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Fast Pyrolysis of ^{13}C-Labeled Cellobioses: Gaining Insights into the Mechanisms of Fast Pyrolysis of Carbohydrates

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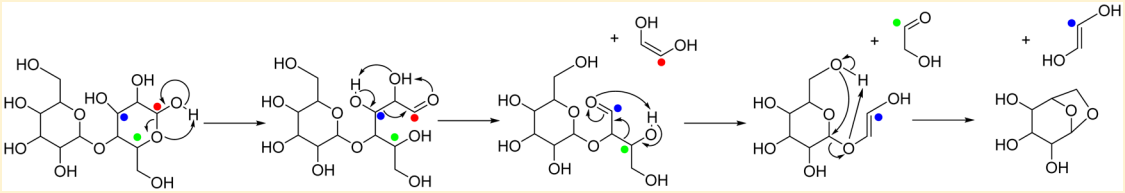
Supporting Information



ABSTRACT: A fast-pyrolysis probe/tandem mass spectrometer combination was utilized to determine the initial fast-pyrolysis products for four different selectively ^{13}C-labeled cellobiose molecules. Several products are shown to result entirely from fragmentation of the reducing end of cellobiose, leaving the nonreducing end intact in these products. These findings are in disagreement with mechanisms proposed previously. Quantum chemical calculations were used to identify feasible low-energy pathways for several products. These results provide insights into the mechanisms of fast pyrolysis of cellulose.

Fast pyrolysis is an efficient method for converting biomass to a low energy-density liquid (bio-oil) that can be further upgraded for use as fuel.[1] Optimization of fast pyrolysis to maximize the yields of compounds containing six or more carbons, which represent some of the most valuable potential end products, requires detailed knowledge of the dominant pyrolysis reactions.[2] However, the pathways and mechanisms (e.g., stepwise radical or ionic or concerted) of fast pyrolysis reactions are still largely unknown even for cellulose, the simplest component of biomass, as well as for analogous di- and oligosaccharides.[2,3] The goal of this study was to explore these mechanisms by using selective ^{13}C isotope labeling since this has not been performed previously.

The inherent capability of mass spectrometers to separate ions that differ in their mass-to-charge ratios (such as those with and without a ^{13}C label) makes the combination of this technique with fast pyrolysis of selectively labeled carbohydrates a powerful approach for mechanistic studies. We report here the results obtained upon examination of the initial fast-pyrolysis products of unlabeled cellobiose (a glucose dimer with the same linkage as in cellulose; see Figure 1) and four selectively ^{13}C-labeled cellobioses by tandem mass spectrometry. It should be noted that "initial" products are defined here as the products that first leave the heated surface of the fast-pyrolysis probe. The experimental studies were complemented by extensive quantum chemical calculations.

The pyrolysis/tandem mass spectrometry technique utilized here has been described in detail in the literature.[4] It combines a pyrolysis probe that can be heated very fast (up to 20 000 °C s^{-1}) with a Thermo Scientific linear quadrupole ion trap (LQIT) mass spectrometer. This technique was used to study the initial fast-pyrolysis products of cellobiose and four selectively ^{13}C-labeled cellobioses: commercially available [1-^{13}C]glucopyranosylglucose and glucopyranosyl[1-^{13}C]-glucose (Omicron Biochemicals, South Bend, IN) as well as glucopyranosyl[3-^{13}C]glucose and glucopyranosyl[5-^{13}C]-glucose (synthesized according to literature procedures[5,6]). The pyrolysis probe uses a resistively heated flat platinum ribbon (2.1 mm × 35 mm × 0.1 mm), which was placed inside the atmospheric-pressure chemical ionization (APCI) source of the LQIT. The ribbon was heated to 600 °C at a rate of 1000 °C s^{-1}. The initial products of pyrolysis evaporated from the heated surface into a nitrogen atmosphere at 100 °C (at atmospheric pressure) in the ion source and were quenched (it should be noted that this approach is very different from that used in laboratory pyrolysis reactors[7]). A soft ionization method, APCI with ammonium hydroxide dopant, was utilized to ionize the products since this method typically generates[4] only one ion (either the NH_4^+ adduct or the protonated molecule) without fragmentation for each product molecule of the type of interest here. The reactor and analysis suites were designed to prevent fragmentation of the pyrolysis products. Mass balances cannot be obtained using this new methodology;[4] however, relative abundances[4] of the products were determined, and much larger pyrolysis products were detected than reported previously.[8,9] Each reported mass spectrum is a result of a weighted average (based on total ion current) of the individual mass spectra measured during pyrolysis. No significant changes were detected in the product distribution

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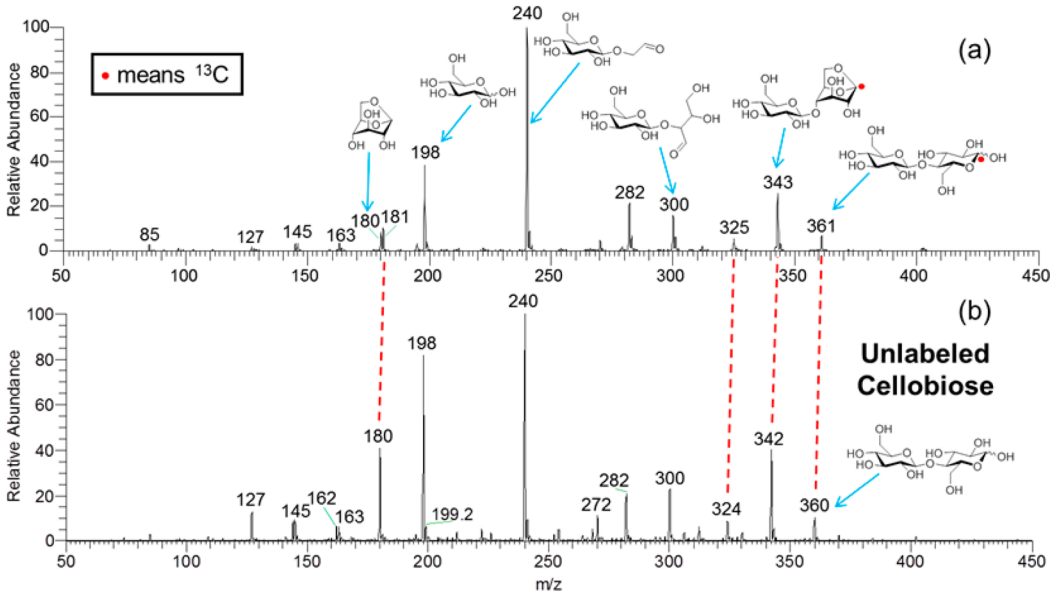

Figure 1. Positive ion-mode mass spectra showing the initial fast-pyrolysis products (either as ammonium adducts or protonated molecules) of (a) glucopyranosyl[1-^{13}C]glucose and (b) unlabeled cellobiose ionized by APCI with ammonium hydroxide dopant. The structures of the intact molecules are shown at the far right in each spectrum. The products that are ^{13}C-labeled in the top spectrum are connected with red dotted lines to the corresponding unlabeled products in the lower spectrum.

during cellobiose pyrolysis; in other words, the relative abundances were constant during pyrolysis.

The structures of most of the ions were examined by using MS^2 experiments (i.e., by isolating them and subjecting them to collision-activated dissociation (CAD)). When necessary, the structures of the fragment ions were examined by isolating them and subjecting them to further CAD (MS^3 experiment). High-resolution data needed to determine the elemental compositions of the ions were collected using an LQIT/Fourier-transform ion cyclotron resonance mass spectrometer coupled with the pyrolysis probe as described above.

The initial fast pyrolysis products of unlabeled cellobiose are shown in Figure 1b, and they are in agreement with those reported previously.[4] The identified products include hydroxymethylfurfural (protonated molecule of m/z 127), levoglucosan (NH_4^+ adduct of m/z 180 and a protonated molecule of m/z 163), glucose (NH_4^+ adduct of m/z 198), and β-D-glucopyranosylglycolaldehyde (NH_4^+ adduct of m/z 240). β-D-Glucopyranosylglycolaldehyde is likely formed upon the loss of two glycolaldehyde (or isomeric) molecules from cellobiose upon fast pyrolysis. On the other hand, the pyrolysis product generating ions of m/z 300 (NH_4^+ adduct) is formed via the loss of a single glycolaldehyde (or isomeric) molecule from cellobiose, and the product generating ions of m/z 282 (NH_4^+ adduct) is formed via the loss of glycolaldehyde (or isomeric) molecule and water from cellobiose. Evidence in support of these products being initial products instead of secondary products was obtained from the previously determined[4] structure of the β-D-glucopyranosylglycolaldehyde product. The glycosidic bond (i.e., the C−O bond at the oxygen atom on the carbon adjacent to the ring oxygen) in this pyrolysis product is still in the same position and has the same stereochemistry (β-1) as in cellobiose. If β-D-glucopyranosylglycolaldehyde were not an initial product but instead the result of recombination of smaller initial products, one would expect a mixture of linkage positions and stereochemistry. These larger products (>162 Da) are typically not seen with pyrolysis/GC−MS methodologies because of their thermal instability and low volatility, which may at least partially explain the incomplete mass balance of previous oligosaccharide pyrolysis studies.[8,9] The results presented here are in agreement with recent mechanistic studies[10−12] suggesting that glycolaldehyde may form directly from cellobiose (or a longer glucosaccharide) rather than through a glucose intermediate.

The ionized initial fast-pyrolysis products of glucopyranosyl-[1-^{13}C]glucose (with the label at the reducing end or the end containing a free hydroxyl group on the carbon adjacent to the ring oxygen) are shown in the mass spectrum in Figure 1a. Comparison of these products to those produced from unlabeled cellobiose (Figure 1b) revealed that only one product is partially ^{13}C-labeled (levoglucosan, NH_4^+ adducts of m/z 180 and 181), while all of the others are either completely labeled or do not contain the label at all. Specifically, cellobiosan (NH_4^+ adduct of m/z 343) and the product formed via loss of water from cellobiosan (NH_4^+ adduct of m/z 325) are completely ^{13}C-labeled, as expected, while all of the other products are unlabeled (Figure 1a). For example, glucose, β-D-glucopyranosylglycolaldehyde and β-D-glucopyranosylerythrose contain intact rings that originate exclusively from the nonreducing end since they have no label. The formation of glucose from the nonreducing end of cellobiose must occur via scission of the aglyconic bond (i.e., the other C−O bond of the oxygen atom involved in the glycosidic linkage) rather than the glycosidic bond as previously proposed.[3,13] In the literature, the only mechanisms that explain formation of glucose involve glycosidic bond cleavage and thermohydrolysis, which form glucose either from the reducing end only or from the reducing and nonreducing ends in equal amounts, respectively.[3,14,15] On the contrary, the present results indicate that the formation of glucose occurs exclusively from the nonreducing end of cellobiose (within the detection limits of our pyrolysis/MS experiment). This observation suggests that there is at least one additional glucose formation pathway that has not been proposed in the literature. Work is underway

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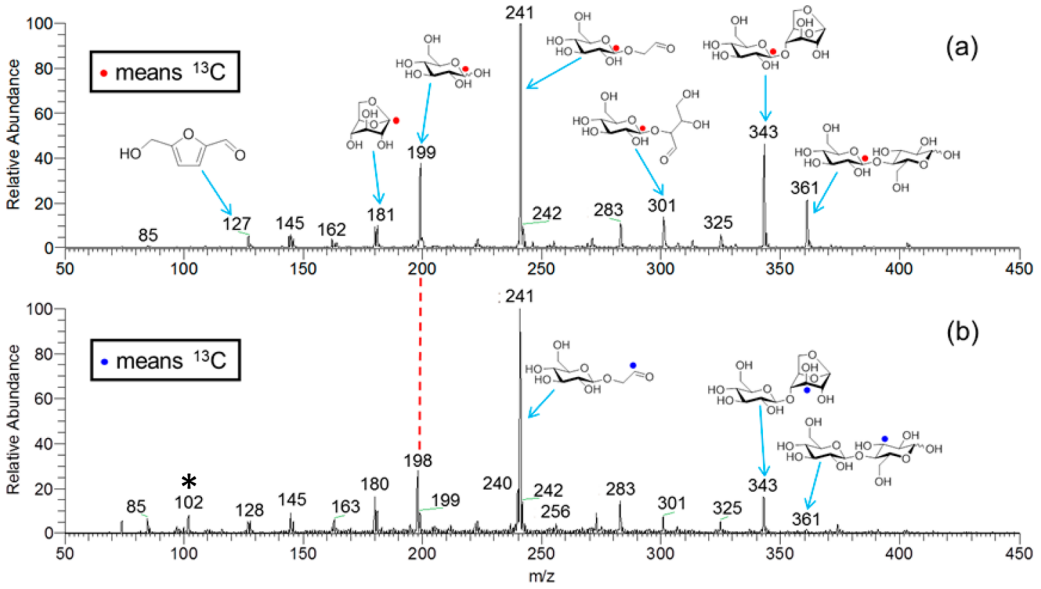

Figure 2. Positive ion-mode mass spectra showing the initial fast-pyrolysis products (either as ammonium adducts or protonated molecules) of (a) [1-^{13}C]glucopyranosylglucose and (b) glucopyranosyl[3-^{13}C]glucose ionized by APCI with ammonium hydroxide dopant. The structures of the intact molecules are shown at the far right in each spectrum. The only product that is labeled in the top spectrum but unlabeled in the bottom spectrum is glucose, as indicated by a red dotted line. The ion labeled with * corresponds to an unknown impurity.

to explore alternate reaction pathways that explain formation of glucose from the nonreducing end.

The results also demonstrate that the first glycolaldehyde (or isomeric) molecule eliminated from cellobiose upon pyrolysis contains the ^{13}C label (NH_4^+ adduct of m/z 300); this process must involve the loss of ^{13}C-1 and most likely C-2 of the reducing end of cellobiose. Identification of the origin of the initially eliminated glycolaldehyde on the basis of carbon labeling indicates that certain mechanisms may be more feasible than others. For example, the retro-aldol mechanism considered here and elsewhere results in the elimination of glycolaldehyde from the C-1 and C-2 positions.[14,15] On the other hand, 1,2-dehydration followed by retro-Diels–Alder reaction would result in elimination of glycolaldehyde from the C-5 and C-6 positions.

Levoglucosan must be formed via at least two pathways since two different ions (NH_4^+ adducts of m/z 180 and 181, corresponding to ammoniated molecules with and without the ^{13}C label) are produced. Hence, levoglucosan is likely formed from both the reducing and nonreducing ends of cellobiose. The ionized initial fast-pyrolysis products of another labeled cellobiose, [1-^{13}C]glucopyranosylglucose, with a ^{13}C label in the nonreducing end, are shown in the mass spectrum in Figure 2a. Comparison of these products to those obtained for unlabeled cellobiose revealed that all of the ionized products with m/z values greater than 180 contain the ^{13}C label. These results support the above conclusion that glucose is produced exclusively from the nonreducing end of cellobiose. Further, both labeled and unlabeled levoglucosan were observed, in agreement with the above proposal that more than one mechanism must lead to levoglucosan and that it is likely to be formed from both the reducing and nonreducing ends. Finally, the results show that the first *two* glycolaldehyde (or isomeric) molecules eliminated from cellobiose come from the reducing end.

In order to explore the mechanism for the elimination of the second glycolaldehyde (or isomeric) molecule from the reducing end of cellobiose (to yield the NH_4^+ adduct of m/z 240 for the unlabeled cellobiose), a third ^{13}C-labeled cellobiose, glucopyranosyl[3-^{13}C]glucose, was synthesized. The ionized initial fast-pyrolysis products of this compound are shown in the mass spectrum in Figure 2b. The presence of a major ion of m/z 301 (containing ^{13}C) supports the above finding that the first glycolaldehyde (or isomeric) molecule eliminated from cellobiose upon pyrolysis contains C-1 and C-2 of the reducing end (i.e., to form the ion of m/z 301). Furthermore, since elimination of the second glycolaldehyde (or isomeric) molecule yields an ion of m/z 241 (containing ^{13}C), this glycolaldehyde unit must contain C-5 and C-6 of the reducing end. Examination of a fourth ^{13}C-labeled compound, glucopyranosyl[5-^{13}C]glucose, confirmed all of the above conclusions. In particular, the elimination of C-5 of the reducing end in the second eliminated glycolaldehyde (or isomeric) molecule was verified by the observation of β-D-glucopyranosylglycolaldehyde with no ^{13}C.

Quantum chemical calculations[16] at 600 °C (see the Supporting Information for calculations performed at 25 °C) at the M06-2X/6-311++G(d,p)//M06-2X/6-311++G(d,p) level of theory[17,18] revealed a low-energy pathway for the consecutive elimination of one glycolaldehyde and two isomeric ethenediol molecules[19] from cellobiose, eventually producing levoglucosan (Figure 3). This pathway is consistent with the ^{13}C-labeling results described above. Work to identify additional pathways leading to levoglucosan and the other observed products is in progress.

In conclusion, identification of the initial fast-pyrolysis products of selectively ^{13}C-labeled cellobioses using a previously reported[4] experimental method has been demonstrated to be a powerful approach for exploring the details of the initial reactions in the fast pyrolysis of cellobiose. Several products that are likely to be produced by consecutive glycolaldehyde (or isomer) eliminations from the reducing end of cellobiose, including levoglucosan, were observed. Literature mechanisms proposed[3] for the fast pyrolysis of

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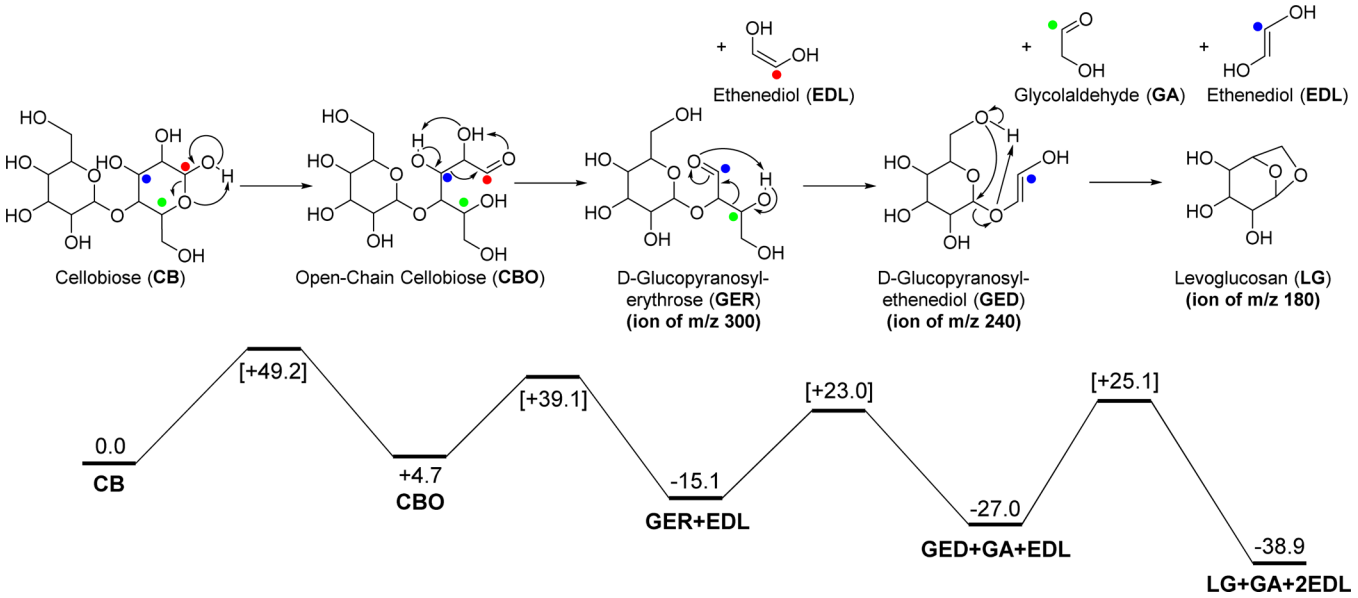

Figure 3. Calculated free energies (kcal mol^{-1}) of intermediates and transition states (square brackets) for the formation of levoglucosan from cellobiose via consecutive losses of one glycolaldehyde (GA) and two ethenediol (EDL) molecules (which are likely to eventually tautomerize to glycolaldehyde) at 600 °C obtained at the M06-2X/6-311++G(d,p)//M06-2X/6-311++G(d,p) level of theory. The location of a ^{13}C label at C-1 in the reducing end is indicated by a red circle, at C-3 by a blue circle, and at C-5 by a green circle. The mass-to-charge (m/z) ratios are for unlabeled cellobiose.

cellobiose are not consistent with the results reported here. Quantum chemical calculations revealed a feasible low-energy pathway for some of the products observed. Since many of the initial products have molecular weights greater than those of the final pyrolysis products reported for cellobiose in the literature,[8,9] these initial products may be intermediates in the formation of the final products, which include light oxygenated hydrocarbons.

Minimization of the production of low-molecular-weight oxygenated hydrocarbons is an important goal for fast pyrolysis of cellulose in order to maximize the production of fuel and high-value chemical products. Knowledge of the fragmentation pathways occurring during fast pyrolysis of smaller carbohydrates will contribute to the knowledge of fast pyrolysis of cellulose and ultimately fast pyrolysis of whole biomass, possibly enabling the tailoring of the product distribution obtained upon fast pyrolysis of whole biomass.

EXPERIMENTAL SECTION

Synthesis of Labeled Cellobioses. 3-^{13}C-β-D-Glucose and [1-^{13}C]glucopyranosylglucose were purchased from Omicron Biochemicals. The synthesized compounds (for the syntheses, see below) were purified by column chromatography on a Teledyne-ISCO CombiFlash system with a silica gel column. These compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry. The reactions were performed under an argon atmosphere if needed. Solvents were purified and/or predried as necessary. Analytical thin-layer chromatography (TLC) was used to monitor the reactions. Visualization was accomplished with UV light (254 nm) and by ethanol/H_2SO_4 TLC stain. $CDCl_3$, CD_3OD, and D_2O were used as NMR solvents. ^{1}H NMR spectra were acquired on a 400 MHz instrument, and the chemical shifts (δ) are reported relative to the residual solvent peak. ^{13}C NMR spectra were acquired on a 100 MHz instrument, and the chemical shifts are reported in parts per million relative to the residual solvent peak. When reporting spectral data, the format chemical shift (integration, multiplicity, J value(s) in Hz, identification) was used with the following multiplicity abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. The elemental compositions were obtained using high-resolution mass spectrometry and ESI in positive-ion mode, and the measured m/z values are reported. The previously synthesized compound 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl trichloroacetimidate (**S7**) was prepared from commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose by following a literature procedure.[20] The synthesis of labeled cellobioses glucopyranosyl[3-^{13}C]glucose (**S9**) and glucopyranosyl[5-^{13}C]glucose (**S17**) are shown in Schemes 1 and 2, respectively.

1,2,3,4,6-Penta-*O*-acetyl-3-^{13}C-β-D-glucopyranoside (S1).[21] To a solution of acetic anhydride (6 mL) was added sodium acetate trihydrate (1.13 g, 8.3 mmol, 3 equiv). The mixture was refluxed at 90 °C for 20 min, and then the labeled D-glucose (0.5 g, 2.8 mmol) was added. The resulting mixture was stirred for 4 h and then concentrated, dissolved in methanol, and recrystallized with cold water. A white solid was then filtered and dried to afford **S2** (1.00 g, 92%). ^{1}H NMR (400 Hz, $CDCl_3$, δ): 2.02, 2.03, 2.04, 2.09, and 2.12 (15H, s, CH_3), 3.84−3.87 (1H, m, CH), 4.10−4.13 (1H, m, CH_2), 4.28−4.32 (1H, dd, J_1 = 8, J_2 = 4, CH), 5.04−5.09 (d, 1H, J = 8, CH), 5.13−5.17 (m, 1H, CH), 5.42−5.47 (m, 1H, CH), 5.72−5.74 (d, 1H, J = 8, CH). ^{13}C NMR (100 Hz, $CDCl_3$, δ): 170.6, 170.1, 169.4, 169.2, 168.9, 91.7, 89.1, 74.0, 69.8, 67.8, 67.5, 61.5, 20.7. HRMS (ESI): calcd for $^{13}CC_{15}H_{22}O_{11}Na$ $[M + Na]^+$, 414.1093; measured, 414.1086.

Benzyl 2,3,4,6-Tetra-*O*-acetyl-3-^{13}C-β-D-glucopyranoside (S2).[6] To a solution of penta-*O*-acetylglucose **S1** (1.00 g, 2.56 mmol) and benzyl alcohol (0.61 mL, 5.60 mmol) in anhydrous CH_2Cl_2 (10 mL) was added $BF_3{\cdot}Et_2O$ (0.41 mL, 3.33 mmol). The reaction mixture was stirred at room temperature (RT) for 24 h and diluted with 5% aqueous $NaHCO_3$ (10 mL). The organic layer was separated, washed sequentially with aqueous $NaHCO_3$ (10 mL) and water (10 mL), dried over Na_2SO_4, and concentrated. The crude product was recrystallized from EtOH to give **S2** (0.55 g, 49%). ^{1}H NMR (400 Hz, $CDCl_3$, δ): 7.39−7.27 (5H, m, Ar−H), 5.13−4.99 (3H, m, CH, H2, H3, and H4), 4.88 (1H, d, J = 12, CH_2), 4.61 (1H, d, J = 12, CH_2), 4.60 (1H, d, J = 8, CH, H1), 4.30 (1H, dd, J = 12, 4, CH, H6), 4.19 (1H, dd, J = 12, 2, CH, H6), 3.67−3.64 (1H, m, CH, H5), 2.09 (3H, s, CH_3), 2.00 (3H, s, CH_3), 1.99 (3H, s, CH_3), 1.98 (3H, s, CH_3). ^{13}C NMR (100 Hz, $CDCl_3$, δ): 170.6, 170.2, 169.3, 169.2, 136.6, 128.4, 127.9, 127.8, 127.7, 99.2, 77.0, 76.7, 72.7, 71.7, 71.4, 70.6, 70.1, 69.7, 68.5, 68.1, 20.5. HRMS (ESI): calcd for $^{13}CC_{20}H_{26}O_{10}Na$ $[M + Na]^+$, 462.1424; measured, 462.1410.

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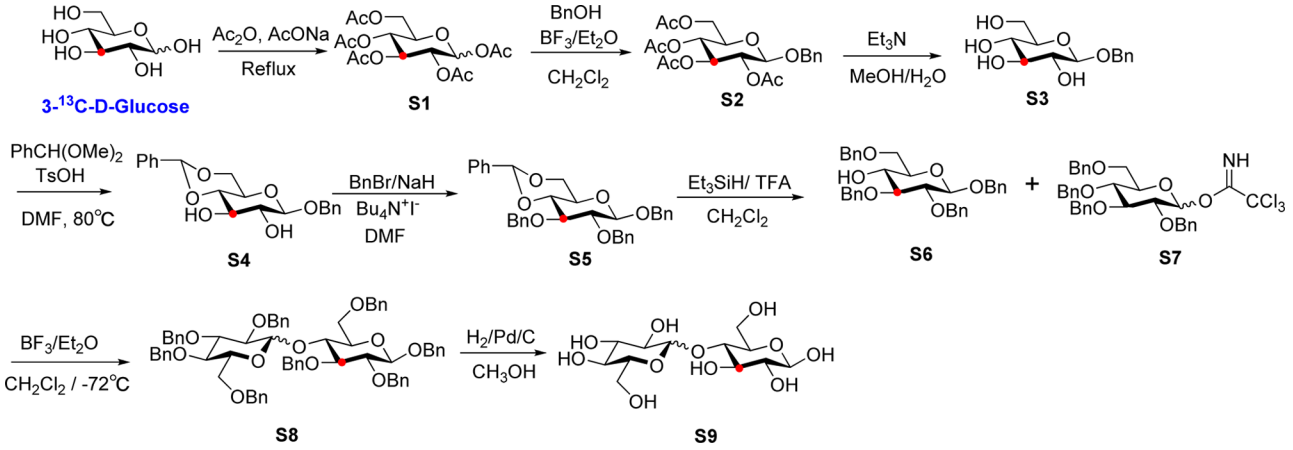
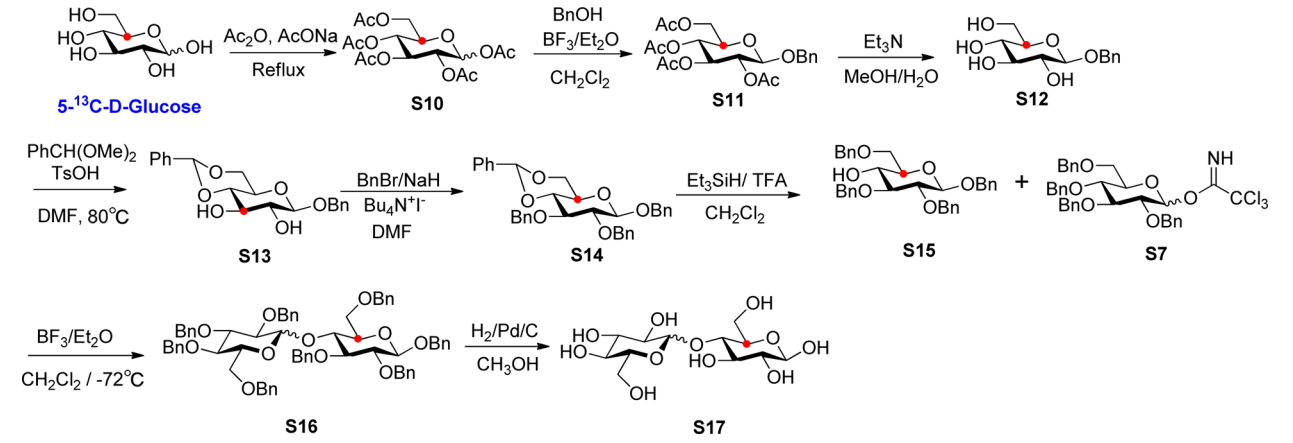
Scheme 1. Synthesis of Glucopyranosyl[3-^{13}C]glucose (S9)



Scheme 2. Synthesis of Glucopyranosyl[5-^{13}C]glucose (S17)



Benzyl 3-^{13}C-*β*-D-Glucopyranoside (S3).[6] A mixture of benzyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside (0.896 g, 2.50 mmol), MeOH (16 mL), triethylamine (2 mL), and H_2O (2 mL) was stirred for 5 h. The reaction was concentrated in vacuo, and the resulting residue was purified by chromatography (5:1 CH_2Cl_2/CH_3OH) to give **S3** (0.44 g, 81%). ^{1}H NMR (400 Hz, CD_3OD, δ): 7.43−7.25 (5H, m, Ar−H), 4.78 (2H, ABq, J = 16, $PhCH_2$), 4.36 (1H, d, J = 8 Hz, CH, H1), 3.89 (1H, dd, J = 12, 2, CH, H6), 3.70 (1H, dd, J = 12, 6, CH, H6), 3.35−3.15 (4H, m, CH, H2, H3, H4, and H5). ^{13}C NMR (100 Hz, $CDCl_3$, δ): 139.1, 129.3, 129.2, 128.7, 103.3, 78.1, 75.1, 74.9, 74.1, 71.8, 71.4, 62.8. HRMS (ESI): calcd for $^{13}CC_{12}H_{18}O_6Na$ [M + Na]$^+$, 294.1030; measured, 294.0988.

Benzyl 4,6-*O*-Benzylidene-3-^{13}C-*β*-D-glucopyranoside (S4).[6] To a mixture of benzyl *β*-D-glucopyranoside (0.21 g, 0.78 mmol) and benzaldehyde dimethyl acetal (0.14 mL, 0.94 mmol) in dimethylformamide (DMF) (2 mL) at RT was added *p*-toluenesulfonic acid (TsOH·H_2O) (37 mg, 0.195 mmol). The reaction mixture was stirred for 5 min, heated to 80 °C, and stirred for 2.5 h. The mixture was cooled to RT and subsequently concentrated at reduced pressure. The resulting residue was partitioned between CH_2Cl_2 (20 mL) and saturated Na_2CO_3 (20 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with water (2 × 10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated. Purification of the resulting residue by flash chromatography (1:1 EtOAc/hexanes) provided the desired product (0.21 g, 75%) as a white solid. ^{1}H NMR (400 Hz, $CDCl_3$, δ): 7.50−7.48 (2H, m, Ar−H), 7.37−7.29 (8H, m, Ar−H), 5.46 (1H, s, benzylidene CH), 4.88 (1H, d, J = 12, CH_2), 4.59 (1H, d, J = 12, CH_2), 4.53 (1H, d, J = 8, CH, H1), 4.31 (1H, dd, J = 8, 4, CH, H4), 3.59−3.76 (2H, m, CH, C6, H3), 3.63−3.58 (4H, m, CH, H6, H2, and 2OH), 3.35−3.30 (1H, m, CH, H5). ^{13}C NMR (100 Hz, $CDCl_3$, δ): 137.0, 136.8, 129.2, 128.5, 128.1, 128.0, 126.3, 102.2, 101.8, 80.6, 80.2, 77.4, 77.0, 76.71, 74.6, 74.2, 73.0, 72.8, 71.2, 68.5, 66.2. HRMS (ESI): calcd for $^{13}CC_{20}H_{22}O_6Na$ [M + Na]$^+$, 382.1314; measured, 382.1310.

4,6-*O*-Benzylidene-1,2,3-tri-*O*-benzyl-3-^{13}C-*β*-D-glucopyranoside (S5).[22] The partially protected **S4** (0.21 g, 0.58 mmol) was added to anhydrous DMF (2 mL), and the mixture was stirred at 0 °C for 30 min. NaH in mineral oil (60%) (94 mg, 2.32 mmol) was then added under argon in small portions over 30 min, with the temperature being maintained at 0 °C. Tetrabutylammonium iodide (54 mg, 0.14 mmol) was then added, and the mixture was stirred for a further 2 h at 0 °C. Benzyl bromide (0.21 mL, 1.74 mmol) was then added slowly. The reaction mixture was stirred at 0 °C for a further 30 min, allowed to warm to room temperature, and stirred for 24 h. Methanol was added slowly to destroy the excess NaH, and the solvents were then removed in vacuo. The residue was subjected to column chromatography with 3:2 hexane/ethyl acetate to furnish **S5** as a pale-yellow oil (0.3 g, 81%). ^{1}H NMR (400 Hz, $CDCl_3$, δ): 7.48−7.34 (20H, m, Ar−H), 5.65 (1H, s, CH, H7), 5.04−4.71 (8H, m, CH, CH_2), 4.48−4.44 (1H, dd, J = 8, 4, CH, H4), 3.92−3.76 (2H, m, CH, H6, H3), 3.66−3.60 (2H, m, CH, H6, H2), 3.53−3.48 (1H, m, CH, H5). ^{13}C NMR (100 Hz, $CDCl_3$, δ): 138.5, 137.1, 129.0, 128.3, 127.6, 126.0, 103.2, 101.1, 80.9, 80.7, 77.4, 76.8, 75.4, 75.1, 71.6, 68.8, 66.1. HRMS (ESI): calcd for $^{13}CC_{33}H_{34}O_6Na$ [M + Na]$^+$, 562.2253; measured, 562.2227.

1,2,3,6-Tetra-*O*-benzyl-3-^{13}C-*β*-D-glucopyranoside (S6).[22] Triethylsilane (0.27 mL, 1.68 mM) and trifluoroacetic acid (0.13 mL, 1.68 mmol) were added to a solution of **S5** (0.3 g, 0.56 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. The solution was stirred at RT for 6 h. The reaction mixture was diluted with EtOAc (approximately 20 mL), neutralized with saturated aqueous $NaHCO_3$ and brine, dried

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over Na_2SO_4, and evaporated to dryness. The residue was purified by flash column chromatography (3:10 EtOAc/hexane) to get **S6** (0.2 g, 60%) as a colorless oil. ^{1}H NMR (400 Hz, $CDCl_3$, δ): 7.45−7.29 (20H, m, Ar−H), 5.02−4.64 (8H, m, CH_2), 4.58 (1H, d, J = 8, CH, H1), 3.86−3.48 (6H, m, CH, CH2, ring CH), 2.60 (1H, s, OH). ^{13}C NMR (100 Hz, $CDCl_3$, δ): 138.6, 138.3, 137.5, 128.1, 127.8, 102.5, 84.3, 84.1, 83.8, 82.5, 81.7, 81.4, 77.3, 77.0, 76.7, 75.2, 74.7, 74.1, 73.6, 717, 71.3, 71.1, 70.2. HRMS (ESI): calcd for $^{13}CC_{33}H_{36}O_6Na$ [M + Na]$^+$, 564.2433; measured, 564.2465.

2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl-(1−4)-1,2,3,6-tetra-*O*-benzyl-3-^{13}C-β-D-glucopyranoside (S8).[22] To a solution of **S6** (0.1 g, 0.18 mmol) and **S7** (0.12 g, 0.18 mmol) in anhydrous CH_2Cl_2 (5 mL) was added $BF_3{\cdot}Et_2O$ (3 μL, 0.018 mmol) at −72 °C. After the solution was stirred at −72 °C for 1 h, the reaction mixture was neutralized with triethylamine. The residue was purified by flash column chromatography (1:8 EtOAc/hexane) to give **S8** as a 1:3 mixture of α and β isomers (0.12 g, 55%) as a colorless syrup. ^{1}H NMR (400 Hz, $CDCl_3$, δ): 7.42−7.19 (40H, m, Ar−H), 5.15−4.46 (18H, m, CH, $PhCH_2$, H1, H1′), 3.92−3.35 (12H, m, CH, CH_2, ring CH). ^{13}C NMR (100 Hz, $CDCl_3$, δ): 139.3, 138.6, 137.6, 128.3, 127.8, 127.5, 102.4, 84.8, 83.1, 78.0, 77.3, 76.7, 75.5, 74.9, 73.2, 70.9, 69.0, 68.2. HRMS (ESI): calcd for $^{13}CC_{68}H_{70}O_{11}Na$ [M + Na]$^+$, 1086.4849; measured, 1086.4846.

Glucopyranosyl[3-^{13}C]glucose (S9).[22] A solution of **S8** (0.12 g, 1.15 mmol) in MeOH (10 mL) was hydrogenated in the presence of 10% Pd/C (15 mg) at atmospheric pressure at RT for 36 h. After the catalyst was filtered off, the reaction mixture was evaporated to give **S9** (α:β = 1:3, 34 mg, 87%) as a colorless solid. ^{1}H NMR (400 Hz, D_2O, δ): 4.56 (1H, d, J = 8, CH, H1′), 4.41 (1H, d, J = 8, CH, H1), 3.83−3.15 (12H, m, CH, CH2, ring CH). ^{13}C NMR (100 Hz, D_2O, δ): 103.1, 96.3, 92.4, 76.8, 76.6, 76.1, 75.4, 74.9, 74.6, 73.8, 72.1, 70.7, 70.0, 61.0, 60.5. HRMS (ESI): calcd for $^{13}CC_{11}H_{22}O_{11}Na$ [M + Na]$^+$, 366.1060; measured, 366.1068.

Glucopyranosyl[5-^{13}C]glucose (S17).[22] The same synthesis strategy as described above for **S9** was employed for the synthesis of glucopyranosyl[5-^{13}C]glucose (**S17**). A solution of **S16** (0.12 g, 1.15 mmol) in MeOH (10 mL) was hydrogenated in the presence of 10% Pd/C (15 mg) at atmospheric pressure at RT for 36 h. After the catalyst was filtered off, the reaction mixture was evaporated to give **S17** (α:β = 1:3, 34 mg, 87%) as a colorless solid. ^{1}H NMR (500 MHz, D_2O, δ): 4.56 (1H, d, J = 5, CH, C1′), 4.41 (1H, d, J = 5, CH, C1), 3.83−3.15 (12H, m, CH, CH2, ring CH). ^{13}C NMR (125 MHz, D_2O, δ): 102.5, 95.6, 91.7, 78.8, 78.5, 78.3, 75.9, 75.4, 74.7, 74.2, 73.8, 71.7, 71.2, 71.1, 70.0, 69.4, 60.5, 60.1, 59.9. HRMS (ESI): calcd for $^{13}CC_{11}H_{22}O_{11}Na$ [M + Na]$^+$, 366.1060; measured, 366.1066.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra, tables of cartesian coordinates, and complete ref 16. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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