Supporting Information

The inositol polyphosphates intersect with protein signaling and metabolic networks *via* two distinct mechanisms

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I. General Information

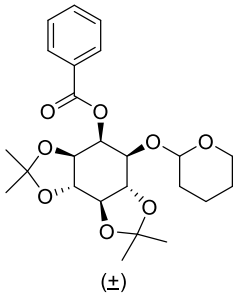
Commercial chemicals were purchased from Sigma-Aldrich or Alfa Aesar and used without further purification. Dichloromethane, diethyl ether and dimethylformamide (DMF) was dried by passing through activated alumina columns[1]. Thin layer chromatography (TLC) was performed on EMD Silica Gel 60 F_{254} plates and visualized by fluorescence quenching or $KMnO_4$ staining.

Automated column chromatography was performed using SiliCycle SiliaFlash F60 (40–53 μm) in RediSep® Rf cartridges and normal-phase silica flash columns on a CombiFlash® Rf from Teledyne Isco. Preparative high-performance liquid chromatography (HPLC) was performed on a Varian instrument with SD-1 analytical to prep solvent delivery modules, a ProStar 325 UV-Vis detector and a 440-LC fraction collector, using a Waters XBridge® column (5 μm, C18, 19 × 150 mm).

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AVANCE III spectrometer (500, 125 and 202 MHz respectively). NMR data are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, td = triplet of doublets, tq = triplet of quartets, m = multiplet), coupling constant (Hz) and integration. Deuterium chloride was added to adjust the pD for recording NMR spectra of compounds **9** and **13**. pD values were calculated from measured pH^* by using the equation: $pD = 0.929pH^* + 0.42$[2]. High resolution mass spectra (HRMS) were obtained on an Agilent 6220 spectrometer using electrospray ionization time-of-flight (ESI-TOF). 3% H_2O in CH_3CN with 0.1% formic acid was used for positive ion detection mode and 3% CH_3CN in H_2O with 0.1% formic acid was used for negative ions.

Affi-Gel® 15 bead and anti-mouse IgG with HRP Conjugate antibody was purchased from Bio-Rad Laboratories, Inc. Anti-GST antibody was purchased from Thermo Scientific. Protease inhibitor (cOmplete ULTRA Tablets), EDTA-free protease inhibitor (cOmplete ULTRA Tablets, EDTA-free) and phosphate inhibitor (PhosSTOP) were purchased from Roche. Restriction enzymes and T4 ligase were purchased from New England Biolabs.

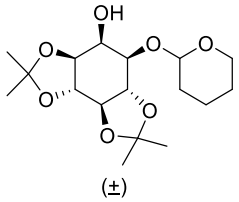
II. Chemical Synthesis



(±)

1(3)-*O*-(2-tetrahydropyranyl)-2-benzoyl-3(1),4(6):5,6(4)-di-*O*-isopropylidene-*myo*-inositol (S2)

To a solution of protected *myo*-inositol **S1**[3] (500 mg, 1.37 mmol) and pyridinium *p*-toluenesulfonate (34.5 mg, 0.137 mmol) was added 3,4-dihydro-2*H*-pyran (231 mg, 2.74 mmol) and the resulting solution was stirred overnight at room temperature. After addition of triethylamine (0.2 mL), the reaction was concentrated under vacuum and purified by flash chromatography (0% to 25% EtOAc in hexane) to give the title compound (566 mg, 92%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, integrations are reported as the sum of the two diastereomers (1:1) when peaks overlap): δ 8.05 (d, J = 7.4 Hz, 2H), 8.02 (d, J = 7.4 Hz, 2H), 7.59 (t, J = 7.5 Hz, 4H), 7.47 (t, J = 7.4 Hz, 2H), 7.46 (t, J = 7.4 Hz, 2H), 7.46 (t, J = 2.6 Hz, 2H), 4.99 (s, 1H), 4.93 (s, 3H), 4.30–4.18 (m, 4H), 4.00–3.90 (m, 4H), 3.74–3.62 (m, 4H), 3.55 (dt, J = 11.2, 3.3 Hz, 1H), 3.48 (dt, J = 11.3, 4.5 Hz, 1H), 1.65–1.33 (m, 36H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.7, 165.6, 133.4, 133.2, 130.1, 129.9, 129.8, 128.62, 128.57, 113.58, 113.56, 112.8, 112.7, 97.8, 95.4, 80.3, 78.7, 78.5, 78.3, 76.5, 76.5, 74.77, 74.75, 72.5, 71.4, 69.3, 65.6, 62.1, 61.6, 30.2, 29.9, 27.0, 26.94, 26.90, 26.88, 26.45, 26.41, 25.6, 25.3, 19.0, 18.1, 14.3; HRMS $[M+Na]^+$ calcd for $C_{24}H_{32}O_8Na^+$ 471.1990, found 471.1985.

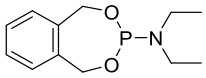


(±)

1(3)-*O*-(2-tetrahydropyranyl)-3(1),4(6):5,6(4)-di-*O*-isopropylidene-*myo*-inositol (4)

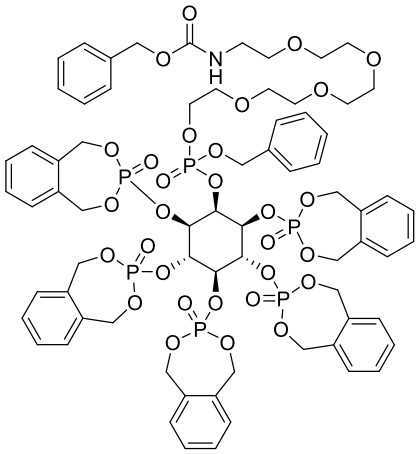
To a mixture of the benzoyl ester **S2** (475 mg, 1.06 mmol) and methanol (12 mL), was added NaOMe (4.24 mL, 0.5 M in methanol, 2.12 mmol) over 3 min, and the mixture was stirred

overnight at room temperature. Sat. aq. NH_4Cl (3 mL) and water (5 mL) were added to quench the reaction and MeOH was removed under vacuum. The mixture was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over Na_2SO_4, filtered, and the solvent was removed under vacuum. The residue was purified by flash chromatography (0% to 35% EtOAc in hexane) to give the title compound (313 mg, 86%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, integrations are reported as the sum of the two diastereomers (1:1) when peaks overlap): δ 4.86 (t, *J* = 3.7 Hz, 1H), 4.80 (dd, *J* = 6.4, 2.2 Hz, 1H), 4.53 (s, 1H), 4.49 (s, 1H), 4.17 (t, *J* = 9.4 Hz, 2H), 4.09–3.87 (m, 6H), 3.66–3.41 (m, 6H), 2.92 (s, 1H), 2.67 (s, 1H), 1.92–1.41 (m, 36H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 113.02, 112.97, 112.4, 112.3, 100.2, 95.5, 79.9, 79.5, 78.8, 77.5, 77.3, 77.0, 76.8, 76.6, 76.5, 76.4, 73.64, 73.60, 73.1, 67.9, 65.4, 64.2, 62.6, 30.9, 30.3, 26.9, 26.8, 26.7, 26.49, 26.47, 25.11, 25.06, 20.5, 19.3; HRMS $[M+Na]^+$ calcd for $C_{17}H_{28}O_7Na^+$ 367.1728, found 367.1722.



***N*,*N*-diethyl-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amine (7)**

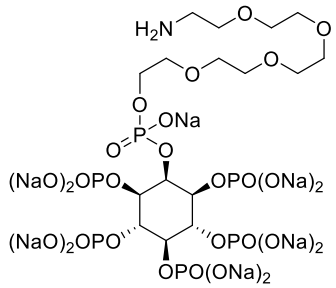
A mixture of hexaethyltriamidophosphite (5.00 g, 20.2 mmol) and 1,2-benzenedimethanol (2.79 g, 20.2 mmol) was heated to 100 ^{o}C to distill off diethylamine. After 30 min, the crude mixture was cooled to room temperature and purified by flash chromatography (2.5% triethylamine in hexane, 20 g silica gel) to give the title compound (2.92 g, 60%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28–7.26 (m, 2H), 7.23–7.21 (m, 2H), 5.19 (dd, *J* = 13.9, 6.9 Hz, 2H), 4.91 (dd, *J* = 19.6, 13.8 Hz, 2H), 3.19 (dq, *J* = 10.1, 7.1 Hz, 4H), 1.11 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.3, 128.2, 127.9, 65.9, 65.8, 38.7, 38.5, 15.12, 15.09; ^{31}P NMR (202 MHz, $CDCl_3$): δ 145.5 (s, 1P); HRMS $[M+H]^+$ calcd for $C_{12}H_{19}NO_2P^+$ 240.1148, found 241.1150.



2-*O*-[(Benzyloxycarbonylamino-pentaethyleneglycol)-benzyloxyphosphoryl]-1,3,4,5,6-penta-*O*-(*o*-xylylene)-phosphoryl-*myo*-inositol (8).

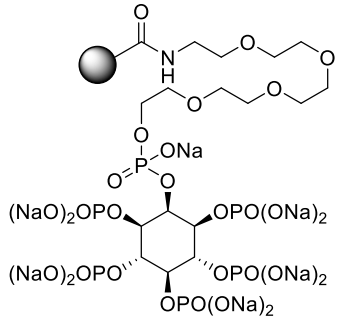
Phosphoramidite **5** (398 mg, 0.653 mmol) in CH_2Cl_2 (1.0 mL) was added to a mixture of alcohol **4** (150 mg, 0.436 mmol) and 5-phenyl-1*H*-tetrazole (95.5 mg, 0.654 mmol) in CH_2Cl_2 (3.0 mL) in an ice bath. The mixture was stirred for 2 h in an ice bath, followed by 18 h at room temperature. The reaction mixture was then placed in a dry ice/acetone bath for 15 min before mCPBA (293 mg, 77%, 1.31 mmol) was added. The mixture was stirred for 30 min in the dry ice/acetone bath, followed by 2 h at room temperature. EtOAc (30 mL) was added to dilute the mixture and the organic phase was washed with 10% $Na_2S_2O_3$ (10 mL), sat. aq. $NaHCO_3$ (10 mL) and sat. aq. NaCl (6 mL). The organic layer was dried over Na_2SO_4, filtered, concentrated under vacuum and purified by flash chromatography (0.5% to 4% MeOH in CH_2Cl_2) to give the crude linker attached to inositol compound **6** as a white solid. The crude intermediate was exposed to a TFA/CH_2Cl_2/H_2O (5:1:1, 3.5 mL) solvent mixture for 30 min at room temperature, concentrated under vacuum and dissolved in CH_2Cl_2 (3.0 mL). 5-phenyl-1*H*-tetrazole (168 mg, 1.15 mmol) and a solution of **7** (276 mg, 1.15 mmol) in CH_2Cl_2 (1.0 mL) was added to the intermediate in CH_2Cl_2 in an ice bath. The mixture was stirred for 2 h at an ice bath, followed by 18 h at room temperature. The reaction mixture was then placed in a dry ice/acetone bath for 15 min before mCPBA (516 mg, 77%, 2.30 mmol) was added. The mixture was stirred for 30 min at –78 ^{o}C followed by 2 h at room temperature. EtOAc (40 mL) was added and the organic phase was washed with 10% $Na_2S_2O_3$ (10 mL), sat. aq. $NaHCO_3$ (10 mL) and sat. aq. NaCl (6 mL). The organic layer was dried over Na_2SO_4, concentrated under vacuum and purified by flash chromatography (0.5% to 4% MeOH in CH_2Cl_2) to give the title compound (30 mg, 34% over

three steps) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.29 (m, 26H), 7.21 (dd, *J* = 7.1, 1.9 Hz, 1H), 7.15–7.11 (m, 3H), 5.71 (dd, *J* = 13.9, 12.1 Hz, 1H), 5.64–4.93 (m, 29H), 4.75 (dd, *J* = 22.8, 13.7 Hz, 1H), 4.36–4.31 (m, 1H), 4.28–4.22 (m, 1H), 4.28–4.22 (m, 16H), 3.33 (q, *J* = 5.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.6, 136.7, 135.9, 135.80, 135.77, 135.42, 135.36, 135.3, 135.19, 135.18, 135.10, 129.7, 129.6, 129.53, 129.45, 129.40, 129.39, 129.30, 129.26, 129.2, 129.0, 128.62, 128.60, 128.5, 128.3, 128.2, 128.0, 127.4, 74.0, 70.6, 70.51, 70.46, 70.4, 70.3, 70.2, 70.11, 70.06, 69.83, 69.77, 69.62, 69.56, 69.5, 69.42, 69.36, 69.30, 69.25, 67.91, 67.85, 66.8, 40.9; ^{31}P NMR (202 MHz, $CDCl_3$) δ –1.59 (s, 1P), –2.19 (s, 1P), –3.66 (s, 1P), –4.44 (s, 1P), –4.52 (s, 1P), –4.75 (s, 1P); HRMS $[M+2H]^{2+}$ calcd for $C_{71}H_{83}O_{30}NP_6^{2+}$ 807.6708, found 807.6714.



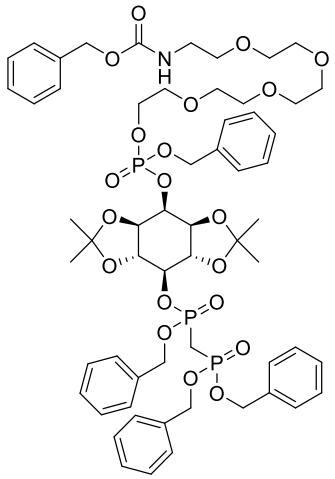
2-(Aminopentaethyleneglycol)-phosphate-1,3,4,5,6-penta-phosphate-*myo*-inositol hendecabasic sodium salt (9).

$NaHCO_3$ (14.9 mg, 0.177 mmol) and palladium black (41.1 mg, 0.388 mmol) were added to a solution of **8** (26.0 mg, 0.0161 mmol) in *t*-BuOH/H_2O (40:7, 3.0 mL) under an N_2 atmosphere before purging with an H_2 atmosphere. The mixture was stirred overnight and filtered through a pad of Celite®. The residue on Celite® was washed with ether (3 × 3 mL) followed by a water wash (4 × 2.5 mL). The water wash was filtered through a 0.2 μm nylon syringe filter and lyophilized to yield the title product (16.5 mg, 91%) as a white solid. ^{1}H NMR (500 MHz, D_2O, pD = 7.2): δ 4.28 (q, *J* = 9.5 Hz, 2H), 4.07–3.97 (m, 5H), 3.64–3.54 (m, 16H), 3.05 (t, *J* = 4.9 Hz, 2H); ^{13}C NMR (125 MHz, D_2O, pD = 7.2) δ 77.4, 76.8, 75.9, 73.2, 70.53, 70.48, 69.7, 69.6, 69.53, 69.47, 69.3, 66.5, 65.2, 65.1, 38.9; ^{31}P NMR (202 MHz, D_2O, pD = 7.2) δ 1.65 (s, 1P), 1.18 (s, 2P), 0.42 (s, 2P), –0.73 (s, 1P); HRMS $[M–2H]^{2–}$ calcd for $C_{16}H_{38}O_{28}NP_6^{2–}$ 438.4969, found 438.4969.



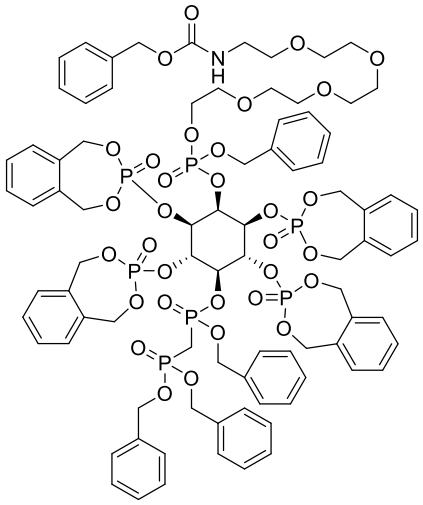
2-(*N*-(Affi-gel 15)aminopentaethyleneglycol)-phosphate-1,3,4,5,6-penta-phosphate-*myo*-inositol hendecabasic sodium salt (1).

Affi-Gel® 15 bead mixture (0.5 mL, 7.5 μmol) was washed with cold water (3 × 1.0 mL) and suspended in water (0.63 mL) at 4 °C. A solution of **9** (1.68 mg, 1.5 μmol) in cold water (150 μL), HEPES (250 mM, pH = 7.4, 200 μL) and Triton X-100 (0.5% (v/v), 20 μL) were added to the bead suspension, and the mixture was shaken overnight at 4 °C. After centrifugation, the supernatant was removed and the beads were washed with cold water (3 × 1.0 mL). The washed beads were treated by ethanolamine (50 mM, pH = 8.0, 1.0 mL) for 1 h at 4 °C and then washed with cold water (3 × 1.0 mL). The synthesized affinity reagent was stored in sodium azide (2% in water) with a total 1.0 mL volume at 4 °C. Supernatants from the reaction and wash were combined (approx. 4 mL in total), concentrated by lyophilization and analyzed by ^{31}P NMR to determine the yield (0.71 μmol, 47%), using tetramethylphosphonium bromide as an internal standard. The affinity reagent could be stored in water with 2% sodium azide at 4 °C for a few months without a significant loss in binding capabilities.



2-*O*-[(Benzyloxycarbonylamino-pentaethyleneglycol)-benzyloxyphosphoryl]-5-*O*-[[bis(benzyl- oxy)phosphorylmethyl]benzyloxyphosphoryl]-1,6:3,4-di-*O*-isopropylidene-*myo*-inositol (11).

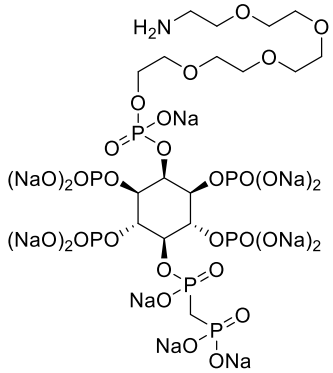
Phosphoramidite **5** (156 mg, 0.256 mmol) in CH_2Cl_2 (1.0 mL) was added to a mixture of the alcohol **10**[4] (118 mg, 0.171 mmol) and 5-phenyl-1*H*-tetrazole (37.6 mg, 0.258 mmol) in CH_2Cl_2 (3.0 mL) in an ice bath. The mixture was stirred for 2 h in an ice bath, followed by 18 h at room temperature. The reaction mixture was placed in a dry ice/acetone bath for 15 min before mCPBA (115 mg, 77%, 0.513 mmol) was added. The mixture was stirred for 30 min at –78 ^oC, followed by 2 h at room temperature. EtOAc (40 mL) was added to dilute the reaction mixture and the organic phase was washed with 10% $Na_2S_2O_3$ (10 mL), sat. aq. $NaHCO_3$ (10 mL) and sat. aq. NaCl (10 mL). The organic layer was dried over Na_2SO_4, concentrated under vacuum and purified by flash chromatography (0.5% to 3.5% MeOH in CH_2Cl_2) to give the title compound (168 mg, 81%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$, integrations are reported as the sum of the two diastereomers when peaks overlap): δ 7.40–7.23 (m, 50H), 5.25–4.47 (m, 26H), 4.28–4.16 (m, 4H), 4.03–3.96 (m, 2H), 3.68–3.52 (m, 38H), 3.37 (q, *J* = 5.3 Hz, 4H), 2.85–2.49 (m, 4H), 1.41–1.27 (m, 24H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.5, 136.64, 136.00, 135.95, 135.88, 135.8, 128.68, 128.66, 128.62, 128.58, 128.55, 128.52, 128.50, 128.44, 128.40, 128.37, 128.32, 128.30, 128.21, 128.13, 128.07, 127.99, 127.94, 127.88, 127.7, 113.3, 113.2, 77.3, 77.1, 76.8, 76.70, 76.66, 76.6, 76.3, 76.1, 75.3, 74.3, 73.51, 73.47, 70.7, 70.6, 70.54, 70.49, 70.4, 70.3, 69.99, 69.94, 69.87, 69.13, 69.09, 68.8, 68.7, 68.02, 67.98, 67.81, 67.76, 67.54, 67.48, 66.9, 66.8, 66.6, 40.9, 31.0, 27.1, 27.1, 27.0, 26.88, 26.85, 26.5, 26.39, 26.36, 26.33, 26.30, 26.04, 24.95; ^{31}P NMR (121 MHz, $CDCl_3$, integrations are reported as the sum of the two diastereomers when peaks overlap) δ 19.98 (s, 1P, minor), 19.89 (s, 1P, minor), 19.68 (d, *J* = 3.7 Hz, 1P, major), 19.60 (d, *J* = 3.7 Hz, 1P, minor), –1.64 (d, *J* = 2.0 Hz, 2P); HRMS $[M+H]^+$ calcd for $C_{59}H_{77}O_{20}NP_3^+$ 1212.4247, found 1212.4237.



2-*O*-[(Benzyloxycarbonylamino-pentaethyleneglycol)-benzyloxyphosphoryl]-5-*O*-[[bis(benzyl- oxy)phosphorylmethyl]benzyloxyphosphoryl]-1,3,4,6-tetra-*O*-(*o*-xylylene)phosphporyl-*myo*-inositol (12).

The protected inositol **11** (160 mg, 0.132 mmol) was treated with a TFA/CH_2Cl_2/MeOH (3:6:1, 5.0 mL) solvent mixture for 3 h in an ice bath, then concentrated under vacuum to yield the crude tetraol product. A solution of **7** (190 mg, 0.794 mmol) in CH_2Cl_2 (1.5 mL) was added to a mixture of the tetraol intermediate and 5-phenyl-1*H*-tetrazole (116 mg, 0.685 mmol) in CH_2Cl_2 (3.0 mL) in an ice bath. The mixture was stirred for 2 h in an ice bath, followed by 18 h at room temperature. The reaction mixture was then placed in a dry ice/acetone bath for 15 min before mCPBA (356 mg, 77%, 1.59 mmol) was added. The mixture was stirred for 30 min in the dry ice/acetone bath, followed by 2 h at room temperature. EtOAc (40 mL) was added to the reaction mixture and the organic phase was washed with 10% $Na_2S_2O_3$ (10 mL), sat. aq. $NaHCO_3$ (10 mL) and sat. aq. NaCl (6 mL). The organic layer was dried over Na_2SO_4, concentrated under vacuum and purified by flash chromatography (0% to 4% MeOH in CH_2Cl_2) followed by preparative HPLC (70% CH_3CN in H_2O for 1 min and a linear gradient from 70% to 85% CH_3CN in H_2O over 19 min, flow rate: 20 mL/min at an absorbance of 220 nm, t_R = 14.8 min) to give the title compound (85 mg, 34% over two steps) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, integrations are reported as the sum of the two diastereomers when peaks overlap): δ 7.48–7.45 (m, 4H), 7.48–7.45 (m, 4H), 7.40–7.14 (m, 72H), 7.13–7.11 (m, 2H), 6.95 (dd, *J* = 7.1, 1.8 Hz, 2H), 6.85 (dd, *J* = 7.6, 3.5 Hz, 2H), 5.74 (dd, *J* = 13.8, 11.7 Hz, 2H), 5.63–4.69 (m, 64H), 4.37–4.32 (m, 2H), 4.29–4.24 (m, 2H), 3.67–3.59 (m, 4H), 3.54–3.43 (m, 28H), 3.35 (d, *J* = 4.8 Hz,

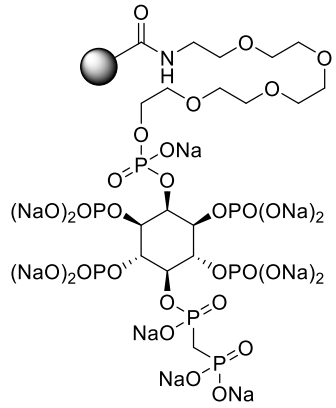
4H), 3.18–2.89 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.6, 136.7, 136.3, 136.24, 136.16, 136.11, 136.05, 136.0, 135.51, 135.46, 135.4, 135.3, 135.21, 135.19, 135.12, 135.09, 135.07, 135.01, 134.99, 134.94, 129.51, 129.50, 129.38, 129.36, 129.28, 129.27, 129.19, 129.16, 129.15, 129.04, 129.01, 128.99, 128.93, 128.87, 128.81, 128.74, 128.71, 128.66, 128.61, 128.59, 128.58, 128.56, 128.47, 128.42, 128.37, 128.34, 128.26, 128.24, 128.20, 128.15, 128.0, 127.2, 127.0, 76.6, 76.3, 76.1, 75.9, 74.2, 73.8, 70.6, 70.52, 70.50, 70.47, 70.45, 70.4, 70.3, 70.09, 70.07, 70.0, 69.9, 69.8, 69.58, 69.55, 69.53, 69.50, 69.46, 69.40, 69.36, 69.31, 69.25, 69.19, 68.84, 68.79, 68.7, 68.40, 68.35, 67.9, 67.8, 66.7, 40.9, 27.2, 26.1, 25.0; ^{31}P NMR (202 MHz, $CDCl_3$): δ 20.87 (s, 1P), 20.81 (s, 1P), 20.40 (s, 1P), 20.38 (s, 1P), –0.47 (s, 1P), –0.74 (s, 1P), –1.26 (s, 1P), –1.54 (s, 1P), –1.90 (s, 1P), –1.94 (s, 1P), –2.29 (s, 1P), –2.31 (s, 1P), –3.79 (s, 1P), –3.81 (s, 1P); HRMS $[M+2H]^{2+}$ calcd for $C_{85}H_{98}O_{32}NP_7^{2+}$ 930.7112, found 930.7102.



2-*O*-(aminopentaethyleneglycol-phosphoryl)-5-*O*-[(phosphoryloxy)phosphoryl]-1,3,4,6-penta-*O*-phosphate-*myo*-inositol dodecabasic sodium salt (13)

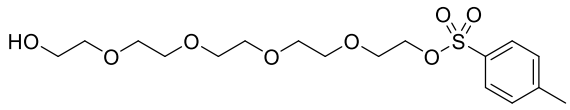
$NaHCO_3$ (21.1 mg, 0.251 mmol) and palladium black (54.5 mg, 0.505 mmol) were added to a solution of **12** (39.0 mg, 0.0210 mmol) in *t*-BuOH/H_2O (40:7, 5.0 mL) under an N_2 atmosphere before purging with an H_2 atmosphere. The mixture was stirred overnight and filtered through a pad of Celite®. The residue on Celite® was washed with ether (3 × 3 mL) followed by a water wash (4 × 2.5 mL). The water wash was filtered through a 0.2 μm nylon syringe filter and lyophilized to yield the title product (17 mg, 99%) as a white solid. ^{1}H NMR (500 MHz, D_2O, pD = 7.0): δ 4.76 (dt, *J* = 9.6, 2.6 Hz, 1H), 4.41 (q, *J* = 9.5 Hz, 2H), 4.32 (q, *J* = 9.4 Hz, 1H), 4.26 (tt, *J* = 9.6, 1.9 Hz, 2H), 3.98 (ddt, *J* = 6.6, 4.6, 2.6 Hz, 2H), 3.61–3.52 (m, 16H), 3.03 (t, *J* = 5.0 Hz, 2H), 2.43 (t, *J* = 20.9 Hz, 2H); ^{13}C NMR (125 MHz, D_2O, pD = 7.0) δ 75.9, 75.0, 73.1,

70.04, 69.98, 69.54, 69.46, 69.41, 69.38, 69.30, 66.26, 65.3, 65.2, 39.0, 28.0, 27.0, 26.0; ^{31}P NMR (202 MHz, D_2O, pD = 7.0): δ 18.92 (s, 2P), 0.12 (s, 2P), –0.31 (s, 2P), –1.34 (s, 1P); HRMS $[M–H]^-$ calcd for $C_{17}H_{41}O_{30}NP_7^-$ 955.9882, found 955.9879.



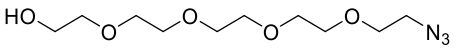
2-*O*-[*N*-(Affi-gel 15)aminopentaethyleneglycol-phosphoryl]-5-*O*-[(phosphoryloxy)phosphoryl]-1,3,4,6-penta-*O*-phosphate-*myo*-inositol dodecabasic sodium salt (2)

Affi-Gel® 15 bead mixture (0.5 mL, 7.5 μmol) was washed with cold water (3 × 1.0 mL) and suspended in water (0.63 mL) at 4 °C. A solution of **13** (1.83 mg, 1.50 μmol) in cold water (150 μL), HEPES (250 mM, pH = 7.4, 200 μL) and Triton X-100 (0.5% (v/v), 20 μL) was added to the bead suspension, and the mixture was shaken overnight at 4 °C. After centrifugation, the supernatant was removed and the beads were washed with cold water (3 × 1.0 mL). The washed beads were treated by ethanolamine (50 mM, pH = 8.0, 1.0 mL) for 1 h at 4 °C and then washed with cold water (3 × 1.0 mL). The synthesized affinity reagent was stored in sodium azide (2% in water) with a total 1.0 mL volume at 4 °C. Supernatants from the reaction and wash were combined (approx. 4 mL in total), concentrated by lyophilization and analyzed by ^{31}P NMR to determine the yield (0.65 μmol, 43%), using tetramethylphosphonium bromide as an internal standard. The affinity reagent could be stored in water with 2% sodium azide at 4 °C for a few months without a significant loss in binding capabilities.



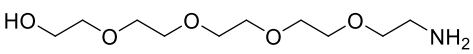
Pentaethyleneglycol-mono-*p*-toluenesulfonate (S3).

p-Toluenesulfonyl chloride (6.12 g, 32.1 mmol) was added in 6 portions to a cold suspension of pentaethylene glycol (7.50 g, 31.5 mmol) and silver oxide (10.9 g, 47.2 mmol) in CH_2Cl_2 (150 mL) in an ice bath. After the addition of potassium iodide (1.04 g, 6.27 mmol), the mixture was stirred for 18 h at room temperature and then filtered through Celite®. After the solvent was removed under vacuum, the compound was purified by flash chromatography (0% to 3.5% MeOH in CH_2Cl_2) to give the title compound (7.32 g, 59%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.73 (d, J = 8.0 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 4.09 (t, J = 4.8 Hz, 2H), 3.65–3.51 (m, 18H), 2.84 (s, 1H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.8, 132.8, 129.8, 127.9, 72.4, 70.6, 70.48, 70.47, 70.4, 70.2, 69.3, 68.5, 21.6; HRMS $[M+H]^+$ calcd for $C_{17}H_{29}O_8S^+$ 393.1578, found 393.1575.



Pentaethyleneglycol-monoazide (S4)

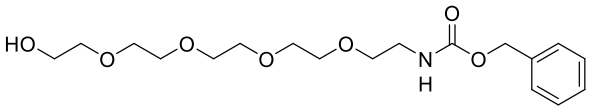
DMF (45 mL) was added to a mixture of the glycol tosylate **S3** (7.32 g, 18.7 mmol) and sodium azide (3.03 g, 46.6 mmol) under an N_2 atmosphere, and the reaction mixture was stirred at 70 °C for 12 h. After allowing the reaction to cool to room temperature, the mixture was concentrated under vacuum and purified by flash chromatography (1% to 2.5% MeOH in CH_2Cl_2) to give the title compound (3.46 g, 70%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.65–3.48 (m, 18H), 3.32–3.27 (m, 2H), 3.02–3.93 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 72.5, 70.6, 70.52, 70.49, 70.45, 70.42, 70.22, 70.20, 69.93, 69.91, 61.6, 61.5, 50.6, 50.5; HRMS $[M+Na]^+$ calcd for $C_{10}H_{21}N_3O_5Na^+$ 286.1374, found 286.1370.



Pentaethyleneglycol-monoamine (S5)

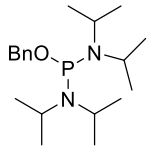
Palladium on carbon (10%, 150 mg) was added to a solution of the azide **S4** (1.50 g, 5.70 mmol) in MeOH (25 mL) under an N_2 atmosphere before purging with an H_2 atmosphere. The mixture was stirred overnight and filtered through a pad of Celite®. The filtrate was concentrated under

vacuum to yield the title compound (1.30 g, 96%) as a colorless oil. ^{1}H NMR (500 MHz, CD_3OD): δ 3.70–3.66 (m, 14H), 3.61–3.59 (m, 2H), 3.54 (t, J = 5.4 Hz, 2H), 2.76 (t, J = 5.4 Hz, 1H); ^{13}C NMR (125MHz, CD_3OD): δ 71.9, 69.8, 69.8, 69.7, 69.7, 69.6, 69.5, 60.5, 39.9; HRMS $[M+H]^+$ calcd for $C_{10}H_{24}NO_5^+$ 238.1649, found 238.1644.



Pentaethyleneglycol-mono(benzyloxycarbonyl)amine (S6)

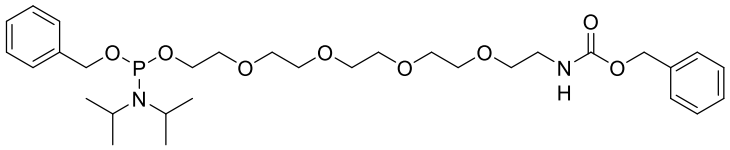
A solution of benzyloxycarbonyl chloride (1.05 mL, 1.26 g, 7.37 mmol) in THF (3.0 mL) was added to a mixture of amine **S5** (1.40 g, 5.90 mmol) in THF/H_2O (1:1, 20 mL) and $NaHCO_3$ (793 mg, 9.44 mmol) in an ice bath. After the mixture was stirred at room temperature overnight, THF was removed under vacuum. The resulting aqueous mixture was extracted with EtOAc (3 × 20 mL), and the combined organic extracts were washed with sat. NaCl (4 mL) and dried over Na_2SO_4. After concentrating under vacuum, the crude product was purified by flash chromatography (1% to 4% MeOH in CH_2Cl_2) to give the title compound (1.47 g, 67%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–7.29 (m, 5H), 5.82 (br s, 1H), 5.10 (s, 2H), 3.69–3.54 (m, 18H), 3.39 (t, J = 5.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.8, 136.9, 128.6, 128.3, 128.2, 72.7, 70.7, 70.6, 70.3, 66.7, 61.7, 41.0; HRMS $[M+Na]^+$ calcd for $C_{18}H_{29}NO_7Na^+$ 394.1837, found 394.1835.



Benzyloxyl-*N*,*N*-diisopropylphosphoramidite (S7)

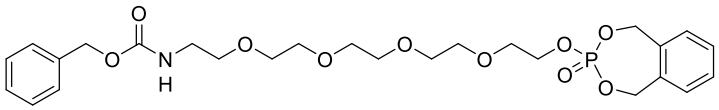
Pyridine (2.88 g, 36.4 mmol) was added to a solution of phosphorus trichloride (5.00 g, 36.4 mmol) in diethyl ether (40 mL) under an N_2 atmosphere. The mixture was cooled in a dry ice/acetone bath and benzyl alcohol (3.94 g, 36.4 mmol) in diethyl ether (20 mL) was added dropwise over 1h. After warming the reaction to room temperature, the mixture was stirred for 3 h and then filtered. The filtrate was cooled in a brine ice bath and diisopropylamine (15.1 g, 149

mmol) was added over 30 min. The reaction mixture was stirred overnight at room temperature and filtered. The filtrate was concentrated under vacuum and purified by flash chromatography (2.5% triethylamine in hexane, 20 g silica gel) to give the title compound (8.06 g, 65%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.42–7.27 (m, 5H), 4.69 (d, J = 7.3 Hz, 2H), 3.67–3.56 (m, 2H), 1.22 (t, J = 6.6 Hz, 24H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.7, 140.6, 128.2, 126.96, 126.95, 66.4, 66.2, 44.6, 44.5, 24.8, 24.7, 24.00, 23.96.; ^{31}P NMR (202 MHz, $CDCl_3$): δ 123.5 (s, 1P); HRMS $[M+H]^+$ calcd for $C_{19}H_{36}N_2OP^+$ 339.2560, found 339.2560.



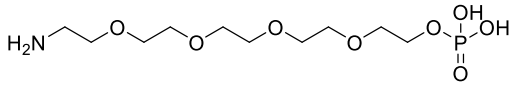
Benzyloxyl-(benzyloxycarbonylamino-pentaethyleneglycol)-*N*,*N*-diisopropylphosphoramidite (5)

A solution of alcohol **S6** (600 mg, 1.62 mmol) and phosphoramidite **S7** (547 mg, 1.62 mmol) in CH_2Cl_2 (3.0 mL) was added dropwise over 5 min to a mixture of 5-phenyl-1*H*-tetrazole (236 mg, 1.62 mmol) and CH_2Cl_2 (6.0 mL) in an ice bath. The reaction was stirred for 1 h at 0 ^{o}C, followed by 2 h at room temperature. After concentrating the reaction under vacuum, the crude product was purified by flash chromatography (1% triethylamine and 33% EtOAc in hexane, 8 g silica gel) to give the title compound (673 mg, 68%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38–7.29 (m, 10H), 5.36 (s, 1H), 5.09 (s, 2H), 4.74 (dd, J = 12.6, 8.2 Hz, 1H), 4.66 (dd, J = 12.6, 8.6 Hz, 1H), 3.82 (ddt, J = 11.1, 7.9, 5.6 Hz, 1H), 3.74 (ddt, J = 10.9, 8.5, 5.5 Hz, 1H), 3.68 – 3.57 (m, 16H), 3.55 (t, J = 5.1 Hz, 2H), 3.38 (q, J = 5.3 Hz, 2H), 1.18 (d, J = 6.8 Hz, 6H), 1.17 (d, J = 6.8 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.6, 139.62, 139.56, 136.7, 128.6, 128.34, 128.26, 128.2, 127.3, 127.1, 71.4, 71.3, 70.74, 70.70, 70.68, 70.60, 70.4, 70.1, 66.8, 65.5, 65.3, 62.7, 62.6, 43.1, 43.0, 41.0, 24.8, 24.7; ^{31}P NMR (202 MHz, $CDCl_3$): δ 147.4 (s, 1P); HRMS $[M+H]^+$ calcd for $C_{31}H_{50}N_2O_8P^+$ 609.3300, found 609.3304.



Benzyloxycarbonylamino-pentaethyleneglycol-*O*-xylylene-phosphate (S8)

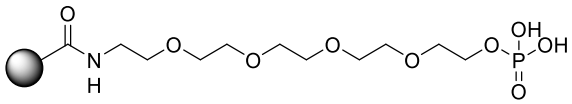
A solution of **7** (129 mg, 0.540 mmol) in CH_2Cl_2 (1.5 mL) was added to a mixture of the alcohol **S6** (100 mg, 0.270 mmol), 5-phenyl-1*H*-tetrazole (78.7 mg, 0.540 mmol) and CH_2Cl_2 (3.0 mL) in an ice bath. The mixture was stirred for 2 h in an ice bath, followed by 18 h at room temperature. The reaction mixture was then placed in a dry ice/acetone bath for 15 min before mCPBA (241 mg, 77%, 1.08 mmol) was added. The reaction was stirred for 30 min in the dry ice/acetone bath, followed by 2 h at room temperature. EtOAc (30 mL) was added and the organic phase was washed with 10% $Na_2S_2O_3$ (5 mL), sat. aq. $NaHCO_3$ (5 mL) and sat. aq. NaCl (5 mL). The organic layer was dried over Na_2SO_4, filtered, concentrated under vacuum and purified by preparative HPLC (20% CH_3CN in H_2O for 2 min and a linear gradient from 20% to 45% CH_3CN in H_2O over 18 min, flow rate: 20 mL/min at an absorbance of 238, t_R = 15.4 min) to give the title compound (60 mg, 40%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.30–7.19 (m, 9H), 5.33 (s, 1H), 5.20 (t, *J* = 13.2 Hz, 2H), 5.07 (dd, *J* = 18.9, 13.6 Hz, 2H), 5.02 (s, 2H), 4.30–4.15 (m, 2H), 3.67 (t, *J* = 4.6 Hz, 2H), 3.61–3.44 (m, 14H), 3.31 (q, *J* = 5.3 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.6, 136.8, 135.7, 129.3, 129.2, 128.6, 128.3, 128.2, 70.72, 70.68, 70.65, 70.6, 70.4, 70.2, 70.1, 70.0, 68.7, 68.6, 67.0, 66.9, 66.8, 41.0; ^{31}P NMR (202 MHz, $CDCl_3$): δ 1.10 (s, 1P); HRMS $[M+H]^+$ calcd for $C_{26}H_{37}O_{10}NP^+$ 554.2150, found 554.2144.



Amino-pentaethyleneglycol-phosphate (S9)

Palladium hydroxide on carbon (20%, 29 mg, 0.041 mmol) was added to a solution of **S8** (29.0 mg, 0.0523 mmol) in MeOH/H_2O (1:1, 3.0 mL) under an N_2 atmosphere before purging with H_2 gas. The mixture was stirred overnight and filtered through a pad of Celite®. The residue on Celite® was washed with MeOH/H_2O (1:1) and water wash was filtered through a 0.2 μm nylon syringe filter and lyophilized to yield the title product (16 mg, 96%) as a colorless oil. ^{1}H NMR (500 MHz, D_2O): δ 3.88 (q, *J* = 5.6 Hz, 2H), 3.67–3.61 (m, 16H), 3.11 (t, *J* = 4.7 Hz, 2H); ^{13}C

NMR (125 MHz, D_2O): δ 70.4, 70.3, 69.6, 69.54, 69.53, 69.49, 69.40, 66.35, 64.12, 64.08, 39.0; ^{31}P NMR (202 MHz, $CDCl_3$): δ 0.91 (s, 1P); HRMS $[M+H]^+$ calcd for $C_{10}H_{25}O_8NP^+$ 318.1313, found 318.1308.



***N*-(Affi-gel 15)amino-pentaethyleneglycol-phosphate (3)**

Affi-Gel® 15 bead mixture (0.5 mL, 7.5 μmol) was washed by cold water (3 × 1.0 mL) and suspended in water (0.63 mL) at 4 °C. A solution of **S9** (0.48 mg, 1.5 μmol) in cold water (150 μL), HEPES (250 mM, pH = 7.4, 200 μL) and Triton X-100 (0.5% (v/v), 20 μL) was added to the bead suspension, and the mixture was shaken overnight at 4 °C. After centrifugation, the supernatant was removed and the beads were washed by cold water (3 × 1.0 mL). The washed beads were treated by ethanolamine (50 mM, pH = 8.0, 1.0 mL) for 1 h at 4 °C and then washed by cold water (3 × 1.0 mL). The synthesized affinity reagent was stored in sodium azide (2% in water) with a total 1.0 mL volume at 4 °C. Supernatants from the reaction and wash were combined (approx. 4 mL in total), concentrated by lyophilization and analyzed by ^{31}P NMR to determine the yield (0.75 μmol, 50%) with tetramethylphosphonium bromide as an internal standard.

III. Cloning, Expression and Purification of Recombinant hDIPP1

hDIPP1 was amplified by PCR from cDNA using primers 5'-CAT GCC CGG GAA TGA AGC TCA AGT CGA ACC AG-3' and 5'-CAT GGA ATT CTC ATC TGA TGC CTG ACA TCG AG-3' containing SmaI and EcoRI restriction sites, respectively. The PCR product and the pGEX-2T vector were digested with SmaI and EcoRI, and ligated using T4 DNA ligase. The resulting construct was transformed into *E. Coli* BL21. The cloning of recombinant poly-His tagged hDIPP1 was described in our previous work[5]. Both GST-hDIPP1 and His-hDIPP1 were overexpressed by inducing with 100 μM isopropylthiogalactoside (IPTG) in LB media, and the proteins were purified from the cell lysate using glutathione beads (GE Healthcare) and Ni-NTA beads (Novagen) respectively.

IV. Peptide Preparation

Following resolution by SDS-PAGE and Coomassie staining (GelCode Blue, ThermoFisher Scientific), gel lanes containing protein eluates from the capture resins were excised, cut into 6 uniform slices per lane, then diced, and subjected to in-gel thiol reduction/alkylation and trypsin digestion using a method adapted from Shevchenko, et al[6]. Briefly, gel cubes were destained and washed extensively in 100 mM ammonium bicarbonate buffer, pH 8.8 (ABC), treated with 50 mM tris(2-carboxyethyl)phosphine (TCEP) in ABC for 1 h at 55°C, washed, subjected to alkylation with 55 mM iodoacetamide in ABC for 30 min at r.t. in the dark, washed, and finally digested overnight with ~10 ug trypsin endoproteinase (Promega Trypsin Gold, Promega, Madison, WI) per gel slice. Peptides were eluted from the gel pieces and then desalted using STAGE-Tips[7] prior to LC-MS analyses.

V. Peptide Sample Analysis Using High-Resolution nano-UPLC-MS

LC-MS/MS analyses were performed on a high-resolution, high-mass-accuracy, reversed-phase nano-UPLC-MS platform, consisting of an Easy nLC Ultra 1000 nano-UPLC system coupled to an Orbi Elite mass spectrometer (ThermoFisher Scientific) equipped with a Flex Ion source (Proxeon Biosystems, Odense, Denmark). LC was conducted using a trapping capillary column (150 μm x ca. 40 mm, packed with 3 μm, 100 Å Magic AQ C18 resin, Michrom, Auburn, CA) at

a flow rate of 5 µL/min for 4 min, followed by an analytical capillary column (75 µm x ca. 45 cm, packed with 3 µm, 100 Å Magic AQ C18 resin, Michrom) under a linear gradient of A and B solutions (solution A: 3% acetonitrile/ 0.1% formic acid; solution B: 97% acetonitrile/ 0.1% formic acid) from 5%-35% B over 180 min at a flow rate of 300 nL/ min. Nanospray was achieved using Picospray tips (New Objective, Woburn, MA) at a voltage of 2.4 kV, with the Elite heated capillary at 275 °C. Full-scan (m/z 335–1800) positive-ion mass spectra were acquired in the Orbitrap at a resolution setting of 120,000. MS/MS spectra were simultaneously acquired using CID in the LTQ for the top 15 most abundant multiply charged species in the full-scan spectra, having signal intensities of >1000 NL. Lockmass on environmental dodecamethylcyclohexasiloxane was employed, maintaining calibration to 2-3 ppm of accurate mass.

VI. Mass Spectrometric Data Analysis

Resultant LC-MS/MS raw data files were processed using Proteome Discoverer (v. 1.4, Thermo Fisher) for database search against the yeast SGD database using the Mascot search engine (v. 2.5, Matrix Science, London, UK.), allowing for error windows of ±6 ppm for parent ions and ± 1.2 Da for fragments, and specifying ≤ 2 missed trypsin cleavages, methionine oxidation, and *N*-terminal protein acetylation as variable modifications and carbamidomethylation of cysteines as a fixed modification. Peptide assignment cutoffs were specified at a high confidence level (<1% FDR), employing the Percolator[8] support vector machines node within the Proteome Discoverer framework, to set a strict peptide-level FDR using reversed database searching and parameter optimization. Relative abundance levels for proteins between experimental conditions were estimated using spectral counting. Raw mass spectra were visualized using Xcalibur (v. 2.2, Thermo Fisher) and peptide spectral matches were visualized using Proteome Discoverer. Identified captured proteins were characterized according to GO categories using the DAVID Bioinformatics Resources 6.7 at the National Institute of Allergy and Infectious Diseases (NIAID) (http://david.abcc.ncifcrf.gov/)[9].

VII. Arginine Composition Analysis

Using Python, each protein isolated with the affinity reagents, as well as the whole yeast proteome provided by UniProt, were analyzed for both the number of arginine residues and the percent composition of arginine per sequence. Wilcoxon signed-rank test and Welch's t-test were performed to compare the yeast proteome to our datasets. To meet the assumption of normally distributed data for Welch's t-test, the percent composition of arginine residues was log transformed. For the results see Dataset S3.

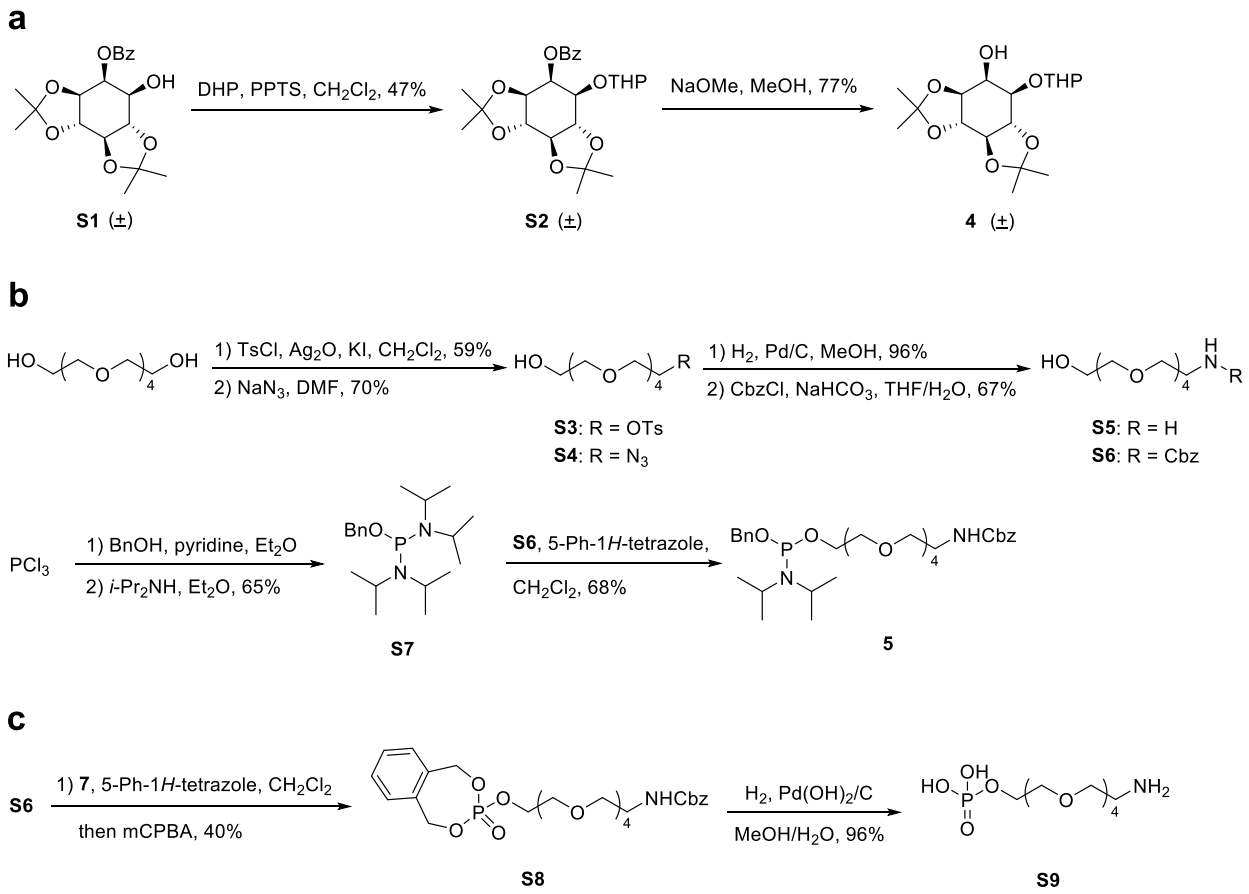
VIII. Phosphosite Analysis

The systematic names of each hit identified with the affinity reagents were individually scanned using Scansite3[10] (http://scansite3.mit.edu/#home) for all yeast motifs using the non-standard reference *S. cerevisiae* proteome with high stringency. The compiled results were then categorized by motif and analyzed for the number of unique proteins that were predicted to contain that motif (see Dataset S4).

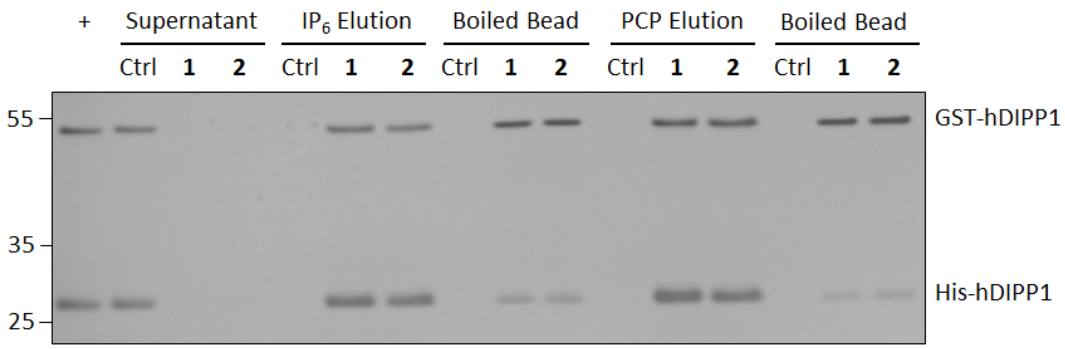
IX. Affinity capture of Vtc4 polyphosphate polymerase

Recombinant His-Vtc4p* protein expression and purification were conducted as previously described[11]. The assay followed the same procedure of the hDIPP1 binding assay. Briefly, 20 μg His-Vtc4* in 500 μL buffer A (50 mM Tris pH 7.5, 150 mM NaCl, 0.05% Triton-X100) was loaded to 100 μL suspension of reagents **1**, **2**, or **C**. The beads were washed with buffer (3 x 1.0 mL) followed by elution with either 10 mM $InsP_6$ or 10 mM 5PCP-$InsP_5$ solution in buffer A. The supernatants from the His-Vtc4* binding step, and the eluted fractions were mixed with SDS sample buffer (4x), boiled, and resolved by SDS-PAGE. Proteins were visualized using silver staining.

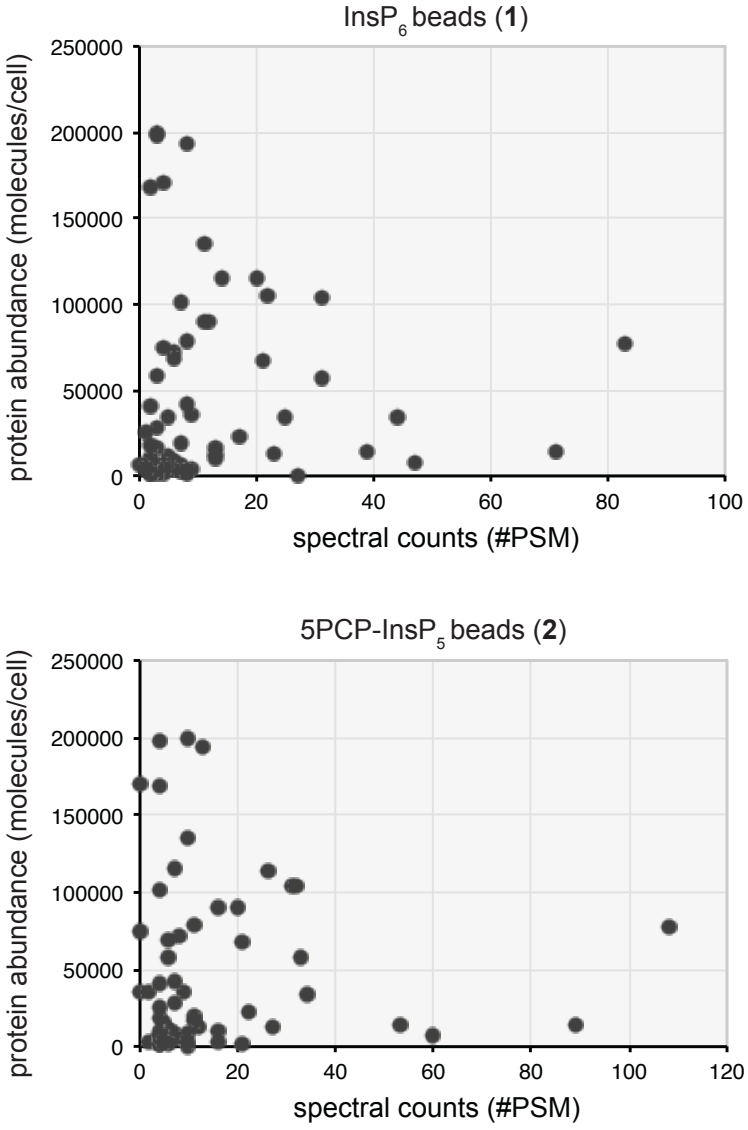
X. Supporting Scheme S1, Figures S1-S6, and Table S1



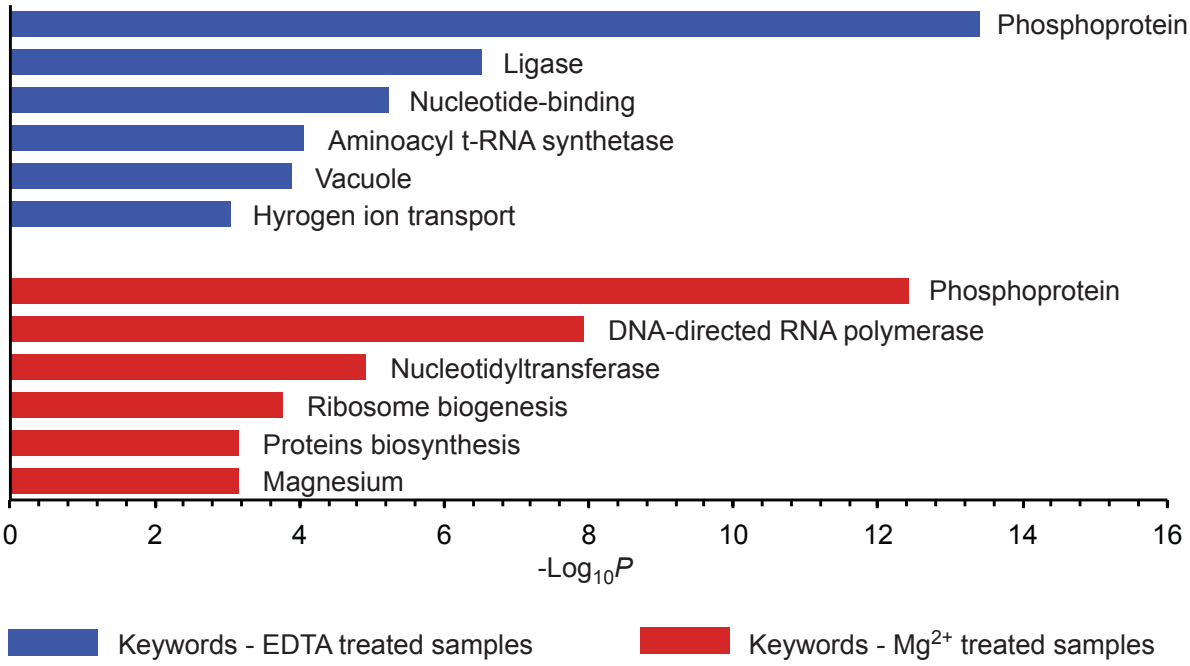
Supplemental Scheme 1 | Additional synthetic routes. (a) Synthesis of the protected inositol intermediate **4**. (b) Synthesis of the PEG linked phosphoramidite **5.** (c) Synthesis of the precursor **S9** for the control affinity reagent.



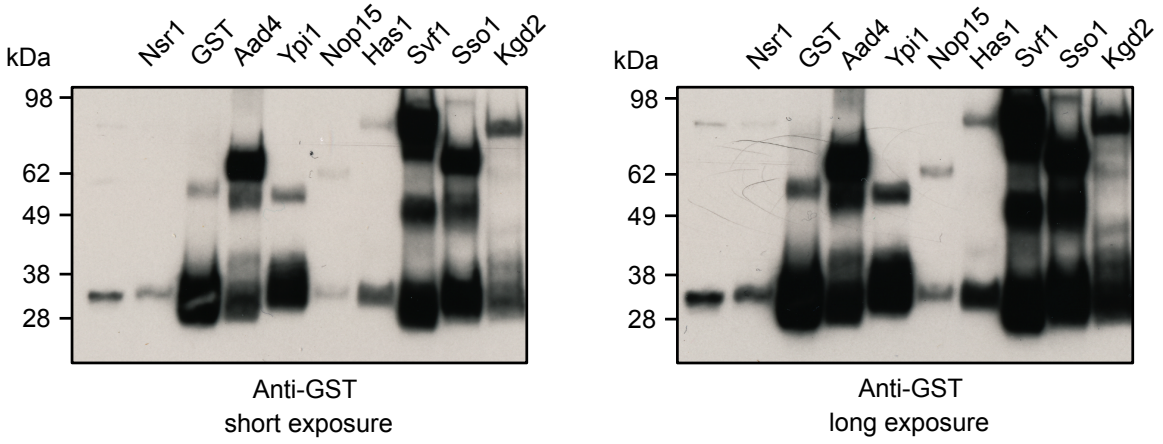
Supplemental Figure 1 | Both $InsP_6$ and 5PCP-$InsP_5$ effectively elute hDIPP1 from the affinity reagents. PCP denotes 5PCP-$InsP_5$.



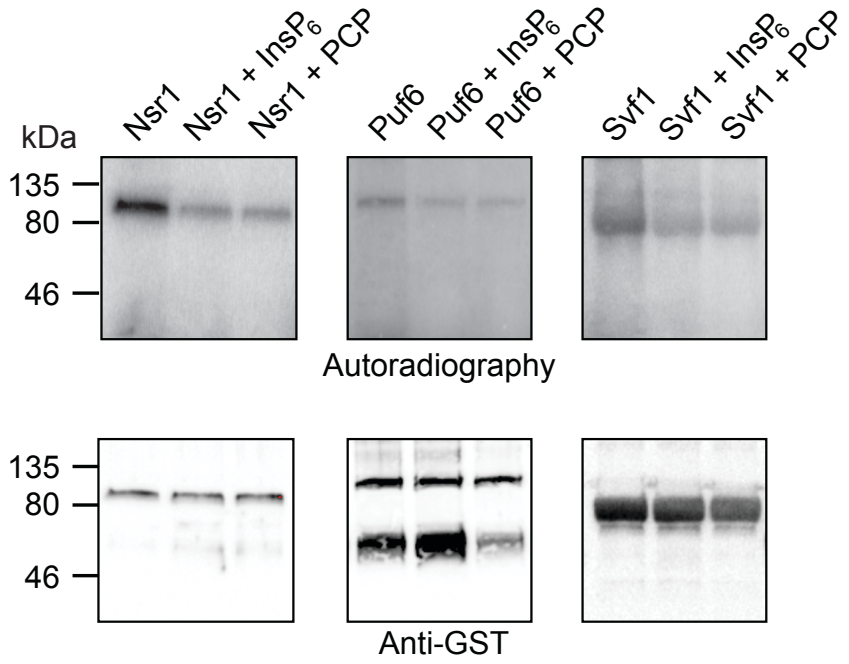
Supplemental Figure 2 | Plot of spectral counts for proteins enriched with either $InsP_6$ affinity reagent **1** (top) or 5PCP-$InsP_5$ affinity reagent **2** (bottom) versus their cellular abundance.



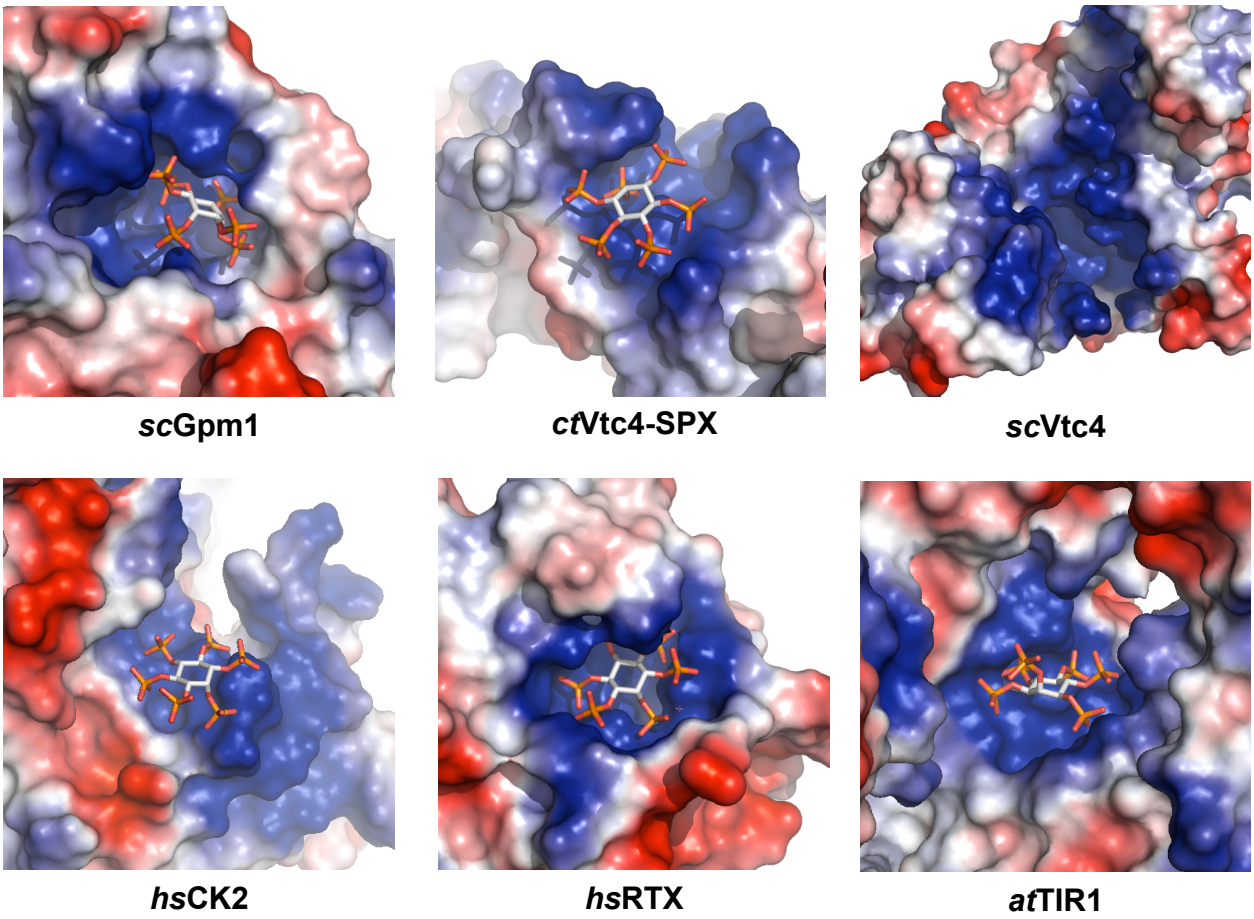
Supplemental Figure 3 | Over-representation of keyword annotations (SwissProt/Uniprot and Protein Information Resource databases) associated with proteins enriched under conditions restricting metal ion availability (blue) or enriched in the presence of magnesium ions (red).



Supplemental Figure 4 | Short and long exposure of Anti-GST blots, following the protein pyrophosphorylation experiments from Figure 4. Protein loading was analyzed by Western blot of the PVDF membrane with an anti-GST antibody. The predicted molecular weights are as follows: GST-Nsr1, 71 kDa; GST, 26 kDa; GST-Aad4, 63 kDa; GST-Ypi1, 44 kDa; GST-Nop15, 51 kDa; GST-Has1, 83 kDa; GST-Svf1, 80 kDa; GST-Sso1, 59 kDa; GST-Kgd2, 76 kDa. The long exposure highlights the presence of full-length GST-Nsr1, which was not visible in the short exposure due to its low concentration.



Supplemental Figure 5 | GST-fusion proteins purified from *S. cerevisiae* were mixed with buffer only, 100 μM $InsP_6$, or 100 μM 5PCP-$InsP_5$ (PCP), and then treated with 1 μM β[^{32}P]5PP-$InsP_5$ (15 μCi) in 25 mM Tris (pH = 7.4, 50 mM NaCl, 6 mM $MgCl_2$, 1 mM DTT) at 37 ^{o}C for 40 min, while still on beads. Reactions were quenched, heated, resolved by SDS-PAGE, transferred to PVDF membranes and visualized by autoradiography. Protein loading was analyzed by Western blot of the PVDF membrane with an anti-GST antibody. The predicted molecular weights are as follows: GST-Nsr1, 71 kDa; GST-Puf6, 101 kDa, GST-Svf1, 80 kDa; The presence of $InsP_6$ and 5PCP-$InsP_5$ notably diminishes the extent of pyrophosphorylation.



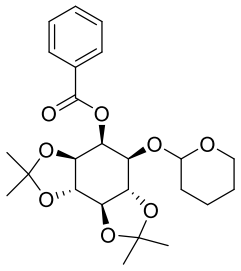
Supplemental Figure 6 | Structures of characterized $InsP_6$ binding proteins, and a putative $InsP_6$/5PP-$InsP_5$ interacting region in *sc*Vtc4. The PDB codes are as follows: scGpm1: 1BQ3, ctVtc4-SPX: 5IJP, scVtc4: 3G3T, hsCK2: 3W8L, hsRTX: 3EEB, atTIR1: 2P1M.

Supplemental Table 1 | Arginine composition analysis of proteins isolated with affinity reagents **1** and **2**, in the presence of magnesium ions (Mg), or in the absence of magnesium ions (EDTA).

p-value

	Mg	EDTA
Wilcoxon	0.8734	0.7561
Welch's	0.9157	0.7443

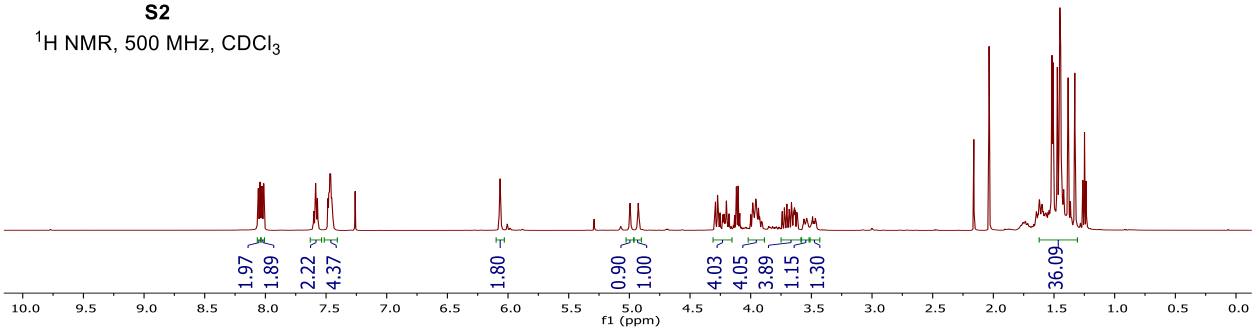
XI. NMR Spectra

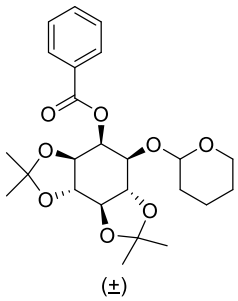


(±)

S2

^{1}H NMR, 500 MHz, $CDCl_3$





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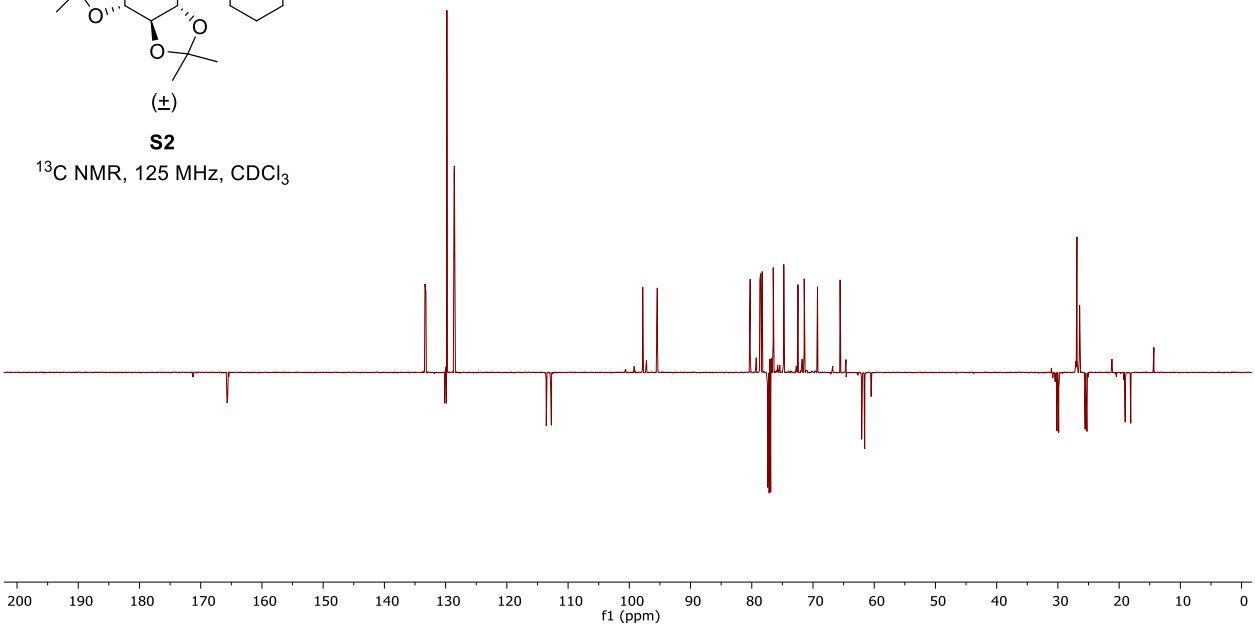
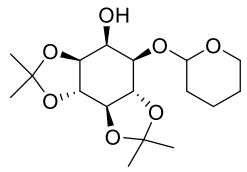
S2

^{13}C NMR, 125 MHz, $CDCl_3$





(±)

4

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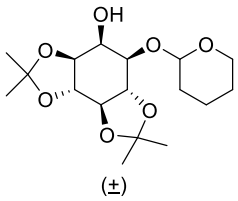
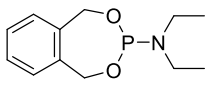


(±)

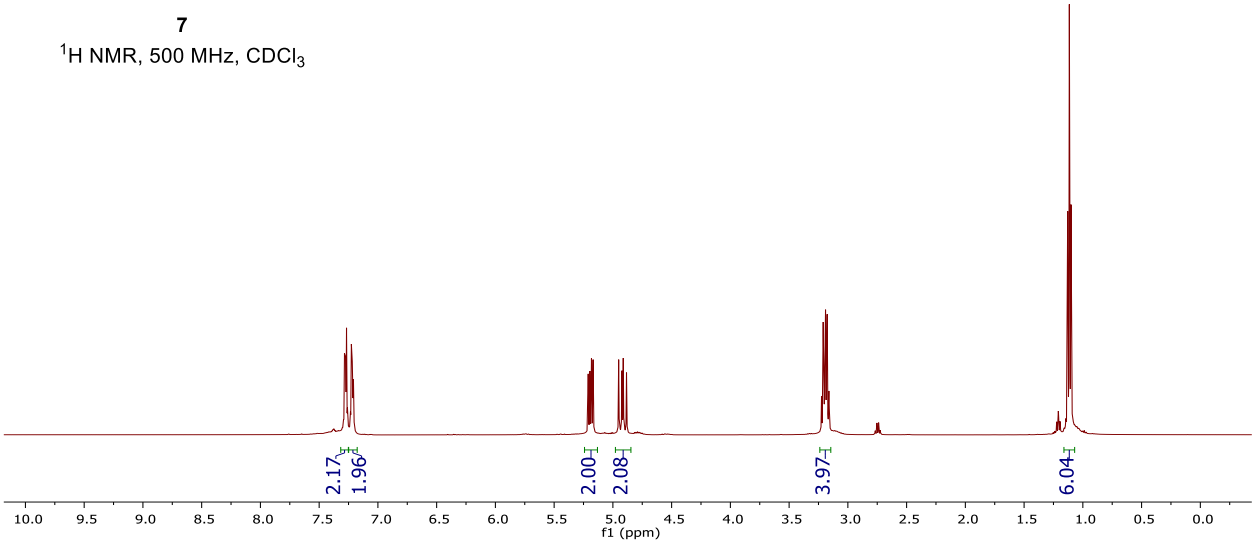
4

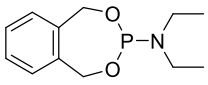
^{13}C NMR, 125 MHz, $CDCl_3$



7

^{1}H NMR, 500 MHz, $CDCl_3$





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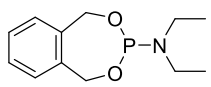
^{13}C NMR, 125 MHz, $CDCl_3$



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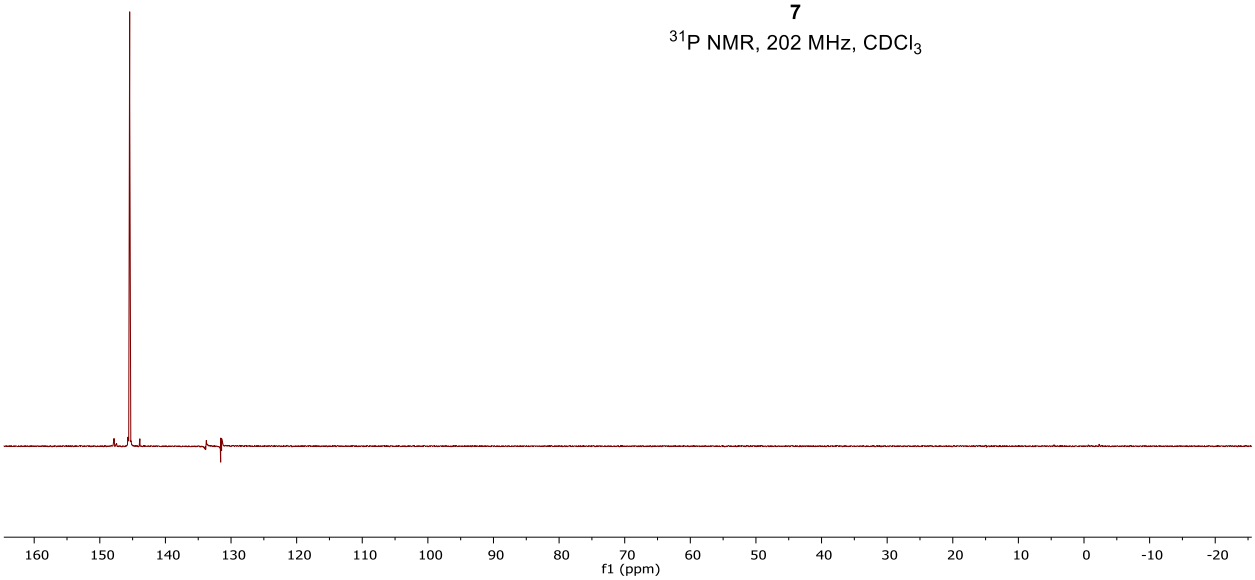
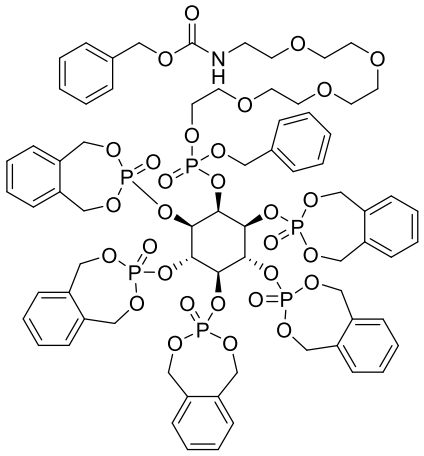
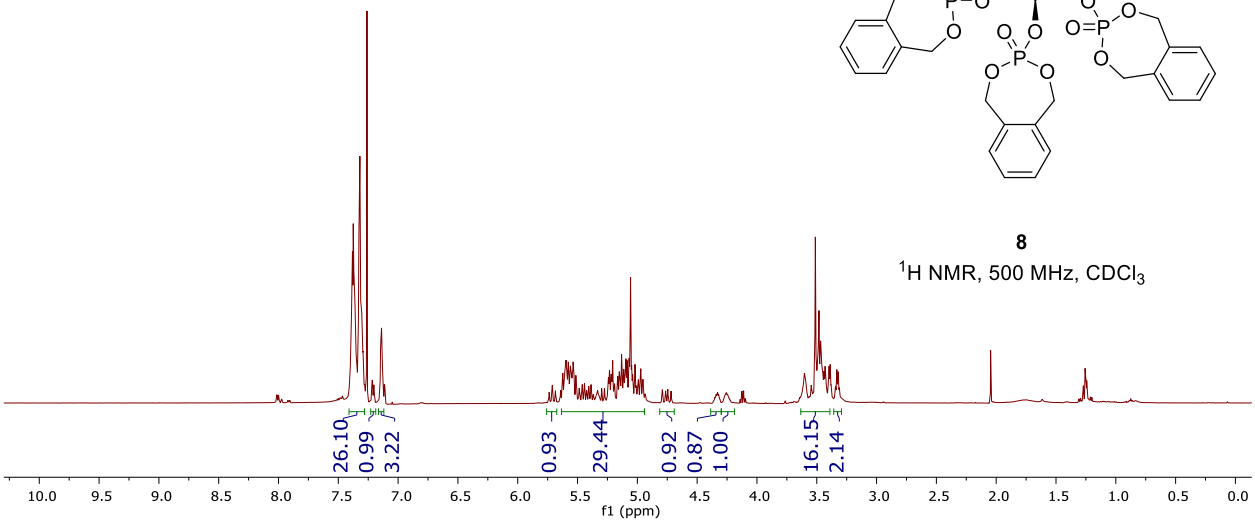
^{31}P NMR, 202 MHz, $CDCl_3$

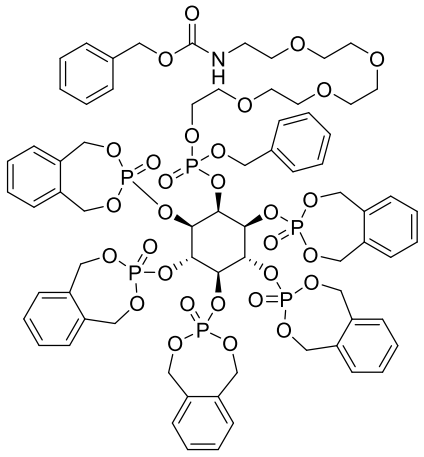




8

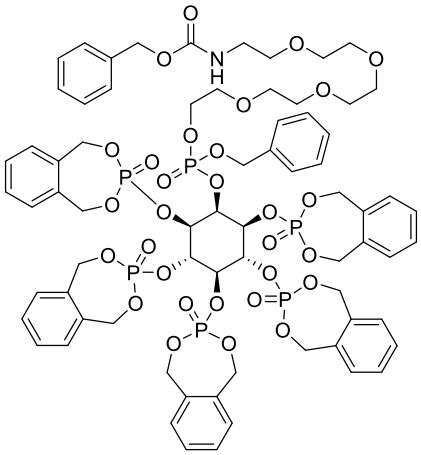
^{1}H NMR, 500 MHz, $CDCl_3$





8

^{13}C NMR, 125 MHz, $CDCl_3$



8

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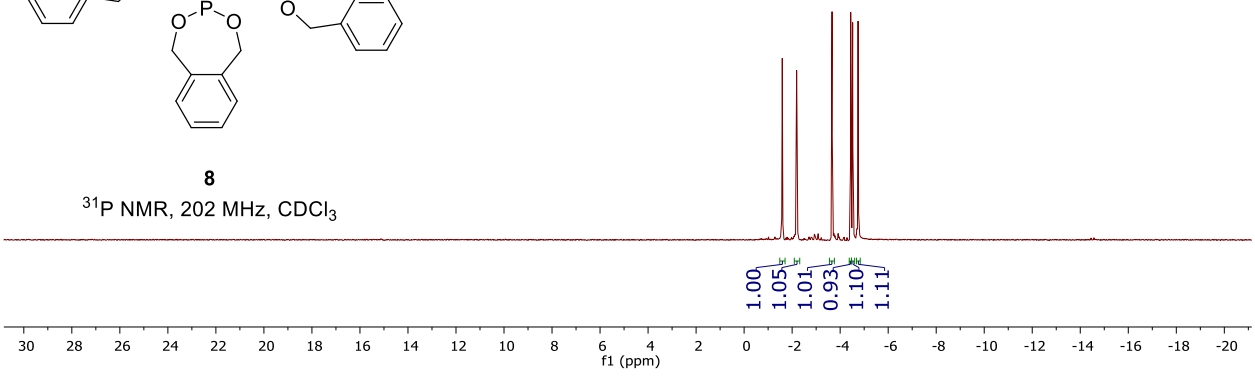

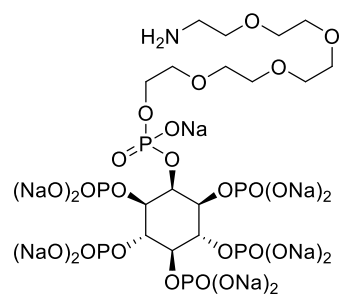

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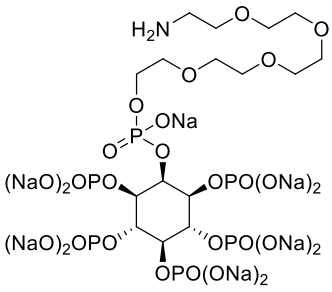

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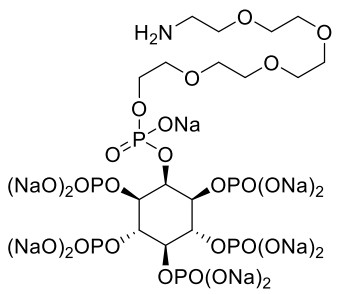

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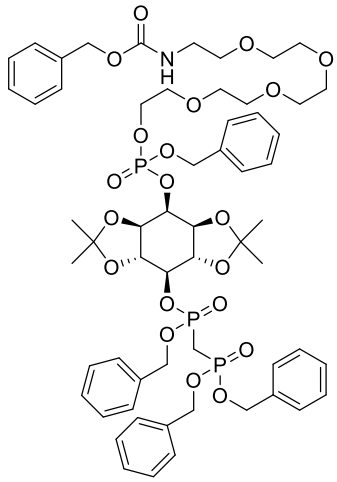

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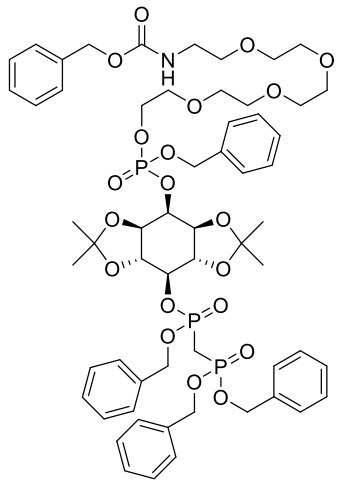

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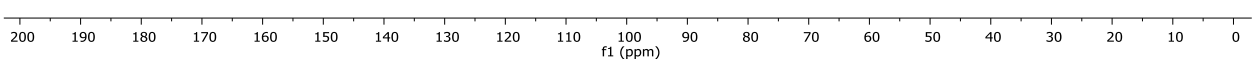

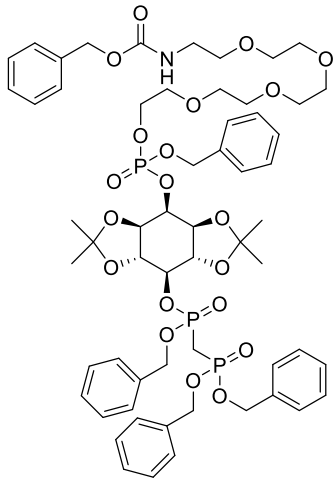

11

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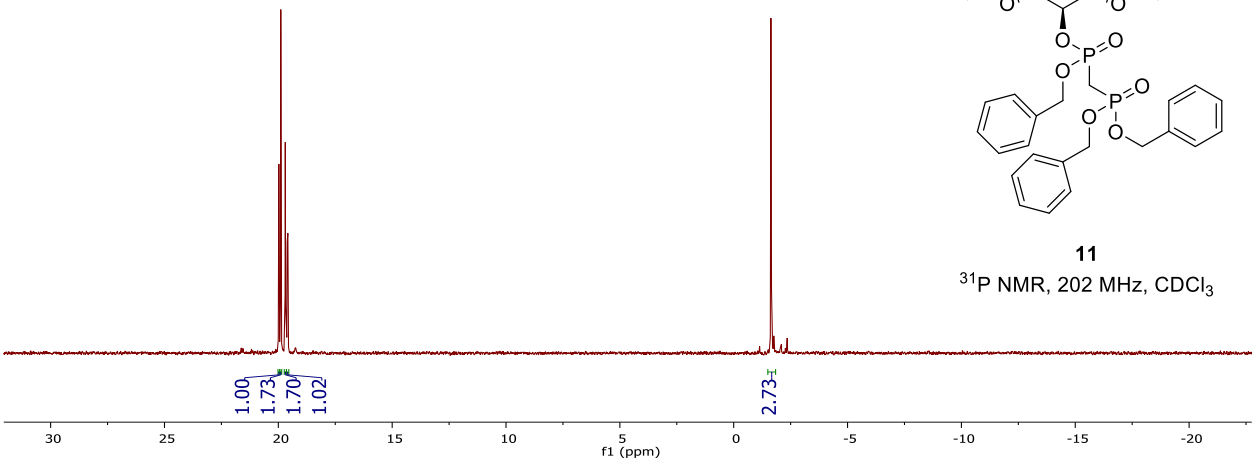

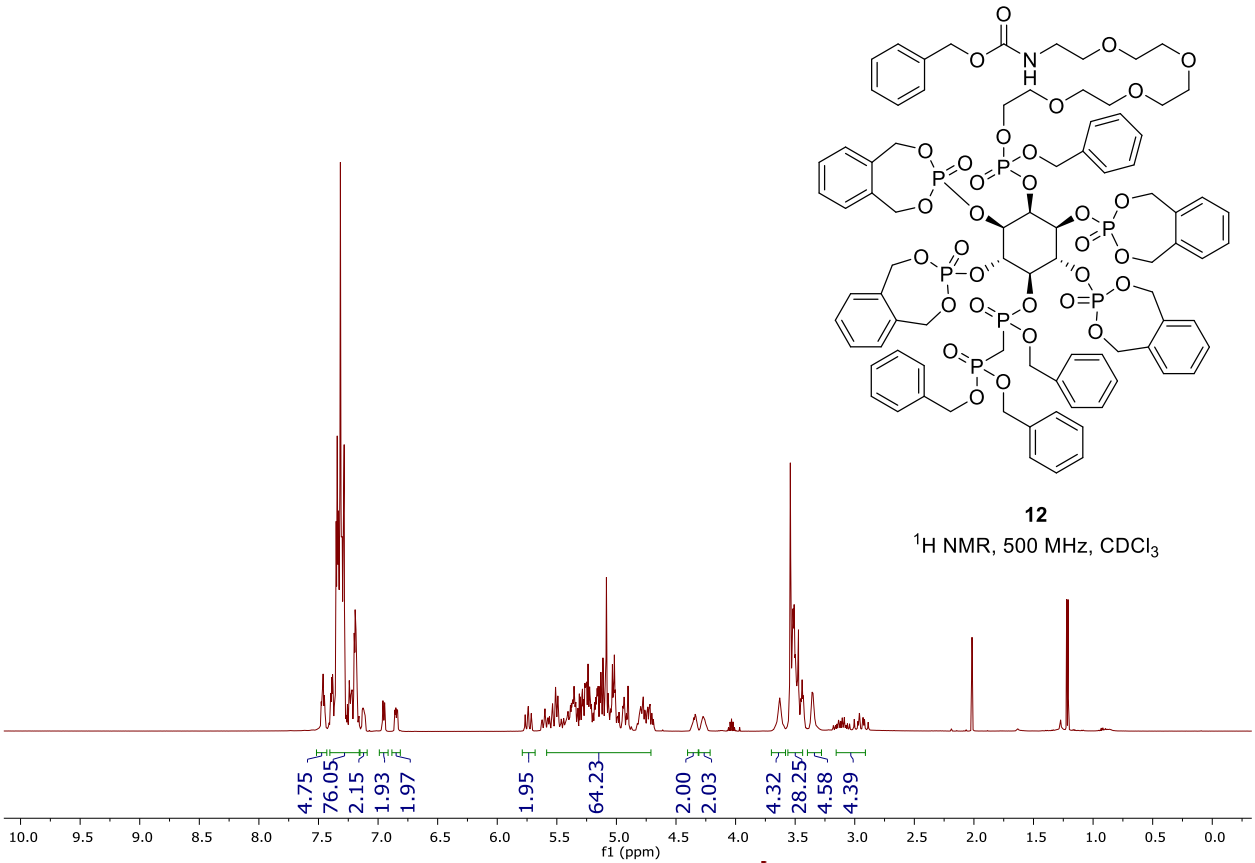

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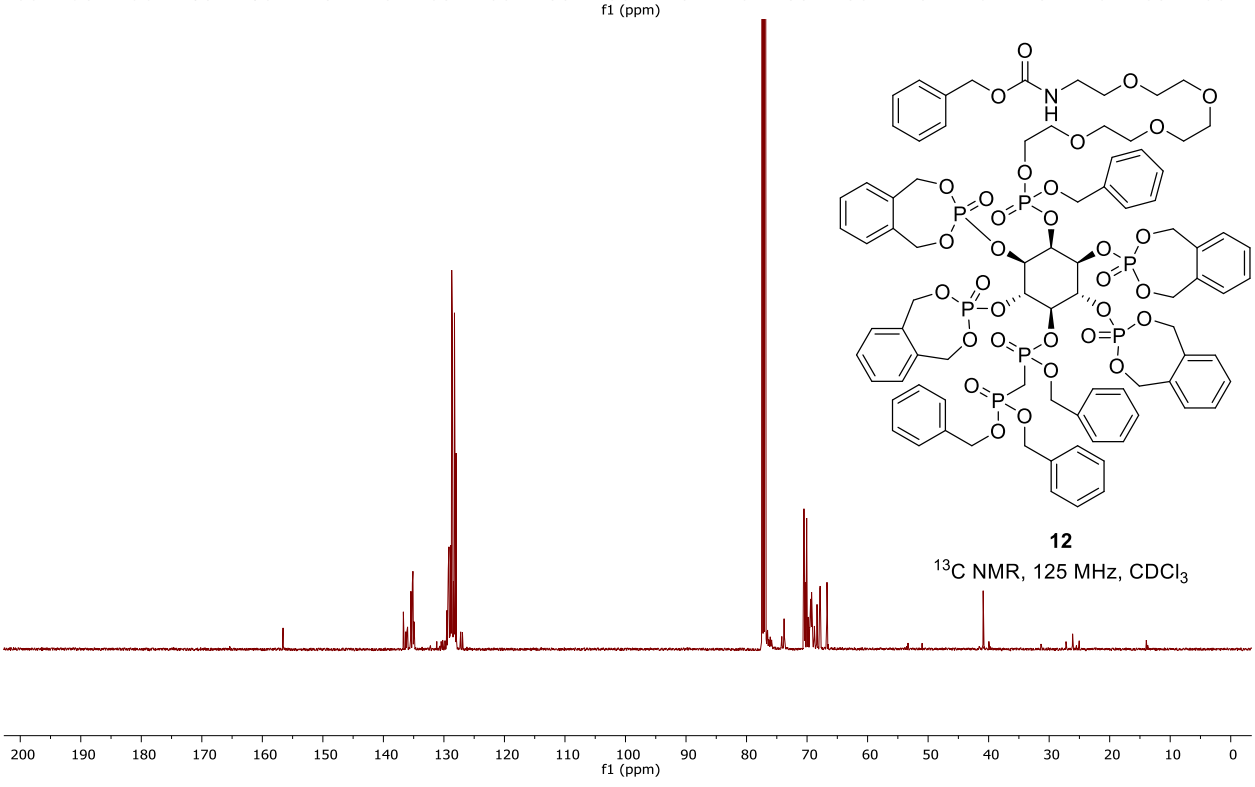

12

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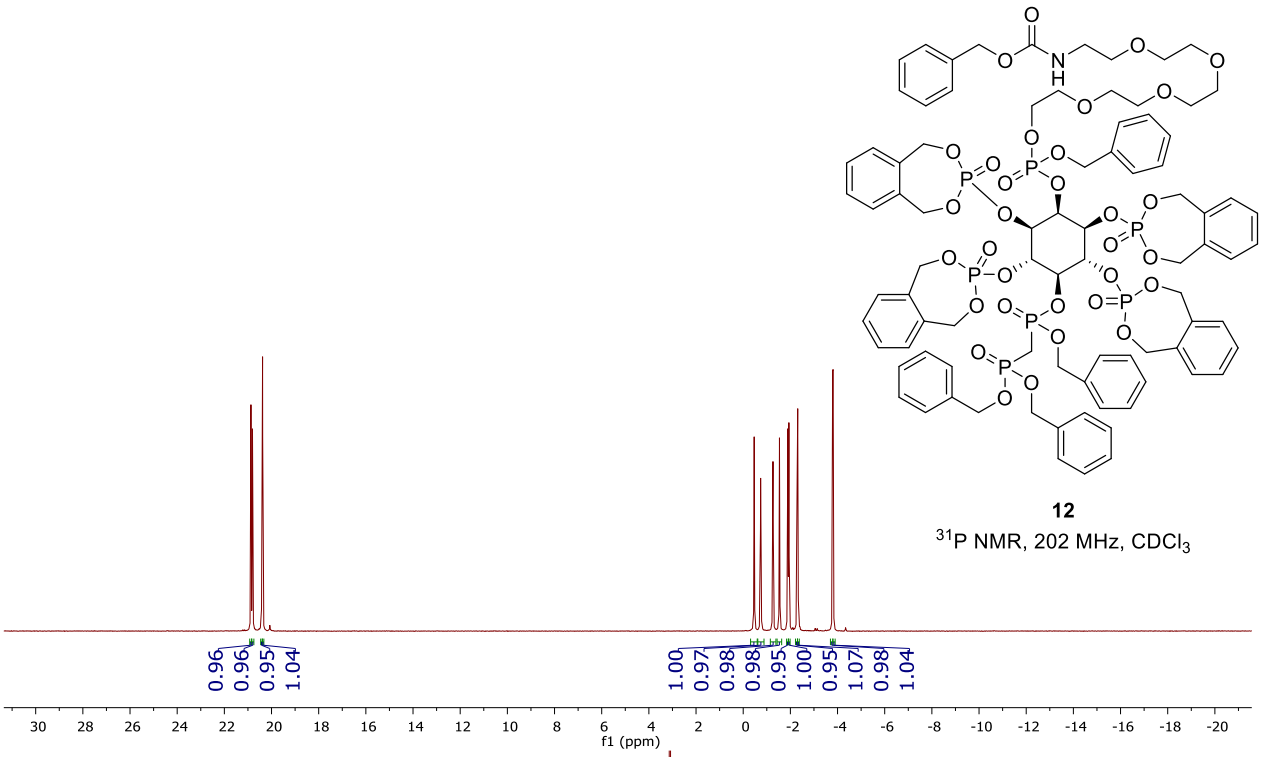

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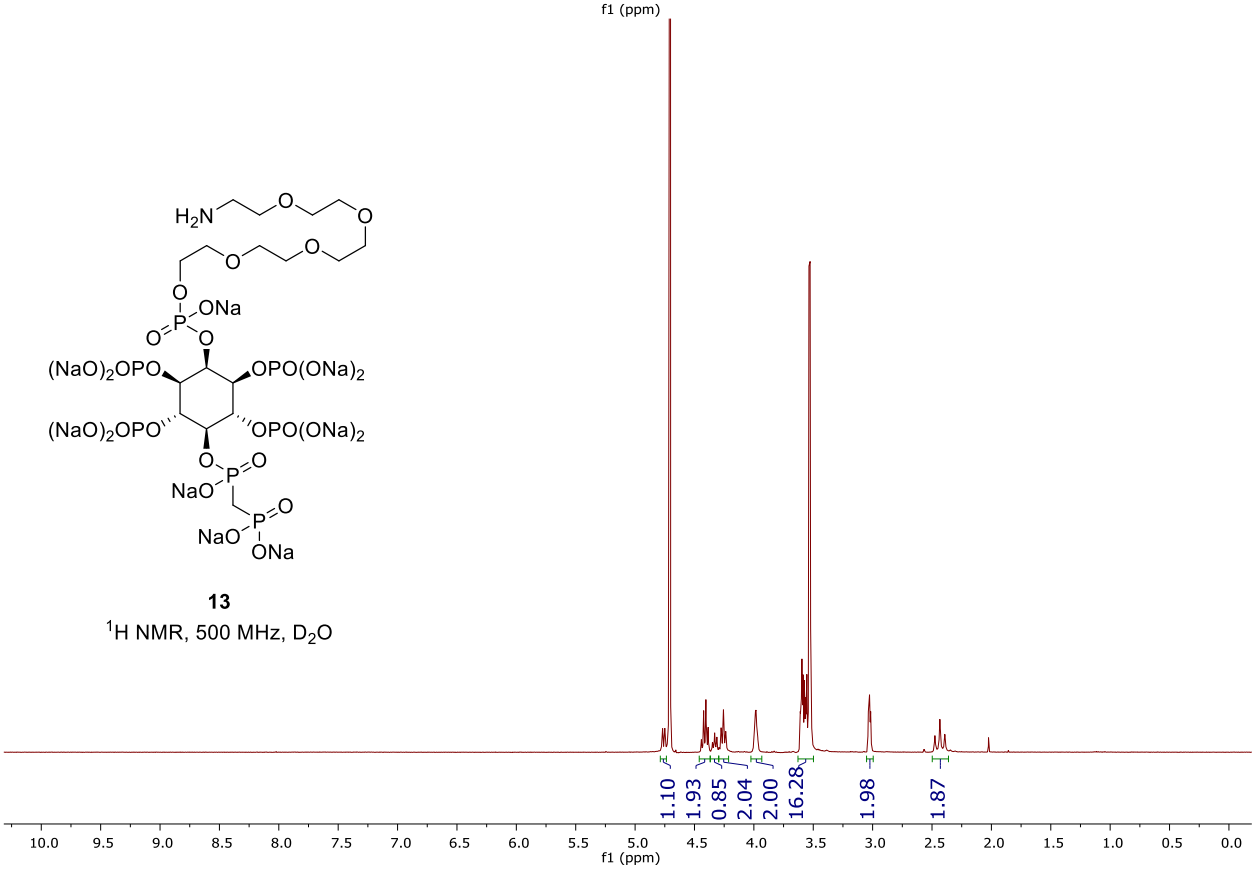

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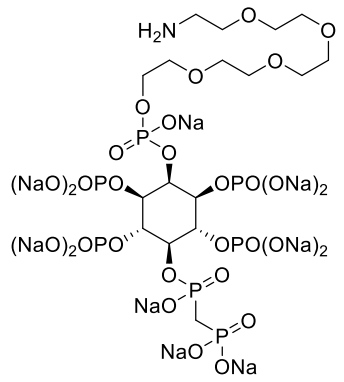

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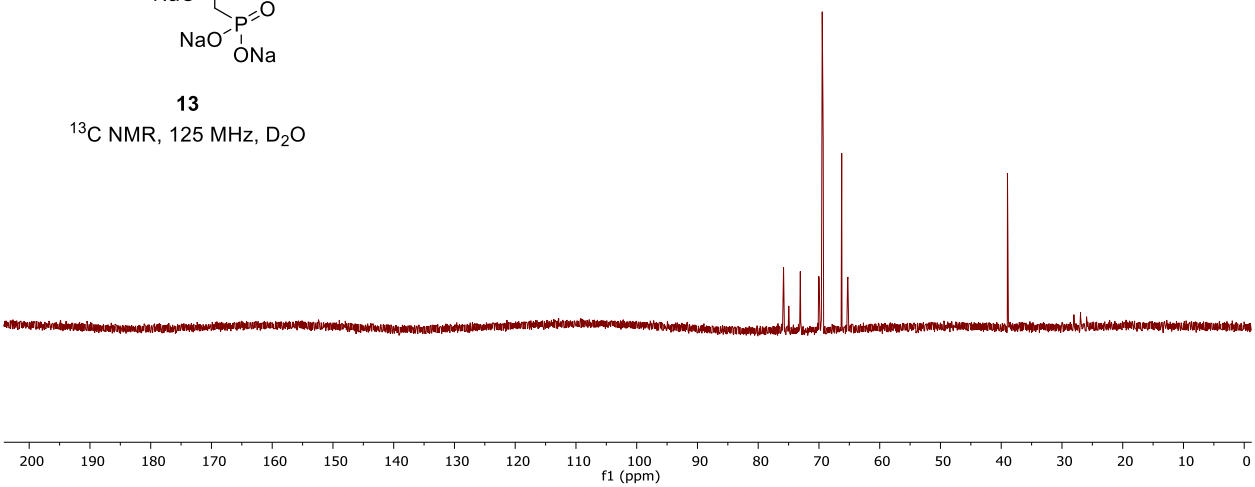

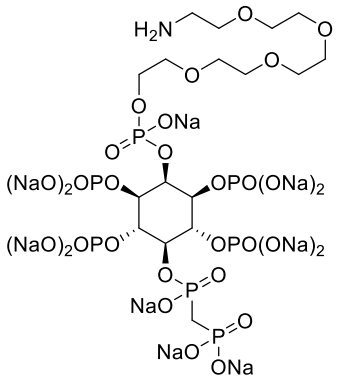

13

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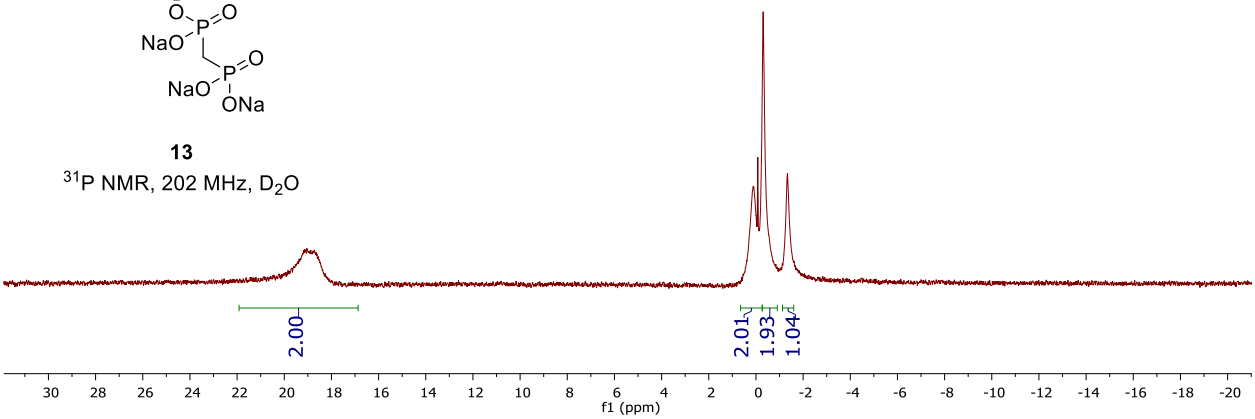

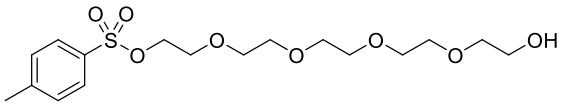

S3

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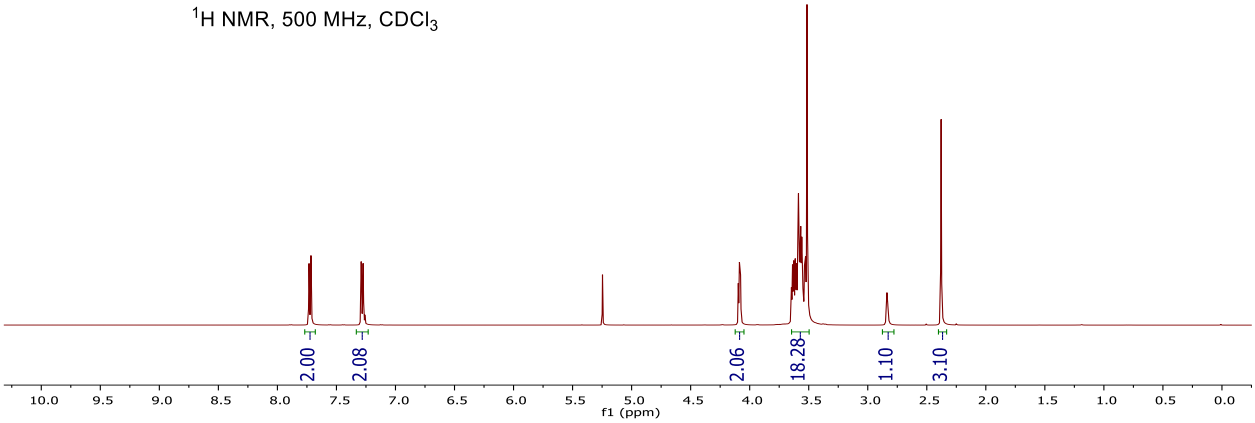

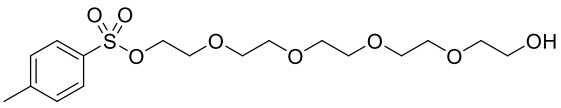

S3

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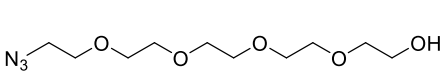

S4

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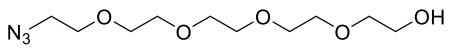

S4

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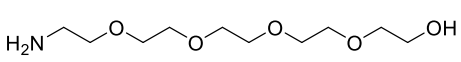
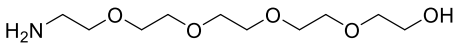


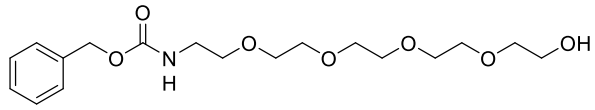
S5

^{1}H NMR, 500 MHz, CD_3OD



S5

^{13}C NMR, 125 MHz, CD_3OD



S6

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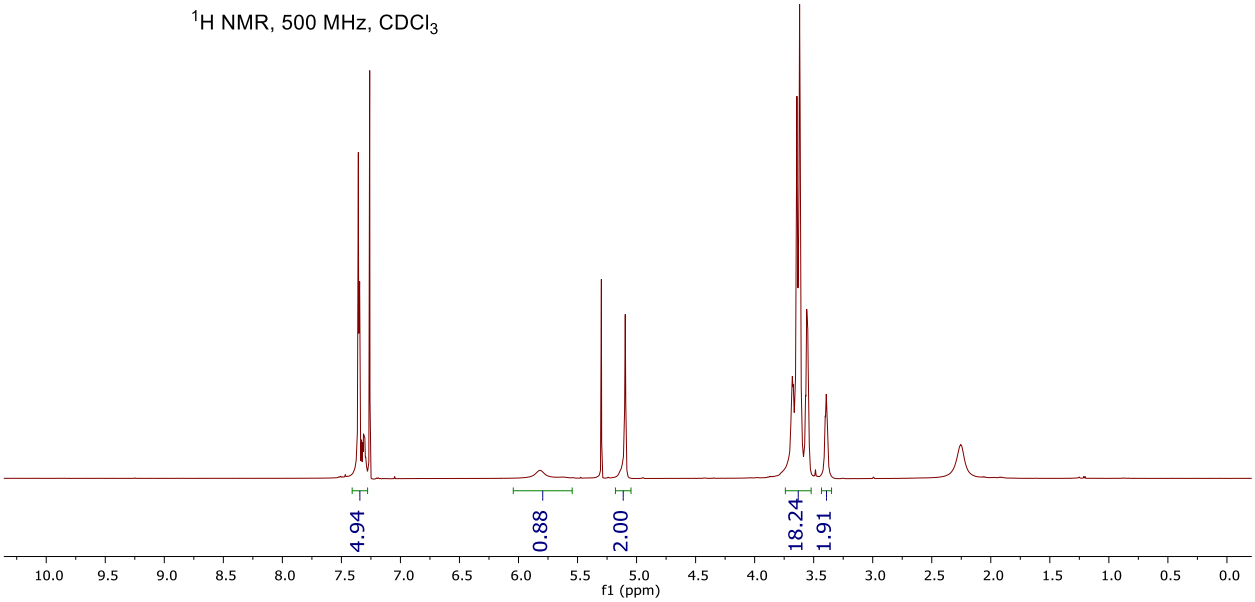
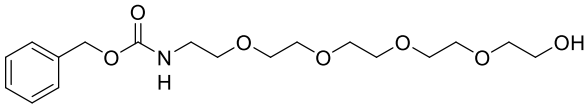
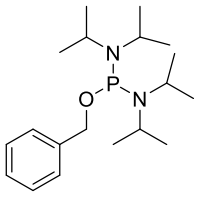




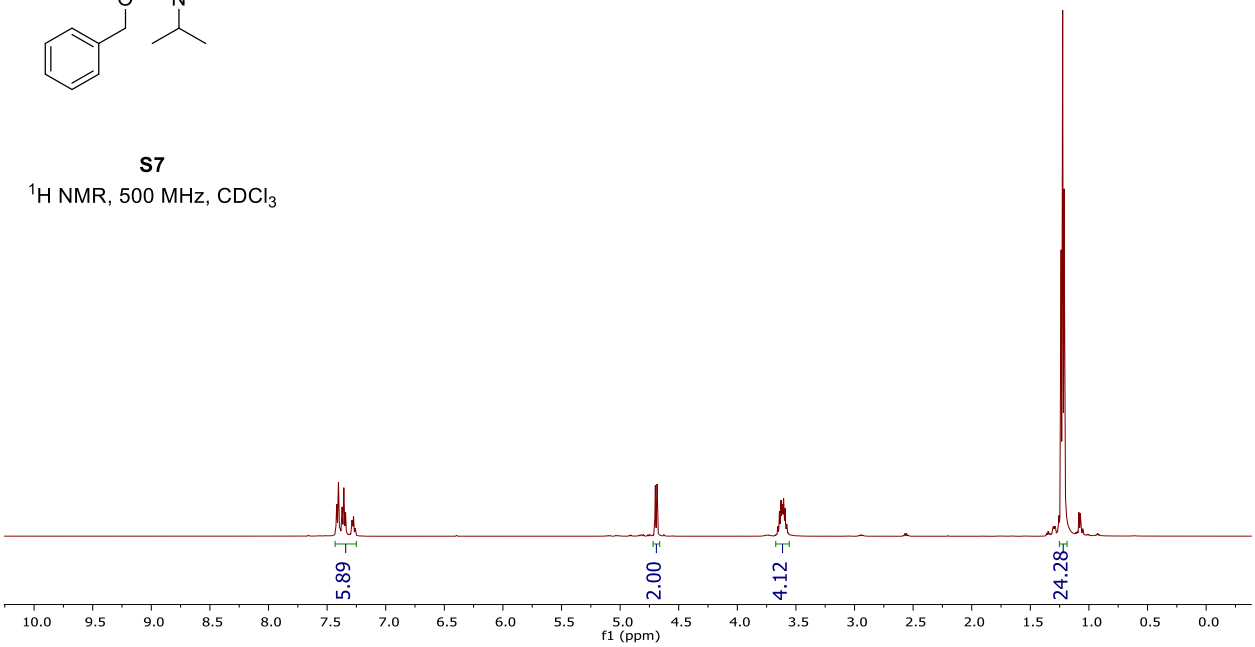
S6

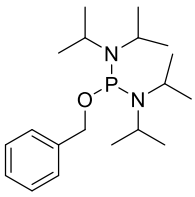
^{13}C NMR, 125 MHz, $CDCl_3$



S7

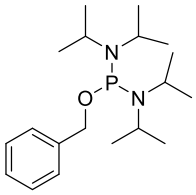
^{1}H NMR, 500 MHz, $CDCl_3$





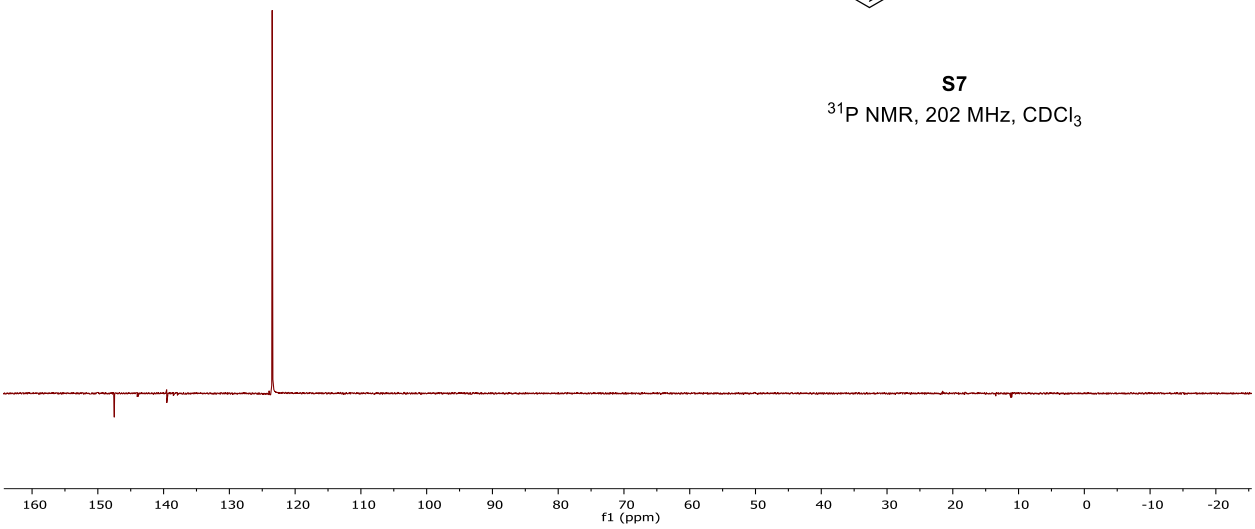
S7

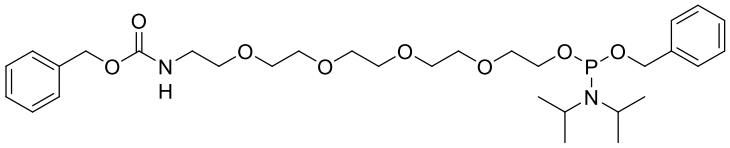
^{13}C NMR, 125 MHz, $CDCl_3$



S7

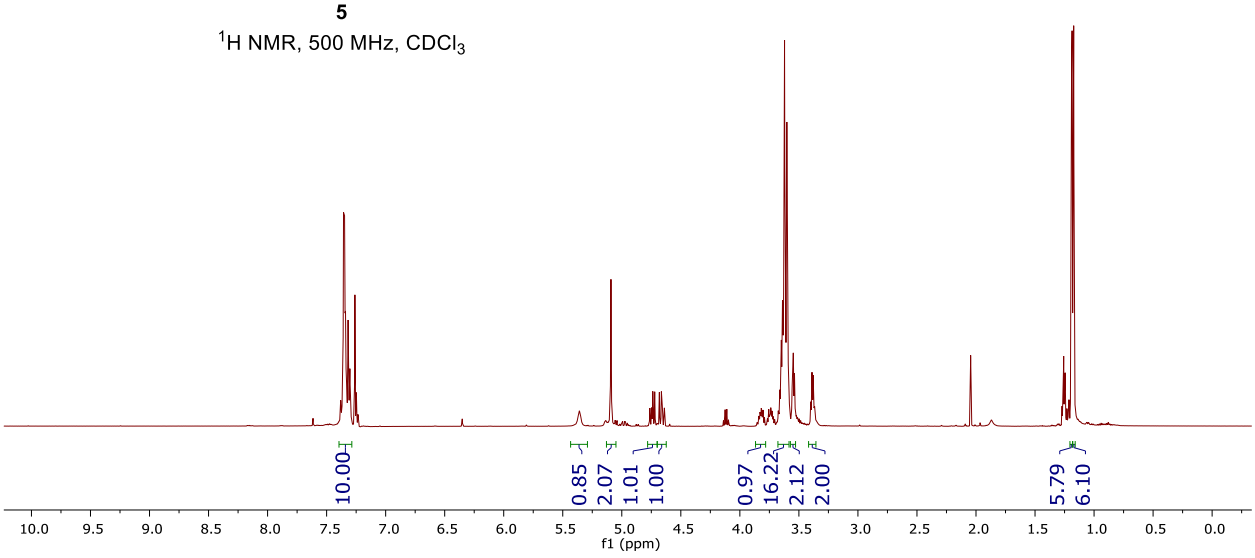
^{31}P NMR, 202 MHz, $CDCl_3$

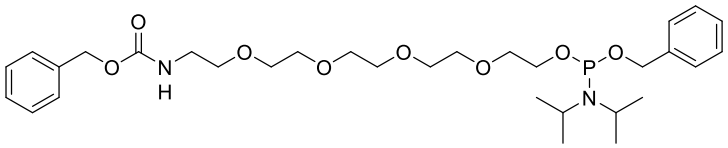




5

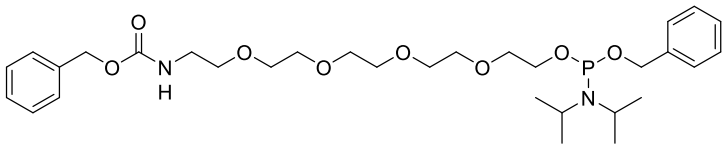
^{1}H NMR, 500 MHz, $CDCl_3$





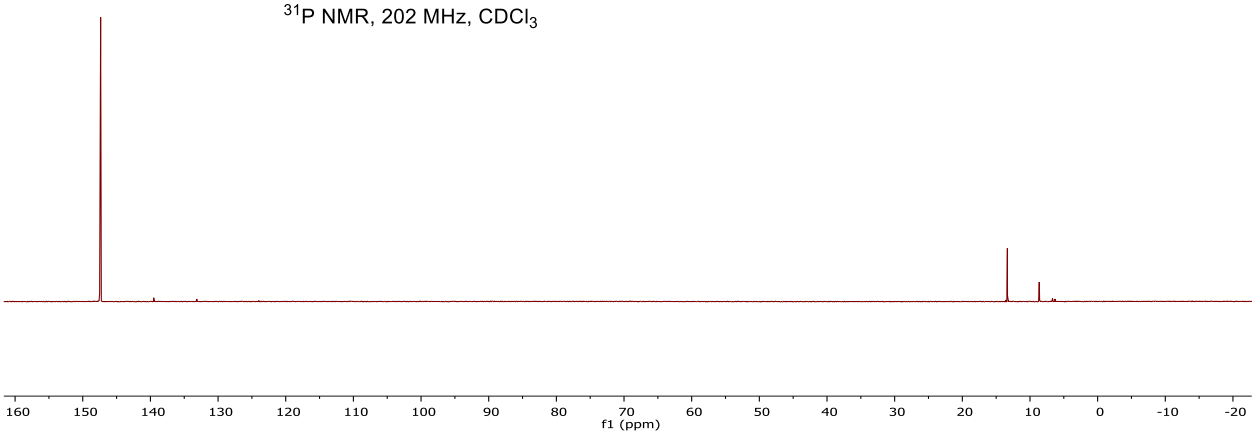
5

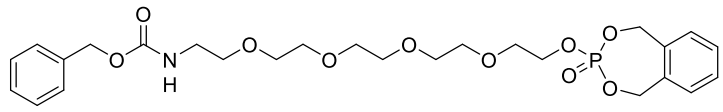
^{13}C NMR, 125 MHz, $CDCl_3$



5

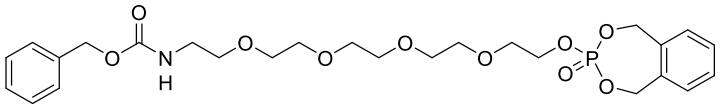
^{31}P NMR, 202 MHz, $CDCl_3$





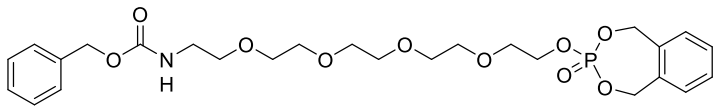
S8

^{1}H NMR, 500 MHz, $CDCl_3$



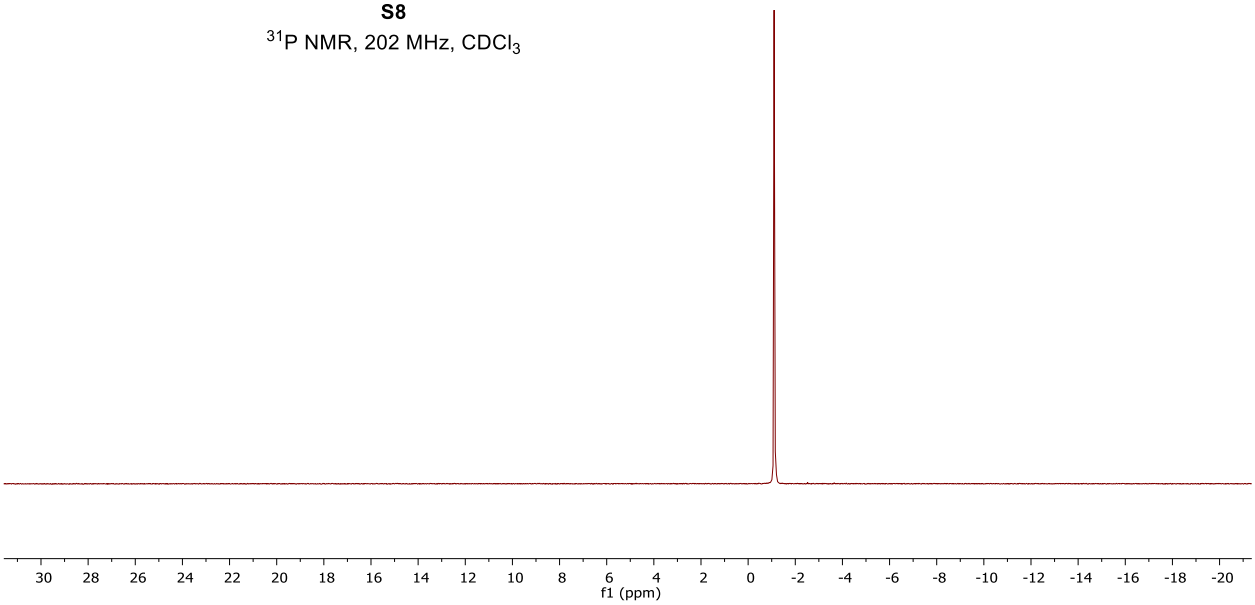
S8

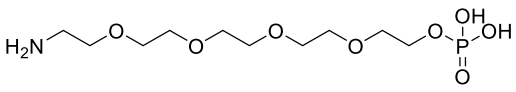
^{13}C NMR, 125 MHz, $CDCl_3$



S8

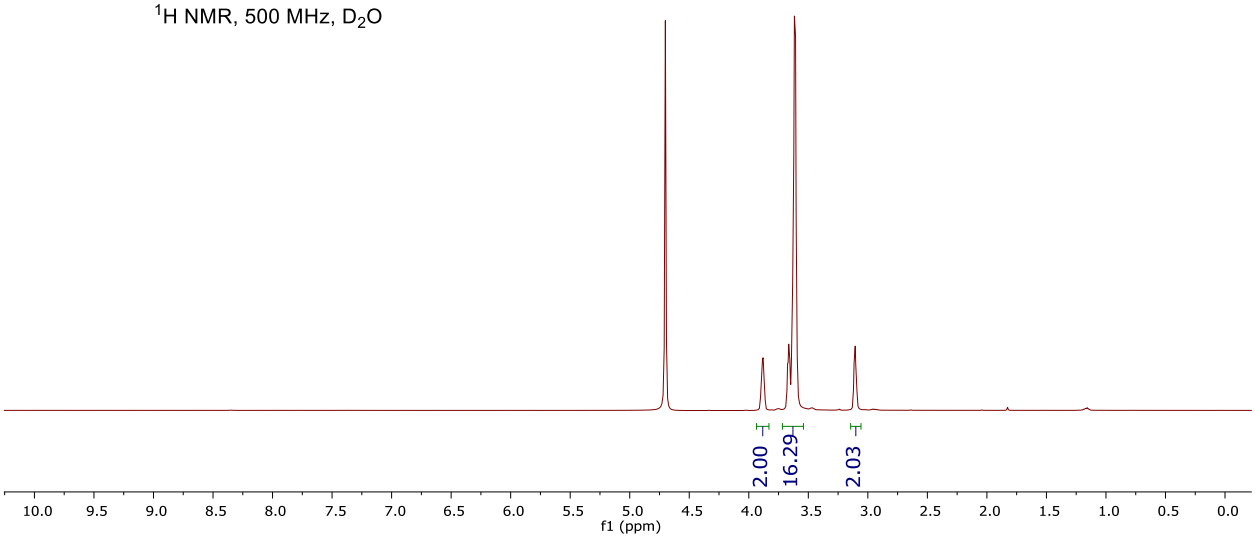
^{31}P NMR, 202 MHz, $CDCl_3$

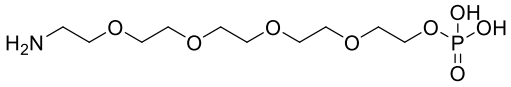




S9

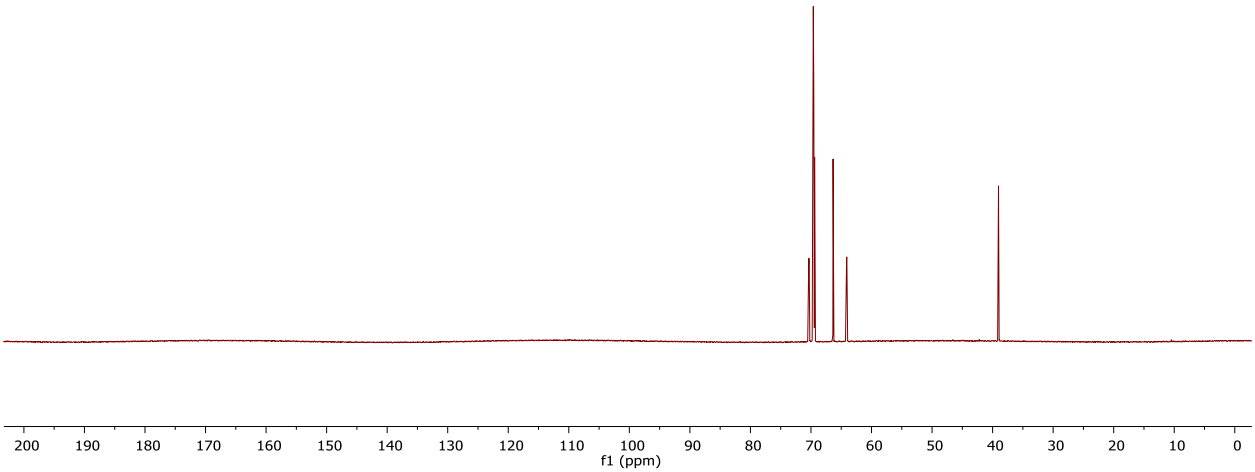
^{1}H NMR, 500 MHz, D_2O

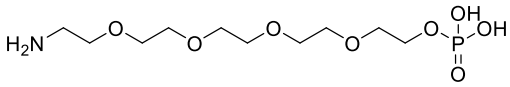




S9

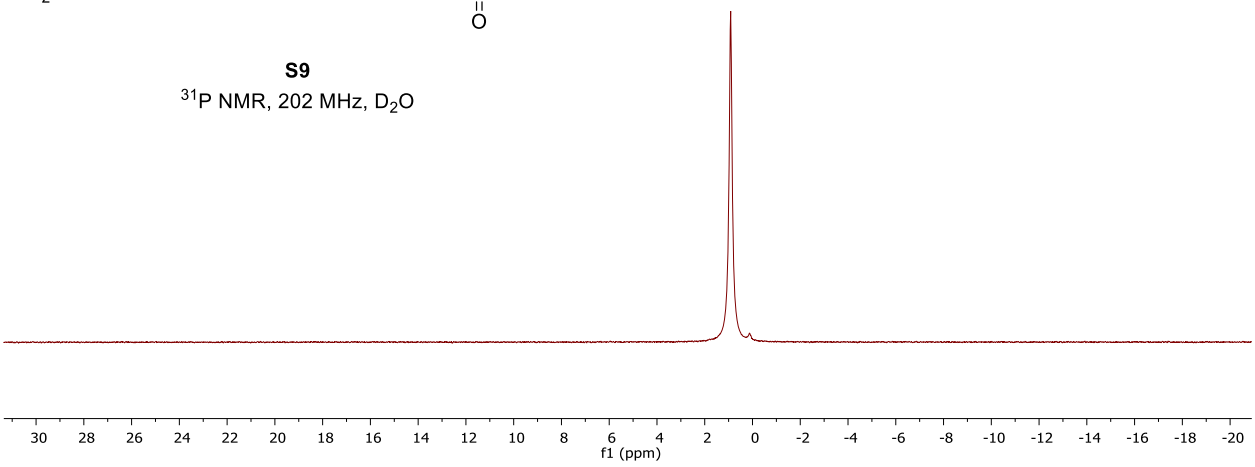
^{13}C NMR, 125 MHz, D_2O





S9

^{31}P NMR, 202 MHz, D_2O



XII. References

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