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Note

Synthesis of methyl 4′-*O*-methyl-$^{13}C_{12}$-β-D-cellobioside from $^{13}C_6$-D-glucose. Part 1: Reaction optimization and synthesis

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Abstract—A high yielding synthetic route for methyl 4′-*O*-methyl-β-D-cellobioside starting from D-glucose was established. The reaction conditions optimized with nonlabeled materials were used for the synthesis of methyl 4′-*O*-methyl-$^{13}C_{12}$-β-D-cellobioside, a compound having more than 99% ^{13}C enrichment at each of the twelve pyranose carbon atoms. The labeled compound is required to study the hydrogen bond network of cellodextrins and cellulose by CPMAS NMR experiments.
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Methyl 4′-*O*-methyl-β-D-cellobioside (**12**) is the first cellodextrin model compound for cellulose which was found to crystallize in two distinct phases.[1] Both allomorphs were comprehensively characterized including X-ray crystallography and ^{13}C CPMAS NMR, which indicated an unexpectedly high effect of the crystal packing on the chemical shifts.[2] The availability of complete solid-phase structural data of **12** prompted us toward the synthesis of ^{13}C-perlabeled material, which would constitute an ideal probe for CPMAS studies of the hydrogen bond network in cellodextrins and elaboration of novel suitable CPMAS techniques. Due to the considerable intensity gain in the ^{13}C domain, optimization of modern two- and multi-dimensional techniques,[3] such as the MAS-*J*-HMQC,[4] and eventually resolution of the H domain will become possible. With the hydrogen bond pattern being known from X-ray studies, these data can be correlated with the CPMAS results. Furthermore, the problem of the assignment of the two sets of six resonances to the two anhydroglucose units of **12**, which remained an open issue so far, can be solved. CPMAS on the ^{13}C-perlabeled **12** is expected to provide novel insights into the dissolution mechanism of higher cellodextrins in cellulose solvents, such as *N*-methylmorpholine-*N*-oxide or *N*,*N*-dimethylacetamide/LiCl, and of the changes in the hydrogen bond network during this process. This will also shed some new light on the dissolution mechanism of cellulose, which is important for a better understanding of technologies requiring cellulose dissolution, for example, in cellulosic fiber manufacturing, such as the viscose or Lyocell production process.

Although a high-yield synthesis of **12** starting from D-cellobiose has already been established,[5] it could not be used for the plotted synthesis of the ^{13}C-perlabeled derivative, as $^{13}C_6$-D-glucose is the only appropriate, commercially available starting material. Due to high costs of this starting material and the requirement of product yields in the multi-100 mg range the whole synthetic scheme was supposed to be comprehensively optimized with nonlabeled D-glucose, before entering 'hot runs' with perlabeled material. In the present first part of this study, we wish to communicate an effective synthetic route toward methyl 4′-*O*-methyl-β-D-cellobioside (**12**) starting from D-glucose and the synthesis of the respective labeled compound, while the second will

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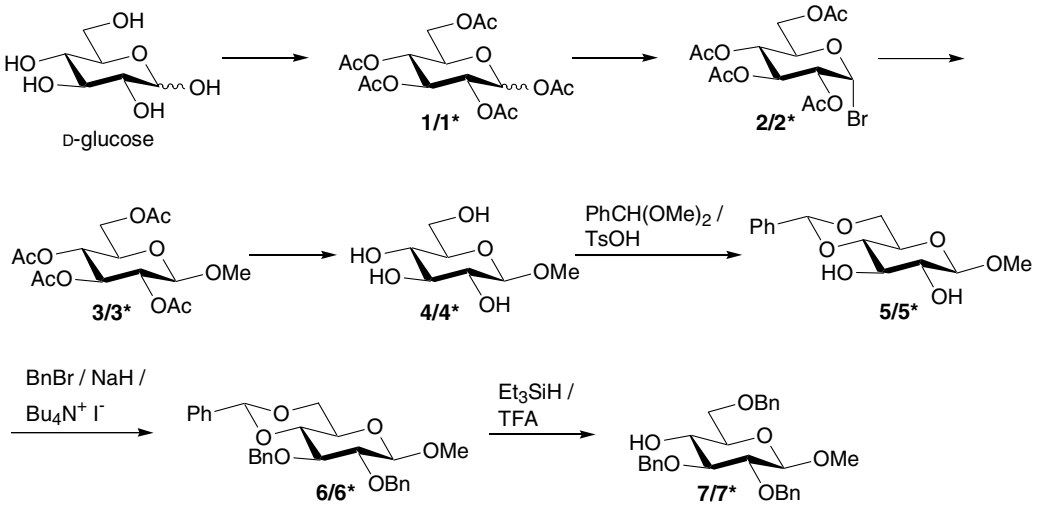
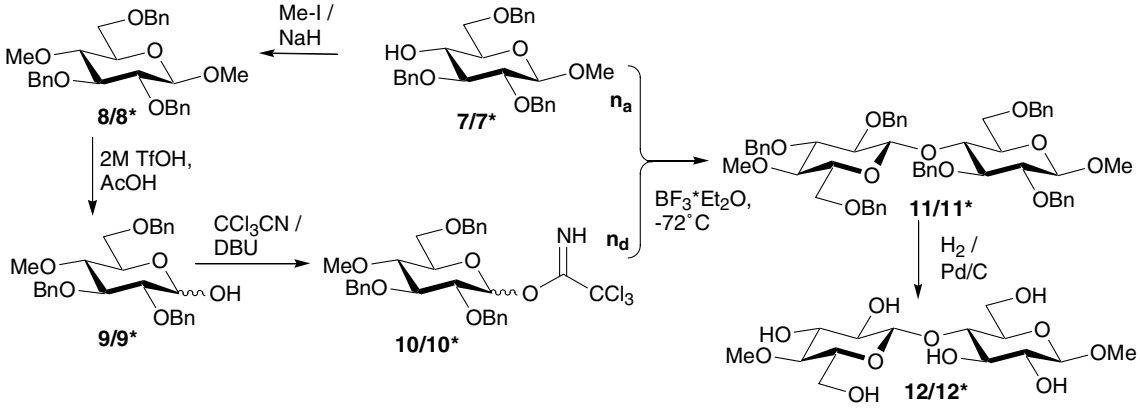


Scheme 1. Synthesis of intermediate **7/7***.



Scheme 2. Synthesis of the target compound **12/12***.

cover an in-depth solid state structural analysis of the two allomorphs, placing emphasis on studies of the hydrogen bond system by CPMAS techniques.

Dictated by the requirement of optimum yields of **12/12***[†]—not necessarily implying to take to shortest synthetic route—the path shown in Schemes 1 and 2 was chosen. From retrosynthetic analysis, methyl 4′-*O*-methyl-β-D-cellobioside (**12**) was to be synthesized through a glycosidation reaction between a methyl 2,3,6-tri-*O*-protected β-D-glucoside and 4-*O*-methyl-2,3,6-tri-*O*-protected β-D-glucosyl donor. Methyl 2,3,6-tri-*O*-benzyl-β-D-glucoside (**7**) was selected as the common intermediate, which could be used directly as a glycosyl acceptor and as precursor of glucosyl imidate **10**. Benzyl group protection was chosen to avoid migration as to be expected in the case of acetyl protecting groups.

Scheme 1 shows the synthetic route to intermediate **7**, starting from D-glucose. Methyl β-D-glucoside (**4**) was prepared via glucose pentaacetate (**1**), acetobromoglucose (**2**) and methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside (**3**). Protection of all hydroxyl groups in **4** except 4-OH was accomplished by a reaction sequence comprising the introduction of a 4,6-*O*-benzylidene group giving **5**, 2,3-di-*O*-benzylation to afford compound **6**, and regioselective opening of the benzylidene ring with triethylsilane (TES)/trifluoroacetic acid (TFA) to obtain compound **7** in 27.2% overall yield relative to D-glucose.

Intermediate **7** can be directly used as the glycosyl acceptor. Preparation of the glycosyl donor—starting from **7** as well—required three additional steps, as shown in Scheme 2. Methylation of 4-OH gave compound **8**, and hydrolysis of the 1-OMe group was achieved by acid treatment. In the case of trichloroacetic acid as the catalyst, no reaction was observed. Trifluoroacetic acid

[†] Here and in the following the nonlabeled compounds are given in bold numbers, while isotopic labeling is indicated with an asterisk following the compound number.

(TFA)[6] and/or trifluoromethanesulfonic acid (TfOH)[7] gave much better results, although a minor side reaction was noted, possibly a hydrolytic cleavage of the 6-*O*-benzyl group. Finally, 2 M triflic acid was chosen as the catalyst. The main product of the hydrolysis, 2,3,6-tri-*O*-benzyl-4-*O*-methyl-α/β-D-glucose (**9**), underwent trichloroacetimidoylation to a mixture of α- and β-imidates **10** in an approximate ratio of 5:1 as estimated by TLC and NMR spectroscopy.

For the glycosidation of benzyl protected trichloroacetimidate **10** no neighbour group participation would be expected, as in the case of acyl protection. Thus, only the α-imidate should be used to construct a β-glycosidic linkage, assuming that the reaction proceeds according to a S_N2-type mechanism. However, preliminary experiments with neat β-imidate indicated also the β-glycoside was produced to some part. We, thus, decided to use the mixture of both imidates in the glycosidation reaction. In addition, imidate mixture **10** proved to be too reactive to withstand purification by flash chromatography on silica gel without significant yield penalties. Therefore, work-up of the imidoylation reaction was done by rapid filtration through a short alumina column to remove the diazabicyclo[5.4.0]undec-7-ene (DBU) catalyst. Evaporation of the solvents afforded a mixture of the anomeric imidates in 92% yield, which was directly used in the glycosylation step. Purification and separation of the α/β-glycosides was performed afterwards, to afford 63% of the desired β-isomer **11**.

The overall yield of imidate mixture **10** from the intermediate **7** was 64%. As the common intermediate **7** is split into glycosyl acceptor and glycosyl donor, 61% of **7** are to be used for the preparation of imidate **10**, while the remaining 39% of **7** are taken directly as the glycosyl acceptor, provided **7** and **10** are to be applied in an equimolar ratio (see Table 1).

The glycosylation reaction was performed at −72 °C in CH_2Cl_2 with BF_3 etherate as the catalyst. Acetonitrile, which possibly would have had a beneficial nitrilium effect on the yield, was not tested as the solvent. The initial molar ratio of imidate **10** and glycosyl acceptor **7** (n_d/n_a) affected the overall yield of cellobiose derivative **11** relative to the common intermediate **7** in a rather complex manner (Table 1). The first trial was conducted with a molar ratio (n_d/n_a) of 1:1. The yield of **11** was 40.4% after purification and separation from the corresponding maltose derivative, while 25% of acceptor **7** remained as nonreacted starting material. The overall yield of cellobiose derivative **11** relative to compound **7** was then $40.4\% \times 0.39 = 15.8\%$. In order to consume acceptor **7** completely, n_d/n_a was set to 1.33:1. As a result, the yield of **11** in the glycosylation increased to 63.2%, and the overall yield to 20.9%, superior to that of the first trial. However, even in the case of excess donor, still 9% of the starting compound **7** remained unused. The third run was conducted with an n_d/n_a of 1.33/(1–0.09). This gave a better glycosylation yield (66.1%) than that of the second trial, but a less optimal overall yield (19.8%) due to the smaller n_a (0.30). In conclusion, an initial molar ratio n_d/n_a of 1.33:1 proved to be the best variant among those examined and was used further. The fully benzyl-protected cellobiose derivative **11** was finally hydrogenated with 10% Pd/C as the catalyst to afford the target compound methyl 4′-*O*-methyl-β-D-cellobioside (**12**) in 90.9% yield.

Having optimized the procedure, the reaction sequence was repeated step-by-step in parallel with nonlabeled and labeled materials. The yields for both runs were generally equal or close to equal. The total yield of **12** (**12***) starting from D-glucose ($^{13}C_6$-D-glucose) was 4.84%, with 5 g of $^{13}C_6$-D-glucose affording 250 mg of ^{13}C-perlabeled methyl 4′-*O*-methyl-$^{13}C_{12}$-β-D-cellobioside: sufficient amounts for the planned analytical studies.

The signal pattern in the ^{13}C soln NMR spectrum of **12*** is rather complex due to primary signal splitting by homonuclear ^{13}C–^{13}C ^1J-couplings, and additional splitting by geminal and vicinal ^{13}C–^{13}C couplings. Figure 1 displays the ^1H-decoupled spectrum which shows four distinct regions of resonances: the anomeric carbons, the C-4 carbons, the C-6 carbons, and the complex region of C-2, C-3, and C-5.

For comparison, the solid-state NMR spectrum of **12*** is given in Figure 2, the trace shown being the result of a single scan—which illustrates the intensity gain achieved by the isotopic enrichment. The spectrum displayed is that of a microcrystalline mixture of both allomorphs, and is thus slightly different from the

Table 1. Optimization of educt ratios and yields for the glycosylation reaction

Molar ratio n_d/n_a	n_a[a]	Yield for glycosylation[b] (%)			Nonreacted **7**	Yield of **11** from **7**
		α[c]	β[d]	α + β		
1/1 = 1	0.39*n_0	11.6	40.4	52.0	25	15.8
1.33/1 = 1.33	0.33*n_0	19.9	63.2	83.1	9	20.9
1.33/0.91 = 1.46	0.30*n_0	24.0	66.1	90.1	Trace	19.8

[a] n_0 starting yield (D-glucose).
[b] Calculated based on n_a.
[c] Maltose derivative.
[d] Cellobiose derivative **11**.

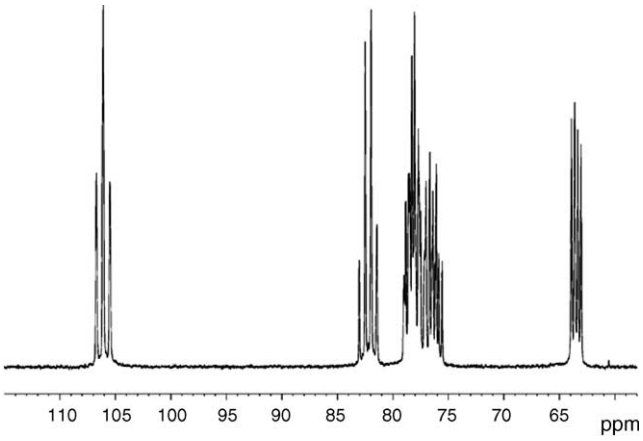


Figure 1. Proton-decoupled ^{13}C NMR solution spectrum of **12***, having >99% ^{13}C enrichment at the 12 cellobiose carbon atoms.

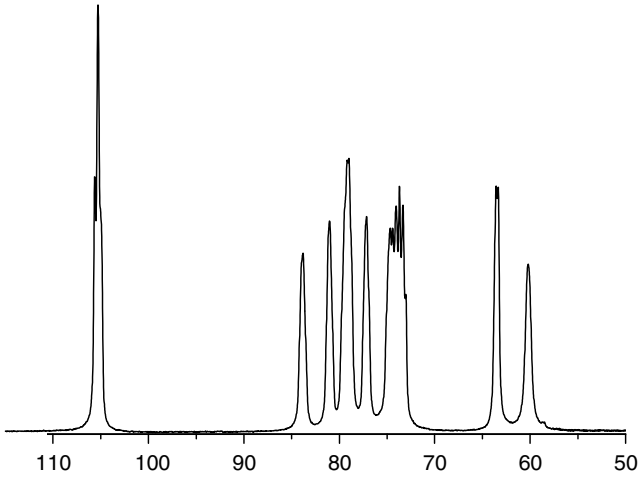


Figure 2. Proton-decoupled ^{13}C NMR CPMAS solid-state spectrum of **12***, having >99% ^{13}C enrichment at the 12 cellobiose carbon atoms.

spectra obtained for the two allomorphs of **12**. For the CPMAS and solid state structural studies, which will be reported in part 2 of this work, this sample as well as both neat, crystalline phases of **12*** will be employed.

1. Experimental

Melting points (mp) are uncorrected. TLC was performed on silica gel plates (Kieselgel 60 F_{254}, E. Merck), flash column chromatography on silica gel (Wakogel FC-40). ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ if not otherwise stated, on a JNM-500 FT-NMR (Jeol) at 500 MHz for ^1H and 125 MHz for ^{13}C, with tetramethylsilane (TMS) as the internal standard. Chemical shifts are given in ppm, coupling constants (J) in hertz. The signals were assigned by homo- and heteronuclear two-dimensional techniques. Compounds **1**–**4** were synthesized according to standard techniques, and the corresponding experimental procedures are not listed in the following. All intermediates and the product showed satisfying purity data (elemental analysis), which in some cases were sustained by HRMS data.

1.1. Methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (5)

To a soln of methyl β-D-glucopyranoside (**4**, 3.57 g, 18.4 mmol) in 1,4-dioxane (80 mL) were added benzaldehyde dimethyl acetal (3.35 mL, 22.1 mmol) and *p*-toluenesulfonic acid monohydrate (35.0 mg, 0.184 mmol) at rt. The soln was stirred under diminished pressure (4 kPa) for 1 h at 30 °C. A second portion of benzaldehyde dimethyl acetal (2.80 mL, 18.4 mmol) was added, and stirring was continued for 2 h. The reaction mixture was neutralized with solid $NaHCO_3$. Solids were filtered off and the filtrate was evaporated to dryness. The filtrate was purified by flash column chromatography (1:19 MeOH–CH_2Cl_2) to give a colorless syrup which spontaneously crystallized to afford **5** (4.12 g, 79.3%) as colorless crystals: R_f 0.74 (1:9 MeOH–CH_2Cl_2); mp 171–173 °C [lit. 174–175 °C];[8] $[\alpha]_D$ −61.4 (*c* 0.8, $CHCl_3$) [lit. $[\alpha]_D$ −53.3 (*c* 0.5, $CHCl_3$)];[8] ^1H NMR: δ 2.70 (d, $J_{2,2\text{-OH}}$ 2.3 Hz, 1H, 2-O*H*), 2.85 (d, $J_{3,3\text{-OH}}$ 2.5 Hz, 1H, 3-O*H*), 3.49 (dt, $J_{5,4} = J_{5,6a} = 9.2$ Hz, $J_{5,6b}$ 5.1 Hz, 1H, H-5), 3.51 (ddd, $J_{1,2}$ 7.6 Hz, $J_{2,3}$ 9.2 Hz, $J_{2,2\text{-OH}}$ 2.3 Hz, 1H, H-2), 3.56 (t, $J_{3,4} = J_{4,5} = 9.2$ Hz, 1H, H-4), 3.59 (s, 3H, 1-O*Me*), 3.80 (t, $J_{5,6a} = J_{6a,6b} = 9.2$ Hz, 1H, H-6a), 3.83 (dt, $J_{2,3} = J_{3,4} = 9.2$ Hz, $J_{3,3\text{-OH}}$ 2.3 Hz, 1H, H-3), 4.34 (d, $J_{1,2}$ 7.6 Hz, 1H, H-1), 4.37 (dd, $J_{5,6b}$ 5.1 Hz, $J_{6a,6b}$ 9.2 Hz, 1H, H-6b), 5.55 (s, 1H, C*H*Ph), 7.36–7.40 (m, 3H, *Ph*), 7.48–7.51 (m, 2H, *Ph*); ^{13}C NMR: δ 57.54 (1-O*Me*), 66.39 (C-5), 68.66 (C-6), 73.18 (C-3), 74.57 (C-2), 80.58 (C-4), 101.93 (*C*HPh), 104.13 (C-1), 126.25, 128.35, 129.30, 136.90 (*Ph*). Anal. Calcd for $C_{14}H_{18}O_6$: C, 59.57; H, 6.43. Found: C, 59.62; H, 6.55. The ^1H and ^{13}C NMR data agree well with one literature report,[8] but disagree with another.[9]

1.2. Methyl 4,6-*O*-benzylidene-$^{13}C_6$-β-D-glucopyranoside (5*)

Yield: 3.238 g (11.23 mmol, 63%) from **4*** (3.563 g, 17.70 mmol); mp 172–177 °C; $[\alpha]_D$ −62.4 (*c* 0.5, $CHCl_3$). Anal. Calcd for $C_8{}^{13}C_6H_{18}O_6$ (288.25): C, 58.35; H, 6.30. Found: C, 58.30; H, 6.38.

1.3. Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (6)

NaH (2.27 g, 56.6 mmol, as 60% dispersion in mineral oil), tetra-*n*-butylammonium iodide (262 mg, 0.708 mmol) and benzyl bromide (4.04 mL, 34.0 mmol) were added to a soln of **5** (4.00 g, 14.2 mmol) in anhyd THF (150 mL) at 0 °C. The soln was refluxed overnight. After the reaction mixture was cooled to rt, excess NaH was

quenched with MeOH. The reaction mixture was diluted with EtOAc (approx. 200 mL), washed neutral with water, washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash column chromatography (*n*-hexane, then 1:49 EtOAc–toluene) to give a colorless syrup. The syrup was crystallized from EtOH/*n*-hexane to afford **6** (4.21 g, 64.1%) as colorless crystals: R_f 0.45 (1:4 EtOAc–*n*-hexane); mp 119–120 °C [lit. 119–120 °C,[10] mp 119–119 °C[11]]; $[\alpha]_D$ −35.0 (*c* 1.0, $CHCl_3$) [lit. $[\alpha]_D$ −35.8 (*c* 3, $CHCl_3$),[10] $[\alpha]_D$ −37 (*c* 1.4, $CHCl_3$)[11]]; ^1H NMR: δ 3.41 (br dt, $J_{4,5}$ 9.7 Hz, $J_{5,6a}$ 10.1 Hz, $J_{5,6b}$ 5.0 Hz, 1H, H-5), 3.45 (br t, $J_{1,2}$ 7.6 Hz, $J_{2,3}$ 9.2 Hz, 1H, H-2), 3.58 (s, 3H, 1-O*Me*), 3.68 (br t, $J_{3,4}$ 9.2 Hz, $J_{4,5}$ 9.7 Hz, 1H, H-4), 3.75 (t, $J_{2,3} = J_{3,4} = 9.2$ Hz, 1H, H-3), 3,79 (br t, $J_{5,6a}$ 10.1 Hz, $J_{6a,6b}$ 10.3 Hz, 1H, H-6a), 4.37 (dd, $J_{5,6b}$ 5.0 Hz, $J_{6a,6b}$ 10.3 Hz, 1H, H-6b), 4.42 (d, $J_{1,2}$ 7.6 Hz, 1H, H-1), 4.76, 4.80, 4.87, 4.91 (four d, 4H, CH_2Ph), 5.57 (s, 1H, C*H*Ph), 7.23–7.50 (m, 15H, *Ph*); ^{13}C NMR: δ 57.44 (1-O*Me*), 65.90 (C-5), 68.74 (C-6), 75.07, 75.23 (*C*H_2Ph), 80.72 (C-3), 81.43 (C-4), 82.11 (C-2), 101.06 (*C*HPh), 105.16 (C-1), 125.95–128.90, 137.25, 138.32, 138.41 (*Ph*). Anal. Calcd for $C_{28}H_{30}O_6$: C, 72.81; H, 6.54. Found: C, 72.69; H, 6.65. ^1H NMR data agree with the literature, which either presented only data for H-4, H-5, and H-6,[12] or gave no signal assignment.[13]

1.4. Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-$^{13}C_6$-β-D-glucopyranoside (6*)

Yield: 2.247 g (4.80 mmol, 44%) from **5*** (3.179 g, 11.03 mmol); mp 121–122 °C; $[\alpha]_D$ −33.1 (*c* 0.7, $CHCl_3$). Anal. Calcd for $C_{22}{}^{13}C_6H_{30}O_6$ (468.49 g mol^{-1}): C, 71.82; H, 6.46. Found: C, 71.77; H, 6.56. HRMS: Calcd for $[C_{22}{}^{13}C_6H_{30}O_6 + Na^+]$: 491.2141 g mol^{-1}. Found: 491.2291 g mol^{-1} ($\Delta M = 0.0150$ Da).

1.5. Methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (7)

Triethylsilane (1.69 mL, 10.6 mM) and trifluoroacetic acid (0.82 mL, 10.6 mmol) were added to a soln of **6** (4.10 g, 8.86 mmol) in anhyd CH_2Cl_2 (100 mL) at 0 °C. The soln was stirred at rt for 2 h. Another charge of triethylsilane (1.42 mL, 8.86 mmol) and trifluoroacetic acid (0.68 mL, 8.86 mmol) was added at 0 °C, and the stirring at rt was continued for 2 h. A third portion of triethylsilane (1.42 mL, 8.86 mmol) and trifluoroacetic acid (0.68 mL, 8.86 mmol) was added again at 0 °C, and the reaction mixture stirred at rt for 2 h. The reaction mixture was diluted with EtOAc (approx. 200 mL), neutralized with saturated aq $NaHCO_3$, washed with 0.05 M aq HCl, neutralized with saturated aq $NaHCO_3$, washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash column chromatography (1:19 EtOAc–toluene) to give a colorless syrup, which was crystallized from EtOH/*n*-hexane to afford **7** (3.32 g, 80.7%) as colorless crystals: R_f 0.49 (1:2 EtOAc–*n*-hexane); mp 71–73 °C [lit. 64–65 °C];[14] $[\alpha]_D$ −12.6 (*c* 1.0, $CHCl_3$) [lit. $[\alpha]_D$ −17 (*c* 1.0, $CHCl_3$),[14] $[\alpha]_D$ −9 (*c* 1.05, $CHCl_3$)[15]]; ^1H NMR: δ 2.61 (d, $J_{4,4\text{-OH}}$ 2.3 Hz, 1H, 4-O*H*), 3.40 (dd, $J_{1,2}$ 7.6 Hz, $J_{2,3}$ 8.9 Hz, 1H, H-2), 3.44 (ddd, $J_{4,5}$ 8.9 Hz, $J_{5,6a}$ 5.3 Hz, $J_{5,6b}$ 3.9 Hz, 1H, H-5), 3,45 (t, $J_{2,3} = J_{3,4} = 8.9$ Hz, 1H, H-3), 3.57 (s, 3H, 1-O *Me*), 3.58 (dt, $J_{3,4} = J_{4,5} = 8.9$ Hz, $J_{4,4\text{-OH}}$ 2.3 Hz, 1H, H-4), 3.70 (dd, $J_{5,6a}$ 5.3 Hz, $J_{6a,6b}$ 10.6 Hz, 1H, H-6a), 3.77 (dd, $J_{5,6b}$ 3.9 Hz, $J_{6a,6b}$ 10.6 Hz, 1H, H-6b), 4.32 (d, $J_{1,2} = 7.6$ Hz, 1H, H-1), 4.56, 4.60, 4.70, 4.72, 4.92, 4.93 (six d, 6H, CH_2Ph), 7.26–7.37 (m, 15H, *Ph*); ^{13}C NMR: δ 57.07 (1-O*Me*), 70.14 (C-6), 71.40 (C-4), 73.59 (*C*H_2Ph), 73.98 (C-5), 74.60, 75.20 (2 × *C*H_2Ph), 81.69 (C-2), 83.89 (C-3), 104.67 (C-1), 127.64–128.47, 137.84, 138.38, 138.51 (*Ph*). Anal. Calcd for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94. Found: C, 72.36; H, 7.06. NMR data agreed with the literature references, which give either only ^{13}C data,[16] or only ^{13}C data without assignment,[14] or a differing carbon signal assignment.[15]

1.6. Methyl 2,3,6-tri-*O*-benzyl-$^{13}C_6$-β-D-glucopyranoside (7*)

Yield: 1.882 g (4.00 mmol, 87%) from **6*** (2.192 g, 4.62 mmol); mp 70–71 °C; $[\alpha]_D$ −19.5 (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{22}{}^{13}C_6H_{32}O_6$ (470.51): C, 71.51; H, 6.86. Found: C, 71.52; H, 6.87. HRMS: Calcd for $[C_{22}{}^{13}C_6H_{32}O_6 + Na^+]$: 493.2298. Found: 493.2512 ($\Delta M = 0.0214$ Da).

1.7. Methyl 2,3,6-tri-*O*-benzyl-4-*O*-methyl-β-D-glucopyranoside (8)

NaH (380 mg, 9.47 mmol, as 60% dispersion in mineral oil) was added to a soln of **7** (2.20 g, 4.74 mmol) in anhyd THF (30 mL) at rt, and the soln was stirred for 30 min. Methyl iodide (590 μL, 9.47 mmol) was added to this soln, which was further stirred at rt for 2 h. After quenching excess NaH with MeOH, the reaction mixture was diluted with EtOAc (approx. 100 mL), neutralized with water, washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash column chromatography (1:19 EtOAc–toluene) to give **8** (2.24 g, 98.9%) as a colorless syrup: R_f 0.56 (1:4 EtOAc–*n*-hexane); $[\alpha]_D$ +14.0 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 3.29 (dd, $J_{3,4}$ 9.2 Hz, $J_{4,5}$ 9.6 Hz, 1H, H-4), 3.37 (ddd, $J_{4,5}$ 9.6 Hz, $J_{5,6a}$ 4.9 Hz, $J_{5,6b}$ 1.9 Hz, 1H, H-5), 3.39 (dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.2 Hz, 1H, H-2), 3.48 (s, 3H, 4-O*Me*), 3.53 (t, $J_{2,3} = J_{3,4} = 9.2$ Hz, 1H, H-3), 3.57 (s, 3H, 1-O*Me*), 3.69 (dd, $J_{5,6a}$ 4.9 Hz, $J_{6a,6b}$ 10.8 Hz, 1H, H-6a), 3.76 (dd, $J_{5,6b}$ 1.9 Hz, $J_{6a,6b}$ 10.8 Hz, 1H, H-6b), 4.28 (d, $J_{1,2}$ 7.8 Hz, 1H, H-1), 4.57,

4.65, 4.69, 4.77, 4.88, 4.90 (6 d, 6H, 3CH_2Ph), 7.24–7.36 (m, 15H, 3*Ph*); ^{13}C NMR: δ 57.05 (1-O*Me*), 60.65 (4-O*Me*), 69.03 (C-6), 73.47, 74.70, 75.53 (*C*H_2Ph), 74.84 (C-5), 79.78 (C-4), 82.10 (C-2), 84.52 (C-3), 104.63 (C-1), 127.56–128.29, 138.22, 138.54, 138.62 (*Ph*). Anal. Calcd for $C_{29}H_{34}O_6$: C, 72.78; H, 7.16. Found: C, 72.61; H, 7.13. The NMR data agree with those available for the 2,3-*p*-methoxybenzylated derivative.[17]

1.8. Methyl 2,3,6-tri-*O*-benzyl-4-*O*-methyl-$^{13}C_6$-β-D-glucopyranoside (8*)

Yield: 1.245 g (2.57 mmol, 98%) from **7*** (1.237 g, 2.63 mmol); $[\alpha]_D$ +14.0 (*c* 1.0, $CHCl_3$). Elemental analysis not available. HRMS: Calcd for [$C_{23}{}^{13}C_6H_{34}O_6$ + Na^+]: 507.2454 g mol^{-1}. Found: 507.2343 g mol^{-1} ($\Delta M = -0.0111$ Da).

1.9. 2,3,6-Tri-*O*-benzyl-4-*O*-methyl-α,β-D-glucopyranose (9)

To a soln of **8** (2.15 g, 4.49 mmol) in acetic acid (45 mL) was added a 2 M soln of TfOH in water (9.0 mL). The soln was heated to 80 °C and then stirred for 3 h. The reaction mixture was diluted with CH_2Cl_2 (approx. 200 mL), neutralized with saturated aq $NaHCO_3$, washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash column chromatography (1:6 EtOAc–toluene) to give a colorless syrup, which was crystallized from EtOH/*n*-hexane to afford **9** (1.68 g, 80.6%) as colorless crystals: R_f 0.33 and 0.38 (1:2 EtOAc/*n*-hexane); mp 96–98 °C; $[\alpha]_D$ +38.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 1.75 (br s, 0.65H, 1α-O*H*), 3.25 (t, $J_{3\beta,4\beta} = J_{4\beta,5\beta} = 9.4$ Hz, 0.35H, H-4β), 3.31 (t, $J_{3\alpha,4\alpha} = J_{4\alpha,5\alpha} = 9.3$ Hz, 0.65H, H-4α), 3.35 (t, $J_{1\beta,2\beta} = J_{2\beta,3\beta} = 8.0$ Hz, 0.35H, H-2β), 3.36 (br s, 0.35H, 1β-O*H*), 3.42 (ddd, $J_{5\beta,6b\beta}$ 0.7 Hz, $J_{5\beta,6a\beta}$ 5.3 Hz, $J_{4\beta,5\beta}$ 9.4 Hz, 0.35H, H-5β), 3.46 (s, 1.05H, 4-O*Me*β), 3.47 (s, 1.95H, 4-O*Me*α), 3.52 (dd, $J_{1\alpha,2\alpha}$ 3.2 Hz, $J_{2\alpha,3\alpha}$ 9.3 Hz, 0.65H, H-2α), 3.53 (dd, $J_{2\beta,3\beta}$ 8.0 Hz, $J_{3\beta,4\beta}$ 9.4 Hz, 0.35H, H-3β), 3.62 (dd, $J_{5\beta,6a\beta}$ 5.3 Hz, $J_{6a\beta,6b\beta}$ 10.6 Hz, 0.35H, H-6aβ), 3.64 (dd, $J_{5\alpha,6a\alpha}$ 0.7 Hz, $J_{6a\alpha,6b\alpha}$ 10.5 Hz, 0.65H, H-6aα), 3.67 (dd, $J_{5\alpha,6b\alpha}$ 3.8 Hz, $J_{6a\alpha,6b\alpha}$ 10.5 Hz, 0.65H, H-6bα), 3.71 (dd, $J_{5\beta,6b\beta}$ 0.7 Hz, $J_{6a\beta,6b\beta}$ 10.6 Hz, 0.35H, H-6bβ), 3.87 (t, $J_{2\alpha,3\alpha} = J_{3\alpha,4\alpha} = 9.3$ Hz, 0.65H, H-3α), 3.94 (ddd, $J_{5\alpha,6a\alpha}$ 0.7 Hz, $J_{5\alpha,6b\alpha}$ 3.8 Hz, $J_{4\alpha,5\alpha}$ 9.3 Hz, 0.65H, H-5α), 4.50, 4.55, 4.611, 4.614, 4.67, 4.74, 4.75, 4.78, 4.82, 4.88, 4.91, 4.93 (12d, 12H, CH_2Ph), 4.60 (d, $J_{1\beta,2\beta}$ 8.0 Hz, 0.35H, H-1β), 5.19 (d, $J_{1\alpha,2\alpha}$ 3.2 Hz, 0.65H, H-1α), 7.24–7.38 (m, 15H, *Ph*); ^{13}C NMR: δ 58.77 (4-O*Me*β), 60.64 (4-O*Me*α), 68.64 (C-6α), 69.03 (C-6β), 70.19 (C-5α), 73.19, 73.43, 73.50, 74.70, 75.58 (*C*H_2Ph), 74.63 (C-5β), 79.50 (C-4α), 79.69 (C-2α), 79.80 (C-4β), 81.61 (*C*-3α), 82.87 (C-2β), 84.39 (C-3β), 91.22 (C-1α), 97.36 (C-1β), 127.83–128.34, 134.77–138.66 (*Ph*). Anal. Calcd for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94. Found: C, 72.39; H, 7.08. The ^{1}H NMR data agree with those of an anomeric mixture (α/β = 1:0.6).[18]

1.10. 2,3,6-Tri-*O*-benzyl-4-*O*-methyl-$^{13}C_6$-α,β-D-glucopyranose (9*)

Yield: 0.920 g (1.96 mmol, 78%) from **8*** (1.217 g, 2.51 mmol), mp 94–97 °C, $[\alpha]_D$ +36.4 (*c* 0.5, $CHCl_3$). Anal. Calcd for $C_{22}{}^{13}C_6H_{32}O_6$ (470.51): C, 71.51; H, 6.86. Found: C, 71.44; H, 6.96. HRMS: calcd for [$C_{22}{}^{13}C_6H_{32}O_6 + Na^+$]: 493.2298 g. Found: 493.2133 g mol^{-1} ($\Delta M = -0.0165$ Da).

1.11. 2,3,6-Tri-*O*-benzyl-4-*O*-methyl-α-D-glucopyranosyl trichloroacetimidate (10α/10α*) and 2,3,6-tri-*O*-benzyl-4-*O*-methyl-β-D-glucopyranosyl trichloroacetimidate (10β/10β*)

To a soln of **9** (1.55 g, 3.34 mmol) in CH_2Cl_2 (20 mL) DBU (200 μL, 1.33 mmol) was added under stirring for 30 min at rt. Then, trichloroacetonitrile (1.34 mL, 13.3 mmol) was added, and the soln was stirred at ambient temperature for 2 h. The reaction mixture was diluted with *n*-hexane (5 mL), and the residue was filtered using a column of alumina (B grade, activity 1, by ICN Biomedicals GmbH, approx. 30 g) to remove DBU. The filtrate was evaporated to give the mixture of **10**α and **10**β (1.63 g, 80.2% recovery) as a pale yellow syrup, which was directly used for the next reaction. For analysis, the residue was purified by flash column chromatography (1:19 EtOAc–toluene containing 1% (v/v) of triethylamine) to separate **10**α and **10**β(α/β = 10/1, w/w).

2,3,6-Tri-*O*-benzyl-4-*O*-methyl-α-D-glucopyranosyl trichloroacetimidate (**10**α); R_f 0.44 (1:4 EtOAc–*n*-hexane); ^{1}H NMR: δ 3.49 (s, 3H, 4-O*Me*), 3.50 (t, $J_{3,4}$ 9.4 Hz, $J_{4,5}$ 9.6 Hz, 1H, H-4), 3.66 (dd, $J_{5,6a}$ 1.9 Hz, $J_{6a,6b}$ 10.9 Hz, 1H, H-6a), 3.70 (dd, $J_{5,6b}$ 3.5 Hz, $J_{6a,6b}$ 10.9 Hz, 1H, H-6b), 3.73 (dd, $J_{1,2}$ 3.3 Hz, $J_{2,3}$ 9.4 Hz, 1H, H-2), 3.89 (ddd, $J_{5,6a}$ 1.9 Hz, $J_{5,6b}$ 3.5 Hz, $J_{4,5}$ 10.9 Hz, 1H, H-5), 3.95 (t, $J_{2,3} = J_{3,4} = 9.4$ Hz, 1H, H-3), 4.49, 4.60, 4.66, 4.72, 4.81, 4.92 (6d, 6H, 3 CH_2Ph), 6.48 (d, $J_{1,2}$ 3.3 Hz, 1H, H-1), 8.57 (s, 1H, N*H*).

2,3,6-Tri-*O*-benzyl-4-*O*-methyl-β-D-glucopyranosyl trichloroacetimidate (**10**β); R_f 0.31 (1:4 EtOAc–*n*-hexane); ^{1}H NMR: δ 3.45 (dd, $J_{3,4}$ 8.8 Hz, $J_{4,5}$ 9.67 Hz, 1H, H-4), 3.50 (s, 3H, 4-O*Me*), 3.54 (ddd, $J_{5,6a}$ 2.1 Hz, $J_{5,6b}$ 3.9 Hz, $J_{4,5}$ 9.7 Hz, 1H, H-5), 3.95 (t, $J_{2,3} = J_{3,4} = 8.8$ Hz, 1H, H-3), 3.73 (dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 8.8 Hz, 1H, H-2), 3.72 (dd, $J_{5,6a}$ 3.9 Hz, $J_{6a,6b}$ 11.2 Hz, 1H, H-6a), 3.76 (dd, $J_{5,6b}$ 2.1 Hz, $J_{6a,6b}$ 11.2 Hz, 1H, H-6b), 4.56, 4.65, 4.74, 4.81, 4.87, 4.92 (6d, 6H, 3CH_2Ph), 5.72 (d, $J_{1,2}$ 7.8 Hz, 1H, H-1), 8.69 (s, 1H, N*H*).

1.12. Methyl 2,3,6-tri-*O*-benzyl-4-*O*-methyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11)

To a soln of **7** (935 mg, 2.01 mmol) and **10** (1.63 g, 2.68 mmol) in anhyd CH_2Cl_2 (50 mL) was added $BF_3{\cdot}Et_2O$ (34 μL, 0.27 mmol) at −72 °C. After the soln was stirred at −72 °C for 1 h, the reaction mixture was neutralized with triethylamine. The residue was purified by flash column chromatography (1:9 EtOAc–toluene) to give **11** (1.16 g, 63.1%) as a colorless syrup: R_f 0.32 (1:9 EtOAc–toluene); $[\alpha]_D$ +24.5 (*c* 1.0, $CHCl_3$) [lit. $[\alpha]_D$ +23 (*c* 0.5, $CHCl_3$)[5]]; ^{1}H NMR: δ 3.18 (dd, $J_{5',6'b}$ 1.6 Hz, $J_{5',6'a}$ 4.6 Hz, $J_{4',5'}$ 9.3 Hz, 1H, H-5′), 3.320 (dd, $J_{1',2'}$ 7.6 Hz, $J_{2',3'}$ 9.3 Hz, 1H, H-2′), 3.324 (t, $J_{3',4'} = J_{4',5'} = 9.3$ Hz, 1H, H-4′), 3.35 (ddd, $J_{5,6a}$ 1.3 Hz, $J_{5,6b}$ 4.1 Hz, $J_{4,5}$ 9.6 Hz, 1H, H-5), 3.39 (dd, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 9.0 Hz, 1H, H-2), 3.41 (t, $J_{2',3'} = J_{3',4'} = 9.3$ Hz, 1H, H-3′), 3.47 (s, 3H, 4′-O*Me*), 3.52 (dd, $J_{5',6'a}$ 4.6 Hz, $J_{6'a,6'b}$ 11.0 Hz, H-6′a), 3.55 (s, 3H, 1-O*Me*), 3.57 (dd, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 9.2 Hz, 1H, H-3), 3.67 (dd, $J_{5',6'b}$ 1.6 Hz, $J_{6'a,6'b}$ 11.0 Hz, 1H, H-6′b), 3.71 (dd, $J_{5,6a}$ 1.3 Hz, $J_{6a,6b}$ 11.0 Hz, 1H, H-6a), 3.82 (dd, $J_{5,6b}$ 4.1 Hz, $J_{6a,6b}$ 11.0 Hz, 1H, H-6b), 4.00 (dd, $J_{3,4}$ 9.2 Hz, $J_{4,5}$ 9.6 Hz, 1H, H-4), 4.28 (d, $J_{1,2}$ 7.7 Hz, 1H, H-1), 4.40, 4.42, 4.43, 4.58, 4.68, 4.72, 4.73, 4.77, 4.78, 4.83, 4.83, 5.05 (12d, 12H, 6CH_2Ph), 4.46 (d, $J_{1',2'}$ 7.6 Hz, 1H, H-1′), 7.17–7.35 (m, 30H, 6*Ph*); ^{13}C NMR: δ 57.00 (1-O*Me*), 60.54 (4′-O*Me*), 68.10 (C-6), 68.88 (C-6′), 73.21 (*C*HPh), 74.86, 74.95, 74.96, 75.50 (C-5, C-5′, *C*HPh), 76.56 (C-4), 79.86 (C-4′), 81.66 (C-2), 82.49 (C-2′), 82.72 (C-3), 84.77 (C-3′), 102.59 (C-1′), 104.59 (C-1), 127.43–128.31, 138.13, 138.45, 138.47, 138.58, 138.59, 139.24 (*Ph*). Elemental analysis not performed. ^{1}H NMR data agreed with the literature.[5]

1.13. Methyl 2,3,6-tri-*O*-benzyl-4-*O*-methyl-$^{13}C_6$-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-$^{13}C_6$-β-D-glucopyranoside (11*)

Yield: 0.781 g (0.846 mmol, 64%) from **7*** (0.623 g, 1.324 mmol) and **10*** (1.186 g, 1.88 mmol). Elemental analysis not performed. HRMS: Calcd for $[C_{44}{}^{13}C_{12}H_{62}O_{11} + Na^+]$: 945.4592 g mol^{-1}. Found: 945.4471 ($\Delta M = -0.0121$ Da).

1.14. Methyl 4-*O*-methyl-$^{13}C_6$-β-D-glucopyranosyl-(1→4)-$^{13}C_6$-β-D-glucopyranoside (12)

A soln of **11** (1.05 g, 1.15 mmol) in MeOH (20 mL) was hydrogenated in the presence of 10% Pd/C (25 mg) at atmospheric pressure at rt for 36 h. After the catalyst was filtered off, the reaction mixture was evaporated. The residue was crystallized from MeOH to give **12** (387 mg, 90.9%) as a colorless solid: R_f 0.58 (3:7 MeOH–CH_2Cl_2); mp 204–210 °C [lit. 196–198 °C[5]]; $[\alpha]_D$ −16.4 (*c* 1.0, water) [lit. $[\alpha]_D$ −11 (*c* 0.4, water)[5]]; ^{1}H NMR (D_2O): δ 3.39 (t, $J_{3',4'} = J_{4',5'} = 9.4$ Hz, 1H, H-4′), 3.47 (broad dd, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 8.9 Hz, 1H, H-2), 3.49 (dd, $J_{1',2'}$ 8.0 Hz, $J_{2',3'}$ 9.4 Hz, 1H, H-2′), 3.65 (ddd, $J_{5',6'b}$ 2.3 Hz, $J_{5',6'a}$ 5.5 Hz, $J_{4',5'}$ 9.4 Hz, 1H, H-5′), 3.72 (s, 3H, 4′-O*Me*), 3.74 (s, 3H, 1-O*Me*), 3.74–3.80 (m, 4H, H-3′, H-4, H-3, H-5), 3.91 (dd, $J_{5',6'a}$ 5.5 Hz, $J_{6'a,6'b}$ 12.4 Hz, 1H, H-6′a), 3.97 (dd, $J_{5,6a}$ 4.8 Hz, $J_{6a,6b}$ 12.5 Hz, 1H, H-6a), 4.07 (dd, $J_{5',6'b}$ 2.3 Hz, $J_{6'a,6'b}$ 12.4 Hz, 1H, H-6′b), 4.16 (dd, $J_{5,6b}$ 2.0 Hz, $J_{6a,6b}$ 12.5 Hz, 1H, H-6b), 4.56 (d, $J_{1,2}$ 8.0 Hz, 1H, H-1), 4.64 (d, $J_{1',2'}$ 8.0 Hz, 1H, H-1′), 4.80 (br s, 6H, O*H*); ^{13}C NMR (D_2O): δ 57.54 (1-O*Me*), 60.30 (4′-O*Me*), 60.45 (C-6), 60.73 (C-6′), 73.22 (C-2), 73.55 (C-2′), 74.69 (C-3), 75.10 (C-5′), 75.35 (C-5), 75.51 (C-3′), 79.08 (C-4), 79.49 (C-4′), 102.80 (C-1′), 103.42 (C-1). Anal. Calcd for $C_{14}H_{26}O_{11}$: C, 45.40; H, 7.08. Found: C, 45.35; H, 7.11. The data are consistent with those previously published.[5]

1.15. Methyl 4-*O*-methyl-$^{13}C_6$-β-D-glucopyranosyl-(1→4)-$^{13}C_6$-β-D-glucopyranoside (12*)

Yield: 0.250 g (0.645 mmol, 64%) from **11*** (0.779 g, 0.844 mmol) 77%, mp 197–203 °C, $[\alpha]_D$ −15.5 (*c* 0.66, water). Anal. Calcd for $C_2{}^{13}C_{12}H_{26}O_{11}$ (382.26): C, 44.00; H, 6.86. Found: C 44.10; H 6.74. HRMS: Calcd for $[C_2{}^{13}C_{12}H_{26}O_{11} + Na^+]$: 405.1775. Found: 405.1625 ($\Delta M = -0.0150$ Da).

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