Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.


**Supporting Information**

# A Biocatalytic Approach Towards the Stereoselective Synthesis of Protected Inositols

*Lloyd H. Sayer, Gordon J. Florence and Terry K. Smith*

*Biomedical Science Research Complex, Schools of Chemistry and Biology, University of St Andrews, North Haugh, St Andrews, KY16 9ST, UK*

**Table of Contents:**

**General Experimental**

Infrared (IR) spectra were recorded on a Shimadzu IRAffinity-1 FTIR spectrometer, using attenuated total reflectance (ATR) as the sampling technique. Absorption maxima are reported in wave numbers ($cm^{-1}$).

$^{1}H$ NMR spectra were recorded on Bruker Avance 300 (300.1 MHz), Bruker Avance II 400 (400.1 MHz) or Bruker Avance III 500 (500.1 MHz) instruments, and referenced to deuterated solvent; $CDCl_3$ = 7.26 ppm; $D_2O$ = 4.79 ppm. Chemical shifts (δ) are reported in parts per million (ppm, $\delta_{TMS} = 0$). For each resonance; the number of protons, multiplicity pattern, coupling constants (*J*) and interpretation are reported. $^{13}C$ NMR spectra were recorded on Bruker Avance 300 (75 MHz) or Bruker Avance III 500 (126 MHz) instruments. The chemical shifts were recorded in ppm on the δ scale ($\delta_{TMS} = 0$) and referenced using deuterated chloroform to 77.00 ppm. The coupling constant (*J*) is measured in Hz and reported to the nearest 0.1 Hz. $^{31}P$ NMR spectra were recorded on a Bruker Avance III 500 (202 MHz) instrument using broadband proton decoupling pulse sequences and deuterium internal lock. The chemical shifts were recorded in ppm on the δ scale ($\delta_{TMS} = 0$).

Optical Rotation were recorded using a Perkin Elmer Model 341 automatic polarimeter instrument and 589 nm (sodium D line), in a cell with a path length of 1 dm and are reported as: $[\alpha]_D^{20}$, concentration (*c* in g / 100 mL) and solvent.

High Resolution Mass Spectrometry (HRMS) were recorded by the EPSRC National Mass Spectrometry Service at Swansea on a Thermo Fisher LTQ Orbitrap XL mass spectrometer using the Electrospray Ionisation (ES) technique.

Analytical Thin Layer Chromatography (TLC) used pre-coated (25 µm) Merck Kieselgel 60 $F_{254}$ plates with visualization by ultraviolet (UV) light at 254 nm and/or heating the plate after staining with a solution of 20 % ceric ammonium molybdate w/v in water. Purification by flash column chromatography was conducted on Merck Silica gel 60 (40-63 µm) under a positive pressure of compressed air.

All reagents/solvents were purchased from Sigma Aldrich UK, Alfa Aesar UK, Acros Organics UK or TCI Europe and were used as received, unless otherwise stated. Diethyl ether ($Et_2O$), dichloromethane (DCM) and tetrahydrofuran (THF) were dried by passage through two columns of alumina using a MBRAUN SPS-800 solvent purification system. Methanol and acetonitrile were distilled from calcium hydride in a recycling still under argon. Pyridine was

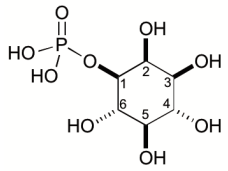
purchased from Fisher Scientific and dried by distillation from 4 Å molecular sieves. Dimethylsulfoxide (DMSO) was dried over calcium hydride, distilled under high vacuum and stored over 4 Å molecular sieves Reactions were performed under anhydrous conditions under a positive pressure of argon. Room temperature (RT) refers to a temperature of approximately 20 °C. The term "brine" refers to a saturated aqueous solution of sodium chloride in deionized water.

**Experimental Procedures**

*IMPase assay for batch reaction conversion*[1]

A sample from the *Tb*INO1 reaction mixture was added to a solution containing Tris-acetate (50 mM, pH 8.0), 1.25 mU of IMPase from bovine brain (Sigma) and $MgCl_2$ (4 mM). The reaction was incubated at 37 °C for 3 hours. The amount of free phosphate was determined by the malachite green method[2] and compared with a standard curve of $KH_2PO_4$ solution.

*Batch reaction conversion of G6P to IP*



**2**

To water (70 µL) were added solutions of ammonium bicarbonate (500 mM, pH 8.5, 15 µL), D-glucose 6-phosphate (**1**, 100 mM, 15 µL), $NAD^+$ (20 mM, 15 µL), DTT (10 mM, 15 µL) and *Tb*INO1 (4.35 µg / µL, 20 µL) in order. The mixture was incubated at 37 °C overnight. The IMPase assay determined the conversion of the reaction as 92 %.

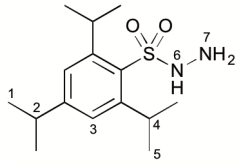
*10 mg Scale*

To D-glucose 6-phosphate (**1**, 10 mg, 38 µmol) in water (458 µL) were added solutions of ammonium bicarbonate (500 mM, pH 8.5, 60 µL), $NAD^+$ (200 mM, 6 µL), DTT (100 mM, 6 µL) and *Tb*INO1 (4.35 µg / µL, 70 µL) in order. The mixture was incubated at 37 °C overnight. The IMPase assay determined the conversion of the reaction as 98 %.

*100 mg Scale*

To D-glucose 6-phosphate (**1**, 100 mg, 384 µmol) in water (1664 µL) were added solutions of ammonium bicarbonate (500 mM, pH 8.5, 240 µL), $NAD^+$ (200 mM, 48 µL), DTT (100 mM, 24 µL) and *Tb*INO1 (4.35 µg / µL, 400 µL) in order. The mixture was incubated for 6 days with shaking at 37 °C in the dark and under an argon atmosphere. The IMPase assay determined the conversion of the reaction as 76 %.

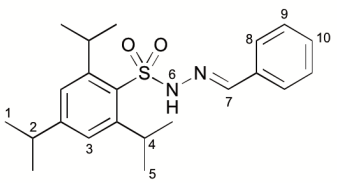
*2,4,6-Triisopropylbenzenesulfonyl hydrazide (***S1***)*[3]




**S1**

To a stirred solution of 2,4,6-triisopropylbenzenesulfonyl chloride (2.00 g, 6.60 mmol) in THF (10 mL) was added hydrazine monohydrate (0.64 mL, 13.21 mmol, 2 equiv.) slowly dropwise at 0 °C. The reaction was stirred for 4 hours at this temperature. The precipitate was dissolved by addition of water (20 mL) and the product was extracted in diethyl ether (2 x 30 mL). The organic phases were combined and washed with water (30 mL) and brine (30 mL) before being dried over magnesium sulfate, filtered and concentrated under reduced pressure to give hydrazide **S1** as a white solid (1.88 g, 6.28 mmol, 95 %). **$^1$H NMR** (300 MHz, $CDCl_3$) δ 7.20 (2H, s, H-3), 5.49 (1H, s, H-6), 4.17 (2H, septet, *J* = 6.8 Hz, H-4), 3.66 (2H, br. s, H-7), 2.92 (1H, septet, *J* = 7.0 Hz, H-2), 1.27 (12H, d, *J* = 6.8 Hz, H-5) and 1.26 (6H, d, *J* = 7.0 Hz, H-1); **$^{13}$C NMR** (75 MHz, $CDCl_3$) δ 154.0, 152.0, 128.7, 124.2, 122.9, 34.4, 30.0, 25.1 and 23.7.

N*'-Benzylidine-2,4,6-triisopropylbenzenesulfonyl hydrazide* (**S2**)[4,5]




**S2**

To a solution of 2,4,6-triisopropylbenzenesulfonyl hydrazide (**S1**, 1.50 g, 5.03 mmol) in methanol (20 mL) was added benzaldehyde (0.51 mL, 5.03 mmol, 1 equiv.). The reaction

was stirred at room temperature for 30 minutes before being stirred at 4 °C for 16 hours. The precipitate was collected and washed with ice-cold methanol before dried *in vacuo*. Recrystallisation in ethanol furnished pure hydrazide **S2** (1.77 g, 4.59 mmol, 91 %). **$^{1}$H NMR** (300 MHz, $CDCl_3$) δ 7.85 (1H, br. s, H-7), 7.60-7.50 (2H, m, H-9), 7.41-7.29 (3H, m, H-8 and H-10), 7.18 (2H, s, H-3), 4.27 (2H, septet, *J* = 6.8 Hz, H-4), 2.89 (1H, septet, *J* = 6.9 Hz, H-2), 1.31 (12H, d, J = 6.8 Hz, H-5) and 1.24 (6H, d, *J* = 6.9 Hz, H-1); **$^{13}$C NMR** (75 MHz, $CDCl_3$) δ 153.5, 151.4, 146.4, 130.3, 128.6, 127.3, 123.9, 34.2, 30.1, 24.9 and 23.5.

*Phenyldiazomethane*[5]

To a stirred suspension of *N*'-benzylidene-2,4,6-triisopropylbenzenesulfonyl hydrazide (**S2**, 1.78 g, 4.61 mmol) in methanol (30 mL) was added potassium hydroxide (0.52 g, 9.22 mmol, 2 equiv.) and the mixture heated to reflux for 30 minutes in apparatus with flame-polished joints. The reaction was allowed to cool to room temperature before ice-cold water (30 mL) was added. The product was extracted into diethyl ether (3 x 30 mL) and subsequently washed with sodium bicarbonate solution (sat., aq., 30 mL), water (30 mL) and brine (30 mL) in glassware with flame-polished joints. The phenyldiazomethane was then used as an ethereal solution in further reactions.

**References**

1. K. L. Martin and T. K. Smith, *Mol. Microbiol.*, 2006, **61**, 89-105.

2. K. Itaya and M. Ui, *Clin. Chim. Acta.*, 1966, **14**, 361-366.

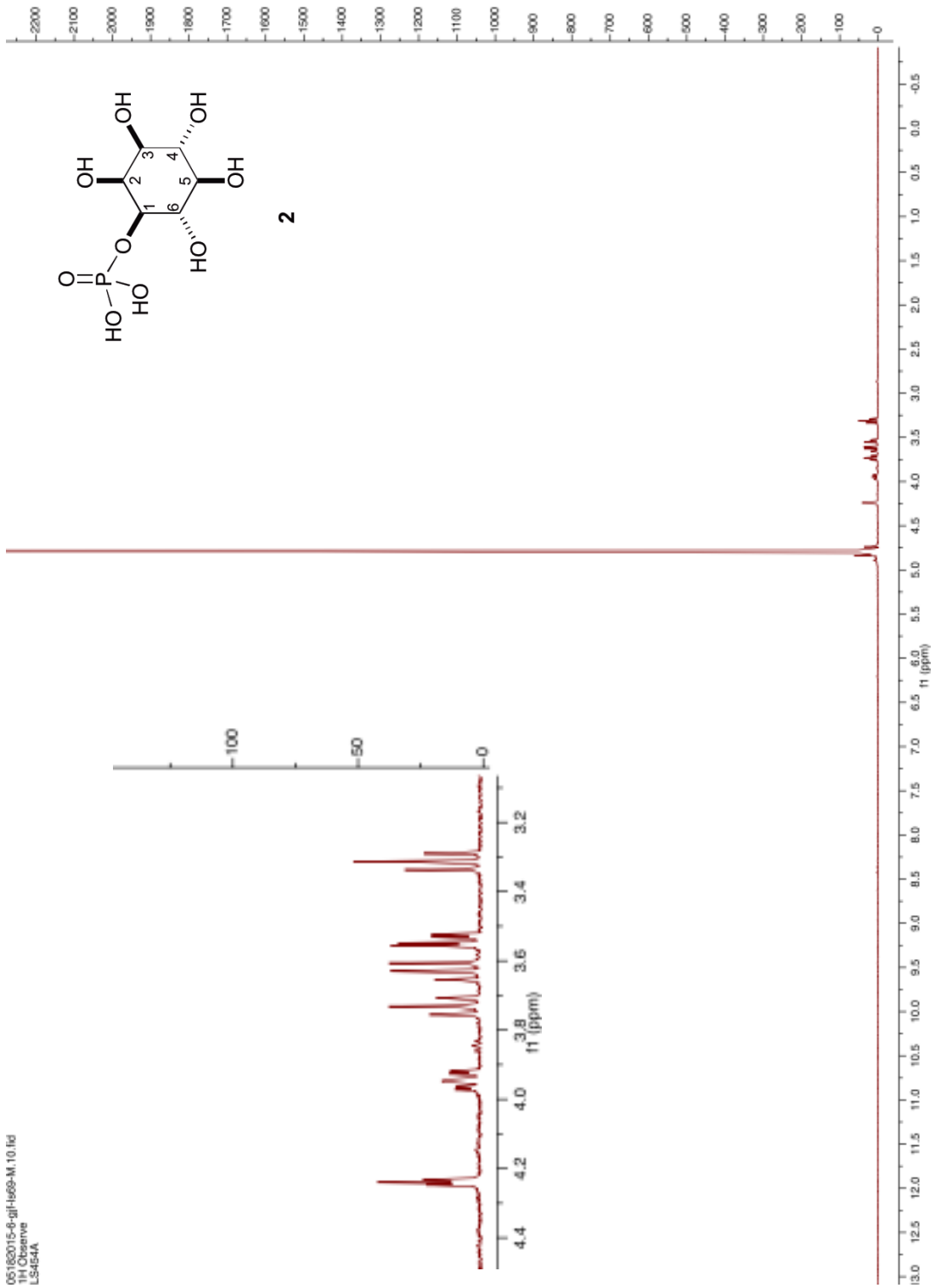
3. P. J. Pedersen, J. Henriksen, C. H. Gotfredsen and M. H. Clausen, *Tetrahedron Lett.*, 2008, **49**, 6220-6223.

4. G. W. Kabalka, J. T. Maddox, E. Bogas and S. W. Kelley, *J. Org. Chem.*, 1997, **62**, 3688-3695.

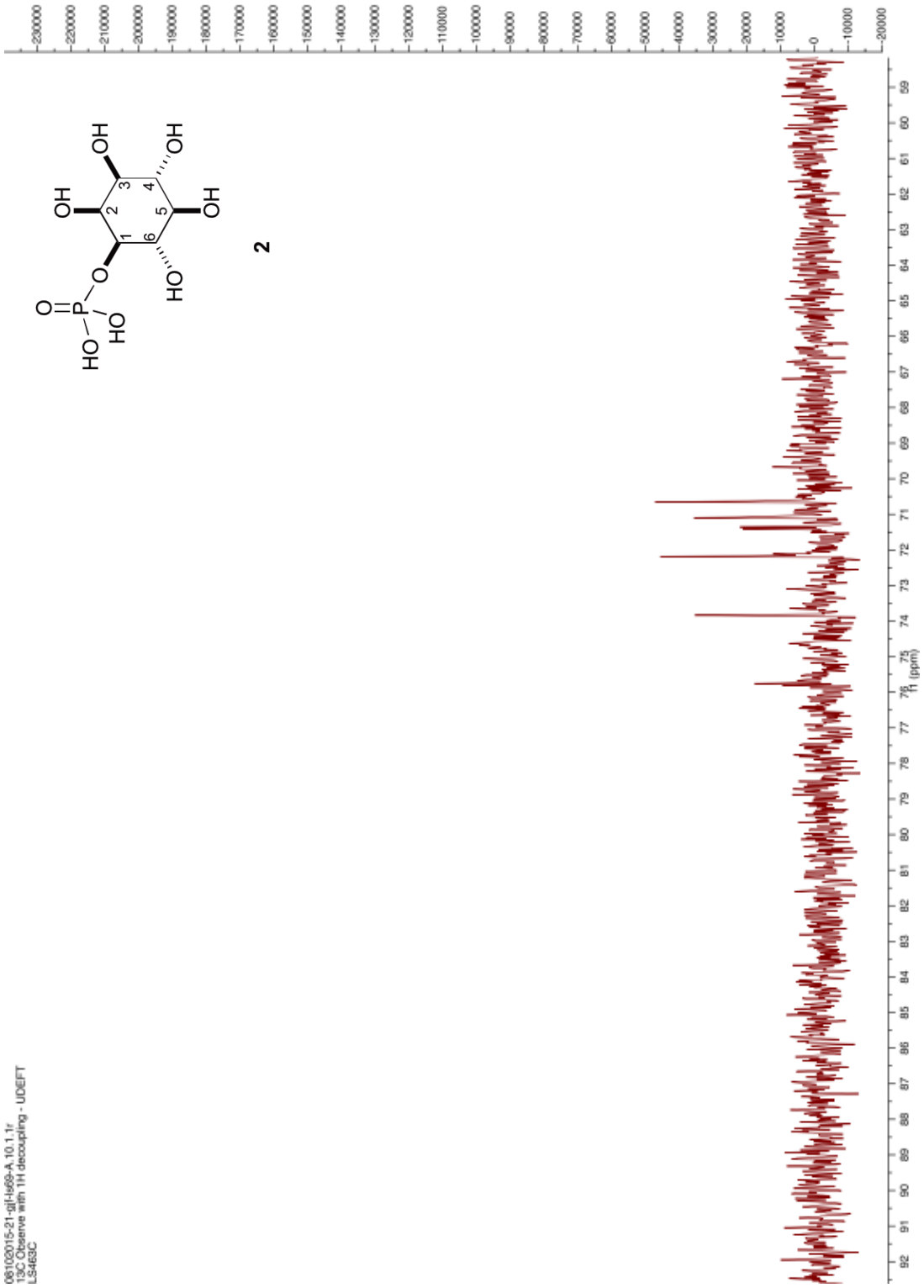
5. C. C. Dudman and C. B. Reese, *Synthesis*, 1982, **1982**, 419-421.

## $^{1}H$ and $^{13}C$ NMR Spectra

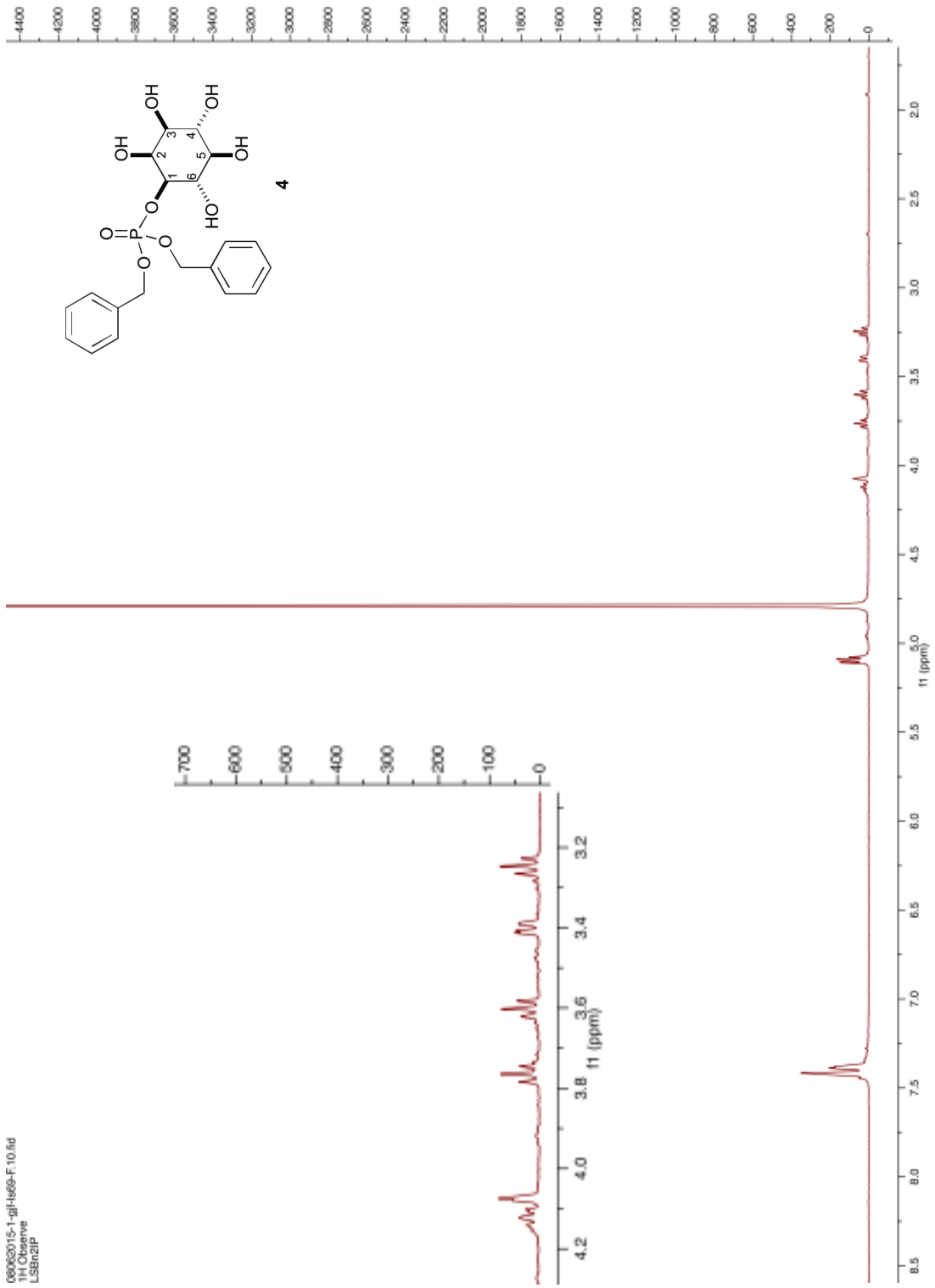
### $^{1}H$ NMR of L-*myo*-Inositol 1-phosphate (2)



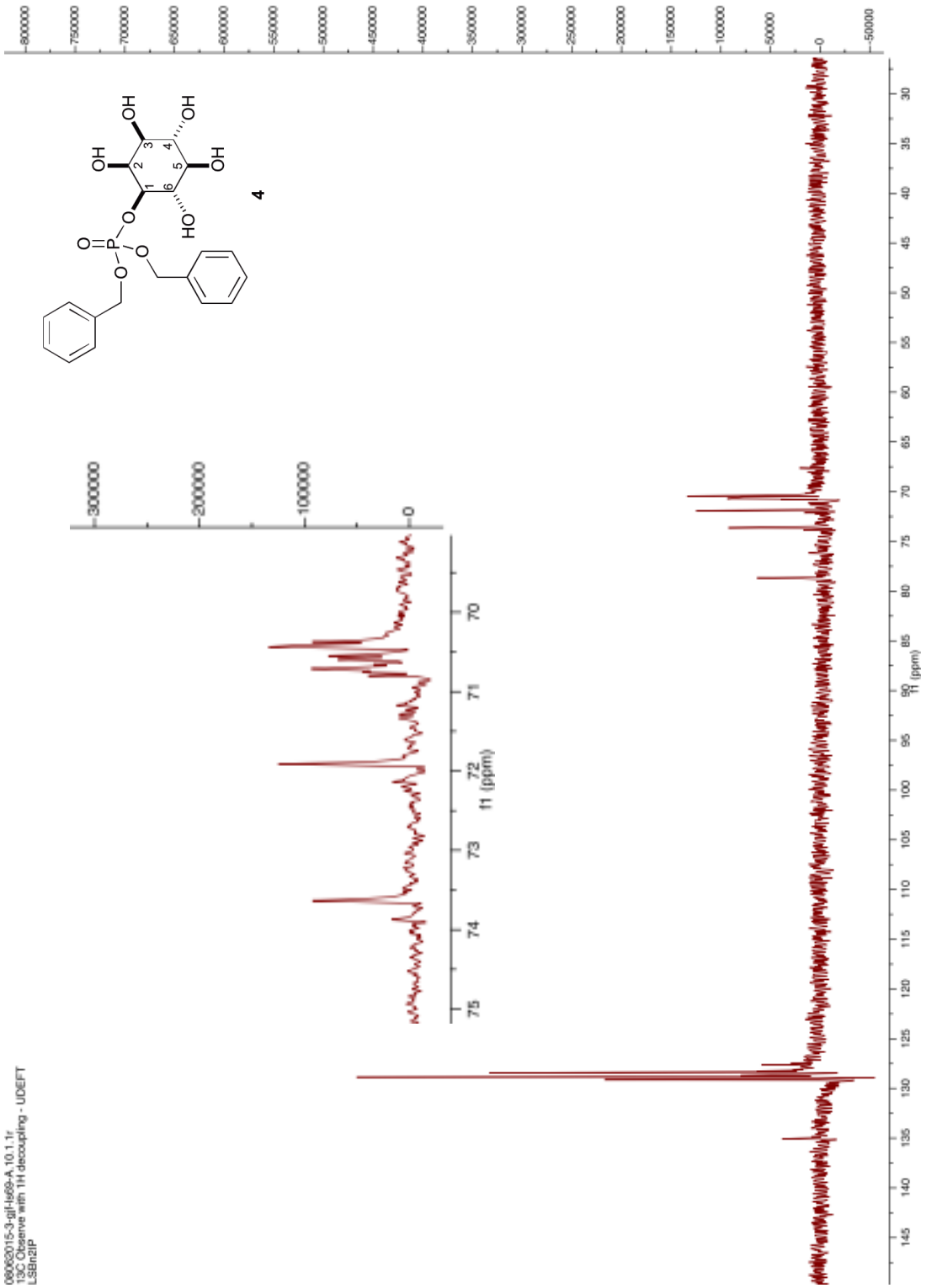
## $^{13}$C NMR of L-*myo*-Inositol 1-phosphate (2)



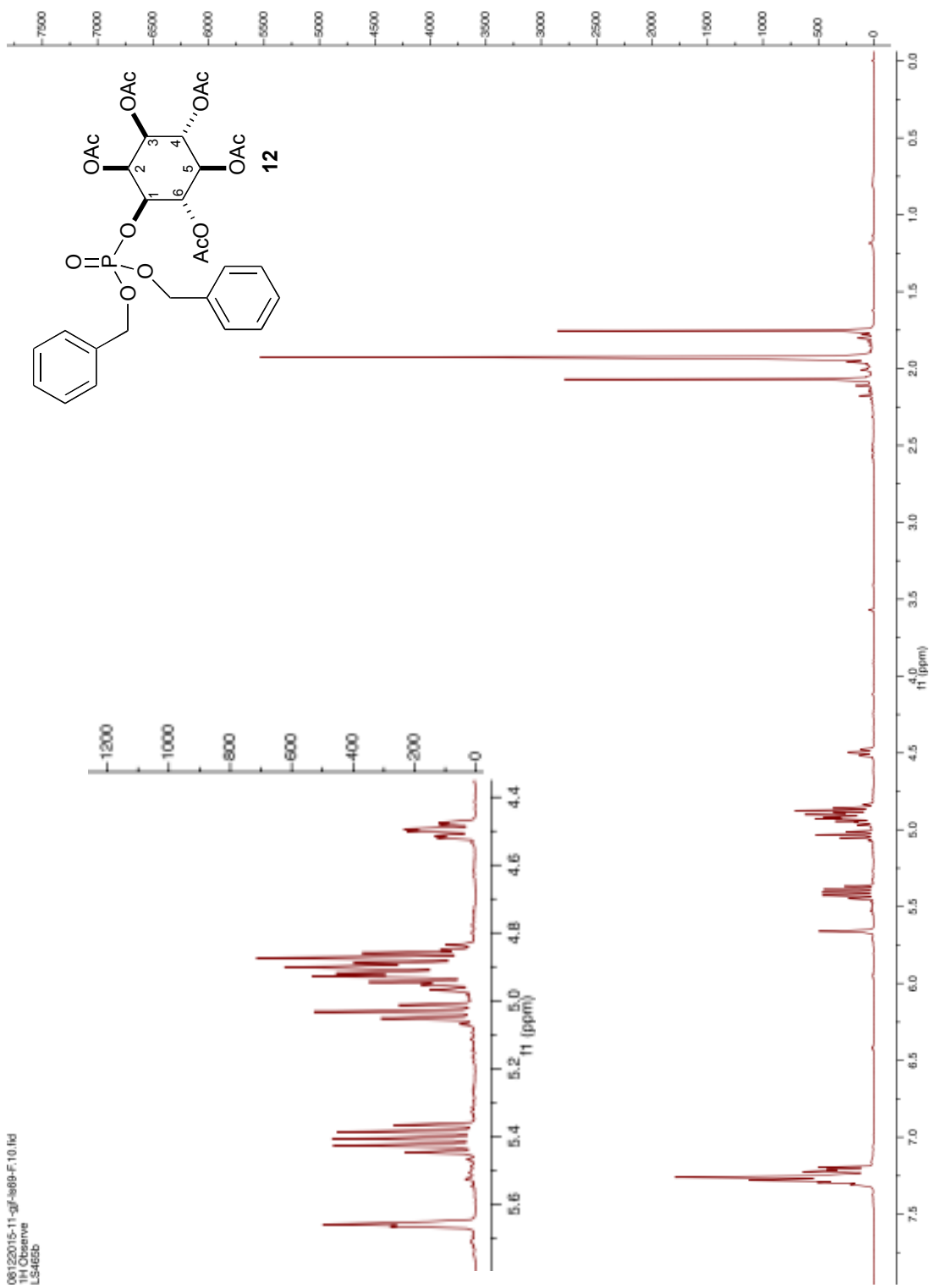
**$^{1}$H NMR of 1-Bis(benzyloxy)phosphoryl L-*myo*-inositol (4)**



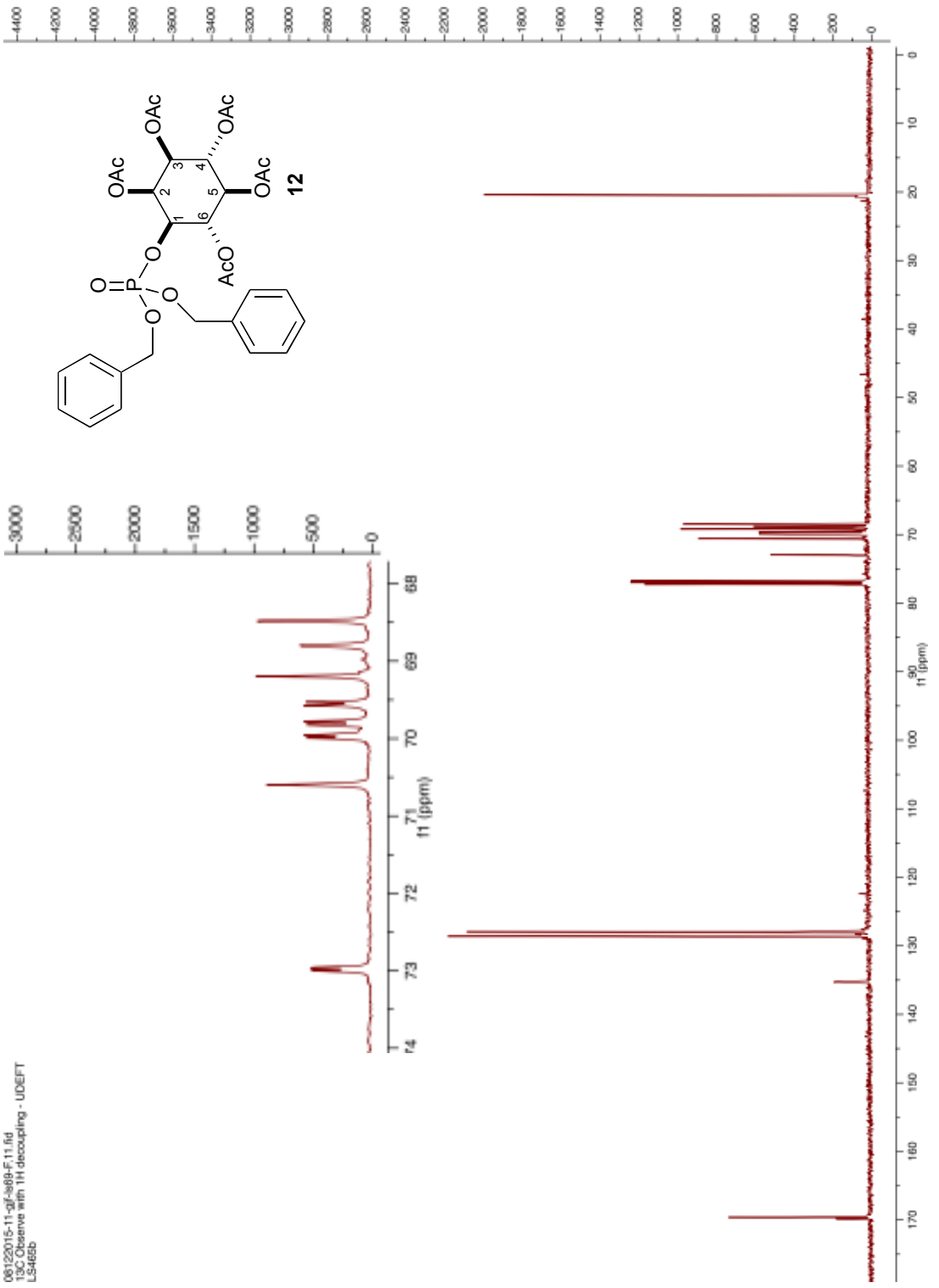
## $^{13}$C NMR of 1-Bis(benzyloxy)phosphoryl L-*myo*-inositol (4)



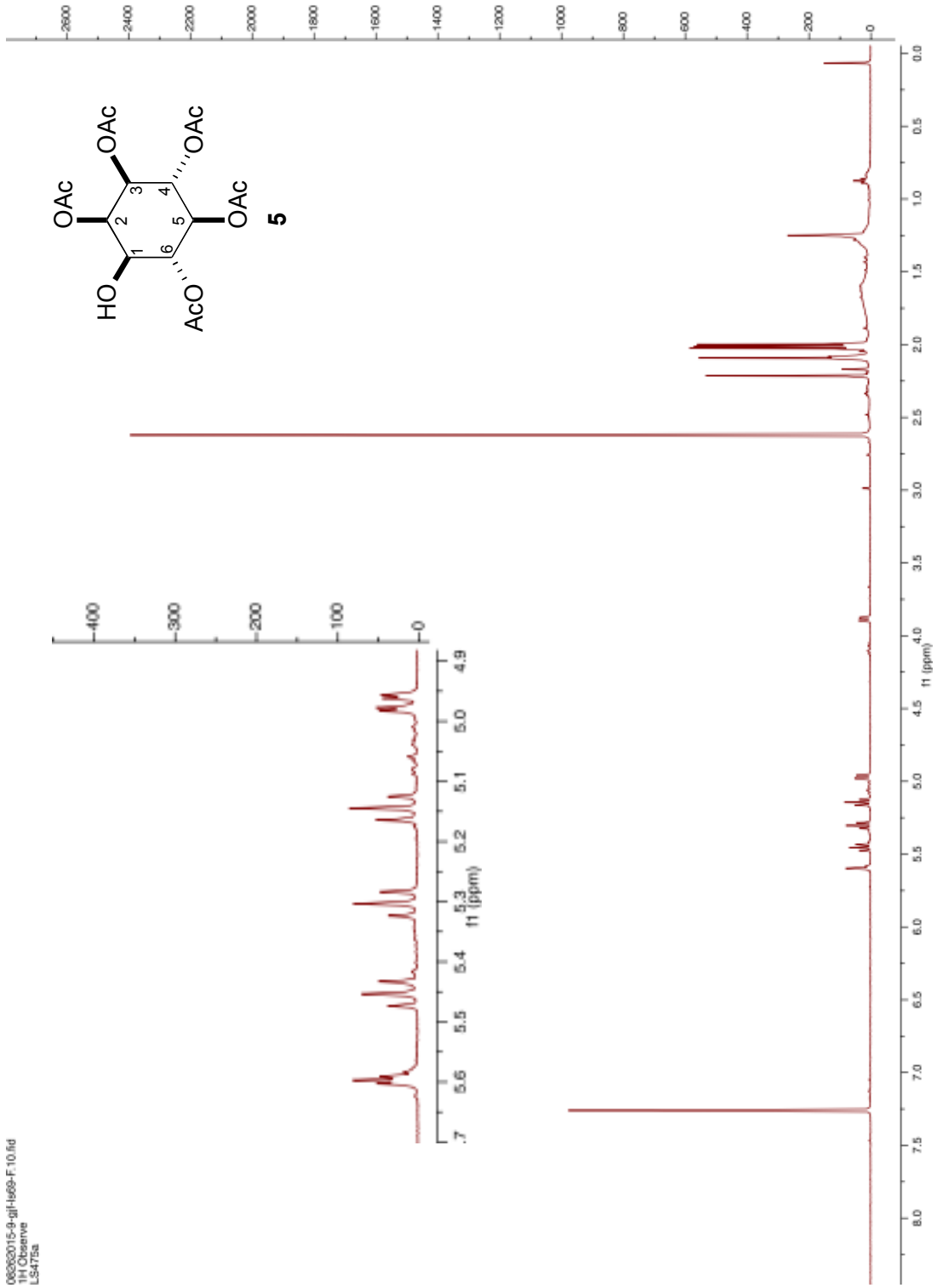
**$^{1}$H NMR of 2,3,4,5,6-Penta-*O*-acetyl 1-bis(benzyloxy)phosphoryl L-*myo*-inositol (12)**



**$^{13}$C NMR of 2,3,4,5,6-Penta-*O*-acetyl 1-bis(benzyloxy)phosphoryl L-*myo*-inositol (12)**



**$^{1}$H NMR of 2,3,4,5,6-penta-*O*-acetyl L-myo-inositol (5)**



**$^{13}$C NMR of 2,3,4,5,6-penta-*O*-acetyl L-myo-inositol (5)**