Supporting Information

Scalable Chemoenzymatic Synthesis of Inositol Pyrophosphates

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Abbreviation

1,5(PP)₂-InsP₄  1,5-bisdiphosphoinositol tetrakisphosphate
1PP-InsP₅  1-diphosphoinositol pentakisphosphate
5PP-InsP₅  5-diphosphoinositol pentakisphosphate
Amp  ampicillin
ATP  adenosine triphosphate
CV  column volume
DTT  dithiothreitol
EDTA  ethylenediaminetetraacetic acid
FPLC  fast protein liquid chromatography
Gen  Gentamycin
HEPES  2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
InsP  inositol polyphosphate
InsP₆  inositol hexakisphosphate
IP6K1  (human) inositol hexakisphosphate kinase 1 (Genbank: AC099668)
IP6KA  inositol hexakisphosphate kinase A from Entamoeba histolytica (Genbank: XP_648490.2)
LIC  ligation independent cloning
Kan  Kanamycin
MBP  maltose binding protein
MES  2-(N-morpholino)ethanesulfonic acid
MOPS  3-morpholinopropane-1-sulfonic acid
NTA  nitriloacetic acid
OD₆₀₀  optical density at 600 nm
PAGE  polyacrylamide gel electrophoresis
PPIP5K₂KD  (human) diphosphoinositol pentakisphosphate kinase 2 kinase domain (Genbank: NP_001263206.1)
rt  room temperature
sumo  small ubiquitin like modifier
TB  Terrific broth medium
TRIS  tris(hydroxymethyl)-aminomethan
Supporting Figures

Figure S1. Superimposed pseudo-2D spin-echo difference spectra at different time points (black: 0 min, red: 8 min, blue: 20 min, green: 30 min). The InsP$_6$ and 5PP-InsP$_5$ peaks are labeled and change over time as indicated by the arrows.
Figure S2. PAGE purified 5PP-InsP₅ is contaminated with buffer components. Pure chemoenzymatically prepared 5PP-InsP₅ was mixed with all kinase reaction components except IP6KA and purified by PAGE followed by gel extraction. (a) $^1$H NMR spectrum shows several highly abundant species that are at least a 100 fold higher in intensity which precludes the observation of the inositol protons. (b) $^{31}$P NMR spectrum shows 5PP-InsP₅ and no other PP-InsP species.
**Figure S3.** *In vitro* pyrophosphorylation of NSR1 and pNSR1 using 5PP-InsP$_5$-β$^{32}$P prepared enzymatically and purified as described in Fig 4b.

**General Information**

All chemicals were purchased from Sigma Aldrich, VWR, Carl Roth, Thermo Fisher Scientific, Alfa Aesar, TCI and used without further purification unless stated otherwise. All dry solvents were purified using a solvent purification system MBRAUN MB-SPS-5 by passing through activated alumina columns. Deuterated solvents were purchased from Euriso-Top. The C18 reversed phase silica was purchased from Carl Roth. Telos® was ordered from Kinesis. Automated flash chromatography was performed using gradient grade solvents on a CombiFlash® Rf from Teledyne Isco using prepacked CombiFlash® columns (40–63 µm). LC-MS analysis was carried out with an Agilent 1260 Infinity Binary LC system connected to an Agilent 6130 Quadrupole LC/MS system with a ZORBAX Rapid Resolution HT Narrow Bore SB-C18 1.8 µm column (2.1 x 50mm) at 30 °C using API-ESI (atmospheric pressure ionization-electrospray) in positive ion mode. The eluent consisted of 10% ACN in water with 0.1% formic acid at 0.7 mL/min flow rate.

NMR spectra were recorded on Bruker spectrometers operating at 300 or 600 MHz for proton nuclei, 75 or 151 MHz for carbon nuclei or 122 and 244 MHz for phosphorous nuclei. NMR data are given as follows: chemical shift δ in ppm (multiplicity, coupling constant(s) J Hz, relative integral) where multiplicity is defined as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad or combinations of the above. Measurement for the determination of enzyme activity was performed on Bruker AV-III spectrometers (Bruker Biospin, Rheinstetten, Germany) at 310 K using cryogenically cooled 5 mm TCI-triple resonance probe equipped with one-axis self-shielded gradients. The software used to control the spectrometer was topspin 3.5 pl6. Temperature had been calibrated using d$_4$-methanol and the formula of Findeisen et al.¹
High-resolution mass spectrometry was performed by direct inject on a Xevo G2-XS QTof Quadrupole Time-of-Flight Mass Spectrometry (Waters), using negative ion mode.

For the purification via FPLC an NGC Quest™ 10 Chromatography System from Bio-Rad was used with an integrated NGC™ Sample Pump Module and a BioFrac™ Fraction Collector. For the spin filtration Amicon Ultra 0.5 mL centrifugal filters with a cut off of 10 kDa or 3 kDa from Merck Millipore were used.

Recombinant Protein Expression

Inositol Hexakisphosphate Kinase 1 (IP6K1)

The procedure is adapted from. In short: The codon optimized human IP6K1-gene was subcloned via LIC into a in a pET-MBP vector and transformed into E. coli arctic express (DE3). An 800 mL expression culture (37 °C) was inoculated at OD₆₀₀ 0.1 in TB-Amp/Gen and grown for 4 h. The culture was switched to 15 °C for 30 min the expression was induced with 0.1 mM IPTG. The Expression occurred overnight. The cells were harvested by centrifugation (3,000 g for 10 min at 4 °C) and washed with ice cold water. The pellet was stored at –80 °C until further use.

The frozen cells were resuspended in lysis buffer (20 mM Tris HCl pH 7.4, 150 mM NaCl). For 1 g wet weight 10 mL lysis buffer was used. The cell suspension was supplemented with lysozyme, DNase I and 1 tablet cOmplete™ protease inhibitor (Roche), and incubated for 15 min on ice. The cells were lysed with a Microfluidizer™ LM10 at 15,000 psi with five iterations. The lysate was clarified by centrifugation (30,000 g for 30 min at 4 °C). The supernatant was adjusted to 0.1 % (v/v) Triton X-100, filtered (VWR® vacuum filter, PES 0.45 µm), and loaded onto a Co-NTA column (GE, 1 mL, HiTrap IMAC HP) that was equilibrated with lysis buffer with a flowrate of 1 mL/min. The column was washed with wash buffer (20 mM Tris HCl pH 7.4, 500 mM NaCl, 50 mM imidazole, 0.1 % (v/v) Triton X-100) until the absorption was constant. IP6K1 was eluted with a gradient of elution buffer (20 mM Tris HCl pH 7.4, 500 mM NaCl, 500 mM imidazole, 0.1 % (v/v) Triton X-100) in wash buffer from 0–100 % over 10 CV. 1 mL fractions were collected. The fractions containing IP6K1 were concentrated and dialyzed overnight against dialysis buffer (20 mM Tris HCl pH 7.4, 500 mM NaCl, 1 mM DTT). The protein was adjusted to 20 % (v/v) glycerol and stored at –80 °C.
Inositol Hexakisphosphate Kinase A (IP6KA)

The procedure was adopted from.² In short: The codon optimized *Entamoeba histolytica* IP6KA-gene in a pET15b plasmid was transformed into *E. coli* BL21 Arctic Express (DE3) and an overnight culture of this strain was diluted into 1 L of TB-Amp/Gen to a final density of OD₆₀₀ 0.1. The cells were grown for 6 h at 37 °C. The culture was then switched to 18 °C for 30 min before induction with 0.1 mM IPTG for 18 hours. The cells were harvested by centrifugation (3,000 g for 10 min at 4 °C) and washed with ice cold water. The cell pellet was resuspended in lysis buffer (25 mM Tris HCl pH 7.4, 500 mM NaCl, 50 mM imidazole). For 1 g wet weight 10 mL lysis buffer was used. The cell suspension was supplemented with lysozyme, DNase I, and 1 tablet cOmplete™ protease inhibitor (Roche), and incubated for 15 min on ice. The cells were lysed with a Microfluidizer™ LM10 at 15,000 psi with five iterations. The lysate was clarified by centrifugation (30,000 g for 30 min at 4 °C). The supernatant was filtered (VWR® vacuum filter, PES 0.45 µm), and loaded onto a Ni-NTA column (GE, 5 mL, HiTrap IMAC HP) equilibrated with lysis buffer with a flowrate of 2.5 mL/min. The column was washed with lysis buffer until the absorption was constant. IP6KA was eluted with a gradient of elution buffer (25 mM Tris HCl pH 7.4, 200 mM NaCl, 500 mM imidazole) in lysis buffer from 0–100 % over 10 CV. 1.5 mL fractions were collected. The fractions containing IP6KA were concentrated by spin filtration (Amicon® Ultra 0.5 mL 10K) and dialyzed overnight against dialysis buffer (20 mM Tris HCl pH 7.4, 200 mM NaCl, 1 mM DTT). The following day the protein was adjusted to 25 % glycerol, frozen in liquid nitrogen and stored at –80 °C.

Diphosphoinositol Pentakisphosphate Kinase 2 Kinase Domain (PPIP5K2⁹⁴)

The codon optimized human PPIP5K⁹⁴-gene was subcloned from a pET21a vector into a pSumo vector using the restriction sites Ndel and Xhol. The resulting vector was transformed into *E. coli* Arctic Express (DE3) and an overnight culture of this strain was diluted into 2 L of TB-Kan/Gen to a final density of OD₆₀₀ 0.1. The cells were grown for 4 h at 37 °C. The culture was then switched to 15 °C for 30 min before induction with 0.2 mM IPTG for 18 hours. The cells were harvested by centrifugation (3,000 g for 10 min at 4 °C) and washed with ice cold water. The cell pellet was resuspended in lysis buffer (20 mM Tris HCl pH 7.5, 500 mM NaCl). For 1 g wet weight 10 mL lysis buffer was used. The cell suspension was supplemented with lysozyme, DNase I, and 2
tablets cOmplete™ protease inhibitor (Roche), and incubated for 15 min on ice. The cells were lysed using a Microfluidizer™ LM10 at 15,000 psi with five iterations. The lysate was clarified by centrifugation (30,000 g for 30 min at 4 °C). The supernatant was filtered (VWR® vacuum filter, PES 0.45 µm), and loaded onto a Ni-NTA column (GE, 5 mL, HiTrap IMAC FF) equilibrated with lysis buffer with a flowrate of 3.5 mL/min. The column was washed with 3 CV lysis buffer and 10 CV of 10 % elution buffer (20 mM Tris HCl pH 7.5, 500 mM NaCl, 600 mM imidazole) in lysis buffer or until the absorption was constant. PPIP5K2 was eluted with 100 % elution buffer over 10 CV. 1.4 mL fractions were collected. The fractions containing PPIP5K2 were dialyzed overnight against dialysis buffer (20 mM Tris HCl pH 7.4, 500 mM NaCl, 1 mM DTT). The following day the protein was adjusted to 20 % glycerol, frozen in liquid nitrogen and stored at −80 °C.
Chemoenzymatic Synthesis of Inositol pyrophosphates

The compound was synthesized as described before in two steps starting from *myo*-inositol. Analytical data were identical with the values reported in the literature.

**General Preparation for Enzymatic Synthesis**

For all enzymatic syntheses the following stock solutions were prepared:

- **Creatine kinase stock solution**: 350 U/mL creatine kinase in 200 mM MOPS pH 6.5, 20 mM MgCl₂, 20 mM DTT
- **IP6KA stock solution**: 10 mg/mL IP6KA in 20 mM Tris HCl pH 7.4, 200 mM NaCl, 1 mM DTT
- **PPIP5K²KD stock solution**: 10 mg/mL 20 mM Tris HCl pH 7.4, 500 mM NaCl, 1 mM DTT, 40% glycerol
- **ATP stock solution**: 100 mM in MilliQ® water pH 6.4; (Note: Concentration was determined via UV-Vis analysis at 259 nm; $\varepsilon_{259} = 15.4$ E/mmol/cm)

**Amberlite IRC-748 resin** was prepared as follows:

500 mL resin was placed on a big fritted filter and washed with 1L MeOH and 1L H₂O and stored at 4 °C in 20 % MeOH. For a 100 mg reaction roughly 30 mL of resin were placed on a filter, washed with 500 mL H₂O and acidified with 250 mL of 1 M HCl. The resin was washed with H₂O (usually 1–2 L) until it reached pH 6–7 and the resin was equilibrated with 250 mL 1 M NH₄HCO₃ pH 7.5–8. The resin was washed with 1 L H₂O and left dry on the filter until use.
Synthesis of 5PP-InsP₅

MilliQ® water was prewarmed to 37 °C by incubation in a water bath. A solution of InsP₆ (250 µM, 350 mg based on free acid 647.9 g/mol), MES (20 mM, pH 6.4), NaCl (50 mM), ATP (disodium salt, 2 mM), creatine phosphate (5 mM), MgCl₂ (7 mM), DTT (1 mM) in 2.16 L prewarmed MilliQ® water was prepared. The solution was evenly split among 3 × 1 L Schott bottles and the bottles were incubated in a water bath at 37 °C for 10 min. IP6KA (0.3 µM) and creatine kinase (1 U/mL) were added and the Schott bottles were gently inverted several times to homogenize and left to react for 30 min without shaking. (Note: The correct temperature was essential to assure full conversion of the starting material within 30 min. Prolonged reaction times above 1 h led to side reactions. However, the speed of the reaction will depend on the batch and quality of the recombinantly expressed IP6KA.)

Purification: The reaction was stopped by cooling the reaction mixture down to 4 °C within 5 min with the help of a dry ice isopropanol bath. A fritted filter was loaded with 21 g of C18 reversed phase silica gel suspended in MeCN and sand was added on top. The C18 plug was washed with 100 mL MeCN and 100 mL H₂O and the complete reaction mixture was passed through the filter under vacuum. The C18 plug was washed with 2 × 100 mL H₂O and all the combined flow through was supplemented with 50 equiv. of MgCl₂ (12.5 mM added; concentration based on the original reaction volume of 2.16 L) The pH was adjusted to 8.8–9.0 by drop wise addition of 10 mM NaOH solution which leads to precipitation of the PP-InsPs as magnesium complex within 1 h at room temperature. (The precipitation can also be performed overnight at room temperature. Note: Higher pH values than 9.3 should be avoided because this can lead to the precipitation of Mg(OH)₂.) The suspension was collected in 4 canonical 50 mL tubes by centrifugation (2 min at 3000 g) and the supernatant was removed.
The precipitate of each tube was washed 3 times with 15 mL MgCl$_2$ solution (8 mM, pH 9 adjusted with NaOH).

The precipitate of each tube was resuspended in 20 mL NH$_4$HCO$_3$ buffer (10 mM, pH 8) and vortexed with Amberlite® IRC-748 (20 mL wetted bed volume, pre-equilibrated with NH$_4$HCO$_3$, pH 7.5–8) until the precipitate dissolved. The buffer/resin suspension of each tube was added to a short Amberlite® IRC-748 column (5 mL bed volume, pre-equilibrated with NH$_4$HCO$_3$, pH 8) to remove excess Mg$^{2+}$. The product was flushed through the column with 50 mL water and all eluents were collected, combined and lyophilized in a round-bottom flask to afford the ammonium salt of the product as white solid. The solids were dissolved in D$_2$O and the concentration of the solution was determined by NMR against a standard (phosphonoacetic acid) to determine yield and purity: 30 mL of 14.3 mM solution were obtained which corresponds to 312 mg (80% yield, purity > 95%) 5PP-InsP$_5$ based on the free acid 726.9 g/mol. Occasionally, an organic impurity leaked from the Amberlite® resin can be observed at 82 ppm in the $^{13}$C NMR spectrum. This impurity can be readily removed by extraction with diethyl ether (3 times).

$^1$H NMR (300 MHz, Deuterium Oxide) δ 4.46 (q, $J = 9.4$ Hz, 2H), 4.27 (q, $J = 9.4$ Hz, 1H), 4.10 (t, $J = 9.6$ Hz, 2H).

$^{13}$C NMR (151 MHz, D$_2$O) δ 77.57, 75.81, 75.69, 73.20.

$^{31}$P NMR (243 MHz, Deuterium Oxide) δ 0.60, 0.53, -0.42, -10.31 (d, $J = 19.9$ Hz), -10.86 (d, $J = 19.9$ Hz).

HRMS (ESI) calculated for C$_6$H$_{17}$O$_7$P$_7$ (M$^+$)738.8204, found 738.8222.
Synthesis of 1PP-InsP₅

MilliQ® water was prewarmed to 37 °C by incubation in a water bath. A solution of InsP₆ (250 µM, 100 mg based on free acid 647.9 g/mol), MES (20 mM, pH 6.4), NaCl (250 mM), ATP (disodium salt, 2 mM), creatine phosphate (5 mM), MgCl₂ (6 mM), DTT (1 mM) in 617 mL prewarmed MilliQ® water was prepared in a 1 L Schott bottles and incubated in a water bath at 37 °C for 10 min. PPIP5K2KD (2 µM) and creatine kinase (1 U/mL) were added and bottle gently inverted several times to homogenize and left to react overnight for 18 h without shaking.

Purification: The reaction was stopped by cooling the reaction mixture down to 4 °C within 5 min with the help of a dry ice isopropanol bath. A fritted filter was loaded with 6 g of C₁₈ reversed phase silica gel suspended in MeCN and sand was added on top. The C₁₈ plug was washed with 30 mL MeCN and 30 mL H₂O and the complete reaction mixture was passed through the filter under vacuum. The C₁₈ plug was washed with 2 × 30 mL H₂O and all the combined flow through was supplemented with 50 equiv. of MgCl₂ (12.5 mM added; concentration based on the original reaction volume of 617 mL). The pH was adjusted to 8.8–9.0 by drop wise addition of 10 mM NaOH solution which leads to precipitation of the PP-InsPs as magnesium complex within 1 h at room temperature. (The precipitation can also be performed overnight at room temperature. Note: Higher pH values than 9.3 should be avoided because this can lead to the precipitation of Mg(OH)₂.) The suspension was collected in 2 canonical 50 mL tubes by centrifugation (2 min at 3000 g) and the supernatant was removed. The precipitates of each tube were washed 3 times with 15 mL MgCl₂ solution (8 mM, pH 9 adjusted with NaOH).

The precipitate of each tube was resuspended in 15 mL NH₄HCO₃ buffer (10 mM, pH 8) and vortexed with Amberlite® IRC-748 (15 mL wetted bed volume, pre-equilibrated with NH₄HCO₃, pH 7.5–8) until the precipitate dissolved. The buffer/resin suspension of each tube was added to a short Amberlite® IRC-748 column (5 mL bed volume, pre-
equilibrated with NH₄HCO₃, pH 8) to remove excess Mg²⁺. The product was flushed through the column with 40 mL H₂O and all eluents were collected, combined and lyophilized in a round-bottom flask. The resulting white solid was purified in 2 runs using a strong anion exchange column (HiPrep™ Q HP 16/10, GE Healthcare) and H₂O as buffer A and 1 M NH₄HCO₃ (pH 7.5–8) as buffer B. The column was washed with 100% B and equilibrated at 1% B. The sample was dissolved and loaded in 1% B and, followed by a gradient from 1% to 20% in 1 CV. The product was eluted in a gradient from 20%–40% over 10 CV. Fractions were analyzed by a metal dye detection assay in a 96-well plate format and product containing fractions were combined and lyophilized in a round-bottom flask. The solids were dissolved in D₂O and the concentration of the solution was determined by NMR against a standard (phosphonoacetic acid) to determine yield and purity: 20 mL of 5.3 mM solution were obtained which corresponds to 77 mg (68% yield, purity > 95%) 1PP-InsP₅ based on the free acid 726.9 g/mol.

**¹H NMR** (600 MHz, Deuterium Oxide) δ 5.08 (d, J = 9.8 Hz, 1H), 4.40 (p, J = 9.7 Hz, 2H), 4.21 (t, J = 9.8 Hz, 1H), 4.15 (q, J = 9.5 Hz, 1H), 4.09 (t, J = 9.2 Hz, 1H).

**¹³C NMR** (151 MHz, D₂O) δ 77.32, 76.11, 75.68, 75.58, 73.70, 73.08.

**³¹P NMR** (243 MHz, Deuterium Oxide) δ 1.64, 0.90, 0.40, 0.32, -0.94, -8.70, -10.89 (d, J = 18.7 Hz).

**HRMS** (ESI) calculated for C₆H₁₇O₂₇P₇ (M⁻)738.8204, found 738.8198.
Synthesis of 1,5(PP)₂-InsP₄

MilliQ® water was prewarmed to 37 °C by incubation in a water bath. A solution of 5PP-InsP₅ (250 µM, 100 mg based on free acid 726.9 g/mol), MES (20 mM, pH 6.4), NaCl (250 mM), ATP (disodium salt, 2 mM), creatine phosphate (5 mM), MgCl₂ (5 mM), DTT (1 mM) in 715 mL prewarmed MilliQ® water was prepared in a 1 L Schott bottles and incubated in a water bath at 37 °C for 10 min. PPIP5K²KD (1.5 µM) and creatine kinase (1 U/mL) were added and bottle gently inverted several times to homogenize and left to react overnight for 5.5 h without shaking.

Purification: The reaction was stopped by cooling the reaction mixture down to 4 °C within 5 min with the help of a dry ice isopropanol bath. A fritted filter was loaded with 6 g of C18 reversed phase silica gel suspended in MeCN and sand was added on top. The C18 plug was washed with 30 mL MeCN and 30 mL H₂O and the complete reaction mixture was passed through the filter under vacuum. The C18 plug was washed with 2 × 30 mL H₂O and all the combined flow through was supplemented with 50 equiv. of MgCl₂ (12.5 mM added; concentration based on the original reaction volume of 715 L). The pH was adjusted to 8.8–9.0 by drop wise addition of 10 mM NaOH solution which leads to precipitation of the PP-InsPs as magnesium complex within 1 h at room temperature. (The precipitation can also be performed overnight at room temperature. Note: Higher pH values than 9.3 should be avoided because this can lead to the precipitation of Mg(OH)₂.) The suspension was collected in 2 canonical 50 mL tubes by centrifugation (2 min at 3000 g) and the supernatant was removed. The precipitates of each tube were washed 3 times with 15 mL MgCl₂ solution (8 mM, pH 9 adjusted with NaOH).

The precipitate of each tube was resuspended in 15 mL NH₄HCO₃ buffer (10 mM, pH 8) and vortexed with Amberlite® IRC-748 (15 mL wetted bed volume, pre-equilibrated
with NH₄HCO₃, pH 7.5–8) until the precipitate dissolved. The buffer/resin suspension of each tube was added to a short Amberlite® IRC-748 column (5 mL bed volume, pre-equilibrated with NH₄HCO₃, pH 8) to remove excess Mg²⁺. The product was flushed through the column with 40 mL H₂O and all eluents were collected, combined and lyophilized in a round-bottom flask to afford the ammonium salt of the product as white solid. The solids were dissolved in D₂O and the concentration of the solution was determined by NMR against a standard (phosphonoacetic acid) to determine yield and purity: 12 mL of 12.7 mM solution were obtained which corresponds to 111 mg (77% yield, purity > 85%) 1,5(PP)₂-InsP₄ based on the free acid 805.9 g/mL. Occasionally, an organic impurity leaked from the Amberlite® resin can be observed at 82 ppm in the ¹³C NMR spectrum. This impurity can be readily removed by extraction with diethyl ether (3 times).

¹H NMR (600 MHz, Deuterium Oxide) δ 5.01 (d, J = 9.6 Hz, 1H), 4.55 (p, J = 9.9 Hz, 2H), 4.36 (q, J = 9.8 Hz, 1H), 4.25 (t, J = 9.4 Hz, 1H).

³¹P NMR (243 MHz, Deuterium Oxide) δ 0.56, 0.33, -0.26, -1.04, -10.34 (dd, J = 29.9, 19.2 Hz), -11.03 (dd, J = 60.0, 19.3 Hz).

¹³C NMR (151 MHz, D₂O) δ 77.42, 76.05, 75.53, 74.78, 73.53, 73.18.

HRMS (ESI) calculated for C₆H₁₇O₂₇P₇ (M⁻) 818.7868, found 818.7865.
Synthesis of 5PP-InsP$_5$-$\beta^{32}$P

InsP$_6$ (0.2 mM), MES (20 mM, pH 6.4), NaCl (50 mM), ATP (disodium salt, 0.2 mM), ATP$_{\gamma^{32}}$P (6000 Ci/mmol, 10 mCi/mL, Perkin Elmer), creatine phosphate (5 mM), MgCl$_2$ (2 mM), DTT (1 mM) in 500 µL MilliQ® was prepared and equilibrated at 37 °C for 10 min. IP6KA (0.3 µM) and creatine kinase (1 U/mL) were added and the tube was gently inverted several times to homogenize and left to react for 60 min without shaking. (Note: This procedure is also compatible with IP6K1 instead of IP6KA. However, the time needs to be adjusted for the lower activity of IP6K1. In our hands this was 90 min.)

For Purification: One short C18 column (SepPak Vac RC tC18 100 mg, Waters, WAT043410) was washed and equilibrated with 5 mL MeCN, then 5 mL H$_2$O. The reaction mixture was filtered through the SepPak column and the column was washed with 0.25 mL water aliquots until no radioactivity (low radioactivity compared to the filtrate) remained on the column. The filtrate and the precipitate from the previous step were combined and 0.25 M MgCl$_2$ was added to a final concentration of 16 mM. The pH was adjusted to 8.5–9 by dropwise addition of a 0.1 M NaOH-solution (The pH was checked with pH-stripes. Note: Higher pH values than 9.3 should be avoided because this can lead to the precipitation of Mg(OH)$_2$). The mixture was left at rt overnight to facilitate complete precipitation.

The next day the suspension was centrifuged (5 min at 5000 g) and the supernatant was removed. The precipitate was washed three times in 0.25 mL MgCl$_2$ solution (8 mM, pH 9 adjusted with NaOH) and the suspensions were centrifuged (5 min at 5000 g). The precipitate was resuspended in 0.1 mL NH$_4$HCO$_3$ buffer (10 mM, pH 8) and mixed with chelax 100 resin (0.3 mL wetted bed volume, pre-equilibrated with 10 mM NH$_4$HCO$_3$, pH 8; column: BD PP reactor 2 ml with PE frit) until the precipitate dissolved (this will take up to one hour). The buffer/resin suspension was added to a short chelex 100 column (1 mL bed volume, pre-equilibrated with 10 mM NH$_4$HCO$_3$, pH 8) to remove
excess Mg$^{2+}$. The product was flushed through the column with water until no radioactivity remained on the column and the eluent was collected (you should have < 3 mL). To the solution 10 X apyrase buffer and 4 U/ml apyrase (NEB) were added and the reaction was incubated overnight at rt. Another precipitation and chelation as described above followed. The elution of the chelex column was collected and concentrated in a speed-vac to afford the ammonium salt of the product as a concentrated solution usually between 50–100 µL. The preparation contained 0.65 µCi/µL and 0.26 Ci/mmol.

References


5PP-InsP₅
3¹P NMR
pD 5.0

1PP-InsP₅
1H NMR
pD 5.0
$1,5(PP)_2$-InsP$_4$

31P NMR

pD 5.0