74.9 (C-5/5'), 73.7 (CH_2arom.), 73.2 (C-5/5'), 73.2 CCH_2arom.), 72.7 (C-4), 72.5 (C-4'), 72.4 (CH_2arom.), 71.6 (C-3), 69.8 (OCH_2), 68.2 (C-6), 68.1 (C-6'), 56.2 (C-2'), 56.0 (C-2), 51.5 (CH_2N_3), 37.6 (CH_2, Lev), 29.7 (CH_3, Lev), 29.4, 28.9, 28.0, 26.6, 25.6 (CH_2, hexyl, Lev) ppm. HRMS $[M+Na]^+$ calcd for $C_{59}H_{65}Cl_6N_5O_{13}Na$: 1284.26077, found 1284.26022.

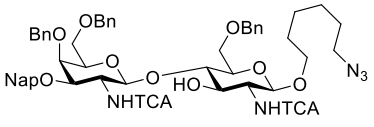
6-azidohexyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2-methylnaphthyl)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (25)



Donor **19** (2.46 g, 3.12 mmol, 1.5 eq.) and acceptor **13** (1.21 g, 2.08 mmol, 1.0 eq.) were co-evaporated thrice with dry toluene, before dissolving in a mixture of dry DCM and dry ACN (10 mL, 0.1M, 4/1,

v/v). Freshly dried molecular sieves (4Å) were added and the reaction mixture was cooled to -40˚C and left to stir for 1 hour at that temperature. TfOH (36 µL, 0.42 mmol, 0.2 eq.) was added and the reaction was warmed to -30˚C and kept at that temperature. TLC analysis showed full consumption of acceptor **13** (~5 hours). Et_3N (0.4 mL) was added and the reaction mixture was diluted in EtOAc. The organic layer was washed with HCl (1M, aq.), sat. $NaHCO_3$ (aq.) and brine. Any traces of water were removed by drying over $MgSO_4$ followed by filtration and concentration *in vacuo*. The title compound **25** was obtained by silicagel chromatography (tol:ACN, 1:0 → 19:1) followed by size exclusion (LH-20, DCM/MeOH, 1/1, v/v) as a viscous colourless oil (1.55 g, 1.28 mmol, 82%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.89 – 7.71 (m, 4H, arom.), 7.53 – 7.46 (m, 2H, arom.), 7.44 – 7.13 (m, 20H, arom.), 6.80 (d, 1H, *J*=7.3 Hz, NH'), 6.73 (d, 1H, *J*=8.6 Hz, NH), 5.10 (t, 1H, *J*=10.6 Hz, H-3), 4.88 (m, 2H, H-1', CH_2arom.), 4.79 (d, 1H, *J*=11.5 Hz, CH_2arom.), 4.70 – 4.59 (m, 2H, CH_2arom.), 4.58 – 4.40 (m, 5H, H-1, CH_2arom.), 4.14 (dd, 1H, *J*=11.0, 2.7 Hz, H-3'), 4.09 – 4.00 (m, 2H, H-4, H-4'), 3.95 (dt, 1H, *J*=10.6, 8.6 Hz, H-2), 3.86 (dt, 1H, *J*=9.5, 6.1 Hz, OCH_2), 3.75 – 3.65 (m, 2H, H-6, H-2'), 3.65 – 3.58 (m, 2H, H-6'), 3.55 (dd, 1H, *J*=8.8, 5.1 Hz, H-6), 3.53 – 3.45 (m, 2H, H-5, H-5'), 3.42 (dt, 1H, *J*=9.5, 6.6 Hz, OCH_2), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.82 (s, 3H, CH_3, Ac), 1.58 – 1.50 (m, 4H, CH_2, hexyl), 1.32 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 126 MHz) δ 171.4 (C=O, Ac), 162.0, 161.9 (C=O, TCA), 138.5, 138.2, 137.8, 135.0, 133.3, 133.2, 128.7, 128.5, 128.5, 128.4, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 126.8, 126.4, 126.3, 125.9 (arom.), 101.1 (C-1), 97.6 (C-1'), 92.6 (C_q, TCA), 77.4 (C-3'), 75.0 (C-5), 74.9 (CH_2arom), 73.7 (CH_2arom), 73.2 (C-5, CH_2arom), 72.6 (C-4), 72.3 (CH_2arom), 72.3 (C-4'), 72.1 (C-3), 69.7 (OCH_2), 68.1 (C-6), 68.0 (C-6'), 56.5 (C-2'), 55.6 (C-2), 51.5 (CH_2N_3), 29.4, 28.9, 26.6, 25.6 (CH_2-hexyl), 20.7 (CH_3, Ac) ppm. HRMS $[M+Na]^+$ calcd for $C_{56}H_{61}Cl_6N_5O_{12}Na$: 1228.23456, found 1228.23401.

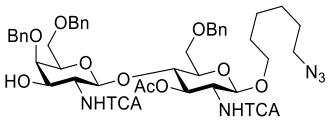
6-azidohexyl 6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-3-*O*-(2-methylnaphthyl)-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (27)



NaOH (83 µL, 0.1 eq., 0.1M, aq.) was added to a solution of disaccharide **25** (1.0 g, 0.83 mmol, 1.0 eq.) in dioxane (4 mL, 0.2M). The solution was left to stir until TLC analysis showed full consumption of the starting material (~8 hours), before the reaction was stopped by addition of AcOH (0.1M in H_2O). The volatiles were evaporated and the residue was taken up in EtOAc and subsequently washed with sat. $NaHCO_3$ (aq.) and brine. The title compound was obtained by silicagel chromatography (tol:ACN, 1:0 → 17:3) as a white solid (0.56 g, 0.48 mmol, 58%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.87 – 7.73 (m, 4H, arom.), 7.53 – 7.47 (m, 2H, arom.), 7.42 (dd, 1H, *J*=8.4, 1.7 Hz, arom.), 7.39 – 7.10 (m, 16H, arom), 6.83 – 6.73 (m,

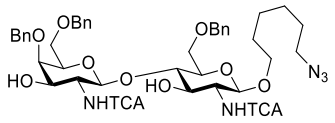
2H, NH, NH'), 4.87 (d, 1H, *J*=11.5 Hz, CH_2arom), 4.83 – 4.73 (m, 3H, H-1, H-1', CH_2arom), 4.70 – 4.61 (m, 2H, CH_2arom), 4.52 (dd, 2H, *J*=11.8, 5.2 Hz, CH_2arom), 4.49 – 4.35 (m, 2H, CH_2arom), 4.09 – 3.95 (m, 3H, H-2, H-4, H-3'), 3.92 (d, 1H, *J*=1.8 Hz, H-4'), 3.86 (dt, 1H, *J*=9.6, 6.1 Hz, OCH_2), 3.75 – 3.57 (m, 5H, H-3, H-6, H-5', H-6'), 3.54 (ddd, 1H, *J*=7.7, 6.0, 4.2 Hz, H-5), 3.51 – 3.35 (m, 3H, H-2', H-6', OCH_2), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.62 – 1.49 (m, 4H, CH_2, hexyl), 1.39 – 1.30 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 162.1, 161.8 (C=O, TCA), 138.5, 138.0, 137.4, 134.7, 133.3, 133.2, 128.7, 128.6, 128.5, 128.5, 128.4, 128.2, 128.2, 128.0, 128.0, 128.0, 127.9, 127.8, 127.0, 126.5, 126.4, 125.9 (arom.), 99.9 (C-1), 99.7 (C-1'), 92.5 (C_q, TCA), 80.7 (C-5'), 77.6 (C-3'), 74.8 (CH_2arom), 74.3 (C-5), 74.0, (C-3) 73.8 (CH_2arom), 73.5 (CH_2arom), 72.5 (CH_2arom), 71.7 (C-4'), 71.4 (C-4), 69.8 (OCH_2), 68.6 (C-6), 68.5 (C-6'), 58.6 (C-2'), 55.5 (C-2), 51.5 (CH_2N_3), 29.5, 28.9, 26.6, 25.7 (CH_2, hexyl) ppm. HRMS $[M+Na]^+$ calcd for $C_{54}H_{59}Cl_6N_5O_{11}Na$: 1186.22399, found 1186.22344.

6-azidohexyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (28)



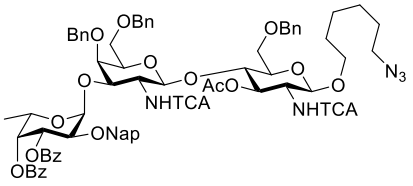
Disaccharide **25** (0.14 g, 0.12 mmol, 1.0 eq.) was dissolved in a mixture of DCM and HFIP (1.2 mL, 0.1M, 1/1, v/v). TES (58 µL, 0.36 mmol, 3.0 eq.) was added followed by 83 µL of HCl (1.0M, in HFIP). After 3 hours TLC analysis showed full conversion of the starting material and the reaction was stopped by addition of Et_3N (0.2 mL). The mixture was taken up in EtOAc and washed with sat. $NaHCO_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mixture was purified by silicagel chromatography (tol:ACN, 1:0 → 17:3), giving disaccharide **28** (0.070 g, 0.065 mmol, 55%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.42 – 7.20 (m, 16H, arom.), 6.96 (d, 1H, *J*=9.2 Hz, NH), 6.67 (d, 1H, *J*=7.6 Hz, NH'), 5.10 (dd, 1H, *J*=10.7, 9.0 Hz, H-3), 4.75 – 4.60 (m, 3H, CH_2Bn), 4.57 – 4.43 (m, 5H, H-1, H-1', CH_2Bn), 4.05 – 3.93 (m, 2H, H-2, H-4), 3.90 – 3.81 (m, 2H, H-4', OCH_2), 3.72 – 3.63 (m, 3H, H-6, H-3'), 3.63 – 3.57 (m, 2H, H-6'), 3.57 – 3.51 (m, 1H, H-2'), 3.51 – 3.38 (m, 3H, H-5, H-5', OCH_2), 3.21 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.36 (d, 1H, *J*=9.7 Hz, OH), 1.89 (s, 3H, CH_3, Ac), 1.59 – 1.46 (m, 4H, CH_2, hexyl), 1.36 – 1.27 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 171.3 (C=O, Ac), 162.5, 162.1 (C=O, TCA), 138.0, 137.8, 137.5, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9 (arom.), 101.0 (C-1), 98.8 (C-1'), 92.6 , 92.6 (C_q, TCA), 75.6 (C-4'), 75.4 (CH_2Bn), 74.6 (C-5), 74.0 (C-4), 73.7 (CH_2Bn), 73.6 (CH_2Bn), 73.2 (C-5'), 72.2 (C-3), 70.9 (C-3'), 69.7 (OCH_2), 68.4 (H-6), 67.8 (H-6'), 57.4 (C-2'), 55.4 (C-2), 51.4 (CH_2N_3), 29.4, 28.8, 26.5, 25.6 (CH_2-hexyl), 20.8 (CH_3, Ac) ppm. HRMS $[M+Na]^+$ calcd for $C_{45}H_{53}Cl_6N_5O_{12}Na$: 1088.17196, found 1088.17141.

6-azidohexyl 6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (29)



Disaccharide **28** (0.185 g, 0.174 mmol, 1.0 eq.) was dissolved in a mixture of THF and MeOH (1.7 mL, 0.1M, 6/1, v/v). NaOMe (2.0 mg, 0.034 mmol, 0.2 eq.) was added and the mixture was stirred heated to 40˚C. When TLC analysis showed full consumption of the starting material (~6 hours) the reaction mixture was neutralized by addition of AcOH (0.1M in H_2O). The reaction mixture was diluted in EtOAc, transferred to a separatory funnel and washed with sat. $NaHCO_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The title compound was obtained after purification by silicagel chromatography (tol:EtOAc, 1:0 → 3:2) as a white amorphous solid (0.12 g, 0.12 mmol, 68%).^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.40 – 7.22 (m, 15H,), 6.90 (d, 1H, *J*=8.2 Hz, NH), 6.80 (d, 1H, *J*=7.9 Hz, NH), 4.77 – 4.57 (m, 4H, H-1', CH_2Bn), 4.55 – 4.37 (m, 4H, H-1, CH_2Bn), 4.04 – 3.90 (m, 2H, H-3', H-2), 3.85 (dt, 1H, *J*=9.6, 6.2 Hz, OCH_2), 3.79 (dd, 1H, *J*=11.4, 4.3 Hz, H-6), 3.75 – 3.66 (m, 2H, H-4, H-6), 3.67 – 3.58 (m, 4H, H-3, H-4', H-5', H-6'), 3.58 – 3.50 (m, 2H, H-5, H-2'), 3.49 – 3.40 (m, 2H, H-6', OCH_2), 3.23 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.44 (s, 1H, OH), 1.81 (s, 1H, OH), 1.63 – 1.50 (m, 4H, CH_2, hexyl), 1.42 – 1.26 (m, 4H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 126 MHz) δ 163.3, 161.9 (C=O, TCA), 138.2, 137.6, 137.3, 128.8, 128.7, 128.7, 128.3, 128.3, 128.2, 128.2 (arom.), 100.7 (C-1), 99.9 (C-1'), 92.8 (C_q, TCA), 92.5 (C_q, TCA), 81.4 (C-3), 75.6 (CH_2Bn), 75.5 (C-4), 74.1 (C-5), 74.0 (C-5'), 73.8 (CH_2Bn), 73.8 (CH_2Bn), 72.2 (C-4'), 71.6 (C-3'), 69.9 (OCH_2), 68.7 (C-6), 68.4 (C-6'), 58.4 (C-2'), 56.4 (C-2), 51.5 (CH_2N_3), 29.5, 28.9, 26.6, 25.7 (CH_2-hexyl) ppm. HRMS $[M+Na]^+$ calcd for $C_{43}H_{51}Cl_6N_5O_{11}Na$: 1046.16139, found 1046.16084.

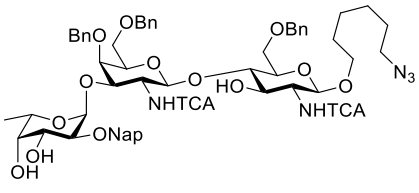
6-azidohexyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2-methylnaphthyl)-α-L-fucopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (30)



Donor **A** (0.121 g, 0.2 mmol, 2.0 eq.) and acceptor **28** (0.107 g, 0.1 mmol) were co-evaporated together thrice with dry toluene, before dissolving them in dry DCM (1 mL, 0.1M). Freshly dried MS (4Å) were added and the mixture was stirred for 15 min. at room temperature. Next NIS (0.049 g, 0.022 mmol, 2.2 eq.) was added and the mixture was cooled to -40˚C, at which temperature it was stirred for an additional 30 min. TMSOTf (100 µL, 0.1 eq., of a 0.1M solution in DCM) was added and the mixture was allowed to warm up to -20˚C and kept at that temperature. After 6 hours the reaction was stopped

by addition of Et_3N (0.05 mL). The reaction mixture was diluted in EtOAc, washed twice with sat. $Na_2S_2O_3$ (aq.), followed by sat. $NaHCO_3$ (aq.) and brine. After drying over $MgSO_4$ and filtration the solvents were removed by evaporation. The title compound was separated by silicagel chromatography (tol:EtOAc, 1:0 → 4:1), followed by size exclusion (LH-20, MeOH/DCM, 1/1, v/v). This gave the title compound as a white solid (0.102 g, 0.065 mmol, 65%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.67 – 7.55 (m, 3H, arom.), 7.55 – 7.46 (m, 5H, arom.), 7.42 – 7.28 (m, 4H, arom.), 7.27 – 6.99 (m, 22H, NH', arom.), 6.84 (d, 1H, *J*=9.3 Hz, NH), 5.54 (dd, 1H, *J*=10.5, 3.3 Hz, H-3''), 5.45 (dd, 1H, *J*=3.4, 1.3 Hz, H-4''), 5.01 – 4.88 (m, 2H, H-3, H-1''), 4.84 – 4.72 (m, 2H, H-1', CH_2arom), 4.64 (s, 2H, CH_2arom), 4.49 – 4.39 (m, 3H, CH_2arom), 4.38 – 4.21 (m, 4H, H-1, H-5'', CH_2arom), 4.13 (dd, 1H, *J*=11.1, 2.9 Hz, H-3'), 4.03 (dd, 1H, *J*=10.5, 3.3 Hz, H-2''), 3.95 – 3.82 (m, 2H, H-2, H-4), 3.80 (d, 1H, *J*=2.9 Hz, H-4'), 3.68 – 3.57 (m, 2H, H-2', OCH_2), 3.57 – 3.41 (m, 5H, H-5, H-6, H-6'), 3.37 (dd, 1H, *J*=8.4, 5.0 Hz, H-5'), 3.12 (dt, 1H, *J*=9.6, 6.6 Hz, OCH_2), 3.04 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.66 (s, 3H, CH_3, Ac), 1.44 – 1.28 (m, 4H, CH_2, hexyl), 1.20 – 1.07 (m, 4H, CH_2, hexyl), 0.86 (d, 3H, *J*=6.5 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 171.2 (C=O, Ac), 166.1, 165.6 (C=O, Bz), 162.1, 162.0 (C=O, TCA), 138.4, 137.7, 135.0, 133.4, 133.2, 133.1, 129.7, 129.6, 129.4, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 128.0, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 126.4, 126.3, 126.3 (arom.), 100.9 (C-1), 97.8 (C-1'), 97.8 (C-1''), 92.8, 92.6 (C_q, TCA), 77.4 (C-3'), 75.1 (C-5), 74.8 (CH_2arom), 73.6 (CH_2arom), 73.5 (CH_2arom), 73.5 (C-4'), 73.3 (C-2''), 73.1 (CH_2arom), 73.0 (C-5'), 72.4 (C-4), 72.3 (C-3), 72.2 (C-4''), 70.8 (C-3''), 69.7 (OCH_2), 68.5 (C-6), 67.8 (C-6'), 66.5 (C-5'), 55.9 (C-2'), 55.3 (C-2), 51.4 (CH_2N_3), 29.4, 28.8, 26.5, 25.6, 20.7 (CH_2-hexyl), 16.0 (C-6'') ppm. HRMS $[M+Na]^+$ calcd for Chemical Formula: $C_{76}H_{79}Cl_6N_5O_{18}Na$: 1582.34490, found 1582.34435.

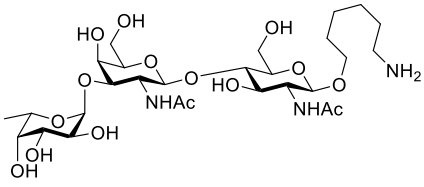
6-azidohexyl 6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2-*O*-(2-methylnaphthyl)-α-L-fucopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (30a)



Trisaccharide **30** (0.080 g, 0.05 mmol, 1.0 eq.) was dissolved in a mixture of THF and MeOH (0.5 mL, 0.1M, 4/1, v/v). NaOMe (100 µL of a 0.1M solution of NaOMe in MeOH, 0.2 eq.) was added and the mixture was left to stir overnight at room temperature. The reaction mixture was diluted with THF (0.5 mL) and neutralized with Dowex H^+ resin. The resin was filtered off and the volatiles were removed *in vacuo*. The crude was purified by size exclusion (DCM/MeOH, 1/1, v/v) to give the title compound as a colourless film (0.043 g, 0.032 mmol, 64%). ^{1}H NMR (CD_3CN, 500 MHz): δ = 7.87 (m, 4H, arom.), 7.58 (d, 1H, *J*=10.5 Hz, NH'), 7.53 – 7.47 (m, 2H, arom.), 7.45 (d, 1H, *J*=8.7 Hz, NH), 7.38 (d, 4H, *J*=5.7 Hz, arom.),

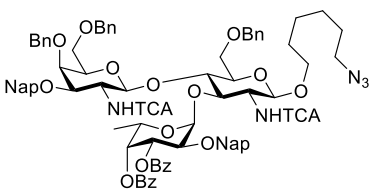
7.35 – 7.22 (m, 11H, arom.), 5.22 (d, 1H, *J*=3.5 Hz, H-1''), 4.91 – 4.81 (m, 3H, CH_2arom), 4.57 – 4.51 (m, 1H, CH_2arom), 4.51 – 4.43 (m, 4H, H-1, CH_2arom), 4.40 – 4.32 (m, 3H, H-1', CH_2arom), 4.17 – 4.09 (m, 1H, H-2'), 4.06 (dd, 1H, *J*=11.2, 2.6 Hz, H-3'), 3.98 (q, 1H, *J*=6.7, 5.7 Hz, H-5''), 3.95 – 3.88 (m, 2H, H-4', H-3''), 3.85 – 3.78 (m, 1H, H-5), 3.78 – 3.70 (m, 2H, H-2'', OCH_2), 3.68 – 3.58 (m, 4H, H-2, H-6, H-5', H-4''), 3.55 – 3.46 (m, 2H, H-6, H-6'), 3.46 – 3.40 (m, 2H, H-4, OCH_2), 3.39 – 3.36 (m, 1H, H-6'), 3.31 (dd, 1H, *J*=9.5, 7.4 Hz, H-3), 3.23 (t, 2H, *J*=7.0 Hz, CH_2N_3), 3.15 (d, 1H, *J*=5.8 Hz, 3''-OH), 3.03 (d, 1H, *J*=4.1 Hz, 4''-OH), 1.51 (m, 4H, CH_2, hexyl), 1.35 – 1.24 (m, 4H, CH_2, hexyl), 1.11 (d, 3H, *J*=6.5 Hz, H-6'') ppm. ^{13}C-APT NMR (CD_3CN, 126 MHz): δ 163.4, 162.9 (C=O, TCA), 139.8, 139.5, 139.0, 137.3, 134.2, 134.0, 129.4, 129.3, 129.2, 129.2, 129.2, 129.0, 128.8, 128.7, 128.7, 128.4, 128.3, 128.0, 127.5, 127.4, 127.1 (arom.), 102.5 (C-1'), 101.1 (C-1), 98.0 (C-1''), 93.8, 93.3 (C_q, TCA), 82.2 (C-3), 78.4 (C-2''), 77.5 (C-3'), 75.6 (CH_2arom), 74.9 (C-4), 74.7 (C-4'), 74.6 (C-5), 74.3 (CH_2arom), 73.9 (CH_2arom), 73.6 (CH_2arom), 73.3 (C-5'), 72.8 (C-4''), 70.4 (C-3''), 70.2 (C-6', OCH_2), 69.6 (C-6), 68.4 (C-5''), 58.2 (C-2), 54.9 (C-2'), 52.0 (CH_2N_3), 30.1, 29.4, 27.1, 26.2 (CH_2-hexyl), 16.7 (C-6'') ppm. HRMS $[M+Na]^+$ calcd for $C_{60}H_{69}Cl_6N_5O_{15}Na$: 1332.28190, found 1332.28135.

6-aminohexyl 2-acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranoside)-β-D-galactopyranoside)-β-D-glucopyranoside (F-LDN)



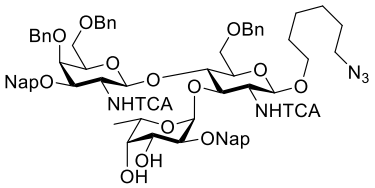
Pd/C (10 mg) was added to a degassed solution of **30a** (0.032 g, 0.024 mmol, 1.0 eq.) in EtOH (0.5 mL). The solution was kept under hydrogen atmosphere for 24 hours. The hydrogen atmosphere was replaced by a nitrogen atmosphere and the solution was filtered over a whatman filter. The filtrate was concentrated *in vacuo*, redissolved in H_2O and purified by HW-40 size exclusion chromatography. After lyophilisation **F-LDN** was obtained as a white solid. (6.1 mg, 9.1 µmol, 38%). ^{1}H NMR (D_2O, 500 MHz): δ = 4.99 (d, 1H, *J*=4.1 Hz, H-1''), 4.58 (d, 1H, *J*=8.5 Hz, H-1'), 4.49 (d, 1H, *J*=8.2 Hz, H-1), 4.11 (q, 1H, *J*=6.6 Hz, H-5''), 4.04 (dd, 1H, *J*=10.9, 8.4 Hz, H-2'), 3.97 (d, 1H, *J*=3.2 Hz), 3.93 – 3.60 (m, 11H), 3.57 (dt, 1H, *J*=10.4, 6.4 Hz, OCH_2), 3.50 (ddd, 1H, *J*=9.8, 5.5, 2.1 Hz), 2.98 (t, 2H, *J*=7.7 Hz, CH_2N_3), 2.04 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac), 1.68 – 1.50 (m, 4H, CH_2, hexyl), 1.42 – 1.32 (m, 4H, CH_2, hexyl), 1.19 (d, 3H, *J*=6.6 Hz, H-6'') ppm. ^{13}C-APT NMR (D_2O, 126 MHz): δ 175.0, 174.4 (C=O, Ac), 101.5 (C-1'', C-1'), 101.1 (C-1), 79.1, 78.4, 75.3, 74.5, 72.6, 71.8, 71.6, 70.4 (OCH_2), 69.3, 68.2, 67.9, 67.2 (C-5''), 60.9 (C-6'), 60.2 (C-6), 54.9 (C-2), 51.7 (C-2'), 39.5 (CH_2N_3), 28.4, 26.7, 25.3, 24.6 (CH_2-hexyl), 22.2 (CH_3, Ac), 15.4 (C-6'') ppm. HRMS $[M+H]^+$ calcd for $C_{28}H_{51}N_3O_{15}H$: 670.33985, found 670. 670.33929.

6-azidohexyl 6-*O*-benzyl-2-deoxy-3-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2-methylnaphthyl)-α-L-fucopyranoside)-4-*O*-(4,6-di-*O*-benzyl-3-*O*-(2-methylnaphthyl)-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (31)



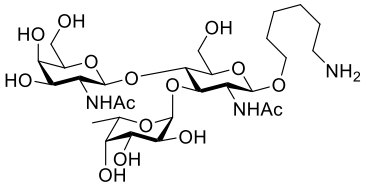
Disaccharide acceptor **27** (0.088 g, 0.075 mmol, 1.0 eq.) and donor **A** (0.091 g, 0.075 mmol, 2.0eq.) were co-evaporated together thrice with dry toluene, before dissolving them in dry DCM (0.75 mL, 0.1M). Freshly dried MS (4Å) were added and the mixture was stirred for 15 min. at room, before addition of NIS (0.037 g, 0.165 mmol, 2.2 eq.). The mixture was cooled to -40˚C, at which temperature it was stirred for an additional 30 min. TMSOTf (150 µL, 0.2 eq. of a 0.1M solution in DCM) was added and the mixture was allowed to warm up to -20˚C and kept at that temperature. After 5 hours the reaction was stopped by addition of Et_3N (0.05 mL). The reaction mixture was diluted in EtOAc, washed twice with sat. $Na_2S_2O_3$ (aq.), followed by sat. $NaHCO_3$ (aq.) and brine. After drying over $MgSO_4$ and filtration the solvents were removed by evaporation. The title compound was separated by silicagel chromatography (tol:EtOAc, 1:0 → 4:1), followed by size exclusion (LH-20, MeOH/DCM, 1/1, v/v). This gave trisaccharide **31** as colourless film (0.086 g, 0.052 mmol, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.03 – 7.71 (m, 11H, arom.), 7.71 – 7.26 (m, 25H, arom.), 7.26 – 7.02 (m, 7H, NH', arom.), 6.52 (d, 1H, *J*=8.1 Hz, NH), 5.82 (dd, 1H, *J*=10.5, 3.4 Hz, H-3''), 5.74 (dd, 1H, *J*=3.5, 1.3 Hz, H-4''), 5.48 (d, 1H, *J*=3.7 Hz, H-1''), 5.05 (q, 1H, *J*=6.1 Hz, H-5'') 4.97 – 4.73 (m, 7H, H-1, H-1', CH_2arom), 4.68 (d, 1H, *J*=11.8 Hz, CH_2arom), 4.64 – 4.48 (m, 4H, CH_2arom), 4.40 (t, 1H, *J*=7.9 Hz, H-3), 4.31 – 4.20 (m, 2H, H-4, H-2''), 4.16 (d, 1H, *J*=2.7 Hz, H-4'), 4.01 (m, 2H, H-2', H-6'), 3.93 (dd, 1H, *J*=9.0, 4.9 Hz, H-6'), 3.90 – 3.71 (m, 5H, H-2, H-6, H-3', OCH_2), 3.59 (dt, 1H, *J*=8.3, 3.3 Hz, H-5), 3.53 – 3.38 (m, 2H, H-5', OCH_2), 3.21 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.61 – 1.46 (m, 4H, CH_2, hexyl), 1.39 – 1.25 (m, 4H, CH_2, hexyl), 1.16 (d, 3H, *J*=6.5 Hz, H-6'') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 166.1, 165.3 (C=O, Bz), 161.9, 161.6 (C=O, TCA), 138.3, 138.1, 138.0, 135.6, 134.9, 133.3, 133.2, 133.2, 133.1, 133.0, 132.9, 130.1, 130.0, 129.8, 129.7, 129.5, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.0, 126.4, 126.3, 126.3, 126.1, 126.0, 125.9, 125.7 (arom.), 99.0 (C-1), 98.9 (C-1'), 97.2 (C-1''), 92.7, 92.5 (C_q, TCA), 77.6 (C-3'), 74.9 (C-5), 74.6 (C-3), 74.5 (CH_2arom), 74.1 (C-2''), 73.5 (C-5'), 73.4 (CH_2arom), 73.3 (CH_2arom), 73.2 (C-4), 73.1 (CH_2arom), 72.9 (C-4''), 71.5 (CH_2arom), 70.9 (C-3''), 70.1 (C-4'), 69.7 (OCH_2), 68.5 (C-6), 67.9 (C-6'), 65.3 (C-5''), 59.1 (C-2), 55.4 (C-2'), 51.4 (CH_2N_3), 29.4, 28.8, 26.5, 25.6 (CH_2-hexyl), 15.9 (CH_3, Ac) ppm. HRMS $[M+Na]^+$ calcd for $C_{85}H_{85}Cl_6N_5O_{17}Na$: 1680.39693, found 1680.39638.

6-azidohexyl 6-*O*-benzyl-2-deoxy-3-*O*-(2-*O*-(2-methylnaphthyl)-α-L-fucopyranoside)-4-*O*-(4,6-di-*O*-benzyl-3-*O*-(2-methylnaphthyl)-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (31a)



NaOMe (100 µL of a 0.1M solution of NaOMe in MeOH, 0.2 eq.) was added to a solution of trisaccharide **31** (0.080 g, 0.048 mmol, 1.0 eq.) in a mixture of THF and MeOH (0.5 mL, 0.1M, 5/2, v/v). The reaction mixture was left to stir overnight at room temperature. The reaction mixture was diluted with THF (1 mL) and neutralized by addition of a solution of AcOH (0.1M in H_2O). The mixture was concentrated and purified by size exclusion (DCM/MeOH, 1/1, v/v) to give the title compound as a colourless film. (0.047 g, 0.032 mmol, 65%) ^{1}H NMR (CD_3CN, 500 MHz): δ = 7.92 – 7.75 (m, 8H, arom.), 7.61 (d, 1H, *J*=9.5 Hz, NH'), 7.55 – 7.45 (m, 5H, arom.), 7.42 (d, 1H, *J*=9.5 Hz, NH), 7.39 – 7.15 (m, 14H, arom.), 5.37 (d, 1H, *J*=3.6 Hz, H-1''), 4.85 (d, 1H, *J*=11.7 Hz, CH_2arom), 4.82 – 4.72 (m, 2H, CH_2arom), 4.68 (d, 1H, *J*=11.7 Hz, CH_2arom), 4.64 – 4.52 (m, 7H, H-1, CH_2arom), 4.50 – 4.39 (m, 2H, H-1', CH_2arom), 4.16 – 3.99 (m, 4H, H-2, H-3', H-4', H-3''), 3.91 (t, 1H, *J*=8.8 Hz, H-3), 3.87 – 3.69 (m, 5H, H-2', H-5', H-6', OCH_2), 3.69 – 3.56 (m, 3H, H-6, H-2''), 3.53 (t, 1H, *J*=6.4 Hz, H-5), 3.48 – 3.36 (m, 3H, H-4, H-4'', OCH_2), 3.23 (t, 2H, *J*=7.0 Hz, CH_2N_3), 2.91 (d, 1H, *J*=5.4 Hz, 3''-OH), 2.39 (d, 1H, *J*=3.9 Hz, 4''-OH), 1.57 – 1.42 (m, 4H, CH_2, hexyl), 1.34 – 1.22 (m, 4H, CH_2, hexyl), 1.06 (d, 3H, *J*=6.6 Hz, H-6'') ppm. ^{13}C-APT NMR (CD_3CN, 126 MHz) δ 163.1, 162.5 (C=O, TCA), 139.6, 139.5, 139.5, 137.6, 136.7, 134.2, 134.2, 134.0, 133.8, 129.8, 129.4, 129.3, 129.2, 129.1, 128.9, 128.8, 128.8, 128.8, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 127.7, 127.3, 127.3, 127.3, 127.2, 127.0, 126.9, 126.7 (arom.), 101.5 (C-1'), 100.9 (C-1), 96.4 (C-1''), 93.8 (C_q, TCA), 79.4 (C-5'), 77.7 (C-2''), 75.8 (C-4), 75.7 (CH_2arom), 74.5 (C-5), 74.4 (C-3), 74.1 (CH_2arom), 73.6 (C-3'), 73.4 (CH_2arom), 73.2 (C-4'), 72.9 (C-4''), 72.6 (CH_2arom), 72.5 (CH_2arom), 70.4 (OCH_2), 69.7 (H-6'), 69.5 (H-6), 69.3 (C-3''), 66.7 (C-5''), 59.2 (C-2'), 55.2 (C-2), 52.0 (CH_2N_3), 30.2, 29.3, 27.1, 26.3 (CH_2-hexyl), 16.6 (C-6'') ppm. HRMS $[M+Na]^+$ calcd for $C_{71}H_{77}Cl_6N_5O_{15}Na$: 1472.34450, found 1472.34395.

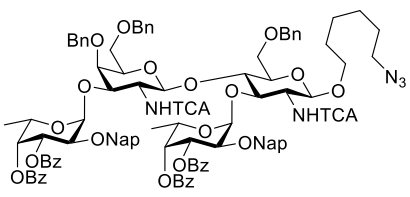
6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranoside)-4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranoside)-β-D-glucopyranoside (LDN-F)



Pd/C (10 mg) was added to a degassed solution of **31a** (0.021 g, 0.014 mmol, 1.0 eq.) in EtOH (0.5 mL). The solution was kept under hydrogen atmosphere for 24 hours. The hydrogen atmosphere was replaced by a nitrogen atmosphere and the solution was filtered over a whatman filter. The filtrate was concentrated *in vacuo*, redissolved in H_2O and purified by HW-40 size exclusion chromatography. After lyophilisation **LDN-F**

was obtained as a white solid (2.3 mg, 0.0034 mmol, 24%). ^{1}H NMR (D_2O, 500 MHz): δ = 5.00 (d, 1H, *J*=3.9 Hz, H-1''), 4.78 – 4.72 (m, 1H, H-5''), 4.43 – 4.32 (m, 2H, H-1, H-1''), 3.89 – 3.70 (m, 11H), 3.70 – 3.49 (m, 9H), 3.49 – 3.41 (m, 1H), 3.41 – 3.34 (m, 1H), 3.07 – 3.01 (m, 1H), 2.86 (t, 2H, *J*=7.7 Hz, CH_2N), 1.93 (s, 3H, CH_3, Ac), 1.90 (s, 3H, CH_3, Ac), 1.58 – 1.50 (m, 2H, CH_2, hexyl), 1.44 (s, 2H, CH_2, hexyl), 1.30 – 1.20 (m, 4H, CH_2, hexyl), 1.15 (d, 3H, *J*=6.6 Hz, H-6'') ppm HRMS $[M+H]^+$ calcd for $C_{28}H_{51}N_3O_{15}H$: 670.33985, found 670.33929.

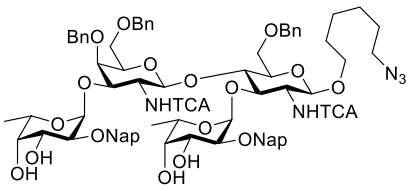
6-azidohexyl 6-*O*-benzyl-2-deoxy-3-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2-methylnaphthyl)-α-L-fucopyranoside)-4-*O*-(4,6-di-*O*-benzyl-3-*O*-(3,4-di-*O*-benzoyl-2-*O*-(2-methylnaphthyl)-α-L-fucopyranoside)-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (32)



Diol acceptor **29** (0.10 g, 0.10 mmol, 1.0 eq.) and donor **A** (0.30 g, 0.5 mmol, 5.0 eq.) were co-evaporated together thrice with dry toluene, before dissolving them in dry DCM (1 mL, 0.1M). Freshly dried MS (4Å) were added and the mixture was stirred for 15 min. at room temperature. Next NIS (0.14 g, 0.6 mmol, 6.0 eq.) was added and the mixture was cooled to -40˚C, at which temperature it was stirred for an additional 30 min. TMSOTf (100 µL, of a 0.1M solution in DCM) was added and the mixture was allowed to warm up to -20˚C and kept at that temperature. After 6 hours the reaction was stopped by addition of Et_3N (0.05 mL). The reaction mixture was diluted in EtOAc, washed twice with sat. $Na_2S_2O_3$ (aq.), followed by sat. $NaHCO_3$ (aq.) and brine. After drying over $MgSO_4$ and filtration the solvents were removed by evaporation. The title compound was separated by silicagel chromatography (tol:EtOAc, 1:0 → 4:1), followed by size exclusion (LH-20, MeOH/DCM, 1/1, v/v). This gave tetrasaccharide **32** as a white solid (0.14 g, 0.069 mmol, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.94 – 7.81 (m, 6H, arom), 7.81 – 7.65 (m, 8H, arom), 7.65 – 7.43 (m, 9H, arom), 7.44 – 7.14 (m, 31H, NH, NH', arom.), 5.76 – 5.54 (m, 5H, H-1'', H-3'', H-4'', H-3''', H-4'''), 5.17 (d, 1H, *J*=3.5 Hz, H-1'''), 4.93 (m, 3H, CH_2arom), 4.81 (m, 2H, CH_2arom), 4.74 – 4.59 (m, 4H, H-1, CH_2arom), 4.54 – 4.30 (m, 6H, H-1', H-5'', H-2''', H-5''', CH_2arom), 4.30 – 4.08 (m, 4H, H-3, H-4, H-2', H-2''), 4.07 – 3.95 (m, 2H, H-2, H-4'), 3.89 – 3.60 (m, 7H, H-5, H-6, H-3', H6', OCH_2), 3.43 (dd, 1H, *J*=8.7, 4.6 Hz, H-5'), 3.35 (dt, 1H, *J*=9.5, 6.7 Hz, OCH_2), 3.13 (t, 2H, *J*=7.0 Hz, CH_2N_3), 1.52 – 1.39 (m, 4H, CH_2, hexyl), 1.28 – 1.18 (m, 4H, CH_2, hexyl), 1.14 – 1.01 (m, 6H, H-6'', H-6''') ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ 166.1, 165.8, 165.5, 165.4 (C=O, Bz), 162.7, 162.1 (C=O, TCA), 138.3, 138.1, 137.9, 135.5, 134.4, 133.4, 133.3, 133.3, 133.3, 133.2, 133.2, 133.0, 132.9, 130.1, 129.8, 129.8, 129.7, 129.7, 129.7, 129.5, 129.1, 128.6, 128.6, 128.6, 128.5, 128.5, 128.3, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.7, 126.8, 126.8, 126.7, 126.1, 126.1, 125.9, 125.8 (arom.),

100.9 (C-1'), 99.5 (C-1), 98.1 (C-1'''), 96.0 (C-1''), 92.8, 92.7 (C_q, TCA), 79.8 (C-3'), 76.4 (C-5), 75.8 (C-3), 74.9 (CH_2arom), 74.9 (C-2'''), 74.6 (CH_2arom), 73.5 (C-5'), 73.5 (CH_2arom), 73.4 (CH_2arom), 73.0 (C-2''), 72.7 (C-4), 72.6 (C-4''), 72.2 (CH_2arom), 71.9 (C-4'''), 71.7 (C-4'), 71.6 (-3'''), 70.5 (C-3''), 69.5 (C-6, C-6'), 67.7 (OCH_2), 66.9 (C-5''), 65.4 (C-5'''), 56.7 (C-2), 53.9 (C-2'), 51.4 (CH_2N_3), 29.5, 28.8, 26.5, 25.6 (CH_2-hexyl), 16.2 (C-6''), 16.1 (C-6''') ppm. HRMS $[M+Na+H]^{2+}$ calcd $C_{105}H_{103}Cl_6N_5O_{23}NaH$: 1017.75755, found 1017.75700.

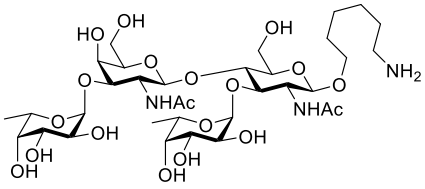
6-azidohexyl 6-*O*-benzyl-2-deoxy-3-*O*-(2-*O*-(2-methylnaphthyl)-α-L-fucopyranoside)-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2-*O*-(2-methylnaphthyl)-α-L-fucopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (32a)



NaOMe (130 µL of a 0.1M solution of NaOMe in MeOH, 0.2 eq.) was added to a suspension of tetrasaccharide **32** (0.14 g, 0.067 mmol, 1.0 eq.) in dry methanol (0.7 mL, 0.1M). The reaction was left to stir overnight, diluted in MeOH (5 mL) and neutralized by addition of Dowex H^+ resin. The resin was filtered off, washed and the filtrate was concentrated and purified by size exclusion (DCM/MeOH, 1/1, v/v) to give tetraol **32a** as a colourless film (0.067 g, 0.042 mmol, 63%). ^{1}H NMR (CD_3CN, 500 MHz): δ = 7.94 – 7.78 (m, 8H, arom.), 7.62 (d, 1H, *J*=9.4 Hz, NH), 7.60 – 7.45 (m, 9H, NH', arom.), 7.41 – 7.36 (m, 2H, arom.), 7.36 – 7.30 (m, 6H, arom.), 7.30 – 7.20 (m, 7H, arom.), 5.34 (d, 1H, *J*=3.6 Hz, H-1''), 5.31 (d, 1H, *J*=3.6 Hz, H-1'''), 4.90 – 4.76 (m, 4H, CH_2arom), 4.65 – 4.54 (m, 3H, CH_2arom), 4.54 – 4.33 (m, 6H, H-1, H-1', H-5'', CH_2arom), 4.25 – 4.14 (m, 1H, H-2'), 4.08 (dd, 1H, *J*=11.1, 2.8 Hz, H-3'), 4.06 – 3.95 (m, 3H, H-3, H-3'', H-5'''), 3.94 – 3.88 (m, 3H, H-4, H-4', H-3'''), 3.83 (q, 1H, *J*=9.2, 8.3, 7.9 Hz, H-2), 3.75 – 3.63 (m, 5H, H-6, H-6', H-2''', OCH_2), 3.63 – 3.59 (m, 3H, H-5', H-6', H-4'), 3.57 (dd, 1H, *J*=10.0, 3.6 Hz, H-2''), 3.50 – 3.43 (m, 1H, H-5), 3.41 – 3.30 (m, 2H, H-4'', OCH_2), 3.21 (t, 2H, *J*=6.9 Hz, CH_2N_3), 3.17 (d, 1H, *J*=5.8 Hz, 3'''-OH), 3.01 (d, 1H, *J*=4.1 Hz, 4'''-OH), 2.91 (d, 1H, *J*=5.3 Hz, 3''-OH), 2.34 (d, 1H, *J*=3.8 Hz, 4''-OH), 1.57 – 1.37 (m, 4H, CH_2, hexyl), 1.26 (h, 4H, *J*=3.4 Hz, CH_2, hexyl), 1.12 (d, 3H, *J*=6.6 Hz, H-6'''), 1.03 (d, 3H, *J*=6.5 Hz, H-6'') ppm. ^{13}C-APT NMR (CD_3CN, 126 MHz): δ 163.0, 162.5 (C=O, TCA), 139.6, 139.5, 137.5, 137.2, 134.2, 134.2, 133.9, 133.8, 129.9, 129.6, 129.4, 129.2, 129.2, 129.0, 128.9, 128.9, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 127.9, 127.4, 127.4, 127.3, 127.1, 127.0, 126.9, 126.7, 126.2 (arom.), 101.6 (C-1'), 101.4 (C-1), 97.8 (C-1'''), 96.3 (C-1''), 93.8, 93.7 (C_q, TCA), 78.1 (C-2'''), 77.6 (C-2''), 76.4 (C-3'), 76.2 (C-5), 76.1 (CH_2arom), 75.4 (C-4'), 74.6 (C-5'), 74.2 (C-3, C-4), 74.1 (CH_2arom), 73.7 (CH_2arom), 73.5 (CH_2arom), 72.9 (C-4''), 72.8 (C-4'''), 72.5 (CH_2arom), 70.4 (C-3'''), 70.3 (OCH_2), 69.8 (C-6), 69.5 (C-6'), 69.4 (C-3''), 68.1 (C-5'''), 66.8 (C-5''), 58.9 (C-2), 55.4 (C-2'), 52.0 (CH_2N_3),

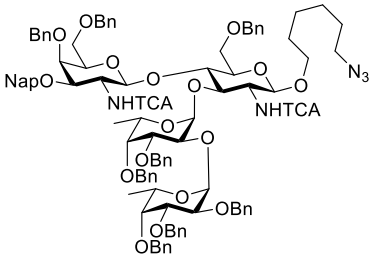
30.2, 29.3, 27.1, 26.2 (CH_2-hexyl), 16.7 (H-6'''), 16.6 (C-6'') ppm. HRMS $[M+Na]^+$ calcd for $C_{77}H_{87}Cl_6N_5O_{19}Na$: 1618.40241, found 1618.40186.

6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranoside)-4-*O*-(2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranoside)-β-D-galactopyranoside)-β-D-glucopyranoside (F-LDN-F)



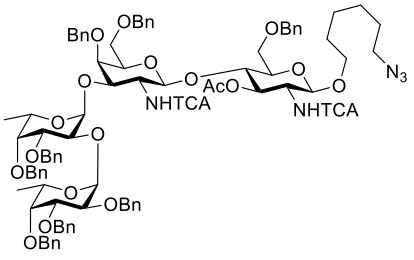
Tetraol **32a** (57 mg, 28 µmol, 1.0 eq.) was dissolved in degassed, sodium treated EtOH (1 mL). Pd/C (20 mg) was added after purging the flask with nitrogen. The reaction was kept under hydrogen atmosphere for 24 hours at room temperature. After this time period the hydrogen atmosphere was replaced by a nitrogen atmosphere and the solution was filtered over a whatman filter. The solvent was removed *in vacuo* and the colourless film was redissolved in degassed H_2O (1 mL). Pd black (10 mg) was added and the reaction was put under hydrogen atmosphere for an additional 24 hours. After this time period the temperature was increased to 50˚C and the mixture was stirred for another 24 hours. Upon completion, indicated by LC-MS analysis, the hydrogen atmosphere was replaced by a nitrogen atmosphere and the solution was filtered over a whatman filter. The mixture was concentrated and purified by HW-40 size exclusion chromatography and lyophilized. This gave fully deprotected tetramer as a fluffy white solid (5.4 mg, 6.6 µmol, 24%). ^{1}H NMR (D_2O, 500 MHz): δ = 5.11 (d, 1H, *J*=4.0 Hz), 4.97 (d, 1H, *J*=4.2 Hz), 4.84 (q, 1H, *J*=6.6 Hz), 4.50 (d, 1H, *J*=8.5 Hz), 4.48 (d, 1H, *J*=8.1 Hz), 4.15 – 4.03 (m, 2H), 3.99 – 3.79 (m, 10H), 3.79 – 3.65 (m, 5H), 3.61 (dd, 1H, *J*=8.1, 4.1 Hz), 3.59 – 3.53 (m, 1H), 3.50 (ddd, 0H, *J*=9.3, 4.9, 2.3 Hz), 2.99 (dt, 2H, *J*=13.5, 7.6 Hz), 2.02 (s, 3H), 2.01 (s, 3H), 1.69 – 1.62 (m, 2H), 1.58 – 1.51 (m, 2H), 1.38 – 1.32 (m, 4H), 1.26 (d, 3H, *J*=6.6 Hz), 1.20 (d, 3H, *J*=6.6 Hz) ppm. ^{13}C-APT NMR (D_2O, 126 MHz) δ 175.1, 174.1 (C=O, Ac), 101.6, 101.0, 100.7, 98.4, 78.7, 75.4, 75.0, 74.3, 73.5, 72.0, 71.8, 70.5, 69.3, 69.2, 68.3, 67.8, 67.8, 67.2, 66.9, 61.4, 60.0, 55.8, 51.4, 47.0, 42.8, 39.5, 28.4, 26.7, 25.5, 25.4, 25.3, 24.7, 22.3, 22.2, 15.4, 15.3, 10.5 ppm. HRMS $[M+Na]^+$ calcd for $C_{34}H_{61}N_3O_{19}H$: 816.39776, found 816.39720.

6-azidohexyl 6-*O*-benzyl-2-deoxy-3-*O*-(3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranose)-α-L-fucopyranose)-4-*O*-(4,6-di-*O*-benzyl-3-*O*-(2-methylnaphthyl)-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (33)



Di-fucosyl donor **35** (0.11 g, 0.13 mmol, 1.5 eq.) and acceptor **27** (0.10 g, 0.086 mmol, 1.0 eq.) were co-evaporated thrice with dry toluene and dissolved in dry DCM (0.9 mL, 0.1M). MS (3Å) were added and the solution was stirred at 0˚C for 1 hour. IDCP (0.12 g, 0.26 mmol, 3.0 eq.) was added and the mixture was allowed to warm to RT. The mixture was stirred for 24 hours. As TLC analysis showed little to no conversion after this time the reaction was diluted with EtOAc and quenched by addition of sat. $Na_2S_2O_3$ (aq.) (0.2 mL). The two-phase system was transferred to a separatory funnel and the water layer was removed. The organic layer was washed with sat. $CuSO_4$ (aq.) and brine, dried over $MgSO_4$, filtered and concentrated. The brown mixture was purified by silicagel chromatography (tol:ACN, 1:0 → 4:1) followed by size exclusion over LH-20 (DCM/MeOH, 1/1. v/v) to give compound **33** as a colourless film (0.012 g, 0.0063 mmol, 7%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.82 (m, 5H), 7.54 – 7.11 (m, 48H), 6.82 (d, 1H, *J*=7.3 Hz), 5.17 (d, 1H, *J*=3.5 Hz), 5.00 – 4.92 (m, 3H), 4.92 – 4.78 (m, 5H), 4.76 – 4.34 (m, 15H), 4.24 – 4.07 (m, 6H), 3.99 (dd, 1H, *J*=10.2, 2.7 Hz), 3.86 (dd, 1H, *J*=10.5, 2.7 Hz), 3.80 – 3.70 (m, 2H), 3.70 – 3.62 (m, 3H), 3.60 – 3.52 (m, 1H), 3.47 – 3.39 (m, 2H), 3.36 – 3.29 (m, 2H), 3.25 (s, 1H), 3.15 (t, 2H, *J*=6.9 Hz), 1.62 – 1.43 (m, 4H), 1.25 (m, 4H), 1.04 (d, 3H, *J*=6.5 Hz), 0.77 (d, 3H, *J*=6.5 Hz) ppm.

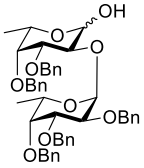
6-azidohexyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-(3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranose)-α-L-fucopyranose)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranoside)-2-(2,2,2-trichloroacetamido)-β-D-glucopyranoside (34)



Acceptor **28** (0.053 g, 0.050 mmol, 1.0 eq.) and di-fucosyl donor **35** (0.085 g, 0.10 mmol, 2.0 eq.) were co-evapotared thrice with dry toluene and dissolved in dry DCM (0.5 mL, 0.1M). MS (3Å) were added and the solution was stirred at 0˚C for 1 hour. IDCP (0.094 g, 0.20 mmol, 4.0 eq.) was added and the mixture was allowed to warm to RT. The mixture was stirred for 24 hours. As TLC analysis showed little to no conversion after this time the reaction was diluted with EtOAc and quenched by addition of sat. $Na_2S_2O_3$ (aq.) (0.2 mL). The two-phase system was transferred to a separatory funnel and the water layer was removed. The organic layer was washed with sat. $CuSO_4$

(aq.) and brine, dried over $MgSO_4$, filtered and concentrated. The brown mixture was purified by silicagel chromatography (tol:ACN, 1:0 → 4:1) followed by size exclusion over LH-20 (DCM/MeOH, 1/1. v/v) to give compound **34** as a colourless film (0.013 g, 0.0072 mmol, 14%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.66 (d, 1H, *J*=8.4 Hz, NH'), 7.46 – 7.09 (m, 38H, NH, arom.), 7.04 (dd, 2H, *J*=6.4, 2.9 Hz, arom.), 5.09 (d, 1H, *J*=3.3 Hz, H-1''), 5.04 – 4.96 (m, 2H, H-3, CH_2Bn), 4.94 – 4.85 (m, 3H, H-1''', CH_2Bn), 4.81 – 4.73 (m, 3H, CH_2Bn), 4.71 – 4.61 (m, 4H, CH_2Bn), 4.57 – 4.32 (m, 7H, H-1, H-2', H-2'', CH_2Bn), 4.30 – 4.06 (m, 6H, H-1', H-5'', H-2''', H-5''', CH_2Bn), 4.05 – 3.91 (m, 4H, H-2, H-4', H-3'', H-3'''), 3.89 – 3.76 (m, 4H, H-4, H-3', H-4'', OCH_2), 3.70 (q, 1H, *J*=5.3 Hz, H-5), 3.59 – 3.50 (m, 2H, H-6, H-6'), 3.50 – 3.33 (m, 4H, H-6, H-6', H-4''', OCH_2), 3.23 (m, 3H, H-5', CH_2N_3), 1.73 (s, 3H, CH_3, Ac), 1.55 (q, 4H, *J*=6.1, 5.0 Hz, CH_2, hexyl), 1.34 (m, 4H, CH_2, hexyl), 1.07 (d, 3H, *J*=6.4 Hz, H-6''), 0.87 (d, 3H, *J*=6.4 Hz, H-6''') ppm. ^{13}C-APT NMR ($CDCl_3$, 126 MHz) δ 170.1 (C=O, Ac), 162.6, 161.9 (C=O, TCA), 138.6, 138.6, 138.5, 138.3, 138.2, 138.0, 137.9, 129.0, 128.7, 128.7, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.3 (arom.), 101.6 (C-1'), 100.9 (C-1), 95.6 (C-1'''), 95.4 (C-1''), 81.0 (C-3'''), 78.1 (C-3''), 77.0 (C-4''), 76.7 (C-5), 76.2 (C-4'''), 75.3 (CH_2Bn), 74.9 (CH_2Bn), 74.6 (CH_2Bn), 73.8 (C-2'''), 73.7 (CH_2Bn), 73.6 (CH_2Bn), 73.3 (CH_2Bn), 73.3 (C-5'), 73.1 (C-4), 72.2 (C-3), 72.1 (C-4'), 71.9 (CH_2Bn), 71.3 (CH_2Bn), 70.8 (C-2''), 69.8 (OCH_2), 68.6 (C-6), 68.3 (C-5''), 67.9 (C-6'), 66.2 (C-5'''), 54.9 (C-2), 53.8 (C-2'), 51.5 (CH_2N_3), 29.4, 28.9, 26.6, 25.6 (CH_2, hexyl), 20.7 (CH_3, Ac), 16.8 (C-6''), 16.3 (C-6''') ppm. HRMS $[M+Na]^+$ calcd for $C_{92}H_{103}Cl_6N_5O_{20}Na$: 1830.52253, found 1830.52198.

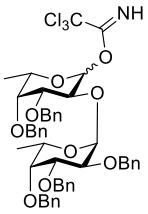
3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranose)-α/β-L-fucopyranose (35a)



Disaccharide **35** (0.050 g, 0.057 mmol, 1.0 eq.) was dissolved in a mixture of acetone and water (0.6 mL, 0.1M, 9/1, v/v). NBS (0.030 g, 0.172 mmol, 3.0 eq.) was added and the mixture was stirred for 10min. when TLC analysis indicated full conversion to two lower running spots. sat. $Na_2S_2O_3$ (aq. 0.5 mL) was added and the solution was stirred until the red solution turned colourless. The mixture was diluted in EtOAc, washed with brine, dried over $MgSO_4$, filtered and concentrated. The title compound was obtained after purification by silicagel chromatography (tol:ACN, 1:0 → 9:1) as a yellow oil (0.025 g, 0.035 mmol, 71%, 5/3, α/β). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.69 – 6.99 (m, 39H, arom.), 5.35 (d, 0.6H, *J*=3.6 Hz, H-1'β), 5.26 (d, 1H, *J*=3.5 Hz, H-1'α), 5.01 – 4.89 (m, 3H, CH_2arom), 4.87 – 4.52 (m, 17H, H-1α, H-1β, CH_2arom), 4.17 (dd, 1H, *J*=9.7, 3.5 Hz, H-2'α), 4.15 – 4.06 (m, 2.6H, H-2'α, H-5'α, H-2β), 4.04 (dd, 1H, *J*=9.2, 3.7 Hz, H-2α), 4.01 – 3.89 (m, 2.8H, H-5α, H-2β, H-3β, H-5'β), 3.87 – 3.78 (m, 2H, H-3α, H-3'α), 3.67 (dd, 1H, *J*=2.8, 1.3 Hz, H-4'α), 3.61 (dd, 1H, *J*=2.9, 1.0 Hz, H-4β), 3.58 – 3.50 (m, 1.2H, H-5β, H-3'β), 3.47 (dd, 1H, *J*=2.9, 1.2 Hz, H-4α), 3.43 (dd, 0.6H, *J*=2.9, 1.2 Hz, H-4β), 1.25 (d, 2H, *J*=6.4 Hz, H-6β), 1.20 (d, 3H, *J*=6.5 Hz, H-6'α), 0.93 (d, 3H, *J*=6.5 Hz, H-6α), 0.88 (d, 2H, *J*=6.4 Hz, H-6'β) ppm. ^{13}C-APT NMR

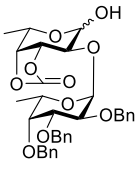
($CDCl_3$, 101 MHz) δ 139.0, 138.8, 138.7, 138.7, 138.6, 138.6, 138.2, 138.0, 138.0, 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.0, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.6, 127.4 (arom.), 98.2 (C-1β), 97.8 (C-1'β), 96.1 (C-1α), 90.7 (C-1'α), 82.2 (C-3'β), 79.7 (C-3α), 79.4 (C-3β), 78.3 (C-3'α), 77.7 (C-4'β), 77.5 (C-2'β), 77.4 (C-4α), 76.8 (C-2β), 76.7 (C-4'α), 76.5 (C-4β), 75.7 (C-2α), 74.9 (CH_2arom), 74.9 (CH_2arom), 74.8 (CH_2arom), 74.7 (CH_2arom), 74.4 (CH_2arom), 73.8 (C-2'α), 73.6 (CH_2arom), 73.1 (CH_2arom), 72.9 (CH_2arom), 72.8 (CH_2arom), 72.6 (CH_2arom), 71.0 (C-5β), 67.3 (C-5'α), 66.9 (C-5α), 66.8 (C-5'β), 17.1, 16.9, 16.5, 16.5 (C-6α, C-6'α, C-6β, C-6'β) ppm. HRMS $[M+Na]^+$ calcd for $C_{47}H_{52}O_9Na$: 783.35090, found 783.35035.

(2,2,2-trichloroacetimidoyl) 3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranose)-α/β-L-fucopyranose (36)



Hemi acetal **35a** (0.025 g, 0.032 mmol, 1.0 eq.) was dissolved in dry DCM (0.5 mL, 0.06M). Cl_3CCN (34 µL, 0.32 mmol, 10 eq.) was added, followed by addition of DBU (33 µL, 3 µmol 0.1 eq. of a 0.1M solution in DCM). The mixture turned brown in 10 min. and the mixture was concentrated *in vacuo*. The dark oil was purified by silicagel chromatography using neutralized silica (tol:ACN, 1:0 → 9:1), which gave the title compound as a colourless oil (0.028 g, 0.031 mmol, 97%, 9/1, α/β). α-product: ^{1}H NMR (CD_3CN, 400 MHz): δ = 9.11 (s, 1H, NH), 7.73 – 7.04 (m, 36H, arom.), 5.81 (d, 1H, *J*=8.2 Hz, H-1), 5.41 (d, 1H, *J*=3.7 Hz, H-1'), 4.95 (d, 1H, *J*=10.7 Hz, CH_2Bn), 4.88 – 4.80 (m, 2H, CH_2Bn), 4.78 – 4.66 (m, 5H, CH_2Bn), 4.62 (d, 1H, *J*=11.2 Hz, CH_2Bn), 4.50 (m, 4H, CH_2Bn), 4.24 (q, 1H, *J*=7.5, 6.0 Hz, H-5), 4.12 – 4.02 (m, 1H, H-3), 3.93 – 3.68 (m, 5H, H-2, H-4, H-5, H-2', H-3'), 3.40 (dd, 1H, *J*=3.0, 1.3 Hz, H-4'), 1.24 (d, 3H, *J*=6.3 Hz, H-6), 0.80 (d, 3H, *J*=6.4 Hz, H-6') ppm. ^{13}C-APT NMR (CD_3CN, 101 MHz) δ 160.8 (C=NH), 140.1, 140.0, 139.7, 139.0, 129.4, 129.3, 129.3, 129.3, 129.2, 129.1, 129.1, 129.1, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3 (arom.), 99.5 (C-1), 96.7 (C-1'), 82.2 (C-3), 79.6 (C-2), 78.5 (C-4'), 77.1, (C-4, C-2') 76.1 (CH_2Bn), 75.6 (CH_2Bn), 73.5 (CH_2Bn), 73.3 (CH_2Bn), 73.1 (CH_2Bn), 72.0 (C-5), 71.4 (C-3), 66.9 (C-5'), 16.9 (C-6), 16.6 (C-6') ppm.

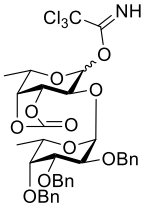
3,4-*O*-carbonate-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranose)-α/β-L-fucopyranose (37a)



NBS (0.035 g, 0.203 mmol, 3.0 eq.) was added to a solution containing disaccharide **37** (0.047 g, 0.067 mmol, 1.0 eq.) and left to stir in the dark for 15 min. Sat. $Na_2S_2O_3$ (aq. 0.5 mL) was added and the solution was stirred until the red solution turned colourless. The mixture was diluted in EtOAc, washed with brine, dried over $MgSO_4$, filtered and concentrated. The title compound was obtained after purification by silicagel chromatography (tol:ACN, 1:0 → 9:1) as a yellow oil (0.026 g, 0.043 mmol, 64%, 5/3, α/β). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.43 – 7.22 (m, 19H, arom.), 5.31 (d, 0.2H, *J*=3.8 Hz,

H-1'β), 5.20 – 5.13 (m, 1H, H-1'α), 4.96 (d, 1H, *J*=11.5 Hz, CH_2Bn), 4.90 (d, 1H, *J*=11.6 Hz, CH_2Bn), 4.86 – 4.79 (m, 3H, H-1α, H-4α, CH_2Bn), 4.79 – 4.74 (m, 3H, H-1β), 4.74 – 4.71 (m, 1H, CH_2Bn), 4.69 – 4.66 (m, 2H, H-4β, CH_2Bn), 4.66 – 4.63 (m, 1H, CH_2Bn), 4.51 (dd, 0.2H, *J*=6.9, 2.1 Hz, H-3β), 4.47 (dd, 1H, *J*=8.1, 1.7 Hz, H-3α), 4.13 – 3.99 (m, 3.4H, H-2α, H-5α, H-2'α, H-5β, H-2'β), 3.90 (dd, 0.2H, *J*=10.1, 2.8 Hz, H-3'β), 3.86 (dd, 0.2H, *J*=6.6, 2.1 Hz, H-5'β), 3.83 – 3.73 (m, 2H, H-3'α, H-5'α), 3.73 – 3.68 (m, 1.4H, H-4'α, H-2β, H-4'β), 1.42 (d, 0.6H, *J*=6.6 Hz, H-6β), 1.29 (d, 3H, *J*=6.5 Hz, H-6α), 1.17 (d, 0.6H, *J*=6.4 Hz, H-6'β), 1.15 (d, 3H, *J*=6.5 Hz, H-6'α) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz): δ 153.8 (C=O), 138.8, 138.6, 138.2, 138.1, 137.3, 129.2, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.3, 128.3, 128.0, 128.0, 128.0, 127.8, 127.7, 127.7, 127.5, 125.4 (arom.), 101.8 (C-1α), 98.1 (C-1'β), 95.6 (C-1β), 89.6 (C-1'α), 79.3 (C-3'β), 78.9 (C-3'α), 78.5 (C-4β), 77.9 (C-3'β), 77.5 (C-4'β), 76.6 (C-4'α), 76.3 (C-2β), 76.0 (C-3α, C-2'β), 75.1 (CH_2Bn), 75.0 (C-2α), 74.8 (CH_2Bn), 73.6 (CH_2Bn), 73.2 (CH_2Bn), 72.5 (C-2'α), 72.5 (CH_2Bn), 68.5 (C-5'β), 68.0 (C-5'α), 67.3 (C-5β), 64.2 (C-5α), 16.8 (C-6α), 16.6 (C-6β), 16.3 (C-6'β), 15.9 (C-6'α) ppm. HRMS $[M+Na]^+$ calcd for $C_{34}H_{38}O_{10}Na$: 629.23627, found 629.23572.

(2,2,2-trichloroacetimidoyl) 3,4-*O*-carbonate-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranose)-α/β-L-fucopyranose (38)



Hemiacetal **37a** (0.025 g, 0.041 mmol, 1.0 eq.) was dissolved in dry DCM (0.5 mL, 0.06M). Cl_3CCN (41 µL, 0.41 mmol, 10 eq.) was added, followed by addition of DBU (61 µL, 4 µmol, 0.1 eq. of a 0.1M solution in DCM). The mixture turned brown in 10 min. and the mixture was concentrated *in vacuo*. The dark oil was purified by silicagel chromatography using neutralized silica (tol:ACN, 1:0 → 9:1), which gave the title compound as a colourless oil (0.034 g, 0.041 mmol, 99%, 5/3, α/β). ^{1}H NMR (CD_3CN, 400 MHz): δ = 9.13 (s, 0.6H, C=NHβ), 8.99 (s, 1H, C=NHα), 7.47 – 7.12 (m, 22H, arom.), 6.40 (d, 1H, *J*=3.6 Hz, H-1α), 6.06 (d, 0.6H, *J*=5.8 Hz, H-1β), 5.33 (m, 0.6H, H-1'β), 5.17 (d, 1H, *J*=2.7 Hz, H-1'α), 4.98 – 4.80 (m, 4H, H-3α, H-3β, CH_2Bn), 4.80 – 4.55 (m, 19H, H-4α, H-4β, CH_2Bn), 4.40 (qd, 1H, *J*=6.6, 2.4 Hz, H-5α), 4.17 (qd, 0.6H, *J*=6.6, 1.9 Hz, H-5β), 4.10 (dd, 1H, *J*=7.4, 3.6 Hz, H-2α), 4.08 – 3.99 (m, 2.2H, H-2β, H-5'α, H-5'β), 3.94 – 3.92 (m, 1.2H, H-2'β, H-4'β), 3.90 – 3.87 (m, 2.6H, H-2α. H-3'α, H-3'β), 3.85 – 3.83 (m, 1H, H-4'α), 1.32 (d, 3H, *J*=6.7 Hz, H-6α), 1.32 (d, 3H, *J*=6.7 Hz, H-6β), 1.20 (d, 2H, *J*=6.4 Hz, H-6'β), 1.18 (d, 3H, *J*=6.5 Hz, H-6'α) ppm. ^{13}C-APT NMR (CD_3CN, 101 MHz): δ 160.8, 160.7 (C=NH), 140.1 (C=O) 129.2, 129.2, 129.1, 129.1, 128.9, 128.9, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2 (arom.), 98.6 (C-1'β), 97.8 (C-1'α), 97.2 (C-1β), 94.1 (C-1α), 79.5 (C-4'β), 79.2, 78.8 (C-4'α), 78.7, 78.4, 77.6, 77.4, 76.5, 76.4, 75.9 (CH_2Bn), 75.8 (CH_2Bn), 73.7 (CH_2Bn), 73.1 (CH_2Bn), 72.9 (CH_2Bn), 72.9 (C-5'β), 72.3 (C-2α), 68.8 (C-5β), 68.1 (C-5'α), 65.8 (C-5α), 16.8, 16.3, 16.0 (C-6α, C-6'α, C-6β, C-6'β) ppm.

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5

*A novel approach to the synthesis of circulating anodic antigen**

Introduction

The circular anodic antigen (CAA) is a highly glycosylated protein excreted by the gut of *Schistosoma Mansoni* fluke worms. The CAA protein is decorated with long polysaccharides consisting of the 6-[GlcA-β-(1-3)-GalNAc-β-(1-] repeating unit (Figure 1).[1] It plays an important role in the survival of the schistoma worms in the hostile environment of the human host. The long negatively charged glycan chains on the CAA protein are one of the methods the worms employs to evade the hosts immune system. As the worms feed on blood their gut is subjected to all components of the immune system.[2] It has been reported that CAA interacts with the C1q protein complex of the complement system.[2], [3] As this protein is at the start of the classic pathway of the complement system, any complementary immune attack might get suppressed, thus ensuring its survival.

*Harvey, M. R., Amponsah, K. B., Hokke. C., Van der Marel, G. M., Codée, J. D. C. were involved in the research described in this chapter.

The CAA carbohydrate structure is unique to schistosoma worms and can therefore be used as a biomarker to monitor the degree of infection by schistosomes. Unlike the multi-fucosylated glycans discussed in previous chapters, CAA glycans are excreted by the host in urine, so analytic samples can be obtained without invasive methods.[4] In addition the excretion of CAA in the bloodstream doesn't fluctuate, thus greatly increasing analytical accuracy. [5]–[7]

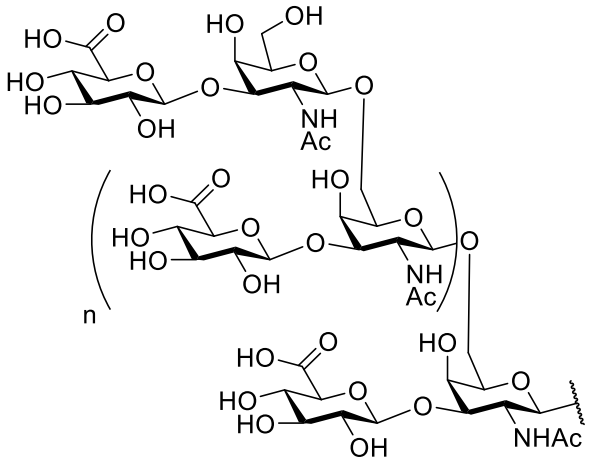


Figure 1: Structure of glycan present on CAA protein.

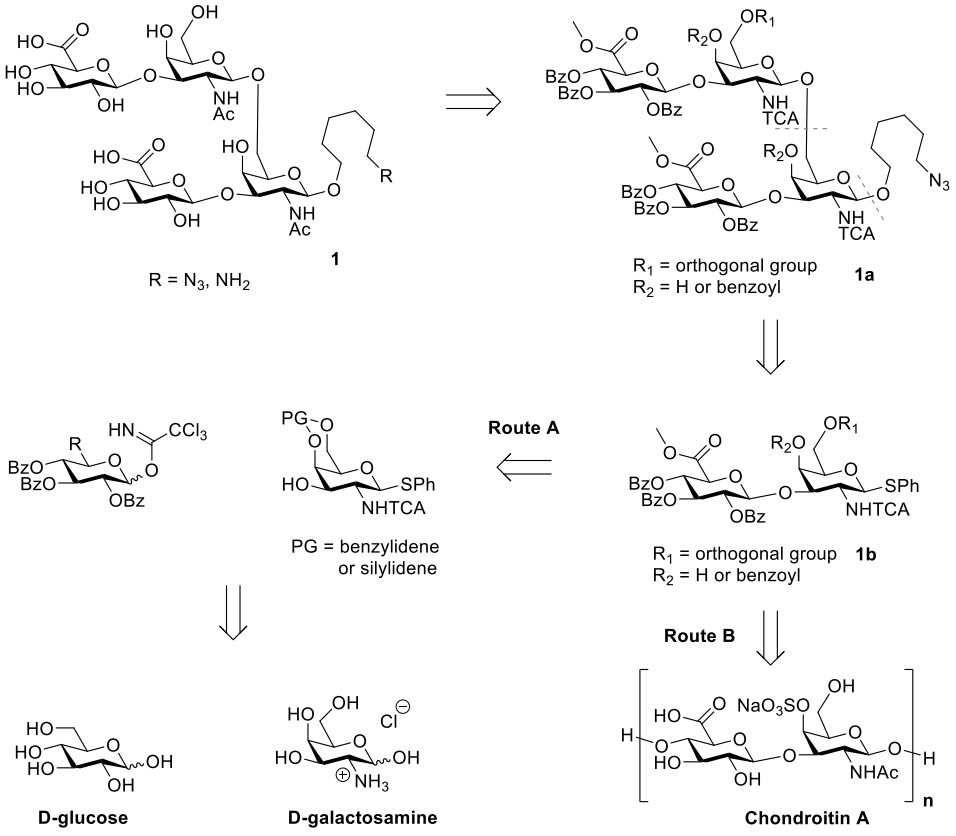
Besides being useful as a biomarker CAA may also be a target for a glycan based vaccine, as recognition of these antigens might help to target the hidden worms.[8] In order to make better diagnostics or a vaccine, well defined glycans are required, which cannot be obtained from biological sources. Vliegenthart and co-workers have previously described the synthesis of CAA oligosaccharides up to a pentasaccharide (see Chapter 1 for a more detailed description of their synthesis).[1], [3], [9]–[13] This chapter describes a feasible and efficient protocol to synthesize oligosaccharide fragments of CAA, that may ultimately lead to production of larger CAA oligomers.[14]

Results and discussion

Two routes (Route A and B) to synthesize CAA oligomers were envisioned and both are tested and evaluated in this chapter. In order to test the validity of these routes CAA tetrasaccharide **1** was selected as the target structure and the retrosynthesis towards this tetrasaccharide is depicted in Scheme 1. It was envisioned that tetrasaccharide **1** can be obtained from its protected precursor **1a**. The use of trichloroacetamide functions in combination with benzoyl and methyl esters should allow for a single step global deprotection, as all these groups can be removed through saponification. This would leave the azide in the spacer untouched and available for subsequent conjugation purposes. The tetrasaccharide can be built from GlcA-GalNAc-disaccharide building

blocks, wherein the galactosamine amine is protected as a trichloroacetamide to ensure 1,2-*trans* selective condensation reactions.[15]–[18] The galactosamine C4- and C6-hydroxyl functions may bear orthogonal protecting groups or the higher reactivity of the C6-OH over that of the C4-OH may be exploited in a regioselective glycosylation reaction.

Scheme 1: Retrosynthesis of selected target CAA tetrasaccharide.



The key disaccharide will be assembled as a thiophenyl glycoside as thioglycosides are stable to a wide range of conditions and the thiophenyl group can be readily activated to provide a glycosylating agent. Alternatively, it can be orthogonally removed or converted into another anomeric leaving group, such as a fluoride or an imidate.[19]

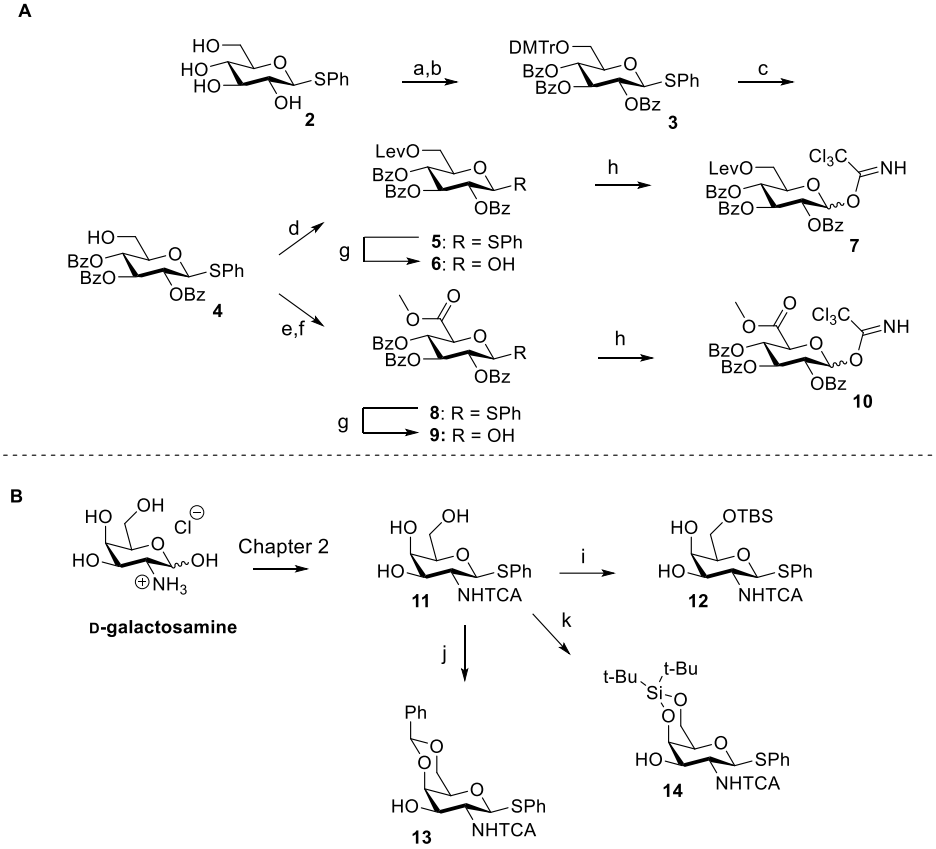
Disaccharide **1b** can be obtained through two different routes. The first, route A represents a flexible assembly route, as a diversity of D-glucose based donors and D-galactosamine based acceptors can be synthesized and evaluated, ensuring an optimization strategy for the synthesis of the core disaccharide. Optimization will include the timing of the oxidation of the glucose to the glucuronic acid and with this route it can

be determined whether it is more advantageous to oxidize the glucose monomer before glycosylation or to first generate a disaccharide and oxidize the primary glucose alcohol at a later stage. A downside of the flexibility is the number of steps required to obtain the disaccharide. Route B is based on the work of Jacquinet and co-workers, who described the assembly of well-defined chondroitin oligomers from GlcA-GalNAc dimer synthons, obtained from the acidic hydrolysis of chondroitin A, a cheap and readily available starting material.[20] As chondroitin and CAA share the same disaccharide motif this route presents a promising alternative to the *de novo* synthesis of route A. The main advantage of route B is the reduction in steps, as the core disaccharide can be obtained from chondroitin A, thus avoiding the glycosylation reaction. A downside in this route is a decrease in flexibility as the acidity of the C5-proton of the glucuronic acid prevents protective group manipulations that require a strong base, such as a benzylation.

Route A: Starting from D-galactosamine and D-glucose.

Scheme 2 depicts the monosaccharide synthons required for the assembly of key disaccharide **1b** following Route A. The synthesis of glucose donors **7** and **10** started from thioglycoside **2**, which was synthesized from D-glucose in three steps according to literature procedures (Scheme 2-A).[21] The primary alcohol of **2** could be selectively protected with a bulky 4,4'-di-methoxytrityl (DMT) group by treating it with DMT-Cl in pyridine at 80˚C.[22] After cooling to room temperature Bz-Cl was added and the mixture was left to stir overnight to provide the fully protected glucose **3**. Several methods were investigated to remove the DMT group. Initially $BF_3{\cdot}OEt_2$ was tested, but this resulted in partial benzoyl migration to the C6-OH.[23] When TFA was used together with TFAA, no benzoyl migration was observed but the reaction didn't go to completion resulting in a low yield. The use of TFA in conjunction with Et_3SiH as a scavenger for the released DMT cation, had none of the aforementioned side reactions and effectively liberated the C6-OH. The primary alcohol was either protected with a levuloyl group **5**, or oxidized to the acid, which in turn was protected as a methyl ester **8**.[24], [25] With these building blocks (**5** and **8**) in hand the imidate donors **7** and **10** were synthesized. The thiophenyl group was hydrolysed using N-Bromosuccinimide (NBS) in acetone/water in a near quantitative yield in both cases.[26] Lastly, the imidate was introduced using DBU and trichloroacetonitrile giving donors **7** and **10** in an overall yield of 26%, 40% respectively from D-glucose.[27]

*Scheme 2: Synthesis of the building blocks required for Route A. **A**) Synthesis of glucose donors **7** and **10**, **B**) Synthesis of galactosamine building blocks **12**, **13** and **14**.*



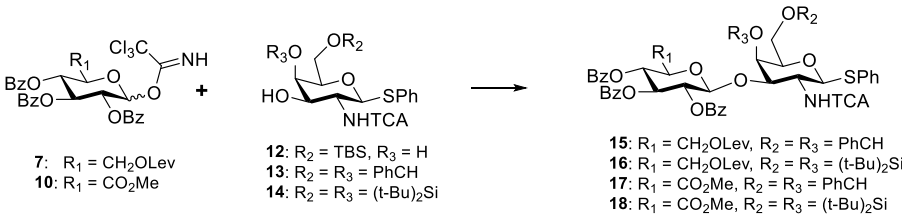
Reagents and conditions: **a**: DMTr-Cl, pyr, 80˚C, **b**: Bz-Cl, pyr, **c**: TFA, Et_3SiH, DCM, -40˚C, 78% (yield over three steps), **d**: Lev-OH, DMAP, EDC·HCl, DCM, 0˚C→RT, 65%, **e**: TEMPO (cat.), BAIB, DCM, 0˚C, **f**: MeI, K_2CO_3, DMF, 82% (over two steps), **g**: NBS, acetone, water, **6** quant., **9** 98%, **h**: Cl_3CCN, DBU, DCM, **9** 71%, **10** 88%, **i**: TBS-Cl, pyr, Et_3N, 70%, **j**: $PhCH(OMe)_2$, CSA (cat.), ACN, 50˚C, 330 mbar, 74%, **k**: $DTBS(OTf)_2$, DMF, -10˚C, 92%.

In order to synthesize the CAA disaccharide the following three acceptors **12**, **13**, and **14** were synthesized (Scheme 2-B). Diol **12** was synthesized to explore whether the reactivity difference between the C3-OH and the C4-OH is sufficient to be exploited in a regioselective glycosylation reaction.[28] Acceptors **13** and **14** are protected by either a benzylidene acetal or a silylidene ketal. The latter has been shown to have a beneficial effect on the reactivity of the C3-OH, however, it will have to be removed from the disaccharide as silylidene protected galactose-type donors react in a highly selective 1,2 *cis* fashion, even if C2-O/N protecting groups are present that are capable of neighbouring group participation.[29]–[32]

The synthesis of **11** from D-galactosamine is described in Chapter 2. Diol **12** was obtained by selective silylation of the primary alcohol using *tert*-butyldimethyl Chloride (TBS-Cl) with pyridine and Et_3N at -30˚C. Acceptors **13** and **14** could be synthesized from triol **11** by using either benzaldehyde dimethyl acetal and a catalytic amount of camphorsulfonic acid (CSA), or di-*tert*-butylsilylditriflate (DTBS$(OTf)_2$) in DMF.

The results of the glycosylation reactions of donors **7** and **10** and acceptors **12**-**14** are summarized below in Table 1. In all cases the reactions were performed in DCM at a concentration of 0.1M with freshly activated molecular sieves (3Å) and with TMSOTf as the activator.

Table 1: Optimization of the glycosylation reaction of disaccharide building blocks.



7: $R_1 = CH_2OLev$
10: $R_1 = CO_2Me$

12: R_2 = TBS, R_3 = H
13: $R_2 = R_3$ = PhCH
14: $R_2 = R_3 = (t\text{-}Bu)_2Si$

15: $R_1 = CH_2OLev$, $R_2 = R_3$ = PhCH
16: $R_1 = CH_2OLev$, $R_2 = R_3 = (t\text{-}Bu)_2Si$
17: $R_1 = CO_2Me$, $R_2 = R_3$ = PhCH
18: $R_1 = CO_2Me$, $R_2 = R_3 = (t\text{-}Bu)_2Si$

entry	donor	acceptor	TMSOTf	T (˚C)	product	yield (%)
1	**7**	**12**	0.1 (eq.)	-40˚C→ -10˚C	Complex mixture	-
2	**10**	**12**	0.1 (eq.)	-40˚C→ -10˚C	Complex mixture	-
3	**7**	**13**	0.1 (eq.)	-40˚C→ -20˚C	**15**	31
4	**10**	**13**	0.1-0.5 (eq.)[a]	-40˚C → RT	No reaction	-
5	**7**	**14**	0.1 (eq.)	-40˚C→ -20˚C	**16**	43[b]
6	**10**	**14**	0.1-0.5 (eq.)[a]	-20˚C→ RT	**18**	68[c]
7	**10**	**14**	0.3 (eq.)	RT	**18**	84

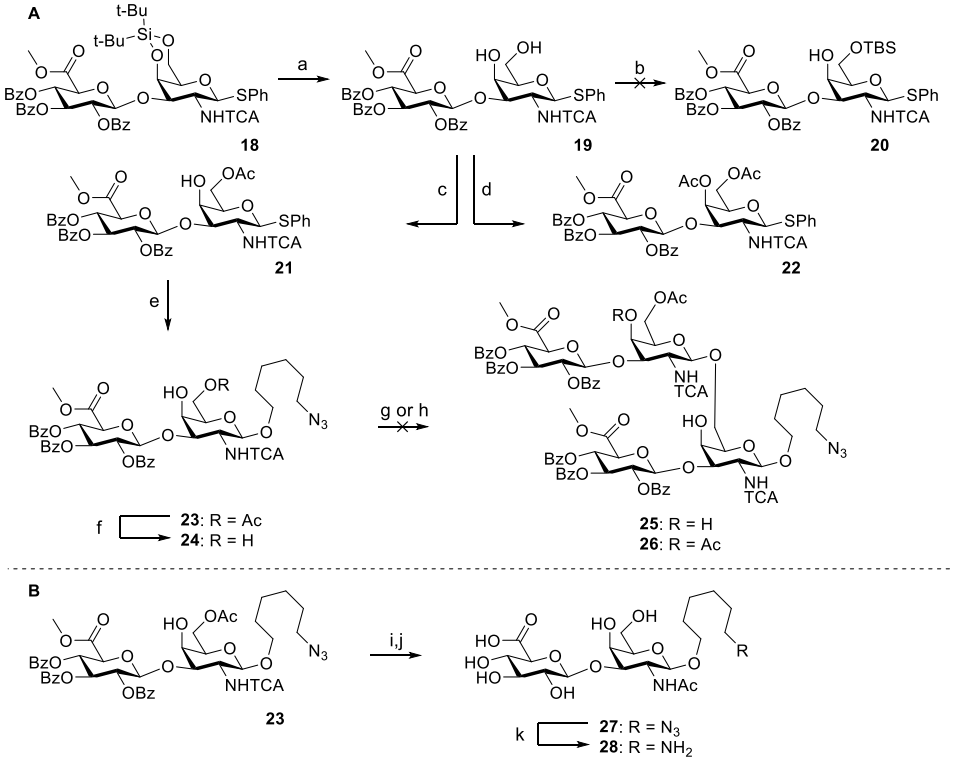
[a]five different experiments each with 0.1 eq. more of TMSOTf.
[b]yield determined after removal of silylidene
[c]This yield was obtained with 0.3 eq. of TMSOTf

Initially diol **12** was tested with donors **7** and **10** (entries 1 and 2). Unfortunately, this reaction led to a complex and inseparable mixture of C3-O and C4-O linked disaccharides. Although there are examples where excellent regioselectivity of the C3-OH over the C4-OH hydroxyl was observed, these building blocks provided poor regioselectivity.[33]–[38] Next benzylidene acceptor **13** was tested. When glucose donor **7** was used disaccharide **15** was formed in a modest yield of 31% (entry 3). Glucuronic acid donor **10** proved to be too unreactive as no reaction was observed even after 60 hours. Increasing the amount of activator did not solve this problem (entry 4). Lastly silylidene **14** was used as the acceptor. The condensation with Lev bearing donor **7** led to an inseparable mixture of disaccharide **16** and hydrolysed donor (entry 5). After removal of the silylidene with HF·pyridine the diol disaccharide **19** could be obtained in a yield of 43% over two steps.

The use of glucuronic acid donor **10** did not lead to this problem and after some optimization disaccharide **18** could be obtained in an excellent yield of 84% (entries 6 and 7).

Although it is known that silylidene donors are more reactive then benzylidene donors, the effect of this protecting group on the reactivity of the acceptor has not been investigated often.[39]–[41] There are examples showing that the silylidene ketal increases the reactivity of the neighbouring hydroxyl in comparison to the analogous benzylidene protected system, although the reason behind this phenomenon is not clear yet.[31] Del Bino *et al*. postulated that the increased flexibility of the silylidene ketal adds to the reactivity of the system.[30] Thollard *et al*. implied that the electron donating effect of the *t*-Bu groups increase the reactivity of the C3-OH.[29] With conditions established for the effective generation of the disaccharide, attention was focused next on the assembly of the tetrasaccharide (see Scheme 3).

*Scheme 3: **A**) Synthesis of tetrasaccharide **26**, **B**) deprotection of disaccharide **23**.*



Reagents and conditions: **a**: HF·pyridine, THF, pyr, 79%, **b**: TBS-Cl, pyr, -30˚C, 0%, **c**: Ac_2O, NaOAc, 50˚C, 92%, **d**: Ac_2O, NaOAc, 110˚C, 82%, **e**: 6-azidohexan-1-ol, NIS, TfOH (cat.), MS (3Å), DCM, 0˚C, 58%, **f**: Ac-Cl, MeOH, DCM, 4˚C, 88%, **g**: **21**, NIS, TfOH, MS (3Å), ACN/DCM, **h**: **22**, NIS, TfOH, MS (3Å), ACN/DCM, **i**: NaOH, dioxane, H_2O, **j**: Ac_2O, Et_3N, MeOH **k**: Pd/C, H_2, H_2O, 35% (over three steps).

In order to install the 6-azidohexan-1-ol spacer β-selectively, the silylidene ketal was

removed first as is it α-directing due its steric bulk. The removal of the silylidene was accomplished by treatment of **18** with HF·pyridine in good yield. Next the primary alcohol in **19** was selectively protected with an orthogonal protecting group. A variety of protective groups were tested and the results are shown in Table 2.

Initially it was attempted to introduce a TBS group on the primary alcohol of **19** (entry 1). The same conditions as used for the preparation of acceptor **12** were used, but these proved ineffective. At elevated temperatures the starting material was consumed, but the desired compound could not be obtained. Next the introduction of the Fmoc group was probed. Similar to the attempted introduction of the TBS protection group the starting material was consumed, but the desired product had not formed (entry 2). Since both the TBS and the Fmoc are quite bulky, a less sterically demanding acetyl group was tried (entry 3). Unfortunately treatment of **19** with Ac_2O in pyridine again resulted in a complex mixture. The common element in all these reactions was the use of pyridine as a solvent and it was reasoned that this basic solvent could be the cause of the complex reaction mixtures. When disaccharide **19** was dissolved in pyridine and left overnight, TLC analysis showed the appearance of multiple spots (entry 4), indicating that **19** was not stable in pyridine. Therefore, different solvents were used for the introduction of other protective groups. The introduction of a levulinoyl ester proceeded extremely sluggish taking 2 weeks to achieve 38% conversion (entry 5). Selective acylation using Taylor's catalyst **31** was successful for installing either an acetyl or a chloroacetyl (entries 6 and 7).[42] Lastly an acetyl was installed by using sodium acetate in acetic anhydride. When heated to 50˚C the primary alcohol could be selectively acetylated in an excellent yield of 92% (entry 8).

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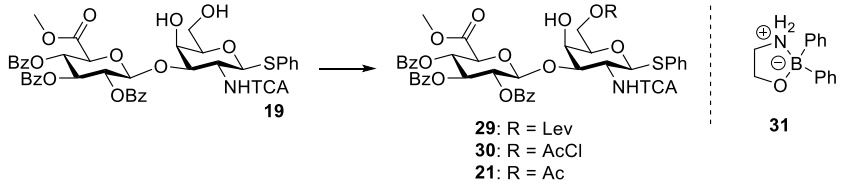
*Table 2: Selective protection of the primary alcohol of **19**.*



entry	reagents*	solvent	T (˚C)	yield
1	TBS-Cl	pyridine	-30 → 80	Complex mixture
2	Fmoc-Cl,	Pyridine	RT	Complex mixture
3	Ac_2O	pyridine	RT	Complex mixture
4	-	pyridine	RT	Complex mixture
5	Lev-OH, DMAP, EDC·HCl, pyr	DCM	RT	38%
6	ClAc-Cl, **31**, K_2CO_3, KI	ACN	RT	60%
7	Ac-Cl, **31**, DIPEA	ACN	RT	68%
8	NaOAc	Ac_2O	50	92%

*1.1 eq. of reagent was added in every reaction

Now that the primary alcohol of **21** was successfully protected, the 6-azidohexan-1-ol could be installed using N-iodosuccinimide (NIS) and triflic acid (TfOH) as the activator giving **23** in 58% yield (Scheme 3A). Subsequently, the acetyl ester was selectively removed with dry HCl in methanol to provide diol acceptor **23**. Since the primary alcohol of galactose is much more reactive than the 4-OH, it was reasoned that a regioselective glycosylation strategy could be employed to construct tetrasaccharide **25**, using disaccharide **24** as the acceptor. First the use of disaccharide donor **21** was explored. The glycosylation, however, proceeded very sluggishly as both the donor and the acceptor were poorly soluble in the mixture of acetonitrile and methylene chloride and the reaction mixture slowly turned into a gel. In order to increase the solubility of the donor, it was decided to synthesize the di-acetyl donor **22** by heating diol **19** to 110˚C in acetic anhydride with sodium acetate. Unfortunately using di-acetyl donor **22** did not proceed any better. Although in both glycosylation reactions the desired tetramer appeared to be the main product in the mixture, it was impossible to separate it from formed byproducts and therefore it was decided to abandon this synthetic route.

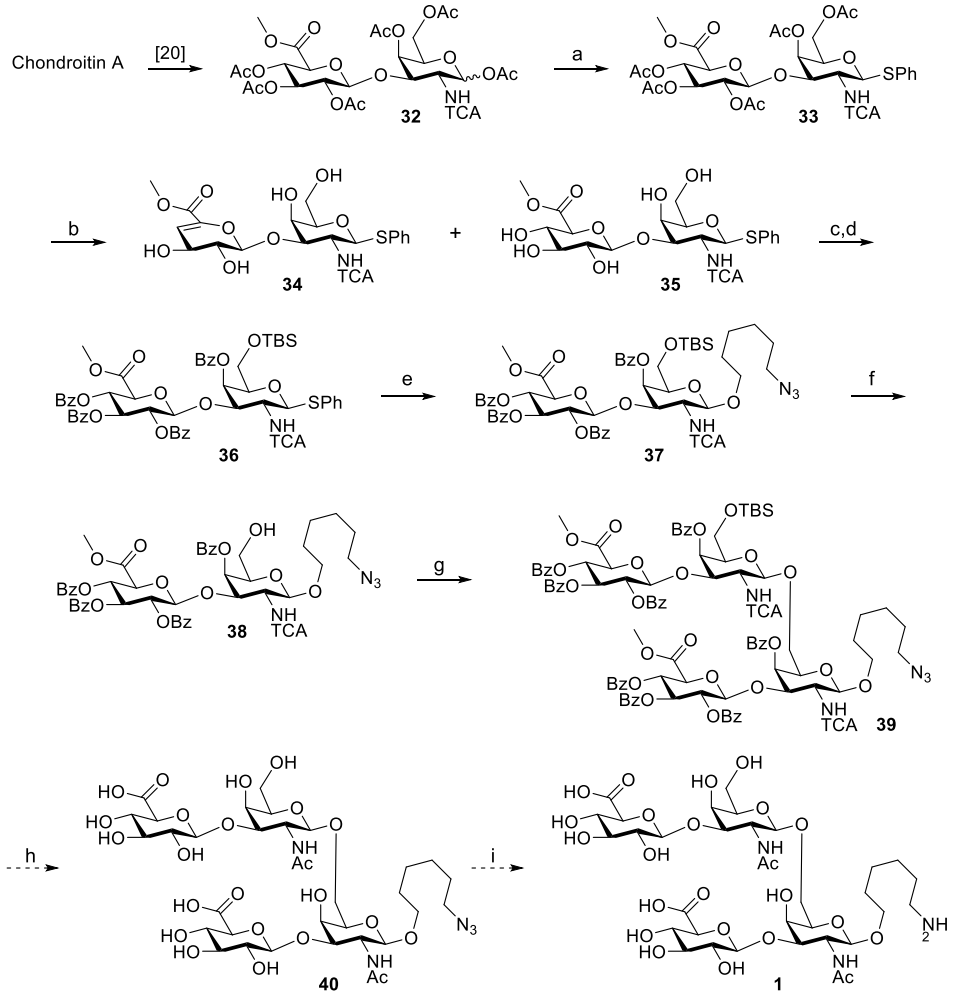
Disaccharide **23** was deprotected by hydrolysis of the esters and the trichloroacetamide using sodium hydroxide, after which the zwitterionic intermediate was purified over sephadex LH-20 size exclusion gel. The amine was then selectively acetylated under Schotten-Baumann conditions, resulting in disaccharide **27**. Finally, the spacer amine was liberated by catalytic hydrogenation of the azide giving rise to fully deprotected disaccharide **28** in 33% yield over three steps.

Route B: Starting from Chondroitin A.

Scheme 4 depicts the assembly of tetrasaccharide **39** using disaccharide synthons obtained from chondroitin A. Thus, chondroitin A was treated according to the protocol of Jacquinet and co-workers to yield acetylated disaccharide **32** on a multi-gram scale.[20] The anomeric centre was functionalized with a thiophenyl group using thiophenol and $BF_3{\cdot}OEt_2$, delivering thioglycoside **33** in 62% yield. Side products formed in this reaction consisted of furanosides, present in the starting material and the α-S-phenyl product. Disaccharide **33** was deacetylated using Zemplén conditions, resulting in **35** as the major compound. Compound **34**, formed by the deprotonation of the C5' by a methoxide ion followed by the elimination of the C4 acetate, was isolated as a minor side product. The primary alcohol was selectively protected with a TBS group using TBSOTf and imidazole in DMF. The same reaction using TBS-Cl proceeded very sluggishly.[43] In route A (Scheme 3) the degradation of diol **19** was observed when pyridine was used as the solvent. Therefore, it was attempted to install benzoyl groups on the disaccharide using benzoic anhydride and sodium benzoate at elevated temperature (110˚C), analogous to the synthesis of compound **22**. Unfortunately, complete benzoylation could not be achieved using these conditions. Therefore, benzoyl chloride was used in the next attempt in combination with a minimal amount of pyridine, 1.5 eq. per free hydroxyl, this did result in less degradation but could not completely prevent it and donor **36** was obtained in a

yield of 44% over two steps. Thioglycoside **36** was condensed with 6-azidohexan-1-ol using the NIS/TMSOTf activator couple to give **37** in 56% yield. In order to prevent migration of the 4-O benzoyl to the primary alcohol, the removal of the TBS group was done using CSA in wet acetonitrile. Acceptor **38** was obtained in 97% yield without any benzoyl migration.

*Scheme 4: Synthesis of CAA tetrasaccharide **39**.*



Reagents and conditions: **a**: PhSH, $BF_3{\cdot}OEt_2$, DCM, 62%, **b**: NaOMe (cat.), MeOH, **35** 82%, **34** 9%, **c**: TBSOTf, imidazole, DMF, **d**: Bz-Cl, pyr, 44% (over 2 steps), **e**: 6-azidohexanol, NIS, TMSOTf (cat.), MS (3Å), DCM, -20˚C, 56%, **f**: CSA, H_2O, ACN, 97%, **g**: **37**, NIS, TMSOTf (cat.), MS (3Å), DCM, -20˚C, 42%, **h**: i) NaOH, 1,4 dioxane, H_2O, ii) Ac_2O, MeOH, Et_3N, **i**: Pd/C, H_2, H_2O.

Tetrasaccharide **39** was then synthesized by condensing donor **36** and acceptor **38** using NIS with catalytic TMSOTf as the activator couple. Although the reaction proceeded sluggishly, tetrasaccharide **39** could be obtained in a yield of 42%. A small quantity of **39** was obtained and the previously employed deprotection strategy was applied. Unfortunately, this was unsuccessful as removal of the esters and TCA groups led to a product that was poorly soluble, which hampered the purification by LH-20 gel permeation chromatography.

Conclusion

This Chapter has described a study to a novel approach of generating well-defined CAA-oligosaccharides. To this end, an assembly strategy was devised that hinges on the use of disaccharide GlcA-GalNAc building blocks. For the generation of the key disaccharide building blocks, two routes were designed. Route A started from D-glucose and D-galactosamine, which were turned into building blocks **10** and **14** using well-established protective group manipulations. Disaccharide **18** was synthesized by condensing these two building blocks in a yield of 84%. It was shown that a silylidene ketal bearing acceptor is more reactive than an acceptor bearing a benzylidene acetal. Further investigations showed that the diol **19**, formed by removal of the silylidene, was instable and therefore difficult to manipulate. The glycosylation reaction forming tetrasaccharide **1a** from the two disaccharide synthons proceeded problematically and therefore this route was ultimately abandoned. Route B started from chondroitin A, a cheap and readily available starting material, which could be hydrolysed to obtain the required [GlcA-β-(1-3)-GalNAc-β] disaccharide repeating unit of CAA. This reduced the total number of steps to get to tetrasaccharide **1a** from **15** (Route A) to only 6 steps. Also in this route, the base lability of the disaccharide intermediate was encountered but a fully protected building block could be obtained rapidly nonetheless. Importantly the crucial glycosylation in which two disaccharides were united to form tetrasaccharide **39** proceeded much better. An initial attempt to deprotect the tetrasaccharide unfortunately failed. However, the chemistry developed here will allow for the generation of sufficient amounts of building blocks to further optimize the glycosylation chemistry and provide sufficient material to develop a deprotection strategy. Eventually the optimized chemistry should lead to the generation of a set of well-defined CAA glycans to be used in diagnostics and perhaps open up possibilities for an anti-schistosome vaccine.

Experimental

General procedures

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

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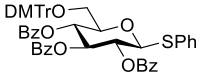
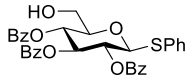
Phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-(4,4'-dimethoxytrityl)-1-thio-β-D-glucopyranose (3)



Compound **2** (11.4 g, 42.0 mmol, 1.0 eq.) was dissolved in anhydrous pyridine (500 mL, 0.08M) and the solution was warmed to 80°C. DMTr-Cl (18.6 g, 55.0 mmol, 1.3 eq.) was slowly added and the reaction was stirred for one hour before increasing the temperature to 110°C. After 3 hours TLC analysis showed full consumption of the starting material. The mixture was cooled to room temperature and BzCl (30 mL, 258 mmol, 6.1 eq.) and DMAP (3.4 g, 27.4 mmol, 0.65 eq.) were added to the solution. After two days TLC analysis showed full conversion. The mixture was quenched by slow addition of water (100 mL) at 0˚C. The mixture was taken up in EtOAc and washed with sat. $CuSO_4$ (aq.), the layers were separated and the water layer was re-extracted with EtOA. The combined organic layers were then washed with sat. $NaHCO_3$ (aq.) until bubbling no longer occurred followed by brine. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to obtain the crude as a dark red oil. The crude was purified by silica gel column chromatography

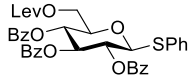
(PE:EtOAc, 9:1 → 1:1) to obtain the product as a yellow foam (25.7 g,28.9 mmol, 69%). ^{1}H NMR (CD_3CN, 400 MHz) δ: 8.22 – 8.18 (m, 2H, arom.), 7.97 (m, 4H, arom.), 7.90 – 7.84 (m, 2H, arom.), 7.73 – 7.68 (m, 2H, arom.), 7.63 – 7.26 (m, 41H, DMTrOH, arom.), 6.90 – 6.84 (m, 4H, arom.), 6.19 (t, 1H, *J*=9.4 Hz, H-3), 6.02 (t, 1H, *J*=9.8 Hz, H-4), 5.89 (t, 1H, *J*=9.7 Hz, H-2), 5.48 (d, 1H, *J*=10.0 Hz, H-1), 4.23 (d, 1H, *J*=10.2 Hz, H-5), 3.75 (3, 6H, OCH_3, DMTr), 3.65 (d, 1H, *J*=9.8 Hz, H-6), 3.41 (dd, 1H, *J*=10.7, 4.5 Hz, H-6) ppm. ^{13}C-APT NMR (CD_3CN, 101 MHz) δ: 166.6, 166.0, 165.7 (C=O, Bz), 159.6, 159.5, 146.1, 138.8, 136.8, 136.4, 134.5, 133.3, 131.0, 130.2, 130.2, 130.1, 129.9, 129.6, 129.2 (arom), 86.3 (C-1), 78.5 (C-5), 75.9 (C-3), 71.9 (C-2), 69.9 (C-4), 62.8 (C-6), 55.8 (OCH_3, DMTr) ppm. HRMS $[M+Na]^+$ calculated for $[C_{54}H_{46}O_{10}SNa]^+$: 909.27094, found 909.2718.

Phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranose (4)



Compound **3** (13.4 g, 15.1 mmol, 1.0 eq.) was dissolved in anhydrous DCM (150 mL, 0.1 M) and the round bottom flask purged with argon gas. The solution was cooled to -40°C. Et_3SiH (10.9 mL, 68.0 mmol, 4.5 eq.) and TFA (1.7 mL, 22.7 mmol, 1.5 eq.) were slowly added to the solution. The colour changed from a yellow solution to a bright red solution. The reaction was stirred for 4 hours at -40°C after which an additional 2.0 eq. of TFA was added. After one hour TLC analysis showed full consumption of the starting material. The mixture was quenched by adding solid $NaHCO_3$ until the evolution of gas ceased. The mixture was allowed to warm to room temperature before washing. The reaction mixture was transferred to a separatory funnel and washed once with water and once with brine. The organic layer was dried over $MgSO_4$, filtered and evaporated *in vacuo* to obtain the crude as a bright red oil. The crude was purified by silica gel column chromatography (PE:EtOAc, 9:1 → 3:7) to obtain the product as yellow foam in 78% yield (6.9 g, 11.8 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.04 – 7.78 (m, 6H, arom.), 7.59 – 7.17 (m, 15H, arom.), 5.98 (t, 1H, *J*=9.5 Hz, H-3), 5.51 (m, 2H, H-2, H-4), 5.10 (d, 1H, *J*=10.0 Hz, H-1), 3.96 – 3.82 (m, 2H, H-5, H-6), 3.75 (dd, 1H, *J*=13.2, 5.5 Hz, H-6), 2.82 (s, 1H, OH) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 165.9, 165.8, 165.1 (C=O, Bz), 133.7, 133.3, 132.9, 129.9, 129.1, 128.8, 128.6, 128.5, 128.3 (arom.), 86.2 (C-1), 78.9 (C-5), 74.1 (C-3), 70.6, 69.3 (C-2, C-4), 61.6 (C-6) ppm.

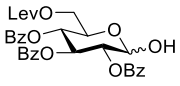
Phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-levulinoyl-1-thio-β-D-glucopyranose (5)



Compound **4** (15.3 g, 26.2 mmol, 1.0 eq.) was dissolved in anhydrous CH_2Cl_2 (250 mL, 0.1M) and cooled to 0°C. DMAP (3.8 g, 31,4 mmol, 1.2 eq.), EDC·HCl (6.8 g, 34.9 mmol, 1.3 eq.) and levulinic acid (3.2 mL, 31.4 mmol, 1.2 eq.) were added to the reaction and stirred for 2 days at room temperature under inert atmosphere. TLC analysis indicated full conversion and the reaction was quenched by adding sat. $NaHCO_3$ (aq.). The layers were separated and the organic layer washed with sat. $NaHCO_3$ (aq.), 1M HCl (aq.), and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain the crude as an off-white solid. The crude was purified by silica gel column chromatography (PE:EtOAc, 19:1

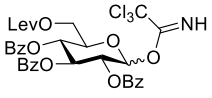
→ 1:4) to obtain the product as a white solid in 65% yield (11.5 g, 16.9 mmol). ^{1}H NMR ($CDCl_3$400 MHz) δ: 8.05 – 7.75 (m, 6H. arom.), 7.59 – 7.23 (m, 15H, arom.), 5.89 (t, 1H, *J*=9.5 Hz, H-3), 5.50 (m, 2H, H-2, H-4), 5.04 (d, 1H, *J*=10.0 Hz, H-1), 4.40 – 4.26 (m, 2H, H-6), 4.11 – 4.03 (m, 1H, H-5), 2.85 – 2.54 (m, 4H, CH_2, Lev), 2.18 (s, 3H, CH_3, Lev) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 172.4, 165.9, 165.3 (C=O, Bz, Lev), 133.2, 132.0, 130.0, 129.3, 129.1, 128.8, 128.6, 128.5, 128.5 (arom.), 86.28 (C-1), 76.3 (C-5), 74.2 (C-3), 70.6, 69.3 (C-2, C-4), 63.0 (C-6), 37.98, 27.92 (CH_2, Lev) ppm. HRMS: $[M+Na]^+$ calculated for $[C_{38}H_{34}O_{10}SNa]^+$: 705.1770, found 705.1782.

2,3,4-tri-*O*-benzoyl-6-*O*-levulinoyl-α/β-D-glucopyranose (6)



Thioglycoside **5** (0.7 g, 1.0 mmol, 1.0 eq.) was dissolved in a mixture of acetone and water (9:1, 10 mL, 0.1M). NBS (0.53 g, 3 mmol, 3.0 eq.) was added and the reaction was stirred under inert atmosphere at room temperature in the absence of light. After 2 hours an additional 2.0 eq. of NBS was added and the reaction stirred for an additional 1.5 hours. TLC analysis showed full consumption of the starting material and the mixture was diluted with EtOAc. The organic layer washed with sat. $Na_2S_2O_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and evaporated *in vacuo* to obtain the crude as a yellow oil. The crude was purified by silica gel column chromatography (PE:EtOAc, 19:1 → 3:2) to obtain the product as a white foam in quantitative yield (0.6 g, 1.0 mmol). α/β ratio 9/1. ^{1}H NMR of α-anomer ($CDCl_3$, 400 MHz) δ: 8.02 – 7.92 (m, 5H, arom.), 7.90 – 7.85 (m, 2H), 7.51 – 7.30 (m, 8H, arom), 6.26 (t,, 1H, *J*=9.9 Hz, H-3), 5.78 (d, 1H, *J*=3.7 Hz, H-1), 5.60 (t, 1H, *J*=9.9 Hz, H-4), 5.33 (dd, 1H, *J*=10.2, 3.5 Hz, H-2), 5.13 (s, 1H, OH), 4.63 – 4.54 (m, 1H, H-5), 4.38 – 4.22 (m, 2H, H-6), 2.77 – 2.69 (m, 2H, CH_2, Lev), 2.62 – 2.53 (m, 2H, CH_2, Lev), 2.15 (s, 3H, CH_3, Lev) ppm.^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 172.5 (C=O, COMe), 165.8, 165.4, 165.3 (C=O, Bz, Lev), 133.5, 133.4, 133.3, 133.1, 130.0, 129.8, 129.7, 129.6, 129.1, 129.0, 128.8, 128.7, 128.4, 128.2, 95.7 (C-1), 72.3 (C-2), 70.3 (C-3), 69.4 (C-4), 67.2 (C-5), 62.7 (C-6), 37.9, (CH_2, lev), 29.7, (CH_3, lev), 27.9, (CH_2, lev) ppm.

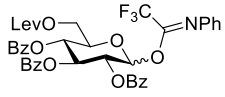
2,3,4-tri-*O*-benzoyl-6-*O*-levulinoyl-1-*O*-(2,2,2-trichloroacetimidoyl)-α/β-D-glucopyranose (7)



Compound **6** (0.6 g, 1.0 mmol, 1.0 eq.) was dissolved in anhydrous DCM (10 mL, 0.1 M) and the round-bottom flask purged with N_2-gas. K_2CO_3 (0.6 g, 4.0 mmol, 4.0 eq.) was added and the mixture cooled to 0°C. Trichloroacetonitrile (0.6 mL, 6.0 mmol, 6.0 eq.) was added slowly to the mixture and the reaction was stirred overnight at room temperature under N_2-atmosphere. TLC analysis showed full conversion and the reaction was diluted in EtOAc. The organic layer was washed with sat. $NaHCO_3$ (aq.) and brine followed by drying over $MgSO_4$. The organic layer was filtered and the volatiles were removed *in vacuo* to obtain crude immidate donor **7** as a yellow oil. The crude was purified by silica gel column chromatography (PE:EtOAc:Et_3N, 90:9:1 → 50:49:1) to obtain the product as a yellow oil in 71% yield (0.52 g, 0.71 mmol). First eluted was the α-anomer: ^{1}H NMR (CD_3CN 300

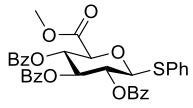
MHz) δ: 9.10 (s, 1H, NH), 7.97 – 7.77 (m, 6H, arom.), 7.62 – 7.31 (m, 9H, arom.), 6.77 (d, 1H, *J*=3.6 Hz, H-1), 6.14 (t, 1H, *J*=9.9 Hz, , H-3), 5.80 – 5.63 (m, 2H, H-2, H-4), 4.60 – 4.49 (m, 1H, H-5), 4.37 – 4.19 (m, 2H, H-6), 2.72 (t, 1H, 2H, CH_2, Lev), 2.50 (t, 2H, *J*=6.8, 5.7 Hz, CH_2, Lev), 2.12 (s, 3H, CH_3, Lev) ppm. ^{13}C-APT NMR (CD_3CN, 75 MHz) δ: 173.1(C=O, COMe), 166.5, 166.0 (C=O, Bz), 160.7 (C=O, NH), 134.8, 134.6, 130.5, 130.3, 129.9, 129.6 (arom.), 93.9 (C-1), 71.6, 71.3 (C-2, C-3, C-4), 69.3 (C-5), 62.6 (C-6), 38.4, 28.7 (CH_2, Lev). Next eluted was the β-anomer. ^{1}H NMR (CD_3CN, 300 MHz): δ:=8.05 (s, 1H, NH), 6.95 – 6.55 (m, 7H, arom.), 6.49 – 6.16 (m, 12H, arom.), 5.17 (d, 1H, *J*=8.2 Hz, H-1), 4.86 (t, 1H, *J*=9.4 Hz, H-3), 4.71 – 4.38 (m, 2H, H-3, H-4), 3.34 – 2.88 (m, 3H, H-5, H-6), 1.53 (t, 2H, *J*=6.3 Hz, CH_2, Lev), 1.33 (td, 2H, *J*=6.8, 3.0 Hz, CH_2, Lev), 0.92 (s, 3H, CH_3, Lev) ppm.

2,3,4-tri-*O*-benzoyl-6-*O*-levulinoyl-1-*O*-(*N*-phenyl-2,2,2-trifluoroacetimidoyl)-α/β-D-glucopyranose (7b)



Compound **6** (0.6 g, 1.0 mmol, 1.0 eq.) and Cs_2CO_3 (1.0 g, 3.0 mmol, 3.0 eq.) were dissolved in anhydrous CH_2Cl_2 (10 mL, 0.1 M). $CF_3C(N=Ph)Cl$ (0.3 mL, 2 mmol, 2.0 eq.) was added and the mixture stirred overnight at room temperature under inert atmosphere. TLC analysis showed complete consumption of the starting material. The mixture was filtered over Celite and the filtrate was concentrated to obtain the crude as a yellow oil. The crude was purified by silica gel column chromatography (PE:EtOAc, 19:1 → 1:1) to obtain the product as a white foam in a 62% yield (0.47 g, 0.62 mmol). ^{1}H NMR (CD_3CN, 400 MHz, 325 K) δ: 9.12 – 8.78 (m, 6H, arom.), 8.76 – 8.11 (m, 14H, arom), 7.34 (s, 1H, H-1), 7.05 (t, 1H, *J*=9.3 Hz, H-3), 6.86 – 6.65 (m, 2H, H-2, H-4), 5.45 – 5.19 (m, 3H, H-5, H-6), 3.80 – 3.70 (m, 2H, CH_2, Lev), 3.63 – 3.50 (m, 2H, CH_2, Lev), 3.16 (3H, CH_3, Lev) ppm. ^{13}C-APT NMR (CD_3CN, 101 MHz) δ: 173.2, 171.4, 166.5, 166.2 (C=O, Bz, Lev), 134.8, 130.2, 130.0, 129.9, 125.8, 120.3 (arom.), 96.3 (C-1), 74.1 , 74.0 (C-3, C-5), 72.4, 70.0 (C-2, C-4), 63.0 (C-6), 38.6 (CH_2, Lev), 28.9 (CH_2, Lev) ppm.

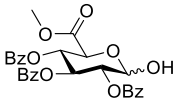
Methyl (phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranosyl uronate) (8)



Compound **4** (2.7 g, 4.9 mmol, 1.0 eq.) was dissolved in a mixture of CH_2Cl_2 and THF (1:2, 50 mL, 0.1 M) and cooled to 0°C. TEMPO (0.15 g, 1.0 mmol, 0.2 eq.) was added and stirred 10 minutes before adding BAIB (1.6 g, 4.9 mmol, 1.0 eq.) and water (5 mL). The reaction was stirred 2 hours before an additional 0.8 eq. of BAIB was added. After an additional 2 hours TLC analysis showed full conversion. The reaction mixture was acidified to pH 1 using 3M aq. HCl. Brine was added to the mixture and the layers were separated. The aqueous layer was extracted thrice with $CHCl_3$ and the organic layers were collected, dried over $MgSO_4$ and evaporated in vacuo. The crude was obtained as a yellow oil and used without further purification. The crude (0.59 g, 0.1 mmol, 1.0 eq.) was dissolved in anhydrous DMF (1 mL, 0.1 M). K_2CO_3 (0.016 g, 0.12 mmol, 1.2 eq.) and iodomethane (0.03 mL, 0.5 mmol, 5.0 eq.) were added sequentially and the reaction was left to stir overnight under inert atmosphere. TLC analysis showed full consumption of the starting material and the

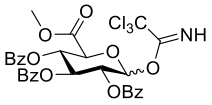
reaction was partitioned between EtOAc and water. The layers were separated and the organic layer was washed with water, sat. $NaHCO_3$(aq.), and brine. The organic layer was dried over $MgSO_4$, filtered and evaporated *in vacuo* to obtain the crude as a yellow oil. The crude was purified over silica gel column chromatography (PE:EtOAc 9:1 → 3:2) to obtain the product obtained as a yellow oil in 82% over 2 steps (0.05 g, 0.082 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.01 – 7.89 (m, 4H, arom.), 7.84 – 7.79 (m, 2H, arom.), 7.58 – 7.24 (m, 14H, arom.), 5.92 (t, 1H, *J*=9.5 Hz, H-3), 5.64 (t, 1H, *J*=9.7 Hz, H-4), 5.49 (t, 1H, *J*=9.6 Hz, H-2), 5.05 (d, 1H, *J*=9.9 Hz, H-1), 4.36 (d, 1H, *J*=9.8 Hz, H-5), 3.71 (s, 3H, OCH_3) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 169.4 (C=O, CO_2Me), 166.6, 166.3, 166.1 (C=O, Bz), 133.7, 133.6, 133.5, 133.5, 130.0, 129.9, 129.2, 128.8, 128.6, 128.4, (arom.), 86.7 (C-1), 76.6 (C-5), 73.5 (C-3), 70.2 (C-2, C-4), 53.1 (OCH_3) ppm. HRMS: $[M+Na]^+$ calculated for $[C_{34}H_{28}O_9SNa]^+$: 635.1352, found 635.1358.

Methyl 2,3,4-tri-*O*-benzoyl-α/β-D-glucopyranosyl uronate (9)



Thioglycoside **8** (1.0 mmol, 0.6 g, 1.0 eq.) was dissolved in a mixture of acetone and water (7:3, 10 ml, 0.1 M) and the mixture was cooled to 0°C. NBS (1.8 g, 5.0 mmol, 5.0 eq.) was added to the reaction. The white suspension was stirred in the absence of light for 45 minutes before the reaction was quenched by adding sat. $Na_2S_2O_3$ (aq.). The mixture was diluted in EtOAc and the layers separated. The organic layer was washed once with $Na_2S_2O_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated.The crude was obtained as a yellow oil and purified by silica gel column chromatography (PE/EtOAc (9:1 → 1:1) to obtain the product as a white solid in 98% yield (0.51 g, 0.98 mmol). α/β ratio 5/1. ^{1}H NMR ($CDCl_3$, 400 MHz): δ:=8.01 – 7.83 (m, 7H, arom.), 7.53 – 7.26 (m, 11H, arom.), 6.30 (t, 1H, *J*=9.7 Hz, H-3α), 5.98 (t, 0.2H, *J*=9.5 Hz, H-3β), 5.89 (d, 1H, *J*=2.7 Hz, H-1α), 5.75 – 5.62 (m, 1.2H, H-4α, H-4β), 5.48 (dd, 0.2H, *J*=9.5, 7.5 Hz, H-2β), 5.36 (dd, 1H, *J*=10.0, 3.5 Hz, H-2α), 5.15 (d, 0.2H, *J*=7.4 Hz, H-1β), 4.91 (d, 1H, *J*=9.9 Hz, H-5α), 4.84 (s, 1H, OH), 4.42 (d, 0.2H, *J*=9.6 Hz, H-5β), 3.64 (s, 0.6H, OCH_3α), 3.61 (s, 3H, OCH_3β) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MH) δ: 169.4 (C=O, CO_2Me), 166.6, 166.3, 166.1 (C=O, Bz), 134.7, 134.6, 134.6, 130.4, 130.4, 130.3, 130.3, 130.3, 130.1, 130.0, 129.7, 129.6 (arom.), 95.7 (C-1β), 91.0 (C-1α), 73.9 (C-2β), 73.6 (C-5β), 73.1 (C-3β), 72.6(C-2α), 71.3 (C-4β), 71.1 (C-4α), 71.0 (C-3α), 69.2 (C-5α), 53.3 (OCH_3α, OCH_3β) ppm.

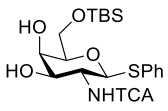
Methyl 2,3,4-tri-*O*-benzoyl-1-*O*-(2,2,2-trichloroacetimidoyl)-α/β-D-glucopyranosyl uronate (10)



Anomeric alcohol **9** (1.1 g, 1.8 mmol, 1.0 eq.) was dissolved in anhydrous DCM (18 mL, 0.1 M). Trichloroacetonitrile (1.8 mL, 18 mmol, 10 eq.) was added and the mixture was cooled to 0°C before slow addition of DBU (80 µL, 0.54 mmol, 0.3 eq.). The reaction was stirred under N_2-atmosphere while slowly warming to room temperature turning it into a dark brown solution. After 30 minutes TLC analysis showed complete consumption of the starting material. The reaction was concentrated *in vacuo* to obtain the crude as a

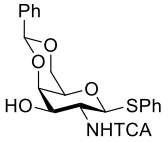
dark brown oil. The crude was purified by silica gel column chromatography (DCM:Et_2O, 9:1 → 1:1) to obtain the product as a yellow foam in 88% yield (1.1 g, 1.6 mmol). α/β ratio 29/1 ^{1}H NMR of the α-product (CD_3CN, 400 MHz) δ: 9.15 (s, 1H, $NHCCl_3$), 7.99 – 7.81 (m, 6H, arom.), 7.63 – 7.31 (m, 9H, arom.), 6.84 (d, 1H, *J*=3.8Hz, H-1), 6.20 (t, 1H, *J*=9.8 Hz, H-3), 5.83 (t, 1H, *J*=9.8, 1.7 Hz, H-4), 5.75 (dd, 1H, *J*=10.1, 3.5, 1.6 Hz, H-2), 4.80 (d, 1H, H-5), 3.61 (s, 3H, OCH_3) ppm. ^{13}C-APT NMR (CD_3CN, 101 MHz) δ: 168.2 (C=O, CO_2Me), 166.4, 166.0 (C=O, Bz), 160.5 (C=NH), 134.8, 130.4, 129.8, 129.7, 129.6 (arom.), 93.7 (C-1), 71.6 (C-5), 70.8 (C-3), 70.3 (C-2, C-4), 53.6 (OCH_3) ppm.

Phenyl 2-deoxy-6-*O*-(*tert*-butyldimethylsilyl)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (12)



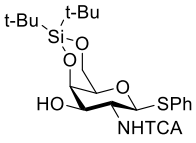
Triol **11** (0.2 g, 0.5 mmol, 1.0 eq.) was dissolved in anhydrous pyridine (5 mL, 0.1 M). TBS-Cl (50%wt in toluene, 1.5 mL, 0.8 mmol, 1.6 eq.) was added slowly to the reaction and the mixture was cooled to -30°C. Et_3N (0.8 mL, 0.6 mmol, 1.1 eq.) was added slowly over the course of 5 minutes. The reaction was allowed to warm to room temperature and stirred overnight under N_2-atmosphere. TLC analysis showed full conversion and the reaction mixture was diluted in methylene chloride. The mixture was poured into ice-cold water and the layers were separated. The aqueous layer was extracted four times with CH_2Cl_2. The organic layers were combined, filtered over Celite® and washed with sat. $NaHCO_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was obtained as a brown oil and purified by silica gel column chromatography (PE:EtOAc, 1:1 → 0:1) to obtain the product as a white solid (0.19 g, 0.35 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.57 – 7.45 (m, 2H, arom.), 7.35 – 7.24 (m, 3H, arom.), 7.20 (d, 1H, *J*=7.6 Hz, NH), 4.94 (d, 1H, *J*=9.6 Hz, H-1), 4.09 (s, 1H, H-5), 4.01 – 3.82 (m, 5H, H-2, H-3, H-4, H-6), 3.73 (s, 1H, OH), 3.56 (t, 1H, OH), 0.91 (s, 9H, *t*-Bu, TBS), 0.10 (s, 3H, CH_3, TBS), 0.9 (s, 3H, CH_3, TBS) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 162.8 (C=O, TCA), 132.9, 132.5, 129.1, 128.06 (arom.), 92.5 (CCl3, TCA), 86.2 (C-1), 78.2 (C-3), 72.2 (C-4), 69.6 (C-5), 63.8 (C-6), 54.3 (C-2), 26.0 (*t*-Bu, TBS), 18.3 (C_q, TBS), -5.3 (CH_3, TBS) ppm.

Phenyl 4,6-*O*-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose. (13)



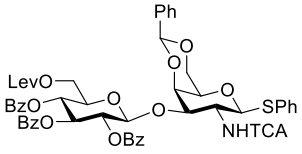
The synthesis and characterization of this compound are described in Chapter 2 compound **3**.

Phenyl 2-deoxy-4,6-*O*-(di-*tert*-butylsilyl)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (14)



Galactopyranose **11** (5.0 g, 12.0 mmol, 1.0 eq.) was co-evaporate twice with anhydrous toluene and dissolved in anhydrous DMF (100 mL, 0.12 M). Activated molecular sieves (4Å) were added and the mixture was stirred one hour under argon gas while cooling to -10°C. Di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (6.0 mL, 18 mmol, 1.5 eq.) was added and the mixture stirred for 3 hours at -10°C under argon gas before TLC analysis indicated full conversion and the reaction was quenched by addition of water (50 mL). The reaction was diluted in Et_2O and the layers separated. The organic layer was washed with twice with water and five times with brine. The organic layer was dried over $MgSO_4$ and filtered before evaporation *in vacuo* to obtain crude silylidene as an orange oil. The crude was purified by silica gel column chromatography (DCM:Et_2O, 99:1 → 7:3) to obtain the product as a white foam in 92% yield (6.2 g, 11.0 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49 – 7.42 (m, 2H, arom.), 7.29 – 7.23 (m, 3H, arom.), 7.10 (d, 1H, *J*=8.9 Hz, NHTCA), 4.88 (d, 1H, *J*=10.5 Hz, H-1), 4.43 (d, 1H, *J*=3.3 Hz, H-4), 4.23 (s, 2H, H-6), 4.09 (q, 1H, *J*=10.0 Hz, H-2), 3.84 (t, 1H, *J*=10.6, 3.2 Hz, H-3), 3.49 (t, 1H, *J*=1.5 Hz, H-5), 2.95 (d, 1H, *J*=11.1 Hz, OH), 1.08 (s, 9H, *t*-Bu, DTBS) 1.04 (s, 9H, *t*-Bu, DTBS) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 162.4 (C=O, TCA), 133.6, 132.2, 129.0, 127.8 (arom.), 92.6 (CCl_3, TCA), 86.7 (C-1), 75.0 (C-5), 72.1 (C-3, C-4), 67.0 (C-6), 54.3 (C-2), 27.5 (*t*-Bu, DTBS), 23.3 (C_q, DTBS), 20.7 (C_q, DTBS) ppm.

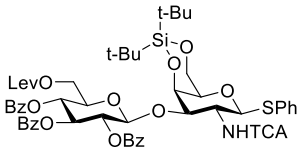
Phenyl 4,6-*O*-benzylidene-3-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-levulinoyl-β-D-glucopyranose)-2-deoxy-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (15)



Acceptor **13** (0.051 g, 0.10 mmol, 1.0 eq.) and donor **7** (0.080 g, 0.15 mmol, 1.5 eq.) were co-evaporated thrice with anhydrous toluene before dissolving in anhydrous DCM (1 mL, 0.1 M). Activated molecular sieves (4Å) were added and the reaction stirred 1 hour under inert atmosphere at room temperature. The reaction was cooled to 0˚C and TMSOTf (0.1 M solution in dry DCM, 0.1 mL, 0.01 mmol, 0.1 eq.) was added. The reaction was stirred as an orange solution at 0°C under N_2 atmosphere. After 5 hours TLC analysis and TLC-MS analysis showed consumption of the starting material and the reaction was quenched by adding Et_3N (0.1 mL) to the solution. The mixture was diluted in EtOAc and the organic layer was washed with sat. $NaHCO_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain the crude as a colourless oil. The crude was purified by size exclusion chromatography (DCM:MeOH, 1:1) to obtain the product as a colourless oil in 31% yield (0.031 g, 0.031 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.99 – 7.73 (m, 10H, arom.), 7.64 – 7.60 (m, 2H, arom.), 7.54 – 7.21 (m, 21H, arom, NH), 5.74 (t, 1H, *J*=9.6 Hz, H-3'), 5.61 – 5.42 (m, 3H, H-2', H-4', PhCH), 5.28 (d, 1H, *J*=10.1 Hz, H-1), 5.19 (d, 1H, *J*=7.9 Hz, H-1'), 4.81 (dd, 1H, *J*=10.8, 3.3 Hz, H-3), 4.64 (dd, 1H, *J*=12.2, 2.1 Hz, H-6), 4.52 (d, 1H, *J*=3.3 Hz, H-4), 4.38 – 4.30 (m, 2H, H-5', H-6'), 4.12 – 3.95

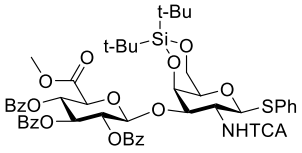
(m, 3H, H-2, H-6, H-6'), 3.62 (s, 1H, H-5), 2.95 – 2.49 (m, 4H, CH_2, Lev), 2.25 (s, 3H, CH_3, Lev) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 172.3 (C=O, COMe), 165.7, 165.5, 165.3 (C=O, Bz, Lev), 161.8 (C=O, TCA), 138.0, 137.9, 132.1, 129.9, 129.2, 129.0, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 126.4 (arom.), 100.8 (PhCH), 99.7 (C-1'), 84.6 (C-1), 75.7 (C-4), 73.7 (C-3), 73.1 (C-3'), 72.7 (C-5'), 71.6 (C-2'), 70.3 (C-5) , 69.4 (C-6'), 68.7 (C-4'), 62.2 (C-6), 52.2 (C-2), 38.3 (CH_2, Lev), 29.9 (CH_3 Lev), 28.5 (CH_2, Lev) ppm.

Phenyl 2-deoxy-3-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-levulinoyl-β-D-glucopyranose)-4,6-*O*-(di-*tert*-butylsilyl)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (16)



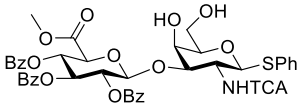
Acceptor **14** (0.056 g, 0.1 mmol, 1.0 eq.) and donor **7** (0.11 g, 0.15 mmol, 1.5 eq.) were co-evaporated thrice with anhydrous toluene before dissolving in anhydrous DCM (1 mL, 0.1 M). Freshly activated molecular sieves (3Å) were added and the reaction stirred for 1 hour under inert atmosphere at room temperature. The reaction was cooled to 0˚C and TMSOTf (0.1 M solution in anhydrous CH_2Cl_2, 0.1 mL, 0.01 mmol, 0.1 eq.) was added after 20 min. The reaction was stirred as an orange solution at 0°C under argon atmosphere. After 35 minutes TLC analysis and TLC-MS showed consumption of acceptor **14** and the reaction was quenched by addition of sat. $NaHCO_3$ (aq.) to the solution. The mixture was diluted in EtOAc and the organic layer was washed with sat. $NaHCO_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$ filtered and the organic layer was evaporated *in vacuo* to obtain the crude as a colourless oil. The crude was purified by size exclusion chromatography (DCM/MeOH, 1/1, v/v) to obtain the product as a colourless oil in 58% yield (0.066 g, 0.058 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 – 7.89 (m, 4H, arom.), 7.78 – 7.75 (m, 2H, arom.), 7.61 – 7.33 (m, 7H, arom.), 7.28 – 7.23 (m, 7H, arom.), 5.75 (t, 1H, *J*=9.7 Hz, H-3'), 5.55 (t, 1H, *J*=9.7, Hz, H-2'), 5.47 – 5.37 (m, 2H, H-1', H-4'), 5.24 (d, 1H, *J*=10.3 Hz, H-1), 4.69 (d, 1H, *J*=2.6 Hz, H-4), 4.63 – 4.54 (m, 2H, H-3, H-6'), 4.30 – 4.18 (m, 3H, H-2, H-6), 4.12 (dd, 1H, *J*=12.1, 6.6 Hz, H-6'), 4.02 (ddd, 1H, *J*=9.0, 6.6, 2.1 Hz, H-5), 3.49 (d, *J*=5.3 Hz, 1H, H-5'), 2.84 – 2.79 (m, 2H, CH_2, Lev), 2.64 – 2.47 (m, 2H, CH_2, Lev), 2.18 (s, 3H, CH_3, Lev), 1.10 (s, 9H, *t*-Bu, DTBS), 0.99 (s, 9H, *t*-Bu, DTBS) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 172.4 (C=O, C=OMe), 165.7, 165.5, 165.4, 161.9 (C=O, Bz, Lev, TCA), 134.4, 133.8, 133.4, 132.7, 130.2, 130.1, 130.0, 129.8, 129.1, 129.0, 128.8, 128.7, 128.6, 128.4, 127.8 (arom.), 99.6 (C-1'), 87.0 (C-1), 75.2 (C-5), 75.0 (C-3), 73.5 (C-4), 73.1 (C-3'), 72.7 (C-5'), 71.7 (C-2'), 69.3 (C-4'), 67.4 (C-6), 63.0 (C-6'), 52.9 (C-2), 38.2 (CH_2, Lev), 29.9 (CH_3, Lev), 28.3 (CH_2, Lev), 27.8 (*t*-Bu, DTBS), 27.6 (*t*-Bu, DTBS), 23.4 (C_q, DTBS), 20.8 (C_q, DTBS) ppm.

Phenyl 2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-4,6-*O*-(di-*tert*-butylsilyl)-2-(2,2,2-trichloroacetamido)-1-thio-β-D-galactopyranose (18)



Acceptor **14** (0.56 g, 1.0 mmol, 1.0 eq.) and donor **10** (1.0 g, 1.5 mmol, 1.5 eq.) were co-evaporated thrice with anhydrous toluene before dissolving in anhydrous DCM (10 mL, 0.1 M). Activated molecular sieves (3Å) were added and the mixture stirred 1 hour under inert atmosphere. TMSOTf (0.1M in anhydrous DCM, 3.0 mL, 0.3 mmol, 0.3 eq.) was added and the mixture stirred for 30 minutes before TLC analysis indicated full consumption of the acceptor. The reaction was quenched by adding sat. aq. $NaHCO_3$ (aq.) to the reaction mixture. The mixture was diluted with EtOAc and the layers were separated. The organic layer washed with sat. $NaHCO_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to obtain crude **18** as a yellow oil. The crude was purified by silica gel column chromatography (toluene/acetone, 19:1 → 1:1) and size exclusion chromatography (MeOH:DCM, 1:1) to obtain the product as a pale yellow solid in 84% yield (0.84 mmol, 0.89 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 – 7.89 (m, 4H, arom.), 7.85 – 7.79 (m, 2H, arom.), 7.63 – 7.21 (m, 14H, arom.), 5.83 (t, 1H, *J*=9.2 Hz, H-3'), 5.62 (t, 1H, *J*=9.5 Hz, H-4'), 5.58 – 5.48 (m, 2H, H-1', H-2'), 5.43 (d, 1H, *J*=10.2 Hz, H-1), 4.76 (d, 1H, *J*=2.7 Hz, H-4), 4.57 (dd, 1H, *J*=10.7, 2.7 Hz, H-3), 4.28 (d, 1H, *J*=9.7 Hz, H-5'), 4.21 (s, 2H, H-6), 3.99 (q, 1H, *J*=10.5, 6.9 Hz, 1H, H-2), 3.68 (s, 3H, OCH_3), 3.46 (s, 1H, H-5), 1.11 (s, 9H, *t*-Bu, DTBS), 1.03 (s, 9H, *t*-Bu, DTBS) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 167.2 (C=O, CO_2Me), 165.7, 165.4, 165.1 (C=O, Bz), 162.1 (C=O, TCA), 133.5, 133.4, 133.1, 130.1, 129.9, 128.8, 128.7, 128.5, 128.1 (arom.), 100.1 (C-1'), 85.6 (C-1), 75.9 (C-3), 75.3 (C-5), 73.6 (C-4), 72.6 (C-3'), 72.5 (C-5'), 71.9 (C-2'), 70.0 (C-4'), 67.4 (C-6), 53.9 (C-2), 53.2 (OCH_3), 27.8 (*t*-Bu, DTBS), 23.4 (C_q, DTBS), 20.8 (C_q, DTBS) ppm.

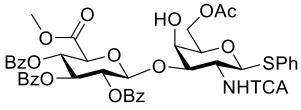
Phenyl 2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-2-(2,2,2-trichloroacetamido)-1-thio-β-D-galactopyranose (19)



Disaccharide **18** (6.5 mmol, 6.9 g, 1.0 eq.) was dissolved in THF (65 mL, 0.1 M). HF·pyridine (26.1 mmol, 2.4 mL, 4.0 eq.) was slowly added and the solution was stirred for 4 hours at which point TLC analysis showed full consumption of the starting material. The reaction mixture was diluted in EtOAc and treated with $CaCO_3$ (36 mmol, 3.6 g, 6.0 eq.) until the evolution of gas ceased. The organic layer was washed with 10% NaCl (aq.), dried over $MgSO_4$, filtered and evaporated *in vacuo* to obtain crude **19** as a yellow solid. The crude was purified by silica gel column chromatography (toluene: acetone 9:1 → 1:1) to obtain the product as a white solid in 75% yield (4.9 mmol, 4.5 g). ^{1}H NMR (400 MHz, CD_3CN) δ: 7.92 – 7.85 (m, 4H), 7.78 – 7.73 (m, 2H), 7.63 – 7.23 (m, 14H), 5.93 (t, 1H, *J*=9.4 Hz, H-3'), 5.62 (t, 1H, *J*=10Hz, H-4'), 5.53 (dd, 1H, *J*=9.4, 7.7 Hz, H-2'), 5.25 (d, 1H, *J*=7.7 Hz, H-1'), 4.96 (d, 1H, *J*=10.3 Hz, H-1), 4.58 (d, 1H, *J*=9.9 Hz, H-5'), 4.26 (t, 1H, *J*=3.3 Hz, H-4), 4.16 (dd, 1H, *J*=10.4, 3.0 Hz, H-3), 4.05 (m, 1H, H-2), 3.79 – 3.67 (m, 2H, H-6), 3.65 – 3.58 (m, 4H, H-5', OCH_3), 3.47 (d, 1H, *J*=3.9,

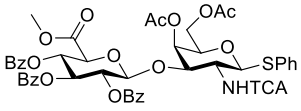
1.1 Hz, 4-OH), 3.05 (t, 1H, *J*=6.0 Hz, 6-OH) ppm. ^{13}C-APT NMR (CD_3CN, 101 MHz) δ: 168.6 (C=O, CO_2Me), 166.2, 166.1, 165.8 (C=O, Bz), 162.6 (C=O, TCA), 135.0, 134.8, 132.0, 130.7, 130.2, 130.0, 130.0, 129.8, 129.5, 129.4, 128.3 (arom.), 101.2 (C-1'), 87.4 (C-1), 80.9 (C-3), 79.6 (C-5), 73.7 (C-3'), 72.7 (C-2'), 72.6 (C-5'), 70.9 (C-4'), 68.7 (C-4), 62.4 (C-6), 53.5 (OCH_3), 52.5 (C-2) ppm. HRMS: $[M+Na]^+$ calculated for $[C_{42}H_{38}Cl_3NO_{14}SNa]^+$: 940.0976, found 940.0992.

Phenyl 6-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (21)



Diol **19** (0.92 g, 1.0 mmol, 1.0 eq.) and NaOAc (0.05 g, 0.6 mmol, 0.6 eq.) were suspended in Ac_2O (10 mL, 0.1 M) and the white suspension was warmed to 50°C. After 1.5 hours TLC analysis showed full consumption of the starting material. The reaction was quenched by adding solid $NaHCO_3$ and the mixture was diluted in EtOAc and washed with sat. $NaHCO_3$ (aq.) followed by brine. The crude was obtained as a yellow oil and purified by silica gel column chromatography (toluene:MeCN, 9:1 → 4:1). The product was obtained as a white solid in 92% yield (0.88 g, 0.92 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 – 7.77 (m, 6H, arom.), 7.67 – 7.33 (m, 9H, arom.), 7.31 – 7.27 (m, 5H, arom.), 6.92 (d, 1H, *J*=7.3 Hz, NH), 5.85 (t, 1H, *J*=8.9 Hz, H-3'), 5.69 (t, 1H, *J*=9.1 Hz, H-4'), 5.53 (dd, 1H, *J*=8.8, 6.8 Hz, H-2'), 5.31 (d, 1H, *J*=12.5 Hz, H-1), 5.11 (d, 1H, *J*=6.9 Hz, H-1'), 4.61 (dd, 1H, *J*=10.3, 3.0 Hz, H-3), 4.39 (m, 3H, H-5', H-6), 4.29 (d, 1H, *J*=2.9 Hz, H-4), 3.83 (t, 1H, *J*=6.3 Hz, H-5), 3.80 – 3.70 (m, 1H, H-2), 3.66 (s, 3H, OCH_3), 3.47 (s, 1H, OH), 2.07 (s, 3H, CH_3, Ac) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 171.0, 167.6, 165.6, 165.3, 165.2, 162.0 (C=O, Ac, Bz, CO_2Me, TCA), 133.7, 132.8, 132.5, 130.1, 129.9, 129.1, 128.7, 128.5 (arom.), 100.9 (C-1'), 84.3 (C-1), 78.4 (C-3), 76.0 (C-5), 72.5 (C-5'), 71.7 (C-4'), 71.6 (C-3'), 69.5 (C-2'), 68.1 (C-4), 63.8 (C-6), 53.2 (C-2, OCH_3, Me), 21.0 (CH_3, Ac) ppm.

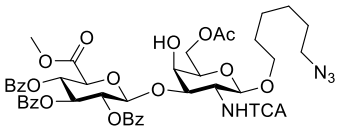
Phenyl 4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (22)



Diol **19** (0.09 g, 0.1 mmol, 1.0 eq.) and NaOAc (0.025 g, 0.3 mmol, 0.3 eq.) were dissolved in Ac_2O (1 mL, 0.1 M) and the solution warmed to 50°C. After two hours to mixture was warmed to 110°C. After one additional hour TLC analysis indicated full conversion and the reaction was quenched with sat. $NaHCO_3$ (aq.). The mixture was diluted in EtOAc and the layers separated. The organic layer was washed three times with sat. $NaHCO_3$ (aq.) and twice with brine. The organic layer was dried over $MgSO_4$ evaporated *in vacuo* to obtain the crude as a yellow oil. The crude was purified silica gel column chromatography (tol:MeCN, 9:1 → 3:2) to obtain the product as a white solid in 82% yield (0.082 g, 0.082 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94 – 7.77 (m, 6H, arom.), 7.56 – 7.25 (m, 16H, arom.), 6.80 (d, 1H, *J*=7.2 Hz, NH), 5.79 (t, 1H, *J*=9.4 Hz, H-3'), 5.67 (t, 1H, *J*=9.6 Hz, H-4'), 5.59 (d, 1H, *J*=3.3 Hz, 1H, H-4), 5.45 (dd, 1H, *J*=9.4, 7.5 Hz,

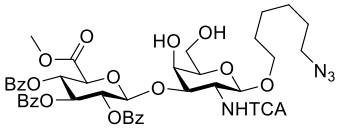
H-2), 5.31 (d, 1H, *J*=10.4 Hz, H-1), 4.99 (d, 1H, *J*=7.5 Hz, H-1'), 4.76 (dd, 1H, *J*=10.4, 3.3 Hz, H-3), 4.29 (d, 1H, *J*=9.7 Hz, H-5'), 4.23 – 4.04 (m, 2H, H-6), 3.95 (dd, 1H, *J*=7.3, 6.2 Hz, H-5), 3.70 (s, 3H, OCH_3), 3.64 (q, 1H, *J*=10.4, 7.1 Hz, H-2), 2.13 (s, 3H, CH_3, Ac), 2.07 (s, 3H, CH_3, Ac) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 172.6, 171.6 (C=O, Ac) 169.9, 166.9, 165.6 (C=O, Bz, CO_2Me), 162.0 (C=O, TCA), 133.6, 133.5, 133.1, 132.0, 130.0, 129.9, 129.9, 129.9, 129.1, 129.0, 128.7, 128.6, 128.4 (arom.), 100.4 (C-1'), 84.4 (C-1), 75.4 (C-5), 74.2 (C-3), 72.9 (C-5'), 72.2 (C-3'), 71.8 (C-2'), 69.9 (C-4'), 69.0 (C-4), 62.7 (C-6), 54.2 (C-2), 53.0 (OCH_3), 20.9 (CH_3, Ac), 20.8 (CH_3, Ac) ppm.

6-azidohexyl 6-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (23)



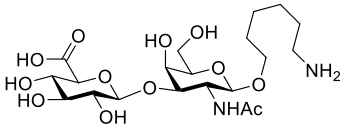
Donor **21** (0.35 g, 0.36 mmol, 1.0 eq.) and 6-azidohexan-1-ol (0.06 g, 0.4 mmol, 1.1 eq.) were co-evaporated with anhydrous toluene thrice and dissolved in anhydrous DCM (3.6 mL, 0.1 M). Activated molecular sieves (3Å) were added and the mixture was stirred for 1 hour at room temperature under inert atmosphere. NIS (0.08 g, 0.36 mmol, 1.0 eq.) was added and the reaction was cooled to -40°C and stirred for an additional 30 min. TfOH (0.1 M in anhydrous DCM, 0.3 mL, 0.03 mmol, 0.08 eq.) was added and the reaction was left to stir as a dark red solution at -40°C. After 30 minutes TLC analysis indicated that the starting material was completely consumed. The reaction was quenched by addition of sat. $NaHCO_3$ (aq.) and diluting the mixture in EtOAc. The organic layer was washed with sat. $Na_2S_2O_3$ (aq.), sat. $NaHCO_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to obtain crude **23** as a dark yellow oil. The crude was purified by size exclusion chromatography (DCM:MeOH, 1:1) to obtain the product as a colourless oil in 56% yield (0.2 g, 0.2 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): δ:=7.92 (ddd, 3H, *J*=10.8, 8.4, 1.4 Hz, arom), 7.84 – 7.78 (m, 2H, arom.), 7.59 – 7.21 (m, 10H, arom.), 6.95 (d, 1H, *J*=6.8 Hz, NH), 5.87 (t, 1H, *J*=9.1 Hz, H-3'), 5.70 (t, 1H, *J*=9.3 Hz, H-4'), 5.57 (dd, 1H, *J*=9.0, 7.1 Hz, H-2'), 5.11 (d, 1H, *J*=7.1 Hz, H-1'), 4.95 (d, 1H, *J*=8.3 Hz, H-1), 4.66 (dd, 1H, *J*=10.7, 3.2 Hz, H-3), 4.45 – 4.36 (m, 3H, H-6, H-5'), 4.25 (d, 1H, *J*=3.3 Hz, H-4), 3.86 (qd, 1H, *J*=6.3, 3.1 Hz, OCH_2), 3.79 (t, 1H, *J*=6.4 Hz, H-5), 3.74 – 3.64 (m, 4H, OCH_3, OH), 3.58 (ddd, 1H, *J*=10.7, 8.2, 6.7 Hz, H-2), 3.52 – 3.40 (m, 1H, OCH_2), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 2.09 (s, 3H, CH_3, Ac), 1.60 – 1.46 (m, 4H, CH_2-hexyl), 1.36 – 1.28 (m, 4H, CH_2-Hexyl) ppm. ^{13}C-APT NMR (101 MHz, $CDCl_3$) δ: 171.0 (C=O, CO_2Me), 167.5 (C=O, Ac), 165.6, 165.3, 165.1 (C=O, Bz), 162.3 (C=O, TCA), 133.7, 133.6, 130.1, 129.9, 129.8, 128.6, 128.6, 128.5, 128.4 (arom.), 101.0 (C-1'), 98.4 (C-1), 92.1 (C_q, TCA), 77.2 (C-3), 72.4 (C-5'), 71.8 (C-5), 71.7 (C-3'), 71.6 (C-2'), 69.9 (OCH_2), 69.7 (C-4'), 68.0 (C-4), 63.3 (C-6), 55.6 (C-2), 53.2 (OCH_3), 51.4 (CH_2N_3), 29.5, 28.8, 26.5, 25.6 (CH_2, hexyl), 20.9 (CH_3, Ac) ppm.

6-azidohexyl 2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (24)



Disaccharide **23** (0.2 g, 0.2 mmol, 1.0 eq.) was dissolved in anhydrous DCM (2 mL, 0.1 M) and the mixture was cooled to 0°C. Acetyl chloride (0.1 M in anhydrous MeOH, 2 mL, 0.2 mmol, 1.0 eq.) was added and the reaction was stirred under inert atmosphere at 4°C for 6 days until TLC analysis showed full conversion of the starting material. The mixture was concentrated *in vacuo* and adsorbed on silica. The crude was purified by silica gel column chromatography (DCM:MeOH, 99:1 → 0:1) and recrystallized from EtOH to obtain **24** as colourless solid in 88% yield (0.16 g, 0.18 mmol). ^{1}H NMR (CD_3CN, 500 MHz): δ:=7.93 (ddd, 1H, *J*=8.5, 5.2, 1.3 Hz, arom.), 7.90 – 7.86 (m, 2H, arom.), 7.86 – 7.82 (m, 2H, arom), 7.76 – 7.71 (m, 2H, arom), 7.65 – 7.27 (m, 10H, arom., NH), 5.89 (t, 1H, *J*=9.4 Hz, H-3'), 5.60 (t, 1H, *J*=9.7 Hz, H-4'), 5.49 (dd, 1H, *J*=9.4, 7.7 Hz, H-2'), 5.21 (d, 1H, *J*=7.7 Hz, H-1'), 4.55 (d, 1H, *J*=9.9 Hz, H-5'), 4.48 (d, 1H, *J*=8.4 Hz, H-1), 4.20 – 4.14 (m, 1H, H-4), 4.07 (dd, 1H, *J*=10.9, 3.1 Hz, H-3), 3.85 (dt, 1H, *J*=11.1, 9.0 Hz, H-2), 3.75 (dt, 1H, *J*=9.7, 6.3 Hz, OCH_2), 3.73 – 3.63 (m, 2H, H-6), 3.59 (s, 3H, OCH_3), 3.49 (ddd, 1H, *J*=6.5, 5.2, 1.2 Hz, H-5), 3.40 (dt, 1H, *J*=9.9, 6.5 Hz, OCH_2), 3.31 (s, 1H, OH), 3.21 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.54 – 1.40 (m, 4H, CH_2-hexyl), 1.32 – 1.19 (m, 4H, CH_2-hexyl) ppm. ^{13}C-APT NMR (CD_3CN, 101 MHz) δ: 168.5 (C=O, CO_2Me), 166.2, 166.1, 165.8 (C=O, Bz), 162.8 (C=O, TCA), 134.7, 134.6, 134.4, 134.0, 130.8, 130.7, 130.4, 130.3, 130.2, 130.1, 130.0, 129.8, 129.7, 129.6, 129.5, 129.5, 129.4 (arom.), 101.5 (C-1'), 101.1 (C-1), 93.4 (C_q, TCA), 80.1 (C-3), 75.4 (C-5), 73.7 (C-3'), 72.6 (C-5'), 72.5 (C-2'), 70.8 (C-4'), 70.0 (OCH_2), 68.5 (C-4), 62.2 (C-6), 54.1 (C-2), 53.4 (OCH_3), 52.0 (CH_2N_3), 30.1, 29.3, 27.1, 26.2 (CH_2, hexyl) ppm.

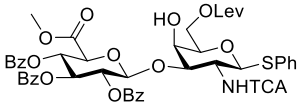
6-aminohexyl 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyrano iduronic acid)-β-D-galactopyranose (28)



Compound **23** (14.2 mg, 15 µmol) was dissolved in a mixture of water and 1,4-dioxane (1:1, 2 mL, 0.01 M) and 1M NaOH (aq.) was added to the mixture until pH 13 was reached. The solution was stirred for 3 days under inert atmosphere at room temperature until LCMS analysis indicated full consumption of the starting material. The mixture was diluted in MeOH (1 mL) and quenched by slow addition of solid CO_2 until pH 7 was reached. The solvents were evaporated *in vacuo* to obtain the crude as a white solid. The crude was purified by size exclusion chromatography (MeOH:H_2O, 9:1) LCMS: $[M+H]^+$ calculated for $[C_{18}H_{32}N_4O_{11}H]^+$: 481.21, found 481.07. The deprotected disaccharide was dissolved in anhydrous MeOH (1.0 mL) and Ac_2O (0.1 mL) was added. The mixture was stirred for 3 hours until LCMS indicated full consumption of the starting material. The solvents were evaporated *in vacuo* and the crude **27** was dissolved in 0.1M NaOH (aq.) (10 mL) and stirred at room temperature. After 30 minutes the solvents were evaporated *in vacuo* and the product was co-evaporated (2x) with D_2O. ^{1}H NMR (D_2O, 400 MHz) δ: 4.51 (2x d,

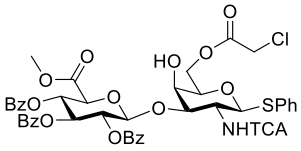
2H, *J*=11.4, 8.2 Hz, H-1, H-1'), 4.18 (d, 1H, *J*=3.2 Hz, H-4), 4.06 – 3.56 (m, 8H, H-2, H-2', H-3, H-3', H-4, H-5', H-6), 3.50 (t, 2H, *J*=8.8 Hz, OCH_2), 3.34 (t, 3H, *J*=6.9 Hz, H-5, CH_2N_3), 2.04 (s, 3H, CH_3, Ac), 1.68 – 1.53 (m, 4H, CH_2-hexyl), 1.39 – 1.36 (m, 6H, CH_2-hexyl). ESI-MS: $[M+H]^+$ calculated for $[C_{20}H_{34}N_4O_{12}H]^+$: 523.23, found 523.00. Compound **27** was dissolved in water (1.0 mL) and the round-bottom flask was purged with N_2-gas. Pd/C (5 mg) was added and the round-bottom flask purged with H_2-gas. Glacial AcOH (0.05 mL) was added and the reaction stirred one hour before LCMS and IR-spectroscopy confirmed full conversion. The round-bottom flask was purged with N_2-gas and the black suspension filtered over Celite® with milliQ water. The crude was purified by size exclusion chromatography (HW-40 gel, H_2O with NH_4HCO_3) and the product was lyophilized to obtain the product as a white solid in 33% yield over 3 steps (5 µmol, 2.7 mg). ^{1}H NMR (D_2O, 400 MHz) δ: 4.54 (dd, *J*=12.7, 8.2 Hz, 2H, H-1, H-1'), 4.21 (d, *J*=3.1 Hz, 1H, H-4), 4.08 – 3.46 (m, 11H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, OH), 3.37 (td, *J*=7.4, 2.5 Hz, 1H, OCH_2), 3.05 – 2.98 (m, 2H, CH_2N_3), 2.11 (s, 3H, CH_3, Ac), 1.74 – 1.57 (m, 4H, CH_2, hexyl), 1.41 (t, *J*=3.6 Hz, 5H, C*H*$_2$, hexyl) ppm. ESI-MS: $[M+H]^+$ calculated for $[C_{20}H_{36}N_2O_{12}H]^+$: 497.23, found 497.17.

Phenyl 2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-6-*O*-levulinoyl-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (29)



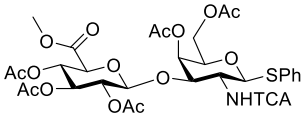
Diol **19** (0.92 g, 1 mmol, 1.0 eq.) was dissolved in a mixture of anhydrous DCM/ACN (10 mL, 9/1, 0.1M) and cooled to 0°C. DMAP (0.15 g, 1.2 mmol, 1.2 eq.), EDC·HCl (0.25 g, 1.3 mmol, 1.3 eq.) and levulinic acid (0.11 mL, 1.1 mmol, 1.1 eq.) were added. The reaction was stirred for 48 hours and stopped by addition of sat. $NaHCO_3$ (aq.). The mixture was diluted in DCM and washed with sat. $NaHCO_3$ (aq.), 1M HCl (aq.) and brine. The organic layer was dried over $MgSO_4$ filtered and the solvents evaporated *in vacuo* to obtain the crude as dark brown oil. The crude was purified by silica gel column chromatography (tol:DCM:Et_2O:CH_3CN, 3:5:1:1) to obtain the product as a yellow oil in 38% yield (0.039 g, 0.38 mmol). ^{1}H NMR (CD_3CN, 400 MHz) δ: 7.92 – 7.73 (m, 6H, arom.), 7.62 – 7.27 (m, 14H, arom.), 5.95 (t, 1H, *J*=9.4 Hz, H-3'), 5.63 (t, 1H, *J*=9.5 Hz, H-4'), 5.59 – 5.53 (m, 1H, H-2'), 5.27 (d, 1H, *J*=7.7 Hz, H-1'), 4.98 (dd, 1H, *J*=10.5, 2.9 Hz, H-1), 4.60 (d, 1H, *J*=9.9 Hz, H-5'), 4.28 (m, 2H, H-6), 4.23 – 3.92 (m, 2H, H-2, H-3), 3.87 – 3.77 (m, 1H, H-5), 3.56 (s, 3H, OCH_3), 3.54 – 3.47 (m, 1H, OH), 2.73 (m 2H, CH_2, Lev), 2.50 (m, 2H, CH_2, Lev), 2.11 (s, 3H, CH_3, Lev). ^{13}C-APT NMR (CD_3CN, 101 MHz) δ: 173.4 (C=O, CO_2Me), 168.5, 165.9, 165.8, 162.7 (C=O, Bz, CO_2Me, Lev), 134.9, 134.4, 130.7, 130.3, 130.2, 130.0, 129.8, 129.7, 129.5 (arom.), 101.1 (C-1'), 87.2 (C-1), 80.5 (C-3), 76.8 (C-5), 73.7 (C-3'), 72.6, 72.5 (C-2', C-5'), 70.8 (C-4'), 68.4 (C-4), 64.5 (C-6), 53.5 (OCH_3), 52.3 (C-2), 38.4 (CH_2, Lev), 29.9 (CH_3, Lev), 28.6 (CH_2, Lev) ppm. HRMS: $[M+Na]^+$ calculated for $[C_{47}H_{44}Cl_3NO_{16}SNa]^+$: 1038.1344, found 1038.1350.

Phenyl 6-*O*-(2-chloroacetyl)-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)- 1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (30)



Compound **19** (0.092 g, 0.1 mmol, 1.0 eq.) was dissolved in a mixture of anhydrous 1,2-dichloroethane and anhydrous pyridine (9:1, 1.0 mL, 0.1M). Chloroacetyl chloride (0.01 mL, 0.12 mmol, 1.2 eq.) was added and the reaction stirred under inert atmosphere. After two hours additional chloroacetyl chloride (6 µL, 0.8 mmol, 0.8 eq.) was added and stirred for an additional 1.5 hours before the reaction was diluted in EtOAc. The organic layer was washed with 1M HCl (aq.), sat. $NaHCO_3$ (aq.), and brine. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to obtain the crude as yellow oil. The crude was purified by silica gel column chromatography (toluene:CH_3CN, 99:1 → 3:1) to obtain the product as a yellow oil in 48% yield (0.48 g, 0.048 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.03 – 7.81 (m, 6H, arom.), 7.59 – 7.30 (m, 14H, arom.), 6.94 (d, 1H, *J*=7.1 Hz, NH 5.88 (t, 1H, *J*=8.9 Hz, H-3'), 5.71 (t, 1H, *J*=9.1 Hz, H-4'), 5.55 (dd, 1H, *J*=8.7, 6.8 Hz, H-2'), 5.35 (d, 1H, *J*=10.3 Hz, H-1), 5.12 (d, 1H, *J*=6.9 Hz, H-1'), 4.64 (dd, 1H, *J*=10.2, 3.1 Hz, H-3), 4.60 – 4.46 (m, 2H, H-6), 4.43 (d, 1H, *J*=9.2 Hz, H-5'), 4.33 (d, 1H, *J*=1.9 Hz, H-4), 4.10 (s, 2H, CH_2Cl), 3.93 – 3.85 (m, 1H, H-5), 3.77 (q, 1H, *J*=10.2, 7.0 Hz, H-2), 3.69 (s, 3H, OCH_3) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 167.7, 167.3, 165.6, 165.4, 165.2 (C=O, Bz, CO_2Me, $COCH_2Cl$), 162.1 (C=O, TCA), 132.9, 132.3, 130.2, 129.2, 128.7, 128.7, 128.7, 128.6 (arom.), 100.9 (C-1'), 84.3 (C-1), 78.3 (C-3), 75.7 (C-5), 72.5 (C-5'), 71.7 (C-4'), 71.6 (C-3'), 69.5 (C-2'), 68.1 (C-4), 65.2 (C-6), 53.3 (C-2, OCH_3), 40.9 (CH_2Cl) ppm.

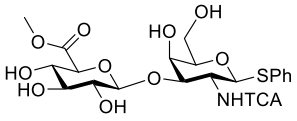
Phenyl 4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl uronate)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (33)



Disaccharide **32** (1.25 g, 1.62 mmol, 1.0 eq.) was dissolved in dry DCM (16 mL, 0.1M) and cooled to 0˚C. Thiophenol (0.18 mL, 1.78 mmol, 1.1 eq.) and $BF_3{\cdot}OEt_2$ (0.40 mL, 3.25 mmol, 2.0 eq.) were added consecutively and the mixture was stirred for 4 hours while letting the temperature increase to RT. The mixture was then diluted in EtOAc and washed with sat. $NaHCO_3$ (aq.) thrice followed by 1M NaOH (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The dark yellow oil was purified by silicagel chromatography (PE:EtOAc, 4:1 → 1:1) to give the title compound in a 62% yield (0.82 g, 1.01 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): δ:=7.57 – 7.47 (m, 2H, Ph), 7.35 – 7.27 (m, 3H, Ph), 7.24 (d, 1H, *J*=8.2 Hz, NH), 5.46 (d, 1H, *J*=3.2 Hz, H-4), 5.22 – 5.06 (m, 3H, H-1, H-3', H-4'), 4.93 (t, 1H, *J*=7.9, Hz, H-2'), 4.77 (d, 1H, *J*=7.7 Hz, H-1'), 4.47 (dd, 1H, *J*=10.4, 3.3 Hz, H-3), 4.20 – 4.06 (m, 2H, H-6), 4.06 – 3.89 (m, 3H, H-2, H-5, H-5'), 3.72 (s, 3H, OCH_3), 2.09 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.98 (s, 3H, Ac) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 170.6, 170.0, 170.0, 169.4, 169.3, 166.9 (C=O, Ac), 161.8 (C=O, TCA), 132.6, 132.4, 129.0, 128.3 (arom.), 99.7 (C-1'), 92.4, CCl_3, TCA), 85.5 (C-1), 75.1 (C-5'), 75.0 (C-3), 72.3 (C-5), 72.0 (C-3'), 70.8 (C-

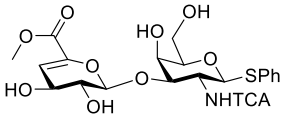
2'), 69.1 (C-4'), 68.5 (C-4), 62.5 (C-6), 53.1 (C-2), 53.0 (OCH_3), 20.8, 20.7, 20.6, 20.5, 20.5 (CH_3, Ac) ppm. HRMS $[M+Na]^+$ calcd for $C_{31}H_{36}Cl_3NO_{16}SNa$: 838.07181, found 838.07126.

Phenyl 2-deoxy-3-*O*-(methyl β-D-glucopyranosyl uronate)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (35)



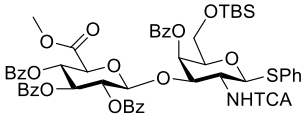
Thioglycoside **33** (0.80 g, 1.0 mmol, 1.0 eq.) was suspended in dry MeOH (10 mL, 0.1M) and NaOMe (11 mg, 0.2 mmol, 0.2 eq.) was added. After 3 hours TLC analysis showed full conversion and the mixture was neutralized by addition of AcOH (0.2 mmol, 0.2 eq.). The neutralized mixture was concentrated *in vacuo*, redissolved in a mixture of $CHCl_3$:*iso*-propanol (4:1) and washed with water twice followed by brine. The organic layer was dried over $MgSO_4$, filtered and concentrated, giving the title compound as an amorphous solid (0.82 mmol, 82%). ^{1}H NMR (MeOD, 400 MHz): δ:=7.61 – 7.46 (m, 2H, Ph), 7.40 – 7.20 (m, 3H, Ph), 5.00 (d, 1H, *J*=10.5 Hz, H-1), 4.54 (d, 1H, *J*=7.3 Hz, H-1'), 4.23 (t, 1H, *J*=10.4 Hz, H-2), 4.12 (d, 1H, *J*=3.0 Hz, H-4), 4.02 (dd, 1H, *J*=10.3, 3.1 Hz, H-3), 3.87 (d, 1H, *J*=9.8 Hz, H-5'), 3.85 – 3.71 (m, 5H, H-6, OCH_3), 3.62 (t, 1H, *J*=6.6, 5.5 Hz, H-5), 3.54 (t, 1H, *J*=9.2 Hz, H-4'), 3.40 – 3.28 (m, 2H, H-2', H-3') ppm. ^{13}C-APT NMR (MeOD, 101 MHz) δ: 171.4(C=O, CO_2Me), 164.0 (C=O, TCA), 135.6, 132.6, 129.9, 128.5 (arom), 105.6 (C-1'), 94.1 (CCl_3), 88.4 (C-1), 80.8 (C-3), 80.5 (C-5), 76.9 (C-3'), 76.7 (C-5'), 74.4 (C-2'), 73.1 (C-4'), 69.5 (C-4), 62.5 (C-6), 53.4 (C-2), 52.9 (OCH_3) ppm. HRMS $[M+Na]^+$ calcd for $C_{21}H_{26}Cl_3NO_{11}SNa$: 628.01898, found 628.01844.

Phenyl 2-deoxy-3-*O*-(methyl 4,5-anhydro-β-D-glucopyranosyl uronate)-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (34)



^{1}H NMR (MeOD, 400 MHz): δ:=7.58 – 7.51 (m, 2H, Ph), 7.37 – 7.24 (m, 3H, Ph), 6.20 (d, 1H, *J*=4.4 Hz, H-4'), 5.27 (d, 1H, *J*=3.1 Hz, H-1'), 4.96 (d, 1H, *J*=10.5 Hz, H-1), 4.25 (t, 1H, *J*=10.2 Hz, H-2), 4.10 – 4.00 (m, 2H, H-3, H-4), 3.97 – 3.90 (m, 1H, H-3'), 3.85 (td, 1H, *J*=3.0, 1.2 Hz, H-2'), 3.82 (s, 3H, OCH_3), 3.81 – 3.75 (m, 1H, H-6), 3.71 (dd, 1H, *J*=11.5, 5.1 Hz, H-6), 3.63 (t, 1H, *J*=6.0 Hz, H-5) ppm.^{13}C-APT NMR (MeOD, 101 MHz) δ: 164.6 (C=O, CO_2Me), 164.0 (C=O, TCA), 141.1, 135.4, 132.7, 130.0, 128.5 (arom), 113.2 (C-4'), 102.8 (C-1'), 88.4 (C-1), 82.1 (C-3), 80.6 (C-5), 70.8 (C-2'), 69.4 (C-4), 66.4 (C-3'), 62.5 (C-6), 53.2 (C-2) , 53.0 (OCH_3) ppm. HRMS $[M+Na]^+$ calcd for

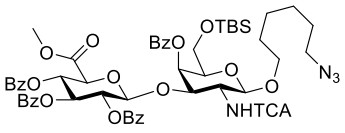
Phenyl 4-*O*-benzoyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-6-*O*-*tert*-butyldimethylsilyl-1-thio-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (36)



Compound **35** (0.20 g, 0.33 mmol, 1.0 eq.) was dissolved in dry DMF (1.0 mL, 0.3M) and cooled to 0˚C, before addition of TBSOTf (84 µL, 0.36 mmol, 1.1 eq.). After 5 min. of stirring imidazole (0.067 g, 0.99 mmol, 3.0 eq.) was added. TLC indicated full conversion after 2 hours and benzoyl chloride (0.31 mL, 2.64

mmol, 8 eq.) and pyridine (1 mL, 0.15 M) were added. The mixture was left stirring overnight at RT. MeOH was added to quench any remaining benzoyl chloride and the mixture was diluted in EtOAc and washed twice with brine, twice with 1M HCl (aq.), twice with sat. $NaHCO_3$ (aq.) and finally once more with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The orange oil was purified using silicagel chromatography (PE:EtOAc 9:1 → 4:1) to obtain the title compound as a white foam (0.164 g, 0.14 mmol, 44%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ:=7.97 – 7.85 (m, 4H, arom.), 7.80 – 7.72 (m, 4H, arom.), 7.64 – 7.54 (m, 3H, arom.), 7.54 – 7.47 (m, 2H, arom.), 7.47 – 7.28 (m, 11H, arom.), 7.28 – 7.18 (m, 4H, arom.), 6.82 (d, 1H, *J*=7.2 Hz, NH), 5.87 (d, 1H, *J*=3.2 Hz, H-4), 5.74 (t, 1H, *J*=9.3 Hz, H-3'), 5.65 (t, 1H, *J*=9.5 Hz, H-4'), 5.43 – 5.31 (m, 2H, H-1, H-2'), 5.05 (d, 1H, *J*=7.4 Hz, H-1'), 4.88 (dd, 1H, *J*=10.4, 3.2 Hz, H-3), 4.29 (d, 1H, *J*=9.5 Hz, H-5'), 3.89 (t, 1H, *J*=6.1 Hz, H-5), 3.78 – 3.67 (m, 3H, H-2, H-6), 3.64 (s, 3H, OCH_3), 0.87 (s, 9H, *t*-Bu, TBS), 0.01 (s, 6H, CH_3, TBS) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 167.0 (C=O, CO_2Me), 165.6 (C=O, Bz), 165.1 (C=O, Bz), 164.8 (C=O, Bz), 161.9 (C=O, TCA), 133.5, 133.4, 133.4, 133.1, 131.7, 130.1, 130.0, 129.9, 129.9, 129.2, 129.1, 128.9, 128.7, 128.5, 128.4, 128.4 (arom.), 99.9 (C-1'), 83.8 (C-1), 79.1 (C-5), 74.5 (C-3), 73.1 (C-5'), 72.3 (C-3'), 72.0 (C-2'), 69.8 (C-4'), 69.5 (C-4), 62.2 (C-6), 54.5 (C-2), 53.0 (OCH_3), 26.0 (*t*-Bu, TBS), 18.4 ($C(CH_3)_3$, TBS), -5.3 (CH_3, TBS) ppm. HRMS $[M+Na]^+$ $C_{55}H_{56}Cl_3NO_{15}SSiNa$: 1158.21032, found 1158.20977.

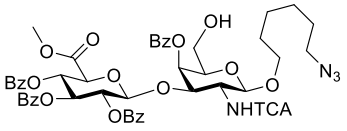
6-azidohexyl 4-*O*-benzoyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-6-*O*-*tert*-butyldimethylsilyl-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (37)



Thiodonor **36** (0.10 g, 0.088 mmol, 1.0 eq.) and azidohexan-1-ol (0.015 g, 0.11 mmol, 1.2 eq.) were co-evaporated together thrice with dry toluene, before being dissolved in dry DCM (0.9 mL, 0.1 M). Freshly activated molecular sieves (3Å) were added followed by NIS (0.029 g, 0.13 mmol, 1.5 eq.) and the solution was left to stir for 1 hour at -20˚C. At this point TMSOTf (88 µL of a 0.1M solution in DCM, 0.1eq.) was added and the cooling bath was removed. After 30 min. TLC indicated full conversion of the starting material and the reaction was quenched by addition of pyridine (0.1 mL). Next the reaction was diluted in EtOAc and washed once with sat. $Na_2S_2O_3$ (aq.) and once with brine. After drying the organic layer over $MgSO_4$, it was filtered and concentrated. The light brown oil was purified using silicagel chromatography (PE:EtOAc 9:1 → 4:1) to obtain the title compound as white solid (0.058 g, 0.050 mmol, 56%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ:=8.08 – 8.02 (m, 2H, arom.), 7.93 – 7.88 (m, 2H, arom.), 7.82 – 7.73 (m, 4H, arom.), 7.61 – 7.30 (m, 10H, arom.), 7.28 – 7.20 (m, 2H, arom.), 6.92 (d, 1H, *J*=7.0 Hz, NH), 5.81 (d, 1H, *J*=3.4 Hz, H-4), 5.77 (t, 1H, *J*=9.4 Hz, H-3'), 5.67 (t, 1H, *J*=9.5 Hz, H-4'), 5.40 (dd, 1H, *J*=9.3, 7.5 Hz, H-2'), 5.08 – 4.98 (m, 2H, H-1, H-1'), 4.89 (dd, 1H, *J*=10.9, 3.5 Hz, H-3), 4.31 (d, 1H, *J*=9.6 Hz, H-5'), 3.92 (dt, 1H, *J*=9.7, 6.2 Hz, OCH_2), 3.88 – 3.75 (m, 2H, H-5, H-6), 3.75 – 3.62 (m, 5H, H-2, H-6, OCH_3), 3.49 (dt, 1H, *J*=9.7, 6.7 Hz, OCH_2), 3.23 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.64 – 1.50 (m, 4H, CH_2-

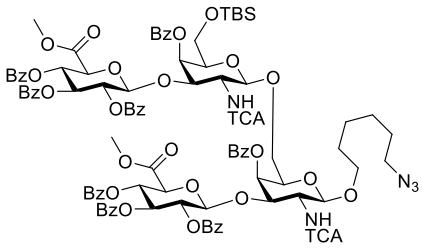
hexyl), 1.43 – 1.29 (m, 4H, CH_2-hexyl), 0.87 (s, 9H, *t*-Bu, TBS), 0.01 (s, 3H, CH_3, TBS), -0.00 (s, 3H, CH_3, TBS) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 167.0 (C=O, CO_2Me), 165.6, 165.4, 165.1, 164.7 (C=O, Bz), 162.2 (C=O, TCA), 133.5, 133.4, 133.0, 130.1, 130.0, 130.0, 129.9, 129.8, 129.1, 128.9, 128.7, 128.5, 128.4, 128.4 (arom.), 100.3 (C-1'), 98.8 (C-1), 92.3 (CCl_3, TCA), 75.0 (C-5), 73.5 (C-3), 73.1 (C-5'), 72.3 (C-3'), 71.8 (C-2'), 70.2 (OCH_2), 69.9 (C-4'), 69.7 (C-4), 62.1 (C-6), 56.9 (C-2), 53.0 (OCH_3), 51.4 (CH_2N_3), 29.5, 28.8, 26.6 (CH_2, hexyl), 25.9 (*t*-Bu, TBS), 25.7 (CH_2, hexyl), 18.3 ($C(CH_3)_3$, TBS), -5.3 (CH_3, TBS), -5.4 (CH_3, TBS) ppm. HRMS $[M+NH_4]^+$ calcd for $C_{55}H_{63}Cl_3N_4O_{16}SiNH_4$: 1186.34176, found 1186.34122.

6-Azidohexyl 4-*O*-benzoyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl urinate)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (38)



Disaccharide **37** (0.050 g, 0.043 mmol, 1.0 eq.) was dissolved in a mixture of H_2O in acetonitrile (1/9, v/v, 4 mL, 0.01M). CSA (0.035 g, 0.150 mmol, 3.5 eq.) was added to pH 2. TLC analysis showed full conversion of the starting material in a lower running spot. The mixture was concentrated to approximately 1 mL at RT and subsequently diluted with EtOAc. The organic layer was washed once with sat. $NaHCO_3$ (aq.) followed by brine. The organic layer was dried over $MgSO_4$ filtered and concentrated. The yellow oil was purified using silicagel chromatography (PE:EtOAc 4:1 → 2:3) to obtain the title compound as colourless oil (0.044 g, 0.042 mmol, 97%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ:=8.13 – 8.06 (m, 2H, arom.), 7.93 – 7.85 (m, 4H, arom.), 7.77 – 7.70 (m, 2H, arom.), 7.64 (t, 1H, *J*=7.4 Hz, arom.), 7.50 (td, 4H, *J*=7.7, 1.9 Hz, arom.), 7.43 – 7.31 (m, 5H, arom), 7.23 (m, 2H, arom.), 6.93 (d, 1H, *J*=6.8 Hz, NH), 5.79 (t, 1H, *J*=9.5 Hz, H-3'), 5.72 (d, 1H, *J*=2.7 Hz, H-4), 5.59 (t, 1H, *J*=9.5 Hz, H-4'), 5.42 (dd, 1H, *J*=9.6, 7.6 Hz, H-2'), 5.11 – 5.01 (m, 2H, H-1, H-1'), 4.92 (dd, 1H, *J*=9.3, 2.4 Hz, H-3), 4.32 (d, 1H, *J*=9.7 Hz, H-5'), 3.94 – 3.82 (m, 2H, H-5, OCH_2), 3.69 (m, 5H, H-2, H-6, OCH_3), 3.60 – 3.40 (m, 3H, H-6, OCH_2, OH), 3.22 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.61 – 1.47 (m, 4H, CH_2-hexyl), 1.38 – 1.28 (m, 3H, CH_2-hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 168.0 (C=O, CO_2Me), 166.6, 165.5, 165.0, 164.8 (C=O, Bz), 162.3 (C=O, TCA), 133.7, 133.5, 133.5, 133.3, 130.3, 130.0, 129.8, 129.7, 129.3, 128.8, 128.7, 128.5, 128.4, 128.3 (arom.), 101.0 (C-1'), 98.7 (C-1), 92.0 (CCl_3, TCA), 74.4 (C-3), 73.5 (C-5), 72.9 (C-5'), 72.1 (C-3'), 71.5 (C-2'), 70.7 (C-4), 70.3 (OCH_2), 69.7 (C-4'), 60.0 (C-6), 56.7 (C-2), 53.0 (OCH_3), 51.3 (CH_2N_3), 29.4, 28.7, 26.5, 25.6 (CH_2, hexyl) ppm. HRMS $[M+Na]^+$ calcd for $C_{49}H_{49}Cl_3N_4O_{16}Na$: 1077.21068, found 1077.21014.

6-azidohexyl [4-*O*-benzoyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-6-*O*-*tert*-butyldimethylsilyl-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose] (1→6) 4-*O*-benzoyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl uronate)-2-(2,2,2-trichloroacetamido)-β-D-galactopyranose (39)



Disaccharide acceptor **38** (0.037 g, 0.035 mmol, 1.0 eq.) and disaccharide donor **36** (0.058 g, 0.051 mmol, 1.4 eq.) were co-evaporated together thrice and afterwards solved in dry DCM (0.35 mL, 0.1M). Freshly dried molecular sieves (3Å) were added and the mixture was stirred at RT. After 30 min. NIS (0.013 g, 0.060 mmol, 1.7 eq.) was added and the mixture was cooled to -20˚C and stirred at this temperature for another 30 min. TMSOTf (35 µL of a 0.1M solution in DCM, 0.1 eq.) was added and the reaction was allowed to warm to 0˚C. After 6 hours the reaction was quenched by addition of pyridine (0.05 mL). The mixture was taking up in EtOAc and washed with once with sat. $Na_2S_2O_3$ (aq.) and once with brine. After drying the organic layer over $MgSO_4$, it was filtered and concentrated. The light brown oil was purified using silicagel chromatography (tol:ACN 1:0 → 9:1), followed by size exclusion of LH-20 (DCM/MeOH, 1/1, v/v) to obtain the title compound as pale white solid (0.028 g, 0.015 mmol, 42%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ:=8.10 – 8.03 (m, 2H, arom.), 8.02 – 7.97 (m, 2H, arom.), 7.96 – 7.84 (m, 5H, arom.), 7.84 – 7.72 (m, 7H, arom.), 7.62 – 7.18 (m, 25H, NH, arom.), 6.85 (d, 1H, *J*=7.0 Hz, NH), 5.81 (d, 1H, *J*=3.4 Hz, H-4''), 5.78 (d, 1H, *J*=2.5 Hz, H-4), 5.77 – 5.73 (m, 2H, H-3'. H-3'''), 5.70 – 5.61 (m, 2H, H-4', H-4'''), 5.45 – 5.35 (m, 2H, H-2', H-2'''), 5.11 – 4.99 (m, 3H, H-1, H-1', H-1'''), 4.98 – 4.93 (m, 2H, H-1'', H-3''), 4.81 (dd, 1H, *J*=10.9, 3.4 Hz, H-3), 4.37 – 4.23 (m, 2H, H-5', H-5'''), 3.99 (dd, 1H, *J*=10.7, 5.0 Hz, H-6), 3.96 – 3.86 (m, 2H, H-5, OCH_2), 3.81 – 3.74 (m, 1H, 5''), 3.71 – 3.60 (m, 9H, H-2, H-6, H-2'', OCH_3', OCH_3'''), 3.60 – 3.56 (m, 1H, H-6''), 3.56 – 3.50 (m, 1H, H-6''), 3.45 (dt, 1H, *J*=9.8, 6.6 Hz, OCH_3), 3.23 (t, 2H, *J*=6.9 Hz, CH_2N_3), 1.62 – 1.48 (m, 4H, CH_2-hexyl), 1.41 – 1.26 (m, 4H, CH_2-hexyl), 0.81 (s, 9H, *t*-Bu, TBS), -0.07 (s, 3H, CH_3, TBS), -0.09 (s, 3H, CH_3, TBS) ppm. ^{13}C-APT NMR ($CDCl_3$, 126 MHz) δ: 167.1, 167.0 (CO_2Me), 165.7, 165.6, 165.4, 165.2, 165.1, 165.1, 164.8, 164.7 (C=O, Bz), 162.4, 162.2 (C=O, TCA), 133.6, 133.5, 133.4, 133.3, 133.3, 133.1, 133.0, 130.2, 130.1, 130.0, 129.9, 129.9, 129.9, 129.8, 129.1, 129.0, 129.0, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3 (arom.), 100.3 (C-1'), 100.2 (C-1'''), 98.7 (C-1''), 98.0 (C-1), 92.2 (CCl_3, TCA), 74.8 (C-5''), 73.4 (C-5), 73.3 (C-3), 73.1 (C-3''), 73.0 (C-5', C-5'''), 72.4 (C-3'), 72.2 (C-3'''), 71.8 (C-2'), 71.6 (C-2'''), 70.2 (OCH_2), 70.0 (C-4'), 69.9 (C-4), 69.9 (C-4'''), 69.4 (C-4''), 67.1 (C-6), 61.6 (C-6'), 56.7 (C-2, C-2''), 53.1 (OCH_3), 53.0 (OCH_3), 51.4 (CH_2N_3), 29.5, 28.8, 26.6 (CH_2, hexyl), 25.9 (*t*-Bu, TBS), 25.7 (CH_2, hexyl), 18.3 ($C(CH_3)_3$, TBS), -5.5 (CH_3, TBS) ppm. HRMS $[M+Na+H]^{2+}$ calcd for Chemical Formula: C98H99Cl6N5O31SiNaH: 1051.71002, found 1051.70947.

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6

Summary & future prospects

1 **Chapter 1** gives a concise overview of schistosomiasis, what causes it, where it occurs, its pathology and, how it is diagnosed and what the current treatment is. Following this is an overview of several syntheses of Schistosoma specific glycans known in literature.

2 **Chapter 2** describes the synthesis of two unique schistosomal glycan motifs, Fuc-α-(1-2)-Fuc-α-(1-3)-GlcNAc and Fuc-α-(1-2)-Fuc-α-(1-3)-GalNAc. A significant difference in yield was observed in the introduction of the second fucosyl residue towards these trisaccharides. DFT calculations were performed on the correspondent protected disaccharide acceptors, which gave insights into their conformational behavior, indicating that the Fuc-GlcNAc acceptor was more hindered than its Fuc-GalNAc counterpart. Analysis of the NMR spectra of the protected trisaccharides showed a remarkable feature, hinting at a stable conformation for the GalNAc trisaccharide that was not available to the GlcNAc trisaccharide. Gold nanoparticles (5 nm) were functionalized with the synthesized sugars and these were subsequently used to screen antibodies and sera by ELISA's techniques. The trisaccharides were recognized by monoclonal antibodies and by antibodies present in sera from schistosoma infected individuals. This shows that these glycan functionalized gold nanoparticles can be used as diagnostic tool for schistosomiasis.

3 The synthesis of α-(1-2)-oligofucosides is described in **Chapter 3**. Two approaches were evaluated; Route A which started the elongation from the reducing end, and Route B that started elongation from the non-reducing end. Although a disaccharide was easily obtained in Route A, subsequent elongation to the tri- and tetramer oligofucosides proved difficult. Route B proved to be a much more viable approach for the extension of the α-(1-2)-oligofucoside chain, although this route was plagued by a low yielding catalytic hydrogenation in the deprotection procedure. Di-, tri- and tetramers were obtained via both routes and they were coupled to gold nanoparticles (5 nm). These gold nanoparticles were characterized by monoclonal antibodies in an ELISA experiment. Subsequently, the gold nanoparticles

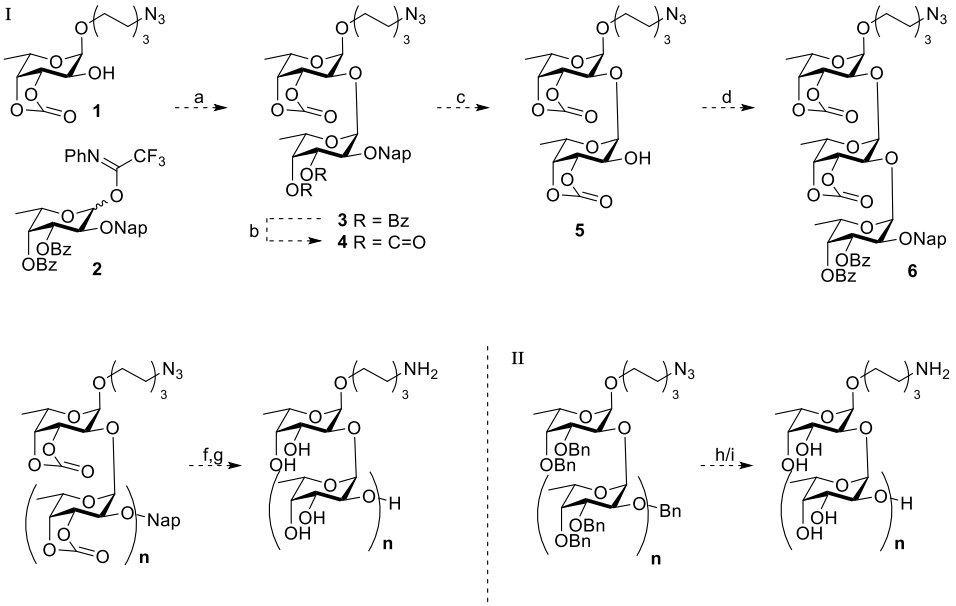
were used to screen sera of people infected with schistosomes. From this data it was concluded that these glycan coated particles, especially the particles coated with the tri- and tetramer can be used as diagnostic tools for schistosomiasis.

As it was shown in this chapter that the tri- and tetramer α-(1-2)-oligofucosides were recognized by antibodies, optimizing the corresponding synthetic routes would be beneficial. To this end, possible improvements are depicted in Scheme 1.

Although the deprotection of the oligofucosides, following route A in chapter 3, proceeded smoothly, the assembly of the fully protected oligofucosides proved problematic. It was hypothesized that the glycosylation reactions were hampered by the steric bulk of the benzoyl groups. Replacement of these groups by smaller protecting groups is not an option as full α-selectivity of the glycosylation depends on the steric bulk of the protecting groups on the C3- and C4-O positions of the donor. In contrast, the acceptor used in route B carried the much smaller cyclic carbonate on the C3- and C4-O positions on the acceptor, which did not appear to hinder the stereoselectivity of the glycosylation reaction. To optimize the assembly of α-(1-2)-oligofucosides the merger of these routes deserves further investigation (Scheme 1-I). Acceptor **1** and donor **2** could be condensed using the optimized conditions described in Chapter 3 giving disaccharide **3**. Hydrolysis of the benzoyl esters and the cyclic carbonate followed by the (re-) installation of the cyclic carbonates would give rise to fully protected dimer **4**. Removal of the naphthyl groups using DDQ in MeOH/DCM would then result in acceptor **5**. Repeating these steps would lead to oligofucosides of the desired length. Deprotection would be achieved using Zemplén conditions, which would leave the oligofucosides with a clickable azide. The free amine can be obtained by catalytic hydrogenation.

Although the method to synthesize the fully protected α-(1-2)-oligofucosides from the non-reducing end upwards (Route B) proved very efficient, removal of the protecting groups in the precursors was problematic, resulting in low yields of the target oligosaccharides. It was reasoned that the solid palladium catalyst could not reach the inner benzyl groups of the protected precursors. Subjecting these oligofucosides to Birch conditions might alleviate this problem (Scheme 1-II).[1] A second alternative would be to oxidize the benzyl ethers using ozone, followed by base assisted hydrolysis of the formed benzoyl esters.[2]

Scheme 1: Suggested improvements to the synthesis of α-(1-2)-oligofucosides for I) from the reducing end and II) from the non-reducing end.



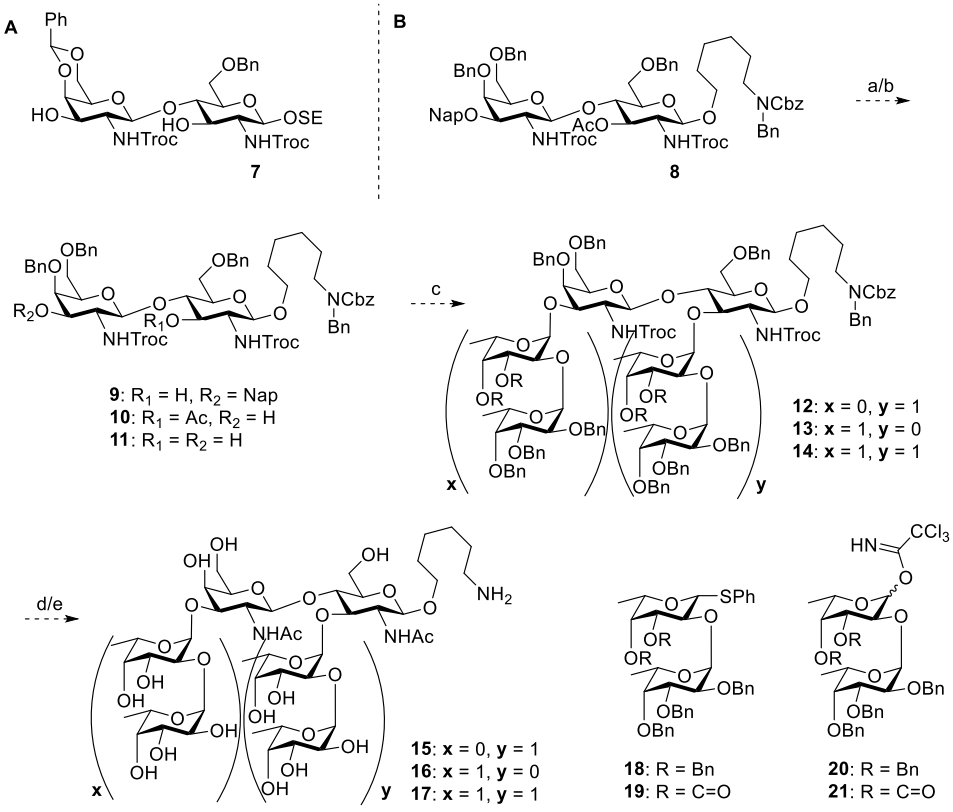
Reagents and conditions: **a**: TMSOTf, MS (3Å), DCM, -30˚C, **b**: i) NaOH, H_2O, THF, 40˚C, ii) triphosgene, pyr. DCM, 0˚C, **c**: DDQ, DCM, MeOH, **d**: **2**, TMSOTf, MS (3Å), DCM, -30˚C, **f**: NaOH, H_2O, THF, 40˚C, **g**: Pd/C, H_2, H_2O, **h**: Na, THF, NH_3, -78˚C, **i**: i) O_3, DCM, ii) NaOMe, MeOH, iii) Pd/C, H_2, H_2O.

4 **Chapter 4** describes the synthesis of fucosylated *N*-acetylgalactosamine-β-(1-4)-*N*-acetylglucosamine disaccharide (GalNAc-β-(1-4)-GlcNAc, LacdiNAc or LDN) fragments, specifically three mono-fucosylated fragments (F)-LDN-(F) and three di-fucosylated fragments (F2)-LDN-(F2). The LDN backbone was constructed from appropriately protected glucosamine and galactosamine synthons. A variety of orthogonal protective groups on the C3-OH positions of both sugars were explored, such as TBS-Nap, Nap-Bz, Nap-Lev and Nap-Ac. Ultimately the Nap-Ac pair proved most successful. After removal of the orthogonal groups the fucosyl residue was introduced, which, after deprotection, resulted in the mono-fucosylated fragments (F)-LDN-(F). The introduction of the di-fucosyl α-(1-2) chain to the LDN fragments led to (F2)-LDN-(F2) in low yields. Unfortunately, optimization of this glycosylation was fruitless.

It would be of interest to optimize the introduction of the α-(1-2) di-fucosyl chain. A possible improvement is depicted in Scheme 2. As several different donors, ranging from activated to deactivated, and several different activation methods have been explored already, it would be prudent to make alterations to the acceptor. Kanaya *et al.* have shown that it is possible to introduce α-(1-2) di- and tri-fucosyl chains on a similar LDN backbone, carrying Troc groups as protection for the amines (**7**).[3] By changing the TCA

protective group to a Troc group and using the chemistry outlined in chapter 4, disaccharide **8** can be synthesized. The change from a TCA to a Troc group does warrant a different protecting group on the amine linker, such as a Cbz. The acetyl and naphthyl group are orthogonal and can be deprotected by either NaOMe in methanol or HCl with HFIP as described in chapter 4. The obtained LDN fragments **9**-**11** can then be condensed with either of donors **18**-**21**. Deprotection would be achieved by reducing the Troc group by zinc followed by acetylation.[4] The remaining protective groups could then be removed by a Birch reaction.[1]

Scheme 2: Proposed synthesis of di-fucusylated LDN fragments.



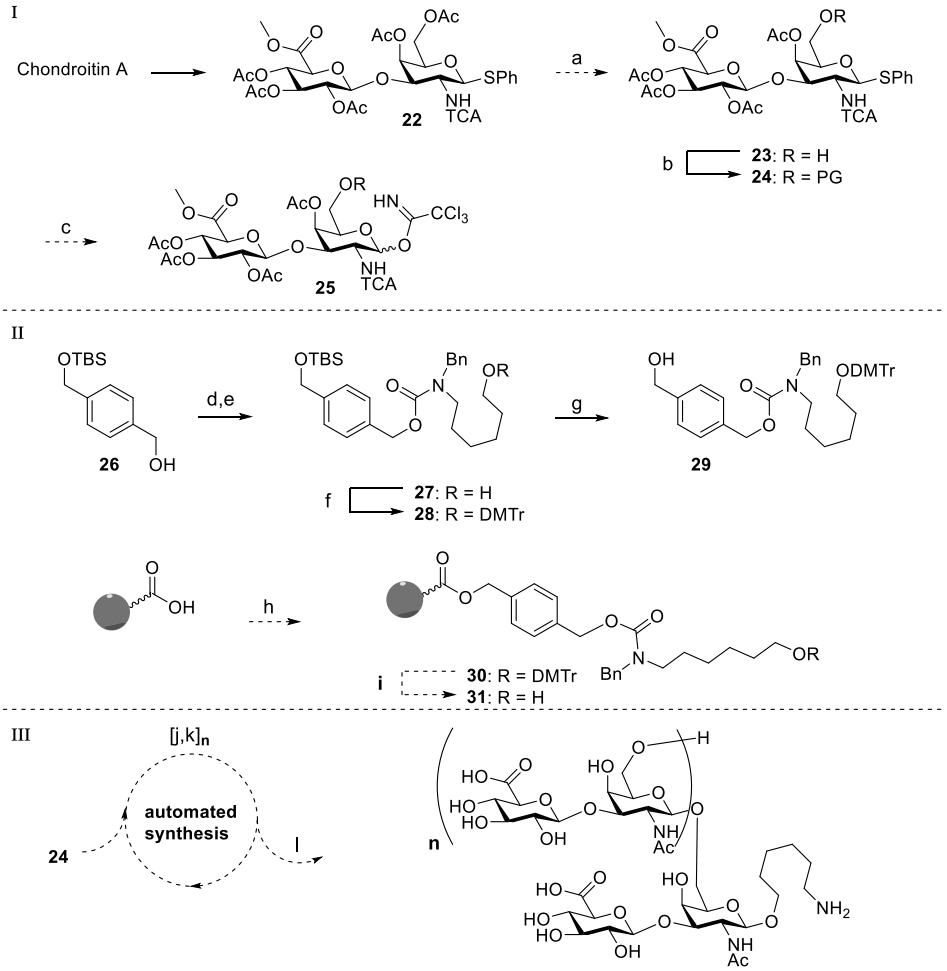
Reagents and conditions: **a**: NaOMe, THF, MeOH, **b**: HCl, HFIP, DCM, **c**: glycosylation conditions, **d**: i) Zn, AcOH, Ac_2O, EtOAc, ii) NaOMe, MeOH, **e**: Na, THF, NH_3.

5 Two novel routes (routes A and B) towards Circulating Anodic Antigen (CAA) oligosaccharides are described in **Chapter 5**. Route A is a flexible assembly route where the disaccharide repeating unit is synthesized from a variety of D-glucose or D-glucuronic donors and D-galactosamine acceptors. It was observed that the glucuronic donor was superior the D-glucose donors. Of the synthesized acceptors the silylidene protected D-galactosamine proved to be the best.

Combining these two glycans resulted in the synthesis of the core disaccharide in 84% yield. Unfortunately, the desired CAA tetramer could not be obtained via this route, due to the formation of several byproducts during the [2+2] glycosylation. Route B is based on the isolation of the repeating unit of CAA from chondroitin A by acid hydrolysis. Using this synthesis the target CAA tetramer was obtained in 40% yield.

It would be of interest to further optimize Route B, especially the glycosylation step (Scheme 3-I). Converting the thiophenyl donor **24** into the more reactive imidate donor **25** would be a good way to start, as the glycosylations done by Vliegenthart and coworkers were performed with imidate donors. Further improvements can be made to shorten the synthetic route towards the desired acceptors and donors. A recent paper by Lecourt *et al.* shows a novel way to selectively remove the acetyl from the primary alcohol with $(Cp)_2ZrCl_2$ in combination with DIBAL-H.[5] This method would shorten Route B to only three steps. The downside with this method is that the acetyl on the C4-O tends to migrate with sugars with the galactose configuration. In this case the migrated product could be subjected again to $(Cp)_2ZrCl_2$ in combination with DIBAL-H yielding a C4- C6-OH diol. It was shown in route A that the selectivity of the primary 6-OH over the axial 4-OH in a glycosylation reaction is complete.

*Scheme 3: **I**) Proposed improvements to the synthesis of disaccharide donor **25**, **II**) Synthesis of a linker suitable for solid state chemistry, **III**) automated synthesis of CAA oligomers.*



Reagents and conditions: **a**: $(Cp)_2ZrCl_2$, DIBAL-H, THF, -20˚C, **b**: reagents to introduce the selected protective group, **c**: i) NBS, acetone, water, ii) Cl_3CCN, DBU, DCM, **d**: *p*-nitrophenylchloroformate, pyridine, DCM, **e**: *N*-benzyl aminohexan-1-ol, DIPEA, DCM, 0˚C, 50% (over two steps), **f**: DMTr-Cl, pyridine, DCM, 86%, **g**: TBAF, THF, 83%, **h**: **29**, DIC, DMAP, DCM, **i**: TCA, DCM, **j**: TMSOTf (cat.), DCM, **k**: removal of PG, **l**: cleavage from resin.

A relevant next step will be to make a building block that is suitable for solid phase synthesis. Even though there are examples of thioglycosides being used in a solid phase synthesis the most used anomeric leaving groups are the imidate and the phosphonate functionalities.[6], [7] Besides this, the TBS group on the primary hydroxyl used in chapter 5 should be replaced by a temporary protective group that is compatible with common solid phase syntheses such as the Lev or the Fmoc group.[8], [9] Both of those groups can be removed fast and orthogonal, where the Fmoc has the advantage that the coupling efficiency can be measured by UV.[10] Care will have to be taken to prevent migration of the C4-O-acetate to the liberated C6-OH. Introduction of these temporary protecting

groups should be possible using the reported protocols to synthesize disaccharide building block **25**.[11], [12]

In solid phase synthesis the linker will be installed on functionalized resin **31** (Scheme 3-II). Starting from silylated compound **26**, *N*-benzyl 6-aminohexanol is introduced by first transforming the free hydroxyl of **26** into an active carbonate, using *p*-nitrophenylchloroformate.[13] This activated carbonate was subsequently left to react with *N*-benzyl 6-aminohexanol giving compound **27**. Besides carbamate compound **27** a small amount of carbonate linked product was also obtained. The free hydroxyl in **27** was masked with a DMTr group giving **28**, followed by the selective removal of the TBS group in **28** with TBAF giving **29**, which can be condensed with carboxylic acid functionalized polystyrene resin to afford functionalized resin **31.**

General future prospects

Nanoparticles

In the research described in this thesis the gold nanoparticles that were synthesized were 2 nm in diameter with only a single type of glycan on its surface. An attractive property of AuNP's is that they offer the possibility to combine different components in a controlled way onto their surface.[14] It is proposed to further improve its use as a diagnostic tool, by coating the AuNP's with a variety of synthesized glycans from different developmental stages of *S. mansoni*. The drawback of 2 nm AuNP's is that they require an ELISA set-up for diagnosis. Alternatively, a fast and easy diagnostic test can be designed on the basis of the colorimetric properties of AuNP's. Dissolved 15 nm AuNP's have a ruby red colour, while aggregated AuNP's have a blue/purple colour.[15], [16] Aggregation can be induced by the binding of antibodies, present in serum. A colourshift then shows if the person is infected with *S. mansoni* parasites.

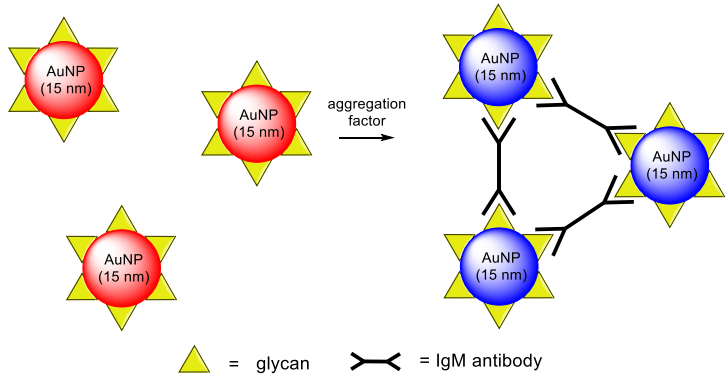
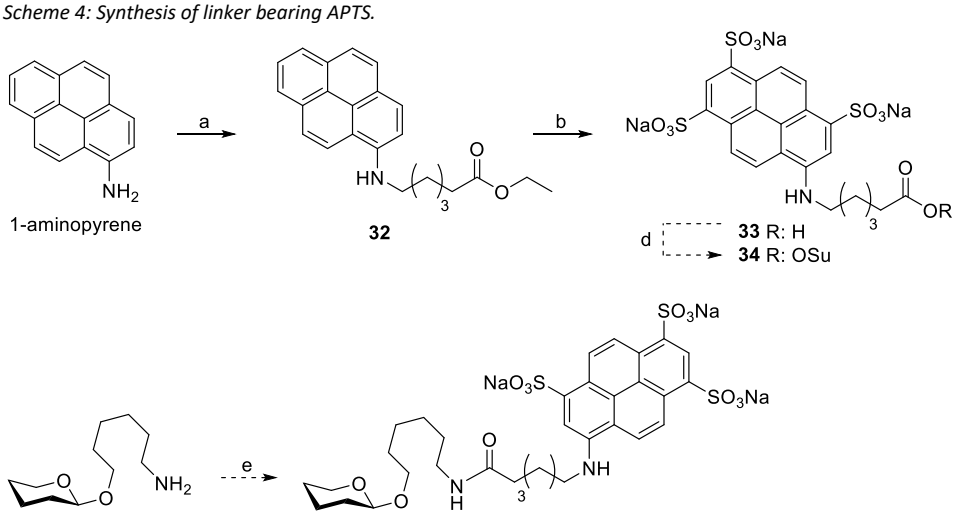


Figure 1: Schematic representation of colour change from red (free AuNP's) to blue (aggregated AuNP's).

Functionalization with a dye (APTS)

The glycans, of which the synthesis is described in this thesis can also be used as a means to discover the, as of yet, unknown enzymes that either remove or extend the α-(1-2)-Fuc chains. In order to achieve this it is proposed to attach a dye to the glycans by which they can function as fluorescent probes. An example of such a dye is 8-aminopyrene-1,3,6-trisulphonic acid (APTS), it is an anionic water soluble bright green fluorophore that is compatible with high resolution capillary electrophoresis.[17] In order to attach APTS to the glycans a linker is required. A linker, having a carboxylic acid function, was chosen to facilitate the coupling to the amine at the spacer of the glycans. The synthesis of this linker, bearing APTS is described below (Scheme 4). The synthesis started from commercially available 1-aminopyrene, which was functionalized by treating it with ethyl 7-bromohexanoic and K_2CO_3 in DMF at 80°C, giving **32** in 52% yield. The sulphate groups were installed following a protocol by Sharret *et al.* using oleum, concentrated sulphuric acid and sodium sulphate at 60°C.[18] In order to remove all the formed sulphate salts compound **33** was purified by adsorbing it on activated carbon. After eluting the dye from the carbon column **33** was obtained as a green solid. In order to conjungate the dye to the glycan, the carboxylic acid moiety on the linker will have to be transformed into an OSu ester, which can then react with the glycan.[19], [20]

Scheme 4: Synthesis of linker bearing APTS.



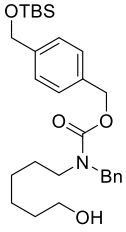
Reagents and conditions: **a**: Ethyl 6-bromohexanoic acid, TBAI, DMF, K_2CO_3, 80°C, 52%, **b**: oleum, H_2SO_4, Na_2SO_4, 60°C, **d**: *N*-hydroxysuccinimide, DCC, DMF, **e**: **34**, $NaHCO_3$, DMSO, H_2O.

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.

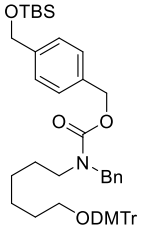
4-(*tert*-butyldimethylsilyl-oxy-methyl-benzyl)-6-(hydroxyhexylaminobenzyl)-carbamate (27)



The TBS protected benzene derivative **26** (7.3 g, 28 mmol, 1.0 eq.) was dissolved in dry DCM (80 mL, 0.35M). Pyridine (4.0 mL, 50 mmol, 1.8 eq.) was added and the mixture was cooled with an ice bath before addition of *p*-nitrochloroformate (6.7 g, 31 mmol, 1.1 eq.) dissolved in DCM (25 mL) was added. After addition of the chloroformate, the ice bath was removed and the reaction mixture was left to stir overnight at RT. When TLC showed full conversion the reaction mixture was poured into icecold water and the organic phase was separated and dried over $MgSO_4$, filtered and concentrated. The obtained brown solid was recrystallized from ether/dichloromethane (1:1) to afford yellow crystals (10.1 g, 24.4 mmol, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.29 – 8.26; 7.45 – 7.26 (m, 8H, arom), 5.29 (s, 2H, $BnCH_2O$), 4.76 (s, 2H, $TBSOCH_2$), 0.95 (s,

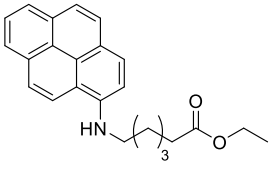
9H, *t*-Bu, TBS), 0.11, (s, 6H, CH_3, TBS) ppm. The formed carbonate (10.1 g, 24.4 mmol, 1.0 eq.) was dissolved in DCM (120 mL, 0.2M), and 6-(benzylamino)-1-hexanol (5.4 g, 26 mmol, 1.02 eq.) and DIPEA (6.8 mL, 39 mmol, 1.5 eq.) were added. The reaction was left to stir overnight at RT. When TLC showed conversion, the mixture was diluted in EtOAc and washed thrice with 1M HCl (aq.), thrice with sat. $NaHCO_3$ (aq.) and once with brine. The organic layer was dried over $MgSO_4$, filtered and the solvents were removed under reduced pressure. The obtained yellow oil was purified via column chromatography (Tol: EtOAc, 1:0 → 6:4), to afford compound **27** in 50% yield (6.21 g, 12.8 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 – 7.20 (m, 9H, arom), 5.19 (s, 2H, $BnCH_2O$), 4.78 (s, 2H, $TBSOCH_2$), 4.53 (s, 2H, NCH_2Bn), 3.59 (t, 2H, *J* = 4.5 Hz, CH_2OH), 3.30 (t, 2H, *J* = 5.1 Hz, CH_2NBn), 1.58 – 1.26 (m, 8H, CH_2, hexyl), 1.02 (s, 9H, *t*-Bu, TBS), 0.16 (s, 6H, CH_3, TBS) ppm.

4-(hydroxymethyl)benzyl benzyl(6-(bis(4-methoxyphenyl)(phenyl)methoxy)hexyl)carbamate (29)



Carbamate **27** (5.8 g, 12 mmol, 1.0 eq.) was dissolved in a mixture of DCM (40 mL) and pyridine (20 mL) and DMTr-Cl (4.5 g, 13.2 mmol, 1.1 eq.) was added and left to stir overnight under nitrogen atmosphere at RT. The reaction was quenched by addition of MeOH and transferred to a seperatory funnel. The organic layer was washed diluted in EtOAc and washed with sat. $NaHCO_3$ (aq.) and brine. The organic layer was dried over $MgSO_4$, filtered and the solvents were removed *in vacuo*. The obtained crude product (**28**) was dissolved in THF (60 mL, 0.2M) and cooled with an icebath. TBAF (12 mL, 12 mmol, 2 eq., 1M in THF) was added and the mixture was left to stir overnight under argon atmosphere at RT. When TLC analysis showed full conversion the mixture was diluted in EtOAc and washed water followed by brine. The organic layer was dried over $MgSO_4$, filtered and the solvents were removed under reduced pressure. The obtained brown oil was purified by column chromatography (PE: EtOAc, 9:1 → 1:1) to afford linker **29** as a yellow oil (4.3 mmol, 2.73 g, 72%). TLC analysis: PE:EtOAc, 6:4). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.55 – 6.83 (m, 22H, arom), 5.19 (s, 2H, $BnCH_2O$), 4.71 (s, 2H, NCH_2Bn), 4.52 (s, 2H, CH_2OH), 3.83 (s, 6H, 2x OCH_3, DMTr) 3.25 (t, 2H, *J* = 6.6 Hz, CH_2ODMTr), 3.03 (t, 2H, *J* = 5.1 Hz, CH_2NBn), 1.50 – 1.20 (m, 8H, CH_2, hexyl) ppm. ^{13}C-APT NMR ($CDCl_3$, 101 MHz) δ: 158.6 (C=O), 145.5, 145.1, 139.1, 138.0, 136.8, 136.3, 130.2, 128.6, 128.3, 128.0, 127.8, 127.1, 113.2, 113.1 (arom.), 67.1 ($BnCH_2O$), 65.4 (CH_2ODMTr), 64.9 ($BnCH_2OH$), 55.3 (2x CH_3, DMTr), 50.5 (CH_2NCH_2Bn), 32.6 (CH_2NCH_2Bn), 30.1 – 25.4 (CH_2, hexyl) ppm.

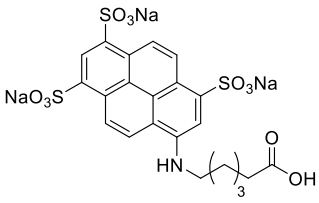
Ethyl 6-(pyren-1-ylamino)hexanoate (32)



1-aminopyrene (0.20 g, 1.0 mmol, 1.0 eq.) was dissolved in DMF (5 mL, 0.2M). K_2CO_3 (0.35 g, 2.5 mmol, 2.5 eq.), ethyl 6-bromohexanoic acid (0.26 mL, 1.5 mmol, 1.5 eq.) and TBAI (0.18 g, 0.5 mmol, 0.5 eq.) were added and the mixture was heated to 60°C in the dark for 48 hours. Next, the reaction mixture was poured into EtOAc and washed with brine (5x), dried over $MgSO_4$, filtered

and concentrated. The obtained dark green oil was purified by silicagel chromatography with toluene to give the title compound in 52% yield (0.19 g, 0.52 mmol). The starting material was isolated in 41% (0.080 g, 0.41 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 8.16 – 7.70 (m, 7H, arom.), 7.30 (d, 1H, *J*=9.9 Hz, arom.), 4.72 (s, 1H, NH), 4.19 (q, 2H, *J*=7.1 Hz, CH_2, Et), 3.40 (s, 2H, CH_2NH), 2.39 (t, 2H, *J*=7.3 Hz, CH_2COO), 1.79 (dp, 4H, *J*=23.1, 7.4 Hz, CH_2, hexyl), 1.66 – 1.45 (m, 2H, CH_2, hexyl), 1.31 (t, *J*=7.1 Hz, CH_3, Et) ppm.

6-((3,6,8-trisulfopyren-1-yl)amino)hexanoic acid (33)



Compound **32** (0.076 g, 0.21 mmol, 1.0 eq.) was added to flask containing concentrated H_2SO_4 (0.50 mL) and Na_2SO_4 (0.12 g, 0.84 mmol, 4.0 eq.). Oleum (0.60 mL) was added and the mixture was heated to 60°C and stirred in the dark for 24h. The solution was cooled to RT and slowly poured onto ice and neutralized by addition of $Ba(OH)_2$. The resulting slurry was filtered and concentrated *in vacuo*, redissolved in sat. $NaHCO_3$ (aq.) and reconcentrated. The palegreen solid was washed with icecold methanol, the methanol was then added to activated carbon and dried. The carbon was packed in a column and the compound was eluted (H_2O:MeOH:dioxane, 1:0:0 → 0:1:1). The title compound was obtained after lyophilization as a green solid (0.022 g, 0.033 mmol, 16%). ^{1}H NMR (D_2O, 400 MHz) δ = 9.07 (s, 1H), 8.96 (d, 1H, *J*=9.7 Hz), 8.81 (d, 1H, *J*=9.6 Hz), 8.69 (d, 1H, *J*=9.7 Hz), 8.25 (d, 1H, *J*=9.6 Hz), 7.78 (s, 1H), 3.03 (t, 2H, *J*=6.8 Hz, CH_2N), 2.15 (t, 2H, *J*=7.3 Hz, CH_2COOH), 1.57 – 1.46 (m, 4H, CH_2, hexyl), 1.39 – 1.20 (m, 2H, CH_2, hexyl) ppm.

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Samenvatting

1 **Hoofdstuk 1** introduceert de ziekte schistosomiasis; Wat het is, waardoor het wordt veroorzaakt, de huidige diagnostische middelen om de ziekte vast te stellen en welke geneesmiddelen ervoor handen zijn om de ziekte te bestrijden. Daaropvolgend worden alle synthese methoden van schistosoom gerelateerde suikers, die in de literatuur gerapporteerd zijn behandeld.

2 In **Hoofdstuk 2** wordt de synthese beschreven van twee unieke schistosoom suikermotieven, Fuc-α-(1-2)-Fuc-α-(1-3)-GlcNAc en Fuc-α-(1-2)-Fuc-α-(1-3)-GalNAc. Gedurende de synthese van deze twee suikers werd een opmerkelijk verschil waargenomen bij de introductie van de tweede fucosyl groep. DFT berekingen werden uitgevoerd om te achterhalen waar dit verschil vandaan zou kunnen komen. Deze berekingen wezen op sterische hindering bij de Fuc-GlcNAc acceptor, die niet aanwezig was bij de Fuc-GalNAc acceptor. De NMR spectra van de twee gesynthetiseerde trimeren toonden een opvallend verschijnsel dat wees op een stabiele conformatie voor het GalNAc trisaccharide, welke niet werd gevonden voor het GlcNAc trisaccharide. Gedurende de ontscherming van de trimeren bleek de labiele Fuc-α-(1-2)-Fuc glycosidische band gemakkelijk te breken door het zuur, dat vrijkwam bij de reductie van de TCA beschermgoep. Om dit te vermijden werd de hydrogenering uitgevoerd in aanwezigheid van $NaHCO_3$. Vervolgens werden goud nanodeeltjes (5nm) gefunctionaliseerd met de gesynthetiseerde trisachariden en gescreend met ELISA technieken. De goud nanodeeltjes werden gekarakteriseerd met behulp van monoklonale antilichamen en konden gebruikt worden voor het screenen sera om de aanwezigheid van anti-schistosoom antilichamen aan te tonen bij personen lijdend aan schistosomiasis. De verkregen resultaten tonen aan dat deze gefunctionaliseerde goud nanodeeltjes gebruikt zouden kunnen worden voor de diagnose van schistosomiasis.

3 De synthese van α-(1-2)-oligofucosides is beschreven in **Hoofdstuk 3**. Twee syntheseroutes werden gevolgd: Route A, waarbij de verlenging van de fucose keten startte vanaf het reducerende eind en Route B, waarbij de synthese startte vanaf het niet-reducerende eind. Middels Route A werd een dimeer in goede opbrengst en stereoselectiviteit verkregen, maar verlenging tot een tri- and tetrameer resulteerde in lage opbrengsten. Analyse van de NMR spectra wees op sterische hindering van de benzoyl groepen op de C3-O en C4-O van de fucose residuen. Ontscherming van het verkregen di-, tri en tetrameer verliep zonder problemen. Route B bleek een beter alternatief te zijn voor de verlenging van de fucose ketens, zowel het di-, tri- en tetrameer werd verkregen in hoge opbrengst en stereoselectiviteit. De ontscherming door middel van een katalytische hydrogenering verliep met een relatief lage opbrengst. De verkregen fucose ketens werden covalent gekoppeld aan goud nanodeeltjes (5 nm) en gebruikt in ELISA experimenten. Aanvankelijk werden de goud nanodeeltjes gescreend tegen monoklonale antilichamen en vervolgens tegen sera van

geïnfecteerde individuen. In beide gevallen werden de fucose ketens selectief herkend en bleken de langere fucose ketens beter in staat om schistosoom specifieke antilichamen te binden. Op basis van deze data werd geconcludeerd dat deze gefunctionaliseerde goud nanodeeltjes, gebruikt kunnen worden als diagnostisch hulpmiddel voor schistosomiasis.

4 **Hoofdstuk 4** beschrijft de synthese van gefucosyleerde *N*-acetylgalactosamine-β-(1-4)-*N*-acetylglucosamine dimer (LacdiNAc ook wel LDN genoemd) fragmenten. De doelmoleculen van dit hoofdstuk waren drie mono- gefucosyleerde (F-LDN, F-LDN-F en LDN-F) en drie di-gefucosyleerde (F2-LDN, F2-LDN-F2 en LDN-F2) LDN-fragmenten. Het LDN-dimeer werd gesynthetiseerd met passend beschermde glucosamine en galactosamine bouwstenen. Hierbij werden diverse orthogonale beschermgroepen op de C3-O van zowel de glucosamine als de galactosamine bouwsteen getest, waaronder de volgende combinaties; TBS/Nap, Nap/Bz, Nap/Lev en Nap/Ac. Het laatste beschermgroepen paar bleek het meest succesvol. Na verwijdering van de orthogonale beschermgroep(en) en fucosylering werden de (F)-LDN-(F) fragmenten in hoge opbrengsten verkregen. Introductie van de di-fucose ketens resulteerde echter in lage opbrengsten, optimalisatie van deze reactie heeft helaas geen vruchten afgeworpen.

5 Twee nieuwe syntheseroutes (A en B) voor oligomeren van het Circulating Anodic Antigen (CAA) staan beschreven in **Hoofdstuk 5**. Route A is een flexibele route waarbij de dimeer, waaruit CAA is opgebouwd, gesynthetiseerd werd met behulp van D-glucose en D-glucuronzuur donoren en een variatie van D-galactosamine acceptoren. Van de geteste donoren bleek de glucuronzuur donor de beste te zijn en van de geteste acceptoren gaf de galactosamine bouwsteen beschermd met een 4,6-silylideen ketaal de hoogste opbrengst. Ondanks dat het repeterende dimeer in hoge opbrengst verkregen werd, kon het CAA tetrameer helaas niet verkregen worden middels een [2+2] glycosylering. Route B is gebaseerd op de isolatie van de repeterende dimeer van CAA uit chondroïtine A door middel van zuur gekatalyseerde hydrolyse. Bescherming van de verkregen dimeer en een koppeling tussen twee beschermde dimeren resulteerde in een CAA tetrameer.

Curriculum vitae

The author of this thesis was born on the 26th of July 1991 in Leeds, United Kingdom. After finishing his secondary education (VWO) at Het Erasmiaans gymnasium in Rotterdam, he commenced with the bachelor Molecular Science & Technology at Leiden University and Technological University Delft. He received his Bachelor degree in 2012 after successfully completing his internship in the group Metals in Catalysis & Inorganic Materials of Prof. dr. E. Bouwman. The master Chemistry, with the research track Design and Synthesis was commenced in that same year at Leiden University. During the master a research internship was carried out at the Bio-Organic Synthesis group under supervision of Dr. J.D.C. Codée. He commenced his Ph.D in November 2014 under supervision of Dr. J.D.C. Codée and Prof. dr. G.A. van der Marel at the Bio-Organic Synthesis group at Leiden University. Parts of the work described in this thesis were presented as poster presentations at the CHemistry As INnovating Science (CHAINS) congress in Veldhoven (2016-2018) and the 19th European Carbohydrate Symposium in Barcelona, Spain. Oral presentations were given at CHAINS in 2017 and at the 29th International Carbohydrate Symposium (ICS) in Lisbon, Portugal in 2018.

De auteur van dit proefschrift werd geboren op 26 juli 1991 te Leeds, Verenigd Koninkrijk. Na het behalen van zijn VWO diploma aan het Erasmiaans gymnasium te Rotterdam, startte hij de Bachelor Molecular Science & Technology aan de Universiteit Leiden en de Technische Universiteit Delft. Het Bachelor diploma werd verkregen in 2012 na het afronden van zijn onderzoeksstage in de groep Metals in Catalysis & Inorganic Materials van Prof. dr. E. Bouwman. In datzelfde jaar begon hij aan de masteropleiding Chemistry, in de richting van Design and Synthesis aan de Universiteit Leiden. Gedurende de masteropleiding werd een onderzoeksstage gedaan in de Bio-Organische Synthese vakgroep onder begeleiding van Dr. J.D.C. Codée. In 2014 begon hij aan het promotieonderzoek dat is beschreven in dit proefschrift onder begeleiding van Dr. J.D.C. Codée en Prof. dr. G.A. van der Marel in de Bio-Organische Synthese vakgroep. Delen van dit onderzoek zijn gepresenteerd als posterpresentatie op het CHemistry As INnovating Science (CHAINS) congres in Veldhoven (2016-2018) en het 19de European Carbohydrate Symposium in Barcelona, Spanje. Mondeling presentaties zijn gegeven op CHAINS in 2017 en het 29th International Carbohydrate Symposium (ICS) in Lissabon, Portugal in 2018.