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## Synthesis of tri-, penta-, and heptasaccharides, functionalized with orthogonally *N*-protected amino residues at the reducing and non-reducing ends

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#### A R T I C L E I N F O

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#### ABSTRACT

The synthesis of four bifunctionalized orthogonally *N*-protected oligosaccharides derived from lactose and mannose, intended as cross-linking derivatives, is described. The aminosugar at the non-reducing end is derivatized with an *N*-Boc-protected glycine moiety, and further connected to either a mannose  $(1 \rightarrow 6)$  disaccharide or  $(1 \rightarrow 3)$  lactose units (one, two or three) resulting in tri-, penta-, or heptasaccharides. All of the synthesized oligosaccharides have an *N*-benzyloxycarbonyl-aminoethyl residue at the reducing end. The orthogonal *N*-Boc/*N*-Cbz protection group pattern enables further conjugation/ derivatization and results in a hydrophilic cross-linking molecule. It was found that the order of the final synthetic steps was crucial to avoid acyl migration. A suitable amide coupling protocol has been applied to introduce the *N*-Boc-protected glycine moiety in alcoholic solvent. The synthesized oligosaccharides will provide a model system to investigate the influence of length, structure and flexibility.

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## 1. Introduction

To study biological processes, appropriate cross-linking molecules are often required to circumvent the synthesis of complex oligosaccharides. A challenge within the field of glycobiology and biotechnology is to achieve controlled immobilization of proteins onto surfaces, while retaining their biological features and properties.<sup>1,2</sup> The design of bifunctional spacers comprises several aspects that have to be taken into consideration; (i) the extent of the synthesis, (ii) homo-/heterobifunctionality (identical or different reactive groups) (iii) length (iv) water-solubility and (v) conformational flexibility.<sup>3–8</sup> One of the most common classes of compounds used for bioconjugation in various biophysical or biological systems is the poly- and oligoethylene glycol (PEG/OEG) chains.<sup>9</sup> Both are known for their protein-resistant properties<sup>10,11</sup> and occur in numerous research fields.<sup>12–15</sup> PEGs and OEGs have also been used as spacers to form glycoconjugates.<sup>16,17</sup> An investigation on how the spacer length and flexibility influence the liposome-phagocyte interaction<sup>18</sup> confirmed the importance of tuning the length of the spacer depending on the biological system.<sup>19</sup> To resemble biomolecules and compensate for weak individual protein-carbohydrate interactions, glycodendrimers are often used, facilitating the effect of multivalency.<sup>20</sup> Dendrimers are constructed using a poly(amido)-,<sup>21,22</sup> ethylene(glycol)<sup>23</sup> or polyether<sup>24</sup> backbone. Saccharides as building blocks in dendrimers are suitable for controlling characteristics, such as flexibility, surface properties and to impart biocompatibility.<sup>25</sup> Several carbohydrate-containing surfaces have also shown protein-resistant properties, <sup>26–28</sup> i.e., an eligible feature for serving as general cross-linking molecules. Oligosaccharide-based spacer molecules, comprised of (oligo-)lactoses<sup>19</sup> appears to be comparable with those containing OEGs, thus demonstrating possible cross-linking features of oligosaccharides in biophysical- or biosensing applications. Herein, we present the synthesis of four oligosaccharides to be used as bifunctionalized spacer molecules (1–4) (Fig. 1) derived from lactose and mannose, containing an *N*-Boc-glycine functionalized aminosugar. The orthogonal N-Boc/N-Cbz protection group pattern allows for further conjugation and derivatization depending on the specific target, thus resulting in a potential cross-linking molecule. The results from the oligosaccharides synthesized by Schmidt and co-workers,<sup>29</sup> suggest that oligo(lactoses) adopts linear rod-like conformations. Using the selected aminosugar from the corresponding gluco series, glycosylated in a  $(1 \rightarrow 3)$  manner (1-3), we hypothesize that the same conformation will be retained, giving fairly rigid rod-like target molecules. Using this analogy, we also synthesized a more flexible mannose  $(1 \rightarrow 6)$  based trisaccharide (4) with the identical terminal aminosugar moiety, allowing for investigations and tuning of the cross-linking property, i.e., length, structure and flexibility.





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Fig. 1. The four synthesized orthogonally N-protected oligosaccharides.

#### 2. Results and discussion

The azido sugar **6** was synthesized starting from the known thioglycoside  $\mathbf{5}^{30}$  using triflic anhydride followed by NaN<sub>3</sub> displacement, giving the azido functionalized derivative **6** as a crystalline compound in 85% yield (Scheme 1).



Scheme 1. Reagents and conditions: (i) Tf<sub>2</sub>O, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, 20 °C; (ii) NaN<sub>3</sub>, DMF, 70 °C.

The synthesis of the lactosides **1–3** (*n*=0, 1, 2) (Scheme 2) started from the commercially available lactose monohydrate. In a straightforward six-step manner, i.e., acetylation, formation of the bromosugar, phase-transfer substitution at the anomeric center, deacetylation, selective introduction of an isopropylidene group at the 3',4'-position and subsequent protection of the remaining alcohols using BzCl, afforded the crystalline building block **7**<sup>31</sup> in 43% yield over six steps. The phase-transfer conditions for introducing the phenyl thioglycosides<sup>32</sup> and the use of TMSCl/2,2-dimethoxypropane in acetone to selectively install the 3',4'-isopropylidene acetal provides a scalable synthetic route to donor **7** without to resort to purification by chromatography.

Utilizing NIS/AgOTf promoted glycosylation of **7** with *N*-Cbz-2aminoethanol afforded the crystalline glycoside **8** (n=0) in 87% yield. Deprotection of the isopropylidene acetal of **8** followed by a regioselective glycosylation with azido sugar **6** and final benzoylation of the 4'-hydroxyl group gave the ( $\beta$  1 $\rightarrow$ 3) azido trisaccharide **9** (n=1) in 81% yield. Although the regioselectivity of the 3-position on galacto-configured epitopes is known,<sup>29,31</sup> we confirmed this by NMR spectroscopic analysis after benzoylation at the remaining 4'-position. The selectivity depends on the glycosyl donor and promotor used, which was earlier found by the group of Oscarson.<sup>33</sup> This extra benzoylation was in agreement with the protection group pattern and also used for the synthesis of compounds **10–13**. The target molecule **1** (n=1) was obtained by deprotection of the benzoates using NaOMe, thereby circumventing intramolecular migration of acyls, followed by subsequent reduction of the azido functionality to the corresponding amine using NaBH<sub>4</sub> and NiCl<sub>2</sub>  $\cdot$  6H<sub>2</sub>O<sup>34,35</sup> in MeOH. It was found that the order of addition strongly affects the outcome of the reaction. The highest yields were obtained when the NiCl<sub>2</sub>·6H<sub>2</sub>O was stirred with the azido sugar for 5 min followed by addition of the NaBH<sub>4</sub> and subsequently quenching the reaction using Dowex<sup>®</sup>-H<sup>+</sup>. The amine was then coupled with N-Boc-glycine using a combination of methods<sup>36,37</sup> with EDC·HCl in MeOH. Initially, the azido group was reduced prior to the deprotection using either PPh3 or NiCl2 · 6H2O, however, the reduction exclusively resulted in acyl migration, which is consistent with the finding made by Lin et al.<sup>38</sup> Tetrasaccharide **10** (n=1) was obtained by deprotection of the isopropylidene acetal of compound 8 followed by an NIS/AgOTf-promoted glycosylation with donor 7 and subsequent benzovlation to afford compound **10** in an overall yield of 69%. Applying the protocol described for 9. tetrasaccharide 10 was used to afford the protected azido pentasaccharide **11** (n=2) in 66% yield. Finally, target compound **2** (n=2) was obtained in 64%, using the previously described synthetic protocol for 1. Product 3 was synthesized in 29% yield from 10, via 12 and 13 (69% and 51% yields, respectively) using the methods described above. It should be noted that H<sub>2</sub>O had to be added in the glycine derivatization due to poor solubility of the deprotected amino heptasaccharide in MeOH. The H<sub>2</sub>O, in combination with the increased steric hindrance of the amine, is probably the reason why a lower yield was obtained for compound **3** (*n*=3) (cf. **1** (65%), **2** (64%), and **3** (29%)) (Scheme 2).

The mannoside **15**<sup>39,40</sup> was obtained in 45% yield by well-known procedures (i.e., tritylation, benzoylation, deprotection) starting from the tetraol **14** (Scheme 3).<sup>41</sup> However, to the best of our knowledge the characterization of compound **15** has not been described previously. Chloroacetylation of the 6-hydroxyl mannoside **15** at -40 °C gave compound **16** in 82% yield. Conversion to the corresponding bromosugar followed Koenigs–Knorr glycosylation using AgOTf as promotor at -30 °C gave disaccharide **17** in 87% yield. A NIS/ TfOH-promoted glycosylation at -30 °C with *N*-Cbz-2-aminoethanol afforded **18** in 82% yield. Treatment with thiourea/2,6-lutidine removed the chloroacetyl group to afford the corresponding 6-hydroxyl acceptor, which was subsequently glycosylated with azido donor **6** using NIS/AgOTf to give compound **19** in 85% yield. The conditions for the final three-step conversion were in accordance with the protocol



Scheme 2. Reagents and conditions: (i) Ac<sub>2</sub>O, HOAc, HClO<sub>4</sub>, 0 °C; (ii) HBr/HOAc, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C; (iii) PhSH, Bu<sub>4</sub>NHSO<sub>4</sub>, 1 M Na<sub>2</sub>CO<sub>3</sub> (aq), CH<sub>2</sub>Cl<sub>2</sub>; (iv) NaOMe, MeOH; (v) Acetone, DMP, TMSCl; (vi) BzCl, DMAP, pyridine; (vii) *N*-Cbz-2-aminoethanol, NIS, AgOTf, 4 Å MS, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C  $\rightarrow$  rt; (viii) TFA (90%), CH<sub>2</sub>Cl<sub>2</sub>; (ix) **7**, NIS, AgOTf, 4 Å MS, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C  $\rightarrow$  rt; (x) BzCl, NaOMe, MeOH; (v) Acetone, DMP, pyridine; (xi) NIS, AgOTf, 4 Å MS, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C  $\rightarrow$  rt; (x) BzCl, NaOMe, MeOH; (x) *N*-(*tert*-butyloxycarbonyl)-glycine, HOAt, *N*-methylmorpholine, EDC·HCl, MeOH.

previously described for the synthesis of **1**, **2**, and **3** resulting in the target trisaccharide **4** in 62% yield.

provide a relevant model system that enables investigations of crosslinker influence on biological studies.

## 3. Conclusions

# The function of biologically conjugated motifs for, e.g., biosensing strongly depends on the cross linker. We have demonstrated the synthesis of oligosaccharides, with variable lengths and flexibility, as possible bifunctional cross-linking structures (1–4). Common problems associated with the synthesis of the traditionally used PEGs/ OEGs are purification and low crystallinity. Efficient synthetic protocols have been developed to produce the crystalline building blocks **6**, **7**, and **8**. The synthetic pathway developed is straightforward, using known transformations and crystalline intermediates and may therefore be applied on larger scale providing alternative compounds to the commonly used OEGs/PEGs. Furthermore, these oligosaccharides have the potential to serve as well-defined, biocompatible spacers for biophysical model systems. Finally these oligosaccharides

#### 4. Experimental

#### 4.1. General procedure

CH<sub>2</sub>Cl<sub>2</sub> and toluene were distilled over calcium hydride and collected onto pre-activated 4 Å MS. Thin layer chromatography (TLC) was carried out on Merck 60 F<sub>254</sub> plates and visualized by UV light and/or developed with PAA [EtOH (95%, 740 mL), H<sub>2</sub>SO<sub>4</sub> (concn, 28 mL), AcOH (100%, 8.4 mL), *p*-anisaldehyde (20 mL)]. Flash column chromatography (FC) was carried out on silica gel Merck 60 (40–63 µm). Reverse phase chromatography (RP) was carried out on Merck LiChroprep<sup>®</sup> (RP-18). Proton nuclear magnetic resonance (<sup>1</sup>H NMR) were recorded on Varian 300 MHz and 600 MHz spectrometers, carbon nuclear magnetic resonance (<sup>13</sup>C NMR) was recorded on Varian 300 (75.4 MHz) spectrometer; multiplicities are quoted as



Scheme 3. Reagents and conditions: (i) TrCl, pyridine; (ii) BzCl, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C; (iii) *p*-TsOH, CHCl<sub>3</sub>/MeOH; (iv) Chloroacetyl chloride, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, 40 °C; (v) Br<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (vi) AgOTf, 4 Å MS, CH<sub>2</sub>Cl<sub>2</sub>, 30 °C; (vii) *N*-Cbz-2-aminoethanol, NIS, 4 Å MS, TfOH, CH<sub>2</sub>Cl<sub>2</sub>, 30 °C; (viii) 2,6-lutidine, thiourea, CH<sub>2</sub>Cl<sub>2</sub>, MeOH; (ix) **6**, NIS, AgOTf, 4 Å MS, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C → rt; (x) NaOMe, MeOH, CH<sub>2</sub>Cl<sub>2</sub>; (xi) NiCl<sub>2</sub>·6H<sub>2</sub>O, NaBH<sub>4</sub>, MeOH; (xii) *N*-(*tert*-butyloxycarbonyl)-glycine, HOAt, *N*-methylmorpholine, EDC·HCl, MeOH.

apparent doublet (ad), apparent singlet (as), broad singlet (bs), singlet (s), doublet (d), doublet of doublets (dd), double doublet of doublets (ddd), triplet (t) and multiplet (m). If overlaps in carbon spectra occur (e.g., anomeric- or carbamate carbons) this is noted. In situations where a doublet of doublets (dd) appears as a triplet (t) due to resolution, this is denoted as a (dd) with identical couplings constants. TMS or the resonances of the deuterated solvent was used as internal standard; CDCl<sub>3</sub> (1H NMR,  $\delta$ =7.26; <sup>13</sup>C NMR,  $\delta$ =77.2); CD<sub>3</sub>OD (<sup>1</sup>H NMR,  $\delta$ =3.31; <sup>13</sup>C NMR,  $\delta$ =49.0); with D<sub>2</sub>O, CH<sub>3</sub>OH (<sup>1</sup>H NMR,  $\delta$ =3.34; <sup>13</sup>C NMR,  $\delta$ =49.5) was used as reference.<sup>42</sup> Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry was recorded on a Voyager-DE STR Biochemistry Workstation, in a positive mode, using  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) or 2,4,6-trihydroxyacetophenone (THAP) as matrices. ESI-MS (recorded at Medivir AB, Huddinge, Sweden) was performed on a Water Synapt HDMS instrument equipped with electrospray interface. Optical rotation measurements were recorded at 20 °C with a Perkin-Elmer 141 polarimeter. FTIR was recorded on a Perkin-Elmer Spectrum 1000 using KBr pellets. Melting points were recorded on a Stuart<sup>®</sup> melting point apparatus.

## 4.2. Ethyl 4-deoxy-4-azido-2,3,6-tri-O-benzoyl- $\beta$ -D-thio-glucopyranoside (6)

To a solution of ethyl 2,3,6-tri-O-benzoyl-β-D-thio-galactopyranoside<sup>30</sup> (**5**) (5.00 g, 9.32 mmol) and dry pyridine (2.25 mL, 28.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) under argon was added Tf<sub>2</sub>O (2.35 mL, 14.0 mmol) at -20 °C. After 30 min the solution was diluted with  $CH_2Cl_2$  (50 mL), washed with  $H_2O$  (2×50 mL), dried over MgSO<sub>4</sub> (s) and evaporated. The crude triflate  $R_{\rm f}$ =0.72 (toluene/EtOAc 4:1) was dissolved in DMF (50 mL) whereupon NaN<sub>3</sub> (2.42 g, 37.3 mmol) was added and the solution was heated to 70 °C. After 2 h the solution was diluted with toluene (100 mL), washed with brine (2×100 mL),  $H_2O$  (2×100 mL), dried over MgSO<sub>4</sub> (s) and evaporated. Crystallization from EtOH (99.5%) gave the azide 6 (4.44 g, 7.91 mmol, 85%) as white needles. *R*<sub>f</sub>=0.78 (toluene/EtOAc 4:1); mp 129–130 °C (EtOH);  $[\alpha]_{D}$ +108 (c 1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>):  $\delta$  14.9, 24.4, 61.0, 63.6, 70.5, 74.9, 76.6, 83.9, 128.2-128.6 (several carbons), 128.9, 129.2, 129.8-130.0 (several carbons), 133.4, 133.6, 165.4, 165.7, 166.2 (note: overlaps occur in aromatic region); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  1.26 (t, 3H, *J*=7.4 Hz), 2.63–2.81 (m, 2H), 3.80 (ddd, 1H, *J*=2.3, 4.7, 10.2 Hz), 3.91 (dd, 1H, *J*=9.6, 10.2 Hz), 4.60 (dd, 1H, *J*=4.7, 12.2 Hz), 4.76 (dd, 1H, *J*=2.3, 12.2 Hz), 4.77 (d, 1H, *J*=9.8 Hz), 5.44 (dd, 1H, *J*=9.6, 9.8 Hz), 5.70 (dd, 1H, *J*=9.6, 9.6 Hz), 7.35–7.40 (m, 4H), 7.46–7.54 (m, 4H), 7.58–7.64 (m, 1H), 7.92–7.97 (m, 4H), 8.08–8.11 (m, 2H); HRMS (ESI): [M+NH<sub>4</sub>]<sup>+</sup> calcd for C<sub>29</sub>H<sub>31</sub>N<sub>4</sub>O<sub>7</sub>S, 579.1835; found 579.1833.

## 4.3. Phenyl 2,6-di-O-benzoyl-3,4-di-O-isopropylidene- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside (7)^{31}

To a stirred solution of lactose monohydrate (75.0 g, 208 mmol), Ac<sub>2</sub>O (190 mL, 2.01 mol), HOAc (375 mL), was added two drops of aq  $HClO_4$  (60%, aq) with 15 min interval at 0 °C (exothermic!). After 1 h the mixture containing the *per*-acetylated lactose ( $R_f=0.52$ , toluene) EtOAc 1:1) was diluted with  $CH_2Cl_2(200 \text{ mL})$  whereupon HBr in HOAc (250 mL, 33 wt %) was added (over 30 min) at 0 °C and stirred for an additional 3 h. The solution was diluted with CH<sub>2</sub>Cl<sub>2</sub> (200 mL) and washed with ice/H<sub>2</sub>O (2×300 mL), NaHCO<sub>3</sub> (satd aq) (2×300 mL), dried over MgSO<sub>4</sub> (s), filtered and concentrated. The crude bromosugar ( $R_f=0.58$ , toluene/EtOAc 1:1) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (250 mL) whereupon PhSH (50.9 mL, 496 mmol),  $Bu_4NHSO_4$  (56.1 g, 165 mmol), and 1 M Na<sub>2</sub>CO<sub>3</sub> (aq) (250 mL) were added. The mixture was stirred vigorously overnight. The mixture was separated and the organic phase was washed with 1 M NaOH (aq)  $(2 \times 100 \text{ mL})$ , H<sub>2</sub>O  $(2 \times 200 \text{ mL})$ , dried over MgSO<sub>4</sub> (s), filtered and concentrated. To a mixture of the crude thioglycoside ( $R_f=0.56$ , toluene/EtOAc 1:1) in MeOH (200 mL) was added NaOMe (8.92 g, 165 mmol) and stirred overnight, whereupon the solution was neutralized with DOWEX-H<sup>+</sup> and filtered. The solution was washed with *n*-heptane (200 mL) followed by evaporation and co-concentration with toluene. To a mixture of the deprotected thioglycoside ( $R_{\rm f}$ =0.24, chloroform/MeOH 7:2) in acetone (400 mL) and 2,2-dimethoxypropane (150 mL) was added TMSCl (150 mL, 1.18 mol) and stirred for 2 h. To the reaction mixture, EtOAc/ *n*-heptane (500 mL, 1:1) was added and the precipitate was filtered and washed with additional *n*-heptane (200 mL). To a solution of pyridine (500 mL), DMAP (1.00 g, 8.19 mmol) and BzCl (112 mL, 1.24 mol) was added (over 30 min) the crude isopropylideneprotected derivative (R<sub>f</sub>=0.60, chloroform/MeOH 7:2) at 0 °C and stirred overnight at rt. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (300 mL) and washed with 1 M HCl (aq) ( $6 \times 1$  L), NaHCO<sub>3</sub> (satd aq)  $(2 \times 2 L)$ , H<sub>2</sub>O  $(2 \times 2 L)$ , dried over MgSO<sub>4</sub>(s), filtered, and concentrated. Crystallization followed by re-crystallization from EtOH (4 L, 99.5%, v/v) gave thiosugar **7** as white crystals (89.2 g, 89.7 mmol, 43%).  $R_{\rm f}$ =0.73 (toluene/EtOAc 1:1); mp 189–191 °C(EtOH); [α]<sub>D</sub>+34(*c* 1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>): *b* 26.3, 27.6, 62.9, 63.0, 70.6, 71.5, 73.3, 73.8, 74.0, 75.5, 77.3, 77.3, 86.1, 100.4, 111.2, 128.2–130.0 (several carbons), 129.4–130.0 (several carbons), 132.0, 133.1–133.5 (several carbons), 165.0, 165.3, 165.7, 166.0, 166.1, (note: overlaps occur in aromatic region); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  1.25 (s, 3H), 1.52 (s, 3H), 3.68 (dd, 1H, J=7.4, 11.4 Hz), 3.80–3.91 (m, 2H), 4.09 (dd, 1H, J=2.1, 5.6 Hz), 4.12 (dd, 1H, J=9.6, 9.6 Hz), 4.21–4.26 (m, 2H), 4.47 (dd, 1H, J=5.0, 12.1 Hz), 4.60 (d, 1H, J=7.7 Hz), 4.66 (dd, 1H, J=1.9, 12.1 Hz), 4.88 (d, 1H, J=9.9 Hz), 5.13 (dd, 1H, J=6.8, 7.7 Hz), 5.40 (dd, 1H, J=9.6, 9.6 Hz), 5.73 (dd, 1H, J=9.2, 9.2 Hz), 7.07–7.12 (m, 2H), 7.17–7.20 (m, 1H), 7.25–7.62 (m, 17H), 7.91–8.01 (m, 8H), 8.05–8.08 (m, 2H); MALDI-TOF (CHCA): [M+Na]<sup>+</sup> calcd for C<sub>56</sub>H<sub>50</sub>NaO<sub>15</sub>S, 1017.27; found 1017.30.

## 4.4. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (2,6-O-benzoyl-3,4-O-isopropylidene- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranoside (8)

Compound **7** (25.0 g, 25.1 mmol) and *N*-Cbz-2-aminoethanol (9.80 g, 50.3 mmol) were dissolved in  $CH_2Cl_2$  (50 mL) and preactivated 4 Å MS were added and stirred for 10 min. NIS (8.48 g,

37.7 mmol) and AgOTf ( $\sim 0.1$  equiv) were added and stirred for 4 h,  $0 \circ C \rightarrow rt$ . The reaction was guenched with Et<sub>3</sub>N, diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and filtered through Celite<sup>®</sup>, washed with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (10 wt %, aq) (2×50 mL), 1 M HCl (aq) (2×50 mL), NaHCO<sub>3</sub> (satd aq) (2×50 mL), H<sub>2</sub>O (2×50 mL) dried over MgSO<sub>4</sub> (s), filtered, and concentrated. FC (toluene $\rightarrow$  toluene/EtOAc 6:1) followed by crystallization from EtOAc/n-heptane gave glycoside 8 (23.5 g, 21.7 mmol, 87%) as white needles. Rf=0.68 (toluene/EtOAc 1:1); mp 153-154 °C (EtOAc/nheptane);  $[\alpha]_{D}$ +39 (*c* 1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>):  $\delta$  26.1, 27.4, 40.9, 62.4, 62.8, 66.4, 69.4, 71.3, 71.9, 72.2, 73.1, 73.2, 73.6, 75.2, 77.1, 100.1, 101.3, 110.8, 127.9-128.6 (several carbons), 129.1-129.8 (several carbons), 132.9, 133.1, 133.2, 133.3, 136.5, 156.2, 164.8, 165.3, 165.5, 165.8, 165.9; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.25 (s, 3H), 1.52 (s, 3H), 3.24–3.32 (m, 2H), 3.56–3.63 (m, 1H), 3.69–3.85 (m, 4H), 4.09 (dd, 1H, J=2.1, 5.6 Hz), 4.17 (dd, 1H, J=9.5, 9.5 Hz), 4.22–4.28 (m, 2H), 4.45 (dd, 1H, J=4.3, 12.1 Hz), 4.58–4.64 (m, 3H), 4.89 (d, 1H, J=12.3 Hz), 4.96 (d, 1H, J=12.3 Hz), 5.09 (bs, 1H), 5.15 (dd, 1H, J=6.8, 7.7 Hz), 5.37 (dd, 1H, *J*=7.8, 9.7 Hz), 5.71 (dd, 1H, *J*=9.4, 9.4 Hz), 7.22–7.37 (m, 13H), 7.42-7.60 (m, 7H), 7.91-8.02 (m, 8H), 8.07-8.11 (m, 2H); HRMS (ESI): [M+Na]<sup>+</sup> calcd for C<sub>60</sub>H<sub>57</sub>NNaO<sub>18</sub>, 1102.3395; found 1102.3372.

## 4.5. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (4-*N*-azido-4-deoxy-2,3,6-tri-*O*-benzoyl- $\beta$ -D -glucopyranosyl)-(1 $\rightarrow$ 3)-(2,4,6-tri-*O*-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-benzoyl- $\beta$ -D-glucopyranoside (9)

Compound 8 (3.50 g, 3.24 mmol) was added to a solution of  $CH_2Cl_2/TFA$  (90% v/v, aq) (50 mL, 4:1) and stirred for 1 h. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and washed with NaHCO<sub>3</sub> (satd aq) (2×200 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. The crude acceptor ( $R_f=0.24$  (toluene/EtOAc 2:1)) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) whereupon 6 (2.00, 3.56 mmol) and preactivated 4 Å MS were added and stirred for 10 min. NIS (1.09 g, 4.86 mmol) and AgOTf ( $\sim$  0.1 equiv) were added and stirred for 1 h,  $(0 \circ C \rightarrow rt)$ . The reaction was quenched with Et<sub>3</sub>N whereupon pyridine (10 mL) and BzCl (1.5 mL, 13 mmol) were added. After 2 h, the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and washed with 1 M HCl (aq)  $(2 \times 100 \text{ mL})$ , NaHCO<sub>3</sub> (satd aq)  $(2 \times 100 \text{ mL})$ , H<sub>2</sub>O  $(2 \times 100 \text{ mL})$ , dried over MgSO<sub>4</sub> (s), filtered, and concentrated. FC (toluene $\rightarrow$ toluene/EtOAc 2:1) gave trisaccharide **9** (3.99 g, 2.62 mmol, 81%) as a colorless syrup.  $R_{\rm f}$ =0.62 (toluene/EtOAc 2:1);  $[\alpha]_{\rm D}$ +75 (c 0.1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>): δ 40.8, 60.1, 62.0, 62.3, 62.7, 66.4, 69.3, 69.4, 71.2, 71.6, 71.9, 72.4, 72.5, 73.0, 73.4, 75.3, 78.2, 100.6, 101.2, 101.3, 127.8-128.7 (several carbons), 129.0-130.1 (several carbons), 132.6-133.4 (several carbons), 156.2, 164.1, 164.4, 165.2, 165.3, 165.4, 165.7, 165.8, 165.9. (Note: overlaps occur in spectra); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 3.16 (dd, 1H, *J*=8.1, 11.4 Hz), 3.19-3.24 (m, 1H), 3.28-3.31 (m, 1H), 3.57-3.60 (m, 1H), 3.62-3.65 (m, 2H), 3.67 (dd, 1H, J=5.1, 7.7 Hz), 3.74–3.77 (m, 1H), 3.78 (dd, 1H, J=10.1, 10.1 Hz), 3.86 (dd, 1H, J=4.7, 11.6 Hz), 4.00 (dd, 1H, J=3.3, 10.0 Hz), 4.06 (dd, 1H, /=9.5 Hz), 4.31 (dd, 1H, /=4.5, 12.0 Hz), 4.42-4.44 (m, 1H), 4.54-4.56 (m, 2H), 4.60-4.63 (m, 2H), 4.79 (d, 1H, J=7.7 Hz), 4.87 (d, 1H, J=12.3 Hz), 4.93 (d, 1H, J=12.3 Hz), 5.08 (bs, 1H), 5.18 (dd, 1H, J=7.7, 9.6 Hz), 5.36 (d, 1H, J=8.0, 9.8 Hz), 5.44 (dd, 1H, J=9.6, 9.6 Hz), 5.51 (dd, 1H, J=8.3, 9.5 Hz), 5.63-5.66 (m, 2H), 6.78–6.83 (m, 2H), 7.01–7.06 (m, 2H), 7.12–7.57 (m, 32H), 7.68-7.77 (m, 5H), 7.88-7.95 (m, 7H), 8.08-8.10 (m, 2H); HRMS (ESI):  $[M+H]^+$  calcd for  $C_{91}H_{79}N_4O_{26}$ , 1643.4903; found 1643.4865.

## 4.6. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (4-deoxy-4-*N*-tert-butyloxycarbonyl-glycyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-( $\beta$ -D-glactopyranosyl)-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranoside (1)

Compound **9** (1.03 g, 0.679 mmol) was added to a solution of  $CH_2Cl_2/MeOH$  (10 mL, 1:4) whereupon NaOMe (660 mg, 12.2 mmol) was added. After 3 h the solution was neutralized

with Dowex<sup>®</sup>-H<sup>+</sup>, filtered, and evaporated. The crude debenzoylated trisaccharide was dissolved in MeOH (30 mL), and washed with *n*-heptane ( $3 \times 50$  mL), and concentrated. Without further purification, the compound was dissolved in MeOH (10 mL) and  $NiCl_2 \cdot 6H_2O$  (~1 mg) was added and stirred for 5 min followed by the addition of NaBH<sub>4</sub> (51 mg, 1.4 mmol). After 15 min, the mixture was neutralized with Dowex®-H+, filtered and concentrated. The crude amine was dissolved in MeOH (10 mL) followed by the addition of N-(tert-butyloxycarbonyl)-glycine (125 mg, 0.713 mmol), HOAt (0.5 M in DMF, 0.14 mL, 68 µmol), 4methylmorpholine (79 µL, 0.71 mmol) and stirred for 10 min whereupon EDC·HCl (0.137 g, 0.713 mmol) was added. After 3 h the mixture was concentrated. RP chromatography  $(H_2O \rightarrow MeOH)$ H<sub>2</sub>O 1:1) gave the title compound **1** (0.370 g, 0.443 mmol, 65%) as a white solid.  $R_{\rm f}$ =0.80 (chloroform/MeOH/H<sub>2</sub>O 7:4:1);  $[\alpha]_{\rm D}$ -8 (c 0.1, H<sub>2</sub>O); <sup>13</sup>C NMR (75.4 MHz, CD<sub>3</sub>OD): δ 27.3, 40.6, 41.5, 43.5, 51.1, 51.8, 60.6, 61.1, 66.1, 68.2, 68.7, 70.2, 73.2, 73.3, 74.5, 74.8, 75.0, 75.3, 79.4, 79.5, 83.1, 102.9, 103.3, 104.2, 127.5, 127.6, 128.1, 136.9, 157.1, 157.5, 172.1; <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD):  $\delta$  1.44 (s, 9H), 3.23-3.46 (m, 6H), 3.53-3.93 (m, 17H), 4.1 (ad, 1H, J=2.6), 4.32 (d, 1H, J=7.8 Hz), 4.44 (d, 1H, J=7.4 Hz), 4.58 (d, 1H, J=7.59 Hz), 5.07 (s, 2H), 7.27–7.35 (m, 5H); HRMS (ESI): [M+H]<sup>+</sup> calcd for C<sub>35</sub>H<sub>56</sub>N<sub>3</sub>O<sub>20</sub>, 838.3379; found 838.3335.

## 4.7. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (2,6-di-*O*-benzoyl-3,4-di-*O*-isopropylidene- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-*O*-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-(2,4,6-tri-*O*-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-*O*-benzoyl- $\beta$ -D-glucopyranoside (10)

To a solution of  $CH_2Cl_2/TFA$  (90% v/v, aq) (50 mL, 4:1) compound 8 (15.0 g, 13.9 mmol) was added. After 2 h the mixture was diluted with  $CH_2Cl_2$  (50 mL) and washed with NaHCO<sub>3</sub> (satd aq) (2×100 mL), dried over MgSO<sub>4</sub>(s), filtered, and concentrated. The crude acceptor ( $R_f=0.23$ ) (toluene/EtOAc 2:1)) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) whereupon 7 (15.2 g, 15.3 mmol) and pre-activated 4 Å MS was added and the reaction mixture was stirred for 10 min. NIS (4.69 g, 20.8 mmol) and AgOTf ( $\sim 0.1$  equiv) were added and the reaction was stirred for 1.5 h,  $(0^{\circ}C \rightarrow rt)$ . The reaction was guenched with Et<sub>3</sub>N, whereupon pyridine (30 mL) and BzCl (6.45 mL, 55.6 mmol) were added. After an additional 1.5 h the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>, (50 mL) filtered through Celite<sup>®</sup> and washed with 1 M HCl (aq) (2×200 mL), NaHCO<sub>3</sub> (satd aq) (2×100 mL), H<sub>2</sub>O (2×100 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. FC (toluene $\rightarrow$  toluene/EtOAc 2:1) gave tetrasaccharide **10** (19.4 g, 9.54 mmol, 69%) as a colorless syrup. *R*<sub>f</sub>=0.63 (toluene/EtOAc 2:1); [α]<sub>D</sub>+38(*c* 1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>): δ 26.1, 27.4, 40.9, 62.0, 62.3, 62.7, 66.4, 69.4, 69.4, 71.2, 71.3, 71.6, 71.7, 72.0, 72.4, 72.5, 72.9. 73.0, 73.2, 73.6, 74.6, 75.3, 77.1, 78.2, 100.1, 100.7, 101.4, 110.8, 127.8-130.0 (several carbons), 132.5-133.4 (several carbons), 136.6, 156.2, 164.1, 164.4, 164.8, 165.2, 165.3, 165.3, 165.4, 165.7, 165.7, 165.8, 165.8. (Note: overlaps occur in spectra); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 1.18 (s, 3H), 1.44 (s, 3H), 3.10 (dd, 1H, J=8.0, 11.4 Hz), 3.19–3.31 (m, 2H), 3.49 (dd, 1H, J=7.4, 11.4 Hz), 3.56–3.67 (m, 4H), 3.73–3.76 (m, 2H), 3.87 (dd, 1H, J=4.6, 11.6 Hz), 3.91 (dd, 1H, J=3.4, 10.1 Hz), 3.97 (dd, 1H, J=2.1, 5.5 Hz), 4.03–4.07 (m, 2H), 4.11–4.16 (m, 2H), 4.29 (dd, 1H, J=4.5, 11.9 Hz), 4.40–4.48 (m, 4H), 4.55 (d, 1H, *J*=7.8 Hz), 4.58 (d, 1H, *J*=7.9 Hz), 4.71 (d, 1H, J=7.6 Hz), 4.87 (d, 1H, J=12.3 Hz), 4.93 (d, 1H, J=12.3 Hz), 5.04 (dd, 1H, J=7.3, 7.3 Hz), 5.07 (bs, 1H), 5.22 (dd, 1H, J=7.6, 9.5 Hz), 5.34 (dd, 1H, J=7.9, 9.8 Hz), 5.48 (dd, 1H, J=9.4, 9.4 Hz), 5.50 (dd, 1H, J=8.0, 9.8 Hz), 5.54 (ad, 1H, J=3.4 Hz), 5.63 (dd, 1H, J=9.5, 9.5 Hz), 6.76-6.78 (m, 2H), 7.02-7.05 (m, 2H), 7.11-7.53 (m, 36H), 7.66-7.68 (m, 4H), 7.77–7.89 (m, 9H), 7.93–8.10 (m, 7H); MALDI-TOF (CHCA): [M+Na]<sup>+</sup> calcd for C<sub>114</sub>H<sub>101</sub>NNaO<sub>34</sub>, 2050.63; found 2050.61.

# 4.8. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (4-*N*-azido-4-deoxy-2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-(2,4,6-tri-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-(2,4,6-tri-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl- $\beta$ -D-glucopyrano

Compound 10 (4.00 g. 1.97 mmol) was added to a solution of  $CH_2Cl_2/TFA$  (90%, v/v, aq) (50 mL, 4:1). After 30 min the solution was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and washed with NaHCO<sub>3</sub> (satd aq) (2×150 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. The crude acceptor ( $R_f=0.22$  (toluene/EtOAc 2:1)) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) whereupon 6 (1.28 g, 2.17 mmol) and pre-activated 4 Å MS were added and the reaction mixture was stirred for 10 min. NIS (890 mg, 3.90 mmol) and AgOTf ( $\sim$ 0.1 equiv) were added and stirred for 1.5 h, (0  $^{\circ}C \rightarrow rt$ ). The reaction was guenched with Et<sub>3</sub>N, whereupon pyridine (5 mL) and BzCl (460 µL, 3.90 mmol) were added. After an additional 1.5 h, the mixture was diluted with  $CH_2Cl_2$ , (~50 mL) filtered through Celite<sup>®</sup> and washed with 1 M HCl (aq) (2×50 mL), NaHCO<sub>3</sub> (satd aq) (2×100 mL), H<sub>2</sub>O (2×200 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. FC (tolue $ne \rightarrow toluene/EtOAc 4:1)$  gave pentasaccharide 11 (3.35 g, 1.29 mmol, 66%) as a colorless syrup.  $R_{\rm f}$ =0.64 (toluene/EtOAc 2:1);  $[\alpha]_{\rm D}$ +66 (c 0.1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>): δ 40.9, 60.2, 61.8, 62.0, 62.3, 62.7, 66.5, 69.3, 69.4, 69.5, 71.1, 71.4, 71.6, 71.7, 71.9, 72.0, 72.4, 72.5, 72.8, 73.1, 73.5, 74.7, 75.3, 78.2, 78.2, 100.6, 100.7, 101.3, 101.4, 126.3, 127.7-128.8 (several carbons), 129.1-130.1 (several carbons), 132.5–133.4 (several carbons), 136.6, 156.3, 164.1, 164.1, 165.2, 165.2, 164.4.164.5.165.3.165.3.165.5.165.7.165.7.165.7.165.9.165.9.(note: several overlaps occur in spectra); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta$  2.92 (dd, 1H, J=8.0, 11.5 Hz), 3.07 (dd, 1H, J=8.1, 11.2 Hz), 3.16-3.31 (m, 2H), 3.46 (d, 1H, J=5.1, 7.8 Hz), 3.51-3.62 (m, 5H), 3.72-3.81 (m, 4H), 3.86-3.91 (m, 2H), 3.95 (dd, 1H, J=9.4, 9.4 Hz), 4.03 (dd, 1H, J=9.5, 9.5 Hz), 4.25-4.33 (m, 3H), 4.40-4.43 (m, 2H), 4.50-4.60 (m, 4H), 4.65 (d, 1H, J=7.6 Hz), 4.74 (d, 1H, J=7.6 Hz), 4.86 (d, 1H, J=12.3 Hz), 4.93 (d, 1H, *I*=12.3 Hz), 5.05 (bs, 1H), 5.15 (dd, 1H, *I*=7.7, 9.5 Hz), 5.19 (dd, 1H, *J*=7.7, 9.8 Hz), 5.33 (dd, 1H, *J*=8.0, 9.7 Hz), 5.39–5.43 (m, 3H), 5.47 (dd, 1H, J=8.5, 9.6 Hz), 5.50 (ad, 1H, J=3.3 Hz), 5.51 (ad, 1H, J=3.3 Hz), 5.62 (dd, 1H, J=9.4, 9.4 Hz), 6.64–6.67 (m, 2H), 6.74–6.77 (m, 2H), 6.98–7.58 (m, 52H), 7.61–7.91 (m, 20H), 7.94–7.98 (m, 2H), 8.02–8.05 (m, 2H); MALDI-TOF (THAP):  $[M+Na]^+$  calcd for C145H122N4NaO42, 2613.74; found 2613.77.

## 4.9. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (4-deoxy-4-*N*-tert-butyloxycarbonyl-glycyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-( $\beta$ -D-glactopyranosyl)-(1 $\rightarrow$ 4)-( $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-( $\beta$ -D-glactopyranosyl)-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranoside (2)

Compound 11 (0.782 g, 0.302 mmol) was added to a solution of CH<sub>2</sub>Cl<sub>2</sub>/MeOH (10 mL, 1:4) whereupon NaOMe (0.489 g, 9.05 mmol) was added and stirred overnight. The solution was neutralized with Dowex<sup>®</sup>-H<sup>+</sup>, filtered and evaporated. The crude debenzoylated derivative was dissolved in MeOH (30 mL), and washed with nheptane (3×50 mL), and concentrated. Without further purification the compound was dissolved in MeOH (10 mL) and NiCl<sub>2</sub>·6H<sub>2</sub>O  $(\sim 1 \text{ mg})$  was added and stirred for 5 min followed by the addition of NaBH<sub>4</sub> (23 mg, 0.60 mmol). After 15 min, the mixture was neutralized with Dowex®-H<sup>+</sup>, filtered and concentrated. The crude amine was dissolved in MeOH (10 mL) followed by the addition of N-(tert-butyloxycarbonyl)-glycine (55 mg, 0.32 mmol), HOAt (0.5 M in DMF, 60 µL, 32 µmol), 4-methylmorpholine (35 µL, 0.32 mmol) and stirred for 10 min whereupon EDC · HCl (61 mg, 0.32 mmol) was added. After 3 h the mixture was concentrated. RP chromatography  $(H_2O/MeOH 95:5 \rightarrow MeOH/H_2O 50:50)$  gave the title compound 2 (0.223 g, 0.192 mmol, 64%) as a white solid.  $R_{f}=0.45$  (chloroform/ MeOH/H<sub>2</sub>O 7:4:1); [α]<sub>D</sub>+74 (*c* 0.1, H<sub>2</sub>O); <sup>13</sup>C NMR (75.4 MHz, D<sub>2</sub>O): δ 28.2, 41.1, 42.6, 44.1, 52.1, 53.3, 60.5, 60.7, 61.3, 61.5, 67.5, 68.8, 69.6, 70.6, 70.7, 73.4, 73.6, 74.7, 74.9, 75.2, 75.3, 75.6, 78.6, 78.9, 82.2, 82.6, 82.7, 102.9, 103.1, 104.2, 104.2, 128.3, 129.0, 129.4, 137.0, 158.4, 158.9, 173.7. (Note: overlaps occur in region 52.1–104.2); <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 1.43 (s, 9H), 3.24–3.37 (m, 4H), 3.39–3.46 (m, 2H), 3.48–3.96 (m, 28H), 4.16–4.17 (m, 2H), 4.40 (d, 1H, *J*=7.5 Hz), 4.47 (d, 1H, *J*=7.5 Hz), 4.50 (d, 1H, *J*=7.4 Hz), 4.65–4.68 (m, 2H) (overlap with HDO), 5.09 (s, 2H), 7.35–7.45 (m, 5H); HRMS (ESI): [M+H]<sup>+</sup> calcd for C<sub>47</sub>H<sub>76</sub>N<sub>3</sub>O<sub>30</sub>, 1162.4436; found 1162.4419.

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To a solution of  $CH_2Cl_2/TFA$  (90% v/v, aq) (50 mL, 4:1) compound 10 (9.25 g, 4.81 mmol) was added. After 1.5 h, the mixture was diluted with  $CH_2Cl_2$  (~50 mL) and washed with NaHCO<sub>3</sub> (satd aq), dried over MgSO<sub>4</sub> (s), filtered and concentrated. The crude acceptor  $(R_{\rm f}=0.24 \text{ (toluene/EtOAc 2:1)})$  was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) followed by the addition of 7 (5.26 g, 5.29 mmol) and pre-activated 4 Å MS and stirred for 10 min at 0 °C. NIS (1.62 g, 7.21 mmol) and AgOTf ( ~0.1 equiv) were added and stirred for 2 h, (0  $^{\circ}$ C $\rightarrow$ rt). The reaction was guenched with Et<sub>3</sub>N followed by the addition of pyridine (30 mL) and BzCl (2.23 mL, 19.2 mmol). After 2 h the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL), filtered through Celite<sup>®</sup> and washed with 1 M HCl (aq)  $(2 \times 200 \text{ mL})$ , NaHCO<sub>3</sub> (satd aq) (2×150 ml), H<sub>2</sub>O (2×200 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. FC (toluene $\rightarrow$ toluene/EtOAc 4:1) gave hexasaccharide **12** (8.45 g, 2.84 mmol, 59%) as a white syrup.  $R_f=0.62$ (toluene/EtOAc 2:1); [a]<sub>D</sub>+32 (c 1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>):  $\delta$  26.1, 27.4, 40.8, 61.7–62.0 (several carbons), 62.2, 62.3, 62.7, 66.5, 69.3, 69.5, 71.0, 71.1, 71.3, 71.6, 71.7, 71.9, 72.3, 72.5, 72.7, 72.8, 72.9, 73.0, 73.1, 73.5, 73.6, 74.5, 74.6, 75.3, 100.1, 100.5, 100.6, 101.3, 101.4, 110.8, 127.6-128.8 (several carbons), 129.1-123.0 (several carbons), 132.5-133.0 (several carbons), 136.6, 156.2, 164.0, 164.3, 164.4, 164.8, 165.1, 165.1, 165.3, 165.3, 165.4, 165.6, 165.6, 165.7, 165.8, 165.8. (note: several overlaps occur in spectra); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta$  1.18 (s, 3H), 1.43 (s, 3H), 2.89 (dd, 1H, J=8.0, 11.5 Hz), 3.02-3.10 (m, 2H), 3,17-3.31 (m, 2H), 3.39 (dd, 1H, J=4.8, 8.1 Hz), 3.45–3.80 (m, 11H), 3.85 (dd, 1H, J=3.5, 9.9 Hz), 3.90–4.05 (m, 5H), 4.09-4.14 (m, 3H), 4.19-4.31 (m, 4H), 4.34-4.44 (m, 5H), 4.30 (dd, 1H, J=3.7, 7.8 Hz), 4.64 (dd, 1H, J=6.9 Hz), 4.46 (d, 1H, J=12.3 Hz), 4.92 (d, 1H, J=12.3 Hz), 5.01 (dd, 1H, J=7.4, 7.4 Hz), 5.04 (bs, 1H), 5.15-5.18 (m, 2H), 5.31 (dd, 1H, J=8.0, 9.9 Hz), 5.36–5.48 (m, 4H), 5.60 (dd, 1H, *I*=9.5, 9.5 Hz), 6.60–6.65 (m, 2H), 6.74–6.80 (m, 3H), 6.94–7.58 (m, 58H), 7.61–8.05 (m, 27H); MALDI-TOF (THAP): [M+Na]<sup>+</sup> calcd for C<sub>168</sub>H<sub>145</sub>NNaO<sub>50</sub>, 2998.87; found 2999.03.

4.11. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (4-*N*-azido-4-deoxy-2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-(2,4,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-(2,4,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri- $\beta$ -2,3,6-tri- $\beta$ -2,3,

Compound **12** (1.02 g, 0.343 mmol) was added to a solution of CH<sub>2</sub>Cl<sub>2</sub>/TFA (90%,  $\nu/\nu$ , aq) (10 mL, 4:1). After 30 min the solution was diluted with CH<sub>2</sub>Cl<sub>2</sub> (25 mL) and washed with NaHCO<sub>3</sub> (satd aq) (2×100 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. The crude acceptor was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) whereupon **6** (0.212 g,

0.377 mmol) and pre-activated 4 Å MS were added and the reaction mixture was stirred for 10 min. NIS (0.154 g, 0.685 mmol) and AgOTf (~0.1 equiv) were added and stirred for 1.5 h,  $(0 \circ C \rightarrow rt)$ . The reaction was guenched with Et<sub>3</sub>N, whereupon pyridine (5 mL) and BzCl (0.160 mL, 1.37 mmol) were added. After an additional 1.5 h, the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (25 mL), filtered through Celite<sup>®</sup> and washed with 1 M HCl (aq) ( $2 \times 50$  mL), NaHCO<sub>3</sub> (satd aq) ( $2 \times 50$  mL), H<sub>2</sub>O (2×50 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. FC (toluene/EtOAc 9:1) gave heptasaccharide 13 (0.615 g, 0.174 mmol, 51%) as a colorless syrup.  $R_f=0.64$  (toluene/EtOAc 2:1);  $[\alpha]_D+55$  (c 0.1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>): δ 40.8, 60.1, 61.8, 61.9, 62.2, 62.7, 66.5, 69.2, 69.2, 69.3, 69.5, 71.0, 71.2, 71.3, 71.5, 71.6, 71.9, 71.9, 72.3, 72.5, 72.7, 72.7, 73.0, 73.4, 74.6, 74.6, 75.3, 77.2, 78.1, 78.1, 100.5, 100.6, 101.2, 101.3, 101.4, 127.6-128.8 (several carbons), 129.1-130.0 (several carbons), 132.4–133.3 (several carbons), 136.5, 156.2, 163.9, 164.0, 164.3, 164.4, 165.1, 165.1, 165.1, 165.2, 165.2, 165.3, 165.4, 165.5, 165.6, 165.6, 165.8, 165.9. (note: several overlaps occur in spectra); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 2.84 (dd, 1H, *J*=8.2, 11.3 Hz), 2.90 (dd, 1H, *J*=8.0, 11.5 Hz), 3.06 (dd, 1H, J=8.1, 11.1 Hz), 3.16-3.31 (m, 2H), 3.36 (dd, 1H, J=5.0, 7.6 Hz), 3.43 (dd, 1H, J=5.3, 7.4 Hz), 3.48-3.63 (m, 6H), 3.67 (dd, 1H, J=4.4, 11.6 Hz), 3.71-3.78 (m, 5H), 3.84-3.94 (m, 4H), 4.01 (dd, 1H, J=9.4, 9.4 Hz), 4.22-4.28 (m, 5H), 4.35-4.40 (m, 4H), 4.49-4.64 (m, 7H), 4.71 (d, 1H, /=7.6 Hz), 4.86 (d, 1H, /=12.3 Hz), 4.92 (d, 1H, J=12.3 Hz), 5.04 (bs, 1H), 5.12–5.17 (m, 3H), 5.30–5.50 (m, 8H), 5.60 (dd, 1H, J=9.5, 9.5 Hz), 6.58-6.68 (m, 4H), 6.73-6.78 (m, 3H), 6.94-7.56 (m, 71H), 7.60-8.03 (m, 32H); MALDI-TOF (THAP):  $[M+Na]^+$  calcd for C<sub>199</sub>H<sub>166</sub>N<sub>4</sub>NaO<sub>58</sub>, 3562.01; found 3562.06.

## 4.12. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (4-deoxy-4-*N*-tertbutyloxycarbonyl-glycyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-( $\beta$ -Dgalactopyranosyl)-(1 $\rightarrow$ 4)-( $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-( $\beta$ -Dgalactopyranosyl)-(1 $\rightarrow$ 4)-( $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 3)-( $\beta$ -Dgalactopyranosyl)-(1 $\rightarrow$ 4)-( $\beta$ -D-glucopyranoside) (3)

Compound 13 (0.500 g, 0.141 mmol) was added to a solution of CH<sub>2</sub>Cl<sub>2</sub>/MeOH (10 mL, 1:4) whereupon NaOMe (0.320 g, 5.92 mmol) was added and stirred overnight. The solution was neutralized with Dowex<sup>®</sup>-H<sup>+</sup>, filtered and evaporated. The crude debenzoylated heptasaccharide was dissolved in MeOH (20 mL), and washed with nheptane (2×30 mL), and concentrated. Without further purification the compound was dissolved in MeOH (10 mL) and NiCl<sub>2</sub>·6H<sub>2</sub>O  $(\sim 1 \text{ mg})$  was added and stirred for 5 min followed by the addition of NaBH<sub>4</sub> (11 mg, 0.28 mmol). After 15 min, the mixture was neutralized with Dowex<sup>®</sup>-H<sup>+</sup>, filtered and concentrated. The crude amine was dissolved in MeOH/H<sub>2</sub>O (10 mL, 1:1) followed by the addition of N-(tert-butyloxycarbonyl)-glycine (26 mg, 0.15 mmol), HOAt (0.5 M in DMF, 28 µL, 14 µmol), 4-methylmorpholine (16 µL, 0.15 mmol) and stirred for 10 min whereupon EDC · HCl (28 mg, 0.15 mmol) was added and stirred overnight followed by concentration. RP chromatography (H<sub>2</sub>O/MeOH 95:5 $\rightarrow$  MeOH/H<sub>2</sub>O 75:25) gave the title compound **3** (61 mg, 41  $\mu$ mol, 29%) as a white solid.  $R_f=0.25$  (chloroform/MeOH/  $H_2O7:4:1$ ;  $[\alpha]_D+17(c0.1, H_2O)$ ; <sup>13</sup>CNMR(75.4 MHz, D<sub>2</sub>O):  $\delta$  28.2, 41.1, 42.6, 44.1, 52.1, 53.3, 60.5, 60.7, 61.3, 61.6, 62.1, 67.5, 68.9, 69.6, 70.7, 73.4, 73.6, 73.7, 73.9, 74.8, 74.8, 74.9, 75.2, 75.3, 75.4, 75.6, 78.6, 78.8, 82.3, 82.6, 82.7, 102.9, 103.2, 104.2, 104.2, 104.3, 128.4, 129.0, 129.4, 137.1, 159.0, 173.8. (Note: several overlaps occur in spectra); <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 1.43 (s, 9H), 3.27–3.46 (m, 8H), 3.49–3.96 (m, 37H), 4.17-4.18 (m, 3H), 4.43-4.51 (m, 4H), 4.63-4.67 (m, 3H), 5.12 (s, 2H), 7.37-7.47 (m, 5H); HRMS (ESI): [M+H]<sup>+</sup> calcd for C<sub>59</sub>H<sub>96</sub>N<sub>3</sub>O<sub>40</sub>, 1486.5492; found 1486.5562.

## 4.13. Ethyl 2,3,4-tri-O-benzoyl-1-thio-α-D-mannopyranoside (15)

To a solution of **14**<sup>41</sup> (24.9 g, 111 mmol) in pyridine (100 mL), TrCl (46.5 g, 167 mmol) was added. After 16 h, the reaction mixture was

diluted with CH<sub>2</sub>Cl<sub>2</sub> (200 mL) and BzCl (58.4 mL, 503 mmol) was added dropwise (over 30 min) at 0 °C. After 4.5 h, the solution was washed with ice/H<sub>2</sub>O (2×500 mL), 1 M HCl (aq) (2×200 mL), NaHCO<sub>3</sub> (satd aq) (2×200 mL), H<sub>2</sub>O (2×200 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. Without further purification, the crude monosaccharide was dissolved in CHCl<sub>3</sub>/MeOH (300 mL, 2:1) and p-TsOH (10.5 g, 55.4 mmol) was added. After 3 h, the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (100 mL), washed with NaHCO<sub>3</sub> (satd aq) (2×150 mL), H<sub>2</sub>O (2×150 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. FC (petroleum ether/EtOAc  $4:1 \rightarrow$  EtOAc) gave 15 (27.0 g, 50.3 mmol, 45%) as a colorless oil. R<sub>f</sub>=0.30 (toluene/ EtOAc 9:1);  $[\alpha]_D = 82 (c \ 1, CHCl_3); {}^{13}C \ NMR (75.4 \ MHz, CDCl_3); \delta \ 15.0,$ 25.8, 61.5, 67.6, 70.2, 71.6, 72.5, 82.6, 128.4, 128.7, 128.8, 128.9, 129.2, 129.5, 129.8, 130.1, 133.4, 133.7, 133.8, 165.5, 165.6, 166.7, (Note: overlap occur aromatic region); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  1.37 (t, 3H, *I*=7.4 Hz), 2.65–2.83 (m, 2H), 3.81–3.83 (m, 2H), 4.42–4.47 (m, 1H), 5.58 (d, 1H, J=1.5 Hz), 5.79 (dd, 1H, J=1.5, 2.9 Hz), 5.85–5.94 (m, 2H), 7.22–7.28 (m, 2H), 7.36–7.64 (m, 7H), 7.80–7.84 (m, 2H), 7.97-8.01 (m, 2H), 8.09-8.13 (m, 2H); MALDI-TOF (CHCA): [M+Na]<sup>+</sup> calcd for C<sub>29</sub>H<sub>28</sub>NaO<sub>8</sub>S, 559.14; found 559.18.

## 4.14. Ethyl 2,3,4-tri-O-benzoyl-6-O-chloroacetyl-1-thio-α-D-mannopyranoside (16)

To a solution of ethyl-2,3,4-tri-O-benzoyl-α-D-thio-mannopyranoside 15 (2.28 g, 4.25 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/Pyridine (30 mL, 14:1), chloroacetyl chloride (1.70 mL, 21.3 mmol) was added at -40 °C. After 1 h, the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and washed with 1 M HCl (aq) ( $2 \times 50$  mL), NaHCO<sub>3</sub> (satd aq) (100 mL), H<sub>2</sub>O (200 mL), dried over MgSO<sub>4</sub> (s), filtered and concentrated. FC (toluene $\rightarrow$ toluene/EtOAc 6:1) gave chloroacetate **16** (2.15 g, 3.50 mmol, 82%) as a colorless syrup.  $R_{\rm f}$ =0.57 (toluene/EtOAc 9:1);  $[\alpha]_{D}$ -57 (c 1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>):  $\delta$  14.9, 25.7, 40.6, 64.2, 67.1, 69.9, 70.4, 72.1, 82.5, 128.4-128.9 (several carbons), 129.7-130.0 (several carbons), 133.3, 133.6, 133.7, 166.4, 165.4, 165.6, 166.9, (Note: several overlaps occur in spectra); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.34 (t, 3H, *J*=7.4 Hz), 2.64–2.83 (m, 2H), 4.09 (d, 1H, J=15.0 Hz), 4.14 (d, 1H, J=15.0 Hz), 4.40-4.45 (m, 1H), 4.55 (dd, 1H, J=4.8, 12.2 Hz), 4.76-4.82 (m, 1H), 5.60 (as, 1H), 5.85-5.89 (m, 2H), 6.04 (dd, 1H, J=9.7, 9.7 Hz), 7.18-7.23 (m, 2H), 7.31-7.36 (m, 3H), 7.44-7.49 (m, 3H), 7.55-7.60 (m, 1H), 7.81-7.84 (m, 2H), 7.96 (m, 2H), 8.11-8.14 (m, 2H); HRMS (ESI): [M+NH<sub>4</sub>]<sup>+</sup> calcd for C<sub>31</sub>H<sub>33</sub>ClNO<sub>9</sub>S, 630.1565; found 630.1595.

## 4.15. Ethyl (2,3,4-tri-O-benzoyl-6-O-chloroacetyl- $\alpha$ -D-mannopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-benzoyl- $\alpha$ -D-mannopyranoside (17)

To a solution of compound 16 (5.36 g, 8.75 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL), Br<sub>2</sub> (0.52 mL, 10.1 mmol) was added. After 30 min, the mixture was evaporated and co-concentrated with toluene. The crude bromosugar was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) whereupon 15 (3.75 g, 6.99 mmol) and pre-activated 4 Å MS were added. AgOTf (4.49 g, 17.5 mmol) was added at -30 °C and the solution was stirred for 30 min. The reaction was quenched with Et<sub>3</sub>N, filtered through Celite<sup>®</sup> and washed with 1 M HCl (aq) (50 mL), NaHCO<sub>3</sub> (satd aq) (100 mL), H<sub>2</sub>O (100 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. FC (toluene  $\rightarrow$  toluene/EtOAc 12:1) gave disaccharide 17 (5.82 g, 5.35 mmol, 77%) as a colorless syrup.  $R_{\rm f}$ =0.53 (toluene/EtOAc 9:1); [α]<sub>D</sub>-82 (*c* 1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>): δ 15.0, 25.6, 40.6, 64.2, 66.8, 66.9, 67.4, 68.7, 69.9, 70.0, 70.4, 70.7, 72.3, 82.3, 97.7, 128.3-129.2 (several carbons), 129.3, 129.4, 129.5, 129.8-130.1 (several carbons), 133.3, 133.3, 133.6, 133.7, 133.7, 133.7, 165.3, 165.4, 165.5, 165.7, 165.7, 165.8, 165.9, (Note: several overlaps occur in spectra); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.47 (t, 3H, J=7.4 Hz), 2.73–2.95 (m, 2H), 3.76 (dd, 1H, J=1.9, 10.8), 3.91 (d, 1H, J=15.0 Hz), 3.96 (d, 1H,  $\begin{array}{l} J{=}15.0~{\rm Hz}), 4.09{-}4.15~({\rm m},1{\rm H}), 4.23{-}4.37~({\rm m},3{\rm H}), 4.79{-}4.85~({\rm m},1{\rm H}), \\ 5.12~({\rm d},1{\rm H},J{=}1.8~{\rm Hz}), 5.63~({\rm d},1{\rm H},J{=}1.1~{\rm Hz}), 5.74~({\rm dd},1{\rm H},J{=}1.8, 3.1~{\rm Hz}), \\ 5.84{-}5.92~({\rm m},3{\rm H}), 5.96~({\rm dd},1{\rm H},J{=}3.1,10.3~{\rm Hz}), 6.04~({\rm dd},1{\rm H},J{=}9.9, \\ 9.9~{\rm Hz}), 7.15{-}7.63~({\rm m},18{\rm H}), 7.81{-}7.86~({\rm m},4{\rm H}), 7.97{-}8.02~({\rm m},4{\rm H}), \\ 8.06{-}8.09~({\rm m},2{\rm H}), 8.16{-}8.19~({\rm m},2{\rm H});~{\rm HRMS}~({\rm ESI}):~[{\rm M}{+}{\rm NH_4}]^+~{\rm calcd} \\ {\rm for}~{\rm C}_{58}{\rm H}_{55}{\rm CINO}_{17}{\rm S}, 1104.2879;~{\rm found}~1104.2859. \end{array}$ 

## 4.16. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (2,3,4-O-benzoyl-6-O-chloroacetyl- $\alpha$ -D-mannopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-benzoyl- $\alpha$ -D-mannopyranoside (18)

Compound 17 (1.99 g, 1.83 mmol) and N-Cbz-2-aminoethanol (720 mg, 50.0 mmol) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and preactivated 4 Å MS were added, and the reaction mixture was stirred for 10 min. The mixture was cooled to -30C° whereupon NIS (0.82 g, 3.7 mmol) and TfOH (~0.1 equiv) were added under nitrogen. After 2 h, the reaction was quenched with Et<sub>3</sub>N, filtered through Celite<sup>®</sup>, and washed with NaHCO<sub>3</sub> (satd aq) (100 mL), H<sub>2</sub>O (100 mL), dried over MgSO<sub>4</sub> (s), filtered and concentrated. FC (toluene/EtOAc 9:1) gave compound 18 (1.83 g, 1.50 mmol, 82%) as a colorless syrup.  $R_f=0.32$  (toluene/EtOAc 6:1);  $[\alpha]_D=82$  (c 1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>): δ 40.5, 40.7, 64.1, 66.6, 66.7, 66.8, 67.1, 67.6, 68.8, 69.8, 70.1, 70.3, 70.5, 97.4, 98.0, 128.0-129.3 (several carbons), 129.8-130.0 (several carbons), 133.2, 133.3, 133.6, 133.6, 133.7, 156.6, 165.3, 165.5, 165.6, 165.6, 165.7, 165.9. (Note: several overlaps occur in spectra); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 3.64–3.86 (m, 4H), 3.96 (d, 1H, J=15.0 Hz), 3.99 (d, 1H, J=15.0 Hz), 4.01-4.14 (m, 2H), 4.25-4.46 (m, 4H), 5.06-5.18 (m, 4H), 5.66 (bs, 1H), 5.75 (dd, 1H, *I*=1.8, 3.0 Hz), 5.80 (dd, 1H, *I*=1.6, 2.9 Hz), 5.90-6.04 (m, 4H), 7.13-7.62 (m, 23H), 7.82-7.87 (m, 4H), 7.97-8.01 (m, 4H), 8.07-8.11 (m, 2H), 8.15-8.18 (m, 2H); HRMS (ESI): [M+H]<sup>+</sup> calcd for C<sub>66</sub>H<sub>59</sub>NO<sub>20</sub>Cl, 1220.3241; found 1220.3234.

## 4.17. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (4-*N*-azido-4-deoxy-2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 6)-(2,3,4-tri-O-benzoyl- $\alpha$ -D-mannopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-benzoyl- $\alpha$ -D-mannopyranoside (19)

To a solution of  $18(500 \text{ mg}, 410 \mu \text{mol})$  in CH<sub>2</sub>Cl<sub>2</sub>/MeOH(10 mL, 1:1) was added 2,6-lutidine (380 µL, 3.28 mmol) and thiourea (440 mg, 5.73 mmol). After 4 days, the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and washed with 1 M HCl (aq) (30 mL), NaHCO<sub>3</sub> (satd aq)  $(2 \times 50 \text{ mL})$ , H<sub>2</sub>O (50 mL), dried over MgSO<sub>4</sub> (s), filtered and concentrated. FC (toluene→toluene/EtOAc 2:1) gave 2-(N-Benzyloxycarbonyl)-aminoethyl (2,3,4-tri-O-benzoyl- $\alpha$ -D-mannopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-benzoyl-α-p-mannopyranoside (370 mg, 330 μmol, 80%). This acceptor was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) followed by the addition of 6 (0.20 g, 0.36 mmol) and pre-activated 4 Å MS. NIS (0.11 g, 0.49 mmol) and AgOTf ( $\sim 0.1$  equiv) were added and stirred for 1 h. (0 °C $\rightarrow$ rt). After 1 h, the reaction was quenched with Et<sub>3</sub>N, filtered through Celite<sup>®</sup> and washed with NaHCO<sub>3</sub> (satd aq) (50 mL), H<sub>2</sub>O (50 mL), dried over MgSO<sub>4</sub> (s), filtered, and concentrated. FC (tolue $ne \rightarrow toluene/EtOAc 6:1)$  gave the trisaccharide 19 (460 mg, 280  $\mu$ mol, 85%) as a colorless syrup.  $R_f=0.27$  (toluene/EtOAc 9:1);  $[\alpha]_D=34$  (c 0.1, CHCl<sub>3</sub>); <sup>13</sup>C NMR (75.4 MHz, CDCl<sub>3</sub>): δ 40.6, 61.0, 63.4, 65.9, 66.8, 67.2, 67.4, 69.4, 69.8, 70.3, 70.4, 70.4, 71.7, 72.5, 73.7, 97.0, 97.8, 101.7, 127.9-128.9 (several carbons), 129.1, 129.1, 129.1, 129.3, 129.4, 129.5, 129.7–129.9 (several carbons), 130.1, 133.1, 133.1, 133.3, 133.4, 133.5, 133.5, 136.6, 156.5, 165.1, 165.3, 165.4, 165.5, 165.5, 165.6, 165.6, 166.0. (Note: several overlaps occur in spectra); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 3.26–3.28 (m, 1H), 3.61–3.77 (m, 7H), 3.84 (dd, 1H, J=5.7, 10.7 Hz), 3.95-3.99 (m, 2H), 4.20-4.23 (m, 2H), 4.48 (dd, 1H, J=4.7, 12.0 Hz), 4.63 (dd, 1H, J=2.0, 12.1 Hz), 4.67–4.70 (m, 2H), 5.06–5.07 (m, 2H), 5.13 (d, 1H, J=12.2 Hz), 5.29 (dd, 1H, J=8.8, 8.8 Hz), 5.58–5.64 (m, 3H), 5.74 (as, 1H), 5.85 (dd, 1H, J=3.2, 10.1 Hz), 5.89 (dd, 1H, J=3.3, 10.1 Hz), 6.01 (dd, 1H, J=10.2, 10.2 Hz), 7.16–7.62 (m, 32H), 7.73–7.76 (m, 2H), 7.83–7.91 (m, 6H), 7.96–8.04 (m, 8H), 8.17–8.21 (m, 2H); HRMS (ESI): [M+NH<sub>4</sub>]<sup>+</sup> calcd for C<sub>91</sub>H<sub>82</sub>N<sub>5</sub>O<sub>26</sub>, 1660.5248; found 1660.5270.

## 4.18. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (4-deoxy-4-*N*-tertbutyloxycarbonyl-glycyl- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 6)-( $\alpha$ -Dmannopyranosyl)-(1 $\rightarrow$ 6)- $\alpha$ -D-mannopyranoside (4)

Compound 19 (0.256 g, 0.156 mmol) was added to a solution of CH<sub>2</sub>Cl<sub>2</sub>/MeOH (10 mL, 1:4) whereupon NaOMe (150 mg, 2.80 mmol) was added and stirred overnight. The solution was neutralized with Dowex<sup>®</sup>-H<sup>+</sup>, filtered, and evaporated. The crude debenzoylated trisaccharide was dissolved in MeOH (30 mL) and washed with n-heptane  $(2 \times 40 \text{ mL})$  and concentrated. Without further purification the compound was dissolved in MeOH (10 mL) and NiCl<sub>2</sub>  $\cdot$  6H<sub>2</sub>O (~1 mg) was added and the reaction mixture was stirred for 5 min followed by the addition of NaBH<sub>4</sub> (12 mg, 0.31 mmol). After 15 min, the mixture was neutralized with Dowex<sup>®</sup>-H<sup>+</sup>, filtered and concentrated. The crude amine was dissolved in MeOH (10 mL) followed by the addition of N-(tert-butyloxycarbonyl)-glycine (27 mg, 0.16 mmol), HOAt (0.5 M in DMF, 31 µL, 16 µmol), 4-methylmorpholine (18 µL, 0.16 mmol) and stirred for 10 min whereupon EDC·HCl (31 mg, 0.164 mmol) was added and stirred overnight followed by concentration. RP chromatography (H<sub>2</sub>O/MeOH 95:5 $\rightarrow$  MeOH/H<sub>2</sub>O 75:25) gave the title compound **4** (81 mg, 97  $\mu$ mol, 62%) as a white solid.  $R_{\rm f}$ =0.25 (chloroform/ MeOH/H<sub>2</sub>O 7:4:1);  $[\alpha]_{D}$ +29 (c 0.1, H<sub>2</sub>O); <sup>13</sup>C NMR (75.4 MHz, D<sub>2</sub>O):  $\delta$  27.5, 40.1, 51.7, 52.7, 60.9, 65.8, 66.4, 66.4, 66.5, 66.8, 68.5, 69.9, 70.0, 70.5, 70.7, 71.0, 71.6, 73.1, 73.5, 74.8, 81.7, 99.7, 99.8, 102.6, 127.6, 128.3, 128.8, 136.5, 158.3, 173.2; <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O); δ 1.36 (s. 9H). 3.26-3.33 (m, 3H), 3.45-3.84 (m, 20H), 4.07-4.10 (m, 1H), 4.42 (d, 1H, *I*=8.0 Hz), 4.71–4.71 (m, 2H) (overlap with HDO), 5.02 (d, 1H, *I*=12.3 Hz), 5.08 (d, 1H, *I*=12.3 Hz), 7.30–7.41 (m, 5H); HRMS (ESI): [M+H]<sup>+</sup> calcd for C<sub>35</sub>H<sub>56</sub>N<sub>3</sub>O<sub>20</sub>, 838.3379; found 838.3356.

#### Supplementary data

Spectroscopic data, <sup>1</sup>H, <sup>13</sup>C NMR and IR for all new compounds are available. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2012.05.118. These data include MOL files and InChiKeys of the most important compounds described in this article.

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