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Chapter 6

Synthesis of the repeating unit of the Capsular Polysaccharide of *Staphylococcus aureus* Strain M

Introduction

*Staphylococcus aureus* is a bacterial pathogen, responsible for a wide array of infections of *inter alia* the skin, lungs and joints, and it can cause life-threatening conditions like endocarditis or toxic shock syndrome. *S. aureus* M is a particular strain whose polysaccharide capsule has been associated with its pathogenicity, and strain M was shown to be both more lethal and more resistant to phagocytosis in mouse models when compared to other *S. aureus* strains.¹

¹ Hagen, B.; Van Dijk, J. H. M.; Zhang, Q.; Overkleeft, H. S.; Van der Marel, G. A.; Codée, J. D. C.; manuscript in preparation.
Hash and co-workers discovered that the strain M Capsular Polysaccharide (CPS) consists of \(N\)-acetylgalactosaminuronic acid (GaNAc), \(N\)-acetylfucosamine (FucNAc) and taurine, in the molar ratio of 4:2:1.\(^2\) A later study established that the repeating unit is the one shown in Scheme 1A.\(^3\) It is thought, that a taurine unit is attached to one out of four GaNAcA residues by an amide bond. The polysaccharide repeating unit structure shows that all glycosidic linkages are \(\alpha\) (or 1,2-cis), the formation of which remains a synthetic challenge. The GaNAcA units are connected through their C-4 alcohol. This presents another synthetic hurdle, because of the low reactivity of the axially oriented C-4-OH. The vicinity to the uronic acid functionality renders it even less reactive.

\textbf{Scheme 1:} A) Trisaccharide repeating unit of \textit{S. aureus} Strain M CPS and \textit{S. typhi} Vi antigen. B) \textit{S. typhi} Vi oligomers and the applied monosaccharide building blocks by the groups of Ye and Lay.

The synthesis of GaNAcA-containing oligosaccharides has been pursued in the context of \textit{Salmonella typhi} Vi antigen oligosaccharides. This CPS, a homopolymer of \(\alpha-(1\rightarrow4)\) linked GaNAcA (Scheme 1B), with irregular C-3-\(O\)-acetyl groups is currently used in a conjugate vaccine against typhoid fever.\(^4,5\) Synthetic efforts towards \textit{S. typhi} Vi antigen oligomers have been published by the groups of Ye and Lay.\(^6,7\) Ye and co-workers relied on a non-oxidized, \(N\)-acetyl oxazolidinone containing GaNAc donor 1\(^6\) to selectively introduce the required \(\alpha\)-glycosidic bonds. The C-6-alcohols were oxidized after formation of all glycosidic linkages, possibly to prevent further reduction of the nucleophilicity of the already poorly nucleophilic galactosyl C-4-OH. The group of Lay used on 4,6-\(O\)-benzylidene protected donor 2A\(^7\) to synthesize a non-\(O\)-acetylated disaccharide, and relied on disaccharide donor 2B to synthesize a trisaccharide. Again, oxidation of the C-6-OH occurred after formation of all glycosidic bonds.

This Chapter describes the synthesis of \textit{a S. aureus} strain M Capsular Polysaccharide trisaccharide repeating unit, relying on a post-glycosylation oxidation strategy. In Chapter 5, the
synthesis and reactivity of 2-azidogalacturonate (GalN$_2$A) donors and their corresponding 6,3-lactones was studied. While the 'normal' GalN$_3$A donors were highly $\beta$-selective, and therefore unsuitable for the construction of 1,2-\textit{cis} glycosidic linkages, the corresponding lactone was found to be $\alpha$-selective. However, the glycosylation outcome proved to be dependent on the acceptor, with weakly nucleophilic acceptors showing diminished $\alpha$-selectivity. Based on these findings, this Chapter describes an approach in which the C-5 carboxylic acid is introduced after each glycosylation event.

\textbf{Results and Discussion}

The retrosynthetic analysis for the assembly of \textit{S. aureus} Strain M type 1 trisaccharide 3 is shown in Scheme 2. The two GalNAcA $\alpha$-glycosidic linkages would be formed using 2-azidogalactosyl donor 5, equipped with a 4,6-di-\textit{tert}-butylsilylene (DTBS) protecting group.$^{8,9}$ Kiso and co-workers found that the use of this protecting group provides highly $\alpha$-selective galactosylations,$^{10-12}$ even in the presence of the normally dominant C-2-$N$/O-acyl neighboring group participation effect of a 2-$O$-acyl protecting group. Furthermore, the DTBS group is easily introduced and can selectively be removed.

\textbf{Scheme 2:} Retrosynthetic analysis of target trisaccharide 1.

After formation of the glycosidic bond and removal of the silylene protecting group, the primary 6-position can be selectively oxidized,$^{13,14}$ leaving the 4-position available for further glycosylation. Although this strategy may render the C-4-OH of the newly formed acceptor less nucleophilic, it circumvents the need for additional protecting group manipulations (\textit{i.e.} introduction and removal of a protecting group on the 6-$O$ position) and the necessity for a challenging late stage oxidation step. The third monosaccharide in the target structure is a rare, $\alpha$-linked d-FucNAc moiety.$^{15}$ To install the 1,2-\textit{cis} d-FucNAc bond, a FucN$_3$ donor 6 would be used,
Chapter 6

the reactivity of which has been explored earlier (Chapters 3 and 4). The TBS protecting group on
the 3-\(O\) position serves as both an \(\alpha\)-directing protecting group, and as an orthogonal, temporary
protecting group for the potential synthesis of higher oligomers.

The synthesis of donor 5 commenced with known 2-azido selenogalactoside 9, made
available by the procedure described in Chapter 5 (Scheme 3). Introduction of the DTBS moiety
proceeded to give 10 in 98% yield. Subsequent benzylation of the remaining 3-\(O\) position gave
donor 5, after crystallization, in 86% yield. This reaction sequence was easily executed on
multigram scale. Synthesis of \(\beta\)-FucN\(_3\) donor 6 was achieved in only one step from previously
described building block 12 (see Chapters 3 and 4) by introduction of the TBS group using TBSOTf
and DMAP in pyridine at elevated temperature, to obtain 6 in 77% yield.

Scheme 3: Synthesis of building blocks 5 and 6.

![Scheme 3](image)

Reagents and conditions: a) (tBu)\(_2\)Si(OTf)\(_2\), DMF, -40 °C; then NEt\(_3\) (98%); b) BnBr, NaH, DMF, 0 °C (86%); c) TBSOTf,
DMAP (cat.), pyridine, 0 → 70 °C (77%).

In order to assess the reactivity of GalN\(_3\) donor 5, low-temperature NMR experiments\(^{16}\)
(see also Chapters 3 and 5) were carried out to determine which reactive species could form upon
activation. Reacting 5 with Ph\(_2\)SO and Tf\(_2\)O in CD\(_2\)Cl\(_2\) at -80 °C led to total conversion of 5 (Figure
1A) into a single new species (Figure 1B), which was, based on its chemical shift and small \(J\)-
coupling constant (\(\delta\): 6.16 ppm, \(J\) = 3.0 Hz), identified as \(\alpha\)-triflate 13. In order to assess the
stability of the newly formed triflate, the NMR sample was warmed by incremental heating. The
anomeric triflate proved to be relatively stable, with decomposition starting at -10 °C.
Next, donor 5 was reacted with a set of model acceptors to determine whether acceptor reactivity influences the stereoselectivity of the glycosylations. Ethanol and its 2-fluorinated derivatives have been used as a screening panel to investigate the influence of acceptor nucleophilicity on glycosylation stereoselectivity (Chapters 3 and 5). As Table 1 shows DTBS-protected donor 5 only provides α-galactosidic linkages, in contrast to the clear dependency of the stereoselectivity of glycosylations involving 2-azidofucosyl (FucN₃) donors, as well as 2-azido-2-deoxygalacturonic acid-[3,6]-lactones, on acceptor nucleophilicity (Chapter 3 and 5). These results further demonstrate the powerful α-directing effect of the 4,6-O-di-tert-butylsilylene group on galactosylation reactions. Based on the complete absence of β-linked product, one can exclude an Sₙ₂-like pathway, in which the covalent anomic triflate 13 is directly displaced by the nucleophile (Scheme 4). Although a β-triflate cannot be ruled out as a reactive intermediate, its existence is deemed unlikely due to the low nucleophilicity of the triflate anion. Kiso and co-workers reported the crystal structure of a DTBS-protected 2-aminogalactoside, noting that the ring adopted a flattened, half-chair like conformation, in which one of the tert-butyl was positioned over the anomic center. Although crystal structures do not necessarily reflect conformational behavior of molecules in solution, it is possible that shielding of the β-face by the
Chapter 6

DTBS group favors α-glycosidic bond formation (Scheme 4). Also in an \(^4\)H\(_2\) half-chair oxocarbenium ion-like intermediate 19, the C2- and C4-substituents are placed in an electronically favorable position\(^11,18\) this shielding can contribute to the overall selectivity. This half chair is preferentially attacked by the incoming nucleophile from the α-face, proceeding through a chair-like transition state to give α-glycoside 20.

Table 1: Model glycosylations of donor 5 with non-carbohydrate acceptors.

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>product</th>
<th>yield (α/β)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Et</td>
<td>14</td>
<td>60% (1:0)</td>
</tr>
<tr>
<td>2</td>
<td>FEt</td>
<td>15</td>
<td>79% (1:0)</td>
</tr>
<tr>
<td>3</td>
<td>F(_2)Et</td>
<td>16</td>
<td>90% (1:0)</td>
</tr>
<tr>
<td>4</td>
<td>F(_3)Et</td>
<td>17</td>
<td>81% (1:0)</td>
</tr>
<tr>
<td>5</td>
<td>Cy</td>
<td>18</td>
<td>54% (1:0)</td>
</tr>
</tbody>
</table>

Having established the highly α-selective performance of 5 in glycosylations, the synthesis of trisaccharide 3 commenced with glycosylation of 5 with 5-aminopentanol spacer 7 (Scheme 5).\(^19\) Both Ph\(_2\)SO/Tf\(_2\)O-mediated pre-activation\(^20,21\) and NIS/TMSOTf-mediated glycosylations\(^22\) gave 21 as the desired α-isomer in high yield. Attempted removal of the DTBS group using Bu\(_4\)NF proceeded in moderate yields, while the use of HF in pyridine gave the diol 22 in quantitative yield. Oxidation of the C-6 alcohol with TEMPO/PhI(OAc)\(_2\) provided the oxidized acceptor 23 in 85% yield, after methylation of the acid.

Scheme 4: Stereochemical rationale for α-glycosidic bond formation.
Glycosylation of 5 with galacturonic acceptor 23 was effected under NIS/TMSOTf conditions (selected on the basis of the operational simplicity compared to the pre-activation protocol), yielding the disaccharide 24 as the sole product in 88% yield, in spite of the low reactivity of the acceptor due to presence of the C-5 carboxylic acid ester. Removal of the DTBS group using HF in pyridine gave diol 25 in quantitative yield.

Scheme 5: Synthesis of trisaccharide 4.

Reagents and conditions: a) 7, NIS, TMSOTf (cat.), 3Å MS, CH₂Cl₂, 0 °C (82%); b) HF, pyridine, THF (quant.) c) TEMPO (cat.), Phl(OAc)₂, AcOH (cat.), CH₂Cl₂, H₂O; then Mel, K₂CO₃, DMF (85%); d) 5, NIS, TMSOTf (cat.), 3Å MS, CH₂Cl₂, 0 °C (88%); e) TEMPO (cat.), Phl(OAc)₂, CH₂Cl₂, THF; NaClO₃, NaH₂PO₄, 2-methylbut-2-ene, tBuOH, H₂O (84%); f) Ph₃SO, TTBP, 3Å MS, CH₂Cl₂; Tf₂O, -80 → -70 °C; 6, -80 → -40 °C (79%, α only).

The oxidation of disaccharide 25, using TEMPO/Phl(OAc)₂ and ensuing methylation, resulted in 35% of dicarboxylate 26. Surprisingly, monosaccharide 23 was isolated in significant quantities, indicating cleavage of the GaLA-GaLA glycosidic bond. Therefore an alternative oxidation protocol was employed, involving a two-step oxidation process. First, the C-6 alcohol was selectively transformed into the corresponding aldehyde, using TEMPO and Phl(OAc)₂ under anhydrous conditions to prevent hydration of the formed aldehyde and overoxidation to the carboxylate. When this oxidation step was complete (as judged by TLC analysis), a mixture of NaClO₃, 2-methylbut-2-ene and NaH₂PO₄ in tert-butanol/water was added. This Pinnick oxidation provided the desired carboxylic acid uneventfully and after methylation of the uronic acid, disaccharide 26 was obtained in 84% yield, with no detectable glycosidic bond cleavage. The final glycosylation, using FucN₃ donor 6 in combination with the Ph₃SO/Tf₂O activation couple, as described in Chapter 3 and 4, proceeded uneventfully to give 4 with complete α-selectivity (Scheme 6). It was observed that quenching of the reaction with NEt₃ can lead to drastically lower
Chapter 6

yields, likely due to β-elimination on the galacturonate moieties, and therefore pyridine was used to stop the glycosylation reaction.

With the fully protected trisaccharide repeating unit in hand, attention was focused on the deprotection of the trimer (Scheme 6). First all azides in 4 were transformed into acetamides, using AcSH and pyridine.25 The reaction proved to be slow, but afforded product 27 in 47% yield. Next the TBS group in 27 was removed using HF-pyridine and the two carboxylic esters were saponified using a mixture of H2O2 and KOH. Dicarboxylate 29 was obtained in 49% over these two steps. An alternative procedure in which it was attempted to remove the TBS-ether concomitantly with the saponification of the two esters proved to be inefficient. Finally, Pd(OH)2-catalyzed hydrogenation delivered fully deprotected target trisaccharide 3 in 69% yield.

**Scheme 6:** Deprotection of trimer 4 towards target 3.

![Scheme 6](image)

Reagents and conditions: a) AcSH, pyridine (47%); b) HF, pyridine, pyridine (56%); c) H2O2, KOH, THF, tBuOH, H2O (87%); d) Pd(OH)2/C, H2 (1 atm.), AcOH, THF, tBuOH, H2O (69%).

Conclusion

In conclusion, the first synthesis of the repeating unit of the *S. aureus* strain M capsular polysaccharide has been described. A post-glycosylation oxidation strategy was employed, using a 4,6-di-tert-butyl silyldene protected 2-azidogalactosyl donor as precursor of the GalNAcA residues. The two glycosylation reactions with these donors provided the corresponding products
with complete α-selectivity and high yields. Desilylation, followed by selective oxidation of the primary position in 2-azidogalactosyl residues to the carboxylic acids set the stage for the next glycosylation. An unprecedented cleavage of the glycosidic bond during the TEMPO/ BAIB oxidation procedure was observed in the disaccharide stage. To circumvent this side reaction, a new two-step, one-pot oxidation procedure was used to transform the primary alcohol into the carboxylic acid. The final glycosylation of a 2-azidofucosyl donor proceeded with complete stereoselectivity, underscoring the usefulness of this donor for α-fucosaminylation reactions. The orthogonal TBS protecting group in the fully protected trisaccharide repeating unit allows for the elongation of the trimer to generate higher oligomers. The assembled trimer described here can be evaluated for its capacity to act as an synthetic antigen in vaccine modalities.

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)₄Ce(SO₄)₂·2H₂O, 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 anomic carbon atoms (J_H,H,C) were determined using HMBC-GATED experiments. Infrared spectra were recorded with a Shimadzu FTIR 8300 instrument. Wavenumbers (ν) are reported in cm⁻¹. LC-MS analyses were performed on a Thermo Finnigan Surveyor HPLC system equipped with a Gemini C-18 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.

**Phenyl 2-azido-2-deoxy-4,6-O-(di-tert-butylsilylene)-1-seleno-α-D-galactopyranoside (10)**

![Structural formula of the title compound](image1)

To a stirred, ice-cooled solution of 9 (5.5 g, 16.0 mmol, 1.0 eq.) in DMF (160 mL, 0.1 M) was added tBu₂Si(OTf)₂ (7.8 mL, 24 mmol, 1.5 eq.). After TLC analysis (PE/Et₂O, 4:1 v/v) indicated complete conversion of the starting material (~30 min.) the mixture was neutralized with NEt₃ (4.9 mL, 35.2 mmol, 2.2 eq.) and stirred for 5 additional minutes. The mixture was then diluted with water and extracted three times with Et₂O. The combined organic fractions were washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (PE/Et₂O, 1:0 → 4:1 v/v) gave the title compound as a slightly yellow oil in 98% yield (7.6 g, 15.7 mmol): ¹H NMR (400 MHz) δ 7.58-7.25 (m, 5H, CH₆); 5.96 (d, 1H, J = 5.2 Hz, H-1); 4.49 (d, 1H, J = 2.8 Hz, H-4); 4.02-4.30 (m, 2H, H-6); 4.19 (s, 1H, H-5); 4.05 (m, 1H, H-2); 3.84 (t, 1H, J = 3.2 Hz, H-3); 1.07 (d, 18H, J = 15.2 Hz, (CH₃)₃Si); ¹³C NMR (100 MHz) δ 134.5 (CH₂); 129.3 (CH); 128.5 (C₆); 128.0 (CH₂); 85.4 (C-1); 72.3 (C-4); 71.8 (C-3); 69.9 (C-5); 66.7 (C-6); 62.1 (C-2); 27.6, 27.4 (CH₃); 23.4, 20.8 (C₁). IR (thin film) ν: 3505, 2934, 2859, 2105, 1474, 1155, 1190, 1059. HRMS: [M+H-N₂]⁺ calculated for C₂₀H₁₃NO₄SeSi: 458.12613; found 458.12573.

**Phenyl 2-azido-3-O-benzyl-2-deoxy-4,6-O-(di-tert-butylsilylene)-1-seleno-α-D-galactopyranoside (5)**

To a stirred solution, ice-cooled solution of 10 (7.6 g, 15.7 mmol, 1.0 eq.) and BnBr (3.7 mL, 31.4 mmol, 2.0 eq.) in DMF (150 mL, 0.1 M) was added NaH (60% dispersion in mineral oil, 0.75 g, 1.2 eq.). After TLC analysis (PE/Et₂O, 19:1 v/v) indicated complete conversion of the starting material (~2 hours), the mixture was quenched with H₂O and extracted three times with Et₂O. The combined organic fractions were washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Crystallization from EtOH gave the title compound as a white powder in 86% yield (7.82 g, 13.5 mmol): ¹H NMR (400 MHz) δ: 7.56-7.26 (m, 10H, CH₂).
5.2 Hz, H-1); 4.79-4.67 (m, 2H, PhCH$_2$); 4.58 (d, 1H, $J$ = 2.8 Hz, H-4); 4.31 (dd, 1H, $J$ = 5.2 Hz, 10.4 Hz, H-2); 4.26-3.98 (m, 2H, H-6); 4.04 (s, 1H, H-5); 3.64 (dd, 1H, $J$ = 2.8 Hz, 10.4 Hz, H-3); 1.06 (s, 9H, (CH$_3$)$_3$Si); 1.03 (s, 9H, (CH$_3$)$_3$Si); $^{13}$C NMR (100 MHz) δ: 137.6 (C$_{q, arom}$); 134.5, 129.2, 128.6 (CH$_{arom}$); 128.5 (C$_{q, arom}$); 128.0, 127.9, 127.9 (CH$_{arom}$); 86.0 (C-1); 78.8 (C-3); 70.6 (PhCH$_2$); 70.0 (C-5); 69.2 (C-4); 67.0 (C-6); 59.6 (C-2) 27.6, 27.3 ((CH$_3$)$_3$Si); 23.4, 20.8 (C$_3$Si). IR (thin film) ν: 2933, 2857, 2112, 1580, 1474, 1163, 1082, 1070. HRMS: [M+H]$^+$ calculated for C$_{27}$H$_{38}$N$_4$O$_4$SeSi: 576.17908; found 576.17879.

**Phenyl 2-azido-4-O-benzyl-2-deoxy-3-O-(tert-butylidimethylsilyl)-1-seleno-α-d-fucopyranoside** (6)

A 3-necked 50 mL flask equipped with a Liebig condenser was charged with a solution of 12 (0.84 g, 2.0 mmol, 1.0 eq.) and DMAP (49 mg, 0.4 mmol, 0.2 eq.) in pyridine (10 mL, 0.2 M). The mixture was cooled to 0 °C and, under a stream of N$_2$ gas was slowly added TBSOTf (0.92 g, 4.0 mmol, 2.0 eq.). The reaction was heated to 70 °C and stirred until TLC analysis (PE/EtOAc, 9:1 v/v) indicated complete conversion of the starting material (~2 hours). The reaction was cooled to room temperature and quenched with MeOH. The mixture was diluted with EtOAc and washed (10% aq. CuSO$_4$5H$_2$O, 2x; H$_2$O, 2x; brine, 1x), dried over MgSO$_4$, filtered and concentrated in vacuo. Purification by column chromatography (PE/Et$_2$O,1:0 → 9:1 v/v) delivered the title compound in 77% yield (0.82 g, 1.54 mmol). $^1$H NMR (400 MHz) δ: 7.58-7.55 (m, 2H, CH$_{arom}$); 7.39-7.26 (m, 8H, CH$_{arom}$); 5.96 (d, 1H, $J$ = 5.2 Hz, H-1); 5.06 (d, 1H, $J$ = 11.2 Hz, PhCH$_2$H); 4.59 (d, 1H, $J$ = 11.2 Hz, PhCH$_2$H); 4.27 (q, 1H, $J$ = 6.4 Hz, H-5); 4.22 (dd, 1H, $J$ = 5.2 Hz, 10.0 Hz, H-2); 3.88 (dd, 1H, $J$ = 2.8 Hz, 10.0 Hz, H-3); 3.54 (d, 1H, $J$ = 2.0 Hz, H-4); 1.15 (d, 3H, $J$ = 6.4 Hz, H-6); 0.99 (s, 9H, (CH$_3$)$_3$Si); 0.25, 0.22 (s, 3H, CH$_2$Si). $^{13}$C-APT NMR (100 MHz) δ: 138.4 (C$_{q, arom}$); 134.2, 129.0, 128.3, 127.8, 127.7, 127.5 (CH$_{arom}$); 85.5 (C-1); 80.1 (C-4); 75.6 (PhCH$_2$); 74.1 (C-3); 69.4 (C-5); 62.9 (C-2); 25.9 ((CH$_3$)$_3$Si); 18.1 (C$_3$Si); 16.4 (C-6); -4.2, -5.0 (CH$_3$Si). IR (thin film) ν: 2953, 2106, 1360, 1254, 1111, 1080, 1063, 1043. HRMS: [M-N$_2$+H]$^+$ calculated for C$_{27}$H$_{38}$N$_4$O$_4$SeSi: 506.16242; found 506.16223.
Chapter 6


![Chemical reaction diagram]

A mixture of 5 (22 mg, 0.038 mmol, 1.0 eq.), Ph$_2$SO (10 mg, 0.049 mmol, 1.3 eq.) was dried by co-evaporation with toluene (3x), followed by 3 vacuum/argon purges. The mixture was dissolved in CD$_2$Cl$_2$ (0.75 mL, 0.05 M) and transferred to a dry NMR tube and subsequently capped with a septum. The tube was placed in the probe of a NMR magnet and cooled to -80 °C, after which a $^1$H NMR spectrum was recorded. The tube was transferred to an acetone/N$_2$ (l) bath (temperature ≤ -80 °C), and Tf$_2$O (8 µL, 0.049 mmol, 1.3 eq.) was added and, after rapid mixing, was placed back in the NMR probe. Observation by $^1$H NMR revealed complete consumption of 5, and after further characterization of the newly formed triflate 13, its stability was assessed by incremental (by 10 °C) heating of the probe until decomposition was observed.

General procedure for Ph$_2$SO/Tf$_2$O-mediated glycosylations with non-carbohydrate acceptors

![Chemical reaction diagram]

To a solution of donor 5 (57 mg, 0.1 mmol, 1.0 eq.), Ph$_2$SO (26 mg, 0.13 mmol, 1.3 eq.) and TTBP (62 mg, 0.25 mmol, 2.5 eq.) in CH$_2$Cl$_2$ (2 mL, 0.05 M) were added flame-dried 3Å molecular sieves. After ~30 minutes, the mixture was cooled to -80 °C, and Tf$_2$O (22 µL, 0.13 mmol, 1.3 eq.) was added. The reaction mixture was allowed to warm to -70 °C over the course of ~15 minutes. The mixture was re-cooled to -80 °C, a solution of the acceptor (0.5 M in CH$_2$Cl$_2$, 0.4 mL) was slowly added via the wall of the flask. The reaction mixture was allowed to warm to -40 °C over the course of ~90 minutes, after which the reaction was quenched by addition of NEt$_3$ (0.1 mL). The reaction mixture was diluted with CH$_2$Cl$_2$, filtered over a bed of celite, washed with brine, dried over MgSO$_4$, filtered and concentrated in vacuo. Purification by column chromatography yielded the corresponding O-glycoside.
**Ethyl 2-azido-3-O-benzyl-2-deoxy-4,6-O-(di-tert-butylsilylene)-α-D-galactopyranoside (14)**

The product was obtained after column chromatography (PE/EtOAc, 1:0 → 9:1) in 60% yield (28 mg, 0.06 mmol). δ: 7.38-7.26 (m, 5H, CH\textsubscript{arom}); 4.94 (d, 1H, J = 3.6 Hz, H-1); 4.76 (d, 1H, J = 11.6 Hz, PhCH\textsubscript{A}); 4.67 (d, 1H, J = 11.6 Hz, PhCH\textsubscript{H}); 4.58 (d, 1H, J = 2.4 Hz, H-4); 4.26 (dd, 1H, J = 2.0 Hz, 12.4 Hz, H-6); 4.14 (dd, 1H, J = 2.0 Hz, 12.6 Hz, H-6); 3.88 (dd, 1H, J = 2.8 Hz, 10.4 Hz, H-3); 3.79 (dd, 1H, J = 3.6 Hz, 10.6 Hz, H-2); 3.75-3.71 (m, 1H, OC\textsubscript{HCHCH\textsubscript{3,Et}}); 3.66 (bs, 1H, H-5); 3.58-3.53 (m, 1H, CH\textsubscript{CH\textsubscript{3}} Et); 1.23 (t, 3H, J = 7.2 Hz, CH\textsubscript{3} Et); 1.07 (s, 9H, (CH\textsubscript{3})\textsubscript{3}Si); 1.05 (s, 9H, (CH\textsubscript{3})\textsubscript{3}Si). \(^{13}\text{C}-\text{APT NMR} (100 \text{ MHz}) \delta: 137.9 (C\textsubscript{q,arom}); 128.5, 127.9, 127.8 (CH\textsubscript{arom}); 98.1 (C-1); 75.7 (C-3); 70.5 (PhCH\textsubscript{2}); 69.9 (C-4); 67.4 (C-5); 67.2 (C-6); 63.9 (OCH\textsubscript{2}CH\textsubscript{3}); 58.3 (C-2); 27.7 ((CH\textsubscript{3})\textsubscript{3}Si); 27.3 ((CH\textsubscript{3})\textsubscript{3}Si); 23.5, 20.7 (C\textsubscript{Si}); 15.0 (CH\textsubscript{3,Et}). \(^{13}\text{C}-\text{GATED NMR} (100 \text{ MHz}) \delta: 98.1 (d, J = 170 Hz, C-1); IR (thin film) ν: 2934, 2859, 2110, 1474, 1175, 1175, 1144, 1099, 1065. HRMS: [M+\textsubscript{NH}+\textsubscript{4}]\textsuperscript{+} calculated for C\textsubscript{23}H\textsubscript{41}N\textsubscript{4}O\textsubscript{3}Si: 481.28407; found 481.28381.

**2-fluoroethyl 2-azido-3-O-benzyl-2-deoxy-4,6-O-(di-tert-butylsilylene)-α-D-galactopyranoside (15)**

The product was obtained after column chromatography (PE/EtOAc, 1:0 → 9:1) in 79% (38 mg, 0.079 mmol). \(^{1}\text{H NMR} (400 \text{ MHz}) \delta: 7.44-7.26 (m, 5H, CH\textsubscript{arom}); 4.98 (d, 1H, J = 3.6 Hz, H-1); 4.75 (d, 1H, J = 11.6 Hz, CH\textsubscript{Bn}); 4.69-4.50 (m, 2H, CH\textsubscript{H}Bn, CH\textsubscript{H}F, H-4) 4.20 (dd, 2H, J = 2.0, 12.8 Hz, H-6); 4.14 (dd, 1H, J = 1.6 Hz, 12.4 Hz, H-6); 3.98-3.69 (m, 4H, H-2, H-3, CH\textsubscript{CH\textsubscript{2}}F); 3.73 (s, 1H, H-5); 1.06 (s, 9H, (CH\textsubscript{3})\textsubscript{3}Si); 1.04 (s, 9H, (CH\textsubscript{3})\textsubscript{3}Si); \(^{13}\text{C}-\text{APT NMR} (100 \text{ MHz}) \delta: 137.8 (C\textsubscript{q,arom}); 131.1, 129.4, 129.3, 128.6, 127.9, 127.9, 127.7, 124.8 (CH\textsubscript{arom}); 98.6 (C-1); 82.5 (d, J = 169 Hz, CH\textsubscript{F}); 75.6 (C-3); 70.5 (PhCH\textsubscript{2}); 69.7 (C-4); 67.5 (C-5); 67.3 (d, J = 20 Hz, CH\textsubscript{2}CH\textsubscript{2}F); 67.1 (C-6); 58.3 (C-2); 27.7, 27.3 ((CH\textsubscript{3})\textsubscript{3}Si); 23.5, 20.7 (C\textsubscript{Si}). \(^{13}\text{C}-\text{GATED NMR} (100 \text{ MHz}) : 98.6 (d, J = 171 Hz, C-1). IR (thin film) ν: 2932, 2859, 2110, 1474, 1173, 1144, 1103, 1005. HRMS: [M + \textsubscript{NH}_4]^\textsuperscript{+} calculated for C\textsubscript{23}H\textsubscript{48}FN\textsubscript{4}O\textsubscript{3}Si: 499.27465; found 499.27431.
Chapter 6

2,2-difluoroethyl 2-azido-3-O-benzyl-2-deoxy-4,6-O-(di-tert-butylsilylene)-α-D-galactopyranoside (16)

The product was obtained after column chromatography (PE/EtOAc, 1:0 → 9:1) in 90% yield (45 mg, 0.09 mmol). ^1H NMR (400 MHz) δ: 7.44-7.26 (m, 5H, CH\textsubscript{arom}); 6.07-5.79 (m, 1H, CH\textsubscript{F2}); 4.97 (d, 1H, J = 1.6 Hz, H-1); 4.75 (d, 1H, J = 11.6 Hz, PhCHH); 4.67 (d, 1H, J = 11.2, PhCH\textsubscript{H}); 4.26 (dd, 1H, J = 2.0 Hz, 12.8 Hz, H-6); 4.15 (d, 1H, J = 1.6 Hz, 12.8 Hz, H-6); 3.86-3.76 (m, 4H, H-2, H-3, CH\textsubscript{CF3H}); 3.68 (s, 1H, H-5); 1.06 (s, 9H, (CH\textsubscript{3})\textsubscript{3}Si); 1.03 (s, 9H, (CH\textsubscript{3})\textsubscript{3}Si). ^13C-APT NMR (100 MHz) δ: 137.7 (C\textsubscript{arom}); 128.6, 128.0, 127.9 (CH\textsubscript{arom}); 113.8 (t, J = 240 Hz, CH\textsubscript{F2}); 99.2 (C-1); 75.4 (C-3); 70.6 (PhCH\textsubscript{3}); 69.6 (C-4); 67.9 (C-5); 67.3 (t, J = 28 Hz, CH\textsubscript{2}CH\textsubscript{F2}); 67.0 (C-6); 58.1 (C-2); 27.6, 27.3 ((CH\textsubscript{3})\textsubscript{3}Si); 23.4, 20.7 (C\textsubscript{Si}); ^13C-GATED NMR (100 MHz): 99.2 (d, J = 172 Hz, C-1); IR (thin film) ν: 2936, 2859, 2114, 1474, 1364, 1171, 1142, 1101. HRMS: [M+H-N\textsubscript{2}]\(^+\) calculated for C\textsubscript{23}H\textsubscript{35}F\textsubscript{3}NO\textsubscript{5}Si: 472.23253; found 472.23239.

2,2,2-trifluoroethyl 2-azido-3-O-benzyl-2-deoxy-4,6-O-(di-tert-butylsilylene)-α-D-galactopyranoside (17)

The product was obtained after column chromatography (PE/EtOAc, 1:0 → 9:1) in 81% yield (42 mg, 0.081 mmol). ^1H NMR (400 MHz) δ: 7.44-7.26 (m, 5H, CH\textsubscript{arom}); 5.01 (s, 1H, H-1); 4.76 (d, 1H, J = 11.6 Hz, PhCH\textsubscript{H}); 4.68 (d, 1H, J = 12.4 Hz, PhCH\textsubscript{H}); 4.61 (s, 1H, H-4); 4.26 (d, 1H, J = 2.0 Hz, 12.8 Hz, H-6); 4.15 (d, 1H, J = 1.6 Hz, 12.8 Hz, H-6); 4.00-3.87 (m, 4H, H-2, H-3, CH\textsubscript{2}CH\textsubscript{F2}); 3.68 (s, 1H, H-5); 1.07 (s, 9H, (CH\textsubscript{3})\textsubscript{3}Si); 1.04 (s, 9H, (CH\textsubscript{3})\textsubscript{3}Si). ^13C-APT NMR (100 MHz) δ: 137.6 (C\textsubscript{arom}); 128.6, 128.0, 127.9 (CH\textsubscript{arom}); 123.5 (q, J = 277 Hz, CF\textsubscript{3}); 99.3 (C-1); 75.3 (C-3); 70.6 (PhCH\textsubscript{3}); 69.6 (C-4); 68.2 (C-5); 66.9 (C-6); 56.2 (q, J = 35 Hz, CH\textsubscript{2}CH\textsubscript{F2}); 57.9 (C-2); 27.6, 27.3 ((CH\textsubscript{3})\textsubscript{3}Si); 23.4, 20.7 (C\textsubscript{Si}); ^13C-GATED NMR (100 MHz): 99.3 (d, J = 170 Hz, C-1); IR (thin film) ν: 2934, 2859, 2112, 1279, 1167, 1148, 1086, 1007. HRMS: [M+H-N\textsubscript{2}]\(^+\) calculated for C\textsubscript{23}H\textsubscript{35}F\textsubscript{3}NO\textsubscript{5}Si: 490.22311; found 490.22291.
Cyclohexyl 2-azido-3-O-benzyl-2-deoxy-4,6-O-(di-tert-butylsilylene)-α-ᴅ-galactopyranoside (18)

The product was obtained after column chromatography (PE/EtOAc, 1:0 → 9:1 v/v) in 54% yield (28 mg, 0.054 mmol). ³¹H NMR (400 MHz) δ: 7.44-7.26 (m, 5H, CH₆arom); 5.08 (d, 1H, J = 3.5 Hz, H-1); 4.76 (d, 1H, J = 11.5 Hz, PhCH₃H); 4.66 (d, 1H, J = 11.5 Hz, PhCH₂H); 4.61 (d, 1H, J = 2.5 Hz, H-4); 4.26 (dd, 1H, J = 2.0, 12.5 Hz, H-6); 4.13 (dd, 1H, J = 2.0, 12.5 Hz, H-6); 3.91 (dd, 1H, J = 3.0, 10.5 Hz, H-3); 3.75 (s, 1H, H-5); 3.72 (dd, 1H, J = 3.5, 11 Hz, H-2); 3.61 (m, 1H, OCH₃); 1.96-1.20 (m, 10H, CH₂C₆); 1.06 (s, 9H, (C₆)₃C); 1.04 (s, 9H, (C₆)₃C); ³¹C-APT NMR (100 MHz) δ: 137.9 (C₆ṣarom); 128.5, 127.9, 127.8 (CH₆arom); 96.8 (C-1); 76.2 (OCH₃); 75.2 (C-3); 70.4 (PhCH₂); 69.9 (C-4); 67.4 (C-5); 67.2 (C-6); 58.1 (C-2); 33.4, 31.4 (CH₂C₆); 27.6, 27.3 ((CH₆)₃C); 25.5, 24.1, 23.8 (CH₂C₆); 23.4, 20.7 (C₆Si); ³¹C-GATED NMR (100 MHz): 96.8 (d, J = 170 Hz, C-1); IR (thin film) ν: 2932, 2859, 2114, 1474, 1364, 1175, 1111. HRMS: [M+H-N₂]⁺ calculated for C₂₇H₄₄NO₅Si: 490.29833; found 490.29813.

5-(benzyl(benzylloxycarbonyl)amino)pentyl 2-azido-3-O-benzyl-2-deoxy-4,6-O-(di-tert-butylsilylene)-α-ᴅ-galactopyranoside (21)

To a solution of donor 5 (3.45 g, 6.0 mmol, 1.0 eq.), N-benzylcarbonyl-N-benzyl-5-aminopentanol 7 (3.93 g, 12.0 mmol, 2.0 eq.) in CH₂Cl₂ (30 mL, 0.2 M) were added flame-dried, rod-shaped 3Å MS. After ~30 minutes, the mixture was cooled to 0 °C and N-iodosuccinimide (1.75 g, 7.8 mmol, 1.3 eq.) and TMSOTf (0.11 mL, 0.6 mmol, 0.1 eq.) were added. After TLC analysis (PE/EtOAc, 9:1 v/v) indicated complete consumption of the starting material (~2 hours), the reaction was quenched with NEt₃ (5 mL) and the mixture was diluted with CH₂Cl₂. After filtration over celite, the mixture was washed with sat. aq. Na₂S₂O₃ (1x), sat. aq. NaHCO₃ (1x) and brine, dried over MgSO₄, filtered and concentrated in vacuo. Column chromatography (PE/EtOAc, 1:0 → 9:1) afforded the title product as the sole isomer in 82% yield (3.67 g, 4.9 mmol): ¹H NMR (500 MHz, 323 K) & 7.40-7.20 (m, 15H, CH₆arom); 5.16 (s, 2H, PhCH₃); 4.85 (s, 1H, H-1); 4.73 (d, 1H, J = 11.5 Hz, PhCH₃H); 4.65 (d, 1H, J = 11.5 Hz, PhCH₂H); 4.57 (s, 1H, H-4); 4.48 (s, 2H, PhCH₂); 4.22 (d,
Chapter 6

1H, /= 12.5 Hz, H-6); 4.11 (d, 1H, /= 12.0 Hz, H-6); 3.83 (d, 1H, /= 10.5 Hz, H-3); 3.76 (d, 1H, /= 10.5 Hz, H-2); 3.60-3.57 (m, 2H, H-5, OCH₂H₃pentylyl); 3.43 (bs, 1H, OCH₂H₃pentylyl); 3.23 (bs, 2H, NC₃H₂pentylyl); 1.54-1.53 (m, 4H, CH₂pentylyl); 1.43-1.40 (m, 2H, CH₂pentylyl); 1.06 (s, 9H, (CH₃)₃CSi); 1.05 (s, 9H, (CH₃)₃CSi). 13C NMR (125 MHz, 323 K) δ: 138.3, 138.2 (C₃H₂pentylyl); 138.1, 137.5 (C₃H₂pentylyl); 128.6, 128.5, 128.0, 127.9, 127.8, 127.3 (CH₃arom); 98.8 (C-1); 75.7 (C-3); 70.5 (PhCH₂); 70.3 (C-4); 68.5 (OCH₂pentylyl); 67.9 (C-5); 67.4 (C-6); 67.3 (PhCH₂); 58.7 (C-2); 50.8 (PhCH₂); 29.2 (CH₂pentylyl); 27.7, 27.5 ((CH₃)₃CSi); 23.4 (CH₂pentylyl); 20.8 (C₆Si). IR (thin film) ν: 2932, 2859, 2108, 1699, 1472, 1229, 1138, 1043. HRMS: [M+N₄H⁺]⁺ calculated for C₄₃H₆₀N₅O₇Si: 762.42565; found 762.42586.

5-(benzyl(benzylxycarbonyl)amino)pentyl 2-azido-3-O-benzyl-2-deoxy-α-D-galactopyranoside (22)

To a solution of 21 (1.68 g, 2.3 mmol, 1.0 eq.) in THF (23 mL, 0.1 M) was added HF-pyridine (70% HF, 0.47 mL, 18 mmol, 8.0 eq.). After TLC analysis (PE/EtOAc, 1:4 v/v) indicated complete conversion of the starting material (~2 hours), the reaction was quenched with NEt₃ (2 mL). The mixture was concentrated, dissolved in EtOAc and subsequently washed with sat. aq. NaHCO₃ and brine. The aqueous layers were then extracted with EtOAc combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo. Column chromatography (PE/EtOAc, 1:0 → 1:4) furnished the title compound in 98% yield. (1.34 g, 2.2 mmol). 1H NMR (500 MHz, 323 K) δ: 7.35-7.20 (m, 15H, CH₃arom); 5.16 (bs, 2H, PhCH₂); 4.87 (s, 1H, H-1); 4.72 (d, 1H, /= 11.5 Hz, PhCH₂); 4.67 (d, 1H, /= 11 Hz, PhCH₂); 4.48 (bs, 2H, PhCH₂); 4.09 (s, 1H, H-4); 3.88-3.84 (m, 2H, H-3, H-6); 3.78 (bs, 2H, H-5, H-6); 3.64-3.62 (m, 2H, H-2, OCH₂H₃pentylyl); 3.43 (bs, 1H, OCH₂H₃pentylyl); 3.24 (bs, 2H, NC₃H₂pentylyl); 1.54 (bs, 4H, CH₂pentylyl); 1.32-1.27 (m, 2H, CH₂pentylyl). 13C-APT NMR (125 MHz, 323 K) δ: 138.1, 137.5 (C₃H₂pentylyl); 128.7, 128.6, 128.5, 128.2, 128.0, 127.9, 127.9, 127.6, 127.3 (CH₃arom); 98.4 (C-1); 76.1 (C-3); 72.2 (PhCH₂); 70.1 (C-5); 68.3 (OCH₂pentylyl); 67.6 (C-4); 67.3 (PhCH₂); 62.8 (C-6); 59.5 (C-2); 50.8 (PhCH₂); 29.1, 27.7, 23.5 (CH₂pentylyl). 13C-GATED NMR (125 MHz, 323 K) δ: 98.4 (d, /= 170 Hz, C-1). IR (thin film) ν: 3456, 3408, 2106, 1693, 1423, 1229, 1138, 1043. HRMS: [M+H]⁺ calculated for C₃₃H₄₁N₄O₇: 605.29698; found 605.29726.
Synthesis of Staphylococcus aureus Strain M CPS

Methyl 5-(benzyl(benzyloxy carbonyl)amino)pentyl 2-azido-3-O-benzyl-2-deoxy-α-D-galactopyranosiduronate (23)

To an ice-cooled solution solution of 22 (0.16 g, 0.26 mmol, 1.0 eq.) in a mixture of CH₂Cl₂, tBuOH and H₂O (4:4:1, 1.3 mL, 0.2 M) were added TEMPO (8 mg, 0.05 mmol, 0.2 eq.), Phl(OAc)₂ (0.21 g, 0.65 mmol, 2.5 eq.) and AcOH (3 drops). The resulting mixture was stirred overnight at 4 °C, after which TLC analysis (PE/EtOAc, 1:1 v/v) indicated complete conversion of the starting material. The reaction was then quenched with sat. aq. Na₂S₂O₃ (10 mL) and aq. H₃PO₄ (1 mL, 1.0 M) was added. The mixture was extracted with CH₂Cl₂ (3x) and the combined organic layers were dried over MgSO₄, filtered, concentrated *in vacuo*, and the residue was co-evaporated with toluene. The residue was then dissolved in DMF (1.3 mL, 0.2 M) and cooled to 0 °C, after which MeI (32 µL, 0.52 mmol, 2.0 eq.) and K₂CO₃ (72 mg, 0.52 mmol, 2.0 eq.) were added. The mixture was stirred 4 °C until TLC analysis indicated complete conversion of the starting material (~16 hours). The reaction was then quenched with H₂O and the mixture was partitioned between H₂O and Et₂O. The aqueous phase was then extracted with Et₂O (3x), the combined organic layers washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 1:0 → 1:1) gave the title compound as a yellow oil in 85% yield (0.14 g, 0.22 mmol): ¹H NMR (500 MHz, 323 K) δ: 7.37-7.20 (m, 15H, CH₃arem); 5.17 (s, 2H, PhCH₂); 4.96 (s, 1H, H-1); 4.74-4.68 (m, 2H, PhCH₂); 4.48 (s, 2H, PhCH₂); 4.40 (s, 1H, H-5); 4.36 (s, 1H, H-4); 3.92 (d, 1H, J = 10.0 Hz, H-3); 3.83 (s, 3H, CO₂CH₃); 3.68-3.63 (m, 2H, H-2, OCH₄pentyl); 3.46-3.45 (m, 1H, OCH₂pentyl); 3.24 (s, 2H, NCH₂pentyl); 1.56-1.51 (m, 4H, CH₂pentyl); 1.38-1.24 (m, 2H, CH₂pentyl). ¹³C NMR (100 MHz) δ: 168.7 (C-6); 138.2, 137.3, 137.1 (C₉arem); 128.7, 128.6, 128.5, 128.3, 128.0, 127.9, 127.9, 127.6, 127.3 (CH₂arem); 98.5 (C-1); 75.6 (C-3); 72.3 (PhCH₂); 70.3 (C-4); 69.1 (OCH₂pentyl); 67.7 (C-5); 67.3 (PhCH₂); 59.0 (C-2); 52.3 (CO₂CH₃); 50.8 (PhCH₂); 50.2 (PhCH₂); 29.7, 29.1, 27.8, 23.4 (CH₂pentyl). IR (thin film) v: 3497, 2926, 2859, 2106, 1762, 1693, 1227, 1140, 1028. ¹³C-GATED NMR (125 MHz, 323 K) δ: 98.5 (d, J = 171 Hz, C-1). HRMS: [M+H]+ calculated for C₃₄H₄₁N₄O₆: 633.29189; found 633.29215.
Methyl (5-(benzyl(benzyloxy carbonyl)amino)pentyl 2-azido-4-O-(2-azido-3-O-benzyl-2-deoxy-
4,6-O-(di-tert-butyl-silylene)-α-D-galactopyranosyl)-3-O-benzyl-2-deoxy-α-D-
galactopyranosidosiduronate) (24)

To a solution of 23 (1.80 g, 2.8 mmol, 1.0 eq.) and 5 (3.26 g, 5.6 mmol, 2.0 eq.) in CH₂Cl₂ (14 mL, 0.2 M) were added flame-dried, rod-shaped 3Å MS. After ~30 minutes, the solution was cooled to 0 °C, and N-iodosuccinimide (1.66 g, 7.4 mmol, 2.6 eq.) and TMSOTf (0.1 mL, 0.56 mmol, 0.2 eq.) were added respectively. After TLC analysis (PE/Et₂O, 9:1 v/v) indicated complete conversion of the starting material (~2 hours), the reaction was quenched by addition of NEt₃ (3 mL) the mixture filtered over celite, washed with sat. aq. Na₂S₂O₅, sat. aq. NaHCO₃ and brine, dried over MgSO₄ filtered and concentrated in vacuo. Column chromatography (PE/Et₂O, 1:0 → 9:1) afforded the product as an oil in 88% yield (2.62 g, 2.49 mmol): ¹H NMR (500 MHz, 323 K) δ: 7.39-7.19 (m, 20H, CH₃); 5.16 (s, 2H, PhCH₂); 5.02 (s, 1H, H-1'); 4.99 (s, 1H, H-1'); 4.76-4.61 (m, 5H, H-4, PhCH₂); 4.51 (s, 1H, H-4'); 4.49-4.47 (m, 2H, PhCH₂); 4.33 (s, 1H, H-5); 3.98 (s, 1H, H-5'); 3.95 (d, 1H, J = 11.0 Hz, H-3); 3.84 (s, 3H, CO₂CH₃); 3.82-3.77 (m, 4H, H-2', H-3', H-6'); 3.64 (bs, 1H, OCH₃); 3.37 (m, 2H, OCH₃); 3.58 (d, 1H, J = 10.5 Hz, H-2); 3.48 (bs, 1H, OCH₃); 3.23 (m, 2H, NCH₃); 1.53-1.52 (m, 4H, CH₂); 1.28 (bs, 2H, CH₂); 1.01 (s, 9H, (CH₃)₃CSi); 1.00 (s, 9H, (CH₃)₃CSi); ¹³C NMR (125 MHz, 323 K) δ: 168.8 (C-6); 138.2, 137.3 (C₃arom); 128.6, 128.6, 128.5, 128.4, 128.0, 127.9, 127.7, 127.5, 127.3 (C₃arom); 99.8 (C-1'); 98.4 (C-1); 75.6 (C-3'); 75.5 (C-3); 74.8 (C-4); 72.4 (PhCH₂); 70.0 (C-4'); 69.1 (OCH₂); 68.2 (C-5'); 67.3 (PhCH₃); 67.1 (C-6'); 59.7 (C-2); 58.8 (C-2'); 52.5 (CO₂CH₃); 50.5 (PhCH₂); 50.3 (PhCH₃); 47.1 (NCH₃); 46.1 (CH₃N linker); 29.0 (CH₂); 27.6; 27.3 (C₃S); 23.3 (CH₂); 23.3, 20.7 (C₃Si). IR (thin film) ν: 2934, 2859, 2106, 1730, 1697, 1474, 1362, 1256, 1140, 1040. HRMS: [M+Na]⁺ calculated for C₅₅H₇₁N₇NaO₇Si: 1072.48222; found 1072.48236.
Methyl (5-(benzyl(benzyloxycarbonyl)amino)pentyl 2-azido-4-O-(2-azido-3-O-benzyl-2-deoxy-α-D-galactopyranosyl)-3-O-benzyl-2-deoxy-α-D-galactopyranosiduronate) (25)

To a solution of compound 24 (1.26 g, 1.2 mmol, 1.0 eq.) in THF (12 mL, 0.1 M) was added HF.pyr (70% HF, 0.12 mL, 4.8 mmol, 4.0 eq.). After TLC analysis (PE/EtOAc, 1:4 v/v) indicated complete conversion of the starting material (~2 hours), the reaction was quenched with NEt₃ (1 mL). The mixture was concentrated, the residue dissolved in EtOAc and washed with sat. aq. NaHCO₃ and brine. The aqueous layers were then extracted with EtOAc and the combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo. Column chromatography (PE/EtOAc, 1:0 → 1:4) yielded the title compound as an oil in quantitative yield. (1.08 g, 1.2 mmol): ¹H NMR (500 MHz, 323 K) δ: 7.40-7.15 (m, 20H, CH₃oph); 5.16 (s, 2H, PhCH₂); 5.05 (s, 1H, H-1); 5.01 (d, 1H, J = 3.0 Hz, H-1'); 4.82 (d, 1H, J = 11.5 Hz, PhCHA); 4.70-4.63 (m, 3H, PhCH₂); 4.60 (s, 1H, H-4); 4.48 (s, 2H, PhCH₂); 4.35 (s, 1H, H-5); 4.09 (s, 1H, H-4'); 4.02 (bs, 1H, H-5'); 3.92 (d, 1H, J = 10.0 Hz, H-3); 3.86-3.83 (m, 4H, H-3', CO₂CH₃); 3.70 (dd, 1H, J = 3.5 Hz, 10.5 Hz, H-2'); 3.63 (bs, 1H, OCH₃penti); 3.62 (dd, 1H, J = 3.5 Hz, 10.5 Hz, H-2); 3.52-3.48 (m, 3H, H-6', OCH₃penti); 3.22 (bs, NCH₂penti); 1.53 (m, 4H, CH₂penti); 1.34-1.26 (m, 2H, CH₂penti). ¹³C-APT NMR (125 MHz, 323 K) δ: 168.5 (C-6'); 138.0, 137.2, 137.2, 136.9 (C₆arom); 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.3 (CH₂arom); 99.5 (C-1'); 98.2 (C-1); 76.0 (C-3'); 75.3, 75.2 (C-3, C-4); 72.4, 71.9 (PhCH₂); 70.2 (C-5); 69.8 (C-5'); 69.0 (OCH₂penti); 67.6 (C-4'); 67.2 (PhCH₂); 62.6 (C-6'); 59.4, 59.3 (C-2, C-2'); 52.6 (CO₂CH₃); 50.5 (NCH₂penti); 29.6, 29.0, 23.3 (CH₂penti). ¹³C-GATED NMR (125 MHz, 323 K) δ: 99.5 (d, J = 173 Hz, C-1'); 98.2 (d, J = 173 Hz, C-1). IR (thin film) ν: 3448, 2936, 2112, 2108, 1730, 1695, 1694, 1454, 1423, 1233, 1040. HRMS: [M+H]+ calculated for C₄₂H₅₀N₁₂O₁₂: 910.39815; found 910.39941.
Chapter 6

Methyl (5-(benzyl(benzylxycarbonyl)amino)pentyl 2-azido-4-O-(methyl (2-azido-3-O-benzyl-2-deoxy-a-D-galactopyranosiduronate))-3-O-benzyl-2-deoxy-a-D-galactopyranosiduronate) (26)

\[
\text{HO} \quad \text{CO}_{2}\text{Me}\hspace{1cm} \text{N} \quad \text{CO}_{2}\text{Me}\hspace{1cm} \text{O} \quad \text{BnO}
\]

To an ice-cooled solution of 25 (0.42 g, 0.46 mmol, 1.0 eq.) in THF and CH₂Cl₂ (3:2 v/v, 4.5 mL, 0.1 M) were added TEMPO (14 mg, 0.09 mmol, 0.2 eq.) and Phl(OAc)₂ (147 mg, 0.46 mmol, 1.0 eq.). After stirring for 30 minutes the mixture was allowed to warm to room temperature. After stirring for an additional 90 minutes a second portion of Phl(OAc)₂ (59 mg, 0.18 mmol, 0.4 eq.) was added. After TLC analysis (PE/EtOAc, 1:1 v/v) indicated complete conversion of the starting material, tBuOH (3.6 mL) and 2-methylbut-2-ene (0.5 mL) were added. The mixture was then cooled to 0 °C again and a solution of NaClO₂ (83 mg, 0.91 mmol, 2.0 eq.) and NaH₂PO₄ (0.11 g, 0.91 mmol, 2.0 eq.) in H₂O (0.45 mL) was added. After ~30 minutes, the reaction mixture was allowed to warm to room temperature. After TLC analysis (PE/EtOAc/AcOH, 10:10:1 v/v/v) indicated complete transformation of the intermediate aldehyde, aq. 1M H₂PO₄ (2 mL) was added to the reaction mixture, after which it was extracted three times with EtOAc. The combined organic layers were dried over MgSO₄ filtered and concentrated in vacuo. The residue was dissolved in DMF (2.4 mL, 0.2M) and MeI (57 μL, 0.91 mmol, 2.0 eq.) and K₂CO₃ (0.13 g, 0.91 mmol, 2.0 eq.) were added. After TLC analysis (PE/EtOAc, 1:1 v/v) indicated complete conversion of the starting material (~2 hours), H₂O was added and the mixture extracted with Et₂O (4x). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated in vacuo. Purification by column chromatography (PE/EtOAc, 1:0 → 1:1) yielded the title compound as an oil in 84% yield (0.36 g, 0.38 mmol). ¹H NMR (500 MHz, 323 K) δ: 7.35-7.19 (m, 20H, CH₃); 5.16-5.14 (m, 3H, H-1, PhCH₂); 5.04 (d, 1H, J = 2.5 Hz, H-1’); 4.85-4.82 (m, 2H, H-5’, PhCHH); 4.71-4.68 (m, 2H, PhCH₂); 4.65 (s, 1H, H-4’); 4.55 (d, 1H, J = 12.0 Hz, PhCH₂H); 4.47 (s, 2H, PhCH₂); 4.39 (d, 1H, J = 1.5 Hz, H-4); 4.35 (s, 1H, H-5); 3.94-3.89 (m, 2H, H-3, H-3’); 3.84 (s, 3H, CO₂CH₃); 3.73 (dd, 1H, J = 3.5 Hz, 10.5 Hz, H-2); 3.65 (bs, 1H, OCH₃penny); 3.55 (dd, 1H, J = 3.5 Hz, 11 Hz, H-2’); 3.48 (bs, 1H, OCH₃penny); 3.43 (s, 3H, CO₂CH₃); 3.21 (bs, 2H, NH₂penny); 1.52-1.51 (m, 4H, CH₂penny); 1.34-1.23 (m, 2H, CH₂penny). ³¹C-APT NMR (125 MHz, 323 K) δ: 168.4, 168.3 (C-6, C-6’); 137.9, 137.0, 136.9 (Cₐrom); 128.6, 128.5, 128.4, 128.4, 128.2, 127.9, 127.9, 127.8, 127.7, 127.3, 127.2 (Cₐrom); 99.6 (C-1); 98.2 (C-1’); 75.6, 75.3, 74.9 (C-3, C-3’, C-4’); 72.1, 71.9 (PhCH₂); 70.6, 70.1 (C-5, C-5’); 69.0 (OCH₂penny); 67.2 (PhCH₂); 67.1 (C-4); 59.0 (C-2’); 58.7 (C-2); 52.6, 52.0 (CO₂CH₃); 50.5 (PhCH₂);
Synthesis of Staphylococcus aureus Strain M CPS

29.6, 28.9, 23.2 (CH$_2$-pentyl). $^{13}$C-GATED NMR (125 MHz, 323 K) δ: 99.6 (d, $J = 173$ Hz, C-1); 98.2 (d, $J = 172$ Hz, C-1'). IR (thin film) ν: 3449, 2936, 2859, 2110, 2106, 1761, 1732, 1695, 1454, 1360, 1219, 1134, 1058. HRMS: [M+H]$^+$ calculated for C$_{46}$H$_{50}$N$_4$O$_{13}$: 938.39306; found 938.39426.

Methyl (5-(benzyl(benzyl氧carboxyl)氨基)pentyl 2-azido-4-O-(methyl (2-azido-4-O-benzyl-2-deoxy-3-O-(tert-butyldimethylsilyl)α-D-fucopyranosyl)-3-O-benzyl-2-deoxy-α-D-galactopyranosiduronate))-3-O-benzyl-2-deoxy-α-D-galactopyranosiduronate) (4)

To a solution of 6 (0.57 g, 1.1 mmol 2.0 eq.) Ph$_2$SO (0.21 g, 1.1 mmol, 2.0 eq.) and TTBP (0.53 g, 2.1 mmol, 4.0 eq.) in CH$_2$Cl$_2$ (20 mL, 0.05 M) and flame-dried, rod-shaped 3ÅMS were added. After ~30 minutes, the mixture was cooled to -80 °C. Tf$_2$O (0.18 mL, 1.1 mmol, 2.0 eq.) was added and the solution was allowed to warm to -70 °C, after which TLC analysis indicated complete activation of the donor. The mixture was re-cooled to -80 °C, and a solution of acceptor 26 (0.50 g, 0.53 mmol, 1.0 eq.) in CH$_2$Cl$_2$ (1.3 mL, 0.4M) was slowly added. The mixture was warmed to -40 °C and then kept at this temperature. After TLC analysis (PE/EtOAc, 4:1 v/v) indicated complete consumption of the acceptor, the reaction was quenched by addition of pyridine (1 mL), and the reaction mixture was diluted with CH$_2$Cl$_2$, filtered over a small bed of celite, washed with brine, dried over MgSO$_4$, filtered and concentrated in vacuo. Purification by size exclusion chromatography (CH$_2$Cl$_2$/MeOH, 1:1 v/v) and subsequent column chromatography (PE/EtOAc, 1:0 → 3:1) yielded the title trisaccharide as a single isomer, in 79% yield (0.55 g, 0.42 mmol): $^1$H NMR (500 MHz, 328 K) δ: 7.36-7.23 (m, 25H, CH$_{arom}$); 5.21 (d, 1H, $J = 3.5$ Hz, H-1); 5.16 (s, 2H, PhCH$_2$); 5.01 (d, 1H, $J = 3.0$ Hz, H-1'); 4.96-4.46 (m, 2H, PhCH$_2$); 4.87-4.52 (m, 2H, PhCH$_2$); 4.85 (s, 1H, H-1''); 4.82-4.52 (m, 2H, PhCH$_2$); 4.78 (s, 1H, H-5'); 4.71 (d, 1H, $J = 2.0$ Hz, H-4'); 4.54 (s, 1H, H-4''); 4.47 (s, 2H, PhCH$_2$); 4.34 (s, 1H, H-5); 4.08 (q, 1H, $J = 6.5$ Hz, H-5''); 4.01 (dd, 1H, $J = 2.8$, 10.3 Hz, H-3''); 3.89 (m, 1H, H-3'); 3.87 (m, 1H, H-3); 3.85 (s, 3H, CO$_2$CH$_3$); 3.69 (m, 1H, H-2''); 3.66 (m, 1H, H-2); 3.65-3.45 (m, 2H, OCH$_2$-pentyl); 3.47 (dd, 1H, $J = 3.8$, 10.8 Hz, H-2'); 3.39 (s, 3H, CO$_2$CH$_3$); 3.34 (d, 1H, $J = 1.5$ Hz, H-4''); 3.21 (m, 2H, NCH$_2$-pentyl); 1.51 (m, 4H, CH$_2$-pentyl); 1.25 (m, 2H, CH$_2$-pentyl); 0.95 (s, 9H, (CH$_2$)$_3$CSi(CH$_3$)$_3$); 0.87 (d, 3H, $J = 6.5$ Hz, H-6''); 0.20 (s, 3H, CH$_3$Si); 0.15 (s, 3H, CH$_3$Si). $^{13}$C-APT NMR (125 MHz, 328 K) δ: 168.5, 168.3 (C-6, C-6''); 138.7 (C$_{q,arom}$); 138.1 (CH$_{arom}$); 138.0, 137.2,
Chapter 6

137.2 (C,arom); 128.6,128.5, 128.5, 128.3, 127.9, 127.9, 127.8, 127.7, 127.6, 127.5, 127.3, 126.9 (CH,arom); 99.4 (C-1); 98.8 (C-1’); 98.3 (C-1’); 81.0 (C-4’); 75.6 (PhCH2); 75.1 (C-3); 75.1 (C-3’); 74.6 (C-4); 73.5 (C-4’); 72.1 (PhCH2); 71.9 (PhCH2); 71.2 (C-3’); 70.8 (C-5’); 70.2 (C-5); 69.0 (OCH2pentyl); 67.6 (C-5’); 67.2 (PhCH2); 62.1 (C-2’); 59.5 (C-2); 59.2 (C-2’); 52.6, 52.0 (CO2CH3); 29.0 (CH2pentyl); 25.9 ((CH3)3CSi); 23.3 (CH2pentyl); 18.1 (C3Si); 16.5 (C-6’); -3.9 (CH3Si). IR (thin film) ν: 2924, 2859, 2110, 2117, 1713, 1693, 1506, 1474, 1474, 1211, 1045. HRMS: [M+Na]+ calculated for C67H86N10NaO16Si: 1335.57282; found 1335.57294.

Methyl (5-(benzyl(benzylloxycarbonyl)amino)pentyl 2-acetamido-4-O-(methyl (2-acetamido-4-O-(2-acetamido-4-O-benzyl-2-deoxy-3-O-(tert-butyldimethylsilyl)-α-D-fucopyranosyl)-3-O-benzyl-2-deoxy-α-D-galactopyranosidurionate)-3-O-benzyl-2-deoxy-α-D-galactopyranosidurionate) (27)

To a solution of 4 (0.28 g, 0.21 mmol, 1.0 eq.) in pyridine (10 mL, 0.02M) was added freshly distilled AcSH (10 mL). The mixture was stirred until LC-MS analysis (MeCN/H2O/TFA, 50:50:0.1 → 90:10:0.1 v/v/v, tR 9.50 min.) indicated completion of the reaction (~9 days). The reaction mixture was concentrated in vacuo and purification by means of size exclusion chromatography (CH2Cl2/MeOH, 1:1 v/v) and column chromatography (DCM/MeOH, 1:0 → 19:1) gave the title compound as a white solid in 47% yield (0.14 g, 0.1 mmol): 1H NMR (500 MHz, methanol-d4, 330 K) δ: 7.37-7.22 (m, 25H, CH,arom); 5.14 (s, 2H, PhCH2); 5.03 (d, 1H, J = 3.5 Hz, H-1); 4.98-4.55 (m, 2H, PhCH2); 4.95 (m, 1H, H-3); 4.92 (d, 1H, J = 3.5 Hz, H-1’); 4.76-4.58 (m, 2H, PhCH2); 4.68 (d, 1H, J = 2.0 Hz, H-4’); 4.578-4.46 (m, 5H, H-1”, H-2’, H-2”, H-5’, PhCH2); 4.31 (q, 1H, J = 6.5 Hz, H-5’'); 4.06 (dd, 1H, J = 2.8, 10.2 Hz, H-3’); 3.98 (dd, 1H, J = 2.5, 11.5 Hz, H-3); 3.84-3.82 (m, 1H, H-3’); 3.76 (s, 3H, CO2CH3); 3.67-3.42 (m, 2H, OC,H2pentyl); 3.46 (d, 1H, J = 1.5 Hz, H-4”); 3.27 (m, 2H, NCH2pentyl); 3.18 (s, 3H, CO2CH3); 2.02 (s, 3H, CH3,NHAc); 2.02 (s, 3H, CH3,NHAc); 1.96 (s, 3H, CH3,NHAc); 1.54 (m, 4H, CH2pentyl); 1.30 (m, 2H, CH2pentyl); 0.90 (s, 9H, (CH3)3CSi); 0.80 (d, 3H, J = 6.5 Hz, H-6’); 0.19 (s, 6H, CH3Si); 13C NMR (125 MHz, methanol-d4, 330 K) δ: 172.5; 172.3, 172.1 (d, CH3,NHAc); 169.2, 168.8 (C-6, C-6’); 139.0, 138.6, 138.4, 138.0 (C,arom); 128.3, 128.2, 128.0, 128.0, 127.8, 127.6, 127.5, 127.2, 127.1, 127.0, 127.0, 126.9, 126.1, (CH,arom); 99.2 (C-1’);
Synthesis of Staphylococcus aureus Strain M CPS

99.2 (C-1); 98.1 (C-1’); 81.0 (C-4’’); 76.4 (C-3’); 75.4 (PhCH₂); 75.2 (C-3); 74.6 (C-4’); 73.9 (C-4); 72.0, 71.8 (PhCH₂); 71.0 (C-5); 70.8 (C-3’’); 69.8 (C-5’’); 68.7 (OCH₂penty1); 67.7 (C-5’’); 67.1 (PhCH₂); 51.5, 51.0 (CO₂H₂); 50.3 (PhCH₂); 48.9 (C-2’); 48.6 (C-2’’); 48.6 (C-2); 28.6 (CH₂penty1); 25.0 ((CH₂)3CSi); 23.1 (CH₂linker); 22.4, 22.0, 21.5 (CH₃,NH₄⁺); 17.4 (Cₛ,Si); 15.7 (H-6’’); -5.0 (CH₃Si).

IR (thin film) ν: 2947, 2835, 1763, 1684, 1676, 1663, 1518, 1454, 1364, 1213, 1128, 1038.

5-(benzyl(benzyloxy carbonyl)amino)pentyl 2-acetamido-4-O-(2-acetamido-4-O-benzyl-2-deoxy-3-O-(tert-butyldimethylsilyl)-α-D-fucopyranosyl)-3-O-benzyl-2-deoxy-α-D-galactopyranosiduronate)-3-O-benzyl-2-deoxy-α-D-galactopyranosiduronic acid (28)

To a plastic tube, containing a solution of 27 (35 mg, 26 μmol, 1.0 eq.) in pyridine (5 mL, 0.005 M) was added HF,pyridine (70% HF, 0.1 mL, 3.8 mmol, 150 eq.). The mixture stirred until LC-MS analysis (MeCN/H₂O/TFA, 50:50:0.1 → 90:10:0.1 v/v/v, t₂: 6.10 min.) indicated that the reaction was complete. The reaction was quenched with H₂O (5 mL) and the mixture was extracted CH₂Cl₂ (10x). The combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo.

Purification by column chromatography (DCM/MeOH, 1:0 → 19:1) gave compound 28 as a white solid in 56% yield (18 mg, 14 μmol): ¹H NMR (500 MHz) δ: 7.38-7.07 (m, 25H, CH₃arom); 5.17 (s, 2H, PhCH₂); 5.01 (d, 1H, J = 3.5 Hz, H-1); 4.95 (s, 1H, H-1’); 4.94 (s, 1H, H-5); 4.90-4.56 (m, 2H, PhCH₂); 4.89-4.63 (m, 2H, PhCH₂); 4.86-4.47 (m, 2H, PhCH₂); 4.70 (s, 1H, H-4’); 4.66 (d, 1H, J = 3.5 Hz, H-1’’); 4.55 (m, 1H, H-2’’); 4.53 (s, 2H, PhCH₂Bn); 4.51 (m, 1H, H-4’’); 4.51 (m, 1H, H-5’’); 4.31 (m, 1H, H-5’’’); 4.30 (m, 1H, H-2’’’); 3.99 (m, 1H, H-3’’); 3.97 (m, 1H, H-3’’’); 3.84 (m, 1H, H-3’’’); 3.78 (s, 3H, CO₂CH₂); 3.67-3.44 (m, 2H, OCH₂penty1); 3.52 (d, 1H, J = 1.5 Hz, H-4’’’); 3.30 (s, 1H, H₂CH₂); 2.05 (s, 3H, CH₃,NH₄⁺); 2.04 (s, 3H, CH₃,NH₄⁺); 1.97 (s, 3H, CH₃,NH₄⁺); 1.58 (m, 4H, CH₂penty1); 1.33 (m, 2H, CH₂penty1); 0.79 (d, 3H, J = 6.5 Hz, H-6’’’). ¹³C NMR (125 MHz) δ: 172.8, 172.6, 172.3 (CD₃,NH₄⁺); 169.0, 168.8 (C-6, C-6’); 138.9, 138.6, 138.4, 138.0, (C₉ arorn); 128.3, 128.2, 128.0, 128.0, 127.8, 127.8, 127.6, 127.1, 127.1, 127.1, 127.0, 126.8, 126.1 (CH₃arom); 98.9 (C-1); 98.6 (C-1’); 98.1 (C-1’’); 80.1 (C-4’’); 76.4 (C-3’); 75.4 (PhCH₂); 75.3 (C-3); 74.3 (C-4’); 73.8 (C-4); 71.9 (PhCH₂); 71.8 (PhCH₂); 70.9 (C-5); 69.8 (C-5’); 69.0 (C-5’’); 68.7 (OCH₂penty1); 67.4
Chapter 6

(C-5’’); 67.1 (PhCH₃); 51.5, 51.0 (CO₂CH₃); 50.3 (PhCH₂); 50.3 (C-2’’); 48.9 (C-2’); 48.7 (C-2); 28.6, 23.1 (CH₃-pentyl); 22.0, 21.9, 21.5 (CH₃,NIHAc); 15.7 (C-6’). IR (thin film) ν: 3422, 3333, 2924, 2855, 1742, 1663, 1653, 1454, 1228, 1043, 1028. HRMS: [M+H]+ calculated for C₆₇H₈₅N₄O₁₉: 1247.56460; found 1247.56524.

5-(benzyl(benzyloxy carbonyl)amino)pentyl 2-acetamido-4-O-(2-acetamido-4-O-benzyl-2-deoxy-α-D-fucopyranosyl)-3-O-benzyl-2-deoxy-α-D-galactopyranosiduronate)-3-O-benzyl-2-deoxy-α-D-galactopyranosiduronic acid (29)

Compound 28 (39 mg, 31 μmol, 1.0 eq.) was dissolved in THF (1.6 mL, 0.02M) after which 0.6 mL of a solution of 0.5 M KOH and 30% wt. H₂O₂ (22:3, v:v) was added to the solution together with t-BuOH (0.6 mL). The resulting mixture was allowed to stir for 2 days after which LC-MS analysis (MeCN/H₂O/TFA, 50:50:0.1 → 90:10:0.1 v/v/v, tᵣ: 4.70 min.) indicated that the reaction was complete. The reaction mixture was neutralized with 1M aq. HCl and subsequently extracted CH₂Cl₂ (10x). Evaporation of the combined organic phases gave the product as a white solid in 87% yield (33 mg, 27 μmol). ¹H NMR (500 MHz, CD₂OD) δ: 7.36-7.21 (m, 25H, CH₃-arom); 5.15 (s, 2H, PhCH₂); 5.14 (s, 1H, H-1); 4.93 (s, 1H, H-5); 4.88-4.83 (m, 4H, H-1’, H-1’’), PhCH₂; 4.75 (d, 1H, J = 12.5 Hz, PhCHH); 4.64 (s, 1H, H-4’); 4.59-4.45 (m, 7H, H-2, H-2’, H-4, PhCH₂); 4.37 (s, 1H, H-5’); 3.85-3.31 (m, 2H, H-2”, H-5’’); 3.98-3.93 (m, 2H, H-3, H-3’’); 3.77 (m, 1H, H-3’); 3.66-3.55 (m, 1H, OCHH₃-pentyl); 3.51 (d, 1H, J = 2.0 Hz, H-4’’); 3.46-3.38 (m, 1H, OCHH₂-linker); 3.26 (t, 2H, J = 6.8 Hz, NCH2-pentyl); 2.03, 1.99, 1.91 (s, 3H, CH₂NIHAc); 1.54-1.51 (m, 4H, CH₂-pentyl); 1.40 (s, 2H, CH₂-pentyl); 0.77 (d, 3H, J = 6.5 Hz, H-6’’); ¹³C NMR (125 MHz, CD₂OD) δ: 174.5, 173.8, 173.5 (C-6, C-6’’, CO₂NIHAc); 140.2, 140.0, 139.5 (C₆-arom); 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 128.9, 128.6, 128.5, 128.5, 128.4, 128.2 (CH₃-arom); 100.5 (C-1); 100.4 (C-1’’); 99.4 (C-1’); 81.5 (C-4’’); 77.0 (C-3’’); 77.0 (C-3’); 75.8 (C-4’’); 75.6 (C-4); 73.2, 73.2 (PhCH₂); 72.1 (C-5); 71.2 (C-5’); 70.7 (C-3’’); 69.8 (OCH₂-linker); 68.8 (C-5’’); 68.5 (PhCH₂); 51.7 (C-2’’); 51.6 (PhCH₂); 50.2 (C-2’); 49.9 (C-2); 29.9, 24.4 (CH₂-pentyl); 23.2, 23.2, 22.8 (CH₂NIHAc); 17.0 (C-6’’). IR (thin film) ν: 3422, 3333, 2924, 2855, 1742, 1663, 1653, 1454, 1228, 1043, 1028. HRMS: [M+H]+ calculated for C₆₅H₇₉N₄O₁₉: 1219.53330; found 1219.53503.
5-amino-pentyl 2-acetamido-4-O-(2-acetamido-4-O-(2-acetamido-2-deoxy-α-D-fucopyranosyl)-2-deoxy-α-D-galactopyranosiduronic acid (3)

To a mixture of 29 (34 mg, 28 μmol, 1.0 eq.), was dissolved in mixture of water, t-BuOH, and THF (3:1:1, 0.56 mL, 0.005M) was added AcOH until pH 3 was reached. Pd(OH)$_2$/C (78 mg, 56 μmol, 2.0 eq.) was then added to the solution, after which H$_2$ was bubbled through the solution. The mixture was then allowed to stir for 3 days under H$_2$ atmosphere, after which the solution was filtered over a Whatman filter, the residue was rinsed with THF/H$_2$O, co-evaporated with H$_2$O and then filtered again. After lyophilization, the product was obtained as a white solid in 69% yield (14 mg, 19 μmol). $^1$H NMR (500 MHz, D$_2$O) δ: 5.01 (d, 1H, H-1'); 4.93 (d, 1H, J = 3.5 Hz, H-1'); 4.85 (d, 1H, J = 3.5 Hz, H-1''); 4.72 (s, 1H, H-5); 4.39 (m, 2H, H-4', H-5''); 4.29 (s, 1H, H-4); 4.21 (m, 2H, H-2, H-5'); 4.11 (m, 4H, H-2", H-3, H-3'); 3.93 (dd, 1H, J = 3.0, 11.0 Hz, H-3''); 3.80 (d, 1H, J = 3.0 Hz, H-4''); 3.65 (d, 1H, J = 7.0 Hz, OCH$_3$ (pentlyl) 3.49 (t, 1H, J = 5.0 Hz, OCH$_3$ (pentlyl)); 2.95 (t, 1H, J = 7.8 Hz, CH$_2$N (pentlyl)); 2.06 (s, 6H, CH$_3$N$_2$H$_4$); 2.00 (s, 3H, CH$_3$NI$_2$H$_4$); 1.69-1.61 (m, 4H, CH$_2$ (pentlyl)); 1.41-1.38 (m, 2H, CH$_2$ (pentlyl)); 1.13 (d, 3H, J = 6.5 Hz, H-6''); $^{13}$C NMR (125 MHz, D$_2$O) δ: 174.9, 174.6, 174.5 (CH$_3$NI$_2$H$_4$, C-6, C-6'); 99.4 (C-1''); 98.4 (C-1'); 96.8 (C-1''); 79.9 (C-4'); 77.4 (C-4''); 72.0 (C-5); 71.2 (C-4''); 70.6 (C-5''); 68.0 (OCH$_2$ (pentlyl)); 67.9 (C-3 '); 67.7 (C-5 '); 67.2 (C-3 ); 67.0 (C-3''); 49.8 (C-2'); 49.5 (C-2'); 49.5 (C-2''); 39.4 (NCH$_2$ (pentlyl)); 28.0, 26.3, 22.4 (CH$_2$ (pentlyl)); 22.3, 22.3, 21.9 (CH$_3$NI$_2$H$_4$); 15.4 (C-6'). IR (thin film) ν: 3352, 3269, 3237, 2918, 1639, 1595, 1377, 1109, 1026. HRMS: [M+H]$^+$ calculated for C$_{29}$H$_{49}$N$_4$O$_{17}$: 725.30872; found 725.30846.

References


