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Synthesis of 1-*O*-Alkyl- and 1-*O*-Acyl-*myo*-inositol 3, 4, 5-trisphosphates as Novel Analogues of Phosphatidyl-*myo*-inositol 3, 4, 5-trisphosphate

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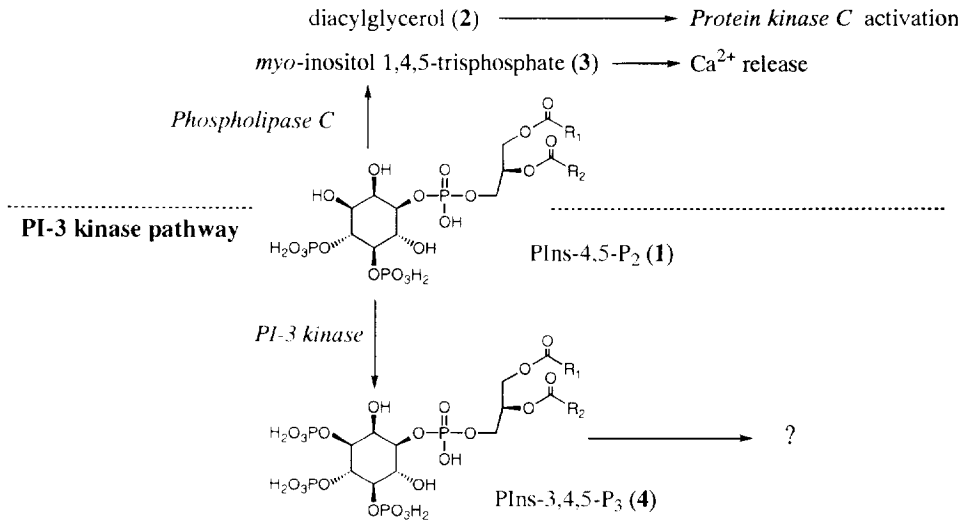
Abstract: Homochiral 1-*O*-alkyl and 1-*O*-acyl derivatives of *myo*-inositol 3,4,5-trisphosphate were designed and synthesized as novel analogues of phosphatidyl-*myo*-inositol 3,4,5-trisphosphate. 1-*O*-Octadecyl and 1-*O*-stearoyl analogues inhibited the synthesis of phosphatidyl-*myo*-inositol 3,4-bisphosphate from phosphatidyl-*myo*-inositol 3,4,5-trisphosphate catalyzed by PIns-3,4,5-P_3 5-phosphatase. These compounds may act as competitors in the enzyme reaction, suggesting that they may be useful in studies of the role of phosphatidyl-*myo*-inositol 3,4,5-trisphosphate as a candidate second messenger in cellular signal transduction.

Polyphosphoinositides are important in cellular signal transduction.[1] Phospholipase C, which is activated upon stimulation with many growth factors, hydrolyzes phosphatidyl-*myo*-inositol 4,5-bisphosphate (**1**) to produce diacylglycerol (**2**), which activates protein kinase C, and *myo*-inositol 1,4,5-trisphosphate (**3**), which releases Ca^{2+} ions from intracellular storage sites. Recently, it has been shown that phosphatidyl-*myo*-inositol (PI) 3 kinase catalyzes phosphorylation of **1** to phosphatidyl-*myo*-inositol 3,4,5-trisphosphate (**4**)[2], which is resistant to known phospholipase Cs. No enzyme that hydrolyzes **4** has been found yet, suggesting that **4** may be metabolized through a different pathway from the classical phosphatidyl-*myo*-inositol turnover. Stephenes et al. reported an enzyme that dephosphorylates phosphatidyl-*myo*-inositol 3,4,5-trisphosphate to produce phosphatidyl-*myo*-inositol 3,4-bisphosphate[3], but no other enzyme that metabolizes this compound has been found so far. Although several reports on the syntheses[4] and roles[5] of **4** have appeared, the role of **4** is not yet clear. In order to obtain some informations on the role and function of **4**, we designed and synthesized the novel analogues of **4**.

We hypothesized that the diacylglycerol moiety of **4** functions for hydrophobic interaction with the cell membrane, and could be substituted with simple lipophilic alkyl or acyl chains, and we also considered that the *myo*-inositol trisphosphate moiety facing the cytosol should play an essential role in the function of **4**. On the basis of this assumption, we synthesized a series of 1-*O*-alkyl- and 1-*O*-acyl-*myo*-inositol 3,4,5-trisphosphates as analogues of **4** (**Scheme 2**).

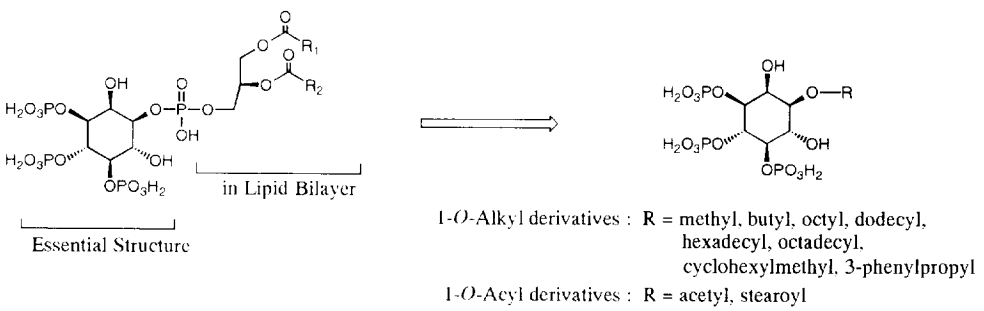
Scheme 1

Classical pathway

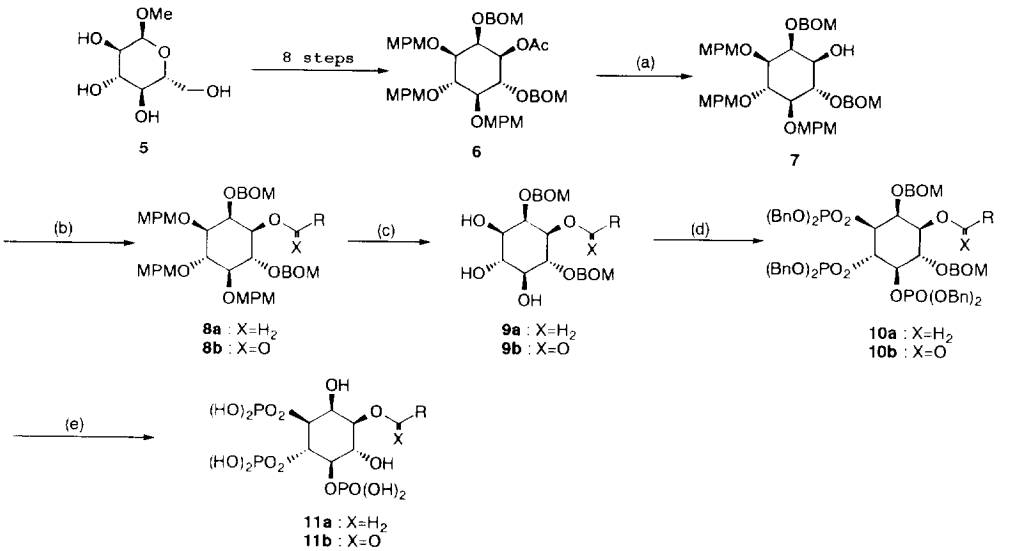


Synthesis of these 1-*O*-alkyl- and 1-*O*-acyl-*myo*-inositol 3,4,5-trisphosphates is illustrated in **Scheme 3**. The common key intermediate, 2,6-di-*O*-benzyloxymethyl-3,4,5-tri-*O*-*p*-methoxybenzyl-*myo*-inositol (**7**), was prepared from methyl-α-D-glucopyranoside according to the reported procedure.[6] The alcohol (**7**) was alkylated with the corresponding alkyl halides (methyl iodide for a 1-*O*-methyl derivative; alkyl bromide for others) in the presence of KOH in dimethylsulfoxide[7] or acylated with stearic anhydride in the presence of 4-(dimethylamino)pyridine in pyridine. The 1-*O*-acetyl derivative was directly obtained from 1-*O*-acetyl-2,6-

Scheme 2



Scheme 3

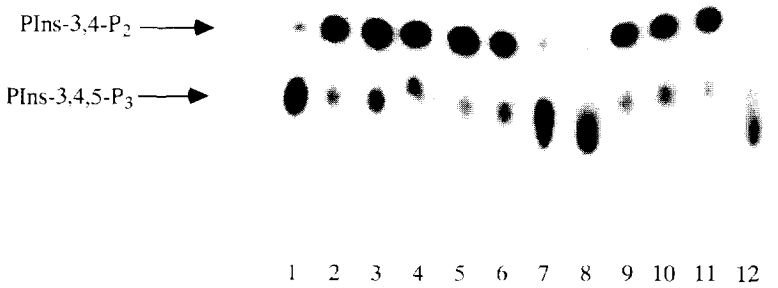


Reagents and conditions (a) NaOH, MeOH, reflux, 1 h (89 %) ; (b) for alkylation : alkyl bromide or iodide, KOH, DMSO, r.t., 18 h (25 -100%) ; for acylation : acid anhydride, DMAP, pyridine, r.t., 36 h (51 %) ; (c) DDQ,CH_2Cl_2, r.t., 1 h (70.5-100 %) ; (d) tetrazole, dibenzyl *N,N*-diethylphosphoramidite, CH_2Cl_2, r.t., 30 min, then *m*CPBA, -40°C to r.t., 18 h (28-96%) ; (e) H_2, 10% Pd-C, 50 psi, 95 % EtOH, r.t., 5.5 h, (56-100%)

di-*O*-benzyloxymethyl-3,4,5-tri-*O*-*p*-methoxybenzyl-*myo*-inositol (**6**), an intermediate for the synthesis of **7**. Removal of the *p*-methoxybenzyl groups with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in wet dichloromethane gave the triols (**9**). These were phosphitylated with dibenzyl *N,N*-diethylphosphoramidite and the resulting trisphosphites were oxidized with *m*-chloroperbenzoic acid to give the desired fully-protected trisphosphates (**10**). All protective groups of **10** were removed by hydrogenolysis (10% Pd/C, 50 psi) and the filtrates were neutralized with diluted ammonium hydroxide to give 1-*O*-alkyl and 1-*O*-acyl-*myo*-inositol 3,4,5-trisphosphate ammonium salts[8].

To see whether or not these derivatives serve as analogues of phosphatidyl-*myo*-inositol 3,4,5-trisphosphate, we examined their effect on PIns-3,4,5-P_3 5-phosphatase (**Figure 1**), recently isolated by us[9]. 1-*O*-Hexadecyl, 1-*O*-octadecyl and 1-*O*-stearoyl derivatives completely inhibited this enzyme activity. In contrast, the 1-*O*-dodecyl derivative showed only a weak effect and others with shorter side chains had no activity. The results suggested that the presence of a long lipophilic side chain is necessary for PIns-3, 4, 5-P_3 5-phosphatase-inhibitory activity. These novel 1-*O*-alkyl and 1-*O*-acyl-*myo*-inositol 3,4,5-trisphosphates may be applicable for the preparation of biochemically useful tools, such as fluorescent probes or photoaffinity labeling reagents, for further studies of the role of phosphatidyl-*myo*-inositol 3,4,5-trisphosphate in cellular signal transduction.

Figure 1



The inhibitory activity of 1-*O*-alkyl and 1-*O*-acyl derivatives on PIns-3,4,5-P_3 5-phosphatase. PIns-3,4,5-P_3 was prepared from phosphatidyl-*myo*-inositol 4,5-bisphosphate and [γ-^{32}P]ATP by purified PI 3-kinase. Effects of various compounds on PIns-3,4,5-P_3 5-phosphatase activity were assayed by using partially purified enzyme from bovine thymus (see ref. 9). The reaction mixtures (30 μl) contained PIns-3,4,5P_3 (20 nM), phosphatidylserine (200 μg/ml), 20 mM Tris/HCl (pH 7.5), 150 mM NaCl, 1 mM $MgCl_2$, 0.3 % octylglucoside, partially purified enzyme, and PIP_3-analogue (0.5 mM). After incubation for 10 min at 25°C, the reaction was stopped by the addition of chloroform / methanol / HCl (200 / 100 / 1). Then, 1N HCl was added, and the mixture was separated into two phases by centrifugation. Organic phase was spotted onto a Silica Gel 60 Plate (Merck), which was developed in chloroform / methanol / acetone / acetic acid / H_2O (40 / 15 / 13 / 12 / 7). Lipids were visualized by autoradiography. Lane 1: no enzyme; 2: control; 3: methyl; 4: butyl; 5: octyl; 6: dodecyl; 7: hexadecyl; 8: octadecyl; 9: 3-phenylpropyl; 10: cyclohexylmethyl; 11: acetyl; 12: stearoyl.

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REFERENCES AND NOTES

1. Potter, B.V.L. *Natural Product Reports*, **1990**, 1.
2. Kapeller, R.; Cantley, L.C. *BioEssays*, **1994**, *16*, 565.
3. Stephenes, L.R.; Hughes, K.T.; Irvine, R.F. *Nature*, **1991**, *351*, 33.
4. Falck, J.R.; Abdali, A. *Inositol Phosphates and Derivatives, Synthesis, Biochemistry and Therapeutic Potential*; Reitz, A.B., Ed.; American Chemical Society: Washington, DC, **1991**; pp 145-154. Watanabe, Y.; Hirofuji, H.; Ozaki, S. *Tetrahedron Lett.*, **1994**, *35*, 123. Gou, D.M.; Chen, C.-S.; *J. Chem. Soc., Chem. Commun.*, **1994**, 2125. Reddy, K.K.; Saaddy, M.; Falck J.R.; Whited, G.; *J. Org. Chem.*, **1995**, *60*, 3385.
5. Singh, S.S.; Chauhan, A.; Brockerhoff, H; Chauhan, V.P.S. *Biochem. Biophys. Res. Commun.*, **1993**, *195*, 104. Nakanishi, H.; Brewer, K.A.; Exton, J.H. *J. Biol. Chem.*, **1993**, *268*, 13. Toker, A.; Meyer, M.; Reddy, K.K.; Falck, J.R.; Aneja, R.; Aneja, S.: Parra, A.; Burns, D.J.; Ballas, L.M.; Cantley, L.C. *J. Biol. Chem.*, **1994**, *269*, 32358.
6. Esteves, V.A.; Prestwich, G.D. *J. Am. Chem. Soc.*, **1991**, *113*, 9885. Bender S.L.; Budhu R.J.; *J. Am. Chem. Soc.*, **1991**, *113*, 9883.
7. Johnstone, R.A.W.; Rose, M.E. *Tetrahedron*, **1979**, *35*, 2169.
8. All synthetic intermediates were characterized by ^{1}H-NMR, FABMS, HRMS, IR and optical rotation measurements. The final products were characterized by ^{1}H-NMR, ^{31}P-NMR, negative FABMS and optical rotation as the corresponding ammonium salts.
   Representative data for selected compound: 1-*O*-dodecyl-*myo*-inositol 3,4,5-trisphosphate, ^{1}H-NMR: (500 MHz, D_2O) δ : 0.76 (t, 7Hz, 3H), 1.14-1.26 (br, 18H), 1.50 (m, 2H), 3.31 (brd, 10Hz, 1H, inositol), 3.44 (brm, 1H), 3.61 (brm, 1H), 3.74 (brm, 1H, inositol), 3.89-4.00 (brm, 2H, inositol), 4.30 (brm, 1H, inositol), 4.34 (br, 1H, inositol). ^{31}P-NMR: (205.5 MHz, D_2O) δ: 0.55, 1.07, 1.34. Negative FABMS: 587 (M-H), 609 (M-2H+Na). $[\alpha]_D^{25}$ =-11.8° (c=0.30, H_2O).
9. Kabuyama, Y.; Homma, Y.; Fukui, Y. Submitted for publication.

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