Improved Synthesis of the Tetrasaccharide Repeat Unit of the O-Antigen Polysaccharide from *Escherichia coli* O77

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The efficient synthesis of a tetrasaccharide, the suitably protected form of the repeat unit, →2)-α-D-Man*p*-(1→2)-β-D-Man*p*-(1→3)-α-D-Glc*p*NAc-(1→6)-α-D-Man*p*-(1→, of the O-antigen polysaccharide of the lipopolysaccharide from *E. coli* O77 has been accomplished. Glycosylation reactions for the coupling of four monosaccharide building blocks of the tetrasaccharide were carried out employing the CB glycoside method, the mannosyl 4-pentenoate/PhSeOTf method, and the glycosyl trichloroacetimidate method with complete stereoselectivities in excellent yields.

Key Words : *Escherichia coli* O77, Glycosylation, 2'-Carboxybenzyl (CB) glycoside, Mannosyl 4-pentenoate/PhSeOTf, Glycosyl trichloroacetimidate

Introduction

Escherichia coli is a facultative anaerobic gram-negative rod and is a predominant species of the human colonic flora. Normal nonpathogenic strains of *E. coli* usually remain harmlessly confined to the intestinal lumen but when the host is immunosuppressed or debilitated, even normal non-pathogenic strains of *E. coli* can cause infection. Moreover, infections due to inherently pathogenic *E. coli* strains result in clinical syndromes such as urinary tract infections, sepsis/meningitis, and enteric/diarrheal disease. The species is sub-divided into different serotypes based on the immunogenicity of bacterial surface structures.[1,2] The *E. coli* O77, which belongs to an O-serotype, causes diarrheal infections[3] and has been identified to produce Shiga-like toxin in both human and animals.[4-7] It is also reported that natural immunity to gram-negative bacteria is often provided by antibodies that recognize lipopolysaccharide (LPS) antigens.[8] Recently, Widmalm and his associates have reported the structure of the O-antigen polysaccharide of the LPS from the *E. coli* O77.[9] As shown in Figure 1, this particular polysaccharide is composed of a series of the repeat unit of a tetrasaccharide with the following structure: →2)-α-D-Man*p*-(1→2)-β-D-Man*p*-(1→3)-α-D-Glc*p*NAc-(1→6)-α-D-Man*p*-(1→ (**A**).

Very recently, we reported the synthesis of tetrasaccharide **1**, the suitably protected form of the repeat unit **A** of the O-antigen polysaccharide of the LPS from *E. coli* O77, employing the 2'-carboxybenzyl (CB) glycoside method for the coupling of four monosaccharide building blocks (Figure 1).[10] Although the CB glycoside methodology[11] has been successfully applied to the synthesis of other oligosaccharides[11c,12] and glycospingolipids,[13] our previous synthesis of the tetrasaccharide **1** employing the CB glycoside method needs to be improved in yields and stereoselectivities in the coupling steps of monosaccharide building blocks. For the eventual synthesis of an octasaccharide or a dodecasaccharide from the suitably protected tetrasaccharide by dimerization or trimerization, more efficient methods for the coupling of four monosaccharide components including more stereoselective α- and β-mannopyranosylations and α-2-azidoglucopyranosylation are highly desirable. Herein we report the synthesis of the suitably protected tetrasaccharide **2**, of which structure is modified slightly from that of our original tetrasaccharide **1** and three glycosyl linkages could be constructed more efficiently using not only the CB glycoside method but also other glycosylation methods.

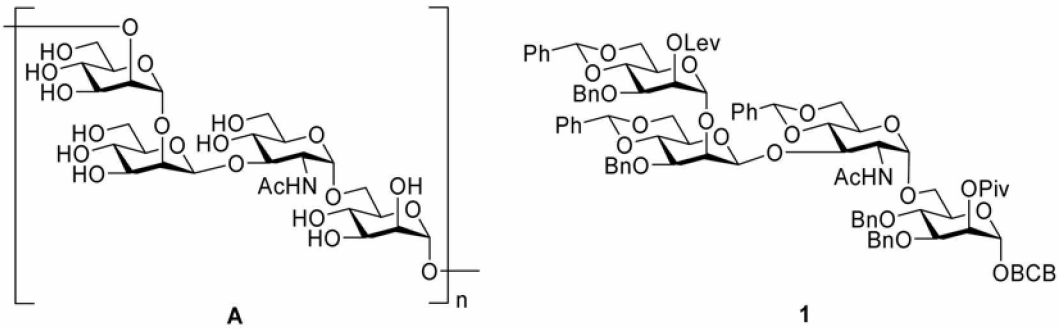


Figure 1

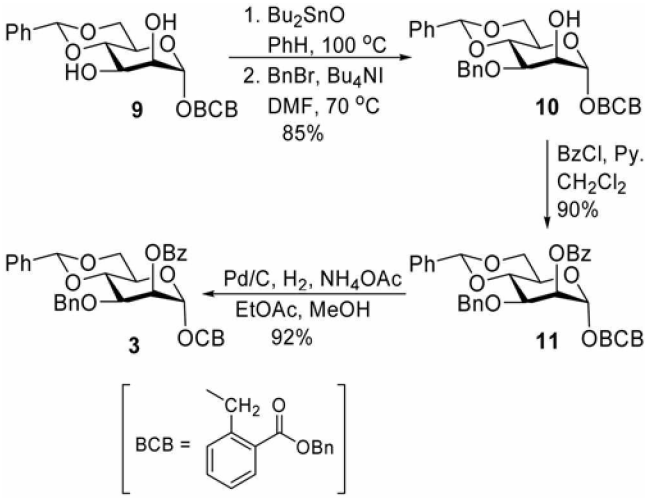
Results and Discussion

Protective groups in the target tetrasaccharide **2** were carefully chosen after consideration of the future synthesis of an octasaccharide or a dodecasaccharide by dimerization or trimerization of **2**. Thus, the thioglycoside in the reducing end of the tetrasaccharide **2** would be employed as a tetrasaccharide donor. The levulinyl (Lev) protective group of **1** is replaced with the benzoyl (Bz) group in **2** in the present synthesis because the levulinyl group resulted in the poor α-stereoselectivity in the glycosylation of the previous synthesis.[10] And the selective deprotection of the benzoyl group would provide a tetrasaccharide acceptor. One of the challenges in the synthesis of **2** would be the elaboration of the β-mannosyl linkage. Although several strategies have been developed for the β-mannopyranosylation.[14] the mannosyl 4-pentenoate/PhSeOTf method.[15] which is one of new glycosylation methods recently developed in our laboratory.[11,16] would be employed. In addition, the glycosyl trichloroacetimidate[17] as a donor would be used for the α-2-azidoglucopyranosylation.

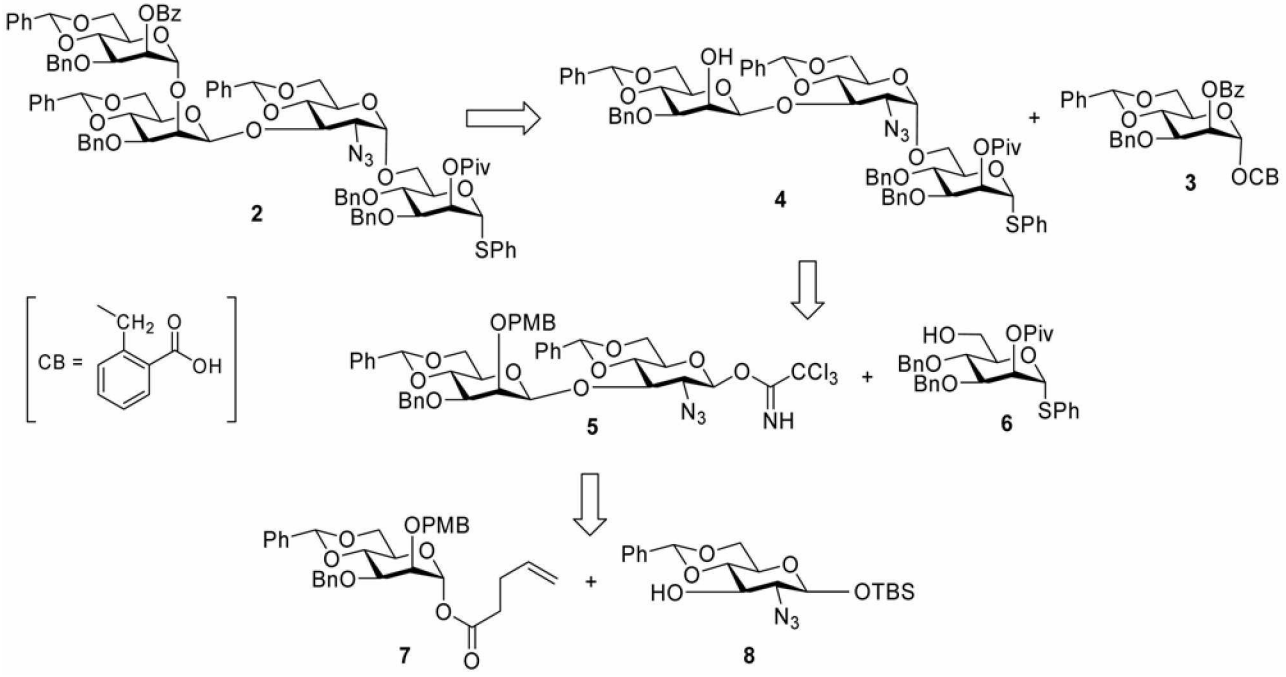
Retrosynthesis of **2**, therefore, leads to benzoyl-protected CB mannosyl donor **3** and trisaccharide acceptor **4**, and then further analysis of **4** provides disaccharyl trichloroacetimidate **5** as a donor and pivaloyl (Piv)-protected thioglycoside **6** as an acceptor (Scheme 1). The disaccharide **5** can be derived from *p*-methoxybenzyl (PMB)-protected mannosyl pentenoate **7** and 2-azido-2-deoxy-glucopyranosyl acceptor **8**.

The linear synthesis of the tetrasaccharide **2** began with the preparation of the monosaccharide building blocks **3**, **6**, **7**, and **8**. Thus, mannosyl 4-pentenoate **7**[15] and 2-azidoglucoside **8**[18] were synthesized according to previously reported procedures. The CB glycoside building block **3** was prepared from known intermediate **9**[12a] (Scheme 2). Treatment of the diol **9** with Bu_2SnO in refluxing benzene and the subsequent reaction of the resulting crude *O*-stannylene acetal with benzyl bromide in the presence of Bu_4NI in DMF afforded C-3 benzyl ether **10** in 85% yield. Benzoylation of **10** followed by the selective hydrogenolysis of resulting BCB glycoside **11** in the presence of NH_4OAc gave the desired CB glycoside **3** in high yields.

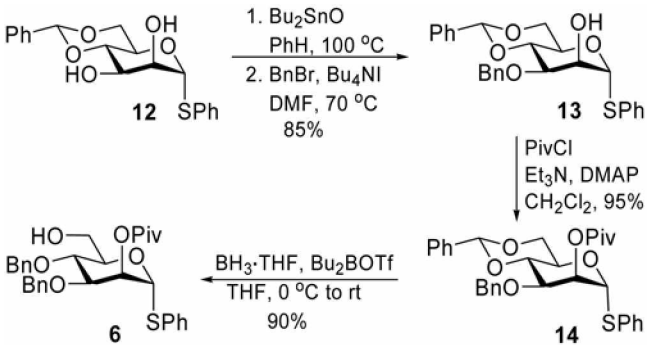
For the synthesis of the monosaccharide building block **6**, known intermediate **12**[19] was used (Scheme 3). Dibutyltin oxide-mediated selective benzylation of the diol **12** afforded C-3 benzyl ether **13** in 85% yield. Pivaloylation of **13** and the subsequent reductive cleavage of resultant benzylidene acetal **14** with borane-dibutylboron triflate[20] provided the desired C-6 alcohol **6** in high yields.



Scheme 2



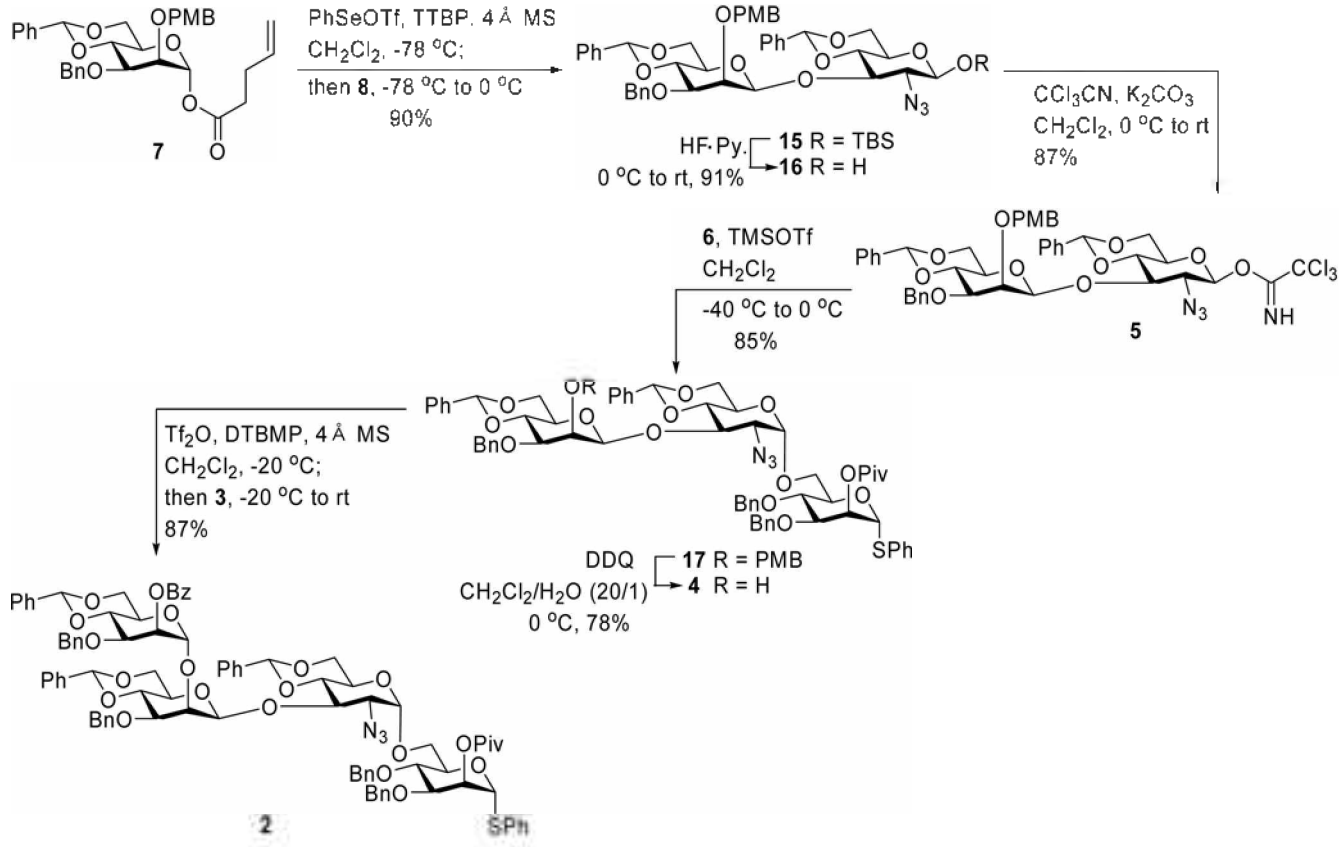
Scheme 1



Scheme 3

The stage was set for the assembly of properly protected monosaccharide building blocks **3**, **6**, **7**, and **8** to make the tetrasaccharide **2**. The crucial stereoselective β-mannopyranosylation was achieved by activation of the mannosyl 4-pentenoate donor **7** with PhSeOTf, which was readily generated *in situ* from PhSeBr and AgOTf, in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP) and 4 Å molecular sieves in CH_2Cl_2 followed by addition of the acceptor **8** at –78 °C. Although the reaction was so efficient that the donor **7** disappeared in 20 min at –78 °C, the reaction mixture was further warmed to 0 °C to make sure the completion of the reaction. Desired β-mannopyranosyl disaccharide **15** was exclusively obtained in 90% yield as shown in Scheme 4. No α-disaccharide was detected at all in the reaction mixture. On the other hand, when the glycosyl sulfoxide method[21] using 4,6-*O*-benzylidene mannosyl sulfoxide donor was employed for this β-mannopyranosylation, the desired disaccharide **15** was obtained in 85% yield (see experimental section), while CB glycoside method gave the β-disaccharide in 59% yield along with the anomeric α-disaccharide as a by-product in 14% yield in our previous synthesis. Removal of the TBS group in **15** with HF·pyridine and the subsequent reaction of resulting disaccharyl lactol **16** with CCl_3CN and K_2CO_3 furnished the β-trichloroacetimidate **5** in high yield. The coupling of the trichloroacetimidate donor **5** and the phenyl thiomannoside acceptor **6** in the presence of a catalytic amount of TMSOTf provided desired α-trisaccharide **17** exclusively in 85% yield. Subsequent removal of the *p*-methoxybenzyl (PMB) group of **17** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave the alcohol **4**. Finally, the glycosylation of the trisaccharide acceptor **4** with the CB monosaccharide donor **3** in the presence of Tf_2O and 2,6-di-*t*-butyl-4-methylpyridine (DTBMP) afforded the desired α-tetrasaccharide **2** as a single isomer in 87% yield. The result of this α-mannopyranosylation with the 2-*O*-benzoyl protected donor **3** is better than that with 2-*O*-levulinyl protected donor, which was used in our previous synthesis (65%, α/β = 2/1),[10] with respect to both yield and stereoselectivity.

In conclusion, we have described the synthesis of suitably protected tetrasaccharide repeat unit **2** of the O-antigen polysaccharide of the LPS from *E. coli* O77. All glycosylation methods employed for the coupling of the mono-



Scheme 4

saccharide building blocks **3**, **6**, **7**, and **8** to make the tetrasaccharide **2** showed the complete stereoselectivities in excellent yields.

Experimental Section

***t*-Butyldimethylsilyl (3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-azido-2-deoxy-β-D-glucopyranoside (15).**

A. Using 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-D-mannopyranosyl pentenoate: A solution of PhSeBr (190 mg, 0.81 mmol) and AgOTf (200 mg, 0.81 mmol) in CH_2Cl_2 (1 mL) in the presence of 4 Å molecular sieves (500 mg) was stirred for 15 min at rt and cooled to −78 °C, then a solution of donor **7** (90 mg, 0.16 mmol) and TTBP (210 mg, 0.84 mmol) in CH_2Cl_2 (2 mL) was added. After the resulting solution was stirred at −78 °C for 15 min, acceptor **8** (80 mg, 0.19 mmol) was added and stirred at −78 °C for 20 min and allowed to warm over 1 hr to 0 °C, then stirred for further 20 min at 0 °C. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (10 mL), and then extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (Hexane/EtOAc/CH_2Cl_2, 7 : 1 : 1) to afford the desired compound **15** (β only, 130 mg, 90%) as a colorless oil.: R_f = 0.55 (Hexane/EtOAc/CH_2Cl_2, 7 : 1 : 1); $[\alpha]_D^{20}$ = −5.4 (*c* 2.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.17 (s, 3H), 0.18 (s, 3H), 0.95 (s, 9H), 3.17-3.24 (m, 1H), 3.26 (dd, *J* = 7.6, 8.0 Hz, 1H), 3.31-3.39 (m, 1H), 3.54-3.58 (m, 1H), 3.59 (d, *J* = 8.8 Hz, 1H), 3.65 (d, *J* = 9.2 Hz, 1H), 3.75 (s, 3H), 3.79 (t, *J* = 10.4 Hz, 1H), 3.86 (dd, *J* = 4.8, 10.6 Hz, 1H), 3.99 (d, *J* = 2.8 Hz, 1H), 4.11-4.20 (m, 2H), 4.28 (dd, *J* = 4.6, 10.6 Hz, 1H), 4.60 (d, *J* = 12.4 Hz, 1H), 4.62 (d, *J* = 7.6 Hz, 1H), 4.73 (d, *J* = 12.4 Hz, 1H), 4.83 (d, *J* = 12.0 Hz, 1H), 4.90 (d, *J* = 11.6 Hz, 1H), 5.52 (s, 1H), 5.55 (s, 1H), 6.82 (d, *J* = 6.4 Hz, 2H), 7.24-7.40 (m, 13H), 7.45-7.52 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.0, −4.2, 25.7, 55.4, 66.9, 67.6, 68.6, 68.8, 68.9, 72.7, 74.7, 76.1, 78.2, 78.7, 79.6, 80.4, 97.9, 101.4, 101.5, 103.0, 113.7 126.20, 126.23, 126.4, 127.65, 127.69, 128.29, 128.32, 128.50, 128.54, 129.0, 129.2, 130.4, 130.8, 137.2, 137.8, 138.6, 159.4. HRMS Calcd for $[M+Na]^+$ 890.3660. Found 890.3664.

B. Using *S*-phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-1-deoxy-1-thio-D-mannopyranoside *S*-oxide: A solution of mannosyl sulfoxide donor (707 mg, 1.21 mmol) and DTBMP (595 mg, 2.89 mmol) in CH_2Cl_2 (40 mL) in the presence of 4 Å molecular sieves (1.0 g) was stirred for 10 min at room temperature and cooled to −78 °C, then Tf_2O (0.24 mL, 1.45 mmol) was added. To the resulting solution was added a solution of acceptor **8** (447 mg, 1.10 mmol) in CH_2Cl_2 (20 mL). After being stirred at −78 °C for 1 hr and allowed to warm over 2 hr to 0 °C, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (10 mL), and then extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (Hexane/EtOAc/CH_2Cl_2, 7 : 1 : 1) to afford the desired compound **15** (β only, 889 mg, 85%).

(3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-azido-2-deoxy-β-D-glucopyranosyl trichloroacetimidate (5). To a solution of **15** (140 mg, 0.16 mmol) in pyridine (2 mL) was added HF·pyridine (0.2 mL) at 0 °C. After the resulting solution was warmed to room temperature, it was stirred for 1 hr at room temperature. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and then extracted with CH_2Cl_2. The combined organic layer was washed with brine and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (Hexane/EtOAc, 3 : 2) to afford the desired compound **16** (110 mg, 91%).

To a solution of **16** (110 mg, 0.15 mmol) and CCl_3CN (0.15 mL, 1.5 mmol) in CH_2Cl_2 (5 mL) was added K_2CO_3 (24 mg, 1.75 mmol) at 0 °C. After the resulting solution was warmed to room temperature and stirred for 30 min at room temperature, it was concentrated *in vacuo*. The residue was purified by flash column chromatography (Hexane/EtOAc/CH_2Cl_2, 5 : 1 : 1) to afford the desired compound **5** (110 mg, 87%) as a colorless oil.: R_f = 0.43 (Hexane/EtOAc/CH_2Cl_2, 5 : 1 : 1); $[\alpha]_D^{20}$ = −2.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.19-3.27 (m, 1H), 3.50-3.69 (m, 4H), 3.70-3.75 (m, 2H), 3.77 (s, 3H), 3.80-3.88 (m, 2H), 3.99 (d, *J* = 2.4 Hz, 1H), 4.11-4.20 (m, 2H), 4.38 (dd, *J* = 4.8, 10.4 Hz, 1H), 4.62 (d, *J* = 12.4 Hz, 1H), 4.67 (s, 1H), 4.77 (d, *J* = 12.4 Hz, 1H), 4.86 (d, *J* = 11.6 Hz, 1H), 4.88 (d, *J* = 11.6 Hz, 1H), 5.53 (s, 1H), 5.54 (s, 1H), 5.74 (d, *J* = 8.4 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 2H), 7.30-7.42 (m, 13H), 7.45-7.54 (m, 4H), 8.82 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.4, 65.9, 67.4, 67.5, 68.3, 68.4, 68.7, 72.7, 74.7, 76.3, 78.2, 78.7, 79.0, 97.0, 101.4, 101.5, 102.8, 126.2, 126.4, 127.6, 127.7, 128.3, 128.4, 128.5, 129.0, 129.3, 129.6, 130.3, 130.7, 137.1, 138.5, 159.3, 160.9. HRMS Calcd for $[M+Na]^+$ 919.1892. Found 919.2224.

Phenyl (3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-*p*-methoxybenzyl-β-D-mannopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4-di-*O*-benzyl-2-*O*-pivaloyl-1-thio-α-D-mannopyranoside (17). A solution of donor **5** (200 mg, 0.22 mmol) and acceptor **6** (140 mg, 0.27 mmol) in CH_2Cl_2 (6 mL) in the presence of 4 Å molecular sieves (500 mg) was stirred for 20 min at −40 °C, then TMSOTf (8 μL, 0.045 mmol) was added. After the resulting solution was stirred at −40 °C for 20 min, it was allowed to warm over 1 hr to 0 °C and stirred for further 20 min at 0 °C. The reaction mixture was quenched with Et_3N (20 μL), and then extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (Hexane/EtOAc/CH_2Cl_2, 6 : 1 : 2) to afford the desired compound **17** (α only, 240 mg, 85%) as a colorless oil.: R_f = 0.18 (Hexane/EtOAc/CH_2Cl_2, 6 : 1 : 2, v/v); $[\alpha]_D^{20}$ = +171.6 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz,

$CDCl_3$) δ 1.22 (s, 9H), 3.14 (dt, J_d = 4.8 Hz, J_t = 9.6 Hz, 1H), 3.35 (dd, J = 3.7, 9.9 Hz, 1H), 3.53 (dd, J = 2.8, 9.7 Hz, 1H), 3.62-3.73 (m, 4H), 3.76 (s, 3 H), 3.78-3.64 (m, 2H), 3.86-3.99 (m, 4H), 4.05 (dd, J = 4.8, 10.4 Hz, 1H), 4.13 (dd, J = 2.8, 9.6 Hz, 1H), 4.17 (dd, J = 2.8, 9.6 Hz, 1H), 4.22 (dd, J = 4.8, 10.4 Hz, 1H), 4.38-4.44 (m, 1H), 4.53 (d, J = 10.8 Hz, 1H), 4.56 (d, J = 12.0 Hz, 1H), 4.63 (d, J = 12.0 Hz, 1H) 4.66 (d, J = 1.6 Hz, 1H), 4.71 (d, J = 10.8 Hz, 1H), 4.78 (d, J = 11.6 Hz, 1H), 4.87 (d, J = 12.0 Hz, 1H), 4.95 (d, J = 11.2 Hz, 1H), 5.10 (d, J = 3.6 Hz, 1H), 5.42 (d, J = 1.2 Hz, 1H), 5.53 (s, 1H), 5.54 (s, 1H), 5.64 (dd, J = 1.6, 2.8 Hz, 1H), 6.78 (d, J = 8.5 Hz, 2H), 7.27-7.53 (m, 32H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.2, 55.4, 63.0, 63.2, 67.0, 67.6, 68.7, 68.9, 69.8, 71.7, 72.1, 72.5, 74.6, 74.8, 75.3, 76.7, 78.1, 78.6, 78.9, 80.6, 86.5, 98.3, 101.5, 101.7, 102.5, 113.5, 127.6, 127.7, 127.8, 127.95, 128.03, 128.2, 128.26, 128.29, 128.33, 128.4, 128.5, 128.6, 129.0, 129.3, 129.3, 130.1, 134.1, 137.3, 137.7, 137.8, 138.2, 138.5, 159.2, 177.6. HRMS Calcd for $[M+Na]^+$ 1294.4922. Found 1294.4934.

Phenyl (3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4-di-*O*-benzyl-2-*O*-pivaloyl-1-thio-α-D-mannopyranoside (4). To a solution of **17** (240 mg, 0.19 mmol) in CH_2Cl_2/H_2O (20 : 1, 5 mL) was added DDQ (85 mg, 0.38 mmol) at 0 °C. After the resulting solution was stirred at 0 °C for 1 hr, it was quenched with saturated aqueous $NaHCO_3$ and then extracted with CH_2Cl_2. The combined organic layer was washed with brine and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (Hexane/EtOAc, 2 : 1) to afford the desired compound **4** (170 mg, 78%) as a colorless oil.: R_f = 0.13 (Hexane/EtOAc, 2 : 1, v/v); $[\alpha]_D^{20}$ = +101.6 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (s, 9H), 2.69 (br s, 1H), 3.24 (dt, J_d = 4.8 Hz, J_t = 9.6 Hz, 1H), 3.42 (dd, J = 3.7, 9.9 Hz, 1H), 3.62 (dd, J = 2.8, 9.7 Hz, 1H), 3.69-3.85 (m, 6H), 3.85-3.95 (m, 3H), 4.06-4.25 (m, 6H), 4.38-4.44 (m, 1H), 4.50 (d, J = 10.8 Hz, 1H), 4.65 (d, J = 10.8 Hz, 1H), 4.71 (d, J = 10.4 Hz, 1H), 4.72 (d, J = 9.6 Hz, 1H), 4.74 (d, J = 1.6 Hz, 1H), 4.82 (d, J = 12.4 Hz, 1H), 5.01 (d, J = 3.2 Hz, 1H), 5.42 (s, 1H) 5.48 (s, 1H), 5.52 (s, 1H), 5.64 (s, 1H), 7.27-7.53 (m, 30H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.1, 63.0, 63.2, 66.8, 67.0, 68.7, 68.8, 69.7, 70.0, 71.6, 72.0, 72.6, 74.5, 75.2, 76.6, 76.7, 78.2, 78.8, 80.4, 86.5, 98.0, 100.9, 101.50, 101.52, 125.4, 126.1, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 129.0, 129.1, 129.3, 129.2, 129.3, 131.8, 132.7, 134.0, 137.2, 137.5, 137.7, 137.9, 138.0, 138.1, 177.5. HRMS Calcd for $[M+Na]^+$ 1174.4347. Found 1174.4347.

Phenyl (3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-benzoyl-α-D-mannopyranosyl)-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4-di-*O*-benzyl-2-*O*-pivaloyl-1-thio-α-D-mannopyranoside (2). A solution of acceptor **4** (100 mg, 0.087 mmol) and DTBMP (53 mg, 0.26 mmol) in CH_2Cl_2 (2 mL) in the presence of 4 Å molecular sieves (400 mg) was stirred for 10 min at rt and cooled to –20 °C. After Tf_2O (19 μL, 0.11 mmol) was added and subsequently a solution of donor **3** (67 mg, 0.11 mmol) in CH_2Cl_2 (5 mL) was added slowly for 30 min using syringe pump, the resulting solution was stirred at –20 °C for 1 hr and allowed to warm to rt. The reaction mixture was quenched with saturated aqueous $NaHCO_3$, and then extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography (toluene/EtOAc/CH_2Cl_2, 30 : 1 : 2) to afford the desired compound **2** (α only, 120 mg, 87%) as a colorless oil.: R_f = 0.33 (Toluene/EtOAc/CH_2Cl_2, 30 : 1 : 2, v/v); $[\alpha]_D^{20}$ = +5.34 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (s, 9H), 3.20-3.40 (m, 3H), 3.65-3.74 (m, 3H), 3.76-3.85 (m, 4H), 3.90-3.95 (m, 2H), 3.97-4.03, (m, 2H), 4.07 (d, J = 10.8 Hz, 1H), 4.13 (d, J = 8.8 Hz, 1H), 4.17 (d, J = 8.8 Hz, 1H), 4.20 (d, J = 11.2 Hz, 1H), 4.27 (d, J = 2.8 Hz, 1H), 4.31 (t, J = 5.2 Hz, 1H), 4.35 (d, J = 9.6 Hz, 1H), 4.39 (d, J = 3.2 Hz, 1H), 4.40 (d, J = 2.8 Hz, 1H), 4.52 (d, J = 11.2 Hz, 1H), 4.61 (d, J = 10.8 Hz, 1H), 4.64-4.70 (m, 2H), 4.71 (d, J = 10.8 Hz, 1H), 4.89 (d, J = 12.0 Hz, 1H), 4.93 (d, J = 11.2 Hz, 1H), 4.99 (d, J = 3.6 Hz, 1H), 5.34 (d, J = 1.6 Hz, 1H), 5.37 (d, J = 1.2 Hz, 1H), 5.40 (s, 1H), 5.63 (dd, J = 1.6, 2.8 Hz, 1H), 5.66 (s, 1H), 5.68 (s, 1H), 5.83 (dd, J = 1.6, 3.6 Hz, 1H), 7.10-7.60 (m, 43H), 8.07 (d, J = 6.9 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.2, 63.4, 63.6, 63.9, 67.2, 67.6, 68.4, 68.8, 69.0, 69.8, 70.3, 71.7, 72.0, 72.4, 73.2, 73.9, 74.4, 74.6, 75.2, 75.3, 78.1, 78.9, 79.0, 79.1, 79.4, 86.7, 98.3, 99.6, 99.8, 100.4, 101.1, 101.7, 126.16, 126.22, 126.3, 127.3, 127.36, 127.40, 127.6, 127.9, 128.0, 128.1, 128.20, 128.24, 128.37, 128.44, 128.46, 128.50, 128.6, 128.87, 128.94, 129.1, 129.4, 130.1, 130.8, 132.2, 133.2, 133.9, 136.0, 137.6, 137.78, 137.83, 137.9, 138.3, 138.6, 165.4, 177.5. HRMS Calcd for $[M+Na]^+$ 1618.5920. Found 1618.5928.

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