## Chemical Synthesis of Residue-Selectively <sup>13</sup>C and <sup>2</sup>H Double-Isotope-Labeled Oligosaccharides as Chemical Probes for the NMR-Based Conformational Analysis of Oligosaccharides

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selectively <sup>13</sup>C-labeled oligosaccharides have provided valuable information concerning their conformation, which would not be possible using nonlabeled oligosaccharides. The amount of accessible information from an atom-selectively labeled molecule, however, is limited. In this work, we report on the chemical synthesis of residue-selectively <sup>13</sup>C- and <sup>2</sup>H-labeled oligosaccharides and their use in conformational analysis. <sup>1</sup>H NMR



measurements of such double isotope-labeled compounds can provide a great deal of information on the dihedral angles across glycosidic linkages. We demonstrated this method in the conformational analyses of some linear and branched  $\beta(1,3)$ -glucan oligosaccharides.

## INTRODUCTION

Cell-surface oligosaccharides play important roles in biological events such as protein trafficking and cellular recognition.<sup>1</sup> To understand these types of structure-activity relationships, conformational analysis of oligosaccharides is highly important. NMR spectroscopy of oligosaccharides is a valuable tool for elucidating their solution conformation by providing structurerelated parameters such as homo- and heteronuclear couplings (J-couplings) and nuclear Overhauser effects (NOEs).<sup>2</sup> However, severe overlaps of <sup>1</sup>H signals and the low abundance of NMR-active [13C] carbon frequently hamper obtaining these parameters. To overcome these difficulties, selectively <sup>13</sup>C-labeled oligosaccharides have been used as chemical probes.<sup>3</sup> The enriched molecules can provide specific Jcouplings in a complex structure. Serianni et al. reported that a set of dihedral angles  $\varphi$  and  $\Psi$  across a glycosidic linkage can be estimated by using two different oligosaccharides containing a <sup>[13</sup>C] carbon at an anomeric or C2 position, which provide inter-residue  ${}^{3}J_{CH}$  and  ${}^{3}J_{CC}$  coupling constants.<sup>4</sup> Seeberger et al. recently reported that the glucose-ring conformation of  $\beta(1,6)$ hexaglucosides can be determined by using hexasaccharides containing a uniformly <sup>13</sup>C-labeled glucose unit at different positions, which provides  ${}^{1}J_{CH}$  at the anomeric center.<sup>5</sup> Uniformly <sup>13</sup>C-labeled sugar units have the potential for providing multiple inter-residue  ${}^{3}J_{CH}$  coupling constants. However, these [ ${}^{13}C$ ] carbons complicate the  ${}^{1}H$  NMR spectrum by coupling with all protons one, two, or three bonds apart. On the other hand, a uniformly <sup>13</sup>C and <sup>2</sup>H double-isotope-labeled glucose is commercially available and has been used in studies related to the biosynthesis of

isotopically labeled compounds.<sup>6</sup> We hypothesized that replacing the [<sup>1</sup>H] hydrogens on the uniformly <sup>13</sup>C-labeled sugar unit with <sup>1</sup>H NMR-silent [<sup>2</sup>H] hydrogens would be effective in simplifying the <sup>1</sup>H NMR spectrum. Herein we report on the synthesis and NMR analysis of some linear and branched oligosaccharides containing uniformly <sup>13</sup>C and <sup>2</sup>H double-isotope-labeled monosaccharide units.

## RESULTS AND DISCUSSION

Our strategy was to analyze <sup>1</sup>H NMR spectra of oligosaccharides containing a uniformly <sup>13</sup>C- and <sup>2</sup>H-labeled monosaccharide unit in order to obtain multiple <sup>3</sup> $J_{CH}$  coupling constants across glycosidic linkages (Figure 1). Double isotope



Figure 1. <sup>13</sup>C-Labeled oligosaccharides for conformational studies.

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labeling would allow one to assign  ${}^{3}J_{\rm CH}$  coupling constants between  $[{}^{1}{\rm H}]$  hydrogens on the attached glycosides and the enriched  $[{}^{13}{\rm C}]$  carbons. The enriched  $[{}^{13}{\rm C}]$  carbon at the anomeric position has a  ${}^{3}J_{\rm CH}$  coupling related to dihedral angles  $\Psi$ . The other enriched  $[{}^{13}{\rm C}]$  carbons have  ${}^{3}J_{\rm CH}$  coupling related to dihedral angles  $\varphi$ .

To demonstrate the utility of this method, we compared inter-residue  ${}^{3}J_{CH}$  coupling constants of the branched and linear  $\beta(1,3)$ -oligosaccharides **5** and **6**, which are partial structures of  $\beta(1,3)$ -glucans (Scheme 1).  $\beta(1,3)$ -Glucans are

Scheme 1. Strategy for the Synthesis of the Isotope-Labeled Branched Tetrasaccharide 5 and Linear Trisaccharide 6



major components of fungal cell walls and certain bacteria and function to stimulate innate immunity. Branched  $\beta$ -glucans such as Schizophyllan (1) containing  $\beta(1,6)$ -glucosidic linkages on every third glucose unit on the backbone are known to exhibit a higher affinity for dectin-1 compared to linear  $\beta(1,3)$ glucans such as Curdlan (2) (Figure 2).<sup>7</sup> The positive effects of the branching glucosides on affinity for Dectin-1 can be attributed to a conformational change in the linear  $\beta(1,3)$ backbone caused by the attachment of  $\beta(1,6)$ -branching glycosides.<sup>8</sup> However, structure–activity relationships remain unclear due to the lack of availability of pure, naturally occurring  $\beta(1,3)$ -glucosides. Therefore, chemical synthesis of



Figure 2. Naturally occurring  $\beta$ -glucans 1 and 2 and the synthetic derivatives 3 and 4.

structurally defined oligosaccharides as model compounds for  $\beta(1,3)$ -glucans would be highly desirable for evaluating the biological activities of such polysaccharides.<sup>9,10</sup> Our recent studies on oligosaccharides **3** and **4** showed that branching  $\beta(1,6)$ -glucosides on a  $\beta(1,3)$ -undecasaccharides did not have positive effects on the binding to dectin-1.<sup>9g</sup> Therefore, a comparison of the <sup>3</sup>J<sub>CH</sub> coupling constants of **5** and **6** would be expected to provide information on the effects of the branching  $\beta(1,6)$ -glucoside on the conformation of the  $\beta(1,3)$ -backbone.

The retrosynthesis of the branched tetrasaccharide 5 and the linear trisaccharide 6 containing a uniformly <sup>13</sup>C and <sup>2</sup>H double-isotope-labeled glucoside was designed to proceed via the same synthetic intermediate 8 (Scheme 1). The tetrasaccharide 5 would be synthesized by the regioselective glycosylation of the diol 8 with the superarmed glycosyl imidate 7.<sup>11</sup> The diol 8 can be prepared by the gold-catalyzed glycosylation of the alcohol  $10^{12}$  with the glycosyl oalkynylbenzoate (ABz) 9 followed by the chemoselective hydrolysis of the 4,6-O-tert-butylsilylene acetal. The benzylidene acetal of 10 would reduce the steric hindrance of the alcohol at the 3 position. The glycosyl donor 9 can be prepared by the chemoselective glycosylation of the glucal 12 with glycosyl imidate 11,<sup>13</sup> followed by anomeric transformation via the stereoselective epoxidation of the vinyl ether.<sup>14</sup> The unhindered alcohol of the glucal 12 would show a high reactivity with respect to its glycosylation. In addition, O-tertbutylsilylene protection would improve the stability of glycals under acidic conditions.<sup>15</sup> The glucal can be synthesized from the commercially available uniformly  $^{13}C$  and  $^{2}H$  double-isotope-labeled glucose (13) in 5 steps.<sup>14</sup>

We first examined the preparation of the branched and linear oligosaccharides by using the nonlabeled glucoside  $14^{14}$  (Scheme 2). Treatment of the glucal 14 (1.00 equiv) and the glycosyl imidate 11 (1.00 equiv) with 10 mol % of TMSOTf at temperatures between -78 to -60 °C gave the disaccharide 15 in a quantitative yield. Increasing the temperature from -78 to -50 °C reduced the yield to 85%. This result can be attributed to the instability of the double bond of glucals under acidic conditions.<sup>16</sup> We also examined the glycosylation of the 4,6-O-tert-butylsilylene-protected thioglycoside  $16^{17}$  with the same donor 11, to provide the





3-O-glycosylated product 17 in 44% yield along with a significant amount of the 2-O-glycosylated product 18 (25%).

The preparation of glycosyl donor 21 from 15 was examined next (Scheme 3). Exposure of the glucal 15 to dimethyldioxirane (DMDO) at temperatures between 0 °C and room temperature, followed by the removal of the oxidant and solvents *in vacuo*, provided the epoxide 19 as a single isomer.

# Scheme 3. Conversion of the Glucal 15 to 2-O-Acyl Glycosyl Donors 21



The epoxide 19 was used in the following reaction without further purification due to its high susceptibility to acids. Treatment of the epoxide 19 with the o-hexylbenzoate (ABzOH) in the presence of 4 Å MS provided the alcohol 20. The subsequent acylation of the resulting alcohol of 20 with benzoyl chloride under basic conditions provided the glycosyl (2-alkynyl)benzoate 21 as an anomeric mixture ( $\alpha/\beta$ = 17:83) in 65% yield based on the glucal 15. We also examined the preparation of the thioglycoside 23 from the epoxide 19. Treatment of the epoxide 19 with ethanethiol in the presence of a catalytic amount of trifluoroacetic acid (TFA) provided the  $\beta$ -thioglycoside **22** in 45% yield based on the glucal 15. The relative stereochemistry at the C1 and C2 positions was confirmed by a coupling constant between H-1 and H-2 (9.8 Hz) indicative of a trans configuration. The production of the  $\beta$ -thioglycoside **22** clearly indicated that the stereoselective epoxidation of the glucal 15 had proceeded at the  $\alpha$ -face. Acylation of the  $\beta$ -thioglycoside **22** with benzoyl chloride under basic conditions was unsuccessful, even at high temperatures with the substrate being recovered. The steric hindrance of the equatorially oriented ethanesulfenyl group and the O-tert-butylsilylene protection likely inhibited the acylation. Finally, we attempted the direct glycosidation of the epoxide 19. The epoxide 19 was treated with the alcohol 10 in the presence of ZnCl<sub>2</sub> at temperatures between -78 °C and room temperature,<sup>18</sup> only to give a complex mixture of products. On the other hand, the use of PPh3·AuOTf as a catalyst gave the  $\alpha$ -product 24 in 25% yield based on the glucal 15.<sup>19</sup> The stereochemistry of the anomeric center was determined by the coupling constant between H-1 and H-2,  ${}^{3}J_{1,2}$  = 3.4 Hz to be  $\alpha$ . The formation of the  $\alpha$ -product 24 implied that an oxocarbenium ion was produced from the epoxide and that the secondary alcohol 10 attacked the cation from the  $\alpha$  face probably because of the bulky groups at the opposite face.

Synthesis of the tetrasaccharide 31 and the trisaccharide 28 from 21 is shown (Scheme 4). Treatment of the donor 21 and 1.2 equiv of the alcohol 10 with 20 mol % of PPh<sub>3</sub>·AuOTf in CH<sub>2</sub>Cl<sub>2</sub> at temperatures between 0 °C and room temperature provided the trisaccharide 25 in 82% yield. The stereochemistry of the anomeric center was determined based on the coupling constant between H-1 and H-2,  ${}^{3}J_{1,2} = 8.0$  Hz to be  $\beta$ . The subsequent removal of the silvlene protecting group by treatment with HF pyridine in tetrahydrofuran gave the diol 26 in 80% yield. Treatment of the diol 26 and 1.2 equiv of the imidate 7 with 20 mol % of TMSOTf from -78 °C to -50 °C provided the tetrasaccharide 29 in 61% yield as a single isomer. The position of the remaining hydroxyl group was confirmed by COSY between the proton at the C4 and 4-OH in COSY spectra. Global deprotection was achieved by the following procedures. Solvolysis of the tetrasaccharide 29 under basic conditions provided the corresponding hydrolysate 30 in 69% yield. Subsequent hydrogenolysis of the benzyl ethers by treatment with 10% Pd/C under a hydrogen atmosphere afforded the tetrasaccharide 31 in 61% yield. The trisaccharide 28 was also prepared from the protected trisaccharide 26 in 33% total yield via solvolysis and hydrogenolysis.

We next examined the labeled tetra- and trisaccharide **5** and **6** prepared from uniformly <sup>13</sup>C and <sup>2</sup>H double-isotope-labeled glucose (**13**) according to the established method (Scheme 5). We further prepared the labeled disaccharide **44** (Scheme 6). The pentaacetyl glucoside **32** was treated with 30% HBr in

AcOH at 0 °C to provide glycosyl bromide 41 in 61% yield.

Scheme 4. Synthesis of the Tetrasaccharide 31 and Trisaccharide 28



Glycosylation of 10 with the glycosyl bromide 41 in the presence of AgOTf at -20 °C provided the disaccharide 42 in 59% yield. Deprotection of the disaccharide 42 was achieved by solvolysis under basic conditions followed by hydrogenolysis to provide the disaccharide 44 in 96% total yield.

We next carried out an NMR analysis of the tetra-, tri-, and disaccharides 5, 6, and 44 containing a uniformly <sup>2</sup>H and <sup>13</sup>C double-isotope-labeled glucose (Figure 3). Figure 4 shows <sup>1</sup>H NMR spectra of the nonlabeled tetrasaccharide 31 and the labeled tetrasaccharide 5, respectively (400 MHz, 25 °C,  $^{2}H_{2}O$ ). In the spectra of the labeled tetrasaccharide 5,  $^{2}H_{2}$ labeling at the center residue reduced the overlap of the <sup>1</sup>H signals, and three inter-residue vicinal coupling constants  ${}^{3}J_{C1H\nu}$  ${}^{3}J_{C3H}$ , and  ${}^{3}J_{C6H}$  between the  ${}^{13}C$  atoms and protons were assigned (Table 1). The corresponding coupling constants of trisaccharide 6 and the disaccharide 44 were also obtained by the same method. The  ${}^{3}J_{C1H}$  (5.0 Hz) for the disaccharide 44 was close to a previously reported value (4.8 Hz).<sup>4</sup> From these coupling constants, the glycosidic torsion angles for the synand anti-conformers were calculated using the Karplus equation  $({}^{3}J_{CH} = 7.49\cos^{2}\theta - 0.96\cos\theta + 0.15)$  (Table 1).<sup>20</sup> The crystal structure of methyl  $\alpha$ -laminarabioside, an unlabeled version of 44 was reported to be the syn-conformer, which was stabilized by an inter-residue hydrogen bond between 4-OH and O5, with  $\varphi$  and  $\psi$  angles of  $+28^{\circ}$  and  $-38^{\circ}$ .<sup>21</sup> In the solution state of methyl  $\alpha$ -laminarabioside, the  $\varphi$  and  $\psi$  angles were also estimated to be  $(+40^\circ, -9^\circ)$ .<sup>4</sup> Hence, the synconformer would be the predominant conformer in  $\beta(1,3)$ glucosidic linkages. Our results showed no significant difference in dihedral angles between the tetra- and trisaccharides 5

and 6, i.e.,  $\varphi_{C3} = \sim + 40^{\circ}$ ,  $\psi_{C1} = \sim -32^{\circ}$ . This consistency suggests that a side chain via  $\beta(1,6)$ -glycosidic linkage would have negligible effects on the conformation across  $\beta(1,3)$ glycosidic linkages. This small conformational change, however, could lead to small differences in biological activities between linear and branched  $\beta(1,3)$ -glucan oligosaccharides, as was reported in our previous study.<sup>9g</sup> Additionally, consistent values between  $\varphi_{C3}$  and  $\varphi_{C6}$ , i.e.,  $\varphi = \sim +40^{\circ}$ , suggested that the  $\varphi$  angles were not affected by the position of the  $\beta$ glucosidic linkages due to exoanomeric effects. These results demonstrate that using residue-specific, double-isotope-labeled oligosaccharides provide a wide range of information on conformations across glycosidic linkages, based on NMR data.

## CONCLUSIONS

We report herein on the chemical synthesis of a residueselectively <sup>13</sup>C-and <sup>2</sup>H-labeled tetra- and trisaccharides 5 and 6 using 4,6-O-tert-butylsilylene-D-glucal (12) as a key intermediate. The TMSOTf-catalyzed glycosylation of the 4,6-Otert-butylsilylene-D-glucal (12) proceeded at low temperatures to provide a 3-O-glycosylated glucal, which was further converted into a glycosyl o-alkynyl benzoate via stereoselective epoxidation. The subsequent gold-catalyzed glycosidation of the glycosyl benzoate gave a trisaccharide structure. On the other hand, a tetrasaccharide structure was constructed by the regioselective glycosylation of a diol, which was prepared by the selective removal of the silylene group of a trisaccharide, with a superarmed glycosyl donor. The partially doubleisotope-labeled oligosaccharides that were synthesized proved to be a practical probe for obtaining some vital heteronuclear coupling constants across glycosidic linkages based on <sup>1</sup>H NMR spectra. From these coupling constants, glycosidic torsional angles were obtained via a Karplus equation, and the findings suggested that a side chain via a  $\beta(1,6)$ -glucosidic linkage had little effect on the conformation across  $\beta(1,3)$ glycosidic linkages. We expect that the strategy using NMR measurements of residue-specific, double-isotope-labeled oligosaccharides will expand the efficiency of conformational studies in glycobiology.

## EXPERIMENTAL SECTION

General Methods. All chemicals used were reagent grade and used as supplied, except where noted. Prior to use, molecular sieves were activated by heating at 350 °C. All reactions were performed in oven-dried glassware under an argon atmosphere. Dichloromethane (DCM), toluene, and tetrahydrofuran (THF) were dispensed from a solvent purification system. All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light, visualized by *p*-anisaldehyde/H<sub>2</sub>SO<sub>4</sub>/ ethanol solution. Column chromatography was carried out using the forced flow of the indicated solvent on Merck silica gel. Preparative HPLC purifications were carried out on an SSC-3461 pump with an SSC-5410 UV detector by using a Senshu Pak column (Silica-3301-N, 8  $\phi$   $\times$  300 mm). NMR spectra were recorded using a Bruker AVANCE III HD 400 in CDCl<sub>3</sub> or D<sub>2</sub>O. Chemical shifts are reported in units of parts per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane in CDCl<sub>3</sub>. <sup>1</sup>H NMR spectrum data is reported as follows: CDCl<sub>3</sub> (7.26 ppm) or HOD (4.79 ppm). <sup>13</sup>C NMR spectrum data is reported as follow: CDCl<sub>3</sub> (77.1 ppm) or acetone- $d_6$  (30.9 or 215.9 ppm) or CD<sub>3</sub>OD (49.5 ppm) as an internal standard for D<sub>2</sub>O. Multiplicities are reported by using the following abbreviations: s; singlet, d; doublet, t; triplet, m; multiplet, J; coupling constants in hertz. Structural assignments were made with additional information from gCOSY experiments. Pure shift NMR (broadband homonuclear-decoupling) of 5, 6, and 44 was measured using an

#### Scheme 5. Synthesis of the Tetrasaccharide 5 and Trisaccharide 6



Scheme 6. Synthesis of the Labeled Disaccharide 44



AVANCE 600 spectrometer equipped with a 5 mm TXI gradient probe (Bruker). The oligosaccharide samples 5, 6, and 44 were dissolved in  $D_2O$  (D 99.9%), and the <sup>1</sup>H chemical shifts were calibrated based on an outer standard of the chemical shift of 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) at 0 ppm. The probe temperature was set to 278 K to avoid the overlapping of the HDO

signal to the anomeric signals. The pulse sequence was based on the previous report,<sup>22</sup> using an RSnob selective 180° pulse of 60 ms under a slice-selection gradient of 0.535 G cm<sup>-1</sup>. 40 t1 increments were acquired with a relaxation delay of 2.3 s. NMR data were processed with TOPSPIN (ver 2.1), and the spectra were displayed using XWINPLOT (ver 3.5). High-resolution mass spectrometry (HRMS) was performed by Waters LCT Premier XE. HRMS (ESI-TOF) was calibrated with leucine enkephalin (SIGMA) as an internal standard. IR spectra were recorded on a JASCO FT/IR-4200 spectrophotometer. Only the strongest and/or structurally important peaks are reported as the IR data given in cm<sup>-1</sup>. Optical rotations were measured on a JASCO model P-1020 polarimeter, with concentrations expressed in g per 100 mL.

3-O-(4,6-O-Benzylidene-2,3-di-O-benzoyl-β-D-glucopyranosyl)-4,6-O-tert-butylsilylidene-D-glucal (15). A solution of 14<sup>14</sup> (0.800 g, 2.79 mmol) and 11<sup>13</sup> (1.77 g, 2.85 mmol) in DCM (57.0 mL) was dried over 4 Å MS at room temperature for 1 h. Then, a catalytic amount of TMSOTf (0.100 equiv, 0.0505 mL, 0.279 mmol) was added to the solution at -78 °C. After being warmed to -60 °C over 4 h, the solution was quenched with NEt<sub>3</sub>, filtered through a pad of Celite. The filtrate was poured into saturated NaHCO<sub>3</sub> (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (toluene/ethyl acetate = 4:1) to give 15 (2.04 g, 2.79 mmol, quant.) as a white solid: [α]<sub>28</sub><sup>28</sup> +0.57 (c 1.11,



Figure 3. Labeled oligosaccharides 5, 6, and 44.



Figure 4. <sup>1</sup>H NMR spectra of the nonlabeled tetrasaccharide 31 (upper side) and isotope-labeled tetrasaccharide 5 (lower side).

 Table 1. Glycosidic Torsion Angles Derived from Coupling Constants

entry	compound	<sup>3</sup> J <sub>C1H</sub> (ψC1(syn/ anti))	<sup>3</sup> J <sub>C3H</sub> (φC3(syn/ anti))	<sup>3</sup> J <sub>С6Н</sub> ( <i>ф</i> Сб(syn/ anti))
1	5	$4.7 \text{ Hz} (-32^{\circ}/+136^{\circ})$	3.8 Hz (+40°/− 130°)	4.2 Hz (+37°/− 132°)
2	6	4.8 Hz (−31°/ +137°)	3.9 Hz (+39°∕− 130°)	
3	44	5.0 Hz (-29°/ +138°)		

CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.00–7.97 (m, 4H), 7.55– 7.47 (m, 2H), 7.42–7.30 (m, 9H), 6.16 (d, 1H, *J* = 6.0 Hz), 5.74 (t, 1H, *J* = 9.0, 9.0 Hz), 5.56 (s, 1H), 5.46 (t, 1H, *J* = 7.7, 7.7 Hz), 5.10 (d, 1H, *J* = 7.2 Hz), 4.49 (d, 1H, *J* = 6.1 Hz), 4.42 (dd, 1H, *J* = 10.5, 4.7 Hz), 4.30–4.28 (m, 1H, H-3), 4.16–4.11 (m, 2H), 4.06 (t, 1H, *J* = 9.4, 9.4 Hz), 3.96 (t, 1H, *J* = 10.5, 10.5 Hz), 3.89 (t, 1H, *J* = 10.2, 10.2 Hz), 3.80–3.70 (m, 2H), 1.10 (s, 9H), 1.03 (s, 9H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  165.8, 165.3, 144.7, 138.0, 137.0, 133.4, 133.2, 130.0, 129.9, 129.6, 129.5, 129.2, 128.6, 128.4, 128.4, 128.4, 126.2, 125.4, 101.6, 101.1, 101.1, 79.2, 78.8, 75.5, 73.7, 72.8, 72.4, 69.0, 66.9, 65.8, 27.6, 27.2, 22.9, 21.6, 20.0; FT-IR (neat) 3067, 2860, 1731, 1271, 709 cm<sup>-1</sup>; HRMS (ESI-TOF) *m*/*z* [M + Na]<sup>+</sup>calcd for C<sub>41</sub>H<sub>48</sub>O<sub>11</sub>SiNa 767.2864, found 767.2841.

Phenyl 3-O-(4,6-O-Benzylidene-2,3-di-O-benzoyl-*D*-glucopyranosyl)-4,6-O-tert-butylsilylidene-1-thio- $\beta$ -*D*-glucoside (17) and Phenyl 2-O-(4,6-O-Benzylidene-2,3-di-O-benzoyl-*D*-glucopyranosyl)-4,6-O-tert-butylsilylidene-1-thio- $\beta$ -*D*-glucoside (18). A solution of 16<sup>17</sup> (0.140 g, 0.339 mmol) and 11 (0.201 g, 0.324 mmol) in DCM (3.24 mL) was dried over 4 Å MS (324 mg) at room temperature for 1 h. Then, a catalytic amount of TMSOTf (0.0058 mL, 0.0324 mmol) was added to the solution at -78 °C. After being warmed to -50 °C over 45 min, the solution was quenched with NEt<sub>3</sub>, filtered through a pad of Celite. The filtrate was poured into saturated NaHCO3 (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane:ethyl acetate = 9:1) to give 17 (0.123 g, 0.141 mmol, 44%.) and 18 (0.0692 g, 0.0794 mmol, 25%) as colorless oils. Compound 17:  $[\alpha]_{D}^{18}$  -0.86 (c 1.06, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>); δ 8.00-7.94 (m, 4H), 7.55-7.46 (m, 2H), 7.42-7.21 (m, 14H), 5.78 (t, 1H, J = 9.3, 9.3Hz), 5.55 (s, 1H), 5.47 (dd, 1H, J = 9.0, 7.5 Hz), 5.26 (d, 1H, J = 7.4 Hz), 4.42 (d, 1H, J = 9.8 Hz), 4.41 (dd, 1H, J = 10.5, 4.8 Hz), 4.17 (dd, 1H, J = 10.3, 5.0 Hz), 3.98 (t, 1H, J = 9.5, 9.5 Hz), 3.90-3.79(m, 3H), 3.67 (dt, 1H, J = 9.6, 9.6, 4.8 Hz), 3.59 (t, 1H, J = 8.6, 8.6 Hz), 3.38 (dt, 1H, J = 9.9, 9.9, 5.0 Hz), 3.34-3.29 (m, 1H), 2.54 (d, 1H, OH, J = 2.4 Hz), 1.08 (s, 9H), 1.01 (s, 9H);<sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 166.1, 165.7, 136.8, 133.4, 133.2, 131.2, 130.0, 129.9, 129.5, 129.4, 129.1, 129.1, 128.5, 128.4, 128.3, 126.2, 101.9, 101.5, 88.0, 85.9, 78.8, 75.7, 74.9, 73.9, 72.2, 71.5, 68.9, 66.8, 66.2, 27.6, 27.2, 22.8, 20.1; FT-IR (neat) 2933, 2860, 1730, 1273, 1179, 1095, 758, 710 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + NH<sub>4</sub>]<sup>+</sup> calcd for  $C_{47}H_{58}NO_{12}SSi$  888.3449, found 888.3438. Compound 18:  $[\alpha]_D^{18}$ -14.5 (c 0.810, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>);  $\delta$  8.04–8.02 (m, 2H), 7.99–7.96 (m, 2H), 7.53–7.47 (m, 4H), 7.44–7.29 (m, 12H), 5.77 (dd, 1H, J = 9.4, 8.6 Hz), 5.57 (s, 1H), 5.54 (t, 1H, J = 8.5, 8.5 Hz), 5.48 (d, 1H, J = 7.4 Hz), 4.59 (d, 1H, J = 9.7 Hz), 4.41 (dd, 1H, J = 10.5, 5.0 Hz), 4.14–4.09 (m, 2H), 3.97 (t, 1H, J = 10.2, 10.2 Hz), 3.82 (t, 1H, J = 10.2 Hz, 10.2 Hz), 3.76-3.68 (m, 2H), 3.56 (t, 1H, J = 9.2, 9.2 Hz), 3.41 (t, 1H, J = 8.7, 8.7 Hz), 3.27 (dt, 1H, J = 10.0, 10.0, 5.2 Hz), 2.65 (br, 1H, OH), 1.01 (s, 9H), 0.85 (s, 9H); ${}^{13}C{}^{1}H$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  165.7, 165.5, 137.0, 133.4, 133.0, 132.7, 130.0, 129.9, 129.7, 129.7, 129.1, 128.9, 128.5, 128.4, 128.3, 128.0, 126.3, 101.6, 100.9, 85.9, 79.1, 78.8, 77.0, 76.5, 74.0, 73.2, 72.5, 68.8, 66.6, 66.0, 27.5, 27.0, 22.8, 20.0; FT-IR (neat) 2933, 2883, 2860, 1732, 1473, 1451, 1274, 1095, 770, 707 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + NH<sub>4</sub>]<sup>+</sup> calcd for C<sub>47</sub>H<sub>58</sub>NO<sub>12</sub>SSi 888.3449, found 888.3472.

2-O-BenzovI-3-O-(4.6-O-benzvlidene-2.3-di-O-benzovI-B-D-alucopyranosyl)-4,6-O-tert-butylsilylidene-D-glucopyranosyl O-1-Hexvnvlbenzoate (21). To a stirred solution of 15 (0.353 g, 0.484 mmol) in DCM (2.42 mL) was added a solution of DMDO in acetone (4.84 mL, 0.484 mmol) at -20 °C. After being warmed to room temperature over 4 h, the solution was concentrated in vacuo to give 19 as a white solid, which was used for the next reaction without further purification:  $[\alpha]_D^{28}$  +7.11 (c 1.06, CHCl<sub>3</sub>), <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.98 (d, 4H, J = 7.2 Hz), 7.56–7.49 (m, 2H), 7.42– 7.31 (m, 9H), 7.56 (t, 1H, J = 8.9, 8.9 Hz), 5.57 (s, 1H), 5.50 (t, 1H, J = 7.5,7.5 Hz), 5.14 (d, 1H, J = 7.2 Hz), 4.74 (br, 1H), 4.42 (dd, 1H, J = 10.4, 4.7 Hz), 4.11-4.01 (m, 3H), 3.90-3.81 (m, 3H), 3.76 (dt, 1H, J = 10.2, 10.2, 4.7 Hz), 3.62 (dt, 1H, J = 10.2, 10.2, 4.7 Hz), 2.82 (br, 1H), 1.09 (s, 9H), 1.00 (s, 9H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz,  $CDCl_3$ )  $\delta$  165.8, 165.3, 136.9, 133.6, 133.3, 130.0, 129.9, 129.6, 129.2, 129.2, 128.7, 128.5, 128.4, 126.2, 101.6, 101.0, 78.8, 78.7, 74.4, 73.4, 72.4, 68.9, 66.8, 66.2, 64.6, 52.9, 31.0, 27.5, 27.1, 22.9, 20.0; FT-IR (neat) 3063, 2934, 2859, 1731, 1603, 1452, 1387, 1272, 1098 cm<sup>-1</sup>; HRMS (ESI-TOF)  $m/z [M + H]^+$  calcd for C<sub>41</sub>H<sub>49</sub>O<sub>12</sub>Si 761.2993, found 761.2989.) A solution of 19 in DCM (9.69 mL) was dried over

4 Å MS at room temperature for 1 h. Then, o-1-hexynyl benzoic acid (196 mg, 0.969 mmol) was added to the solution at -40 °C. After being stirred at room temperature overnight, the solution was filtered through a pad of Celite. The filtrate was poured into saturated NaHCO<sub>3</sub>(aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (toluene/ethyl acetate = 14:1) to give **20** as a colorless oil (368 mg, 0.382 mmol, 79%,  $\alpha/\beta = 17.83$ ), which was used for the next reaction without further purification. To a stirred solution of 20 in pyridine (4.84 mL) were sequentially added BzCl (0.169 mL, 1.45 mmol) and a catalytic amount of DMAP (5.9 mg, 0.0382 mmol) at 0 °C. After being stirred in an oil bath at 60 °C for 5 h, the solution was poured into 3 M HCl (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with saturated NaHCO<sub>3</sub> (aq) and brine, dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 7:1) to give 21 as a yellow oil (335 mg, 0.315 mmol, 65% in 3 steps,  $\alpha/\beta = 17.83$ ). The major product, the  $\beta$ -isomer, was separated from the mixture of  $\alpha$ - and  $\beta$ -isomers by HPLC chromatography for structure confirmation:  $[\alpha]_{D}^{28}$  +7.11 (c 1.06, CHCl<sub>3</sub>); <sup>-1</sup>H NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.85 (d, 2H, J = 7.8 Hz), 7.74 (d, 1H, J = 8.0 Hz), 7.70 (d, 2H, J = 7.8 Hz), 7.57 (d, 2H, J = 7.8 Hz), 7.51-7.27 (m, 14H), 7.23-7.14 (m, 3H), 5.95 (d, 1H, J = 8.1 Hz), 5.61 (t, 1H, J = 9.4, 9.4 Hz), 5.54 (s, 1H), 5.50–5.41 (m, 2H), 5.17 (d, 1H, J = 7.2 Hz), 4.44 (dd, 1H, J = 10.4, 4.4 Hz), 4.26 (dd, 1H, J = 10.0, 4.5 Hz), 4.13-4.11 (m, 2H), 3,94 (t, 2H, J = 9.8, 9.8 Hz), 3.85 (t, 1H, J = 10.2, 10.2 Hz), 3.73-3.68 (m, 2H), 2.42 (t, 2H, J = 7.0, 7.0 Hz), 1.61-1.53 (m, 2H), 1.47-1.42 (m, 2H), 1.13 (s, 9H), 1.07 (s, 9H), 0.92 (t, 3H, J = 7.4, 7.4 Hz);  ${}^{13}C{}^{1}H$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  165.7, 165.1, 164.8, 163.6, 136.9, 134.6, 133.3, 133.1, 133.0, 132.5, 130.9, 129.8, 129.8, 129.8, 129.4, 129.2, 129.1, 128.5, 128.3, 128.2, 127.3, 101.5, 101.0, 97.3, 92.7, 81.0, 79.1, 78.7, 75.5, 73.2, 72.6, 72.2, 71.7, 69.0, 66.6, 66.3, 30.8, 27.6, 27.2, 22.9, 22.2, 20.1, 19.6, 13.8; FT-IR (neat) 2933, 1736, 1270, 1095 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + Na]<sup>+</sup> calcd for C<sub>61</sub>H<sub>66</sub>O<sub>15</sub>SiNa 1089.4069, found 1089.4073.

Ethyl 3-O-(4,6-O-Benzylidene-2,3-di-O-benzoyl-β-D-qlucopyranosyl)-4,6-O-tert-butylsilylidene-1-thio- $\beta$ -D-glucoside (**22**). To a stirred solution of 19 prepared from 15 (51.7 mg, 0.0723 mmol) according to the established method, and ethanethiol (0.0261 mL, 0.361 mmol) in DCM (0.600 mL) was added TFAA (0.500 equiv, 0.005 mL, 0.0362 mmol). After being stirred at room temperature overnight, the solution was quenched with triethylamine and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 3:1) to give 22 as a colorless oil (26.5 mg, 0.0322 mmol, 45%):  $[\alpha]_D^{24}$  +1.52 (c 0.683, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.99 (d, 2H, J = 8.0 Hz), 7.96 (d, 2H, J = 8.0 Hz), 7.53–7.47 (m, 2H), 7.42–7.30 (m, 9H), 5.78 (t, 1H, J = 9.3, 9.3 Hz), 5.56 (s, 1H), 5.49 (t, 1H, J = 8.0 Hz, 8.0 Hz), 5.28 (d, 1H, J = 7.4 Hz), 4.41 (dd, 1H, J = 10.6, 4.8 Hz), 4.26 (d, 1H, J = 9.8 Hz), 4.15 (dd, 1H, J = 10.2 Hz, 4.9 Hz), 4.01 (t, 1H, J = 9.5, 9.5 Hz), 3.87 (t, 3H, J = 9.8, 9.8 Hz), 3.69 (dt, 1H, J = 9.6, 9.6, 4.8 Hz), 3.59 (t, 1H, J = 8.6, 8.6 Hz), 3.40–3.34 (m, 2H), 2.67–2.54 (m, 2H), 2.47 (br, –OH), 1.21 (t, 3H, J = 7.4, 7.4 Hz), 1.09 (s, 9H), 1.03 (s, 9H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ 166.2, 165.8, 136.9, 133.4, 133.3, 130.0, 130.0, 129.6, 129.6, 129.2, 128.5, 128.5, 128.4, 126.2, 101.9, 101.6, 86.1, 85.8, 78.8, 75.8, 75.0, 74.0, 72.3, 72.3, 69.0, 66.8, 66.3, 27.6, 27.2, 24.4, 22.9, 20.1, 15.3; FT-IR (neat) 2920, 1732, 1272, 1220, 1098 cm<sup>-1</sup>; HRMS (ESI-TOF) m/  $z [M + NH_4]^+$  calcd for  $C_{43}H_{58}NO_{12}SSi 840.3449$ , found 840.3453.

Methyl 3-O-[3-O-(4,6-O-Benzylidene-2,3-di-O-benzoyl- $\beta$ -D-glucopyranosyl)-4,6-O-tert-butylsilylidene- $\alpha$ -D-glucopyranosyl]-2-Obenzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranoside (24). A solution of 19 prepared from 15 (36.7 mg, 0.0494 mmol) according to the established method and 10<sup>12</sup> (18.4 mg, 0.0494 mmol) in DCM (1.48 mL) was dried over 4 Å MS at room temperature for 1 h. Then, a solution of PPh<sub>3</sub>AuOTf in DCM (0.200 equiv, 0.0988 mL, 0.00988 mmol) was added to the solution at room temperature. After being stirred at room temperature for 30 min, the solution was filtered through a pad of Celite. The filtrate was purified by column chromatography on silica gel (hexane/ethyl acetate = 1:1) to give 24 (14.0 mg, 0.0124 mmol, 25%) as a colorless oil:  $[\alpha]_{D}^{22}$  +9.00 (c 0.42, CHCl<sub>2</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>2</sub>)  $\delta$  8.01–7.98 (m, 4H), 7.52– 7.14 (m, 21H), 5.70 (t, 1H, J = 9.2, 9.2 Hz), 5.55 (s, 1H), 5.50 (t, 1H, *J* = 7.9, 7.9 Hz), 5.30 (s, 1H), 5.18 (d, 1H, *J* = 7.3 Hz), 5.07 (d, 1H, *J* = 3.9 Hz), 4.85 (d, 1H, J = 12.6 Hz), 4.50 (d, 1H, J = 12.6 Hz), 4.41 (d, 1H, J = 3.4 Hz), 4.38 (dd, 1H, J = 10.8, 4.9 Hz), 4.21 (dd, 1H, J = 10.5, 4.5 Hz), 4.13-3.46 (m, 12H), 3.35 (dd, 1H, I = 8.9, 3.3 Hz), 3.28 (s, 3H), 3.25 (t, 1H, J = 9.3, 9.3 Hz), 1.07 (s, 9H), 1.00 (s, 9H); $^{13}C{^{1}H}$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  165.9, 165.5, 138.1, 137.0, 136.8, 133.2, 133.1, 130.1, 130.1, 130.0, 129.7, 129.5, 129.1, 128.8, 128.6, 128.5, 128.4, 128.4, 128.1, 126.2, 126.1, 102.2, 101.6, 101.5, 100.0, 98.9, 82.6, 81.8, 79.0, 78.2, 76.0, 73.8, 73.6, 72.6, 72.1, 69.1, 69.0, 67.3, 66.8, 66.7, 61.9, 55.4, 27.7, 27.3, 22.9, 20.1; FT-IR (neat) 3020, 2934, 1732, 1454, 1373, 1271, 1219, 1091 cm<sup>-1</sup>; HRMS (ESI-TOF)  $m/z [M + NH_4]^+$  calcd for  $C_{62}H_{76}NO_{18}Si$  1150.4832, found 1150.4803.

Methyl 3-O-[2-O-Benzov]-3-O-(4.6-O-benzvlidene-2.3-di-O-ben $zoyl-\beta-d-glucopyranosyl)-4,6-O-tert-butylsilylidene-\beta-d-glucopyra$ nosyl]-2-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranoside (25). A solution of 21 (180 mg, 0.169 mmol) and 10 (75.5 mg, 0.203 mmol) in DCM (3.37 mL) was dried over 4 Å MS at room temperature for 1 h. Then, a solution of 0.100 M solution of PPh<sub>3</sub>AuOTf in DCM (0.337 mL, 0.0337 mmol) was added to the solution at 0 °C. After being warmed to room temperature overnight, the solution was filtered through a pad of Celite. The filtrate was poured into saturated  $NaHCO_3$  (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (toluene/ethyl acetate = 14:1) to give 25 (169 mg, 0.137 mmol, 82%) as a colorless oil:  $[\alpha]_{\rm D}^{28}$  -5.16  $(c 0.670, CHCl_3);$ <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.83 (d, 2H, J = 7.9 Hz), 7.77 (d, 2H, J = 7.8 Hz), 7.58 (d, 2H, J = 7.9 Hz), 7.53-7.16 (m, 24H), 5.55 (t, 1H, J = 8.0, 8.0 Hz), 5.51 (s, 1H), 5.43 (s, 1H), 5.37 (t, 1H, J = 7.9, 7.9 Hz), 5.27 (t, 1H, J = 8.4, 8.4 Hz), 5.08 (d, 1H, J = 7.2 Hz), 4.88 (d, 1H, I = 8.0 Hz), 4.43–4.40 (m, 2H), 4.15–3.79 (m, 10H), 3.72-3.58 (m, 3H), 3.43 (t, 1H, J = 9.2, 9.2 Hz), 3.33-3.27(m, 1H), 3.22-3.19 (m, 4H), 1.09 (s, 9H), 0.97 (s, 9H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 165.7, 165.2, 164.6, 138.4, 137.5, 136.9, 133.1, 132.8, 129.9, 129.9, 129.8, 129.8, 129.5, 129.4, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 127.9, 126.2, 126.2, 125.4, 101.4, 101.2, 101.1, 100.7, 99.0, 81.1, 79.9, 79.7, 78.8, 75.5, 74.1, 73.9, 73.2, 72.6, 70.7, 69.1, 66.5, 66.4, 62.2, 55.3, 27.5, 27.2, 22.8, 20.1; FT-IR (neat) 1736, 1269, 1178 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + H]<sup>+</sup> calcd for C<sub>69</sub>H<sub>77</sub>O<sub>19</sub>Si 1237.4828, found 1237.4825.

Methyl 3-O-[2-O-Benzoyl-3-O-(4,6-O-benzylidene-2,3-di-O-ben $zoyl-\beta-d-glucopyranosyl)-\beta-d-glucopyranosyl]-2-O-benzyl-4,6-O$ benzylidene- $\alpha$ -D-glucopyranoside (26). To a stirred solution of 25 (169 mg, 0.137 mmol) in THF (2.77 mL) was added HF·pyridine (0.139 mL) at 0 °C. After being stirred at 0 °C for 2 h, the solution was poured into saturated NaHCO3 (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate) to give 26 (122 mg, 0.111 mmol, 80%) as a colorless oil:  $[\alpha]_{\rm D}^{28}$  +0.451 (c 0.650, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.82 (d, 2H, J = 8.0 Hz), 7.55 (t, 4H, J = 8.7, 8.7 Hz), 7.48–7.18 (m, 22H), 7.11 (t, 2H, J = 7.5, 7.5 Hz), 6.91–6.89 (m, 2H), 5.67 (t, 1H, J = 9.6, 9.6 Hz), 5.52 (s, 1H), 5.45 (t, 1H, J = 8.5, 8.5 Hz), 5.44 (s, 1H), 5.24 (t, 1H, J = 8.4, 8.4 Hz), 4.87 (d, 1H, J = 6.7 Hz), 4.85 (d, 1H, J = 7.1 Hz), 4.46 (dd, 1H, J = 10.2, 4.5 Hz), 4.31 (d, 1H, J = 12.6 Hz), 4.18–4.14 (m, 2H), 4.06 (t, 1H, H-3, J = 9.2, 9.2 Hz), 3.94–3.57 (m, 9H), 3.44–3.32 (m, 3H), 3.21 (s, 3H), 3.17 (dd, 1H, J = 9.0, 3.2 Hz);  ${}^{13}C{}^{1}H$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  165.6, 165.3, 164.5, 138.3, 137.5, 136.6, 133.3, 133.1, 132.8, 129.8, 129.8, 129.6, 129.2, 129.1, 129.0, 128.5, 128.4, 128.3, 128.2, 127.9, 127.6, 126.2, 126.1, 102.0, 101.7, 101.5, 100.8, 99.0, 84.7, 80.0, 79.8, 78.5, 77.4, 76.7, 74.9, 74.0, 73.0, 72.3, 71.8, 69.3, 69.0, 68.3, 66.9, 62.4, 60.5, 55.3, 21.2; FT-IR

(neat) 2925, 1733, 1271, 1220 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + Na]<sup>+</sup> calcd for C<sub>61</sub>H<sub>60</sub>O<sub>19</sub>Na 1119.3627, found 1119.3618.

Methyl 3-O-[2-O-Benzovl-6-O-(2-O-benzovl-3,4,6-tri-O-benzvl-B-D-glucopyranosyl)-3-O-(4,6-O-benzylidene-2,3-di-O-benzoyl- $\beta$ -Dqlucopyranosyl)-β-D-qlucopyranosyl]-2-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranoside (29). A solution of 26 (66.5 mg, 0.0606 mmol) and 7<sup>11</sup> (50.8 mg, 0.0727 mmol) in DCM (1.82 mL) was dried over 4 Å MS (121 mg) at room temperature for 1 h. Then, a catalytic amount of TMSOTf (0.0022 mL, 0.00121 mmol) was added to the solution at -78 °C. After being warmed to -50 °C over 90 min, the solution was quenched with NEt<sub>3</sub>, filtered through a pad of Celite. The filtrate was poured into saturated NaHCO<sub>3</sub> (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (toluene/ethyl acetate = 10:1) to give **29** (60.9 mg, 0.0373 mmol, 61%) as a colorless oil:  $[\alpha]_{D}^{26}$  +3.53 (c 0.760, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.14 (d, 2H, J = 7.7 Hz), 7.80 (d, 2H, J = 7.8 Hz), 7.53-7.08 (m, 46H), 5.61 (t, 1H, J = 9.4, 9.4 Hz), 5.49 (s, 2H), 5.39 (t, 1H, J = 8.5, 8.5 Hz), 5.30 (t, 1H, J = 7.9, 7.9 Hz), 5.16 (t, 1H, J = 8.6, 8.6 Hz), 4.84 (d, 1H, J = 8.1 Hz), 4.79–4.75 (m, 3H,), 4,70 (d, 1H, J = 10.9 Hz), 4.64 (d, 1H, J = 11.0 Hz), 4.58 (d, 1H, J = 12.1 Hz), 4.54 (d, 1H, J = 11.1 Hz), 4.44-4.35 (m, 3H), 4.22 (d, 1H, J = 3.4 Hz), 4.15-4.02 (m, 4H), 3.85 (t, 1H, J)= 9.3, 9.3 Hz), 3.80-3.63 (m, 8H), 3.58 (t, 1H, J = 9.3, 9.3 Hz), 3.48-3.44 (m, 3H), 3.32-3.20 (m, 3H), 3.14 (dd, 1H, J = 9.1, 3.4 Hz), 3.12 (s, 3H);  ${}^{13}C{}^{1}H$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  165.6, 165.4, 165.2, 164.4, 138.7, 138.5, 138.4, 138.1, 138.0, 137.7, 136.6, 133.2, 133.0, 132.7, 130.2, 130.2, 129.8, 129.8, 129.6, 129.4, 129.2, 129.2, 129.2, 129.0, 128.9, 128.6, 128.4, 128.4, 128.3, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 126.3, 126.2, 125.4, 101.7, 101.7, 101.1, 100.9, 100.3, 99.4, 84.1, 83.5, 80.0, 79.7, 78.5, 77.6, 77.4, 75.9, 75.0, 75.0, 74.5, 74.1, 73.8, 73.5, 72.9, 72.2, 71.9, 69.1, 68.9, 68.3, 68.2, 66.7, 62.6, 55.6, 21.6; FT-IR (neat) 2869, 1733, 1269, 1092 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + NH<sub>4</sub>]<sup>+</sup> calcd for C<sub>95</sub>H<sub>96</sub>NO<sub>25</sub> 1650.6271, found 1650.6265.

Methyl 2-O-Benzyl-4,6-O-benzylidene-3-O-[3-O-(4,6-O-benzylidene- $\beta$ -p-qlucopyranosyl)-6-O-(3,4,6-tri-O-benzyl- $\beta$ -p-qlucopyranosyl)- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside (**30**). To a stirred solution of 29 (52.9 mg, 0.0324 mmol) in MeOH (1.30 mL) was added NaOMe (7.0 mg, 0.130 mmol) at room temperature. After being stirred in an oil bath at 85 °C for 5 h, the solution was quenched with Dowex 50W-4X, filtered through a pad of Celite, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (toluene/ethyl acetate = 1:2) to give **30** (26.8 mg, 0.0220 mmol, 69%) as a colorless oil:  $\left[\alpha\right]_{D}^{29}$  +0.428 (c 1.20, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.50–7.45 (m, 4H), 7.36-7.24 (m, 24H), 7.18-7.14 (m, 2H), 5.53 (s, 1H), 5.50 (s, 1H), 4.95 (d, 1H, J = 11.2 Hz), 4.81-4.76 (m, 2H), 4.73 (d, 1H, J = 11.6 Hz), 4.65 (d, 1H, J = 11.7 Hz), 4.59-4.47 (m, 6H), 4.31-4.13 (m, 5H), 3.83–3.32 (m, 20H), 3,32 (s, 3H, CH<sub>3</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 139.1, 138.4, 138.3, 137.3, 137.2, 137.0, 130.3, 129.4, 129.2, 128.9, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 126.4, 126.2, 105.4, 104.3, 104.1, 102.1, 101.1, 98.4, 87.8, 84.7, 80.4, 80.2, 79.1, 78.4, 77.4, 77.4, 75.2, 75.2, 75.0, 75.0, 74.5, 73.9, 73.6, 73.5, 73.3, 69.3, 69.0, 68.9, 68.8, 68.5, 66.9, 62.5, 55.5; FT-IR (neat) 3464, 2922, 1373, 1086 cm<sup>-1</sup>; HRMS (ESI-TOF)  $m/z [M + Na]^+$  calcd for  $C_{67}H_{76}O_{21}Na$  1239.4777, found 1239.4761.

Methyl 3-O-[3,6-Di-O-( $\beta$ -D-Glucopyranosyl)- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside (**31**). To a stirred solution of **30** (16.9 mg, 13.9  $\mu$ mol) in MeOH (1.40 mL) was added 10% Pd/C (21.0 mg) at room temperature. After being stirred at room temperature under hydrogen overnight, the solution was filtered through a pad of Celite and concentrated *in vacuo*. The residue was purified by reverse-phase column chromatography on Bond Elut-C18 (methyl alcohol) and diluted with water. Freeze-drying of the aqueous solution gave **31** (5.7 mg, 8.55  $\mu$ mol, 61%) as a white solid:  $[\alpha]_{D}^{21}$  +4.63 (*c* 0.340, H<sub>2</sub>O); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  4.84 (d, 1H, H-1, J<sub>1,2</sub> = 3.2 Hz), 4.78 (d, 1H), 4.73 (d, 1H, J = 8.0 Hz), 4.53 (d, 1H, J = 8.0 Hz), 4.24 (d, 1H, J = 11.5 Hz), 3.95–3.30 (m, 23H);  ${}^{13}C{}^{1}H$  NMR (100 MHz, D<sub>2</sub>O + CD<sub>3</sub>OD as an internal standard)  $\delta$  103.7, 103.7, 100.1, 85.0, 84.1, 77.0, 76.8, 76.6, 76.5, 75.4, 74.4, 74.1, 72.3, 71.5, 70.6, 70.5, 69.7, 69.1, 69.0, 61.7, 61.5, 55.9, 49.8; FT-IR (neat) 3894, 1575, 1263, 1199 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + Na]<sup>+</sup> calcd for C<sub>25</sub>H<sub>44</sub>O<sub>21</sub>Na 703.2273, found 703.2246.

Methyl 2-O-Benzyl-4,6-O-benzylidene-3-O-[3-O-(4,6-O-benzylidene- $\beta$ -D-glucopyranosyl)- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside (27). To a stirred solution of 26 (54.3 mg, 0.0495 mmol) in MeOH (1.98 mL) was added NaOMe (10.7 mg, 0.198 mmol) at room temperature. After being stirred in an oil bath at 70 °C for 3 h, the solution was quenched with Dowex 50W-4X, filtered through a pad of Celite, and concentrated in vacuo to give 27 (38.5 mg, 0.0491 mmol, 99%) as a colorless oil:  $[\alpha]_{D}^{27}$  -5.92 (c 0.995, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.48-7.44 (m, 4H), 7.37-7.34 (m, 11H), 5.50 (s, 1H), 5.48 (s, 1H, benzylidene), 4.74 (d, 1H, J = 11.9 Hz), 4.63 (d, 1H, J = 11.9 Hz), 4.58–4.56 (m, 2H), 4,49 (d, 1H, J = 7.7 Hz), 4.30-4.16 (m, 3H), 3.81-3.41 (m, 15H), 3.33 (s, 3H), 3.28–3.24 (m, 1H);  ${}^{13}C{}^{1}H$  NMR (100 MHz, CDCl<sub>2</sub>)  $\delta$  137.2, 137.0, 129.4, 129.4, 128.8, 128.6, 128.6, 128.5, 126.4, 126.1, 105.3, 104.3, 102.0, 101.6, 98.4, 88.3, 80.2, 80.2, 79.1, 78.6, 75.8, 74.9, 73.5, 73.4, 73.3, 68.9, 68.8, 68.4, 66.8, 62.6, 62.2, 55.5, 50.9; FT-IR (neat) 3329, 2912, 1078 cm<sup>-1</sup>; HRMS (ESI-TOF)  $m/z [M + Na]^+$  calcd for C40H48O16Na 807.2840, found 807.2821.

Methyl 3-O-[3-O-( $\beta$ -D-Glucopyranosyl)- $\beta$ -D-glucopyranosyl]- $\alpha$ -Dglucopyranoside (28). To a stirred solution of 27 (38.5 mg, 0.0491 mmol) in MeOH (0.981 mL) was added 10% Pd/C (98.1 mg) at room temperature. After being stirred at room temperature under hydrogen, the solution was filtered through a pad of Celite and concentrated in vacuo. The residue was purified by reverse-phase column chromatography on Bond Elut-C18 (methyl alcohol) and diluted with water. Freeze-drying of the aqueous solution gave 28 (8.5 mg, 0.0164 mmol, 33%) as a white solid:  $[\alpha]_{D}^{24}$  +1.85 (*c* 0.610, H<sub>2</sub>O); <sup>1</sup>H NMR (400 MHz,  $D_2O$ )  $\delta$  4.83 (d, 1H, J = 3.7 Hz), 4.76 (d, 1H, J = 7.9 Hz), 4.75 (d, 1H, J = 8.0 Hz), 3.95-3.87 (m, 4H), 3.81-3.67 (m, 6H), 3.59-3.47 (m, 6H), 3.44 (s, 3H), 3.44 (m, 2H);  ${}^{13}C{}^{1}H$ NMR (100 MHz,  $D_2O+CD_3OD$  as an internal standard)  $\delta$  103.8, 103.5, 100.1, 85.2, 83.3, 76.9, 76.5, 76.5, 74.4, 74.2, 72.3, 71.8, 70.5, 69.0, 68.9, 61.6, 61.6, 61.4, 55.9; FT-IR (neat) 3380, 2925, 1635, 1373, 1156, 1075,1043 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + Na]<sup>+</sup> calcd for C<sub>19</sub>H<sub>34</sub>O<sub>16</sub>Na 541.1745, found 541.1749.

Penta-O-acetyl-*D*-glucose<sup>-13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (**32**). To a stirred solution of **13** (5.0 g, 25.9 mmol) in Ac<sub>2</sub>O (53.6 mL) was addde Cu(OTf)<sub>2</sub> (0.468 g, 1.29 mmol) at room temperature. After being stirred at room temperature for 1 h, the solution was poured into saturated NaHCO<sub>3</sub> (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 2:1) to give **32** (10.4 g, 25.9 mmol, quant.,  $\alpha\beta$  = 2:1) as a white solid:  $[\alpha]_{12}^{29}$ +20.5 (*c* 1.08, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  2.23 (s), 2.18 (s), 2.12 (s), 2.09 (s), 2.09 (s), 2.04 (s), 2.03 (s), 2.03 (s), 2.02 (s); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>): 170.5, 170.1, 169.5, 169.3, 168.7, 166.3, 91.7–90.7 (m, C-1 $\alpha$ ), 89.1–88.1 (m, C-1 $\beta$ ), 72.8–71.3 (m), 70.4–66.7 (m), 61.4–60.3 (m), 20.7, 20.7, 20.6, 20.5, 20.4, 20.3; HRMS (ESI-TOF) *m*/*z* [M + Na]<sup>+</sup> calcd for C<sub>10</sub><sup>13</sup>C<sub>6</sub>H<sub>15</sub>D<sub>7</sub>O<sub>11</sub>Na 426.1701, found 426.1696.

*Tri-O-acetyl-D-glucal-*<sup>13</sup> $C_{67}$  <sup>2</sup> $H_7$  (**33**). To a stirred solution of **32** (1.5 g, 3.72 mmol) in DCM (11.2 mL) was added 30% HBr/AcOH (3.72 mL) at 0 °C. After being stirred at room temperature overnight, the solution was concentrated *in vacuo* to give a residue, which was used for the next reaction without further purification. To a stirred solution of the residue in water (7.44 mL) and AcOH (10.2 mL) were sequentially added NaOAc (3.89 g, 47.4 mmol), CuSO<sub>4</sub>·SH<sub>2</sub>O (0.315 mg, 1.30 mmol), and Zn dust (8.46 g, 129 mmol) at 0 °C. After being stirred at room temperature overnight, the solution was poured into saturated NaHCO<sub>3</sub> (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/ethyl

acetate = 1:1) to give **33** (0.908 g, 3.18 mmol, 86% in 2 steps) as a yellow oil:  $[\alpha]_D^{23}$  -6.91 (c 0.270, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  2.10 (s, 3H, acetyl), 2.08 (3H, s, acetyl), 2.05 (3H, s, acetyl); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  170.6, 170.3, 169.6, 146.0–144.7 (m, 1C, C-1), 99.2–97.5 (m, 1C, C-2), 77.3, 74.0–72.7 (m, 1C), 67.5–66.0 (m, 2C), 61.4–60.1 (m, 1C, C-6), 21.0, 20.8, 20.7; FT-IR (neat) 1741, 1637, 1371, 1244 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + NH<sub>4</sub>]<sup>+</sup> calcd for C<sub>6</sub><sup>-13</sup>C<sub>6</sub>H<sub>13</sub>D<sub>7</sub>NO<sub>7</sub> 303.1881, found 303.1868.

4,6-O-tert-Butylsilylidene-D-glucal- ${}^{13}C_{6r}$   ${}^{2}H_{7}$  (12). To a stirred solution of 33 (0.908 g, 3.18 mmol) in methyl alcohol (17.5 mL) was added NaOMe (18.9 mg, 0.318 mmol) at room temperature. After being stirred at room temperature for 3 h, the solution was quenched with Dowex 50W-4X and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (chloroform/ methyl alcohol = 9:1) to give a glucal. To a stirred solution of the glucal in DMF (8.03 mL) was added 'Bu<sub>2</sub>Si(OTf)<sub>2</sub> (0.817 mL, 2.52 mmol) at -45 °C. After being stirred at -45 °C for 1 h, pyridine (0.370 mL, 4.58 mmol) was added to the reaction mixture. Then, the solution was warmed to 0 °C over 90 min and poured into saturated  $NaHCO_3$  (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 2:1) to give 12 (0.561 g, 1.87 mmol, 82%) as a colorless oil:  $[\alpha]_D^{23}$  +4.47 (c 0.100, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.07 (s, 9H, butylsilylidene), 0.99 (s, 9H, butylsilylidene); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 144.2–142.9 (m, 1C, C-1), 103.4–101.7 (m, 1C, C-2), 77.3-76.2 (m, 1C), 72.4-71.1 (m, 1C), 70.4-69.1 (m, 1C), 65.7-64.4 (m, 1C), 27.6, 27.1, 22.9, 20.0; FT-IR (neat) 3005, 1473, 1260, 1070 cm<sup>-1</sup>; HRMS (ESI-TOF)  $m/z [M + NH_4]^+$  calcd for C<sub>8</sub><sup>13</sup>C<sub>6</sub>H<sub>23</sub>D<sub>7</sub>NO<sub>4</sub>Si 317.2585, found 317.2566.

3-O-(4,6-O-Benzylidene-2,3-di-O-benzoyl-β-D-glucopyranosyl)-4,6-O-tert-butylsilylidene-*D*-glucal- $^{13}C_{6}$ ,  $^{2}H_{7}$  (**34**). A solution of **12** (0.561 g, 1.87 mmol) and 11 (1.22 g, 1.97 mmol) in DCM (37.5 mL) was dried over 4 Å MS (1.87 g) at room temperature for 1 h. Then, a catalytic amount of TMSOTf (0.0416 mL, 0.187 mmol) was added to the solution at -78 °C. After being warmed to -50 °C over 30 min, the solution was quenched with NEt<sub>3</sub>, filtered through a pad of Celite. The filtrate was poured into saturated NaHCO<sub>3</sub> (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (toluene/ethyl acetate = 9:1) to give 34 (1.21 g, 1.60 mmol, 85%) as a white solid:  $[\alpha]_{D}^{28}$  +2.85 (c 0.800, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.00–7.97 (m, 4H), 7.56–7.30 (m, 11H), 5.74 (t, 1H, J = 9.0, 9.0 Hz), 5.56 (s, 1H), 5.45 (dd, 1H, J = 8.6, 7.5 Hz), 5.13 (dd, 1H, J = 7.2, 4.9 Hz), 4.44 (dd, 1H, J = 10.5, 4.8 Hz), 4.08 (t, 1H, J = 9.5, 9.5 Hz), 3.91 (t, 1H, J = 10.2, 10.2 Hz), 3.77-3.72 (dt, 1H, J = 9.8, 9.8, 4.7 Hz), 1.10 (s, 9H), 1.03 (s, 9H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 165.8, 165.3, 145.1–143.8 (m, 1C, C-1), 133.4, 133.2, 130.0, 129.9, 129.2, 128.6, 128.4, 128.4, 126.2, 125.4, 101.6, 101.3-99.6 (m, 1C, C-2), 79.2-77.9 (m, 1C), 75.4-74.2 (m, 1C), 72.8-71.6 (m, 1C), 65.2-64.8 (m, 1C), 27.6, 27.2; FT-IR (neat) 2961, 1731, 1271, 1093, 1071 cm<sup>-1</sup>; HRMS (ESI-TOF) *m/z* [M + Na]<sup>+</sup> calcd for  $C_{35}^{13}C_6H_{41}D_7O_{11}$ SiNa 780.3505, found 780.3479.

3-O-(4,6-O-Benzylidene-2,3-di-O-benzoyl- $\beta$ -D-glucopyranosyl)-4,6-O-tert-butylsilylidene- $\beta$ -D-glucopyranosyl o-1-hexynylbenzoate-<sup>13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (**36**). To a stirred solution of 34 (961 mg, 1.27 mmol) in DCM (6.34 mL) was added a solution of DMDO in acetone (12.7 mL, 1.27 mmol) at -20 °C. After being warmed to room temperature overnight, the solution was concentrated *in vacuo* to give **35** as a white solid, which was directly used for the next reaction:  $[\alpha]_{D}^{23}$  +8.48 (*c* 0.390, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.98–7.95 (m, 4H), 7.55–7.48 (m, 2H), 7.42–7.29 (m, 9H), 5.74 (dd, 1H, *J* = 9.4, 8.6 Hz), 5.56 (s, 1H), 5.49 (dd, 1H, *J* = 8.5, 7.2 Hz), 5.13 (dd, 1H, *J* = 7.2, 4.3 Hz), 4.41 (dd, 1H, *J* = 10.4, 4.8 Hz), 4.08 (t, 1H, *J* = 9.4, 9.4 Hz), 3.86 (t, 1H, *J* = 10.2, 10.2 Hz), 3.75 (dt, 1H, *J* = 9.6, 9.6, 4.8 Hz), 1.08 (s, 9H), 0.99 (s, 9H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ 165.7, 165.3, 133.6, 133.3, 130.0, 129.9, 129.5, 129.2, 129.1, 128.7,

128.6, 128.5, 128.4, 126.2, 101.6, 78.7-75.9 (m, 2C), 74.3-73.1 (m, 1C), 72.4, 66.8-63.7 (m, 2C), 53.0-51.7 (m, 1C), 31.0, 27.6, 27.5, 27.1, 22.9, 20.0; FT-IR (neat) 2962, 2859, 2252, 1731, 1373, 1272, 1096 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + H]<sup>+</sup> calcd for  $C_{35}^{13}C_6H_{42}D_7O_{12}Si$  774.3634, found 774.3607. A solution of 35 in DCM (12.7 mL) was dried over 4 Å MS (2.54 g) at room temperature for 1 h. Then, o-1-hexynylbenzoic acid (282 mg, 1.39 mmol) was added to the solution at -20 °C. After being stirred at room temperature overnight, the solution was filtered through a pad of Celite. The filtrate was poured into saturated NaHCO<sub>3</sub> (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 5:1) to give 36 (491 mg, 0.503 mmol, 40% in 2 steps) as a colorless oil:  $\left[\alpha\right]_{D}^{28}$ +6.24 (c 0.955, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.97-7.94 (m, 4H), 7.52-7.27 (m, 15H), 5.79 (t, 1H, J = 9.4, 9.4 Hz), 5.57 (s, 1H), 5.51 (t, 1H, J = 7.6, 7.6 Hz), 5.29 (dd, 1H, J = 7.2, 4.3 Hz), 4.45 (dd, 1H, J = 10.4, 4.7 Hz), 4.03 (t, 1H, J = 9.5, 9.5 Hz), 3.90 (t, 1H, J = 10.3 Hz, 10.3 Hz), 3.72 (m, 1H), 2.38 (t, 2H, J = 7.0, 7.0 Hz), 1.61-1.42 (m, 4H), 1.11 (s, 9H), 1.05 (s, 9H), 0.93 (t, 3H, J = 7.2, 7.2 Hz);  ${}^{13}C{}^{1}H$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  166.3, 165.7, 136.8, 134.6, 134.3, 133.5, 133.2, 132.3, 130.9, 130.0, 129.9, 129.5, 129.3, 129.2, 128.6, 128.4, 128.3, 127.2, 126.2, 125.3, 102.1, 101.5, 96.9, 94.6-93.5 (m, 1C), 84.9-84.1 (m, 1C), 79.4, 78.7, 75.7-74.9 (m, 1C), 74.1, 72.2-70.2 (m, 2C), 69.0, 66.9, 65.3 (br, 1C), 30.7, 27.5, 27.2, 22.8, 22.1, 20.1, 19.5, 13.8; FT-IR (neat) 2933, 1732, 1272, 1099, 1069 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + Na]<sup>+</sup> calcd for C48<sup>13</sup>C6H55D7O14SiNa 998.4447, found 998.4431.

2-O-Benzoyl-3-O-(4,6-O-benzylidene-2,3-di-O-benzoyl- $\beta$ -D-qlucopyranosyl)-4,6-O-tert-butylsilylidene- $\beta$ -D-glucopyranosyl o-1-Hexynylbenzoate-<sup>13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (**9**). To a stirred solution of **36** (471 mg, 0.483 mmol) in pyridine (5.03 mL) were sequentially added BzCl (0.526 mL, 4.52 mmol) and DMAP (6.10 mg, 0.0483 mmol) at room temperature. After being stirred in an oil bath at 80 °C for 1 h, the solution was poured into 3 M HCl (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with saturated NaHCO3 (aq) and brine, dried over MgSO4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 7:1) to give 9 (433 mg, 0.401 mmol, 83%) as a yellow oil:  $[\alpha]_D^{28}$  +11.8 (c 0.965, CHCl<sub>2</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>2</sub>)  $\delta$  7.86 (d, 2H, J = 7.2 Hz), 7.75 (d, 1H, J = 8.0 Hz), 7.71 (d, 2H, J = 7.2 Hz), 7.58 (d, 2H, J = 7.2 Hz), 7.51–7.13 (m, 17H), 5.61 (t, 1H, J = 9.4, 9.4 Hz), 5.54 (s, 1H), 5.43 (dd, 1H, J = 8.8, 7.5 Hz), 5.17 (dd, 1H, J = 7.4, 3.5 Hz), 4.44 (dd, 1H, J = 10.5, 4.8 Hz), 3.94 (t, 1H, J = 9.5, 9.5 Hz), 3.85 (t, 1H, J = 10.2, 10.2 Hz), 3.69 (dt, 1H, J = 9.6, 9.6, 4.8 Hz), 2.41 (t, 2H, J = 7.0, 7.0 Hz), 1.60–1.53 (m, 2H), 1.49–1.42 (m, 2H), 1.13 (s, 9H), 1.07 (s, 9H), 0.92 (t, 3H, J = 7.3, 7.3 Hz);  ${}^{13}C{}^{1}H$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  165.7, 165.1, 136.9, 134.6, 133.7, 133.3, 133.1, 132.9, 132.5, 130.9, 130.3, 129.8, 129.8, 129.4, 129.2, 129.1, 128.6, 128.5, 128.3, 128.2, 127.3, 126.2, 125.8, 101.5, 100.9, 97.3, 92.8-91.9 (m, 1C), 80.9-80.0 (m, 1C), 79.0, 78.7, 77.4, 75.4-74.2 (m, 1C), 72.5-70.6 (m, 2C), 69.0, 66.6, 65.5 (br, 1C), 30.8, 27.5, 27.2, 22.8, 22.2, 20.1, 19.6, 13.8; FT-IR (neat) 1736, 1273, 1104, 1069 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + Na]<sup>+</sup> calcd for C<sub>55</sub><sup>13</sup>C<sub>6</sub>H<sub>59</sub>D<sub>7</sub>O<sub>15</sub>SiNa 1102.4709, found 1102.4664.

Methyl 3-O-[2-O-Benzoyl-3-O-(4,6-O-benzylidene-2,3-di-O-benzoyl- $\beta$ -D-glucopyranosyl)-4,6-O-tert-butylsilylidene- $\beta$ -D-glucopyranosyl]-2-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranoside-<sup>13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (**37**). A solution of **9** (413 mg, 0.382 mmol) and **10** (171 mg, 0.459 mmol) in toluene (7.65 mL) was dried over 4 Å MS (383 mg) at room temperature for 1 h. Then, a solution of PPh<sub>3</sub>-AuOTf in DCM (0.765 mL, 0.0765 mmol) was added to the solution at -20 °C. After being stirred at room temperature overnight, the solution was filtered through a pad of Celite. The filtrate was poured into saturated NaHCO<sub>3</sub> (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (toluene/ethyl

acetate = 20:1) to 37 (369 mg, 0.295 mmol, 77%) as a colorless oil:  $[\alpha]_{D}^{28}$  -3.86 (c 1.02, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.84 (d, 2H, J = 8.1 Hz), 7.77 (d, 2H, J = 8.0 Hz), 7.59 (d, 2H, J = 8.0 Hz), 7.54-7.20 (m, 24H), 5.55 (t, 1H, J = 9.3 Hz, 9.3 Hz), 5.51 (s, 1H), 5.43 (s, 1H), 5.38 (dd, 1H, J = 8.8, 7.3 Hz), 5.08 (dd, 1H, J = 7.4, 3.4 Hz), 4.43-4.39 (m, 2H), 4.15-4.12 (m, 2H), 4.06 (dt, 1H, J = 9.2, 9.2, 4.0 Hz), 4.18 (d, 1H, J = 12.7 Hz), 3.91 (t, 1H, J = 9.4, 9.4 Hz), 3.81 (t, 1H, J = 10.2, 10.2 Hz), 3.72-3.58 (m, 3H), 3.42 (t, 1H, J = 9.2, 9.2 Hz), 3.22-3.19 (m, 4H), 1.09 (s, 9H), 0.97 (s, 9H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 165.7, 165.2, 164.6, 138.4, 138.0, 137.5, 136.9, 133.1, 133.1, 132.8, 129.9, 129.8, 129.8, 129.4, 129.4, 129.2, 129.1, 129.1, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 127.9, 126.2, 125.4, 101.4-100.4 (m, 1C), 99.0, 81.1-79.9 (m, 1C), 79.7, 78.7, 77.4, 75.5-73.0 (m, 2C), 72.6, 70.4-69.5 (m, 1C), 69.0, 66.4, 65.9 (m, 1C), 62.2, 55.3, 27.5, 27.2, 22.8, 21.6, 20.0; FT -IR (neat) 2933, 2859, 1736, 1274, 1091 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + H] calcd for C<sub>63</sub><sup>13</sup>C<sub>6</sub>H<sub>70</sub>D<sub>7</sub>O<sub>19</sub>Si 1250.5469, found 1250.5457.

Methyl 3-O-[2-O-Benzovl-3-O-(4.6-O-benzvlidene-2.3-di-O-benzoyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside- ${}^{13}C_{6}$ ,  ${}^{2}H_7$  (8). To a stirred solution of 37 (350 mg, 0.280 mmol) in THF (5.60 mL) was added HFpyridine (0.280 mL) at 0 °C. After being stirred at 0 °C for 1 h, the solution was poured into saturated NaHCO<sub>3</sub> (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/ ethyl acetate = 1:1) to give 8 (150 mg, 0.135 mmol, 48%) as a colorless oil:  $[\alpha]_{D}^{26}$  +1.04 (c 1.16, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.81 (d, 2H, J = 7.2 Hz), 7.55 (t, 4H, J = 8.4, 8.4 Hz), 7.48-7.19 (m, 22H), 7.10 (t, 2H, J = 7.8, 7.8 Hz), 5.66 (t, 1H, J = 9.6, 9.6 Hz), 5.51 (s, 1H), 5.44 (dd, 1H, J = 9.5, 7.8 Hz), 5.43 (s, 1H), 4.87 (dd, 1H, J = 7.8, 3.5 Hz), 4.45 (dd, 1H, J = 10.3, 4.7 Hz), 4.32-4.29 (d, 1H, J = 12.6 Hz), 4.17-4.10 (m, 2H), 4.05 (dt, 1H, J = 9.3, 9.3, 5.0 Hz), 3.92–3.82 (m, 3H), 3.78–3.66 (m, 2H), 3.58 (t, 1H, J = 10.2, 10.2 Hz), 3.43-3.38 (m, 2H), 3.21-3.15 (m, 4H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 165.5, 165.2, 164.4, 138.2, 137.4, 136.5, 133.2, 133.1, 132.7, 129.8, 129.7, 129.6, 129.2, 129.1, 128.9, 128.6, 128.4, 128.3, 128.3, 128.1, 127.9, 127.6, 126.2, 126.0, 101.8, 101.6, 101.4, 100.5-100.0 (m, 1C), 98.9, 84.5-83.7 (m, 1C), 79.9, 79.7, 78.5, 77.0, 74.6; FT-IR (neat) 3475, 2868, 1733, 1452, 1276, 1179, 1092 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + Na]<sup>+</sup> calcd for C<sub>55</sub><sup>13</sup>C<sub>6</sub>H<sub>53</sub>D<sub>7</sub>O<sub>19</sub>Na 1132.4267, found 1132.4315.

Methyl 3-O-[2-O-Benzoyl-6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl- $\beta$ -D-glucopyranosyl)-3-O-(4,6-O-benzylidene-2,3-di-O-benzoyl- $\beta$ -Dglucopyranosyl)- $\beta$ -D-glucopyranosyl]-2-O-benzyl-4,6-O-benzyli-dene- $\alpha$ -D-glucopyranoside-<sup>13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (**38**). A solution of **8** (132 mg, 0.119 mmol) and 7 (156 mg, 0.223 mmol) in DCM (2.70 mL) was dried over 4 Å MS at room temperature for 1 h. Then, a catalytic amount of TMSOTf (6  $\mu$ L, 0.238 mmol) was added to the solution at -78 °C. After being stirred at -60 °C for 40 min, the solution was quenched with NEt<sub>3</sub>, filtered through a pad of Celite ,and concentrated in vacuo. The residue was purified by column chromatography on silica gel (toluene/ethyl acetate = 9:1) to give 38 (153 mg, 0.0929 mmol, 78%) as a colorless oil:  $[\alpha]_{\rm D}^{29}$  +4.11 (c 0.970, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.15 (d, 2H, J = 7.1 Hz), 7.81 (d, 2H, J = 7.3 Hz), 7.53-7.08 (m, 44H), 6.97-6.95 (m, 2H), 5.61 (t, 1H, J = 9.5, 9.5 Hz), 5.49 (s, 2H), 5.39 (dd, 1H, J = 9.2, 7.8 Hz), 5.30 (t, 1H, J = 9.2, 9.2 Hz), 4.84 (dd, 1H, J = 8.0, 4.2 Hz), 4.79 (dd, 1H, J = 7.8, 3.4 Hz), 4.77 (d, 1H, J = 11.3 Hz), 4.70 (d, 1H, J = 11.0 Hz, 4.64 (d, 1H, J = 11.0 Hz), 4.58 (d, 1H, J = 12.2 Hz), 4.54 (d, 1H, J = 11.2 Hz), 4.44–4.35 (m, 3H), 4.23 (d, 1H, J = 3.8Hz), 4.15–4.09 (m, 2H), 4.04 (d, 1H, J = 12.8 Hz), 3.87–3.63 (m, 7H), 3.48-3.43 (m, 2H), 3.33-3.21 (m, 3H), 3.14 (dd, 1H, J = 9.2, 3.7 Hz), 3.12 (s, 3H, CH<sub>3</sub>);  ${}^{13}C{}^{1}H{}$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ 165.6, 165.4, 165.2, 138.7, 138.5, 138.4, 138.1, 137.7, 136.6, 133.2, 133.0, 132.7, 130.2, 130.2, 129.8, 129.8, 129.6, 129.4, 129.2, 129.1, 129.0, 128.9, 128.6, 128.4, 128.3, 128.3, 128.3, 128.1, 128.0, 127.9, 127.7, 127.7, 127.6, 127.6, 127.6, 126.3, 126.2, 125.4, 101.6, 101.1, 100.9, 100.0-99.4 (m, 1C), 83.9-83.1 (m, 1C), 80.0, 79.7, 78.5, 77.6, 77.4, 75.6–74.5 (m, 1C), 74.0, 73.8, 73.5, 72.9–71.9 (m, 1C), 68.9–

66.7 (m, 2C), 62.5, 55.5; FT-IR (neat) 2868, 1733, 1274, 1093, 1070 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + Na]<sup>+</sup> calcd for C<sub>89</sub><sup>13</sup>C<sub>6</sub>H<sub>85</sub>D<sub>7</sub>O<sub>25</sub>Na 1668.6466, found 1668.6493.

Methyl 2-O-Benzyl-4,6-O-benzylidene-3-O-[3-O-(4,6-O-benzylidene- $\beta$ -D-glucopyranosyl)-6-O-(3,4,6-tri-O-benzyl- $\beta$ -D-glucopyranosyl)- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside-<sup>13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (**39**). To a stirred solution of 38 (101 mg, 0.0613 mmol) in MeOH (2.45 mL) was added NaOMe (4.00 equiv, 13.3 mg, 0.245 mmol) at room temperature. After being stirred in an oil bath at 60 °C overnight, the solution was quenched with Dowex 50W-4X, filtered through a pad of Celite, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (chloroform/methyl alcohol = 95:5) to give 39 (73.0 mg, 0.0443 mmol, 97%) as a colorless oil:  $[\alpha]_{D}^{26}$  +0.370 (c 0.810, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.50–7.22 (m, 28H), 7.16-7.13 (m, 2H), 5.51 (s, 1H, benzylidene), 5.45 (s, 1H, benzylidene), 4.95 (d, 1H, benzyl, J = 11.2 Hz), 4.80-4.74 (m, 3H), 4.62 (d, 1H, benzyl, J = 11.8 Hz), 4.58–4.46 (m, 5H), 4.29–4.22 (m, 3H), 4.15 (dd, 1H, J = 10.2 Hz, J = 4.8 Hz), 3.81–3.38 (m, 19H), 3.29–3.25 (m, 4H, CH<sub>3</sub>);  ${}^{13}C{}^{1}H{}$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ 139.0, 138.4, 138.2, 137.4, 137.3, 137.0, 133.0, 129.7, 129.3, 129.1, 128.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 127.9, 127.9, 127.7, 127.7, 127.6, 126.4, 126.2, 105.1, 103.6-103.2 (m, 1C), 102.0, 101.1, 98.5, 87.4-86.7 (m, 1C), 84.6, 80.2, 79.2, 78.0, 77.4, 75.2-72.7 (m, 2C), 68.8-68.0 (m, 2C), 66.9, 62.5, 55.4, 52.2; FT-IR (neat) 3470, 2869, 1374, 1078 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + Na]<sup>+</sup> calcd for C<sub>61</sub><sup>13</sup>C<sub>6</sub>H<sub>69</sub>D<sub>7</sub>O<sub>21</sub>Na 1252.5418, found 1252.5385.

Methyl 3-O-[3,6-Di-O-( $\beta$ -D-Glucopyranosyl)- $\beta$ -D-glucopyrano-syl]- $\alpha$ -D-glucopyranoside-<sup>13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (5). To a stirred solution of 39 (64.8 mg, 0.0527 mmol) in MeOH (1.05 mL) and THF (1.05 mL) was added 10% Pd/C (105 mg) at room temperature. After being stirred at room temperature under hydrogen overnight, the solution was filtered through a pad of Celite and concentrated in vacuo. The residue was purified by reverse-phase column chromatography on Bond Elut-C18 (methyl alcohol) and diluted with water. Freezedrying of the aqueous solution gave 5 (8.7 mg, 0.0128 mmol, 24%) as a white solid:  $[\alpha]_{D}^{25}$  +0.861 (c 0.720, H<sub>2</sub>O); <sup>1</sup>H NMR (400 MHz,  $D_2O$ )  $\delta$  4.84 (d, 1H, J = 3.7 Hz), 4.77 (dd, 1H, J = 8.0, 3.8 Hz), 4.53 (dd, 1H, J = 7.9, 4.2 Hz), 3.95–3.85 (m, 4H), 3.81–3.68 (m, 5H), 3.56–3.30 (m, 12H);  ${}^{13}C{}^{1}H$  NMR (100 MHz, D<sub>2</sub>O)  $\delta$  103.7– 103.1 (m, 1C), 100.1, 84.3 (br, 1C), 77.0, 76.8, 76.5, 74.8-73.5 (m, 2C), 72.3, 71.5, 70.6, 69.1-68.0 (m, 2C), 61.7, 55.9; FT-IR (neat) 3351, 1076, 1038 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + Na]<sup>+</sup> calcd for C<sub>19</sub><sup>13</sup>C<sub>6</sub>H<sub>37</sub>D<sub>7</sub>O<sub>21</sub>Na 716.2914, found 716.2891.

Methyl 2-O-Benzyl-4,6-O-benzylidene-3-O-[3-O-(4,6-O-benzylidene- $\beta$ -D-glucopyranosyl)- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranosi $de^{-13}C_{6r}^{2}H_{7}$  (40). To a stirred solution of 8 (85.0 mg, 0.0766 mmol) in MeOH (3.09 mL) was added NaOMe (16.7 mg, 0.309 mmol) at room temperature. After being stirred in an oil bath at 70 °C for 4 h, the solution was quenched with Dowex 50W-4X, filtered through a pad of Celite, and concentrated in vacuo. The residue was recrystallized from methyl alcohol to give 40 (33.8 mg, 0.0424 mmol, 55%) as a colorless oil:  $[\alpha]_{D}^{29}$  +2.52 (c 0.980, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.52-7.46 (m, 4H), 7.40-7.35 (m, 11H), 5.53 (s, 1H), 5.53 (s, 1H), 4.73-4.66 (m, 3H), 4.48 (dd, 1H, J = 7.8, 5.0 Hz), 4.31 (dd, 1H, J = 10.5, 4.8 Hz), 4.27 (dd, 1H, J = 10.0, 4.6 Hz), 4.17 (dt, 1H, J = 9.5, 9.5, 5.1 Hz), 3.86-3.68 (m, 5H), 3.63 (dd, 1H, J = 9.5, 3.6 Hz), 3.59-3.48 (m, 4H), 3.37 (s, 3H), 3.30 (d, 1H, J = 1.5 Hz), 3.21-3.18 (m, 1H), 2.70 (d, 1H, J = 2.2 Hz), 1.84-1.83 (m, 1H);  ${}^{13}C{}^{1}H$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  137.1, 137.0, 129.4, 128.9, 128.7, 128.6, 128.5, 126.4, 126.0, 105.6, 104.6-103.7 (1C), 102.1, 101.6, 98.3, 88.5-87.3 (1C), 80.4, 80.2, 79.1, 75.7-74.9 (1C), 73.5-72.9 (1C), 69.0-68.2 (1C), 66.9, 62.7, 62.2-61.0 (1C), 55.5; FT-IR (neat) 3452, 1376, 1176, 1086 cm<sup>-1</sup>; HRMS (ESI-TOF)  $m/z [M + Na]^+$  calcd for  $C_{34}^{13}C_6H_{41}D_7O_{16}Na$  820.3481, found 820.3499

Methyl 3-O-[3-O-( $\beta$ -D-Glucopyranosyl)- $\beta$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside-<sup>13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (6). To a stirred solution of 40 (20.0 mg, 0.0251 mmol) in MeOH (1.00 mL) and THF (2.0 mL) was added 10% Pd/C (50.0 mg) at room temperature. After being stirred at room temperature under hydrogen overnight, the solution was filtered

through a pad of Celite and concentrated *in vacuo*. The residue was purified by reverse-phase column chromatography on Bond Elut-C18 (methyl alcohol) and diluted with water. Freeze-drying of the aqueous solution gave **6** (5.0 mg, 0.00964 mmol, 38%) as a white solid:  $[\alpha]_D^2$  +7.03 (*c* 0.110, H<sub>2</sub>O); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  4.83 (d, 1H, J<sub>1,2</sub> = 3.7 Hz), 4.76 (dd, 1H, *J* = 8.0, 3.9 Hz), 3.95–3.86 (m, 3H), 3.81–3.67 (m, 4H), 3.56–3.34 (m, 8H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, D<sub>2</sub>O)  $\delta$  103.7–102.5 (m, 1C), 100.1, 84.6 (m, 1C), 76.9, 76.5–75.4 (m, 1C), 74.4–72.9 (m, 1C), 72.3, 71.7, 70.5, 68.9–67.9 (m, 1C), 61.6, 60.9 (m, 1C), 55.9; FT-IR (neat) 3538, 3198, 2952, 2841, 1668, 1451, 1410, 1016 cm<sup>-1</sup>; HRMS (ESI-TOF) *m*/*z* [M + Na]<sup>+</sup> calcd for C<sub>13</sub><sup>13</sup>C<sub>6</sub>H<sub>27</sub>D<sub>7</sub>O<sub>16</sub>Na 554.2386, found 554.2338.

Tetra-O-acetyl-α-D-glucopyranosyl Bromide- ${}^{13}C_{6'}{}^{2}H_7$  (41). To a stirred solution of **32** (0.553 g, 1.37 mmol) in DCM (4.11 mL) was added 30% HBr/AcOH (1.37 mL) at 0 °C. After being stirred at room temperature overnight, the solution was poured into saturated NaHCO<sub>3</sub> (aq) and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 2:1) to **41** (0.352 g, 0.830 mmol, 61%) as a yellow oil:  $[\alpha]_D^{26}$  +57.5 (*c* 1.17, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 2.10 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 170.6, 169.9, 169.9, 169.6, 86.8–85.9 (m, 1C, C-1), 72.3–71.0 (m, 1C), 70.7–69.1 (m, 2C), 67.6–66.2 (m, 1C), 61.1–59.8 (m, 1C, 6-C), 20.8, 20.7, 20.7, 20.6; FT-IR (neat) 1748, 1372, 1241 cm<sup>-1</sup>; HRMS (ESI-TOF) *m*/*z* [M + NH<sub>4</sub>]<sup>+</sup> calcd for C<sub>8</sub><sup>13</sup>C<sub>6</sub>H<sub>16</sub>D<sub>7</sub>BrNO<sub>9</sub> 441.1197, found 441.1156.

Methyl 2-O-Benzyl-4,6-O-benzylidene-3-O-(tetra-O-acetyl- $\beta$ -Dglucopyranosyl)- $\alpha$ -*D*-glucopyranoside-<sup>13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (42). A solution of 41 (0.229 g, 0.540 mmol) and 10 (0.261 g, 0.701 mmol) in DCM (5.39 mL) was dried over 4 Å MS at room temperature for 1 h. Then, a stoichiometric amount of AgOTf (1.10 equiv, 0.152 g, 0.592 mmol) was added to the solution at -40 °C. After being warmed to -20 °C over 2 h, the solution was quenched with NEt<sub>3</sub> and filtered through a pad of Celite. The filtrate was poured into saturated water and extracted with two portions of ethyl acetate. The combined organic layer was washed with brine, dried over MgSO4, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (toluene/ethyl acetate = 9:1) to give 42 (0.228 g, 0.319 mmol, 59%) as a colorless oil:  $[\alpha]_D^{29}$  -11.1 (c 0.900, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.48-7.45 (m, 2H), 7.37-7.35 (m, 8H), 5.52 (s, 1H), 4.77 (d, 1H, J = 12.1 Hz), 4.49 (d, 1H, J = 12.1 Hz), 4.47 (d, 1H, J = 3.8 Hz), 4.23–4.17 (m, 2H), 3.79 (dt, 1H, J = 9.7, 9.7, 4.4 Hz), 3.70 (t, 1H, J = 9.9, 9.9 Hz), 3.60 (t, 1H, J = 9.2, 9.2 Hz), 3.51 (dd, 1H, J = 9.3, 3.8 Hz), 3.35 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 170.7, 170.4, 169.6, 169.4, 138.0, 137.4, 129.3, 128.6, 128.4, 128.3, 128.2, 126.1, 101.5, 100.9-100.0 (m), 98.9, 80.6, 78.9, 78.9, 78.3, 74.1, 73.0–70.6 (m), 69.1, 68.4–67.1 (m), 62.1, 61.4–61.0 (m), 55.4, 20.8, 20.8, 20.7, 20.7; FT-IR (neat) 2913, 1751, 1371, 1244, 1050 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + NH<sub>4</sub>]<sup>+</sup> calcd for C<sub>29</sub><sup>13</sup>C<sub>6</sub>H<sub>39</sub>D<sub>7</sub>NO<sub>15</sub> 733.3508, found 733.3457.

Methyl 2-O-Benzyl-4,6-O-benzylidene-3-O- $(\beta$ -D-glucopyranosyl)- $\alpha$ -D-glucopyranoside-<sup>13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (43). To a stirred solution of 42 (207.1 mg, 0.289 mmol) in MeOH (0.868 mL) and THF (0.868 mL) was added NaOMe (1.00 equiv, 15.6 mg, 0.289 mmol) at room temperature. After being stirred at room temperature overnight, the solution was quenched with Dowex 50W-4X, filtered through a pad of Celite and concentrated in vacuo to give 43 (152.1 mg, 0.278 mmol, 96%) as a colorless oil:  $[\alpha]_D^{27}$  +5.37 (c 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.46–7.44 (m, 2H), 7.37–7.28 (m, 8H), 5.47 (s, 1H), 4.77 (d, 1H, J = 12.1 Hz), 4.57 (d, 1H, J = 12.1 Hz), 4.78 (d, 1H, J = 3.5 Hz), 4.22–4.12 (m, 2H), 3.79–3.50 (m, 8H), 3.30 (s, 3H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 137.6, 137.2, 129.3, 128.8, 128.6, 128.4, 126.2, 103.7-103.3 (m, 1C, C-1'), 101.5, 98.6, 80.2, 79.3, 78.0, 76.1-74.8 (m, 2C), 74.1-73.3 (m, 1C), 69.8-68.6 (m, 1C), 62.6, 60.9 (br, 1C), 55.4; FT-IR (neat) 3361, 1387, 1090, 1053 cm<sup>-1</sup>; HRMS (ESI-TOF) m/z [M + NH<sub>4</sub>]<sup>+</sup> calcd for C<sub>21</sub><sup>13</sup>C<sub>6</sub>H<sub>31</sub>D<sub>7</sub>NO<sub>11</sub> 565.3086, found 565.3086.

Methyl 3-O-( $\beta$ -D-Glucopyranosyl)- $\alpha$ -D-glucopyranoside-<sup>13</sup>C<sub>6</sub>, <sup>2</sup>H<sub>7</sub> (44). To a stirred solution of 43 (125 mg, 0.228 mmol) in MeOH (2.50 mL) and THF (2.50 mL) was added 5% Pd/C (228 mg) at room temperature. After being stirred at room temperature under hydrogen overnight, the solution was filtered through a pad of Celite and concentrated in vacuo. The residue was purified by reverse-phase column chromatography on Bond Elut-C18 (methyl alcohol) and diluted with water. Freeze-drying of the aqueous solution gave 44 (84.3 mg, 0.228 mmol, quant.) as a white solid:  $\left[\alpha\right]_{D}^{25}$  +3.14 (c 0.600,  $H_2O$ ; <sup>1</sup>H NMR (400 MHz,  $D_2O$ )  $\delta$  4.85 (d, 1H, J = 3.5 Hz), 3.91-3.87 (m, 2H), 3.82-3.76 (m, 2H), 3.72-3.68 (m, 1H), 3.54 (t, 1H, J = 9.2, 9.2 Hz), 3.46 (s, 3H);  ${}^{13}C{}^{1}H$  NMR (100 MHz, D<sub>2</sub>O + acetone-d<sub>6</sub>)  $\delta$  104.2-103.3 (m, 1C, C-1'), 100.5 (C-1), 84.0, 77.3-75.8 (m, 2C), 74.9-73.7 (m, 1C), 72.8, 72.1, 71.0-69.8 (m, 1C), 69.5, 61.9-60.8, 56.3; FT-IR (neat) 3368, 1366, 1046 cm<sup>-1</sup>; HRMS (ESI-TOF)  $m/z [M + Na]^+$  calcd for  $C_7^{13}C_6H_{17}D_7O_{11}Na$  392.1857, found 392.1864.

## ASSOCIATED CONTENT

## **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c01939.

<sup>1</sup>H and <sup>13</sup>C NMR spectra for all new compounds (PDF)

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#### Notes

The authors declare no competing financial interest.

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#### REFERENCES

(1) (a) Ritchie, G. E.; Moffatt, B. E.; Sim, R. B.; Morgan, B. P.; Dwek, R. A.; Rudd, P. M. Glycosylation and the Complement System. *Chem. Rev.* 2002, 102, 305–320. (b) Davis, B. G. Synthesis of Glycoproteins. *Chem. Rev.* 2002, 102, 579–601. (c) Ohtsubo, K.; Marth, J. D. Glycosylation in cellular mechanisms of health and disease. *Cell* 2006, 126, 855–867. (d) Bishop, J. R.; Schuksz, M.; Esko, J. D. Heparan sulphate proteoglycans fine-tune mammalian physiology. *Nature* 2007, 446, 1030–1037.

(2) Battistel, M. D.; Azurmendi, H. F.; Yu, B.; Freedberg, D. I. Prog. Nucl. Magn. Reson. Spectrosc. 2014, 79, 48-68.

(3) (a) Klepach, T.; Zhang, W. H.; Carmichael, I.; Serianni, A. S. C-13-H-1 and C-13-C-13 NMR *J*-couplings in C-13-labeled *N*-acetylneuraminic acid: correlations with molecular structure. *J. Org. Chem.* **2008**, 73, 4376–4387. (b) Olsson, U.; Sawen, E.; Stenutz, R.; Widmalm, G. Conformational flexibility and dynamics of two  $(1\rightarrow 6)$ linked disaccharides related to an oligosaccharide epitope expressed

on malignant tumour cells. Chem. - Eur. J. 2009, 15, 8886-8894. (c) Barb, A.; Freedberg, D. I.; Battistel, M.; Prestegard, J. NMR detection and characterization of sialylated glycoproteins and cell surface polysaccharides. J. Biomol. NMR 2011, 51, 163-171. (d) Norris, S. E.; Landström, J.; Weintraub, A.; Bull, T. E.; Widmalm, G.; Freedberg, D. I. Transient hydrogen bonding in uniformly <sup>13</sup>C, <sup>15</sup>N labeled carbohydrates in water. *Biopolymers* 2012, 97, 145-154. (e) Battistel, M. D.; Shangold, M.; Trinh, L.; Shiloach, J.; Freedberg, D. I. Evidence for helical structure in a tetramer of  $\alpha 2$ -8 sialic acid: unveiling a structural antigen. J. Am. Chem. Soc. 2012, 134, 10717-10720. (f) Zhu, T.; Yamaguchi, T.; Satoh, T.; Kato, K. A Hybrid Strategy for the Preparation of <sup>13</sup>C-labeled High-mannosetype Oligosaccharides with Terminal Glucosylation for NMR Study. Chem. Lett. 2015, 44, 1744-1746. (g) Huang, T.-Y.; Irene, D.; Zulueta, M. M. L.; Tai, T.-J.; Lain, S.-H.; Cheng, C.-P.; Tsai, P.-X.; Lin, S.-Y.; Chen, Z.-G.; Ku, C.-C.; Hsiao, C.-C.; Chyan, C.-L.; Hung, S.-C. Structure of the Complex between a Heparan Sulfate Octasaccharide and Mycobacterial Heparin-Binding Hemagglutinin. Angew. Chem., Int. Ed. 2017, 56, 4192-4196.

(4) Olsson, U.; Serianni, A. S.; Stenutz, R. Conformational Analysis of  $\beta$ -Glycosidic Linkages in <sup>13</sup>C-Labeled Glucobiosides Using Interresidue Scalar Coupling Constants. *J. Phys. Chem. B* **2008**, *112*, 4447–4453.

(5) Delbianco, M.; Kononov, A.; Poveda, A.; Yu, Y.; Diercks, T.; Jimenez-Barbero, J.; Seeberger, P. H. Well-Defined Oligo- and Polysaccharides as Ideal Probes for Structural Studies. *J. Am. Chem. Soc.* **2018**, *140*, 5421–5426.

(6) Yamaguchi, Y.; Takizawa, T.; Kato, K.; Arata, Y.; Shimada, I. <sup>1</sup>H and <sup>13</sup>C NMR assignments for the glycans in glycoproteins by using  ${}^{2}\text{H}/{}^{13}\text{C}$ -labeled glucose as a metabolic precursor. *J. Biomol. NMR* **2000**, *18*, 357–360.

(7) Adachi, Y.; Ishii, T.; Ikeda, Y.; Hoshino, A.; Tamura, H.; Aketagawa, J.; Tanaka, S.; Ohno, N. Characterization of beta-glucan recognition site on C-type lectin, dectin 1. *Infect. Immun.* **2004**, *72*, 4159–4171.

(8) Okobira, T.; Miyoshi, K.; Uezu, K.; Sakurai, K.; Shinkai, S. Molecular Dynamics Studies of Side Chain Effect on the  $\beta$ -1,3-d-Glucan Triple Helix in Aqueous Solution. *Biomacromolecules* **2008**, *9*, 783–788.

(9) (a) Tanaka, H.; Kawai, T.; Adachi, Y.; Ohno, N.; Takahashi, T.  $\beta(1,3)$  Branched heptadeca- and linear hexadeca-saccharides possessing an aminoalkyl group as a strong ligand to dectin-1. Chem. Commun. 2009, 46, 8249-8251. (b) Adamo, R.; Tontini, M.; Brogioni, G.; Romano, M. R.; Costantini, G.; Danieli, E.; Proietti, D.; Berti, F.; Costantino, P. Synthesis of Laminarin Fragments and Evaluation of a  $\beta$ -(1,3) Glucan Hexasaccaride-CRM197 Conjugate as Vaccine Candidate against Candida albicans. J. Carbohydr. Chem. 2011, 30, 249-280. (c) Tanaka, H.; Kawai, T.; Adachi, Y.; Hanashima, S.; Yamaguchi, Y.; Ohno, N.; Takahashi, T. Synthesis of  $\beta(1,3)$  oligoplucans exhibiting a Dectin-1 binding affinity and their biological evaluation. Bioorg. Med. Chem. 2012, 20, 3898-3914. (d) Liao, G.; Burgula, S.; Zhou, Z.; Guo, Z. A Convergent Synthesis of 6-O-Branched  $\beta$ -Glucan Oligosaccharides. Eur. J. Org. Chem. 2015, 2015, 2942-2951. (e) Yashunsky, D. V.; Tsvetkov, Y. E.; Nifantiev, N. E. Synthesis of 3-aminopropyl glycoside of branched  $\beta$ -(1  $\rightarrow$  3)-dglucooctaoside. Carbohydr. Res. 2016, 436, 25-30. (f) Weishaupt, M. W.; Hahm, H. S.; Geissner, A.; Seeberger, P. H. Automated glycan assembly of branched  $\beta$ -(1,3)-glucans to identify antibody epitopes. Chem. Commun. 2017, 53, 3591-3594. (g) Hamagami, H.; Adachi, Y.; Ohno, N.; Tanaka, H. Convergent Synthesis of Linear and Branched  $\beta(1,3)$ -Glucans and Evaluation of their Binding Affinities to Dectin-1. Asian J. Org. Chem. 2019, 8, 411-416.

(10) (a) Kinnaert, C.; Daugaard, M.; Nami, F.; Clausen, M. H. Chemical Synthesis of Oligosaccharides Related to the Cell Walls of Plants and Algae. *Chem. Rev.* **2017**, *117*, 11337–11405. (b) Miyagawa, A. Chemical Synthesis of  $\beta$ -(1,3)-Glucan Oligosaccharide and Its Application. *Trends Glycosci. Glycotechnol.* **2018**, *30*, 91–101.

(11) Mydock, L. K.; Demchenko, A. V. Superarming the S-Benzoxazolyl Glycosyl Donors by Simple 2-O-Benzoyl-3,4,6-tri-O-benzyl Protection. Org. Lett. 2008, 10, 2103–2106.

(12) Meng, S.; Zhong, W.; Yao, W.; Li, Z. Stereoselective Phenylselenoglycosylation of Glycals Bearing a Fused Carbonate Moiety toward the Synthesis of 2-Deoxy- $\beta$ -galactosides and  $\beta$ -Mannosides. *Org. Lett.* **2020**, *22*, 2981–2986.

(13) Lefeber, D. J.; Kamerling, J. P.; Vliegenthart, J. F. G. Synthesis of Streptococcus pneumoniae Type 3 Neoglycoproteins Varying in Oligosaccharide Chain Length, Loading and Carrier Protein. *Chem. - Eur. J.* **2001**, *7*, 4411–4421.

(14) Schimmel, J.; Eleuterio, M. I. P.; Ritter, G.; Schmidt, R. R. Synthesis of Saponins with Cholestanol, Cholesterol, and Friedelanol as Aglycones. *Eur. J. Org. Chem.* **2006**, 2006, 1701–1721.

(15) (a) Fraser-Reid, B.; Wu, Z.; Andrews, C. W.; Skowronski, E.; Bowen, J. P. Torsional effects in glycoside reactivity: saccharide couplings mediated by acetal protecting groups. J. Am. Chem. Soc. **1991**, 113, 1434–1435. (b) Crich, D.; Sun, S. Direct chemical synthesis of  $\beta$ -mannopyranosides and other glycosides via glycosyl triflates. Tetrahedron **1998**, 54, 8321–8348. (c) Chong, P. Y.; Roush, W. R. Concerning the Origin of the High  $\beta$ -Selectivity of Glycosidation Reactions of 2-Deoxy-2-iodo-glucopyranosyl Trichloroacetimidates. Org. Lett. **2002**, 4, 4523–4526. (d) Crich, D.; Smith, M. Solid-Phase Synthesis of  $\beta$ -Mannosides. J. Am. Chem. Soc. **2002**, 124, 8867–8869. (e) Nukada, T.; Berces, A.; Whitfield, D. M. Can the stereochemical outcome of glycosylation reactions be controlled by the conformational preferences of the glycosyl donor? Carbohydr. Res. **2002**, 337, 765–774.

(16) Shao, N.; Guo, Z. Solution-Phase Synthesis with Solid-State Workup of an O-Glycopeptide with a Cluster of Cancer-Related T Antigens. Org. Lett. 2005, 7, 3589–3592.

(17) Wisse, P.; Gold, H.; Mirzaian, M.; Ferraz, M. J.; Lutteke, G.; van den Berg, R. J. B. H. N.; van den Elst, H.; Lugtenburg, J.; van der Marel, G. A.; Aerts, J. M. F. G.; Codee, J. D. C.; Overkleeft, H. S. Synthesis of a Panel of Carbon-13-Labelled (Glyco)Sphingolipids. *Eur. J. Org. Chem.* **2015**, 2015, 2661–2677.

(18) (a) Liu, K. K. C.; Danishefsky, S. J. A striking example of the interfacing of glycal chemistry with enzymatically mediated sialylation: a concise synthesis of ganglioside GM3. J. Am. Chem. Soc. 1993, 115, 4933-4934. (b) Gervay, J.; Peterson, J. M.; Oriyama, T.; Danishefsky, S. J. An unexpected sialylation: total syntheses of ganglioside GM4 and a positional isomer. J. Org. Chem. 1993, 58, 5465-5468. (c) Randolph, J. T.; Danishefsky, S. J. Application of the glycal assembly strategy to the synthesis of a branched oligosaccharide: the first synthesis of a complex saponin. J. Am. Chem. Soc. 1993, 115, 8473-8474. (d) Randolph, J. T.; McClure, K. F.; Danishefsky, S. J. Major Simplifications in Oligosaccharide Syntheses Arising from a Solid-Phase Based Method: An Application to the Synthesis of the Lewis b Antigen. J. Am. Chem. Soc. 1995, 117, 5712-5719. (e) Halcomb, R. L.; Danishefsky, S. J. Total syntheses of ML-236A and compactin by combining the lactonic (silyl) enolate rearrangement and aldehyde-diene cyclocondensation technologies. J. Am. Chem. Soc. 1989, 111, 6661-6666. (f) Danishefsky, S. J.; McClure, K. F.; Randolph, J. T.; Ruggeri, R. B. A strategy for the solid-phase synthesis of oligosaccharides. Science 1993, 260, 1307-1309.

(19) Li, Y.; Tang, P.; Chen, Y.; Yu, B. Gold(I)-Catalyzed Glycosidation of 1,2-Anhydrosugars. J. Org. Chem. 2008, 73, 4323–4325.

(20) Cloran, F.; Carmichael, I.; Serianni, A. S. Density Functional Calculations on Disaccharide Mimics: Studies of Molecular Geometries and Trans-O-glycosidic  ${}^{3}J_{\text{COCC}}$  and  ${}^{3}J_{\text{COCC}}$  Spin-Couplings. J. Am. Chem. Soc. **1999**, 121, 9843–9851.

(21) (a) Takeda, H.; Yasuoka, N.; Kasai, N. The crystal and molecular structure of a 3:2 mixture of laminarabiose and O- $\alpha$ -d-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -d-glucopyranose. *Carbohydr. Res.* **1977**, 53, 137–152. (b) Noguchi, K.; Okuyama, K.; Kitamura, S.; Takeo, K. Crystal structure of methyl3-O- $\beta$ -d-glucopyranosyl- $\beta$ -d-glucopyranoside (methyl  $\beta$ -D-laminarabioside) monohydrate. *Carbohydr. Res.* **1992**, 237, 33–43.

(22) Aguilar, J. A.; Faulkner, S.; Nilsson, M.; Morris, G. A. Pure shift <sup>1</sup>H NMR: a resolution of the resolution problem? *Angew. Chem., Int. Ed.* **2010**, *49*, 3901–3903.