Development of inositol-based antagonists for the D-*myo*-inositol 1,4,5-trisphosphate receptor

Neil S. Keddie,[a] Yulin Ye,[b] Tashfeen Aslam,[a] Tomas Luyten,[c] Davide Bello,[a] Clive Garnham,[d] Geert Bultynck,[c] Antony Galione[d] and Stuart J. Conway*[b]

[a] School of Chemistry and Centre for Biomolecular Sciences, University of St Andrews, North Haugh, St Andrews, Fife, KY16 9ST, UK.
[b] Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford, OX1 3TA, UK.
[c] Laboratory of Molecular Signalling, Campus Gasthuisberg O/N1, K.U. Leuven, Herestraat 49, Bus 802, B-3000 Leuven, Belgium.
[d] Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, OX1 3QT, UK.

Electronic Supplementary Information

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Additional biological data

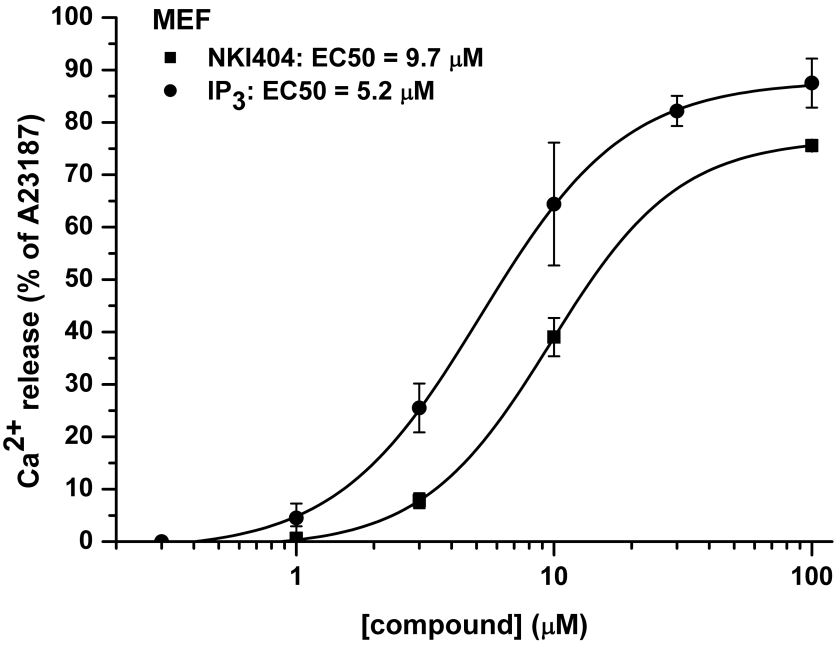


Fig. S1 Dose response curve for activity of the methyl phosphate ester **2** (NKI404) and InsP_3 **1** against the InsP_3Rs in 2MEFwt cells. The curves are fitted using a sigmoidal model in Kalidegraph. EC_{50} values are calculated based on the maximum Ca^{2+} releasable by the calcium ionophore A23187.

It is possible that compound **2** acts as a "prodrug" for InsP_3 and the methyl ester is hydrolysed *in vitro* to give InsP_3. However, simple stability studies (data not shown) indicate that **2** is hydrolytically stable at room temperature over a period of two days. Previous work on the synthesis of membrane permeant InsP_3 derivatives indicates that esters have to be distant from the steric bulk of the inositol ring in order to be removed enzymatically.[1-3]

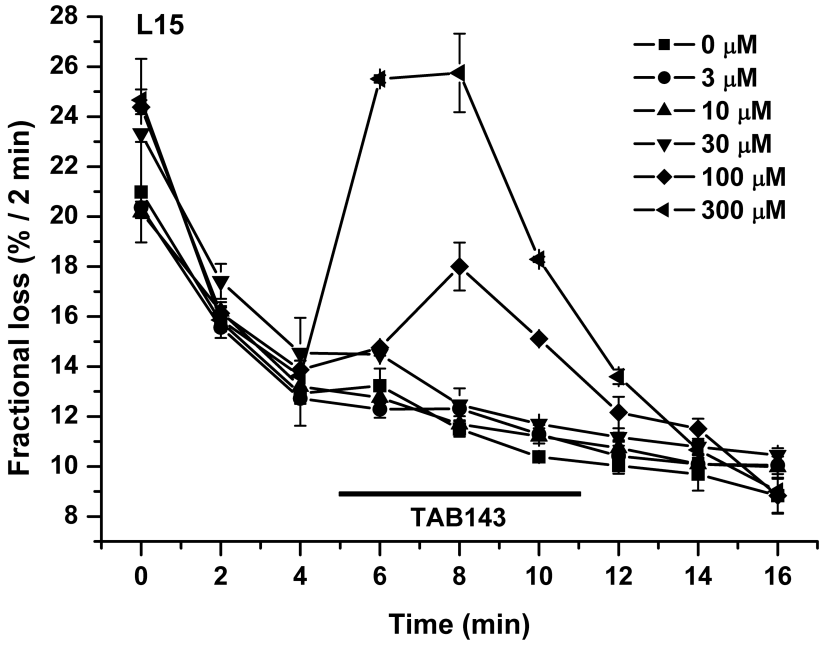


Fig. S2 Fractional loss plots of Ca^{2+} in L15 cell lines when treated with the 5-position sulfate analogue **5** (TAB143). The lower horizontal bar indicates the time at which **5** was administered.

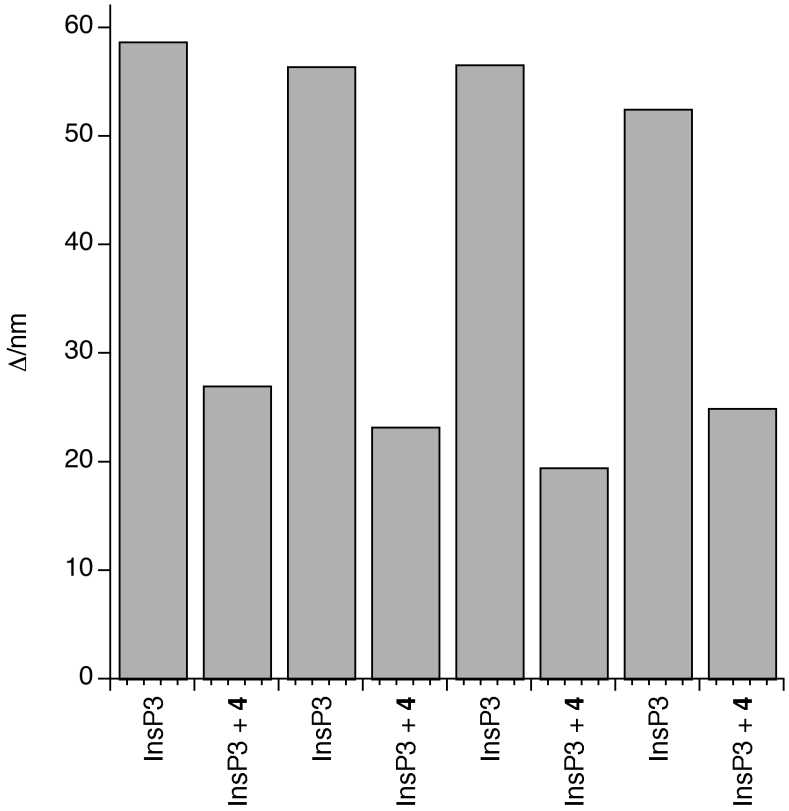


Fig. S3 Sequential experiments conducted using the sea urchin egg homogenate assay. It can be seen that the concentration of Ca^{2+} released upon application of InsP_3 (400 nM) over time is approximately consistent and that application of InsP_3 (400 nm) in the presence of compound **4** (5.00 mM) results in a significant reduction in the concentration of Ca^{2+} released.

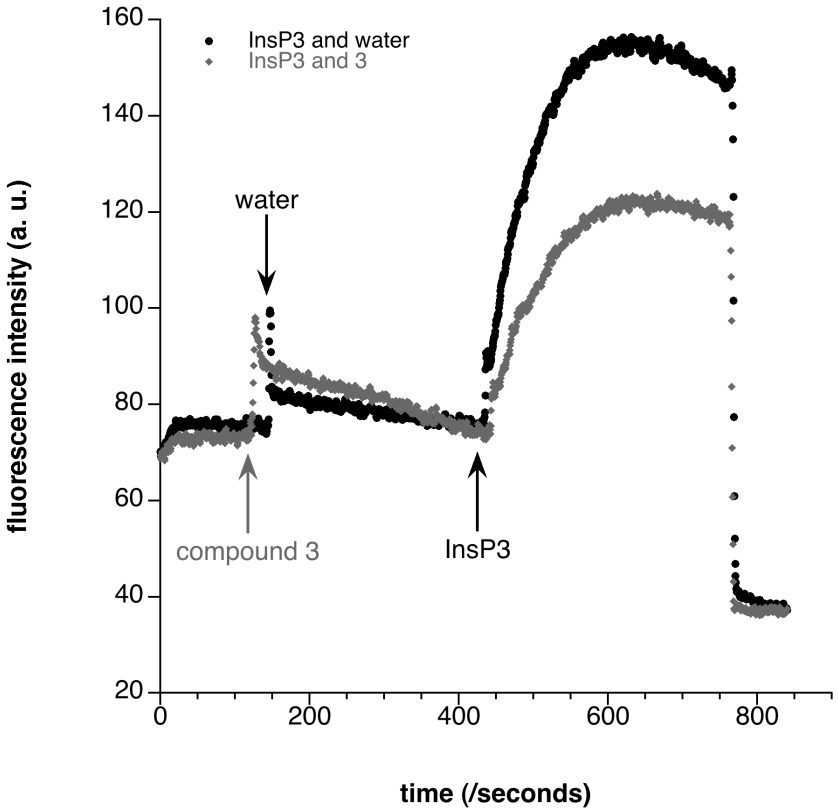


Fig. S4 A representative trace obtained from sea urchin egg homogenate loaded with the fluorescent Ca^{2+} dye Fluo-3. Application of InsP_3 (400 nM) evokes an increase in fluorescence due to Ca^{2+} release from InsP_3Rs (black trace). Application of compound **3** (1.67 mM) alone does not cause Ca^{2+} release, however, application of InsP_3 (400 nM) in the presence of **3** evokes significantly reduced Ca^{2+} release, demonstrating that **3** is an InsP_3R antagonist (grey trace).

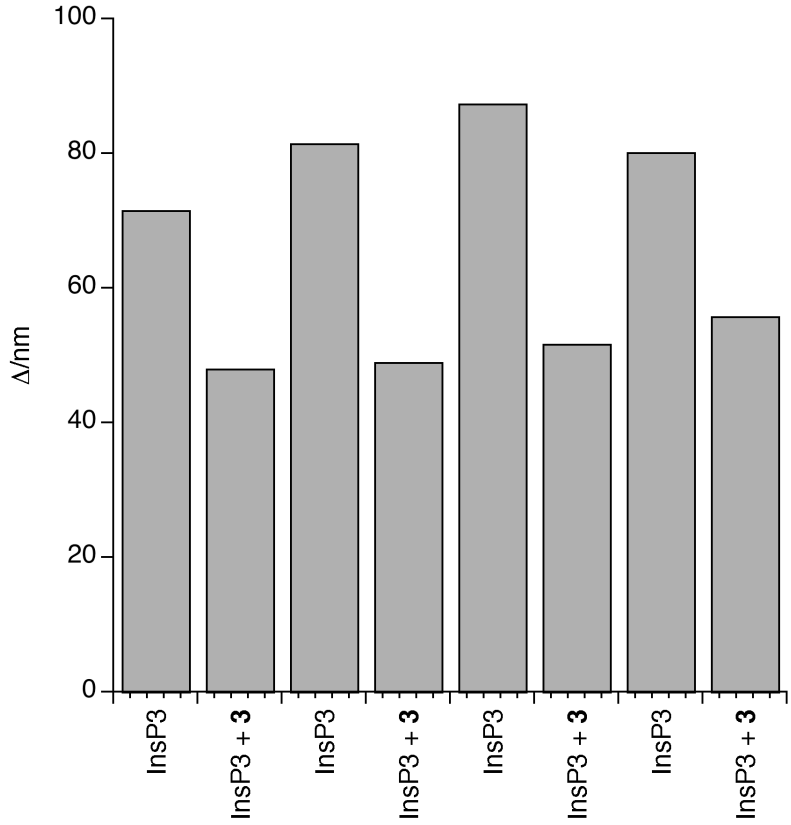


Fig. S5 Sequential experiments conducted using the sea urchin egg homogenate assay. It can be seen that the concentration of Ca^{2+} released upon application of InsP_3 (400 nM) over time is approximately consistent and that application of InsP_3 (400 nm) in the presence of compound **3** (1.67 mM) results in a significant reduction in the concentration of Ca^{2+} released.

General experimental procedures

^{1}H NMR spectra were recorded on Bruker DPX250 (250 MHz); Bruker Avance 300 (300 MHz); Bruker Avance 400 (400 MHz); Bruker Avance II 400 (400 MHz); Bruker DRX500 (500 MHz); Bruker Avance 500 (500 MHz); or Bruker Avance III 500 (500 MHz) using deuterochloroform (unless indicated otherwise) as a reference for internal deuterium lock. The chemical shift data for each signal are given as δ_H in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); q (quartet); dd (doublet of doublets); ddd (doublet of doublet of doublets); dddd (doublet of doublet of doublet of doublets); ddt (doublet of doublet of triplets); sp (septet); or m (multiplet). The number of protons (n) for a given resonance signal is indicated by nH. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. Identical proton coupling constants (*J*) are averaged in each spectrum and reported to the nearest 0.1 Hz. The coupling constants are determined by analysis using Bruker TopSpin software.

^{13}C NMR spectra were recorded on Bruker Avance 300 (75 MHz); Bruker Avance II 400 (100 MHz); or Bruker Avance III 500 (125 MHz) spectrometers using the PENDANT or DEPT Q pulse sequences with broadband proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_C (TMS) = 0.00 ppm. Where appropriate, coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz.

^{1}H and ^{13}C spectra were assigned using 2D NMR experiments including COSY, HSQC, HMBC and DEPT Q.

^{31}P NMR spectra were recorded on Bruker Avance II 400 (162 MHz) or Bruker DRX500 (202 MHz) spectrometers using broadband proton decoupling pulse sequences and deuterium internal lock. The chemical shift data for each signal are given as δ_P in units of parts per million (ppm) relative to 85% phosphoric acid as an external reference.

Mass spectra. Electrospray ionisation spectra were obtained on Micromass LCT; Micromass LCT Premier; and Bruker MicroTOF spectrometers, operating in positive or negative mode, from solutions of methanol, acetonitrile or water. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses.

Melting points were determined from recrystallised samples using an Electrothermal 9100 melting point apparatus or Kofler hot stage microscope and are uncorrected. The solvent(s) from which the sample was crystallised is given in parentheses.

Infrared Specta were obtained either as: **a** thin film on sodium chloride discs; **b** potassium bromide disc; **c** nujol mull as indicated in parentheses. The spectra were recorded on Perkin Elmer GX FT-IR or Bruker Tensor 27 spectrometers. Absorption maxima are reported in wavenumbers (cm^{-1}).

Specific Optical Rotations were measured using Perkin Elmer Model 241 and 341 polarimeters, in cells with a path length of 1 dm. The light source was maintained at 589 nm. The concentration (*c*) is expressed in g/100 mL (equivalent to g/0.1 dm^3). Specific rotations are denoted $[\alpha]_D^T$ and are given in implied units of 10^{-1} deg cm^2 g^{-1} (T = ambient temperature in ˚C).

Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} aluminum-supported thin layer chromatography sheets. Visualisation was by absorption of UV light (λ_{max} 254 or 365 nm), or thermal development after dipping in one of: **a** ethanolic solution of phosphomolybdic acid (PMA); **b** aqueous solution of potassium permanganate, potassium carbonate and sodium hydroxide; **c** ethanolic solution of 4-anisaldehyde, sulfuric acid and acetic acid.

Flash Column chromatography was carried out on Apollo Scientific Ltd silica gel 40-63 micron or Merck silica gel 60 (240-400 mesh), eluting with solvents as supplied, under a positive pressure of compressed air (unless otherwise stated).

Anhydrous solvents were obtained under the following conditions: dry acetonitrile was distilled from calcium hydride in a recycling still; dry *N*,*N*-dimethylformamide was purchased from SigmaAldrich UK in a SureSeal^{TM} bottle and used without further purification or was distilled under reduced pressure from activated 4 Å molecular sieves and stored over 4 Å molecular sieves under an N_2 atmosphere; anhydrous 1,4-dioxane was distilled from sodium and benzophenone in a recycling still and stored over activated 3 Å molecular sieves under an Ar atmosphere. Anhydrous dichloromethane, diethyl ether, toluene, hexane and tetrahydrofuran were obtained using a MBRAUN GmbH MB SPS-800 solvent purification system, where solvent was dried by passage through filter columns and dispensed under an atmosphere of N_2 or Ar gas.

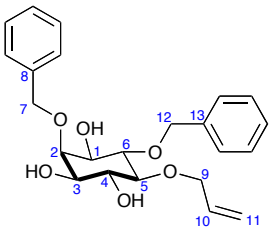
Chemicals were purchased from Acros UK, Sigma Aldrich UK, Alfa Aesar UK, Fisher UK, Fluka UK, Fluorochem, Merck or TCI-Europe. All solvents and reagents were purified, when necessary, by standard techniques.[4] Where appropriate and if not stated otherwise, all non aqueous reactions were performed in a flame-dried flask under an inert atmosphere of nitrogen or argon, using a double vacuum manifold with the inert gas passing through a bed of activated 4 Å molecular sieves and self indicating silica gel.

In vacuo refers to the use of a rotary evaporator attached to a diaphragm pump. Brine refers to a saturated aqueous solution of sodium chloride. Rochelle's Salt refers to a saturated aqueous solution of potassium sodium tartrate tetrahydrate. Hexane refers to a mixture of hexane isomers and petroleum ether to the fraction boiling between 40-60 ˚C.

Millipore Frit refers to a Millipore Millex-GP Filter Unit (model SLGP033RS), which has a 33 mm filter membrane (polyethersulfone) with a pore size of 0.22 µm. The solution is passed through the frit under pressure from a syringe.

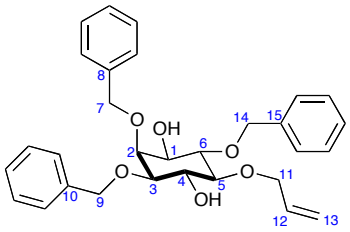
Lypholisation refers to the removal of water by using a Christ Alpha 2-4 LD-2 Freeze Drier attached to a rotary-vane oil pump. The water solution is frozen with liquid nitrogen, then attached to the freeze drier, which removes the water by sublimation under high vacuum (>0.1 mbar).

(-)-D-5-*O*-Allyl-2,6-bis-*O*-benzyl-*myo*-inositol



To a solution of (-)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-3-*O*-*endo*-4-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidene)-*myo*-inositol **7** (2.50 g, 4.68 mmol, 1 eq) in CH_2Cl_2 (20 mL) and methanol (12 mL) was added acetyl chloride (220 mg, 200 µL, 2.81 mmol, 0.6 eq) dropwise. The mixture was stirred for 18 h at RT after which time TLC analysis indicated consumption of the starting material. The reaction was quenched by the careful addition of triethylamine (1 mL) and stirred for 20 min at RT. The solvent was the removed *in vacuo*, affording a yellow oil, which was adsorbed onto silica gel. Purification of the material by silica gel chromatography, eluting with chloroform and methanol (98:2), afforded a colourless solid. Crystallisation of the solid from diethyl ether afforded *(-)-D-5-O-allyl-2,6-bis-O-benzyl-myo-inositol* (1.56 g, 83%) as a microcrystalline solid: R_f 0.20 (chloroform/methanol 98:2); $[\alpha]_D^{20}$ -22.5 (c 1.00 in $CHCl_3$); mp 110-111 °C (from diethyl ether); ν_{max} (KBr disc) cm^{-1} 3388 (s), 3065 (w), 3031 (w), 2922 (m), 1646 (w), 1497 (w), 1454 (m), 1359 (m), 1151 (m), 1131 (m), 1054 (s), 928 (m), 697 (m) and 620 (m); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.41-7.29 (10H, m, Ar *H*), 5.98 (1H, dddd, *J* 17.2, 10.5, 5.8, 5.6, *H*-10), 5.32 (1H, dddd, *J* 17.2, 1.6, 1.5, 1.5, CH_XH_Y *H*-11), 5.20 (1H, dddd, *J* 10.5, 1.6, 1.4, 1.3, CH_XH_Y *H*-11'), 4.93 (1H, d, *J* 11.1, CH_AH_B *H*-12), 4.89 (1H, d, *J* 11.6, $CH_{A'}H_{B'}$ *H*-7), 4.79 (1H, d, *J* 11.6, $CH_{A'}H_{B'}$ *H*-7'), 4.76 (1H, d, *J* 11.1, CH_AH_B *H*-12'), 4.39 (1H, dddd, *J* 12.5, 5.6, 1.5, 1.4, $CH_{A''}H_{B''}$ *H*-9), 4.33 (1H, dddd, *J* 12.5, 5.8, 1.5, 1.3, $CH_{A''}H_{B''}$ *H*-9'), 4.00 (1H, dd, *J* 2.8, 2.7, *H*-2), 3.81 (1H, dd, *J* 9.6, 9.4, *H*-4), 3.74 (1H, dd, *J* 9.6, 9.4, *H*-6), 3.57 (1H, ddd, *J* 9.6, 5.2, 2.7, *H*-1), 3.45 (1H, ddd, *J* 9.6, 6.8, 2.8, *H*-3), 3.22 (1H, dd, *J* 9.4, 9.4, *H*-5), 2.88 (1H, d, *J* 1.4, O*H*-4), 2.62 (1H, d, *J* 6.8, O*H*-3) and 2.45 (1H, d, *J* 5.2, O*H*-1); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 423.1771. $C_{23}H_{28}O_6Na$ requires M^+, 423.1778]; *m/z* (ES^+) 459 ($[M+MeCN+NH_4]^+$, 100%). The appropriate data are in good agreement with the literature values for the racemic compound.[5]

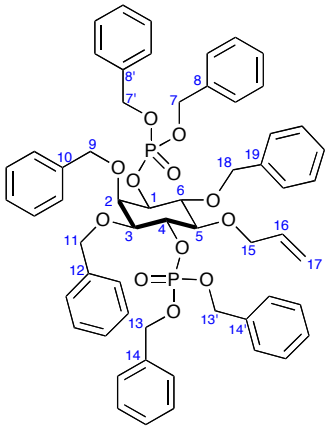
(-)-D-5-*O*-Allyl-2,3,6-tris-*O*-benzyl-*myo*-inositol 8



To a solution of (-)-D-5-*O*-allyl-2,6-*O*-bis-benzyl-*myo*-inositol (1.40 g, 3.50 mmol, 1 eq) in acetonitrile (50 mL) was added di-*n*-butyl tin oxide (0.96 g, 3.85 mmol, 1.1 eq), TBABr (1.13 g, 3.50 mmol, 1 eq) and benzyl bromide (1.79 g, 1.25 mL, 10.49 mmol, 3 eq). The mixture was heated under reflux for 20 h, with azeotropic removal of water *via* a Soxhlet apparatus filled with activated 3 Å molecular sieves. After this time TLC analysis indicated consumption of the starting material. The solvent was concentrated *in vacuo*, affording a thick yellow oil, which was partitioned between water (60 mL) and ethyl acetate (60 mL). The layers were separated and the aqueous phase was re-extracted with ethyl acetate (2 × 60 mL). The combined organic phases were treated with saturated aqueous sodium bicarbonate solution (60 mL), precipitating the residual

tin salts as a fine colourless suspension. The mixture was filtered through a pad of Celite and the layers were separated again. The organic phase was washed with brine (60 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* providing a light brown oil. Purification of the oil by silica gel chromatography, eluting with petroleum ether and ethyl acetate (85:15, 80:20), afforded *(-)-D-5-O-allyl-2,3,6-tris-O-benzyl-myo-inositol* **8** (1.35 g, 79%) as a colourless oil: R_f 0.59 (petroleum ether/ethyl acetate 50:50), $[\alpha]_D^{24}$ -3.5 (*c* 1.15 in $CHCl_3$); ν_{max} (KBr disc) /cm^{-1} 3424 (s), 3088 (w), 3030 (m), 2891 (m), 1644 (w), 1496 (m), 1454 (s), 1360 (s), 1244 (m), 1211 (s), 1152 (s), 1067 (s), 1025 (m), 930 (w), 815 (s), 701 (s) and 606 (w); ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.40-7.28 (15H, m, Ar *H*), 5.99 (1H, dddd, *J* 17.2, 10.5, 5.8, 5.7, C*H*-12), 5.31 (1H, dddd, *J* 17.2, 1.5, 1.5, 1.4, CH_XH_Y *H*-13), 5.18 (1H, dddd, *J* 10.5, 1.5, 1.4, 1.3, CH_XH_Y *H*-13'), 4.93 (1H, d, *J* 11.6, CH_AH_B *H*-7), 4.91 (1H, d, *J* 11.1, $CH_{A'}H_{B'}$ *H*-14), 4.77 (1H, d, *J* 11.1, $CH_{A'}H_{B'}$ *H*-14'), 4.72 (1H, d, *J* 11.6, CH_AH_B *H*-7'), 4.70 (1H, d, *J* 11.7, $CH_{A''}H_{B''}$ *H*-9), 4.61 (1H, d, *J* 11.7, $CH_{A''}H_{B''}$ *H*-9'), 4.39 (1H, dddd, *J* 12.5, 5.8, 1.5, 1.4, $CH_{A'''}H_{B'''}$ *H*-11), 4.35 (1H, dddd, *J* 12.5, 5.7, 1.4, 1.3, $CH_{A'''}H_{B''}$ *H*-11'), 4.11 (1H, ddd, *J* 9.7, 9.4, 1.7, *H*-4), 4.07 (1H, dd, *J* 2.7, 2.5, *H*-2), 3.75 (1H, dd, *J* 9.5, 9.3, *H*-6), 3.49 (1H, ddd, *J* 9.5, 6.3, 2.7, *H*-1), 3.28 (1H, dd, *J* 9.7, 2.5, *H*-3), 3.27 (1H, dd, *J* 9.4, 9.3, *H*-5), 2.56 (1H, d, *J* 1.7, O*H*-4) and 2.29 (1H, d, *J* 6.3, O*H*-1); ^{13}C NMR (125 MHz; $CDCl_3$) δ 138.6 (*C*-15 Ph), 138.5 (*C*-8 Ph), 137.8 (*C*-10 Ph), 135.2 (*C*H *C*-12), 128.6, 128.5, 128.3, 128.1, 127.9, 127.8, 127.77, 127.75, 127.6, 116.9 (CH_2 *C*-13), 82.9 (*C*H-5), 81.8 (*C*H-6), 80.3 (*C*H-3), 76.1 (*C*H-2), 75.5 (CH_2 *C*-14), 74.7 (CH_2 *C*-7), 74.1 (CH_2 *C*-11), 73.0 (*C*H-4), 72.5 (CH_2 *C*-9) and 72.49 (*C*H-1); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 513.2244. $C_{30}H_{34}O_6Na$ requires M^+, 513.2248]; *m/z* (ES^+) 549 ($[M+MeCN+NH_4]^+$, 100%); Anal. Calcd. For $C_{30}H_{34}O_6$: C, 73.45; H, 6.99. Found: C, 73.39; H, 7.05.

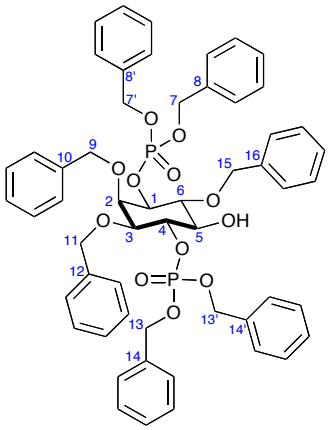
(-)-D-5-*O*-Allyl-2,3,6-tris-*O*-benzyl-*myo*-inositol 1,4-O-bis(dibenzyl phosphate)



To a solution of bis(benzyloxy)-*N,N*-diisopropylamino phosphine (2.35 g, 6.81 mmol, 2.8 eq) in CH_2Cl_2 (50 mL) was added a solution of 1*H*-tetrazole in acetonitrile (0.43 M, 15.1 mL, 6.81 mmol, 2.8 eq), turning the mixture cloudy after 15 min stirring. A solution of (-)-D-5-*O*-allyl-2,3,6-*O*-tris-benzyl-*myo*-inositol **8** (1.20 g, 2.45 mmol, 1 eq) in CH_2Cl_2 (50 mL) was added dropwise over 30 min *via* a pressure equalised dropping funnel. The resulting solution stirred for 16 h at RT, forming a colourless precipitate. TLC analysis of the mixture showed consumption of the starting material. The mixture was cooled to -78 ˚C and 3-chloroperoxybenzoic acid (1.18 g, 6.81 mmol, 2.8 eq) was added in a single portion. The mixture stirred for 20 min at -78 ˚C then was allowed to warm to RT over 90 min, after which TLC analysis indicated consumption of the intermediate (presumed P(III) species). The solution was diluted with CH_2Cl_2 (100 mL) and the reaction was quenched by addition of aqueous sodium sulfite solution (10% *w/v*, 100 mL). The

layers were separated and the aqueous phase was re-extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with aqueous saturated sodium bicarbonate solution (100 mL) and brine (100 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* affording a colourless oil, which solidified on standing. Purification of the solid, eluting with petroleum ether and ethyl acetate (70:30, 50:50), furnished a colourless solid. Crystallisation of the solid from diethyl ether, afforded *(-)-D-5-O-allyl-2,3,6-tris-O-benzyl-myo-inositol 1,4-O-bis(dibenzyl phosphate)* (2.00 g, 81%) as colourless needles: R_f 0.42 (petroleum ether/ethyl acetate 50:50); $[\alpha]_D^{24}$ -0.9 (*c* 0.86 in $CHCl_3$); mp 90-91 °C (from diethyl ether); ν_{max} (KBr disc) /cm^{-1} 3032 (w), 2930 (w), 1498 (m) 1455 (m), 1357 (m), 1264 (s), 1215 (w), 1024 (s), 999 (s), 966 (m), 745 (s), 696 (s), 603 (w) and 500 (w); ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.35-7.15 (35H, m, Ar *H*), 5.85 (1H, dddd, *J* 17.2, 10.6, 5.7, 5.6, C*H*-16), 5.15 (1H, dddd, *J* 17.2, 1.6, 1.6, 1.5, CH_XH$_Y$ *H*-17), 5.03 (1H, dddd, *J* 10.6, 1.5, 1.5, 1.4, CH$_X$*H*$_Y$ *H*-17'), 5.00-4.84 (9H, m, *H*-4 and 4 × CH$_A$H$_B$Ph), 4.73 (1H, d, *J* 11.4, C*H*$_A$H$_B$ *H*-9), 4.81 (1H, d, *J* 10.7, C*H*$_{A'}$H$_{B'}$ *H*-18), 4.74 (1H, d, *J* 10.7, CH$_{A'}$*H*$_{B'}$ *H*-18'), 4.67 (1H, d, *J* 11.4, CH$_A$*H*$_B$ *H*-9'), 4.52 (2H, s, C*H*$_2$ *H*-11,11'), 4.33-4.25 (3H, m, *H*-2 and C*H*$_2$ *H*-15,15'), 4.22 (1H, ddd, *J* 9.8, 7.7, 2.5, *H*-1), 4.03 (1H, dd, *J* 9.8, 8.5, *H*-6) and 3.39-3.34 (2H, m, *H*-3 and *H*-5); ^{13}C NMR (125 MHz; $CDCl_3$) δ 138.3 (*C*-10 Ph), 138.2 (*C*-19 Ph), 137.4 (*C*-12 Ph), 136.2 (d, *J* 7.5), 136.18 (d, *J* 7.4), 135.7 (d, *J* 7.6), 135.65 (d, *J* 7.8), 134.8 (*C*H-16), 128.54, 28.5, 128.49, 128.4, 138.34, 138.32, 138.31, 128.2, 128.17, 128.1, 128.05, 127.8, 127.78, 127.72, 127.7, 127.66, 127.6, 127.54, 127.5, 127.4, 116.8 (*C*H$_2$ *C*-17), 80.9 (*C*H-5), 79.7 (d, *J* 7.2, *C*H-6), 79.4 (d, *J* 7.3, *C*H-4), 78.5 (d, *J* 2.6, *C*H-3), 78.1 (d, *J* 6.3, *C*H-1), 75.5 (*C*H$_2$ *C*-18), 75.4 (*C*H$_2$ *C*-2), 75.1 (*C*H$_2$ *C*-9), 74.4 (*C*H$_2$ *C*-15), 72.3 (*C*H$_2$ *C*-11), 69.4 (d, *J* 5.5, *C*H$_2$Ph), 69.24 (d, *J* 5.7, *C*H$_2$Ph), 69.2 (d, *J* 5.6, *C*H$_2$Ph) and 69.0 (d, *J* 5.1, *C*H$_2$Ph); ^{31}P NMR (202 MHz; $CDCl_3$) δ -0.28 and -0.36; isotope distribution *m/z* (ES$^+$) [Found: (M+Na)$^+$ 1033.3271 (100), 1034.3310 (64), 1035.3340 (20), 1036.3353 (5). $C_{58}H_{60}O_{12}P_2Na$ requires M$^+$, 1033.3452 (100), 1034.3486 (64), 1035.3519 (23), 1036.3553 (6)]; *m/z* (ES$^+$) 1070 ([M+MeCN+NH$_4$]$^+$, 100%); Anal. Calcd. For $C_{58}H_{60}O_{12}P_2$: C, 68.90; H, 5.98. Found: C, 68.99; H, 5.93.

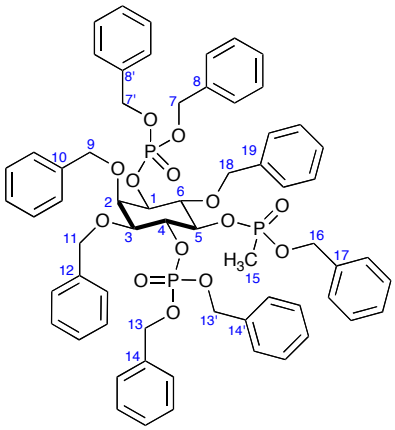
(+)-D-2,3,6-Tris-*O*-benzyl-*myo*-inositol 1,4-*O*-bis(dibenzyl phosphate) 9



Six reactions were performed in parallel (total scale 1.20 g), then combined after workup for purification. To a stirred solution of (-)-D-5-*O*-allyl-2,3,6-tris-*O*-benzyl-*myo*-inositol 1,4-O-bis(dibenzyl phosphate) (200 mg, 0.20 mmol, 1 eq) in methanol (8 mL) was added palladium dichloride (44 mg, 0.25 mmol, 1.25 eq) in a single portion. The heterogeneous mixture was vigorously stirred for 2.5 h at RT, after which time TLC analysis indicated consumption of the starting material. The solvent was removed *in vacuo* and the resulting dark residue was partitioned between CH_2Cl_2 (25 mL) and hydrogen peroxide solution (15% *v/v*, 25 mL), stirring for 15 min until effervescence subsided. The layers were separated and the aqueous phase was re-

extracted with CH_2Cl_2 (2 × 30 mL). The organic phases from all six experiments were combined, washed with water (100 mL) and brine (100 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*, affording a pale yellow oil that solidified to a waxy solid on standing. Purification of the solid by silica gel chromatography, eluting with petroleum ether and ethyl acetate (50:50, 0:100), afforded a colourless solid. Crystallisation of the solid from diethyl ether furnished *(-)-D-2,3,6-tris-O-benzyl-myo-inositol 1,4-O-bis(dibenzyl phosphate)* **9** (970 mg, 84%) as a microcrystalline colourless solid: R_f 0.30 (petroleum ether/ethyl acetate 50:50); $[\alpha]_D^{24}$ +7.2 (*c* 1.10 in $CHCl_3$); mp 103-104 °C (from diethyl ether); ν_{max} (KBr disc) /cm^{-1} 3362 (m), 3032 (m), 2889 (m), 1497 (m), 1456 (s), 1382 (m), 1266 (s), 1217 (m), 1011 (s), 886 (m), 734 (s), 696 (s), 622 (w); ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.39-7.35 (2H, m, Ar *H*), 7.34-7.21 (29H, m, Ar *H*), 7.20-7.17 (4H, m, Ar *H*), 5.05-5.00 (3H, m, 1.5 × CH_AH_BPh), 5.00-4.90 (6H, m, CH_AH_B *H*-15 and 2.5 × CH_AH_BPh), 4.81 (1H, d, *J* 11.6, $CH_{A'}H_{B'}$ *H*-9), 4.77-4.69 (3H, m, *H*-4, CH_AH_B *H*-15' and $CH_{A'}H_{B'}$ *H*-9'), 4.51 (1H, d, *J* 11.5, $CH_{A''}H_{B''}$ *H*-11), 4.44 (1H, d, *J* 11.5, $CH_{A''}H_{B''}$ *H*-11'), 4.34 (1H, dd, *J* 2.5, 2.5, *H*-2), 4.26 (1H, d, *J* 2.5, ex. in D_2O, O*H*-5), 4.23 (1H, ddd, *J* 9.8, 7.8, 2.5, *H*-1), 3.98 (1H, dd, *J* 9.8, 9.1, *H*-6), 3.71 (1H, ddd, *J* 9.1, 8.9, 2.5, *H*-5) and 3.38 (1H, dd, *J* 9.7, 2.5, *H*-3); ^{13}C NMR (125 MHz; $CDCl_3$) δ 138.5 (*C*-16 Ph), 138.3 (*C*-10 Ph), 137.4 (*C*-12 Ph), 135.7 (d, *J* 7.5), 135.66 (d, *J* 7.3), 135.6 (d, *J* 7.4), 135.56 (d, *J* 7.7), 128.53, 128.5, 128.49, 128.33, 128.3, 128.22, 128.2, 128.0, 127.82, 127.8, 127.72, 127.7, 127.6, 127.5, 127.47, 81.0 (d, *J* 5.9, *C*H-4), 79.6 (d, *J* 7.4, *C*H-6), 78.0 (d, *J* 6.2, *C*H-3), 77.7 (d, *J* 5.9, *C*H-1), 75.7 (*C*H-2), 75.4 (CH_2 *C*-15), 75.1, (CH_2 *C*-9), 74.8 (*C*H-5), 72.5 (CH_2 *C*-11), 69.73 (d, *J* 5.8, CH_2Ph), 69.7 (d, *J* 5.6, CH_2Ph), 69.4 (d, *J* 5.6, CH_2Ph) and 69.3 (d, *J* 5.5, CH_2Ph); ^{31}P NMR (202 MHz; $CDCl_3$) δ 1.61 (*P*-4) and -0.46 (*P*-1); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 993.3162. $C_{55}H_{56}O_{12}P_2$ requires M^+, 993.3139]; *m/z* (ES^+) 1029 ($[M+MeCN+NH_4]^+$, 100%); Anal. Calcd. For $C_{55}H_{56}O_{12}P_2$: C, 68.03; H, 5.81. Found: C, 68.13; H, 5.73.

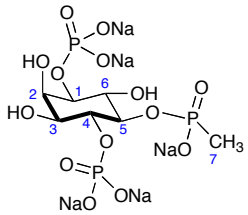
(-)-D-2,3,6-Tris-*O*-benzyl-5-(benzyl methylphosphonate)-*myo*-inositol 1,4-O-bis(dibenzyl phosphate)



To a stirred solution of (+)-D-2,3,6-tris-*O*-benzyl-*myo*-inositol 1,4-*O*-bis(dibenzyl phosphate) **9** (180 mg, 0.19 mmol, 1 eq) in pyridine (600 µL) was added activated 3 Å molecular sieves. A solution of bis(1-[6-trifluoromethyl]benzotriazolyl) methylphosphonate in dioxane (0.2 M, 1.85 mL, 0.37 mmol, 2 eq) was added and the resulting mixture was stirred for 10 min at RT. Anhydrous benzyl alcohol (80 mg, 77 µL, 0.74 mmol, 4 eq) was added dropwise and the mixture stirred for a further 90 min, after which time TLC analysis indicated consumption of the starting material. The reaction was quenched by the addition of triethylammonium bicarbonte buffer (1 M, 10 mL, pH 8.5) and CH_2Cl_2 (10 mL). The layers were separated and the aqueous layer was re-extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with triethylammonium bicarbonate buffer (10 mL), water (10 mL) and brine (10 mL), dried over

magnesium sulfate, filtered and concentrated *in vacuo*, affording a colourless oil. Purification of the oil by silica gel chromatography, eluting with petroleum ether and ethyl acetate (40:60, 30:70, 20:80, 0:100), afforded *(-)-D-2,3,6-tris-O-benzyl-5-(benzyl methylphosphonate)-myo-inositol 1,4-O-bis(dibenzyl phosphate)* (78 mg, 36%) as a colourless oil and 2:1 mixture of diastereoisomers (ds1:ds2, adjudged by ^{1}H NMR): R_f 0.30 (petroleum ether/ethyl acetate 30:70); $[\alpha]_D^{25}$ -4.9 (*c* 0.48 in $CHCl_3$); ν_{max} (KBr disc) /cm^{-1} 3449 (w), 3033 (w), 2925 (w), 2361 (w), 2341 (w), 1636 (w), 1498 (m), 1456 (m), 1378 (m), 1270 (s), 1015 (s), 919 (m), 736 (s) and 697 (s); ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.43-6.99 (40H, m, Ar *H*), 5.11-4.80 (12H, m, 6 × CH_AH_B), 4.77 (1H, d, *J* 11.2, CH_AH_B *H*-18'), 4.69 (1H, d, *J* 11.4, $CH_{A'}H_{B'}$ *H*-9' diastereoisomer 2), 4.69 (1H, d, *J* 11.4, $CH_{A'}H_{B'}$ *H*-9' diastereoisomer 1), 4.60-4.51 (2H, m, *H*-5 and $CH_{A''}H_{B''}$ *H*-11), 4.48 (1H, d, *J* 11.6, $CH_{A''}H_{B''}$ *H*-11' diastereoisomer 1), 4.47 (1H, d, *J* 11.6, $CH_{A''}H_{B''}$ *H*-11' diastereoisomer 2), 4.40 (1H, ddd, *J* 9.6, 9.6, 9.5, diastereoisomer 2, *H*-5), 4.37-4.34 (1H, m, *H*-2), 4.30 (1H, ddd, *J* 9.8, 7.5, 2.5, *H*-1 diastereoisomer 1), 4.26 (1H, ddd, *J* 9.8, 7.5, 2.5, *H*-1 diastereoisomer 2), 4.08 (1H, dd, *J* 9.8, 9.6, *H*-6), 3.44 (1H, dd, *J* 9.9, 2.2, *H*-3 diastereoisomer 1), 3.43 (1H, dd, *J* 9.8, 2.3, *H*-3 diastereoisomer 2), 1.49 (3H, d, *J* 18.0, CH_3 *H*-15 diastereoisomer 1) and 1.33 (3H, d, *J* 18.0, CH_3 *H*-15 diastereoisomer 2); ^{13}C NMR (125 MHz; $CDCl_3$) δ 138.3 (*C*-10 Ph), 138.1 (*C*-19 Ph diastereoisomer 1), 137.7 (*C*-19 Ph diastereoisomer 2), 137.3 (*C*-12 Ph), 136.7 (d, *J* 6.8, diastereoisomer 2), 136.6 (d, *J* 6.4, diastereoisomer 1), 136.1 (d, *J* 7.8), 136.0 (d, *J* 7.5), 135.53 (d, *J* 7.2), 135.5 (d, *J* 7.2), 135.46 (d, *J* 6.7), 128.6, 128.54, 128.5, 128.4, 128.3, 128.24, 128.2, 128.12, 128.1, 128.0, 127.93, 127.9, 127.84, 127.8, 127.76, 127.74, 127.7, 127.62, 127.6, 127.55, 127.5, 127.4, 127.37, 127.2, 127.1, 78.0 (*C*H-4 diastereoisomer 2), 77.97 (*C*H-4 major), 77.84 (*C*H-3), 77.8 (*C*H-6), 77.74 (d, *J* 6.7, *C*H-1), 76.5 (*C*H-5 diastereoisomer 2), 75.8 (*C*H-5 diastereoisomer 1), 75.4 (*C*H-2 diastereoisomer 1), 75.3 (CH_2 *C*-9), 75.2 (*C*H-2 diastereoisomer 2), 74.6 (CH_2 *C*-18 diastereoisomer 2), 74.5 (CH_2 *C*-18 diastereoisomer 1), 72.5 (CH_2 *C*-11 diastereoisomer 1), 72.4 (CH_2 *C*-11 diastereoisomer 2), 69.6 (d, *J* 5.7, CH_2Ph diastereoisomer 1), 69.55 (d, *J* 4.9, CH_2Ph diastereoisomer 2), 69.5 (d, *J* 5.7, CH_2Ph diastereoisomer 1), 69.4 (d, *J* 5.7, CH_2Ph diastereoisomer 2), 69.36 (d, *J* 6.1, CH_2Ph diastereoisomer 2), 69.3 (d, *J* 5.8, CH_2Ph diastereoisomer 1), 69.2 (d, *J* 5.5, CH_2Ph diastereoisomer 1), 69.16 (d, *J* 5.8, CH_2Ph diastereoisomer 2), 67.3 (d, *J* 5.9, CH_2Ph diastereoisomer 2), 67.0 (d, *J* 5.7, CH_2Ph diastereoisomer 1), 12.4 (d, *J* 145.7, CH_3 *C*-15 diastereoisomer 2) and 12.2 (d, *J* 146.1, CH_3 *C*-15 diastereoisomer 1); ^{31}P NMR (202 MHz; $CDCl_3$) δ 33.10 (*P*-5 diastereoisomer 1), 31.43 (*P*-5 diastereoisomer 2), -1.54 (diastereoisomer 2), -1.59 (diastereoisomer 1), -1.62 (diastereoisomer 1) and -1.84 (diastereoisomer 2); isotope distribution *m/z* (ES$^+$) [Found: (M+Na)$^+$ 1161.3456 (100), 1162.3456 (68), 1163.3524 (22), 1164.3595 (4), 1165.3462 (1). $C_{63}H_{65}O_{14}P_3Na$ requires M$^+$, 1161.3479 (100), 1162.3513 (70), 1163.3546 (27), 1164.3580 (7), 1165.3613 (2)]; *m/z* (ES$^+$) 1197 ([M+MeCN+NH$_4$]$^+$, 100%).

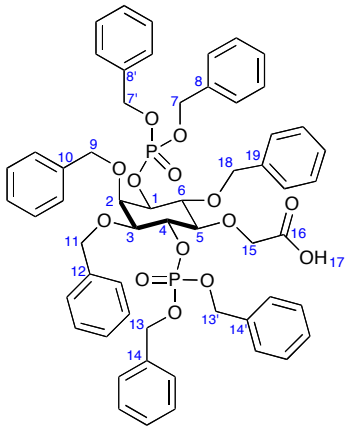
(-)-D-5-(Methylphosphonate)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt 3



To a solution of (-)-D-2,3,6-tris-*O*-benzyl-5-(benzyl methylphosphonate)-*myo*-inositol 1,4-O-bis(dibenzyl phosphate) (63 mg, 0.055 mmol, 1 eq) in a mixture of *t*-butanol (4.0 mL) and water (0.7 mL) under Ar was added sodium bicarbonate (23.2 mg, 0.276 mmol, 5 eq) and palladium black (118 mg, 1.106 mmol, 20 eq).

The flask was evacuated and the atmosphere was purged with hydrogen gas (~12 L). The heterogeneous mixture was vigorously stirred under a hydrogen atmosphere for 12 h. The mixture was filtered through a pad of Celite and the residue was washed with diethyl ether (3 × 5 mL); the filtrates were discarded. The palladium residues were then washed with water (6 × 5 mL). The aqueous filtrates were passed through a Millipore frit and lypholised to furnish (-)-D-5-(methylphosphonate)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt **2** (25.8 mg, 93%) as a colourless solid: $[\alpha]_D^{25}$ -10.4 (*c* 0.63 in H_2O); ν_{max} (KBr disc) /cm^{-1} 3410 (s), 1637 (m), 1377 (w), 1154 (s), 1123 (s) and 1019 (s); ^{1}H NMR (500 MHz; D_2O) δ 4.17 (1H, dd, *J* 2.8, 2.7, *H*-2), 4.11 (1H, ddd, *J* 9.2, 8.7, 8.1, *H*-4), 3.90-3.83 (2H, m, *H*-1 and *H*-5), 3.78 (1H, dd, *J* 9.5, 9.5, *H*-6), 3.64 (1H, dd, *J* 9.2, 2.8, *H*-3) and 1.34 (3H, d, *J* 16.9, C*H*$_3$ *H*-7); ^{13}C NMR (125 MHz; D_2O) δ 77.6 (dd, *J* 6.6, 6.6, *C*H-5), 74.8 (dd, *J* 4.3, 4.3, *C*H-4), 74.0 (d, *J* 5.2, *C*H-1), 71.6 (*C*H-3), 71.3 (d, *J* 4.7, *C*H-6), 70.8 (*C*H-2) and 12.9 (d, *J* 138.3, *C*H$_3$ *C*-7); ^{31}P NMR (202 MHz; D_2O) δ 28.6 (*P*-5), 4.1 and 3.8; HRMS *m/z* (ES$^-$) [Found: (M-2Na+H)$^-$ 482.9215. $C_7H_{13}O_{14}P_3Na_3$ requires M$^-$, 482.9217]; *m/z* (ES$^-$) 483 ([M-2Na+H]$^-$, 5), 461 ([M-3Na+2H]$^-$, 23), 439 ([M-4Na+3H]$^-$, 19), 417 ([M-5Na+4H]$^-$, 24), 359 (41), 337 (100) and 259 (13).

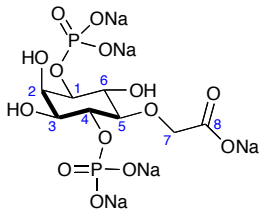
(+)-D-2,3,6-Tris-*O*-benzyl-5-*O*-(carboxymethyl)-*myo*-inositol 1,4-*O*-bis(dibenzyl phosphate)



To a solution of (-)-D-5-*O*-allyl-2,3,6-tris-*O*-benzyl-*myo*-inositol 1,4-*O*-bis(dibenzyl phosphate) (250 mg, 0.25 mmol, 1.0 eq) in a biphasic mixture of carbon tetrachloride (2 mL), acetonitrile (2 mL) and water (3 mL) was added sodium periodate (217 mg, 1.04 mmol, 4.1 eq) and ruthenium trichloride hydrate (1 mg, 0.005 mmol, 0.02 eq). The dark mixture was stirred for 8 h at RT, after which time TLC analysis indicated consumption of the starting material. The volatile components were removed *in vacuo*, and the resulting aqueous phase was partitioned between CH_2Cl_2 (30 mL) and hydrogen peroxide solution (15% *v/v*, 30 mL). The mixture was stirred vigorously for 30 min, then passed through a pad of Celite. The dark residue was washed with CH_2Cl_2 (30 mL) and water (30 mL). The filtrate layers were separated and the aqueous phase was re-extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with hydrogen peroxide solution (15% *v/v*, 20 mL) by vigorously stirring for 20 min. The mixture was passed through a fresh pad of Celite and the layers were separated. The organic phase was washed with brine (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*, affording a purple/brown oil that was adsorbed onto silica gel. Purification of the material by silica gel chromatography, eluting with ethyl acetate and acetic acid (100:0, 99:1), provided *(+)-D-2,3,6-tris-O-benzyl-5-O-(carboxymethyl)-myo-inositol 1,4-O-bis(dibenzyl phosphate)* (114 mg, 44%) as a colourless gum: $[\alpha]_D^{25}$ +7.1 (*c* 2.65 in $CHCl_3$); ν_{max} (KBr disc) /cm^{-1} 3442 (m), 3064 (m), 3033 (m), 2952 (m), 2921 (m), 1750 (s), 1635 (w), 1498 (s), 1455 (m), 1377 (m), 1263 (s),

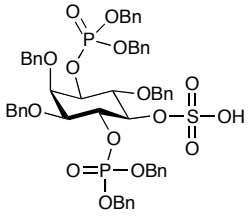
1214 (w), 1169 (m), 1016 (s), 917 (w), 738 (s), 697 (s) and 601 (w); ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.35-7.16 (35H, m, Ar *H*), 5.05-4.91 (8H, m, 4 × C*H*$_2$Ph), 4.80 (1H, d, *J* 11.4, C*H*$_A$H$_B$ *H*-9), 4.80 (1H, d, *J* 11.0, C*H*$_{A'}$H$_{B'}$ *H*-18), 4.75 (1H, ddd, *J* 9.7, 9.3, 9.2, *H*-4), 4.71 (1H, d, *J* 11.4, CH$_A$*H*$_B$ *H*-9'), 4.64 (1H, d, *J* 11.0, CH$_{A'}$*H*$_{B'}$ *H*-18'), 4.52 (1H, d, *J* 14.7, C*H*$_{A''}$H$_{B''}$ *H*-15), 4.51 (1H, d, *J* 11.7, C*H*$_{A'''}$H$_{B'''}$ *H*-11), 4.45 (1H, d, *J* 11.7, CH$_{A'''}$*H*$_{B'''}$ *H*-11'), 4.35 (1H, dd, *J* 2.3, 2.2, *H*-2), 4.18 (1H, ddd, *J* 9.4, 7.6, 2.3, *H*-1), 4.03 (1H, dd, *J* 9.4, 9.1, *H*-6), 4.02 (1H, d, *J* 14.7, CH$_{A''}$*H*$_{B''}$ *H*-15'), 3.32 (1H, dd, *J* 9.7, 2.2, *H*-3) and 3.20 (1H, dd, *J* 9.2, 9.1, *H*-5); ^{13}C NMR (125 MHz; $CDCl_3$) δ 170.0 (C=O, *C*-16), 138.1 (*C*-10 Ph), 137.1 (*C*-12 Ph), 137.0 (*C*-19 Ph), 135.5 (d, *J* 5.7), 135.4 (d, *J* 5.7), 135.39 (d, *J* 5.6), 135.2 (d, *J* 6.6), 128.7, 128.65, 128.62, 128.6, 128.57, 128.5, 128.47, 128.43, 128.4, 128.3, 128.1, 128.0, 127.95, 127.93, 127.9, 127.86, 127.7, 127.67, 127.6, 127.58, 82.0 (*C*H-5), 79.5 (d, *J* 7.6, *C*H-6), 78.2 (d, *J* 5.9, *C*H-1), 77.9 (d, *J* 5.9, *C*H-4), 77.7 (d, *J* 4.8, *C*H-3), 75.5 (*C*H$_2$ *C*-9), 75.4 (*C*H$_2$ *C*-18), 75.3 (*C*H-2), 72.6 (*C*H$_2$ *C*-11), 71.6 (*C*H$_2$ *C*-15), 70.1 (d, *J* 5.6, *C*H$_2$Ph), 69.9 (d, *J* 5.3, *C*H$_2$Ph), 69.7 (d, *J* 5.6, *C*H$_2$Ph) and 69.5 (d, *J* 5.6, *C*H$_2$Ph); ^{31}P NMR (202 MHz; $CDCl_3$) δ -0.21 and -1.23; HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 1051.3199. $C_{57}H_{58}O_{14}P_2Na$ requires M^+, 1051.3194]; *m/z* (ES^-) 1027 ([M-H]$^-$, 100%).

(+)-D-5-*O*-(Carboxymethyl)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt 4



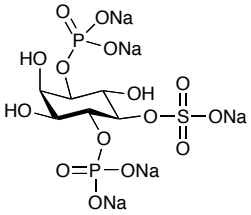
To a solution of (+)-D-2,3,6-tris-*O*-benzyl-5-*O*-(carboxymethyl)-*myo*-inositol 1,4-*O*-bis(dibenzyl phosphate) (114 mg, 0.11 mmol, 1 eq) in a mixture of *t*-butanol (6 mL) and water (1 mL) under Ar was added sodium bicarbonate (46.5 mg, 0.55 mmol, 5 eq) and palladium black (236 mg, 2.22 mmol, 20 eq). The flask was evactuated and the atmosphere was purged with hydrogen gas (~10 L). The heterogeneous mixture was stirred vigorously under a hydrogen atmosphere for 14 h, then vented. The mixture was filtered through a pad of Celite and the residue was washed with diethyl ether (2 × 5 mL); the filtrates were discarded. The palladium residue was then washed with water (6 × 5 mL) to release the product. The aqueous filtrates were passed through a Millipore frit and lypholised, affording *(+)-D-5-O-(carboxymethyl)-myo-inositol 1,4-bisphosphate pentakis sodium salt* **4** (54.9 mg, 98%) as a colourless solid: $[\alpha]_D^{25}$ +2.7 (*c* 0.53 in H_2O); ν_{max} (KBr disc) /cm^{-1} 3424 (s), 1609 (s), 1427 (m), 1377 (m), 1095 (s), 974 (s), 850 (m), and 695 (s); ^{1}H NMR (500 MHz; D_2O) δ 4.35 (1H, d, *J* 16.3, C*H*$_A$H$_B$ *H*-7), 4.21-4.14 (2H, m, *H*-2 and *H*-4), 4.07 (1H, d, *J* 16.3, CH$_A$*H*$_B$ *H*-7'), 3.84 (1H, ddd, *J* 9.4, 6.7, 2.4, *H*-1), 3.81 (1H, dd, *J* 9.4, 9.3, *H*-6), 3.61 (1H, dd, *J* 9.7, 2.9, *H*-3) and 3.23 (1H, dd, *J* 9.3, 9.0, *H*-5); ^{13}C NMR (125 MHz; D_2O) δ 82.8 (d, *J* 6.2, *C*H-5), 76.0 (d, *J* 5.0, *C*H-4), 74.4 (d, *J* 5.2, *C*H-1), 71.6 (*C*H-3), 71.4 (*C*H$_2$ *C*-7), 71.0 (d, *J* 6.0, *C*H-6) and 70.8 (*C*H-2); ^{31}P NMR (202 MHz; D_2O) δ 4.23 and 3.60; HRMS *m/z* (ES^-) [Found: $(M-Na)^-$ 484.9222. $C_8H_{11}O_{14}P_2Na_4$ requires M^-, 484.9220]; *m/z* (ES^-) 441 ([M-3Na+2H]$^-$, 2), 419 ([M-4Na+3H]$^-$, 9), 397 ([M-5Na+4H]$^-$, 48) and 317 (100).

(-)-D-2,3,6-Tris-*O*-benzyl-5-*O*-(sulfono)-*myo*-inositol 1,4-*O*-bis(dibenzyl phosphate)



A solution of (-)-D-5-*O*-allyl-2,3,6-tris-*O*-benzyl-*myo*-inositol 1,4-*O*-bis(dibenzyl phosphate) (39.6 mg, 0.04 mmol, 1.0 eq) and sulfur trioxide-pyridine complex (80.2 mg, 0.40 mmol, 10 eq) in DMF (1.8 mL) was heated to 50 °C for 16 h. After this time, the reaction was adjudged to be complete by TLC analysis. Triethylamine (1 mL) and methanol (2 mL) was added and the solvent was removed *in vacuo*. Purification by silica gel column chromatography, eluting with hexane and ethyl acetate (4:1, 6:4 then 0:1) and then methanol and chloroform (5:95), gave *(-)-D-2,3,6-tris-O-benzyl-5-O-(sulfono)-myo-inositol 1,4-O-bis(dibenzyl phosphate)* (22 mg, 52% yield) as a colourless oil: R_f 0.23 (ethyl acetate); $[\alpha]_D^{20}$ -5.6 (*c* 0.48 in $CHCl_3$); ν_{max} (neat)/cm^{-1} 3033 (m), 2828 (m), 1455.6 (m), 1264 (s), 1018 (s), 789 (m), 696 (m); ^{1}H NMR (300 MHz; MeOD) δ 7.49-7.44 (2H, m, Ph), 7.29-7.14 (33H, m, Ph), 5.15 (1H, d, *J* 10.3, OC*H*$_2$Ph), 5.06-5.00 (5H, m, OC*H*$_2$Ph), 4.87-4.75 (4H, m, OC*H*$_2$Ph), 4.70 (1H, d, *J* 11.5, OC*H*$_2$Ph), 4.65-4.57 (2H, m, OC*H*$_2$Ph), 4.46 (1H, d, *J* 10.3, OC*H*$_2$Ph), 4.40-4.39 (2H, m, 2 × inositol ring), 4.37-4.28 (2H, m, 2 × inositol ring), 3.98 (1H, dd, *J* 9.5, 9.5, inositol ring), 3.58 (1H, dd, *J* 9.8, 2.1, H-3 inositol ring); ^{13}C NMR (125 MHz; MeOD) δ 139.8 (C), 139.7 (C), 139.0 (C), 137.3 (d, *J* 7.4, *C*), 137.2 (C), 137.04 (d, *J* 6.8, *C*), 137.02 (d, *J* 7.4, *C*), 130.0 (CH), 129.64 (CH), 129.59 (CH), 129.50 (CH), 129.48 (CH), 129.4 (CH), 129.3 (CH), 129.2 (CH), 129.1 (CH), 129.0 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.5 (CH), 81.1 (CH), 80.2 (d, *J* 7.6, *C*H), 79.7-79.4 (m, *C*H), 79.1 (CH), 79.0 (CH), 76.7 (CH_2), 76.3 (CH), 76.2 (CH_2), 73.4 (CH_2), 71.5 (d, *J* 5.6, PO*C*H$_2$), 71.4 (d, J_{CP} 5.6, PO*C*H$_2$), 71.0 (d, *J* 5.8, PO*C*H$_2$), 70.8 (d, *J* 5.8, PO*C*H$_2$); ^{31}P NMR (162 MHz; $CDCl_3$) δ -2.6, -4.9; HRMS *m/z* (ES^+) [Found: $(M-H+2Na)^+$ 1095.2516. $C_{55}H_{55}O_{15}Na_2P_2S$ requires M^+, 1095.2527]; *m/z* (ES^-) 1050.3 ($[M-H]^-$, 100%).

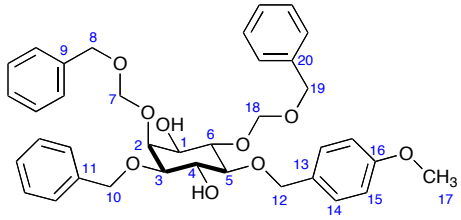
(-)-1D-5-*O*-(Sulfono)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt 5



Palladium black (36.2 mg, 0.34 mmol, 20 eq) and sodium bicarbonate (7.1 mg, 0.08 mmol, 5 eq) were added to a solution of (-)-D-2,3,6-tris-*O*-benzyl-5-*O*-(sulfono)-*myo*-inositol 1,4-*O*-bis(dibenzyl phosphate) (17.7 mg, 0.017 mmol, 1 eq) in *t*-butanol (3 mL) and water (0.6 mL) under nitrogen. The flask was flushed three times with hydrogen, then stirred the mixture under an atmosphere of hydrogen (atmospheric pressure) for 16 h. The organic phase was decanted by pipette and water added to the black residue. The palladium was removed by filtration using a pad of 3-4 filter papers, and the aqueous filtrate was lyophilised to give *(-)-1D-5-O-(sulfono)-myo-inositol 1,4-bisphosphate pentakis sodium salt* **5** (8.5 mg, 94% yield) as a colourless solid. $[\alpha]_D^{20}$ -2.3 (*c* 0.51 in water); ν_{max} (KBr disc)/cm^{-1} 3399 (s), 1655 (m), 1383 (w), 1096 (s), 979 (m), 540 (m); ^{1}H NMR (400 MHz; D_2O) δ 4.11-4.07 (3H, m, inositol ring), 4.84-4.78 (2H, m, inositol ring), 3.63-3.60 (1H, m, inositol ring) and 3.70-3.64 (1H, m, *H*-3 inositol ring); ^{31}P NMR (162 MHz; D_2O) δ 4.23, 3.74; HRMS *m/z*

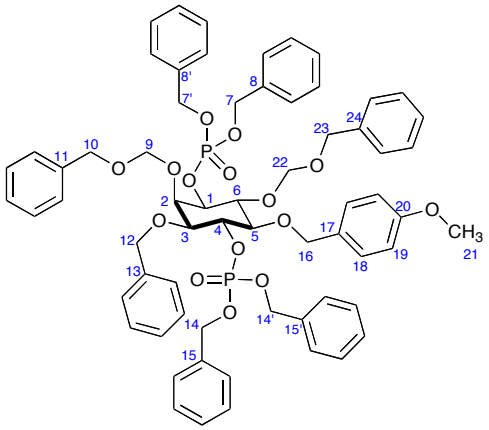
(ES^-) [Found: $(M-H)^-$ 484.8910. $C_6H_{10}O_{15}Na_3SP_2$ requires M^-, 484.8914]; *m/z* (ES^-) 484.9 ($[M-H]^-$, 5%), 462.9 ($[M-H-Na]^-$, 10), 231.0 (30), 220.0 (100), 209.0 (70).

(-)-D-3-*O*-Benzyl-2,6-bis-*O*-benzyloxymethyl-5-*O*-(4-methoxybenzyl)-*myo*-inositol



To a solution of (-)-D-1-*O*-acetyl-3-*O*-benzyl-2,6-bis-*O*-benzyloxymethyl-5-*O*-(4-methoxybenzyl)-4-*O*-triisopropylsilyl-*myo*-inositol **11** (234 mg, 0.30 mmol, 1 eq) in THF (5 mL) was added a solution of tetrabutylammonium fluoride in THF (1.0 M, 5.95 mL, 20 eq). The solution was stirred at RT for 18 h, after which time TLC analysis showed consumption of the starting material. The reaction solution was diluted with water (20 mL), then extracted with diethyl ether (30 mL). The aqueous phase was re-extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*, providing a colourless solid. Purification of this solid by silica gel chromatography, eluting with hexane and ethyl acetate (70:30, 50:50), provided *(-)-D-3-O-benzyl-2,6-bis-O-benzyloxymethyl-5-O-(4-methoxybenzyl)-myo-inositol* (167 mg, 89%) as a colourless solid: R_f 0.13 (hexane/ethyl acetate 70:30); $[\alpha]_D^{20}$ -16.9 (*c* 0.54 in $CHCl_3$); mp 105-106 °C (from hexane/ethyl acetate); ν_{max} (KBr disc) /cm^{-1} 3465 (s), 2892 (w), 1611 (m), 1514 (s), 1498 (m), 1454 (m), 1370 (m), 1251 (s), 1175 (s), 1065 (s), 1029 (s), 818 (w), 735 (s), 700 (m) and 586 (w); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.37-7.27 (17H, m, Ar *H*), 6.92-6.88 (2H, m, *H*-15,15' Ar), 5.00 (1H, d, *J* 6.8, OCH_AH_BO *H*-7), 5.00 (1H, d, *J* 6.7, $OCH_{A'}H_{B'}O$ *H*-18), 4.93 (1H, d, *J* 6.7, $OCH_{A'}H_{B'}O$ *H*-18'), 4.87-4.59 (9H, m, 4.5 × CH_AH_B), 4.30 (1H, dd, *J* 2.4, 2.3, *H*-2), 4.11 (1H, ddd, *J* 9.7, 9.3, 1.9, *H*-4), 3.86 (1H, dd, *J* 9.6, 9.3, *H*-6), 3.83 (3H, s, OCH_3 *H*-17), 3.80 (1H, d, *J* 4.8, O*H*-1, ex. in D_2O), 3.51 (1H, ddd, *J* 9.6, 4.8, 2.4, *H*-1), 3.38 (1H, dd, *J* 9.3, 9.3, *H*-5), 3.32 (1H, dd, *J* 9.7, 2.3, *H*-3) and 2.55 (1H, d, *J* 1.9 O*H*-4, ex. in D_2O); ^{13}C NMR (100 MHz; $CDCl_3$) δ 159.2 (*C*-16 Ar), 137.7 (*C*-11 Ph), 137.6 (*C*-9 Ph), 137.3 (*C*-20 Ph), 130.9 (*C*-13 Ph), 129.5 (*C*H-14,14' Ar), 128.6, 128.5, 128.4, 128.0, 127.96, 127.9, 127.7, 113.9 (*C*H-15,15' Ar), 96.7 (OCH_2O *C*-18), 95.7 (OCH_2O *C*-7), 82.9 (*C*H-6), 82.4 (*C*H-5), 79.2 (*C*H-3), 75.2 (*C*H-2), 75.0 (CH_2 *C*-12), 73.0 (*C*H-4), 72.1 (CH_2 *C*-10), 71.5 (*C*H-1), 70.2 (CH_2 *C*-19), 69.7 (CH_2 *C*-8) and 55.3 (OCH_3 *C*-17); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 653.2723. $C_{37}H_{42}O_9Na$ requires M^+, 653.2727]; *m/z* (ES^+) 653 ($[M+Na]^+$, 100%); Anal. Calcd. for $C_{37}H_{42}O_9$: C, 70.46; H, 6.71. Found: C, 70.58; H, 6.62.

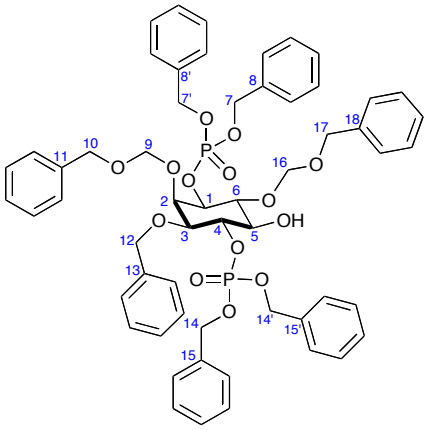
(-)-D-3-*O*-Benzyl-2,6-bis-*O*-benzyloxymethyl-5-*O*-(4-methoxybenzyl)-*myo*-inositol 1,4-bis(dibenzyl phosphate) 12



To a solution of bis(benzyloxy)-*N,N*-bisisopropylamine phosphine (455 mg, 1.32 mmol, 5 eq) in CH_2Cl_2 (10 mL) was added a solution of 1*H*-tetrazole in acetonitrile (0.43 M, 3.07 mL, 1.32 mmol, 5 eq). The solution was stirred for 15 min, turning opaque. To this solution was added a solution of (-)-D-3-*O*-benzyl-2,6-bis-*O*-benzyloxymethyl-5-*O*-(4-methoxybenzyl)-*myo*-inositol (166 mg, 0.26 mmol, 1 eq) in CH_2Cl_2 (10 mL) dropwise *via* a pressure equalised dropping funnel over 1 h. The resulting solution was stirred for 18 h at RT, after which time TLC indicated consumption of the starting material. The mixture was cooled to -78 ˚C and 3-chloroperoxybenzoic acid (228 mg, 1.32 mmol, 5 eq) was added in a single portion. The mixture was stirred at -78 ˚C for 15 min, then allowed to warm to RT over 90 min, after which TLC indicated consumption of the intermediate (presumed P(III) species). The solution was diluted with aqueous sodium sulfite solution (10% *w/v*, 20 mL) and CH_2Cl_2 (20 mL) and the layers were separated. The aqueous phase was re-extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*, providing a colourless oil. Purification of this oil by silica gel chromatography, eluting with hexane and ethyl acetate (80:20, 70:30, 60:40, 50:50), provided *(-)-D-3-O-benzyl-2,6-bis-O-benzyloxymethyl-5-O-(4-methoxybenzyl)-myo-inositol 1,4-bis(dibenzyl phosphate)* **12** (263 mg, 89%) as a colourless solid: R_f 0.24 (hexane/ethyl acetate 60:40); $[\alpha]_D^{20}$ -8.2 (*c* 0.67 in $CHCl_3$); mp 59-61 ˚C (from diethyl ether); ν_{max} (KBr disc) /cm^{-1} 3063 (w), 3034 (w), 2959 (w), 2892 (w), 1612 (w), 1513 (s), 1498 (m), 1455 (m), 1372 (m), 1259 (s), 1163 (m), 1116 (m), 1025 (s), 824 (w), 735 (s), 696 (s) and 513 (m); ^{1}H NMR (500 MHz; $CDCl_3$) δ 7.32-7.16 (33H, m, Ar *H*), 7.13-7.06 (4H, m, Ar *H*), 6.76-6.73 (2H, m, *H*-19,19'), 5.02 (2H, dd, *J* 7.8, 3.1, C*H*$_2$Ph), 4.99-4.96 (3H, m, 1.5 × CH_AH_BPh), 4.95-4.78 (9H, m, 4.5 × CH_AH_BPh), 4.70 (1H, d, *J* 10.3, CH_AH_B H-16'), 4.66 (1H, d, *J* 12.1, C$H_{A'}H_{B'}$ H-23), 4.61 (2H, s, CH_2 *H*-10,10'), 4.55 (1H, d, *J* 11.5, C$H_{A''}H_{B''}$ H-12), 4.53 (1H, d, *J* 12.1, C$H_{A'}H_{B'}$ H-23'), 4.48-4.46 (1H, m, *H*-2), 4.47 (1H, d, *J* 11.5, C$H_{A''}H_{B''}$ H-12'), 4.24 (1H, dd, *J* 9.9, 9.3, *H*-6), 4.18 (1H, ddd, *J* 9.9, 8.1, 2.1, *H*-1), 3.75 (3H, s, OC*H*$_3$ *H*-21), 3.46 (1H, dd, *J* 9.3, 9.1, *H*-5) and 3.36 (1H, dd, *J* 9.9, 2.1, *H*-3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 159.0 (*C*-20 Ar), 138.0 (*C*-24 Ph), 137.8 (*C*-11 Ph), 137.2 (*C*-13 Ph), 136.2 (d, *J* 8.3), 136.1 (d, *J* 8.3), 135.7 (d, *J* 7.2), 130.3 (*C*-17 Ar), 129.2 (*C*H-18,18' Ar), 128.6, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.7, 127.5, 127.4, 113.6 (*C*H-19,19' Ar), 96.2 (O*C*H$_2$O *C*-22), 95.3 (O*C*H$_2$O *C*-9), 81.2 (*C*H-5), 79.5 (d, *J* 7.0, *C*H-4), 77.6 (*C*H-3), 77.2 (d, *J* 5.5, *C*H-1), 76.0 (d, *J* 6.5, *C*H-6), 74.7 (*C*H$_2$ *C*-16), 72.8 (*C*H-2), 72.0 (*C*H$_2$ *C*-12), 70.2 (*C*H$_2$ *C*-23), 69.7 (*C*H$_2$ *C*-10), 69.6 (d, *J* 5.3, *C*H$_2$Ph), 69.4 (d, *J* 5.2, *C*H$_2$Ph), 69.2 (d, *J* 5.3, *C*H$_2$Ph), 69.1 (d, *J* 5.2, *C*H$_2$Ph), and 55.2 (O*C*H$_3$ *C*-21); ^{31}P NMR (162 MHz; $CDCl_3$) δ -0.86 (*P*-1) and -1.41 (*P*-4); isotope distribution *m/z*

(ES^+) [Found: $(M+Na)^+$ 1173.3645 (100), 1174.3678 (72), 1175.3709 (27), 1176.3727 (6.4), 1177.3731 (1). $C_{65}H_{68}O_{15}P_2Na$ requires M^+, 1173.3926 (100), 1174.3959 (72), 1175.3992 (28), 1176.4026 (8), 1177.4060 (2)]; *m/z* (ES^+) 1173 ($[M+Na]^+$, 100%); Anal. Calcd. For $C_{65}H_{68}O_{15}P_2$: C, 67.82; H, 5.95. Found: C, 67.51; H, 5.89.

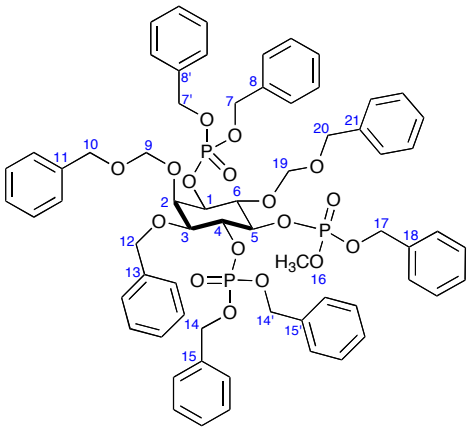
(+)-D-3-*O*-Benzyl-2,6-bis-*O*-benzyloxymethyl-*myo*-inositol 1,4-bis(dibenzyl phosphate)



To a solution of (-)-D-3-*O*-benzyl-2,6-bis-*O*-benzyloxymethyl-5-*O*-(4-methoxybenzyl)-*myo*-inositol 1,4-bis(dibenzyl phosphate) **12** (262 mg, 0.23 mmol, 1 eq) in a mixture of acetonitrile (12 mL) and water (3 mL) was added ceric ammonium nitrate (437 mg, 0.80 mmol, 3.5 eq) in a single portion, turning the solution orange. The mixture was stirred for 40 min, after which time TLC showed consumption of the starting material. The volatile components were removed *in vacuo* and the resulting residue was reconstituted with water (20 mL) and ethyl acetate (20 mL). The layers were separated and the aqueous phase was re-extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo* affording a colourless oil. Purification of the oil by silica gel chromatography, eluting with hexane and ethyl acetate (60:40, 55:45, 50:50, 40:60), provided *(+)-D-3-O-benzyl-2,6-bis-O-benzyloxymethyl-myo-inositol 1,4-bis(dibenzyl phosphate)* (178 mg, 75%) as a colourless waxy solid. A small portion was recrystallised from diethyl ether, affording a colourless microcrystalline solid: R_f 0.16 (hexane/ethyl acetate 60:40); $[\alpha]_D^{20}$ +24.0 (*c* 0.6 in $CHCl_3$); mp 88-89 °C (from diethyl ether); ν_{max} (KBr disc) /cm^{-1} 3283 (m), 3035 (w), 2894 (m), 1498 (m), 1456 (s), 1381 (m), 1267 (s), 1164 (w), 1024 (s), 899 (m), 764 (s), 696 (s), 603 (w) and 500 (m); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.33-7.17 (35H, m, Ar *H*), 5.09-4.95 (8H, m, 4 × CH_AH_BPh), 4.92 (1H, d, *J* 6.8, CH_AH_B *H*-16), 4.87 (1H, d, *J* 7.1, $CH_{A'}H_{B'}$ *H*-9), 4.85 (1H, d, *J* 7.1, $CH_{A'}H_{B'}$ *H*-9'), 4.82 (1H, d, *J* 6.8, CH_AH_B *H*-16'), 4.75 (1H, ddd, *J* 9.5, 9.1, 9.0, *H*-4), 4.73 (1H, d *J* 11.8, $CH_{A''}H_{B''}$ *H*-17), 4.62 (2H, s, CH_2 *H*-10,10'), 4.61 (1H, d, *J* 11.8, $CH_{A''}H_{B''}$ *H*-17'), 4.58 (1H, d, *J* 11.7, $CH_{A'''}H_{B'''}$ *H*-12), 4.53 (1H, d, *J* 2.0, O*H*-5), 4.46-4.43 (1H, m, *H*-2), 4.44 (1H, d, *J* 11.7, $CH_{A'''}H_{B'''}$ *H*-12'), 4.25 (1H, ddd, *J* 9.5, 9.2, 2.1, *H*-1), 4.03 (1H, dd, *J* 9.2, 9.1, *H*-6), 3.62 (1H, ddd, *J* 9.1, 9.0, 2.0, *H*-5) and 3.39 (1H, dd, *J* 9.5, 2.1, *H*-3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 137.8 (*C*-11 Ph), 137.4 (*C*-18 Ph), 137.3 (*C*-12 Ph), 136.0 (d, *J* 7.5), 135.9 (d, *J* 7.8), 135.7 (d, *J* 7.5), 135.66 (d, *J* 7.2), 128.6, 128.5, 128.45, 128.4, 128.35, 128.3, 128.0, 127.98, 127.9, 127.8, 127.79, 127.7, 127.67, 127.5, 96.2 (OCH_2O *C*-16), 95.3 (OCH_2O *C*-9), 80.4 (d, *J* 6.0, *C*H-4), 79.5 (d, *J* 5.6, *C*H-6), 77.3 (d, *J* 5.2, *C*H-3), 76.9 (d, *J* 5.8, *C*H-1), 73.6 (*C*H-5), 73.0 (*C*H-2), 72.2 (CH_2 *C*-12), 70.2 (CH_2 *C*-17), 69.7 (CH_2 *C*-10), 69.54 (d, *J* 4.9, CH_2Ph), 69.5 (d, *J* 5.1, CH_2Ph), 69.45 (d, *J* 5.2, CH_2Ph) and 69.4 (d, *J* 5.3, CH_2Ph); ^{31}P NMR (162 MHz; $CDCl_3$) δ -0.16 and -1.06; isotope distribution *m/z* (ES^+) [Found: $(M+Na)^+$ 1053.3103 (100), 1054.3133 (60), 1055.3164 (19), 1056.3182

(5). $C_{57}H_{60}O_{14}P_2Na$ requires M^+, 1053.3351 (100), 1054.3384 (63), 1055.3417 (22), 1056.3451 (6), 1057.3485 (1)]; *m/z* (ES^+) 1053 ([M+Na]$^+$, 100%).

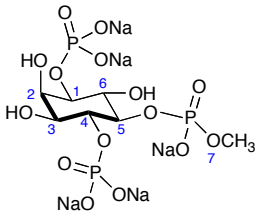
(-)-D-3-*O*-Benzyl-5-*O*-(benzyl methylphophono)-2,6-bis-*O*-benzyloxymethyl-*myo*-inositol 1,4-bis(dibenzyl phosphate) 13



To a solution of benzyl methyl (*N,N*-diisopropylamino)phosphoramidite (77 mg, 0.244 mmol, 3 eq) in CH_2Cl_2 (5 mL) was added a solution of 1*H*-tetrazole in acetonitrile (0.43 M, 567 µL, 0.244 mmol, 3 eq). The solution was stirred for 10 min, then a solution of (+)-D-3-*O*-benzyl-2,6-bis-*O*-benzyloxymethyl-*myo*-inositol 1,4-bis(dibenzyl phosphate) (84 mg, 0.081 mmol, 1 eq) in CH_2Cl_2 (5 mL) was added dropwise *via* a pressure equalised dropping funnel over 30 min. The resulting mixture was stirred for 18 h, after which time TLC showed consumption of the starting material. The mixture was cooled to -78 ˚C, then 3-chloroperoxybenzoic acid (56 mg, 0.325 mmol, 4 eq) was added in a single portion. The mixture was stirred for 15 min at -78 ˚C, then allowed to warm to RT over 1 h. TLC analysis of the reaction mixture indicated consumption of the intermediate (presumed P(III) species). The reaction was quenched by the addition of aqueous sodium sulfite solution (10% *w/v*, 20 mL) and CH_2Cl_2 (20 mL), then the layers were separated. The aqueous phase was re-extracted with CH_2Cl_2 (3 × 20 mL), then the combined organic phases were washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*, providing a colourless oil. Purification of the oil by silica gel chromatography, eluting with hexane and ethyl acetate (65:35, 60:40, 50:50, 40:60, 0:100), provided *(-)-D-3-O-benzyl-5-O-(benzyl methylphophono)-2,6-bis-O-benzyloxymethyl-myo-inositol 1,4-bis(dibenzyl phosphate)* **13** (91 mg, 92%) as a colourless oil containing a 1:1 mixture of diastereoisomers (adjudged by ^{1}H NMR): R_f 0.30 (hexane/ethyl acetate 30:70); $[\alpha]_D^{20}$ -16.9 (*c* 0.51 in $CHCl_3$); ν_{max} (KBr disc) /cm^{-1} 3449 (m), 3033 (w), 2955 (w), 1497 (m), 1456 (m), 1381 (m), 1278 (s), 1215 (m), 1025 (s), 882 (m), 738 (s), 697 (s), 602 (m) and 499 (m); ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.34-7.18 (38H, m, Ar *H*), 7.15-7.09 (2H, m, Ar *H*), 5.21-4.80 (15.5H, m), 4.73 (0.5H, d, *J* 12.1, C*H*$_A$H$_B$ *H*-20 diastereoisomer 1), 4.67-4.65 (2H, m, C*H*$_2$ *H*-10,10'), 4.66 (0.5H, d, *J* 12.0, CH$_A$*H*$_B$ *H*-20' diastereoisomer 2), 4.56 (0.5H, d, *J* 12.1, CH$_A$*H*$_B$ *H*-20' diastereoisomer 1), 4.53-4.51 (1H, m, *H*-2), 4.50-4.48 (2H, m, C*H*$_2$ *H*-12,12'), 4.47-4.36 (1H, m, *H*-5), 4.30 (0.5H, dd, *J* 9.5, 9.5, *H*-6 diastereoisomer 1), 4.29 (0.5H, dd, *J* 9.6, 9.6, *H*-6 diastereoisomer 2), 4.18-4.12 (1H, m, *H*-1), 3.66 (1.5H, d, *J* 4.9, OC*H*$_3$ *H*-16 diastereoisomer 1), 3.63 (1.5H, d, *J* 4.9, OC*H*$_3$ *H*-16 diastereoisomer 2) and 3.38 (1H, dd, *J* 9.9, 2.0, *H*-3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 138.1 (*C*-21 Ph), 137.8 (*C*-11 Ph), 137.0 (*C*-13 Ph), 136.2 (d, *J* 7.7), 136.18 (d, *J* 4.4), 136.15 (d, *J* 7.8), 136.1 (d, *J* 7.3), 136.0 (d, *J* 7.2), 135.64 (d, *J* 7.1), 135.6 (d, *J* 7.5), 128.6, 128.5, 128.45, 128.4, 128.3, 128.26, 128.2, 128.1, 128.08, 128.0, 127.9, 127.86, 127.83, 127.8, 127.7, 127.69,

127.5, 127.4, 96.6 (O*C*H$_2$O *C*-19 diastereoisomer 1), 96.5 (O*C*H$_2$O *C*-19 diastereoisomer 2), 95.5 (O*C*H$_2$O *C*-9), 79.0 (m, *C*H-5), 77.5 (m, *C*H-4), 76.8 (*C*H-3), 76.4 (m, *C*H-1), 75.1 (m, *C*H-6), 72.6 (d, *J* 3.7, *C*H-2), 72.0 (*C*H$_2$ *C*-12), 70.5 (*C*H$_2$ *C*-20 diastereoisomer 1), 70.4 (*C*H$_2$ *C*-20 diastereoisomer 2), 69.9 (*C*H$_2$ *C*-10), 69.7 (d, *J* 5.9, *C*H$_2$Ph), 69.67 (d, *J* 4.5, *C*H$_2$Ph), 69.5 (d, *J* 4.8, *C*H$_2$Ph), 69.4 (d, *J* 4.8, *C*H$_2$Ph), 69.2 (d, *J* 5.1, *C*H$_2$Ph), 54.8 (d, *J* 6.3, O*C*H$_3$ *C*-16 diastereoisomer 1) and 54.5 (d, *J* 5.4, O*C*H$_3$ *C*-16 diastereoisomer 2); ^{31}P NMR (162 MHz; CDCl$_3$) δ -0.07, -0.12, -1.09, -1.11, -1.64 and -1.73; *m/z* (ES$^+$) 1237 ([M+Na]$^+$, 100%); Anal. Calcd. For $C_{65}H_{69}O_{17}P_3$: C, 64.25; H, 5.72. Found: C, 63.91; H, 5.83.

D-5-*O*-(Methyl phosphate)-*myo*-inositol 1,4-*O*-bisphosphate pentakis sodium salt 2



To a solution of (-)-D-3-*O*-benzyl-5-*O*-(benzyl methylphophono)-2,6-*O*-bisbenzyloxymethyl-*myo*-inositol 1,4-bis(dibenzyl phosphate) **13** (80 mg, 0.066 mmol, 1 eq) in *t*-butanol (6.7 mL) and water (0.7 mL) was added palladium black (140 mg, 1.320 mmol, 20 eq) and aqueous sodium bicarbonate solution (0.5 M, 658 μL, 0.329 mmol, 5 eq). The flask was evacuated and the atmosphere purged with hydrogen gas three times, then the heterogeneous mixture was stirred vigorously under an atmosphere of hydrogen for 6.5 h. The mixture was filtered through a pad of Celite and the residue was washed with water (2 mL). This filtrate was lypholised, providing a colourless solid (31.8 mg), shown to be incomplete deprotected material. The remaining dark palladium residue was washed with ethyl acetate (2 × 4 mL), discarding the organic layer, and water (5 × 10 mL). The aqueous filtrate was then lypholised, providing a fine solid (> 0.1 mg). The initial product (31.8 mg) was resubmitted to hydrogenolysis under the above conditions for a further 16 h. The heterogeneous mixture was filtered through a pad of Celite and the residue was washed with ethyl acetate (2 × 4 mL). The organic filtrates were discarded and the remaining residue was washed with water (5 × 10 mL). The filtrate was passed through a Millipore frit then lypholised, affording *D-5-O-(methyl phosphate)-myo-inositol 1,4-O-bisphosphate pentakis sodium salt* **2** (18.2 mg, 51%) as a light brown solid: $[\alpha]_D^{20}$ -7.2 (*c* 0.50 in H$_2$O); ν_{max} (KBr disc) /cm^{-1} 3423 (s), 1655 (m), 1457 (s), 1230 (w), 1089 (m), 974 (m), 881 (w), 866 (w) and 556 (br w); ^{1}H NMR (400 MHz; CDCl$_3$) δ 4.13 (1H, br s, *H*-2), 4.13-4.06 (1H, m, *H*-4), 3.88-3.77 (3H, m, *H*-1, *H*-5 and *H*-6), 3.61 (1H, dd, *J* 9.7, 2.5, *H*-3) and 3.54 (3H, d, *J* 10.8, O*CH*$_3$ *H*-7); ^{13}C NMR (125 MHz; CDCl$_3$) δ 79.1 (*C*H-5), 74.7 (*C*H-4), 74.0 (d, *J* 4.9, *C*H-6), 71.5 (2C by HSQC, *C*H-1 and *C*H-3), 70.8 (*C*H-2) and 53.4 (d, *J* 5.8, O*C*H$_3$); ^{31}P NMR (162 MHz; CDCl$_3$) δ 4.16 (*P*-4), 3.71 (*P*-1) and 1.60 (*P*-5); HRMS *m/z* (ES$^-$) [Found: (M-2Na+H)$^-$ 498.9170. $C_7H_{13}O_{15}P_3Na_3$ requires M$^-$, 498.9166]; *m/z* (ES$^-$) 499 ([M-2Na+H]$^-$, 6%), 477 ([M-3Na+2H]$^-$, 17), 455 ([M-4Na+3H]$^-$, 18), 433 ([M-5Na+4H]$^-$, 19), 375 (18) and 353 (100).

General procedure for Ca^{2+}-flux assay

i. Cells (2MEFwt, L15 or Lvec) are seeded into the bottom of wells, forming a confluent monolayer. The culture is allowed to grow for 6-8 days prior to the flux experiments.

ii. Cells are incubated with saponin, which permeabilises the cell membranes, allowing the free passage of Ca^{2+} and the inositol analogues across the membrane.

iii. The non-mitochondrial Ca^{2+} stores are then loaded with free Ca^{2+}, containing $^{45}Ca^{2+}$.

iv. Cells are washed twice with a medium containing thapsigargin, which inhibits the ER Ca^{2+} pumps, ensuring that any release of Ca^{2+} that is observed is a result of inositol-induced Ca^{2+}-release (IICR).

v. The run is commenced, then the efflux medium is replaced every 2 min over the duration of the run.

vi. After a set period (indicated on the fractional loss plots), the cells are challenged with the compound of interest for several minutes.

vii. InsP_3 is applied to the cells later in the experiment, to induce IICR.

viii. At the end of the run, the Ca^{2+} stores are emptied by treatment with 2% sodium dodecyl sulfate (SDS) to allow the entire remaining Ca^{2+} content to be measured.

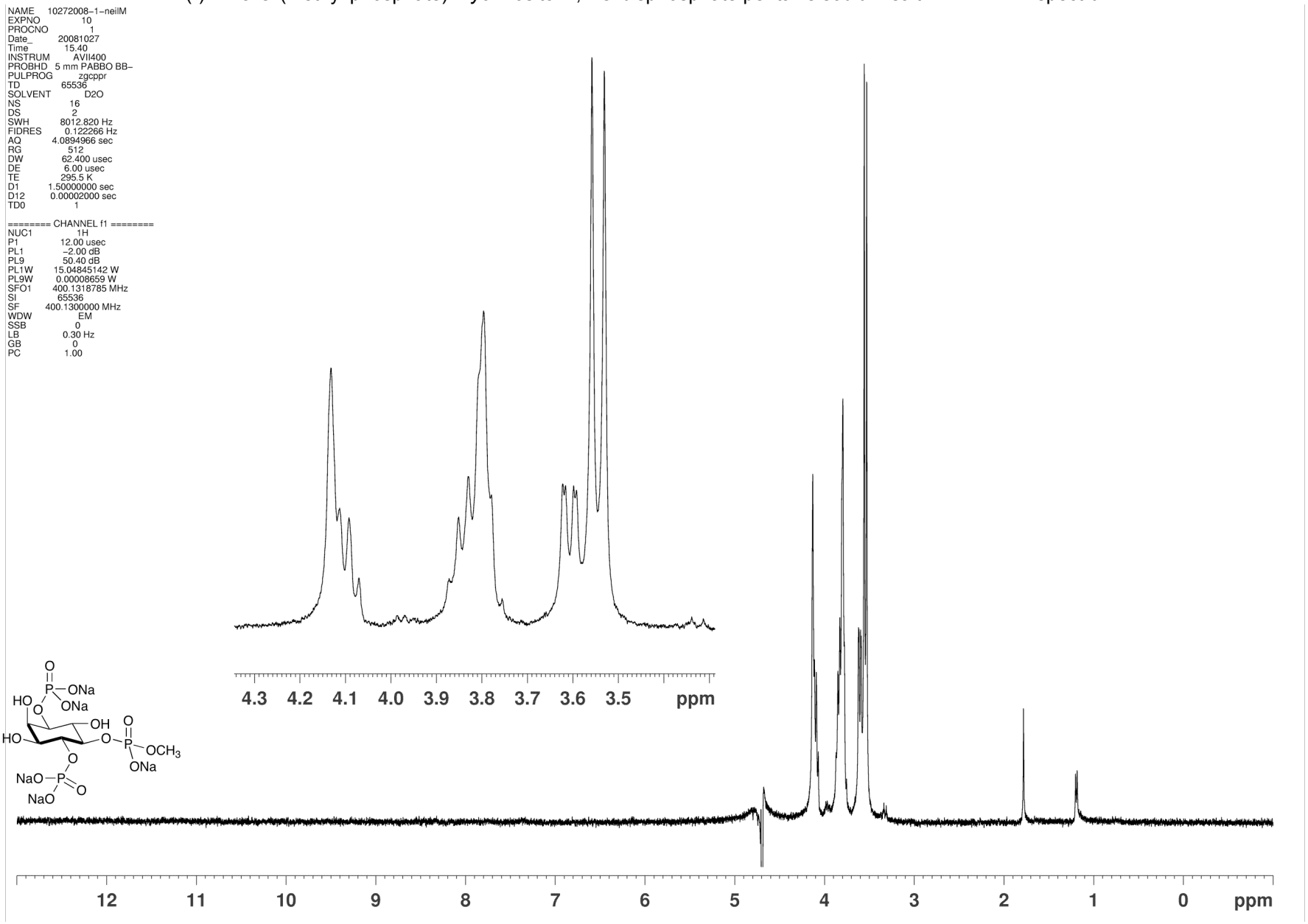
ix. $^{45}Ca^{2+}$ efflux from the ER membranes over each 2 min period is measured in a scintillation counter.

x. Ca^{2+} release is plotted as % fractional loss: $\frac{\text{amount of } Ca^{2+} \text{ released in 2 min}}{\text{total } Ca^{2+} \text{ store content at timepoint}} \times 100$

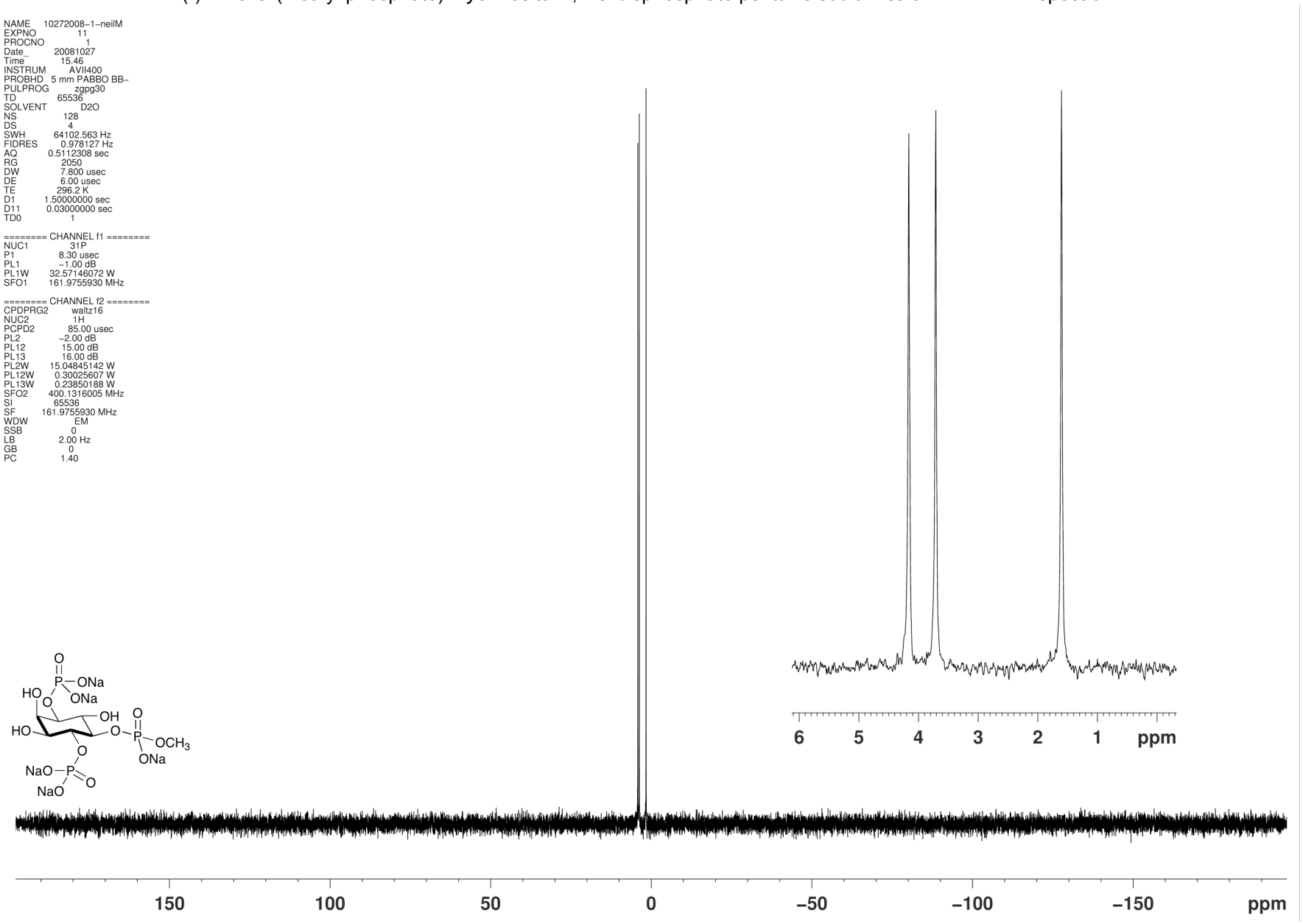
xi. The results are normalised to the potential maximum releasable quantity of Ca^{2+} by the calcium ionophore, A23187.

The sea urchin egg homogenate assay was performed as described previously.[6]

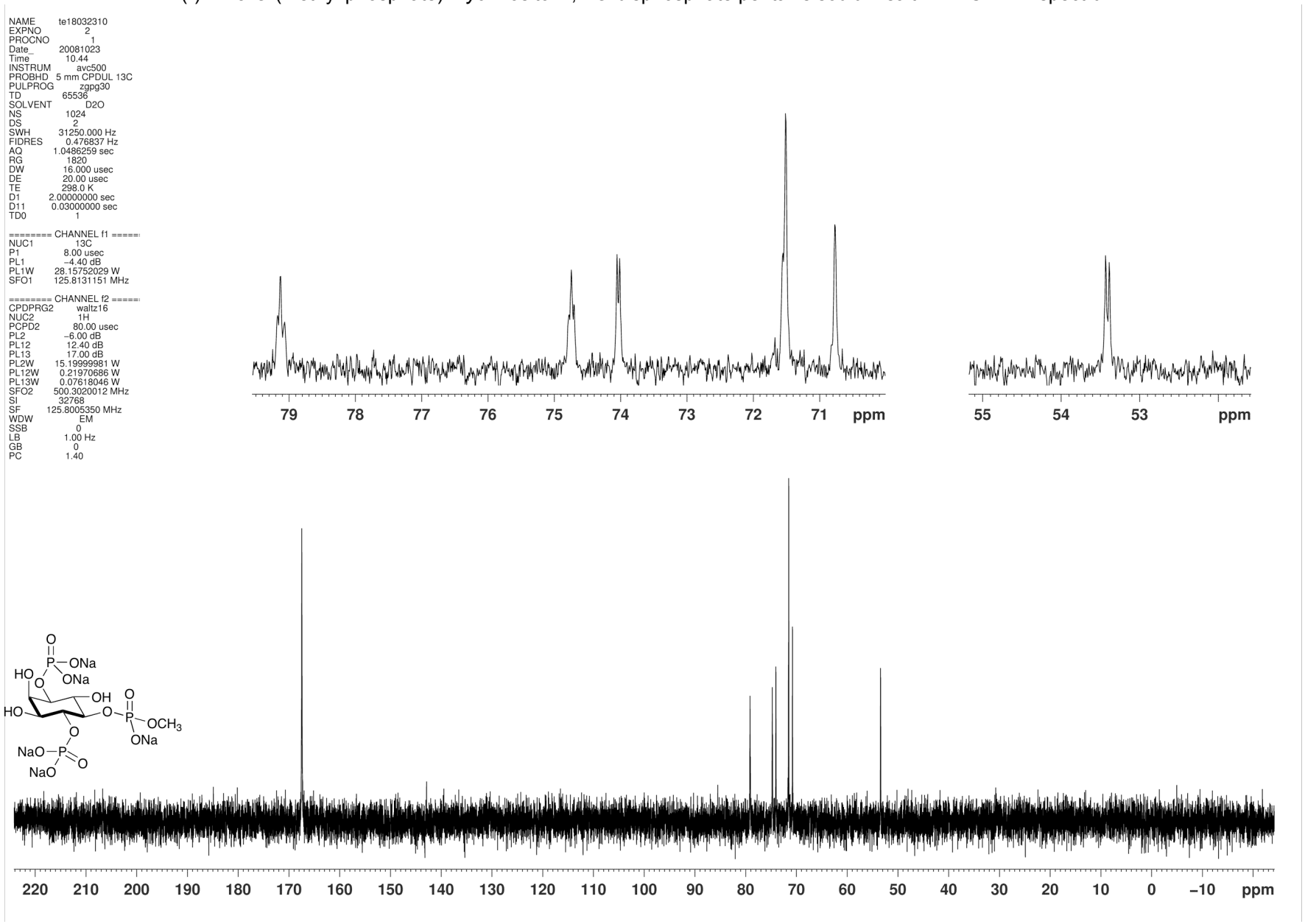
(-)-1D-5-*O*-(Methyl phosphate)-*myo*-inositol 1,4-*O*-bisphosphate pentakis sodium salt **2** – ^{1}H NMR spectrum



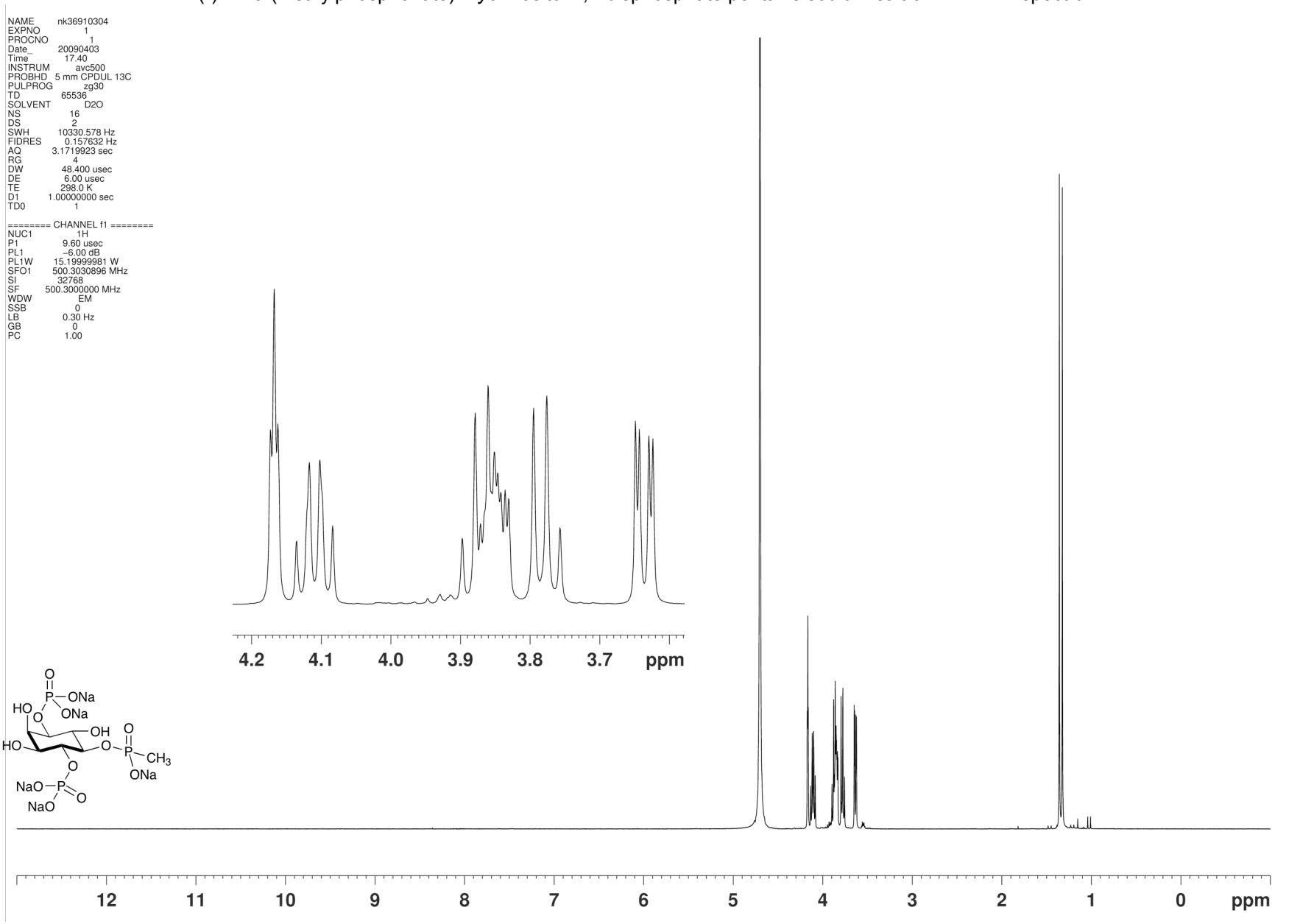
(-)-1D-5-*O*-(Methyl phosphate)-*myo*-inositol 1,4-*O*-bisphosphate pentakis sodium salt **2** – ^{31}P NMR spectrum



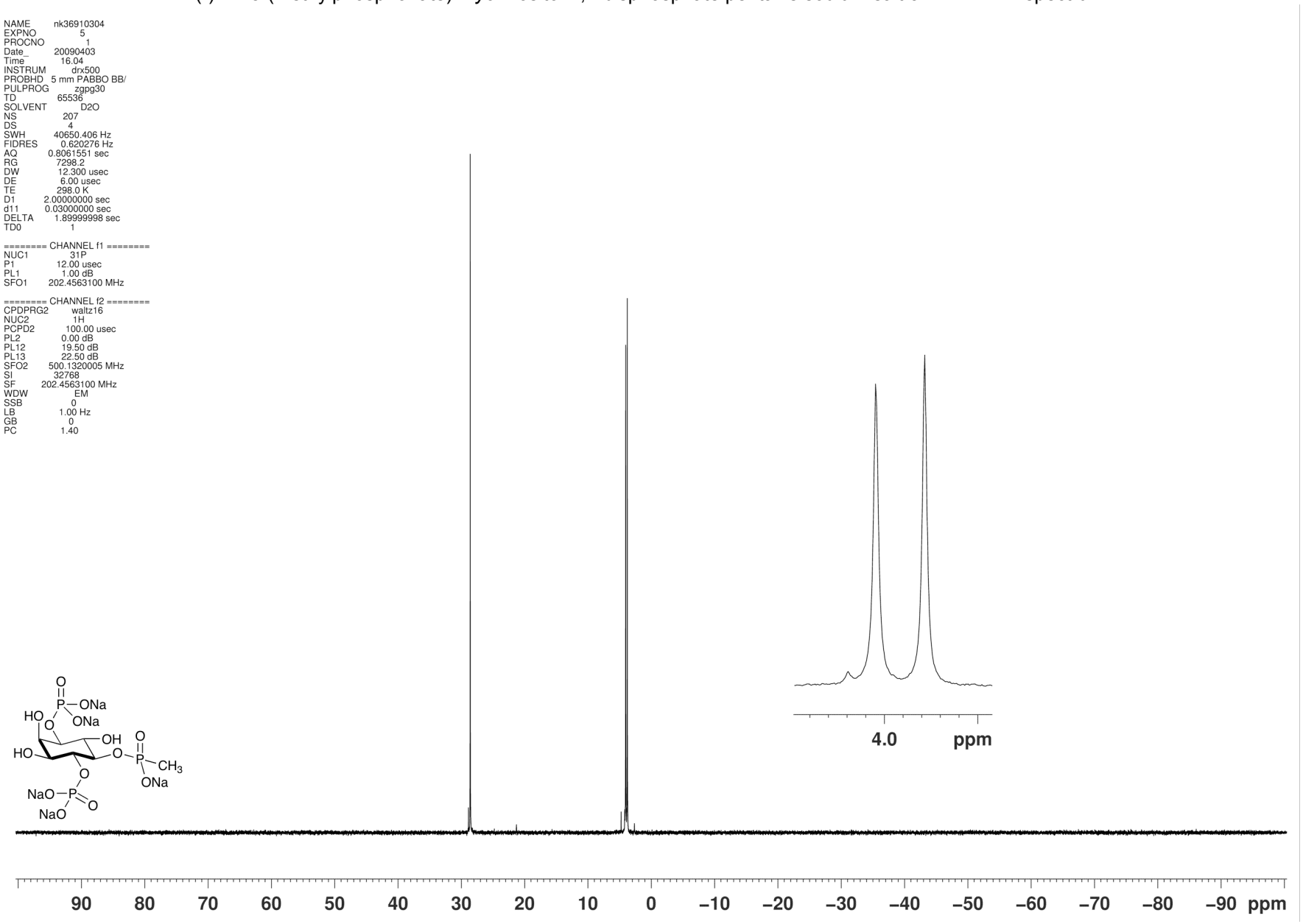
(-)-1D-5-*O*-(Methyl phosphate)-*myo*-inositol 1,4-*O*-bisphosphate pentakis sodium salt **2** – ^{13}C NMR spectrum



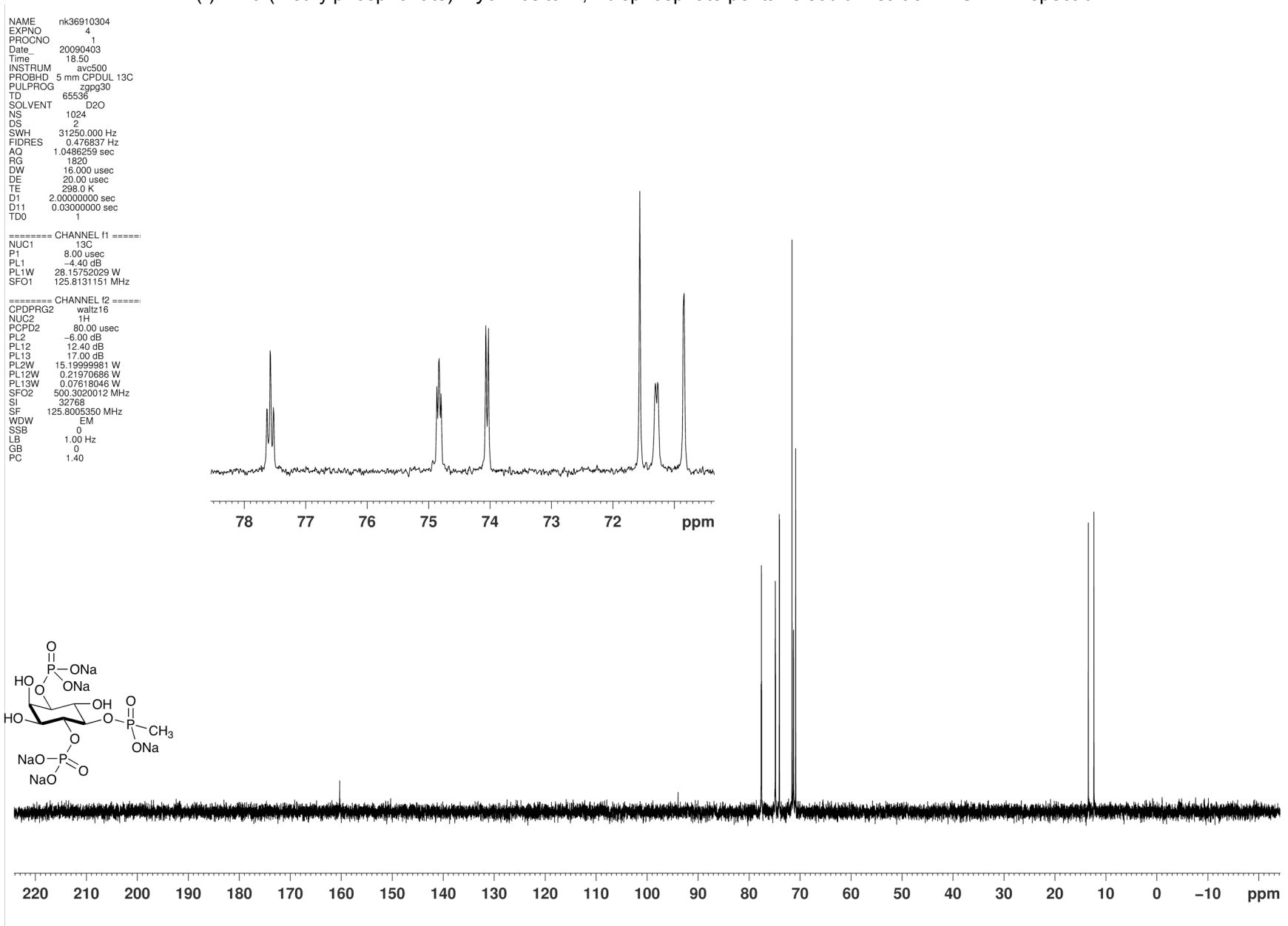
(-)-1D-5-(Methylphosphonate)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt **3** – ^{1}H NMR spectrum



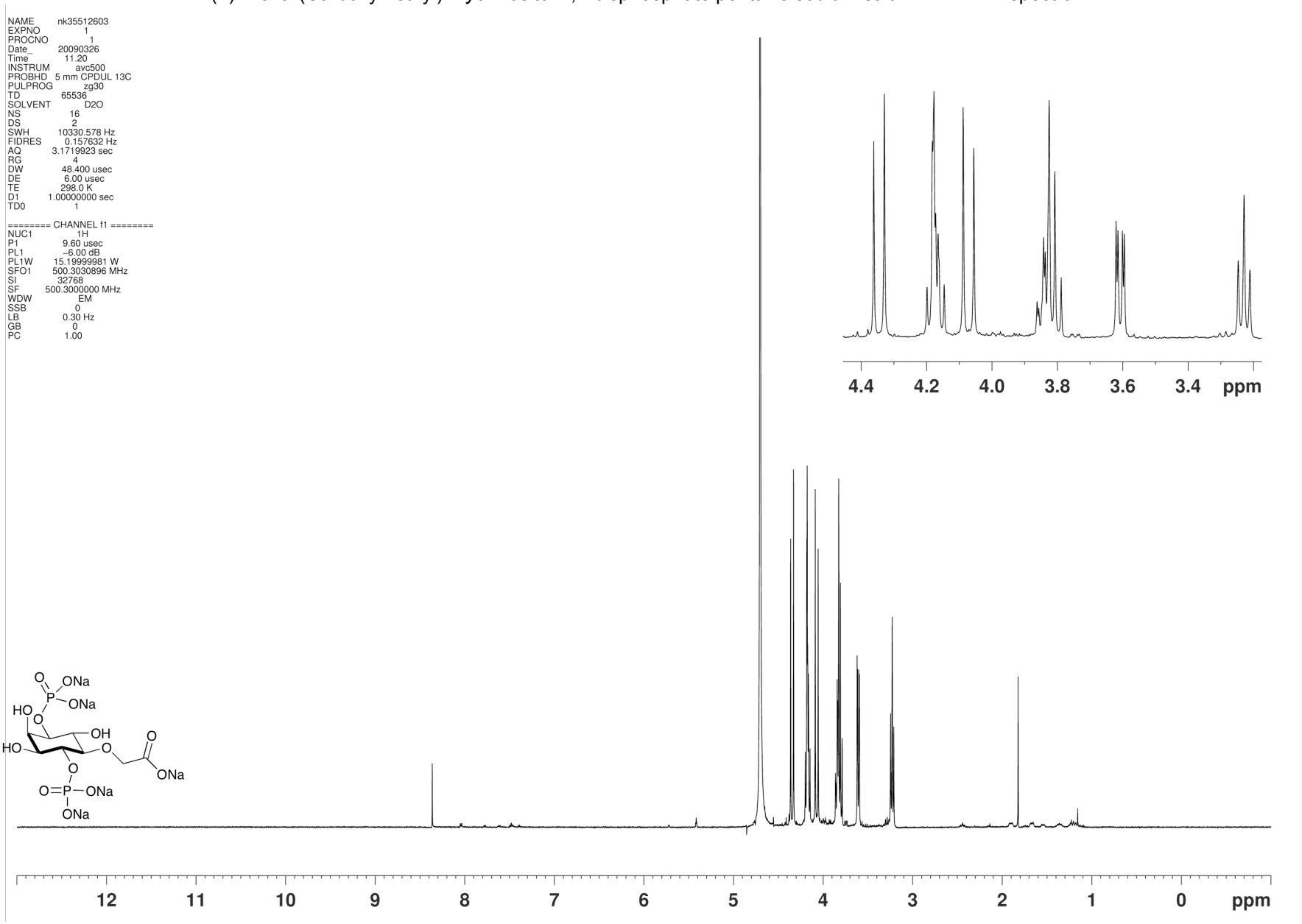
(-)-1D-5-(Methylphosphonate)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt **3** – ^{31}P NMR spectrum



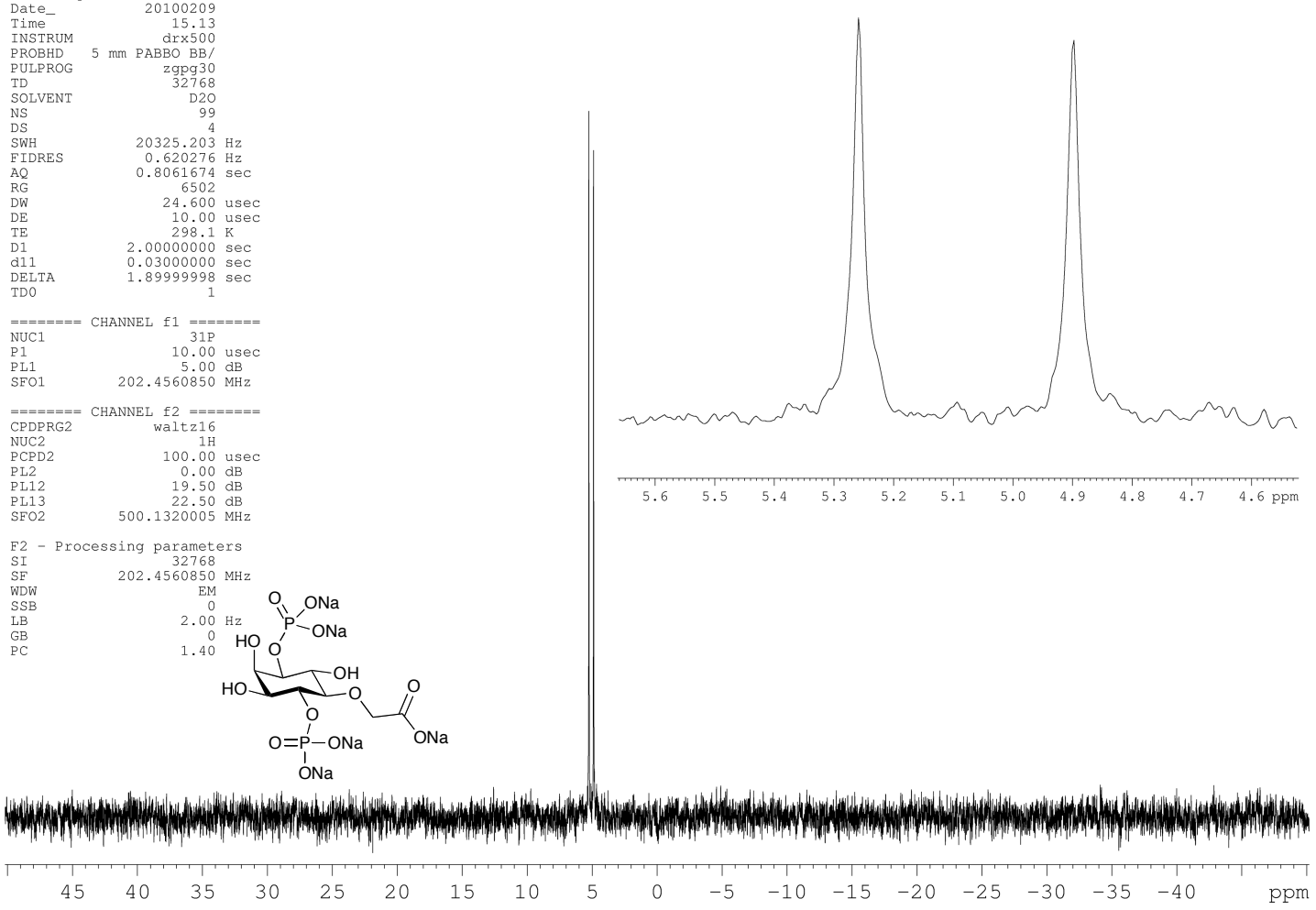
(-)-1D-5-(Methylphosphonate)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt **3** – ^{13}C NMR spectrum



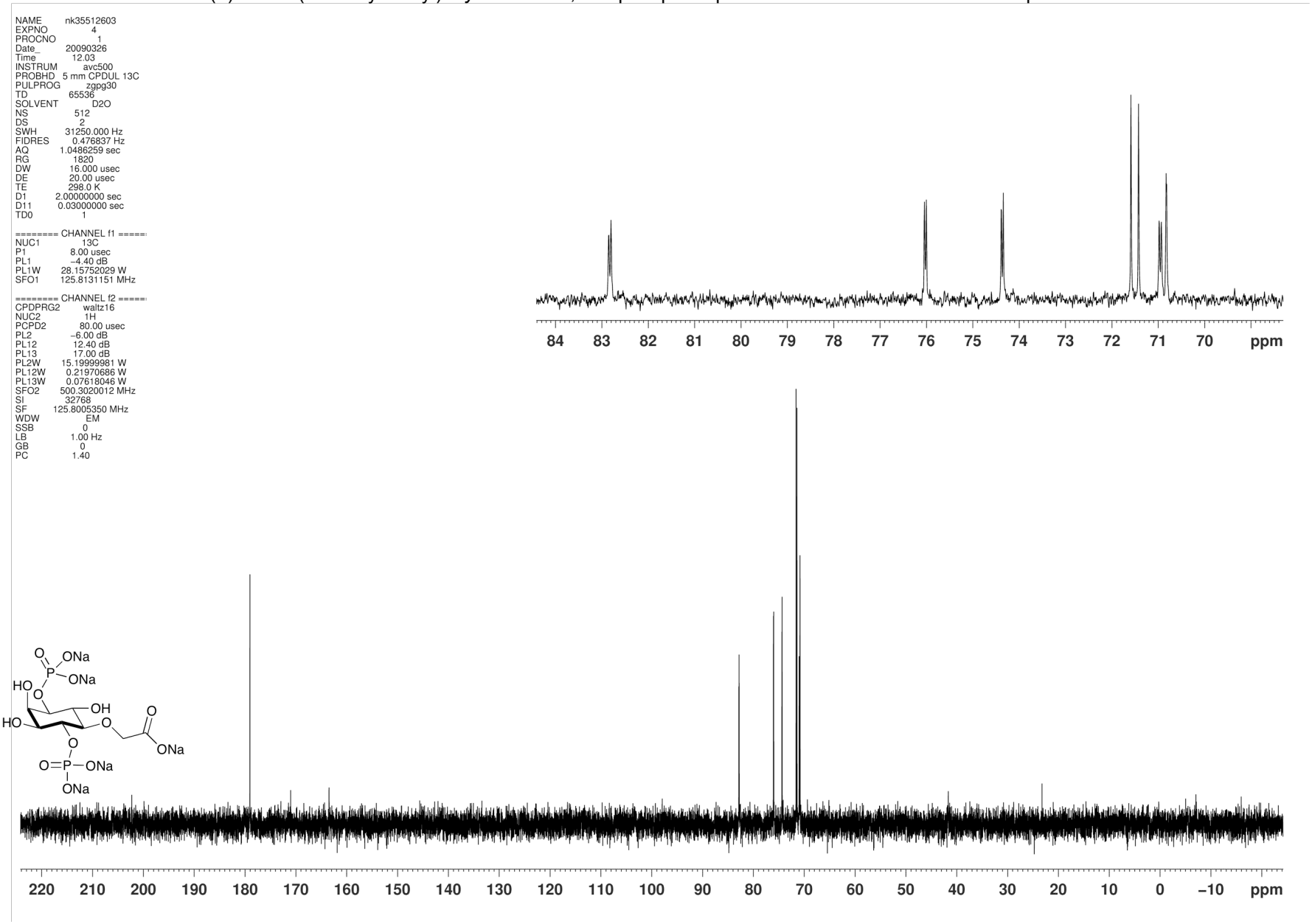
(+)-D-5-*O*-(Carboxymethyl)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt **4 –** ^{1}H NMR spectrum



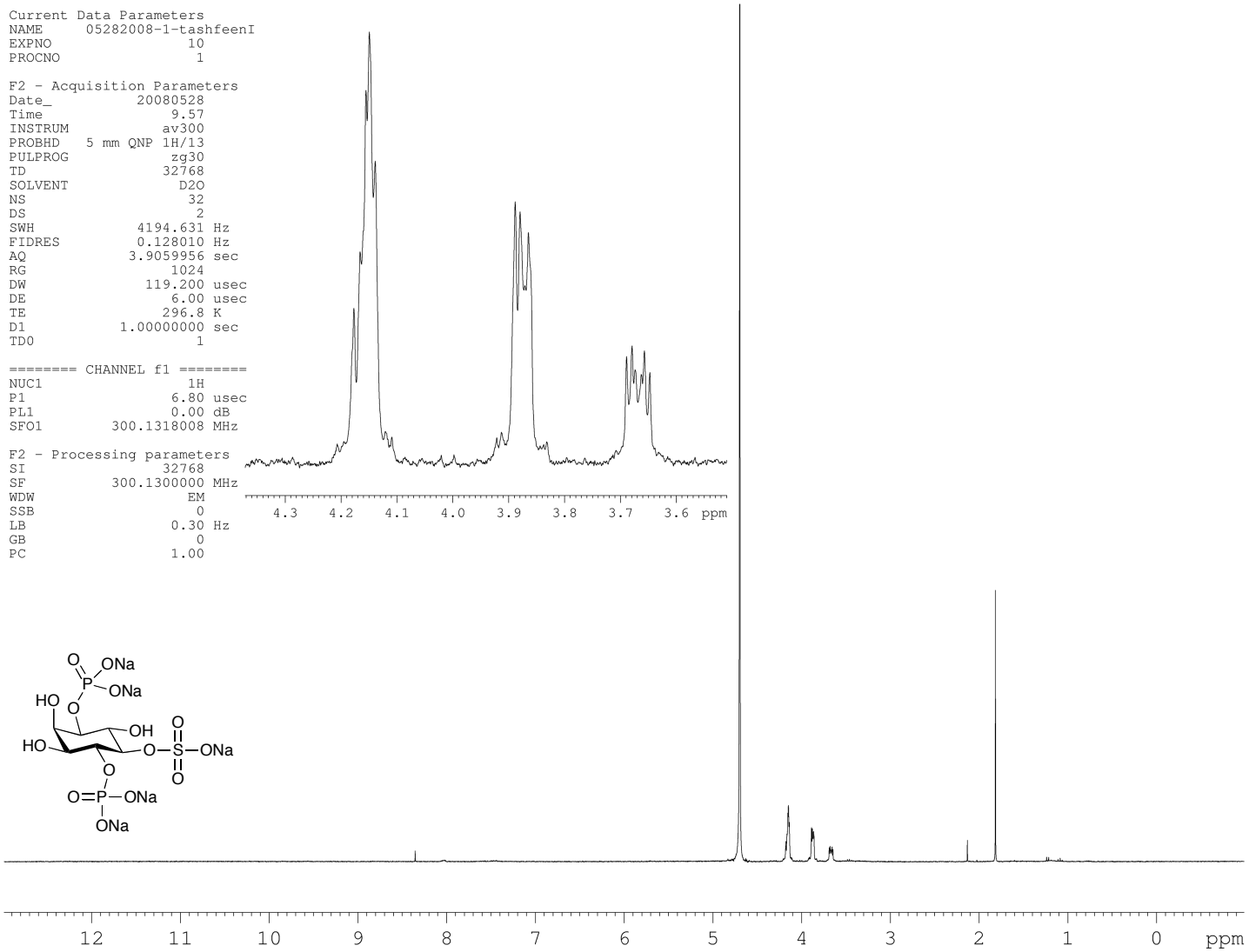
(+)-D-5-*O*-(Carboxymethyl)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt **4 –** ^{31}P NMR spectrum



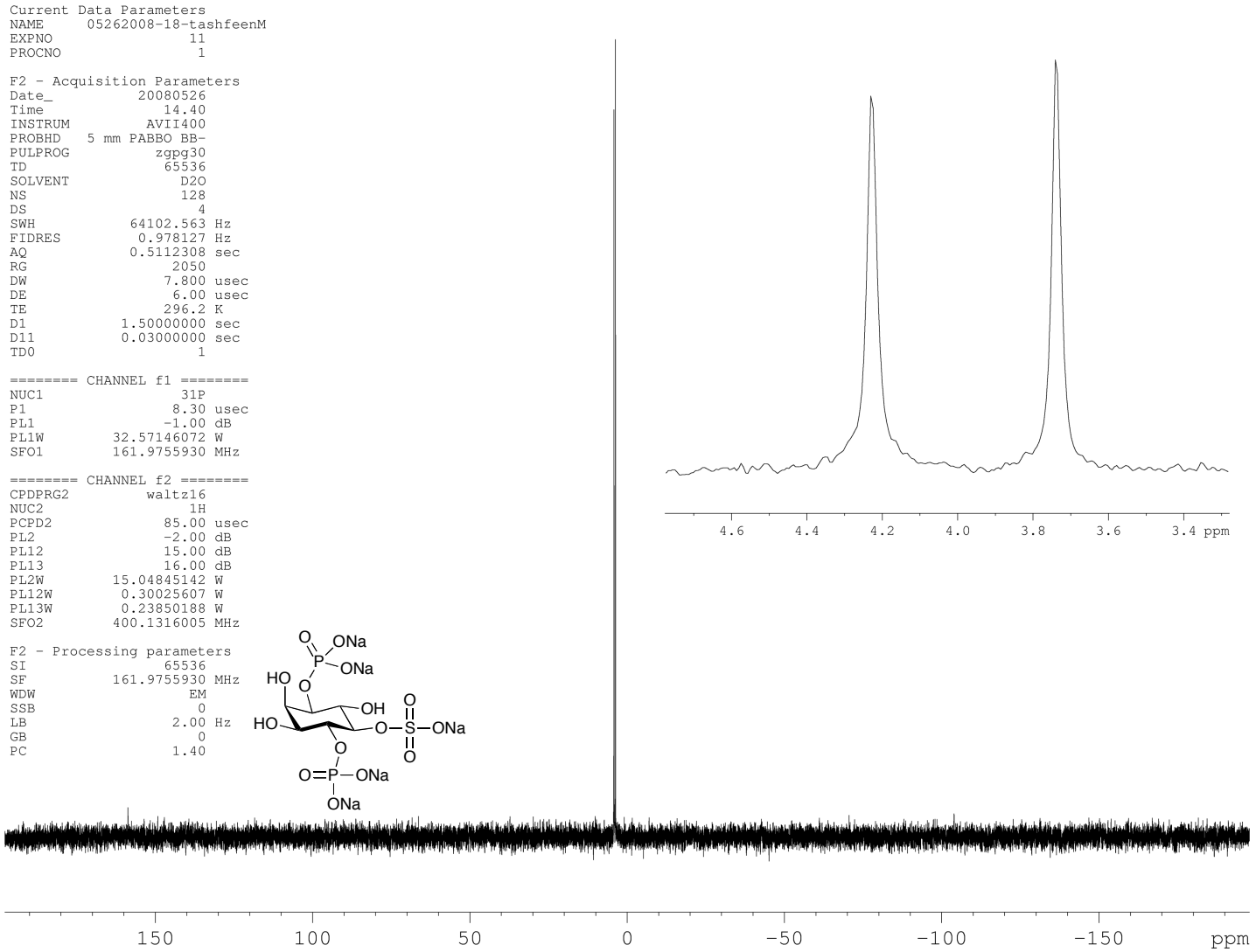
(+)-D-5-*O*-(Carboxymethyl)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt **4 –** ^{13}C NMR spectrum



(+)-1D-5-*O*-(Sulfono)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt **5** – ^{1}H NMR spectrum



(+)-1D-5-*O*-(Sulfono)-*myo*-inositol 1,4-bisphosphate pentakis sodium salt **5** – ^{31}P NMR spectrum



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