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

Del Bino, L.

# Chapter 8

*Summary and future perspectives*

The design of innovative glycoconjugate vaccines, for which a robust correlation between epitope structure and immunogenicity can be established, implies availability of structurally defined oligosaccharide fragments[1]. In some cases, such oligosaccharides can be obtained by depolymerization of the capsular polysaccharide[2] and subsequent careful selection of the shortest DPs (DP=Degree of Polymerization). In other cases, this approach is not efficient since neither chemical nor enzymatic depolymerization methods of the capsular polysaccharide are available. This is the challenge, for example, with GBS serogroup Ia and Ib, for which chemical synthesis is the only tool to prepare defined short oligosaccharides to be used for epitope mapping studies. In Chapter 1, an overview of glycoconjugate vaccines is provided, including their efficacy and mechanism of action compared to polysaccharide vaccines. Traditional approaches to the preparation of glycoconjugate vaccines are also described. Finally, the focus shifts to enzymatic and synthetic methods currently available for antigen preparation.
The epidemiology of Group B streptococcus (GBS) is described in Chapter 2: clinical symptoms, differences between Early-Onset Disease and Late-Onset Disease, main prevention strategies and serotype distribution according to the age of the patient are described. It is underlined how a multivalent vaccine composed of the most representative serotypes (Ia, Ib, II, III, IV, V) is needed to prevent the disease in an efficacious way both in infants and in elderly and immunocompromised patients[3, 4]. Finally, a literature review of the syntheses of the repeating units of GBS Ia and III is provided: chemo-enzymatic methods, one-pot reactions, pre-activation and regioselective strategies are synthetic approaches that have all been used to obtain the target complex oligosaccharides of GBS, showing how progress in carbohydrate chemistry can give access to biologically relevant structures for implementation of existing vaccines or development of new ones.

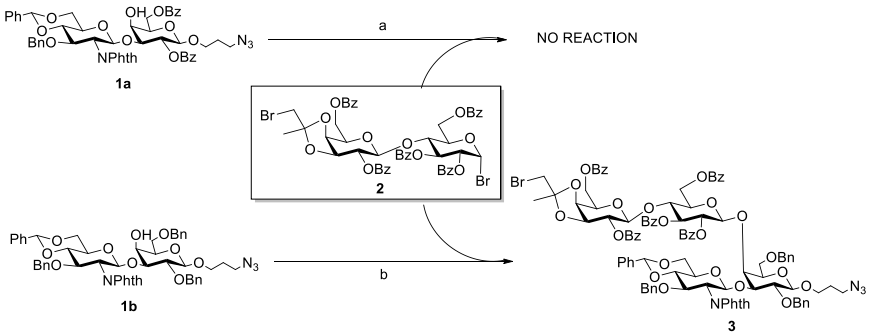
In Chapter 3, a regioselective glycosylation strategy to the synthesis of the biologically relevant GlcNAcβ1-3Gal disaccharide synthon is presented. Efforts to optimize the regioselective glycosylation of galactose 3-OH were pursued with the aim to obtain the disaccharide with high yield and complete regioselectivity to have faster access to branched oligosaccharide structures from GBS type Ia and Ib. Indeed, PSIa and Ib share a very similar structure, since the repeating unit is a pentasaccharide composed by the same sugar residues with, as the only difference, the linkage of the side chain galactose to the glucosamine, which is β-(1→4) in type Ia and β-(1→3) in type Ib[5]. Given this structural analogy, with a limited number of building blocks and a common synthetic design, glycans from both serotypes can be prepared. Optimization of the regioselective glycosylation involved the screening of glycosylation conditions using several glucosamine donors with different protecting group patterns allowing the possibility to elongate the glycosylation products at either the glucosamine C4 or C3. Benzoyl esters and benzyl ethers were installed as protecting groups on the galactose diol acceptor to investigate their arming/disarming effect. The results showed that when 2,6-benzoyl galactose was used as the glycosyl acceptor only the target β(1→3) disaccharide was isolated, indicating that the electron withdrawing effect of the

2,6-benzoyl groups has a beneficial effect on the regioselectivity. In addition, the outcome of the glycosylation reaction seems to benefit from the torsional disarming effect of the 4,6-benzylidene ring, locking the glucosamine 4,6-hydroxyls thereby disarming the donor glycoside. Disaccharide synthons that could be elongated at the galactose C-4 and either at the glucosamine C-3 or C-4 were obtained and their use as intermediates for GBS related glycans is discussed in the following Chapters of this Thesis.

The disaccharide bearing a temporary protecting group at C-4, that was obtained with the highest yield and full regioselectivity, was selected as key intermediate for the synthesis of a panel of fragments from GBS type Ia capsular polysaccharide as outlined in Chapter 4. This small library comprises the pentasaccharide repeating unit (both in the linear and a branched frameshift form), a non-sialylated tetrasaccharide, two hexasaccharides and one heptasaccharide. All compounds were synthesized using a convergent approach and an aminopropyl linker was installed at the non-reducing end to be exploited for further manipulation of the final oligosaccharide or conjugation to protein. Thanks to the developed regioselective glycosylation strategy, the synthesis of GBS PSIa repeating unit was optimized in terms of the number of reaction steps and protecting groups employed. Furthermore, fragments longer than one single repeating unit could be prepared with high efficiency, using a limited number of building blocks.
With the aim to translate the same approach to the synthesis of GBS type Ib glycans a similar GlcNAcβ(1→3)Gal disaccharide bearing a temporary protecting group at glucosamine C-3 was selected for elongation as described in Chapter 5. The β(1→3) connection between the Neu5Acα2-3Gal disaccharide and glucosamine 3-OH proved to be particularly challenging. For this reason, the reactivity of this position needed to be modulated by installation of different protecting groups on the nearby amino and hydroxyl groups. The *N*-phthaloyl group was replaced with the less electron withdrawing *N*-trichloroethoxy carbonyl group and a silylidene ketal (which is more flexible than its benzylidene counterpart) was installed to lock glucosamine 4,6-OH. These glucosamine modifications gave access to the branched and linear frameshifts of GBS type Ib repeating unit.
The branched repeating units of GBS type Ia and Ib were used for structural studies employing NMR and Molecular Dynamics to give insight in the conformational preferences of the corresponding polysaccharides. Conformational analysis, using the individual repeating units, highlighted as main difference the presentation of the protruding Neu5Ac-α-(2→3)-Gal moieties, with a major *exo-anti-Φ* population for Ia and *exo-syn-Φ* for Ib. Both polysaccharides have a relaxed helix-shaped backbone, with a more rigid and repetitive structure for PSIa. PSIb has a less defined preferred conformation and a larger solvent exposure. Potentially, these features also allow the exposure of different epitopes for the two polysaccharides, which would justify the serotype-specifity of the immune response.
To investigate this hypothesis in more detail, a study of the interactions between anti PS Ia and Ib mAbs and the prepared synthetic oligosaccharides is presented in Chapter 6. Competitive SPR

experiments were set up to verify the affinity of the fragments for the serotype specific functional mAbs. While for GBS type Ia none of the synthetic glycans showed high affinity for the mAb and the hexasaccharides showed a better inhibition activity than the single repeating unit, the branched pentasaccharide from type Ib was able to deplete the binding of the anti PSIb mAb to PSIb-HSA conjugate attached on the chip. To map the interactions of this fragment to the mAb, STD-NMR experiments were undertaken, underlining a high saturation transfer to the protons of the Neu5Ac-Gal branching and of the glucose moiety in the backbone. These positions therefore appear to be involved in the binding with the mAb. This also holds true for the protons of the sialic acid acetamide group which, when compared to the GlcNAc acetamide present in the molecule, shows a higher STD-signal.

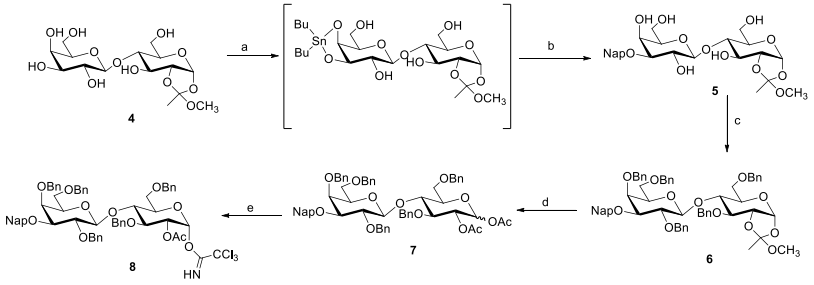
While for PSIb the repeating unit already represents a promising antigen, the type Ia synthetic glycans prepared so far probably do not cover the minimal epitope able to interact with a protective mAb. For this reason, a synthetic route giving access to longer oligosaccharide structures by elongation at the galactose 3-OH with a second glucosamine moiety or even with a trisaccharide to obtain a DP2 structure was designed and its feasibility tested. The key step of the synthesis was to identify a lactose donor which could glycosylate the poorly reactive galactose 4-OH of the previously described GlcNAcβ(1→3)Gal **1a** while carrying a temporary protecting group at C-3 of galactose moiety. To this aim, the lactose bromide donor **2**[6], described in Chapter 7, was tested but unfortunately, glycosylation reaction under AgOTf activation was unsuccessful and the target tetrasaccharide was not obtained (Scheme 1). In contrast, the benzylated disaccharide counterpart **1b** was successfully glycosylated by lactose bromide **2**, confirming again how the presence of electron withdrawing benzoyl groups plays a key role in reducing the nucleophilicity of the galactose 4-OH.




**Scheme 1**. Glycosylation trial with lactose bromide. *Reagents and conditions*: a) AgOTf, DCM, MS 3Å, -40°C to rt, no reaction; b) AgOTf, DCM, MS 3Å, -40°C to rt, 65%.

As discussed in Chapter 3, disaccharide **1b** was prepared in only 40% yield due to poor regioselectivity in the glycosylation reaction between the glucosamine donor and the galactose diol acceptor. To avoid using a route commencing with a non-selective reaction, the research of a more reactive lactose donor was undertaken.

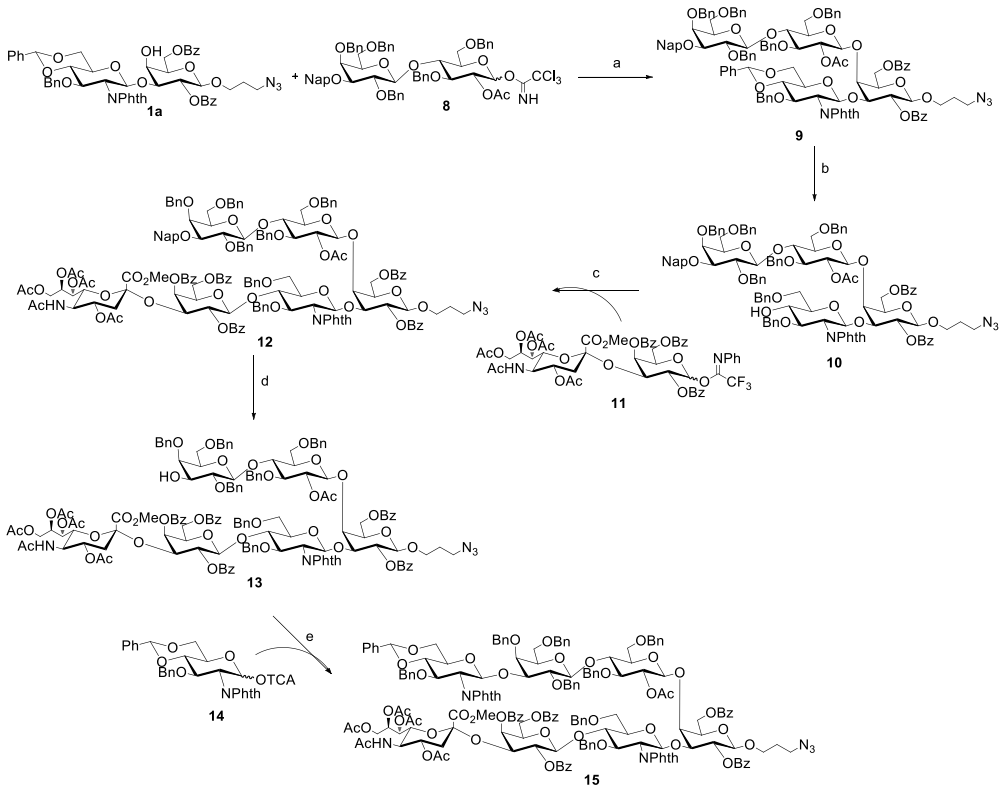
Therefore, an armed lactose analogue of the benzylated compound used for the synthesis of the branched GBS Ia hexasaccharide and heptasaccharide (Chapter 4) bearing an orthogonal protective group on galactose 3-OH was designed (Scheme 2). The synthesis started from lactose orthoester **4**, which was treated with dibutyl tin oxide to give the C3'-C4'-stannylene acetal. Subsequent treatment with an electrophile such as NapBr gave the regioselective alkylation of the 3-OH of the galactose moiety. The resulting compound **5** was then benzylated to afford lactose **6**, which was subjected to ortho ester hydrolysis followed by acetylation with $Ac_2O$ and pyridine. The anomeric was selectively removed and the resulting lactol was directly converted into trichloroacetimidate **8**, which was used as glycosyl donor with disaccharide **1a** acceptor.



**Scheme 2**. Synthetic pathway to lactose **8**. *Reagents and conditions*: a) 3Å MS, $Bu_2SnO$, toluene, reflux; b) NapBr, TBAI, THF, 50% c) NaH, BnBr, TBAI, DMF; 80%; d) TFA/$H_2O$ 1:1, DCM; $Ac_2O$/Py, 0°C to rt, 86%; e) $H_2NNH_2$, AcOH, THF; $CCl_3CN$; DBU, DCM, 70%.

When lactose donor **8** was coupled with disaccharide **1a** under influence of TMSOTf activation, the target branched tetrasaccharide **9** was obtained in satisfactory 60% yield (Scheme 3). After regioselective benzylidene opening, the obtained tetrasaccharide **10** was glycosylated with the already described Neu5Ac-Gal disaccharide donor **11**, leading to the formation of hexasaccharide **12** in 72% yield. Oxidative cleavage of Nap protecting group with DDQ in a DCM/MeOH mixture yielded the hexasaccharide acceptor **13**, key intermediate for the synthesis of longer and more complex GBS Ia glycans.

To test whether glycosylation of this intermediate could be effective, heptasaccharide **15** was targeted. To this aim, trichloroacetimidate glucosamine donor **14** reacted with hexasaccharide **13** under TMSOTf activation, providing the desired oligosaccharide **15** in 68% yield.



**Scheme 3**. Synthetic pathway to heptasaccharide **15**. *Reagents and conditions*: a) TMSOTf, 4Å MS, DCM, 0°C to rt, 60%; b) $Me_3N{\cdot}BH_3$, $BF_3{\cdot}Et_2O$, ACN, 0°C, 55%; c) TMSOTf, DCM dry, 72%; d) DDQ, DCM/MeOH 4:1, 75%; e) TMSOTf, DCM dry, 68%.

The successful assembly of heptasaccharide **15** indicates that the route is also suitable to acquire even more complex structures such as oligomers encompassing multiple repeating units. The heptasaccharide that can be obtained after global deprotection of **15** will be used to assess the impact of the additional upstream glucosamine on antibody recognition.
Moreover, key building block **8** can be used to approach the synthesis of PSIb glycans longer than one single repeating unit, in case the structures synthesized so far prove not immunogenic *in vivo*.

To test the immunogenicity of synthetic oligosaccharides, conjugation to a carrier protein through covalent linkage is needed. Indeed, the protein provides the T-cell epitopes required for the development of the immunological memory[7]. To evaluate whether the GBS type Ia and Ib synthetic fragments could elicit an immune response *in vivo*, conjugation to the classical carrier $CRM_{197}$ was performed as described in Chapter 6. A saccharide/protein ration of at least 10:1 was targeted because a high glycodensity is pivotal to achieve a robust immune response with short OS-conjugates, as was recently shown for GBS type III. Characterization of the conjugates by SDS-Page, Western Blot, microBCA to measure the protein content and HPAEC-PAD for the

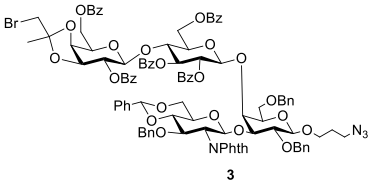
total saccharide amount calculation was performed and the target saccharide/protein ratio was reached for almost all glycoconjugates. Ongoing immunological studies will tell whether the multivalent display of these short oligosaccharides (corresponding to optimal or suboptimal epitopes) allows to achieve antigenicity characteristics of larger PS glycans.
Moreover, the Ia and Ib glycoconjugates will be used to complete the epitope mapping, by studying their affinity (in terms of $K_d$) with serotype specific functional mAbs or Fab fragments.

With the aim to demonstrate that identification of antigenic determinants from highly complex polysaccharide is propaedeutic for the rational design of conjugates based on synthetic carbohydrates[8, 9], Chapter 7 reports a structure-guided approach to obtain an innovative anti GBS type III glycoconjugate vaccine. A hexasaccharide was designed starting from the X-ray crystal structure of a semisynthetic GBS III DP2 fragment with a protective Fab[10]. This oligosaccharide comprises all moieties interacting with the Fab and consists of a full branched repeating unit and a second glucosamine. Structural and conformational studies confirmed that the hexasaccharide is recognized by a functional anti-PSIII rabbit mAb similarly to the dimer previously obtained by PS depolymerization. Conjugation to the carrier protein led to obtainment of two glycoconjugates with different glycodensity. When tested for immunogenicity, only the conjugate with a saccharide/protein ratio of about 8 elicited a robust and functional immune response, non inferior to the polysaccharide conjugate, indicating that this structure is the minimal epitope of GBSIII.
Of note, the synthetic glycan based conjugate showed a predominant IgG vs IgM production, which is relevant for a vaccine intended to elicit primarily IgG antibodies crossing the placenta.
While literature reports have suggested GBSIII to be an extremely complex epitope and while it has been pointed that long polysaccharide portions are required to present immunogenic epitopes, the described structure guided approach changes this paradigm, demonstrating that a small synthetic glycan can be designed to contain all the structural features required for a robust immune response.

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



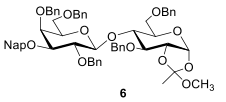
**3**

Lactose bromide donor **2** (0.137 g, 0.131 mmol) and disaccharide acceptor **1b** (0.080 g, 0.088 mmol) with 4Å MS were dissolved in 3.0 mL of dry DCM under nitrogen atmosphere. The resulting suspension was cooled down to -10°C and AgOTf (0.045 g, 0.176 mmol) was added. The resulting reaction mixture was stirred in the darkness for 2 hours, then (TLC: Tol/EtOAc 8:2) it was quenched by addition of TEA. The solvent was removed under reduced pressure and the crude was purified by column chromatography. Pure fractions were collected and evaporated to dryness, affording the target compound **3** as a colourless oil (0.107 g, 65% yield).

$^1$H NMR (400 MHz, $CDCl_3$) δ 8.22-6.88 (m, 49H, Ar-H), 6.16 (t, 1H, J=9.5 Hz), 5.61 (s, 1H, CHPh), 5.55 (d, 1H, J=8.2 Hz), 5.48 (d, 1H, J=8.3 Hz), 5.46-5.36 (m, 1H), 5.26-5.18 (m, 1H), 4.92 (d, 1H, J=7.4 Hz), 4.78 (d, 1H, J=12.1 Hz), 4.70 (d, 1H, J=12.1 Hz), 4.61-4.53 (m, 2H), 4.52-4.46 (m, 2H), 4.43 (t, 1H, J=6.1 Hz), 4.38-4.13 (m, 7H), 4.08 (d, 1H, J=7.5 Hz), 3.92-3.80 (m, 4H), 3.76-3.62 (m, 3H), 3.62-3.45 (m, 7H), 3.36 (dd, 2H, $J_{1,2}$=10.9 Hz, $J_{1,3}$=17.2 Hz, $CH_2$Br), 3.10-2.96 (m, 3H), 1.61 (s, 3H, $CH_3$), 1.67-1.51 (m, 2H, C*H*$_2$CH$_2$N$_3$).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 167.9, 166.0, 165.8, 165.6, 165.5, 165.1, 165.0, 138.3, 1398.0, 137.2, 134.4-126.1 (m, Ar-H), 109.5, 103.3, 101.2, 100.0, 99.0, 98.8, 82.9, 79.0, 78.5, 78.4, 76.3, 74.6, 74.5, 74.2, 74.0, 73.8, 73.7, 73.5, 73.1, 72.6, 72.5, 72.3, 71.1, 70.9, 69.3, 68.5, 66.1, 65.8, 62.5, 56.7, 48.1, 46.1, 37.3, 29.7, 29.1, 24.5, 22.9,

**2,4,6-tri-*O*-benzyl-3-*O*-(2-naphtyl methyl)-*β*-D-galactopyranosyl-(1→4)-1,2-(methyl orthoacetate)-3,6-di-*O*-benzyl-*α*-D-glucopyranose 6.**



6

3Å molecular sieves (5.0 g) were activated under nitrogen and then poured into a flask containing 5.5 g (13.8 mmol) of lactose orthoester **4** suspended in toluene. $Bu_2SnO$ (5.1 g, 20.7 mmol) was added and the reaction mixture was stirred in refluxing toluene for 2 h. After 2 hr toluene was evaporated under reduced pressure, the crude was redissolved in 100.0 mL of THF, Nap-Br (4.3 g, 20.7 mmol) and TBAI (700 mg) were added. The resulting reaction mixture was stirred at 50°C overnight. After 16 hr (TLC: EtOAc/MeOH 85:15) the solvent was evaporated under reduced pressure and the crude was purified by column chromatography using a gradient from 0 to 50% of MeOH in EtOAc (containing 3% of TEA). Pure fractions were collected and evaporated under reduced pressure affording compound **5** (3.0 g, 50% yield).

Lactose 3-Nap orthoester **5** (0.600 g, 1.11 mmol) was dissolved in 10.0 mL of DMF and the resulting solution was cooled down to 0°C in an ice-water bath. NaH (0.445 g, 11.14 mmol) was added under nitrogen atmosphere and the resulting suspension was stirred for 20 minutes. After 20 minutes the ice-water bath was removed and BnBr (2.0 mL, 16.7 mmol) was added dropwise under nitrogen atmosphere, followed by TBAI (100 mg). The reaction mixture was stirred overnight at rt.

After 16 h (TLC: Hexane/EtOAc) the reaction was quenched with MeOH, then it was diluted with DCM and washed with $NaHCO_3$ and $H_2O$. The organic phase was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure affording a crude product, which was purified by column chromatography. The column was eluted with a gradient from 0 to 100% of EtOAc in Cyclohexane (containing 3% of TEA).

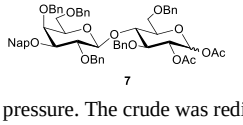
Pure fractions were collected and evaporated affording compound **6** (857 mg, 80% yield) as a colorless oil.

$[\alpha]_D^{25} = +23.98^\circ$ (c 0.185, $CHCl_3$). ESI MS ($C_{61}H_{64}O_{12}$) *m/z* $[M+H]^+$ found 992.46; calcd 988.4398.

$^1$H NMR (400 MHz, $CDCl_3$) δ 7.86-7.11 (m, 32H, Ar-H), 5.69 (d, 1H, J=5.2 Hz, H-1$^A$), 4.96 (d, 1H, J=11.8 Hz), 4.84 (s, 2H, OC*H*$_2$Ph), 4.77 (s, 2H, OC*H*$_2$Ph), 4.75-4.67 (m, 2H, OC*H*$_2$Ph), 4.66-4.49 (m, 3H, OC*H*$_2$Ph), 4.37 (d, 1H, J=12.2 Hz), 4.34-4.30 (m, 2H), 4.28 (s, 1H, J=7.8 Hz, H-1$^B$), 4.15 (t, 1H, J=2.0 Hz), 3.98 (d, 1H, J=9.3 Hz), 3.89 (d, 1H, J=2.7 Hz, H-4$^B$), 3.70-3.58 (m, 2H), 3.54 (dd, 1H, $J_{1,2}$=7.2 Hz, $J_{1,3}$=8.5 Hz), 3.50-3.40 (m, 3H), 3.24 (s, 3H, $OCH_3$), 1.62 (s, 3H, $CH_3$).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.3, 169.6, 139.2-125.6 (C-Ar), 103.1 (C-1$^B$), 102.7 (C-1$^A$), 91.3, 90.4, 82.3, 80.3, 80.0, 79.9, 77.5, 75.5, 75.4, 75.3, 75.1, 74.7, 73.6, 73.5, 75.5, 73.2, 73.2, 73.1, 73.0, 72.9, 72.5, 70.6, 70.4, 68.1, 67.5, 21.2, 20.9.

**2,4,6-tri-*O*-benzyl-3-*O*-(2-naphtyl methyl)-*β*-D-galactopyranosyl-(1→4)-1,2-di-*O*-acetyl-3,6-di-*O*-benzyl-*α*-D-glucopyranose 7.**



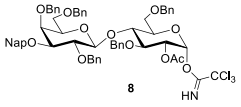
7

To a solution of compound **6** (567 mg, 0.57 mmol) in $CH_2Cl_2$ (5.0 ml), TFA (0.025 ml) and water (0.025 ml) were added and the mixture was stirred 3 hr at rt. After 16 h (TLC: Cyclohexane/EtOAc 7:3) the reaction mixture was concentrated under reduced pressure. The crude was redissolved in 3.0 mL of pyridine, then cooled to 0°C and $Ac_2O$ (3.0 mL) was added. After 2 hr, analytical TLC (Cyclohexane/EtOAc) the reaction was quenched by addition of MeOH at 0°C, co-evaporated with toluene and then purified by medium pressure column chromatography. The column was eluted with a gradient from 0 to 100% of EtOAc in cyclohexane. Pure fractions were collected and evaporated to dryness affording compound **7** as a clear syrup (500 mg, 86% yield, only α anomer). $[\alpha]_D^{25} = +21.40^\circ$ (c 0.145 $CHCl_3$). ESI MS ($C_{62}H_{64}O_{13}$) m/z $[M+NH_4]^+$ found 1034.47; calcd 1034.4685.

$^1$H NMR (400 MHz, $CDCl_3$) δ 7.86-7-15 (m, 32H, Ar-H), 6.30 (d, 1H, J=3.7 Hz, H-1$^A$), 5.07-4.97 (m 3H), 4.91-4.79 (m, 4H, incl. H-2$^A$), 4.60 (d, 1H, J=11.4 Hz), 4.55 (d, 1H, J=12.0 Hz), 4.46-4.21 (m, 4H, incl. H-1$^B$), 4.08 (t, 1H, J=9.5 Hz), 3.97 (d, 1H, J=2.9 Hz), 3.91-3.73 (m, 4H), 3.62-3.43 (m, 4H), 3.42-3.32 (m, 2H), 2.17 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 169.9 (CO), 169.2 (CO), 139.1, 139.0, 138.8, 138.0, 137.9, 135.9, 133.3, 132.9, 128.4-125.6 (C-Ar), 102.9 (C-$1^B$), 89.8 (C-$1^A$), 82.4, 79.9, 77.7, 76.1, 75.3, 74.9, 74.7, 73.5, 73.4, 73.3, 73.2, 73.0, 72.5, 71.1, 68.1, 67.5, 56.6, 21.1 ($CH_3$), 20.7 ($CH_3$).

**2,4,6-tri-*O*-benzyl-3-*O*-(2-naphtyl methyl)-*β*-D-galactopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-*α*-D-glucopyranosyl trichloroacetimidate 8.**



**8**

Compound **7** (0.633 g, 0.622 mmol) was dissolved in dry THF (6.0 mL). Ethylenediamine (0.062 mL, 0.933 mmol) was added, followed by acetic acid (0.053 mL, 0.933 mmol).

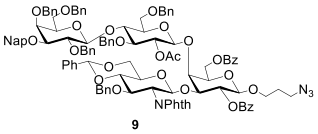
The reaction was stirred overnight at rt. After 16 hr (TLC: Cyclohexane/EtOAc) the reaction mixture was evaporated under reduced pressure, then the crude was purified by medium pressure column chromatography using a gradient from 0 to 100% of EtOAc in Cyclohexane. Pure fractions were collected and evaporated to dryness affording the lactose 1-OH, which was directly progressed to the next step.

Lactose 1-OH (0.5 g, 0.512 mmol) was dissolved in dry DCM (5 mL) under nitrogen and trichloroacetonitrile (0.257 mL, 2.56 mmol) and 1,8-diazobicyclo[5.4.0]undec-7-ene (0.023 mL, 0.15 mmol) were added. After stirring for 2h at rt, TLC (7:3 cyclohexane:EtOAc) showed complete reaction. The solvent was removed at reduced pressure and the crude was purified by flash chromatography (cyclohexane:EtOAc) to afford **8** (0.487 g) in 70% yield over two steps. ESI MS ($C_{62}H_{62}Cl_3NO_{12}$) *m/z* $[M\text{-}CNHCCl_3+NH_4]^+$ found 992.46; calcd 992.4580.

$^1$H NMR (400 MHz, $CDCl_3$) δ 8.6 (s, 1H, NH), 7.89-7.14 (m, 32H, Ar-H), 6.5 (d, 1H, J=3.4 Hz, H-$1^A$), 5.01-4.99 (m, 3H), 4.92-4.85 (m, 3H), 4.81 (t, 1H, J=12.6 Hz), 4.67 (d, 1H, J=11.2 Hz), 4.62 (d, 1H, J=11.3 Hz), 4.55 (d, 1H, J=12.0 Hz), 4.42 (d, 1H, J=7.4 Hz), 4.36 (t, 2 H, J=11.7 Hz), 4.28 (d, 1H, J=11.6 Hz), 4.21-4.11 (m, 1H), 4.04-3.87 (m, 4H), 3.83 (t, 1H, J=8.5 Hz), 3.60-3.52 (m, 2H), 3.49-3.36 (m, 3H), 1.99 (s, 3H, $COCH_3$).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.0 (CO), 160.9 (CO), 139.1-125.6 (C-Ar), 102.9 (C-$1^A$), 100.0, 93.9 (C-$1^B$), 82.4, 79.8, 75.8, 75.3, 75.0, 74.7, 73.6, 73.5, 73.4, 73.1, 73.0, 72.5, 71.6, 68.1, 67.3, 20.6 ($CH_3$).

**3-Azidopropyl 4,6-*O*-benzilidene-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[(2,4,6-tetra-*O*-benzyl-3-*O*-(2-naphtyl methyl)-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 9.**



**9**

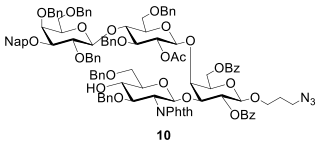
A solution of donor **8** (0.200 g, 0.191 mmol) and disaccharide acceptor **1a** (0.116 g, 0.127 mmol) with activated 4 Å molecular sieves (0.200 g) in dry DCM (3 mL) was stirred for 20 min under nitrogen. TMSOTf (4.6 µL, 0.025 mmol) was added at 0°C. After 4 h (TLC: 8:1 Tol: EtOAc) the reaction was quenched with TEA, the solid filtered off and the solvent removed under reduced pressure. The crude was purified by flash chromatography (Tol:EtOAc) to afford the tetrasaccharide **9** in 60% yield (0.146 g) as a white amorphous solid.

$[\alpha]_D^{25} = +9.15^\circ$ (c 0.185, $CHCl_3$). ESI MS ($C_{111}H_{108}N_4O_{25}$) *m/z* $[M+NH_4]^+$ found 1915.77; calcd 1914.7641.

$^1$H NMR (400 MHz, $CDCl_3$) δ 8.13-6.77 (m, 56H, Ar-H), 5.55 (s, 1H, CHPh), 5.40 (d, 1H, J=8.2 Hz), 5.19-5.12 (m, 2H), 5.05-4.80 (m, 7H), 4.75 (d, 1H, J=11.5 Hz), 4.73 (d, 1H, J=12.1 Hz), 4.69-4.59 (m, 2H), 4.53 (d, 1H, J=7.6 Hz), 4.51-4.29 (m, 10H), 4.24 (d, 1H, J=11.3 Hz), 4.02-3.70 (m, 11H), 3.67-3.58 (m, 1H), 3.57-3.48 (m, 3H), 3.46-3.32 (m, 3H), 3.11-2.90 (m, 2H, $CH_2N_3$), 1.91 (s, 3H, $COCH_3$), 1.70-1.50 (m, 2H, $CH_2CH_2N_3$)

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.4, 167.6, 166.9, 166.4, 164.5, 139.6, 139.1, 138.9, 138.4, 138.1, 137.6, 137.1, 136.0, 133.5-122.9 (m, C-Ar), 103.1, 101.4, 100.9, 100.3, 99.9, 83.0, 82.5, 81.3, 80.0, 79.2, 75.7, 75.3, 74.7, 74.6, 74.4, 74.3, 73.8, 73.6, 73.5, 73.2, 73.1, 73.0, 72.6, 72.6, 72.3, 70.7, 68.7, 68.5, 68.2, 66.1, 65.1, 64.6, 55.9, 47.9, 29.7, 28.9, 20.5.

**3-Azidopropyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-[(2,4,6-tetra-*O*-benzyl-3-*O*-(2-naphtyl methyl)-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 10.**



**10**

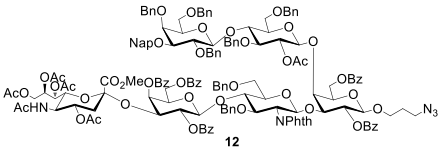
A solution of compound **9** (0.146 g, 0.076 mmol) in ACN (3 mL) was cooled at 0°C. $Me_3NBH_3$ (0.028 g, 0.38 mmol) and $BF_3{\cdot}OEt_2$ (0.047 mL, 0.38 mmol) were added and the reaction was stirred for 2 h under nitrogen. First TEA and then MeOH were added until neutral pH. The solvent was removed at reduced pressure and the crude was purified by flash chromatography (toluene:EtOAc) to afford the target tetrasaccharide acceptor **10** in 55% yield (0.080 g).

$[\alpha]_D^{25}$ = +3.95° (c 0.7, $CHCl_3$). ESI MS ($C_{111}H_{110}N_4O_{25}$) *m/z* $[M+NH_4]^+$ found 1917.78; calcd 1916.7797.

$^1$H NMR (400 MHz, $CDCl_3$) δ 8.11-6.81 (m, 56H, Ar-H), 5.32 (d, 1H, J=7.9 Hz), 5.19-5.10 (m, 2H), 5.04-4.80 (m, 7H), 4.71 (d, 1H, J=11.7 Hz), 4.66-4.58 (m, 3H), 4.55-4.44 (m, 4H), 4.41 (d, 1H, J=4.2 Hz), 4.38 (d, 1H, J=4.0 Hz), 4.34 (t, 1H, J=4.5 Hz), 4.32-4.28 (m, 2H), 4.22 (d, 1H, J=11.7 Hz), 4.14 (d, 1H, J=7.8 Hz), 4.12 (d, 1H, 7.8 Hz), 4.01 (d, 1H, J=9.4 Hz), 3.99-3.96 (m, 1H), 3.93 (dd, 1H, $J_{1,2}$=2.6 Hz, $J_{1,3}$=9.8 Hz), 3.89-3.71 (m, 10H), 3.67 (d, 1H, J= 11.1 Hz), 3.64-3.58 (m, 1H), 3.55-3.47 (m, 3H), 3.44-3.38 (m, 2H), 3.37-3.30 (m, 1H), 3.09-2.93 (m, 2H, $CH_2N_3$), 1.78 (s, 3H, $COCH_3$), 1.70-1.50 (m, 2H, $CH_2CH_2N_3$).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.2, 166.3, 164.4, 139.7, 139.2, 139.0, 138.5, 138.1, 137.9, 137.4, 136.0, 133.5-123.0 (C-Ar), 102.9, 100.9, 100.3, 99.1, 82.5, 81.3, 80.0, 78.7, 78.5, 76.9, 75.5, 75.2, 74.7, 74.6, 74.5, 74.3, 74.0, 73.7, 73.6, 73.5, 73.4, 73.2, 73.0, 72.6, 72.5, 72.4, 70.8, 70.6, 68.5, 68.2, 64.9, 64.8, 55.5, 47.9, 29.7, 28.9, 20.4.

**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)-[(2,4,6-tetra-*O*-benzyl-3-*O*-(2-naphtyl methyl)-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 12.**



**12**

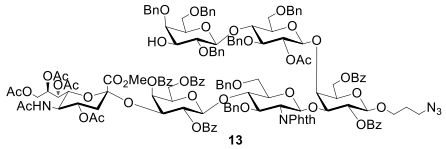
A solution of disaccharide donor **11** (0.071 g, 0.063 mmol) and tetrasaccharide acceptor **10** (0.080 g, 0.042 mmol) with 4 Å molecular sieves (0.100 g) in dry DCM (5.0 mL) was stirred for 20 min under nitrogen. TMSOTf (1.5 μL, 0.0084 mmol) was added at -10°C. After 4 h (TLC; 6:4 Toluene: Acetone) the reaction was quenched with TEA, the solid filtered off and the solvent removed under reduced pressure. The crude was purified by flash chromatography (Toluene:Acetone) to afford hexasaccharide **12** in 72% yield (86 mg).

$[\alpha]_D^{25}$ = -1.26° (c 0.7, $CHCl_3$). ESI MS ($C_{158}H_{159}N_5O_{45}$) *m/z* $[M+NH_4]^+$ found 2865.07; calcd. 2864.0645.

$^1$H NMR (400 MHz, $CDCl_3$) δ 8.33-6.57 (m, 71H, Ar-H), 5.73-5.65 (m, 1H), 5.48 (dd, 1H, $J_{1,2}$=7.8 Hz, $J_{1,3}$=9.9 Hz), 5.33 (d, 1H, J=3.2 Hz), 5.22 (dd, 1H, $J_{1,2}$=2.6 Hz, $J_{1,3}$=9.7 Hz), 5.18-5.05 (m, 3H), 5.01-4.91 (m, 3H), 4.91-4.75 (m, 7H), 4.66 (d, 1H, J=11.7 Hz), 4.61-4.54 (m, 3H), 4.54-4.39 (m, 5H), 4.35-4.27 (m, 3H), 4.25 (d, 1H, J=2.9 Hz), 4.24-4.09 (m, 6H), 4.08-3.91 (m, 5H), 3.90-3.75 (m, 9H, incl. $COOCH_3$), 3.75-3.64 (m, 3H), 3.59 (dd, 1H, $J_{1,2}$=2.7 Hz, $J_{1,3}$=10.7 Hz), 3.55 (d, 1H, J=10.6 Hz), 3.52-3.46 (m, 2H), 3.44 (d, 1H, J=8.4 Hz), 3.42-3.32 (m, 3H), 3.32-3.26 (m, 1H), 3.08-2.90 (m, 2H, $CH_2N_3$), 2.43 (dd, 1H, $J_{1,2}$=4.7 Hz, $J_{1,3}$=12.7 Hz, H-$3^F_{eq}$), 2.14 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.91 (s, 3H, $COCH_3$), 1.79 (s, 3H, $COCH_3$), 1.66 (t, 1H, J=12.4 Hz, H-$3^F_{ax}$), 1.62 (s, 3H, $COCH_3$), 1.50 (s, 3H, $COCH_3$), 1.54-1.42 (m, 2H, $CH_2CH_2N_3$).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 133.3-123.0 (C-Ar), 103.0, 100.8, 100.5, 100.4, 98.5, 82.6, 81.2, 79.9, 77.6, 77.5, 77.2, 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, 72.0, 71.8, 71.7, 70.5, 69.4, 68.2, 68.0, 67.1, 66.5, 64.7, 62.4, 61.5, 55.9, 53.2, 48.8, 48.0, 37.4, 29.7, 28.9, 23.2, 21.4, 20.8, 20.4, 20.2.

**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)-[(2,4,6-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 13.**



13

Hexasaccharide **12** (0.080 g, 0.028 mmol) was dissolved in a 4:1 DCM/MeOH mixture (5.0 mL). Then, DDQ (0.032 g, 0.140 mmol) was added and the resulting reaction mixture was stirred for 2 hr at rt. After two hours (TLC: Toluene/Acetone 6:4) the reaction was quenched by addition of aqueous $NaHCO_3$, then washed with $NaHCO_3$ (20 mL) for three times.

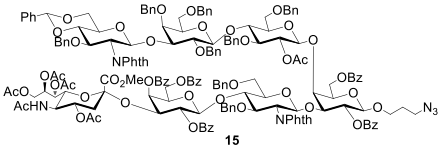
The organic phase was dried over $Na_2SO_4$, then evaporated to dryness. The crude was purified by column chromatography (Toluene:Acetone). Pure fractions were collected and evaporated under reduced pressure affording heptasaccharide **13** in 75% yield (50 mg).

$[\alpha]_D^{25}$ = -1.26° (c 0.7, $CHCl_3$). ESI MS ($C_{147}H_{151}N_5O_{45}$) *m/z* $[M+NH_4]^+$ found 2725.01; calcd. 2724.0019

$^1$H NMR (400 MHz, $CDCl_3$) δ 8.33-6.58 (m, 64H, Ar-H), 5.72-5.65 (m, 1H), 5.48 (dd, 1H, $J_{1,2}$= 7.7 Hz, $J_{1,3}$=9.9 Hz), 5.33 (d, 1H, J=3.2 Hz), 5.22 (dd, 1H, $J_{1,2}$=2.6 Hz, $J_{1,3}$=9.9 Hz), 5.16 (d, 1H, J=7.9 Hz), 5.13-5.05 (m, 2H), 4.99-4.78 (m, 7H), 4.76 (d, 1H, J=8.6 Hz), 4.71 (d, 1H, J=11.3 Hz), 4.65 (d, 1H, J=10.6 Hz), 4.62-4.56 (m, 3H), 4.55-4.52 (m, 1H), 4.51-4.39 (m, 4H), 4.36-4.26 (m, 4H), 4.25-4.08 (m, 6H), 4.07-3.93 (m, 4H), 3.90-3.75 (m, 9H, incl. $COOCH_3$), 3.75-3.66 (m, 3H), 3.60 (d, 1H, J=2.7 Hz), 3.59-3.53 (m, 2H), 3.52-3.44 (m, 3H), 3.44-3.34 (m, 3H), 3.32 (dd, 1H, $J_{1,2}$=2.6 Hz, $J_{1,3}$=6.5 Hz), 3.08-2.90 (m, 2H, $CH_2N_3$), 2.43 (dd, 1H, $J_{1,2}$=4.3 Hz, $J_{1,3}$=12.7 Hz, H-$3^F_{eq}$), 2.13 (s, 3H), 1.98 (s, 3H), 1.91 (s, 3H), 1.94-1.88 (m, 2H, $CH_2CH_2N_3$), 1.78 (s, 3H), 1.65 (t, 1H, J=12.7 Hz, H-$3^F_{ax}$), 1.62 (s, 3H). 1.50 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 133.9-127.6 8, C-Ar), 103.5, 101.6, 101.2, 101.0, 99.3, 81.9, 81.3, 78.4, 78.0, 77.8, 77.6, 77.5, 76.7, 75.9, 75.8, 75.7, 75.7, 75.6, 75.5, 75.4, 75.3, 75.2, 75.1, 75.0, 74.6, 72.8, 72.6, 72.4, 71.6, 71.2, 70.1, 69.0, 68.9, 68.8, 68.7, 68.7, 68.3, 67.7, 67.3, 65.9, 65.5, 63.1, 62.2, 56.6, 54.1, 53.8, 48.5, 38.2, 29.7, 23.9, 22.0, 21.7, 21.6, 21.2, 20.9.

**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)-[4,6-*O*-benzilidene-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-(2,4,6-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 15.**



15

A solution of glucosamine donor **14** (0.018 g, 0.028 mmol) and acceptor **10** (0.050 g, 0.018 mmol) with 4 Å molecular sieves (0.070 g) in dry DCM (3.0 mL) was stirred for 20 min under nitrogen. TMSOTf (0.7 μL, 0.0036 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 reduced pressure. The crude was purified by flash chromatography (Toluene:Acetone) to afford heptasaccharide **15** in 60% yield (35 mg).

$[\alpha]_D^{25}$ = +14.95° (c 0.125, $CHCl_3$). ESI MS ($C_{175}H_{174}N_6O_{51}$) *m/z* $[M+Na]^+$ found 3199.13; calcd. 3198.1099

$^1$H NMR (400 MHz, $CDCl_3$) δ 8.30-6.56 (m, 78H, Ar-H), 5.76-5.68 (m, 1H), 5.63 (s, 1H, CHPh), 5.54-5.43 (m, 2H), 5.38 (d, 1H, J=2.9 Hz), 5.13 (d, 1H, J=7.7 Hz), 5.08 (d, 1H, J=8.2 Hz), 5.06-4.92 (m, 3H), 4.92-4.69 (m, 7H), 4.66-4.40 (m, 9H), 4.40-4.23 (m, 7H), 4.23-4.12 (m, 5H), 4.12-3.99 (m, 5H), 3.96 (d, 1H, J=12.2 Hz), 3.93-3.74 (m, 11H, incl. $COCH_3$), 3.73-3.56 (m, 7H), 3.55-3.25 (m, 7H), 3.18-3.07 (m, 1H), 3.06-2.91 (m, 2H, $CH_2N_3$), 2.45 (dd, 1H, $J_{1,2}$=4.9 Hz, $J_{1,3}$=12.6 Hz, H-$3^E_{eq}$), 2.20 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 1,92 (s, 3H, $COCH_3$), 1.80 (s, 3H, $COCH_3$), 1.78 (s, 3H, $COCH_3$), 1,68 (t, 1H, J=12.6 Hz, H-$3^E_{ax}$), 1.64-1.56 (m, 2H, $CH_2CH_2N_3$), 1.53 (s, 3H, $COCH_3$).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 133.5-123.2 (C-Ar), 102.6, 101.4, 101.2, 100.8, 100.7, 100.5, 100.2, 83.1, 81.6, 81.0, 77.6, 77.2, 76.7, 76.6, 75.9, 75.6, 74.9, 74.7, 74.3, 74.2, 73.3, 73.0, 72.9, 72.7, 72.4, 72.0, 71.8, 71.3, 71.0, 70.6, 70.5, 69.4, 68.8, 68.3, 67.9, 67.6, 67.2, 66.5, 65.8, 64.7, 62.4, 61.5, 56.3, 56.0, 55.9, 55.0, 53.2, 48.9, 47.9, 37.4, 28.9, 23.2, 21.4, 20.8, 20.7, 20.4, 20.0.

## References


1. Adamo, R. *Accounts Chem. Res.* **2017**, *50*, 1270-1279.
2. Michon, F.; Uitz, C.; Sarkar, A.; D'Ambra, A.J.; Laude-Sharp, M.; Moore, S.; Fusco, P.C: *Clin. Vacc. Immunol.* **2006**, *13*, 936-943.
3. Song, J.Y.; Lim, J.H.; Lim, S.; Yong, Z.; Seo, H.S. *Hum. Vacc. Immunother.* **2018**, *14*, 2669-2681.
4. Lin, S.M.; Zhi, Y.; Ahn, K.B.; Lim, S.; Seo, H.S. *Clin. Exp. Vaccine Res.* **2018**, *7*, 76-81.
5. Jennings, H.J.; Katzenellenbogen, E.; Lugowski, C.; Kasper, D.L *Biochemistry*, **1983**, *22*, 1258-1264.
6. Sundgren, A.; Lahmann, M.; Oscarson, S. *J. Carb. Chem.* **2005**, *24*, 379-391.
7. Berti, F.; Adamo, R. *ACS Chem. Biol.* **2013**, *8*, 1653-1663.
8. Broecker, F.; Hanske, J.; Martin, C.E.; Baek, J.Y.; Wahlbrink, A.; Wojcik, F.; Hartmann, L.; Rademacher, C.; Anish, C.; Seeberger, P.H. *Nat. Commun.* **2016**, *7*, 11224.
9. Carboni, F.; Angiolini, F.; Fabbrini, M.; Brogioni, B.; Corrado, A; Berti, F.; Adamo, R.; Margarit, I. *J. Infect. Dis.* **2019**, DOI: 10.1093/infdis/jiz551.
10. Carboni, F.; Adamo, R.; Fabbrini, M.; De Ricco, R.; Cattaneo, V.; Brogioni, B.; Veggi, D.; Pinto, V.; Passalacqua, I.; Oldrini, D.; Rappuoli, R.; Malito, E.; Margarit Y Ros, I.; Berti, F. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 5017-5022.