



## Synthesis of the Lewis b pentasaccharide and a HSA-conjugate thereof

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### ABSTRACT

*Helicobacter pylori*, a gastric pathogen, binds to various blood group antigens, including the Lewis types, present in the gastric tissue and a relation between the presentation of the ligands and the overall strength of binding has been assumed. Synthetic Lewis b tetra- and hexasaccharide conjugates are available but not the analogous pentasaccharide. An efficient synthesis of the amino spacer equipped Lewis b pentasaccharide, 3-aminopropyl  $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -L-fucopyranosyl-(1 $\rightarrow$ 4)]-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranoside, is presented to enable further investigation of the carbohydrate recognition process of *H. pylori*.

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### 1. Introduction

The spiral-shaped Gram-negative bacterium *Helicobacter pylori*, a gastric pathogen, binds to various blood group antigens, including the Lewis types, present in the gastric tissue.<sup>1,2</sup> In developed countries, approximately 50% of the population over the age of 50 are infected with this bacterium, while, in contrast, such infection is uncommon in children. In developing nations, 70–90% of the population are carrying *H. pylori* and virtually everyone gets infected during childhood.<sup>3,4</sup>

The adhesion process of *H. pylori* bacteria to Lewis b structures is mediated by a membrane lectin, the blood group antigen binding adhesion protein (BabA).<sup>5</sup> Synthetic glycoconjugates containing the Lewis b hexasaccharide ligand are used in affinity chromatography for purification of the BabA, but analogous Lewis b tetrasaccharide conjugates are not effective for this purpose, although the free tetrasaccharide can inhibit the adhesion process. Reductive amination, the conjugation technique preferably used for preparing glycoconjugates from native Lewis b hexasaccharide, destroys the reducing end glucose unit. These glycoconjugates are feasible for purification purposes, although only a pentasaccharide unit is available for recognition. Kojima et al.<sup>6</sup> investigated the adhesion properties of Lewis b oligosaccharide conjugates linked to Bovine serum albumin (BSA), and polyacrylamide (PAA) and

palmitoylphosphatidylethanolamine (DPPE), concluding that the recognition is affected by the carrier. The DPPE conjugate was synthesised via reductive amination from the hexasaccharide, but no further information was given for the purchased conjugates (Lewis b hexasaccharide BSA-conjugate, Funakoshi, Tokyo, Japan; Lewis b tetrasaccharide PAA-conjugate, Seikagaku Kogyuo Co., Tokyo, Japan). All conjugates were recognised by *H. pylori*, but the BSA-conjugate was clearly the best recognised conjugate.

Syntheses of both the Lewis b tetrasaccharide and the hexasaccharide have been published. The synthesis of the Lewis b tetrasaccharide has been elaborated many times, e.g., as the free reducing oligosaccharide by Matta et al.<sup>7</sup> and as methyl glycoside, first by Lemieux and Spohr<sup>8</sup> and later by Kahne and Yan.<sup>9</sup> Solid phase synthesis has been employed by Danishefsky's group<sup>10</sup> and a large scale approach, reducing chromatography to a bare minimum, was achieved by Norberg et al.<sup>11</sup> Recently an enzymatic approach has been established.<sup>12</sup> Also for the Lewis b hexasaccharide several approaches have been investigated and human serum albumin (HSA) conjugates have been prepared.<sup>13–16</sup> The pentasaccharide structure (Fig. 1) has been prepared earlier as a protected intermediate, e.g., in Danishefsky's glycal approach, but has never been deprotected and used for synthetic conjugates. To further investigate the binding specificity of BabA, we envisaged using synthetic Lewis pentasaccharide glycoconjugates. To obtain comparable parameters, we intended to use the same spacer and conjugation techniques as in earlier studies with the hexa- and tetrasaccharides.

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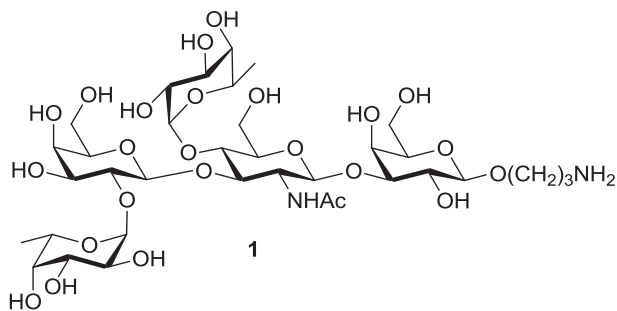
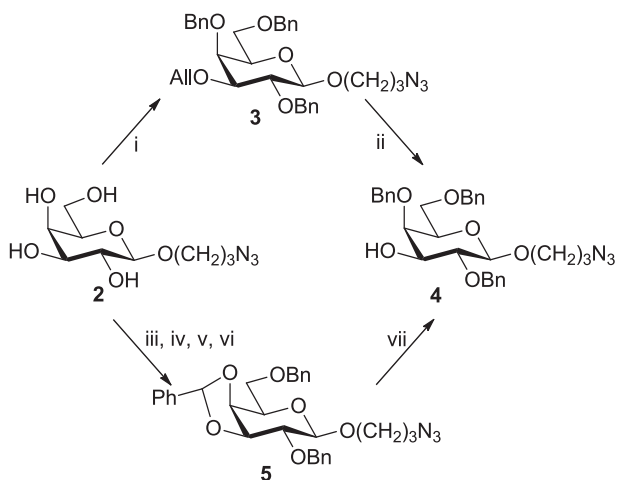


Figure 1. The target Lewis b pentasaccharide.

## 2. Results and discussion

Attempts to adapt the synthetic approach used for our previous synthesis of the Lewis b hexasaccharide,<sup>16</sup> where a suitable protected lacto-*N*-tetraose unit was assembled by a 2+2 approach to gain quick access to the desired target pentasaccharide **1** were hampered by the loss of regioselectivity during the coupling of the disaccharide donor to the analogous 3-azidopropyl 2,6-di-*O*-benzyl galactoside acceptor. Glycosylations, using the 4-*O*-benzyl protected derivative **4** as glycosyl acceptor instead, produced unsatisfactory yields. Therefore, we decided to trial a stepwise synthesis starting from the reducing-end with acceptor galactoside **4**.

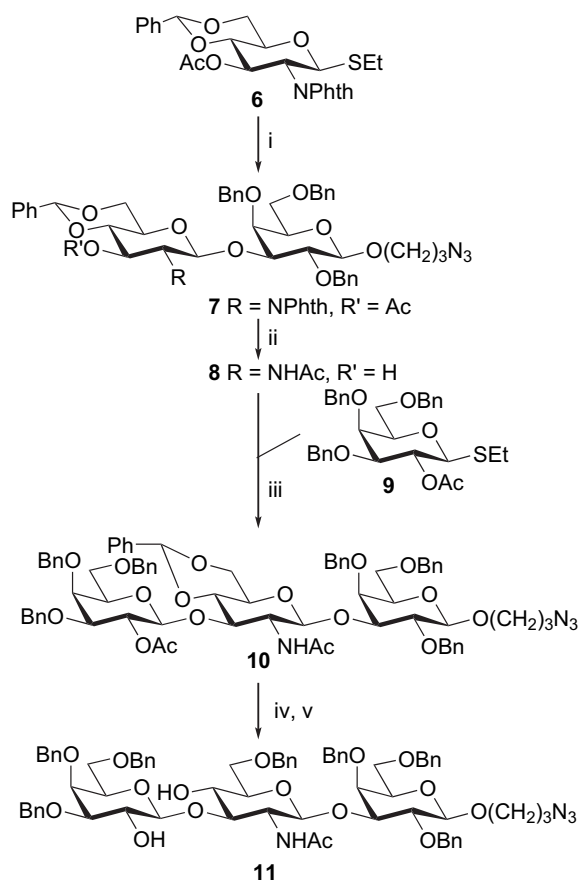
At first, galactoside **4** (Scheme 1) was prepared from 3-azidopropyl  $\beta$ -D-galactopyranoside **2**.<sup>17</sup> Regioselective allylation was achieved by activating the OH-3 group using dibutyltin oxide followed by treatment with allyl bromide and CsF in DMF to give 3-azidopropyl 3-*O*-allyl- $\beta$ -D-galactopyranoside. This material was benzylated in the same reaction vessel using sodium hydride and benzyl bromide to give **3** in 74% yield. When we tried to reproduce this sequence on a multi-gram scale, substantially lower yields were obtained. Also, the 3,6-di-*O*-allylated galactoside was produced as major product when the reaction mixture was co-evaporated with toluene after stannylidene formation in MeOH. Furthermore, removal of the allyl group was not straightforward. The use of Wilkinson's catalyst for the isomerisation step produced only base-line material on the TLC-plates, while PdCl<sub>2</sub> in THF or methanol was very slow. Using a methanol/ethanol (1:1) mixture gave better results but unidentified side products appeared during prolonged reaction times. Eventually, acceptor **4** could be isolated



Scheme 1. (i) (1) Bu<sub>2</sub>SnO, MeOH, reflux, 4 h; (2) AllBr, CsF, DMF, 30 °C, overnight; (3) BnBr, NaH, DMF, rt, 3 h, 74%; (ii) PdCl<sub>2</sub>, MeOH/EtOH (1:1), rt, 24 h, 15–66%; (iii) 2, 2-dimethoxypropane, CSA, 56%; (iv) BnBr, NaH, DMF, 66%; (v) TFA (90% aq), CH<sub>2</sub>Cl<sub>2</sub>, 95%; (vi) PhCH(OMe)<sub>2</sub>, pTSA, THF, 95%; (vii) NaBH<sub>3</sub>CN, HCl/Et<sub>2</sub>O, THF, 90%.

in a maximum yield of 66% after 24 h. Since large amounts of compound **4** were required, we decided to employ a longer but more reproducible route. The key step in this approach is the formation of the *endo*-3,4-benzylidene acetal, which under reductive conditions can be opened regioselectively to give the unprotected 3-OH product as major regioisomer.<sup>18</sup> However, the 3,4-benzylidene acetal cannot be introduced directly into tetraol **2**, therefore the spacer galactoside **2** was first treated with 2,2-dimethoxypropane and a catalytic amount of camphorsulfonic acid (CSA) to form the interim 3,4-acetonide, followed by benzylation and cleavage of the isopropylidene group, a procedure adapted from the known 5-azidopentyl galactoside.<sup>19</sup> Formation of the *endo*-3,4-benzylidene acetal using benzaldehyde dimethyl acetal (PhCH(OMe)<sub>2</sub>) and *p*-toluenesulfonic acid (pTSA) in THF was followed by reductive opening using standard conditions to give the desired acceptor **4** as major regioisomer.<sup>20,21</sup>

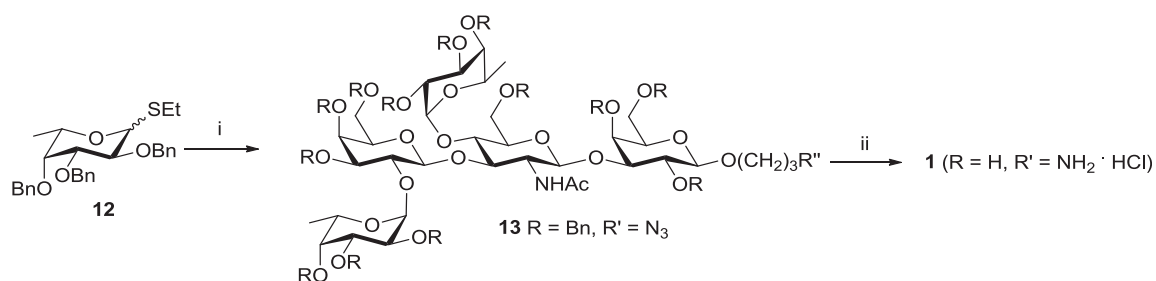
Coupling of acceptor **4** with the donor ethyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside **6**,<sup>22</sup> using *N*-iodosuccinimide (NIS)/silver trifluoromethanesulfonate (AgOTf) as promoter system in CH<sub>2</sub>Cl<sub>2</sub>, gave disaccharide **7** in excellent (93%) yield (Scheme 2). The phthaloyl protecting group ensured good solubility of the donor and complete  $\beta$ -selectivity during the reaction. Upon removal of the acetyl group, this disaccharide could serve as glycosyl acceptor, but to avoid problems with late removal of the phthaloyl group, experienced in the deprotection sequence of the hexasaccharide,<sup>15</sup> we decided to exchange it for the target acetyl amido group already at this stage. Treating disaccharide **7** first with ethylenediamine in ethanol at reflux temperature, followed by addition of sodium methoxide to ensure complete removal of the 3-acetyl group,



Scheme 2. i) **4**, NIS, AgOTf, 4 Å MS, CH<sub>2</sub>Cl<sub>2</sub>, –20 °C, 30 min, 93%; (ii) (1) EtOH/ethylenediamine (10:1), reflux, 2 h; (2) NaOMe/MeOH, reflux, 2 h; (3) Ac<sub>2</sub>O, MeOH, 0 °C, 1 h, 83% over sequence; (iii) NIS, AgOTf, 4 Å MS, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 30 min, 84%; (iv) (1) NaBH<sub>3</sub>CN, HCl/Et<sub>2</sub>O, 3 Å MS, THF, rt, 2 h; (2) NaOMe, MeOH, rt, 74% over two steps.

and subsequent *N*-acetylation with acetic anhydride in methanol at 0 °C produced **8** (83%). Coupling between the disaccharide acceptor **8** and the suitably protected thioethyl galactoside donor **9** afforded the trisaccharide **10** (84%). All three  $\beta$ -anomeric linkages were confirmed by the coupling constants between the C-1's and H-1's (162.1 Hz, 162.5 Hz and 158.0 Hz). Regioselective opening of the benzylidene acetal with  $\text{NaBH}_3\text{CN}$  and  $\text{HCl}/\text{Et}_2\text{O}$  in THF followed by Zemplén deacetylation provided diol **11** (74% over two steps).

The subsequent coupling (Scheme 3) under halide-assisted coupling conditions between the diol trisaccharide acceptor **11** and the halide donor 2,3,4-tri-*O*-benzyl- $\alpha$ -*L*-fucopyranosyl bromide, prepared in situ by addition of bromine to the glycosylation mixture already containing acceptor **11**, the thioethyl fucoside **12** and tetraethyl ammonium bromide, furnished the protected pentasaccharide **13** in 67% yield. Catalytic hydrogenolysis under atmospheric hydrogen pressure, using Pd/C in the presence of 1 equiv of 1 M HCl in EtOAc/EtOH/H<sub>2</sub>O (2:2:1), gave complete removal of the benzyl ethers and concomitant reduction of the azido group in 1 day as verified by MALDI-TOF spectrometry (**1**-HCl, 82%).



**Scheme 3.** i) 1.  $\text{Et}_4\text{NBr}$ ,  $\text{CH}_2\text{Cl}_2/\text{DMF}$  (10:1), 4 Å MS, rt; (2)  $\text{Br}_2$ , rt, 72 h, 67%; (ii)  $\text{H}_2$  (1 atm), 10% Pd/C, HCl, EtOAc/EtOH/H<sub>2</sub>O, rt, 24 h, 82%.

Since disuccinimidyl suberate (DSS) has been successfully employed for the conjugation of the Lewis b hexasaccharide to HSA in our group,<sup>16</sup> we decided to follow the same two-step methodology. An eightfold excess, to prevent dimerisation, of DSS, prepared from suberic acid and *N*-hydroxy succinimide was reacted with pentasaccharide **1** in DMSO, in the presence of triethylamine. After complete conversion (30 min–1 h), the crude product was applied onto a C18 column, pre-treated with DCM. Residual reagents and DMSO were removed by elution with DCM, while the DSS/active-ester conjugate was retained due to its poor solubility in the eluting solvent. Elution with water provided eventually the DSS/active-ester conjugate. This material was conjugated—monitored by MALDI-TOF—with HSA in a buffer (pH 10) to give the Lewis b pentasaccharide/HSA conjugate, with an average loading of 10 sugar residues per protein molecule, ready for biological investigation.

In conclusion, a short and efficient way was developed for the synthesis of the Lewis b pentasaccharide. DSS methodology was successful to provide the Lewis b pentasaccharide/HSA conjugate.

### 3. Experimental

#### 3.1. General methods

Organic solutions were dried over  $\text{Na}_2\text{SO}_4$  before concentration, which was performed under reduced pressure at <40 °C (bath). NMR spectra were recorded at 25 °C at 300 or 400 MHz (Varian) or 500 MHz (Bruker) (<sup>1</sup>H) or 75, 100 or 125 MHz (<sup>13</sup>C), respectively,  $\text{CDCl}_3$ ,  $\text{D}_2\text{O}$  or at ambient temperature if not otherwise stated. All proton and carbon NMR spectra in  $\text{CDCl}_3$  were referenced to the chloroform signal (<sup>1</sup>H  $\delta$  7.27 ppm, <sup>13</sup>C  $\delta$  77.17 ppm) if not otherwise

stated. No reference was used for the spectrum in  $\text{D}_2\text{O}$ . TLC was performed on Silica Gel F<sub>254</sub> (E. Merck) with detection by UV light and/or charring with 8% sulfuric acid. Silica gel (0.041–0.063 mm, Amicon) was used for column chromatography. Mass spectra were recorded on a Bruker Daltonics MicroTOF using electrospray technique. MALDI-TOF spectra were recorded on a Bruker Reflex IV using 2',4',6'-trihydroxy-acetophenone monohydrate (THAP) as matrix. For conjugation reactions a borate buffer was prepared from 0.0125 M  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  solution that was adjusted to pH 10 with 0.10 M NaOH.

#### 3.2. Synthetic procedures

##### 3.2.1. 3-Azidopropyl 2,4,6-tri-*O*-benzyl- $\beta$ -*D*-galactopyranoside (**4**).

3-Azidopropyl  $\beta$ -*D*-galactopyranoside **2** (2.58 g, 9.8 mmol) and dibutyltin oxide (4.88 g, 19.6 mmol) were refluxed in dry MeOH (50 mL) until the mixture became clear (4 h). The solvent was evaporated and the residue was dissolved in dry DMF (50 mL). Allyl bromide (1.02 mL, 11.8 mmol) and CsF (1.93 g, 12.7 mmol) were added to this solution and the mixture was stirred overnight. After

that, NaH (60%; 2.35 g, 58.8 mmol) and BnBr (5.2 mL, 44.1 mmol) were added and the reaction mixture was again left overnight. After concentration and co-evaporation with toluene (3  $\times$  15 mL), the residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (300 mL), washed with 10% KI (2  $\times$  50 mL) and water (2  $\times$  50 mL), dried, concentrated and purified by silica gel flash chromatography (95:5 toluene/EtOAc) to produce 3-azidopropyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl- $\beta$ -*D*-galactopyranoside (**3**) (4.16 g, 74%);  $[\alpha]_{\text{D}}^{22}$  –10 (c 1,  $\text{CHCl}_3$ ); NMR ( $\text{CDCl}_3$ ): <sup>13</sup>C,  $\delta$  29.4 ( $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ), 48.5 ( $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ), 66.6 (C-6), 69.0 ( $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ), 72.0 ( $\text{OCH}_2\text{CH}=\text{CH}_2$ ), 73.4 (C-4), 73.6 (C-5), 73.7, 74.6 and 75.4 (3 $\text{OCH}_2\text{Ph}$ ), 79.6 (C-2), 82.2 (C-3), 104.0 (C-1), 116.8 ( $\text{OCH}_2\text{CH}=\text{CH}_2$ ), 127.7–138.9 (aromatic C), 135.1 ( $\text{OCH}_2\text{CH}=\text{CH}_2$ ); <sup>1</sup>H,  $\delta$  1.86 (m, 2H,  $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ), 3.38 (t, 2H,  $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ), 3.41 (dd, 1H,  $J_{3,4}=2.8$  Hz, H-3), 3.51–3.61 (m, 4H, H-5,6' and  $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ), 3.74 (dd, 1H,  $J_{2,3}=8.0$  Hz, H-2), 3.85 (d, 1H, H-4), 3.95 (m, 1H, H-6), 4.18 (m, 2H,  $\text{OCH}_2\text{CH}=\text{CH}_2$ ), 4.31 (d, 1H,  $J_{1,2}=7.6$  Hz, H-1), 4.40 and 4.35 (2  $\times$  d, 2H,  $\text{OCH}_2\text{Ph}$ ), 4.60 and 4.92 (2  $\times$  d, 2H,  $\text{OCH}_2\text{Ph}$ ), 4.77 and 4.83 (2  $\times$  d, 2H,  $\text{OCH}_2\text{Ph}$ ), 5.18 and 5.32 (2  $\times$  dq, 2H,  $\text{OCH}_2\text{CH}=\text{CH}_2$ ), 5.93 (m, 1H,  $\text{OCH}_2\text{CH}=\text{CH}_2$ ), 7.25–7.39 (m, 15H, aromatic H). Compound **3** (3.90 g, 6.80 mmol) was then dissolved in MeOH/EtOH (1:1, 40 mL) and a catalytic amount of  $\text{PdCl}_2$  was added to the solution. The reaction mixture was stirred for 24 h at room temperature. After removal of the catalyst by filtration, the mixture was concentrated and purified by silica gel flash chromatography (9:1 toluene/EtOAc) to yield **4** (2.39 g, 66%);  $[\alpha]_{\text{D}}^{22}$  +4 (c 1,  $\text{CHCl}_3$ ); NMR ( $\text{CDCl}_3$ ): <sup>13</sup>C,  $\delta$  29.4 ( $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ), 48.5 ( $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ), 66.6 (C-6), 68.8 ( $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ), 73.6, 74.9 and 75.1 (3 $\text{OCH}_2\text{Ph}$ ), 73.8, 74.2 and 79.8 (C-2,3,5), 75.6 (C-4), 103.9 (C-1), 127.9–138.5 (aromatic C); <sup>1</sup>H,  $\delta$  1.84–1.97 (m, 2H,  $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ), 2.26 (d, 1H,  $J_{3,\text{OH}}=5.0$  Hz, OH), 3.41 (t, 2H,  $J=6.8$  Hz,  $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$ ),

3.562–3.68 (m, 6H, H-2,3,5,6' and OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.88 (d, 1H, J<sub>3,4</sub>=3.2 Hz, H-4), 3.98–4.02 (m, 1H, H-6), 4.35 (d, 1H, J<sub>1,2</sub>=7.6 Hz, H-1), 4.47 (d, 2H, J=11.8 Hz, OCH<sub>2</sub>Ph), 4.51 (d, 2H, J=11.8 Hz, OCH<sub>2</sub>Ph), 4.66 (d, 2H, J=11.4 Hz, OCH<sub>2</sub>Ph), 4.79 (d, 2H, J=11.7 Hz, OCH<sub>2</sub>Ph), 4.93 (d, 2H, J=11.7 Hz, OCH<sub>2</sub>Ph), 7.27–7.36 (m, 15H, aromatic H); HR-ESI calcd for C<sub>30</sub>H<sub>35</sub>N<sub>3</sub>O<sub>6</sub>Na [M+Na]<sup>+</sup> 556.2418. Found 556.2419.

**3.2.1.1. Alternative preparation.** 3-Azidopropyl 2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside (**4**) via 3-azidopropyl 2,6-di-*O*-benzyl-3,4-*O*-benzylidene-β-*D*-galactopyranoside (**5**). A mixture of 3-azidopropyl 2,6-di-*O*-benzyl-3,4-dihydroxy-β-*D*-galactopyranoside (1.33 g, 3.01 mmol), benzaldehyde dimethyl acetal (2.3 mL, 15 mmol) and *p*TSA (172 mg, 0.9 mmol) in dry THF (30 mL) was stirred at room temperature. After 2 h, TLC (toluene/EtOAc 6:1) showed complete conversion and the reaction was quenched with Et<sub>3</sub>N (1 mL). After concentration, the crude mixture was purified by silica gel chromatography (9:1→6:1 toluene/EtOAc) to give 3-azidopropyl 2,6-di-*O*-benzyl-3,4-*O*-benzylidene-β-*D*-galactopyranoside (**5**) (1.526 g, 95%); [α]<sub>D</sub><sup>22</sup>+24 (c 1, CH<sub>2</sub>Cl<sub>2</sub>); NMR (CDCl<sub>3</sub>): <sup>13</sup>C, δ 29.5 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 48.6 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 66.6 (C-6), 69.6 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 73.2, 73.7, 73.9, 76.4, 79.0 and 80.4 (2OCH<sub>2</sub>Ph and C-2,3,4,5), 103.1 and 104.8 (C-1 and CHPh), 125.6–138.4 (aromatic C); <sup>1</sup>H, δ 1.91–2.03 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.47 (t, 2H, J=6.8 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.57 (dd, 1H, J=7.7, 6.5 Hz), 3.71 (ddd, 1H, J=10.1, 6.9, 5.5 Hz, H-5), 3.92 (dd, 1H, J=10.1, 6.9 Hz), 3.97 (dd, 1H, J=10.1, 5.5 Hz), 4.03–4.12 (m, 2H), 4.31 (dd, 1H, J=6.2, 2.1 Hz), 4.42 (t, 1H, J=6.3 Hz), 4.47 (d, 1H, J<sub>1,2</sub>=7.8 Hz, H-1), 4.66 (d, 2H, J=12.0 Hz, OCH<sub>2</sub>Ph), 4.72 (d, 2H, J=12.0 Hz, OCH<sub>2</sub>Ph), 4.84 (s, 2H, OCH<sub>2</sub>Ph), 5.98 (s, 1H, CHPh), 7.23–7.52 (m, 15H, aromatic H); HR-ESI calcd for C<sub>30</sub>H<sub>33</sub>N<sub>3</sub>O<sub>6</sub>Na [M+Na]<sup>+</sup> 554.2263. Found 554.2472. A solution of **5** (509 mg, 960 μmol) in THF (5 mL) was stirred at room temperature with powdered 3 Å molecular sieves. After 2 h, NaBH<sub>3</sub>CN (362 mg, 5.76 mmol) and more molecular sieves were added and the stirring continued for a further hour. HCl/Et<sub>2</sub>O was added dropwise until the evolution of gas ceased. The reaction was monitored by TLC (toluene/EtOAc 6:1) and quenched with Et<sub>3</sub>N after completion. The mixture was concentrated and purified by flash column chromatography (toluene→toluene/EtOAc 6:1) to give **4** (459 mg, 90%).

**3.2.2. 3-Azidopropyl (3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside (**7**).** A mixture of ethyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-*D*-glucopyranoside **6** (508 mg, 1.05 mmol), acceptor **4** (400 mg, 750 μmol) and powdered molecular sieves (4 Å, 2 g) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was stirred under argon at room temperature. After 1 h, the reaction mixture was cooled to –20 °C, AgOTf (29 mg, 113 μmol) and NIS (337 mg, 1.50 mmol) were added. After a further 30 min, the reaction was quenched with Et<sub>3</sub>N (50 μL). The mixture was filtered through Celite, which was washed with CH<sub>2</sub>Cl<sub>2</sub> (3×10 mL). The combined filtrate was washed with 10% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (2×10 mL) and water (2×10 mL), dried, concentrated and purified by silica gel chromatography (9:1 toluene/EtOAc) to produce **7** (663 mg, 93%); [α]<sub>D</sub><sup>22</sup>–27 (c 1, CHCl<sub>3</sub>); NMR (CDCl<sub>3</sub>): <sup>13</sup>C, δ 20.7 (OCOCH<sub>3</sub>), 29.2 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 48.3 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 55.9 (C-2'), 66.2, 66.6, 68.8, 68.9, 69.7, 73.4, 73.6, 74.0, 74.8, 76.0, 78.6, 79.5 and 81.7 (C-2,3,4,5,6, C-3',4',5',6', OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub> and 3OCH<sub>2</sub>Ph), 100.0, 101.8 and 104.0 (C-1, C-1' and CHPh), 126.4–138.9 (aromatic C), 170.2 (OCOCH<sub>3</sub>). <sup>1</sup>H, δ 1.61–1.72 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 1.88 (s, 3H, OCOCH<sub>3</sub>), 3.05–3.15 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.39 (m, 1H), 3.50–3.59 (m, 4H), 3.63–3.86 (m, 4H), 3.92 (d, 1H, J=2.9 Hz), 4.16 (d, 1H, J=11.8 Hz), 4.23 (d, 1H, J=7.6 Hz), 4.37 (dd, 1H, J=10.2, 8.3 Hz), 4.38–4.48 (m, 4H), 4.60 (d, 1H, J=11.6 Hz, OCH<sub>2</sub>Ph), 4.91 (d, 1H, J=11.6 Hz, OCH<sub>2</sub>Ph), 5.55 (s, 1H, CHPh), 5.77 (d, 1H, J<sub>1,2</sub>=8.3 Hz, H-1'), 5.93 (dd, 1H, J=10.2, 9.1 Hz, H-3'), 7.30–7.41 and 7.45–7.52 (m, 24H, aromatic H), 7.56 (s<sub>b</sub>, 1H), 7.69 (s<sub>b</sub>, 1H); HR-ESI calcd for C<sub>53</sub>H<sub>54</sub>N<sub>4</sub>O<sub>13</sub>Na [M+Na]<sup>+</sup> 977.3580. Found 977.3513.

**3.2.3. 3-Azidopropyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside**

(**8**). Disaccharide **7** (550 mg, 576 μmol) in EtOH/ethylenediamine (10:1, 18 mL) was stirred at reflux temperature for 2 h and then treated with NaOCH<sub>3</sub>/MeOH (1 M, 1 mL) for an additional 2 h. The reaction mixture was concentrated, then the residue was dissolved in MeOH (20 mL), cooled to 0 °C and acetic anhydride (2 mL) was added. After 1 h, the solution was concentrated and purified by silica gel flash chromatography (1:1 toluene/EtOAc) to give **8** (394 mg, 83%); [α]<sub>D</sub><sup>22</sup>–32 (c 1, CHCl<sub>3</sub>); NMR (CDCl<sub>3</sub>): <sup>13</sup>C, δ 22.9 (NHCOCH<sub>3</sub>), 29.3 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 48.4 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 59.4 (C-2'), 66.6 (2C), 68.6, 68.8, 72.8, 73.7 (2C), 74.1, 74.7, 75.7, 79.3, 81.6 and 81.7 (C-2,3,4,5,6, C-3',4',5',6', OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub> and 3OCH<sub>2</sub>Ph), 102.1, 102.8 and 104.0 (C-1, C-1' and CHPh), 126.5–138.7 (aromatic C), 172.7 (NHCOCH<sub>3</sub>); <sup>1</sup>H, δ 1.54 (s, 3H, NCOCH<sub>3</sub>), 1.80–1.93 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.35 (t, 2H, J=6.7 Hz, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.43–3.50 (m, 1H), 3.52 (dd, 1H, J=8.6, 6.2 Hz), 3.56–3.65 (m, 5H), 3.74 (dd, 1H, J=9.9, 3.0 Hz), 3.77–3.86 (m, 3H), 3.93 (d, 1H, J=2.8 Hz), 3.95–4.01 (m, 1H), 4.37 (d, 1H, J<sub>1,2</sub>=7.5 Hz, H-1), 4.38 (dd, 1H, J=10.3, 5.0 Hz), 4.41 (d, 1H, J=11.8 Hz, OCH<sub>2</sub>Ph), 4.45 (d, 1H, J=11.8 Hz, OCH<sub>2</sub>Ph), 4.61 (d, 1H, J=11.7 Hz, OCH<sub>2</sub>Ph), 4.65 (d, 1H, J=12.6 Hz, OCH<sub>2</sub>Ph), 4.76 (d, 1H, J<sub>1,2</sub>=8.3 Hz, H-1'), 4.85 (d, 1H, J=11.7 Hz, OCH<sub>2</sub>Ph), 5.02 (s<sub>b</sub>, 1H), 5.12 (d, 1H, J=12.6 Hz, OCH<sub>2</sub>Ph), 5.53 (d, 1H, J=5.0 Hz), 5.59 (s, 1H, CHPh), 7.28–7.43 (m, 2H, aromatic H), 7.50–7.54 (m, 18H, aromatic H); HR-ESI calcd for C<sub>45</sub>H<sub>52</sub>N<sub>4</sub>O<sub>11</sub>Na [M+Na]<sup>+</sup> 847.3525. Found 847.3300.

**3.2.4. 3-Azidopropyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside (**10**).** A solution of **8** (272 mg, 330 μmol) and ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-β-*D*-galactopyranoside **9** (247 mg, 460 μmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL) was stirred with powdered 4 Å molecular sieves under argon for 1 h. After cooling it to 0 °C, AgOTf (8 mg, 33 μmol) and NIS (148 mg, 660 μmol) were added and the mixture was stirred for an additional 15 min. The reaction was quenched with Et<sub>3</sub>N (250 μL) and concentrated. The residue was purified by column chromatography (toluene→6:1 toluene/EtOAc) to yield **10** (362 mg, 84%); [α]<sub>D</sub><sup>22</sup>–7 (c 1, CHCl<sub>3</sub>); NMR (CDCl<sub>3</sub>): <sup>13</sup>C, δ 21.1 (OCOCH<sub>3</sub>), 23.3 (NHCOCH<sub>3</sub>), 29.3 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 48.4 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 58.7 (C-2'), 65.9, 66.6, 68.5, 68.9, 69.0, 71.9, 72.2, 72.3, 73.1, 73.4, 73.5, 73.6, 74.5, 74.6, 74.8, 75.8, 76.3, 79.2, 80.5, 81.1 and 81.9 (C-2,3,4,5,6, C-3',4',5',6', C-2'',3'',4'',5'',6'', OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub> and 6OCH<sub>2</sub>Ph), 100.7 (J<sub>C1,H1</sub>=162.1 Hz), 101.3 (J<sub>C1,H1</sub>=162.5 Hz) and 104.0 (J<sub>C1,H1</sub>=158.0 Hz) (C-1, C-1' and C-1''), 101.0 (CHPh), 126.3–139.1 (aromatic C), 169.6 and 170.6 (OCOCH<sub>3</sub> and NHCOCH<sub>3</sub>); <sup>1</sup>H, δ 1.58 (s, 3H, NCOCH<sub>3</sub>), 1.78–1.90 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 1.96 (s, 3H, OCOCH<sub>3</sub>), 3.14–3.23 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.30–3.80 (m, 15H), 3.90–4.00 (m, 4H), 4.27 (d, 1H, J=5.0 Hz), 4.32 (d, 1H, J<sub>1,2</sub>=7.3 Hz, H-1), 4.38–4.71 (m, 10H), 4.87 (d, 1H, J=12.1 Hz, OCH<sub>2</sub>Ph) 4.89 (d, 1H, J=12.0 Hz, OCH<sub>2</sub>Ph), 4.90 (d, 1H, J=11.8 Hz, OCH<sub>2</sub>Ph), 5.25 (dd, 1H, J=10.0, 8.0 Hz), 5.31 (d, 1H, J<sub>1,2</sub>=8.3 Hz, H-1), 5.59 (s, 1H, CHPh), 7.24–7.38 (m, 40H, aromatic H); HR-ESI calcd for C<sub>74</sub>H<sub>82</sub>N<sub>4</sub>O<sub>17</sub>Na [M+Na]<sup>+</sup> 1321.5567. Found 1321.5567.

**3.2.5. 3-Azidopropyl (3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-(1→3)-(2-acetamido-6-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside (**11**).** Compound **10** (50 mg, 38 μmol) in dry THF (1 mL), NaBH<sub>3</sub>CN (17 mg, 269 μmol) and crushed molecular sieves (3 Å) were added and stirred at room temperature. After 2 h, HCl/Et<sub>2</sub>O was added dropwise until the evolution of gas ceased. The reaction was stirred at room temperature and followed by TLC (toluene/ethyl acetate 3:2). At completion, the reaction was quenched with Et<sub>3</sub>N (100 μL), evaporated and purified on a silica gel column (9:1→3:1 toluene/EtOAc) to give 3-azidopropyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-(1→3)-(2-acetamido-6-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-*D*-galactopyranoside [α]<sub>D</sub><sup>22</sup>+4 (c 1,

CH<sub>2</sub>Cl<sub>2</sub>); NMR (CDCl<sub>3</sub>): <sup>13</sup>C, δ 21.3 (OCOCH<sub>3</sub>), 23.5 (NHCOCH<sub>3</sub>), 29.4 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 48.4 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 57.5, 66.8, 68.0, 69.5, 70.1, 70.4, 71.7, 72.7, 72.8, 73.8, 73.8, 73.9, 74.0, 74.0, 74.1, 74.9, 75.0, 75.4, 76.3, 79.5, 80.3, 81.7 and 83.6 (C-2,3,4,5,6, C-3<sup>I</sup>,4<sup>I</sup>,5<sup>I</sup>,6<sup>I</sup>, C-2<sup>II</sup>,3<sup>II</sup>,4<sup>II</sup>,5<sup>II</sup>,6<sup>II</sup>, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub> and 7OCH<sub>2</sub>Ph), 101.1, 101.3 and 103.9 (C-1, C-1<sup>I</sup> and C-1<sup>II</sup>), 127.2–139.5 (aromatic C), 170.2 and 171.1 (OCOCH<sub>3</sub> and NHCOCH<sub>3</sub>); HR-ESI calcd for C<sub>74</sub>H<sub>84</sub>N<sub>4</sub>O<sub>17</sub>Na [M+Na]<sup>+</sup> 1323.5724. Found 1323.6362. The obtained compound was then stirred at room temperature in methanol (5 ml) with NaOMe (1 M, 1 mL) for 6 h. After neutralization with Dowex H<sup>+</sup> ion-exchange resin, the reaction mixture was concentrated to give **11** (36 mg, 74%, over two steps); [α]<sub>D</sub><sup>22</sup> +3 (c 1, CH<sub>2</sub>Cl<sub>2</sub>); NMR (CDCl<sub>3</sub>): <sup>13</sup>C, δ 23.0 (NHCOCH<sub>3</sub>), 29.0 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 48.2 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 55.6 (C-2<sup>I</sup>), 66.3, 68.7, 69.1, 69.4, 69.9, 71.1, 72.8, 73.2, 73.3, 73.4, 73.5, 73.9, 74.2, 74.4, 74.5, 75.1, 75.7, 77.2, 79.1, 81.3, 81.7 and 86.8 (C-2,3,4,5,6, C-3<sup>I</sup>,4<sup>I</sup>,5<sup>I</sup>,6<sup>I</sup>, C-2<sup>II</sup>,3<sup>II</sup>,4<sup>II</sup>,5<sup>II</sup>,6<sup>II</sup>, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub> and 7OCH<sub>2</sub>Ph), 101.6, 103.5 and 104.7 (C-1, C-1<sup>I</sup> and C-1<sup>II</sup>), 126.6–139.0 (aromatic C), 171.8 (NHCOCH<sub>3</sub>); <sup>1</sup>H, δ 1.62 (s, 3H, NCOCH<sub>3</sub>), 1.78–1.86 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.22 (sb, 1H), 3.32 (t, 2H, J=6.8 Hz), 3.39–3.45 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.48–3.68 (m, 10H), 3.75–3.79 (m, 4H), 3.87–3.99 (m, 4H), 4.09 (d, 1H, J<sub>1,2</sub>=7.7 Hz, H-1), 4.31–4.33 (m, 1H), 4.37–4.47 (m, 5H), 4.54 (s, 1H), 4.56 (d, 1H, J=11.8 Hz, OCH<sub>2</sub>Ph), 4.60 (d, 1H, J=11.7 Hz, OCH<sub>2</sub>Ph), 4.66 (d, 1H, J=12.3 Hz, OCH<sub>2</sub>Ph), 4.72 (d, 1H, J=12.2 Hz, OCH<sub>2</sub>Ph), 4.79–4.82 (m, 2H), 4.90 (d, 1H, J=11.5 Hz, OCH<sub>2</sub>Ph), 4.91 (d, 1H, J=11.7 Hz, OCH<sub>2</sub>Ph), 4.97 (d, 1H, J=12.1 Hz, OCH<sub>2</sub>Ph), 5.49 (d, 1H, J=8.2 Hz), 7.25–7.39 (m, 35H, aromatic H); HR-ESI calcd for C<sub>72</sub>H<sub>82</sub>N<sub>4</sub>O<sub>16</sub>Na [M+Na]<sup>+</sup> 1281.5618. Found 1281.5574.

**3.2.6. 3-Azidopropyl (2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→3)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→4)]-(2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-O-benzyl-β-D-galactopyranoside (13).** A mixture of **10** (100 mg, 77 μmol), ethyl 2,3,4-tri-O-benzyl-1-thio-α-L-fucopyranoside **12** (111 mg, 231 μmol) and Et<sub>3</sub>NBr (24 mg, 116 μmol) in dry CH<sub>2</sub>Cl<sub>2</sub>/DMF (10:1, 1.5 mL) was stirred with 4 Å molecular sieves for 16 h. Bromine (20 μL, 39 μmol) was then added and the reaction stirred at room temperature. After 48 h, TLC (toluene/ethyl acetate 6:1) and Maldi-Tof showed complete conversion of the starting trisaccharide. The crude mixture was concentrated and purified on a silica gel column (toluene → 6:1 toluene/EtOAc) to give **13** (112 mg, 67%); [α]<sub>D</sub><sup>22</sup> –44 (c 1, CHCl<sub>3</sub>); NMR (CDCl<sub>3</sub>): <sup>1</sup>H, δ 1.10–1.19 (m, 6H, H-6<sup>III</sup> and H-6<sup>IV</sup>) 1.55 (s, 3H, NCOCH<sub>3</sub>), 1.69–1.82 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.15 (s, 1H), 3.25 (d, 2H, J=6.8 Hz), 3.31 (db, 1H J=6.8 Hz), 3.39–4.02 (m, 26H), 4.23 (d, 1H, J=7.3 Hz), 4.26–4.79 (m, 28H), 4.87 (d, 1H, J=11.4 Hz, OCH<sub>2</sub>Ph), 4.89–4.95 (m, 1H), 5.48 (d, 1H, J=3.7 Hz, NH), 6.88–7.33 (m, 65H, aromatic H); <sup>13</sup>C, δ 16.3 and 16.5 (C-6<sup>III</sup> and C-6<sup>IV</sup>) 23.6 (NHCOCH<sub>3</sub>), 29.4 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 48.5 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 53.6 (C-2<sup>I</sup>), 66.6, 66.7, 67.1, 67.5, 68.7, 69.2, 71.3, 71.8, 71.9, 72.8, 73.0, 73.1, 73.3, 73.4, 73.7, 73.8, 74.0, 74.2, 74.6, 74.7, 74.9 (2C), 75.0, 75.2, 75.6, 75.8 (3C), 77.4, 78.1, 78.2, 79.3, 79.5, 80.5, 80.9 and 83.9 (C-2,3,4,5,6, C-3<sup>I</sup>,4<sup>I</sup>,5<sup>I</sup>,6<sup>I</sup>, C-2<sup>II</sup>,3<sup>II</sup>,4<sup>II</sup>,5<sup>II</sup>,6<sup>II</sup>, C-2<sup>III</sup>,3<sup>III</sup>,4<sup>III</sup>,5<sup>III</sup>, C-2<sup>IV</sup>,3<sup>IV</sup>,4<sup>IV</sup>,5<sup>IV</sup>, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub> and 13OCH<sub>2</sub>Ph), 98.1, 98.7, 101.6, 102.0 and 103.9 (C-1, C-1<sup>I</sup>, C-1<sup>II</sup> C-1<sup>III</sup> and C-1<sup>IV</sup>), 126.3–139.7 (aromatic C), 169.4 (NHCOCH<sub>3</sub>); HR-ESI calcd for C<sub>126</sub>H<sub>138</sub>N<sub>4</sub>O<sub>24</sub>Na [M+Na]<sup>+</sup> 2114.9632. Found 2114.9703.

**3.2.7. 3-Aminopropyl (α-L-fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[(α-L-fucopyranosyl)-(1→4)]-(β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (1-HCl).** Pentasaccharide **13** (102 mg, 10.0 μmol) was dissolved in EtOAc/EtOH (95%)/H<sub>2</sub>O (2:2:1, 10 mL), then HCl (1 M, 10 μL) and Pd/C (10%, 100 mg) were added. Hydrogenolysis was carried out under H<sub>2</sub> at atmospheric pressure for 1 day. The suspension was filtered through a filter sandwich (5 μm/10 μm/20 μm pore size), then the filtrate was concentrated and the

residue purified on a Biogel P2 column eluting with H<sub>2</sub>O (1% n-BuOH) to give **1** (36 mg, 82%) after lyophilization; [α]<sub>D</sub><sup>22</sup> –6 (c 0.1, H<sub>2</sub>O); NMR (D<sub>2</sub>O): <sup>1</sup>H, δ 1.25–1.28 (m, 6H, H-6<sup>III</sup> and H-6<sup>IV</sup>), 1.99–2.04 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 2.06 (s, 3H, NHCOCH<sub>3</sub>), 3.14–3.19 (m, 2H, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 3.51–3.63, 3.68–3.95, (m, 22H), 4.02–4.06 (m, 2H), 4.12–4.16 (m, 2H), 4.34 (dd, 1H, J=13.5, 6.7 Hz), 4.38 (d, 1H, J<sub>1,2</sub>=8.0 Hz), 4.61 (d, 1H, J<sub>1,2</sub>=8.5 Hz), and 4.66 (d, 1H, J<sub>1,2</sub>=7.7 Hz) (H-1, H-1<sup>I</sup> and H-1<sup>II</sup>), 4.88 (t, 1H, J=6.4 Hz), 5.03 (d, 1H, J<sub>1,2</sub>=3.8 Hz), and 5.16 (d, 1H, J<sub>1,2</sub>=4.0 Hz) (H-1<sup>III</sup> and H-1<sup>IV</sup>); <sup>13</sup>C, δ 15.4 and 15.4 (C-6<sup>III</sup> and C-6<sup>IV</sup>), 22.3 (NHCOCH<sub>3</sub>), 26.8 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 37.7 (OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 55.8 (C-2<sup>I</sup>), 59.5, 61.0, 61.6, 66.3, 67.1, 67.8, 67.9, 68.3, 68.6, 68.8, 69.1, 69.5, 70.0, 71.8, 72.0, 72.1, 73.7, 74.5, 74.7, 75.2, 76.5, and 81.8 (C-2,3,4,5,6, C-3<sup>I</sup>,4<sup>I</sup>,5<sup>I</sup>,6<sup>I</sup>, C-2<sup>II</sup>,3<sup>II</sup>,4<sup>II</sup>,5<sup>II</sup>,6<sup>II</sup>, C-2<sup>III</sup>,3<sup>III</sup>,4<sup>III</sup>,5<sup>III</sup>, C-2<sup>IV</sup>,3<sup>IV</sup>,4<sup>IV</sup>,5<sup>IV</sup>, OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 97.8, 99.6, 100.7, 103.1 and 103.2 (C-1, C-1<sup>I</sup>, C-1<sup>II</sup> C-1<sup>III</sup> and C-1<sup>IV</sup>), 174.3 (NHCOCH<sub>3</sub>); HR-ESI calcd for C<sub>35</sub>H<sub>63</sub>N<sub>2</sub>O<sub>24</sub>Na [M+H]<sup>+</sup> 895.3765. Found 895.3750.

**3.2.8. 3-Aminopropyl (α-L-fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[(α-L-fucopyranosyl)-(1→4)]-(β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside conjugates.** **3.2.8.1. General procedure for conjugation with DSS.** Compound **1** (2 mg, 2.2 μmol) and DSS (6.5 mg, 17.6 μmol) were dissolved in dry DMSO (100 μL) and Et<sub>3</sub>N (1 μL) was added. The mixture was gently swirled and monitored by MALDI-TOF spectrometry. When all **1** was converted into the active-ester (1 h), the reaction mixture was transferred onto a C18 column (2 g) that had been pre-washed with CH<sub>2</sub>Cl<sub>2</sub> (8 mL). DMSO was washed off with CH<sub>2</sub>Cl<sub>2</sub> (8 mL) and then the CH<sub>2</sub>Cl<sub>2</sub> was removed from the column with a stream of nitrogen. The active ester was released from the column with double-distilled cold water and product-containing fractions were pooled and freeze-dried to produce the ester (2.1 mg, 82%).

**3.2.9. General procedure for coupling to HSA.** HSA (4.1 mg, 0.06 μmol, Sigma) was treated with a borate buffer (pH 10, 200 μL) and added to a solution of the DSS/active-ester conjugate (2.1 mg, 1.8 μmol) in double-distilled water (1 mL). The mixture was stirred for 24 h at room temperature and then transferred to a centrifugal tube (30 kD, omega membrane, Microsep™, Pall) and centrifuged (3×1.5 h) after addition of double-distilled water (2×1 mL). The filter residue was dissolved in water (3×400 μL) and freeze-dried to yield the conjugate (4.0 mg, 85% calcd with respect to the protein). MALDI-TOF: 78,570, corresponds to 10 incorporated receptor saccharides.

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## Supplementary data

Supplementary data associated with this article can be found in online version, at doi:10.1016/j.tet.2010.07.036. These data include MOL files and InChIKeys of the most important compounds described in this article.

## References and notes

- Aspholm-Hurtig, M.; Dailide, G.; Lahmann, M.; Kalia, A.; Ilver, D.; Roche, N.; Vikstroem, S.; Sjoestroem, R.; Linden, S.; Baeckstroem, A.; Lundberg, C.; Arngqvist, A.; Mahdavi, J.; Nilsson, U. J.; Velapattino, B.; Gilman, R. H.; Gerhard, M.; Alarcon, T.; Lopez-Brea, M.; Nakazawa, T.; Fox, J. G.; Correa, P.; Dominguez-

- Bello, M. G.; Perez-Perez, G. I.; Blaser, M. J.; Normark, S.; Carlstedt, I.; Oscarson, S.; Teneberg, S.; Berg, D. E.; Boren, T. *Science* **2004**, *305*, 519–522.
2. Blaser, M. J. *Lancet* **1997**, *349*, 1020–1022.
3. Logan, R. H. *Lancet* **1994**, *344*, 1078–1079.
4. Dunn, B.; Cohen, H.; Blaser, M. *Clin. Microbiol. Rev.* **1997**, *10*, 720–741.
5. Ilver, D.; Arnqvist, A.; Ogren, J.; Frick, I.-M.; Kersulyte, D.; Incecik, E. T.; Berg, D. E.; Covacci, A.; Engstrand, L.; Boren, T. *Science* **1998**, *279*, 373–377.
6. Kojima, N.; Seino, K.; Sato, Y.; Mizuochi, T. *FEBS Lett.* **2002**, *517*, 32–36.
7. Rana, S. S.; Barlow, J. J.; Matta, K. L. *Carbohydr. Res.* **1981**, *96*, 231–239.
8. Spohr, U.; Lemieux, R. U. *Carbohydr. Res.* **1988**, *174*, 211–237.
9. Yan, L.; Kahne, D. J. *Am. Chem. Soc.* **1996**, *118*, 9239–9248.
10. Randolph, J. T.; McClure, K. F.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 5712–5719.
11. Eklind, K.; Gustafsson, R.; Tiden, A. K.; Norberg, T.; Aberg, P. M. J. *Carbohydr. Chem.* **1996**, *15*, 1161–1178.
12. Yi, W.; Shao, J.; Zhu, L.; Li, M.; Singh, M.; Lu, Y.; Lin, S.; Li, H.; Ryu, K.; Shen, J.; Guo, H.; Yao, Q.; Bush, C. A.; Wang, P. G. J. *Am. Chem. Soc.* **2005**, *127*, 2040–2041.
13. Randolph, J. T.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1470–1473.
14. Danishefsky, S. J.; Behar, V.; Randolph, J. T.; Lloyd, K. O. *J. Am. Chem. Soc.* **1995**, *117*, 5701–5711.
15. Chernyak, A.; Oscarson, S.; Turek, D. *Carbohydr. Res.* **2000**, *329*, 309–316.
16. Lahmann, M.; Buelow, L.; Teodorovic, P.; Gybaeck, H.; Oscarson, S. *Glycoconjugate J.* **2004**, *21*, 251–256.
17. Joosten, J. A. F.; Loimaranta, V.; Appeldoorn, C. C. M.; Haataja, S.; ElMaate, F. A.; Liskamp, R. M. J.; Finne, J.; Pieters, R. J. J. *Med. Chem.* **2004**, *47*, 6499–6508.
18. Dahmén, J.; Gnosspelius, G.; Larsson, A.-C.; Lave, T.; Noori, G.; Pålsson, K.; Frejd, T.; Magnusson, G. *Carbohydr. Res.* **1985**, *138*, 17–28.
19. Halkes, K. M.; Lefeber, D. J.; Fransen, C. T. M.; Kamerling, J. P.; Vliegthart, J. F. G. *Carbohydr. Res.* **1998**, *308*, 329–338.
20. Garegg, P. J.; Hultberg, H. *Carbohydr. Res.* **1981**, *93*, C10–C11.
21. An analytical sample of **4** was benzoylated to prove the position of the hydroxyl group by its significant downfield shift. **4OBz** ( $^1\text{H NMR}$ ,  $\text{CDCl}_3$ ,  $\delta$  5.05 ppm (dd,  $J_{2,3}=10.2$  Hz,  $J_{3,4}=3.1$  Hz, H-3)).
22. Kihlberg, J. O.; Leigh, D. A.; Bundle, D. R. *J. Org. Chem.* **1990**, *55*, 2860–2863.