Synthesis and application of glycans unique to S. mansoni

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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

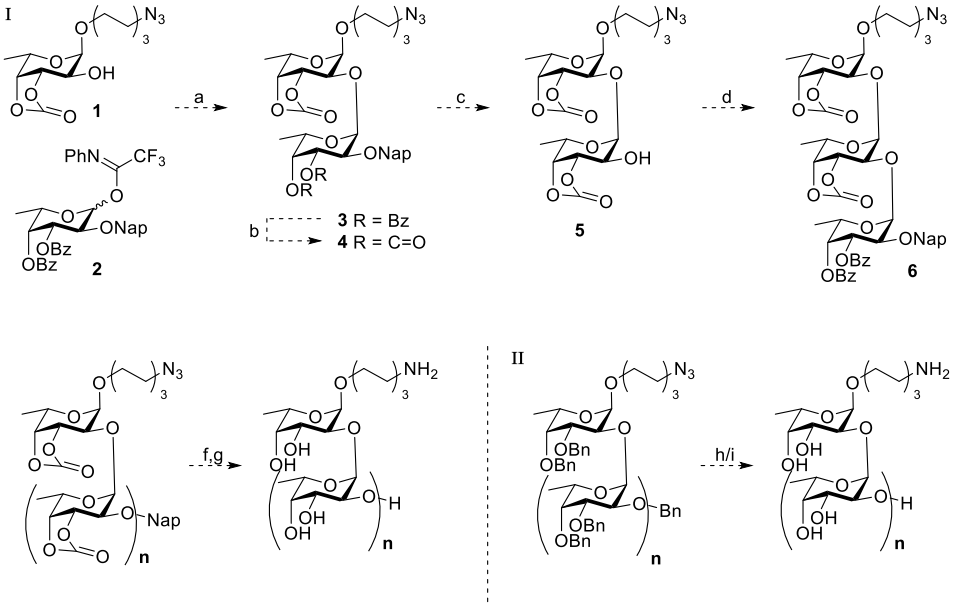
were used to screen sera of people infected with schistosomes. From this data it was concluded that these glycan coated particles, especially the particles coated with the tri- and tetramer can be used as diagnostic tools for schistosomiasis.

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.



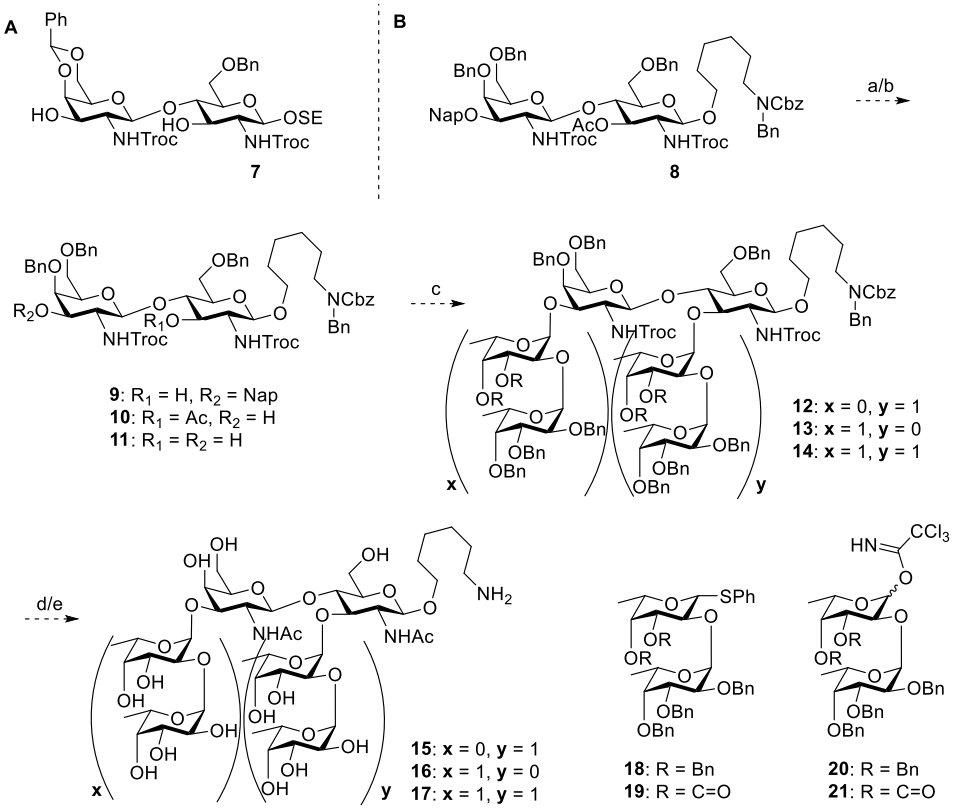
Reagents and conditions: **a**: TMSOTf, MS (3Å), DCM, -30˚C, **b**: i) NaOH, H_2O, THF, 40˚C, ii) triphosgene, pyr. DCM, 0˚C, **c**: DDQ, DCM, MeOH, **d**: **2**, TMSOTf, MS (3Å), DCM, -30˚C, **f**: NaOH, H_2O, THF, 40˚C, **g**: Pd/C, H_2, H_2O, **h**: Na, THF, NH_3, -78˚C, **i**: i) O_3, DCM, ii) NaOMe,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



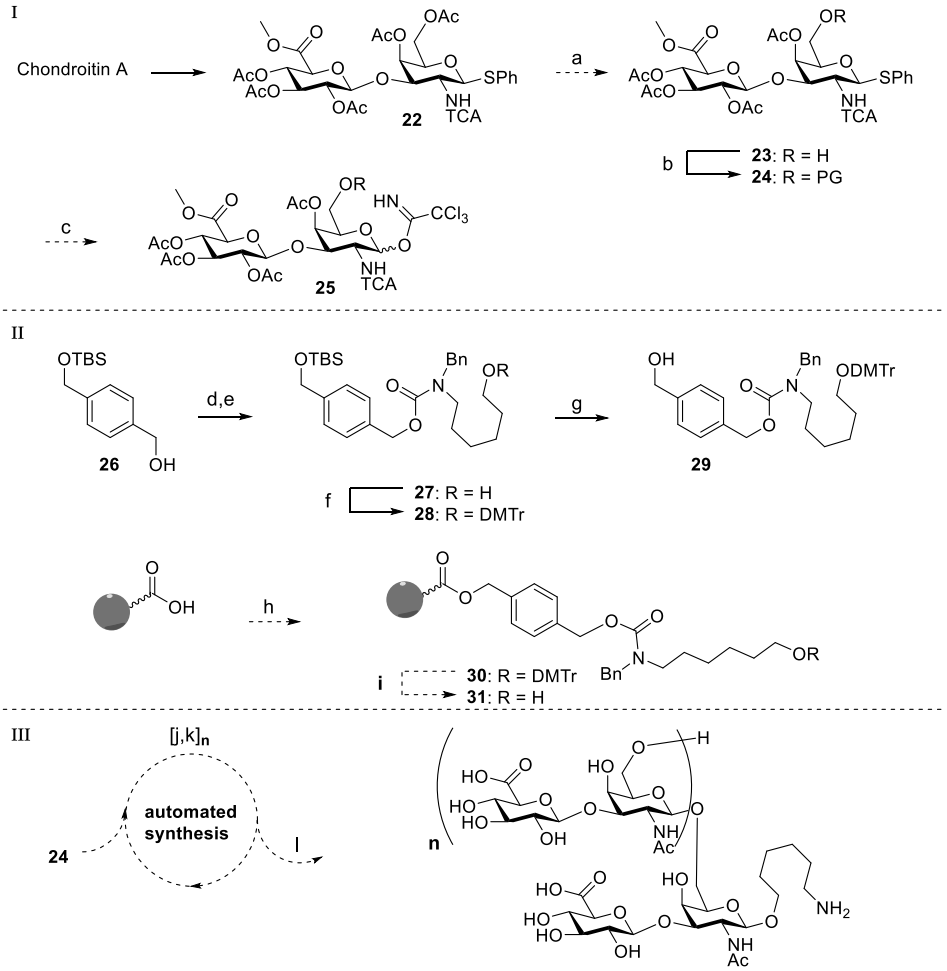
Reagents and conditions: **a**: NaOMe, THF, MeOH, **b**: HCl, HFIP, DCM, **c**: glycosylation conditions, **d**: i) Zn, AcOH, Ac_2O, EtOAc, ii) NaOMe, MeOH, **e**: Na, THF, NH_3.

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.

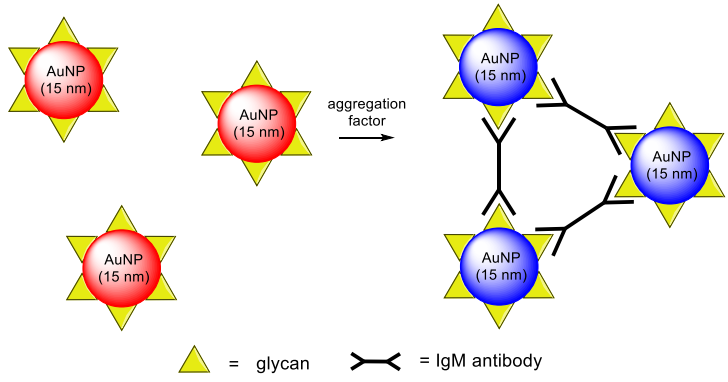
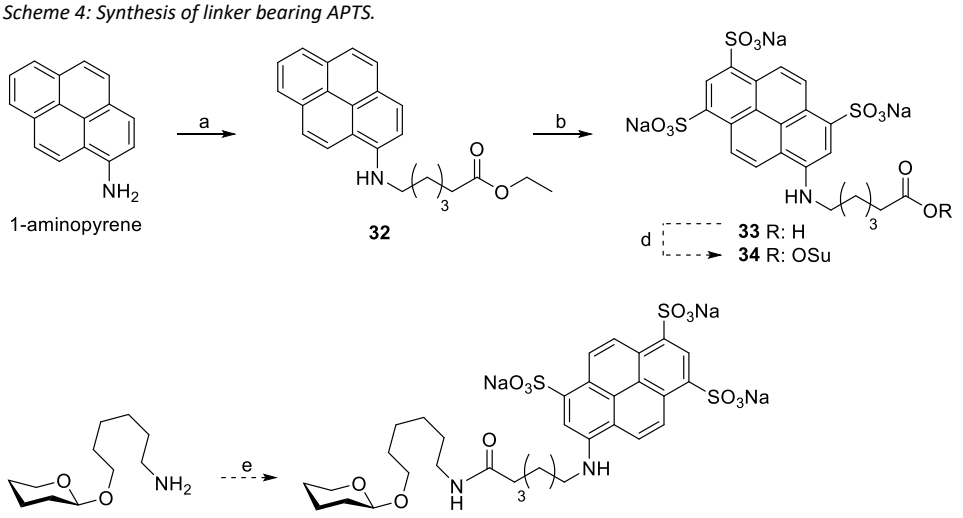


Figure 1: Schematic representation of colour change from red (free AuNP's) to blue (aggregated AuNP's).

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.



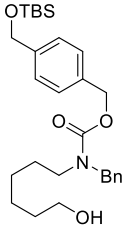
Reagents and conditions: **a**: Ethyl 6-bromohexanoic acid, TBAI, DMF, K_2CO_3, 80°C, 52%, **b**: oleum, H_2SO_4, Na_2SO_4, 60°C, **d**: *N*-hydroxysuccinimide, DCC, DMF, **e**: **34**, $NaHCO_3$, DMSO, H_2O.

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.

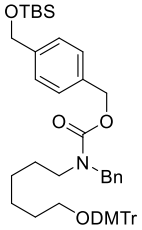
4-(*tert*-butyldimethylsilyl-oxy-methyl-benzyl)-6-(hydroxyhexylaminobenzyl)-carbamate (27)



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,

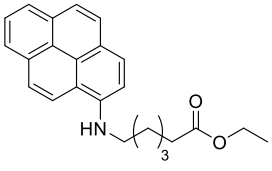
9H, *t*-Bu, TBS), 0.11, (s, 6H, CH_3, TBS) ppm. The formed carbonate (10.1 g, 24.4 mmol, 1.0 eq.) was dissolved in DCM (120 mL, 0.2M), and 6-(benzylamino)-1-hexanol (5.4 g, 26 mmol, 1.02 eq.) and DIPEA (6.8 mL, 39 mmol, 1.5 eq.) were added. The reaction was left to stir overnight at RT. When TLC showed 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.

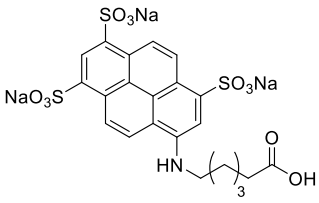
Ethyl 6-(pyren-1-ylamino)hexanoate (32)



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.

References

[1] R. Dettmann and T. Ziegler, "Synthesis of octyl S-glycosides of tri- to pentasaccharide fragments related to the GPI anchor of Trypanosoma brucei," *Carbohydr. Res.*, vol. 346, no. 15, pp. 2348–2361, 2011.

[2] I. Jefferies and B. R. Bowen, "Synthesis of inhibitors of α-1,3-fucosyltransferase," *Bioorganic Med. Chem. Lett.*, vol. 7, no. 9, pp. 1171–1174, 1997.

[3] T. Kanaya, F. Schweizer, T. Takeda, F. Kiuchi, and N. Hada, "Synthetic studies on glycosphingolipids from protostomia phyla: Synthesis of glycosphingolipids and related carbohydrate moieties from the parasite Schistosoma mansoni," *Carbohydr. Res.*, vol. 361, pp. 55–72, 2012.

[4] R. K. H. V, K. Naruchi, R. Miyoshi, and H. Hinou, "A New Approach for the Synthesis of Hyperbranched N - Glycan Core Structures from Locust Bean Gum," *Org. Lett.*, no. 5, pp. 21–24, 2013.

[5] M. Gavel, T. Courant, A. Y. P. Joosten, and T. Lecourt, "Regio- A nd chemoselective deprotection of primary acetates by zirconium hydrides," *Org. Lett.*, vol. 21, no. 7, pp. 1948–1952, 2019.

[6] A. Geert Volbeda, J. Van Mechelen, N. Meeuwenoord, H. S. Overkleeft, G. A. Van Der Marel, and J. D. C. Codée, "Cyanopivaloyl Ester in the Automated Solid-Phase Synthesis of Oligorhamnans," *J. Org. Chem.*, vol. 82, no. 24, pp. 12992–13002, 2017.

[7] M. Guberman, M. Bräutigam, and P. H. Seeberger, "Automated glycan assembly of Lewis type i and II oligosaccharide antigens," *Chem. Sci.*, vol. 10, no. 21, pp. 5634–5640, 2019.

[8] M. T. C. Walvoort *et al.*, "Automated solid-phase synthesis of β-mannuronic acid alginates," *Angew. Chemie - Int. Ed.*, vol. 51, no. 18, pp. 4393–4396, 2012.

[9] S. Eller, M. Collot, J. Yin, H. S. Hahm, and P. H. Seeberger, "Automated solid-phase synthesis of chondroitin sulfate glycosaminoglycans," *Angew. Chemie - Int. Ed.*, vol. 52, no. 22, pp. 5858–5861, 2013.

[10] C. -D Chang and J. Meienhofer, "Solid-Phase Peptide Synthesis Using Mild Base Cleavage of Nαfluorenylmethyloxycarbonylamino Acids, Exemplified By a Synthesis of Dihydrosomatostatin," *Int. J. Pept. Protein Res.*, vol. 11, no. 3, pp. 246–249, 1978.

[11] H. M. Kim, I. J. Kim, and S. J. Danishefsky, "Total syntheses of tumor-related antigens N3: Probing the feasibility limits of the glycal assembly method," *J. Am. Chem. Soc.*, vol. 123, no. 1, pp. 35–48, 2001.

[12] D. A. Argunov, V. B. Krylov, and N. E. Nifantiev, "The Use of Pyranoside-into-Furanoside Rearrangement and Controlled O(5) → O(6) Benzoyl Migration as the Basis of a Synthetic Strategy to Assemble (1→5)- and (1→6)-Linked Galactofuranosyl Chains," *Org. Lett.*, vol. 18, no. 21, pp. 5504–5507, 2016.

[13] P. Czechura, N. Guedes, S. Kopitzki, N. Vazquez, M. Martin-Lomas, and N. C. Reichardt, "A new linker for solid-phase synthesis of heparan sulfate precursors by sequential assembly of monosaccharide building blocks," *Chem. Commun.*, vol. 47, no. 8, pp. 2390–2392, 2011.

[14] M. Marradi, F. Chiodo, I. García, and S. Penadés, "Glyconanoparticles as multifunctional and multimodal carbohydrate systems," *Chem. Soc. Rev.*, vol. 42, no. 11, pp. 4728–4745, 2013.

[15] H. Aldewachi, T. Chalati, M. N. Woodroofe, N. Bricklebank, B. Sharrack, and P. Gardiner, "Gold nanoparticle-based colorimetric biosensors," *Nanoscale*, vol. 10, no. 1, pp. 18–33, 2018.

[16] M. H. Jazayeri, T. Aghaie, A. Avan, A. Vatankhah, and M. R. S. Ghaffari, "Colorimetric detection based on gold nano particles (GNPs): An easy, fast, inexpensive, low-cost and short time method in detection of analytes (protein, DNA, and ion)," *Sens. Bio-Sensing Res.*, vol. 20, no. May, pp. 1–8, 2018.

[17] R. Kottler *et al.*, "Development of a high-throughput glycoanalysis method for the characterization of oligosaccharides in human milk utilizing multiplexed capillary gel electrophoresis with laser-induced fluorescence detection," *Electrophoresis*, vol. 34, no. 16, pp. 2323–2336, 2013.

[18] Z. Sharrett *et al.*, "Exploring the use of APTS as a fluorescent reporter dye for continuous glucose sensing," *Org. Biomol. Chem.*, vol. 7, no. 7, pp. 1461–1470, 2009.

[19] R. Y. Zhao *et al.*, "Synthesis and evaluation of hydrophilic linkers for antibody-maytansinoid conjugates," *J. Med. Chem.*, vol. 54, no. 10, pp. 3606–3623, 2011.

[20] L. Huang and R. J. Kerns, "Diversity-oriented chemical modification of heparin: Identification of charge-reduced N-acyl heparin derivatives having increased selectivity for heparin-binding proteins," *Bioorganic Med. Chem.*, vol. 14, no. 7, pp. 2300–2313, 2006.