



## Synthesis of an Fmoc-threonine bearing core-2 glycan: A building block for PSGL-1 via Fmoc-assisted solid-phase peptide synthesis

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### ABSTRACT

Selectins (L, E, and P) are vascular endothelial molecules that play an important role in the recruitment of leukocytes to inflamed tissue. In this regard, P-Selectin glycoprotein-1 (PSGL-1) has been identified as a ligand for P-Selectin. PSGL-1 binds to P-Selectin through the interaction of core-2 O-glycan expressing sialyl Lewis<sup>x</sup> oligosaccharide and the three tyrosine sulfate residues. Herein, we report the synthesis of threonine-linked core-2 O-glycan as an amino acid building block for the synthesis of PSGL-1. This building block was further incorporated in the Fmoc-assisted solid-phase peptide synthesis to provide a portion of the PSGL-1 glycopeptide.

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### 1. Introduction

Selectins (L, E, and P) are a family of proteins that mediate the adhesion of leukocytes and platelets to vascular surfaces.<sup>1,2</sup> They are responsible for the initial steps of extravasation of leukocytes during an inflammatory response by the recognition of the specific carbohydrate-binding ligands.<sup>3</sup> Inhibitors of selectins may act as potential therapeutic reagents targeting diseases such as asthma, psoriasis, rheumatoid arthritis, acute respiratory distress syndrome (ARDS), lupus, cancer metastasis, septic shock, or reperfusion syndrome diseases.<sup>4</sup> Although there are many candidates for selectins as ligands, only P-selectin glycoprotein ligand-1 (PSGL-1) has been demonstrated to mediate the adhesion of leukocytes to endothelial cells.<sup>5</sup> P-Selectin binds to the extreme N-terminal region of PSGL-1 through a properly positioned core-2 O-glycan expressing sialyl Lewis<sup>x</sup> (sLe<sup>x</sup>) oligosaccharide at Thr57 and at least one of the three sulfated tyrosine residues at Tyr46, Tyr48, and Tyr51.<sup>5,6</sup> It has also been demonstrated that the truncated N-terminal PSGL-1 monomer exhibits high affinity toward P-selectin.<sup>7</sup> Therefore, the development of efficient synthetic methods for the generation of truncated PSGL-1 glycosulfopeptides remains essen-

tial both for the study of the structure and function of P-selectin and for the discovery of new anti-inflammatory agents.

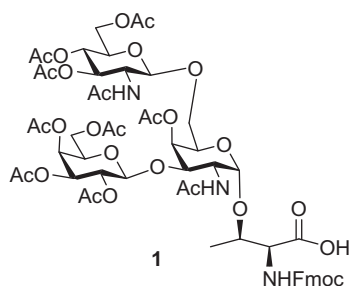
Leppanen et al. employed a chemoenzymatic method to synthesize a series of glycosulfopeptides and to define the important structural moieties that mediate P-selectin and L-selectin interactions with PSGL-1.<sup>7,8</sup> Subsequent to that report, a number of efforts have been directed at the synthesis of glycosulfopeptides with only limited success in improving overall reaction efficiency and yield.<sup>9</sup> As a consequence, many investigative groups continue to pursue the synthesis of oligosaccharide components as intermediates in the solid-phase synthesis of a truncated PSGL-1 monomer.<sup>10</sup> In this report, we describe the synthesis of an Fmoc-threonine bearing trisaccharide **1** (Fig. 1) and demonstrate its facile incorporation in solid-phase peptide synthesis. The trisaccharide-linked threonine **1** represents the core-2 O-glycan on which sLe<sup>x</sup> is to be constructed through a combination of chemical and enzymatic approaches.<sup>11</sup> Sialylation is generally challenging to install chemically, while efficient sialyltransferases have been cloned and are readily available. However, sialyltransferases generally have low efficiency toward a fucosylated Le<sup>x</sup> structure; therefore, it is more efficient to install fucose enzymatically after sialylation. While installing  $\beta$ -(1→4)-D-galactose is relatively easier chemically, the highly efficient  $\beta$ -(1→4)-galactosyltransferase is commercially available.

The synthesis of a similar Fmoc-threonine bearing core-2 as building block for the peptide synthesis has been previously reported.<sup>12</sup> However, the oligosaccharide component was protected

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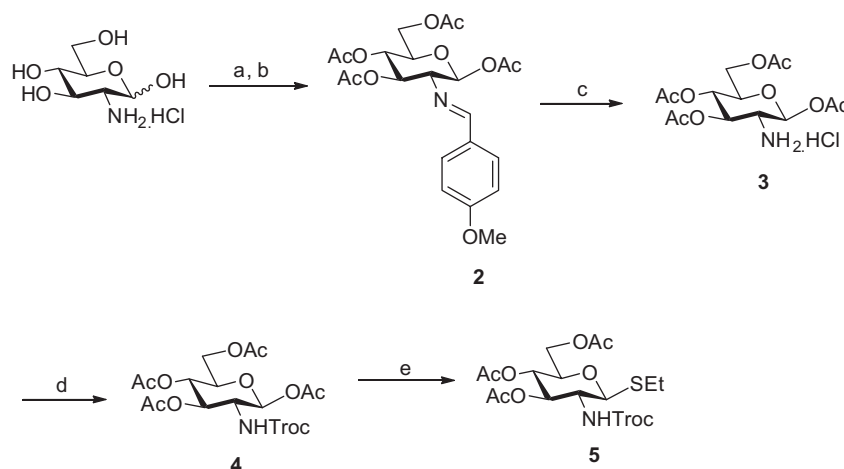


**Figure 1.** Fmoc-threonine bearing core-2 O-glycan as a building block for PSGL-1.

as an *O*-benzoate that requires more severe basic conditions for removal of the benzoyl protecting groups, thereby rendering them less suitable for use in glycopeptide synthesis.<sup>13</sup> Our intention was to synthesize an appropriate building block where the protecting groups in core-2 are fully compatible with solid-phase peptide synthesis, including cleavage conditions necessary for further enzymatic reactions required to obtain the full-length PSGL-1. Thus, we designed Fmoc-threonine bearing core-2 with acetyl protecting groups **1**, as a building block for PSGL-1. In the Fmoc protocol, the iterative removal of the *N*<sup>z</sup>-Fmoc group with piperidine,<sup>14</sup> together with the coupling reagents HBTU/HOBt<sup>15</sup> for peptide elongation, can be achieved without affecting the *O*-acetyl groups of the trisaccharide or inducing  $\beta$ -elimination of the glycan.<sup>16</sup> The use of electron-withdrawing *O*-acetyl protecting groups stabilizes the glycosidic linkage during TFA cleavage of the peptide from solid support, and the acetyl groups can be subsequently removed by treatment with dilute sodium methoxide in methanol or hydrazine hydrate.<sup>8,17</sup>

## 2. Results and discussion

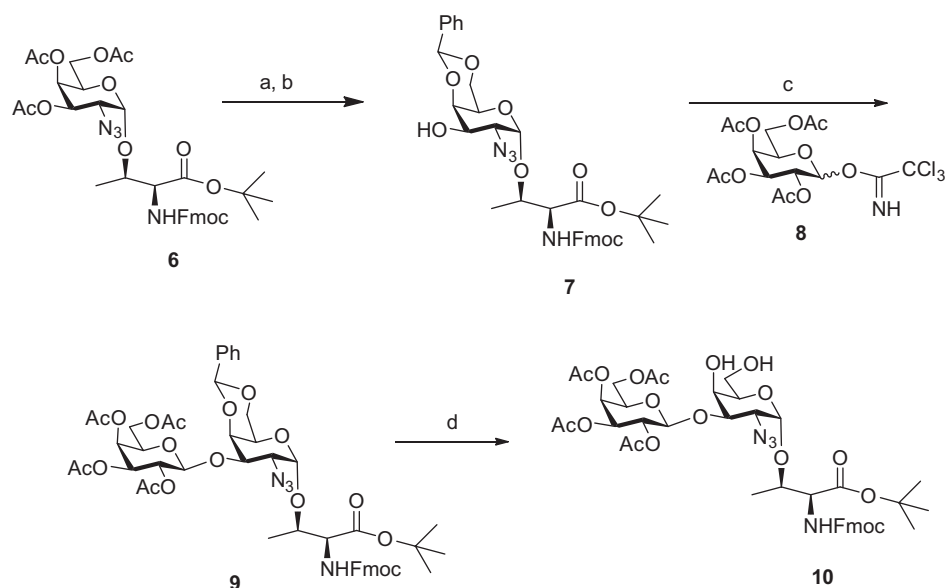
The synthesis of **1** commenced with the preparation of donor **5**. Condensation of glycosamine hydrochloride with *p*-anisaldehyde generated an aldimine that was then peracetylated to obtain **2**.<sup>18</sup> The aldimine was hydrolyzed, and the resulting amine **3** was protected with Troc-Cl, generating **4**.<sup>19</sup> It was decided to use a thioglycoside for the glycosylation due to its inherent stability and mild reaction conditions. Therefore, the anomeric acetate was selectively converted to thioether **5**<sup>20</sup> via reaction with ethanethiol in the presence of  $\text{BF}_3 \cdot \text{OEt}_2$  and 4 Å molecular sieves (Scheme 1).



**Scheme 1.** Synthesis of donor **5**. Reagents and conditions: (a) *p*-anisaldehyde 4 M NaOH, 1 h, 71%; (b)  $\text{Ac}_2\text{O}$ , DMAP (cat.), 16 h, 80%; (c) 5 M HCl, acetone, 0.5 h, 92%; (d) Troc-Cl, pyridine, DIPEA, 16 h, 80%; (e) EtSH,  $\text{BF}_3 \cdot \text{OEt}_2$ , 4 Å MS,  $\text{CH}_2\text{Cl}_2$ , 2 h, 80%.

The synthesis of **10** began from the known galactose derivative **6**.<sup>21</sup> Hydrolysis of the acetates, followed by addition of benzaldehyde dimethylacetal in the presence of PTSA, generated the desired benzylidene acetal **7** in an overall yield of 53%.<sup>12</sup> The imidate **8**,<sup>22</sup> which was generated in two steps from commercially available  $\beta$ -D-galactose pentaacetate, was added to acceptor **7** to smoothly generate the disaccharide **9**. Finally, acid hydrolysis of acetal **9** in refluxing acetic acid generated diol **10** in moderate 54% yield along with 28% recovery of **9**. All efforts aimed to improve the yield via increasing reaction time led to the hydrolysis of the *tert*-butyl ester in **10** (Scheme 2). After activation of the donor **5** using *N*-iodosuccinimide at 0 °C, addition to acceptor **10** generated two products, which can be separated by flash chromatography. The first product was the desired trisaccharide **11**, and the second product was found to be an undesired tetrasaccharide **11a** generated from glycosylation of the donor **5** to both hydroxyl groups in **10**. The structure of the trisaccharide **11** was confirmed by the combination of 1D proton and 2D gCOSY, TOCSY, gHSQC, gHMBC, and NOESY NMR spectra. In the gHMBC NMR spectrum of **11** (see Supplementary data), the cross-peaks from 101.3 ppm (C-1 of the glucosamine residue, A-C1) and 4.02 ppm (H-6 of the galactosamine residue, B-H6) and from 4.70 ppm (H-1 of the glucosamine residue, A-H1) and 69.5 ppm (C-6 of the galactosamine residue, B-C6), suggest that the *O*-6 of the galactosamine residue is glycosylated. The (1→6)-linkage between the galactosamine and the glucosamine was further confirmed by the NOESY spectrum, which displayed a cross-peak between H-1 of the glucosamine residue and H-6 of the galactosamine residue. The combination of these 2D NMR analyses allowed the complete structural assignment of **11** which is listed in Table 1. Next, the trisaccharide **11** was acetylated with acetic anhydride in pyridine in the presence of catalytic DMAP, and the acetylated derivative was subsequently taken to the Troc deprotection step. Thus, addition of freshly activated zinc dust in the presence of acetic anhydride as a solvent reduced both the azide and NH-Troc group along with concomitant protection of both amines as corresponding acetates to generate **12** in an overall yield of 47%. Finally, hydrolysis of the *tert*-butyl ester on the threonine residue **12** was accomplished by treatment with trifluoroacetic acid in dichloromethane at room temperature affording the trisaccharide-linked threonine **1** in 72% yield (Scheme 3).

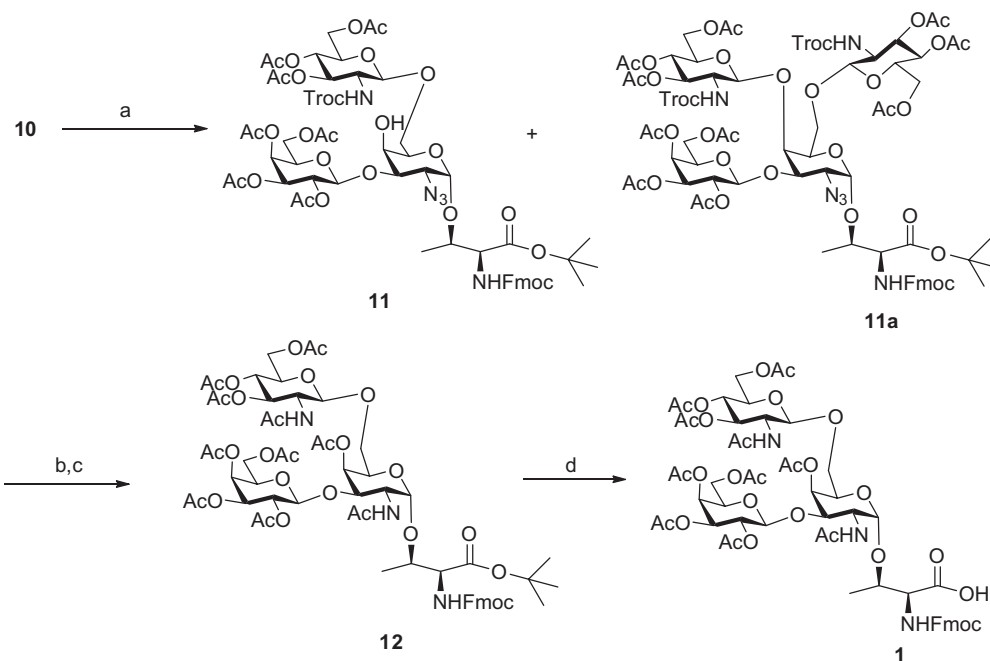
Next, we investigated the incorporation of the trisaccharide-linked threonine residue **1** into an Fmoc-based SPPS strategy using a Rink amide functionalized resin. The iterative amino acid coupling using the HBTU/HOBt method provided the glycosylated



**Scheme 2.** Synthesis of disaccharide **10**. Reagents and conditions: (a) NaOMe, MeOH, 3 h; (b) benzaldehyde dimethyl acetal, PTSA, MeNO<sub>2</sub>, 2 h, 53%; (c) TMSOTf, **8**, CH<sub>2</sub>Cl<sub>2</sub>, 4 Å MS, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 1 h, 61%; (d) 80% HOAc, 70 °C, 2 h (**10**: 54%, **9**: 28%).

**Table 1**  
Structural assignment of trisaccharide **11**

Residue	Nucleus	1	2	3	4	5	6
Gal (C)	<sup>1</sup> H	4.74	5.26	5.03	5.61	3.97	4.19/4.08
	<sup>13</sup> C	102.1	68.6	68.8	67.0	71.3	61.5
Gal-N <sub>3</sub> (B)	<sup>1</sup> H	4.99	3.54	3.98	4.13	3.99	4.02/3.78
	<sup>13</sup> C	99.3	58.8	77.9	68.5	69.3	69.5
GlcNTroc-1,6 (A)	<sup>1</sup> H	4.70	3.63	5.28	5.04	3.69	4.24/4.14
	<sup>13</sup> C	101.3	56.4	72.1	70.9	71.9	62.2



**Scheme 3.** Synthesis of trisaccharide-linked Fmoc-threonine **1**. Reagents and conditions: (a) **5**, NIS, TfOH, 4 Å MS, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 12 h (**11**: 51%, **11a**: 19%); (b) Ac<sub>2</sub>O, DMAP, pyridine, 1 h, 47%; (c) Zn dust, Ac<sub>2</sub>O–AcOH–THF, 3 h, 47%; (d) TFA, CH<sub>2</sub>Cl<sub>2</sub>, 2 h, 72%.

peptide **13** on solid support. Subsequent treatment of **13** with 9.5:0.2:0.2:0.1 TFA-EDT-TES-H<sub>2</sub>O cleaved the peptide from resin without damaging the glycan. These conditions also cleaved the *tert*-butyl ester and trityl protecting groups, thereby, yielding the desired glycopeptide **14** (Scheme 4) in 50% yield (determined by HPLC). Product **14** was characterized by MALDI-TOFMS and reversed-phase HPLC, both of which confirmed the expected glycopeptide. The second product **14a** (43% yield determined HPLC) was identified as a variant of glycopeptide **14** in which the C-terminal cysteine underwent a disulfide linkage with ethanedithiol, the reagent used in the final cleavage step of glycopeptide **14** from the resin.<sup>23,24</sup>

In summary, the Fmoc-threonine bearing core-2 O-glycan **1** was successfully synthesized, and the GlcNAc $\beta$ 1,6-GalNAc linkage was verified by 2D NMR methods. This building block can be readily incorporated into solid-phase peptide synthesis to provide a portion of the PSGL-1 glycopeptide that can be further modified by chemoenzymatic means to obtain the full-length PSGL-1.

### 3. Experimental

#### 3.1. General

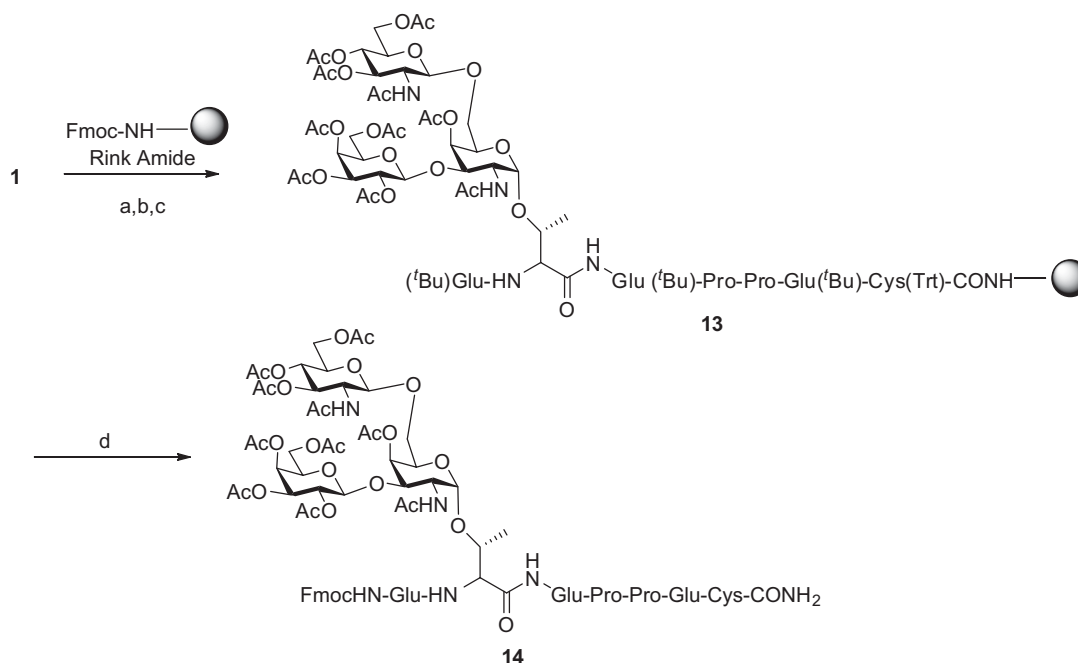
All reagents were purchased from commercial sources and used as received, unless otherwise indicated. All solvents were dried and distilled by standard protocols. All reactions were performed under an inert atmosphere of argon or nitrogen, unless otherwise indicated. For peptide synthesis, Rink amide resin (resin loading: 0.4 mmol/g), DMF (Biotech grade), and piperidine were purchased from Novabiochem. HOBt, PyBOP, and all Fmoc-protected amino acids were purchased from Peptides International. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded with an Inova 600-MHz spectrometer. High-resolution ESI-Mass spectra (HRESIMS) were recorded by The Emory University Mass Spectrometry Center using a JEOL JMS-SX102 instrument. Optical rotation values were recorded using a Perkin-Elmer polarimeter.

#### 3.2. Preparation of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-p-methoxybenzylideneamino- $\beta$ -D-glucopyranose (**2**)

Glucosamine hydrochloride (5 g, 23.3 mmol) was dissolved in 24 mL of 1 M aq NaOH to give a colorless solution. *p*-Anisaldehyde (2.85 mL, 23.3 mmol) was added by syringe under intense stirring, to form a turbid solution. After several min of agitation a white precipitate was formed. The system was kept in an ice bath for 1 h to ensure complete precipitation. The solid was then collected by filtration and washed with water (2  $\times$  20 mL) and a mixture of 1:1 MeOH-Et<sub>2</sub>O (2  $\times$  20 mL). The precipitate was dried under vacuum to yield 2-deoxy-*p*-methoxybenzylideneamino- $\beta$ -D-glucopyranose (4.93 g, 71%) as a white solid. The precipitate was dissolved in 27 mL of pyridine and cooled to 0 °C. To this was added catalytic DMAP (50 mg, 4.1 mmol) and Ac<sub>2</sub>O (15 mL, 157.7 mmol). The solid slowly went into solution, and the reaction mixture was left at room temperature overnight. The starting material dissolved slowly over time to give a clear solution. After 16 h, the reaction mixture was poured into ice forming a white crystalline solid. The crystals were collected by filtration, washed with water (2  $\times$  10 mL) and ether (2  $\times$  10 mL), and dried under vacuum to afford the title compound **2**<sup>18</sup> (6.21 g, 80%) as a white crystalline solid. The NMR data for **2** matched those previously reported.<sup>18</sup> <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$ <sub>H</sub> 1.88 (3H, s), 2.01 (3H, s), 2.03 (3H, s), 2.10 (3H, s), 3.44 (1H, t, *J* 9.6 Hz), 3.84 (3H, s), 3.97 (1H, ddd, *J* 2.4 Hz, 4.8 Hz, 9.6 Hz), 4.12 (1H, dd, *J* 2.1 Hz, 12.6 Hz), 4.37 (1H, dd, *J* 4.5 Hz, 12.3 Hz), 5.14 (1H, t, *J* 9.6 Hz), 5.42 (1H, t, *J* 9.3 Hz), 5.94 (1H, d, *J* 8.1 Hz), 6.91 (2H, d, *J* 8.7 Hz), 7.64 (2H, d, *J* 8.4 Hz), 8.15 (s, 1H).

#### 3.3. Preparation of 2-amino-1,3,4,6-tetra-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl hydrochloride (**3**)

Compound **2** (5 g, 10.8 mmol) was dissolved in 25 mL of refluxing acetone, and to this solution was added dropwise 2.5 mL of 5 N HCl. After 30 min, a thick white precipitate formed, and the system was cooled to room temperature. The precipitate was filtered and



**Scheme 4.** Incorporation of trisaccharide-linked threonine **1** into Fmoc-assisted solid-phase peptide synthesis. Reagents and conditions: (a) Fmoc-AA-OH; HBTU/HOBt; (b) 20% piperidine in DMF; (c) repeat steps a and b for subsequent amino acid coupling; (d) 9.5:0.1:0.2:0.2 TFA-H<sub>2</sub>O-EDT-Et<sub>3</sub>SiH, 2 h.

washed with acetone (10 mL) and ether (2 × 25 mL). This was subsequently dried under vacuum overnight to provide **3**<sup>18</sup> (3.45 g, 92%) as a white powder. The NMR data for **3** matched those previously reported.<sup>18</sup> <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ<sub>H</sub> 2.03 (3H, s), 2.05 (3H, s), 2.09 (3H, s), 2.23 (3H, s), 3.62 (1H, t, *J* 9.3 Hz), 4.03–4.11 (2H, m), 4.25 (1H, dd, *J* 3.9 Hz, 12 Hz), 4.99 (1H, t, *J* 9.3 Hz), 5.42 (1H, t, *J* 9.9 Hz), 5.97 (1H, d, *J* 8.7 Hz), 8.93 (br s, 2H).

### 3.4. Preparation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-*D*-glucopyranose (**4**)

Compound **3** (1.5 g, 4.3 mmol) was dissolved in 20 mL of pyridine and cooled to 0 °C. To this were added DIPEA (0.75 mL, 4.3 mmol) and Troc-Cl dropwise over a period of 30–45 min. The reaction was allowed to stir at room temperature overnight. After 16 h, the reaction was neutralized by adding 15 mL of MeOH. The solvents were removed under reduced pressure, and the resulting residue was purified by chromatography over silica gel (1:1 EtOAc–hexanes) to obtain the title compound **4**<sup>19</sup> (1.7 g, 80%) as white foam. The NMR data for **4** matched those previously reported.<sup>19</sup> <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 2.01–2.21 (9H, m, 3 × OAc), 3.65 (1H, s, –OH), 4.01–4.29 (4H, m), 4.61–4.83 (2H, m), 5.09–5.48 (4H, m).

### 3.5. Preparation of ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-(2,2,2-trichloroethoxycarbonyl)amino-1-thio-*D*-glucopyranoside (**5**)

Compound **4** (1.68 g, 3.45 mmol) was dissolved in dichloromethane (10 mL), and 4 Å molecular sieves (1.6 g) were added. The reaction mixture was cooled to –10 °C, to which ethanethiol (0.51 mL, 6.9 mmol) and BF<sub>3</sub>·Et<sub>2</sub>O (0.87 mL, 6.9 mmol) were added in succession. After stirring at room temperature for 2 h, the suspension was filtered with Celite, washed with satd aq NaHCO<sub>3</sub> (10 mL), and concentrated under reduced pressure. The resulting oil was purified by chromatography over silica gel (1:1 EtOAc–hexanes) to yield **5**<sup>20</sup> (1.37 g, 80%) as a white foam. The NMR data for **5** matched those previously reported.<sup>20</sup> <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 1.24 (3H, t, *J* 6.4 Hz), 2.04 (9H, 3 × s), 2.69 (2H, m), 3.68 (1H, m), 3.75 (1H, dd, *J* 10.3 Hz, 1H, H-2), 4.10 (1H, dd, *J* 12.3 Hz, 2.2 Hz), 4.22 (1H, dd, *J* 12.3 Hz, 5.1 Hz, H-6), 4.60 (1H, d, *J* 10.3 Hz, H-1), 4.78 (2H, dd, *J* 12.1 Hz), 5.05 (1H, dd, *J* 9.6 Hz), 5.19 (1H, d, *J* 9.1 Hz, –NH), 5.21 (1H, dd, *J* 9.6 Hz, H-3).

### 3.6. Preparation of *N*<sup>α</sup>-(fluoren-9-ylmethoxycarbonyl)-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-α-*D*-galactopyranosyl)-*L*-threonine *tert*-butyl ester (**7**)

The galactose derivative **6** (2.99 g, 0.84 mmol) was dissolved in MeOH (84 mL), and the flask was flushed with argon. To this solution was added a freshly prepared 1 M NaOMe solution in MeOH (1 mL). The reaction mixture was stirred at room temperature for 3 h. Upon completion of the reaction the mixture was neutralized with Amberlite IR-120 (H<sup>+</sup>) resin. The reaction mixture was filtered through Celite, and the solvent was removed under reduced pressure to afford the crude diol. The diol was redissolved in nitromethane (70 mL), and flushed with argon. Benzaldehyde dimethyl acetal (1.27 mL, 8.42 mmol) was added, followed by PTSA (0.12 g, 0.63 mmol). The reaction mixture was stirred for 2 h, neutralized with TEA, azeotroped with toluene, and dried. Column chromatography on silica gel (1:5 acetone–toluene) gave acetal **7**<sup>12</sup> (103 mg, 53%) as a white foam. The NMR data for **7** matched those previously reported.<sup>12</sup> <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 1.31 (3H, d, *J* 6.4 Hz, Thr –CH<sub>3</sub>), 1.51 (9H, s, Bu), 2.31 (1H, d, *J* 4.0 Hz, –OH), 3.57 (1H, dd, 2-H), 3.78 (1H, s, Fmoc CH), 4.07 (1H, dd, *J* 10.7 Hz, 3-H), 4.16 (1H, ddd, *J* 3.6 Hz, 7.1 Hz, 7.2 Hz, 5-H), 4.22–4.30 (4H, m, 4-H, 6-H<sub>2</sub> and Thr CH<sub>2</sub>), 4.34 (1H, dd, *J* 2.5 Hz, 7.6 Hz, Thr –CH), 4.43 (2H, m, Fmoc CH<sub>2</sub>), 5.10 (1H, d, *J* 3.6 Hz, 1-H), 5.57 (1H, s, PhCH), 5.75 (1H, d, *J* 9.1 Hz,

–NH), 7.25–7.82 (13H, m, –Fmoc Ar); HRESIMS: Calcd for C<sub>36</sub>H<sub>40</sub>N<sub>4</sub>O<sub>9</sub> [M+Na], *m/z* 695.7140; found 695.2688.

### 3.7. Preparation of 2,3,4,6-tetra-*O*-acetyl-*D*-galactopyranosyl trichloroacetimidate (**8**)

Galactose pentaacetate (0.20 g, 0.51 mmol) was dissolved in DMF (2 mL) and flushed with argon, hydrazine acetate (0.047 g, 0.51 mmol) was added, and the reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with EtOAc (25 mL) and washed with water (10 mL). The EtOAc layer was dried and concentrated under reduced pressure to give a yellow residue (140 mg). This was subsequently dissolved in anhyd CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and flushed with argon. To this solution were added K<sub>2</sub>CO<sub>3</sub> (0.081 g, 0.59 mmol) and trichloroacetonitrile (0.11 mL, 1.11 mmol). The reaction mixture was stirred at room temperature for 8 h. Upon completion, the mixture was filtered through Celite and concentrated. Subsequent purification by chromatography on silica gel (1:1 EtOAc–hexanes) afforded the imidate **8**<sup>22</sup> (0.161 g, 82%) as a white foam. The NMR data for **8** matched those previously reported.<sup>22</sup> <sup>1</sup>H NMR (400 MHz): δ<sub>H</sub> 2.01 (3H, s), 2.02 (3H, s), 2.03 (3H, s), 2.17 (3H, s), 4.08 (1H, dd, *J* 6.7 Hz, 11.3 Hz), 4.17 (1H, dd, *J* 6.6 Hz, 11.3 Hz), 4.44 (1H, m), 5.36 (1H, dd, *J* 10.8 Hz, 3.4 Hz), 5.43 (1H, dd, *J* 10.8 Hz, 3.0 Hz), 5.56 (1H, dd, *J* 3.0 Hz, 1.4 Hz), 6.61 (1H, d, *J* 3.4 Hz), 8.67 (1H, s).

### 3.8. Preparation of *N*<sup>α</sup>-(fluoren-9-ylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-*D*-galactopyranosyl]-*L*-threonine *tert*-butyl ester (**9**)

Trichloroacetimidate **8** (42.7 mg, 0.087 mmol), acceptor derivative **7** (36.5 mg, 0.054 mmol), and freshly activated 4 Å molecular sieves (150 mg) were added together. The solids were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL), flushed with argon, and stirred for 2 h at 0 °C. TMSOTf (2 μL, 8.7 μmol) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h, and then quenched with Hunig's base (diisopropylethylamine, DIEA). The reaction mixture was filtered through Celite, concentrated, and dried. Column chromatography on silica gel (1:5 acetone–toluene) afforded the disaccharide **9**<sup>12</sup> (30 mg, 61%) as a clear oil. The NMR data for **9** matched those previously reported.<sup>12</sup> <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 1.33 (3H, d, *J* 6.4 Hz), 1.51 (9H, s), 1.99 (3H, s), 2.07 (3H, s), 2.17 (3H, s), 2.36 (3H, s), 3.74 (1H, s), 3.80 (1H, dd, *J* 3.6 Hz, 10.8 Hz), 3.95 (1H, t, *J* 6.4 Hz), 4.06–4.16 (3H, m), 4.20–4.32 (4H, m), 4.40–4.52 (3H, m), 4.81 (1H, d, *J* 7.6 Hz), 5.04 (1H, dd, *J* 3.2 Hz, 10.4 Hz), 5.13 (1H, d, *J* 3.6 Hz), 5.32 (1H, dd, *J* 7.6 Hz, 10.2 Hz), 5.42 (1H, d, *J* 2.8 Hz), 5.57 (1H, s), 5.76 (1H, d, *J* 9.6 Hz), 7.15–7.45 (7H, m), 7.54 (2H, dd, *J* 1.9 Hz, 7.9 Hz), 7.64 (2H, dd, *J* 3.2 Hz, 7.2 Hz), 7.78 (2H, d, *J* 7.6 Hz); HRESIMS: Calcd for C<sub>50</sub>H<sub>58</sub>N<sub>4</sub>O<sub>18</sub> [M+Na], *m/z* 1026.0013; found, 1025.3638.

### 3.9. Preparation of *N*<sup>α</sup>-(fluoren-9-ylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→3)-2-azido-2-deoxy-α-*D*-galactopyranosyl]-*L*-threonine *tert*-butyl ester (**10**)

Benzylidene acetal **9** (2.45 g, 2.44 mmol) was dissolved in a mixture of HOAc (88 mL) and H<sub>2</sub>O (22 mL) and stirred at 80 °C for 2 h. The reaction mixture was cooled to room temperature and azeotroped with toluene (2 × 45 mL). The combined organic layer was dried, concentrated, and purified by chromatography over silica gel (1:1 EtOAc–hexanes) to afford the diol **10**<sup>12</sup> (54 mg, 54%) as a white amorphous solid. The NMR data for **10** matched those previously reported.<sup>12</sup> [ $\alpha$ ]<sub>D</sub><sup>22</sup> + 66.0 (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 1.33 (3H, d, *J* 6.7 Hz, Thr –CH<sub>3</sub>); 1.51 (9H, s, –NH(Boc)), 2.00 (3H, s, OAc), 2.06 (3H, s, OAc), 2.10 (3H, s, OAc),

2.17 (3H, s, OAc), 3.57 (1H, dd, *J* 3.3 Hz, 10.5 Hz, H-2), 3.81 (1H, d, *J* 7.6 Hz, H-5), 3.93 (1H, s, H-5'), 3.95 (2H, m, 6-H<sub>2</sub>), 4.02 (1H, dd, *J* 2.9 Hz, 10.5 Hz, H-3), 4.11 (1H, d, *J* 5.3 Hz, H-6'), 4.17 (1H, dd, *J* 4.1 Hz, 11.7 Hz), 4.21 (1H, d, *J* 2.5 Hz, H-4), 4.26 (1H, *J* 7.1 Hz, 7.6 Hz, Fmoc -CH<sub>2</sub>), 4.30 (1H, d, *J* 8.4 Hz, Thr -CH<sup>α</sup>), 4.45 (1H, s, Fmoc -CH), 4.47 (1H, dd, *J* 1.5 Hz, 6.1 Hz, Thr -CH<sup>β</sup>), 4.76 (1H, d, *J* 7.6 Hz, H-1'), 5.04 (1H, dd, *J* 3.3 Hz, 10.5 Hz, H-3'), 5.08 (1H, d, *J* 3.8 Hz, H-1), 5.30 (1H, dd, *J* 8.1 Hz, 10.5 Hz, H-2'), 5.41 (1H, d, *J* 3.3 Hz, H-4'), 5.70 (1 H, d, *J* 9.5 Hz, -NH), 7.31 (2H, at, *J* 7.6 Hz, 7.1 Hz, Fmoc -Ar), 7.40 (2H, dt, *J* 3.8 Hz, 7.1 Hz, Fmoc -Ar), 7.63 (2H, d, *J* 6.7 Hz, Fmoc -Ar), 7.77 (2H, d, *J* 7.2 Hz, Fmoc -Ar), <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>C</sub> 19.2, 20.7, 20.8, 20.9, 28.2, 47.3, 58.7, 59.2, 61.8, 62.9, 67.5, 68.5, 69.4, 69.7, 70.8, 71.5, 76.0, 77.0, 77.2, 77.4, 78.0, 83.2, 99.4, 102.1, 120.2, 124.5, 125.4, 127.2, 127.3, 127.9, 141.5, 143.9, 144.1, 156.9, 169.4, 169.8, 170.2, 170.3, 170.7; HRESIMS: Calcd for C<sub>43</sub>H<sub>53</sub>N<sub>4</sub>O<sub>18</sub> [M+Na]<sup>+</sup>, *m/z* 937.3325; found, 937.3335.

**3.10. N<sup>α</sup>-(Fluoren-9-ylmethoxycarbonyl)-O- $\{$ O-(2',3',4',6'-tetra-O-acetyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 3)-O-[3,4,6-tri-O-acetyl-2-deoxy-2,2,2-trichloroethoxycarbonylamino]- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)]-2-azido-2-deoxy- $\alpha$ -D-galactopyranosyl-L-threonine tert-butyl ester (11)**

Donor **5** (130 mg, 0.24 mmol) and acceptor **10** (180 mg, 0.20 mmol) were mixed with freshly activated 4 Å molecular sieves (500 mg). The reaction mixture was suspended in CH<sub>2</sub>Cl<sub>2</sub> (8.0 mL) and stirred at room temperature for 2 h, then cooled to 0 °C. *N*-Iodosuccinimide (0.09 g, 0.40 mmol) was added, and the suspension was stirred for 15 min. Trifluoromethanesulfonic acid (3.5 μL, 0.04 mmol) was added, and stirring was continued overnight at room temperature. After 12 h, the reaction mixture was filtered through Celite into an aqueous solution of sodium thiosulfate with disappearance of the dark red color as the solution became colorless. The organic phase was separated, dried, and concentrated under reduced pressure. Subsequent purification by chromatography over silica gel (3:2 EtOAc-hexane), afforded the desired trisaccharide **11** (142 mg, 51% yield) and tetrasaccharide **11a** (53 mg, 19% yield). [ $\alpha$ ]<sub>D</sub><sup>22</sup> +45.4 (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.31 (3H, d, *J* 6.0 Hz, Thr -CH<sub>3</sub>), 1.51 (9H, s, -NHBoc), 1.98 (3H, s, OAc), 2.00 (3H, s, OAc), 2.01 (3H, s, OAc), 2.02 (3H, s, OAc), 2.05 (3H, s, OAc), 2.08 (3H, s, OAc), 2.10 (3H, s, OAc), 3.54 (1H, d, *J* 10.8 Hz, Gal-N<sub>3</sub> H-2), 3.67 (1H, m), 3.74 (1H, m), 4.20–4.40 (12 H, m), 4.50–4.61 (2H, m, Fmoc CH), 4.67 (1H, d, *J* 8.4 Hz, Thr CH<sup>β</sup>), 4.70 (1H, d, *J* 12.0 Hz, GlcNTroc-1,6 H-1), 4.74 (1H, d, *J* 7.8 Hz, Gal H-1), 4.83 (1H, d, *J* 12.6 Hz), 4.99 (1H, d, *J* 9.0 Hz, Gal-N<sub>3</sub> H-1), 5.11 (3H, m), 5.26 (1H, ddd, *J* 8.4 Hz, 12 Hz, 22.5 Hz, Gal H-2), 5.28 (1H, d, *J* 3.0 Hz, GlcNTroc-1,6 H-3), 5.52 (1H, d, *J* 8.4 Hz), 5.61 (1H, d, *J* 9.6 Hz, Gal H-4), 6.07 (1H, d, *J* 9.6 Hz, Thr -NH), 7.16 (1H, d, *J* 6.6 Hz), 7.32 (2H, dd, *J* 7.2 Hz, 7.8 Hz, -Fmoc Ar), 7.43 (2H, m, -Fmoc Ar), 7.63 (2H, d, *J* 7.2 Hz, -Fmoc Ar), 7.77 (2H, d, *J* 7.8 Hz, -Fmoc Ar); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>C</sub> 19.2, 20.7, 20.8, 20.9, 20.9, 28.2, 47.3, 56.5, 58.7, 59.2, 61.5, 62.2, 67.0, 67.7, 68.6, 68.7, 69.2, 69.3, 70.9, 71.3, 72.5, 72.1, 74.6, 75.6, 83.4, 95.6, 99.2, 101.3, 102.2, 120.2, 125.1, 125.3, 127.2, 127.3, 127.9, 141.5, 143.9, 144.0, 154.2, 157.1, 158.7, 159.3, 169.5, 169.7, 169.9, 170.3, 170.4, 170.6, 170.8, 170.9; HRESIMS: Calcd for C<sub>58</sub>H<sub>72</sub>Cl<sub>3</sub>N<sub>5</sub>O<sub>27</sub> [M+K]<sup>+</sup>, *m/z* 1414.31144; found, 1414.31119.

Analytical data for **11a**: [ $\alpha$ ]<sub>D</sub><sup>22</sup> +7.5 (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 1.33 (3H, d, *J* 6.6 Hz, Thr -CH<sub>3</sub>), 1.50 (9H, s, -NHBoc), 2.04 (27H, s, 9 × 3 OAc's), 2.18 (3H, s), 3.54 (1H, dd, *J* 3.6 Hz, 10.8 Hz), 3.61 (1H, m), 3.70 (1H, d, *J* 7.2 Hz), 3.80 (1H, t, *J* 7.8 Hz), 3.95–4.4 (18H, m), 4.41 (1H, d, *J* 6.0 Hz), 4.48 (1H, dd, *J* 7.2 Hz, 9.6 Hz), 4.58 (1H, d, *J* 12.0 Hz), 4.73 (1H, d, *J* 19.2 Hz), 4.76 (1H, d, *J* 7.8 Hz), 4.86 (1H, d, *J* 12.0 Hz), 5.00 (1H, d, *J* 4.2 Hz), 5.10–5.03 (4H, m), 5.30 (2H, m), 5.41 (1H, d, *J* 3.0 Hz), 5.49 (1H, d, *J* 8.4 Hz), 5.65 (1H, d, *J* 9.6 Hz), 6.05 (1H, d, *J* 8.5 Hz, 1H), 7.17 (1H, d, *J*

7.2 Hz), 7.31 (2H, ddd, *J* 2.4 Hz, 7.2 Hz, 7.2 Hz, -Fmoc Ar), 7.41 (2H, ddd, *J* 3.7 Hz, 7.2 Hz, 7.2 Hz, -Fmoc Ar), 7.63 (2H, d, *J* 7.8 Hz, -Fmoc Ar), 7.77 (2H, d, *J* 7.8 Hz, -Fmoc Ar); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ<sub>C</sub> 19.2, 20.7, 20.8, 20.9, 21.1, 28.2, 47.3, 55.6, 56.2, 59.1, 59.6, 60.8, 61.8, 62.2, 67.0, 67.5, 68.5, 69.0, 69.2, 69.8, 71.4, 72.0, 72.5, 72.6, 73.4, 74.5, 75.4, 75.6, 83.3, 97.6, 96.0, 98.7, 101.2, 102.1, 120.2, 125.3, 125.4, 125.5, 127.2, 127.3, 127.9, 128.4, 129.2, 154.2, 155.0, 155.8, 169.4, 169.6, 170.1, 170.3, 170.6, 170.7, 170.8; HRESIMS: Calcd for C<sub>73</sub>H<sub>90</sub>Cl<sub>6</sub>N<sub>6</sub>O<sub>36</sub>Cl<sub>6</sub> [M+Na]<sup>+</sup>, *m/z* 1859.3425; found, 1859.34469. Calcd for [M+K]<sup>+</sup>, *m/z* 1875.31645; found, 1875.31979.

**3.11. N<sup>α</sup>-(Fluoren-9-ylmethoxycarbonyl)-O- $\{$ O-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 3)-O-[2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)]-2-acetamido-4-O-acetyl-2-deoxy- $\alpha$ -D-galactopyranosyl-L-threonine tert-butyl ester (12)**

The trisaccharide **11** (61 mg, 0.04 mmol) was dissolved in 3:2:1 THF-AcOH-Ac<sub>2</sub>O (10 mL). Zinc powder (1 g) activated in 2% aq CuSO<sub>4</sub> was added, and the reaction mixture was stirred at room temperature for 1 h. The solid was filtered off through a pad of Celite, and the residue was diluted with CH<sub>2</sub>Cl<sub>2</sub>, and then filtered through a pad of Celite. The CH<sub>2</sub>Cl<sub>2</sub> layer was subsequently washed with 10% aq HCl (25 mL), collected, and dried over anhyd Na<sub>2</sub>SO<sub>4</sub>. The solvents were removed under reduced pressure, and the residue was subsequently dissolved in pyridine (1 mL) and cooled to 0 °C. Excess Ac<sub>2</sub>O (3 mL) was added to this solution, followed by the addition of a catalytic amount of DMAP (10 mg). The reaction mixture was allowed to warm to room temperature and stir for an additional 3 h. After 3 h the solvents were removed under reduced pressure, and the resulting oil was purified by column chromatography (6:1 EtOAc-hexanes) over silica gel to yield **12** as a white amorphous solid (34 mg, 54%). [ $\alpha$ ]<sub>D</sub><sup>22</sup> +38.3 (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 1.33 (3H, d, *J* 6.6 Hz, Thr -CH<sub>3</sub>); 1.51 (9H, s, -NHBoc), 2.04 (24 H, 8 s, OAc), 3.46 (2H, m), 3.55 (1H, dd, *J* 3.6 Hz, 10.2 Hz, Gal-N<sub>3</sub> H-2), 3.68 (1H, d, *J* 8.4 Hz), 3.91 (2H, m), 4.20–4.00 (8H, m), 4.28 (5H, m), 4.39 (1H, m), 4.49 (1H, dd, *J* 7.2 Hz, 10.2 Hz), 4.57 (1H, d, *J* 11.4 Hz), 4.73 (2H, t, *J* 7.8 Hz, Gal H-1), 4.87 (1H, d, *J* 12.0 Hz, GlcNTroc-1,6 H-1), 5.05–5.00 (4H, m), 5.18 (1H, dd, *J* 7.8 Hz, 10.2 Hz), 5.36 (2H, m), 5.42 (1H, d, *J* 2.4 Hz), 5.45 (1H, d, *J* 7.8 Hz), 5.65 (1H, d, *J* 9.6 Hz, Gal H-4), 7.32 (2H, ddd, *J* 3.0 Hz, 7.8 Hz, 7.8 Hz, -Fmoc Ar), 7.41 (2H, ddd, *J* 4.2 Hz, 7.8 Hz, 7.8 Hz, -Fmoc Ar), 7.63 (2H, d, *J* 7.2 Hz, -Fmoc Ar), 7.77 (2H, d, *J* 6.6 Hz, -Fmoc Ar); HRESIMS: C<sub>61</sub>H<sub>79</sub>N<sub>3</sub>O<sub>28</sub> Calcd for [M+Na]<sup>+</sup>, *m/z* 1324.47478; found, 1324.47592. Calcd for [M+K]<sup>+</sup>, *m/z* 1340.44872; found, 1340.45010.

**3.12. N<sup>α</sup>-(Fluoren-9-ylmethoxycarbonyl)-O- $\{$ O-(2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 3)-O-[2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)]-2-acetamido-4-O-acetyl-2-deoxy- $\alpha$ -D-galactopyranosyl-L-threonine (1)**

The protected trisaccharide **12** (27 mg) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) and cooled to 0 °C. Trifluoroacetic acid (2 mL) was added, and the reaction mixture was allowed to warm slowly to room temperature. After 2 h the solvent was concentrated under reduced pressure, and the residue was loaded onto a reversed-phase HPLC column with gradient eluting from 70:30 to 30:70 of H<sub>2</sub>O-CH<sub>3</sub>CN at a flow rate of 6 mL/min. The fraction eluting at a retention time of 58 min was collected and concentrated under reduced pressure to obtain **1** as a white amorphous solid (19 mg, 72% yield). [ $\alpha$ ]<sub>D</sub><sup>22</sup> +35.7 (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ<sub>H</sub> 1.21 (3H, d, *J* 6.6 Hz, Thr -CH<sub>3</sub>), 2.04 (30H, s, 10 × 3 OAc's), 2.48 (1H, br s, -OH), 3.43 (1H, t, *J* 8.4 Hz), 3.66–3.69 (2H, m), 3.79–3.83 (4H, m), 4.04–4.13 (5H, m), 4.22–4.29 (5H, m), 4.42 (1H, d, *J* 6.0 Hz), 4.56–4.51 (3H, m), 4.68 (1H, d, *J* 7.8 Hz), 4.84 (1H, m), 4.92 (1H, m), 4.97–5.03 (2H, m), 5.25

(2H, m), 5.32 (1H, d,  $J$  3.6 Hz), 7.30 (2H, ddd,  $J$  3.0 Hz, 7.8 Hz, 7.8 Hz, -Fmoc Ar), 7.39 (2H, ddd,  $J$  4.2 Hz, 7.8 Hz, 7.8 Hz, -Fmoc Ar), 7.50 (1H, br s, -NH), 7.60 (2H, d,  $J$  7.2 Hz, -Fmoc Ar), 7.76 (2H, d,  $J$  6.6 Hz, -Fmoc Ar);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  18.5, 20.7, 20.8, 20.9, 22.9, 23.1, 29.8, 47.4, 49.3, 49.4, 49.5, 49.7, 49.8, 50.0, 54.8, 58.5, 61.1, 62.1, 66.7, 66.9, 68.5, 68.6, 68.7, 68.8, 69.5, 70.7, 70.9, 72.0, 72.5, 76.1, 98.9, 99.0, 100.5, 101.0, 120.1, 125.0, 127.3, 127.4, 127.9, 141.5, 143.9, 144.0, 157.0, 157.2, 169.7, 169.8, 170.6, 170.8, 171.0, 171.1, 172.1; HRESIMS: Calcd for  $\text{C}_{57}\text{H}_{71}\text{N}_3\text{O}_{28}$   $[\text{M}+\text{H}]^+$ ,  $m/z$  1246.1769; found, 1246.4297.

### 3.13. Glycopeptide 14

The peptide **14** was synthesized manually on a Rink amide resin using a standard Fmoc amino acid coupling strategy. Briefly, Fmoc-Cys-Rink-amide resin (0.4 mg, 0.5 mmol/g) was loaded into a polypropylene centrifuge filter tube (0.22  $\mu\text{m}$ , Corning International) equipped with a plastic cap. The resin was swelled by stirring gently with  $\text{CH}_2\text{Cl}_2$  ( $4 \times 500 \mu\text{L}$ ) for 10 min and filtered. The coupling reaction was performed with Fmoc amino acid (1.6  $\mu\text{mol}$ , 8  $\mu\text{L}$ , 0.2 M), HBTU (1.6  $\mu\text{mol}$ , 8  $\mu\text{L}$ , 0.2 M), HOBt (1.6  $\mu\text{mol}$ , 8  $\mu\text{L}$ , 0.2 M), and DIEA (2.4  $\mu\text{mol}$ , 8  $\mu\text{L}$ , 0.3 M). Fmoc deprotection was performed twice with 20% piperidine in DMF (100  $\mu\text{L}$ ). Cleavage of the peptide and removal of the side chain protecting group from the resin were accomplished by gently stirring in 500  $\mu\text{L}$  of 1:2:2:95 of  $\text{H}_2\text{O}$ -EDT-Et<sub>3</sub>SiH-TFA for 2 h. The solvent was evaporated, and the yield of glycopeptide **14** was determined to be 50% (yield was determined by HPLC). The yield of the second product **14a** was determined to be 43% (yield was determined by HPLC). The molecular weights of glycopeptide **14** and **14a** were determined by MALDI-TOFMS analysis. Calcd for **14**  $\text{C}_{85}\text{H}_{112}\text{N}_{10}\text{O}_{39}\text{S}$   $[\text{M}+\text{Na}]^+$ ,  $m/z$  1952.6546; found, 1952.706. Calcd for **14a**  $\text{C}_{87}\text{H}_{116}\text{N}_{10}\text{O}_{39}\text{S}_3$   $[\text{M}+\text{H}]$ ,  $m/z$  2021.66415; found, 2022.677. Calcd for  $[\text{M}+\text{Na}]$ ,  $m/z$  2043.6461; found, 2044.685.

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### Supplementary data

Supplementary data (1D and 2D NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2010.05.004.

### References

- Ley, K.; Laudanna, C.; Cybulsky, M. I.; Nourshargh, S. *Nat. Rev. Immunol.* **2007**, *7*, 678–689.
- Vestweber, D.; Blanks, J. E. *Physiol. Rev.* **2009**, *79*, 181–213.

- (a) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.; Paulson, J. C. *Science* **1990**, *250*, 1130–1132; (b) Walz, G.; Arrufo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132–1135; (c) McEver, R. P.; Moore, K. L.; Cummings, R. D. *J. Biol. Chem.* **1995**, *270*, 11025–11028.
- Welply, J. K.; Keene, J. L.; Schmuke, J. J.; Howard, S. C. *Biochim. Biophys. Acta* **1994**, *1197*, 215–226.
- (a) Wilkins, P. P.; Moore, K. L.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem.* **1995**, *270*, 22677–22680; (b) DeLuca, M.; Dunlop, L. C.; Andrews, R. K.; Flannery, J. V., Jr.; Ettling, R.; Cumming, D. A.; Veldman, G. M.; Berndt, M. C. *J. Biol. Chem.* **1995**, *270*, 26734–26737; (c) Sako, D.; Comess, K. M.; Barone, K. M.; Camphausen, R. T.; Cumming, D. A.; Shaw, G. D. *Cell* **1995**, *83*, 323–331; (d) Pouyani, T.; Seed, B. *Cell* **1995**, *83*, 333–343.
- (a) Cummings, R. D. *Braz. J. Med. Biol. Res.* **1999**, *32*, 519–528; (b) Moore, K. L.; Stults, N. L.; Diaz, S.; Smith, D. F.; Cummings, R. D.; Varki, A.; McEver, R. P. *J. Cell Biol.* **1992**, *118*, 445–456.
- Leppanen, A.; Mehta, P.; Ouyang, Y.-B.; Ju, T.; Helin, J.; Moore, K. L.; van Die, I.; Canfield, W. M.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem.* **1999**, *274*, 24838–24848.
- (a) Leppanen, A.; White, S. P.; Helin, J.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem.* **2000**, *275*, 39569–39578; (b) Leppanen, A.; Penttila, L.; Renkonen, O.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem.* **2002**, *277*, 39749–39759.
- (a) Koeller, K. M.; Smith, M. E. B.; Huang, R. F.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 4241–4242; (b) Huang, K. T.; Wu, B. C.; Lin, C. C.; Luo, S. C.; Chen, C. P.; Wong, C.-H.; Lin, C. C. *Carbohydr. Res.* **2006**, *341*, 2151–2155; (c) Baumann, K.; Kowalczyk, D.; Gutjahr, T.; Pieczyk, M.; Jones, C.; Wild, M. K.; Vestweber, D.; Kunz, H. *Angew. Chem., Int. Ed.* **2009**, *48*, 1–6.
- (a) Vohra, Y.; Buskas, T.; Boons, G.-J. *J. Org. Chem.* **2009**, *74*, 6064–6071; (b) Baumann, K.; Kowalczyk, D.; Kunz, H. *Angew. Chem., Int. Ed.* **2008**, *47*, 3445–3449; (c) Hanashima, S.; Castagner, B.; Esposito, D.; Nokami, T.; Seeburger, P. H. *Org. Lett.* **2007**, *9*, 1777–1779; (d) Otsubo, N.; Ishida, H.; Kiso, M. *Tetrahedron Lett.* **2000**, *41*, 3879–3882.
- For chemical synthesis of sialyl Le<sup>x</sup>, see: (a) Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1991**, *209*, C1–C4; (b) Nicolaou, K. C.; Hummel, C. W.; Bockovich, N. J.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1991**, *13*, 870–872; (c) Nicolaou, K. C.; Hummel, C. W.; Iwabuchi, Y. *J. Am. Chem. Soc.* **1992**, *114*, 3126–3128; for enzymatic synthesis of sialyl Le<sup>x</sup>, see: (d) Huang, K. T.; Wu, B. C.; Lin, C. C.; Luo, S. C.; Chen, C.; Wong, C.-H.; Lin, C. C. *Carbohydr. Res.* **2006**, *341*, 2151–2155; (e) Palcic, M. M.; Venot, A.; Ratcliffe, R. M.; Hinds Gaul, O. *Carbohydr. Res.* **1989**, *190*, 1–11; (f) Dumas, D. P.; Ichikawa, Y.; Wong, C.-H.; Lowe, J. B.; Nair, R. P. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 425–428.
- Mathieux, N.; Paulsen, H.; Meldal, M.; Bock, K. J. *Chem. Soc., Perkin Trans. 1* **1997**, *13*, 2359–2368.
- (a) Paulsen, H.; Schultz, M.; Klamann, J. D.; Waller, B.; Paal, M. *Liebigs Ann. Chem.* **1985**, *66*, 2028–2048; (b) Reimer, K. B.; Meldal, M.; Kusumoto, S.; Fukase, K.; Bock, K. J. *Chem. Soc., Perkin Trans. 1* **1993**, 925–932.
- Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404–3409.
- Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927–1930.
- (a) Kihlberg, J.; Vuljanic, T. *Tetrahedron Lett.* **1993**, *34*, 6135–6138; (b) Vuljanic, T.; Bergquist, K.-E.; Clausen, H.; Roy, S.; Kihlberg, J. *Tetrahedron* **1996**, *52*, 7983–8000.
- (a) Koeller, M. K.; Smith, M. E. B.; Huang, R. F.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 4241–4242; (b) Sjolín, P.; Elofsson, M.; Kihlberg, J. *J. Org. Chem.* **1996**, *61*, 560–565.
- Silva, D. J.; Wang, H.; Allanson, N. M.; Jain, K. R.; Sofia, M. J. *J. Org. Chem.* **1999**, *64*, 5926–5929.
- Dullenkopf, D.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296*, 135–147.
- Schultz, M.; Kunz, H. *Tetrahedron: Asymmetry* **1993**, *4*, 1205–1220.
- Koeller, M. K.; Smith, M. E. B.; Wong, C.-H. *Bioorg. Med. Chem.* **2000**, *8*, 1017–1025.
- (a) Schmidt, R. R.; Stumpp, M. *Liebigs Ann. Chem.* **1983**, *49*, 4734–4737; (b) Cheng, H.; Cao, X.; Xian, M.; Fang, L.; Cai, T. B.; Ji, J. J.; Tunac, J. B.; Sun, D.; Wang, P. G. *J. Med. Chem.* **2005**, *48*, 645–652.
- Lukszo, J.; Patterson, D.; Albericio, F. *Let. Pept. Sci.* **1996**, *3*, 157–166.
- Singh, P. R.; Rajopadhye, M.; Clark, S. L.; Williams, N. E. *Tetrahedron Lett.* **1996**, *37*, 4117–4120.