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

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Chapter 4

Synthesis of GBS CPS serotype Ia oligosaccharides library

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Introduction

GBS is Gram positive bacterium causing infections in infants and newborns, having as main clinical manifestations sepsis, pneumonia and meningitis, as extensively described in Chapter 2.[1] Intrapartum Antibiotic Prophylaxis (IAP) is the chosen strategy to combat GBS infections in developed countries, but there are rising concerns about antibiotic resistance[2]. For this reason, a GBS vaccine represents an appealing alternative. The GBS capsular polysaccharide (CPS) is a main virulence factor and is a promising target for vaccine development[1, 3, 4].

On the basis of the serology and the structure of the capsular polysaccharide, ten GBS serotypes have been described (Ia, Ib, II-IX)[4-6]. All identified CPSs are high-molecular weight polymers with a sialic acid residue on the side chain; among them, type Ia, Ib, and III CPS share all the same sugar residues and differ only for one linkage position[7]. The most studied of GBS polysaccharides is type III. This CPS is known to form a helical structure[8, 9], and this feature has an impact on epitope exposure[10].

In the past, serotypes Ia, Ib, II, and III were equally prevalent in normal vaginal carriage and early-onset sepsis (developing at less than 7 days of age). However, recent European studies (2008-2010) showed that serotype Ia, III and V together accounted for 88%, 96%, and 67% of strains isolated from neonates with early-onset disease, neonates with late-onset disease, and vaginal-rectal swabs of colonized pregnant women, respectively[11]. These results reaffirm the clinical importance of types Ia and III[12-14].

Furthermore, serotype Ia, together with serotype V, is responsible of most GBS infections in elderly and immunocompromised patients[14]. Given its clinical relevance, a monovalent GBS Ia polysaccharide CRM-conjugate vaccine has been tested in clinical trials[15, 16], as well as a trivalent formulation including type Ia, Ib and III polysaccharide[17]. These vaccines proved to be safe, well-tolerated and able to induce serotype-specific IgGs.

Despite this, GBS polysaccharide vaccines, as many other CPS-based glycoconjugate vaccines, present some issues. Among them, the difficult analytical characterization due to their heterogeneous composition and the potential presence of bacterial contamination which generates quality control issues and safety concerns[18, 19].

Structurally defined glycoconjugate vaccines composed of synthetic oligosaccharide antigens represent an attractive solution to address this problem. Furthermore, availability of synthetic fragments representing parts of the bacterial CPS allows to investigate the structure-immunogenicity relationship for vaccine optimization.

In 2015, Guo et al. published the first synthesis of the repeating unit of GBS type Ia[20]. Recently, the same group extended the synthetic approach to the synthesis of the dimer (DP2) and prepared CRM_{197} conjugates of both oligosaccharide structures in order to evaluate their immunogenicity[21]. However, in order to design efficacious well-defined semisynthetic vaccines, information on the identity of protective glycotopes expressed on the bacterial CPS are required[18, 22]. So far, no structural studies of GBS type Ia oligosaccharides have been carried out to shed lights on the

carbohydrate epitopes expressed on the corresponding CPS. Indeed, such study relies on the availability of structurally defined oligosaccharides, but neither enzymatic nor chemical methods are currently available to depolymerize type Ia CPS in order to obtain short fragments. Chemical synthesis is therefore the only tool able to provide the target glycans from GBS type Ia capsular polysaccharide. It is to be noted that in a polysaccharide there is a plethora of potential glycotopes and this challenges traditional chemical synthesis because a number of different structures are needed[23]. Having an expeditious and efficient approach to the synthesis of these fragments is key to determine the structure-immunogenicity relationship.

To this aim, this Chapter describes the application of the regioselective synthesis of the key synthon GlcNAc-β-(1→3)-Gal*p* (Chapter 3) to prepare a library of GBS serotype Ia related oligosaccharides. In particular, using an innovative synthetic tactic the repeating unit (both in the linear and branched frameshifts) was prepared, together with fragments longer than the single repeating unit.

These fragments can be used in structural studies, comprising competitive Surface Plasmon Resonance (SPR), STD-NMR and competitive ELISA, to gain molecular insights into the sugar-mAb interactions and help to identify glycotopes expressed on the bacterial capsular polysaccharide. Moreover, availability of oligosaccharide structures from both GBS type Ia and Ib (whose synthesis will be described in Chapter 5) could also help understanding what are the features that cause (despite the very close similarities of these two repeating units as they only differ for the connection between a galactose and the glucosamine) the productions of serotype-specific monoclonal antibodies in response to monovalent and multivalent conjugated vaccines[7] (immunospecificity).

Results and Discussion

Synthesis of oligosaccharide fragments

The repeating unit of GBS serotype Ia is a pentasaccharide structurally very similar to the one of GBS type Ib and III, with a α-(2→3)-sialylated disaccharide side chain and a trisaccharide backbone[7].

On the basis of the structure of the CPS, a small library of target glycans with an increasing structural complexity was designed (Figure 1). This library comprises: the pentasaccharide repeating unit of GBS type Ia both in the branched (**1**) and in the linear frameshift forms (**2**), the non-sialylated tetrasaccharide **3**, the two hexasaccharide structures **4** and **5** (which will be called Y-shape and branched hexasaccharide from now on), and the heptasaccharide **6**. All glycans were designed with an aminopropyl linker at the reducing end amenable for future conjugation to a carrier protein.

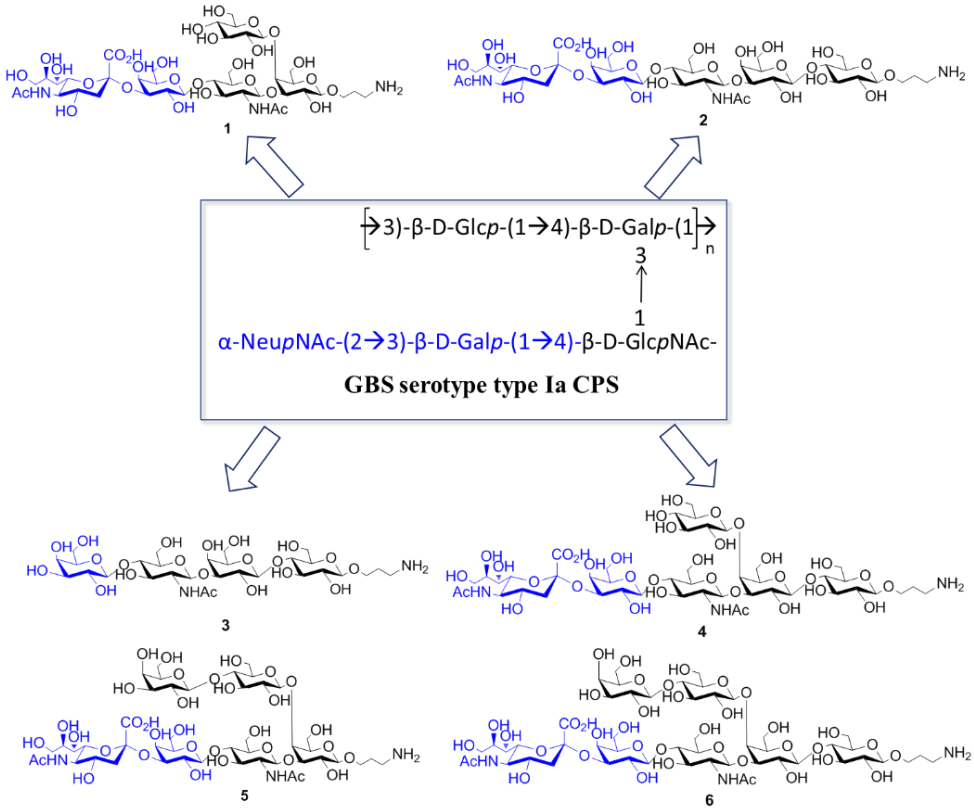
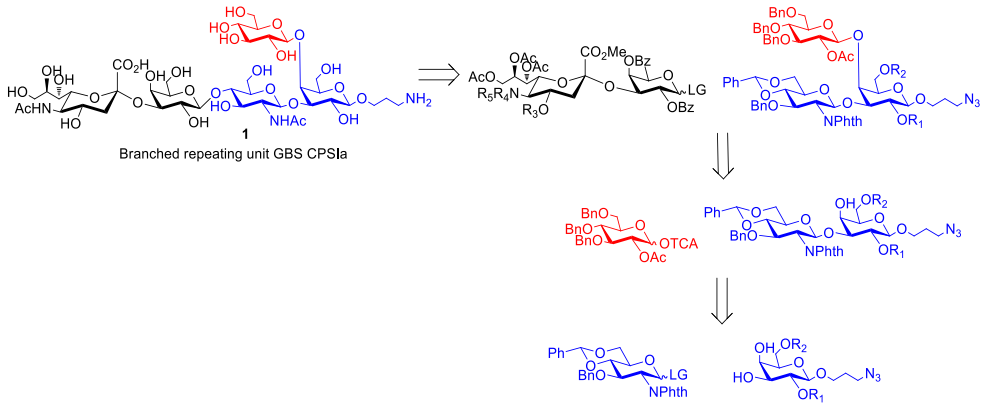


Figure 1. Structure of the GBS type Ia capsular polysaccharide and the library of related glycans to be synthesized.

Starting from the preparation of the pentasaccharide **1**, two main challenges were identified:
(i) the installation of the α-sialic acid connection to the upstream Gal residue. To address this, a convergent [3+2] regioselective approach employing a sialogalactoside donor and a trisaccharide acceptor was chosen.
(ii) the synthesis of the branched trisaccharide Glc*p*NAc-β-D-(1→3)-Gal*p*-β-D-(1→4)-Glc*p* acceptor in an efficient and expeditious way. To this end, the trisaccharide acceptor was prepared starting from the disaccharide synthon Glc*p*NAc-β-D-(1→3)-Gal*p* obtained by means of a regioselective β-(1→3) galactose glycosylation (see Chapter 3). Using this approach allowed to reduce the number of protecting groups employed in the synthesis, as well as the protection/deprotection steps. The glycosylation partners to provide the disaccharide were chosen from the glucosamine donors bearing a temporary protecting group at C-4 and galactose acceptors screened in Chapter 3.



Scheme 1. Retrosynthetic approach to pentasaccharide **1**

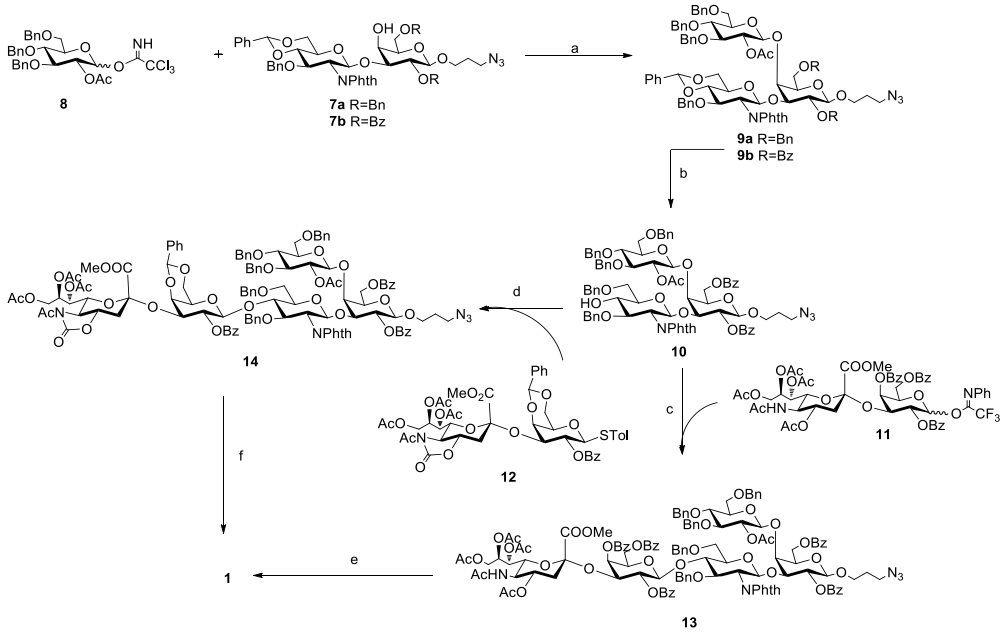
According to our retrosynthetic design (Scheme 1) the target glycan **1** can be obtained through a [2+3] convergent strategy based on the glycosylation of a suitable trisaccharide acceptor with a Neu5Acα2-3Gal donor. This approach envisages the challenging stereoselective α-sialylation of the upstream galactose at an early stage of the synthesis. In this design fast and efficient access to a GlcNAcβ1-3Gal disaccharide building block plays a central role to obtain the trisaccharide acceptor without a temporary protection at Gal 4-OH for further assembly of GBS CPS Ia fragments.

Having identified two disaccharide synthons obtained through an optimized regioselective glycosylation (Chapter 3), the retrosynthesis to the pentasaccharide repeating unit of GBS CPS Ia could be put into practice (Scheme 1). To this end, glucose donor **8**[24] was reacted with benzylated disaccharide **7a** and the benzoylated counterpart **7b** to furnish trisaccharides **9a** and **9b** in 75% and 68% yield, respectively (See Scheme 2).

Despite the deactivating effect of the 6-*O*-benzoyl ester compared to the 6-*O*-benzyl ether, the reaction proceeded with almost identical efficiency using the imidate donor **8**. In contrast, a peracetylated trichloroacetimidate glucose donor with TMSOTf activation was ineffective for glycosylation of the 4-OH. Considering the higher regioselectivity and yield achieved in making disaccharide **7b**, the resulting trisaccharide **9b** was advanced for the construction of the GBS CPS Ia repeating unit and subjected to regioselective opening of the 4,6-*O*-benzylidene acetal with $BF_3{\cdot}Et_2O$ and $Me_3N{\cdot}BH_3$ to provide the acceptor **10** (70%). In order to complete the pentasaccharide construction, the elongation of trisaccharide acceptor **10** with the known donors sialogalactosyl trifluoroacetimidate **11**[25, 26] and thioglycoside **12**[27] were tested. Of these two disaccharides, **12** can be prepared with a higher stereoselective control, whereas **11** is easily accessible from a commercial disaccharide precursor[25].

Glycosylation of trisaccharide **10** with **11** under TMSOTf activation gave the protected pentasaccharide **13** in 75% yield. The use of disaccharide **12** in presence of NIS/TfOH led to the protected pentasaccharide **14** in a similar yield (73%). Pentamer **13** was deprotected by a five-step procedure[28], including (i) removal of the methyl ester of Neu5Ac with lithium iodide in pyridine;

(ii) reaction with ethylenediamine in refluxing ethanol for concomitant removal of the *O*-acetates and the *N*-Phth protecting group; (iii) reacetylation with acetic anhydride/pyridine to install the acetamide group of the GlcNAc residue along with acetyl esters; (iv) methanolysis and (v) final catalytic hydrogenation over Pd/charcoal to provide the crude pentasaccharide **1**. After purification by size-exclusion chromatography, target pentasaccharide **1** was isolated in 40% yield. Pentamer **14** was deprotected by a three steps procedure comprising i) saponification with NaOH in refluxing THF, ii) amine reacetylation using a 2:3 acetic anhydride/methanol mixture, iii) hydrogenation over Pd/charcoal to afford crude pentasaccharide **1**. After purification by size exclusion chromatography, the target pentasaccharide **1**, equipped with an aminopropyl linker, was isolated in a yield of 45%. Yields of **1** obtained via fully protected **13** and **14** was estimated by spectrophotometric quantification of the sialic acid content[29].

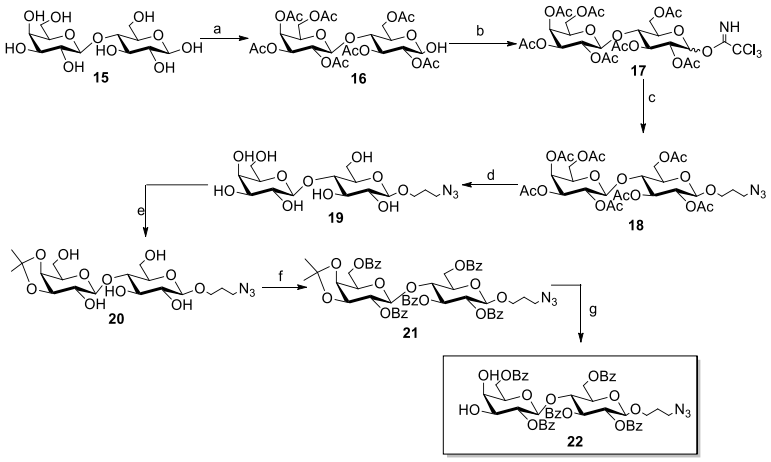


Scheme 2. Assembly of GBS CPSIa repeating unit. *Reagents and conditions*: a) TMSOTf, DCM dry, -10°C, 75% from **7a**; 68% from **7b**; b) $Me_3N{\cdot}BH_3$, $BF_3{\cdot}Et_2O$, ACN, 0°C, 70% ; c) TMSOTf, DCM dry, 0°C, 75%; d) TfOH, NIS, DCM dry, -40°C, 73%; e) LiI, Py, 120°C; $H_2NCH_2CH_2NH_2$, EtOH, 90°C; Ac_2O, Py; MeONa, MeOH; H_2, Pd-C, 40% (over five steps) ; f) 3M NaOH, THF, reflux; Ac_2O, MeOH, H_2, Pd-C, 45% (over three steps).

Next, a similar regioselective approach was employed for the synthesis of the linear frameshift of the serotype Ia repeating unit **2** and to its non-sialylated tetrasaccharide counterpart **3** (Scheme 4). To achieve this, the benzoylated lactose acceptor **22** and the glucosamine donor **23** were chosen as glycosylation partners to obtain a linear trisaccharide synthon common to both target structures. Lactose **22** was selected as acceptor because the benzoyl protecting groups present decreases the nucleophilicity of the axial 4-OH of galactose, making this hydroxyl group completely unreactive

towards the glucosamine donors, thereby enhancing the regioselectivity of the glycosylation reaction at C-3, as previously demonstrated (Chapter 3).

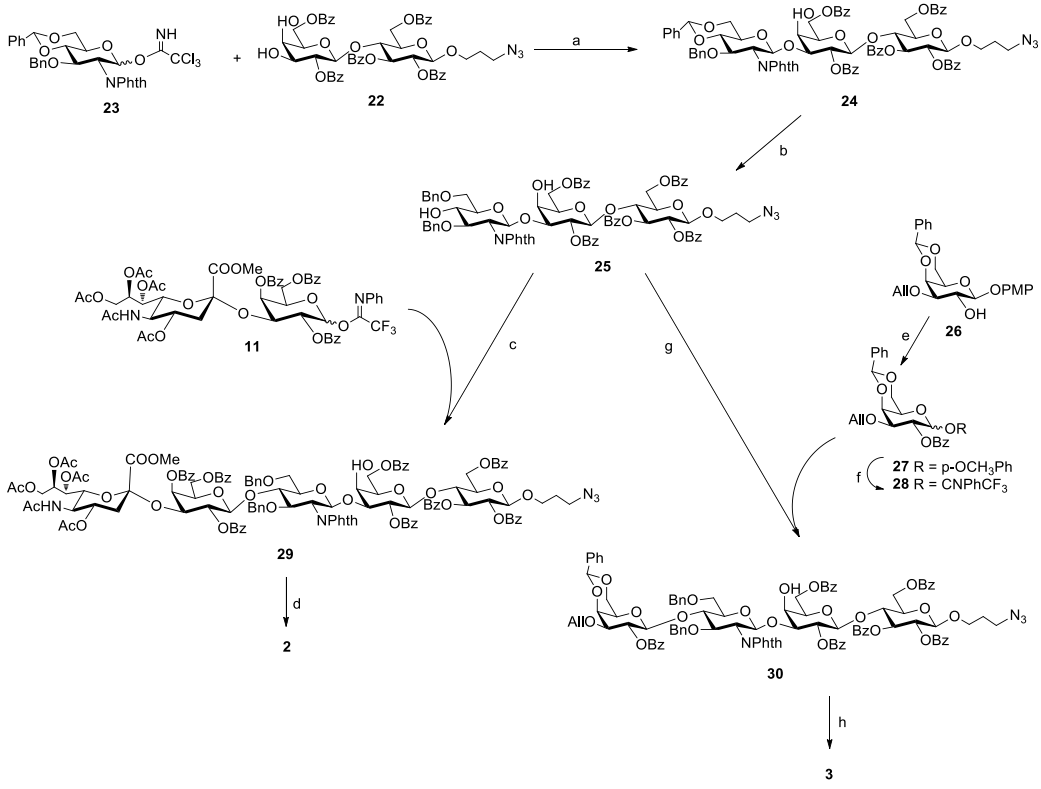
The preparation of lactose acceptor **22** is depicted in Scheme 3 and starts with the acetylation of commercially available D-Lactose (**15**) with acetic anhydride and pyridine, followed by the selective removal of the anomeric acetyl group with ethylenediamine and acetic acid in THF. Conversion of obtained **16** in trichloroacetimidate **17** (2:1 α/β mixture) and subsequent TMSOTf mediated coupling of **17** with the azidopropanol linker afforded acetylated dimer **18**. De-*O*-acetylation using Zemplen conditions and installation of the isopropylidene protective group by dissolving **19** in 2,2-dimethoxypropane in the presence of catalytic amount of *p*-toluenesulfonic acid followed by treatment with 9:1 MeOH: H_2O at 90°C gave compound **20**. Benzoylation of compound **20** using benzoyl chloride and pyridine and, finally, removal of the isopropylidene group afforded the target lactose building block **22** with the free 3-OH and 4-OH positions of the galactose.



Scheme 3. Synthetic pathway to lactose **22**. *Reagents and conditions*: a) Ac_2O/Py; ethylenediamine, AcOH, THF, 75% over two steps; b) CCl_3CN, DBU, DCM dry, 57%; c) azidopropanol, TMSOTf, DCM dry, 74%; d) MeONa/MeOH, 85%; e) *p*-toluenesulfonic acid, 2,2-dimethoxypropane, DMF; MeOH/H_2O, 90°C, 57% over two steps; f) BzCl/Py, 71%; g) AcOH/H_2O, 90°C, 70%.

In line with previous results, glycosylation of lactose **22** and glucosamine **23** afforded the linear trisaccharide acceptor **24** in 68% yield with complete regioselectivity (Scheme 4). Following benzylidene opening in **24**, the coupling of trisaccharide acceptor **25** with both donor **11**[26] and **12**[27] was tested. Reaction of **11** with **24** under influence of TMSOTf at 0°C afforded the target linear pentasaccharide **29** in 65% yield. To demonstrate the regioselectivity the presence of the free galactose 4-OH throughout all stages of the synthesis, from trisaccharide **24** to pentasaccharide **29**, was monitored by following the signal of the Gal H-4, which appeared at 3.97 ppm (d, J = 2.7 Hz) in the ^{1}H NMR and HSQC of all synthetic intermediates. Unexpectedly,

reaction of **25** with the tolyl thioglycoside **12** under NIS/TfOH activation at -40°C was unsuccessful and yielded only traces of the corresponding pentasaccharide, while mainly decomposition of the glycosyl donor was observed, as revealed by LC-MS analysis. The linear pentasaccharide **29** was subjected to the five-step deprotection protocol previously described for compound **13** and after purification by size exclusion chromatography the target oligosaccharide **2** was obtained in 33% overall yield (Scheme 4). Acceptor **25** was also used to obtain a non-sialylated CPS Ia linear fragment for future epitope mapping studies by glycosylation (72% yield) with the trifluoroacetimidate **27**, prepared from the known lactol **26**[28]. After global deprotection tetrasaccharide **3** was obtained in 42% yield (Scheme 4).



Scheme 4. Assembly of linear GBS PSIa fragments **2** and **3**. *Reagents and conditions:* a) TMSOTf, DCM dry, -10°C, 68%; b) $Me_3N{\cdot}BH_3$, $BF_3{\cdot}Et_2O$, ACN, 0°C, 65%; c) TMSOTf, DCM dry, 0°C, 65%; d) LiI, Py, 120°C; $H_2NCH_2CH_2NH_2$, EtOH, 90°C; Ac_2O, Py; MeONa, MeOH; H_2, Pd-C, 33% (over five steps); e) BzCl, Py, 0° to rt, 40%; f) CAN, ACN/water 4:1, 0°C, 77%; TFACl, Cs_2CO_3, DCM, 61%; g) TMSOTf, -20°C, DCM, 72%; h) $PdCl_2$, MeOH; $H_2NCH_2CH_2NH_2$, EtOH, 90°C; Ac_2O, Py; MeONa, MeOH; H_2, Pd-C, 42% (over five steps).

NMR data of the synthesized fragments **1** and **2** showed an excellent agreement with CPS Ia samples (see Table 1 and Figure 2)[30].

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

Residue		**Compound 1**		**Compound 2**		**PS Ia**[a]	
		^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
Gal	1	4.39/ *J* 8.1 Hz	103.1	4.43/J 8.2	103.8	4.44	102.3
	2	3.66	70.0	3.57	70.7	3.61	71.2
	3	3.78	82.2	3.72	82.9	3.73	82.5
	4	4.37	74.8	4.16	69.1		
	5	3.68	74.2	3.66	75.1	3.65	73.6
	6	3.72	60.8	3.65	63.2		
	6’	3.72		3.88			
GlcNAc	1	4.69/ *J* 8.4 Hz	103.1	4.69/J 8.2 Hz	103.7	4.70	102.6
	2	3.79	55.3	3.81	55.9	3.75	56.0
	3	3.71	72.1	3.73	72.8	3.62	72.9
	4	3.73	78.2	3.76	78.5	3.63	78.7
	5	3.58	74.6	3.72	75.7	3.48	75.3
	6	3.98	60.1	3.95	68.2		
	6’	3.85		3.95			
Glc	1	4.88/ *J* 7.7 Hz	102.0	4.50/J 8.5 Hz	102.7	4.89	103.2
	2	3.26	73.6	3.32	73.5	3.20	74.2
	3	3.51	75.9	3.64	75.4	3.45	75.3
	4	3.37	69.7	3.65	78.6	3.52	78.8
	5	3.42	75.6	3.66	75.4	3.59	75.0
	6	3.89	60.7	3.81	60.6		
	6’	3.74		3.96			
Gal’	1	4.54/ *J* 8.4 Hz	102.5	4.56/J 9.0 Hz	103.0	4.56	102.4
	2	3.55	69.3	3.57	70.2	3.46	70.1
	3	4.09	75.5	4.12	76.2	4.06	76.2
	4	3.94	67.5	3.92	68.8	3.84	68.2
	5	3.70	75.2	3.71	75.6	3.61	75.9
	6	3.66	60.8	3.74	61.9		
	6’	3.66		3.71			
Neu5Ac	3	2.74	39.6	2.76	40.4	2.72	40.1
	3’	1.78		1.80		1.77	
	4	3.67	68.3	3.68	69.1	3.57	69.0
	5	3.83	51.7	3.85	52.4	3.79	52.2
	6	3.61	72.9	3.62	73.7	3.53	73.7
	7	3.57	68.1	3.65	68.8	3.49	68.8
	8	3.88	71.8	3.87	72.6	3.84	72.6
	9	3.84	62.6	3.88	63.3	3.77	63.3
	9’	3.62		3.65		3.54	

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

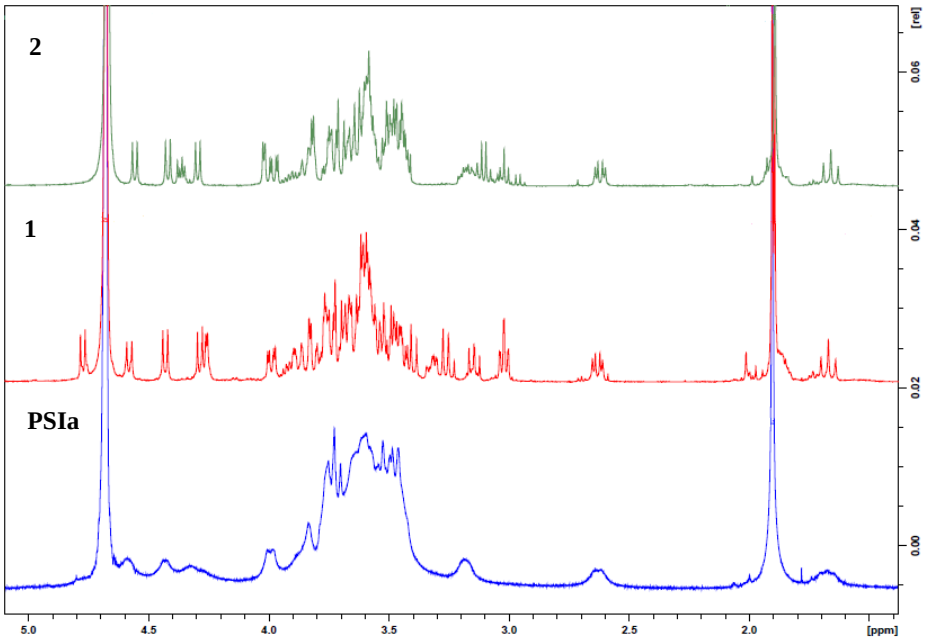
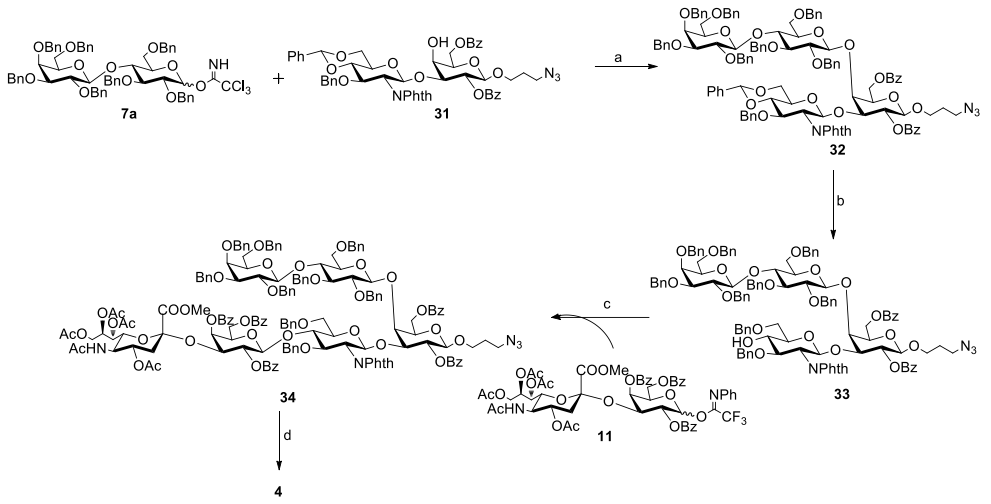


Figure 2. ^{1}H NMR spectra of pentasaccharides **1**–**2** in comparison to PSIa (D_2O, 400 MHz, 298 k)

Once the conditions for the synthesis of GBS PSIa repeating unit were optimized, the key synthons, disaccharide **7a** and trisaccharide **24**, were used to prepare the hexasaccharides **4** and **5** and the heptasaccharide **6** (Scheme 5 and 6).

To prepare the branched hexasaccharide **4,** disaccharide **7a** was condensed with lactose acceptor **31**, affording tetrasaccharide **32** in 60% yield (Scheme 5). Regioselective opening of the benzylidene ring in **32** to give tetrasaccharide acceptor **33**, with the unmasked glucosamine 4-OH, was followed by glycosylation with imidate donor **11** affording the protected hexasaccharide **34** in 78% yield. Compound **34** was subjected to the previously described 5-step deprotection protocol to provide the target branched hexasaccharide **4** in 35% yield.

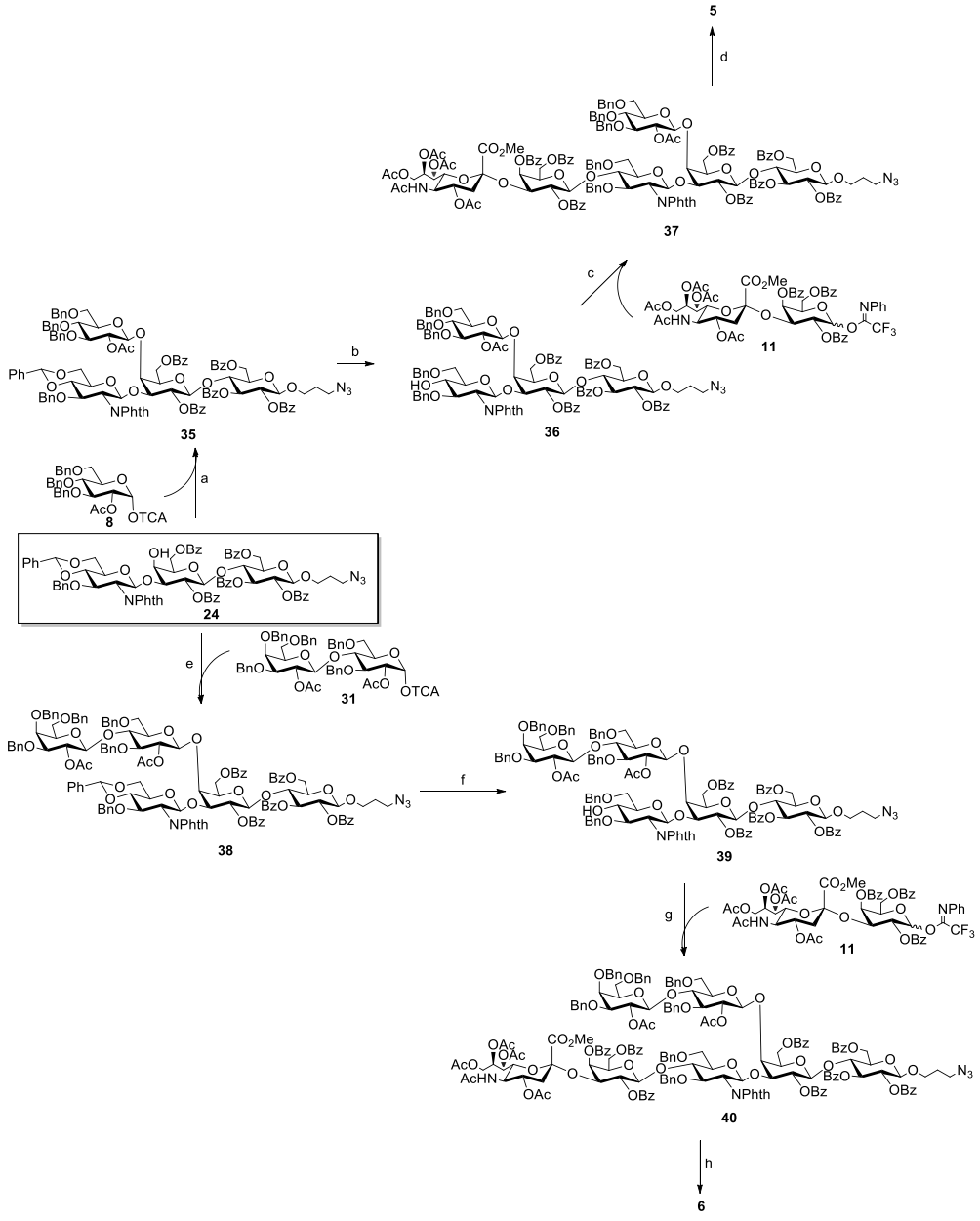


Scheme 5. Synthetic pathway to hexasaccharide **4**. *Reagents and conditions*: a) TMSOTf, DCM dry, 60%; b) $Me_3N{\cdot}BH_3$, $BF_3{\cdot}Et_2O$, ACN, 0°C, 52%; c) TMSOTf, DCM dry, 78%; d) LiI, Py, 120°C; $H_2NCH_2CH_2NH_2$, EtOH, 90°C; Ac_2O, Py; MeONa, MeOH; H_2, Pd-C, 35% (over five steps).

Finally, the synthesis of the Y-shape hexasaccharide **5** and of the heptasaccharide **6** was accomplished starting from the common linear trisaccharide synthon **24** as depicted in Scheme 6. To obtain Y-shaped **5**, the trisaccharide was glycosylated with glucosyl imidate **8**, affording tetrasaccharide **35** in 65% yield. After regioselective opening of the benzylidene ring, glycosylation of glucosamine 4-OH with the sialogalactoside imidate donor **11** afforded the target protected hexasaccharide **37** in 60% yield. On the other hand, to obtain the heptasaccharide **6**, trisaccharide **24** was glycosylated with the armed lactose trichloroacetimidate **31**, yielding the intermediate pentasaccharide **38** (55%). After treatment with trimethylaminoborane and boron trifluoride etherate, the obtained pentasaccharide acceptor **39** was elongated by glycosylation with the disaccharide donor **11** to the protected heptasaccharide **40** (60% yield).

Both compound **37** and **40** were deprotected, according to the previously described protocol, to afford oligosaccharides **5** and **6** in multimilligram quantities (40% yield)[29].

The ^{1}H NMR spectra of compounds **3**-**6** are depicted in Figure 3 and confirmed the integrity of the obtained structures.



Scheme 6. Assembly of oligosaccharides **5** and **6**. *Reagents and conditions*: a) TMSOTf, DCM dry, 65%; b) $Me_3N{\cdot}BH_3$, $BF_3{\cdot}Et_2O$, ACN, 0°C, 55%; c) TMSOTf, DCM dry, 60%; d) LiI, Py, 120°C; $H_2NCH_2CH_2NH_2$, EtOH, 90°C; Ac_2O, Py; MeONa, MeOH; H_2, Pd-C, 40% (over five steps); e) TMSOTf, DCM dry, 55%; f) $Me_3N{\cdot}BH_3$, $BF_3{\cdot}Et_2O$, ACN, 0°C, 55%; g) TMSOTf, DCM dry, 60%; h) LiI, Py, 120°C; $H_2NCH_2CH_2NH_2$, EtOH, 90°C; Ac_2O, Py; MeONa, MeOH; H_2, Pd-C, 40% (over five steps).

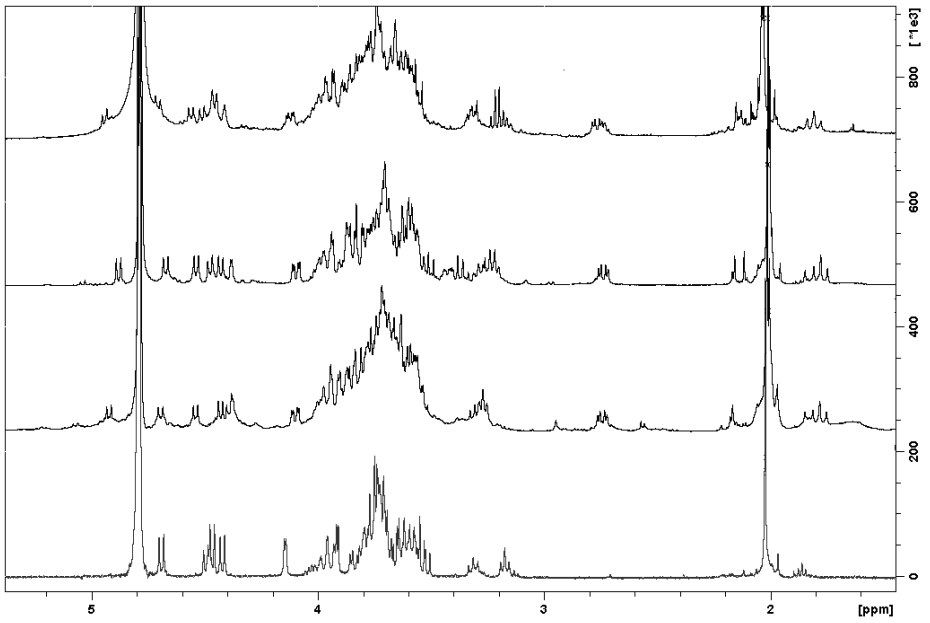


Figure 3. ^{1}H NMR spectra of synthetic oligosaccharides. From the bottom to the top: 1H NMR of: the linear tetrasaccharide **3**, the branched hexasaccharide **4**, the Y-shape hexasaccharide **5** and the heptasaccharide **6**.

Conformational studies

The branched pentasaccharide repeating unit of GBS serotype Ia was used to investigate the conformational behaviour of the type Ia capsular polysaccharide and compare its structure to that of the Ib polymer. Indeed, these two capsular polysaccharides differ exclusively in the connection of the sialogalactoside disaccharides to the GlcNAc residue, which is β-(1→4) in type Ia and β-(1→3) in type Ib. This small difference is crucial to the immunospecificity (anti PSIa and Ib serotype specific mAbs have been isolated and have shown no cross-reactivity).

By a combination of NMR and modelling experiments[31], a model of the natural CPS was built (10 repeating units, 50 monosaccharides) and compared to compound **1**, the branched repeating unit of PSIa. On the basis of this model, the impact of the GlcNAcβ1-3Gal *vs* GlcNAcβ1-4Gal connectivity in the orientation of the Neu5Acα2-3Gal branching was shown and a different shape for the Ia polysaccharide as compared to Ib was revealed. The main difference was the presentation of the protruding Neu5Ac-α-(2→3)-Gal moieties, with a major *exo-anti-Φ* population for Ia and a *exo-syn-Φ* conformation for Ib (see Figure 4, 5 and Chapter 5). The Neu5Acα2-3Gal branches of GBS PSIII have been shown to be strongly engaged in antibody recognition[22], therefore these unique structural features are expected to influence antibody recognition and immunospecificity also in case of GBS PSIa and PSIb.

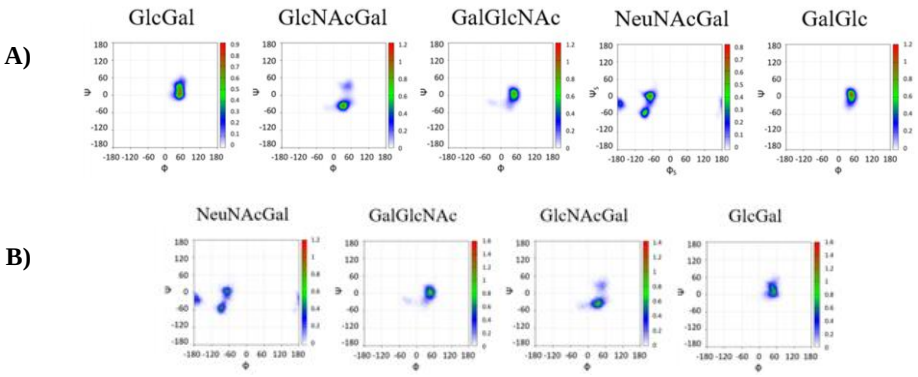


Figure 4. A) Glycosidic linkage analysis for GBS Ia polysaccharide: φ/ψ plots for representative glycosidic bonds of a 10 repeating unit model along the 2.5 μs MD simulation B) Glycosidic linkage analysis for GBS Ia pentasaccharide 1: φ/ψ plots for representative glycosidic bonds along 200 ns of MD simulation.

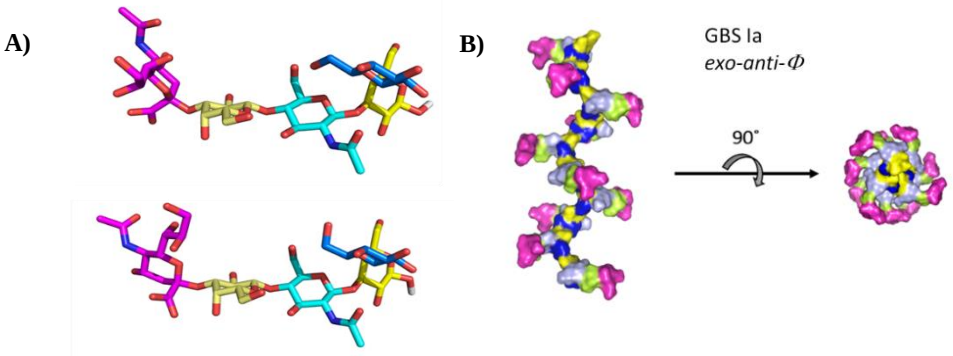


Figure 5. A) Perspectives of the two populations, deduced by ROESY NMR experiments, which define the conformational behaviour of the pentasaccharide repeating unit of GBS Ia. (a) the φ_S torsion angle adopts the major trans (t) geometry. (b) the φ_S torsion angle shows the minor -g conformation. B) Model structures for the GBS Ia polysaccharide with the major exo-anti-φ conformation around all the Neu5Acα2-3Gal linkages

Conclusion

In this Chapter the optimized regioselective β-(1→3)-glycosylation of appropriate galactose synthons was employed to synthesize oligosaccharide fragments from Group B streptococcus serotype Ia capsular polysaccharide. In particular, a small library of six fragments with an increasing degree of complexity (from a non-sialylated linear tetrasaccharide to a Y-shaped heptasaccharide) was designed. Thanks to the regioselective approach described in Chapter 3, the synthesis of the GBS PSIa repeating unit was optimized, reducing the number of synthetic steps and protecting groups employed. Furthermore, fragments longer than one single repeating unit were prepared with high efficiency and using a limited number of building blocks.

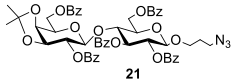
Having access to structurally defined GBS type Ia related glycans is a key step forward to investigate the structural-immunogenicity relationship of the capsular polysaccharide and to gain insights into its conformation.

To this aim, the different conformational behaviour of GBS PSIa compared to the structurally close PSIb was established. The GBS type Ia repeating unit **1** was used to probe the conformation and molecular dynamics of the corresponding capsular polysaccharide, highlighting the different presentation of the protruding Neu5Acα2-3Gal moieties and a reduced flexibility of Ia polymer compared to Ib. These differences are expected to impact epitope exposure.
The synthesized fragments **1**-**6** will be employed to confirm this prediction and to investigate the structural features determining the antibody recognition (Chapter 6).
Moreover, since all the oligosaccharides have been designed and synthesized with an aminopropyl chemical handle, they will be conjugated to carrier proteins for evaluation of their immunogenicity *in vivo*.

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 30–50ºC 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. ^{1}H NMR spectra were measured at 400 MHz and 298 K with a Bruker AvanceIII 400 spectrometer; ^{1}H values are reported in ppm, relative to internal Me_4Si (^{1}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}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 2,6-di-*O*-benzoyl-3,4-di-*O*-isopropylidene-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside 21.



21

Lactose Linker perOAc **18** (5.4 g, 7.6 mmol), prepared according to the literature[28], was dissolved in MeOH (40 mL) and then MeONa/MeOH was added dropwise until pH = 9. After 16 h (TLC 9:1 DCM/MeOH) the reaction was quenched by addition of Dowex, then the solution was filtered and the solvent evaporated under reduced pressure, affording the lactose linker deacetylated. (2.7 g, 6.4 mmol, 85%).

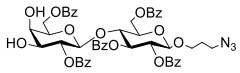
The resulting compound (2.7 g, 6.4 mmol) was dissolved in 30 ml of 9:1 mixture of 2,2-dimethoxypropane:DMF. Catalytic PTSA (0.7 g, 4.5 mmol) was added and the reaction warmed at 50°C for 3 h. After 3 h (TLC 9:1 DCM:MeOH) the reaction was quenched with TEA until neutral pH, and the solvent removed under reduced pressure. The crude was redissolved in 55 ml of 9:1 MeOH/H_2O and stirred at 90°C for 2 h, when the presence of one major spot was detected on TLC. The solvent was removed under reduced pressure, and the crude purified by chromatography (DCM/MeOH) to give the isopropylinated lactose **72** in 57% yield (1.7 g, 3.7 mmol).

Lactose linker isopropylidene **20** (1.7 g, 3.7 mmol) was dissolved in 20 mL of pyridine, then the resulting solution was cooled down to 0°C in a water/ice bath and BzCl (4.3 mL, 37.2 mmol) was added dropwise. The reaction was stirred at rt for 2 h (TLC 6:4 cyclohexane/EtOAc), then the crude mixture was purified on silica gel affording the desired compound **21** (2.6 g, 2.6 mmol, 71% yield). $[\alpha]_D^{25}$ = +39.86° (c 1.7, $CHCl_3$). ESI HR-MS ($C_{53}H_{51}N_3O_{16}$) *m/z* $[M+Na]^+$ found 1008.3178; calcd 1008.3167.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11-7.91 (m, 10H, H-Ar) 7.66-7.26 (m, 15H, H-Ar), 5.71 (t, J = 9.4 Hz, 1H, H3^A), 5.39 (dd , $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2^A), 5.13 (t, J = 7.6 Hz, 1H, H2^B), 4.64- 4.57 (m, 3H, H6a^A, H1^A, H1^B) 4.45 (dd, $J_{5,6a}$ = 4.6 Hz, $J_{6a,6b}$ = 12.1 Hz, 1H, H6b^A) 4.27-4.08 (m, 3H, H5^A, H3^B, H6a^B), 4.07 (dd, $J_{3,4}$ = 2.0, $J_{4,5}$ =5.5, 1H, H-4^A), 3.91-3.77 (m, 3H, H4^B, H5^B, OCH_{2a}), 3.66 (dd, $J_{5,6b}$ = 7.4 Hz, $J_{6a,6b}$= 11.4 Hz, 1H, H6b^B), 3.53-3.43 (m, 1H, OCH_{2b}), 3.21-3.13 (m, 2H, CH_2N$_3$), 1.82-1.45 (m, 5H, CH_2CH$_2$N$_3$, CH$_3$), 1.26-1.23 (m, 3H, CH$_3$).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 166.0, 165.9, 165.6, 165.2, 164.9 (5 x CO), 133.5-128.6 (C-Ar), 100.6 (C1^A, C1^B), 76.9 (C-3^B), 75.4 (C-5^A), 73.6 (C-2^B),73.0 (C-4^A), 72.6 (C-3^A, C-5^B), 71.9 (C-2^A), 71.7 (C-4^B), 66.8 (O*C*H$_2$), 62.8 (C-6^B), 62.5 (C-6^A), 47.9 (*C*H$_2$N$_3$), 29.2 (*C*H$_2$CH$_2$N$_3$), 27.4 (*C*H$_3$), 26.2 (*C*H$_3$).

3-Azidopropyl 2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside 22.



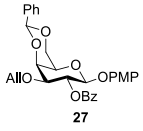
22

Lactose 3,4-isopropylidene 2,6-OBz **21** (2.6 g, 2.6 mmol) was dissolved in 20.0 mL of a 4:1 AcOH/H_2O mixture and the resulting reaction mixture was heated to 90°C. After 2 h (TLC 8:2 Toluene/EtOAc) the solvent was coevaporated with toluene. The crude was purified by column chromatography affording compound **22** (1.6 g, 1.7 mmol, 70% yield). $[\alpha]_D^{25}$ = +37.04° (c 0.7, $CHCl_3$). ESI- HR MS **(**$C_{50}H_{47}N_3O_{16}$) *m/z* $[M+H]^+$ found 946.30, calcd 946.30.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11-7.29 (m, 25H, H-Ar), 5.69 (t, *J* = 9.4 Hz, 1H, H-3^A), 5.44-5.36 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.8 Hz, 1H, H-2^A), 5.30-5.23 (dd, $J_{1,2}$ = 7.9 Hz, $J_{3,4}$ = 9.7 Hz, 1H, H-2^B), 4.65-4.56 (m, 3H, H-1^A, H-1^B, H-6a^A), 4.54-4.48 (dd, $J_{5,6a}$ = 5.1 Hz, $J_{6a,6b}$ = 12.2 Hz, 1H, H-6b^A), 4.16-4.09 (t, *J* = 8.8 Hz, 1H, H-4^A), 4.06-3.99 (dd, $J_{5,6a}$ = 6.6 Hz, $J_{6a,6b}$ =11.3 Hz, 1H, H6$^B{}_a$), 3.90-3.76 (m, 3H, OCH$_{2a}$, H-5^A, H-4^B), 3.71-3.63 (d, *J* = 9.3 Hz, 1H, H-3^B), 3.61-3.43 (m, 3H, OCH$_{2b,}$ H-5^B, H-6b^B), 3.29-3.11 (m, 2H, C*H*$_2$N$_3$), 1.85-1.59 (m, 2H, C*H*$_2$CH$_2$N$_3$).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 166.5, 166.2, 166.0, 165.8, 165.2 (5 x CO), 133.4-128.5 (C-Ar), 101.0, 100.8 (C-1^A, C-1^B), 76.1 (C-4^A), 73.7 (C-2^B), 73.0, 72.9 (C-3^A, C-4^B), 72.5, 72.6 (C-3^B, C-5^B), 71.6 (C-2^A), 68.4 (C-5^A), 66.6 (O*C*H$_2$), 62.5 (C-6^A), 61.7 (C-6^B), 47.8 (*C*H$_2$N$_3$), 28.9 (CH$_2$*C*H$_2$N$_3$).

***p*-Methoxyphenyl 3-*O*-allyl-2-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranoside 27.**



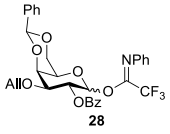
27

Compound **26** (1.0 g, 2.4 mmol) was dissolved in 20 mL of 1:4 pyridine and DCM mixture, then the resulting solution was cooled down to 0°C in a water/ice bath and BzCl (1.0 g, 7.2 mmol) was added dropwise. The reaction stirred at rt overnight, then the reaction was checked by TLC (4:6 cyclohexane:EtOAc), which showed full conversion of the starting material. The solvent was co-evaporated with toluene and purified by column chromatography (cyclohexane:EtOAc) affording **27** (500 mg, 40% yield). $[\alpha]_D^{25}$ = +2.69° (c 0.2, $CHCl_3$). ESI HR-MS ($C_{30}H_{30}O_8$) *m/z* $[M+H]^+$ found 519.20; calcd 519.20.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.15-6.7 (m, 14H, H-Ar), 5.86-5.73 (m, 2H, H-2, C*H*=CH$_2$), 5.59 (s, 1H, C*H*Ph), 5.21 (dd, 1H, *J* = 1.5 Hz, *J* =17.1 Hz, C*H*$_2$=CH), 5.09 (dd, 1H, *J* = 1.3 Hz, *J* = 10.4 Hz, C*H*$_2$=CH), 5.05 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.44-4.33 (m, 2H, H-4, H-6a), 4.20-4.05 (m, 3H, C*H*$_2$=CH, H-6b), 3.81 (dd, $J_{3,4}$ = 3.5, $J_{2,3}$ = 10.1 Hz, 1H, H-3), 3.71 (s, 3H, OCH$_3$), 3.57 (s, 1H, H-5).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.6 (CO), 165.1-126.5 (C-Ar), 119.34 (Cq), 117.5 (CH=*C*H$_2$), 114.33 (*C*H=CH$_2$), 101.53 (C-1), 101.31 (*C*H$_2$Ph), 77.10 (C-3), 73.58 (C-4), 70.81 (*C*H$_2$-CH=CH$_{2,}$ C-2), 69.12 (C-6), 66.88 (C-5), 55.59 (O*C*H$_3$).

***p*-Methoxyphenyl 3-*O*-allyl-2-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranosyl trifluoroacetimidate 28.**



28

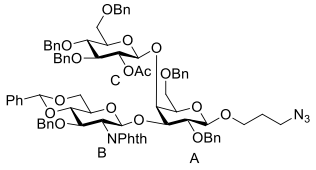
Compound **27** (0.4 g, 0.8 mmol) was dissolved in 10 mL of a 4:1 mixture of acetonitrile and water and the resulting solution was cooled down to 0°C. Cerium ammonium nitrate (0.8 g, 1.5 mmol) was added and the resulting reaction mixture stirred for 2 h at 0°C. After two hours analytical TLC (3:2 cyclohexane/EtOAc) showed full conversion of the starting material and formation of a new spot with lower R_f. The reaction mixture was poured into iced aqueous $NaHCO_3$ and extracted with DCM (5 x). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude was purified by column chromatography (cyclohexane/EtOAc), affording the target compound in 77% yield.

Then the obtained compound (0.2 g, 0.5 mmol) and 2,2,2-trifluoro-*N*-phenyl-acetoimidoyl-chloride (0.4 g, 1.8 mmol) were dissolved in 30.0 mL of dry DCM under nitrogen atmosphere. The resulting solution was cooled down to 0°C and Cs_2CO_3 (0.2 mmol, 0.6 mmol) was added. The resulting reaction mixture was stirred at rt for 2 hours, then analytical TLC (6:4 cyclohexane/EtOAc) showed complete conversion of the starting material and formation of a new spot with higher R_f. The reaction mixture was quenched with TEA and the solid was filtered off, then the reaction mixture was concentrated in vacuum and applied to a chromatography column (cyclohexane/EtOAc). Pure fractions were collected and evaporated under reduced pressure affording the trichloroacetimidate **28** (0.260 g, 55%). $[\alpha]_D^{25}$ = +60.93° (c 1.65, $CHCl_3$). ESI HR-MS ($C_{31}H_{28}F_3NO_7$) *m/z* $[M\text{-}CNPhCF_3+Na]^+$ found 435.1413; calcd 435.1414.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.13-7.01 (m, 14H, H-Ar), 6.72 (s, 2H, H-1, H-Ar), 5.86-5.72 (m, 2H, H-2, C*H*=CH_2), 5.58 (s, 1H, C*H*Ph), 5.22 (dd, 1H, *J* = 1.2 Hz, *J* = 17.3 Hz, C*H*$_2$=CH), 5.12 (dd, 1H, *J* = 0.8 Hz, *J* = 9.3 Hz, C*H*$_2$=CH), 4.44-4.29 (m, 2H, H-4, H-6a), 4.21-4.04 (m, 3H, C*H*$_2$-CH=CH_2, H6b), 3.91-3.41 (m, 2H, H-3, H-5).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.9 (C=O), 143.38-124.30, (C-Ar), 119.28 (*C*H=CH_2), 117.80 (CH=*C*H$_2$), 101.27 (*C*H$_2$Ph), 73.30 (C-4), 70.94 (*C*H$_2$-CH=CH_2), 69.93 (C-2), 68.79 (C-6), 67.69 (C-5).

3-Azidopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl)-(1→4)-[3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)]-2,6-di-*O*-benzyl-*β*-D-galactopyranoside 9a.



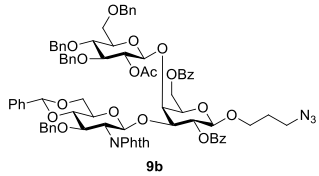
9a

A solution of donor **8** (50 mg, 0.08 mmol) and disaccharide acceptor **7a** (61 mg, 0.067) with activated 4 Å molecular sieves in dry DCM (4 mL) was stirred for 20 min under nitrogen. TMSOTf (3 μL, 0.013 mmol) was added at –10°C. After 4 h (TLC 9:1 Tol:EtOAc) the reaction was quenched with TEA, the solid filtered off and the solvent removed under pressure. The crude was purified by flash chromatography (Tol:EtOAc) to afford the trisaccharide **9a** in 75% yield (68 mg) as a pale yellow solid. $[\alpha]_D^{25}$ = +24.86° (c 0.8, $CHCl_3$). ESI HR-MS ($C_{80}H_{82}N_4O_{18}$): *m/z* = $[M+ Na]^+$ found 1409.5419; calcd 1409.5522.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55-6.85 (m, 39H, H-Ar), 5.66 (s, 1H, C*H*Ph), 5.56 (d, $J_{1,2}$ = 8.8Hz, 1H, H-1^B), 5.00-4.98 (m, 2H, H-1,2^C), 4.91-4.81 (m, 3H, 3 C*H*HPh), 4.60 (d, ^2J = 10.3 Hz, 1H, C*H*HPh), 4.53-4.36 (m, 8H, 7 C*H*HPh, H-6a^C), 4.28 (t, *J* = 9.2 Hz, 1H, H-2^B), 4.19 (d, $J_{1,2}$ = 7.6 Hz, 1H, H-1^A), 4.16 (d, $J_{3,4}$ = 2.3 Hz, 1H, H-4^A), 4.11 (d, ^2J = 11.5 Hz, 1H, C*H*HPh), 3.94-3.89 (m, 1H, H-3^C), 3.86-3.49 (m, 13H, H-3^{A,B}, H-4^{B,C}, H-5^{A-C}, H-6^{A,B}, H-6b^C, OCH$_{2a}$), 3.42-3.37 (m, 1H, OCH$_{2b}$), 3.30 (t, *J* = 8.8 Hz, 1H, H-2^A), 3.13-3.10 (m, 2H, CH_2N_3), 1.79 (s, 3H, CH_3CO), 1.73-1.62 (m, 2H, $CH_2CH_2N_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9 (CO), 133.7-123.1 (C-Ar), 103.5 (C-1^A), 101.3 (PhCH), 100.3 (C-1^C), 100.2 (C-1^B), 83.4 (C-3^C), 83.1, 81.2, 78.6 (C-2^A), 77.8, 75.2, 75.0, 74.9 (3 x PhCH$_2$), 74.5, 74.4 (C-4^A), 74.1, 73.7, 73.5, 73.4, 73.1 (3 PhCH$_2$), 69.8, 69.1, 68.7 (C-6^{A-C}), 66.3 (OCH$_2$), 65.9, 56.3 (C-2^B), 48.2 (*C*H$_2$N$_3$), 29.1 (*C*H$_2$CH$_2$N$_3$), 20.8 (*C*H$_3$CO).

3-Azidopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→4)-[4,6-*O*-benzylidene-3-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)]-2,6-di-*O*-benzoyl-*β*-D-galactopyranoside 9b.



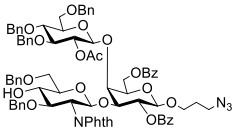
9b

A solution of donor **8** (48 mg, 0.077 mmol) and disaccharide acceptor **7a** (60m g, 0.064 mmol) with activated 4 Å molecular sieves (0.100 g) in dry DCM (4 mL) was stirred for 20 min under nitrogen. TMSOTf (2 μL, 0.013 mmol) was added at –10°C. After 4 h (TLC 9:1 Tol:EtOAc) the reaction was quenched with TEA, the solid filtered off and the solvent removed under pressure. The crude was purified by flash chromatography (Tol:EtOAc) to afford the trisaccharide **9b** in 68% yield (58 mg) as a pale yellow solid. $[\alpha]_D^{25}$ = +18.18° (c 1.45, $CHCl_3$). ESI HR-MS ($C_{80}H_{78}N_4O_{20}$) *m/z* $[M+Na]^+$ found 1437.5027; calcd 1437.5107.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08-6.80 (m, 39H, H-Ar), 5.66 (s, 1H, C*H*Ph), 5.43 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1^B), 5.15 (t, *J* = 8.3 Hz, 1H, H-2^A), 5.03 (t, *J* = 7.4 Hz, 1H, H-2^C), 4.99 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1^C), 4.91 (br. s, 2H, 2 C*H*HPh), 4.86 (d, ^2J = 10.7 Hz, C*H*HPh), 4.75 (d, ^2J = 10.7 Hz, C*H*HPh), 4.71 (d, $J_{5,6a}$ = 4.5 Hz, 12.3 Hz, 1H, H-6a^A), 4.62-4.37 (m, 6H, 4 C*H*HPh, H-6b^A, H-6a^B), 4.35 (d, *J* = 2.7 Hz, 1H, H-4^A), 4.32 (t, *J* = 8.5 Hz, 1H, H-3^B), 4.22 (t, *J* = 9.3 Hz, 1H, H-2^B), 3.94 (t, *J* = 9.5 Hz, 1H, H-3^C), 3.90-3.61 (m, 9H, H-3^A, H-4^{B,C}, H-5^{A,B}, H-6b^B, H-6^C, OCH_{2a}), 3.55-3.52 (m, 1H, H-5^C), 3.44-3.39 (m, 1H, OCH_{2b}), 3.08-2.96 (m, 2H, CH_2N_3), 2.02 (s, 3H, CH_3CO), 1.71-1.55 (m, 2H, CH_2CH$_2$N$_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.2, 166.4, 164.7 (3 x CO), 133.5-122.9 (C-Ar), 101.3 (Ph*C*H), 101.1 (C-1^A), 100.4 (C-1^C), 100.3 (C-1^B), 83.4 (C-3^C), 83.0 (C-4^B), 80.0 (C-3^A), 78.0, 75.5 (C-5^C), 75.4, 75.3, 74.3 (3 x *C*H$_2$Ph), 74.2 (C-3^B), 74.0 (C-4^A), 73.5 (Ph*C*H$_2$), 73.3 (C-2^C), 72.2, 70.6 (C-2^A), 69.2, 68.7 (C-6^{A,B}), 66.1 (C-5^A), 65.3 (O*C*H$_2$), 64.4 (C-6^C), 55.9 (C-2^B), 47.9 (*C*H$_2$N$_3$), 28.9 (*C*H$_2$CH$_2$N$_3$), 20.7 (CH$_3$CO).

3-Azidopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→4)-[3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)]-2,6-di-*O*-benzoyl-*β*-D-galactopyranoside 10.



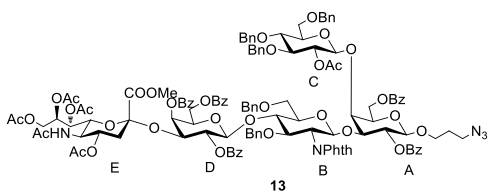
10

A solution of trisaccharide **9b** (258 mg, 0.181 mmol) in acetonitrile was cooled down to 0°C. Trimethylamino borane complex (53 mg, 0.72 mmol) was added, followed by $BF_3{\cdot}Et_2O$ (0.090 mL, 0.72 mmol). The reaction mixture was stirred at 0°C for 3 h, when TLC (4:1 Tol/EtOAc) showed formation of a new spot with lower R_f. The reaction was quenched by addition of Et_3N and MeOH, then evaporated under vacuum. The crude was purified by column chromatography (Tol:EtOAc). Clean fractions were collected and evaporated to dryness affording trisaccharide **10** (0.180 g, 70% yield) as a colorless oil. $[\alpha]_D^{25}$ = + 204.32° (c 0.39, $CHCl_3$). ESI HR-MS ($C_{80}H_{80}N_4O_{20}$) *m/z* ([*M*+ Na]$^+$ found 1439.5051; calcd (1439.5264)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15-6.54 (m, 39H, H-Ar), 5.29 (d, $J_{1,2}$ = 7.4 Hz, 1H, H-1^B), 5.08 (t, *J* = 9.1 Hz, 1H, H-2^A), 5.18-4.9 (m, 2H, incl. H-1^C, H-2^C), 4.81 (s, 2H, C*H*$_2$Ph), 4.76 (d, ^2J = 11.1, 1H, PhC*H*H), 4.66-4.56 (m, 2H, incl. PhC*H*H, H-6a^A), 4.56-4.33 (m, 7H, incl. H-6b^A), 4.32-4.26 (m, 2H, incl. H-1^A, H-4^A), 4.13-4.01 (m, 2H, incl. H-2^B, H-3^B), 3.87-3.66 (m, 6H, incl. H-3^C, H-3^A, H-5^A, H-4^B, H-6^B$_{a,b}$, OCH_{2a}), 3.66-3.52 (m, 4H, incl. H-4^C, H-5^B, H-6a,b^C), 3.49-3.40 (m, 1H, H-5^C), 3.40-3.29 (m, 1H, OCH_{2b}), 3.02-2.85 (m, 2H, CH_2N_3), 1.87 (s, 3H, CH_3CO), 1.66-1.42 (m, 2H, CH_2CH$_2$N$_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 166.4, 164.5 (3 x CO), 138.7-122.9 (C-Ar), 101.0 (C-1^A), 100.5 (C-1^C), 99.7 (C-1^B), 83.4 (C-3^C), 79.7 (C-3^A), 78.5 (C-3^B), 77.9 (C-4^C), 75.3 (C-5^C), 75.2, 75.0, 74.6 (3 x *C*H$_2$Ph), 74.3 (C-4^A), 74.2 (C-5^A), 73.7 (C-5^B), 73.7, 73.5 (2 x *C*H$_2$Ph), 73.1 (C-2^C), 72.3 (C-4^B), 70.7 (C-6^B), 70.6 (C-2^A), 69.2 (C-6^C), 65.1 (O*C*H$_2$), 64.8 (C-6^A), 55.5 (C-2^B), 47.9 (*C*H$_2$N$_3$), 28.9 (*C*H$_2$CH$_2$N$_3$), 20. 6 (*C*H$_3$CO).

3-Azidopropyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-[2,4,6-tri-*O*-benzoyl-3-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-*N*-5-acetamido-3,5-dideoxy-D-glycero-*α*-D-galacto-non-2-ulopyranosylonate)-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)]-2,6-di-*O*-benzoyl-*β*-D-galactopyranoside 13.



13

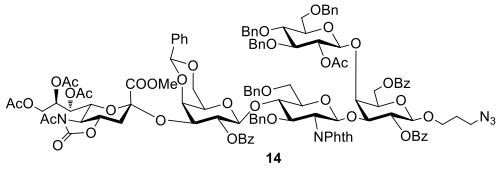
A solution of disaccharide donor **11** (124 mg, 0.109 mmol) and acceptor **10** (110 mg, 0.078 mmol) with 4 Å molecular sieves (0.200 g) in dry DCM (5.0 mL) was stirred for 20 min under nitrogen. TMSOTf (2.8 μL, 0.0156 mmol) was added at -20°C. After 4 h (TLC; 6:4 Tol:Acetone) the reaction was quenched with TEA, the solid filtered off and the solvent removed under reduced pressure. The crude was purified by flash chromatography (Tol:Acetone) to afford pentasaccharide **13** in 75% yield

(138 mg). $[\alpha]_D^{25}$ = +41.05° (c 0.75, $CHCl_3$). ESI HR-MS ($C_{127}H_{129}N_5O_{40}$) *m/z* ([*M*+ Na]$^+$ found 2386.8462; calcd 2386.8106.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39-6.56 (m, 54H, Ar-H), 5.77-5.70 (m, 1H, H-8^E), 5.51 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ =9.8 Hz, 1H, H-2^D), 5.34 (d, $J_{3,4}$ = 3.0 Hz, 1H, H-4^D), 5.26-5.19 (m, 2H, H-2^C, H-1^C), 5.12 (d, 1H, H-1^D), 5.09 (t, *J* = 9.8, 8.3 Hz, 1H, H-2^A), 4.97-4.72 (m, 7H, incl. H-1^B, C*H*HPh), 4.70-4.42 (m, 7H, C*H*HPh), 4.42-4.30 (m, 2H, C*H*HPh), 4.30-4.09 (m, 7H, incl. H-1^A, H-6^E), 4.08-3.92 (m, 2H), 3.92-3.55 (m, 14, incl. $COOCH_3$, H-5^E, H-2^B, OCH_{2a}), 3.54-3.32 (m, 3H, incl. OCH_{2b}), 3.14-2.88 (m, 2H, CH_2N_3), 2.51-2.41 (dd, 1H, $J_{3e,4}$ = 12.7 Hz, $J_{3a,3e}$ = 4.3 Hz, H-3e^E), 2.13, 2.01, 1.92, 1.87, 1.79 (5 x s, 3H each, 5 x CH_3CO), 1.73-1.57 (m, 3H, C*H*$_2$CH$_2$N$_3$, H-3a^E) , 1.50 (s, 3H, CH_3CO).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 170.7, 170.5, 170.4, 170.3, 170.2, 168.2, 167.6, 167.0, 165.6, 165.5, 165.0, 164.5 (14 x CO), 138.1-122.8 (C-Ar), 100.9, 100.7, 100.4, 99.4, 97.0, 83.4, 79.5, 77.8, 75.2, 75.1, 74.9, 74.7, 74.6, 74.4, 73.5, 73.4, 73.1, 72.7, 72.3, 72.0, 71.9, 71.8 (C-2^A), 71.6, 71.5, 71.3, 70.6, 70.6, 69.4, 68.9, 68.7, 68.1 (C-4^D), 67.5, 67.1 (C-8^E), 66.7 (C-2^D), 65.0, 64.8, 62.5, 61.6, 55.9, 53.2, 48.8, 47.9 (*C*H$_2$N$_3$), 37.4 (C-3^E), 28.9 (*C*H$_2$CH$_2$N$_3$), 21.5, 21.3, 20.8, 20.7, 20.6, 20.4 (6 x CO*C*H$_3$).

3-Azidopropyl 2-*O*-acetyl-3,4,6-tri-O-benzyl-*β*-D-glucopyranosyl-(1→4)-[4,6-*O*-benzylidene-2-*O*-benzoyl-3-*O*-(methyl 7,8,9-tri-*O*-acetyl-5-*N*-acetamido,4-*O*-oxazolidinone-3,5-dideoxy-D-glycero-*α*-D-galacto-non-2-ulopyranosylonate)-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)]-2,6-di-*O*-benzoyl-*β*-D-galactopyranoside 14.



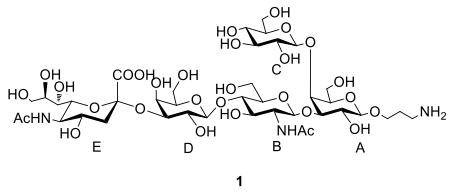
14

A solution of donor **12** (60 mg, 0.063 mmol) and acceptor **10** (60 mg, 0.042 mmol) with 4 Å molecular sieves in dry DCM (3.0 mL) was stirred for 20 min under nitrogen. *N*-Iodosuccinimide (0.019 g, 0.084 mmol) and TfOH (0.7 µL, 0.0084 mmol) were added at -40°C. After 3 h (TLC: 6:4 Toluene/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 **14** in 73% yield (68 mg). $[\alpha]_D^{25}$ = +0.70° (c 0.25, $CHCl_3$). ESI HR-MS ($C_{119}H_{121}N_5O_{38}$): *m/z* = ([*M*+ K]$^+$ found 2267.7361; calcd 2266.7321.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17-6.57 (m, 49H, Ar-H), 5.61-5.55 (m, 2H), 5.52 (dd, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 9.8, Hz, 1H, H-2^D), 5.33 (s, 1H, C*H*Ph), 5.21 (d, $J_{1,2}$ = 7.2 Hz, 1H, H-1^B), 5.05 (dd, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 9.9, Hz, 1H, H-2^A), 5.02-4.95 (m, 2H, incl. H-2^C), 4.91 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1^C), 4.91 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1^D), 4.83 (s, 2H, C*H*$_2$Ph), 4.78 (d, ^2J = 10.9 Hz, 1H, C*H*HPh), 4.58-4.51 (m, 2H), 4.50-4.42 (m, 6H), 4.42-4.29 (m, 4H, incl. H-1^A), 4.28-4.23 (m, 2H, incl. H-2^B), 4.21-3.95 (m, 7H), 3.90-3.61 (m, 9H), 3.58-3.31 (m, 10H), 3.05-2.85 (m, 3H, H-3e^E, OCH_2), 2.46 (s, 3H, $NCOCH_3$), 2.18, 1,99, 1,90, 1.83 (5 x s, 3H each, 5 x CH_3CO), 1.79, (t, $J_{3a,3e}$ = 6.2 Hz, 1H, H-3a^E), 1.63-1.46 (m, 2H, C*H*$_2$CH$_2$N$_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 170.9, 170.5, 170.3, 170.0, 168.4, 167.5, 166.9, 166.2, 164.9, 164.4, 153.5 (12 x CO), 137.7-122.7 (C-Ar), 100.9 (*C*HPh), 100.9 (C-1^B), 100.7 (C-1^C), 100.4 (C-1^A), 99.6 (C-1^D), 97.2, 83.4, 79.9, 77.9, 77.8, 77.2, 75.3, 75.2, 75.1, 75.0, 74.9, 74.9, 74.6, 74.4, 73.5, 73.0, 72.9, 72.8, 72.2, 71.4, 71.1, 70.4, 68.9, 68.8,68.7, 67.9, 66.0, 64.9, 63.7, 58.9, 55.9, 52.9 (COO*C*H$_3$), 47.9 (*C*H$_2$N3), 37.0 (C-3^E), 28.9 (*C*H$_2$CH$_2$N$_3$), 24.7, 21.4, 20.8, 20.7, 20.6 (5 x *C*H$_3$CO).

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



1

Protocol A: A mixture of protected pentasaccharide **13** (0.1 mmol) and LiI (3 mmol) in pyridine (5 ml) was heated for 24h at 120°C. The reaction mixture was concentrated under vacuum, and the residue was purified by silica gel column chromatography (gradient 2% MeOH in DCM) to afford the demethylated product. This material was dissolved in ethanol (4 ml), and ethylenediamine (400 µl) was added. After being stirred for 16 h at 90 °C, the reaction mixture was then concentrated in vacuum, and the residue was coevaporated from toluene (2 x 10 mL) and EtOH (2 x 5 mL). The crude mixture was re-dissolved in pyridine (5 ml), and acetic anhydride (5 ml) was added. After being stirred for 16 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in MeOH and MeONa was added until pH=13. After 48 h the reaction was neutralized and the solvent removed under vacuum. 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 H_2 (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 **1** as an amorphous powder (40% yield).

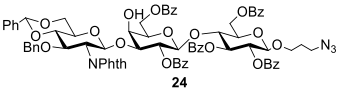
Protocol B: Protected pentasaccharide **14** (0.03 mmol) was dissolved in THF (3 ml) to which 3 M NaOH (0.3 ml) was added. After refluxing for 2 days, the mixture was neutralized with 0.1% HCl and concentrated. The residue was re-dissolved in 2:3 Ac_2O-MeOH (5 ml) and stirred overnight, when C18-TLC (2:3 H_2O/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 H_2 (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 freeze-dried to afford the deprotected oligosaccharide **1** as an amorphous powder (45% yield).

$[\alpha]_D^{25}$ = +1.24° (c 0.2, H_2O). ESI HR -MS ($C_{40}H_{69}N_3O_{29}$) *m/z* $[M-H]^-$ 1054.3971; found 1054.3866.

^{1}H NMR (400 MHz, D_2O) δ 4.88 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1^C), 4.69 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1^B), 4.54 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1^D), 4.39 (d, $J_{1,2}$ = 8.1 Hz, 1H, H-1^A), 4.37-4.35 (m, 1H), 4.09 (dd, $J_{3,4}$ = 2.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-3^A), 4.02-3.47 (m, 30H), 3.47-3.32 (m, 2H), 3.26 (dd, 1H, $J_{2,3}$ =9.4 Hz, H-2^C), 3.13 (dt, *J* = 8.2, 6.7 Hz, 1H), 2.74 (dd, $J_{3e,4}$ = 12.3 Hz, $J_{3e,3a}$ = 4.6, Hz, 1H, H-3e^E), 2.02 (s, 3H, $NCOCH_3$), 2.01 (s, 3H, CH_3CO), 2.04-1.93 (m, 2H, $CH_2CH_2NH_2$), 1.78 (t, 1H, H-3a^E).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 175.0, 174.8, 173.8 (3 x CO), 103.1 (C-1^A), 102.9 (C-1^D), 102.5 (C-1^C), 102.0 (C-1^B), 82.1, 78.1, 75.8, 75.6, 75.5, 75.2, 74.8, 74.6, 74.2, 73.6, 72.8, 72.1, 71.7, 70.0, 69.7, 69.3, 68.3, 68.0, 67.9, 67.4, 62.5, 61.0, 60.6, 60.1, 55.3, 51.6, 39.6 (CH_2N_3), 37.6 (C-3^E), 26.7 ($CH_2CH_2N_3$), 22.2, 22.0 (2 x CH_3CO). See also Table 1.

3-Azidopropyl 4,6-*O*-benzilidene-3-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)-2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside 24.



24

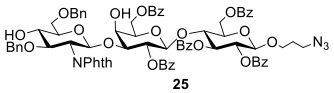
A solution of donor **23** (450 mg, 0.7 mmol) and lactose acceptor **22** (518 mg, 0.548 mmol) with activated 4 Å molecular sieves (500 mg) in dry DCM (7 mL) was stirred for 20 min under nitrogen. TMSOTf (19 µL, 0.1 mmol) was added at –10°C. After 4 h (TLC: 9:1 DCM/EtOAc) the reaction was quenched with TEA, the solid filtered off and the solvent removed under pressure. The crude was

purified by flash chromatography (DCM/EtOAc) to afford the trisaccharide **24** in 68% yield. $[\alpha]_D^{25}$ = + 36.64° (c 0.1, $CHCl_3$). ESI HR-MS ($C_{78}H_{70}N_4O_{22}$) *m/z* $[M+H]^+$ found 1415.46; calcd 1415.45.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.14-6.72 (m, 39H, H-Ar), 5.62 (t, *J* = 9.5 Hz, 1H, H-3^A), 5.59 (s, 1H, C*H*Ph), 5.35 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.6 Hz, H-2^A), 5.31 (d, *J* = 7.5 Hz, 1H, H-1^C), 5.26 (dd, $J_{2,3}$ = 8.1 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-2^B), 4.70 (d, ^2J = 12.4, 1H, C*H*HPh), 4.55 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1^A), 4.45 (d, $J_{1,2}$ =8.0 Hz, 1H, H-1^B), 4.39 (d, *J* = 12.4 Hz, 1H, CH*H*Ph), 4.42-4.10 (m, 6H, incl. H-6a^A, H-6b^A, H-6a^B, H-6a^C, H-3^C, H-2^C), 4.05 (t, *J* = 9.5 Hz, 1H, H-4^A), 3.97 (d, *J* = 3.0 Hz, 1H, H-4^B), 3.85-3.72 (m, 3H, incl. H-5^A, H-6b^C, OCH_{2a}), 3.69 (dd, J = 3.3, 9.8 Hz, 1H, H-3^B), 3.66-3.55 (m, 3H, incl. H-4^C, H-6^{B}b, H-5^C), 3.55-3.49 (m, 1H, H-5^B), 3.49-3.41 (m, 1H, OCH_{2b}), 3.21-3.09 (m, 2H, CH_2N_3), 1.80-1.58 (m, 2H, CH_2CH$_2$N$_3$).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 166.0, 165.8, 165.5, 165.2, 164.0 (5 x CO), 137.6-126.1 (m, C-Ar), 101.3 (CHPh), 101.0 (C-1^A), 100.6 (C-1^B), 99.8 (C1-^{C}), 82.6, 80.6, 75.3, 74.2, 74.0, 72.9, 72.5, 72.1, 71.7, 70.6, 68.5, 68.3, 66.5, 66.1, 62.6, 62.3, 55.5 (C-2^C), 47.8 (*C*H$_2$N$_3$), 28.9 (*C*H$_2$CH$_2$N$_3$).

3-Azidopropyl 3,6-di-*O*-benzyl-2-deoxy-2-phtalimido-*β*-D-glucopyranosyl-(1→3)-[2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)]-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside 25.



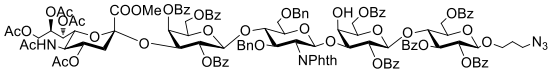
25

Trisaccharide **24** (100 mg, 0.071 mmol) was dissolved in 5.0 mL of acetonitrile and the resulting solution was cooled down to 0°C. Trimethylamino borane complex (26 mg, 0.355 mmol) was added, followed by $BF_3{\cdot}Et_2O$ (43 µL, 0.355 mmol). The reaction mixture was stirred at 0°C for 3 h (TLC 4:1 Tol:EtOAc), then quenched by addition of Et_3N and MeOH, evaporated and purified by flash chromatography (Tol:EtOAc) affording trisaccharide **25** (65% yield). $[\alpha]_D^{25}$ = -4.99° (c 0.05, $CHCl_3$). ESI HR-MS ($C_{78}H_{72}N_4O_{22}$) *m/z* $[M+Na]^+$ found 1439.45; calcd 1439.45.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.14-6.87 (m, 39H, H-Ar), 5.62 (t, *J* = 9.4 Hz, 1H, H-3^A), 5.34 (dd, $J_{1,2}$ = 7.8 Hz, 1H, H-2^A), 5.28-5.21 (m, 2H, H-2^B, H-1^C), 4.64-4.50 (m, 2H, H-1^A, C*H*$_2$Ph), 4.50-4.34 (m, 6H, H-6a^A, 2 x C*H*$_2$Ph, H-1^B), 4.22 (dd, ^2J = 4.7 Hz, 12.2 Hz, 1H, H-6b^A), 4.18-4.01 (m, 4H, including H-2^C, H-6a^B, H-4^A), 3.98 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4^B), 3.84-3.76 (m, 1H, OCH_{2a}), 3.75-3.40 (m, 9H, including H-3^{B,C}, H-5^B, H-6^C, H-6b^B, OCH_{2b}), 3.20-3.10 (m, 2H, CH_2N_3), 1.79-1.58 (m, 2H, CH_2CH$_2$N$_3$).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 165.9, 165.8, 165.4, 165.2, 163.9, 137.8 (7 x CO), 133.4-125.3 (C-Ar), 101.0 (C-1^A), 100.0 (C-1^B), 98.9 (C-1^C), 80.9, 78.4, 75.2, 74.2 (*C*H$_2$Ph), 73.8 (*C*H$_2$Ph), 73.6, 73.5 (*C*H$_2$Ph), 72.9, 72.5 (C-3^A), 72.1, 71.7 (C-2^A), 70.6 (C-2^B), 70.2 (C-6^C), 67.8 (C-4^B), 66.5 (O*C*H$_2$), 62.8 (C-6^B), 62.3 (C-6^A), 54.9 (C-2^C), 47.8 (*C*H$_2$N$_3$), 29.7 (*C*H$_2$CH$_2$N$_3$).

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→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside-(1→3)-2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside 29.



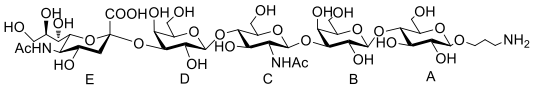
29

A solution of trisaccharide acceptor **25** (103 mg, 0.073 mmol) and disaccharide donor **11** (124 mg, 0.117 mmol) with activated 4 A molecular sieves (100 mg) in DCM (5 mL) was stirred for 20 min under nitrogen. TMSOTf (2.65 µL, 0.015 mmol) was added at 0 °C. After the reaction mixture was stirred for 2h at rt, TLC (6:4 Tol:Acetone) showed complete reaction. TEA was added until neutral pH, the solid filtered off and the solvent removed at reduced pressure. The crude was purified by flash chromatography (Tol:Acetone) to afford **29** (112 mg, 65% yield). $[\alpha]_D^{25}$ = +23.9° (c 0.1, $CHCl_3$). ESI HR-MS *m/z* ($C_{125}H_{121}N_5O_{42}$) *m/z* $[M+Na]^+$; found 2387.34, calcd: 2387.73.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.29-6.55 (m, 54H, H-Ar), 5.73-5.66 (m, 1H, H-8^E), 5.60-5.53 (m, 1H, H-3^A), 5.47 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{3,4}$= 9.9 Hz, H-2^D), 5.31-5.25 (m, 2H, H-2^A, H-7^E), 5.22-5.15 (m, 2H, H-2^B, H-4^D), 5.09 (d,

$J_{1,2}$ = 7.5, 1H, H-1^{D}), 5.05 (t, $J_{1,2}$ = 7.5, 1H, H-1^{C}), 4.87-4.77 (m, 3H, incl. CH_2Ph, H-3^{D}), 4.51-4.42 (m, 3H, incl. CH_2Ph, H-1^{A}), 4.37-4.28 (m, 4H, H-1^{B}), 4.19-43.93 (m, 11H, include. H-2^{C}, H-4^{B}, H-4^{A}), 3.85-3.74 (m, 6H, include. COOCH_3, H-5^{E}), 3.61-3.53 (m, 4H), 3.49-3.45 (dd, 1H, $J_{3,4}$ = 3.3 Hz, $J_{2,3}$ = 9.6 Hz, H-3^{B}), 3.44-3.29 (m, 3H), 3.15-3.05 (m, 2H, CH_2N$_3$), 2.45-2.39 (m, 1H, H-3$_{eq}^{E}$), 2.14 (s, 3H, CH_3CO), 2.06-2.03 (m, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.89 (s, 3H, CH_3CO), 1.76 (s, 3H, CH_3CO), 1.68-1.58 (m, 3H, CH_2CH_2N$_3$), 1.50 (s, 3H, CH_3CO).
^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.78-163.87 (CO), 138.17-125.31 (C–Ar), 101.01 (C-1^{A}), 100.48 (C-1^{B}), 100.37 (C-1^{C}), 98.75 (C-1^{D}), 96.94, 80.99 (C-3^{B}),75.11, (C-4^{A}), 74.44, 74.34 (*C*H_2Ph), 72.98, 72.78, 72.42 (C-3^{A}), 72.17, 71.90 (C-2^{D}), 71.83, 71.78 (C-7^{E}), 71.63 (C-3^{D}), 70.72 (C-4^{B}), 70.52, 69.38, 68.17 (C-2^{A}), 68.04, 67.61, 67.10 (C-8^{E}), 66.66 (C-2^{B}), 66.50, 63.06, 62.58, 62.31, 61.79, 55.23 (C-2^{C}), 53.22 (C-5^{E}), 48.77 (COO*C*H_3), 47.81 (*C*H_2N$_3$), 37.37 (C-3^{E}), 29.70, 28.86 (*C*H_2CH_2N$_3$), 23.15, 21.38, 20.74, 20.67, 20.43 (6 × *C*H_3CO).

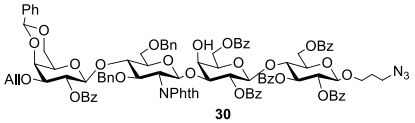
3-Aminopropyl 3-*O*-(5-*N*-acetamido-3,5-dideoxy-D-glycero-*α*-D-galacto-non-2-ulopyranosyl)-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranoside 2.



2

A mixture of protected pentasaccharide **28** (0.1 mmol) and LiI (3 mmol) in pyridine (5 ml) was heated for 24 h at 120°C. The reaction mixture was concentrated under vacuum, and the residue was purified by silica gel column chromatography (gradient 2% MeOH in DCM) to afford the demethylated product. This material was dissolved in ethanol (4 ml), and ethylenediamine (400 µl) was added. After being stirred for 16 h at 90 °C, the reaction mixture was then concentrated in vacuum, and the residue was coevaporated from toluene (2 x 10 mL) and EtOH (2 x 5 ml). The crude mixture was re-dissolved in pyridine (5 ml), and acetic anhydride (5 ml) was added. After being stirred for 16 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in MeOH and MeONa was added until pH=13. After 48h the reaction was neutralized and the solvent removed under vacuum. The residue was dissolved in MeOH and Pd/C (1:1 w/w in respect to the sugar) was added. The reaction mixture was stirred under pressure of H_2 (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 **10** as an amorphous powder (33% yield). $[\alpha]_D^{25}$ = +46.0° (c 0.03, H_2O). ESI HR-MS *m/z* $C_{40}H_{69}N_3O_{29}$ $[M+H]^+$ 1056.3971; found 1056.3966. The compound was identical to the one previously reported in the literature[26].

3-Azidopropyl 3-*O*-allyl-4,6-*O*-benzylidene-3-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)-2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside 30.

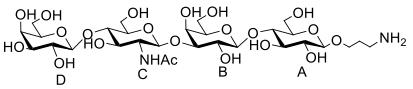

30

A solution of donor **28** (37 mg, 0.063 mmol) and acceptor **25** (60 mg, 0.042 mmol) with activated 4 Å molecular sieves (50 mg) in dry DCM (3 mL) was stirred under nitrogen. TMSOTf (1.5 µL, 0.008 mmol) was added at –5°C. After 2 h the analytical TLC (8:2 Toluene/EtOAc) showed the presence of a lower spot. The reaction was quenched with TEA, the solid filtered off and the solvent removed under reduced pressure. The crude was purified by column chromatography (Tol/EtOAc) to afford the tetrasaccharide **30** (56 mg, 0.031 mmol) in 72% yield. $[\alpha]_D^{25}$ = +11.2° (c 0.15, $CHCl_3$). ESI HR-MS **(**$C_{101}H_{94}N_4O_{28}$) *m/z* $[M+Na]^+$ found 1834.60, calcd 1834.69. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.14-6.56 (m, 49H, H-Ar), 5.83-5.68 (m, 1H, C*H*=CH_2), 5.60-5.50 (m, 2H, H-3^{D}, H-3^{A}), 5.46 (s, 1H, C*H*Ph), 5.28 (t, *J* = 9.0 Hz, 1H, H-2^{A}), 5.22-5.14 (m, 2H, H-2^{B}, C*H*H=CH), 5.09 (d, 2H, C*H*H=CH, incl. $J_{1,2}$ = 8.6 Hz, H-1^{C}), 4.93 (d, ^2J = 12.0 Hz, 1H, *CH*H-CH=CH_2), 4.72 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1^{D}),

4.49 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1^{A}), 4,43 (d, J = 12.0 Hz, 1H, *CH*H-CH=CH$_2$), 4.42 (d, $J_{1,2}$ = 12.8 Hz, 1H, CH*H*Ph), 4.37 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1^{B}), 4.33-4.05 (m, 9H, including H-2^{C}), (m, 7H, including H-4^{A}), 3.84 (s, 1H, H-4^{B}), 3.78-3.71 (m, 1H, OCH$_{2a}$), 3.60-3.48 (m, 5H, incl. H-3^{B}, H-3^{D}), 3.39-3.33 (m, 3H, incl. OCH$_{2b}$), 3.27 (s, 1H, OH-4^{B}), 3.13-3.07 (m, 2H, CH$_2$N$_3$), 1.69-1.54 (m, 2H, C*H*$_2$CH$_2$N$_3$).

^{13}C-NMR (101 MHz, CDCl$_3$) 165.89-163.90 (CO), 138.48-126.45 (C-Ar, *CH*=CH$_2$), 117.38 (*C*H$_2$CH=CH$_2$), 101.24 (*C*HPh), 101.07 (C-1^{D}), 100.99 (C-1^{A}), 100.45 (C-1^{B,C}), 98.80, 80.99, 78.31, 77.36, 77.27, 76.97, 75.21, 75.10 (*C*H$_2$Ph), 74.60, 73.34, 73.25, 72.96, 72.49, 72.07 (C-2^{B}, C-3^{A}), 71.76 (C-2^{A}), 71.42, 70.58, 70.46 (C-2^{D}), 67.55 (C-4^{B}), 66.50, 66.47, 62.86, 55.32 (C-2^{C}), 47.81 (*C*H$_2$N$_3$), 29.71, 28.86 (CH$_2$CH$_2$N$_3$).

3-Aminopropyl *β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranoside 5.



5

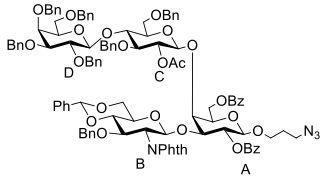
Compound **33** was treated with $PdCl_2$ (2 eq) in methanol (2.5 mL) for 6 h. After filtration, the crude material was dissolved in ethanol (3 mL), and ethylenediamine (400 µL) was added. After being stirred for 16 h at 110 °C, the reaction mixture was concentrated under vacuum, and the residue was coevaporated with toluene (2 x 10 mL) and EtOH (2 x 5 mL). The crude mixture was re-dissolved in pyridine (1.5 mL), and acetic anhydride (1.5 mL) was added at 0°C. After stirring for 16 h at rt, the reaction mixture was quenched with MeOH and concentrated under reduced pressure. The mixture was dissolved in MeOH (2 mL) and MeONa was added until pH = 12. After 48h the reaction was neutralized and the solvent removed under vacuum. The compound was purified by flash chromatography in silica C-18 (MeOH/H_2O), and the obtained product was dissolved in *t*BuOH (2 mL), to which Pd/C (1:1 w/w in respect to the sugar) was added. The reaction mixture was stirred under pressure of H_2 (5 bar) for 72 h. Then, the catalyst was filtered off, the filtrate concentrated under reduced pressure and the crude was purified by size exclusion column chromatography affording compound **5** with a yield of 42%.

$[\alpha]_D^{25}$ = -28.20˚ (c 0.1, $CHCl_3$). ESI-HR MS (**(**$C_{29}H_{52}N_2O_{21}$) *m/z* $[M+Na]^+$ found 765.3136, calcd 765.3135.

^{1}H-NMR (400 MHz, D_2O) δ 4.57 (d, $J_{1,2}$ = 8.1 Hz, 1H, H-1^{C}), 4.37 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1^{B}), 4.34 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1^{A}), 4.30 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1^{D}), 4.02 (d, 1H, J = 3.1 Hz), 3.95-3.77 (m, 4H), 3.76-3.36 (m, 20H, includ. H-3^{A}, OC*H*$_2$), 3.19 (t, J = 8.2 Hz, 1H, H-2^{A}), 3.05 (t, 1H, J = 7.2 Hz, C*H*$_2$N$_3$), 1.9 (s, 3H), 1.96-1.81 (m, 1H, C*H*$_2$CH$_2$N$_3$).

^{13}C-NMR (101 MHz, D_2O) δ 102.9 (C-1^{D}), 102.8 (C-1^{C}), 102.7 (C-1^{A}), 102.0 (C-1^{B}), 81.9, 78.5, 78.4, 75.2, 74.7, 72.8, 72.6, 72.1, 70.7, 70.1, 68.5, 68.2, 67.8, 61.0, 60.1, 55.2, 37.5, 25.8, 22.2.

3-Azidopropyl 4,6-*O*-benzilidene-3-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→4)]-2,6-di-*O*-benzoyl-*β*-D-galactopyranoside 32.



32

A solution of donor **30** (0.900 g, 0.85 mmol) and disaccharide acceptor **7a** (0.500 g, 0.53 mmol) with activated 4 Å molecular sieves (0.500 g) in dry DCM (7 mL) was stirred for 20 min under nitrogen. TMSOTf (19 µL, 0.109 mmol) was added at –10°C. After 4 h (TLC; 9:1 DCM: EtOAc) the reaction was quenched with TEA, the solid filtered off and the solvent removed under pressure. The crude was purified by flash chromatography (Tol:EtOAc) to afford the tetrasaccharide **32** in 60% yield (0.590 g) as a white amorphous solid.

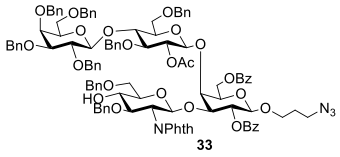
$[\alpha]_D^{25}$ = +11.27˚ (c 0.5, $CHCl_3$). ESI HR-MS ($C_{107}H_{106}N_4O_{25}$) *m/z* $[M-Na]^+$ 1869.7146; found 1869.7047.

^{1}H NMR (400 MHz, CDCl3) δ 8.17-6.74 (m, 54H, H-Ar), 5.59 (s, 1H; CHPh), 5.44 (d, 1H, J=8.3 Hz, H-1^{B}), 5.21 (dd, 1H, J=8.3, 10.1, H-2^{C}), 5.18 (d, 1H, J=11.6 Hz, C*H*HPh), 5.08-5.00 (m, 3H, incl. H-1^{C}, H-2^{C}), 4.95 (d, 2H, J=2.5 Hz, CH$_2$Ph), 4.82-4.74 (m, 4H), 4.70 (dd, 1H, J=3.3, 12.0 Hz, H-6$^{A}_{a}$), 4.65-4.31 (m, 10H, incl. H-6$^{A}_{b}$, H-1^{D},

H-1^{A}), 4.30-4.22 (m, 2H, incl. H-2^{B}), 4.07-3.72 (m, 11H, incl. H-3^{A}, H-3^{C}, OC*H*H), 3.70-3.61 (m, 1H), 3.58-3.49 (m, 3H), 3.48-3.34 (m, 4H, incl. OCH*H*), 3.14-2.95 (m, 2H, CH_2N_3), 1.93 (s, 3H, $COCH_3$), 1.74-1.53 (m, 2H, $CH_2CH_2N_3$)

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 167.6, 166.9, 166.4, 164.5 (5xCO), 139.6-122.9 (m, 68C, C-Ar), 103.1 (C-1^{D}), 101.4 (CHPh), 100.9 (C-1^{A}), 100.3 (C-1^{C}), 99.8 (C-1^{B}), 83.1, 82.6, 81.4, 80.1, 79.2, 75.8, 75.3, 74.7, 74.6, 74.5, 74.3, 73.8, 73.7, 73.5, 73.2, 73.1, 72.6 (C-2^{C}), 72.6, 72.3, 70.7 (C-2^{A}), 68.7, 68.5, 68.2 (CH_2N_3), 66.1, 65.0, 64.6 (C-6^{A}), 55.9 (C-2^{B}), 47.9 (OCH_2), 28.9 ($CH_2CH_2N_3$), 20.5 (CH_3).

3-Azidopropyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→4)]-2,6-di-*O*-benzoyl-*β*-D-galactopyranoside 33.



33

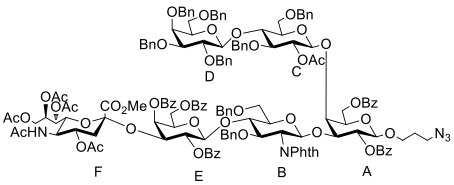
A solution of **32** (0.380 g, 0.21 mmol) in ACN (10 mL) was cooled at 0°C. Me_3NBH_3 (0.075 g, 1.03 mmol) and $BF_3{\cdot}OEt_2$ (0.127 mL, 1.03 mmol) were added and the reaction was stirred for 2 h under nitrogen. TLC showed complete reaction (8:2 toluene:EtOAc). First TEA and then MeOH were added until neutral pH. The solvent was removed at reduced pressure and the crude was purified by flash chromatography (toluene:EtOAc) to afford **33** in 52% yield (0.200 g).

$[\alpha]_D^{25}$ = +29.32° (c 0.25, $CHCl_3$). ESI HR -MS ($C_{107}H_{108}N_4O_{25}$) *m/z* $[M+Na]^+$ 1871.7303; found 1871.7026.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-6.77 (m, 54H, H-Ar), 5.31 (d, 1H, J=7.7 Hz, H-1^{B}), 5.18-5.08 (m, 2H, incl. H-2^{A}), 5.04-4.84 (m, 6H, incl. H-1C, H-2C, 3xCH_2Ph), 4.78-4.26 (m, 16H, incl.H-1D, H-6^{A}a, H-6^{A}b,), 4.26-4.05 (m, 3H), 3.99 (t, 1H, J=9.4 Hz), 3.95-3.89 (m, 2H), 3.89-3.69 (m, 7H), 3.69-3.54 (m, 2H), 3.54-3.27 (m, 6H), 3.10-2.91 (m, 2H), 1.77 (s, 3H), 1.66-1.49 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3, 166.3, 164.6 (3xCO), 139.7-126.8 (m, 68 C, C-Ar), 102.9 (C-1^{D}), 100.9 (C-1^{A}), 100.3 (C-1^{C}), 99.1 (C-1^{B}), 82.6, 81.3, 80.0, 78.7, 78.6, 75.5, 75.1, 74.7, 74.6, 74.5, 74.3, 74.0, 73.7, 73.6, 73.6, 73.4, 73.2, 73.0, 72.6, 72.5, 72.4 (C-2^{C}), 70.8, 70.6 (C-2^{A}), 68.5, 68.2 (CH_2N_3), 64.9, 64.8, 55.6 (C-2^{B}), 48.0 (OCH_2), 28.9 ($CH_2CH_2N_3$), 20.4 (CH_3).

3-Azidopropyl 3-*O*-(5-acetamido-3,5-dideoxy-D-glycero-*α*-D-galacto-non-2-ulopyranosyl)-2,4,6-tri-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl)-(1→4)]-2,6-di-*O*-benzoyl-*β*-D-galactopyranoside 34.



34

A solution of donor **11** (0.162 g, 0.143 mmol) and tetrasaccharide acceptor **33** (0.176 g, 0.095 mmol) with activated 4 Å molecular sieves (0.200 g) in dry DCM (3 mL) was stirred for 20 min under nitrogen. TMSOTf (3.4 µL, 0.019 mmol) was added at –10°C. After 4 h (TLC; 6:4 Toluene:acetone) the reaction was quenched with TEA, the solid filtered off and the solvent removed under pressure. The crude was purified by flash chromatography (Tol:acetone) to afford the tetrasaccharide **34** in 78% yield (0.206 g) as a white amorphous solid.

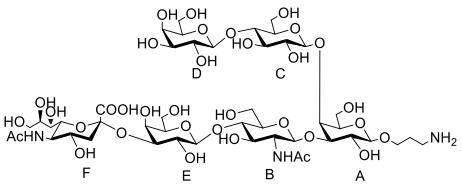
$[\alpha]_D^{25}$ = +26.62° (c 0.25, $CHCl_3$). ESI HR -MS ($C_{154}H_{157}N_5O_{45}$) *m/z* $[M+Na]^+$ 2819.0151; found 2819.0154.

^{1}H NMR (400 MHz, CDCl3) δ 8.36-6.54 (m, 69H, H-Ar), 5.74-5.66 (m, 1H, H-8^{F}), 5.48 (dd, 1H, J=7.4, 9.6 Hz, H-2^{E}), 5.33 (d, 1H, J=3.2 Hz, H-4^{E}), 5.22 (dd, 1H, J=2.3, 9.6 Hz, H-7^{F}), 5.18-5-04 (m, 4H, incl. H-2^{A}, H-1^{E}), 4.99-4.90 (m, 3H, incl. H-3^{E}, H-1^{B}), 4.90-4.77 (m, 4H), 4.75 (s, 2H, OCH_2Ph), 4.72-4.63 (m, 3H), 4.61-4.38 (m, 8H, incl. H-1^{A}), 4.35-4.08 (m, 10H), 4.09-3.93 (m, 4H, incl. H-9^{F}), 3.92-3.64 (m, 12H, COOCH3), 3.64-3.31 (m, 8H, incl.

C*H*HN$_3$), 3.30-3.24 (m, 1H, CH*H*N$_3$), 3.10-2.89 (m, 2H, OCH$_2$), 2.43 (dd, 1H, J=4.5, 12.4 Hz, H-3$^F_{eq}$), 2.14 (s, 3H, COCH$_3$), 1.98 (s, 3H, COCH$_3$), 1.91 (s, 3H, COCH$_3$), 1.78 (s, 3H, COCH$_3$), 1.70-1.57 (m, 7H, incl. C*H*$_2$CH$_2$N$_3$, H-3$^F_{ax}$, COCH$_3$), 1.50 (s, 3H, COCH$_3$).

^{13}C NMR (101 MHz, CDCl$_3$) 170.8, 170.7, 170.5, 170.1, 168.1, 167.5, 167.1, 166.2, 165.6, 165.5, 164.9, 164.7 (12xC=O), 139.8-122.9 (C-Ar), 102.9 (C-1^A), 100.8, 100.5, 100.4 (C-1^B), 100.0, 98.6 (C-1^E), 97.0, 82.7, 81.2, 79.9, 77.6, 77.5, 77.2, 76.9, 76.8, 75.3, 75.1, 74.7, 74.5, 74.3, 73.7, 73.4, 73.1, 72.9, 72.7, 72.5, 72.4, 71.9, 71.8 (C-2^E), 71.7 (C-2^A), 70.9 (C-3^E), 70.5, 69.4, 68.1 (C-4^E), 67.2 (C-8^F), 66.5 (C-7^F), 64.8, 64.7, 62.4, 61.5, 55.9 (C-2^B), 53.2 (COOCH$_3$), 48.8, 47.9 (OCH$_2$), 37.4 (C-5^F), 28.9 (*C*H$_2$CH$_2$N$_3$), 23.2, 21.4, 20.8, 20.4, 20.2 (6xCOCH$_3$).

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



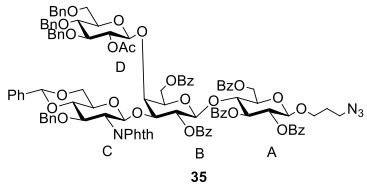
4

A mixture of protected hexasaccharide **34** (0.1 mmol) and LiI (3 mmol) in pyridine (5 ml) was heated for 24h at 120°C. The reaction mixture was concentrated under vacuum, and the residue was purified by silica gel column chromatography (gradient 2% MeOH in DCM) to afford the demethylated product. This material was dissolved in ethanol (4 ml), and ethylenediamine (400 μl) was added. After being stirred for 16 h at 90 °C, the reaction mixture was then concentrated in vacuum, and the residue was coevaporated with toluene (2 x 10 mL) and EtOH (2 x 5 mL). The crude mixture was re-dissolved in pyridine (5 ml), and acetic anhydride (5 ml) was added. After being stirred for 16 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in MeOH and MeONa was added until pH=13. After 48 h the reaction was neutralized and the solvent removed under vacuum. 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 H_2 (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).

^{1}H NMR (400 MHz, D$_2$O) δ 4.93 (d, 1H, $J_{1,2}$= 8.0 Hz, H-1^C), 4.70 (d, 1H, $J_{1,2}$=8.1 Hz, H-1^B), 4.54 (d, 1H, $J_{1,2}$=7.9 Hz, H-1^E), 4.43 (d, 1H, $J_{1,2}$=7.6 Hz, H-1^D), 4.40 (d, 1H, $J_{1,2}$=8,1 Hz, H-1^A), 4.38 (d, 1H, $J_{1,2}$=2.7 Hz, H-4^A), 4.10 (dd, 1H, $J_{1,3}$=9.3 Hz, $J_{1,2}$=2.7 Hz, H-3^E), 4.03-3.51 (m, 36H), 3.30 (t, 1H, J=8.6 Hz, H-2^C), 3.27 (t, 2H, J=7.1 Hz, C*H*$_2$NH$_2$), 2.74 (dd, 1H, $J_{3e,4}$ = 12.3 Hz, $J_{3e,3a}$ = 4.6, Hz, H-3e^F), 2.07-1.95 (m, incl. 2xNHCH$_3$, C*H*$_2$CH$_2$NH$_2$), 1.78 (t, 1H, $J_{3e,4}$ =12.2 Hz, H-3a^F).

^{13}C NMR (101 MHz, D$_2$O) δ 103.1 (C-1^B), 102.9 (C-1^A), 102.8 (C-1^D), 102.5 (C-1^E), 101.7 (C-1^C), 82.1 (C-4^A), 78.4, 78.1, 75.5, 75.3, 75.2, 74.7, 74.4, 74.2, 73.3 (C-2^C), 72.9, 72.5, 72.1, 71.7, 70.9, 70.0, 69.3, 68.5, 68.3, 68.1, 67.5, 67.4 (OCH$_2$), 67.2, 62.6 (C-9^F), 61.0, 60.7, 60.1, 55.8 (CH$_2$NH$_2$), 55.3 (C-2^B), 51.6 (C-5^F), 39.6 (C-3^F), 24.3 (*C*H$_2$CH$_2$NH$_2$), 22.2 (NHCH$_3$), 22.0 (NHCH$_3$).

3-Azidopropyl 4,6-*O*-benzilidene-3-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)-[(2-*O*-*acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→4)]-2,6-di-O-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-*tri-*O*-benzoyl-*β*-D-glucopyranoside 35.



35

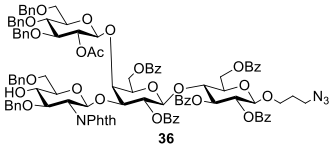
A solution of donor **8** (0.303 g, 0.476 mmol) and trisaccharide acceptor **24** (0.450 g, 0.317 mmol) with activated 4 Å molecular sieves (0.400 g) in dry DCM (7 mL) was stirred for 20 min under nitrogen. TMSOTf (11 µL, 0.063 mmol) was added at –10°C. After 4 h (TLC; 9:1 Toluene:EtOAc) the reaction was quenched with TEA, the solid filtered off and the solvent removed under pressure. The crude was purified by flash chromatography (Toluene:EtOAc) to afford tetrasaccharide **35** in 65% yield (0.380 g) as a white amorphous solid.

$[\alpha]_D^{25}$ = +19.14° (c 1.5, $CHCl_3$). ESI HR -MS ($C_{107}H_{102}N_4O_{28}$) *m/z* $[M+Na]^+$ 1913.6681; found 1913.6892.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17-6.73 (m, 54H, H-Ar), 5.66-5.57 (m, 2H, incl CHPh, H-3A), 5.38 (dd, 1H, J=7.9, 9.6 Hz, H-2^A), 5.33 (d, 1H, J=8.2 Hz, H-1^C), 5.08 (dd, 1H, J=7.9, 10.0, H-2^B), 4.96-4.83 (m, 5H, incl. H-1^D, H-2^D, 3xCH_2OBn), 4.75-4.63 (m, 3H, incl. 3xCH_2OBn), 4.55 (d, 1H, J=7.7 Hz H-1^A), 4.51 (d, 1H, J=12.0 Hz, 1xCH_2OBn), 4.44, 4.04 (m, 8H, incl. 1xCH_2OBn, H-4^B, H-2^C, H-3^C), 4.01-3.87 (m, 2H, H-4^A, H-3^D), 3.85-3.65 (m, 7H, incl. C*H*HN$_3$, H-5^B, H-5^A, H-3^B, 2xCH_2OBn), 3.64-3.51 (m, 3H), 3.51-3.38 (m, 3H, incl. CH*H*N$_3$), 3.23-3.07 (m, 3H, incl. OCH_2), 2.09 (s, 3H, $COCH_3$), 1.81-1.58 (m, 2H, C*H*$_2$CH$_2$N$_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.4, 167.5, 166.8, 165.93, 165.8, 165.3, 164.0, 163.4 (8 x CO), 138.6-122.8 (m, C-Ar), 101.3 (CHPh), 101.1 (C-1^A), 100.6 (C-1^B), 100.1 (s, 2C, C-1^C, C-1^D), 83.4 (C-3^D), 82.9 (C-5^B), 79.7, 77.9, 75.6 (C-4^A), 75.4, 75.3, 75.1, 74.3, 74.2 (C-4^B), 73.5 (C-3^C), 73.4, 73.1 (C-2^D), 72.9, 72.4 (C-3^A), 72.3, 71.5 (C-2^A), 70.9 (C-3^B), 69.1, 68.5, 66.6 (CH_2N_3), 66.0, 63.1, 62.4, 55.8 (C-2^C), 47.9 (OCH_2), 28.9 ($CH_2CH_2N_3$), 20.6 (CH_3).

3-Azidopropyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[(2-O-acetyl-3,4,6-tri-O-benzyl-β-D- glucopyranosyl)-(1→4)]-2,6-di-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside 36



36

A solution of **35** (0.370 g, 0.195 mmol) in ACN (10 mL) was cooled at 0°C. Me_3NBH_3 (0.071 g, 0.98 mmol) and $BF_3{\cdot}OEt_2$ (0.120 mL, 0.98 mmol) were added and the reaction was stirred for 2 h under nitrogen. TLC showed complete reaction (8:2 toluene:EtOAc). First TEA and then MeOH were added until neutral pH. The solvent removed at reduced pressure and the crude was purified by flash chromatography (toluene:EtOAc) to afford **36** in 55% yield (0.200 g).

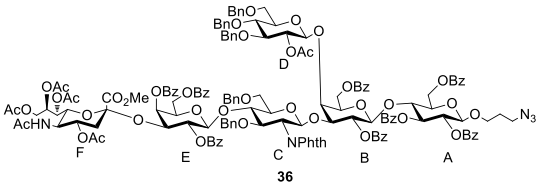
$[\alpha]_D^{25}$ = +20.07° (c 1.5, $CHCl_3$). ESI HR -MS ($C_{107}H_{100}N_4O_{28}$) *m/z* $[M+Na]^+$ 1911.6524; found 1911.6460.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36-6.63 (m, 54H Ar-H), 5.59 (t, 1H, J=9.4 Hz, H-3^A), 5.37 (t, 1H, J=8.2 Hz, H-2^A), 5.24 (d, 1H, 7.3 Hz, H-1^C), 5.05 (dd, 1H, J=8.6, 9.8 Hz, H-2^B), 4.93-4.80 (m, 5H, incl. H-1^D, H-2^D, 3xOCH_2Ph), 4.70-4.58 (m, 2H, 2xOCH_2Ph), 4.58- 4.23 (m, 8H, incl. H-1^A, H-1^B, H-6$^A{}_a$, 5xCH_2OPh), 4.23-4.00 (m, 6H, incl. H-6$^A{}_b$, H-2C), 3.95 (t, 1H, J=9.1 Hz, H-4^A), 3.91-3.82 (m, 1H, H-3^D), 3.83-3.32 (m, 14H, incl. CH_2N_3, H-4^D, H-5^A, H-3^B), 3.23-3.01 (m, 3H, incl.OCH_2), 2.00 (s, 3H. $COCH_3$), 1.80-1.55 (m, 2H, $CH_2CH_2N_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 165.9, 165.8, 165.5, 165.3, 164.0 (6xCO), 138.7-122.9 (m, C-Ar), 101.1 (C-1^A), 100.6 (C-1^B), 100.1 (C-1^D), 99.5 (C-1^C), 83.4 (C-3^D), 79.4, 78.4, 77.8, 75.5 (C-4^A), 75.4, 75.2, 75.0, 74.2, 73.6, 73.5, 73.4, 73.0 (C-2^D), 72.4 (C-3^A), 71.5 (C-2^A), 71.0 (C-2^B), 70.6, 69.1, 66.6 (CH_2N_3), 63.4, 62.5 (C-6^A), 55.4 (C-2^C), 47.9 (OCH_2), 28.9 (*C*H$_2$CH$_2$N$_3$), 20.5 (CH_3).

3-Azidopropyl 3-*O*-(5-acetamido-3,5-dideoxy-D-glycero-*α*-D-galacto-non-2-ulopyranosyl)- 2,4,6-tri-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-*β*-D-glucopyranosyl-(1→3)-

[*O*-acetyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→4)]-2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl}-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside 37.



36

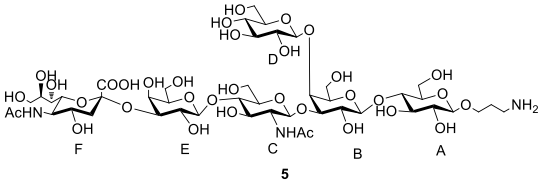
A solution of donor **11** (0.900 g, 0.85 mmol) and disaccharide acceptor **36** (0.500 g, 0.53 mmol) with activated 4 Å molecular sieves (0.500 g) in dry DCM (7 mL) was stirred for 20 min under nitrogen. TMSOTf (19 μL, 0.109 mmol) was added at –10°C. After 4 h (TLC; 9:1 Toluene:EtOAc) the reaction was quenched with TEA, the solid filtered off and the solvent removed under pressure. The crude was purified by flash chromatography (Toluene:EtOAc) to afford the tetrasaccharide **37** in 60% yield (0.590 g) as a white amorphous solid.

$[\alpha]_D^{25}$ = +40.07° (c 0.2, $CHCl_3$). ESI HR -MS ($C_{154}H_{151}N_5O_{48}$) *m/z* $[M+Na]^+$ 2860.9529; found 2860.9487.

^{1}H NMR (400 MHz, CDCl3) δ 8.28-6.55 (m, 69H, H-Ar), 5.71-5.63 (m, 1H, H-8^F), 5.54 (t, 1H, J=9.0 Hz, H-3^A), 5.46 (dd, 1H, J=8.0, 10.0 Hz, H-2^E), 5.34 (t, 1H, J=8.5 Hz, H-2^A), 5.29 (d, 1H, J=3.3 Hz, H-4^E), 5.20 (dd, 1H, J=2.2, 9.7, H-7^F), 5.07 (dd, 2H, J=5.9, 7.8 Hz, H-1^E, H-1^D), 5.02-4.73 (m, 9H, incl. H-2^B, H-3^E, H-1^C, H-4^F, H-5^E), 4.60 (d, 1H, J=10.9 Hz, OC*H*HPh), 4.55-4.36 (m, 5H, incl. H-1^A), 4.33-4.23 (m, 4H, incl. H-1^B), 4.23-3.88 (m, 10H, incl. H-9^F, C*H*HN$_3$), 3.88-3.61 (m, 10H, incl. H-2^C, $COOCH_3$), 3.61-3.53 (m, 3H), 3.50 (bd, 1H, J=7.8 Hz, H-3^C), 3.46-3.34 (m, 3H), 3.33-3.25 (m, 2H), 3.19-3.08 (m, 2H, OCH_2); 3.01 (dd, 1H, J=7.7, 11.6 Hz, CH*H*N$_3$), 2.41 (dd, 1H, J=4.4, 12.4, H-3$^F_{eq}$), 2.09 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 1.93 (s, 3H, CH_3), 1.90 (s, 3H, CH_3), 1.78 (s, 3H, CH_3), 1.65-1.54 (m, 4H, H-3$^F_{ax}$, $CH_2CH_2N_3$), 1.47 (s, 3H, CH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 133.4-122.8, C-Ar), 100.5 (C-1^A), 100.3 (C-1^B), 100.3 (C-1^C), 99.3 (C-1^E, C-1^D), 83.4, 79.2, 77.7, 77.2, 77.1, 76.7, 75.4, 75.3, 75.1, 74.9, 74.7, 74.3, 73.9, 73.5, 72.9, 72.6, 72.4, 71.9 (C-3^A), 71.8 (C-2^E), 71.6, 71.5 (C-2^A) 71.1, 70.5, 69.4, 68.8, 68.5, 68.0 (C-4^E), 67.0 (C-8^F), 66.5 (C-7^F), 63.5, 62.5, 62.4, 61.5, 55.8 (C-2^C), 53.2 ($COOCH_3$), 48.8, 47.9 (OCH_2), 37.3 (C-3^F), 28.9 (*C*$H_2CH_2N_3$), 23.2, 21.3, 20.8, 20.7, 20.5, 20.4 (6x$COCH_3$).

3-Aminopropyl 3-*O*-(5-*N*-acetamido-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosyl)-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→4)]-*β*-D-galactopyranosyl-(1→4)- *β*-D-glucopyranoside 5.



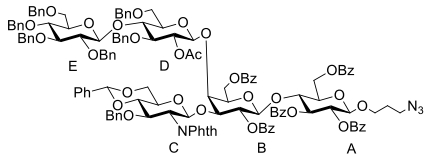
5

A mixture of protected hexasaccharide **37** (0.1 mmol) and LiI (3 mmol) in pyridine (5 ml) was heated for 24h at 120°C. The reaction mixture was concentrated under vacuum, and the residue was purified by silica gel column chromatography (gradient 2% MeOH in DCM) to afford the demethylated product. This material was dissolved in ethanol (4 ml), and ethylenediamine (400 μL) was added. After being stirred for 16 h at 90 °C, the reaction mixture was then concentrated in vacuum, and the residue was coevaporated with toluene (2 x 10 mL) and EtOH (2 x 5 mL). The crude mixture was re-dissolved in pyridine (5 ml), and acetic anhydride (5 ml) was added. After being stirred for 16 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in MeOH and MeONa was added until pH=13. After 48 h the reaction was neutralized and the solvent removed under vacuum. 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 H_2 (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 **5** as an amorphous powder (40% yield).

^{1}H NMR (400 MHz, D_2O) δ 4.88 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{D}), 4.68 (d, 1H, $J_{1,2}$=8.2 Hz, H-1^{C}), 4.54 (d, 1H, $J_{1,2}$=7.8 Hz, H-1^{B}), 4.48 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{D}), 4.43 (d, 1H, $J_{1,2}$=7.8 Hz, H-1^{A}), 4.38 (d, 1H, $J_{1,2}$=2.7 Hz, H-4^{B}), 4.10 (dd, 1H, $J_{1,3}$=9.8 Hz, $J_{1,2}$=2.9 Hz, H-3^{E}), 4.03-3.49 (m, 33H), 3.46-3.33 (m, 2H), 3.32-3.17 (m, 4H, H-2^{D}, H-2^{A}, CH_2NH_2), 2.74 (dd, 1H, $J_{3e,4}$=12.3 Hz, $J_{3e,3a}$=4.6 Hz, H-3e^{F}), 2.01 (s, 3H, $NHCH_3$), 2.01 (s, 3H, $NHCH_3$), 2.07-1.95 (m, 2H, $CH_2CH_2NH_2$), 1.78 (t, 1H, J=12.1 Hz).

^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 174.8, 173.8 (3xCO), 103.2 (C-1^{C}), 102.9 (C-1^{B}), 102.5 (C-1^{E}), 102.0 (C-1^{A}), 102.0 (C-1^{D}), 100.0, 99.8, 81.9 (C-3^{B}), 78.1, 75.9, 75.6, 75.5 (C-3^{E}), 75.2, 75.0 (C-4^{B}), 74.8, 74.6, 74.3, 73.6, 72.8, 72.6, 72.1, 71.7 (C-8^{F}), 70.2, 69.7, 69.3, 68.3, 68.0, 67.5 (OCH_2), 67.4 (C-4^{E}), 62.5 (C-9^{F}), 61.0, 60.8, 60.7, 60.1, 59.9, 55.6 (CH_2NH_2), 55.2 (C-2^{C}), 51.6 (C-5^{F}), 39.6 (C-3^{F}), 24.3 ($CH_2CH_2NH_2$), 22.2 ($NHCH_3$), 22.0 ($NHCH_3$).

3-Azidopropyl 4,6-*O*-benzilidene-3-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)-[(2,3,4,6-tetra-*O*-benzyl- *β*-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→4)]-2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside 38.



38

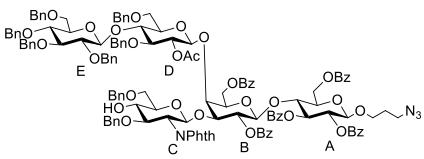
A solution of glycosyl donor **30** (0.545 g, 0.51 mmol) and glycosyl acceptor **24** (0.361 g, 0.26 mmol) with activated 4 Å molecular sieves (0.400 g) in dry DCM (5 mL) was stirred for 20 min under nitrogen. The resulting suspension was cooled down to -5°C and TMSOTf (9.4 µL, 0.052 mmol) was added. After 1 h (TLC: Toluene/EtOAc 9:1) the reaction was quenched with Et_3N, then evaporated to dryness and the crude was purified by column chromatography (gradient from 0 to 50% of EtOAc in toluene). Pure fractions were collected and evaporated, affording the target pentasaccharide **38** in 55% yield.

$[\alpha]_D^{25}$ = +17.02° (c 0.15, $CHCl_3$). ESI HR -MS ($C_{134}H_{128}N_4O_{33}$) *m/z* $[M+Na]^+$ 2343.8461; found 2343.8342.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-6.70 (m, 69H , Ar-H), 5.57 (t, 1H, $J_{1,2}$=9.2 Hz, H-3^{A}), 5.49 (s, 1H, CHPh), 5.36 (dd, 1H, $J_{1,3}$=9.7 Hz, $J_{1,2}$=7.9 Hz, H-2^{A}), 5.29 (d, 1H, $J_{1,2}$=8.2 Hz, H-1^{C}), 5.16 (d, 1H, $J_{1,2}$=11.4 Hz), 5.08 (dd, 1H, $J_{1,3}$=9.8 Hz, $J_{1,2}$=7.9 Hz, H-2^{B}), 5.00 (d, 1H, $J_{1,2}$=11.3 Hz), 4.89 (s, 2H, CH_2OPh), 4.88-4.81 (m, 2H, H-1^{D}, H-2^{D}), 4.76 (d, 2H, $J_{1,2}$=10.9 Hz), 4.73-4.66 (m, 4H), 4.60-4.49 (m, 4H, incl. H-1^{E}, H-1^{A}), 4.44 (d, 1H, $J_{1,2}$=12.1 Hz), 4.40-4.30 (m, 4H, incl. H-1^{B}), 4.30-4.21 (m, 3H), 4.21-4.11 (m, 2H, incl. H-2^{C}), 4.05-3.66 (m, 11H, incl. H-3^{B}, H-3^{D}, H-3^{C}, H-4^{A}), 3.62-3.33 (m, 9H), 3.14 (dt, 2H, $J_{1,3}$=6.4 Hz $J_{1,2}$=3.0 Hz), 3.04 (dd, 1H, $J_{1,3}$=11.9 Hz, $J_{1,2}$=7.5 Hz), 1.95 (s, 3H, $COCH_3$), 1.74-1.59 (m, 2H, C$H_2$$CH_2N_3$).

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.1, 167.5, 166.8, 165.9, 165.8, 165.5, 165.3, 164.1 (8xCO), 145.7, 139.5-122.9 (C-Ar), 106.6, 102.9 (C-1^{A}), 101.3 (C-1^{E}), 101.1 (CHPh), 100.7 (C-1^{B}), 100.1 (C-1^{D}), 100.0 (C-1^{C}), 99.7, 83.0, 82.6, 81.5, 80.0, 78.9, 77.3, 75.8, 75.7, 75.3, 74.7, 74.4, 74.3, 73.8, 73.6, 73.5, 73.4, 73.2, 73.0, 72.9, 72.7, 72.4 (C-3^{B}), 72.3 (C-2^{D}), 71.5 (C-3^{A}), 71.1 (C-3^{A}), 68.6, 68.5, 68.3, 66.5, 66.0, 63.2, 62.5, 55.8 (C-2^{C}), 47.8 (OCH_2), 28.9 ($CH_2CH_2N_3$), 20.4 ($COCH_3$).

3-Azidopropyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→4)]-2,6-di-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside 39.

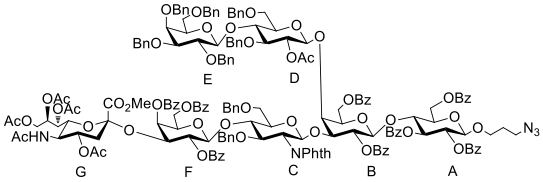


39

A solution of compound **38** (0.265 g, 0.114 mmol) in ACN (6 mL) was cooled at 0°C. Me_3NBH_3 (0.042 g, 0.57 mmol) and $BF_3{\cdot}OEt_2$ (0.070 mL, 0.57 mmol) were added and the reaction was stirred for 2 h under nitrogen. First TEA and then MeOH were added until neutral pH. The solvent was removed at reduced pressure and the crude was purified by flash

chromatography (toluene:EtOAc) to afford the target pentasaccharide acceptor **39** in 55% yield (0.140 g).
$[\alpha]_D^{25}$ = +20.41° (c 0.15, $CHCl_3$). ESI HR -MS ($C_{134}H_{130}N_4O_{33}$) *m/z* $[M+Na]^+$ 2345.8617; found 2345.8594.
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12-6.84 (m, 69H, Ar-H), 5.57 (t, 1H, $J_{1,2}$=9.4 Hz, H-3^A), 5.36 (dd, 1H, $J_{1,3}$=9.8 Hz, $J_{1,2}$=7.9 Hz, H-2^A), 5.22 (d, 1H, $J_{1,2}$=7.8 Hz, H-1^C), 5.16 (d, 1H, $J_{1,2}$=11.6 Hz), 5.06 (dd, 1H, $J_{1,3}$=10.0 Hz, $J_{1,2}$=7.9 Hz, H-2^B), 4.99 (d, 1H, $J_{1,2}$=11.4 Hz), 4.90-4.80 (m, 4H, incl. H-1^D, H-2^D), 4.79-4.65 (m, 3H), 4.61 (d, 1H, $J_{1,2}$=5.6 Hz), 4.59-4.54 (m, 2H, incl. H-1^E), 4.52 (d, 1H, $J_{1,2}$=7.7 Hz, H-1^A), 4.46 (d, 2H, $J_{1,2}$=12.7 Hz), 4.38-4.30 (m, 4H), 4.29-4.14 (m, 4H), 4.12-3.98 (m, 4H), 3.96-3.88 (m, 2H), 3.88-3.66 (m, 8H, incl. H-2^E, H-3^D), 3.60-3.32 (m, 10H), 3.14 (dt, 2H, $J_{1,3}$=6.5 Hz, $J_{1,2}$=3.1 Hz), 3.01-2.98 (m, 1H), 1.85 (s, 3H, $COCH_3$), 1.75-1.59 (m, 2H, C*H*$_2$$CH_2N_3$).
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 165.9, 165.8, 165.4, 165.3, 164.1 (6xCO), 139.7-123-0 (C-Ar), 102.8 (C-1^E), 101.1 (C-1^A), 100.7 (C-1^B), 100.1 (C-1^D), 99.1 (C-1^C), 82.6, 81.4, 80.0, 78.5, 78.4, 75.6, 75.3, 75.1, 74.7, 74.6, 74.6, 74.3, 73.8, 73.6, 73.5, 73.4, 73.3, 73.2, 73.0, 72.6, 72.5 (C-2^D), 72.3 (C-3^A), 71.5 (C-2^A), 71.3 (C-2^B), 70.5, 68.4, 68.3, 66.6, 63.4, 62.5, 55.5 (C-2^C), 47.9 (OCH_2), 28.9 ($CH_2CH_2N_3$), 20.3 ($COCH_3$).

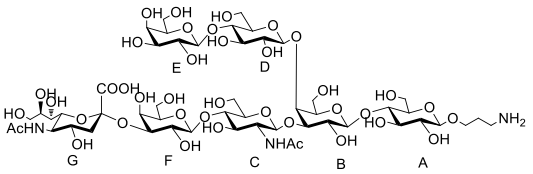
3-Azidopropyl 3-*O*-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl)-2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→4)-*O*-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→4)]-2,6-di-*O*-benzoyl-β-D-galactopyranosyl}-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside 40.



40

A solution of disaccharide donor **11** (0.080 g, 0.070 mmol) and pentasaccharide acceptor **39** (0.109 g, 0.047 mmol) with activated 4 Å molecular sieves (0.100 g) in dry DCM (3 mL) was stirred for 20 min under nitrogen. TMSOTf (1.7 µ L, 0.0094 mmol) was added at 0°C. After 4 h (TLC; 6:4 Toluene:acetone) the reaction was quenched with TEA, the solid filtered off and the solvent removed under pressure. The crude was purified by flash chromatography (Toluene:acetone) to afford the target heptasaccharide **40** as a yellow amorphous solid (0.090 g, 60% yield).
$[\alpha]_D^{25}$ = +20.23° (c 0.5, $CHCl_3$). ESI HR-MS ($C_{181}H_{173}N_5O_{53}$) *m/z* $[M+Na]^+$ 3293.1357; found 3295.14.
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25-6.53 (m, 84H, Ar-H), 5.70, 5.62 (m, 1H, H-8^G), 5.52 (t, 1H, $J_{1,2}$=9.6 Hz, H-3^A), 5.44 (dd, 1H, $J_{1,3}$=9.8 Hz, $J_{1,2}$=7.7 Hz), 5.32 (dd, 1H, $J_{1,3}$=9.7 Hz, $J_{1,2}$=7.9 Hz, H-2^A),5.30-5.27 (m, 1H), 5.21 (dd, 1H, $J_{1,3}$=9.6 Hz, $J_{1,2}$=2.6 Hz, H-7^G), 5.15 (d, 1H, $J_{1,2}$=11.7 Hz), 5.07 (d, 1H, $J_{1,2}$=7.8 Hz), 5.03 (d, 1H, $J_{1,2}$=7.8 Hz), 5.01-4.87 (m, 3H), 4.84-4.70 (m, 6H), 4.70-4.64 (m, 2H), 4.56-4.53 (m, 2H), 4.53-4.37 (m, 5H incl. H-1^E, H-1^A), 4.34-4.29 (m, 5H), 4.20-3.90 (m, 12H), 3.90-3.77 (m, 6H), 3.76 (s, 3H, $COOCH_3$), 3.71-3.63 (m, 3H), 3.57 (dd, 1H, $J_{1,3}$=10.6 Hz, $J_{1,2}$=2.7 Hz), 3.53-3.24 (m, 9H), 3.16-3.06 (m, 2H, OCH_2), 3.00 (dd, 1H, $J_{1,3}$=12.1 Hz, $J_{1,2}$=8.1 Hz), 2.40 (dd, 1H, $J_{3e,4}$=12.7 Hz, $J_{3e,3a}$=4.6 Hz, H-3e^G), 2.11 (s, 3H, $COCH_3$), 1.94 (s, 3H, $COCH_3$), 1.89 (s, 3H, $COCH_3$), 1.77 (s, 3H, $COCH_3$), 1.69 (s, 3H, $COCH_3$), 1.68-1.57 (m, 3H, incl. $CH_2CH_2N_3$, H-3a^G), 1.48 (s, 3H, $COCH_3$).
^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 170.7, 170.4, 170.3, 170.1, 169.8, 168.1, 167.4, 167.0, 165.7, 165.5, 165.4, 165.3, 164.9, 164.1 (15xCO), 139.9-114.4 (C-Ar), 102.9 (C-1^E), 101.2 (C-1^A), 101.1 (C-1^B), 100.6 (C-1^D, C-1^F), 100.3 (C-1^C), 100.0, 98.6, 97.0, 96.9, 82.7, 82.4, 81.3, 79.9, 77.4, 77.2, 76.7, 76.6, 75.5, 75.4, 75.1, 74.6, 73.8, 73.4, 73.1, 72.7, 72.5, 72.2, 72.1 (C-3^A), 71.9, 71.8, 71.7, 71.5 (C-2^A), 71.4, 71.3, 70.4, 69.4, 68.3, 68.1, 67.8, 67.4 (C-8^G), 67.1, 66.6 (C-7^{1qw}), 63.3, 62.6, 62.5, 62.3, 61.5, 55.8 (C-2^C), 53.1 ($COOCH_3$), 48.9, 47.9 (OCH_2), 37.4 (C-3^G), 29.4 ($CH_2CH_2N_3$), 21.5, 21.3, 20.7, 20.4, 20.3, 20.1 (6x$COCH_3$).

3-Aminopropyl 3-*O*-(5-*N*-acetamido-3,5-dideoxy-D-glycero-*α*-D-galacto-2-nonulopyranosyl)-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→3)-[*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→4)]-*β*-D-galactopyranosyl-(1→4)- *β*-D-glucopyranoside 6.



6

A mixture of protected heptasaccharide **40** (0.1 mmol) and LiI (3 mmol) in pyridine (5 ml) was heated for 24h at 120°C. The reaction mixture was concentrated under vacuum, and the residue was purified by silica gel column chromatography (gradient 2% MeOH in DCM) to afford the demethylated product. This material was dissolved in ethanol (3 ml), and ethylenediamine (300 µL) was added. After being stirred for 16 h at 90 °C, the reaction mixture was then concentrated in vacuum, and the residue was coevaporated with toluene (2 x 10 mL) and EtOH (2 x 5 mL). The crude mixture was re-dissolved in pyridine (5 ml), and acetic anhydride (5 ml) was added. After being stirred for 16 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in MeOH and MeONa was added until pH=13. After 48 h the reaction was neutralized and the solvent removed under vacuum. 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 H_2 (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 **6** as an amorphous powder (40% yield).

^{1}H NMR (400 MHz, D_2O) δ 4.95 (s, 1H, $J_{1,2}$=7.7 Hz, H-1^D); 4.71 (d, 1H, $J_{1,2}$=8.4 Hz, H-1^C), 4.56 (d, 1H, $J_{1,2}$=7.7 Hz, H-1^F), 4.52 (d, 1H, $J_{1,2}$=7.7 Hz, H-1^B), 4.49-4.39 (m, 3H, incl. H-4^B, H-1^A, H-1^E), 4.12 (dd, 1H, $J_{1,3}$=9.9, $J_{1,2}$=3.1 Hz), 4.08-3.44 (m, 40H), 3.38-3.27 (m, 2H, H-2^A, H-2^D), 3.25-3.13 (m, 2H, CH_2NH_2), 2.77 (dd, 1H, $J_{3e,4}$=12.3 Hz, $J_{3e,3a}$=4.5 Hz, H-3e^G); 2.04 (s, 3H, $NHCH_3$), 2.03 (s, 3H, $NHCH_3$), 2.09-1.95 (m, 2H, $CH_2CH_2NH_2$), 1.81 (t, 1H, $J_{1,2}$=11.9 Hz, H-3a^G).

^{13}C NMR (101 MHz, D_2O) δ 104.1 (C-1^C), 103.6 (C-1^A, C-1^E), 103.4 (C-1^F), 103.2 (C-1^B), 102.5 (C-1^D), 82.8, 79.3, 79.0, 78.9, 78.8, 76.1, 75.8 (C-4^B), 75.7, 75.5, 74.0, 73.9, 73.6, 73.2, 73.1, 72.6, 71.5, 69.3, 69.2, 69.2, 68.6, 63.4, 61.9, 61.8, 61.5, 61.4, 61.0, 56.0, 52.5, 47.7 (CH_2NH_2), 40.2 (C-3^G), 23.2 (CH_3), 23.2 (CH_3), 21.1 ($CH_2CH_2NH_2$).

Quantitative estimation of sialic acid[29].

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 µg/mL were prepared to build the calibration curve. GBS PSIa 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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