Electronic Supplementary Material (ESI) for ChemComm.
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Supplementary Information

Perdeuterated and ^{13}C-enriched myo-inositol for DNP assisted monitoring of enzymatic phosphorylation by inositol-3-kinase

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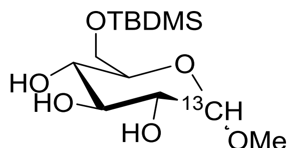
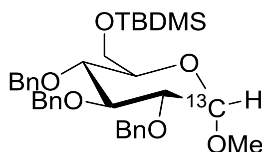
Synthesis

General methods and materials

D-Glucose-1-^{13}C and reagents were obtained from Sigma-Aldrich and used as purchased, unless otherwise noted. Organic solvents were purchased anhydrous and used without further purification. Unless otherwise noted, all reactions were carried out at room temperature (RT) in glassware with magnetic stirring. Organic solutions were concentrated under reduced pressure with bath temperatures < 30 ºC. Flash column chromatography was carried out on silica gel G60 (Silicycle, 60-200 µm, 60 Å). Thin-layer chromatography (TLC) was carried out on Silica gel 60 F_{254} (EMD Chemicals Inc.) with detection by UV excitation (254 nm) after spraying with a solution of $(NH_4)_6Mo_7O_{24}H_2O$ (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150 ºC. ^{1}H NMR, and ^{13}C NMR spectra were recorded on Varian Inova (300/75 MHz) or Varian Inova-500 (500/125 MHz) spectrometers equipped with sun workstations. Multiplicities are quoted as singlet (s), doublet (d), doublet of doublet (dd), triplet (t) or multiplet (m). All NMR signals were assigned on the basis of ^{1}H NMR, ^{13}C NMR, COSY and HSQC experiments. All chemical shifts are quoted on the δ-scale in parts per million (ppm). Residual solvent signals were used as an internal reference. Mass spectra were recorded on an Applied Biosystems 5800 MALDI-TOF or Shimadzu LCMS-IT-TOF mass spectrometer. The matrix used was 2,5-dihydroxy-benzoic acid (DHB). Molecular sieves (MS, 4 Å) were flame activated *in vacuo*. All moisture sensitive reactions were carried out under an argon atmosphere.

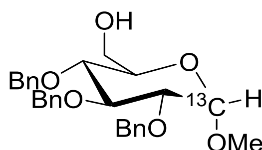


Methyl 6-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranoside-1-^{13}C (4).[1, 2] D-Glucose-1-^{13}C **2** (2.0 g, 11.1 mmol) was suspended in MeOH (50 mL) and acetyl chloride (1.78 mL, 25 mmol) was added dropwise. The solution was stirred for 2 h until glucose had been consumed. The reaction was quenched by addition of trimethylamine (3.48 mL, 25 mmol). The solvents were evaporated under reduced pressure and α-methyl-D-glucopyranoside-1-^{13}C **3** was dried *in vacuo* to give a solid. This compound was used in the next reaction step without purification. TBDMSCl (2.6 g, 16.7 mmol) was added to a stirred solution of **3** (2.2 g, 11.1 mmol), triethylamine (5.5 mL, 38.9 mmol), and DMAP (70 mg, 0.55 mmol) in DMF (38 mL) at 0 ºC. The reaction was allowed to warm up to room temperature and stirring was continued for 12 h. The reaction mixture was poured into ice-water (10 mL) and extracted with dichloromethane (50 mL), and the organic mixture was washed with ammonium chloride and water (10 mL, each). The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexanes/ethyl acetate 1:4), to afford **4** as an amorphous solid (3.1 g, 90%). ^{1}H NMR (300 MHz, MeOD) δ α, β anomers: Two doublets at 4.91 and 4.35 ppm along the diagonal H-1α ($^1J_{C,H}$ = 167.6, $^1J_{H,H}$ = 3.5 Hz, H-1α), two doublets at 4.40 and 3.87 ppm along the diagonal H-1β ($^1J_{C,H}$ = 157.9, $^1J_{H,H}$ = 7.8 Hz, H-1β), 3.98-3.91 (m, 2H, H-6), 3.82-3.75 (m, 2H, H-4), 3.60 (t, J = 9.3 Hz, H-3), 3.50 (d, J = 4.4 Hz, 3H, OCH_3, anomer α), 3.39 (d, J = 4.4 Hz, 3H, OCH_3, anomer β), 3.34-3.25 (m, 3H, H-2 anomer α, H-5), 3.18-3.10 (m, 1H, H-2 anomer β), 0.91 (s, 9H), 0.09 (s, 6H). ^{13}C NMR (300 MHz, MeOD) δ 109.6, 103.9 (C-1β), 99.8 (C-1α), 76.6, 73.8, 73.3, 72.4, 71.8, 70.2, 70.0, 62.6, 55.8, 54.0, 25.0, 17.9, -6.4, -6.5. MALDI-MS: m/z for $C_{12}{}^{13}CH_{28}NaO_6Si$ $[M+Na]^+$: 332.1581; found 332.1011.



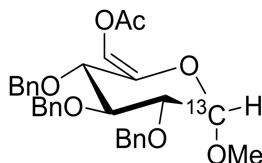
Methyl 6-*O*-*tert*-butyldimethylsilyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside-1-^{13}C (5).[3] Compound **4** (3.1g, 10 mmol) was dissolved in DMF (150 mL) and the resulting solution was

cooled to 0 ºC. Tetrabutylammonium iodide (7.2 g, 2 mmol) and NaH (1.7 g, 43 mmol) was added and the resulting suspension was stirred for 15 min. To the stirred solution, benzyl bromide (5.2 mL, 43 mmol) was added. The reaction was then allowed to warm up to room temperature and stirring was continued for 18 h. After completion of the reaction, the reaction mixture was cooled in an ice-water bath and quenched with methanol. The organic layer was extracted four times with CH_2Cl_2 and the combined extracts were washed with brine (3 x 100 mL), and water (3 x 100 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using hexane/ethyl acetate (9:1) as eluent to give **5** as a colorless syrup (4.6 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ data for α-anomer. 7.40-7.27 (m, 15H), two doublets at 4.90 and 4.35 along the diagonal H-1α ($^1J_{C,H}$ = 177.5, $^1J_{H,H}$ = 3.5 Hz, H-1α), 4.99 (d, J = 10.8 Hz, 1H, *CH₂*Ph), 4.90 (d, J = 9.0 Hz, 1H, CH_2Ph), 4.85 (d, J = 7.7 Hz, 1H, CH_2Ph), 4.81 (d, J = 8.9 Hz, 1H, CH_2Ph), 4.75 (d, J = 9.0 Hz, 1H, CH_2Ph), 4.65 (d, J = 7.9 Hz, 1H, CH_2Ph), 4.02 (t, J = 9.1 Hz, 1H, H-3), 3.81 (d, J = 3.0 Hz, 2H, H-6), 3.68-3.61 (m, 1H, H-5), 3.59-3.45 (m, 2H, H-4, H-2), 3.39 (d, J = 4.4 Hz, 3H, OCH_3), 0.91 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 138.8, 138.5, 138.3, 128.4, 128.1, 127.8, 127.7, 127.6, 104.9, 104.5, 97.9 (C-1α), 82.2 (C-3), 80.5 (C-4), 79.9 (C-2), 75.9, 75.0, 73.3, 71.5 (C-5), 62.3 (C-6) 54.9 (OCH_3) 25.9 (3C, *C*H_3), 18.3 (Si-*C*), -5.1, -5.4. MALDI-MS: m/z for $C_{33}{}^{13}CH_{46}NaO_6Si$ $[M+Na]^+$: 602.2989; found 602.2598.

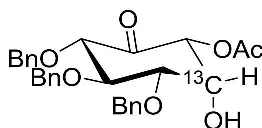


Methyl 2,3,4-tri-*O*-benzyl-α- D-glucopyranoside-1-^{13}C (6). Tetrabutylammonium fluoride (23 mL, 1M in THF) was added to a stirred solution of silylated compound **5** (4.6 g, 7.9 mmol), and MS 4 Å (100 mg) in THF (30 mL) at 0 ºC. The reaction mixture was then allowed to warm up to room temperature and stirring was continued for 6 h. Upon completion of the reaction, the reaction mixture was concentrated *in vacuo* and diluted with ethyl acetate (100 mL). The organic mixture was washed with sodium bicarbonate (30 mL) and water (30 mL). The aqueous extract was washed with ethyl acetate, and the combined organic extracts were dried ($MgSO_4$), filtered and the filtrated was concentrated under reduced pressure. The concentrated residue was purified by silica gel column chromatography (hexanes/ethyl acetate 1:1) to afford the compound **6** (2.5 g, 67%) as a colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44-7.34 (m, 15H), only one of the doublets

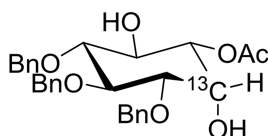
founded at 4.37 along the diagonal H-1α, other one overlapped with *CH₂*Ph groups ($^{1}J_{C,H}$ = 163.0, $^{1}J_{H,H}$ = 3.1 Hz, H-1α), 5.09-4.82 (m, 6H, CH_2Ph), 4.10 (t, *J* = 9.2 Hz, 1H, H-3), 3.76-3.67 (m, 1H, H-5, H-6), 3.64-3.56 (m, 2H, H-4, H-2), 3.41 (d, *J* = 4.2 Hz, 3H, OCH_3), 2.25 (bs, 1H, OH). ^{13}C NMR (300 MHz, $CDCl_3$) δ 138.8, 138.3, 138.2, 128.5, 128.1, 128.0, 127.9, 127.7, 104.9, 104.5, 98.2 (C-1α), 82.0 (C-3), 80.4 (C-4), 79.8 (C-2), 77.5, 75.8, 75.1, 73.4, 70.9 (C-5), 61.7 (C-6) 55.2 (OCH$_3$). MALDI-MS: m/z for $C_{27}{}^{13}CH_{32}NaO_6$ $[M+Na]^+$: 488.2125; found 488.1503.



(Z)-Methyl 6-*O*-acetyl-2,3,4-tri-benzyl-α-D-gluco-hex-5-enopyranoside-1-^{13}C (8). Dess-Martin periodinane (770 mg, 5.83 mmol) was added to a solution of **6** (2.5 g, 5.3 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred for 3 h at room temperature. The residue was filtrated over celita, concentrated and dried 3 times with toluene to afford a yellow syrup. The crude **7** was used in the next reaction step without purification. Compound **7** was treated with triethylamine (4.5 mL, 31.8 mmol,), acetic anhydride (2.5 mL, 26.5 mmol) and 4-(dimethylamino)pyridine (32 mg, 0.265 mmol) in dichloethane (36 mL) and the resulting reaction mixture was stirred at 45 ºC for 18 h. The reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with sodium bicarbonate and water, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (Hexane/DCM/EtOAc 5:2:1) to afford an ***8E*** and ***8Z*** diastereomixture (1.3 g, 50%, ***8E*/*8Z*** 10:90) as orange syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41-7.20 (m, 15H), 7.20 (s, 1H, H-6, Z diastereoisomer), two doublets at 5.01 and 4.45 along the diagonal H-1α ($^{1}J_{C,H}$ = 171.2, $^{1}J_{H,H}$ = 3.3 Hz, H-1α), 4.94-4.66 (m, 6H, CH_2Ph), 4.03-3.96 (m, 2H, H-3, H-4), 3.64-3.58 (m, 1H, H-2), 3.50 (d, *J* = 4.5 Hz, 3H, OCH_3), 2.17 (s, 3H, CH_3CO). ^{13}C NMR (300 MHz, $CDCl_3$) δ 167.3, 138.5, 138.0, 137.6, 135.0 (C-5), 128.5, 128.4, 128.1, 128.0, 127.9, 127.7, 99.8 (C-1α), 98.8, 98.5, 98.2, 81.3 (C-3), 79.4 (C-2), 78.8 (C-4), 77.8, 75.7, 74.5, 73.7, 56.3 (OCH$_3$), 20.6 (CH$_3$CO). MALDI-MS: m/z for $C_{29}{}^{13}CH_{32}NaO_7$ $[M+Na]^+$: 528.2074; found 528.2010.

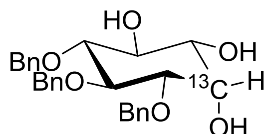


(1R,2R,3S,4R,5S)-2-Hydroxy-3,4,5-tri-*O*-benzyl-6-oxocyclohexyl Acetate (9). $Hg(OCOCF_3)_2$ (1.3 g, 3.05 mmol) was added to a stirred solution of **8** (1 g, 2.03 mmol) in acetone-water (42.5 mL, 34:8.5). The mixture was stirred for 24 h at room temperature. After the reaction was finished, saturated sodium chloride was added at 0 ºC and stirred for 2 h. After addition of dichloromethane, the solution was washed with $Na_2S_2O_3$, dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by silicagel chromatography (hexane/EtOAc 6:4) to afford the product **9** (319 mg, 32%) as a syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42-7.31 (m, 15H), the doublets along the diagonal H-1α and H-2α overlapped with the other signals, 4.97-4.53 (m, 7H, *CH$_2$*Ph, H-1), 4.63-4.53 (m, 2H, H-4), 4.19-4.07 (m, H-3, H-2), 3.89-3.86 (m, 1H, H-5), 2.55 (s, 1H, OH), 2.25 (s, 3H, *CH$_3$*CO). ^{13}C NMR (300 MHz, $CDCl_3$) δ 138.4, 137.3, 128.6, 128.4, 128.1, 128.0, 127.7, 98.4, 83.5 (C-5), 81.8 (C-4), 73.4, 71.0 (C-6), 69.4 (C-2), 20.5 (*C*H$_3$CO). MALDI-MS: m/z for $C_{28}{}^{13}CH_{30}NaO_7$ $[M+Na]^+$: 514.1917; found 513.0220.

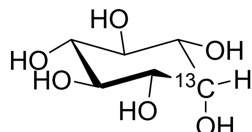


D-1-*O*-Acetyl-3,4,5-benzyl-*myo*-inositol-2-^{13}C (10).[4] $Me_4NBH(OAc)_3$ (795.7 mg, 2.87 mmol) was dissolved in CH_3CN-AcOH (9.0 mL, 4.5:4.5 v/v) and the resulting solution was cooled to -40 ºC. At this temperature, **9** (141.2 mg, 0.287 mmol) in CH_3CN-AcOH (9.0 mL, 4.5:4.5 v/v) was added, and the mixture was allowed to warm up to room temperature and stirring was continued for 24 h. Saturated aqueous Rochelle salt (4 mL) was added and the mixture was stirred for an additional 30 min. The mixture was extracted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:1) to afford **10** (130 mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.29 (m, 15H), 4.97-4.53 (m, 7H, *CH$_2$*Ph, H-1), two broad triplets at 4.54 and 4.04 along the diagonal H-2α ($^1J_{C,H}$ = 150.4 Hz, $^1J_{H,H}$ = 2.52, $^2J_{H,H}$ = 2.40 Hz, H-2α), 4.11 (t, J = 9.7 Hz, 1H, H-6), 3.93 (t, J = 9.4 Hz, 1H, H-4), 3.59-3.53 (m, 1H, H-3), 3.4 (t, J = 9.3 Hz, 1H, H-5), 2.46 (s, 1H, OH), 2.28 (s, 1H, OH), 2.16 (s, 3H, *CH$_3$*CO). ^{13}C NMR (300

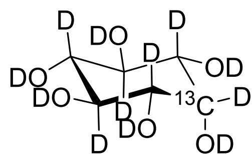
MHz, $CDCl_3$) δ 170.4, 138.4, 138.3, 137.4, 128.6, 128.4, 128.1, 127.9, 127.7, 99.8, 82.9 (C-5), 80.9 (C-4), 80.3 (C-3), 79.9, 75.8, 75.7 (C-1), 73.4, 70.5 (C-6), 70.3, 67.8 (C-2), 21.1 ($\mathit{C}H_3CO$). MALDI-MS: m/z for $C_{28}{}^{13}CH_{32}NaO_7$ $[M+Na]^+$: 516.2074; found 516.1781.



3,4,5-benzyl-*myo*-inositol-2-^{13}C (11).[5] Guanidinium chloride/Na solution (2.14 mL) was added to a solution of **10** (130 mg, 0.263 mmol) in CH_2Cl_2 (2.8 mL). The solution was allowed to stir at room temperature for 5 h. The mixture was neutralized with Amberlite IRP-64 ion exchange resin, filtrated and concentrated to give the amorphous solid **11** (115 mg, 97 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39-7.26 (m, 15H), 4.95-4.68 (m, 6H, CH_2Ph), one broad peak at 4.38 along the diagonal H-2α ($^1J_{C,H}$ and $^1J_{H,H}$ could not defined, H-2α), 3.96-3.85 (m, 2H, H-6, H-4, H-2α), 3.45 (m, H-3), 3.35-3.26 (m, 2H, H-1, H-5), 3.18 (bs, 1H, OH), 3.06 (bs, 1H, OH), 2.87 (bs, 1H, OH). ^{13}C NMR (300 MHz, $CDCl_3$) δ 138.6, 138.5, 137.8, 128.5, 128.4, 127.9, 127.9, 127.8, 127.6, 82.7 (C-5), 81.2, 80.3, 79.8 (C-3), 75.8, 75.4, 72.7 (C-4), 71.9 (C-1), 71.0 (C-6), 69.3 (C-2), 67.8. MALDI-MS: m/z for $C_{26}{}^{13}CH_{30}NaO_6$ $[M+Na]^+$: 474.1968; found 474.0999.



Myo-Inositol-2-^{13}C (12). 20% $Pd(OH)_2$ (50 mg, 0.05 mmol) was added to a solution of **11** (320 mg, 0.709 mmol) in MeOH (9 mL). The mixture was stirred at room temperature under an atomosphere of hydrogen for 20 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness to give **12** (128 mg, 0.702 mmol, 99%) as a amorphous solid. ^{1}H NMR (300 MHz, D_2O) δ two triplets at 4.17 and 3.67 along the diagonal H-2α ($^1J_{C,H}$ = 148.7, $^1J_{H,H}$ = 2.7 Hz, H-2α), 3.52-3.43 (m, H-4, H-6), 3.41-3.38 (m, H-1, H-3), 3.14 (t, J = 9.0 Hz, H-5). ^{13}C NMR (300 MHz, D_2O) δ 74.3 (C-5), 72.3 (C-4, C-6), 72.0 (C-2), 71.0 (C-1, C-3). MALDI-MS: m/z for $C_5{}^{13}CH_{12}KO_7$ $[M+K]^+$: 220.0299; found 219.9157.



Myo-Inositol-2-^{13}C-*d*6 1.

Deuterium-labeling of myo-inositol. Myo-Inositol-2-^{13}C **12** (100 mg, 0.552 mmol) was three times dissolved in deuterium oxide (1 mL) and dried. A suspension of the sugar and 5% Ru/C (10 mol%) in deuterium oxide (3 mL) was stirred at 80 ºC for 24 h under hydrogen atmosphere (balloon). After completion of the reaction, the reaction mixture was cooled to room temperature, filtered by membrane filter (Milipore, MillexR-LH, 0.45 um) and washed 3 times with hot deuterium oxide. The filtrate and washings were combined and evaporated to dryness to give **1** (90 mg, 84 %). ^{1}H NMR (300 MHz, D_2O) δ No signal. ^{13}C NMR (300 MHz, D_2O) δ 72.3 (C-4, C-6), 72.0 (C-2), 71.0 (C-1, C-3). MALDI-MS: m/z for $C_5{}^{13}CD_{12}O_6$ $[M+H]^+$: 193.1421; found 192.8606.

Enzymology

Enzyme production—The gene TK2285 of myo-inositol 3-kinase [6] from *Thermococcus kodakarensis* was optimized for *E. coli* codon expression and synthesized by Genscript (Piscataway, NJ). The restriction sites *NcoI* and *NotI* were used to insert the DNA into pET28b vector. The vector was transformed to *E. coli* BL21 (DE3) for expression. IPTG of the final concentration at 0.2 mM was added when OD_{600} reached 0.6 to induce expression and cells were harvested in 16 hr. The protein was purified using HiTrap Talon crude 5 ml column (GE healthcare), followed by a gel filtration step using Superdex 75 (prep grade) column (GE Healthcare). The yield of the protein was approximately 50 mg per liter culture.

Enzyme activity test—The enzyme activity was assessed using 1D ^{1}H NMR (VNMR 600 MHz spectrometer, Varian instruments, Palo Alto, CA). The reaction was initiated by adding 100 µg enzyme to 10 mM myo-inositol (Sigma-Aldrich) in 50 mM NH_4Ac buffer (10% D_2O) containing 20 mM ATP and 20 mM $MgCl_2$. The temperature and pH screening was conducted by varying the conditions in pH 6.0, 6.5, 7.0 and 7.5, or at 25, 37, 50, 65 and 85ºC. A pH near 6.5 was chosen for comparison with literature values of activity. Reactions went to completion by 30 min at all temperatures and no significant differences between 25 and 37 could be detected at 5

min. However, there may be some temperature variation in activity at the shorter times observable in DNP enhanced measurements.

Spectroscopy

DNP and NMR measurements of enzyme kinetics—To prepare the substrate stock, the u-D,$^{13}C_2$-myo-inositol was dissolved in D_2O at the final concentration of 1 M. The DNP sample was prepared as 20 µL myo-inositol stock, 20 µL DMSO and 15 mM OXO63 trityl radical (GE Healthcare, UK). The sample was loaded into HyperSense DNP polarizer (Oxford Instruments, Abingdon, UK) and cooled to 1.4 K. Transfer of polarization to ^{13}C enriched sites was accomplished by irradiation at 93.997 GHz with 100 mW power in a field of 3.35 T for approximately 4 hr. Dissolution of the polarized sample was manually initiated after reaching desired polarization levels in the HyperSense polarizer. 3 mL of reaction buffer containing 50 mM NH_4Ac, 20 mM $MgCl_2$, pH 6.6 in 100% H_2O was injected to reach a final concentration of myo-inositol of approximately 6.7 mM and this solution was captured in a receiving vessel. The sample transfer took approximately 3 sec. 2.75 mL polarized and dissolved substrate was then transferred to an 8-mm NMR tube containing 260µL of the enzyme (2.6 mg) and ATP (60 µmol) preloaded in the magnet, with the aid of air actuated injection device; this took about 3 sec. Data collection started automatically on an electronic trigger from the polarizer once the dissolution process was complete. Real time enzyme kinetics were then monitored using 1D ^{13}C NMR by INOVA 500 MHz spectrometer (Varian Instruments, Palo Alto, CA) equipped with an 8 mm HX probe at approximately 30°C. 1D carbon spectra were acquired repetitively using a standard one-pulse sequence (a 1 sec delay followed with a 20° pulse and 0.5 sec acquisition with deuterium decoupling).

Data Analysis—NMR experimental data were processed using Mnova (version 8.1, Mestrelab Research, Santiago de Compostela, SPAIN). Exponential multiplication with a time constant approximating transverse decay of magnetization was applied to increase S/N. The peak heights of C2-myo-inositol and its product were extracted from each spectrum in the acquisition array for further data fitting. Differential equations (1-2) describing kinetic processes were solved in MATLAB (version R2016b) using the dsolve routine. Simultaneous curve fitting, for both the

substrate and product (described by Equations 3-4), was accomplished using its nonlinear least squares solver (lsqnonlin). This provided optimum values for kinetic constants and other variables.

$$\frac{dP_{sub}(t)}{dt} = {P_{sub}}^{ini}\exp\left(-\right) - P_{sub}(t)\left(k_{enz} + \frac{1}{T_1\,(sub)}\right) \tag{1}$$

$$\frac{dP_{pro}(t)}{dt} = P_{sub}(t)k_{enz} - P_{pro}(t)\frac{1}{T_1\,(pro)} \tag{2}$$

$$S_{sub}(n) = P_{sub}\big(t = (n-1)\,T_n\big)\,Cos^{n-1}(\theta) + {P_{sub}}^{ini}T_n exp(-n\,T_n/T_{in}) \tag{3}$$

$$S_{pro}(n) = P_{pro}\big(t = (n-1)\,T_n\big)\,Cos^{n-1}(\theta) + {P_{sub}}^{ini}T_n\,k_{enz}k_T\,exp(-n\,T_n/T_{in}) \tag{4}$$

The first term in the equation 1 allows for a decrease in effective rate of buildup of substrate after initial injection over a period of time, T_{in} due to mixing processes. P_{sub}^{ini} is a measure of polarization in substrate being added per second during injection. The T_1s are spin relaxation times for substrate (sub) and product (pro), and k_{enz} is an effective first order rate constant for conversion of substrate to product by enzymes. Initial concentrations of substrate and product are assumed to be zero. The model assumes that the total experiment time (~60 s) is short enough to neglect subsequent reactions involving the initial product and reversal of substrate conversion, something that would become significant if equilibrium were approached. The two differential equations were solved simultaneously to get a time course for the polarization of substrate and the product ($P_{pro}(t)$) in terms of the several unknowns.

The signals observed in NMR observation at time points (n) are given in equations (3) and (4) for substrate and product ($S_{sub}(n)$ and $S_{pro}(n)$, respectively). The fraction of polarization remaining after sampling by an *rf* pulse of angle θ is given by cos(θ). This happens repetitively for material in the NMR tube, but not for newly added material during injection of substrate; hence the correction terms at the end of equations 3 and 4. These equations were fit to experimental data using a non-linear regression routine to evaluate variables. Since the time between pulses was short in our application and the pulse angle relatively large, the decay of substrate signal is primarily due to loss of magnetization on pulsing and not due to T_1 decay. Hence, T_1s could not be determined precisely.

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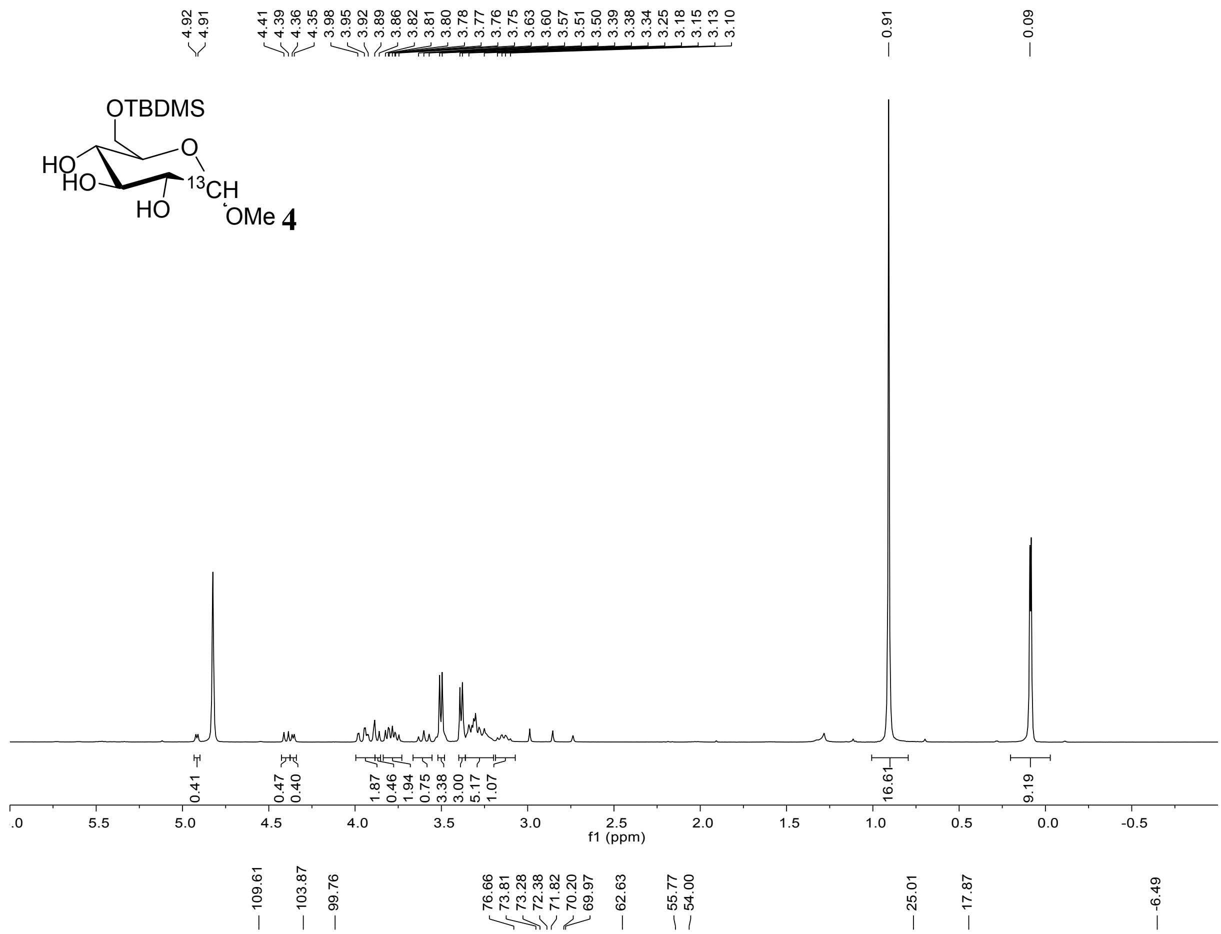
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3.81
3.80
3.78
3.77
3.76
3.75
3.63
3.60
3.57
3.51
3.50
3.39
3.38
3.34
3.25
3.18
3.15
3.13
3.10
0.91
0.09
OTBDMS
HO
HO
HO
13CH
OMe 4
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0.47
0.40
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0.46
1.94
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3.00
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1.07
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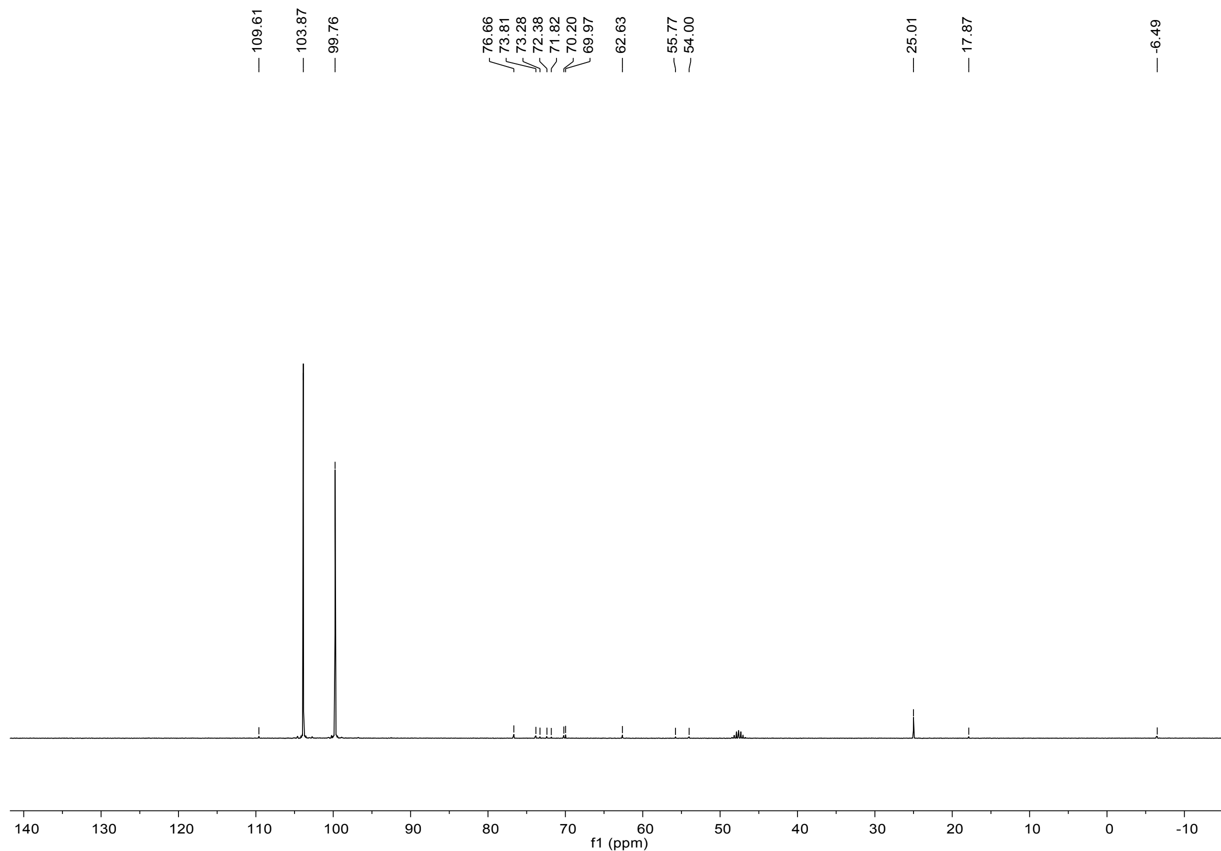
f1 (ppm)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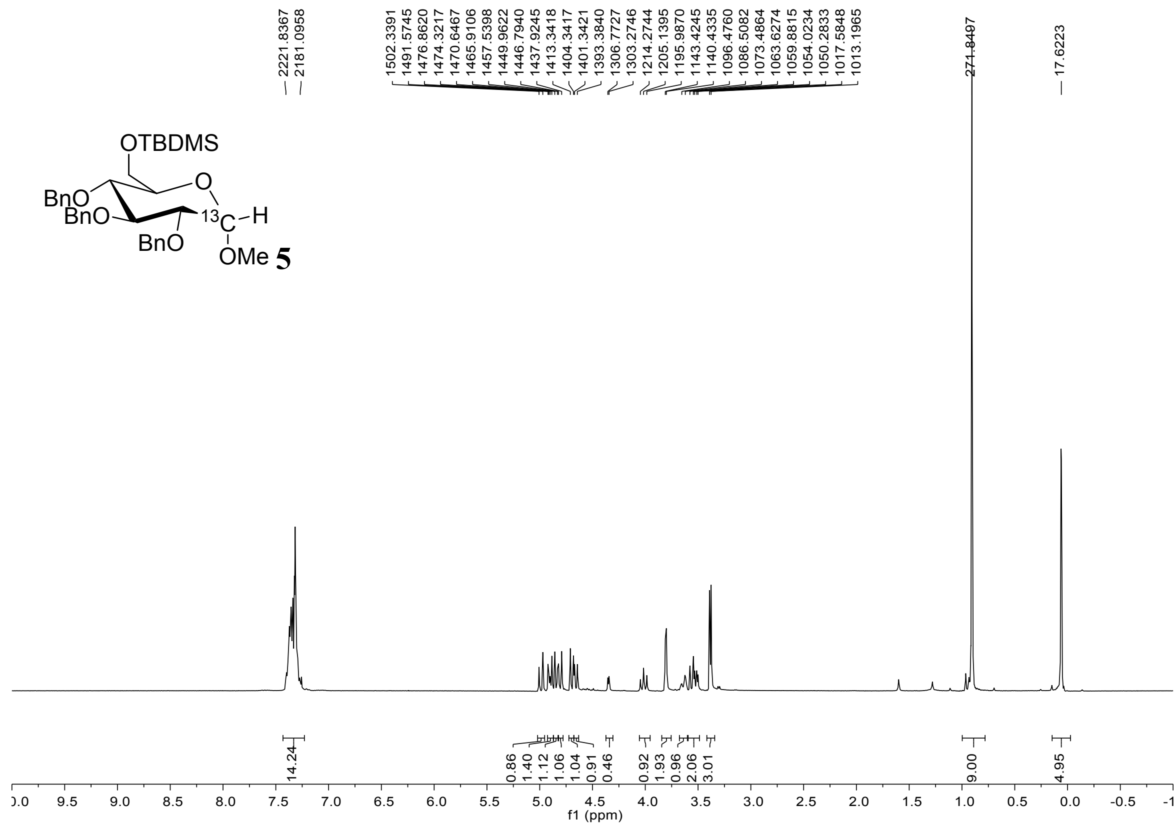

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H
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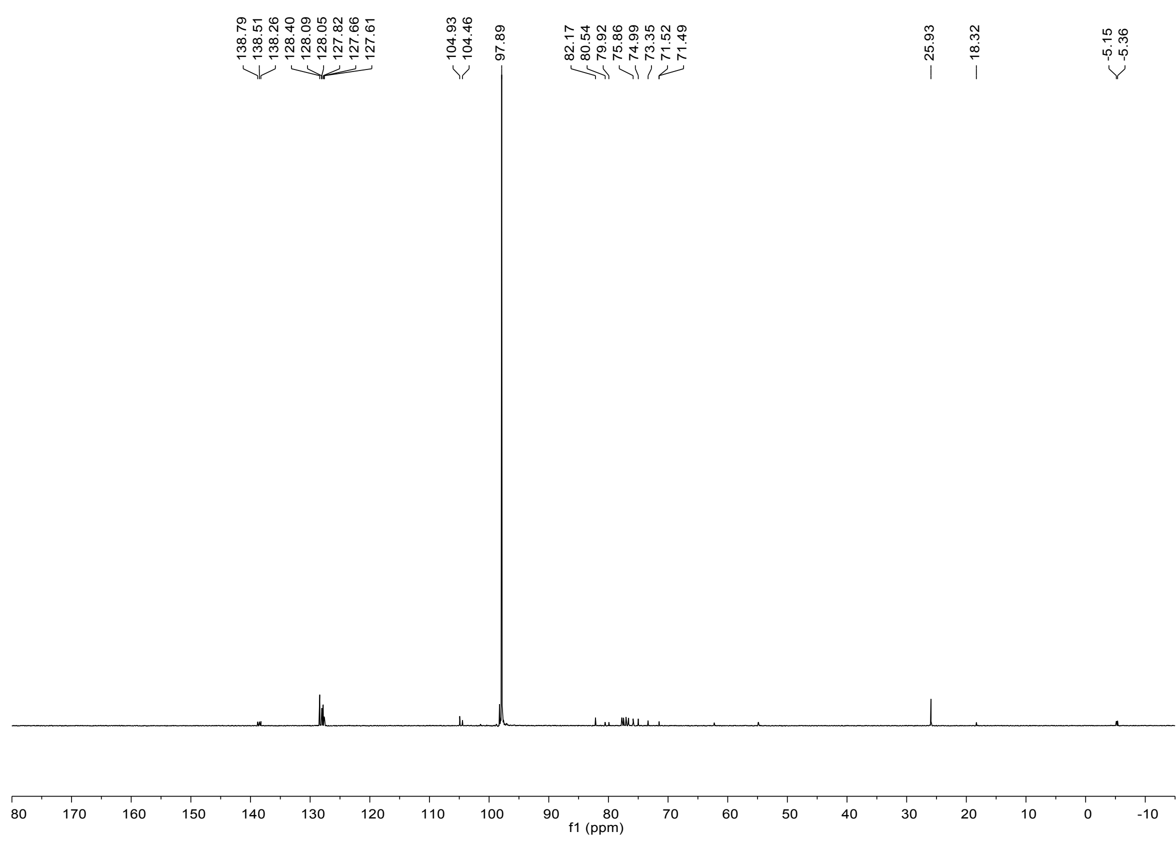

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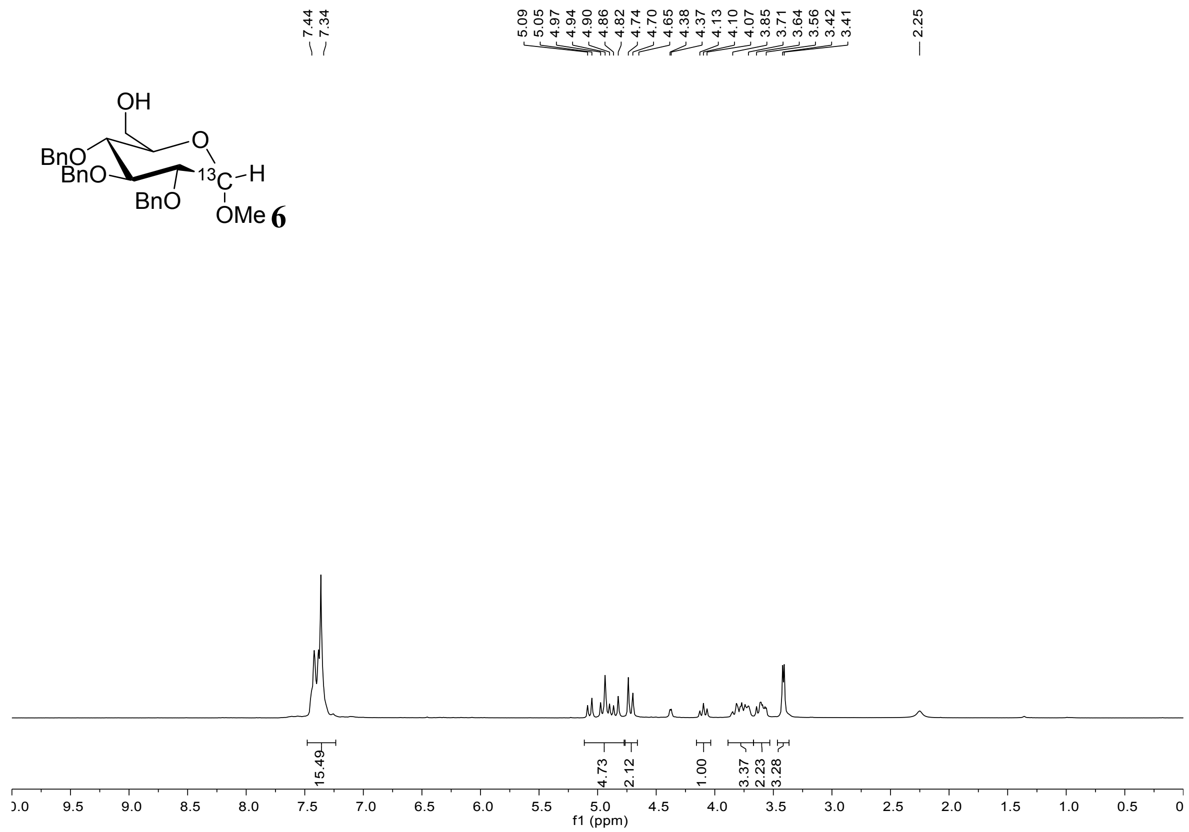
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4.37
4.13
4.10
4.07
3.85
3.71
3.64
3.56
3.42
3.41
2.25
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BnO
BnO
BnO
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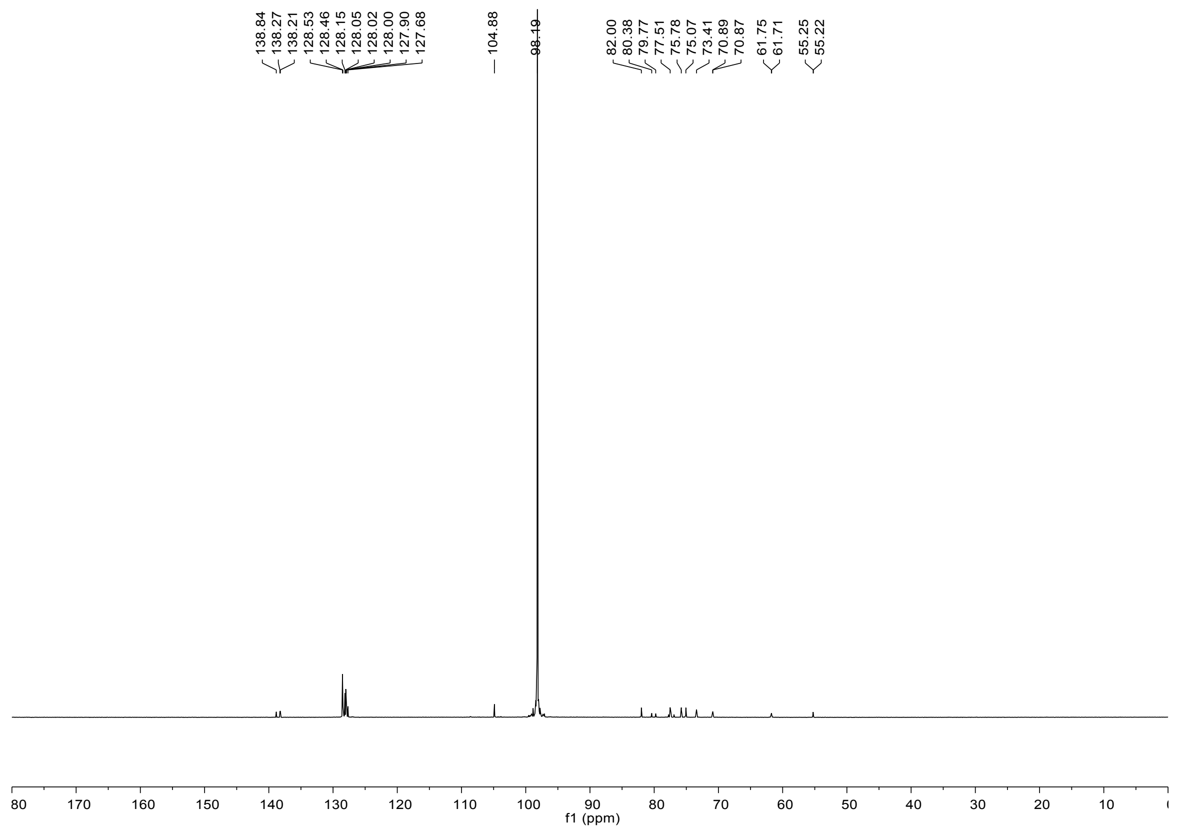
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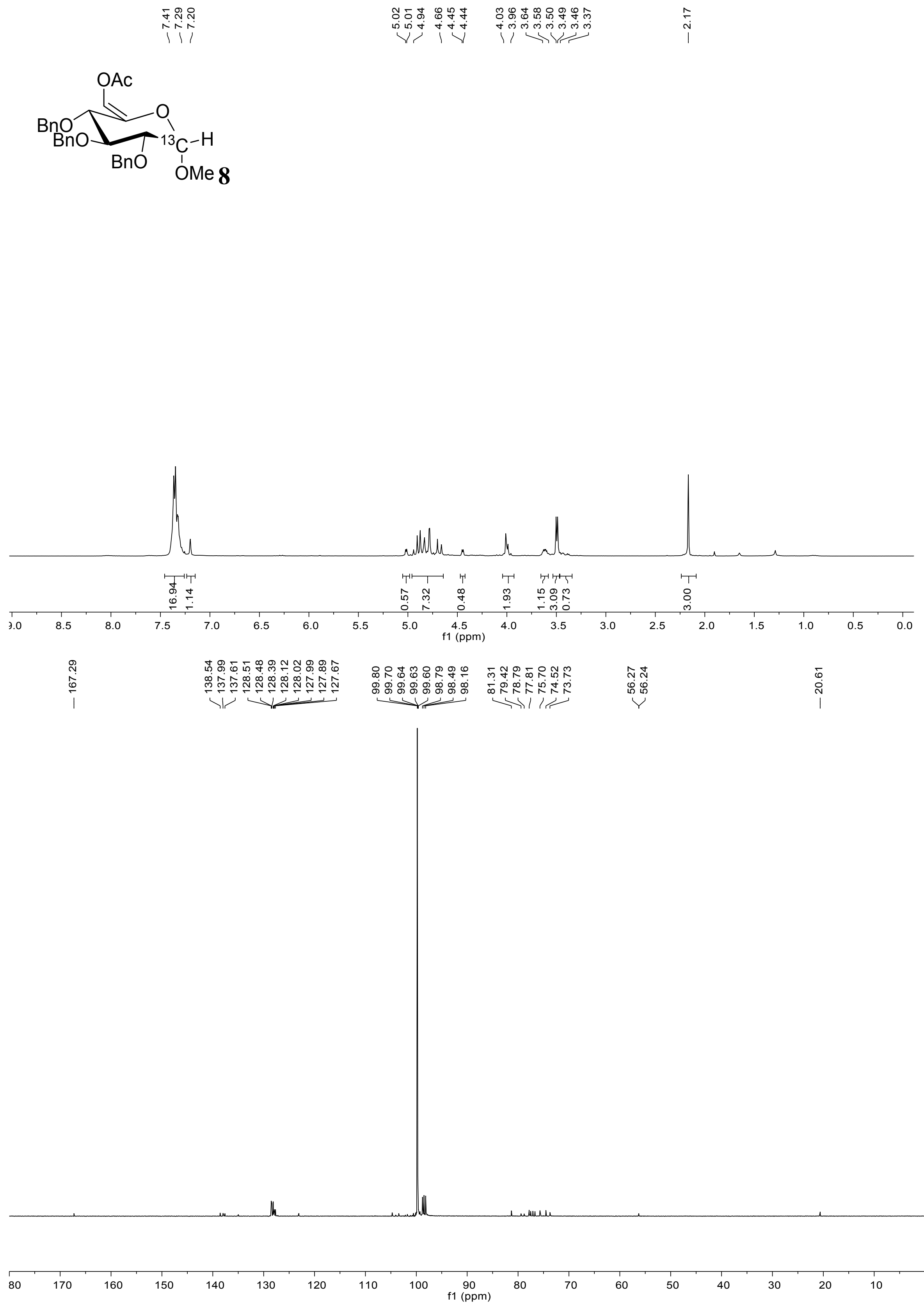

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3.46
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2.17
OAc
BnO
BnO
BnO
O
13C
H
OMe
8
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0.48
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1.15
3.09
0.73
3.00
f1 (ppm)
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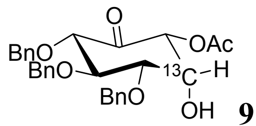

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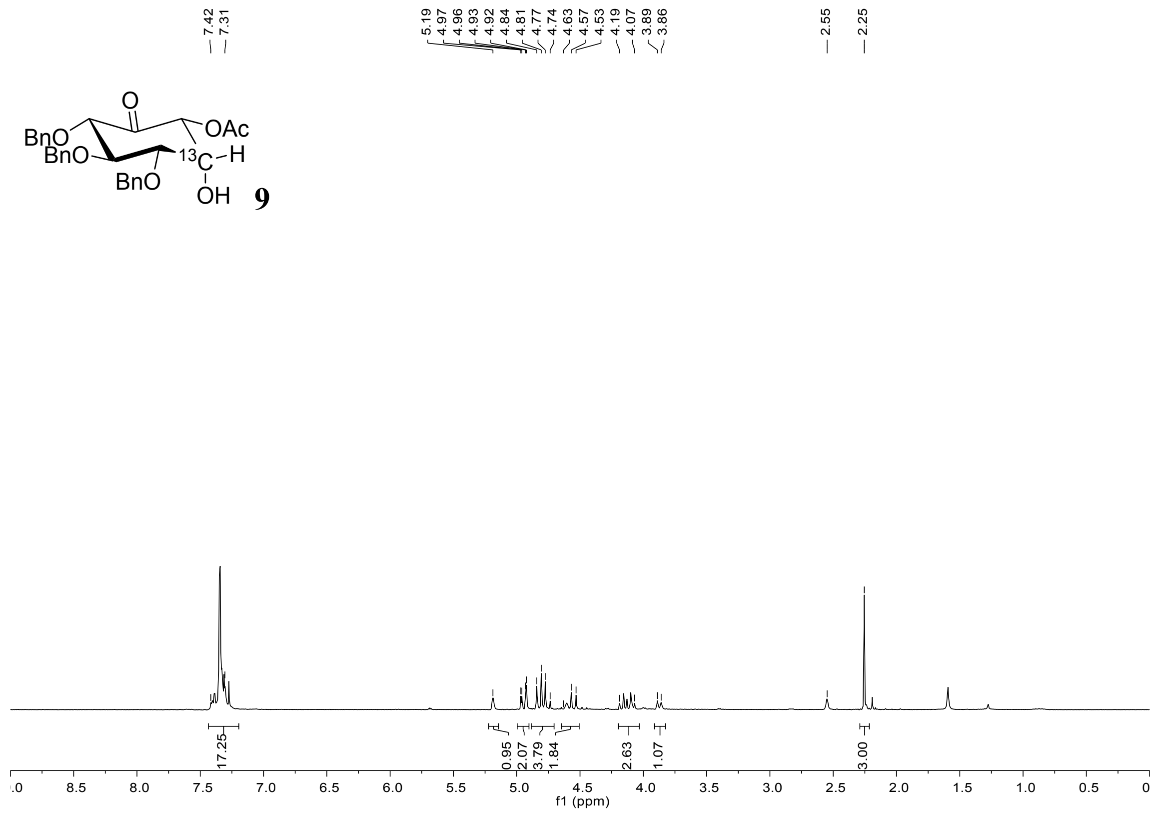

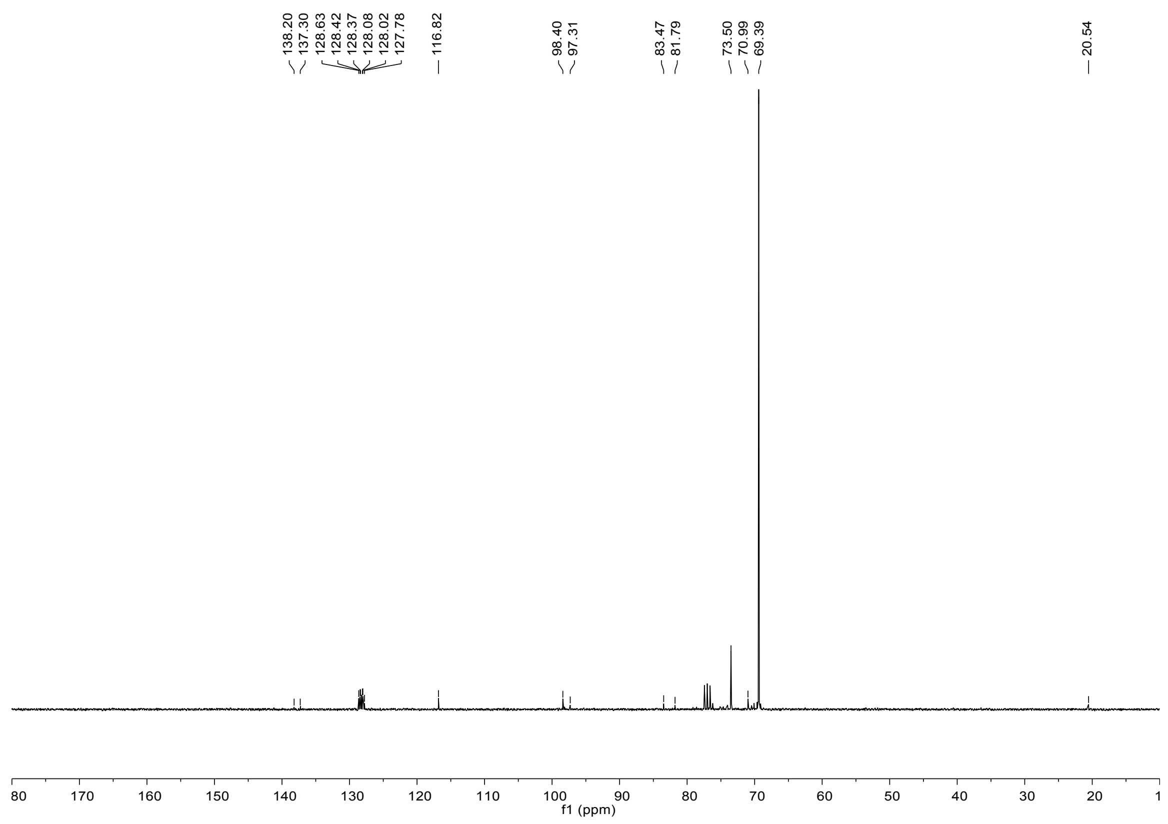

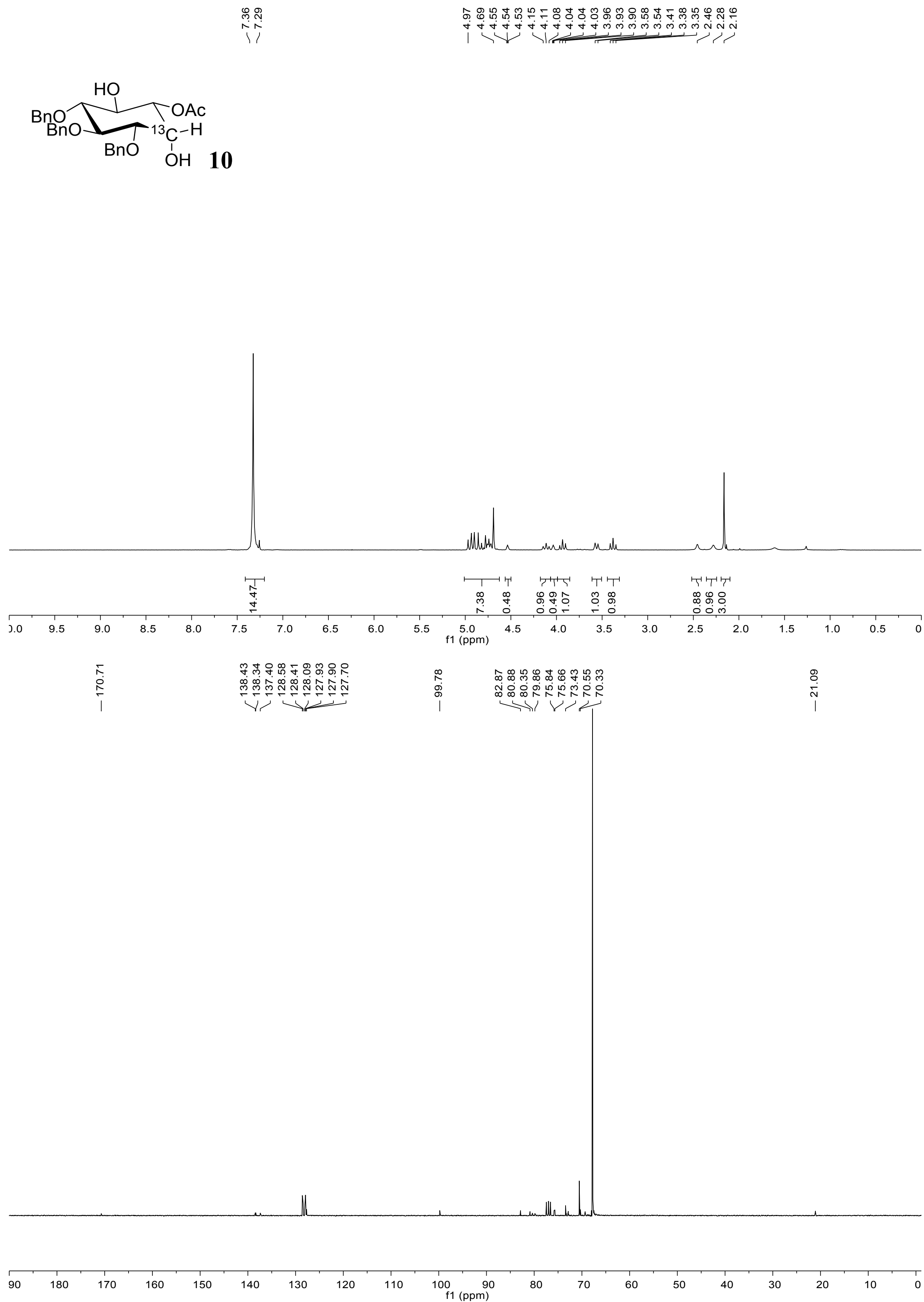

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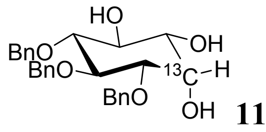
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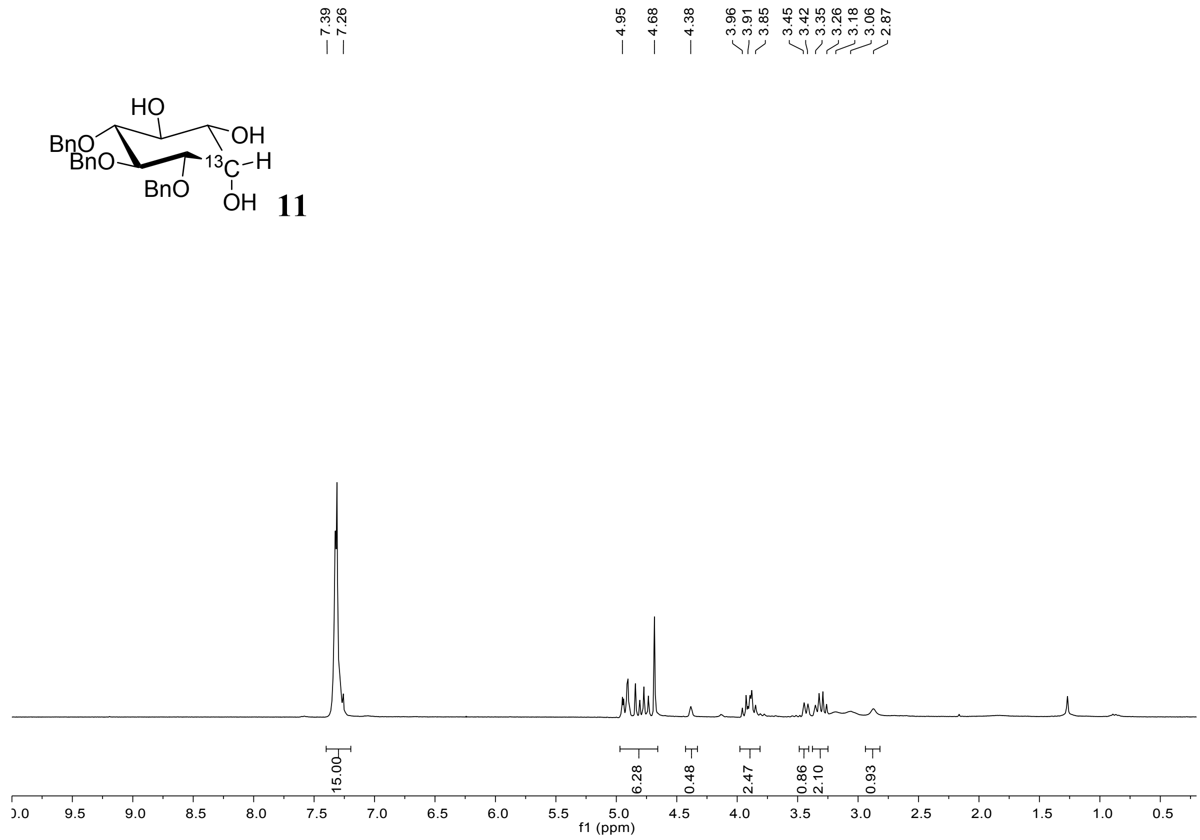
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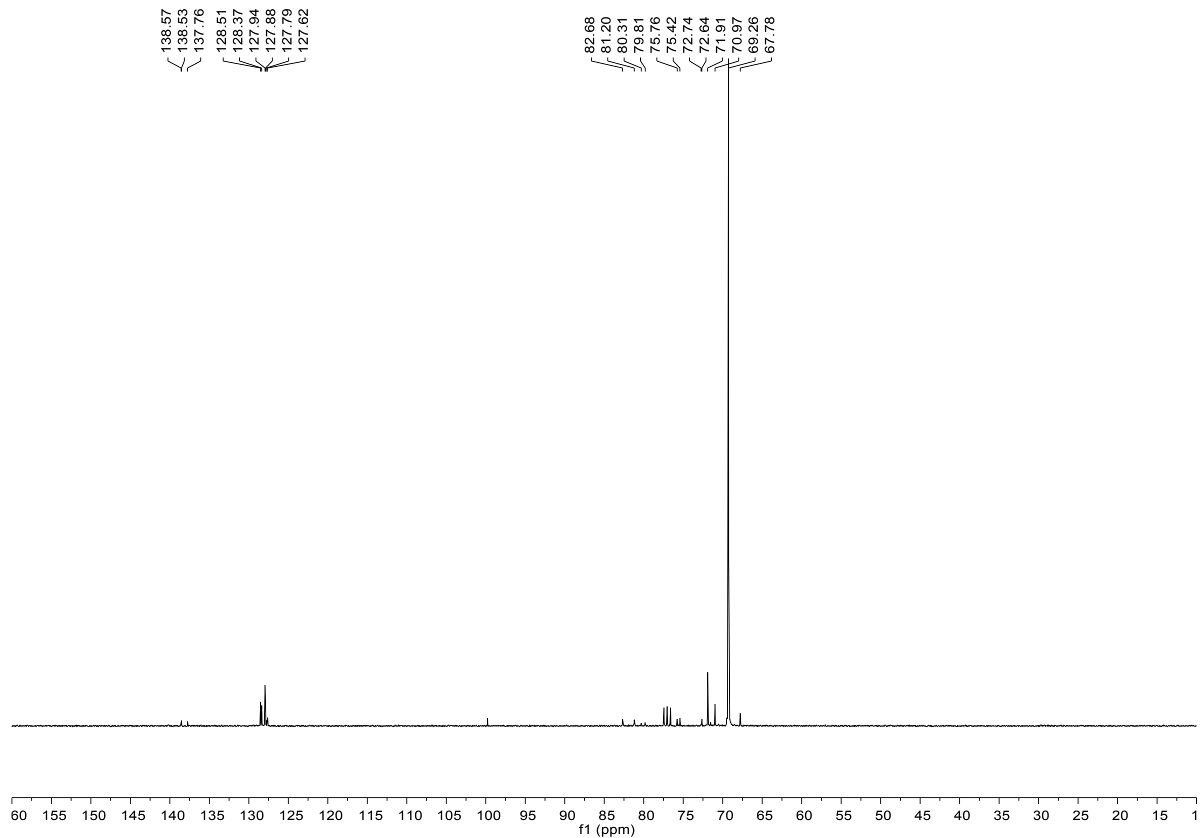
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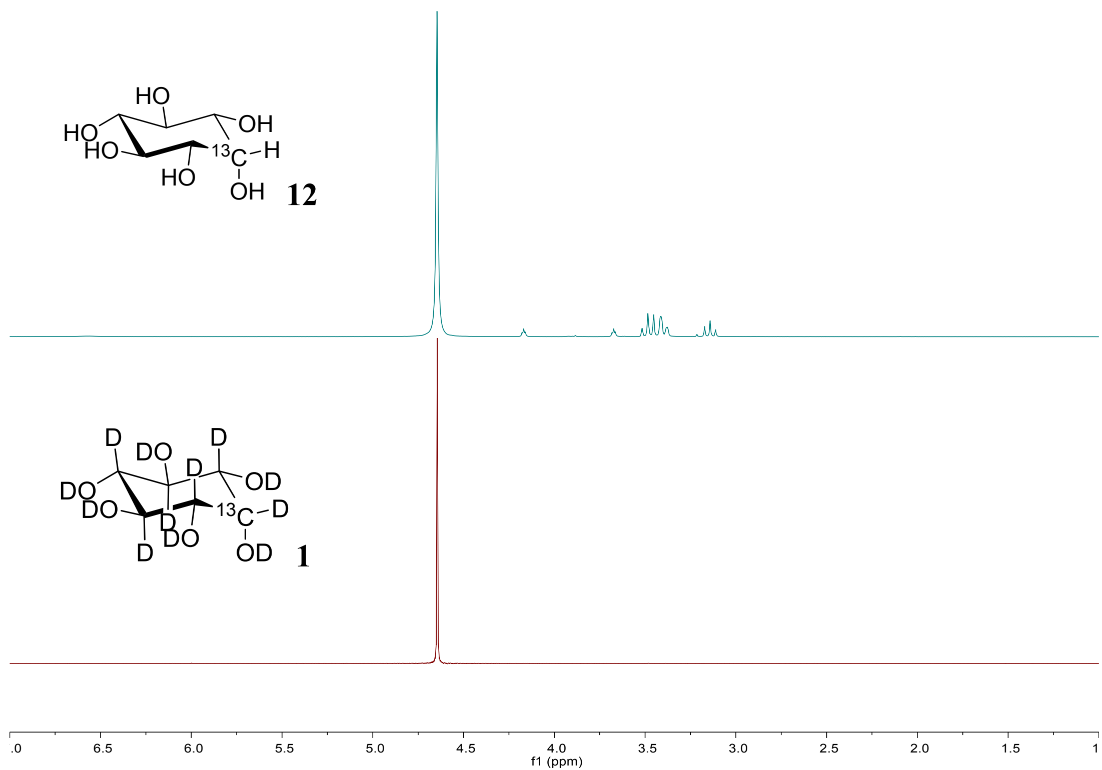
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OD
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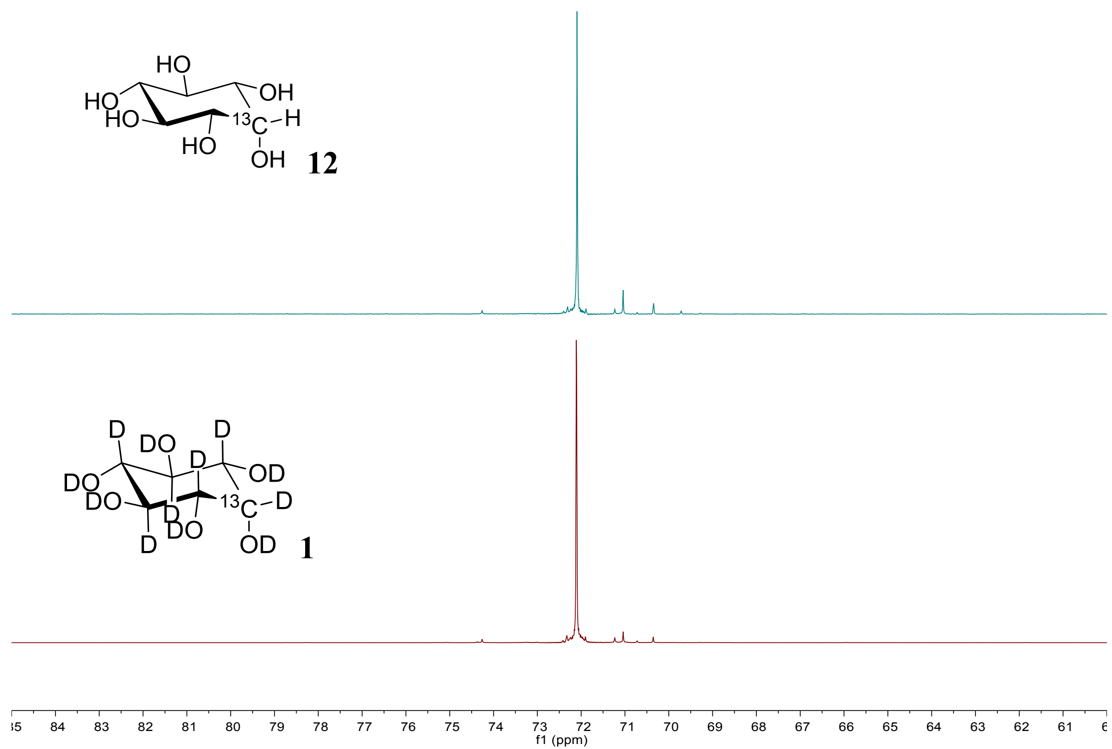
f1 (ppm)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f1 (ppm)