Pergamon

Tetrahedron Letters, Vol. 38, No. 27, pp. 4729-4730, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00950-7

Concise Synthesis of L-α-Phosphatidyl-D-*myo*-inositol 3,4-Bisphosphate, An Intracellular Second Messenger

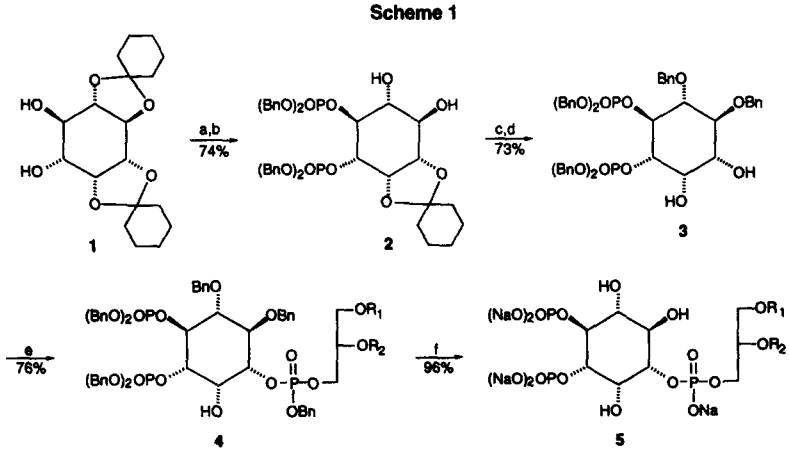
K. Kishta Reddy, Josep Rizo, and J.R. Falck*
Departments of Biochemistry and Pharmacology,
University of Texas Southwestern Medical Center
Dallas, Texas 75235

Abstract: A highly efficient, asymmetric total synthesis of the title phospholipid as well as short chain diester and cross-linkable diether analogs was achieved in six steps from the readily available cyclitol **1**. © 1997 Elsevier Science Ltd.

Recent studies[1] indicate phosphatidylinositol (PtdIns) 3-kinase plays a crucial role in a variety of cellular responses ranging from mitogenesis[2] and vesicular traffic[3] to actin polymerization[4] and glucose uptake.[5] However, in contrast to the canonical PtdIns turnover pathway[6] which generates the second messengers inositol 1,4,5-trisphosphate and diacylglycerol, the 3-phosphoinositide products of PtdIns 3-kinase are not hydrolyzed by phospholipase C, but rather act directly as intracellular mediators. Furthermore, the homologous 3-phosphoinositides are apparently regulated separately, and thus, may subserve different physiologic functions. Frech et al,[7] for instance, found PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 have opposite effects on the activity of RAC/PKB leading to the suggestion that these protein kinases are regulated by the cellular 3-phosphoinositide composition.

Herein, we report the asymmetric synthesis of dihexadecanoyl L-α-phosphatidyl-**D**-*myo*-inositol 3,4-bisphosphate[8] (**5a**) via a highly efficient route which compliments our prior preparation of the higher homologue PtdIns-3,4,5-P_3.[9] To expedite the identification of cellular targets and regulatory agents, we also describe the short chain diester[10] and cross-linkable diether analogs **5b** and **5c** (R = H), respectively.

Scheme 1



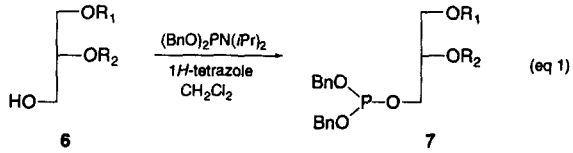
a : R_1 = C(O)$(CH_2)_{14}CH_3$, R_2 = C(O)$(CH_2)_{14}CH_3$ **b** : R_1 = C(O)$(CH_2)_6CH_3$, R_2 = C(O)$(CH_2)_6CH_3$ **c** : R_1 = $(CH_2)_7CH_2NHR$, R_2 = $(CH_2)_7CH_3$

Reagents and conditions: (a) *i*-$Pr_2NP(OBn)_2$ (4 equiv), 1*H*-tetrazole (8 equiv), CH_2Cl_2, 24°C, 2 h; *m*-CPBA (6 equiv), -40°C, 0.5 h. (b) Dry HCl (0.01 equiv), MeOH/CH_2Cl_2 (2:5), 0°C, 16 h. (c) $PhCH_2OC(NH)CCl_3$, Ph_3CBF_4 (5 mol%), Et_2O, 24°C 32 h. (d) CF_3CO_2H/CH_2Cl_2/MeOH (1.5:3:0.5), 0°C, 0.5 h. (e) 7, py-HBr_3, CH_2Cl_2/py/Et_3N (1:0.1:0.05), -20°C, 2 min; 0°C, 0.5 h. (f) H_2 (50 psi), Pd black, tBuOH/H_2O (7:1), 24°C, 14 h.

Chiral cyclitol **1**, prepared in 3 steps from *myo*-inositol according to Wyss[11] and Chen,[12] was phosphatidylated with O,O-dibenzyl-N,N-diisopropylphosphoramidite (Scheme 1), then subjected to *in situ* oxidation at low temperature by *m*-chloroperoxybenzoic acid (*m*-CPBA). Mild acidic hydrolysis selectively removed the *trans*-cyclohexylidene ketal to give **2** which was advanced to diol **3** by trityl cation promoted[13]

benzylation of the C(5)- and C(6)-alcohols using benzyltrichloroacetimidate followed by facile cleavage of the remaining cyclohexylidene unit at 0°C.

The decisive step, i.e., conversion of **3** into **4**, exploited Watanabe's pyridinium perbromide methodology[14] for the *in situ* activation of 1,2-di-O-hexadecanoyl-*sn*-glyceryl dibenzylphosphite (**7a**) and regioselective phosphorylation of the C(1)-alcohol. The corresponding phosphoramidite was less satisfactory under a variety of conditions and often gave rise to mixtures of regioisomeric phosphate esters and bis-derivatized products. The structure of **4**[15] was cogently confirmed by extensive NMR analysis (2D double-quantum filtered correlation, total correlation, and heteronuclear single quantum correlation spectroscopy experiments). Finally, exhaustive debenzylation by catalytic hydrogenolysis over Pd black in *t*-BuOH/H_2O afforded **5a**, isolated as its sodium salt.



6 **7** (eq 1)

Phosphite **7a** (eq 1) was conveniently prepared by condensation (1*H*-tetrazole, 23°C, 0.5 h; 90%) of 1,2-dihexadecanoyl-*sn*-glycerol[9] (**6a**) with O,O-dibenzyl-N,N-diisopropylphosphoramidite (1.8 equiv); after aqueous workup, the phosphite was sufficiently pure to be used in the next step. Likewise, the known[9] glycerols **6b** and **6c** (R = Cbz) provided access to **5b** and **5c** (R = H) following the above sequence.

Acknowledgment: Supported by the Robert A. Welch Foundation (I-782) and NIH (GM31278, NS33731).

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15. Spectral data for **4**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, J = 6.7 Hz, 3 H), 0.87 (t, J = 6.7 Hz, 3 H), 1.16-1.32 (m, 48 H), 1.45-1.58 (m, 4 H), 2.16-2.25 (m, 4 H), 3.44-3.52 (m, 1 H), 3.81 (dd, J = 5.5, 7.9 Hz, 1 H), 3.86-3.92 (m, 1 H), 3.94 (dd, J = 6.2, 8.3 Hz, 1 H), 3.90-4.12 (m, 2 H), 4.22-4.34 (m, 2 H), 4.62-4.68 (m, 2 H), 4.73-5.08 (m, 15 H), 7.02-7.06 (m, 2 H), 7.14-7.33 (m, 33 H). PtdIns **5a**: ^{1}H NMR (250 MHz, D_2O) δ 0.80 (t, J = 6.9 Hz, 6 H), 1.14-1.33 (m, 48 H), 1.45-1.64 (m, 4 H), 2.34 (t, J = 7.3 Hz, 2 H), 2.38 (t, J = 7.3 Hz, 2 H), 3.49 (t, J = 9.0 Hz, 1 H), 3.75 (t, J = 9.7 Hz, 1 H), 3.81-3.99 (m, 2 H), 4.00-4.16 (m, 3 H), 4.23 (dd, J = 7.3, 12.4 Hz, 1 H), 4.42 (d, J = 14.6 Hz, 2 H), 5.20-5.34 (m, 1 H); ^{31}P NMR (202 MHz, D_2O, 85% H_3PO_4 as external reference) δ 0.20, 4.20, 5.62.

(*Received in USA* 8 *April* 1997; *revised* 2 *May* 1997; *accepted* 5 *May* 1997)