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Synthesis of 2- and 5-Diphospho-*myo*-inositol Pentakisphosphate (2- and 5-PP-InsP$_5$), Intracellular Mediators

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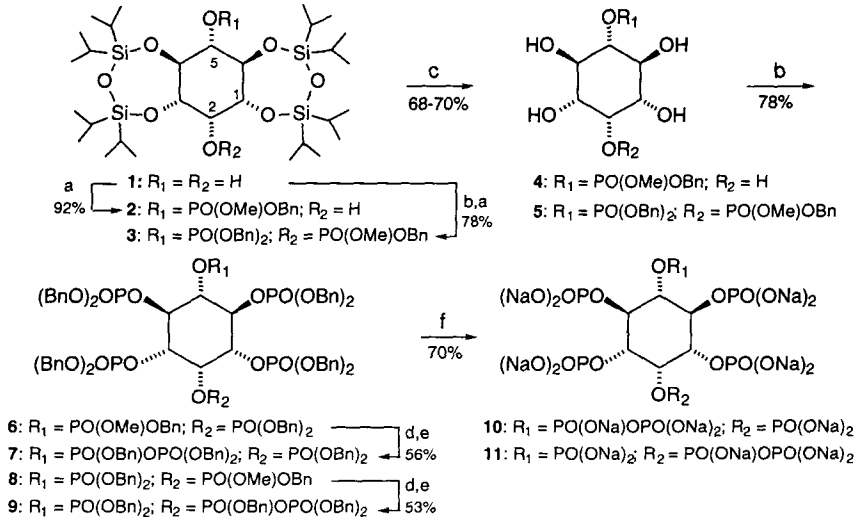
Abstract: The title pyrophosphates were prepared in good overall yield from a readily available bis-disiloxanylidene derivative of *myo*-inositol. © 1997 Elsevier Science Ltd.

The intensive structural and functional studies of the *myo*-inositol cycle have led recently to the discovery of a ubiquitous family of pyrophosphoryl *myo*-inositol pentaphosphates (PP-InsP$_5$) and related bis-pyrophosphates.[1] Although present in most mammalian cells at 1 μM or less, the PP-InsP$_5$ have an unusually rapid metabolic turnover[2] consistent with speculation that they act as intracellular autacoids and/or as the phosphate donor for an unidentified kinase(s).[3] Their levels are sensitive[4] to changes in cellular Ca^{2+} and they display high affinity binding to several key regulatory proteins including coatomer, AP-2, and AP-3.[5]

In *Dictyostelium*, the most extensively studied organism to date, the structure of the predominate PP-InsP$_5$ was initially assigned as 1-PP-**D**-*myo*-InsP$_5$ or its enantiomeric 3-PP-**D** regioisomer.[3a] The regiochemistry was revised to either 4- or 6-PP-**D** by Vogel[6] based on 2D $^1H/^{31}P$ NMR and was finally confirmed as the latter using synthetic standards.[7] More recent investigations, however, have cogently demonstrated the principal PP-InsP$_5$ isoform produced by several mammalian cell lines is distinct from the above chiral PP-InsP$_5$ isomers, *viz.*, 1-/3-/4-/6-PP-**D**-*myo*-InsP$_5$.[8] As a consequence of the greater attention now focused on the two remaining regioisomers,[9] we describe herein a stereocontrolled route to 5-PP-**D**-*myo*-InsP$_5$ (**10**) and 2-PP-**D**-*myo*-InsP$_5$ (**11**).

Scheme 1



Reagents and conditions: (a) **12** (1 equiv), 1*H*-tetrazole (2 equiv), CH_2Cl_2, 0°C, 3 h; *m*-CPBA, -78°C, 15 min. (b) *i*-$Pr_2NP(OBn)_2$, 1*H*-tetrazole, CH_2Cl_2, 0°-23°C, 4 h; *m*-CPBA, -78°C, 15 min. (c) py•HF/THF (1:2.5), 24°C, 3 h; $NaHCO_3$. (d) LiCN (1 equiv), DMF, 23°C, 12 h. (e) $(BnO)_2POCl$, Et_3N, CH_2Cl_2, 0°-23°C, 2 h. (f) Pd black/$NaHCO_3$, H_2 (50 psi), *t*-BuOH/H_2O (6:1), 4 h.

Access to these achiral isomers was achieved from the readily available *myo*-inositol bis-disiloxanylidene **1**, prepared according to Ozaki[10,11] in 68% yield, as summarized in Scheme 1. Selective phosphorylation at the less hindered equatorial C(5)-alcohol in **1** via initial derivatization with benzyl methyl N,N-diisopropylphosphoramidite (**12**), freshly prepared from chloro-(N,N-diisopropylamino)methoxyphosphine and benzyl alcohol using a modification of Caruthers' procedure,[12] followed by low temperature peracid oxidation *in situ* furnished phosphate triester **2**[13] as an ~1:1 diastereomeric mixture. Mild de-silylation of **2** liberating pentaol **4** as expected required carefully defined conditions and was best performed with hydrogen fluoride-pyridine complex at ambient temperature. More basic/acidic reagents, e.g., n-Bu_4NF, HF, and CF_3CO_2H, afforded complex product mixtures. Dibenzylphosphorylation proceeded smoothly to give hexakisphosphate **6** which was advanced by specific LiCN mediated cleavage[9] of the phosphate methyl ester. After extractive isolation, the resultant lithium salt (mp 48°C) was coupled immediately with dibenzyl chlorophosphonate as described previously[9] to give the protected pyrophosphate **7**. Catalytic hydrogenolysis, purification by ion exchange chromatography (Q Sepharose), and bicarbonate neutralization led to the sodium salt of 5-PP-*myo*-$InsP_5$ (**10**).

Alternatively, prior dibenzylphosphorylation of **1** at the C(5)-alcohol and subsequent derivatization with **12** gave rise to **3** whose conversion to 2-PP-*myo*-$InsP_5$ (**11**) via tetraol **5**, hexaphosphate **8**, protected pyrophosphate **9**, and catalytic hydrogenolysis exactly paralleled the above sequence. The comparisons of **10** and **11** with natural material and the results of pharmacologic testing will be presented elsewhere.

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References and Notes

1. Reviews: Olszewski, J. D.; Prestwich, G. D. *Chemtracts-Org. Chem.* **1993**, *6*, 139-144. Menniti, F. S.; Oliver, K. G.; Putney, J. W., Jr.; Shears, S. B. *Trends Biol. Sci.* **1993**, *18*, 53-56.
2. Menniti, F. S.; Miller, R. N.; Putney, J. W., Jr.; Shears, S. B. *J. Biol. Chem.* **1993**, *268*, 3850-3856.
3. (a) Stephens, L.; Radenberg, T.; Thiel, U.; Vogel, G.; Khoo, K.-H.; Dell, A.; Jackson, T. R.; Hawkins, P. T.; Mayr, G. W. *J. Biol. Chem.* **1993**, *268*, 4009-4015. (b) Voglmaier, S M.; Bembenek, M. E.; Kaplin, A. I.; Dorman, J. D.; Olszewski, J. D.; Prestwich, G. D.; Snyder, S. H. *Proc. Natl. Acad. Sci.U.S.A.*, **1996**, *93*, 4305-4310.
4. Glennon, M. C.; Shears, S. B. *Biochem. J.* **1993**, *293*, 583-590.
5. Coatomer: Fleisher, B.; Xie, J.; Mayrleitner, M.; Shears, S. B.; Fleisher, S. *J. Biol. Chem.* **1994**, *269*, 17826-17832. AP-2/AP-3: Ye, W.; Ali, N.; Bembenek, M. E.; Shears, S. B.; Lafer, E. M.; *J. Biol. Chem.* **1995**, *270*, 1564-1568.
6. Laussmann, T.; Eujen, R.; Weisshuhn, C. M.; Thiel, U.; Vogel, G. *Biochem. J.* **1996**, *315*, 715-720.
7. Laussmann, T.; Reddy, K. M.; Reddy, K. K.; Falck, J. R.; Vogel, G. *Biochem. J.* **1997**, *322*, 31-33.
8. Shears, S. B.; Mayr, G. W., personal communication.
9. Asymmetric synthesis of 1-/3-PP-**D**-*myo*-$InsP_5$: Falck, J. R.; Reddy. K. K.; Ye, J.; Saady, M.; Mioskowski, C.; Shears, S. B.; Tan, Z.; Safrany, S. *J. Am. Chem. Soc.* **1995**, *117*, 12172-12175. Synthesis of the 4-/6-regioisomers by modification of this route has been completed: manuscript submitted.
10. Watanabe, Y.; Mitani, M.; Morita, T.; Ozaki, S. *J. Chem. Soc., Chem. Comm.* **1989**, 482-483.
11. The related bis-BBA protected inositol showed similar specificity, but the ketals could not be removed without loss/migration of the phosphate esters: Montchamp, J.-L.; Tian, F.; Hart, M.E.; Frost, J. W. *J. Org. Chem.* **1996**, *61*, 3897-3899.
12. Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859-1862.
13. Spectral data for **2**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.45 (m, 5H), 4.85-5.25 (m, 2H), 4.26 (q, J = 12 Hz, 1H), 4.15 (dt, J = 6, 9.2 Hz, 2H), 4.01 (t, J = 2.4 Hz, 1H), 3.67 (dd, J = 2.8, 8.8 Hz, 2H), 3.55 (d, J = 11.2 Hz, 3H), 2.58 (br s, 1H), 0.80-1.25 (m, 56H). **10**: ^{31}P NMR (D_2O, 162 MHz, 85% H_3PO_4 external ref.) δ 2.61(s, 3P), 1.21-2.15 (br s, 2P), -5.25 (d, J = 14.7 Hz, 1P), -9.92 (br s, 1P). **11**: ^{31}P NMR (D_2O, 162 MHz, 85% H_3PO_4 external ref.) δ 3.36 (s, 2P), 1.63 (br s, 3P), -4.90 (d, J = 16.2 Hz, 1P), -9.67 (br s, 1P).

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