Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1711–1713

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Preparation of L-α-Phosphatidyl-D-*myo*-inositol 3-Phosphate (3-PIP) and 3,5-Bisphosphate (3,5-PIP_2)

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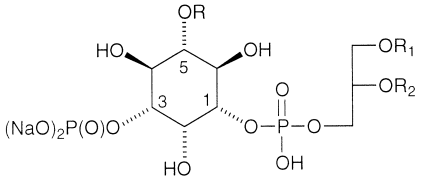
Received 10 January 2000; accepted 30 May 2000

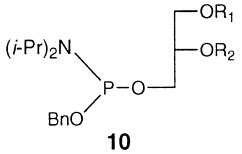
Abstract—Practical, asymmetric total syntheses of the title phospholipids from a readily available *myo*-inositol derivative as well as short chain and cross-linkable aminoether analogues are described.

Phosphoinositide (PI) 3-kinases[1] constitute a critical link in membrane traffic and agonist-activated intracellular signalling in eukaryotes.[2,3] Recent, intensive investigations of these pathways at the molecular level have revealed a novel PI 3-kinase metabolite, L-α-phosphatidyl-D-*myo*-inositol 3,5-bisphosphate (**2**; 3,5-PIP_2), in a wide variety of cells including lymphocytes, platelets, fibroblasts, COS-7, yeast, and plants.[4–10] In select cases,[7–9] a specific PI 3-phosphate 5-kinase that transforms 3-PIP (**1**) to 3,5-PIP_2 (**2**) has been characterized, although alternative pathways for the biogenesis of **2** are possible.[9] Despite considerable speculation,[11] no definitive role(s) for **1** and **2** have been elucidated to date.[7] To expedite current investigations of the physiological significance of these metabolites and their biochemical interconversions,[12] we report herein practical, asymmetric total syntheses of 3-PIP (**1**) and 3,5-PIP_2 (**2**), as well as some useful glyceryl lipid analogues.[13]



1: R = H
2: R = P(O)(ONa)$_2$

Mild acidic hydrolysis of orthoformate **3** (mp 123–25 °C), obtained in two steps from *myo*-inositol as described by Lee and Kishi,[14] and ketalization with cyclohexanone afforded diol **4**[15,16] (Scheme 1). Following several unsuccessful attempts to resolve **4** into its enantiomers via esterase/lipase differentiation and via derivatization with chiral acids, the hydroxyls were masked as MPM ethers to give **5** from which ±-**6** was obtained by removal of the cyclohexylidene. The diastereomeric bis-camphanoyl esters of the latter, however, were readily separable by SiO_2 chromatography (Et_2O/CH_2Cl_2 (5/95), R_f ~0.32 and 0.35).[17] Saponification of the more polar isomer provided (−)-**6**, mp 122–24 °C, $[\alpha]_D^{23}$ −23.5° (*c* 0.69, $CHCl_3$). Regioselective *O*-allylation of the C(1)-alcohol of **6** by way of the in-situ generated tin ester,[18] benzylation of the remaining C(2)-hydroxyl, and Rh-mediated deprotection[19] of the allylic ether gave rise to **7**. The lipid side chain was introduced by phosphatidylation of **7** with freshly prepared 1,2-di-*O*-hexadecanoyl-*sn*-glycerobenzyl (*N,N*-diisopropylamino)phosphoramidite[12c] (**10a**) and low temperature, in situ peracid oxidation; subsequent DDQ cleavage of the MPM ethers led to the key intermediate diol **8a**.[20] Treatment of **8a** with excess *O,O*-dibenzyl-*N,N*-diisopropylphosphoramidite[21] and adjustment of the phosphorus oxidation level afforded tris-phosphate **9a.** Exhaustive catalytic debenzylation of **9a** in the presence of $NaHCO_3$ led to 3,5-PIP**2** (**2a**), isolated as an amorphous solid by sequential trituration of the residue with EtOAc, Et_2O, and MeOH, and characterized by $^1H/^{31}P$ NMR.



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

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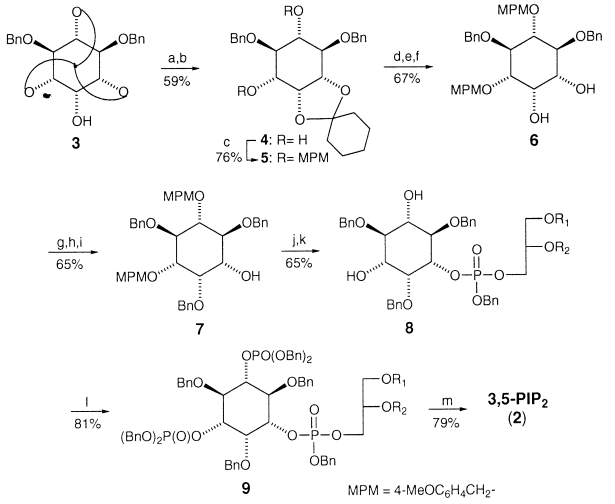
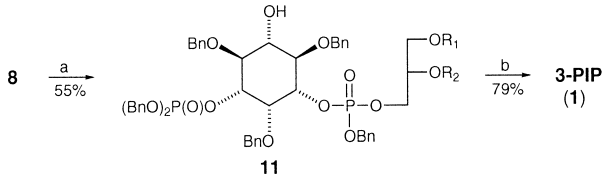


Scheme 1. (a) MeOH/10 N HCl (12.5/1), 65 °C, 0.45 h (87%); (b) cyclohexanone (2 equiv), PTSA, DMF/$PhCH_3$ (1/4), 140 °C, 12 h (68%); (c) MPM-Cl, NaH, DMF, 23 °C, 6 h (76%); (d) MeOH/CH_2Cl_2, 12 N HCl (4/1/1), 23 °C, 1 h (83%); (e) *S*-(−)-camphanic chloride, DMAP, EI_3N, CH_2Cl_2, 23 °C. 12 h (91%); (f) MeOH, KOH, 23 °C, 14 h (88%); (g) *n*-Bu_2SnO, PhH, 85 °C, 6 h; CH_2=$CHCH_2Br$, CsF, DMF, 23 °C, 12 h (86%); (h) BnBr, NaH, DMF, 23 °C, 4 h (86%); (i) $Rh(Ph_3P)Cl$, DABCO, EtOH, 78 °C, 4 h; 1 N HCl/acetone (1/9), 0.5 h (88%); (j) Phosphoramidite **10,** 1*H*-tetrazole, CH_2Cl_2, 23 °C, 2 h; *m*-CPBA, −40 °C, 1 h (81%); (k) DDQ, CH_2Cl_2/H_2O (9/1), 23 °C, 4 h (80%); (l) $(iPr)_2NP(OBn)_2$, 1*H*-tetrazole, CH_2Cl_2, 23 °C, 2 h; *m*-CPBA, −40 °C, 1 h (81%); (m) Pd black, H_2 (52 psi), $NaHCO_3$ (5 equiv), EtOH/H_2O (6/1), 23 °C, 6 h (79%).



Scheme 2. (a) $(iPr)_2NP(OBn)_2$ (1 equiv), 1*H*-tetrazole, CH_2Cl_2, 23 °C, 2 h; *m*-CPBA, −40 °C, 1 h (55%); (b) Pd black, H_2 (52 psi), $NaHCO_3$ (5 equiv), EtOH/H_2O (6/1), 23 °C, 6 h (79%).

Alternatively, selective phosphorylation of the C(3)-alcohol in **8a** utilizing a limited amount of reagent provided convenient access to 3-PIP (**1a**) following the standard deprotection procedure described above (Scheme 2).

Repetition of the phosphorylation of **7** using **10b,c**[12c] and final elaboration of the adducts **8b,c** as outlined in Schemes 1 and 2 afforded **1b,d** and **2b,d,** respectively. The dioctanoyl glyceryl analogues (**b** series) are more water soluble than the fatty acid versions (**a** series) and have proven more tractable in some assays;[19] both are able to compete with natural material for binding to PIP binding proteins. The ω-aminoalkyl analogues (**d** series) have been useful for the introduction of fluorescent, radioactive, and affinity labels.[12c]

Acknowledgements

Supported financially by the Robert A. Welch Foundation and NIH (GM31278, GM37922).

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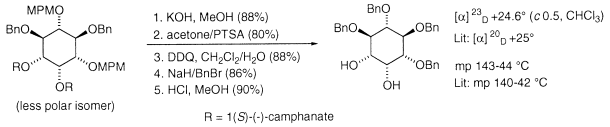

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