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Chapter 8

Summary and future directions

The glycosylation reaction is the central reaction in carbohydrate chemistry and despite the big advancements made in the past few decades, it remains relatively poorly understood, in terms of yield and stereoselectivity. Understanding the glycosylation reaction necessitates investigation of its reactive species. While covalent intermediates, such as anomeric triflates, are readily observable using low-temperature NMR, the nature of the oxocarbenium ion is elusive, owing to its highly ephemeral nature. Chapter 2 provides an overview of research into the nature and reactivity of oxocarbenium ions, from model glycosylation studies to computational methods.
Also provided are literature examples of oligosaccharide syntheses, which (likely) involve oxocarbenium ion intermediates.

Chapter 3 describes a detailed mechanistic study of the reactivity and selectivity of 2-azido-2-deoxyfucosyl (FucN₃) donors in glycosylations. Whereas a lot is known on glycosylations with L-fucosyl donors, the behavior of FucN₃ donors is relatively unexplored. Six donors, protected with benzyl (Bn), benzoyl (Bz) or tert-butyldimethylsilyl groups were synthesized and reacted with a panel of acceptors, varying in nucleophilicity by virtue of electronic and/or steric effects. The results showed that Bn or TBS protecting groups on the FucN₃ donor increased the α-selectivity of glycosylations compared to Bz groups. The nature of the acceptor was shown to be of decisive influence on glycosylation outcome; weakly nucleophilic acceptors, including secondary carbohydrate acceptors, led to α-selective glycosylations, while highly nucleophilic acceptors, such as ethanol, preferentially gave the β-products. Low-temperature NMR studies were carried out on Bn- and Bz-protected FucN₃ donors to identify reactive intermediates. Activation at -80 °C led to formation of two species in each case, which were identified as glycosyl triflates and -oxosulphonium triflates.

Based on the results of the NMR experiments and the model glycosylations, a mechanistic picture was drawn. Reactive acceptors can react with the detected covalent species, which predominantly have an α-anomeric configuration, in a S₉2-like fashion to lead to the β-glycosides. The covalent intermediates are in equilibrium with dissociated intermediates, i.e. oxocarbenium ion-like species. Less reactive acceptors react preferentially with these transient, but more reactive species. The conformation of the oxocarbenium ion and the trajectory of the incoming acceptor determine the stereoselectivity (or lack thereof) of the addition reaction. For the FucN₃ oxocarbenium ion nucleophilic attack of the acceptor on a 4H₃-oxocarbenium ion-like species can account for the formation of the α-linked products. The nature of the protecting groups influences the stability of the reactive intermediates, and thus the outcome of the glycosylations; electron-withdrawing Bz groups destabilize the electron-depleted oxocarbenium ions, shifting the equilibrium between the covalent intermediates and the oxocarbenium ion like species to the side of the former reactive species. This leads to enhanced β-selectivity for reactions involving the benzoylated FucN₃ donors. The higher stability of the covalent triflates and oxosulphonium triflates of the Bz-protected FucN₃ donors compared to their Bn-protected counterparts is reflected by the increased temperature of decomposition of these species.

The equilibrium between the covalent (i.e. anomeric triflates) and ionic (i.e. oxocarbenium ions) species is influenced by the nature of the counterion. To investigate whether non-nucleophilic anions can influence the selectivity of glycosylations of dibenzylated FucN₃ donor 1 with cyclohexanol (CyOH), a set of glycosylations was performed using N-iodosuccinimide (NIS)
in combination with different silver(I) salts (Table 1). While TFOH (entry 1) and AgOTf (entry 2) gave moderately β-selective glycosylations, activation of 1 with the hexafluorophosphate (PF$_6^-$, entry 3) and hexafluoroantimoniate (SbF$_6^-$, entry 4) salts of Ag(I) resulted in highly β-selective glycosylations ($\alpha/\beta$ 1:10). This indicates that under these conditions the generation of oxocarbenium ions is less likely. Possibly, direct substitution of the activated selenodonor (charged with the relatively weak activating systems) can account for the observed selectivity. Conversely, the glycosylation of 1 with 2,2,2-trifluoroethanol (F$_3$EtOH) gave adduct 3 with a strong preference for the $\alpha$-anomer (entries 5-7). These results are in line with the results obtained in Chapter 3 and point to the involvement of an oxocarbenium ion like reactive intermediate as the product forming species.

Table 1: Glycosylations of 1 with NIS/Ag(I) conditions.

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>activator</th>
<th>product</th>
<th>yield ($\alpha/\beta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cy</td>
<td>TFOH</td>
<td>2</td>
<td>82% (1:4)</td>
</tr>
<tr>
<td>2</td>
<td>Cy</td>
<td>AgOTf</td>
<td>2</td>
<td>73% (1:6)</td>
</tr>
<tr>
<td>3</td>
<td>Cy</td>
<td>AgPF$_6^-$</td>
<td>2</td>
<td>73% (1:10)</td>
</tr>
<tr>
<td>4</td>
<td>Cy</td>
<td>AgSbF$_6^-$</td>
<td>2</td>
<td>71% (1:10)</td>
</tr>
<tr>
<td>5</td>
<td>F$_3$Et</td>
<td>TFOH</td>
<td>3</td>
<td>33% (19:1)</td>
</tr>
<tr>
<td>6</td>
<td>F$_3$Et</td>
<td>AgOTf</td>
<td>3</td>
<td>60% (13:1)</td>
</tr>
<tr>
<td>7</td>
<td>F$_3$Et</td>
<td>AgSbF$_6^-$</td>
<td>3</td>
<td>31% (19:1)</td>
</tr>
</tbody>
</table>

In Chapter 4, the synthesis of the *Staphylococcus aureus* type 5 Capsular Polysaccharide repeating unit is described. This complex trisaccharide contains two N-acetyl fucosamine units and an β-N-acetyl mannanuronic acid residue. The repeating unit was built up using FucN$_3$ donors, building upon the reactivity study described in the previous Chapter. The β-glycosidic linkage between the first d-FucN$_3$ and a 5-aminopentyl spacer was installed using a FucN$_3$ donor bearing electron-withdrawing Bz group and the use of Et$_2$O as a solvent. These conditions led to increased β-selectivity, likely due to the decreased polarity of the medium, which disfavors formation of charged intermediates such as oxocarbenium ions. The stereoselectivity of the formation of the
linkage between the two FucN<sub>3</sub> units strongly depended on the protecting groups on the donor, in agreement with the results in Chapter 3. The best result was obtained with a di-TBS protected FucN<sub>3</sub> donor, which gave the disaccharide in 76% yield and with complete α-selectivity. Installation of the final β-mannuronic acid linkage bond was achieved using a large excess of donor (4.0 eq.) and led to the protected trisaccharide in 75% yield and complete β-selectivity. During deprotection of the trisaccharide, an undesired lactamization occurred between the mannuronic acid’s C-5 carboxylate and C-2 amine, during a two-step reduction/acetylation of the azides. This side-reaction could be circumvented by a one-step transformation of the azides into acetamides, using thioacetic acid (AcSH).

The *S. aureus* type 8 Capsular Polysaccharide is structurally very similar to the type 5 Capsular Polysaccharide, also containing two *N*-acetylglucosamine and one *N*-acetylmannosaminuronic acid units.<sup>2,3</sup> Preliminary synthetic studies towards the repeating unit trisaccharide are shown in Scheme 1. The first synthetic challenge was the glycosylation of the d-FucN<sub>3</sub> unit 4 with 5-aminopentanol linker 13, to install the desired α-linkage. The use of additives was explored to modulate the outcome of the glycosylation reaction.<sup>4</sup> Bennett and co-workers reported that the use of excess Bu₄NI in Ph₂SO/Tf₂O-mediated glycosylations dramatically increases the α-selectivity in comparison to unmodified conditions.<sup>5</sup> The addition of excess iodide anion to the reaction mixture leads to establishment of an equilibrium between anomeric α- and a β-iodides, with the latter being displaced in an S₉2-like fashion by the acceptor.<sup>6,7</sup> Applying these conditions to the coupling of 4 and 13 provided the desired α-linked d-FucN<sub>3</sub> unit 5 in high yield with an α/β ratio of 7:1.* Removal of the TBS group allowed separation of the two anomers, yielding acceptor 6 in 63% yield. Synthesis of the disaccharide, using L-4 as the donor and unmodified Ph₂SO/Tf₂O conditions, gave the disaccharide 7 in 73% yield with an α/β ratio of 7:1. Again, removal of the temporary TBS protecting group allowed separation of the anomers, yielding the disaccharide acceptor 8 in 71% yield. The final glycosylation between 8 and known ManN<sub>3</sub>A donor 14 proceeded slowly. This was unexpected because of the perceived higher reactivity of acceptor 8 compared to the acceptor used in the synthesis of the *S. aureus* type 5 CPS trisaccharide. Even when an excess of donor was used, the trisaccharide 9 was obtained in modest yield (but complete β-selectivity). Further studies are needed to elucidate the origin of the poor performance of this donor/acceptor couple.

Initial studies towards the deprotection of 9 were also conducted. Saponification using H₂O₂ and KOH in THF/H₂O proceeded uneventfully to give the free uronic acid 10. Acetylation of the non-reducing end C-4-OH using Ac₂O/pyridine did not lead to desired product 11. Instead, α,β-unsaturated carboxylic acid 12 was isolated as the main product. This byproduct likely arises

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*The addition of *N*-methylmaleimide was necessary to scavenge nucleophilic byproducts. See ref. (5).
from the transient formation of a mixed anhydride at the uronic acid functionality, which renders the proton at C-5 sufficiently acidic for a β-elimination to occur. Other acetylation methods using metal triflate catalysts, such as Sc(O Tf)₃ or Bi(O Tf)₃, were unsuccessful. To circumvent this elimination reaction an alternative protecting group for the mannuronic carboxylic acid should be used, that does not require the use of basic deprotection conditions. The use of an allyl or tert-butyl ester could be explored. Another way to circumvent this problem would be to use a non-oxidized mannosazide donor, although this represents a significant revision of the synthetic route.

**Scheme 1:** Synthesis of the protected S. aureus type 8 CPS trisaccharide.

Reagents and conditions: a) Ph₅SO, TTBP, N-methylmaleimide, 3 Å MS, CH₂Cl₂; Ti(IV) -80 → -70 °C; Bu₄NI, -80 °C; 13, 1,4-dioxane, -80 °C → rt (85%, α/β 7:1); b) Bu₄NF, THF (63% for 6; 71% for 8); c) Ph₅SO, TTBP, 3 Å MS, CH₂Cl₂; Ti(IV) -80 °C → -70 °C; 1-4, -80 → -50 °C (73%, α/β 7:1); d) 14 TBSOTf, 3 Å MS, CH₂Cl₂, -80 → -55 °C (34%); e) H₂O₂, KOH, THF, H₂O (74%); f) Ac₂O, pyridine.

**Chapter 5** describes a study into the reactivity and selectivity of 2-azidogalacturonic acid (GalNAc) donors. The α-selective glycosylation of galacturonic acid (GalA) donors can be effected by use of galacturonic acid-3,6-lactone donors. This lactone has been successfully used in the synthesis of oligosaccharides related to *Streptococcus pneumoniae*. Further studies have shown
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that the high $\alpha$-selectivity of the GalA lactone arises from a covalent, axial triflate, which is displaced in a $S_N2$-like fashion to give the $\alpha$-product. In order to allow $\alpha$-glycosylation of the analogous 2-azidogalacturonic acid (GalN$_3$A) donors, the corresponding lactone has been synthesized. This donor was generated after regioselective protection of the C-4-OH, which was achieved by a reductive opening of a suitably protected 4,6-\textit{O}-\textit{para}-methoxybenzylidene masked selenophenyl galactosazide synthon. An ensuing tandem oxidation/cyclization provided the lactone donor. Its performance as a glycosylating agent was assessed by comparing it with a conformationally non-restricted GalN$_3$A donor, using a range of acceptors, similar to that in Chapter 3. The ‘normal’ GalN$_3$A donor gave highly $\beta$-selective condensation reactions with most acceptors. The corresponding lactone was moderately $\alpha$-selective, but the selectivity strongly depended on the reactivity of the acceptor, with more reactive acceptors providing better $\alpha$-selectivity. The moderate selectivity obtained with carbohydrate acceptors unfortunately limits its use in oligosaccharide synthesis. The GalA lactone was also assessed with the set of acceptors. In accordance with previous reports, this lactone donor provided highly $\alpha$-selective glycosylation reactions. Activation of the GalN$_3$A lactone donor led to the formation of an axial triflate as indicated by low temperature NMR experiments. The triflate derived from the GalN$_3$A lactone donor proved to be less stable than the anomeric triflate formed from the GalA lactone, which provides an explanation for the diminished stereoselectivity observed with the GalN$_3$A lactone donors.

The choice of solvent can be critical in a glycosylation reaction. Some solvents, such as nitrile or ether solvents, have well-known ‘participating’ effects which affect the stereoselective outcome of glycosylations. A solvent’s polarity can also influence the reaction outcome - more polar solvents can better accommodate charge separation, and thus an $S_N1$-like reaction, than nonpolar solvents. In order to promote an $S_N2$-like pathway in glycosylations with GalN$_3$A lactones, the use of nonpolar solvents, such as toluene and 1,1,2-trichloroethylene, should be investigated. The latter was shown by Woerpel and co-workers to be superior in promoting $S_N2$-like reactions.

In Chapter 6, the synthesis of the \textit{Staphylococcus aureus} Strain M Capsular Polysaccharide repeating unit is described. This trisaccharide consists of two $\alpha$-linked $N$-acetyl galactosaminuronic acid (GalNAcA) and an $\alpha$-linked $N$-acyethylfucosamine unit. The $\alpha$-GalNAcA glycosidic linkages were installed using a non-oxidized 4,6-\textit{O}-\textit{di-tert}-butylsilylene (DTBS)-protected 2-azidogalactosyl donor (GalN$_3$). This donor was shown to be completely $\alpha$-selective using a variety of acceptors. Post-glycosylation removal of the DTBS group, followed by selective oxidation of the primary position to the corresponding uronic acids gave the GalNAcA mono- and disaccharides. Installation of the final $\alpha$-FucN$_3$ linkage, using an $\alpha$-directing protecting group pattern (Chapter 3), gave the protected trisaccharide in good yield. The orthogonal TBS group on the FucN$_3$ 3-\textit{O} position makes this approach
amenable to higher oligosaccharides. Stepwise deprotection gave the target trisaccharide repeating unit.

The proposed synthesis of a taurine-bearing hexasaccharide is shown in Scheme 2A, as Hash and co-workers have determined that the Strain M CPS bears a taurine residue for every 4th GalNAcA. Elongation of trisaccharide 15 by glycosylation with donor 16, followed by desilylation, oxidation and methylation leads to tetrasaccharide 17. Elongation to the pentamer can be achieved following another glycosylation-deprotection-oxidation sequence to give uronic acid 18. The installation of the taurine residue, which is likely affixed to a GalNAcA residue by an amide bond, can be accomplished with protected taurine 19, bearing a trichloroethyl protecting group on the sulfonate moiety, using carbodiimide coupling chemistry. Installation of the second FucN₃ unit using donor 4, followed by deprotection then yields the hexasaccharide 22. A more convergent approach to the synthesize higher oligosaccharides may involve block glycosylations involving di- or trisaccharide donors. To allow for the installation of the required glycosidic linkages the donor building blocks cannot be equipped with a GalNAcA donor moiety, and therefore the use of GalN₃A-FucN₃ disaccharide 23 in combination with the highly α-selective GalN₃ building block 16, or the use of the GalN₃A-GalN₃A-FucN₃ trisaccharide 24 is projected.

Chapter 7 describes the synthesis of glycan fragments related to the parasite Schistosoma mansoni, the causative pathogen of schistosomiasis. This parasite expresses a complex array of glycans, and antibodies raised against S. mansoni are glycan-specific. The general structure of these glycans possess a backbone consisting of N-acetylgalactosamine (GlcNAc) and N-acetylgalactosamine (GalNAc) units, decorated with fucosyl chains. The fucose units are interconnected by α-(1,2)-glycosidic linkages, and the fucosyl chains are thought to be a major epitope in anti-glycan antibodies against S. mansoni. In order to explore the chemistry to synthesize a library of S. mansoni glycan fragments, spacer-equipped Fuc-GlcNAc and Fuc-GalNAc disaccharides were synthesized. The GlcNAc and GalNAc acceptors were synthesized by glycosylations of the corresponding C-3-O unprotected donors with a spacer alcohol. The disaccharides were synthesized with a 2-O-naphthylmethyl-protected fucosyl donor, using NIS/TMSOTf-mediated glycosylations. A two-step deprotection, consisting of a saponification, followed by hydrogenation, delivered the conjugation-ready disaccharides. Additionally, the two corresponding protected difucosyl trisaccharides were synthesized, albeit in modest yield. Applying the same deprotection sequence of the Fuc-Fuc-GlcNAc trisaccharide was unsuccessful, implying that a revision of the protecting group strategy may be necessary.
Scheme 2: A) Proposed synthesis of a S. aureus Strain M CPS hexasaccharide bearing a taurine unit. B) Possible disaccharide (23) and trisaccharide (24) building blocks for synthesis of S. aureus Strain M oligosaccharides.
The synthesized described in Chapter 7 can be elaborated upon in order to synthesize a large library of \textit{S. mansoni} glycan fragments (Figure 1A). The length and composition of the GlcNAc/GalNAc backbone can be varied, as well as the number and length of fucosyl branches. The library can be assembled with a limited set of appropriately protected monosaccharide building blocks (Figure 1B). The sites of elongation on the GlcNAc and GalNAc monosaccharides are protected with temporary protecting groups: Fmoc groups in 25, 26, 28 and 30 for the backbone sequence, and Lev groups on 26, 29 and 30 on the branching sites. The fucosyl donor can be modified, so that the sterically bulky pivaloyl esters on the C-3-O- and C-4-O-positions are replaced by benzyl ethers, giving donor 31. Due to the decreased size and electron-depleted nature of the donor, the efficiency of constructing the fucosyl chains may be enhanced. It has been shown that fucosyl donors, bearing only benzyl protecting groups are strongly $\alpha$-selective.\textsuperscript{19,20} This was corroborated by the results obtained in Chapter 3, in which benzyl-protected 2-azidofucosyl donors were more $\alpha$-selective than their benzoyl-protected analogs. Additionally, the presence of the benzyl ethers eliminates the need for a saponification step, meaning that the oligosaccharides can be deprotected in one step by catalytic hydrogenation.
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**Experimental**

**General procedures**

All reactions were carried out in oven-dried glassware (85 °C). Prior to reactions, traces of water and solvent were removed by co-evaporation with toluene where appropriate. Reactions sensitive to air or moisture were carried out under an atmosphere of argon (balloon). Solvents for reactions were of reagent grade and stored over 4Å molecular sieves (3Å for CH₂Cl₂, MeOH and MeCN), except pyridine and DMF. NEt₃ was stored over KOH pellets. Tf₂O used in glycosylations was dried over P₂O₅ (~3 hours), followed by distillation, and stored in a Schlenk flask at -20 °C. All other chemicals were used as received. Reaction progress was monitored using aluminium-supported silica gel TLC plates (Merck, Kieselgel 60, with fluorescent indicator); visualization was carried out by irradiation with UV light (λ: 254 nm), followed by spraying with 20% H₂SO₄ in EtOH (w/v) or Hanessian’s stain ((NH₄)₆Mo₇O₂₄.4H₂O, 25 g/L; (NH₄)₂Ce(SO₄)₃.2H₂O, 10 g/L; in 10%aq. H₂SO₄). Column chromatography was carried out using silica gel (Screening Devices, 0.040-0.063 mm). Size-exclusion chromatography was carried out using Sephadex LH-20 (GE Healthcare). NMR spectra were recorded on Bruker AV-400, DMX-400 or AV-500 instruments. Chemical shifts (δ) are reported in ppm relative to Me₄Si (δ: 0.00 ppm) or residual solvent signals. NMR spectra were recorded at ambient temperature, and samples were prepared in CDCl₃ unless noted otherwise. ¹³C-APT spectra are ¹H decoupled. Structural assignment was achieved using HH-COSY and HSQC 2D experiments. Coupling constants of anomeric carbon atoms (J_H,H_C) were determined using HMBC-GATED experiments. Infrared spectra were recorded with a Shimadzu FTIR 8300 instrument. Wavenumbers (v) are reported in cm⁻¹. LC-MS analyses were performed on a Thermo Finnigan Surveyor HPLC system equipped with a Gemini C18 column (250 x 10 mm), connected to a Thermo Finnigan LCQ Advantage Max Ion-trap mass spectrometer with (ESI⁺). Eluents used were MeCN, H₂O with addition of TFA (0.1%). HRMS spectra were recorded on a Thermo Finnigan LCQ Orbitrap instrument.

**General procedure for the glycosylation of 1 with CyOH or F₂EtOH using NIS/Ag(I) salts**

To a solution of donor 1 (51 mg, 0.10 mmol, 1.0 eq.) in CH₂Cl₂ (5 mL, 0.02 M) were added a solution of CyOH or 2,2,2-trifluoroethanol (0.3 mL of a 0.5 M solution in CH₂Cl₂, 1.5 eq.) and flame-dried 3Å molecular sieves. After ~30 minutes, the reaction mixture was cooled to -50 °C, and under a stream of N₂ gas, were added NIS (38 mg, 0.15 mmol, 1.5 eq.) and the activator (0.15 mmol, 1.5 eq.) and the maroon reaction mixture was stirred at -50 °C until TLC analysis (PE/EtOAc, 9:1 v/v) indicated complete conversion of the starting material (~20 minutes). The reaction was quenched by addition of NEt₃ (0.1 mL), filtered over a pad of celite, washed with Na₂S₂O₃ (sat.,aq.) and brine,
dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography delivered the corresponding O-glycoside(s).

5-(benzyl(benzzyloxycarbonyl)amino)pentyl 2-azido-4-O-benzyl-2-deoxy-a-D-fucopyranoside (6)

To a stirred solution of donor 4 (0.80 g, 1.5 mmol, 1.0 eq.), Ph₂SO (0.39 g, 1.95 mmol, 1.3 eq.), N-methylmaleimide (0.25 g, 2.25 mmol, 1.5 eq.) and TTBP (0.93 g, 3.75 mmol, 2.5 eq.) in CH₂Cl₂ (30 mL, 0.05 M) were added, flame-dried, rod-shaped 3Å MS. After cooling to -80 °C, Tf₂O (0.33 mL, 1.95 mmol, 1.3 eq.) was added and the mixture was warmed to -70 °C, after which TLC analysis (toluene/EtOAc, 9:1 v/v) indicated complete activation of the donor. The mixture was re-cooled to -80 °C, after which Bu₄NI (as a 1M solution in CH₂Cl₂, 7.5 mL, 7.5 mmol, 5.0 eq.) was added, upon which the reaction mixture assumed a maroon color. After 5 minutes at -80 °C, a solution of acceptor 13 (as a 0.5 M solution in CH₂Cl₂/1,4-dioxane (1:1 v/v), 3.0 mmol, 2.0 eq.) and the mixture was allowed to warm to room temperature. After stirring for ~18 hours, TLC analysis (toluene/EtOAc, 9:1 v/v) indicated complete conversion of the starting material. The reaction was quenched by addition of NEt₃, diluted with CH₂Cl₂, filtered over a pad of celite, washed (brine, 1x), dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (toluene/Et₂O, 1:0 → 9:1) delivered the products (α/β ~7:1) as an inseparable mixture in 85% yield (0.90 g, 1.27 mmol). To a stirred solution of 5 (0.90 g, 1.27 mmol, 1.0 eq.) in THF (4 mL, 0.3 M) was added Bu₄NF (as a 1M solution in THF, 2.5 mL, 2.5 mmol, 2.0 eq.). After TLC analysis (PE/EtOAc, 7:3 v/v) indicated complete consumption of the starting material (~2 hours) the reaction was quenched by addition of NaHCO₃ (sat., aq.), and extracted with EtOAc (3x). The combined organic phases were washed (H₂O 1x, brine 1x), dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 19:1 → 4:1 v/v) delivered the title compound as an oil in 63% yield (0.47 g, 0.80 mmol). ¹H NMR (500 MHz, 323 K) δ: 7.36-7.20 (m, 15H, CH₃arom); 5.17 (s, 2H, PhCH₂); 4.82 (d, 1H, J = 3.0 Hz, H-1); 4.77 (d, 1H, J = 11.5 Hz, PhCH⁻H); 4.70 (d, 1H, J = 11.5 Hz, PhCH⁻H); 4.48 (s, 2H, PhCH₂); 4.03 (bd, 1H, J = 8.0 Hz, H-3); 3.92 (q, 1H, J = 6.0 Hz, H-5); 3.62-3.56 (m, 2H, H-4, OCH₂penty); 3.15-3.39 (m, H-2, OCH₂penty); 3.24 (bs, NC₃H₂penty); 1.54 (bs, 4H, CH₂penty); 1.31 (bs, 2H, CH₂penty); 1.24 (d, 3H, J = 6.5 Hz, H-6). ¹³C-APT NMR (125 MHz, 323 K) δ: 138.0, 136.9 (C₃arom); 128.6, 128.5, 128.4, 128.0, 127.9, 127.8, 127.5, 127.2, 126.9 (CH₃arom); 98.2 (C-1); 80.3 (C-4); 76.1 (PhCH₂); 68.5 (C-3); 68.1 (OCH₂penty); 67.2 (PhCH₂); 66.5 (C-5); 61.0 (C-2); 50.5 (NCH₂penty); 29.1, 23.4 (CH₂penty); 16.7 (C-
6). $^{13}$C-GATED NMR (125 MHz, 323 K) δ: 98.2 (d, J = 170 Hz, C-1). IR (thin film) ν: 2934, 2108, 1690, 1454, 1421, 1229, 1171, 1067. HRMS: [M+H]$^+$ calculated for C$_{33}$H$_{41}$N$_4$O$_6$: 589.30206; found 589.30218.

5-(benzyl(benzyloxy carbonyl) amino)pentyl 2-azido-3-O-(2-azido-4-O-benzyl-2-deoxy-α-L-fucopyranosyl)-4-O-benzyl-2-deoxy-α-D-fucopyranoside (8)  

![Diagram of 5-(benzyl(benzyloxy carbonyl) amino)pentyl 2-azido-3-O-(2-azido-4-O-benzyl-2-deoxy-α-L-fucopyranosyl)-4-O-benzyl-2-deoxy-α-D-fucopyranoside (8)](image)

To a stirred solution of donor L-4 (0.43 g, 0.80 mmol, 2.0 eq.), Ph$_2$SO (0.16 g, 0.80 mmol, 2.0 eq.) and TTBP (0.40 g, 1.60 mmol, 4.0 eq.) in CH$_2$Cl$_2$ (8 mL, 0.1 M relative to donor) were added flame-dried, rod-shaped 3Å MS. After stirring for ~30 minutes, the mixture was cooled to -80 °C and Tf$_2$O (0.13 mL, 0.80 mmol, 2.0 eq.) was added. After allowing the mixture to warm to -70 °C, the mixture was re-cooled to -80 °C and a solution of acceptor 6 (0.40 mmol, 1.0 eq., in 0.8 mL CH$_2$Cl$_2$, dried by triple co-evaporation with toluene) was added via the wall of the flask. The mixture was warmed to -60 °C, left at this temperature for 15 minutes, and the reaction was quenched by addition of NEt$_3$. The bright yellow solution was filtered over celite, washed (brine, 1x), dried over MgSO$_4$, filtered and concentrated in vacuo. Purification by size-exclusion chromatography (CH$_2$Cl$_2$/MeOH, 1:1 v/v) yielded a mixture of disaccharides 7 (α/β ~ 7:1) in 73% yield (0.27 g, 0.28 mmol). The disaccharides 7 was dissolved in dry THF (1.4 mL, 0.2 M), and Bu$_4$NF (as 1 M solution in THF, 0.34 mL, 0.34 mmol, 1.2 eq.) was added. After 4 hours, TLC analysis (PE/EtOAc, 7:3 v/v) indicated complete consumption of the starting material and the appearance of two more polar products. The reaction was quenched by addition of sat. aq. NaHCO$_3$, the mixture was extracted (CH$_2$Cl$_2$, 3x), the combined organics were washed (H$_2$O, 1x; brine, 1x), dried over MgSO$_4$ and concentrated in vacuo. Purification by column chromatography (hexane/EtOAc, 19:1 → 4:1 v/v) furnished the title disaccharide in 71% yield (0.17 g, 0.20 mmol). 1H NMR (500 MHz, 323 K) δ: 7.36-7.19 (m, 20H, $CH_{arom}$); 5.20 (d, 1H, J = 4.0 Hz, H-1); 5.17 (bs, 2H, PhCH$_2$); 4.89 (d, 1H, J = 3.0 Hz, H-1); 4.79 (d, 1H, J = 11.5 Hz, PhCH$_2$); 4.75 (d, 1H, J = 11.5 Hz, PhCH$_2$); 4.72-4.67 (m, 2H, 2x PhCH$_2$); 4.49 (bs, 2H, PhCH$_2$); 4.04 (dd, 1H, J = 3.0 Hz, 10.8 Hz, H-3); 3.93-3.90 (m, 3H, H-3', H-5, H-5'); 3.84 (dd, 1H, J = 3.5 Hz, 10.5 Hz, H-2); 3.59 (bs, 1H, OC$_3$H$_7$pentyl); 3.55 (d, 1H, J = 2.5 Hz, H-4); 3.52 (d, 1H, J = 2.5 Hz, H-4'); 3.49 5(dd, 1H, J = 3.5 Hz, 10.8 Hz, H-2'); 3.41 (bs, 1H, OCH$_3$pentyl); 3.24 (bs, 2H, NCH$_2$pentyl); 1.55 (bs, 4H, 2x CH$_2$pentyl); 1.31 (bs, 2H, CH$_2$pentyl); 1.23-1.20 (m, 6H, H-6, H-6'). $^{13}$C-APT NMR (125 MHz, 323 K) δ: 138.4, 138.0, 137.9, 136.9 (C$_{arom}$); 128.6, 128.5, 128.4, 128.4, 128.1, 128.1, 127.9, 127.8, 127.7, 127.6, 127.3 (CH$_{arom}$); 99.8 (C-1'); 98.2 (C-1); 80.4 (C-4); 79.8 (C-4').
Summary and future directions

76.2 (C-3); 76.0, 75.5 (PhCH₂); 68.7 (C-3’, C-5 or C-5’); 68.2 (OCH₂₂ᵖᵉｎᵗʸl); 67.4 (C-3’, C-5 or C-5’); 67.2 (PhCH₂); 67.0 (C-3’, C-5 or C-5’); 61.0 (C-2’); 60.4 (C-2’); 29.1 (2x CH₂₂ᵖᵉⁿᵗʸl); 23.4 (CH₂₂ᵖᵉⁿᵗʸl); 16.8, 16.8 (C-6, C-6’). ¹³C-GATED NMR (125 Hz) δ: 99.8 (d, J = 170 Hz, C-1’); 98.2 (d, J = 168 Hz, C-1). IR (thin film) ν: 2936, 2106, 1694, 1454, 1422, 1092, 1036, 1028. HRMS: [M+NH₄]⁺ calculated for C₄₆H₅₀N₂O₉: 867.43995; found 867.44027.

5-(benzyl(benzyloxycarbonylamino)pentyl 2-azido-3-O-(2-azido-4-O-benzyl-2-deoxy-3-O-(methyl 4-O-acetyl-2-azido-3-O-benzyl-2-deoxy-β-D-mannopyranosiduronate)-α-L-fucopyranosyl)-4-O-benzyl-2-deoxy-α-D-fucopyranoside (9)

To a solution of acceptor 8 (0.144 g, 0.17 mmol, 1.0 eq.), donor 14 (0.364 g, 0.68 mmol, 4.0 eq.) in CH₂Cl₂ (1.7 mL, 0.1 M) were added flame-dried, rod-shaped 3Å molecular sieves. After ~30 minutes, the mixture was cooled to -80 °C and TBSOTf (32 μL, 0.14 mmol, 0.8 eq.) was added. The reaction mixture was allowed to warm to -55 °C and stirred at this temperature for 6 hours. The reaction was quenched by addition of NEt₃ (0.1 mL), filtered over a bed of celite, washed (brine, 1x), dried over MgSO₄, filtered and concentrated in vacuo. Purification by size-exclusion chromatography (CH₂Cl₂/Methanol, 1:1 v/v) and column chromatography (toluene/EtOAc, 1:0 → 9:1 v/v) delivered the title trisaccharide in 34% yield (70 mg, 0.058 mmol). ¹H NMR (500 MHz, 323 K) δ: 7.37-7.21 (m, 25H, CH₉arom); 5.39 (t, 1H, J = 9.0 Hz, H₄’); 5.32 (d, 1H, J = 3.5 Hz, H-1’); 5.16 (bs, 2H, PhCH₂); 4.88 (d, 1H, 2.5 Hz, H-1); 4.82 (d, 1H, J = 11.5 Hz, PhCHH); 4.70-4.60 (m, 5H, PhCH₂); 4.58 (s, 1H, H-1’’); 4.84 (bs, 2H, PhCH₂); 4.20 (dd, 1H, J = 2.5 Hz, 10.5 Hz, H-3’); 4.04 (dd, 1H, J = 2.0 Hz, 10.5 Hz, H-3); 3.92-3.89 (m, 2H, H-5, H-5’); 3.85-3.78 (m, 3H, H-2, H-2’, H-5’’); 3.62 (s, 3H, OCH₃); 3.61-3.54 (m, 5H, H-2’, H-3’); 3.41 (bs, 1H, OCH₃); 3.41 (bs, 1H, OCH₃); 3.23 (bs, 2H, NCH₂₃ːpᵉⁿᵗʸl); 2.00 (s, 3H, CH₃₉ac); 1.55-1.27 (m, 6H, CH₂₂pᵉⁿᵗʸl); 1.23-1.16 (m, 6H, H-6, H-6’). ¹³C-APT NMR (125 MHz, 323 K) δ: 169.1, 167.1 (C-6’, CO₉ac); 138.6, 138.1, 138.0, 137.3, 136.9 (C₉arom); 128.6, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 127.9, 127.8, 127.8, 127.6, 127.6, 127.2 (CH₉arom); 99.7 (C-1’); 98.1 (C-1’’); 97.5 (C-1’’’); 80.0 (C-3’’’); 77.5, 77.2 (C-4, C-4’); 76.7 (C-3); 75.5 (C-3’’); 75.4, 75.2 (PhCH₂); 73.8 (C-5’’’); 72.4 (PhCH₂); 68.2 (C-4’’); 68.2 (OCH₂₂pᵉⁿᵗʸl); 67.5 (C-5 or C-5’); 67.1 (PhCH₂); 66.9 (C-5 or C-5’); 61.5 (C-2’’); 60.2, 58.9 (C-2, C-2’); 52.5 (OCH₃); 50.5 (PhCH₂); 29.6, 29.1, 23.4 (CH₂₂pᵉⁿᵗʸl); 20.6 (CH₃₉ac); 16.8, 16.6 (C-6, C-6’). IR (thin film) ν: 2924,
To a solution of 9 (40 mg, 0.033 mmol, 1.0 eq.) in THF (1.4 mL, 0.02 M) was added a freshly prepared solution of KOOH (0.3 mL, prepared by adding H₂O₂ (0.56 mL, 30%aq. w/w) to 4.4 mL 0.5 Maq. KOH solution) and the mixture was stirred overnight at room temperature, after which TLC analysis (toluene/EtOAc/AcOH, 10:10:1 v/v/v) indicated complete conversion of the starting material. The reaction mixture was acidified to pH 3 with HCl (1M, aq.) and subsequently extracted with CH₂Cl₂ (5x). The combined organic layers were washed with brine, dried over MgSO₄ and concentrated in vacuo. Purification by column chromatography (toluene/EtOAc/AcOH, 16:4:1 → 10:10:1 v/v/v) furnished the title compound in 74% yield (29 mg, 0.025 mmol). ¹H NMR (500 MHz, 323 K, CD₃CN + acetic acid-d₄) δ: 7.39-7.23 (m, 25H, CH₉芳); 5.17 (d, 1H, J = 4.0 Hz, H-1’); 5.12 (s, 2H, PhCH₂); 4.90-4.84 (m, 3H, H-1, H-1”, PhCH₃); 4.75 (d, 1H, J = 11.5 Hz, PhCH₃); 4.69 (s, 2H, PhCH₂); 4.60 (d, 1H, J = 11.5 Hz, PhCHH); 4.47 (s, 2H, PhCH₂); 4.23 (dd, 1H, J = 3.0 Hz, 11.0 Hz, H-3’); 4.02-3.93 (m, 3H, H-3, H-5, H-5’); 3.90 (d, 1H, J = 3.0 Hz, H-2’); 3.85 (t, 1H, J = 9.5 Hz, H-4’); 3.79 (d, J = 2.0 Hz, H-4’); 3.76-3.73 (m, 2H, H-2, H-5’); 3.66 (d, 1H, J = 2.0 Hz, H-4); 3.60-3.56 (m, 3H, H-2’, H-3”, OC₃H₃penny); 3.39 (bs, 1H, OC₃H₃penny); 3.24 (t, 2H, J = 7.5 Hz, NCH₂penny); 1.53-1.50 (m, 4H, CH₂penny); 1.31-1.27 (m, 2H, CH₂penny); 1.19-1.16 (m, 6H, H-6, H-6’). ¹³C-APT NMR (125 MHz, 323 K, CD₃CN + acetic acid-d₄) δ: 174.4 (C-6’’); 140.1, 139.7, 139.5, 139.2 (C₉芳); 129.6, 129.6, 129.5, 129.3, 129.3, 129.1, 129.0, 128.9, 128.9, 128.8, 128.3 (CH₉芳); 101.0 (C-1’); 99.0, 98.2 (C-1, C-1’’); 81.2 (C-3’’); 80.9 (C-4’); 78.5 (C-4’’); 76.9 (C-3); 76.8 (PhCH₂); 76.6 (C-5’’); 76.5 (PhCH₂); 76.1 (C-3’); 73.3 (PhCH₂); 68.9 (C-4’’); 68.9 (PhCH₂); 68.4, 68.0 (C-5, C-5’); 68.0 (OC₃H₃penny); 63.0 (C-2’’); 61.8 (C-2); 59.6 (C-2’); 30.0, 24.3 (CH₂penny); 17.2, 17.1 (C-6, C-6’).
α,β-unsaturated uronic acid 12

To a solution of 10 (29 mg, 0.025 mmol, 1.0 eq.) in pyridine (1 mL, 0.025 M) was added Ac₂O (0.2 mL, 2.1 mmol, 84 eq.) and the mixture was stirred until TLC analysis (toluene/EtOAc/AcOH, 12:8:1 v/v/v) indicated complete conversion of the starting material (several days). The reaction was quenched by slow addition of H₂O (~0.5 mL) and after ~1 hour, the reaction mixture was extracted with CH₂Cl₂ (6x). The combined organic phases were washed with brine, dried over MgSO₄ and concentrated in vacuo to furnish 13 as the main product. ¹H NMR (500 MHz, 323 K, CD₃CN + acetic acid-d₄) δ: 7.38-7.23 (m, 25H, CH₃om); 6.02 (d, 1H, J = 2.5 Hz, H-4’); 5.19 (d, 1H, J = 3.5 Hz, H-1’); 5.11 (s, 2H, PhCH₂); 4.89-4.82 (m, 3H, H-1”, PhCH₂H); 4.65 (s, 2H, PhCH₂); 4.63-4.60 (m, 2H, PhCH₂); 4.52 (dd, 1H, J = 3.0 Hz, 5.0 Hz, H-3”); 4.47 (s, 3H, PhCH₂); 4.28 (dd, 1H, J = 3.0 Hz, 11.0 Hz, H-3’); 4.00-3.93 (m, 3H, H-3, H-5, H-5’); 3.80 (d, 1H, J = 5.0 Hz, H-2”); 3.81 (d, 1H, J = 2.5 Hz, H-4’); 3.75-3.70 (m, 2H, H-2, H-2’); 3.66 (d, 1H, J = 2.5 Hz, H-4); 3.58 (bs, 1H, OCCH₃penty); 3.39 (bs, 1H, OCH₃penty); 3.24 (t, 2H, J = 7.5 Hz, NCCH₂penty); 1.55-1.51 (m, 4H, CH₂penty); 1.34-1.27 (m, 2H, CH₂penty); 1.18-1.15 (m, 6H, H-6, H-6’).

References

Chapter 8


