Supplementary Information

Synthesis of an α-phosphono-α,α-difluoroacetamide analogue of the diphosphoinositol pentakisphosphate 5-InsP₇

Andrew M. Riley, a Huanchen Wang, b Stephen B. Shears and Barry V. L. Potter a

a Medicinal Chemistry & Drug Discovery, Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT.
b Inositol Signaling Group, Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina, USA

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### Data collection and structure refinement statistics

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#### Data collection
- **Resolution (Å)**: 50-1.95 (1.98)
- **Space group**: P 2₁ 2₁ 2₁
- **Cell dimensions a, b, c (Å)**: 88.60 111.03 41.43
- **R_{meas}***: 0.098 (0.835)
- **CC1/2 in the highest shell**: 0.820
- **I/σI***: 23.38 (2.63)
- **Completeness (%)**: 96.87 (95.45)
- **Redundancy**: 6.1 (6.1)

#### Refinement
- **Resolution (Å)**: 1.95 (2.00)
- **R_{work}***: 0.175 (0.220)
- **R_{free}***: 0.214 (0.262)
- **No. atoms Protein**: 2539
- **No. atoms Ligands**: 108
- **No. atoms Solvent**: 296
- **B-factors (Å²) Overall**: 34.15
- **B-factors (Å²) Protein**: 32.79
- **B-factors (Å²) Ligands**: 43.77
- **B-factors (Å²) Solvent**: 42.30
- **R.m.s.d. Bond length(Å)**: 0.008
- **R.m.s.d. Bond Angle (°)**: 1.563
- **Ramachandran favored (%)**: 96.83
- **Ramachandran allowed (%)**: 2.86
- **Ramachandran outliers (%)**: 0.32
- **Rotamer outliers (%)**: 1.42
- **Clash score**: 2.30

*The numbers in parentheses are for the highest resolution shell.*
**Figure S1.** $^{31}$P NMR spectra of 5-PCF$_2$Am-InsP$_5$ (1) and 5-PCH$_2$Am-InsP$_5$ (1a) (triethylammonium salts in D$_2$O, 162 MHz, $^1$H-decoupled). The P-5 signal in 1 appears as a triplet, due to phosphorus-fluorine coupling. It has previously been reported$^1$ that the pKas for the second dissociation of phosphonic acids correlate well with the $^{31}$P NMR shift of the phosphonate P atom. Thus, the upfield shift of P-5 in 1 is consistent with increased acidity of its phosphonate group compared to the phosphonate in 1a. For the synthesis of 1a, see below.

Synthesis of 5-deoxy-5-(phosphonoacetamido)-myo-inositol 1,2,3,4,6-pentakisphosphate (1a)

Scheme. Synthesis of 5-PCH₂Am-InsP₅ (1a). Reagents and conditions: a. (EtO)₂P(O)CH₂COOH, EDAC, CH₂Cl₂, 79%; b. H₂ (50 p.s.i.) Pd(OH)₂/C, MeOH, THF, AcOH, 80%; c. i. (BnO)₂PNiPr₂, 5-phenyl-1H-tetrazole, CH₂Cl₂; ii. mCPBA, 67%; d. i. TMSBr, CH₂Cl₂; ii. MeOH; iii. aqueous triethylammonium bicarbonate, 85%. Bn, benzyl.

Compound 1a, the non-fluorinated equivalent of compound 1 was synthesised by reaction of amine 7 with commercially available diethyl phosphonoacetic acid in the presence of EDAC, giving phosphonoacetamide 8a. The five benzyl protecting groups were then removed by hydrogenolysis over Pd(OH)₂ on carbon to give pentaol 9a. When NMR data of 9a were obtained in D₂O, the ¹H NMR spectrum (see data below) showed that the CH₂ protons of the phosphonoacetamide group had partially exchanged with deuterium from D₂O over the time course of the NMR experiments. When this sample was used in the next step (phosphorylation), the deuterium was retained in the product 10a. After deprotection to give 1a, the extent of deuterium incorporation present did not change, even though the NMR sample of 1a in D₂O was kept for several days. This suggest that, even though deuterium exchange took place for the ethyl protected phosphonoacetamide in pentaol 9a, and was retained in 10a, no further exchange occurred in the deprotected product 1a, in which the phosphonate group is ionised and presumably any required enolisation is disfavoured. Indeed, mass spectrometry (see data below) showed that samples of 1a remained deuterated, even though 1a had been left to stand in (undeuterated) milliQ water, lyophilised, dissolved in milliQ water once more, distributed into vials and concentrated in a vacuum centrifuge over several hours. Attempts to exchange the deuterium in samples of 1a back to hydrogen by leaving samples in aqueous solution at high or low pH for extended periods have so far been unsuccessful. The incorporation of deuterium into the phosphonoacetamide unit of 1a may suggest strategies for developing tritiated versions of 1a and related analogues of PP-InsPs that may be useful in distinguishing potential receptor-mediated roles for such PP-InsPs in contrast to those involving phosphoryl transfer.
1,2,3,4,6-penta-O-benzyl-5-deoxy-5-(diethylphosphonoacetamido)-myo-inositol (8a). To a solution of amine 7 (178 mg, 0.283 mmol) and EDAC (82 mg, 0.428 mmol) in dry dichloromethane (3 mL) under N₂ was added a solution of diethyl phosphonoacetic acid (0.1 mL, 0.6 mmol) in dry dichloromethane (2 mL). The solution was stirred at room temperature for 3 h, after which time TLC (dichloromethane: methanol: triethylamine 1:1:1) showed total conversion of amine (Rf 0.23) into a slightly more polar product (Rf 0.13). Dichloromethane (15 mL) was added and the solution was washed with saturated NaHCO₃, 1.0 M HCl and brine (15 mL each), then dried (MgSO₄) and concentrated. The residue was purified by flash chromatography on silica (ethyl acetate in petrol, 0 to 100%) to give 8a as a white solid (181 mg, 0.224 mmol, 79%); Rf 0.30 (ethyl acetate:petrol 2:1); crystals from boiling disopropyl ether, m.p. 134–135 °C; ¹H NMR (400 MHz, CDCl₃) δ 1.11 (6 H, t, J 7.1, POCH₂CH₃), 2.57 (2 H, d, 2JCP 20.4 Hz, PCH₂), 3.42 (2 H, dd, J 9.3, 2.3 Hz, H-1 and H-3), 3.83–3.98 (6 H, m, POCH₂CH₃, H-4 and H-6), 4.02 (1 H, t, J 2.3 Hz, H-2), 4.10 (1 H, q, J 9.8 Hz, H-5), 4.54, 4.58 (4 H, AB quartet, JAB 11.7 Hz, 2 × OCH₂Ph), 4.71, 4.85 (4 H, AB quartet, JAB 11.5 Hz, 2 × OCH₂Ph), 4.86 (2 H, s, OCH₂Ph), 6.64 (1 H, d, J 9.3 Hz, NH), 7.20–7.33 (23 H, m, Ph), 7.40–7.42 (2 H, m, Ph); ¹³C NMR (101 MHz, CDCl₃) δ 16.11 (²JCP 6.0 Hz, POCH₂CH₃), 34.89 (d, ¹JCP 129.6 Hz, CH₂P), 54.28 (C-5), 62.55 (²JCP 6.4 Hz, POCH₂CH₃), 72.72 (2 × OCH₂Ph), 74.11 (OCH₂Ph), 74.54 (C-2), 74.66 (2 × OCH₂Ph), 79.33 (C-4 and C-6), 81.46 (C-1 and C-3), 127.22, 127.41, 127.60, 127.63, 127.67, 127.87, 128.20, and 128.33 (CH of Ph), 138.26 (2 × ipso-C of Ph), 138.89 (ipso-C of Ph), 138.99 (2 × ipso-C of Ph), 163.88 (d, ²JCP 3.1 Hz, C=O), ³¹P NMR (CDCl₃, 162 MHz, ¹H-decoupled) δ 22.67 (P-5); HRMS (m/z) [M + H]+ calcd. for C₄₇H₅₄NO₅P, 808.3609; found 808.3591.

5-deoxy-5-(diethylphosphonoacetamido)-myo-inositol (9a). To a solution of 8a (107 mg, 0.132 mmol) in methanol (5 mL), THF (5 mL), deionised water (1 mL) and acetic acid (1 mL) was added palladium hydroxide on activated charcoal (20%, 50% water, 50 mg). The suspension was shaken in a Parr hydrogenator under H₂ (50 p.s.i.) for 72 h. The catalyst was removed by filtration through a PTFE syringe filter and the resulting colourless solution was concentrated, then dried under vacuum to give the title compound as a white solid (38 mg, 0.106 mmol, 80%); TLC (dichloromethane/methanol 3:1): Rf 0.06; ¹H NMR (400 MHz, D₂O) δ 1.31 (6 H, t, J 7.1 Hz, POCH₂CH₃), 3.05–3.12 (0.2 H, m, CH₂P partially deuterated), 3.56–3.70 (5 H, m, H-1, H-3, H-4, H-5 and H-6), 4.06 (1 H, t, J 2.6 Hz, H-2), 4.17 (4 H, apparent pentet, J 7.2 Hz, POCH₂CH₃); ¹³C NMR (101 MHz, D₂O) δ 15.50 (²JCP 6.0 Hz, POCH₂CH₃), 56.04 (C-5), 64.15 (²JCP 6.5 Hz, POCH₂CH₃), 70.51 (2 × inositol ring CH), 71.92 (C-2 and 2 × inositol ring CH), 167.12 (d, ²JCP 5.9 Hz, C=O); ³¹P NMR (162 MHz, D₂O, ¹H-
decoupled) δ 24.47 (1 P, s, P-5); HRMS (m/z) [M + Na]^+ calcd. for C_{12}H_{22}D_{2}NO_{9}P (deuterated), 382.1206; found 382.1192.

5-deoxy-5-(diethylphosphonooacetamido)-myo-inositol 1,2,3,4,6-O-pentakis(dibenzylphosphate) (10a). To a stirred suspension of pentaol 9a (38 mg, 0.106 mmol) and 5-phenyl-1H-tetrazole (116 mg 0.794 mmol) in dry dichloromethane (3 mL) under N₂ at room temperature was added bis(benzyloxy)-diisopropylaminophosphine (0.25 mL, 0.72 mmol). The mixture was stirred under N₂ at room temperature for 3 h and then cooled to −78 °C, before mCPBA (57%, 320 mg, 1.06 mmol) was added. The mixture was allowed to warm to room temperature and then diluted with EtOAc (30 mL). The clear, colourless solution was washed with 10% w/v aq. Na₂SO₃ solution (2 × 35 mL), dried over MgSO₄ and concentrated. The residue was purified by flash chromatography (MeOH in dichloromethane, 0 to 5%) to give 10a as a colourless oil (117 mg, 0.071 mmole, 67%); TLC (dichloromethane:MeOH, 30:1): Rf 0.30; ¹H NMR (CDCl₃, 400 MHz) δ 1.19 (6 H, t, J 7.1 Hz, POCH₂CH₃), 2.85–2.91 (~0.3 H, m, CH₂P partially deuterated), 3.98–4.12 (4 H, m, 2 × POCH₂CH₃), 4.23 (1 H, broad m, H-5), 4.39 (2 H, tt, J 9.4, 2.1 Hz, H-1 and H-3), 4.86–5.10 (20 H, m, POCH₂Ph, H-4 and H-6), 5.15–5.19 (2 H, POCH₂Ph), 5.65 (1 H, dt, J 9.1, 2.4 Hz, H-2), 6.87(1 H, d, J 9.1 Hz, NH); 7.14–7.28 (50 H, m, Ph); ¹³C NMR (101 MHz, CDCl₃) δ 16.28 (d, J_CP 3.1 Hz, POCH₂CH₃), 53.04 (broad, C-5), 62.28 (d, J_CP 6.0 Hz, POCH₂CH₃), 69.67–70.05 (overlapping signals with J_CP couplings, POCH₂Ph), 74.49 (with J_CP couplings, C-1 and C-3), 74.68 (with J_CP couplings, C-4 and C-6), 76.21 (with J_CP couplings, C-2), 127.85, 127.89, 128.08, 128.12, 128.16, 128.24, 128.37, 128.44, 128.47 and 128.50 (CH of Ph), 135.58–135.79 (overlapping signals with J_CP couplings, ipso-C of Ph), 166.31 (J_CP 3.1 Hz, C=O); ³¹P NMR (162 MHz, CDCl₃, ¹H-decoupled) δ −2.35 (1 P, P-2), −1.68 (2 P), −0.81 (2 P), 21.57 (1 P, P-5).

5-deoxy-5-(phosphonoacetamido)-myo-inositol 1,2,3,4,6-pentakisphosphate (1a). A stirred solution of 10a (68 mg, 41 μmole) in dry dichloromethane (2 mL) was cooled to 0 °C under N₂ and trimethylsilyl bromide (1 mL) was added dropwise over 5 min. The solution was allowed to warm gradually to room temperature, and stirring was continued for 48 h. The solution was concentrated and methanol (5 mL) was added to the residue. The resulting colourless solution was stirred at room temperature for a further 1 h, then concentrated to give a white gum. The gum was washed with diethyl ether (3 × 2 mL), then taken up in aqueous TEAB (1.0 moldm⁻³, pH 7.6, 5 mL). The solution was then washed with diethyl ether (3 × 5 mL) and concentrated. The residue was re-dissolved in MilliQ water and lyophilised to give the triethylammonium salt of the title compound 11 as a white powder (46 mg, 35 μmole, 85 %); ¹H NMR (500 MHz,
D$_2$O) $\delta$ 1.27 (~56 H, t, $J$ 7.3 Hz, CH$_3$ of TEA$^+$), 2.77–2.82 (~0.3 H, m, CH$_2$P, partially deuterated), 3.18 (~34 H, q, $J$ 7.3 Hz, CH$_2$ of TEA$^+$), 4.06 (1 H, broad t, $J$ 9.6 Hz, H-5), 4.28 (2 H, t, $J$ = 9.5 Hz, H-1 and H-3), 4.45 (2 H, q, $J$ 9.8 Hz, H-4 and H-6), 4.85 (1 H, d, $J$ 10.0 Hz, H-2). $^{13}$C NMR (100 MHz, D$_2$O) $\delta$ 8.21 (CH$_3$ of TEA$^+$), 46.60 (CH$_2$ of TEA$^+$), 53.80 (C-5), 74.42 (with $J_{CP}$ couplings, C-1 and C-3), 74.89 (with $J_{CP}$ couplings, C-4 and C-6), 76.03 (with $J_{CP}$ couplings, C2), 170.56 (d, $^2J_{CP}$ approx. 6 Hz, C=O); $^{31}$P NMR (162 MHz, D$_2$O, $^1$H-decoupled) $\delta$ 0.24 (1 P, P-2), 0.72 (2 P), 1.03 (2 P), 14.11 (1 P, P-5); HRMS ($m/z$) [M – H]$^-$ calcd. for C$_6$H$_{19}$D$_2$NO$_{24}$P$_6$ (deuterated), 701.8932; found 701.8972.
Compound 1: $^1$H NMR (400 MHz, D$_2$O)

$^1$H NMR spectrum of Compound 1 showing the resonance peaks for H-1, H-2, H-3, H-4, and H-5.

Compound 1: $^{13}$C NMR (126 MHz, D$_2$O)

$^{13}$C NMR spectrum of Compound 1 showing the resonance peaks for C-1, C-2, C-3, C-4, C-5, C-6, and the TEA$^+$ and CF$_3$ groups.
Compound 1; $^{31}$P NMR (126 MHz, D$_2$O)

Compound 1; $^{19}$F NMR (471 MHz, D$_2$O)
Compound 1a; $^1$H NMR (500 MHz, D$_2$O)

Compound 1a; $^{13}$C NMR (100 MHz, D$_2$O)
Compound 1a; $^{31}$P NMR (162 MHz, D$_2$O, $^1$H-decoupled)

Compound 4; $^1$H NMR (400 MHz, CDCl$_3$)
Compound 4; $^{13}$C NMR (100 MHz, CDCl$_3$)

Compound 5; $^1$H NMR (400 MHz, CDCl$_3$)
Compound 5; $^{13}$C NMR (100 MHz, CDCl₃)

![Compound 5; $^{13}$C NMR (100 MHz, CDCl₃)](image)

Compound 6; $^1$H NMR (400 MHz, CDCl₃)

![Compound 6; $^1$H NMR (400 MHz, CDCl₃)](image)
Compound 6; $^{13}$C NMR (100 MHz, CDCl$_3$)

Compound 7; $^1$H NMR (400 MHz, CDCl$_3$)
Compound 7; $^{13}$C NMR (100 MHz, CDCl$_3$)

Compound 8; $^1$H NMR (400 MHz, CDCl$_3$)
Compound 8; $^{13}$C NMR (100 MHz, CDCl$_3$)

Compound 8; $^{31}$P NMR (162 MHz, CDCl$_3$, $^1$H-decoupled)
Compound 8; $^{19}$F NMR (471 MHz, CDCl$_3$)

Compound 8a; $^1$H NMR (400 MHz, CDCl$_3$)
Compound 8a; $^{13}$C NMR (100 MHz, CDCl$_3$)

Compound 8a; $^{31}$P NMR (162 MHz, CDCl$_3$, $^1$H-decoupled)
Compound 9; $^1$H NMR (400 MHz, D$_2$O)

Compound 9; $^{13}$C NMR (100 MHz, D$_2$O)
Compound 9; $^{31}$P NMR (162 MHz, D$_2$O, $^1$H-decoupled)

Compound 9; $^{19}$F NMR (471 MHz, D$_2$O)
Compound 9a; $^1$H NMR (400 MHz, D$_2$O)

Compound 9a; $^{13}$C NMR (100 MHz, D$_2$O)
Compound 9a; $^{31}$P NMR (162 MHz, D$_2$O, $^1$H-decoupled)

![NMR spectrum of 9a](image)

Compound 10; $^1$H NMR (400 MHz, CDCl$_3$)

![NMR spectrum of 10](image)
Compound 10; $^{13}$C NMR (100 MHz, CDCl$_3$)

Compound 10; $^{31}$P NMR (162 MHz, CDCl$_3$, $^1$H-decoupled)
Compound 10; $^{19}$F NMR (471 MHz, CDCl$_3$)

Compound 10a; $^1$H NMR (162 MHz, CDCl$_3$)
Compound 10a; $^{13}$C NMR (100 MHz, CDCl$_3$)

Compound 10a; $^{31}$P NMR (162 MHz, CDCl$_3$, $^1$H-decoupled)