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Synthesis of oligosaccharide libraries from GBS capsular polysaccharides for structure-based selection of vaccine candidates

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Chapter 5

Synthesis of GBS CPS serotype Ib branched and linear repeating units

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Introduction

Group B *Streptococcus* (GBS) capsular polysaccharide is, as mentioned, a main target for vaccine development¹⁻³ and, on the basis of its structure, ten GBS serotypes have been classified and described^{4, 5}, and some of them share the same composition in terms of sugar residues differing only for the linkage position. GBS type Ib structure, for instance, is structurally very similar to GBS type Ia, which has been described in Chapter 4. Rebecca Lancefield was the first to notice serologic cross reactions between strains bearing Ia and Ib antigens, although she did not know what cellular structure contained the differing epitopes. NMR analysis showed that the native CPSs of GBS types Ia and Ib are indeed structurally similar, differing only in the linkage of the side chain galactose to *N*-acetylglucosamine⁶, the type Ia CPS has a β -(1 $\rightarrow$ 4) linkage, and the type Ib CPS has a β -(1 $\rightarrow$ 3) linkage in this position. Full characterization of the CPS antigen revealed that the repeating unit of Ia and Ib CPSs is a pentasaccharide with a disaccharide backbone and a trisaccharide side chain. Like all GBS CPSs, each possesses an α -(2 $\rightarrow$ 3)-linked sialic acid as a terminal side chain saccharide (Figure 1).

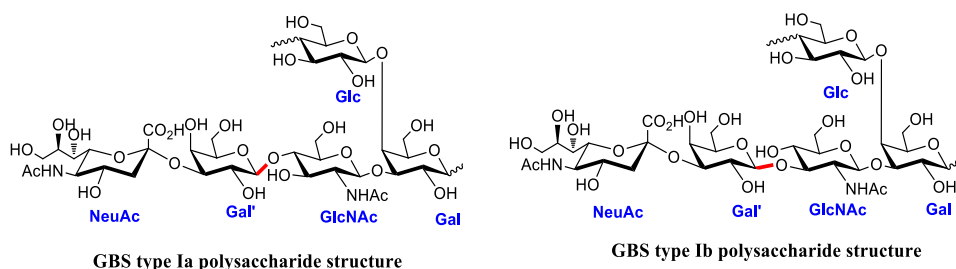


Figure 1. Structure of GBS serotypes Ia and Ib CPS repeating units

Recent European studies (2008-2010) indicated that serotype Ib is cause of 7.3% of the Early-onset and 5.4% of the Late-Onset diseases and together with serotype Ia is the most common strain colonizing the gastro-intestinal and vaginal tracts⁷. Moreover, more than 12% of GBS infections in elderly or immunocompromised patients can be related to type Ib⁸.

Given these data, an effective GBS vaccine should include also serotype Ib. Indeed, monovalent conjugate vaccine prepared with Ib polysaccharide has been tested in phase I/II clinical trials, as well as a trivalent combination (Ia, Ib, III) in order to develop a maternal vaccination strategy⁹⁻¹¹. Immunization with conjugated PSIIa and PSIIb vaccines led to isolation of serotype-specific antibodies, showing that the different linkage in the side chain is determining the immunospecificity of the polysaccharides.

As for GBS type Ia, neither chemical nor enzymatic depolymerization method are available for CPS Ib and therefore, chemical synthesis is the only viable option to obtain homogeneous oligosaccharides from the CPS, which can be used to explore interactions with serotype specific

monoclonal antibodies and to elucidate the mechanism of action of the polysaccharide conjugates^{12, 13}.

While a synthesis of the pentasaccharide corresponding to the branched GBS PSIIa repeating unit was recently described¹⁴, there is no synthesis currently reported for GBS type Ib repeating unit. With the ultimate goal to identify the structural features of the CPS relevant for antibody recognition, this Chapter describes how the best GlcpNAc- β -D-(1 $\rightarrow$ 3)-Galp synthons among those described in Chapter 3 were chosen for elongation to give the pentasaccharide repeating unit of GBS serotype Ib. The synthesized oligosaccharide fragments will be used to map the interaction to an anti PSIIb monoclonal antibody and to highlight (thanks to NMR and Molecular Dynamics simulation) the conformational properties of the corresponding polysaccharide resulting in immunospecificity with respect to the very similar type Ia CPS, reported in the previous Chapter. All targeted synthetic structures are designed with a free amino group at the oligosaccharide reducing end in order to facilitate further manipulation and conjugation to carrier protein for immunological evaluation.

Results and discussion

Synthesis of GBS serotype Ib repeating unit

As mentioned, the repeating unit of GBS serotype Ib is a pentasaccharide with a disaccharide backbone and a trisaccharide side chain, structurally very similar to the one of GBS type Ia and III.

This structure can be described by the branched and the linear frameshifts **1** and **2** depicted in Figure 2.

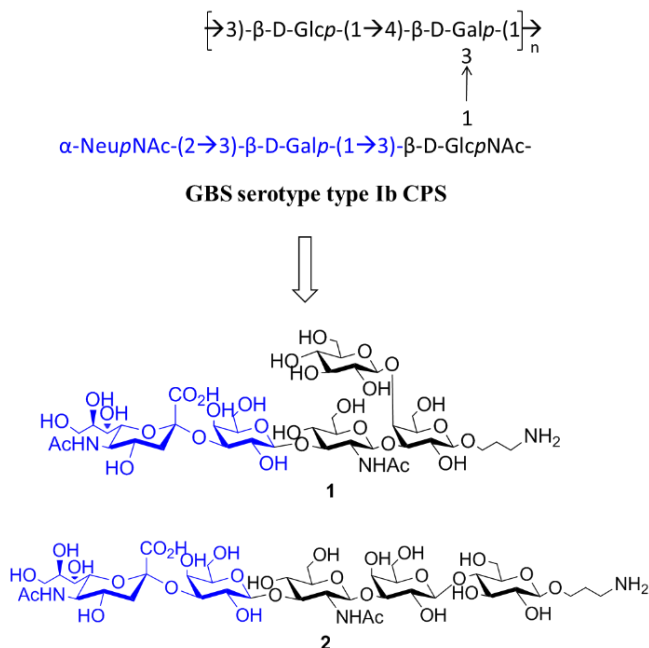
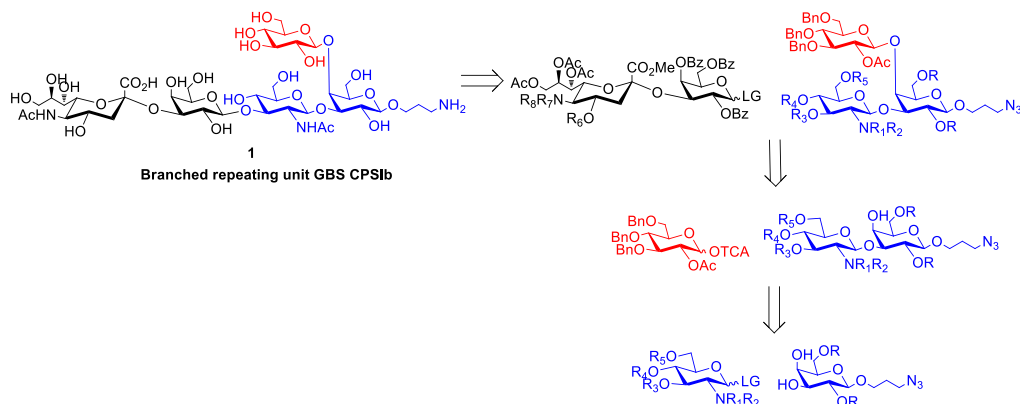


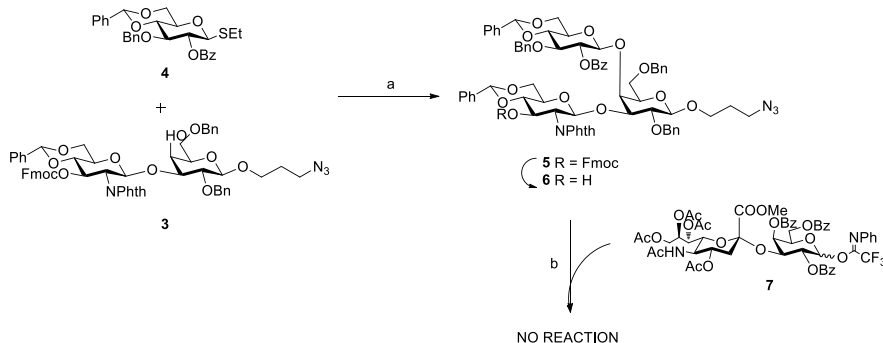
Figure 2. Structure of type Ib GBS CPS and the synthetic targets: its branched (**1**) and linear (**2**) repeating units.

As already mentioned for GBS serotype Ia oligosaccharides (Chapter 4), two main challenges in the synthesis of pentasaccharide **1** can be identified: the presence of the difficult α sialic acid connected to the upstream Gal residue and an effective approach to the branched trisaccharide GlcpNAc- β -D-(1 $\rightarrow$ 3)-Galp- β -D-(1 $\rightarrow$ 4)-Glcp. The stereoselectivity of a late stage α -sialylation could be challenging and impact on the overall yield of the process and therefore, a convergent [3+2] regioselective approach employing a sialogalactoside donor and a trisaccharide acceptor was selected. The trisaccharide acceptor was deriving in turn from the disaccharide synthon GlcpNAc- β -D-(1 $\rightarrow$ 3)-Galp obtained by means of a regioselective β -(1 $\rightarrow$ 3) galactose glycosylation (see Chapter 3). Moreover, the synthetic design to pentasaccharide **1** and **2** anticipated the installation of an azidopropyl linker (converted into aminopropyl during the deprotections) at the reducing end to make these structures suitable for further manipulation and conjugation to carrier proteins (Scheme 1).



Scheme 1. Retrosynthetic scheme to GBS serotype Ib branched repeating unit

Differently than the GBS CPS Ia pentasaccharides, the two Ib frameshifts **1-2** required a glucosamine building block bearing a temporary protecting group at its C3-OH and the creation of the Gal β 1-3GlcNAc linkage, which had a strong impact on the synthetic design. At first, in line with the synthesis of the CPS Ia branched oligosaccharides, attempts to prepare the branched pentasaccharide **1** started from the NPhth protected trisaccharide acceptor **6** which was prepared by Fmoc removal from **5**, obtained by the coupling of disaccharide **3** and glucose donor **4**. Unfortunately, the reaction of **6** with the sialogalactoside donor **7** was unsuccessful (Scheme 2).

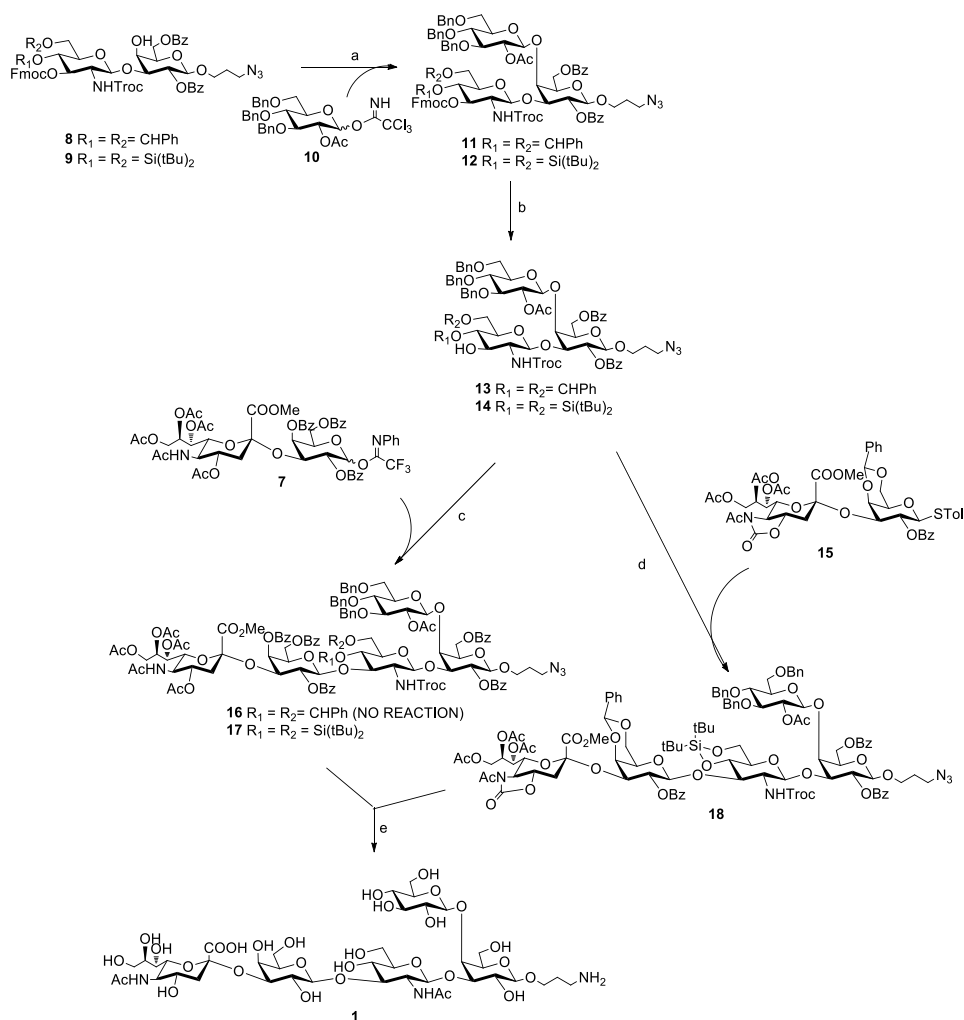


Scheme 2. Reagents and conditions: a) TfOH, NIS, DCM dry, 80%; b) TMSOTf, DCM dry, 0°C.

From this trial, it was realized that the C3-OH of the glucosamine was significantly less reactive than the C4-OH, likely due to the presence of the bulky NPhth group that could hinder the glycosylation reaction at the neighbouring alcohol. It was anticipated that replacement of the NPhth by the Troc group could result in a higher nucleophilicity of the vicinal hydroxyl. To test this hypothesis, disaccharides **8** and **9**, obtained with good yields by means of a regioselective glycosylation and differing only in the cyclic protecting group blocking the glucosamine C4,6-OH, were selected to be elongated to the branched pentasaccharide **1** (Scheme 3). Glycosylation of the two acceptors with the armed Glc donor **10** under TMSOTf activation at 0°C afforded the

trisaccharides **11** and **12** in 63% and 70% yield, respectively. After Fmoc removal by treatment with 10% piperidine in DCM (92%), glycosylation with the sialogalactoside donor **7** of the two acceptors **13** and **14** was undertaken. Reaction of the 4,6-*O*-benzylidene trisaccharide **13** and **7** with TMSOTf as promoter failed to afford the target pentasaccharide, leading to complete recovery of the unreacted acceptor. In contrast, reaction of acceptor **14**, bearing the more flexible 4,6-*O*-silylidene ketal¹⁵, with **7** in the presence of TMSOTf gave the target pentasaccharide **17** in 80% yield (Scheme 3). This result suggests that the glycosylation of **13** was prevented by the steric and torsional constrain of the 4,6-*O*-benzylidene ring. Trisaccharide **14** was also efficiently glycosylated with disaccharide donor **15** by NIS/TfOH activation, affording **18** in 65% yield (Scheme 3). Despite a slightly lower yield in this step, the overall efficiency of the synthesis of GBS serotype Ib branched repeating unit was superior using the thioglycoside **15**^{16, 17}.

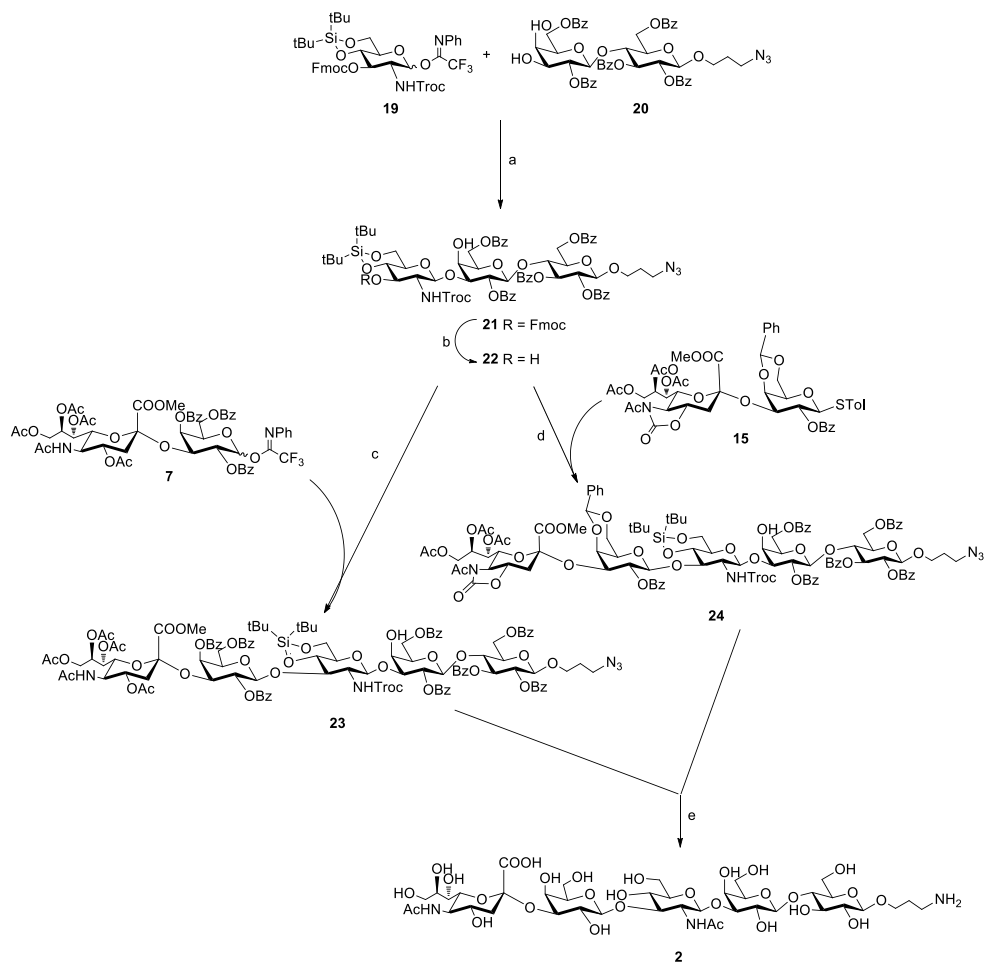
Pentasaccharides **17** and **18** were then deprotected by a four-steps protocol: (i) desilylation by treatment with HF·pyridine, (ii) saponification with NaOH in refluxing THF, for concomitant hydrolysis of the acyl esters, the Troc group and the 5-*N*,4-*O*-oxazolidinone protecting group and Neu5Ac methyl ester; (iii) reacetylation of the amines using a 2:3 acetic anhydride/methanol mixture; (iv) hydrogenation over Pd/charcoal. The target branched pentasaccharide **1** was obtained in 40% yield as estimated by spectrophotometric quantification of the sialic acid content¹⁸.



Scheme 3 Assemble of GBS CPSIa repeating unit. *Reagent and conditions:* a) TMSOTf, DCM dry, -10°C , 75% from **8**; 68% from **9**; b) $\text{Me}_3\text{N}\cdot\text{BH}_3$, $\text{BF}_3\cdot\text{Et}_2\text{O}$, ACN, 0°C , 70% ; c) TMSOTf, DCM dry, 0°C , 75%; d) TfOH, NIS, DCM dry, -40°C , 73%; e) LiI, Py, 120°C ; NaOH 3M, THF, reflux; Ac_2O , MeOH; H_2 , Pd-C, 40% (over four steps).

At this point, the linear frameshift **2** of the GBS serotype Ib repeating unit was targeted and the feasibility of the regioselective approach was assessed again (Scheme 4). For this purpose, benzoylated lactose acceptor **20**, previously described for the synthesis of the linear repeating unit of GBS PSIIa (Chapter 4), was glycosylated with the 4,6-*O*-silylidene glucosamine imidate **19** under TMSOTf activation to give the target trisaccharide **21** with full stereo- and regioselectivity (55%). Following Fmoc deprotection with piperidine in DCM, the obtained acceptor **22** was glycosylated with imidate **7** to attain the linear protected pentasaccharide **23** (66%). Reaction with thioglycoside **15** in TfOH and NIS reaction conditions provided the analogous pentasaccharide **24** (40%). The obtained pentasaccharides were deprotected and purified with the four-step

deprotection protocol described above. NMR data of the synthesized CPS Ib fragments were in excellent agreement with NMR data from samples of the bacterial polysaccharide¹⁹ (Table 1 and Figure 3).



Scheme 4. Synthetic route to GBS type Ib linear repeating unit. *Reagents and conditions:* a) TMSOTf, DCM dry, 0°C, 55%; b) Piperidine, DCM, 90%; c) TMSOTf, DCM dry, 0°C, 66%; d) TfOH, NIS, DCM dry, -40°C, 40%; e) HF/pyridine; 3 M NaOH, THF, reflux; Ac₂O/MeOH; H₂/Pd-C, 40%.

Table 1. Chemical shift (ppm) of ^1H and ^{13}C NMR signals of compound 1-2 in D_2O

Residue		Compound 1		Compound 2		PS Ib ^a	
		^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR
Gal	1	4.38/ <i>J</i> 7.6 Hz	103.3	4.48/ <i>J</i> 8.3	103.3	4.49	101.9
	2	3.69	70.2	3.55	69.8	3.70	71.0
	3	3.80	82.0	3.76	81.9	3.80	82.7
	4	4.36	74.8	4.11	68.2	4.11	69.4
	5	3.69	64.2	3.63	74.9	3.69	75.5
	6	3.74	60.8	3.75	60.5		
	6'	3.74		3.75			
GlcNAc	1	4.72/ <i>J</i> 8.1 Hz	102.7	4.68/ <i>J</i> 8.3 Hz	102.0	4.73	102.9
	2	3.88	54.8	3.85	54.6	3.89	55.3
	3	3.78	82.2	3.69	81.6	3.80	82.7
	4	3.56	68.6	3.53	68.5		
	5	3.48	75.4	3.43	75.2	3.45	75.9
	6	3.91	60.8	3.93	60.1		
	6'	3.91		3.93			
Glc	1	4.87/ <i>J</i> 7.6 Hz	102.0	4.48/ <i>J</i> 8.3 Hz	102.9	4.90	103.3
	2	3.23	73.6	3.28	72.5	3.28	74.2
	3	3.51	75.9	3.59	72.8	3.55	75.2
	4	3.36	69.8	3.59	78.4	3.60	69.4
	5	3.42	75.6	3.65	74.9	3.66	75.4
	6	3.87	60.8	3.94	60.0		
	6'	3.77		3.76			
Gal'	1	4.47/ <i>J</i> 8.1 Hz	102.9	4.41/ <i>J</i> 7.5 Hz	102.5	4.49	102.3
	2	3.51	69.0	3.50	68.9		
	3	4.07	75.5	4.04	75.5	4.06	73.4
	4	3.92	67.3	3.89	67.3	3.91	68.2
	5	3.70	74.2	3.67	74.8	3.64	75.5
	6	3.71	61.0	3.68	60.8		
	6'	3.71		3.68			
Neu5Ac	3	2.73	39.7	2.72	39.7	2.74	40.9
	3'	1.75		1.75		1.78	
	4	3.66	68.4	3.63	68.3		
	5	3.82	51.7	3.80	51.5	3.82	52.4
	6	3.61	72.8	3.59	74.5	3.59	73.5
	7	3.58	68.1	3.54	68.2		
	8	3.86	71.9	3.83	71.6	3.86	72.3
	9	3.83	62.6	3.80	62.4	3.83	63.3
	9'	3.62		3.60		3.62	

[a]. Not assigned peaks are due to overlapping in the HSQC spectrum of the polysaccharide

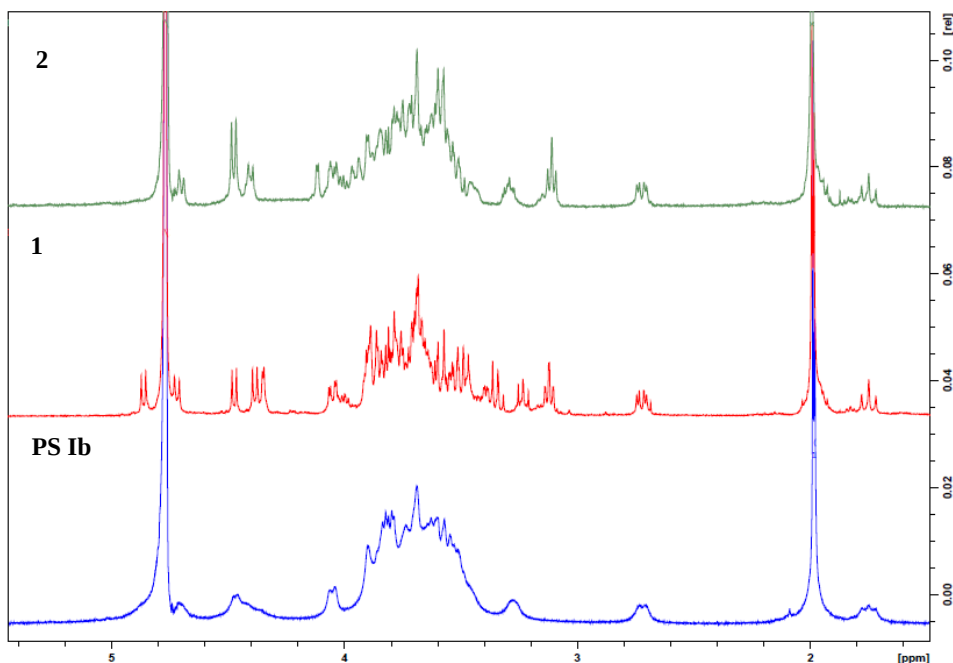


Figure 3. ^1H NMR spectra of pentasaccharides **1–2** in comparison to PS Ib (D_2O , 400 MHz, 298 K)

Conformational studies

Having access to structurally defined GBS type Ib related glycans is a key step forward to investigate the structural-immunogenicity relationship of the capsular polysaccharide and to gain insights into its conformation and understanding how the conformational behaviour of type Ib polysaccharide could influence the exposure of different glycotopes compared to the type Ia polysaccharide. Considering that Ia and Ib CPS differ exclusively in the connection of a Gal residues to the GlcNAc-Gal, which is β -(1 $\rightarrow$ 4) in type Ia and β -(1 $\rightarrow$ 3) in type Ib, this linkage appears to be crucial for the immunospecificity^{11, 19}.

To elucidate the impact of this linkage on the shape of the polysaccharides, conformational properties of the branched repeating unit pentasaccharides **1** were studied by a combination of NMR and modelling tools²⁰, to build up a model of the natural CPS. This model was then compared to the one of GBS type Ia (see Chapter 4). These studies revealed a different preferential shape for Ib polysaccharide compared to Ia was revealed; in particular, the main difference highlighted regards the presentation of the protruding Neu5Ac- α -(2 $\rightarrow$ 3)-Gal moieties, with a major *exo-anti- Φ* population for Ia and *exo-syn- Φ* for Ib, which results in a higher flexibility of the Ib polymer (Figure 5 and 6). These unique structural features are expected to influence antibody recognition and immunospecificity.

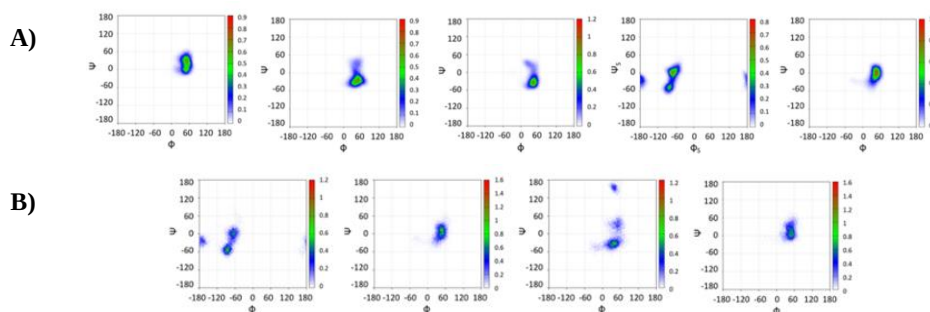


Figure 4. A) Glycosidic linkage analysis for GBS Ia polysaccharide: ϕ/ψ plots for representative glycosidic bonds of a 10 repeating unit model along the 2.5 μ s MD simulation B) Glycosidic linkage analysis for GBS Ia pentasaccharide 1: ϕ/ψ plots for representative glycosidic bonds along 200 ns of MD simulation.

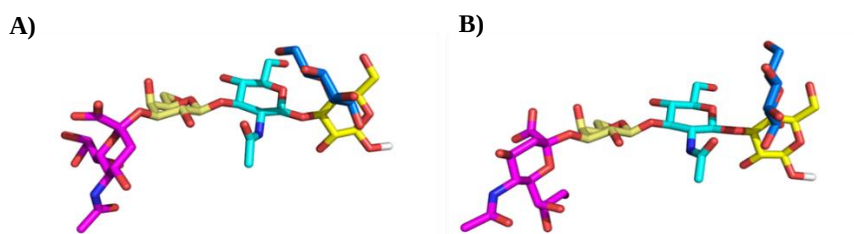


Figure 5. Perspectives of the two populations, deduced by ROESY NMR experiments, which define the conformational behaviour of the pentasaccharide repeating unit of GBS Ib. A) the ϕ_s torsion angle adopts the major trans (t) geometry. B) the ϕ_s torsion angle shows the minor -g conformation.

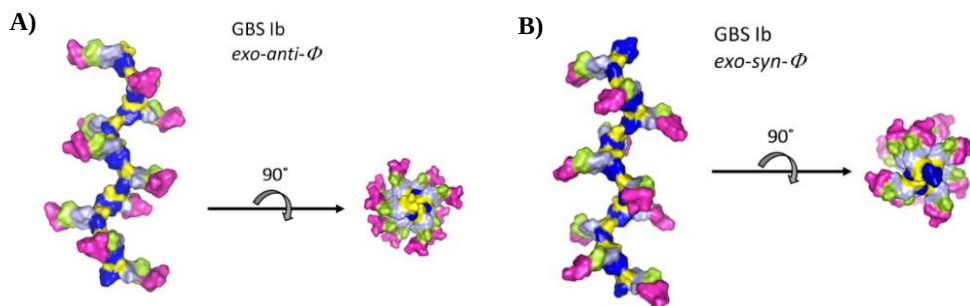


Figure 6. Model structures for the GBS Ib polysaccharide: A) with exo-anti- Φ conformation around all Neu5Aca2-3Gal linkages and B) Ib with the major exo-syn- Φ conformation around all Neu5Aca2-3Gal linkages.

Conclusion

This Chapter describes the application of regioselective strategies to the synthesis of Group B streptococcus serotype Ib repeating unit, which can be represented both by a linear and a branched pentasaccharide. The synthetic design was based on a [2+3] glycosylation strategy employing a sialogalactoside donor and a trisaccharide acceptor, which in turn was obtained by a regioselective

β -(1 $\rightarrow$ 3)-glycosylation of galactose and lactose for the branched and the linear repeating unit, respectively.

Pentasaccharides **1** and **2** helped to investigate the conformational behaviour of the corresponding capsular polysaccharide, especially compared to the structurally close GBS serotype Ia CPS. Indeed, Ia and Ib CPS differ exclusively in the connection of a Gal residues to the GlcNAc-Gal but despite these similarities, no immune cross reactivity was observed between the two serotypes^{11, 19}.

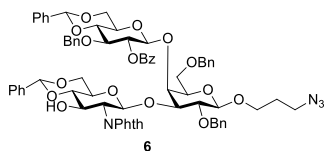
Thanks to a combination of NMR and computational tools²⁰, the branched repeating unit **1** was used as probe to build a model to of the natural CPS. On the basis of this model, a higher flexibility of the Ib polymer compared to the Ia was revealed, which can result in the exposure of different epitopes.

The synthetic glycans **1** and **2** will be used to map the relevant glycotopes by a combined approach including competitive ELISA, SPR and STD-NMR. Moreover, the presence of the aminopropyl handle will be exploited for conjugation to carrier proteins and immunological evaluation of the resulting glycoconjugates.

Experimental

General Methods and Procedures. Reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 F254 (Sigma Aldrich); after exam under UV light, compounds were visualized by heating with 10% (v/v) ethanolic H_2SO_4 . In the work up procedures, organic solutions were washed with the amounts of the indicated aqueous solutions, then dried with anhydrous Na_2SO_4 , and concentrated under reduced pressure at 30–50°C on a water bath. Column chromatography was performed on Silica Gel 60 (Sigma Aldrich, 0.040–0.063 nm) or using pre-packed silica cartridges RediSep (Teledyne-Isco, 0.040–0.063 nm) or Biotage SNAP Ultra (Biotage, silica 0.050 nm). Unless otherwise specified, a gradient 0–100% of the elution mixture was applied in a Combiflash Rf (Teledyne-Isco) or Biotage Isolera instrument. Solvent mixtures less polar than those used for TLC were used at the onset of separation. ^1H NMR spectra were measured at 400 MHz and 298 K with a Bruker AvanceIII 400 spectrometer; ^1H values are reported in ppm, relative to internal Me_4Si ($^1\text{H} = 0.00$, CDCl_3); solvent peak for D_2O was calibrated at 4.79 ppm. ^{13}C NMR spectra were measured at 100 MHz and 298 K with a Bruker AvanceIII 400 spectrometer; ^{13}C values are reported in ppm relative to the signal of CDCl_3 ($^{13}\text{C} = 77.0$, CDCl_3). Assignments of NMR signals were made by homonuclear and heteronuclear 2-dimensional correlation spectroscopy, run with the software supplied with the spectrometer. Assignment of ^{13}C NMR spectra of some compounds was aided by comparison with spectra of related substances reported previously from this laboratory or elsewhere. When reporting assignments of NMR signals, sugar residues in oligosaccharides are indicated with capital letters. Exact masses were measured by electron spray ionization cut-off spectroscopy, using a Q-Tof micro Macromass (Waters) instrument. Structures of these compounds follow unequivocally from the mode of synthesis, NMR data and m/z values found in their mass spectra.

3-Azidopropyl 3-O-benzyl-4,6-O-benzylidene-2-O-benzoyl- β -D-glucopyranosyl-(1-4)-[4,6-O-benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranosyl-(1-3)]-2,6-di-O-benzoyl- β -D-galactopyranoside 6.



Compound **4** (41 mg, 0.08 mmol) and **3** (62 mg, 0.06 mmol) were dissolved in dry DCM (4 mL) with 4 Å activated molecular sieves and the mixture was stirred for 15 min under nitrogen. NIS (36 mg, 0.16 mmol) and TFOH (1.7 mg, 0.18 mmol) were added at -40°C, and the reaction was stirred overnight at rt, when TLC (7:3 Tol:EtOAc) showed complete reaction. The

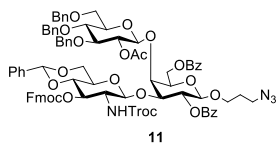
reaction was quenched with TEA, molecular sieves were filtered off and the solvent was removed at reduced pressure. The crude was purified by flash chromatography (Tol: EtOAc) to afford **5** (73 mg, 81 % yield).

Trisaccharide **5** (73 mg, 0.05 mmol) was dissolved in dry DCM (4 mL) and 10% of piperidine (0.4 mL) were added at the solution. 10 min later, TLC (Tol:EtOAc) showed complete conversion, and the reaction was concentrated under reduced pressure. Purification of the crude material by flash chromatography (Tol:EtOAc) gave **6** (57 mg) in 90% yield. $[\alpha]_{\text{D}}^{25} = -7.89^\circ$ (c 0.05, CHCl_3). ESI HR-MS ($\text{C}_{62}\text{H}_{59}\text{N}_4\text{O}_{17}$) m/z $[\text{M}+\text{H}]^+$ found 1267.3391; calcd 1267.3315.

^1H NMR (400 MHz, CDCl_3) δ 7.86–6.63 (m, 34 H, H-Ar), 5.55 (s, 1H, CHPh), 5.52 (s, 1H, CHPh), 5.43 (d, $J_{1,2} = 8.4$ Hz, 1H, H-1^B), 5.40 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1^C), 5.24 (t, $J = 7.9$ Hz, 1H, H-2^C), 4.87 (d, $^2J = 12.7$ Hz, 1H, CHHPh), 4.73 (d, $^2J = 12.7$ Hz, 1H, CHHPh), 4.57 (t, $J = 9.4$ Hz, 1H H-3^B), 4.47 (s, 2H, CH_2Ph), 4.31–4.21 (m, 5H, H-2^B, H-4^A, H-6a^A, CH_2Ph), 4.10 (t, $J = 8.7$ Hz, 1H, H-3^C), 4.02 (d, $J = 7.7$ Hz, 1H, H-1^A), 3.79 (t, $J = 9.2$ Hz, 1H, H-4^C), 3.72–3.51 (m, 10H, H-3^A, H-4^B, H-5^B, H-5^C, H-6b^A, H-6^B, H-6^C OCH_{2a}), 3.44 (dd, $J_{4,5} = 2.5$, $J_{5,6a} = 8.8$ Hz, 1H, H-5^A), 3.22–3.16 (m, 1H, OCH_{2b}), 2.98–2.86 (m, 3H, H-2^A, CH_2N_3), 1.54–1.41 (m, 2H, $\text{CH}_2\text{CH}_2\text{N}_3$).

^{13}C NMR (101 MHz, CDCl_3) δ 165.0 (C=O), 134.1–123.4 (C-Ar), 103.3 (C-1^A), 102.02 (CHPh), 101.4 (CHPh), 100.4 (C-1^B), 99.9 (C-1^C), 82.1, 81.8, 78.9, 78.3, 74.2, 73.8, 73.7, 73.6, 72.8, 72.57, 69.0, 68.9, 68.6, 68.3, 66.4, 66.1, 65.7, 57.0 (C-2^C), 48.1 (CH_2N_3), 29.0 ($\text{CH}_2\text{CH}_2\text{N}_3$).

3-Azidopropyl 2-O-acetyl-3,4,6-tri-O-benzyl- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-[4,6-O-benzylidene-3-O-(9H-fluoren-9-ylmethylcarbonate)-2-deoxy-2-((2,2,2-trichloroethoxycarbonyl)amino)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)-2,6-di-O-benzoyl- β -D-galactopyranoside 11.

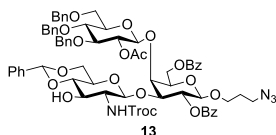


A solution of trichloroacetimidate donor **10** (50 mg, 0.078 mmol) and acceptor **8** (0.073 g, 0.065 mmol) with 4 Å molecular sieves (100 mg) in dry DCM (5.0 mL) was stirred for 20 min under nitrogen. TMSOTf (2.4 μ L, 0.013) was added at -20°C. After 4 h (TLC; 4:1 Tol: EtOAc) the reaction was quenched with TEA, the solid filtered off and the solvent removed under reduced pressure. The crude was purified by flash chromatography (Tol:EtOAc) to afford trisaccharide **11** in 63% yield (0.130 g). $[\alpha]_D^{25} = +16.32^\circ$ (c 0.25, CHCl₃). ESI HR-MS (C₈₅H₈₁Cl₃N₄O₂₂) m/z ($[M + Na]^+$) found 1613.4491; calcd 1613.4306.

¹H NMR (400 MHz, CDCl₃) δ 8.17-7.08 (m, 38H, H-Ar), 5.55 (s, 1H, CHPh), 5.40 (dd, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 10.1$, 1H, H-2^A), 5.34 (t, $J = 10.1$, 1H, H-2^C), 5.06 (d, $J_{1,2} = 8.1$ Hz, 1H, H-1^B), 5.00 (d, $J = 8.2$ Hz, 1H, H-1^C), 4.96 (t, $J = 8.7$ Hz, 1H, H-2^C), 4.91-4.78 (m, 3H, CH₂Ph, CHHPh), 4.74 (dd, 1H, $J = 12.2$ Hz, $J = 4.2$ Hz, CHHCCl₃), 4.62 (d, $J = 10.7$ Hz, 1H, CHHPh), 4.59-4.48 (m, 4H, incl. H-1^A, CHHCCl₃, CH₂Ph), 4.40-4.33 (m, 4H, incl. CH₂^{Fmoc}, H-4^A, H-6a^B), 4.33-4.26 (m, 1H, H-6a^A), 4.26-4.16 (m, 1H, CH^{Fmoc}), 4.01-3.8 (m, 5H, incl. H-3^C, H-3^A, H-6b^A, H-4^C, OCH_{2a}), 3.8-3.6 (m, 5H, incl. H-6b^B, 2H-6^C, H-4^B, H-5^A, OCH_{2a}), 3.62-3.40 (m, 5H, incl. H-2^B, OCH_{2b}, H-5^C, H-5^B), 3.30-3.08 (m, 2H, CH₂N₃), 2.24 (s, 3H, CH₃CO), 1.86-1.64 (m, 2H, CH₂CH₂N₃).

¹³C NMR (101 MHz, CDCl₃) δ 170.5, 166.4, 164.9, 154.7, 153.5 (5 x CO), 143.2-120.0 (C-Ar), 102.1 (C-1^C), 101.6 (CHPh), 101.1 (C-1^A), 100.0 (C-1^B), 82.6 (C-3^C), 80.3 (C-3^A), 78.7 (C-5^A), 78.2 (C-4^B), 75.3 (C-5^B), 75.2, 74.9 (2 x CH₂Ph), 74.2 (C-4^A), 74.1 (C-3^B), 74.0 (C-4^A), 73.7 (C-2^C), 73.5 (CH₂Ph), 72.3 (C-4^C), 70.8 (C-2^A), 70.3 (CH₂^{Fmoc}), 69.2 (C-6^B), 68.4 (C-6^C), 66.2 (C-5^C), 65.6 (OCH₂), 64.5 (CH₂CCl₃), 57.5 (C-2^B), 48.0 (CH₂N₃), 46.5 (CH^{Fmoc}), 29.0 (CH₂CH₂N₃), 21.2 (CH₃ CO).

3-Azidopropyl 2-O-acetyl-3,4,6-tri-O-benzyl- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-[4,6-O-benzylidene-2-deoxy-2-((2,2,2-trichloroethoxycarbonyl)amino)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)]-2,6-di-O-benzoyl- β -D-galactopyranoside 13.

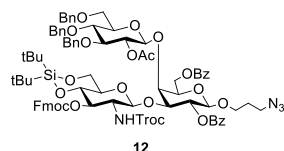


Trisaccharide **11** was (60 mg, 0.038 mmol) was dissolved in 2.0 mL of dry DCM and piperidine (0.2 mL) was added. After 1 h (TLC 4:1 Tol:EtOAc) the solvent was evaporated under reduced pressure and the crude was purified by flash chromatography (Tol:EtOAc) affording compound **13** (48 mg, 92% yield). $[\alpha]_D^{25} = -59.72^\circ$ (c 0.155, CHCl₃). ESI HR-MS (C₆₈H₇₁Cl₃N₄O₂₀) $m/z = ([M + Na]^+)$ found 1391.2691; calcd 1391.2695.

¹H NMR (400 MHz, CDCl₃) δ 8.12-7.01 (m, 30H, Ar-H), 5.54 (s, 1H, CHPh), 5.35 (dd, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 10.1$ Hz, 1H, H-2^A), 5.00-4.86 (m, 3H, incl. H-1^C, H-2^C, H-1^B), 4.85-4.67 (m, 4H, incl. CH₂Ph, CHHPh, CHHCCl₃), 4.61-4.43 (m, 5H, incl. H-1^A, 3 x CHHPh, CHHCCl₃), 4.43-4.34 (m, 1H, H-6a^A), 4.34-4.26 (m, 2H, incl. H-4^A, H-6a^B), 4.15 (t, 1H, $J=8.99$ Hz, H-3^B), 3.95-3.81 (m, 4H, incl. H-3^C, H-3^A, H-4^C, OCH_{2a}), 3.75-3.60 (m, 5H, incl. H-5^C, H-6^C, H-6b^A, H-6b^B), 3.57-3.38 (m, 4H, incl. H-5^A, H-4^B, H-5^B, OCH_{2b}), 3.25-3.07 (m, 3H, incl. H-2^B, CH₂N₃), 2.18 (s, 3H, CH₃CO), 1.80-1.57 (m, 2H, CH₂CH₂N₃).

¹³C NMR (101 MHz, CDCl₃) δ 170.4, 166.4, 165.1 (3 x CO), 138.3-126.3 (C-Ar), 101.9 (CHPh), 101.8 (C-1^B), 101.1 (C-1^A), 100.3 (C-1^C), 82.6 (C-3^C), 81.4 (C-5^A), 80.0 (C-3^A), 78.1 (C-5^C), 75.3 (C-4^B), 75.2, 75.00 (2 x CH₂Ph), 74.6 (C-4^A), 74.0 (C-2^C), 73.8 (CH₂Ph), 73.5 (C-6^A), 72.3 (C-4^C), 71.0 (C-2^A), 69.7 (C-3^B), 69.1 (C-6^C), 68.5 (C-6^B), 66.1 (C-5^B), 65.6 (OCH₂), 64.5 (CH₂CCl₃), 59.4 (C-2^B), 47.9 (CH₂N₃), 29.0 (CH₂CH₂N₃), 21.1 (COCH₃).

3-Azidopropyl 2-O-acetyl-3,4,6-tri-O-benzyl- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-[4,6-O-di-*tert*-butylsilylidene-3-O-(9H-fluoren-9-ylmethyl carbonate)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)]-2,6-di-O-benzoyl- β -D-galactopyranoside 12.

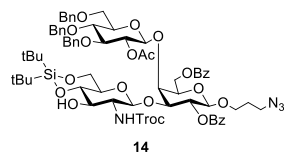


A solution of acceptor **9** (175 mg, 0.150 mmol) and donor **10** (190 mg, 0.300 mmol) in dry DCM (5.0 mL) with 4 Å molecular sieves was stirred for 20 min under nitrogen atmosphere. The resulting suspension was then cooled down to 0° C and TMSOTf (5.4 μ L, 0.03 mmol) was added. The reaction was stirred for 2 h and left to slowly reach rt. After 2 h (TLC 9:1 Tol/EtOAc) the reaction was quenched by addition of TEA, then evaporated to dryness and the crude purified by medium pressure column chromatography using a gradient from 0 to 70% of EtOAc in toluene. Pure fractions were collected and evaporated under reduced pressure affording the target compound **12** 173 mg, 70% yield) as a clear syrup. $[\alpha]_D^{25} = -17.01^\circ$ (c 0.4, CHCl₃). ESI HR-MS (C₈₄H₉₃Cl₃N₄O₂₂Si) m/z ([M+ Na]⁺ found 1665.4995; calcd 1665.5009.

¹H NMR (400 MHz, CDCl₃) δ 8.08-7.28 (m, 33H, Ar-H); 5.36 (dt, $J_{2,3} = 10.0$ Hz, $J_{3,4} = 7.9$ Hz, 1H, H-3^A), 5.09 (t, 1H, $J = 9.9$ Hz, H-3^B), 5.01 (d, $J_{N,H} = 7.5$ Hz, 1H, NH), 4.97-4.88 (m, 3H, H-1^B, H-1^C, H-2^C), 4.86-4.73 (m, 3H, CH₂CCl₃, CHHPh), 4.69 (dd, $J_{5,6a} = 4.2$ Hz, $J_{6a,6b} = 12.1$, Hz, 1H, H-6a^A), 4.62-4.37 (m, 7H, incl. H-6a^B, CH₂Ph, H-1^A), 4.27 (d, $J_{3,4} = 2.1$ Hz, 1H, H-4^A), 4.24-4.15 (m, 4H); 3.96-3.82 (m, 7H, incl. H-4^B, OCH_{2a}), 3.73-3.62 (m, 2H, incl. OCH_{2b}), 3.56-3.38 (m, 4H, incl. H-3^C, H-2^B), 3.23-3.08 (m, 2H, CH₂N₃), 2.18 (s, 3H, CH₃CO), 1.80-1.65 (m, 2H, CH₂CH₂N₃), 1.03 (s, 9H, tBu), 0.94 (s, 9H, tBu).

¹³C NMR (101 MHz, CDCl₃) δ 170.5, 166.4, 164.9, 154.9, 153.5 (5 x CO), 143.3-120.1 (C-Ar), 101.1 (C-1^B, C-1^C), 100.2 (C-1^A), 82.5, 78.1, 75.5, 75.3, 75.2, 75.0, 74.9, 74.4 (C-4^A), 74.0, 73.7, 73.4, 72.3, 70.8 (C-3^A), 70.6, 70.3, 69.0, 66.2, 65.6 (OCH₂), 64.4 (C-6^A), 56.7 (C-2^B), 47.9 (CH₂N₃), 46.6 (CH^{Fmoc}), 29.0 (CH₂CH₂N₃) 27.4 (tBu), 26.9 (tBu), 22.6 (CH₃CO), 21.1 (C(CH₃)₃), 20.0 (C(CH₃)₃).

3-Azidopropyl 2-O-acetyl-3,4,6-tri-O-benzyl- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-[4,6-O-di-*tert*-butylsilylidene-3-O-(9H-fluoren-9-ylmethyl carbonate)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)]-2,6-di-O-benzoyl- β -D-galactopyranoside 14.

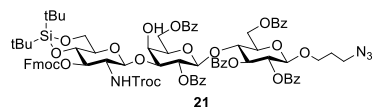


Compound **12** (173 mg, 0.105 mmol) was dissolved in 5.0 mL of DCM and 500 μ L of piperidine were added. After 30 min, analytical TLC (toluene/EtOAc 8:2) showed full consumption of the starting material. The reaction was evaporated under reduced pressure and the crude was purified by column chromatography using a gradient from 0 to 70% of EtOAc in toluene. Pure fractions were collected and evaporated to dryness, affording compound **14** as a pale solid (137 mg, 92% yield). $[\alpha]_D^{25} = -5.96$ (c 1.25, CHCl₃). ESI HR-MS (C₆₉H₈₃Cl₃N₄O₂₀Si) m/z ([M+ Na]⁺ found 1443.4331; calcd 1443.4328.

¹H NMR (400 MHz, CDCl₃) δ 8.08-7.11 (m, 25H, Ar-H), 5.33 (dd, $J_{2,3} = 7.9$ Hz, $J_{3,4} = 9.7$ Hz, 1H, H-3^A), 5.05 (d, $J_{N,H} = 7.4$ Hz, 1H, NH), 4.92-4.80 (m, 2H, H-1^C, H-2^C), 4.82 (d, $J_{1,2} = 8.2$ Hz, 1H, H-1^B), 4.80-4.68 (m, 3H, CH₂CCl₃, CHHPh), 4.65 (dd, $J_{5,6a} = 4.2$ Hz, $J_{6a,6b} = 12.1$, Hz, 1H, H-6a^A), 4.57-4.39 (m, 6H, incl. H-1^A, H-6b^A, CH₂Ph), 4.20 (d, $J_{3,4} = 2.3$ Hz, 1H, H-4^A), 4.14 (dd, 1H, $J = 10.0$, 4.9 Hz), 3.91-3.75 (m, 7H, incl. CHHN₃, H-3^A, H-3^C, H-3^B), 3.70-3.57 (m, 4H), 3.53-3.44 (m, 1H), 3.44-3.34 (m, 2H), 3.20-3.06 (m, 3H, H-2^B, CH₂N₃), 2.14 (s, 3H, CH₃CO), 1.78-1.59 (m, 2H, CH₂CH₂N₃), 1.04 (s, 9H, tBu), 0.95 (s, 9H, tBu).

¹³C NMR (101 MHz, CDCl₃) δ 170.4, 166.4, 165.0, 153.9 (4 x CO), 138.3-127.5 (C-Ar), 101.5 (C-1^B), 101.1 (C-1^A), 100.4 (C-1^C), 82.6, 79.7, 78.1, 77.7, 75.2, 75.0, 74.9, 74.6 (C-4^A), 73.9 (C-2^C), 73.5, 72.8, 72.3, 71.0 (C-2A), 70.4, 69.0, 66.2 (OCH₂), 65.5 (C-6^B), 64.4 (C-6A), 58.3 (C-2^B), 48.0 (CH₂N₃), 29.0 (CH₂CH₂N₃), 27.5 (tBu), 27.0 (tBu), 22.7 (C(CH₃)₃), 21.1 (CH₃CO), 20.0 (C(CH₃)₃).

3-Azidopropyl 4,6-O-di-*tert*-butylsilylidene-3-O-deoxy-(9H-fluoren-9-ylmethylcarbonate)-2-(2,2,2-trichloroethoxycarbonylamino)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)-2,6-di-O-benzoyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzoyl- β -D-glucopyranoside 21.

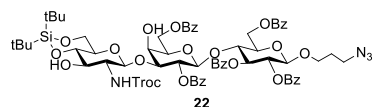


A solution of don trifluoroacetimidate donor **19** (0.337 g, 0.380 mmol) and acceptor **20** (200 mg, 0.211 mmol) in dry DCM (5.0 mL) in presence of 4 Å molecular sieves was stirred under nitrogen atmosphere for 20 min.

The reaction mixture was cooled down to -10°C and TMSOTf (7.6 μL , 0.0422 mmol) was added dropwise. The resulting reaction mixture was stirred and allowed slowly to reach 5°C , when analytical TLC (Tol/EtOAc 9:1) showed formation of a new spot with intermediate. The reaction mixture was evaporated under reduced pressure and the crude was purified by medium pressure column chromatography using a gradient from 0 to 30% of EtOAc in toluene. Pure fractions were collected and evaporated under reduced pressure affording compound **21** as a pale solid (190 mg, 55% yield).

$[\alpha]_{\text{D}}^{25} = -12.99^{\circ}$ (c 0.15, CHCl_3). ESI HR-MS ($\text{C}_{82}\text{H}_{85}\text{Cl}_3\text{N}_4\text{O}_{24}\text{Si}$) m/z ($[M + \text{Na}]^+$ found 1665.4262; calcd 1665.4281. ^1H NMR (400 MHz, CDCl_3) δ 8.14-7.20 (m, 33H, Ar-H), 5.68 (t, $J = 9.2$ Hz, 1H, H-3^A), 5.42-5.33 (m, 2H, H-2^A, H-2^B), 4.97-4.85 (m, 2H, incl. H-3^C), 4.77 (d, $J = 7.8$ Hz, 1H, H-1^C), 4.59 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1^B), 4.54 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1^A), 4.51-4.45 (m, 1H), 4.42-4.24 (m, 3H), 4.22-4.07 (m, 4H, incl. H-4^A), 4.07-3.98 (m, 2H), 3.91-3.77 (m, 4H), 3.77-3.69 (m, 2H, incl. H-3^B), 3.64 (dd, $J = 11.1, 7.1$ Hz, 1H), 3.54-3.32 (m, 4H, incl. H-2^C), 3.21-3.08 (m, 2H, CH_2N_3), 1.79-1.56 (m, 2H, $\text{CH}_2\text{CH}_2\text{N}_3$), 0.97 (s, 9H, tBu), 0.87 (s, 9H, tBu). ^{13}C NMR (101 MHz, CDCl_3) δ 166.0, 165.5, 165.2, 164.6, 155.0 (5 x CO), 143.3-120.0 (C-Ar), 101.1 (C-1^C), 100.6 (C-1^B), 100.0 (C-1^A), 77.2, 75.5, 74.0, 73.8, 73.0, 72.6 (H-3^A), 72.2, 71.8, 71.0, 70.5, 70.3, 68.2, 66.6, 65.9, 62.5, 56.5 (H-2^C), 47.8 (CH_2N_3), 46.5 (CH^{Fmoc}), 28.9 ($\text{CH}_2\text{CH}_2\text{N}_3$), 27.3 (tBu), 26.8 (tBu), 22.6 ($\text{C}(\text{CH}_3)_3$), 19.9 ($\text{C}(\text{CH}_3)_3$).

3-Azidopropyl 4,6-O-di-*tert*-butylsilylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)-2,6-di-O-benzoyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzoyl- β -D-glucopyranoside 22.

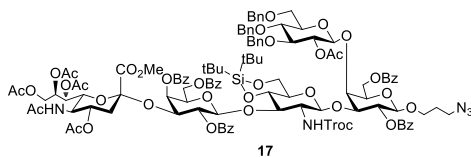


Compound **21** (140 mg, 0.085 mmol) was dissolved in 5.0 mL of DCM and 500 mL of piperidine were added. The resulting reaction mixture was stirred for 30 min at rt, when analytical TLC (Tol/EtOAc 85:15) showed full consumption of the starting material. The reaction

mixture was evaporated under reduced pressure and the crude was purified by medium pressure column chromatography using a gradient from 0 to 80% of EtOAc in toluene. Pure fractions were collected and evaporated under reduced pressure affording compound **22** a pale solid (110 mg, 90% yield). $[\alpha]_{\text{D}}^{25} = -13.62^{\circ}$ (c 0.2, CHCl_3). ESI HR-MS ($\text{C}_{82}\text{H}_{85}\text{Cl}_3\text{N}_4\text{O}_{24}\text{Si}$) m/z ($[M + \text{Na}]^+$ found 1443.3568; calcd 1443.3600.

^1H NMR (400 MHz, CDCl_3) δ 8.08-7.21 (m, 25H, Ar-H); 5.68 (t, $J = 9.3$ Hz, 1H, H-3^A), 5.42-5.32 (m, 2H, H-2^A, H-2^B), 5.02 (bs, 1H, NH), 4.75 (d, 1H, $J_{1,2} = 7.3$ Hz, H-1^C), 4.58 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1^B), 4.54 (d, $J_{1,2} = 7.90$ Hz, 1H, H-1^A), 4.43-4.35 (m, 2H, CHHCCl_3), 4.17-4.06 (m, 2H, H-4^A), 4.03-3.94 (m, 2H), 3.91-3.66 (m, 6H, incl. H-3^{B,C}), 3.66-3.54 (m, 2H), 3.54-3.42 (m, 2H), 3.28 (dt, 1H, $J = 9.7, 5.0$ Hz), 3.20-3.09 (m, 3H, CH_2N_3 , H-2^C), 1.79-1.62 (m, 2H, $\text{CH}_2\text{CH}_2\text{N}_3$), 0.99 (s, 9H, tBu), 0.90 (s, 9H, tBu). ^{13}C NMR (101 MHz, CDCl_3) δ 166.0, 165.9, 165.6, 165.3, 164.7 (5 x CO), 133.5-128.2 (C-Ar), 101.1 (C-1^B), 100.6 (C-1^C), 100.0 (C-1^A), 79.9, 77.3, 75.6, 73.9, 73.1, 72.6 (C-3A), 72.3, 71.8, 71.2, 70.3, 68.3, 66.6, 65.9, 62.5, 57.8 (C-2C), 47.8 (CH_2N_3), 28.9 ($\text{CH}_2\text{CH}_2\text{N}_3$), 27.4 (tBu), 26.9 (tBu), 22.6 ($\text{C}(\text{CH}_3)_3$), 19.9 ($\text{C}(\text{CH}_3)_3$).

3-Azidopropyl 2-O-acetyl-3,4,6-tri-O-benzyl- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-[2,4,6-tri-O-benzoyl-3-O-(methyl 4,7,8,9-tetra-O-acetyl-5-N-acetamido-3,5-dideoxy-D-glycero- α -D-galacto-non-2-ulopyranosylonate)- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-4,6-O-di-*tert*-butylsilylidene-3-O-(9H-fluoren-9-ylmethylcarbonate)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)]-2,6-di-O-benzoyl- β -D-galactopyranoside 17.

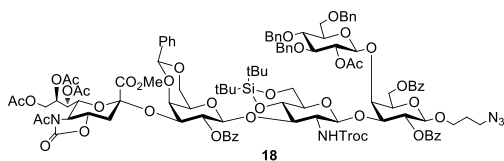


A solution of trisaccharide acceptor **14** (114 mg, 0.080 mmol) and disaccharide donor **7** (135 mg, 0.120 mmol) with 4 Å molecular sieves in dry DCM (3.0 mL) was stirred for 20 min. TMSOTf (2.9 μ L, 0.016 mmol) was added at 0°C and the reaction was stirred for 2 h and allowed to slowly reach rt. After 2 h (TLC 3:2 Tol/Acetone) the reaction was quenched by addition of TEA, and the crude purified by column chromatography using a gradient from 0 to 80% of acetone in toluene. Pure fractions were collected and evaporated affording **17** (150 mg, 80% yield). $[\alpha]_D^{25} = -28.20^\circ$ (c 0.1, CHCl_3). ESI HR-MS ($\text{C}_{116}\text{H}_{132}\text{Cl}_3\text{N}_5\text{O}_{40}\text{Si}$) m/z $[\text{M}+\text{Na}]^+$ found 2390.7193, calcd 2390.7176.

^1H NMR (400 MHz, ACN-d_3) δ 8.13-7.12 (m, 40H, Ar-H), 5.97 (d, $J_{\text{N,H}} = 9.8$ Hz, 1H, NH), 5.60-5.51 (m, 2H), 5.45-5.38 (m, 1H), 5.31-5.14 (m, 3H, H-1^C, H-2^{C,D}), 5.08 (t, $J = 9.0$ Hz, 1H, H-2^A), 4.85 (d, $J_{1,2} = 7.5$ Hz, 1H, H-1^D), 4.80-4.59 (m, 6H, incl. H-1^B, H-3^B), 4.58-4.40 (m, 6H, incl. H-1^A), 4.40-4.31 (m, 3H), 4.24 (bs, 1H), 4.22-3.98 (m, 7H), 3.9 (dd, $J = 9.4, 2.0$ Hz, 1H), 3.91-3.81 (m, 2H), 3.81-3.79 (m, 4H, incl. H-2^B), 3.66-3.58 (m, 4H, incl. COOCH_3), 3.58-3.49 (m, 3H), 3.48-3.39 (m, 2H), 3.39-3.32 (m, 1H), 3.14-2.98 (m, 2H, OCH_2), 2.24 (dd, $J_{3e,4} = 4.5$ Hz, $J_{3a,3e} = 12.2$ Hz, 1H, H-3^E), 2.01 (s, 3H, CH_3CO), 1.96 (s, 6H, 2 x CH_3CO), 1.78 (s, 3H, CH_3CO), 1.69-1.56 (m, 8H, 2 x CH_3CO , $\text{CH}_2\text{CH}_2\text{N}_3$), 1.35 (t, 1H, H-3^{aE}), 1.04 (s, 9H, tBu), 0.87 (s, 9H, tBu).

^{13}C -NMR (101 MHz, ACN-d_3) δ 170.5, 170.4, 169.9, 169.8, 169.7, 169.5, 165.9, 165.7, 165.6, 165.1, 165.0, 153.7 (13 x CO), 138.6-127.4 (C-Ar), 102.7 (C_q), 101.3 (C-1^B), 100.6 (C-1^A), 98.9 (C-1^D), 97.3 (C-1^C), 95.8 (C_q), 82.4, 80.2, 78.2, 78.0, 75.3, 74.7, 74.5, 74.4, 73.5, 73.2, 72.9, 72.0, 71.8, 71.4, 70.9, 70.8, 70.4, 69.1, 68.9, 67.5, 66.6, 66.1, 63.9, 62.4, 61.9, 58.0, 52.7 (COOCH_3), 48.2, 47.6 (CH_2N_3), 37.4 (C-3^E), 28.6 ($\text{CH}_2\text{CH}_2\text{N}_3$), 27.0 (tBu), 26.4 (tBu), 22.2, 22.1, 20.7 (3 x CH_3CO), 20.5 ($\text{C}(\text{CH}_3)_3$), 20.2, 20.1, 20.0 (3 x CH_3CO), 19.5 ($\text{C}(\text{CH}_3)_3$).

3-Azidopropyl 2-O-acetyl-3,4,6-tri-O-benzyl- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-[3-O-(methyl 7,8,9-tri-O-acetyl-5-N-acetoamido,4-O-oxazolidinone-3,5-dideoxy-D-glycero- α -D-galacto-non-2-ulopyranosylonate)- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-4,6-O-di-*tert*-butylsilylidene-3-O-(9H-fluoren-9-ylmethylcarbonate)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)]-2,6-di-O-benzoyl- β -D-galactopyranoside 18.



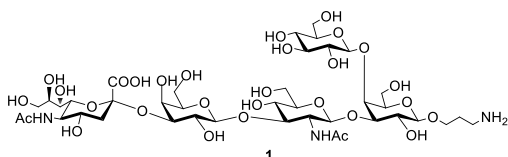
A solution of donor **15** (0.050 g, 0.053 mmol) and acceptor **14** (0.050 g, 0.035 mmol) with 4 Å molecular sieves in dry DCM (3.0 mL) was stirred for 20 min under nitrogen. N-iodosuccinimide (0.016 g, 0.074 mmol) and TfOH (0.6 μ L, 0.007 mmol) were added at -40°C. After 3 h (TLC 7:3 Tol/Acetone) the reaction was quenched with TEA, the solid was filtered off and the solvent removed under reduced pressure. The crude was purified by flash chromatography (Tol:Acetone) to afford pentasaccharide **47** in 65% yield (0.053 g). $[\alpha]_D^{25} = +9.7^\circ$ (c 1.6, CHCl_3). ESI HR-MS ($\text{C}_{108}\text{H}_{124}\text{Cl}_3\text{N}_5\text{O}_{38}\text{Si}$) m/z $[\text{M}+\text{Na}]^+$ found 2254.6687, calcd 2254.6651.

^1H NMR (400 MHz, ACN-d_3) δ 8.13-7.31 (m, 35H, Ar-H), 5.90 (d, $J = 9.0$ Hz, 1H), 5.61 (s, 1H, CHPh), 5.47-5.40 (m, 1H), 5.29 (d, $J_{\text{N,H}} = 8.2$ Hz, 1H, NH), 5.24 (dd, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 9.9$ Hz, 1H, H-2^D), 5.11 (dd, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 10.0$ Hz, 1H, H-2^A), 5.01 (d, $J_{1,2} = 7.7$ Hz, 1H, H-1^D), 4.98 (d, $J_{1,2} = 8.3$ Hz, 1H, H-1^B), 4.92 (d, $J_{1,2} = 8.1$ Hz, 1H, H-1^C), 4.87-4.80 (m, 3H), 4.67 (t, $J = 10.7$ Hz, 2H), 4.62 (d, $J_{1,2} = 9.6$ Hz, 1H), 4.58 (d, $J = 5.4$ Hz, 1H), 4.56-4.51 (m, 3H), 4.47 (d, $J = 12.0$ Hz, 1H), 4.42 (dd, $J = 12.3, 2.8$ Hz, 1H), 4.38 (d, $J = 12.3$ Hz, 1H), 4.35-4.30 (m, 2H),

4.30-4.12 (m, 5H), 4.12-4.06 (m, 2H), 4.06-3.80 (m, 6H), 3.77-3.60 (m, 6H), 3.61-3.55 (m, 4H, incl. COOCH₃), 3.54-3.47 (m, 2H), 3.34 (t, *J* = 8.0 Hz, 1H, H-2^B), 3.22-3.06 (m, 2H, CH₂N₃), 2.47-2.41 (m, 1H, H-3e^F), 2.43 (s, 3H, COCH₃), 2.17 (s, 3H, COCH₃), 2.05 (s, 6H, 2 x COCH₃), 1.97 (t, 1H, *J*_{3a,3e} = 12.7 Hz, H-3a^F), 1.92 (s, 3H, COCH₃), 1.75-1.65 (m, 2H, CH₂CH₂N₃), 1.17 (s, 9H, tBu), 1.09 (s, 9H, tBu).

¹³C-NMR (101 MHz, ACN-d₃) δ 171.2, 170.6, 170.2, 169.8, 169.7, 167.8, 165.8, 165.1, 164.8, 153.8, 153.4 (11xCO), 138.7-126.6 (C-Ar), 101.8 (C-1^B), 101.2 (C-1^A), 100.8 (CHPh), 100.5 (C-1^C), 100.0 (C-1^D), 98.6 (C_q), 95.9 (C_q, CCl₃), 82.2, 80.1, 79.2, 78.1, 75.6, 75.4, 74.7, 74.6, 74.5, 74.5, 74.4, 73.5, 73.0, 72.9, 71.7, 71.1, 70.9, 70.5, 70.3, 69.0, 68.6, 68.2, 66.1, 63.8, 62.9, 58.3, 58.1 (C-2^B), 52.8 (COOCH₃), 47.6 (CH₂N₃), 34.4 (C-3^E), 28.5 (CH₂CH₂N₃), 26.9 (tBu), 26.5 (tBu), 23.9, 20.5, 20.5, 20.4 (5 x CH₃CO), 20.1 (C(CH₃)₃), 19.5 (C(CH₃)₃).

2-Aminopropyl β-D-glucopyranosyl-(1→4)-[3-O-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)]-β-D-galactopyranoside 1.



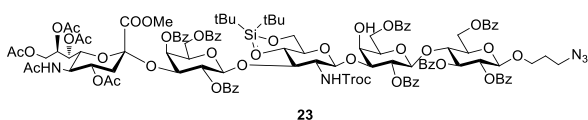
Protocol followed for both compound **17** and compound **18**: the protected pentasaccharide (0.07 mmol) was dissolved in THF (5 mL) to which 3 M NaOH (0.5 mL) was added. After refluxing for 2 d, the mixture was neutralized with 0.1% HCl and concentrated. The residue was re-dissolved in 2:3

Ac₂O-MeOH (5 mL) and stirred overnight, when C18-TLC (2:3 H₂O/MeOH) showed disappearance of the starting material. After concentration, the residue was dissolved in *t*BuOH and Pd/C (1:1 w/w in respect to the sugar) was added. The reaction mixture was stirred under pressure of H₂ (5 bar) for 72 h. Then, the catalyst was filtered off and the filtrate concentrated under reduced pressure. The reaction mixture was purified by G-10 size-exclusion column chromatography using water for elution. Fractions containing the sugar were quantified by sialic acid assay and freeze-dried to afford the deprotected oligosaccharide **3** as an amorphous powder (40% yield). [α]_D²⁵ = +1.95° (c 0.1, H₂O). ESI HR-MS (C₄₀H₆₉N₃O₂₉) *m/z* [M-H]⁺ 1054.3971; found 1054.3847.

¹H NMR (400 MHz, D₂O) δ 4.87 (d, *J*_{1,2} = 7.6 Hz, 1H, H-1^C), 4.72 (d, *J*_{1,2} = 8.1 Hz, 1H, H-1^B), 4.47 (d, *J*_{1,2} = 8.1 Hz, 1H, H-1^D), 4.38 (d, *J*_{1,2} = 7.6 Hz, 1H, H-1^A), 4.35 (d, *J*_{3,4} = 2.9 Hz, 1H, H-4^A), 4.05 (dd, 1H, *J*_{2,3} = 9.9 Hz, H-3^B), 4.03-3.97 (m, 1H), 3.94-3.44 (m, 27H), 3.41-3.30 (m, 2H), 3.23 (dd, 1H, *J*_{2,3} = 9.2 Hz, H-2^C), 3.12 (dt, *J* = 6.9, 8.2 Hz, 2H), 2.73 (dd, *J*_{3e,4} = 4.7, 12.5 Hz, 1H, H-3e^E), 2.03-1.93 (m, 2H, CH₂CH₂NH₂), 1.99 (s, 3H, CH₃CO), 1.98 (s, 3H, CH₃CO), 1.75 (t, 1H, H-3^E_{ax}).

¹³C-NMR (101 MHz, D₂O) δ 174.9, 174.8, 173.8 (3 x CO), 103.3 (C-1^A), 102.9 (C-1^D), 102.7 (C-1^C), 102.0 (C-1^B), 100.0 (C_q), 99.6 (C_q), 82.1, 81.9, 75.8, 75.6, 75.3, 75.1, 74.8, (C-4^A) 74.2, 73.6 (C-2^C), 72.8, 71.8, 70.1, 69.7, 69.0, 68.6, 68.3, 68.0, 67.9, 67.2, 62.4, 61.0, 60.8, 60.7, 60.6, 54.7, 51.6, 39.7 (C-3^E), 37.7 (CH₂N₃), 26.6 (CH₂CH₂NH₂), 22.3 (CH₃CO), 22.0 (CH₃CO). See Table 1 for full assignments.

3-Azidopropyl 2,4,6-tri-O-benzoyl-3-O-(methyl-4,7,8,9-tetra-O-acetyl-5-N-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)-β-D-galactopyranosyl-(1→3)-4,6-O-di-*tert*-butylsilylidene-3-O-(9H-fluoren-9-ylmethyl carbonate)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-2,6-di-O-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside 23.



A solution of trisaccharide acceptor **22** (95 mg, 0.067 mmol) and disaccharide donor **7** (114 mg, 0.100 mmol) with 4 Å molecular sieves (0.100 g) in dry DCM (3.0 mL) was stirred for 20 min. TMSOTf (2.4 mL,

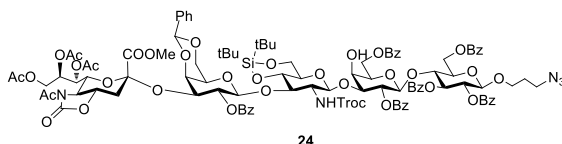
0.0134 mmol) was then added at 0°C. The reaction was stirred for 2 h and let slowly reach rt. The reaction was quenched by addition of TEA (TLC 6:4 Tol/acetone) and the crude purified by column chromatography using a

gradient from 0 to 70% of acetone in toluene. Pure fractions were collected and evaporated affording 110 mg (66% yield) of compound **23** as a pale solid. $[\alpha]_D^{25} = +11.27^\circ$ (c 0.45, CHCl_3). ESI HR-MS ($\text{C}_{114}\text{H}_{124}\text{Cl}_3\text{N}_5\text{O}_{42}\text{Si}$) m/z $[\text{M}+\text{Na}]^+$ found 2290.6468, calcd 2290.6556.

^1H NMR (400 MHz, ACN-d_3) δ 8.18–7.10 (m, 40H, Ar-H), 5.95 (t, $J = 9.3$ Hz, 1H), 5.67–5.58 (m, 2H, incl. H-3^{A}), 5.57–5.32 (m, 3H), 5.27–5.07 (m, 4H, incl. H-1^{D} , H-2^{A}), 4.86–4.65 (m, 3H, incl. H-1^{A} , H-4^{E}), 4.61 (d, 2H, incl. $J_{1,2} = 8.2$ Hz, H-1^{C}), 4.48–4.26 (m, 6H), 4.21 (t, $J = 9.5$ Hz, 1H), 4.17–4.09 (m, 2H), 4.03–3.85 (m, 5H), 3.84–3.78 (m, 1H), 3.78–3.73 (m, 3H), 3.73–3.65 (m, 3H), 3.65–3.55 (m, 4H, incl. COOCH_3), 3.53–3.45 (m, 1H, H-2^{C}), 3.43–3.28 (m, 1H), 3.10 (t, $J = 6.6$ Hz, 2H, OCH_2), 2.22 (dd, 1H, $J_{3e,4} = 4.5$ Hz, $J_{3a,3e} = 12.5$ Hz, H-3e^{E}), 2.17 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.78 (s, 3H, CH_3CO), 1.65 (s, 3H, CH_3CO), 1.67–1.59 (m, 2H, $\text{CH}_2\text{CH}_2\text{N}_3$), 1.34 (t, $J_{3a,3e} = 12.2$ Hz, 1H, H-3a^{E}), 0.99 (s, 9H, tBu), 0.82 (s, 9H, tBu).

^{13}C -NMR (101 MHz, ACN-d_3) δ 170.5, 169.9, 169.8, 169.8, 169.7, 169.5, 168.0, 165.7, 165.6, 165.5, 165.4, 165.1, 165.0, 164.9, 153.9 (15 x CO), 133.8–125.3 (C-Ar), 101.5 (C-1^{A,B}), 100.8 (C-1^C), 100.2 (C-1^D), 98.7, 97.2, 79.7, 77.6, 75.6, 74.7, 73.2, 73.0, 72.8, 72.4, 72.2, 72.1, 71.9, 71.9, 71.8, 71.4, 70.9, 70.6, 70.2, 70.0, 69.8, 69.6, 69.2, 69.1, 68.9, 68.0, 68.0, 67.9, 67.5, 67.0, 66.6, 65.8, 62.9, 62.6, 62.5, 62.0, 61.9, 61.8, 57.5, 52.7 (COOCH_3), 47.7 (CH_2N_3), 37.3 (C-3^E), 28.5, ($\text{CH}_2\text{CH}_2\text{N}_3$), 26.9 (tBu), 26.3 (tBu), 22.2 (CH_3CO), 22.1 (CH_3CO), 20.7 (CH_3CO), 20.5 ($\text{C}(\text{CH}_3)_3$), 20.1 (CH_3CO), 20.0 (CH_3CO), 19.4 ($\text{C}(\text{CH}_3)_3$).

3-Azidopropyl 2,4,6-tri-*O*-benzoyl-3-*O*-(methyl-4,7,8,9-tetra-*O*-acetyl-5-*N*-acetamido-3,5-dideoxy-*D*-glycero- α -*D*-galacto-non-2-ulopyranosylonate)- β -*D*-galactopyranosyl-(1 $\rightarrow$ 3)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- β -*D*-glucopyranosyl-(1 $\rightarrow$ 3)-2,6-di-*O*-benzoyl- β -*D*-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-benzoyl- β -*D*-glucopyranoside **24.**



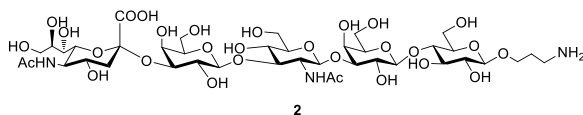
A solution of donor **15** (40 mg, 0.042 mmol) and acceptor **22** (40 mg, 0.028 mmol) with 4 Å molecular sieves (0.100 g) in dry DCM (3.0 mL) was stirred for 20 min under nitrogen. N-iodosuccinimide (0.013 g, 0.056 mmol) and

TfOH (0.5 μL , 0.0056 mmol) were added at -40°C . After 3 h (TLC 6:4 Tol:Acetone) the reaction was quenched with TEA, the solid was filtered off and the solvent removed under reduced pressure. The crude was purified by flash chromatography (Tol:Acetone) to afford pentasaccharide **51** in 40% yield (0.025 g). $[\alpha]_D^{25} = +20.93^\circ$ (c 0.3, CHCl_3). ESI HR-MS ($\text{C}_{106}\text{H}_{116}\text{Cl}_3\text{N}_5\text{O}_{40}\text{Si}$) m/z $[\text{M} + \text{Na}]^+$ found 2253.63; calcd 2254.59.

^1H NMR (400 MHz, CDCl_3) δ 7.67–7.27 (m, 35H, Ar-H), 5.80 (dd, $J = 9.1, 1.2$ Hz, 1H), 5.61 (t, 1H, $J = 9.5$ Hz, H-3^{A}), 5.50 (s, 1H, CHPh), 5.40 (d, 1H, $J_{\text{N,H}} = 8.2$ Hz, NH), 5.37–5.30 (m, 1H), 5.23 (dd, $J_{1,2} = 8.1$ Hz, 1H, H-2^{A}), 5.11–5.05 (m, 2H, H-2^{B} , H-2^{D}), 4.91 (d, $J_{1,2} = 8.0$ Hz, 1H, H-1^{D}), 4.79–4.71 (m, 2H, H-1^{C} , H-1^{A}), 4.59 (d, $J_{1,2} = 8.1$ Hz, 1H, H-1^{B}), 4.56–4.49 (m, 1H), 4.45–4.40 (m, 1H), 4.40–4.29 (m, 3H), 4.26–4.20 (m, 2H), 4.19–4.09 (m, 4H), 4.08–3.97 (m, 3H), 3.97–3.89 (m, 2H), 3.84–3.79 (m, 1H), 3.79–3.71 (m, 4H), 3.58–3.50 (m, 2H), 3.49 (s, 3H, COOCH_3), 3.48–3.43 (m, 1H), 3.38 (dt, $J = 4.9, 9.8$ Hz, 1H, Hz), 3.19–3.09 (m, 3H, H-2^{C} , OCH_2), 2.34 (s, 3H, COCH_3), 2.33 (s, 3H, COCH_3), 2.35–2.30 (m, 1H, $\text{H-3}^{\text{E}}_{\text{eq}}$), 2.08 (s, 3H, COCH_3), 1.96 (s, 3H, COCH_3), 1.87 (t, 1H, $J = 12.3$ Hz, $\text{H-1}^{\text{E}}_{\text{ax}}$), 1.68–1.58 (m, 2H, $\text{CH}_2\text{CH}_2\text{N}_3$), 1.04 (s, 9H, tBu), 0.96 (s, 9H, tBu).

^{13}C -NMR (101 MHz, ACN-d_3) δ 172.1, 171.5, 170.7, 170.6, 168.7, 166.6, 166.5, 166.3, 166.0, 165.8, 154.7, 154.6, 139.4 (13 x CO), 134.3–127.4 (C-Ar), 101.6, 101.3 (CHPh), 101.6 (C-1^B), 101.3 (CHPh), 101.1 (C-1^A), 100.9 (C-1^C), 100.7 (C-1^D), 99.5 (C_q), 80.0, 79.7, 76.5, 75.4, 75.4, 75.3, 74.1, 73.9, 73.7, 73.1, 73.0, 72.0, 71.6, 71.4, 71.0, 69.4, 69.1, 68.8, 67.5, 67.1, 66.8, 63.8, 63.4, 59.2, 58.2 (C-2^C), 53.7, 48.7 (OCH_2), 35.3 (C-3^E), 29.4 ($\text{CH}_2\text{CH}_2\text{N}_3$), 27.7 (tBu), 27.4 (tBu), 24.8 (CH_3CO), 23.0 ($\text{C}(\text{CH}_3)_3$), 21.4 (COCH_3), 21.3 (CH_3CO), 21.0 (CH_3CO), 20.3 ($\text{C}(\text{CH}_3)_3$).

3-Aminopropyl 3-O-(5-N-acetamido-3,5-dideoxy-D-glycero- α -D-galacto-non-2-ulopyranosyl)- β -D-galactopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 3)- β -D-galactopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside 2.



2

Protocol followed for both compound **23** and compound **24**: the protected pentasaccharide (0.07 mmol) was dissolved in THF (5 ml) to which 3 M NaOH (0.5 ml)

was added. After refluxing for 2 d, the mixture was neutralized with 0.1% HCl and concentrated. The residue was re-dissolved in 2:3 Ac₂O-MeOH (5 mL) and stirred overnight. After concentration, the residue was dissolved in *t*BuOH and Pd/C (1:1 w/w in respect to the sugar) was added. The reaction mixture was stirred under pressure of H₂ (5 bar) for 72 h. Then, the catalyst was filtered off and the filtrate concentrated under reduced pressure. The reaction mixture was purified by G-10 size-exclusion column chromatography using water for elution. Fractions containing the sugar were quantified by sialic acid assay and freeze-dried to afford the deprotected oligosaccharide **4** as an amorphous powder (40% yield). $[\alpha]_D^{25} = -4.37^\circ$ (c 0.05, H₂O). ESI HR-MS (C₄₀H₆₉N₃O₂₉) m/z [M-H]⁻ 1054.3971; found 1054.3867.

¹H NMR (400 MHz, D₂O) δ 4.68 (d, $J_{1,2} = 8.3$ Hz, 1H, H-1^C), 4.48 (d, $J_{1,2} = 8.3$ Hz, 2H, H-1^{A,B}), 4.41 (d, $J_{1,2} = 7.5$ Hz, 1H, H-1^D), 4.12 (d, $J_{3,4} = 2.9$ Hz, 1H, H-4^B), 4.07-3.46 (m, 33H), 3.33-3.26 (m, 1H, H-2^A), 3.11 (t, $J = 6.8$ Hz, 2H, CH₂N₃), 2.72 (dd, $J_{3e,4} = 4.1$ Hz, $J_{3e,6a} = 11.9$ Hz, 1H, H-3e^E), 1.99 (s, 6H, 2 x CH₃CO), 2.03-1.91 (m, 2H, CH₂CH₂N₃), 1.75 (t, 1H, H-3a^E).

¹³C-NMR (101 MHz, D₂O) δ 174.9 (CO), 173.9 (CO), 103.3 (C-1^A), 102.9 (C-1^B), 102.5 (C-1^D), 102.0 (C-1^C), 81.9, 81.7, 78.3, 75.5, 75.0, 74.8, 74.7, 74.3, 72.6, 71.8, 69.9, 69.0, 68.4, 68.3, 67.8 (OCH₂), 67.2, 62.4, 61.0, 60.9, 60.4, 59.9, 54.5, 51.6, 39.7 (C-3^E), 37.5 (CH₂N₃), 26.6 (CH₂CH₂N₃), 22.2 (CH₃CO), 22.0 (CH₃CO). See Table 1 for full assignments.

Quantitative estimation of sialic acid¹⁸.

Yields of the synthetic oligosaccharide was calculated on the basis of the sugar quantification, which was done by quantitative estimation of the sialic acid content with a colorimetric resorcinol-hydrochloric acid method. Standard samples of sialic acid at concentrations 5, 10, 15, 20, 25 μ g/mL were prepared to build the calibration curve. GBS PSIIa samples were prepared targeting the calibration curve midpoint. The reference and analytical samples were treated with a solution of Resorcinol/HCl. The reagent resorcinol/HCl was prepared by mixing 10 mL of a 2% resorcinol solution, 0.25 mL of a 0.1 M Copper (II) Sulphate solution in 20 mL of water with 37% hydrochloric acid up to a volume of 100 mL. After treatment of samples and references with the resorcinol/HCl reagent, the resulting mixtures were heated in a oil bath at 110°C for 20 min. After that, absorbance was read at 564 nm.

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