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Concise syntheses of L-α-phosphatidyl-D-*myo*-inositol 3-phosphate (3-PIP), 5-phosphate (5-PIP), and 3,5-bisphosphate (3,5-PIP_2)

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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.

Keywords: cyclitols; phospholipids; inositols; phosphoramidites.

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 precursors[2] 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-PIP_2) as potential new members of the PI cascade[4] 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 program[6] 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-PIP_2 (**4**) as well as some useful glyceryl lipid analogs.[7]

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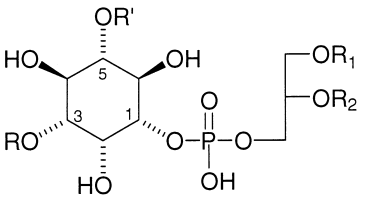
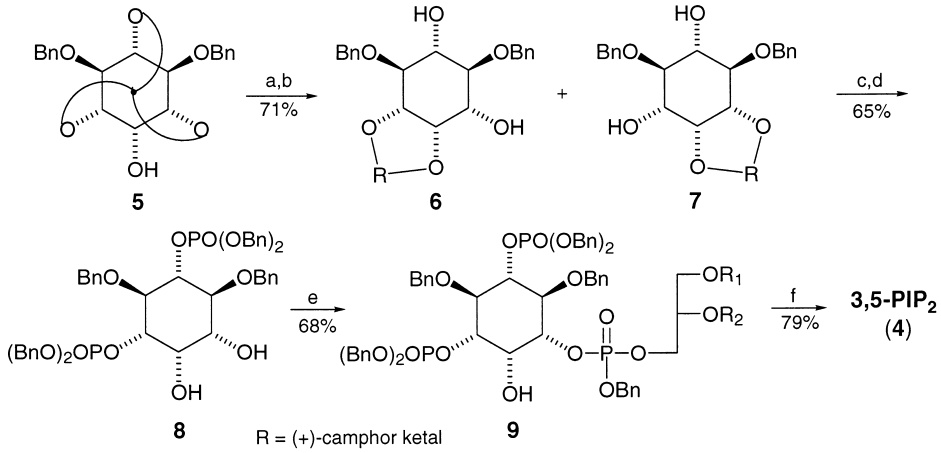


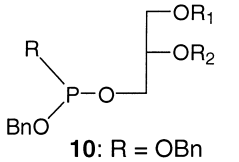
1 : R = R' = H
2 : R = $P(O)(OH)_2$, R' = H
3 : R = H, R' = $P(O)(OH)_2$
4 : R = R' = $P(O)(OH)_2$

Mild acidic hydrolysis of orthoformate **5** (mp 123–25°C), readily available[8] in two steps from *myo*-inositol, followed by *p*-toluenesulfonic acid (PTSA) catalyzed exchange with (+)-camphor dimethyl ketal[9] provided convenient access to the chromatographically separable ketals **6** and **7** [TLC: Et_2O:CH_2Cl_2 (5:95), $R_f \approx 0.32$ and 0.35, respectively] in good overall yield (Scheme 1).[10,11] Treatment of diastereomer **7** with excess *O*,*O*-dibenzyl-*N*,*N*-diisopropylphosphoramidite[12] 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 methodology[15] for the activation of 1,2-di-*O*-hexadecanoyl-*sn*-glyceryl dibenzylphosphite[6] **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%), CH_2Cl_2, 23°C, 4 h (82%); (c) $(iPr)_2NP(OBn)_2$ (2.5 equiv.), 1*H*-tetrazole, CH_2Cl_2, 23°C, 2 h; *m*-CPBA, –40°C, 1 h (88%); (d) CF_3CO_2H:CH_2Cl_2:MeOH (1.5:3:0.5), 0°C, 0.5 h (77%); (e) phosphite **10a–c** (2 equiv.), py·HBr_3 (2.25 equiv.), CH_2Cl_2:py:Et_3N (5:1:0.1), –20°C to 0°C, 0.5 h (63%); (f) Pd black, H_2 (52 psi), $NaHCO_3$ (5 equiv.), EtOH:H_2O (6:1), 23°C, 6 h (79%)

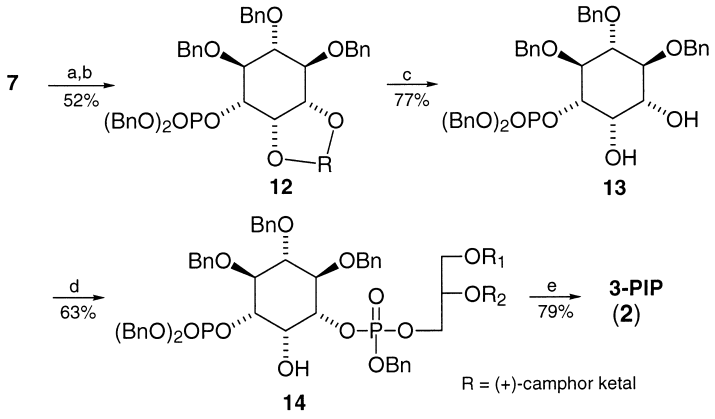
Phosphoramidite[6] **11a** was less satisfactory under a variety of conditions and often resulted in mixtures of regioisomers and/or bis-derivatized products. Exhaustive debenzylation by catalytic hydrogenation over Pd black in EtOH/H_2O in the presence of $NaHCO_3$ afforded 3,5-PIP_2 (**4a**), isolated as its sodium salt.[16]



10: R = OBn
11: R = $(i\text{-Pr})_2N$

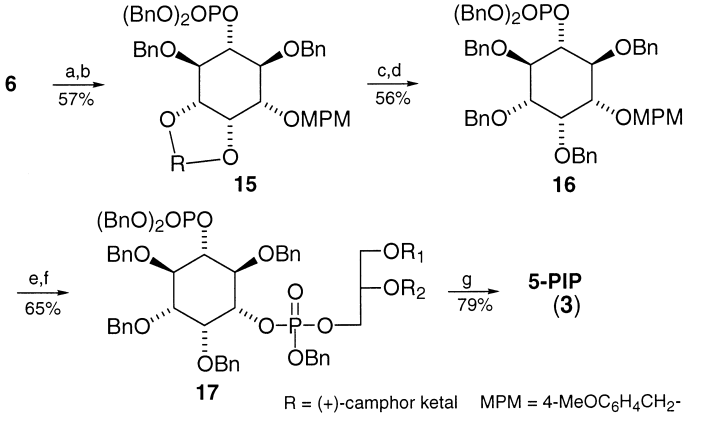
a : $R_1 = R_2 = C(O)(CH_2)_{14}CH_3$ **c** : $R_1 = (CH_2)_8NHCbz$, $R_2 = (CH_2)_7CH_3$
b : $R_1 = R_2 = C(O)(CH_2)_6CH_3$ **d** : $R_1 = (CH_2)_8NH_2$, $R_2 = (CH_2)_7CH_3$

Alternatively, phosphorylation of the C(3)-alcohol in **7** using a limited amount of reagent and trityl cation mediated[17] benzylation 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) $(i\text{Pr})_2\text{NP(OBn)}_2$ (1 equiv.), 1*H*-tetrazole, CH_2Cl_2, 23°C, 2 h; *m*-CPBA, –40°C, 1 h (71%); (b) $PhCH_2OC(NH)CCl_3$, Ph_3CBF_4 (5 mol%), Et_2O, 23°C, 36 h (73%); (c) CF_3CO_2H:CH_2Cl_2:MeOH (1.5:3:0.5), 0°C, 0.5 h (77%); (d) phosphite **10a–c** (2 equiv.), py·HBr_3 (2.25 equiv.), CH_2Cl_2:py:Et_3N (5:1:0.1), –20°C to 0°C, 0.5 h (63%); (e) Pd black, H_2 (52 psi), $NaHCO_3$ (5 equiv.), EtOH:H_2O (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 benzylation. Liberation



Scheme 3. Reaction conditions: (a) $(n\text{-}Bu_3Sn)_2O$ (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) $(i\text{Pr})_2\text{NP(OBn)}_2$, 1*H*-tetrazole, CH_2Cl_2, 23°C, 2 h; *m*-CPBA, –40°C, 1 h (81%); (c) CF_3CO_2H:CH_2Cl_2:MeOH (1.5:3:0.5), 0°C, 0.5 h (77%); (d) $PhCH_2OC(NH)CCl_3$, Ph_3CBF_4 (5 mol%), Et_2O, 23°C, 36 h (73%); (e) DDQ, CH_2Cl_2:H_2O (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.), EtOH:H_2O (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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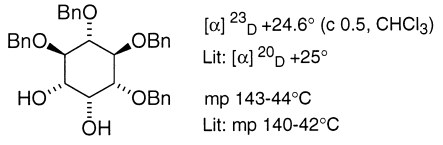

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13. Intermediate **9** was somewhat labile and variable amounts of phosphate migration products were noted. This could be minimized by rapid purification at neutral pH and storage at low temperatures.

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.
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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.
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