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LETTERS

Concise syntheses of L- α -phosphatidyl-D-*myo*-inositol 3-phosphate (3-PIP), 5-phosphate (5-PIP), and 3,5-bisphosphate (3,5-PIP₂)

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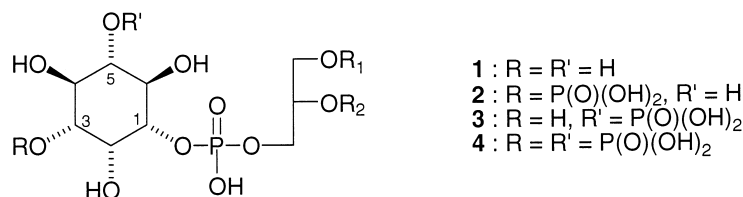
Abstract

Highly efficient, asymmetric total syntheses of the title phospholipids as well as short chain and cross-linkable aminoether analogs were achieved in five to seven steps from a readily available *myo*-inositol derivative. © 2000 Elsevier Science Ltd. All rights reserved.

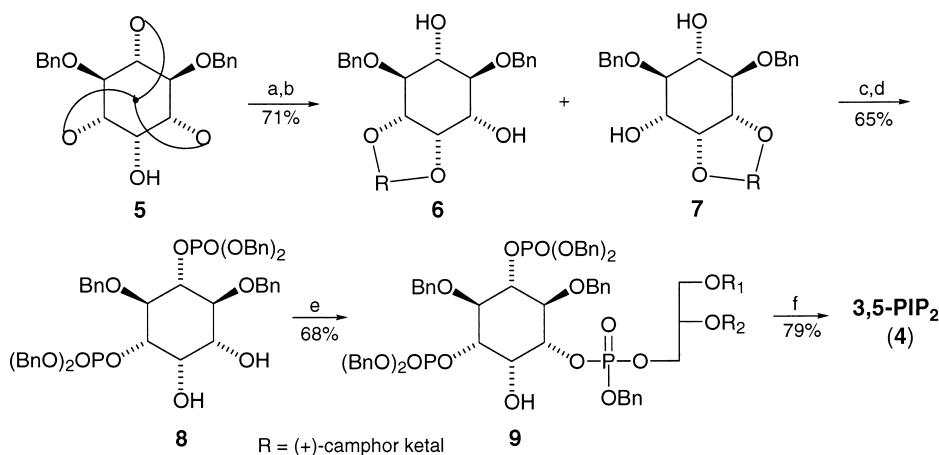
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The minor cellular lipid L- α -phosphatidyl-D-*myo*-inositol (**1**; PI) and its phosphorylated progeny have been implicated in a vast array of essential physiologic processes including mitogenesis, calcium regulation, vesicle trafficking, apoptosis, and cytoskeleton assembly.¹ They function as precursors² to low molecular weight second messengers or act directly via regional recruitment and/or regulation of macromolecules.³ Recent investigations by several laboratories, however, have identified L- α -phosphatidyl-D-*myo*-inositol 5-phosphate (**3**; 5-PIP) and L- α -phosphatidyl-D-*myo*-inositol 3,5-bisphosphate (**4**; 3,5-PIP₂) as potential new members of the PI cascade⁴ and revealed additional pathways for the biosynthesis of PIP_ns in eukaryotes.⁵ The physiologic role(s) of these novel PI metabolites and their interconversions, e.g. **2** ↔ **4** ↔ **3**, are areas of intense, worldwide scrutiny. As part of our continuing program⁶ to provide comprehensive access to all components of the PI cycle, we report herein concise, total syntheses of 3-PIP (**2**), 5-PIP (**3**), and 3,5-PIP₂ (**4**) as well as some useful glyceryl lipid analogs.⁷

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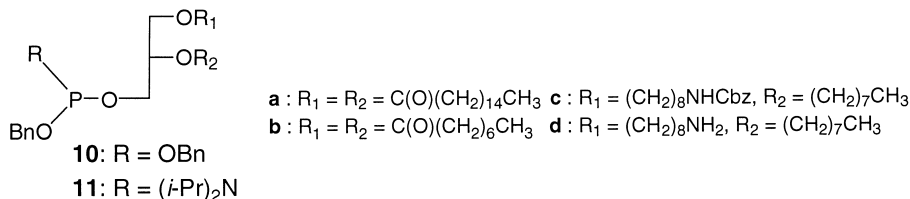


Mild acidic hydrolysis of orthoformate **5** (mp 123–25°C), readily available⁸ in two steps from *myo*-inositol, followed by *p*-toluenesulfonic acid (PTSA) catalyzed exchange with (+)-camphor dimethyl ketal⁹ provided convenient access to the chromatographically separable ketals **6** and **7** [TLC: Et₂O:CH₂Cl₂ (5:95), *R_f* ≈ 0.32 and 0.35, respectively] in good overall yield (Scheme 1).^{10,11} Treatment of diastereomer **7** with excess *O,O*-dibenzyl-*N,N*-diisopropylphosphoramidite¹² and in situ peracid oxidation generated the corresponding bis-phosphate triester. Subsequent cleavage of the camphor ketal at 0°C using trifluoroacetic acid smoothly led to diol **8** that was converted to tris-phosphate **9a**^{13,14} utilizing Watanabe's pyridinium perbromide methodology¹⁵ for the activation of 1,2-di-*O*-hexadecanoyl-*sn*-glyceryl dibenzylphosphite⁶ **10a** and regioselective phosphorylation of the C(1)-alcohol.

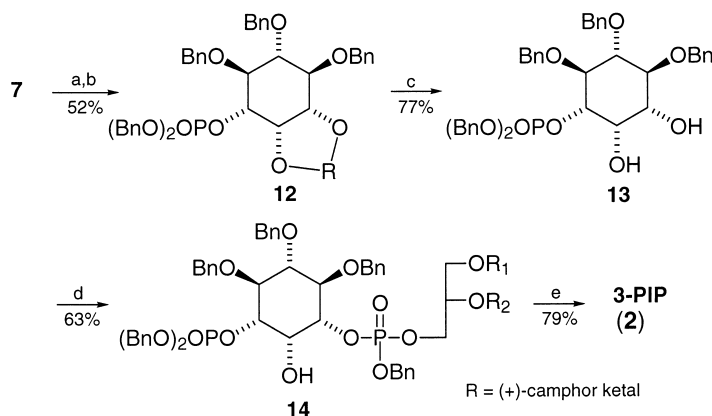


Scheme 1. Reaction conditions: (a) MeOH:10N HCl (12.5:1), 65°C, 0.45 h (87%); (b) (+)-camphor dimethyl ketal (3 equiv.), PTSA (2 mol%), CH₂Cl₂, 23°C, 4 h (82%); (c) (*i*Pr)₂NP(OBn)₂ (2.5 equiv.), 1*H*-tetrazole, CH₂Cl₂, 23°C, 2 h; *m*-CPBA, –40°C, 1 h (88%); (d) CF₃CO₂H:CH₂Cl₂:MeOH (1.5:3:0.5), 0°C, 0.5 h (77%); (e) phosphite **10a–c** (2 equiv.), py·HBr₃ (2.25 equiv.), CH₂Cl₂:py:Et₃N (5:1:0.1), –20°C to 0°C, 0.5 h (63%); (f) Pd black, H₂ (52 psi), NaHCO₃ (5 equiv.), EtOH:H₂O (6:1), 23°C, 6 h (79%)

Phosphoramidite⁶ **11a** was less satisfactory under a variety of conditions and often resulted in mixtures of regioisomers and/or bis-derivatized products. Exhaustive debenzilation by catalytic hydrogenation over Pd black in EtOH/H₂O in the presence of NaHCO₃ afforded 3,5-PIP₂ (**4a**), isolated as its sodium salt.¹⁶

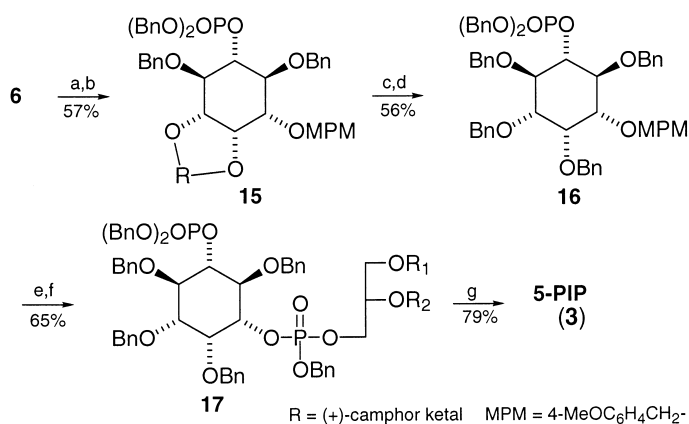


Alternatively, phosphorylation of the C(3)-alcohol in **7** using a limited amount of reagent and trityl cation mediated¹⁷ benzylolation of the remaining C(5)-hydroxyl with benzyltrichloroacetimidate gave rise to phosphate **12** (Scheme 2). The latter's transformation to 3-PIP (**2a**) via **13** and **14a** proceeded as described above in comparable yields.



Scheme 2. Reaction conditions: (a) $(i\text{Pr})_2\text{NP}(\text{OBn})_2$ (1 equiv.), 1*H*-tetrazole, CH_2Cl_2 , 23°C, 2 h; *m*-CPBA, -40°C, 1 h (71%); (b) $\text{PhCH}_2\text{OC}(\text{NH})\text{CCl}_3$, Ph_3CBF_4 (5 mol%), Et_2O , 23°C, 36 h (73%); (c) $\text{CF}_3\text{CO}_2\text{H}:\text{CH}_2\text{Cl}_2:\text{MeOH}$ (1.5:3:0.5), 0°C, 0.5 h (77%); (d) phosphite **10a-c** (2 equiv.), $\text{py}\cdot\text{HBr}_3$ (2.25 equiv.), $\text{CH}_2\text{Cl}_2:\text{py}:\text{Et}_3\text{N}$ (5:1:0.1), -20°C to 0°C, 0.5 h (63%); (e) Pd black, H_2 (52 psi), NaHCO_3 (5 equiv.), $\text{EtOH}:\text{H}_2\text{O}$ (6:1), 23°C, 6 h (79%)

The remaining acetal **6** was exploited for the preparation of 5-PIP (**3**) via regioselective etherification of the in situ generated stannyl ester of the C(1)-hydroxyl and phosphorylation of the residual C(5)-alcohol (Scheme 3). The resultant phosphate triester **15** yielded the differentially protected inositol **16** when subjected to acid ketal hydrolysis and trichloroacetimidate benzylolation. Liberation



Scheme 3. Reaction conditions: (a) $(n\text{-Bu}_3\text{Sn})_2\text{O}$ (1 equiv.), PhH , 80°C, 6 h with Dean–Stark; MPM-Cl (1.1 equiv.), CsF (4 equiv.), DMF, 23°C, 12 h (71%); (b) $(i\text{Pr})_2\text{NP}(\text{OBn})_2$, 1*H*-tetrazole, CH_2Cl_2 , 23°C, 2 h; *m*-CPBA, -40°C, 1 h (81%); (c) $\text{CF}_3\text{CO}_2\text{H}:\text{CH}_2\text{Cl}_2:\text{MeOH}$ (1.5:3:0.5), 0°C, 0.5 h (77%); (d) $\text{PhCH}_2\text{OC}(\text{NH})\text{CCl}_3$, Ph_3CBF_4 (5 mol%), Et_2O , 23°C, 36 h (73%); (e) DDQ, $\text{CH}_2\text{Cl}_2:\text{H}_2\text{O}$ (9:1), 23°C, 4 h (80%); (f) phosphoramidite **11a-c**, 1*H*-tetrazole, CH_2Cl_2 , 23°C, 2 h; *m*-CPBA, -40°C, 1 h (81%); (g) Pd black, H_2 (52 psi), NaHCO_3 (5 equiv.), $\text{EtOH}:\text{H}_2\text{O}$ (6:1), 23°C, 6 h (79%)

of the C(1)-alcohol by DDQ induced deprotection, phosphatidylation with **11a**, and oxidation furnished **17a** from which 5-PIP (**3a**) was obtained as its sodium salt by standard catalytic hydrogenolysis.

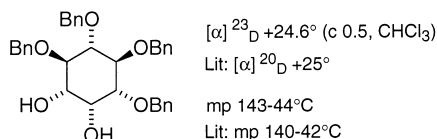
Repetition of the final condensations in Schemes 1–3 using **10/11b,c**⁶ afforded **4/2/3b,d** as appropriate. The dioctanoyl glyceryl analogs (**b-series**) are more water soluble than the fatty acid versions (**a-series**) and have proven more tractable in some assays. The ω -aminoalkyl analogs (**d-series**) can be derivatized with fluorescent, radioactive, and affinity labels; their application in the isolation of several specific PIP binding proteins will be reported elsewhere.

Acknowledgements

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References

- For a review, see: Rameh, L. E.; Cantley, L. C. *J. Biol. Chem.* **1999**, *274*, 8347–8350.
- Nishizuka, Y. *Science* **1992**, *258*, 607–614.
- Cantley, L. C.; Auger, K. R.; Carpenter, C.; Duckworth, B.; Graziani, A.; Kapeller, R.; Soltoff, S. *Cell* **1991**, *64*, 281–302. Martin, T. F. J. *Ann. Rev. Cell Develop. Biol.* **1998**, *14*, 231–264.
- Auger, K. R.; Serunian, L. A.; Soltoff, S. P.; Libby, P.; Cantley, L. C. *Cell*, **1989**, *57*, 167–175. Dove, S. K.; Cooke, F. T.; Douglas, M. R.; Sayers, L. G.; Parker, P. J.; Michell, R. H. *Nature* **1997**, *390*, 187–192. Jones, D. R.; González-García, A.; Díez, E.; Martínez-A., C.; Carrera, A. C.; Mérida, I. *J. Biol. Chem.* **1999**, *274*, 18407–18413.
- Rameh, L. E.; Tolias, K. F.; Duckworth, B. C.; Cantley, L. C. *Nature* **1997**, *390*, 192–196. Whiteford, C. C.; Brearley, C. A.; Ulug, E. T. *Biochem. J.* **1997**, *323*, 597–601.
- 3,4-PIP₂: Reddy, K. K.; Rizo, J.; Falck, J. R. *Tetrahedron Lett.* **1997**, *38*, 4729–4730. Reddy, K. K.; Ye, J.; Falck, J. R.; Capdevila, J. H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2115–2116. 3,4,5-PIP₃: Reddy, K. K.; Saady, M.; Falck, J. R.; Whited, G. *J. Org. Chem.* **1995**, *60*, 3385–3390. 4,5-PIP₂: Falck, J. R.; Krishna, U. M.; Capdevila, J. H. *Tetrahedron Lett.* **1999**, *40*, 8771–8774.
- Previous syntheses of 5-PIP and/or 3,5-PIP₂: Peng, J.; Prestwich, G. D. *Tetrahedron Lett.* **1998**, *39*, 3965–3968. Riley, A. M.; Potter, B. V. L. *Tetrahedron Lett.* **1998**, *39*, 6769–6772.
- Lee, H. W.; Kishi, Y. *J. Org. Chem.* **1985**, *50*, 4402–4404.
- Bruzik, K. S.; Tsai, M. D. *J. Am. Chem. Soc.* **1992**, *112*, 6361–6374.
- Careful spectral and chromatographic analyses revealed **6** and **7** are each single stereoisomers. However, it was not possible to assign the configuration as either *exo* or *endo*. Cf., Grove, S. J. A.; Gilbert, I. H.; Holmes, A. B.; Painter, G. F.; Hill, M. L. *Chem. Commun.* **1997**, 1633–1634.
- The absolute configuration of ketal **6** was established by perbenzylation (BnCl, NaH), ketal cleavage with methanolic HCl, and comparison with the standards reported by Ozaki, S.; Kohno, M.; Nakahira, H.; Bunya, M.; Watanabe, Y. *Chem. Lett.* **1988**, 77–80.



- Tegge, W.; Ballou, C. E. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 94–98.
- Intermediate **9** was somewhat labile and variable amounts of phosphate migration products were noted. This could be minimized by rapid purification at neutral pH and storage at low temperatures.

14. Consists of an ~1:1.6 diastereomeric mixture by ^{31}P NMR analysis as a consequence of the newly created tetrahedral phosphorus, but is typically used in the next step without separation.
15. Watanabe, Y.; Inada, E.; Jinno, M.; Ozaki, S. *Tetrahedron Lett.* **1993**, *34*, 497–500.
16. Spectral data for **7**: ^1H NMR (CDCl_3 , 400 MHz) δ 0.87 (s, 3H), 0.88 (s, 3H), 1.04 (s, 3H), 1.20–1.27 (m, 2H), 1.42–1.48 (m, 2H), 1.73–1.80 (m, 2H), 1.90–2.08 (m, 1H), 2.46 (d, J = 3.1 Hz, 1H), 2.66 (d, J = 2.8 Hz, 1H), 3.68 (ddd, J = 2.8, 6.1, 9.2 Hz, 1H), 3.73 (apparent t, J = 5.8 Hz, 1H), 3.88 (dd, J = 6.4, 9.2 Hz, 1H), 4.03–4.06 (m, 1H), 4.20–4.27 (m, 2H), 4.66 (d, J = 11.6 Hz, 1H), 4.72 (d, J = 11.6 Hz, 1H), 4.81 (d, J = 11.6 Hz, 1H), 4.89 (d, J = 11.6 Hz, 1H), 7.21–7.39 (m, 10H). Compound **2b**: ^1H NMR (D_2O , 400 MHz) δ 0.84 (t, J = 6.2 Hz, 6H), 1.14–1.38 (m, 16H), 1.48–1.62 (m, 4H), 2.12–2.39 (m, 4H), 3.38–3.42 (m, 1H), 3.75–3.82 (m, 2H), 3.94–4.01 (m, 2H), 4.06–4.13 (m, 2H), 4.25–4.31 (m, 1H), 4.37–4.46 (m, 2H), 5.29–5.36 (m, 1H); ^{31}P NMR (121.4 MHz, D_2O , 85% H_3PO_4 external reference) δ 1.82, –3.0. Compound **3b**: ^1H NMR (D_2O , 400 MHz) δ 0.86 (t, J = 6.8 Hz, 6H), 1.19–1.38 (m, 16H), 1.49–1.68 (m, 4H), 2.32–2.48 (m, 4H), 3.62 (dd, J = 2.5, 9.6 Hz, 1H), 3.76–3.85 (m, 3H), 4.01–4.09 (m, 3H), 4.21 (t, J = 2.6 Hz, 1H), 4.23–4.29 (m, 1H), 4.41–4.49 (m, 1H), 5.29–5.36 (m, 1H); ^{31}P NMR (121.4 MHz, D_2O , 85% H_3PO_4 external reference) δ 1.90, –3.0.
17. Nakajima, N.; Horita, K.; Abe, R.; Yonemitsu, O. *Tetrahedron Lett.* **1988**, *29*, 4139–4142.