Synthesis and Structure of Cellular Mediators: Inositol Polyphosphate Diphosphates[1]

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Abstract: The first total syntheses of 1-*O*-[(phosphonooxy)hydroxyphosphinyl]-2,3,4,5,6-pentakis-*O*-phosphono-D-*myo*-inositol (**10**) and its antipode (**11**) were achieved from enantiopure 2,3:5,6-di-*O*-cyclohexylidene-D-*myo*-inositol (**6**). The critical pyrophosphate function was introduced, in the presence of flanking phosphate triesters, by mild LiCN-mediated cleavage of a mixed phosphate methyl ester, isolation of the resultant lithium salt, and addition to dibenzyl chlorophosphonate. The benzyl esters were removed by catalytic hydrogenolysis over Pd in *t*-BuOH/H_2O in the presence of $NaHCO_3$. Comparisons of synthetic and enzyme-devived pyrophosphates with two phosphatases suggests natural material has the stereochemical configuration in **10**.

Introduction

Many of the dozens of inositol derivatives found in Nature have gained prominence for their varied and integral roles in cellular regulation and homeostasis.[2] Among the more recent additions to this burgeoning list is a novel family of pyrophosphate-containing inositol polyphosphates[3] (PP-InsP_n) which have been detected in organisms spanning a wide phylogenetic spectrum including most mammalian cell types. Of these, the most extensively studied are the two major PP-InsP_n isomers present in the amoebae *Dictyostelium discoideum*.[4] They were identified as mono- and bis-pyrophosphates of *myo*-inositol hexakisphosphate (phytic acid) and were tentatively assigned as the C(1)- and C(1),(4)-regioisomers, i.e., 1-PP-InsP_5 and 1,4-$(PP)_2$-InsP_4, respectively, based on extensive chromatographic, degradative (chemical and enzymatic), FAB-mass spectroscopic, and ^{31}P NMR analyses.[5] However, the absolute configurations of both isomers remain obscure. Further studies suggest that other regio- and/or stereoisomers may also exist.[6]

The inositol pyrophosphates are thought to arise through the actions of specific, high energy kinases on InsP_5 and InsP_6 precursors.[4] While the pools of pyrophosphates are relatively small compared with the total amount of cellular InsP_5/InsP_6, they manifest a remarkably rapid metabolic turnover consistent with speculation that they function as intracellular autacoids or, alternatively, as a new form of phosphate donor in an as yet uncharacterized set of phosphotransferase reactions.[7] Fleisher et al.[8] have demonstrated that PP-InsP_5 binds the Golgi protein coatomer and modulates its activity on K^+-selective channels. Additionally, Ye and colleagues[9] reported inhibition of clathrin assembly via high affinity binding of PP-InsP_5 (K_d = 22 nM) with AP-3, a synapse-specific protein; in comparison, binding by InsP_6 was 5−10 fold lower. Current investigations in these and other laboratories will undoubtedly lead to further insights, but it is already clear that the inositol pyrophosphates have all the hallmarks of an essential metabolic cycle.

To help resolve the outstanding questions regarding the regio and absolute configurations of the *myo*-inositol polyphosphate diphosphates and to expedite their biological evaluations, we report herein the first total syntheses of 1-PP-D-*myo*-InsP_5 (**10**) and its enantiomer (**11**). Our strategy exploits a readily available, chiral precursor derived from inexpensive *myo*-inositol and utilized an efficient protocol for the introduction of protected pyrophosphates that is mild enough to be compatible with many acid/base-labile functionalities.[10]

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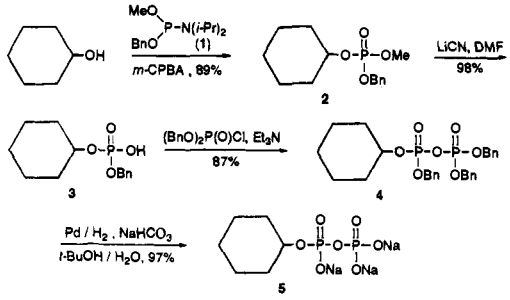
Scheme 1



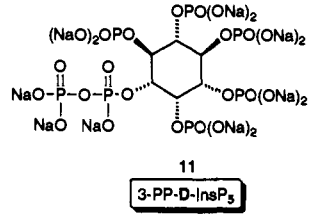
Pyrophosphate Protocol

The synthesis of protected pyrophosphates was initially explored using simple cyclic alcohols as illustrated in Scheme 1. Benzyl methyl *N,N*-diisopropylphosphoramidite (**1**), freshly prepared from chloro-(*N,N*-diisopropylamino)methoxyphosphine and benzyl alcohol using a modification of Caruthers' procedure,[11] was added to 1 equiv of cyclohexanol in the presence of 1*H*-tetrazole followed by peracid oxidation to furnish the mixed phosphate triester **2**. Exclusive demethylation of **2** was accomplished with LiCN[12] (1.1 equiv) in DMF at room temperature overnight. Cleavage in other solvents such as CH_3CN or THF was unacceptably slow and led to polar byproducts when heated. Following evaporation of the DMF, the residue was dissolved in H_2O, acidified to pH~2 with 1 N HCl, extracted into EtOAc, washed with water, and concentrated *in vacuo* to give diester **3** in almost quantitative yield. Coupling of **3** with dibenzyl chlorophosphonate[13] afforded pyrophosphate tetraester **4** that was purified by SiO_2 column chromatography. Catalytic debenzylation using Pd black in the presence of $NaHCO_3$ (3 equiv) uneventfully generated salt **5**. Reduction in the absence of base resulted in additional phosphorus(V) products.

Synthesis of 1-PP-D-*myo*-InsP$_5$ and 3-PP-D-*myo*-InsP$_5$

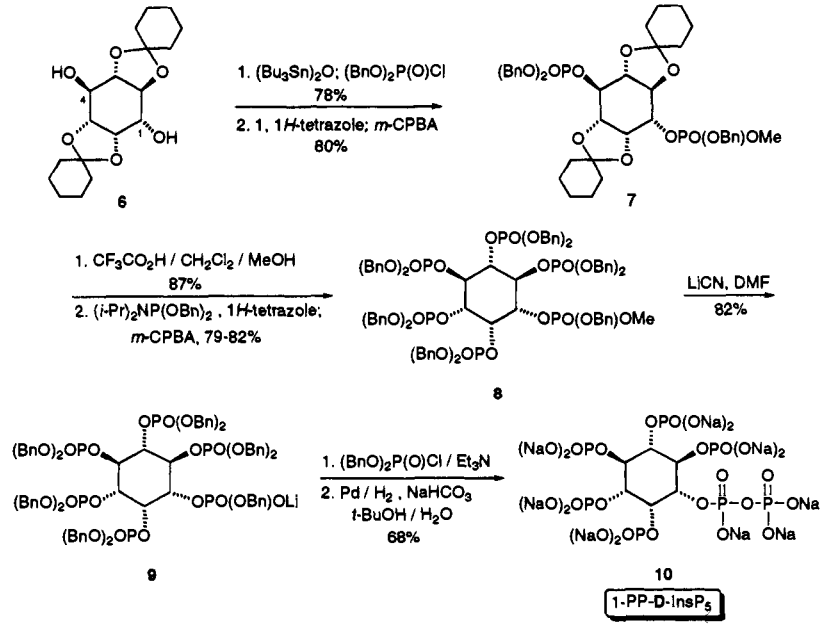
Application of the above methodology to the synthesis of 1-PP-D-*myo*-InsP$_5$ (**10**) is summarized in Scheme 2. Enantiopure **6**, readily derived in three steps from commercial *myo*-inositol,[14] was regioselectively phosphonoated at C(4) via its *in situ*-generated stannyl ester; subsequent introduction of **1** at the remaining hydroxyl and oxidation gave bisphosphate **7** as an inseparable ~1:1 mixture of phosphate diastereomers. Acidic hydrolysis of the cyclohexylidene protecting groups on **7** and exhaustive dibenzylphosphorylation[15] of the liberated tetraol proceeded smoothly to give hexakisphosphate **8**. Selective cleavage of the phosphate methyl ester as described above furnished **9** in good yield as its lithium salt which could be directly coupled with dibenzyl chlorophosphonate.[13] The resultant pyrophosphate, in stark contrast with **4**, proved rather labile (most likely reflecting the more congested steric environment) and was typically used in the next step without delay. Catalytic hydrogenolysis and purification by ion exchange chromatography led to the sodium salt of 1-PP-D-*myo*-InsP$_5$ (**10**), that displayed ^{31}P NMR resonances (D_2O) at −7.7 and −3.6 ppm characteristic of pyrophosphates and that comigrated on TLC and column chromatography with a biological standard. **10** was also easily distinguished from phytic acid, its degradation product.

Repetition of the foregoing sequence using the enantiomer of **6**[14] provided 3-PP-D-*myo*-InsP$_5$ (**11**), the antipode of **10**. These



were compared in two bioassays with commercial PP-InsP$_5$ (DuPont NEN) which was prepared using the InsP$_6$ kinase activity of rabbit brain. Firstly, the ability of all three inositol pyrophosphates, i.e., **10**, **11**, and biosynthesized PP-InsP$_5$, to individually inhibit [^{3}H]Ins(1,3,4,5)P$_4$ dephosphorylation by a multiple inositol polyphosphate phosphatase[4a,16] (MIPP) was ascertained. The IC$_{50}$ plot for **10** was comparable to that for commercial PP-InsP$_5$ whereas **11** was about 4-fold less potent as an inhibitor (Figure 1). A similar ranking of the three pyrophosphates was obtained for inhibition of [β-^{32}P]-PP-InsP$_5$ dephosphorylation by a purified PP-InsP$_5$ phosphatase.[17] Based on these results, biosynthesized PP-InsP$_5$ was provisionally assigned the absolute stereochemistry represented in **10**.

Scheme 2



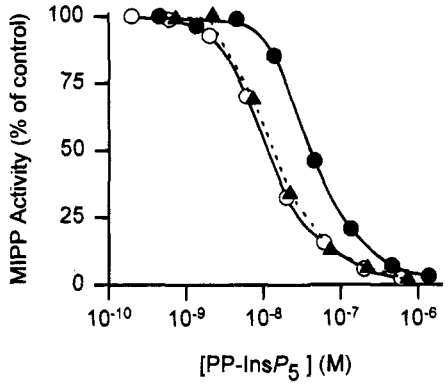


Figure 1. Inhibition of MIPP activity by PP-InsP$_5$. A multiple inositol polyphosphate phosphatase (MIPP) was purified and incubated with [^{3}H]Ins(1,3,4,5)P$_4$ as previously described,[16] in the presence of the indicated concentrations of either **10** (○—○), **11** (●—●), or commercial PP-InsP$_5$ (▲—▲). The extent of MIPP-mediated hydrolysis of [^{3}H]Ins-(1,3,4,5)P$_4$ is plotted as a percentage of the control activity in the absence of any PP-InsP$_5$.

Experimental Section

General. Chromatography, compositional and optical analyses, and routine laboratory manipulations were as reported.[18] Unless otherwise stated, ^{1}H/^{13}C NMR spectra were measured in $CDCl_3$ on a Bruker AC-250 spectrometer and reported relative to tetramethylsilane as internal reference. For TLC, ammonium molybdate reagent was used for visualization of phosphate-containing compounds.[19] High resolution mass spectroscopy was provided by The Midwest Center for Mass Spectrometry, University of Nebraska—Lincoln, Lincoln, NE.

Benzyl Methyl *N,N*-Diisopropylphosphoramidite (1). To a −20 °C solution of methoxydichlorophosphine[20] (1.8 mL, 19.04 mmol) in anhydrous ether (70 mL) was added dropwise a solution of diisopropylamine (5.39 mL, 38.08 mmol) in ether (30 mL) over 2 h.[11] After stirring for 2 h at room temperature, the white precipitate was filtered off under an argon atmosphere and washed with ether (30 mL). The combined filtrates were cooled to −20 °C, and *N,N*-diisopropylethylamine (3.3 mL, 19.04 mmol) was added slowly followed by benzyl alcohol (1.77 mL, 17.13 mmol). The reaction mixture was warmed to room temperature over 1 h and stirred for 2 h. The salts were removed by filtration, the filter cake was washed with ether (30 mL), and the combined filtrates were evaporated under reduced pressure. The residue was purified by flash chromatography (SiO_2) using hexane/EtOAc/Et_3N (8:2:0.1) to give **1** (4.2 g, 82%) as a colorless oil: TLC (SiO_2) 20% EtOAc/hexane, $R_f \sim 0.78$; ^{1}H NMR δ 1.17 (d, J = 2.6 Hz, 6H), 1.20 (d, J = 2.6 Hz, 6H), 3.42 (d, J_{HCOP} = 13.1 Hz, 3H), 3.54−3.77 (m, 2H), 4.60−4.80 (m, 2H), 7.17−7.42 (m, 5H); ^{13}C NMR δ 24.47, 24.58, 24.66, 42.67, 42.87, 50.49 (d, J_{COP} = 17.6 Hz), 65.22 (d, J_{COP} = 18.9 Hz), 126.86, 127.10, 128.10, 139.38 (d, J_{COP} = 7.5 Hz); HRMS (EI) calcd for $C_{14}H_{24}NO_2P$ *m/e* 269.1545, found 269.1549.

Benzyl Cyclohexyl Methyl Phosphate (2). To a suspension of cyclohexanol (200 mg, 2.0 mmol) and 1*H*-tetrazole (560 mg, 8.0 mmol) in anhydrous CH_2Cl_2 (3 mL) was added a solution of **1** (1.076 g, 4.0 mmol) in CH_2Cl_2 (2 mL). After 2 h, the reaction mixture was cooled to −40 °C, and a solution of 3-chloroperoxybenzoic acid (85%, 1.2 g, 6.0 mmol) in CH_2Cl_2 (3 mL) was added slowly. After 1 h, the mixture was diluted with CH_2Cl_2 (30 mL), washed with 5% aqueous $Na_2S_2O_5$ (2 × 10 mL), 10% aqueous $NaHCO_3$ (2 × 10 mL), and brine, and dried. The solvent was removed *in vacuo*, and the residue was purified by chromatography (SiO_2) to give **2** (505 mg, 89%) as a colorless syrup: TLC (SiO_2) ether, $R_f \sim 0.49$; ^{1}H NMR δ 1.14−1.40 (m, 3H), 1.40−1.63 (m, 3H), 1.63−1.81 (m, 2H), 1.83−2.02 (m, 2H), 3.72 (d, J_{HCOP} = 10.9 Hz, 3H), 4.28−4.46 (m, 1H), 5.07 (d, J_{HCOP} = 7.9 Hz, 2H), 7.28−7.47 (m, 5H); ^{13}C NMR δ 23.44, 25.02, 33.18, 33.24, 53.99 (d, J_{COP} = 6.3 Hz), 68.92 (d, J_{COP} = 5.7 Hz), 77.56 (d, J_{COP} = 5.7 Hz), 127.76, 128.37, 128.49, 136.10 (d, J_{COP} = 4.6 Hz).

Benzyl Cyclohexyl Phosphate (3). To a stirred solution of **2** (515 mg, 1.81 mmol) in DMF (1 mL) was added LiCN (0.5M solution in DMF, 4.35 mL). After 20 h, the solvent was evaporated under reduced pressure at room temperature. The residue was dissolved in water (10 mL), acidified to pH ~ 2 with 1 N HCl, and extracted with EtOAc (2 × 20 mL). The combined organic extracts were washed with water (5 mL) and brine (5 mL) and dried. Solvent evaporation *in vacuo* gave **3** (451 mg, 98%) as an off-white solid: ^{1}H NMR δ 1.13−1.40 (m, 3H), 1.40−1.65 (m, 3H), 1.65−1.84 (m, 2H), 1.86−2.02 (m, 2H), 4.23−4.41 (m, 1H), 5.05 (d, J_{HCOP} = 8.3 Hz, 2H), 7.24−7.45 (m, 5H), 7.88 (br. s, 1H); ^{13}C NMR (D_3COD) δ 25.06, 26.70, 35.05, 35.07, 68.30 (d, J_{COP} = 17.0 Hz), 75.66 (d, J_{COP} = 19.1 Hz), 128.31, 128.65, 129.43, 139.88 (d, J_{COP} = 7.5 Hz).

Cyclohexyl Tribenzyl Pyrophosphate (4). To a 0 °C solution of **3** (95 mg, 0.35 mmol) and triethylamine (63 μL, 0.46 mmol) in anhydrous CH_2Cl_2 (2 mL) was added dibenzyl chlorophosphonate[13] (125 mg, 0.42 mmol) in CH_2Cl_2 (1 mL). After 1 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with water (5 mL) and brine (5 mL), and dried. All volatiles were removed *in vacuo*, and the residue was purified by flash chromatography (SiO_2) using ether as eluant to give **4** (162 mg, 87%) as a white low melting solid: TLC (SiO_2) ether, $R_f \sim 0.42$; ^{1}H NMR δ 1.10−1.39 (m, 1H), 1.39−1.79 (m, 5H), 1.72−1.98 (m, 2H), 4.40−4.57 (m, 1H), 5.07−5.25 (m, 6H), 7.29−7.53 (m, 15H); ^{13}C NMR δ 23.32 (2 C), 24.97, 32.80−33.28 (m, 2 C), 69.12−70.45 (m, 3 C), 78.97−79.78 (m, 1 C), 127.86−128.76 (m, 15 C), 135.05−135.89 (m, 3 C).

Cyclohexyl Pyrophosphate Trisodium Salt (5). A mixture of **4** (52 mg, 0.098 mmol), $NaHCO_3$ (29 mg, 0.343 mmol), and Pd black (20 mg) in 7 mL of t-BuOH/H_2O (6:1) was stirred under hydrogen (1 atm) for 4 h. The catalyst was removed by filtration over a pad of Celite 577, and the filter cake was washed with water (5 mL), EtOH (5 mL), and EtOAc (5 mL). The combined filtrates were evaporated *in vacuo* at room temperature, and the residue was triturated with ether to give **5** (31 mg, 97%) as a white amorphous solid: ^{1}H NMR (D_2O) δ 0.90−1.41 (m, 6H), 1.45−1.62 (m, 2H), 1.73−1.91 (m, 2H), 3.88−4.04 (m, 1H); ^{13}C NMR (D_2O) δ 26.32, 27.38, 35.87, 35.93, 78.09 (d, J_{COP} = 56.0 Hz); ^{31}P NMR (D_2O, 81.0 MHz, H_3PO_4 in D_2O as external reference) δ −10.06 (d, J_{POP} = 20.8 Hz), −5.31 (d, J_{POP} = 20.8 Hz).

2,3:5,6-Di-*O*-cyclohexylidene-1-*O*-(benzoxymethoxyphosphono)-4-*O*-dibenzoxyphosphono)-D-*myo*-inositol (7). (+)-2,3:5,6-Dicyclohexylidene-D-*myo*-inositol[14] (**6**) (500 mg, 1.47 mmol) and bis(tributyl)tin oxide (625.9 mg, 1.05 mmol) in toluene (10 mL) were heated under reflux with water removal by activated 4 Å molecular sieves. After 3 h, the reaction mixture was cooled to 0 °C, and neat dibenzyl chlorophosphonate[13] (576.9 mg, 2.2 mmol) was added. The mixture was warmed to room temperature and after 2 h was concentrated under reduced pressure. Flash chromatography (SiO_2) using hexane/ethyl acetate (4:6) gave 2,3:5,6-di-*O*-cyclohexylidene-4-*O*-(dibenzoxyphosphono)-D-*myo*-inositol (688 mg, 78%), mp 133 °C; $[\alpha]^{20}_D$ +16.1° (*c* 2.2, $CHCl_3$); TLC (SiO_2) ether, $R_f \sim 0.33$; ^{1}H NMR δ 1.21−1.80 (m, 20H), 2.49 (d, J = 8.7 Hz, 1H D_2O exchangeable), 3.44 (dd, J = 9.2, 10.7 Hz, 1H), 3.87 (dd, J = 9.2, 10.0 Hz, 1H), 3.96−4.05 (m, 1H), 4.18 (dd, J = 5.0, 6.5 Hz, 1H), 4.46 (t, J = 4.6 Hz, 1H), 4.62−4.73 (m, 1H), 5.10−5.15 (m, 4H), 7.25−7.44 (m, 10H); ^{13}C NMR δ 23.60, 23.66, 23.87, 24.82, 35.13, 36.25, 36.35, 37.41, 68.95−69.02 (m), 69.70, 76.23 (d, J_{CCOP} = 3.5 Hz) 77.25, 77.66, 79.69 (d, J_{CCOP} = 3.5 Hz), 80.90 (d, J_{COP} = 5.5 Hz), 111.28, 113.52, 127.68, 127.73, 128.22, 128.25, 128.38, 136.00, 136.13; HRMS (FAB, NBA) calcd for $C_{32}H_{42}O_9P$ $(M + H)^+$ *m/e* 601.2566, found 601.2570.

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To a suspension of 1*H*-tetrazole (186 mg, 2.66 mmol) and the above monophosphate triester (400 mg, 0.67 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added **1** (375.8 mg., 1.33 mmol). After 1 h at room temperature, the reaction was recooled to −78 °C, and a solution of *m*-CPBA (85%; 458.2 mg, 2.66 mmol) in CH_2Cl_2 (5 mL) was added. After 2 h, the reaction mixture was diluted with more CH_2Cl_2 (20 mL) and washed with 5% aqueous $Na_2S_2O_3$ (30 mL × 2) and saturated aqueous $NaHCO_3$ (30 mL × 2). The aqueous layer was extracted a second time with CH_2Cl_2 (30 mL). The combined organic extracts were washed with brine (50 mL), dried, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2) to give **7** (421 mg, 80%) as an *ca.* 1:1 mixture of phosphate diastereomers: TLC (SiO_2) EtOAc/hexane (1:1), $R_f \sim 0.24$; ^{1}H NMR δ 1.21−1.82 (m, 20H), 3.47 (d, J = 10.45 Hz, 1H), 3.80 (d, J = 11.4 Hz, 1.5H), 3.81(d, J = 11.4 Hz, 1.5H), 4.07−4.15 (m, 2H), 4.58 (t, J = 4.4 Hz, 1H), 4.65−4.80 (m, 2H), 5.08−5.20 (m, 6H), 7.30−7.43 (m, 15H); ^{13}C NMR δ 23.59, 23.81, 24.76, 35.06, 36.20, 37.42, 54.38−54.70 (m), 69.30 (d, J_{COP} = 11.87 Hz), 69.38 (d, J_{COP} = 11.9 Hz), 74.19, 74.27; 74.97, 75.09, 76.23, 79.57, 79.62, 80.19, 80.28, 111.52, 113.74, 127.66, 127.72, 127.79, 128.20, 128.34, 128.45, 135.50, 135.62, 135.92, 136.07; MS (FAB, NBA) *m/z* 785 (M + 1), 695, 597, 409, 355, 281, 221, 147; HRMS (FAB, NBA) calcd for $C_{40}H_{51}O_{12}P_2$ *m/z* 785.2856, found 785.2856.

1-*O*-(Benzoxymethylphosphono)-2,3,4,5,6-penta-*O*-(dibenzoxyphosphono)-D-*myo*-inositol (8). Diphosphate **7** (300mg, 0.382 mmol) was dissolved in CF_3COOH/CH_2Cl_2/MeOH (1.5:3:0.5, 10 mL) at 0 °C. After 4 h, the solvents were evaporated, and the residue was triturated with ether (1 mL × 3), ethyl acetate (1 mL × 3), and methylene chloride (1 mL × 3) to give 1-*O*-(benzoxymethoxyphosphono)-4-*O*-(dibenzoxyphosphono)-D-*myo*-inositol (198 mg, 87%), mp 173−174 °C, sufficiently pure to be used without further purification: TLC (SiO_2) 10% MeOH/CH_2Cl_2, $R_f \sim 0.35$; ^{1}H NMR (CD_3OD) δ 3.41 (t, J = 10.2 Hz, 1H), 3.58−3.62 (m, 1H), 3.72 (d, J = 11.0 Hz, 3H), 3.83−3.95 (m, 1H), 4.15−4.23 (m, 2H), 4.41−4.46 (m, 1H), 5.00−5.04 (m, 6H), 7.19−7.25 (m, 15H); HRMS (FAB, NBA) calcd for $C_{28}H_{35}O_{12}P_2$ (M + H)$^+$ *m/e* 625.1604, found 625.1601.

To a suspension of the above tetraol (150 mg, 0.24 mmol) and 1*H*-tetrazole (203 mg, 2.9 mmol) in CH_2Cl_2 (10mL) was added dibenzyl *N,N*-diisopropylphosphoramidite (666 mg, 1.94 mmol). After 2 h, the reaction mixture was cooled to −78 °C and a solution of *m*-CPBA (85%; 416.2 mg, 2.42 mmol) in CH_2Cl_2 (3 mL) was added slowly. The reaction mixture was stirred at −20 °C for 4 h, diluted with CH_2Cl_2 (15 mL), and washed with 5% aqueous $Na_2S_2O_3$ (25 mL × 2) and saturated $NaHCO_3$ (25 mL × 2). The aqueous solution was extracted with CH_2Cl_2 (25 mL). The combined organic extracts were washed with brine (30 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by chromatography (SiO_2) to give **8** (310 mg, 79%) as an *ca.* 1:1 diastereomeric mixture: TLC (SiO_2) CH_2Cl_2/EtOAc/acetone (2:1:1), $R_f \sim 0.57$; ^{1}H NMR δ 3.61 (d, J = 11 Hz, 1.5H), 3.65 (d, J = 11 Hz, 1.5H), 4.38−4.50 (m, 6H), 4.80−5.30 (m, 22H), 7.05−7.40 (m, 55H); ^{13}C NMR δ 54.6 (d, J_{COP} = 14 Hz), 54.7 (d, J_{COP} = 14 Hz), 69.48 (m), 69.56 (m), 69.84 (m), 73.18 (b), 74.29(b), 74.90(b), 127.64, 127.72, 127.85, 127.91, 127.98, 127.09, 128.08, 128.17, 128.28, 135.42, 135.58, 135.71; ^{31}P NMR δ ($CDCl_3$, 85% H_3PO_4 external standard) −2.04 (0.5P), −1.98 (0.5P), −1.01 (1P), −0.38 to −0.25 (m, 3P), −0.22 (0.5P), 0.13 (0.5P), MS (FAB, NBA) *m/z* 1687 (M + Na)$^+$, 1665 (M + H)$^+$, 1597, 1575, 1485, 361, 271, 181. HRMS (FAB, NBA) calcd for (M + 1) $C_{84}H_{87}O_{24}P_6$ *m/e* 1665.4013, found 1665.3985.

Lithium Salt 9. To a solution of **8** (266 mg, 0.16 mmol) in anhydrous DMF (3 mL) was added LiCN (0.5 M solution in DMF, 340 μL). After 24 h at ambient temperature the solvent was removed *in vacuo* at ambient temperature. The residue was chromotagraphed (SiO_2) to yield lithium salt **9** (217 mg, 82%) as a colorless syrup: TLC (SiO_2) 10% MeOH/CH_2Cl_2, $R_f \sim 0.4$; ^{1}H NMR δ 3.03−3.41 (m, 2H), 3.90−4.20 (m, 1H), 4.20−4.52 (m, 1H), 4.57−5.58 (m, 23H), 6.02−6.15 (m, 1H), 6.75−7.50 (m, 55H); ^{13}C NMR δ 67.45−67.90 (m), 69.10−71.25 (m), 73.00−73.56 (m), 74.75−75.90 (m), 126.70−129.00 (m), 134.90−136.40 (m).

1-*O*-[(Phosphonooxy)hydroxyphosphinyl]-2,3,4,5,6-pentakis-*O*-phosphono-D-*myo*-inositol Sodium Salt (10). To a 0 °C solution of **9** (120 mg, 0.074 mmol) and triethylamine (13 μL, 0.088 mmol) in anhydrous CH_2Cl_2 (2 mL) was added dibenzyl chlorophosphonate[13,21] (26 mg, 0.088 mmol) in CH_2Cl_2 (1 mL). After 2 h at ambient temperature the volatiles were removed *in vacuo*. The residue was dissolved in t-BuOH/H_2O (6:1; 21 mL) and shaken under H_2 (50 psi) in a Parr apparatus for 4 h in presence of $NaHCO_3$ (86 mg, 1.032 mmol) and Pd black (100 mg). The catalyst was removed by filtration over a pad of Celite 577, and the filter cake was washed with water (10 mL), EtOH (10 mL), and EtOAc (5 mL). The combined filtrates were evaporated *in vacuo* at room temperature. The crude material was purified by chromatography (Q sepharose fast flow) eluting with a gradient from 0.5 to 2.0 M ammonium acetate (pH 5.0). The pyrophosphate fractions were lyophilized to afford **10** as its sodium salt (51 mg, 68% overall from **9**) as a white amorphous solid: TLC (PEI-cellulose) 1.5 N HCl, $R_f \sim 0.42$; ^{1}H NMR (D_2O) δ 4.06−4.25 (m, 1H), 4.25−4.56 (m, 5H); ^{31}P NMR (202 MHz, D_2O) δ -7.71 (br s, 1P), −4.20 to −2.80 (m, 1P), 2.30−6.40 (m, 5P); HRMS (neg ion FAB, TEA) calcd for (M − Na)$^-$ $C_6H_6Na_{12}O_{27}P_7$ *m/e* 1002.6032, found 1002.6050.

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Supporting Information Available: ^{1}H and/or ^{13}C NMR spectra for all isolated compounds and ^{31}P NMR spectra for **5**, **8**, and **10** (22 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(21) The use of 3 equiv of dibenzyl chlorophosphonate increased the overall yield of **10** to 80% but also favored the production of a chromatographically similar byproduct that made the purification tedious.